is difficult to estimate since it would only be apparent in the correlations or in the determination of log P values. The rather good agreement between log P_0 and π_0 for both aliphatic and the more inflexible aromatic compounds does not reveal any discontinuity. From some preliminary work measuring partition coefficients, it would appear that at least for some systems π for each CH_2 unit is constant up to at least 10 carbon atoms. Of course this holds only as long as no electronic or dipolar interactions promote intramolecular hydrophobic bonding.[6b] The extreme difficulty in measuring partition coefficients of apolar groups larger than this leaves some uncertainty about the very large aliphatic compounds in Table I. This presents no problem for the results with gram-negative bacteria shown in Table III. While there are several instances where folding could occur with long chains of the molecules on which the data of Table V are based, comparison of the log P_0 for the rigid phenols with the flexible aliphatic amines does not reveal a significant difference in log P_0. For the six more rigid structures we find a mean log P_0 of 5.8 and, for the five flexible examples (including the quinine derivatives), we find a mean log P_0 of 6.0.

As mentioned above, it must be strictly borne in mind that the log P values we have used are for the neutral un-ionized form of the molecules. This poses no problem for the compounds of Table III; however, for the molecules of Table V we are comparing quite basic amines, of which only a very small fraction would be in the neutral form under test conditions, with relatively un-ionized phenols. The fact that we find the same log P_0 for these amines as we do for the phenols and ureas would indicate that the un-ionized form is more suitable to consider in correlation studies. The partition coefficient of the ionized molecule would be greatly different from that of the un-ionized form. Exactly why one finds very similar log P_0 values for highly ionized and un-ionized molecules as well as rather rigid aromatic and flexible aliphatic compounds is not apparent and suggests an important area for further study.

In summary, one can say that octanol–water partition coefficients constitute a very useful reference system for comparative biochemical and pharmacological studies where hydrophobic bonding is involved. Log P_0 also appears to be a useful constant for the study of the movement of organic compounds through biophases.

Acknowledgment.—C. Hansch wishes to express his gratitude to the Guggenheim Foundation for a supporting fellowship, and to Smith Kline and French for a research associateship for Susan Anderson.

Molecular Orbital Calculations of the Preferred Conformations of Histamine and a Theory on Its Dual Activity

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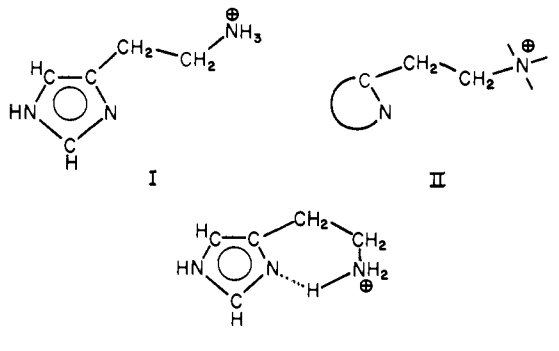
Extended Hückel molecular orbital calculations on the histamine molecule reveal two conformations of nearly equal preference, on the basis on calculated minimum energy. Neither conformation involves intramolecular hydrogen bonding. Population analyses reveal the charge-density pattern of the imidazole ring. The dual activity of histamine is proposed to be a consequence of the existence of two preferred conformations in equilibrium. One of these conformations places the quaternary nitrogen and the ($tr^2trtr\pi$) nitrogen of histamine 4.55 Å apart, which is quite comparable to the 4.8 Å estimated for the internitrogen distance in the antihistaminic triprolidine. An assignment of each histamine conformation to one of two histamine effects is provisionally made on this basis. This explanation of dual activity is comparable with that offered for a similar situation found in previous calculations on acetylcholine, muscarine, and nicotine.

Histamine (I) is known to produce a series of well-characterized biological responses when it is released from storage cells by the influence of trauma or chemical agents. A number of other molecules are known to produce these responses, but histamine is the most active compound known and remains the prototype of histaminic activity. It is evident that the histamine molecule must present near-optimal electronic features to its receptor. To date, several studies have been directed toward elucidating the features of the molecule that are necessary to elicit biological activity. Lee and Jones[1] have suggested that an important structural feature is the fragment II, in which the ring is a small aromatic nucleus. Neimann and Hays[2] have suggested that the univalent cation (the predominant form at body pH) will exist in a hydrogen-bonded form, III. These authors felt that the ability to form this hydrogen bond is a necessary condition for histaminic activity. Lee and Jones,[1] however, observed that, although all of the active compounds they studied were



I II

III

(1) H. M. Lee and R. G. Jones, *J. Pharmacol. Expll. Therap.*, **95**, 71 (1949).

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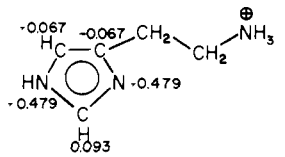
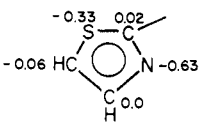
capable of forming such a bond, many compounds that could conceivably form such a bond were inactive. The question of the necessity of this hydrogen bond for activity is one of the problems to which the present study was addressed.

Alterations in the imidazole ring have led generally to abrupt lowering of activity.[1,3,4] However, these studies have not revealed any evident relationship between structure and activity. π electron density calculations of the imidazole ring IV by Brown[5] and of the thiazole ring V by Pullman and Metzger[6] failed to explain the different levels in the activities of IV and



IV



V

histamine. These calculations, however, did not consider σ electrons, were made with different parameters, were made on the aromatic ring only, and did not consider possible stereochemical differences in the chain. Stereochemical differences seem to emerge from all of the work done as a structural feature just as important as atom placement in the small aromatic ring. A recent review by Barlow touches on this.[7]

The intuitive assessment of the preferred conformation becomes less reliable as molecules increase in size and "flexibility." What is needed is a more fundamental method to evaluate conformational preference. The beginnings of this approach can be found in recent advances in quantum mechanics. A particularly promising method has been devised by Hoffmann in which Hückel molecular orbital formalism is extended to all valence electrons, a method referred to as extended Hückel theory (EHT).[8] All overlap integrals are considered, hence the eigenvalues are geometry dependent. The method is based on the use of a single parameter, the ionization potential, to define the Coulomb integral and to calculate the appropriate resonance integrals. The method has met with significant success in calculating the preferred conformation of hydrocarbons, both aliphatic and aromatic and in the cation form. In a recent study, we have extended the method, for the first time, to the prediction of the preferred conformation of a heteroatom-containing molecule.[9] This success has prompted us to use this method in the study of the preferred conformation of biologically important molecules. We have also calculated the preferred conformations of acetylcholine, muscarine, and muscarone.[10] Our calculations predicted preferred conformations for acetylcholine and muscarine practically identical with the conformations reported for these molecules by X-ray analysis of their crystals. These calculations showed that the heteroatoms in each molecule were of similar distance from each other in each case. It is not possible to state conclusively that these molecular orbital calculations predict the preferred conformation in solution in the biophase. Nevertheless, the striking coincidence of similar biological activity and similar heteroatom placement in the crystals and in the calculated preferred conformations is highly suggestive that the approach has considerable potential value. This is particularly true if it can be shown, as was the case with muscarine and muscarone, that the barriers to rotation are quite high. It would be suspected then that the same preferred conformation would be found even in a solvating medium or under the perturbing influence of a receptor.

In addition, we have used the EHT method to study nicotine, calculating a preferred conformation of the nicotinium ion that is in agreement with nmr solution studies.[11] From the calculated electron densities of the atoms of nicotine, we have concluded that the secondary binding site in the molecule is the negatively charged nitrogen atom of the pyridine ring. The position and charge of this atom corresponds quite closely with the carbonyl oxygen of acetylcholine in its preferred conformation.

Using the same method, Giordano, *et al.*, have recently studied the conformations of 2-formyl-*N*-methylpyridinium oxime.[12] Their results also indicate the possible utility of EHT calculations for predicting preferred conformation of drug molecules.

We endeavored in this study to apply these quantum chemical techniques to the study of histamine to deduce the preferred conformation of the molecule and to gain some insight into the total electron density ($\sigma + \pi$) of the aromatic ring atoms.

Experimental Section

It is probable that at physiological pH, histamine exists as a monovalent cation with the side-chain amino group protonated. This group has a pK_a of 9.70, whereas the ring ($tr^2tr1r\pi$) nitrogen has a pK_a of 5.90.[13] Accordingly, the present calculations were made on the protonated histamine molecule. The bonds were assumed to be of standard length, using the values suggested by Pople and Gordon.[14] The Coulomb integrals, H_{ii}, were approximated as the valence-state ionization potentials.[15,16] The off-diagonal matrix elements, H_{ij}, were evaluated by the approximation developed by Mulliken[17] and Wolfsberg and Helmholtz[18]

$$H_{ij} = -0.5K(H_{ii} + H_{jj})S_{ij}$$

where S_{ij} is the overlap integral and K is a constant set equal to 1.75. The total energy was calculated as twice the sum of the energies of all occupied orbitals, to account for double electron occupancy. The conformer having the lowest calculated total energy was assumed to be the preferred one.

Results

The major variation in total energy occurred with the rotation of the C–C side-chain bond. Rotation of this

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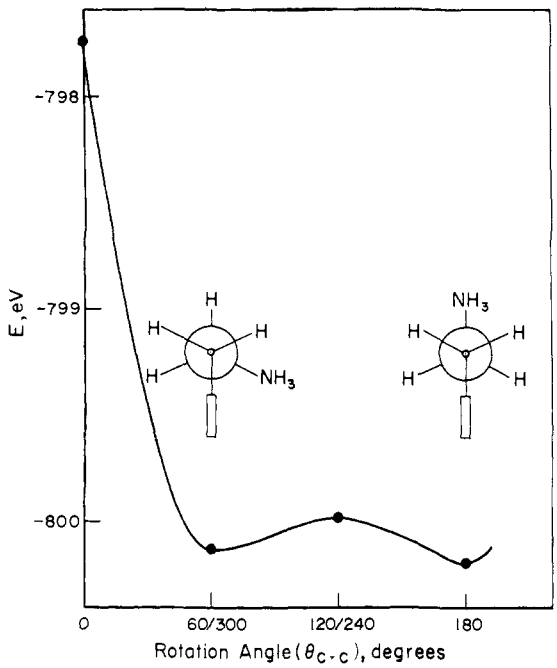


Figure 1.—Angle of histamine side-chain rotation, θ_{C-C}, and calculated total energies.

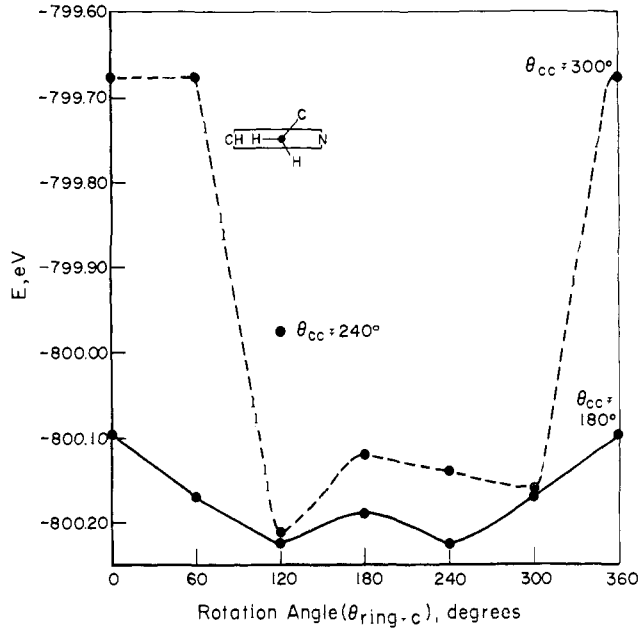


Figure 2.—Angle of histamine ring to side-chain bond, θ_{ring-C}, and calculated total energies.

bond through 180° by 60° increments produced the total energy *vs.* angle relationship illustrated in Figure 1. The minimum energy was found for the conformer in which the side-chain atoms are all staggered from the ring, where $\theta_{CC} = 180°$. Table I compares the

TABLE I
SIDE-CHAIN ROTATION ANGLE, θ_{CC}, WITH ENERGIES AND DISTANCES

		Interatomic distances, Å		
θ_{CC}, deg	Total E, eV	N^+ to N ≤	$^+$NH to N ≤	$^+$N to N ≤
0	−797.737	2.13	1.79	4.34
60/300	−800.120	2.86	2.07	4.83
120/240	−799.872	3.93	3.76	5.70
180	−800.189	4.37	4.78	6.08

total energy values for each rotamer and for key interatomic distances. It is evident that the rotamer corresponding to the postulated quasi-bicyclic system III is the least energetically preferred. The energy between $\theta_{CC} = 0°$ and $\theta_{CC} = 180°$ is 2.45 eV, which is substantial, even allowing for the exaggerated values characteristic of EHT-calculated energy barriers. This finding would argue against a hydrogen bond between the N^+ and the $(tr^2trtr\pi)N$ having this conformation.

It is to be noted that a secondary minimum occurs for the $\theta_{CC} = 60°$ or $\theta_{CC} = 300°$ rotamer, as illustrated in Figure 1. Numerical values shown in Table I reveal an energy difference of 0.069 eV (1.61 kcal) between these two minima, which are separated by a barrier of approximately 0.28 eV (6.5 kcal). Although precise energy values are not possible with EHT calculations, one can derive reason from the relative calculated energies. At this point in the calculations, it would appear that the major conformer involving the side-chain C–C bond would be the $\theta_{CC} = 180°$ rotamer, with some small probability of the $\theta_{CC} = 60°/300°$ rotamer participating.

An examination of the $\theta_{CC} = 60°/300°$ rotamer interatomic distances, given in Table I, shows the N^+-to-$(tr^2trtr\pi)N$ distance to be 2.86 Å, easily within $N\cdots H—N^+$ hydrogen bonding distance. However, the geometry of the $^+N—H$ hydrogen atoms will not permit an approach any closer than about 2.0 Å between the H and the $(tr^2trtr\pi)N$. Hence, it is unlikely that a hydrogen bond bridges the two nitrogen atoms in this conformation, because of an unfavorable geometry and less than minimal energy considerations.

To search further for the minimum energy, the rotamers possible with the ring–C bond must be considered. Since the C–C bond rotation produced two minima of reasonably close energy values, it seemed desirable to calculate further for a possibly lower energy minimum occurring with each of these C–C conformations. Accordingly, both the $\theta_{CC} = 180°$ and $\theta_{CC} = 300°$ conformations were subjected to calculations involving rotation of the ring through a full cycle. Figure 2 shows the calculated energy for the various ring–C rotamers for both the $\theta_{CC} = 180°$ and $\theta_{CC} = 300°$ conformations. Because of symmetry, the variation of the $\theta_{CC} = 180°$ conformation repeats twice per cycle. With both C–C bond conformations, a deeper energy minimum is found at $\theta_{ring-C} = 180°$. Tables II and III present the numerical values of energies and key atomic distances. The lowest energy value is found for the $\theta_{CC} = 180°$, $\theta_{ring-C} = 120°$ conformation VI. But this conformation is only

TABLE II
RING-ROTATION ANGLE θ_{ring-C} FOR $\theta_{CC} = 180°$ WITH ENERGIES AND DISTANCES

		Interatomic distances, Å		
θ_{ring-C}, deg	Total E, eV	$^+$N to N ≤	$^+$NH to N ≤	$^+$N to N ≤
0	−800.095	5.07	5.22	5.74
60	−800.171	4.90	4.95	5.83
120(VI)	−800.224	4.55	4.72	6.00
180	−800.189	4.37	4.78	6.08

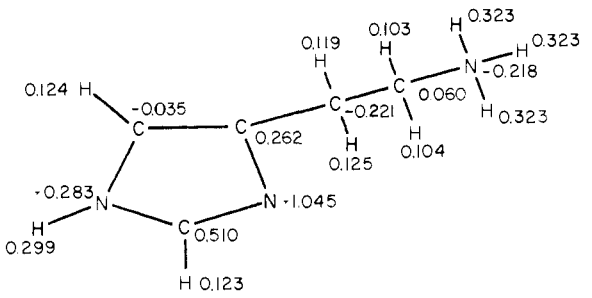


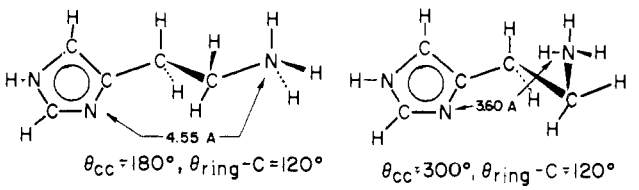
Figure 3.—Histamine monocation total ($\sigma + \pi$) net charges.

TABLE III

RING-ROTATION ANGLE, θ_{ring-C}, FOR $\theta_{CC} = 300°$ WITH ENERGIES AND DISTANCES

θ_{ring-C}, deg	Total E, eV	Interatomic distances, Å: $^+$N to N ≤	$^+$NH to N ≤	$^+$N to N ≤
0	−799.676	4.23	3.75	4.17
60	−799.686	4.22	3.74	4.17
120 (VII)	−800.213	3.60	3.02	4.52
180	−800.120	2.86	2.07	4.83
240	−800.139	2.87	2.09	4.83
300	−800.161	3.62	3.04	4.51

0.011 eV (0.25 kcal) lower than the $\theta_{CC} = 300°$, $\theta_{ring-C} = 120°$ conformation VII.



The N^+-to-$(tr^2trtr\pi)N$ distances in neither conformation are close enough to expect any hydrogen bonding to occur. Conformation VII has an internitrogen distance of 3.60 Å but the closest hydrogen atom on the N^+ is over 3 Å from the $(tr^2trtr\pi)N$; hence, hydrogen bonding is ruled out in these cases.

The choice as to which conformation VI or VII prevails remains problematical. The very slight calculated energy difference between the two minima, 0.25 kcal, is possibly exaggerated.[8] Very likely an equilibrium exists between them. In Figure 2 we have shown the calculated energy for the $\theta_{CC} = 240°$, $\theta_{ring-C} = 120°$ conformer. This conformer is intermediate between VI and VII when the C–C bond is rotated and represents an energy barrier between VI and VII of about 0.24 eV (5.5 kcal). (See Table IV.)

TABLE IV

SIDE-CHAIN ROTATION FOR $\theta_{ring-C} = 120°$ WITH ENERGIES

θ_{CC}, deg	Total E, eV
180 (VI)	−800.224
240	−799.976
300 (VII)	−800.213

If the calculated energy difference of 0.25 kcal between VI and VII is assumed to be approximately correct, then it follows that VI would prevail to an extent of about 60% in equilibrium with VII.

Population analyses were performed on the preferred conformation VII and are reproduced for the ring as total ($\sigma + \pi$) net charges in Figure 3. The $(tr^2trtr\pi)N$ bears a very substantial negative charge, whereas the $(trtrtr\pi^2)N$ is much less electron rich. A substantial positive charge is generated on the carbon atom flanked by the two nitrogen atoms. If the quaternary nitrogen atom of the side chain is regarded as a primary binding site to a receptor, it is quite likely, in view of the common occurrence of the fragment II among histaminics, that the secondary site is the $(tr^2trtr\pi)N$ and it is highly electron rich.

Discussion

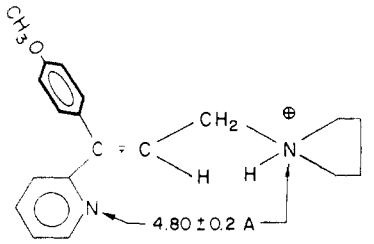
The finding of two significantly different but nearly equally preferred conformations for the histamine cation, by these quantum chemical calculations, raises an extremely interesting question. Can the histamine molecule function *in vivo* to elicit two types of biological responses from two different receptors, one for each conformation? Experimental evidence suggests that this is quite possibly the case. This dualistic behavior manifests itself as guinea pig ileum stimulation on one hand and gastric secretory stimulation on the other. Ash and Schild have obtained evidence supporting the view that histamine receptors may be differentiated into at least these two classes.[19] In their study of histamine analogs, they observed structural requirements for the histamine receptor in the guinea pig ileum (H_1) different from those for the rat uterus and stomach. For distinction, we shall designate the latter receptor as H_2.

Further evidence of the existence of two types of histamine receptors is found in the fact that antihistaminics fail to suppress the action of histamine in stimulating secretion of gastric acid, although they do block the action of histamine on guinea pig ileum.

The question arises then as to which histamine conformation is associated with which receptor in eliciting the characteristic response.

As an approach to this question, we considered the more potent antihistaminics in an effort to find one in which the spatial relationship of key atoms could be reasonably well established. These key interatomic distances in the antihistaminic molecule were then compared with those in VI and VII in order to ascertain which histamine was reasonably close enough to be designated as the conformation associated with the H_1 receptor.

Such an antihistaminic molecule is found in the potent antihistaminic triprolidine VIII. This molecule has been shown by uv absorption to have the pyridyl ring coplanar with the olefinic bond.[20] If the



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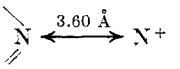
molecule is placed in a conformation permitting the closest approach of the two nitrogen atoms (as depicted in VIII), an internitrogen distance of 4.8 ± 0.2 Å is found, depending upon the choice of angle taken between the pyridyl ring and the double bond. This conformation is not an unreasonable one, although quantum chemical calculations on this molecule as well as physical evidence to support it are lacking. Nevertheless, the internitrogen distance in triprolidine is quite close to the comparable internitrogen distance of 4.55 Å calculated for histamine in conformation VI.

From these calculations and the assumptions made, we propose that VI is the histamine molecule specific for the histamine H_1 receptor. It is not possible at this time to conclude that histamine VII is the prototype of histamine-receptor H_2 activity, since no H_2 blockers are well established. However, it is offered as a possible conformation, pending further work.

A comparison of VI and VII with triprolidine makes it evident why this antihistaminic could competitively inhibit only one preferred conformer of histamine. The close internitrogen distance of 3.60 Å in VII could not be effectively simulated by triprolidine. An antagonist of histamine-receptor H_2 activity would have to have an internitrogen distance of about 3.60 Å, among other features, based upon our calculations.

For these considerations it is possible to theorize that the histamine molecule, by virtue of having the ability to exist in two distinctly different but nearly equally preferred conformations, can exert two distinct biological responses, depending on the presence of one or the other complementary receptors. The H_1 receptor is predicted to be complementary to the internitrogen relationship

and the H_2 receptor is predicted to be complementary to the internitrogen relationship



This explanation of the dual action of an agonist is applicable also to acetylcholine, based on our previous calculations on acetylcholine, muscarine, muscarone,[10] and nicotine.[11] Two calculated preferred conformations were found for actylcholine. One was comparable to the calculated preferred conformations of muscarine and muscarone and could be called the "muscarinic" acetylcholine conformation. The other was comparable to the calculated preferred conformation of nicotine and could be called the "nicotinic" acetylcholine conformation. This same proposal of conformational isomerism to explain acetylcholine's dual activity has been advanced by Martin-Smith, Smail, and Stenlake.[21] The generality of this dual conformation–dual action concept awaits further studies.

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Appendix

In Figure 3, we have displayed the calculated net charge densities for all of the atoms in the histamine cation. Of particular interest is the calculation of a negative charge for the quaternary nitrogen atom. It has been conventional to regard and to represent the nitrogen atom of a quaternary amine as having a positive charge. In order to test the validity of our assignment, we have examined the charge on the nitrogen of the simplest quaternary amine, the ammonium ion. This was chosen because of its symmetry, ease of more sophisticated MO treatment, and because a wave function for NH_4^+ has been published by Krauss which yields about 99.9% of the true Hartree–Fock energy.[22] We have performed an EHT calculation on NH_4^+ and have compared the charges with those from an SCF calculation using Pople and Segal's CNDO/2 MO method[23] (Table V). A third comparative set of charges was obtained from Krauss' *ab initio* work on which a Mulliken population analysis was performed.[24] This latter calculation was performed by Dr. James Hoyland of the Theoretical Chemistry Division of Battelle Memorial Institute.

TABLE V
NET CHARGES ON ATOMS OF NH_4^+

Method	Charge, eV N	H
Extended Hückel	−0.31	+0.33
CNDO/2	−0.36	+0.34
Ab initio	−0.41	+0.35

The results indicate clearly that the nitrogen atom of NH_4^+ is negatively charged. It would be expected that the nitrogen atom of alkyl quaternary salts would also be negatively charged. In the case of the histiminium ion in Figure 3, the net positive charge of the group lies on the attached atoms, thus the unit $C-NH_3$ possesses a net charge of +0.811. It is encouraging to note the degree of agreement of numerical values in using three levels of quantum chemical sophistication. The finding of a negatively charged quaternary nitrogen atom from EHT calculations is also consistent with an SCF calculation by Veillard, *et al.*,[25] for BH_3–NH_3 and a detailed SCF calculation on NH_3HCl by Clementi.[26] The significance of this finding to the interpretation of drug action remains to be seen.

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