# The Mode of Binding of Phenylalkylamines to the Serotonergic Receptor

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## INTRODUCTION

In recent years there has been an increasing interest in the study and analysis of the relation between molecular structure and biological activity in phenylethylamines and phenylisopropylamines. For the sake of simplicity, we shall present the main results of those studies divided in three parts:

### Experimental and Theoretical Conformational Studies

The analysis of the crystalline structure of some $\beta$-phenylethylamines (PEA) and phenylisopropylamines (AMP) clearly shows that the ethyl and isopropyl side chains are almost perpendicular to the plane of the aromatic ring (1-6). Also, it was noted that when the molecule has three methoxy groups that are not contiguous, these groups are coplanar with the ring (3). In the case where there are three contiguous groups, the central one is perpendicular to the aromatic ring (6).

On the other hand, $^{13}C$ Chemical shift and spin-lattice relaxation time measurements were employed to study the conformations around the Ar-$OCH_3$ bond of the arylmethoxy groups in a series of substituted PEA (7). The results indicate that the -$OCH_3$ groups with two ortho substituents are in the out-of-plane conformation, and that those with one or no ortho substituents exist in the planar conformation.

Also, NMR studies on amphetamine and some of its derivatives (8) showed that in the rotamer populations (P) there is a high preference of the trans-phenylamino rotamer ($P_1 = 0.45$, $P_2 = 0.50$, $P_3 = 0.05$ in Fig. 1). NMR data for PEA shows that the rotamer population is $P_2 = 0.56$, $P_1 + P_3 = 0.44$ (9). These results are in agreement with PMR studies (10) and theoretical calculations (11, 12).

INDO calculations on the mescaline cations give two minimal energy conformations for $\tau_1 = 90^\circ$, $\tau_2 = 60^\circ$ and $\tau_1 = 90^\circ$ and $\tau_2 = 180^\circ$ (for the definitions of the torsional angles see Fig. 2 and (13).

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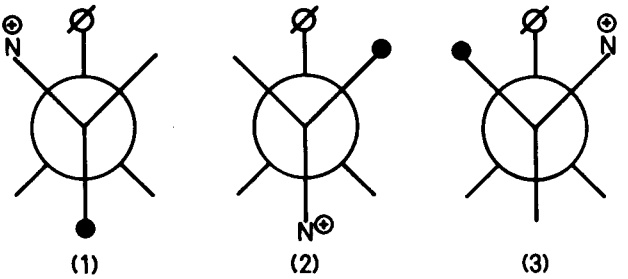


Fig. 1. Rotamers of the phenylisopropylamine.

PCILO calculations (14) showed that energy minima appears for conformers for which $\tau_1 \sim \pm 90^\circ$. In the case of PEA, the prefered conformers are the trans and gauche ones, and in AMP the most stable rotamer is the gauche one. These calculations are in agreement with *ab initio* studies (15).

Weintraub *et al.* (16) analyzed the conformational properties of a number of methyl-substituted phenethylamines with a set of empirical potential functions. Their results show that the molecules adopt one of the two stable conformers: a folded one with the amine chain perpendicular to the aromatic ring and the amine group nearest to the ring and another conformation with the amine chain perpendicular to the ring and the amine group far from the ring. This last conformation is preferred by the cation in aqueous solution.

Phenethylamine, in both neutral and protonated ($PEAH^+$) forms, was studied with *ab-initio* (STO-3G) and semiempirical methods (EHT, CNDO/2, INDO and PCILO) (17). For PEA, all the methods give conformational minima for values of $(\tau_1, \tau_2, \tau_3)$ equal to $(90, -60, -60)$, $(90, 60, 60)$, $(90, 180, 60)$ and $(90, 180, 180)$. For $\tau_1 . \neq 90^\circ$, the PCILO method gives a large flat basin located at (65-135, 180, 60). For $PEAH^+$, the EHT method gives the absolute minimum at (90, 180, 60), *i.e.* the extended form. Another minimum appears at (90, 60, 60), and the conformations with $\tau_2 < 60^\circ$ are not favored.

Recently (18), Anderson *et al.* reexamined, with the PCILO method, the applicability of the above conformational predictions for a series of 2-X-substituted-4,5-dimethoxy-phenylisopropylamines. Their calculations predict that in the gas phase or in an inert solvent, the folded gauche conformation ($\tau_1 = 90^\circ, \tau_2 = 60^\circ$) is about 1 Kcal/mole more stable than the extended trans conformation ($\tau_1 = 90^\circ, \tau_2 = 180^\circ$).

Kumbar (19) investigated with the empirical method the effect of the dialectric constant on the conformational behavior of six phenethylamines (PEA and AMP between them). He concluded that the nature of the solvent medium is critical in assessing percent population but not in describing the energy minima.

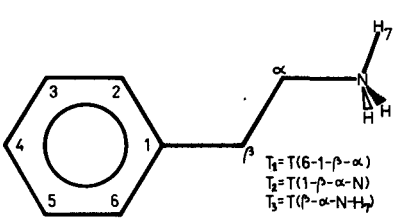


Fig. 2. Definition of the torsional angles.

The comparison of the energies of the trans and gauche conformers (18) and the examination of the minimum energy path connecting the minima of PEA and $PEAH^+$ (17) indicate that the side chain is conformationally quite unrestricted. Also, the rotational barriers for the ring substituents are of the order of several Kcal/mole (20).

Considering that it is reasonable to accept that all the rotamers which have an energy up to 6 Kcal/mole over the absolute minima are possible candidates to interact with the receptor (21), the main conclusion of the conformational studies in this case is that the theoretical results are not able to discard large portions of the conformational space such that we can restrict our attention to only a few conformers.

**Experimental and Theoretical Studies of Electronic Structure**

Domelsmith *et al.* have obtained and analyzed the photoelectron spectra of some PEA and AMP (22-24). The principal result is that PEA has three low-energy ionization potentials. Two of them are more or less localized in the aromatic ring and the other is identifiable as the amine lone pair. The difference between the energies of the Highest Occuppied Molecular Orbital (HOMO) and the next HOMO (NHOMO) is small. This analysis is in agreement with CNDO/2 calculations for PEA, 3,4-dimethoxy-PEA and mescaline, showing that the HOMO and NHOMO are localized in the aromatic ring (22). Also the substitution of powerful donors, as methoxy groups, shifts the degeneracies so that the HOMO has the largest LCAO coefficient at the site of methoxylation.

Progressive methoxylation was found to correlate with an increase of HOMO energy and with the superdelocalizability (25). There was a negative correlation between the number of methoxy groups and the energy of the Lowest Empty Molecular Orbital (LEMO), suggesting that progressive methoxylation decreases the capacity of those compounds to act as electron donors (25).

Mulliken population analysis for PEA and $PEAH^+$ (17) shows that the N atom is negatively charged. In the protonated species, two thirds of the positive charge are on the $-NH_3$ group, the N being still negative (17).

**Structure-Biological Activity Studies**

The main effort has been centered on the relation between electronic structure and hallucinogenic effects. As we shall not analyze them, the reader is referred to (26) for details.

Recently, Glennon *et al.* have studied the binding affinities ($pA_2$) of several PEA and AMP derivatives for the 5-HT receptor in the rat fundus stomach (27-32). They found that in general 4-methylation, 4-ethylation and 4-bromination enhance receptor affinity, while N,N-dimethylation of the terminal amine decreases affinity (27). Also, in disubstituted compounds, methoxy groups at the 2 and 5 positions are optimal for giving a high affinity.

Although it is possible to appreciate some trends, in Glenon's words, "There does not appear to be any simple and straight-forward SAR which would explain the affinities of the compounds examined" (27).

Kang and Green noted that the structure of LSD possesses both the phenylalkylamine and the indolealkylamine molecular subfragments (33). For this reason, it has been suggested that phenylalkylamines and tryptamine derivatives mimic partially or totally the LSD structure during the interaction with the receptor (Fig. 3). This particular position demands that the amine side chain of these molecules adopts a conformation in which it remains close to the aromatic ring's plane. In tryptamines there are no problems, but for phenethylamines, one interesting fact appears. Some of the o- and m-substituted

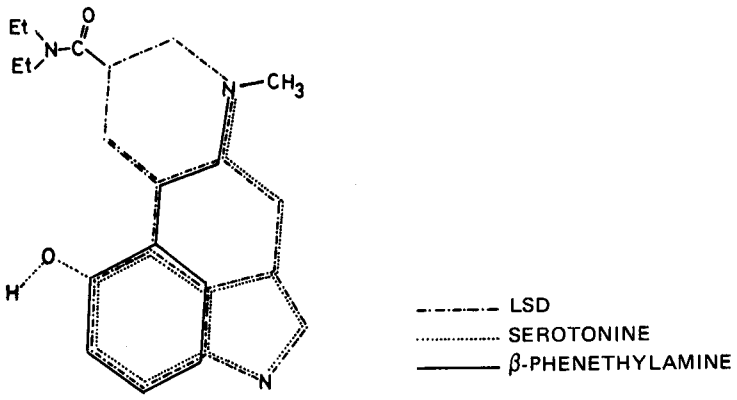


Fig. 3. Phenylethylamine and tryptamine structures superimposed over a molecule of LSD.

molecules may adopt two different positions (A and B in Fig. 4) to interact with the receptor. Strictly, there is no reason *a priori* to consider one of these positions more aceptable than the other.

With the aim to provide evidence in favour of one of these conformations, we have undertaken a semiempirical quantum-chemical study of a group of phenethylamines.

Our interests are the following:

1. To analyze the effect of the protonation on the electrostatic potential (EP) and on the electronic density distribution of the NHOMO, HOMO and LEMO for different rotamers.

2. The study and comparison of the EP maps for rotamers A and B of Figure 4 in a set of phenylethylamines.

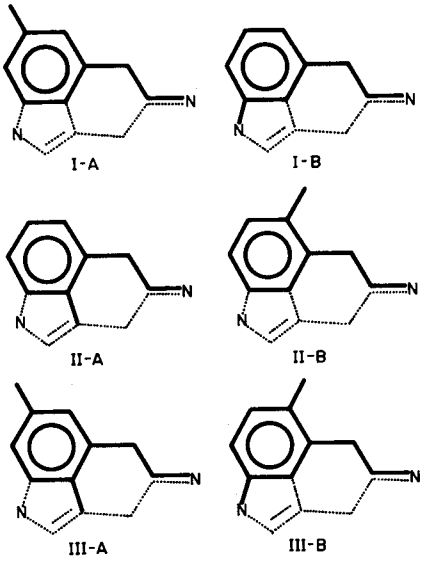


Fig. 4. Possible conformers for mono – and disubstituted phenylalkylamines.

3. The analysis of the electronic distribution for rotamers of type A and B.

4. Considering that the rat fundus is an *in vitro* preparation, the measured binding affinities will reflect only the binding energy between the drugs and the receptor. Also, given that there is a certain similarity between the ability of the phenylalkylamines to interact with the rat fundus 5-HT receptor and the brain's 5-HT binding sites (34-37), the study and the analysis of the relationship between the $pA_2$ and the molecular electronic structure would be of a great help to choose between rotamers A and B, and also to provide data for a rational design of new phenylethylamine derivatives.

With this purpose, we carried out an analysis of the relationship between the variation of electronic structure parameters and the variation of the $pA_2$, employing a new non-empirical quantum-statistical approach.

## METHODS

The study was carried out on the cations given that at physiological conditions the protonated species is the most abundant one. The geometry employed is depicted in Figure 5. The bond distances for the ring's substituents were taken from the work of Niemeyer (36). As we do not know the relative position of the substituents and the amine chain, we shall work with the hypothesis that the phenethylamines mimic more or less the LSD structure during the interaction with the receptor. With this consideration, and for the sake of simplicity, we placed the amine chain in the aromatic plane. The ring's substituents were placed coplanar to the ring.

The electrostatic potential maps represent contours connecting points at which the interaction energy of the unperturbed molecule with a proton is identical. To compute the maps, we obtained first the CNDO/2 molecular wave function (37). Next, we deorthogonalized this wave function (38), carried out a Mulliken population analysis (39) and the resulting atomic charges were employed to obtain the map within a point charge model, where the potential at point r, V(r), is given by:

$$V(\vec{r}) = \sum_i \frac{Q_i}{|\vec{R}_i - \vec{r}|} \qquad (1)$$

$Q_i$ being the net atomic charge at atom i and $|\vec{R}_i - \vec{r}|$ the distance between atom i and point r. Beyond the Van der Waals radius, the map is qualitatively correct (40). This model is useful when we search for similarities in large molecules. We computed the EP in the aromatic plane and, for the sake of comparison, we also obtained the EP map of serotonin.

To examine the effects of the protonation and of a particular position of the substituents on the map's features, we computed the EP map of six conformers of the p-OH-β- phenylethylamine. This conformational study is neccesary because recently we

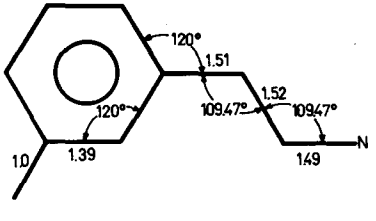


Fig. 5. Skeleton geometry for CNDO/2 calculations.

showed that in the case of protonated molecules the values of the nucleophilic superdelocalizability calculated with the CNDO/2 method are highly dependent on the relative positions of the various substituents and the amine chain (41).

To study the electronic distribution of the NHOMO, HOMO and LEMO, we selected a group of basic and protonated phenethylamines and some tryptamine derivatives for comparison. The computation of the electronic distribution was made according to the formula (42):

$$D_i(\vec{r}) = \sum_{Piq} C_{Pi}\, C_{qi}\, X_P(\vec{r})\, X_q(\vec{r}) \qquad (2)$$

where $C_{pi}$ is the coefficient of the real Slater function $X_p(\vec{r})$ of the i-th one-electron orbital. The MAPDEN program was employed (43).

The search for a relationship between the equilibrium constant and the electronic structure factors was carried out within the following model. Let us consider the weak interaction of a drug, $D_i$, and a macromolecular receptor R. If we accept as a working hypothesis that (44): a) the receptor's conformation is so strongly preferred that the binding energy is accounted for entirely in terms of local atomic interactions, b) the total molecular partition functions can be factorized in terms of independent and uncoupled translational, rotational, vibrational and electronic partition functions, c) only the electronic ground state is important in the electronic partition function, it is possible to show formally that the drug-receptor equilibrium constant, $K_i$, may be expressed as (44):

$$\log(K_i) = a + b \log(M_i) + c \log(\sigma_i/(ABC)_i^{3/2}) + d\,\Delta E_i \qquad (3)$$

where a, b, c, d are constants, M, $\sigma$ and (ABC) are, respectively, the mass, the symmetry number and the product of the three moments of inertia about the three principal axes of rotation, "i" refers to the i-th drug molecule and $\Delta E_i$ is the difference between the ground state energy of the complex and the energies of $D_i$ and R:

$$\Delta E_i = E_{D_i R} - (E_{D_i} + E_R) \qquad (4)$$

In a first approach, we shall accept that the only important component of $\Delta E_i$ is the change in the electronic energy, $\Delta E_i^e$. Considering that the drug-receptor interaction is weak, we may perform a perturbational treatment for the evaluation of $\Delta E_i^e$ (45). In this way, and after some approximations coming from the fact that we do not know the molecular structure of the receptor, Peradejordi *et al.* showed that $\Delta E_i^e$ may be approximated as (46):

$$\Delta E_i^e = q + \sum_{P_i} (f_{P_i} Q_{P_i} + g_{P_i} S_{P_i}^E + h_{P_i} S_{P_i}^N) \qquad (5)$$

where q, $f_{Pi}$, $g_{Pi}$, $h_{Pi}$ are constants, $Q_{P_i}$, $S_{P_i}^E$ and $S_{P_i}^N$ are, respectively, the net charge, the total electrophilic superdelocalizability and the total nucleophilic superdelocalizability of atom "p" in molecule "i" (47). The summation on p is over a set of atoms common to all the drugs that interact with the receptor.

Equation 5 has been employed with success in the analysis of structure-activity relationships in a variety of biologically active compounds (46, 48, 49).

Recently, we showed that eq 5 was incomplete, and we proposed as a more exact

expression the following (J.S. Gómez-Jeria, submitted for publication):

$$\Delta E_i^e = a + \sum_{p_i} (e_{P_i} Q_{P_i} + f_{P_i} S_{P_i}^P + g_{P_i} S_{P_i}^N) +$$
$$\sum_{p_i} \sum_{m=z}^{occ} \left( h_{p_i}(m) D_{m p_i} + j_{p_i}(m) S_{p_i,m}^E \right) +$$
$$\sum_{p_i} \sum_{m'=1}^{x} \left( r_{p_i}(m') D_{m',p_i} + t_{p_i}(m') S_{p_i,m'}^N \right) \qquad (6)$$

where, a, e, f, g, h, j, r and t are constants, $S_{p_i,m}^E$, $S_{p_i,m'}^N$, $D_{m p_i}$ are, respectively, the electrophilic orbital superdelocalizability of MO m in atom $P_i$, the nucleophilic orbital superdelocalizability of MO m' in atom $P_i$, and the orbital electron density of MO m in atom $P_i$. The summation on m indicates the inclusion of a group of the MO's close to the HOMO and the HOMO itself. The summation on m' includes the LEMO and a group of low-lying MO's. These two groups are to be determined with the help of the statistical analysis.

The physical interpretation of the terms appearing in eq 6 is:

1. The net charges are related to the strength of the electrostatic interaction between the drug and the receptor.

2. The total electrophilic and nucleophilic superdelocalizabilities (TS) serve to compare the reactivity of different molecules and indicate the stabilization energy in the formation of a complex with the receptor.

3. The orbital electron densities (FED) are employed as a measure of the ~~relativity~~ reactivity of the various positions of a molecule. They are related to the availability of an electron density or to an absence, for a partial charge transfer between the interacting molecules. Of particular interest are the FED of the HOMO and LEMO.

4. The orbital superdelocalizabilities (OS) reflect the degree of contribution of the frontier orbitals to the total superdelocalizabilities (see point 2 above). If the frontier orbitals play a significant role in the course of the interaction, the OS's must be better index of reactivity than the TS's.

5. The molecular mass and the term related to the moment of inertia are connected to the steric effect (they will appear when molecules differ in the nature of an atom at a certain position or when molecules differ in the position of an atom).

Inserting eq 6 into eq 3, we obtain a general equation relating the equilibrium constant to molecular structure factors. If this equation is to be satisfied, there must exist a common set of atomic reactivity indexes in all the similar drugs interacting with the same receptor. This allows us to analize the interaction at various levels by the inclusion of very different sets of atoms and properties. The coefficients will be determined by means of multiple regression analysis. From the CNDO/2 molecular wave function, we computed all the properties appearing in eq 6.

We have taken the $pA_2$ value as a good approximation to the equilibrium constant of the drug-receptor complex (50). The $pA_2$ values were obtained from the work of Glennon *et al.* (27-32).

## RESULTS AND DISCUSSION

### Molecular Electrostatic Potentials

As an example, we present in Figures 6 and 7 the EP maps of two basic rotamers of the p-OH-β-phenylethylamine (we ommitted the others for the sake of simplicity), and

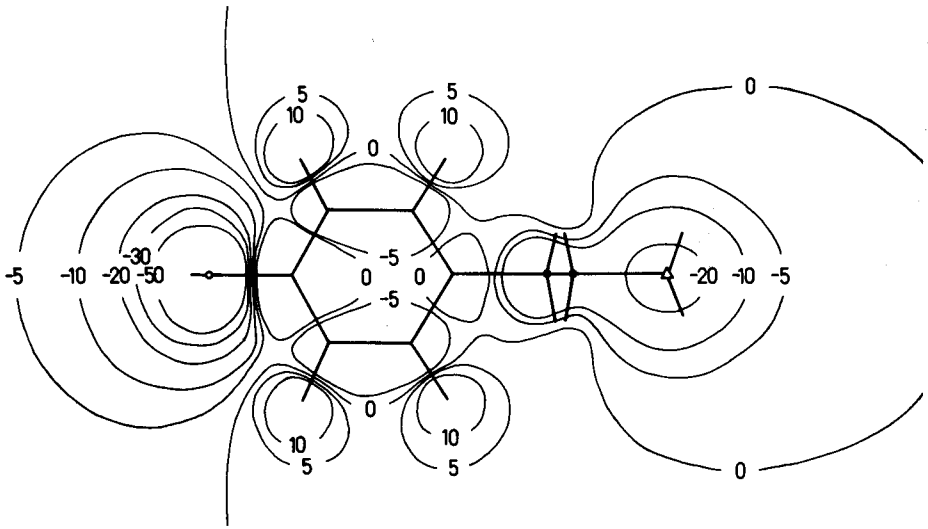


Fig. 6. EP map of the p-OH-β-PEA with the two substituents perpendicular to the ring's plane (the units are Kcal/mol).

in Figures 8 and 9 the EP maps of the same rotamers in their cationic form.

The first fact noted in the maps of the basic rotamers is that the maps' features are highly dependent on the position of the substituents and the amine chain.

The analysis of the EP maps for the basic rotamers leads to a general conclusion: in the basic rotamers, the effects of the position of the various substituents on the map's structure are only of a local character. It may be noted that strong alterations to the EP maps come from lone pairs from the N and O atoms.

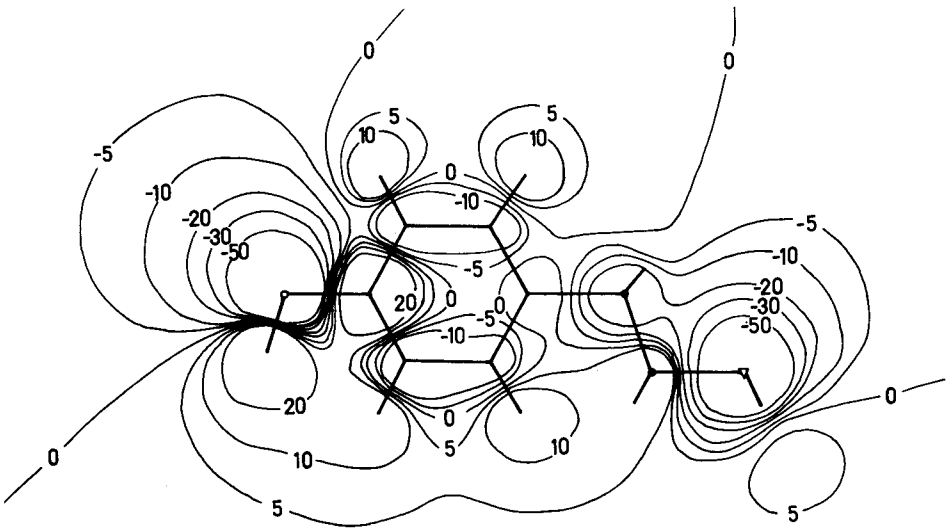


Fig. 7. EP map of the p-OH-β-PEA. The substituents are coplanar with the ring (cis).

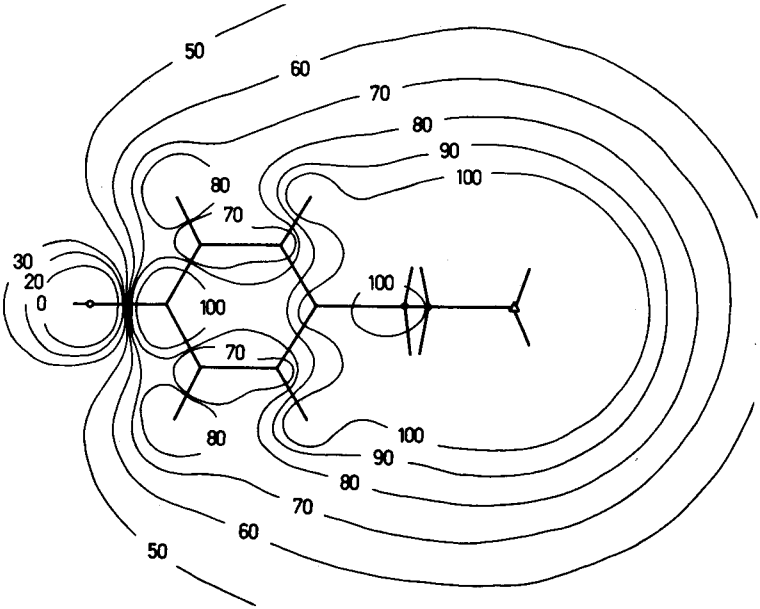


Fig. 8. EP map of the protonated rotamer of Fig. 6.

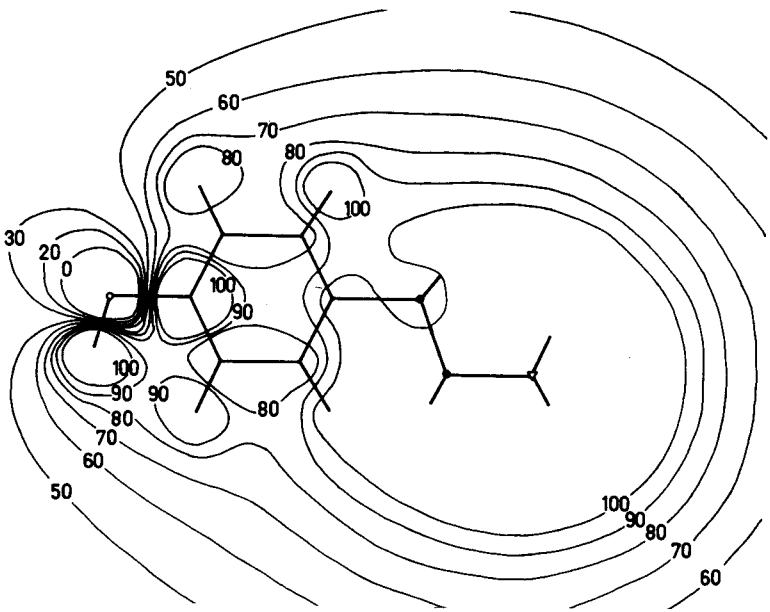


Fig. 9. EP map of the protonated rotamer of Fig. 7.

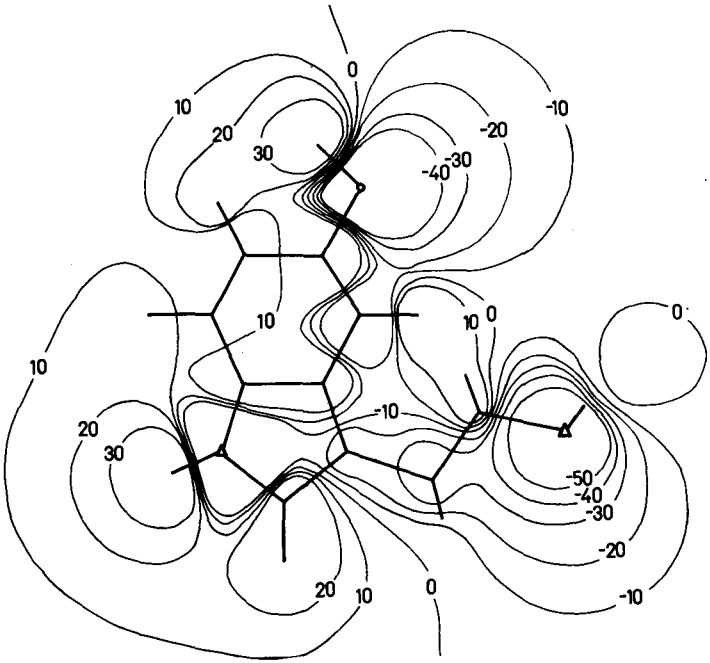


Fig. 10. EP map of the basic serotonin.

Figure 10 shows the EP map of the basic serotonin with the amine chain coplanar to the ring. It is not possible to find similar regions in serotonin and any of the basic rotamers of the p-OH-β-PEA. Nevertheless, if the N lone pairs of the basic rotamers and of serotonin are rotated 180°, it appears a common potential region.

The protonation of the different rotamers produces a very interesting effect, leading to a homogenization of the EP maps (Figs. 8, 9). The EP region around the cationic head shows that in this area there is a large zone of positive potential and that the set of equipotential curves are similar in all the rotamers, the position of the chain having only a very small influence. Analogous results have been obtained for protonated tryptamines (51).

The effect of the –OH group on the EP map continues to have a local character.

Figure 11 displays the EP map of protonated serotonin. Immediately, we see that the EP around the cationic head is very similar to the one in the protonated rotamers of Figures 8 and 9. Based on this similarity, we have suggested (51) that the main effect of protonation could be the formation of a potential zone around the amine chain that can be recognized by the receptor at a distance greater to that at which conformational and electronic perturbation ensue. This recognition may be achieved in Zone II of the Ariën's Model (52).

On the other hand, to analize the effect of the protonation on the EP maps of conformers of type A and B (Fig. 4), we obtained the maps of a group of them. As an example, we present here the following pairs (for the substituent's number, refer to Fig. 2):

a. 3-Methoxy-PEA (Fig. 12)/5-Methoxy-PEA (Fig. 13).

b. 2-Hydroxy-5-Methoxy-PEA (Fig. 14)/3-Methoxy-6-Hydroxy – PEA (Fig. 15).

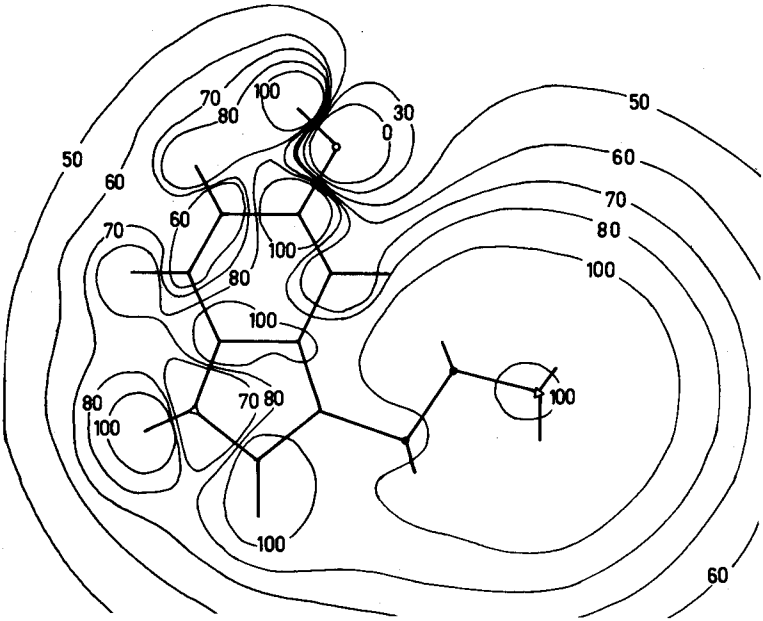


Fig. 11. EP map of the protonated serotonin.

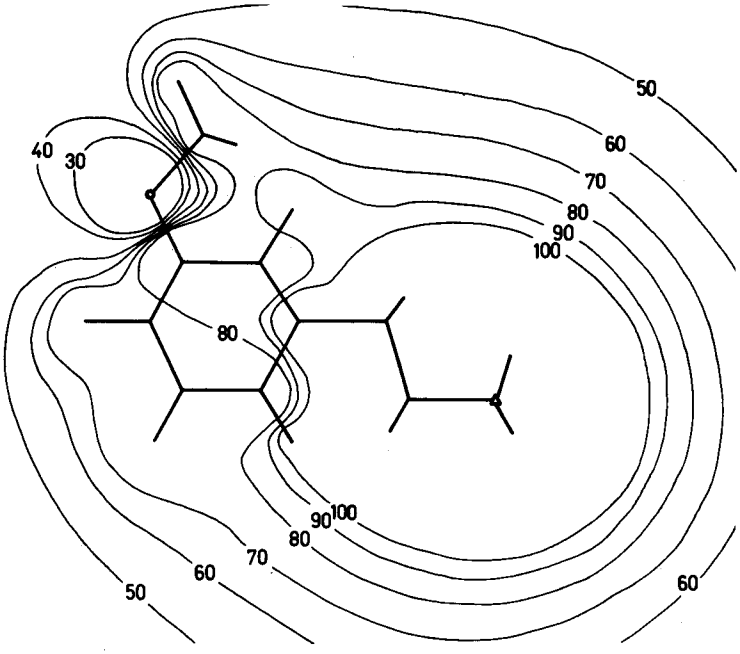


Fig. 12. EP map of the protonated 3-methoxy-β-PEA.

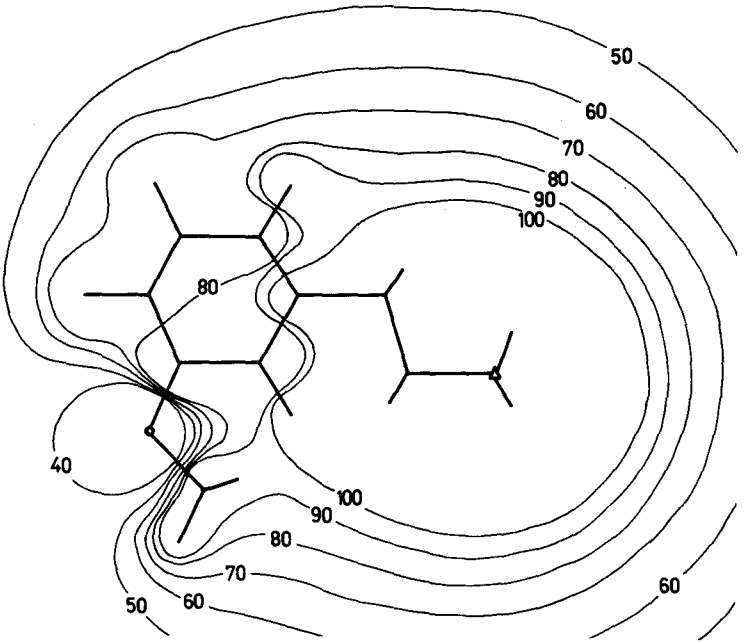


Fig. 13. EP map of the protonated 5-methoxy-β-PEA.

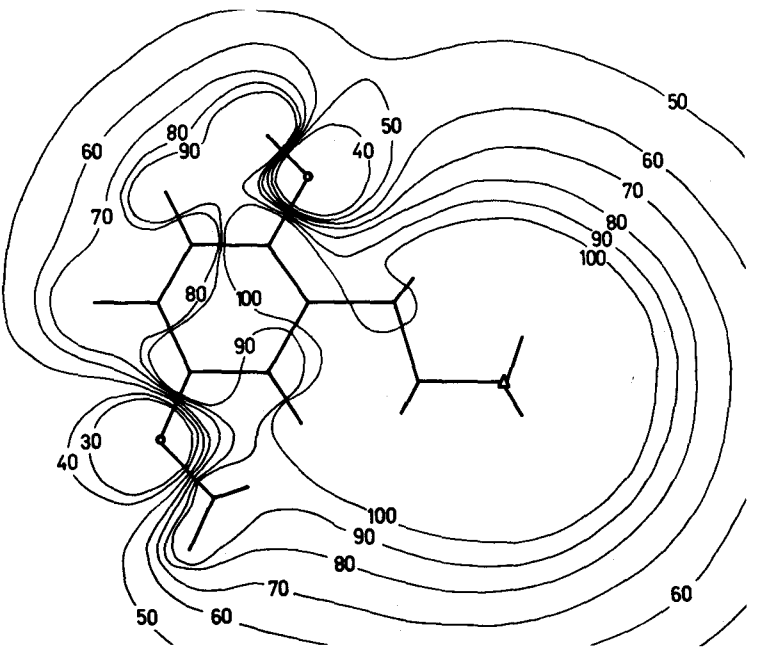


Fig. 14. EP map of the protonated 2-hydroxy-5-methoxy-β-PEA.

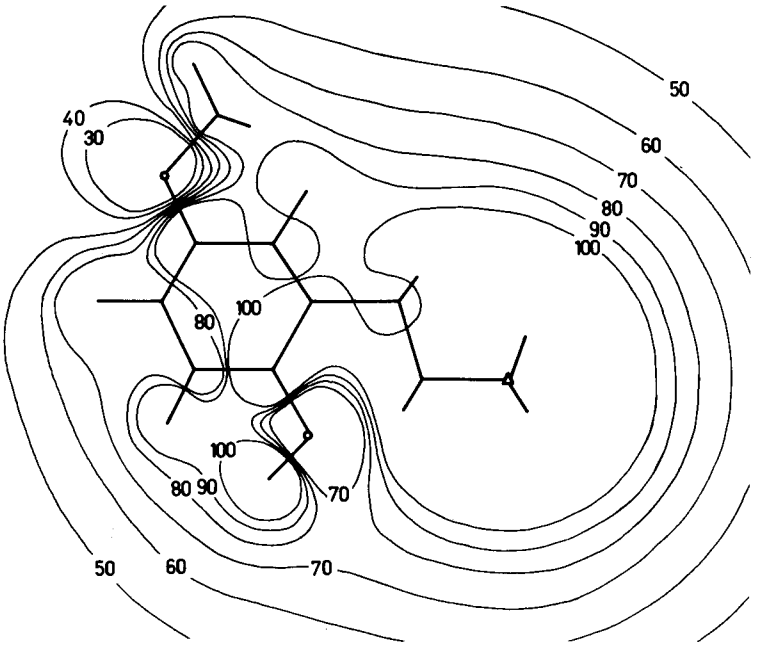


Fig. 15. EP map of the protonated 3-methoxy-6-hydroxy-β-PEA.

Given that serotonin is the natural ligand of the serotonergic receptor, we shall compare the rotamer's maps with the protonated serotonin's EP map.

The comparison of the EP maps in couple (a) indicates that the rotamer that imitates best the serotonin's map is the 5-Methoxy-PEA (Figs. 12, 13).

In the last couple, the situation appears more clear. The comparison of the EP map of the 2-Hydroxy-5-Methoxy-PEA (Fig. 14) with that of the serotonin, shows that the maps are very similar, especially in the region around the –OH groups (Figs. 11, 14). The EP map of the other rotamer (Fig. 15) shows great differences with the serotonin's map around the 6-OH group region.

This short analysis strongly suggests, but does not prove, that the 2,5-disubstituted molecules may interact with the serotonergic receptor in such a way that the o-substituent mimics the 5-OH group of serotonin. The suggestion is supported by the fact that these two molecules have a $pA_2$ of 6.84 and 7.10 respectively (28) (The $pD_2$ of serotonin is 7.46).

The 3,4-Dimethoxy-PEA and the 3-Methoxy-PEA have a $pA_2$ of 5.36 and 5.89 respectively (27).

In conclusion, the EP maps for the 2,5-disubstituted phenylalkylamines suggest that such molecules probably bind to the receptor as the III-B rotamer of Figure 4. As corollary, the o-substituted molecules bind as the II-B rotamers of Figure 4.

Another conclusion of these comparisons is that in the case of the m-substituted compounds, the EP map cannot provide neccesary information to choose any of the two conformers I-A and I-B (Fig. 4) as the possible one at the receptor's level.

**Electronic Density Distribution Analysis**

Electronic density maps provide a useful tool to study bonding, orbital shape, and an insight into the places from where a charge transfer may occur.

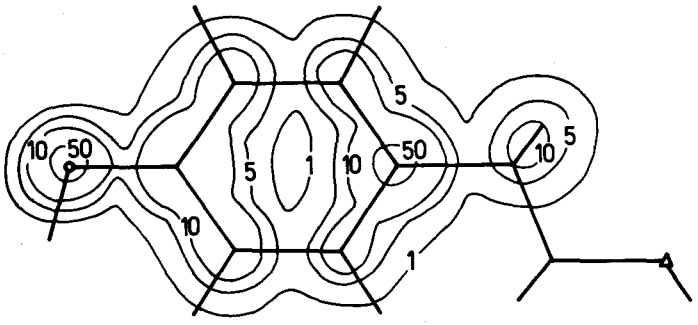


Fig. 16. EDD map of the HOMO of a basic rotamer of the p-OH-β-PEA (in the plane of the ring).

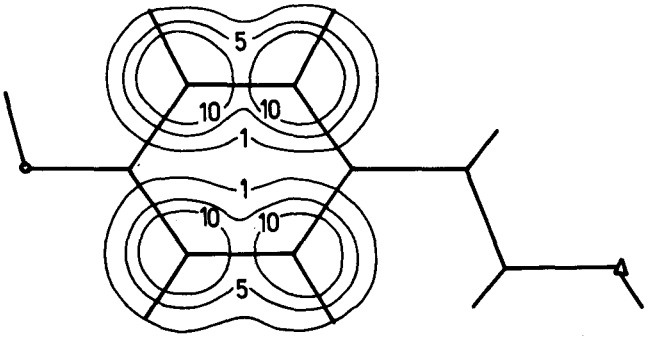


Fig. 17. EDD map of the LEMO of a basic rotamer of the p-OH-β-PEA (in the plane z = 0.5 Å).

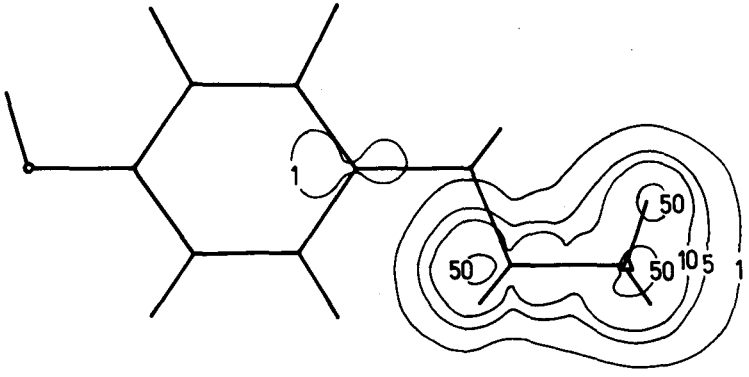


Fig. 18. EDD map of the LEMO of a protonated rotamer of the p-OH-β-PEA (in the plane z = 0.0 Å).

To study the influence of the conformation of the elctronic density distribution (EDD) of the NHOMO, HOMO and LEMO, we obtained these distribution for the six basic rotamers of the p-OH-β-PEA. In all the maps the values must be multiplied by $10^{-3}$ e.

Figure 16 shows the EDD of the HOMO of one rotamer. The electronic density is concentrated principally on the O and C-1 atoms and the form of this distribution is independent of the conformation.

Figure 17 displays the EDD for the LEMO of one rotamer. The LEMO has a π character and it is centered in the aromatic ring. The protonation of the rotamers produces interesting effects. The HOMO EDD of the protonated rotamers does not show variation in relation to the basic rotamer.

Figure 18 shows the EDD of the LEMO. Here, protonation has produced a striking effect. The LEMO lost the π character and transformed itself in a σ MO located on the cationic head and α-C atom. These effects have been found in all the phenylalkylamines analized (36 in all).

In Figures 19 and 20 we present, for comparison, the HOMO and LEMO EDD for the basic serotonin. In the case of the HOMO, it also has a π character and is distributed in almost all the conjugated system. The LEMO is a π MO centered in all the aromatic system and the α-C atom of the side chain.

Figures 21-22 show the result of the protonation of the HOMO and LEMO of serotonin. The LEMO converts itself in a σ MO centered, as in phenylalkylamines, in the cationic head. The NHOMO and HOMO do not experience strong variations in relation to the deprotonated serotonin.

The most important results of the Mulliken population analysis for the protonated molecules are the following:

1. The cationic head posseses between 48 and 61% of the positive charge.
2. The N atoms are negatively charged. This is in agreement with *ab initio* results (17).
3. When the quaternary N atom is substituted with three H atoms, its net charge is around − 0.42. When it contains one methyl group, its charge descends to approximately − 0.31 and with two methyl groups its charge becomes around − 0.21 e.

The EDD of the HOMO belonging to the 2-OH-5-$OCH_3$-PEA and 3-$OCH_3$-6-OH-PEA are displayed in Figures 23 and 24. It is clear from these figures that the 2-OH-5-$OCH_3$-PEA electronic density distribution is very similar to that of the HOMO of serotonin. Also, we can observe that the two EDD maps are very similar in the sense that if we rotate one of them 180° about the side chain, the resulting map is almost similar to the other. From the inspection of the maps of the HOMO it is possible to observe that around the O atoms there is a high electronic density available for a charge transfer. These great similarities and the analogous EP map, could explain the high binding affinity of this molecule ($pA_2 = 7.10$).

To analyze the case of rotamers type I-A and II-B, we obtained the EDD maps of the 3-methoxy-β-PEA and 5-methoxy-β-PEA. The EDD maps of the HOMO of these conformers are displayed in Figures 25 and 26.

Figure 25 shows that the 3-methoxy group does not imitate the similar region of serotonin. On the other hand, Figure 26 shows that the 5-methoxy group has a similarity with the equivalent region of the indole nucleus of serotonin.

In Figure 25, it may be appreciated that the electron density around the O atom of the methoxy group is analogous to the electron density around the N atom of the indole ring in serotonin (Fig. 21). If this N atom participates directly in the drug-receptor interaction, the 5-methoxy rotamer will interact better than the 3-methoxy conformer

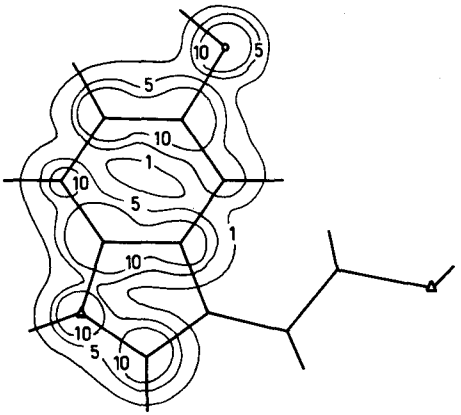


Fig. 19. EDD map of the HOMO of the basic serotonin (in the plane z = 0.0).

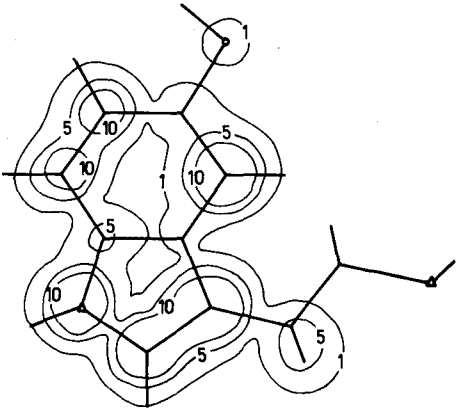


Fig. 20. EDD map of the LEMO of the basic serotonin (in the plane z = 0.5 Å).

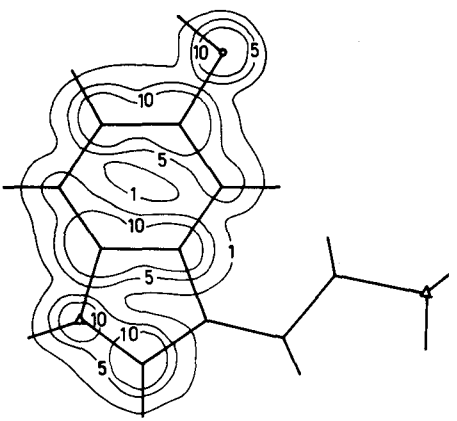


Fig. 21. EDD map of the HOMO of the protonated serotonin (in the plane z = 0.5 Å).

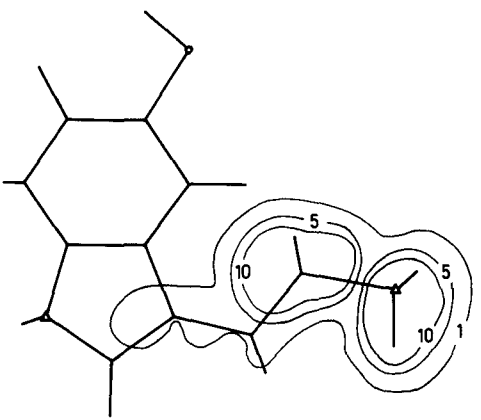


Fig. 22. EDD map of the LEMO of the protonated serotonin (in the plane z = 0.0 Å).

(Fig. 25). Also, all the phenylalkylamines analyzed (36 in all) show a similar pattern of electronic density distributions for the LEMO that is analogous to that of serotonin.

This analysis also strongly suggests that between rotamers III-A and III-B, the latter best mimics the serotonin's electronic features.

In the case of the m-substituents, the EDD analysis cannot help us to decide clearly in favour of one or other rotamers, but it suggests that possibly the m-substituents might occupy a place similar to the pentagonal ring of serotonin.

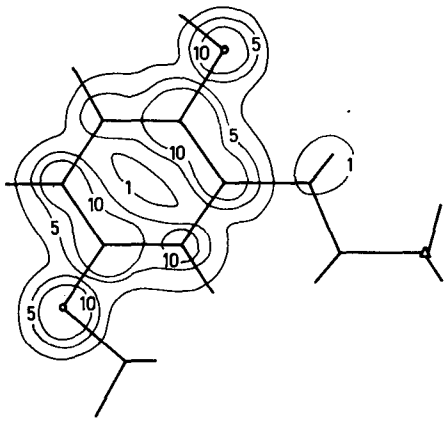


Fig. 23. EDD map of the HOMO of the protonated 2-OH-5-$OCH_3$-$\beta$-PEA (in the plane z = 0.5 Å).

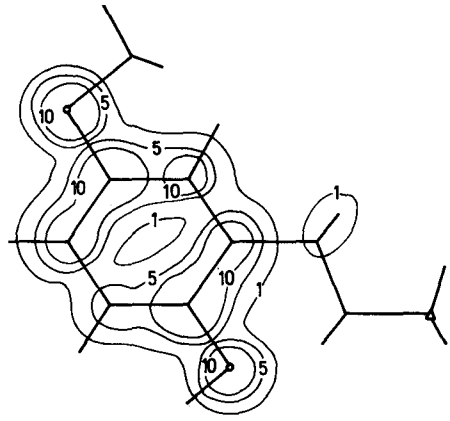


Fig. 24. EDD map of the HOMO of the protonated 3-$OCH_3$-6-OH-$\beta$-PEA (in the plane z = 0.5 Å).

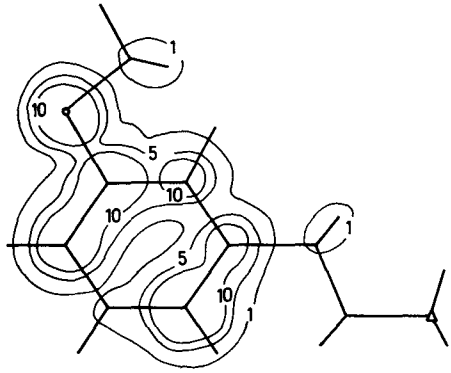


Fig. 25. EDD map of the HOMO of the protonated 3-$OCH_3$-$\beta$-PEA (in the plane z = 0.5 Å).

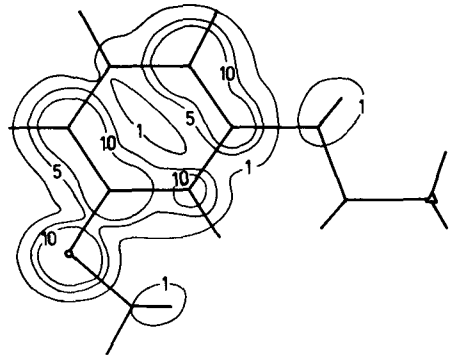


Fig. 26. EDD map of the HOMO of the protonated 5-$OCH_3$-$\beta$-PEA (in the plane z = 0.5 Å).

**Relation Between $pA_2$ and Electronic Structure**

To carry out the search for a relation between the variation of the $pA_2$ and the variation of electronic structure factors, we separated the conformers in five groups (Table 1):

1. In the first group, we placed the molecules with para or no substituents in the ring (molecules 1 to 10 in Table 1). Also, we included those molecules with a symmetrical 3,4,5-substitution pattern (*i.e.* molecule 7).

2. In the second, we placed the molecules with substituents at the positions 4, 5 and 5 (molecules 11 to 15 in Table 1). They are the rotamers of type I-B in Figure 4.

3. In the third there are molecules with substituents at the 2-and 2,5-positions (molecules 16 to 23 in Table 1). This is the group of rotamers type II-B and III-B of Figure 4.

4. The fourth group contains those molecules with substituents at the 6- and 3,6-positions (molecules 24 to 31 in Table 1). This is the group of rotamers II-A and III-A of Figure 4.

**TABLE 1. Experimental $pA_2$ of Some Rotamers of Substituted Phenylalkylamines**

| | Molecule[a] | Exp. $pA_2$ |
|---|---|---|
| 1 | R(−)−AMP | 5.16 |
| 2 | S(+)−AMP | 5.35 |
| 3 | 4−Methoxy−PEA | 5.10 |
| 4 | R(−)−4−Methoxy−AMP | 5.38 |
| 5 | S(+)−4−Methoxy−AMP | 5.16 |
| 6 | PEA | 5.26 |
| 7 | 3,4,5−Trimethoxy−PEA | 5.65 |
| 8 | 4−Methyl−PEA | 5.51 |
| 9 | 3,4,5−Trimethoxy−N−Methyl−PEA | 5.28 |
| 10 | 4−Hydroxy−PEA | 5.07 |
| 11 | 4,5−Dimethoxy−PEA | 5.36 |
| 12 | 5−Methoxy−PEA | 5.89 |
| 13 | 4,5−Methylendioxy−PEA | 6.10 |
| 14 | R(−)−4,5−Methylendioxy−AMP | 6.61 |
| 15 | S(+)−4,5−Methylendioxy−AMP | 6.08 |
| 16 | 2−Methoxy−PEA | 5.52 |
| 17 | 2,5−Dimethoxy−PEA | 6.85 |
| 18 | 2−Hydroxy−5−Methoxy−PEA | 7.10 |
| 19 | R(−)−2,5−Dimethoxy−4−Methyl−AMP | 7.15 |
| 20 | S(+)−2,5−Dimethoxy−4−Methyl−AMP | 6.41 |
| 21 | 2−Hydroxy−5−Methoxy−N,N−Dimethyl−PEA | 6.84 |
| 22 | 2,5−Dimethoxy−N,N−Dimethyl−PEA | 6.52 |
| 23 | S(+)−2,5−Dimethoxy−4−Br−AMP | 6.93 |
| 24 | 3,6−Dimethoxy−PEA | 6.85 |
| 25 | 3−Methoxy−6−Hydroxy−PEA | 7.10 |
| 26 | 6−Methoxy−PEA | 5.52 |
| 27 | R(−)−3,6−Dimethoxy−4−Methyl−AMP | 7.15 |
| 28 | S(+)−3,6−Dimethoxy−4−Methyl−AMP | 6.41 |
| 29 | 3−Methoxy−6−Hydroxy−N,N−Dimethyl−PEA | 6.84 |
| 30 | 3,6−Dimethoxy−N,N−Dimethyl−PEA | 6.52 |
| 31 | S(+)−3,6−Dimethoxy−4−Br−AMP | 6.83 |
| 32 | 3,4−Dimethoxy−PEA | 5.36 |
| 33 | 3−Methoxy−PEA | 5.89 |
| 34 | 3,4−Methylendioxy−PEA | 6.10 |
| 35 | R(−)−3,4−Methylendioxy−PEA | 6.61 |
| 36 | S(+)−3,4−Methylendioxy−PEA | 6.08 |

[a]PEA = Phenylethylamine; AMP = Phenylisopropylamine.

5. In the fifth group we placed the rotamers with substituents at the 3- and 3,4-positions (molecules 32 to 36 in Table 1), They correspond to the rotamers of type I-A in Figure 4.

The reader must keep in mind that the difference between groups 3 and 4 is only created by the particular position of the amine chain. The same holds for groups 2 and 5.

These five groups of rotamers give raise to four possibilities of interaction with the receptor:

Set I : Groups 1, 2 and 3.
Set II : Groups 1, 2 and 4.
Set III : Groups 1, 4 and 5.
Set IV : Groups 1, 3 and 5.

For each one of these sets, we performed the statistical analysis at four levels by the inclusion of more and more variables. The levels are:

**First level.** Includes the net charges, the total atomic superdelocalizabilities (electrophilic and nucleophilic), the molecule's mass and the term related to the moment of inertia. The atoms included are those belonging to the aromatic ring and the amine side chain.

**Second level.** Inclusion of the variables of the first level, plus the frontier orbital atomic densities and superdelocalizabilities of the HOMO and the LEMO. The atoms considered are those of the first level.

**Third level.** Inclusion of the variables of the above levels, plus the atomic orbital densities and the atomic orbital electrophilic superdelocalizabilities of the NHOMO. The atoms are the same as the second level.

**Fourth level.** Includes the variables of the third level, plus the net charges and the total atomic electrophilic superdelocalizabilities of the ring's substituents.

As many possible combinations of parameters must be considered, for each of the above sets we employed regression analysis to obtain statistical parameters for all the combinations of parameters studied. These statistical parameters (the F-test, the Student's t-test, the square of the correlation coefficient, *etc.*), will help us to reject those relationships which are not statistically significant and choose the one which best explains the variation of the $pA_2$.

In the following, we shall present only the results of the statistical analysis for the different levels. Later, we shall discuss and compare them.

*Results for the First Level*

For set I of molecules, the best regression equation is:

$$pA_2 = 14.8645 + 2.4328S_2^E + 5.5463Q_5 + 8.3527Q_8 \qquad (7)$$

with

$$R = 0.9297,\ R^2 = 0.8643,\ SD = 0.2856 \text{ and } F(3, 19) = 42.47\ (p < 0.0001).$$

The best equation for set II of molecules is:

$$pA_2 = 23.6646 + 2.5141Q_3 + 6.5875S_5^E + 4.5514S_6^E + 11.874Q_8 \qquad (8)$$

with

$$R = 0.8784,\ R^2 = 0.7715,\ SD = 0.3804 \text{ and } F(4, 18) = 16.05\ (p < 0.0005).$$

For set III, the best equation found is:

$$pA_2 = 14.8319 + 5.5070Q_3 + 2.4421S_6^E + 8.9559Q_8 \qquad (9)$$

with

$$R = 0.9272,\ R^2 = 0.8596,\ SD = 0.2906 \text{ and } F(3, 19) = 40.84\ (p < 0.0001).$$

For set IV, the best equation is:

$$pA_2 = 16.5400 - 5.624S_1^E + 3.9676S_2^E + 4.0283S_3^E + 2.8954Q_5 \qquad (10)$$

with

$R = 0.8873$, $R^2 = 0.7883$, $SD = 0.3669$ and $F(4, 18) = 17.54$ ($p < 0.0005$).

The experimental and calculated $pA_2$ for sets I to IV are shown in Table 2.

**TABLE 2. Experimental and Calculated $pA_2$ of sets I-IV with Variables of the First Level**

| Molecule | Exp. $pA_2$ | Set I Eq. (7) | Set II Eq. (8) | Set III Eq. (9) | Set IV Eq. (10) |
|---|---|---|---|---|---|
| 1 | 5.16 | 5.44 | 5.56 | 5.44 | 5.27 |
| 2 | 5.35 | 5.44 | 5.56 | 5.44 | 5.27 |
| 3 | 5.10 | 5.03 | 5.01 | 5.03 | 5.17 |
| 4 | 5.38 | 5.22 | 5.22 | 5.17 | 5.21 |
| 5 | 5.16 | 5.22 | 5.24 | 5.23 | 5.21 |
| 6 | 5.26 | 5.24 | 5.33 | 5.23 | 5.22 |
| 7 | 5.65 | 5.55 | 5.75 | 5.55 | 5.86 |
| 8 | 5.51 | 5.09 | 5.08 | 5.09 | 5.07 |
| 9 | 5.28 | 5.51 | 5.81 | 5.57 | 5.79 |
| 10 | 5.07 | 5.05 | 5.09 | 5.12 | 5.28 |
| 11 | 5.36 | 5.90 | 5.61 | – | – |
| 12 | 5.89 | 6.05 | 5.83 | – | – |
| 13 | 6.10 | 5.88 | 5.77 | – | – |
| 14 | 6.61 | 6.08 | 6.00 | – | – |
| 15 | 6.08 | 6.08 | 6.00 | – | – |
| 16 | 5.52 | 5.88 | – | – | 6.43 |
| 17 | 6.85 | 6.72 | – | – | 6.68 |
| 18 | 7.10 | 6.82 | – | – | 6.93 |
| 19 | 7.15 | 6.79 | – | – | 6.58 |
| 20 | 6.41 | 6.79 | – | – | 6.58 |
| 21 | 6.84 | 6.69 | – | – | 6.88 |
| 22 | 6.52 | 6.58 | – | – | 6.61 |
| 23 | 6.93 | 7.11 | – | – | 6.80 |
| 24 | 6.85 | – | 6.62 | 6.72 | – |
| 25 | 7.10 | – | 6.79 | 6.80 | – |
| 26 | 5.52 | – | 6.36 | 5.89 | – |
| 27 | 7.15 | – | 6.68 | 6.81 | – |
| 28 | 6.41 | – | 6.68 | 6.81 | – |
| 29 | 6.85 | – | 6.56 | 6.67 | – |
| 30 | 6.52 | – | 6.38 | 6.58 | – |
| 31 | 6.93 | – | 7.23 | 7.13 | – |
| 32 | 5.36 | – | – | 5.97 | 5.71 |
| 33 | 5.89 | – | – | 6.02 | 5.73 |
| 34 | 6.10 | – | – | 5.88 | 5.93 |
| 35 | 6.61 | – | – | 6.08 | 5.98 |
| 36 | 6.08 | – | – | 6.08 | 5.98 |

*Results for the Second Level*

For set I, the best equation is:

$$pA_2 = 13.7127 + 2.0866S_2^E + 5.6975S_5^E - 0.0391S_8^{N,L} \quad (11)$$

where $S_8^{N,L}$ means the orbital nucleophilic superdelocalizability of the LEMO in atom 8. This equation has $R = 0.9316$, $R^2 = 0.8678$, $SD = 0.2829$ and $F(3, 18) = 41.57$ ($p < 0.0001$)

For set II, the regression equation is:

$$pA_2 = 7.6713 - 1.4054S_4^E + 2.0973S_6^E + 20.5177Q_8 + 0.7746D_9^L + 1.8854S_1^{E,H} + 174.9650S_8^{E,H} \quad (12)$$

where $D_9^L$ means the orbital electronic density of the LEMO in atom 9 and $S_1^{E,H}$ means the orbital electrophilic superdelocalizability of the HOMO at atom 1. This equation has $R = 0.9653$, $R^2 = 0.9145$, $SD = 0.2461$ and $F(6, 16) = 30.28$ ($P < 0.0005$)

For set III, the best equation is:

$$pA_2 = -0.4305 - 1.2680S_4^E + 5.01710Q_6 + 16.5358Q_8 + 1.2124D_8^L + 1.6910S_1^{E,H} + 186.0341S_8^{E,H} \quad (13)$$

with

$R = 0.9489$, $R^2 = 0.9004$, $SD = 0.2677$ and $F(6, 15) = 24.12$ ($p < 0.0005$).

For set IV, the regression equation is:

$$pA_2 = 13.4306 + 5.5693Q_5 - 4.8926S_7^E - 1.3717D_5^H - 3{,}3001D_4^H + 3.4641D_8^L \quad (14)$$

with

$R = 0.9336$, $R^2 = 0.8716$, $SD = 0.2947$ and $F(5, 16) = 23.07$ ($p < 0.0005$).

The $pA_2$ calculated with eqs (11) – (14) are shown in Table 3, together with the experimental ones.

*Results of the third level.*

For set I, the best equation is:

$$pA_2 = 14.5708 + 2.3102S_2^E + 6.4263S_5^E - 1.1555D_2^{E,NH} - 2.3542S_3^{E,NH} \quad (15)$$

with

$R = 0.9464$, $R^2 = 0.8956$, $SD = 0.2568$ and $F(4, 18) = 40.81$ ($p < 0.0001$). $S_3^{E,NH}$ means the electrophilic superdelocalizability of the NHOMO in atom 3.

For set II, the regression equation is:

$$pA_2 = 13.9428 + 2.0264S_6^E + 14.6453Q_8 + 2.4473S_1^{E,H} + 124.5956S_8^{E,H} - 2.2989S_3^{E,NH} \quad (16)$$

with

$R = 0.9580$, $R^2 = 0.9177$, $SD = 0.2360$ and $F(5, 16) = 37.95$ ($p < 0.0001$).

For set III, the best regression equation is:

$$pA_2 = 4.7779 + 6.2992Q_6 + 15.9070Q_8 + 1.7582S_1^{E,H} + 197.5052_8^{E,H} + 1.4457D_6^{E,NH} + 1.9365D_3^{E,NH} \quad (17)$$

with

$$R = 0.9600,\ R^2 = 0.9216,\ SD = 0.2356 \text{ and } F(6,26) = 33.32\ (p < 0.0005).$$

For set IV, the equation is:

$$pA_2 = 9.6893 + 7.0819Q_5 - 3.7731S_7^E - 1.5028D_5^H - 2.6772D_4^H + 3.2434D_8^L - 1.4198S_4^{E,NH} \quad (18)$$

with

$$R = 0.9635,\ R^2 = 0.9283,\ SD = 0.2251 \text{ and } F(6,16) = 36.71\ (p < 0.0001).$$

The $pA_2$ calculated with eqs 15-18 and the experimental ones are shown in Table 4.

**TABLE 3. Experimental and Calculated $pA_2$ of Sets I-IV with Variables of the Second Level**

| Molecule | Exp. $pA_2$ | Set I Eq. (11) | Set II Eq. (12) | Set III Eq. (13) | Set IV Eq. (14) |
|---|---|---|---|---|---|
| 1 | 5.16 | 5.40 | 5.15 | 5.21 | 5.40 |
| 2 | 5.35 | 5.40 | 5.15 | 5.06 | 5.40 |
| 3 | 5.10 | 5.06 | 5.07 | 5.10 | 5.04 |
| 4 | 5.38 | 5.18 | 5.43 | 5.41 | 5.38 |
| 5 | 5.16 | 5.18 | 5.41 | 5.39 | 5.38 |
| 6 | 5.26 | 5.28 | 5.34 | 5.32 | 4.88 |
| 7 | 5.65 | 5.66 | 5.43 | 5.43 | 5.90 |
| 8 | 5.51 | 5.16 | 5.36 | 5.40 | 5.15 |
| 9 | 5.28 | 5.22 | 5.26 | 5.33 | 5.29 |
| 10 | 5.07 | 5.06 | 5.15 | 5.15 | 5.20 |
| 11 | 5.95 | 5.62 | – | – | – |
| 12 | 5.89 | 6.13 | 6.23 | – | – |
| 13 | 6.10 | 5.96 | 5.79 | – | – |
| 14 | 6.61 | 6.08 | 6.24 | – | – |
| 15 | 6.08 | 6.08 | 6.24 | – | – |
| 16 | 5.52 | 5.85 | – | – | 5.86 |
| 17 | 6.85 | 6.71 | – | – | 6.83 |
| 18 | 7.10 | 6.79 | – | – | 6.80 |
| 19 | 7.15 | 6.75 | – | – | 6.77 |
| 20 | 6.41 | 6.75 | – | – | 6.77 |
| 21 | 6.84 | 6.86 | – | – | 6.62 |
| 22 | 6.52 | 6.79 | – | – | 6.64 |
| 23 | 6.93 | 6.85 | – | – | 7.06 |
| 24 | 6.85 | – | 6.77 | 6.83 | – |
| 25 | 7.10 | – | 6.91 | 6.88 | – |
| 26 | 5.52 | – | 5.56 | 5.60 | – |
| 27 | 7.15 | – | 6.79 | 6.85 | – |
| 28 | 6.41 | – | 6.81 | 6.87 | – |
| 29 | 6.85 | – | 6.82 | 6.68 | – |
| 30 | 6.52 | – | 6.67 | 6.61 | – |
| 31 | 6.93 | – | 6.94 | 6.89 | – |
| 32 | 5.36 | – | – | 5.61 | 5.63 |
| 33 | 5.89 | – | – | 6.32 | 6.10 |
| 34 | 6.10 | – | – | 5.75 | 5.82 |
| 35 | 6.61 | – | – | 6.24 | 6.11 |
| 36 | 6.08 | – | – | 6.24 | 6.11 |

**TABLE 4. Experimental and Calculated $pA_2$ for Sets I-IV with Variables of the Third Level**

| Molecule | Exp. $pA_2$ | Set I Eq. (15) | Set II Eq. (16) | Set III Eq. (17) | Set IV Eq. (18) |
|---|---|---|---|---|---|
| 1 | 5.16 | 5.23 | 5.14 | 5.21 | 5.21 |
| 2 | 5.35 | 5.23 | 5.14 | 5.03 | 5.21 |
| 3 | 5.10 | 5.13 | 5.18 | 5.11 | 5.08 |
| 4 | 5.38 | 5.06 | 5.39 | 5.38 | 5.32 |
| 5 | 5.16 | 5.09 | 5.40 | 5.36 | 5.34 |
| 6 | 5.26 | 5.32 | 4.98 | 5.05 | 5.13 |
| 7 | 5.65 | 5.50 | 5.53 | 5.70 | 5.76 |
| 8 | 5.51 | 5.44 | 5.40 | 5.43 | 5.35 |
| 9 | 5.28 | 5.53 | 5.53 | 5.18 | 5.32 |
| 10 | 5.07 | 5.11 | 5.38 | 5.32 | 5.32 |
| 11 | 5.36 | 5.60 | 5.21 | – | – |
| 12 | 5.89 | 6.06 | 5.91 | – | – |
| 13 | 6.10 | 6.03 | 5.91 | – | – |
| 14 | 6.61 | 6.19 | 6.43 | – | – |
| 15 | 6.08 | 6.19 | 6.43 | – | – |
| 16 | 5.52 | 5.91 | – | – | 5.56 |
| 17 | 6.85 | 6.81 | – | – | 6.89 |
| 18 | 7.10 | 6.81 | – | – | 6.88 |
| 19 | 7.15 | 6.92 | – | – | 6.87 |
| 20 | 6.41 | 6.96 | – | – | 6.86 |
| 21 | 6.84 | 6.70 | – | – | 6.63 |
| 22 | 6.52 | 6.68 | – | – | 6.64 |
| 23 | 6.93 | 6.68 | – | – | 6.86 |
| 24 | 6.85 | – | 6.81 | 6.77 | – |
| 25 | 7.10 | – | 7.01 | 6.88 | – |
| 26 | 5.52 | – | 5.67 | 5.66 | – |
| 27 | 7.15 | – | 6.75 | 6.82 | – |
| 28 | 6.41 | – | 6.77 | 6.85 | – |
| 29 | 6.85 | – | 6.84 | 6.79 | – |
| 30 | 6.52 | – | 6.63 | 6.67 | – |
| 31 | 6.93 | – | 6.75 | 6.75 | – |
| 32 | 5.36 | – | – | 5.43 | 5.22 |
| 33 | 5.89 | – | – | 6.04 | 6.17 |
| 34 | 6.10 | – | – | 5.95 | 6.04 |
| 35 | 6.61 | – | – | 6.40 | 6.27 |
| 36 | 6.08 | – | – | 6.40 | 6.27 |

*Results for the Fourth Level*

For set I, the regression equation is:

$$pA_2 = 4.1087 - 1.2441S_6^{E,H} - 5.6435S_7^{N,L} + 1.7260D_4^{E,NH} - 3.0683Q_2^{PER} - 4.4781Q_5^{PER} + 0.1465S_9^{E,PER} \quad (19)$$

with

$$R = 0.9593,\ R^2 = 0.9202,\ SD = 2375 \text{ and } F(6,16) = 32.70\ (p < 0.0001).$$

$Q_2^{PER}$ and $S_9^{E,PER}$ mean, respectively, the net charge at the substituent's atom directly attached to atom 2 and the total electrophilic superdelocalizability of the substituent's atom directly attached to atom 9.

For set II, the best regression equation is:

$$pA_2 = 4.7201 + 15.717Q_8 + 1.8752S_1^{E,H} + 144.0538S_8^{E,H} + 3.3316D_3^{E,NH} - 3.3832Q_6^{PER} \quad (20)$$

with

$$R = 0.9601,\ R^2 = 0.9217,\ SD = 0.2288 \text{ and } F(5, 17) = 42.41\ (p < 0.0001).$$

For set III, the best regression equation is:

$$pA_2 = 4.0558 + 95.2178S_8^{E,H} - 0.3927S_3^{N,L} - 2.6278S_3^{E,NH} - 0.0826S_3^{E,PER} - 0.1941S_6^{E,PER} \quad (21)$$

with

$$R = 0.9693,\ R^2 = 0.9395,\ SD = 0.2011 \text{ and } F(5, 17) = 55{,}93\ (p < 0.0001).$$

For set IV, the equation is:

$$pA_2 = 4.3156 - 4.2126D_5^H + 1.5042D_8^L - 1.8505S_4^{E,NH} - 0.2491S_2^{E,PER} - 0.1498S_5^{E,PER} \quad (22)$$

with

$$R = 0.9647,\ R^2 = 0.9306,\ SD = 0.2153 \text{ and } F(5, 16) = 48.25\ (p < 0.0001).$$

The $pA_2$ calculated with eqs 19-22 and the experimental ones are shown in Table 5.

For the first level, we may appreciate that the two best equations are 7 and 9. The other two have too low a correlation coefficient and a high standard deviation.

The results of the t-test and the correlation matrices for the independent variables show that it is not possible to say that one equation is more significant than the other from a statistical point of view. Interestingly, these equations are those corresponding to the set A and set B separately. The two equations that mix the sets are not statistically significant. Also, it is possible to appreciate that the atoms and properties involved in eqs 7 and 9 are equivalent: $Q_5$ of eq (7) corresponds to $Q_3$ is eq 9 *etc.* This leads to four important facts:

1. At this level, it is not possible to differentiate sets A and B.
2. Atom 2 (Fig. 2) seems to participate in the drug-receptor interaction through a charge transfer acting as a donor.
3. Atom 3 (or 5) interact electrostatically with the receptor.
4. Atom 8 seems to participate in the interaction, but the t-test (Table 2) suggests that the property involved seems to hide a more subtle effect.

The second level shows only set I gives a significant regression equation. Here, the participation of atom 2 seems confirmed. Atom 5 also appears participating as an electron donor. In relation to atom 8, it appears here acting as an acceptor through the LEMO. The correlation matrices for eqs 2 and 11, show that in the last, $S_8^N$ shows a high correlation with $S_2^E$ (0.42), while $Q_8$ correlates only in a little extent with $S_2^E$ (0.05). Therefore, we must prefer for set I, eq 1 to eq 11.

The third level is more complicated. From the point of view of the results of the F-test, all the four equations are good. But analyzing the t-test we see immediatly that it is possible to discard eqs 17 and 18.

**TABLE 5. Experimental and Calculated $pA_2$ for Sets I-IV with Variables of the Fourth Level**

| Molecule | Exp. $pA_2$ | Set I Eq. (19) | Set II Eq. (20) | Set III Eq. (21) | Set IV Eq. (22) |
|---|---|---|---|---|---|
| 1 | 5.16 | 5.31 | 5.17 | 5.17 | 5.27 |
| 2 | 5.35 | 5.30 | 5.17 | 5.17 | 5.27 |
| 3 | 5.10 | 5.07 | 5.11 | 5.19 | 5.09 |
| 4 | 5.38 | 5.25 | 5.38 | 5.13 | 5.27 |
| 5 | 5.16 | 5.26 | 5.38 | 5.14 | 5.29 |
| 6 | 5.26 | 5.20 | 5.06 | 5.19 | 5.24 |
| 7 | 5.65 | 5.54 | 5.68 | 5.82 | 5.59 |
| 8 | 5.51 | 5.27 | 5.44 | 5.56 | 5.31 |
| 9 | 5.28 | 5.18 | 5.62 | 5.33 | 5.32 |
| 10 | 5.07 | 5.18 | 5.26 | 5.23 | 5.32 |
| 11 | 5.36 | 5.70 | 5.24 | – | – |
| 12 | 5.89 | 6.08 | 5.89 | – | – |
| 13 | 6.10 | 6.15 | 5.79 | – | – |
| 14 | 6.61 | 6.19 | 6.34 | – | – |
| 15 | 6.08 | 6.19 | 6.34 | – | – |
| 16 | 5.52 | 5.62 | – | – | 5.50 |
| 17 | 6.85 | 6.78 | – | – | 6.87 |
| 18 | 7.10 | 6.91 | – | – | 6.83 |
| 19 | 7.15 | 6.94 | – | – | 6.93 |
| 20 | 6.41 | 6.94 | – | – | 9.92 |
| 21 | 6.84 | 6.73 | – | – | 6.59 |
| 22 | 6.52 | 6.62 | – | – | 6.63 |
| 23 | 6.93 | 6.76 | – | – | 6.99 |
| 24 | 6.85 | – | 6.77 | 6.78 | – |
| 25 | 7.10 | – | 6.99 | 6.84 | – |
| 26 | 5.52 | – | 5.57 | 5.69 | – |
| 27 | 7.15 | – | 6.82 | 6.81 | – |
| 28 | 6.41 | – | 6.84 | 6.66 | – |
| 29 | 6.85 | – | 6.90 | 6.84 | – |
| 30 | 6.52 | – | 6.67 | 6.77 | – |
| 31 | 6.93 | – | 6.75 | 6.95 | – |
| 32 | 5.36 | – | – | 5.21 | 5.31 |
| 33 | 5.89 | – | – | 5.93 | 5.99 |
| 34 | 6.10 | – | – | 6.23 | 6.16 |
| 35 | 6.61 | – | – | 6.27 | 6.23 |
| 36 | 6.08 | – | – | 6.27 | 6.23 |

Equation 15 for set I appears here having a variable that is not significant ($D_2^{E,NH}$), and that is highly correlated to $S_3^{E,NH}$ ($r = -0.60$). Equation 16 is good, containing less variables than its equivalent for the second level.

The results for the fourth level are franky bad, with the exception of eq 20, that seems to be the best for set II.

Now, if we select the best regression equations for each set, we obtain:

For set I : eq 7
For set II : eq 16 ✓
For set III : eq 9
For set IV : eq 10 ✓

Examining these four equations, from the correlation matrices we may easily discard eq 10 because it contains independent variables that are highly correlated. From the

other three, eq 16 can be discarded considering that its variables are correlated to a greater than in the other two equations.

From the results of the statistical indexes, we cannot choose one or other of the remaining two equations.

The following are possible reasons for this failure:

1. It may be that in the drug-receptor interaction some atoms of the side chain participate as electron acceptors. If this is true, the total nucleophilic superdelocalizability will be involved. We showed that the CNDO/2 method gives $S^N$ values that do not show relation with the nature and the position of the substituents and the side chain (41).

2. Analyzing the dependence of the variation of the $pA_2$ on the electronic structure, we found that in tryptamines, the best regression involves the $S^E$ index for atoms 2 and 6 of Figure 2 (53). Given that this index appears in eq 7 as $S_2^E$ and in eq 9 as $S_6^E$, a possibility arises that the phenylalkylamines bind in any of the two ways depending on the substituents they have (this is only acceptable if we do not consider the results of the EP maps and the electronic density distribution).

## CONCLUSIONS

The main conclusions of this work are:

1. The EP map analysis strongly suggests that phenylalkylamines bind to the serotonergic receptor as rotamers II-B of Figure 4.

2. This suggestion is suported by the results of the electronic density distribution analysis.

3. The quantum-statistical method is not able to distinguish between conformers (II-B, III-B) and (II-A, III-A) in Figure 4.

4. Nevertheless it seems well established that atoms 2, 5 and 8 participate in the drug-receptor interaction, these results not being in opposition with those for thyptamines (53).

5. It is necessary to carry out more statistical analysis, including more molecules and to examine other binding possibilities.

6. Also, it would be of interest to replace the CNDO/2 net charges for the ones obtained from Mulliken population analysis.

7. For the case of the m-substituted phenylalkylamines, the results from the EP maps and EDD maps suggest that possibly these molecules bind to the receptor in a way such that the m-substituent mimics partially the five-membered ring of serotonin.

8. Also, given the importance of atom 2 (or atom 6) as an electron donor, phenylaklylamines substituted in ortho with groups such $-COCH_3$, $-COC_2H_5$, *etc.*, must have a high $pA_2$ value.

9. In any case, the equations explain about 87% of the variation of the $pA_2$. This fact shows the great utility of the quantum-statistical approach employed.

A general equation for both, phenylalkylamines and tryptamine derivatives, is in progress.

The tables with the results of the t-test and the squared correlation matrices for the independent variables are available on request.

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