Structure-Activity Relationships of Phenethylamine. A Comparison of Quantum Mechanical SCF "*Ab Initio*" and Semiempirical Calculations

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Abstract: *"Ab initio"* (STO-3G) and semiempirical calculations have been carried out for phenethylamine in both neutral and protonated forms, in order to obtain information about structure-activity relationships of this molecule and related structures. EH and *"ab initio"* most stable extended conformations are in agreement with experimental results, but zero differential overlap approximations (CNDO, INDO, PCILO) give more stable folded conformations. In the biological media both extended and folded configurations, which in any theoretical method show slight energy differences, may play a similar role. Mulliken population analysis, interaction with an H^- ion, and electrostatic molecular potentials, calculated with *"ab initio"* wave functions, show that substrate-receptor interaction may be independent of the extended and folded molecular configuration and stress the importance of the charge cloud of the amino group and phenyl moiety in the biological activity of the studied molecule.

1. Introduction

In this paper we will present the results of a calculation of the conformational surface and of reactivity properties for the neutral and protonated form of the phenethylamine molecule, which for the sake of brevity will be indicated as PEA and $PEAH^+$, respectively.

The phenethylamine is the precursor of a large set of molecules of biological and pharmacological interest: neurotransmitters, sympathomimetics, hallucinogens. It is, in general, accepted that such drugs reveal their action through an intimate and highly specific contact with an appropriate portion of the biophase: the receptor. For evident experimental difficulties, the receptors are not generally as yet separated and even identified, so the investigations on the mechanism of such interactions must rely on more indirect means than the *in vitro* experiments on the isolated partners. Very probably the onium form of the compounds of the phenethylamine family is the main form responsible for their pharmacological responses by means of ionic interactions between the cationic head and some anionic receptor site, usually identified as the phosphate groups of ATP.[2] Moreover, the protonated species is also, by far, the most abundant one at the physiological value of pH 7.4; for the pK_A value of PEA at 25°,[3] its population is about 99.6%. The study of the neutral molecule may give, however, some further information on the structure-activity relationship (SAR); to this purpose we have performed comparative research on both forms.

The theoretical approach to this problem seems to be of some interest. It is possible at present to calculate wave functions for such drugs in several different approximations. The first step on the theoretical study of the SAR is represented by a conformational analysis which will give the isolated molecule preferred rotamers and the barriers separating them, also some information on the geometrical arrangement of the receptor reactive groups will be obtained. Obviously, the calculated preferred conformations *in vacuo* may be different from those which engage the receptor; the following theoretical study of the reactivity must be performed, therefore, on several rotamers suitably selected from the calculated conformational surface.

The drug-receptor interaction generally involves rather weak noncovalent forces such as ionic interactions, hydrogen bonds, dispersion, and hydrophobic bonds.[2b] The electrostatic forces take place between permanent local multipoles of the two partners, the dispersion and hydrophobic ones being predominant in the hydrocarbonic regions of the drug. In this work, we have tried only to approach the study of the electrostatic interactions by means of the molecular electrostatic potential calculation of the isolated drug and of a very simple model of the drug-receptor complex. Indeed, the analysis of these bonds may be propitiously performed, at least for closed shells, with the monodeterminantal SCF approximation, here used at the molecular potential level.[4] The dispersion forces, on the contrary, may be studied only with a much greater computational effort, by means of the explicit introduction of the electronic correlation; on the other hand, such bonds are probably not very important in the unsubstituted phenethylamine molecule.

2. Experimental Section

The phenethylamine has three torsional degrees of freedom, in both neutral and protonated forms, here expressed by means of the dihedral angles $\tau_1(C_6\text{-}C_1\text{-}C_\beta\text{-}C_\alpha)$, $\tau_2(C_1\text{-}C_\beta\text{-}C_\alpha\text{-}N)$ and $\tau_3(C_\beta\text{-}C_\alpha\text{-}N\text{-}H_7)$ (see Figure 1, where the starting conformation is also indicated). In the present study, all three angles were explicitly considered and the usual convention for torsion angles was adopted. (The torsion angle τ of the bonded atoms A-B-C-D is the angle between the planes ABC and BCD. This angle is positive for clockwise rotations around B-C, when looking from B to C. The values $\tau = 0, \pm 60, \pm 120$, and 180° correspond respectively to the syn-planar, ±syn-clinal, ±anti-clinal, and anti-planar conformations. The more usual names cis and trans correspond to $\tau = 0$ and 180°, while the gauche configurations collect the value ±60 and ±120°.) Using standard values for bond lengths and angles, a complete span of the conformational surface for PEA, as well for $PEAH^+$, with a grid of 30°, was performed with all the semiempirical methods mentioned below.

Four well-known semiempirical methods (EHT, CNDO/2, INDO, and PCILO) all in their standard versions, have been employed. By a parallel perusal of all the four sets of results, a possible conformation path connecting rotameric minima was detected. Along such a path, a few *"ab initio"* SCF-LCAO-MO points were calculated, using a STO-3G basis set.[5]

For a conformational study, performed as a preliminary step in an investigation on the pharmacological reactivity of a relatively large drug, which is supposed to develop its activity only in a very specific conformation, the most advisable way, at the present state of the art, seems to be to use different semiempirical (or empirical) methods in parallel. In fact, the final aim of such a study should be to get the conformation of the molecule, while it interacts with the

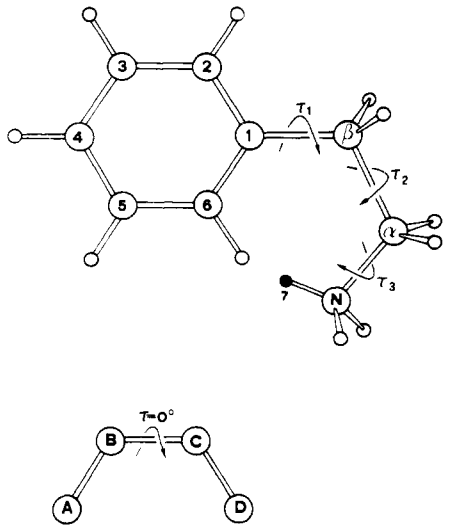

Figure 1. Starting conformation of the two phenethylamine species. The blackened atom is the extra proton on the $PEAH^+$ molecule. At the bottom the definition of the torsion angle is also reported.

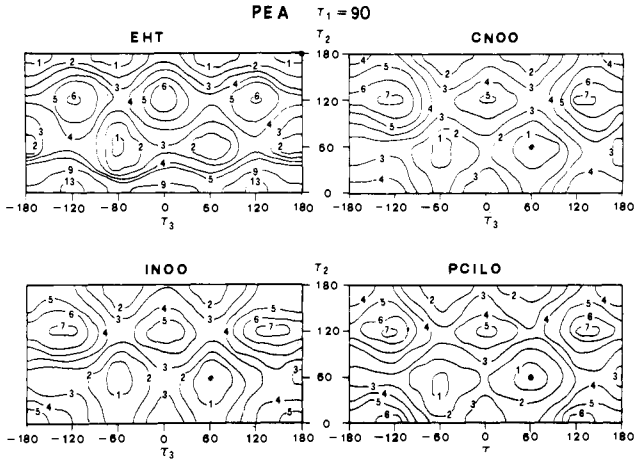

Figure 2. Conformational map of PEA at $\tau_1 = 90°$ for the four semiempirical methods. The energy values are calculated in every case with respect to his own absolute minimum (represented by a star) and expressed in kcal/mol.

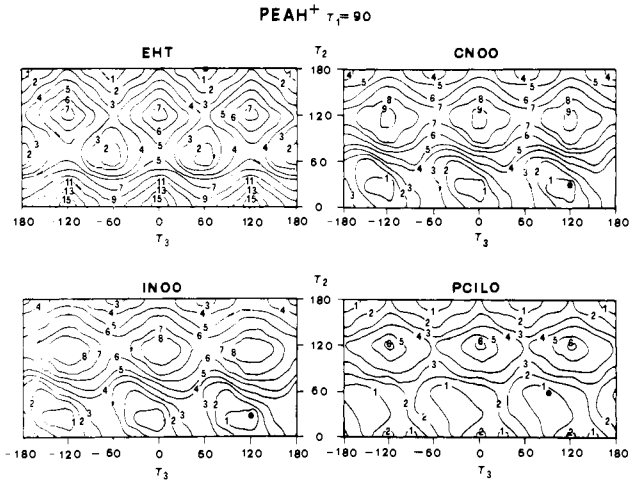

Figure 3. The same as Figure 2 for $PEAH^+$.

corresponding receptor. Conformational studies, either experimental or theoretical, give answers for physical situations (*in vacuo*, aqueous solution, solid state) which could be significantly different from that of interest. A theoretical investigation should lead to discard, if possible, large portions of the conformational space, and to restrict the attention for further studies to only a few conformations. The comparison of the results, obtained by different methods, gives a reasonable confidence that all the conformations of some interest have been evidenced. The more reliable *ab initio* calculations are, at present, too costly to be extensively used to get conformational surfaces and can be limited to those few, useful for a further check of the semiempirical results.

For an attempt to shed some light on the mechanism of the drug-receptor interaction, *via* the examination of some properties of the isolated molecule in the most "interesting" conformations, the *ab initio* wave functions are, of course, the most suitable ones.

In the present paper we have adopted such a strategy, enlarging the analysis of some properties of the wave function also to the semiempirical ones.

The four semiempirical methods are representative of the most used approximations: (a) the approximation of the total Hamiltonian as a sum of one-electron effective Hamiltonians, (b) the zero differential overlap (ZDO) approximation (in two versions), (c) the ZDO approximation on localized orbitals, corrected by the inclusion of a portion of the correlation energy. All four methods are widely known and a further discussion on their basic assumptions is not necessary here. Only a few words on the practical use of the PCILO procedure are perhaps useful. As it is well known, such a method uses as zeroth-order approximation a completely localized description of the molecule. In the case of substituted benzenes, the two Kekulé formulas are not equivalent: the difference in energy, in a large number of cases, is of the order of the conformational energy differences[6] (1 kcal/mol). The most correct way to overcome such inconvenience should be to use a multiconfigurational zeroth-order function; in such a case, however, one of the outstanding features of the method (its velocity) should be lost. Diner, *et al.*,[7] suggest, as a practical criterion, choosing as a starting wave function that one having the best energy. Such a proposal sounds correct, because the zeroth-order energy is an upper bound to the final energy, but the final PCILO energy is, generally, very far from the starting one, while at least for the molecules here considered, the differences between the two starting energies are small, nearly of the same order as the computation errors.

Other criteria could be devised, for instance, to perform the computations on both Kekulé structures and to use as final energy: (a) the best third-order (final) energy; (b) the arithmetical mean between the two third-order energies. While in the free basis (PEA) the three criteria give conformational results in satisfactory accord, in the protonated species the shape of the conformational surface shows some differences (the position of the minima does not change substantially, however). A comparison with the few SCF *ab initio* energies shows, however, that the best fitting is obtained, in the present case, with the original Diner criterion and, accordingly, such a criterion was selected for the results displayed here.

3. Conformational Analysis

(a) Results. In Figures 2 and 3 are reported, for PEA and $PEAH^+$, respectively, the sections of the conformational surfaces corresponding to $\tau_1 = 90°$. For the sake of brevity we limit ourselves to show only such sections of the conformational hyperspace which contain the most important conformational minima. Some information on configurations having $\tau_1 \neq 90°$ will be given in the text.

It is interesting to point out that the molecular symmetry produces equivalence among different portions of the conformational surface; for instance, in both molecules it is sufficient to consider τ_1 and τ_2 ranging only between 0 and 180° and, in the protonated form, the C_{3v} symmetry of the onium group reduces the variation of τ_3 between 0 and 120°.

In both molecules, and especially in $PEAH^+$, some differences among the results of the different methods are evident; more remarkable are the differences between EHT results and those pertaining to the other methods, *i.e.*, CNDO, INDO, and PCILO.

In the PEA molecule, all the methods give conformation-

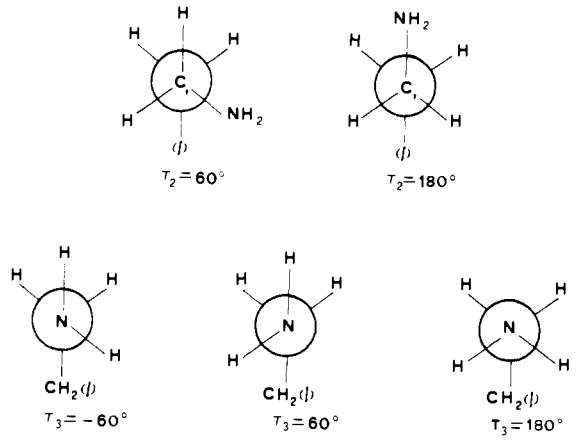

Figure 4. Newman projections along the C_α-C_β and N-C_α axes. ϕ = Ph.

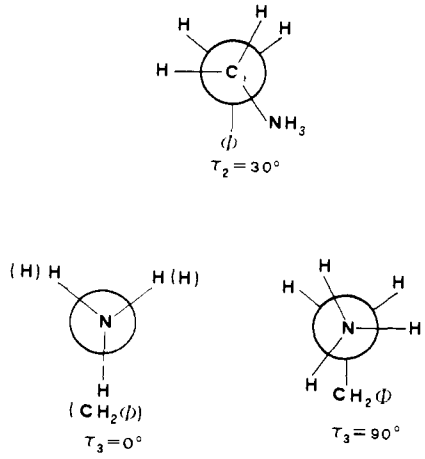

Figure 5. Newman projections along the C_α-C_β and N-C_α axes.

al minima for values of (τ_2,τ_3) equal to (60,−60), (60,60), (180,60), and (180,180). The first two points correspond to folded conformations, completely staggered with the phenyl ring (Ph) and the N group in gauche arrangement (see the Newman projections along the C_α-C_β axis in Figures 4 and 5). The first rotamer has both H atoms of the NH_2 group pointing outward from the phenyl ring, while in the second, a hydrogen points toward the aromatic region. The second couple of points corresponds to extended conformations all staggered: Ph trans with respect to the N atom. In the point (180,60) the hydrogens of NH_2 bear a gauche relationship, while in the conformer (180,180) they are in a trans arrangement. With the EHT method one obtains also a fifth minimum, corresponding to another folded conformation: (60,180).

The main differences between the EHT map and the others are due to the relative values of the minima and barriers, rather than in their locations. The EHT surface is steeper, with higher barriers; the preferred conformations lie at (180,60) and (180,180), corresponding to extended forms. The ZDO methods, which show a more similar trend of the surface, have lower barriers, with preferred conformation at the point (60,60), corresponding to a folded conformation of the side chain.

As anticipated above, the minima occur at $\tau_1 = 90°$. In general, such minima are well defined in τ_1, the broadest curves being those of the PCILO method, which for the conformation (60,−60) gives, in fact, two nearly equivalent minima, one at $\tau_1 = 90°$ and a second at $\tau_1 = 135°$. Such a second minimum appears only when one adopts the Diner

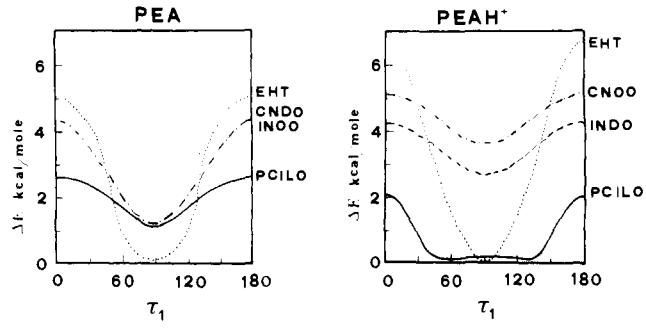

Figure 6. Comparison of the variation of the conformational energy, according to the four methods, with respect to the τ_1 angle for conformations having $\tau_2 = 180°$ and $\tau_3 = 60° - \Delta E$ values in kcal/mol.

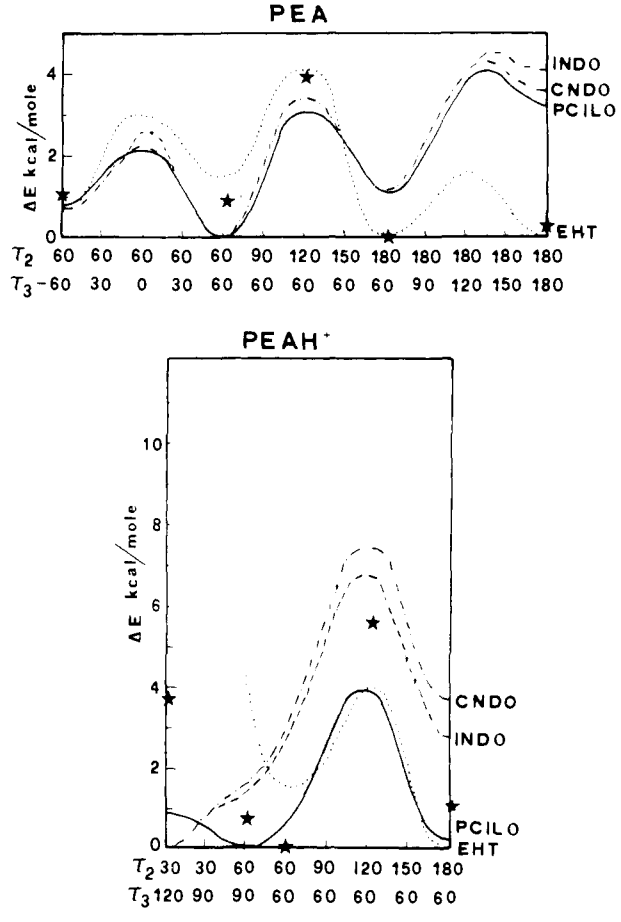

Figure 7. Minimum energy path connecting the rotameric minima. The stars refer to the *a priori* STO-3G calculations.

criterion; with the other two criteria the second minimum disappears.

As said above, the differences between EHT and ZDO results are more marked in the $PEAH^+$ case. The EHT method gives the absolute minimum at (180,60), *i.e.*, at the same extended form as for the free base. A secondary minimum at (60,60) is still present, while conformers with $\tau_2 < 60°$ are decidedly unfavored. Also, the other semiempirical methods give a secondary minimum at (180,60), but the absolute minimum lies in the region of $\tau_2 < 90°$ (folded conformations). Such a region presents a somewhat flat minimum (see in the maps the region surrounded by the isoenergy curve of 1 kcal/mol), such a portion of space being smaller in the CNDO and INDO maps, larger in the PCILO one. Also the location of the true minimum is different, CNDO and INDO giving as preferred rotamer the

Table I. Values of the Conformational Energies for Some Selected Points[a]

	PEA					$PEAH^+$				
Method	90,60,−60	90,60,60	90,120,60	90,180,60	90,180,180	90,30,120	90,60,90	90,60,60	90,120,60	90,180,60
EHT	0.72	1.54	4.12	0.14	0	11.7	3.88	1.52	3.93	0
CNDO	0.72	0	3.45	1.15	3.62	0	1.57	2.96	7.44	3.59
INDO	0.73	0	3.43	1.12	4.10	0	1.40	2.82	6.69	2.74
PCILO	0.75	0	3.07	1.13	3.22	0.88	0	0.56	3.92	0.23
A priori[b]	1.07	0.94	3.97	0	0.28	3.59	0.74	0	5.62	1.14

[a] The conformations here selected can be identified by means of the torsion angles (τ_1,τ_2,τ_3) reported at the top of the table. The values are in kcal/mol. [b] The absolute values of the energy for the best conformations of PEA and $PEAH^+$ are respectively −359.3601 and −359.7934 au.

Table II. Resumé of the Preferred Conformations According to Various Methods

Method	EHT	CNDO	INDO	PCILO	*A priori*	Empirical[a]	Nmr	X-Rays[d]
PEA	Extended	Folded	Folded	Folded	Extended	Folded	Extended[b]	
$PEAH^+$	Extended	Folded	Folded	Ext–fold	Folded	Folded	Ext–fold[c]	Extended

[a] *In vacuo* calculations.[9] [b] Amphetamine in D_2O.[10] [c] Amphetamine·HCl in D_2O.[10] [d] Phenethylamine·HCl.[11]

(30,0) one, equivalent, for symmetry reasons, to the point (30,120); such a minimum corresponds to a folded conformation, with the CH_2-CH_2 group half-way between the eclipsed and the staggered positions, CH_2-NH_3 eclipsed, Ph and N in gauche form (see Figure 5). The PCILO calculations give a very flat minimum; the lower energy seems, however, to be near the (60,90) conformation, which corresponds to a folded form with CH_2-CH_2 staggered, CH_2-NH_3 half-way between the eclipsed and the staggered position, and Ph and N in the gauche position. Such a minimum is, however, practically equivalent from the energetic point of view to the secondary one at (180,60) (ΔE = 0.2 kcal/mol); the PCILO method considers extended and folded conformations as having the same energy.

For conformations having $\tau_1 \neq 90°$, only PCILO again gives insights of other minima One example is given by the conformation (180,60), which in the PCILO description is placed in a large flat basin, ranging from $\tau_1 = 65°$ to $\tau_1 = 135°$. The corresponding section of the energy surface is reported in Figure 6 and compared with those pertaining to the other methods. In the same figure, the corresponding sections for the PEA molecule are also displayed.

By comparing the whole set of results, it is possible to draw, tentatively, a minimum energy path connecting the minima in both species. Such a path is reported in Figure 7 and the corresponding numerical values are collected in Table I.

In the same figure and table are also reported the results of *ab initio* SCF calculations, performed, as said above, with a minimal STO-3G basis set (the calculations have been made with the program GAUSSIAN 70[8]). In this manner, the points, corresponding to the four semiempirical minima and to the main barrier, between the folded and the extended forms, have been checked.

In the PEA molecule, the points at (180,60) and (180,180) are nearly equivalent and about 1 kcal/mol below the couple of points at (60,−60) and (60,60); in the free base, the extended forms are the preferred ones. In $PEAH^+$ the situation is reversed, because the folded form (60,60) represents the absolute minimum. For clarity's sake, a concise resumé of the preferred conformations, obtained *via* different methods, is reported in Table II.

(b) Discussion. The results, displayed above, confirm the tendency of the EHT method to prefer open structures. Such a characteristic is presumably due to the concomitant action of two features of the method, namely the important role assigned to the overlap, which ultimately brings apart the nonbonded atoms, and to an underevaluation of the nucleus-electron attraction energy terms, which are not completely well balanced by the other approximations introduced in the Hamiltonian. Such features of the method lead also to high barriers.

On the other hand the CNDO and INDO methods, which, in turn, discard completely the overlap between nonbonded atoms, favor closed structures, where the attractive nucleus-electron terms are larger.

The rotamers (60,60) of PEA and (30,0) of $PEAH^+$ result presumably stabilized by an interaction, essentially electrostatic in nature, of the type

$$Ph^{\delta-}\cdots H^{\delta+}\text{-}N$$

where the phenyl ring is represented by the carbon atom in ortho position with respect to the ethylamine side chain, *i.e.*, the most negatively charged atom of the ring (see the next section). Such attractive interaction overcomes the steric repulsion, and is a feature of CNDO and INDO methods, clearly evidenced by numerous previous calculations. Limiting ourselves to phenethylamine derivatives, in protonated isoproterenol and INPEA,[2b] eclipsed conformations of the ethanolaminic portion are stabilized by another intramolecular hydrogen bond, involving the alcoholic function and the onium group: *i.e.*,

$$O^{\delta-}\cdots H^{\delta+}\text{-}N$$

In this case, the phenyl ring is not involved, as can be verified by comparing the conformational surface of the related compound 2-aminoethanol.[2b] The occurrence of phenyl ring may be, of its own, another source of error; other authors[9] have also evidenced that CNDO favors folded structures for benzaldehyde, benzoic acid, biphenyl, 2,2′-difluorobiphenyl, etc. Such weakness of the method is generally ascribed to an underevaluation of the conjugative effect, which may be interpreted as being due to the isotropic approximation, introduced in the evaluation of the two-center two-electron integrals. Something unsatisfactory in CNDO and INDO calculations on π systems may also be evidenced in the related calculations of the electrostatic potentials (see below) and, probably, for such that are observable the failure is due again to the approximation in using only *S* functions for calculating two-electron two-center integrals.

Coming back to the examination of the conformational maps, the analysis of the PCILO results is somewhat more complicated. The PCILO method gives, in general, a better description than CNDO or INDO on conjugated systems, being able, for instance, to prefer trans-planar forms rather than the folded ones, orthogonal to the ring.[10] For the two molecules here considered and especially for PEA, on the contrary, the PCILO results are in accord with the CNDO

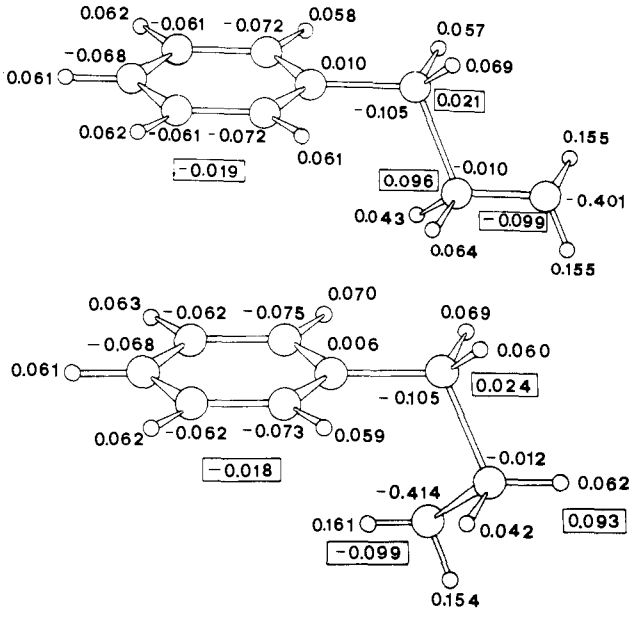

Figure 8. *A priori* STO-3G atomic and group charges for PEA: (a) extended form (90,180,60); (b) folded form (90,60,60).

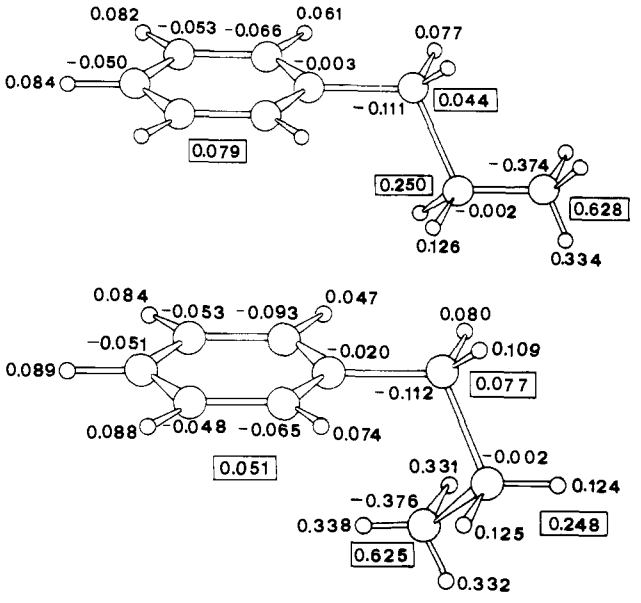

Figure 9. The same as Figure 8 for $PEAH^+$.

and INDO ones and disagree with the *ab initio* calculations.

Some explanations of such behavior may be put forward. Population analysis shows that the aminic head, in both molecules, has highly localized atomic charges, and, because no bulky groups, responsible for steric hindrances, are present here, the tendency of all the ZDO methods to emphasize the attraction among nonbonded atoms (Ph and aminic moiety) works at its best. In the cases where the charge density is more evenly distributed, as in hydrocarbons and secondary and tertiary amines (or onium ions), the PCILO method should work better. The acetylcholine results[11,12] may be considered as a positive check of such a hypothesis.

Another explanation is related to the role played by the delocalization energy. Better performances of PCILO with respect to CNDO and INDO have been found in molecules, like benzaldehyde, benzoic acid, biphenyl, para-substituted perbenzoic acids, etc., where the delocalization energy, explicitly included in the PCILO method, as a contribution to the second-order corrections to the energy, tends to favor open forms with respect to the closed ones. For PEA and $PEAH^+$, which does not contain π electrons in the side chain, the delocalization energy plays the opposite role, and an analysis of the pertinent second-order corrections shows that they favor a closed structure with respect to the open ones. In addition, however, in $PEAH^+$ there is a third-order contribution to the energy (interaction energy between two delocalized electrons), which favors open structures and leads, finally, the energies of both conformations to be nearly equivalent.

(c) Comparison with Other Conformational Results. Several compounds of the phenethylamine family have been investigated experimentally, either in aqueous solution, using nmr techniques,[13] or in the solid state with X-ray methods.[14] Extended conformations, with the side chain nearly perpendicular to the phenyl ring, are the preferred ones. The interaction with the polar molecules of the solvent, or the crystal field energies, will presumably favor extended forms, but, at least in the aqueous solution, where more information is available, the relative stabilities of the folded and extended rotamers do not appear to be too different. In the amphetamine (α-methylphenethylamine), the populations for the neutral form are 50% for the extended conformation and 39% for the folded one; while for the onium form the populaions are 50 and 45%, respectively[13] (see Table II). It seems, as a consequence, quite plausible that *in vacuo* the stability order of $PEAH^+$ could be reversed with respect to the solution, because, in this case, the intramolecular attraction between the cationic head and the aromatic region will not be overwhelmed by intermolecular interactions.

Among the preceding theoretical studies, particular attention should deservedly be paid to two papers, which deal with the determination of the conformations of a set of phenethylamine derivatives, containing the parent molecule.

The most recent paper[15] uses empirical potential functions. For the free base, the folded one is found to be the most stable rotamer *in vacuo*, in accord with our CNDO, INDO, and PCILO calculations, and in disagreement with the EHT and *ab initio* ones. The addition of further parameters, mimicking the molecule-solvent interaction, leads to a situation where there is no preferred conformation. For $PEAH^+$ *in vacuo*, the folded conformation is found to be preferred, and in solution the situation is reversed, the extended one being favored.

Pullman, *et al.*,[16] used the PCILO method for a set of ammonium ions, including $PEAH^+$. The third torsion angle, τ_3, was kept fixed at the staggered position (60–120°), while the conformational energy map in the τ_1 and τ_2 plane is explicitly given. Our Figure 3 can thus be considered as a supplement to that of ref 16, giving further conformational information. The two sets of results fit exactly, being obtained in the same approximation. The explicit introduction of the third torsional parameter, τ_3, confirms the staggered position of the onium group and the value $\tau_1 = 90°$ for the principal rotameric minima.

Our EHT results on $PEAH^+$ agree with the corresponding ones of Bustard and Egan[17] for the onium form of the dopamine. The two phenolic groups of such a molecule influence, only slightly, the conformational energy of the phenethylamine skeleton. In fact, for both compounds, the EHT method points out to a threefold rotational barrier with respect to the τ_2 torsion angle, the extended form being the preferred one.

In conclusion, the *ab initio* STO-3G method gives conformational results which are in reasonable agreement with

Table III. Group Charges for the Selected Conformations According to Various Methods[a]

Group	Extended (90,180,60)					Folded (90,60,60)				
	EHT	CNDO	INDO	PCILO	*Ab initio*	EHT	CNDO	INDO	PCILO	*Ab initio*
					PEA					
Ph	−0.025	0	0.009	0.002	−0.019	−0.022	0	0.004	0.004	−0.018
β-CH_2	0	−0.008	−0.012	−0.014	0.021	0.001	−0.010	−0.013	−0.016	0.024
α-CH_2	0.266	0.085	0.106	0.066	0.096	0.266	0.081	0.103	0.062	0.093
NH_2	−0.241	−0.076	−0.097	−0.055	−0.099	−0.244	−0.070	−0.093	−0.049	−0.099
					$PEAH^+$					
Ph	−0.024	0.154	0.144	0.111	0.079	−0.021	0.111	0.101	0.086	0.051
β-CH_2	0.002	0.041	0.037	0.024	0.044	0.002	0.080	0.075	0.054	0.077
α-CH_2	0.260	0.232	0.227	0.198	0.250	0.260	0.231	0.227	0.193	0.248
NH_2	0.762	0.571	0.593	0.668	0.628	0.759	0.578	0.598	0.667	0.625

[a] After Löwdin's deorthogonalization of the CNDO and INDO eigenvectors.

the experimental ones (see Table II). Such a fit is better for the neutral form. For $PEAH^+$, nevertheless, the difference between the theoretical results (the preferred rotamer is the folded one) and the experimental ones (which prefer the extended rotamer) seems to be not too drastic, in view of the probable presence of intramolecular forces in the gaseous phase with respect to intermolecular ones, which are predominant in the condensed medium. Further information on this point will be given in a following section, by means of the study of the associate $PEAH^+$-H^-. As regards the comparison between the semiempirical methods and the *a priori* one, the EHT is the best one for PEA. In the onium form, the semiempirical methods are in greater defect; nevertheless the EHT and PCILO give conformational results more similar to the nonempirical ones.

4. Mulliken Population Analysis

In Figures 8 and 9 there are reported the *ab initio* atomic charges for the two most important conformations of PEA and $PEAH^+$, namely the extended (90,180,60) and the folded (90,60,60) structures.

The *ab initio* STO-3G wave functions give a charge distribution in accord with the trends found in similar previous calculations. In the free base, all the heavy atoms, with the exception of the ring carbon atom bearing the side chain, are negatively charged, while the H atoms are positive. Such charge transfer, internal to a given group, is more marked in the NH_2 group, in accord with the greater electronegativity of N. The ortho-para directing effect of the ethylamine chain results evidently from the values of the charges on the carbon atoms of the ring. The intergroup charge transfers concern both methylene groups, the electron transfer from α-CH_2 to NH_2 being larger than the transfer from β-CH_2 to Ph (see also Table III); the two terminal groups of the molecule result, thus, negatively charged with respect to the alkane fragment. A comparison between the two rotamers does not show great differences; the largest difference is in the hydrogen attached to the C_2 atom of the ring, which is more positively charged in the folded form.

In the protonated species, two thirds of the positive charge are on the NH_3 group, the nitrogen being still negative, in agreement with the results found for other similar compounds. The intergroup charge transfers are influenced, of course, by the presence of the cationic head; also the Ph group bears a positive charge. The differences between the two rotamers concern mainly the phenyl ring; the electron transfer is smaller in the folded conformation and the C_2 atom (the nearest to the ethylamine chain) shows a larger negative charge, with respect to the other rotamer and the neutral species. In particular, the remarkable negative charge of C_2 can explain the preference of $PEAH^+$ *in vacuo* for the folded form, this being stabilized by an electrostatic attraction between the π region of C_2 and a positively charged hydrogen of the onium group, which overcomes steric repulsions.

A few words on the charges obtained with the semiempirical methods. A synthetic comparison is offered in Table III. The CNDO charges, obtained through a Löwdin deorthogonalization of the AO basis,[18] gives the best accord with the *ab initio* results. The CNDO method, however, underevaluates intragroup charge transfers, mainly between C and H. Such an inconvenience is particularly evident in the phenyl ring (which is more positively charged) and leads to some troubles in the following analysis of the reactivity properties (see later). INDO charges have the same trend as the CNDO ones, with a still larger underevaluation of the H–C charge transfer. As pointed out previously, such a failure of both methods may be due to the isotropic approximation. Both methods, however, give a correct representation of the charges of onium group, with N negatively charged (for similar results, see ref 19).

EHT and PCILO, on the contrary, give a nitrogen atom in the NH_3^+ group positively charged and also too large a positive charge for the α carbon in $PEAH^+$. The description of the charges of the phenyl carbon atoms is, however, satisfactorily given by EHT.

5. Structure-Activity Relationships (SAR)

(a) A Model of Drug–Receptor Complex. The interaction pattern of the drug molecules with the receptor sites is usually represented in terms of the conformational and populational results, along a set of homologous compounds with various pharmacological activities. Such an analysis is related to the inspection of the interatomic distances between the drug centers most frequently implicated in the drug-receptor interaction and of the atomic charges of such centers itself. Therefore, the supplied information rises from atomic considerations, rather than from the electronic properties, and it is obviously related only to a primary phase of receptor recognition. A further step in the study of the interaction pattern is represented either by an explicit inspection of the molecular electronic properties *via* the calculations, for example, of the electrostatic potential, or by the study of some simplified model of the overall associate drug-receptor.

In the phenethylamine family, two groups, the aminic head and the aromatic portion, are common to all the drugs and are most probably responsible for the main interactions. In the onium form, which at the physiological values of pH is, by far, the most abundant species, the highly ionic interaction, between the cationic head and some corresponding anionic receptor site, is a preliminary and fundamental qualification for the binding of the drug to the receptor. Several authors[2] have shown that the anionic receptor site may be characterized by a phosphate group of the ATP

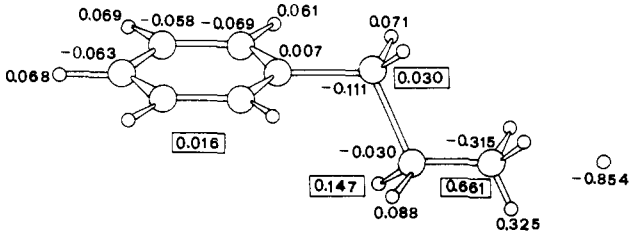

Figure 10. *A priori* STO-3G atomic and group charges for the extended form of $PEAH^+$-H^- complex.

molecule. More recently X-ray studies on the crystal structure of the ephedrine-phosphate associate[2a] have pointed out the presence of strong hydrogen bonds, between the alcoholic and onium groups of the ethanolamine moiety and the oxygen atoms of the phosphate anion, thus the conformations of the drug side chain favoring such a type of interaction.

Therefore, it may be of some interest to add some further data to the previous ones, considering also an ultrasimplified model of the drug-receptor associate, *via* the ionic interactions between the cationic head and an assumed anionic group. We have, thus, calculated the *a priori* wave functions for the associate $PEAH^+$-H^-, where the $PEAH^+$ has either the representative conformations (90,180,60) or (90,60,60). Such a model is surely a very rough one, because the H^- ion cannot be a good description of the receptor anionic site. Nevertheless, the aim of using such a model was to see if it was possible to shed more light on the relative energies of the different rotamers, when the molecule is engaged in an interaction of this kind, and to see when there appear changes in the reactivity features of the other site, *i.e.*, the aromatic portion. In these calculations the H^- ion was placed along the C_α-N axis, at the distance of the largest stabilization energy, which was found to be 2.02 Å from the nitrogen atom.

By comparing the energies of the two forms of the associate with the corresponding ones of $PEAH^+$, it comes out that the strong interaction with an anionic site (as the extended form ΔE is of the order of -135 kcal/mol for this model) does not change drastically the relative conformational energies. However, the calculations show that the extended and the folded form of the associate are nearly equivalent, with a hint that the extended form is slightly preferred, by 0.20 kcal/mol, with respect to the folded one. Such a feature is in agreement with the experimental evidence, which shows that the extended rotamer is the preferred one in the liquid or solid phase, where the intermolecular forces between the drug and the surrounding medium are prevalent.[10,20] As it is reported in Figure 10, the cationic head bears a still more positive charge with respect to $PEAH^+$, while in the other groups the positive charges, already present, decrease to some extent. Such a partial neutralization of the positive charge may have some influence on the reactivity of the phenyl ring. The capability of the aromatic moiety to undergo electrophilic attack or to form charge transfer complexes increases, as it is shown also by the change in the value of the HOMO energy; the mean value, for the two conformations here considered, of such orbital energies decreases from -10.8 eV for $PEAH^+$ to -8.1 eV for $PEAH^+$-H^-. In other words, the π electrons become less tightly bonded in the ionic complex.

(b) The Electrostatic Molecular Potential. For molecular interactions, involving molecules having net charges or permanent dipoles, it was shown that some useful information may be drawn also by the examination of the electrostatic potential, arising from one of the partners and by simple electrostatic calculations, involving such a potential, and a simplified description of the charge distribution of the other molecule, involved in the interaction.[4] We will use here the correct definition of the electrostatic potential, *i.e.*

$$V(r) = \sum_{\alpha}^{\text{nuclei}} Z_\alpha / |r_\alpha - r| - \int dr_1 \rho(r_1) / |r_1 - r|$$

as given by the electron distribution $\rho(r_1)$, derived from molecular wave functions, and by the distribution of the nuclear charges Z_α, specific of a given molecular geometry, without further simplification. $V(r)$ has the characteristics of a true observable, in the quantum-mechanical meaning, and gives more detailed and less ambiguous information than a population analysis, it being a function computed in the overall molecular surrounding space. From the potential definition, the first-order interaction energy, between the molecular charge distribution and a point charge distribution, is easily obtained as

$$W(\{r_i\}) = \sum_i q(r_i) V(r_i)$$

As pointed out previously, such an expression may be used, at least for comparative purposes, for the study of the interaction between two molecular species, by computing the electrostatic potential of the first partner, and by assuming some point charge model as representative of the charge distribution of the second partner.[4]

For an easier lecture of the results, in this work we will report the electrostatic interaction isoenergy maps with a positive unit point charge. Moreover, in the hypothesis of hydrogen bond interactions between the drug and some polar moieties of the receptor, we will also report the electrostatic interaction energy with a finite dipole, represented by two point charges. Previous calculations on typical hydrogen bonded systems showed that the interaction energy, in such a first approximation of the partner, may give account of over the 70% of the total interaction energy.[21] In the present case, lacking more detailed hypotheses on such interaction, we have chosen a model dipole consisting of two point charges at the same distance as the nuclei in a typical N-H bond (1.01 Å) and having as their moment a standard value found for the N-H bonds (1.02 D).

A few words on the values of $V(r)$ obtainable from the semiempirical wave functions. It was already shown that even the electrostatic potential arising from CNDO and INDO wave functions may give some hints on the reactivity properties, although some limitations have very recently been pointed out.[2b,22-25] For example, from unpublished results of one of us (C.P.), the CNDO (or INDO) potential for aromatic compounds is very defective, as it does not take into account the nucleophilic properties of the π electron distribution. To the best of our knowledge, no calculations of EHT and PCILO potential have thus far been published. Therefore, in this paper we will limit ourselves only to the *ab initio* potential with no further approximation.

In PEA molecule, the nonempirical calculations show that, in every rotamer, there are two regions of the space, where the potential is negative (*i.e.*, attractive with respect to the approach of positive charges): the first, and more negative, corresponds to the nitrogen lone pair; the second corresponds to the π regions of the phenyl ring. In Figures 11 and 12 there are reported for the extended rotamer (90,180,60) the isoelectrostatic energy curves for two different planes, which contain the potential minima. The first one contains the atoms C_α and N and the center of the nitrogen lone pair (assumed to be in a tetrahedral arrangement). The second plane is parallel to the phenyl ring at a distance of 1.55 Å on the half-space, which does not contain the side chain. The values of the minima are also reported in Table IV.

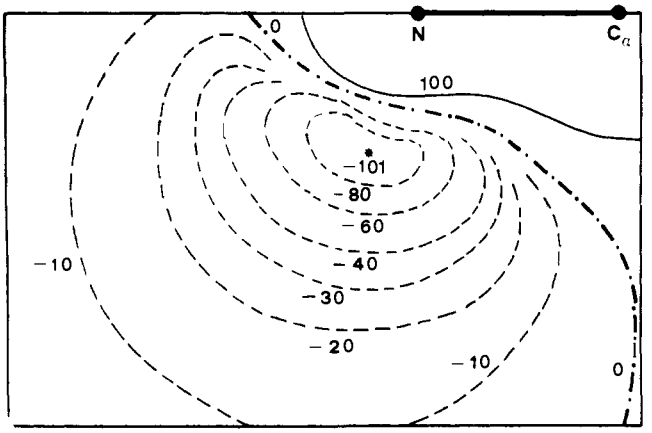

Figure 11. Electrostatic potential map for PEA; extended form (90,180,60); values in kcal/mol; nitrogen lone-pair region, plane containing the three points C_α, N, and nitrogen lone pair (assumed to be in tetrahedral arrangement).

Table IV. Minima Values of the Potential (kcal/mol)

Molecule	Conformation	E_{conf}	V(N)	V(Ph)
PEA	90,60,−60	1.07	−101.9	−12.36
	90,60,60	0.94	−100.8	−9.43
	90,120,60	3.97	−100.2	−9.33
	90,180,60	0	−100.7	−9.83
	90,180,180	0.28	−100.8	−8.56
$PEAH^+$ [a]	90,60,60	0	185.9	61.9
	90,120,60	5.62	186.9	55.7
	90,180,60	1.14	186.8	53.9
$PEAH^+-H^-$	90,60,60	0.20		1.13
	90,180,60	0		2.74

[a] V(N) is calculated at a distance of 1 Å from the extra proton of the onium form. V(Ph) is calculated in the same point as in PEA.

The negative region near the heteroatom shows a well-evidenced hole with a deep minimum, in accord with the marked proton affinity of the aliphatic amines. The minimum lies at ~1 Å from the N nucleus, *i.e.*, at a distance practically equivalent to the standard bond length of the N–H bond (1.01 Å). Also, in this case, as it was found for other compounds, the electrostatic potential gives a fair description of the conformational aspects of the protonation process. The numerical value of V_{min} is, of course, fairly different from the ΔE value of the reaction PEA + H^+ → $PEAH^+$, as given by SCF calculations (for the extended conformation (90,180,60), the values are $V = -101$ and ΔE = 271 kcal/mol). In fact, also the polarization and the charge transfer effects play an important role, but a linear relationship between V and ΔE may be found, as it was verified on other compounds.[26] A possibility of drug–receptor interaction, which is not convenient to discard *a priori*, is that the species actually involved in the reaction is the less abundant neutral one. In such a case, the protonation reaction should evidently represent a poor description of the interaction in the amine region. By introducing the model dipole above mentioned, the values of the interaction energies are of the same order of magnitude, as those obtained with more refined methods.[4] The most favorable disposition of the dipole, in its electrophilic arrangement, is along the direction of the N lone pair, at a distance such as to allow a penetration of the van der Waals spheres of about 10%. The numerical results, reported for the best positions, are shown in Table V and may give a qualitative description of hydrogen-bond like interactions.

In the aromatic moiety of the molecule, the negative zone of the potential spreads over the entire ring, with a shallow minimum slightly displaced from the center. In other aromatic compounds, the potential maps have been able to individuate the carbon atoms on which an electrophilic attack is easier.[4,27] In the present case, all the carbon atoms, with the exception of C_1 and C_6, look alike. Being interested, at present, in milder interactions, we have also investigated the energetics of the interaction with the model dipole, since it may be noted also that charge transfer complexes seem to turn out rather electrostatic.[28] The energy of interaction (see Table V), for an electrophilic approach, along the six-fold rotation axis of the phenyl ring, from the opposite site of the side chain, is decidedly smaller with respect to the interaction with the nitrogen lone pair, in agreement with the chemical evidence.

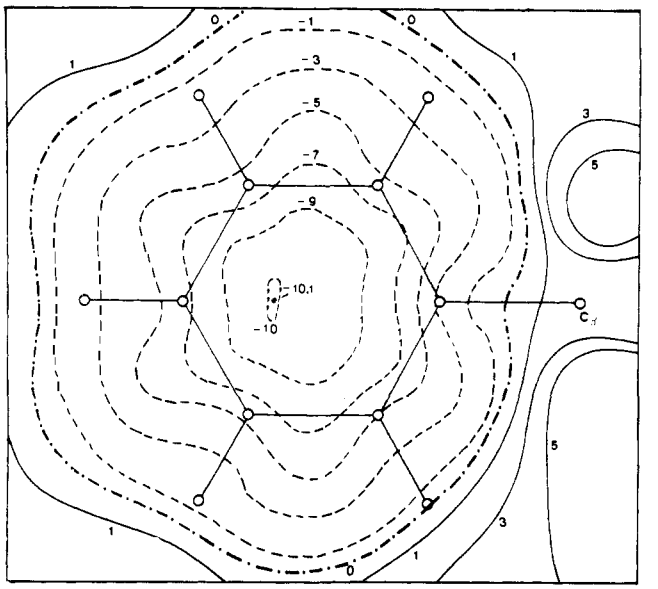

Figure 12. Electrostatic potential map for PEA; extended form (90,180,60); values in kcal/mol; aromatic π region, plane parallel to the phenyl ring at a distance of 1.55 Å at the opposite site of the side chain.

Table V. Electrostatic Interactions with the Dipole for the Best Positions (kcal/mol)

Molecule	Conformation	E_{conf}	W(N)	W(Ph)	E_{stab} [a]
PEA	90,60,−60	1.07	−6.08	−1.17	−6.18
	90,60,60	0.94	−6.78	−0.95	−6.79
	90,120,60	3.97	−6.65	−0.95	−3.63
	90,180,60	0	−6.73	−0.98	−7.71
	90,180,180	0.28	−6.69	−1.00	−7.41
$PEAH^+$ [b]	90,60,60	0	−7.47	1.03	−6.44
	90,120,60	5.62	−7.52	0.60	−1.30
	90,180,60	1.14	−7.51	0.49	−5.88
$PEAH^+-H^-$	90,60,60	0.20		−0.52	−0.32
	90,180,60	0		−0.46	−0.46

[a] $E_{stab} = E_{conf} - W$(N) − W(Ph). [b] In the same position as in PEA molecule.

The differences in the electrostatic potential among the various rotamers of PEA are not particularly impressive, as may be seen from the values reported in Tables IV and V. In Table V, the E_{stab} column gives the total stabilization energy, with respect to the best conformational one, calculated in the hypothesis that two dipoles interact simultaneously with the N and Ph regions. On the whole, it can be stated that neither the general outline of reactivity, drawn from the examination of $V(r)$, depends on the conformation, nor does the introduction of electrostatic interactions with dipoles, placed in the most favorable arrangement, change significantly the shape of the conformational energy surface.

The molecular potential of $PEAH^+$ is everywhere posi-

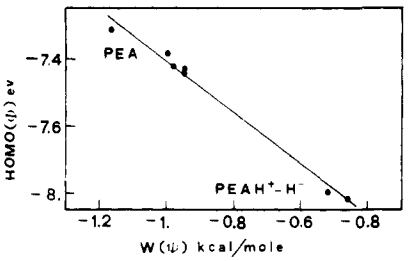

Figure 13. Correlation between the HOMO π energies and the phenyl electrostatic interaction with the dipole.

tive, particularly in the onium region. The predominant effect being due, in this case, to the monopole term, the most likely types of interaction with the biophase, if this very species is actually responsible for the pharmacological action, are the monopole-monopole and monopole-dipole ones. For the last, using the same model of dipole as before, of course with reversed polarity, there are found interaction energies of the order of −7.5 kcal/mol for both conformations. (Because the electrostatic potential of $PEAH^+$ in this region is monotonically positive, it is not possible to find an optimum distance for the dipole. The value quoted in the paper intends to give only a rough idea of the order of magnitude. The dipole was placed at the same distance, found as the most energetic for the neutral species, corresponding approximatively to a 10% penetration of the van der Waals spheres, along the direction of the extra proton of the onium form.) The monopole-monopole interaction is represented, in the present calculation, by the adducts $PEAH^+$-H^-. Such a model is probably rather unrealistic, and the explicit introduction of more representative approximations of the receptor anionic site should presumably lead to different deformations of the charge cloud of $PEAH^+$. However, the present model is sufficient to show, even at the electrostatic level, that when the onium compound interacts with a negatively charged group, the phenyl ring is able to bind electrophilic dipoles as in a neutral molecule. In fact, the potential in the aromatic region of the associate, still being positive, owing to the partial neutralization of the cationic charge of $PEAH^+$ (see Table IV), is less repulsive and presents a small minimum; the interaction energies with the usual dipole are reported in Table V.

The reactivity of the phenyl ring deserves further comment. The occurrence of the aromatic moiety appears to be a structural necessity to elicit pharmacological activity, *e.g.*, β-sympathomimetic[29] or hallucinogenic,[30] but substitution on the ring is also essential for this purpose. The most active adrenergics are, for instance, found in the series of the catecholamines and the occurrence of methoxy groups in the phenyl ring is of paramount importance in the production of hallucinogenic response in amphetamine (α-methylphenethylamine) derivatives. In both examples, the phenyl substitution induces modifications on the π charge cloud and its activity; well known are, for instance, the variation in the HOMO π energy along the set of methoxy derivatives of amphetamine and phenethylamine and the correlation found with the hallucinogenic activity.[31] The phenolic OH groups of the catecholamines are probably involved in direct interactions with a receptor of its own, but they also reinforce the binding capability of the ring.

It may be of some interest to point out that there exists a correlation between the HOMO π energies and the electrostatic propension toward binding with our model dipole in the phenyl region. The relevant data are reported in Figure 13. It may be tentatively inferred that a correlation between the potential values around the ring and the hallucinogenic potency in the amphetamine derivatives could be found.

6. Conclusions

A comparative research on the neutral (PEA) and protonated ($PEAH^+$) phenethylamine molecule has been carried out, in order to find some information about the nature of the biological role of this structure and its derivatives of medicinal interest.

Conformational analysis, using various theoretical methods (EH, CNDO, INDO, PCILO, and "*ab initio*" STO-3G), leads to a possible competence between extended and folded forms of the side chain. From all the approximate methods used, EH provides the results most equivalent to the "*ab initio*" ones. Both methods also give the best fit with experimental conformations. This is not surprising, because EH is known[32] to predict correctly the preferred conformation of a wide variety of biological molecules.

Then, experimental data may be related with the present theoretical results, although interactions of the molecule with the surrounding media may provoke an easy interconversion between both possible forms.

Overlap population analysis carried out on the "*ab initio*" wave functions, provides the first insight on the possible substrate-receptor interactions of the phenethylamine structure. Small differences are obtained between charge distributions on extended and folded forms for PEA and $PEAH^+$, when group charges are observed. The more accused differences are obtained in $PEAH^+$ on α-CH_2 and phenyl groups: a small charge transfer from the first to the second is apparent when a transformation from folded to extended form is made.

Interaction of $PEAH^+$ and H^- in the "*ab initio*" framework has been also calculated on the extended form, placing the H^- ion along the CN axis; a bonded structure has been found, with a considerable charge transfer from H^- to the phenyl moiety of the molecule. This situation can explain a primary substrate–receptor interaction pathway. The most striking feature is the practical equivalence, from the energetical point of view, of the extended and folded forms of the $PEAH^+$-H^- complex.

Electrostatic molecular potentials of both PEA and $PEAH^+$ have also been evaluated. PEA shows a deep minimum in the lone pair region of the amino group and a shallow one in the center of the phenyl ring. $PEAH^+$ electrostatic potential was found positive everywhere, as shall be expected from a positively charged molecule, by inspection of the global charge distribution. Again no striking differences were found between the folded and extended forms of both molecules.

Electrostatic interaction between a dipole and the neutral and protonated forms has also been investigated. Interaction minima were found in both forms on the phenyl moiety; this can indicate another possible receptor feature involving the phenyl ring charge cloud.

It is known that biological action varies with phenyl ring substituents in some classes of related structures, and that empirical structure-activity relationships can be found by use of phenyl related quantum mecanical parameters.[33]

One can conclude, supposing a substrate-receptor interaction of electrostatic nature,[34] that the receptor recognizes qualitatively the molecule through two types of binding: a monopolar one, involving the protonated amino group, and another, multipolar, involving the phenyl ring, this being partly responsible for the quantitative amount of the biological action strength. It can be supposed in the light of the present results, that, probably, the receptor site may be shaped as an electrostatic counterpart of the electrostatic molecular potential of phenethylamines.

In this sense, high correlations should be expected between electrostatic potential minima and biological activity in phenethylamine derivatives.

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Ab Initio Calculations on Large Molecules Using Molecular Fragments. Nitroxide Spin Label Characterizations[1]

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Abstract: An *ab initio* procedure, designed for investigation of large molecules and based upon studies of molecular fragments, is used to characterize various nitroxide spin labels. Several structural parameters are examined through calculations on nitroxide, methyl nitroxide, and dimethyl nitroxide. The calculated values of R_{NO} agree well with values determined experimentally in larger nitroxides. Also, the potential associated with out-of-plane motion is shown to be extremely shallow. Finally, the staggered conformations around the CN bonds are calculated to be preferred in both methyl nitroxide and dimethyl nitroxide. Spin densities and isotropic hyperfine coupling constants are evaluated for the above molecules and several other molecules, *i.e.*, di-*tert*-butyl nitroxide, 2,2,5,5-tetramethylpyrrolidin-1-oxyl, and 2,2,6,6-tetramethylpiperidin-1-oxyl, that are useful as spin labels in biological systems. As expected, the results indicate that essentially all the π-electron spin density is localized in the nitroxide group. However, the calculated spin density distribution between N and O shows a marked basis set dependence, with very small basis sets giving reasonable estimates of trends, but unreliable estimates of absolute magnitudes of spin densities. The effect of an external applied electric field on the spin density and ^{14}N isotropic hyperfine coupling constants is also assessed, in order to simulate the environment found in excitable membranes. The results indicate that rather large fields (>500,000 V/cm) are required to produce measurable changes in the ^{14}N isotropic hyperfine coupling constants. Finally, MO ordering of H_2NO is examined and compared to other available data.

I. Introduction

An *ab initio* procedure (the molecular fragment procedure), designed for the investigation of large molecular systems and based upon studies of molecular fragments, has been developed over the past several years and has been used to investigate problems involving various types of closed-shell molecular systems.[2a] The utility and applicability of the molecular fragment procedure for closed-shell systems have been analyzed recently,[2b] and its extension to open-shell systems has been described. Specifically, investigations have been carried out on several hydrocarbons,[3] and on a number of excited states of formaldehyde and its radical ions.[4] This paper continues the characterization of the molecular fragment procedure in open-shell systems, reporting the results of a study of nitroxide free radicals.

The first stable nitroxide was reported in 1959,[5] although their existence was postulated earlier in 1956 by Rogers, Johnson, and Trappe.[6,7] Since that time, these molecules have developed as important probes in both biological[8] and excited state studies.[9] Despite their great utility as probes and spin labels, the literature on the basic properties of the nitroxides is still quite sparse and contains much conflicting information, especially concerning the geometry of the nitroxides and the localization or lack thereof of the unpaired electron.

This study examines several important nitroxides in order to characterize the electronic and geometric features of these molecules and to assess their effect upon properties such as the spin density distribution. In addition, an estimate of the effect on the spin density and associated proper-