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# Molecular Orbital Study of the Conformation of Histamine: the Isolated Molecule and Solvent Effect

B. PULLMAN AND J. PORT

*Institut de Biologie Physico-Chimique, Laboratoire de Biochimie Théorique, Associé au Centre National de la Recherche Scientifique, Paris 5e, France*

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## SUMMARY

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Extended Hückel theory and the method of perturbative configuration interaction using localized orbitals (PCILO) predict strikingly different results for the conformation of histamine cations. Extend Hückel theory predicts that both mono- and dications should exist as mixtures of *trans* and *gauche* forms, as observed with NMR studies in solution, whereas PCILO predicts a strong preference of the monocation for the *gauche* form and of the dication for the *trans* form, as shown by X-ray crystallography. In order to resolve this dilemma, nonempirical *ab initio* computations have been performed for the two species. They confirm the results of the PCILO calculations and indicate that the intrinsic conformational preferences of the isolated molecule correspond closely to the crystal structure data. In order to elucidate the situation prevailing in solution, the principal hydration sites of the histamine cations have been determined by calculations *ab initio*, and new conformational energy maps have been constructed for the hydrated species by the "supermolecule" approach within the PCILO method. The results indicate that hydrated cations should have a much weaker tendency than free ones for an exclusive conformation and should exist in solution as a mixture of *gauche* and *trans* forms, in close agreement with NMR results. Similar computations are reported for the rare $N_1$—H tautomer of histamine. Conformational and electronic differences appear between the two monocationic tautomers, which may be of significance for their activity at different receptors.

## INTRODUCTION

Histamine (Fig. 1) is a molecule of primary importance in pharmacology, exerting powerful and specific physiological effects. There has recently been much interest, both theoretical and experimental, in the conformational properties of this molecule, since, as with other pharmacologically active compounds, an understanding of its conformational behavior is expected to be important for elucidation of its mechanism of action.

Surprisingly, different theoretical methods have led to strikingly different results—a relatively rare phenomenon. The first molecular orbital calculation was performed by Kier (1), using extended Hückel theory for monocation *a* of Fig. 1, which is the prevailing tautomeric and ionic form of histamine at physiological pH (2). The

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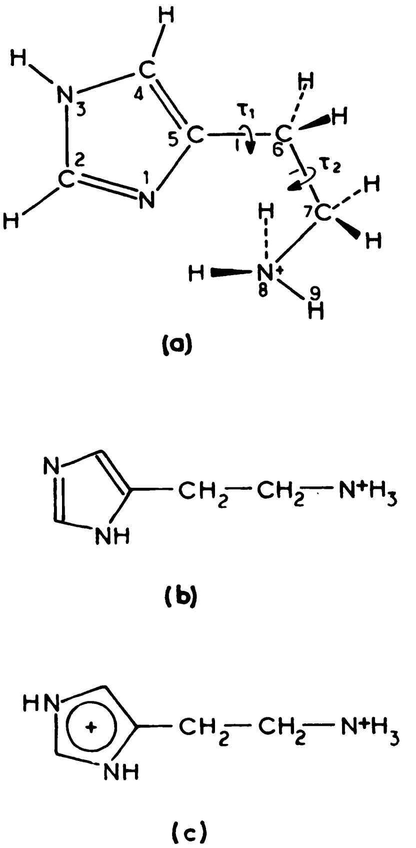

FIG. 1. *Cations of histamine*

*a*, the predominant monocation at physiological pH, with torsion angles $\tau_1$ and $\tau_2$ indicated (the form shown corresponds to $\tau_1 = \tau_2 = 0°$); *b*, the rare monocation; *c*, the dication.

torsion angles considered were $\tau_1 = \tau$ ($N_1$—$C_5$—$C_6$—$C_7$) and $\tau_2 = \tau$ ($C_5$—$C_6$—$C_7$—$N_8$), previous studies on similar cases having shown that the third torsion angle, $\tau_3 = \tau$ ($C_6$—$C_7$—$N^+$—$H_9$), may be fixed at 60°, 180°, or 300°, corresponding to a staggered position of the $N^+H_3$ group with respect to the $C_6$—$C_7$ bond. (The torsion angle $\tau$ between the bonded atoms *A*—*B*—*C*—*D* is the angle between the planes *ABC* and *BCD*. Viewed from the direction of *A*, $\tau$ is positive for clockwise and negative for counterclockwise rotations. The value $\tau = 0°$ corresponds to the planar-*cis* arrangement of bonds *AB* and *CD*.) The first of these torsion angles defines the position of the plane of the side chain with respect to the plane of the ring, and the second, the orientation of the cationic head with respect to the ring, e.g., *trans* or *gauche*. The results indicate a slight preference for a *trans* conformation ($\tau_1 = 60°$, $\tau_2 = 180°$) with two secondary *gauche* energy minima: one at $\tau_1 = 60°$, $\tau_2 = 60°$, 0.5 kcal/mole above the global minimum, and the second at $\tau_1 = 90°$, $\tau_2 = -60°$, 1 kcal/mole above the global minimum, with the plane of the side chain always oblique to that of the ring. These extended Hückel theory results were confirmed recently by Ganellin and collaborators (2, 3), who estimated that the *trans* conformer is 55–60% at equilibrium. Ganellin *et al.* also extended their computations to the tautomeric monocationic form *b* (Fig. 1), which they estimate to be 20% at physiological pH, and to the diprotonated form *c*, which should prevail at low pH and for which they predicted a slight further favoring ($\approx$65%) of the *trans* form: global energy minimum at $\tau_1 = 70°$, $\tau_2 = 180°$, again with two *gauche* minima, one at $\tau_1 = 70°$, $\tau_2 = 60°$, 0.5 kcal/mole above the global one, and another at $\tau_1 = 110°$, $\tau_2 = -60°$, 1.3 kcal above the global one.

Strikingly different results are obtained by the PCILO[1] method (4). The PCILO conformational energy map for the principal tautomeric form of the monocation (Fig. 1*a*) is reproduced in Fig. 2 (where the minima of extended Hückel theory computations are also shown for comparison). The results indicate a very neat global energy minimum for a *gauche* form, with the plane of the side chain only moderately inclined with respect to that of the ring at $\tau_1 = 30°$, $\tau_2 = -60°$, which is clearly strongly stabilized by a close approach between the negatively charged $N_1$ atom of the ring and the ammonium group. (For distribution of electronic charges, see Fig. 12.) The *trans* form is calculated to be about 11 kcal/mole above this global minimum, which means that the compound is expected to exist almost completely in the *gauche* form in its isolated state. The PCILO results for the diprotonated form (Fig. 1*c*), presented in

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[1] The abbreviations used are: PCILO, perturbative configuration interaction using localized orbitals; CNDO, complete neglect of differential overlap; SCF, self-consistent field.

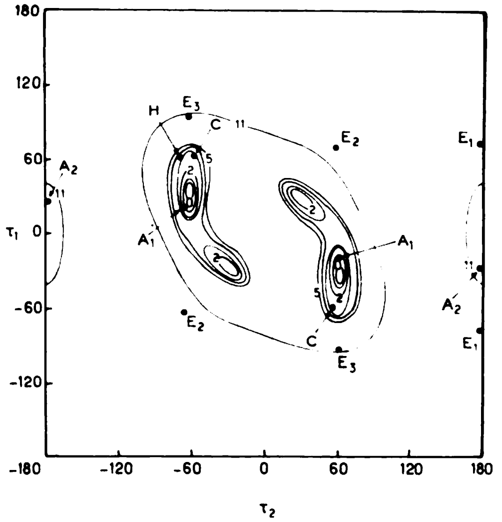

FIG. 2. *PCILO conformational energy map of histamine monocation (Fig. 1a)*

Isoenergy curves in kilocalories per mole are shown with respect to the global energy minimum, considered as zero energy. $E_1$, $E_2$, and $E_3$ are energy minima of extended Hückel theory calculations, in order of increasing energy. $C$ is the global minimum of CNDO computations. $A_1$ is the global energy minimum *ab initio* of the *gauche* forms; $A_2$, the local energy minimum *ab initio* of the *trans* forms, 21 kcal/mole above $A_1$. $H$ is the crystal conformation of histidine (5).

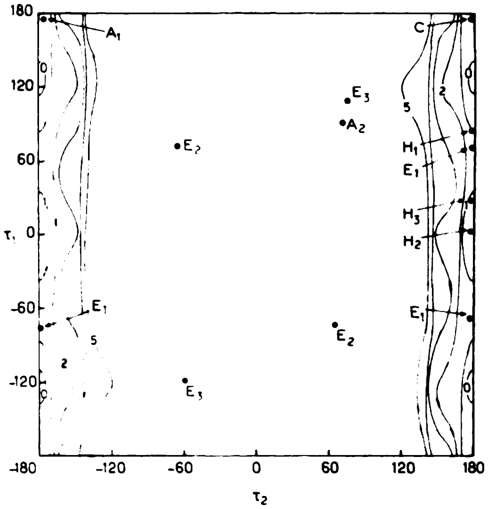

FIG. 3. *PCILO conformational energy map of histamine dication (Fig. 1c)*

Isoenergy curves in kilocalories per mole are shown with respect to the global energy minimum, considered as zero energy. $E_1$, $E_2$, and $E_3$ are energy minima of the extended Hückel theory calculations, in order of increasing energy. $C$ is the global minimum of the CNDO computations. $A_1$ is the global energy minimum *ab initio* of the *trans* forms; $A_2$, the energy minimum *ab initio* of the *gauche* forms, 8 kcal/mole above $A_1$. $H_1$, $H_2$, and $H_3$ are crystal conformations of the dication of histamine (6–8).

Fig. 3, indicate just the opposite. They predict a very strong preference of the compound for a *trans* conformation, with the global energy minimum at $\tau_1 = 120°$, $\tau_2 = 180°$, and a close local energy minimum at $\tau_1 = 0° \pm 30°$, $\tau_2 = 180°$, 1 kcal/mole above the global one. In fact, when $\tau_2 = 180°$, the whole range of the *trans* forms at all values of $\tau_1$ is enclosed by the 2 kcal/mole contour line, the stability of these forms clearly being due to minimization of the repulsion between the two positive centers in this conformation. The *gauche* forms are predicted to be about 8 kcal/mole higher in energy than the *trans* form, which may therefore be expected to represent almost uniquely the isolated state of the compound.

It is thus evident that the two procedures, extended Hückel theory and PCILO, lead to substantially different results, the first predicting the coexistence of *gauche* and *trans* forms for both cations with a slight predominance of the *trans* form, somewhat greater in the dication than in the monocation, and the second predicting a strong preference of the two cations for different forms: of the monocation for the *gauche* form, and of the dication for a *trans* form. The PCILO method is more highly refined, both conceptually and technically, than extended Hückel theory, and its results may thus be considered more plausible a priori. Moreover, calculations carried out by the methods of complete (2, 3) and intermediate (9) negelect of differential overlap tend to agree with the PCILO predictions. The results of these calculations are included in Figs. 2 and 3.

Although the computations have been carried out for isolated molecules, it is customary to seek confirmation by comparison with available experimental data. In the present case two types of data are available, one for crystal structure, obtained from X-ray and neutron diffraction studies, and the other for the situation in solution, obtained by NMR studies.

Strikingly, and perhaps again surprisingly, the experimental situation parallels the discrepancy between the theoretical predictions. X-ray results for histamine are available only for the diprotonated form, which was studied in three different crystals: as the diphosphate monohydrate (6), tetrachlorocobaltate (7), and bromide (8). The three crystals show a *trans* arrangement of the ammonium group relative to the ring ($\tau_2$ = 180°) but three different values of $\tau_1$, equal, respectively, to 90°, 0°, and 30°, thus corresponding closely to the general prediction of the PCILO map (Fig. 3). No X-ray data are available for histamine monocation. The closely related histidine monocation, however, has been abundantly investigated by several groups (10–14); in this case the $NH^+{}_3$ group always appears, in both orthorhombic and monoclinic crystals, to be *gauche* with respect to the ring (and the $COO^-$ group *trans*), with $\tau_1 \approx 57°$ and $\tau_2 \approx -59°$, which is very close to the global energy minimum of Fig. 2. This *gauche* arrangement corresponds to an intramolecular interaction between the $NH^+{}_3$ group and the lone pair-bearing atom $N_1$ of the ring. Indeed, a recent neutron diffraction study, which indicated the positions of the hydrogen atoms (5), explicitly showed this interaction as a bent hydrogen bond $N_1 \cdots H{-}N^+{}_8\langle$. It was assumed over 30 years ago that histamine itself could form the same type of intramolecular bond; indeed, it was considered a prerequisite for activity (15).

Although these crystal structures obviously represent selected stable conformations and cannot be extended to other possible forms, they clearly imply that intramolecular electrostatic forces, evident in the results of the PCILO computations, are dominant at least in these cases, with a resulting net preference of the *gauche* form for monoprotonated histamine and of the *trans* form for diprotonated histamine.

The second group of experimental data refers to the situation in aqueous solution, as indicated by measurements of NMR coupling constants for the ethane-like protons on atoms $C_6$ and $C_7$ of the side chain of histamine. On the assumption that *trans* and *gauche* forms are the only ones present, it is possible to estimate their relative proportions from these measurements. Two such studies have been performed for histamine, one by Ganellin and co-workers (2, 3), and the other by Ham, Casy, and Ison (16, 17). Both predict essentially equal proportions of the *trans* and *gauche* forms in monoprotonated histamine, with significant favoring of the *trans* form in the diprotonated species. The second proton increases the proportion of the *trans* conformer from 47% to 67% according to Ham *et al.* (17), and from 45% to 55% according to Ganellin *et al.* (2). The energy differences between the *trans* and *gauche* conformers in solution are thus predicted to be extremely small (less than 0.5 kcal/mole). The data provide no information on the torsion angle $\tau_1$. These solution results seem in very close agreement with the predictions of the extended Hückel theory calculations, an impression emphasized by the fact that this apparent agreement extends to studies on different methylated histamines (18, 19).

Whereas X-ray crystallographic results favor the PCILO computations, NMR studies in solution support the extended Hückel theory computations. It is generally assumed that the situation in an inert solvent corresponds more closely than in the crystal to computations for isolated molecules. Here, however, we are dealing with an aqueous solution, and therefore it is difficult to determine on the basis of the existing data which of the two divergent computations better represents the intrinsic conformational preferences of histamine mono- and dications.

To resolve this dilemma, a third, more refined method is desirable. Both the extended Hückel and PCILO procedures are semiempirical all-valence electron methods introducing different assumptions and simplifications. Although the PCILO procedure is much more sophisticated, it is doubtful that the tenants of the extended Hückel theory would abandon their proposals on this basis alone. We therefore applied to the problem the *ab initio* nonempirical SCF procedure.

### AB INITIO SCF STUDY OF HISTAMINE MONO- AND DICATIONS

We used the Gaussian 70 program (20) on an STO3G basis (21), which has been applied successfully to a number of conformational studies (22-27). For the histamine dication the geometry of ref. 6 was used, and for the monocation that of ref .12, suitably modified, since it relates to histidine. Rather than re-evaluating the whole conformational map, which is expensive in the treatment *ab initio*, we simply calculated the energies of all the *trans* and *gauche* conformers by rotating the ring (varying $\tau_1$) as the side chain is fixed, with $\tau_2 = 180°$ and 60°, respectively. This seemed a justifiable approach, since no study has yet suggested that a form other than *trans* or *gauche* (i.e., eclipsed) should be at an energy minimum.

The variation in *ab initio* energy of the *trans* and *gauche* forms of histamine monocation as the ring is rotated is shown in Fig. 4. The energy of the *trans* form varies little as the ring is rotated, not surprisingly, since in this form the ring–side chain interactions are minimal. The *gauche* form, on the other hand, shows a pronounced minimum in the region of $\tau_1 = -30°$, corresponding clearly to the interaction of the $N^+H_3$ group with the lone pair of atom $N_1$ of the ring. This interaction stabilizes the *gauche* form over the most stable *trans* form ($\tau_1 = -30°$ to 30°, $\tau_2 = 180°$) to the extent of 21 kcal/mole. These two minima lie close to the corresponding PCILO minima of Fig. 2.

The variation in *ab initio* energy of the *trans* and *gauche* forms of diprotonated histamine is shown in Fig. 5. Here again the energy of the *trans* form varies little as the ring rotates, but has a small minimum toward $\tau_2 = 180°$. The *gauche* form is always higher in energy than the *trans* one, with a minimum in the region of $\tau_1 = \pm 90°$. This minimum, however, is about 11 kcal/mole higher in energy than the lowest *trans* form. Again this situation agrees with the PCILO results of Fig. 3.

The *ab initio* results therefore support the PCILO predictions of extremely strong favoring of the *gauche* form of the histamine monocation and the *trans* form of the dication. The energy differences are approximately as large as in the PCILO predictions, indicating that as far as the theoretical methods are concerned this is indeed the situation in the isolated molecule, which obviously is also preserved in the crystal. The greatly reduced energy differences observed in NMR experiments in solution, as indicated by the coexistence of different forms, presumably result from strong solvent effects. In order to verify this assumption

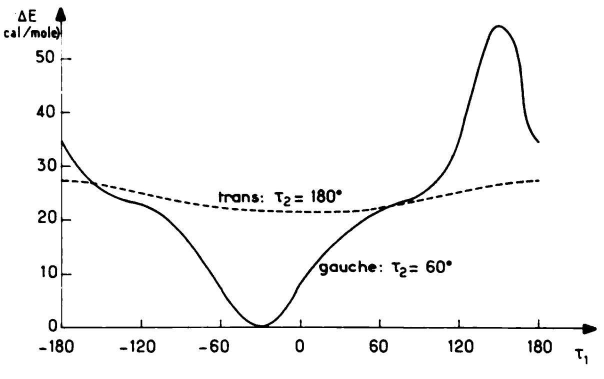

FIG. 4. *Computations ab initio for monoprotonated histamine (Fig. 1a)*

The energies of the *gauche* ($\tau_2 = 60°$) and *trans* ($\tau_2 = 150°$) forms are calculated as functions of $\tau_1$ at angular increments of 30°. The energies are expressed in kilocalories per mole with respect to the global minimum ($\tau_1 = -30°$, $\tau_2 = 60°$; total energy, −353.9110 atomic units), which is taken as zero energy.

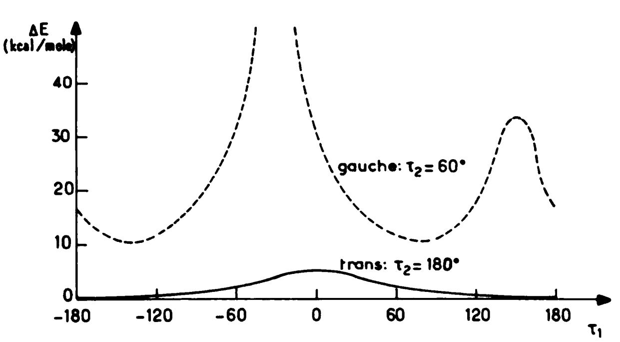

FIG. 5. *Computations ab initio for diprotonated histamine (Fig. 1c)*

The energies of the *gauche* ($\tau_2 = 60°$) and *trans* ($\tau_2 = 180°$) forms are calculated as functions of $\tau_1$ at angular increments of 30°. The energies are expressed in kilocalories per mole with respect to the global minimum ($\tau_1 = 180°$, $\tau_2 = 180°$; total energy, −354.2018 atomic units), which is taken as zero energy.

we attempted to introduce a solvent effect into the computations.

## INFLUENCE OF SOLVENT

The influence of solvent on the conformation of histamine cations was studied by the "microscopic supermolecular" approach, which consists of fixing water molecules at the most favorable hydration sites of the cation and calculating the conformational map of the new "supermolecule." The most favorable hydration sites are determined by studies *ab initio* on model compounds, following the procedure indicated in refs. 28–30. The conformational map of the new supermolecule, representing hydrated histamine, was constructed by the PCILO method, as the hydrated compound is too large for computations *ab initio*. As the PCILO results have been shown above to reflect correctly the *ab initio* results, the procedure is justifiable.

Although we cannot expect that the entire solution behavior of histamine can be explained by such a reduced treatment, we expect to obtain a reasonable indication of the direction and magnitude of changes in conformational preferences of the isolated molecule when it enters aqueous solution. From this point of view the inclusion in the computations of the essential water molecules of the first hydration shell should be particularly significant. To our knowledge this is the first attempt at such an extension in the field of quantum molecular conformational studies.

Calculations *ab initio* for the ethylammonium cation (29) and histamine suggest four principal hydration sites, which are shown in Fig. 6, together with the energies of interaction calculated on an STO3G basis by the supermolecule approach. In both mono- and diprotonated histamine the ammonium group can form three very

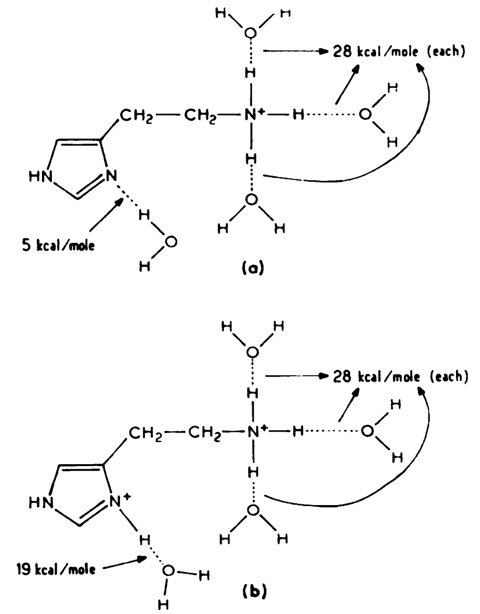

FIG. 6. *Hydration sites in mono- (a) and diprotonated (b) histamine*

The interaction energies, in kilocalories per mole were calculated *ab initio* (STO3G) at N—O separations of 2.7 Å on the $NH_3^+$ group and 2.85 Å on the imidazole ring (e.g., refs. 29, 30).

energetically favorable hydrogen bonds to water (28 kcal/mole each). In the monoprotonated molecule a fourth water molecule can act as proton donor to the lone pair on $N_1$ (energy, 5 kcal/mole), while in the diprotonated species it can act as a proton acceptor from the $N_1{}^+H$ bond (energy, 19 kcal/mole). The high hydrogen-bond energy when the nitrogen is quaternized is a notable feature.

With this water fixation scheme as a starting point, the PCILO conformational energy maps for hydrated cations are presented in Figs. 7 and 8. Figure 7 gives the results for the hydrated monocation (Fig. 6*a*) and indicates a profound change with respect to the results for the isolated molecule depicted in Fig. 2. In place of one deep minimum for the *gauche* form in Fig. 2, we now observe *gauche* ($\tau_1 = \pm 60°$, $\tau_2 = \pm 60°$) and *trans* ($\tau_1 = \pm 30°$, $\tau_2 = 180°$) minima of equal energy. The *trans* minima are more extended and will thus be statistically favored. The results suggest the presence of a mixture of conformers in solution, with a predominance of the *trans* form. No hydrogen bond is noew expcted between the cationic head and the imidazole ring, in agreement with recent experimental studies on the ionization constants of histamine and derivatives in aqueous solution (31).

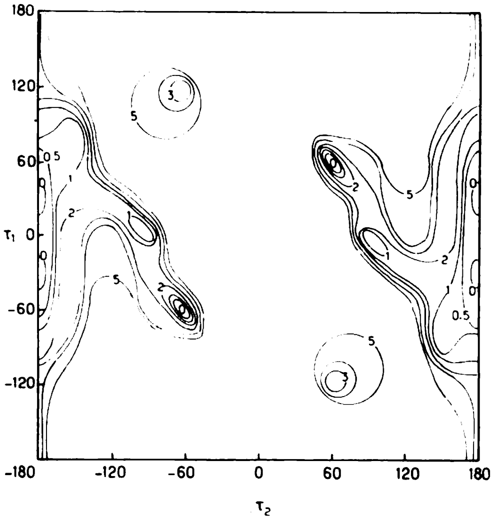

FIG. 7. *PCILO conformational energy map of hydrated monocation of histamine (Fig. 6a)*

Isoenergy curves in kilocalories per mole are shown with respect to the global energy minimum, taken as zero energy.

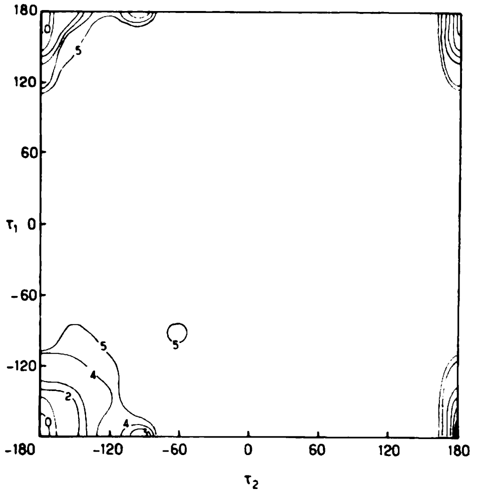

FIG. 8. *PCILO conformational energy map of hydrated dication of histamine (Fig. 6b)*

Isoenergy curves in kilocalories per mole are shown with respect to the global energy minimum, taken as zero energy.

Figure 8 presents the results for the hydrated dication (Fig. 6*b*). In this case also substantial changes are observed in the results for the isolated molecule depicted in Fig. 3. Although the global energy minimum still corresponds to a *trans* form ($\tau_1 = 180°$, $\tau_2 = 180°$), it is now limited to narrow values of $\tau_1$ associated with the antiplanar arrangement of the side chain with respect to the ring. Moreover, a local energy minimum appears 3 kcal/mole above the global one, for a *gauche* conformer at $\tau_1 = 180°$, $\tau_2 = 90°$, and another one 5 kcal/mole above the global one at $\tau_1 = -90°$, $\tau_2 = -60°$, suggesting the possible occurrence of such conformers in equilibrium with the predominant *trans* one, but in a smaller proportion than expected for the monocation.

Altogether the results for the hydrated cations indicate a striking influence of the solvation effect on molecular conformation. Moreover, this effect, which tends to reduce the extreme situation prevalent for isolated molecules (a very strong predominance of one of the conformers, *trans* or *gauche*), brings the computed results into qualitative agreement with the NMR data for the corresponding molecules in solution (a mixture of tautomers with the *trans* form predominating, to a greater degree in the dication than in the monocation).

Although the trend predicted for the solution effect is certainly significant, we are reluctant to assign it a quantitative value because of the approximation involved in the mode of approach. Thus it seems probable that the energy variations as a function of $\tau_1$ and $\tau_2$ may be smoother than depicted, because, for practical reasons, we did not optimize the positions of the water molecules for each value of $\tau_1$ and $\tau_2$; nor did we take into account the effect of the next hydration shell. Nevertheless the essential features of the trend certainly depict correctly the evolution from isolated molecules to the situation in a water solvent. The trend is a buffering one, diminishing the intramolecular attractive interactions prominent in the isolated monocation and reducing the intramolecular repulsive interactions prominent in the isolated dication, thus bringing the two species closer together from the viewpoint of conformational possibilities. Under these circumstances it seems necessary to admit that the solvent effect has a stronger influence on the molecular conformation than crystal packing forces, which seem to alter very little the intrinsic preferences of isolated cations.

The results also permit a rational evaluation of the significance of the extended Hückel theory computations and of their apparent agreement with the situation in solution. This agreement has to be considered as largely accidental; perhaps a better explanation is that, by undervaluing both attractive and repulsive intramolecular interactions in isolated molecules to the point of finding no significant differences between the structures of the mono- and dications, the extended Hückel method corresponds to some degree to the situation produced by the intervention of solvent molecules and thus satisfactorily describes the situation in water. It could be said that extended Hückel theory takes the solvent effects into account without allowing for them, but this gives undue credit to the method. This unwitting "correction" for the solvent effect, once included for the basic histamine skeleton, is systematically carried over to its derivatives, accounting for the satisfactory correlation found by Ganellin *et al.* (2, 3) between the extended Hückel theory predictions and the NMR results for a series of methylhistamines.

### *Rare Monocation Tautomer* (*Fig. 1b*)

Our discussion of the monocation of histamine was limited to the $N_3H$ tautomer (Fig. 1*a*), which seems to be the predominant species at physiological pH. In view of the $pK_a$ study of Ganellin *et al.* (2), however, the tautomer $N_1$—H (Fig. 1*b*) may be present in aqueous solution in the proportion of about 20%. It must therefore be considered a possible active species of histamine, at least in some of its manifestations, particularly as histamine is known to act at a number of receptors. We therefore extended our studies to this tautomeric form, in both its isolated and hydrated states.

Figure 9 presents the conformational energy map of the monocation of the isolated $N_1$—H tautomer. Its distinctive feature is the location of the global energy minimum at coordinates representing a *gauche* conformer ($\tau_1 = 180°$, $\tau_2 = \pm 60°$) with a secondary energy minimum, 0.5 kcal/mole above the global one, at $\tau_1 = 120°$, $\tau_2 = -60°$ (and symmetrically at $\tau_1 = -120°$, $\tau_2 = 60°$). This conformer differs from the predominating $N_3$—H one, however, in the stereochemical features related to the torsion angle $\tau_1$. As already stated in the INTRODUCTION to this paper, the terminology *gauche* or *trans* relates to the value of the torsion angle $\tau_2$. An important second conformational feature, however, is given by $\tau_1$, whose value determines the over-all plane of location of the side chain with respect to the ring. $\tau_1 = 0°$ or 180° corresponds to the $C_6$—$C_7$ bond located in the plane of the imidazole ring on the side of atom $N_1$ or $C_4$, respectively, and $\tau_2 = 90°$, to its location perpendicular to this plane. The preferred conformations of the

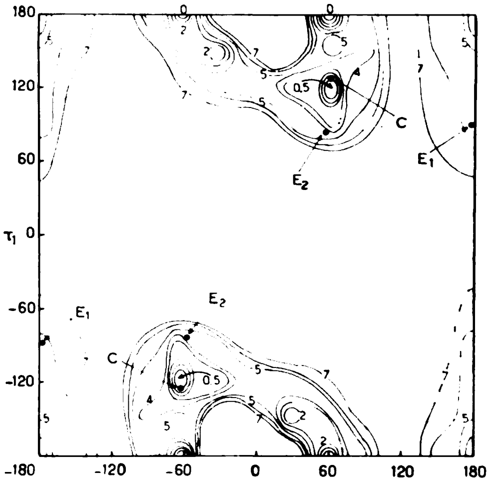

FIG. 9. *PCILO conformational energy map of monocation of rare $N_1$—H tautomer of histamine (Fig. 1b)*

Isoenergy curves in kilocalories per mole are shown with respect to the global energy minimum, taken as zero energy. $E_1$, $E_2$, and $E_3$ are energy minima of the extended Hückel theory calculations, in order of increasing energy. *C* is the global energy minimum of CNDO calculations.

two tautomers, $N_3$—H and $N_1$—H, differ essentially by the value of $\tau_1$: whereas in the $N_3$—H tautomer atom $C_7$ is positioned slightly above the plane of the imidazole ring on the side of $N_1$, it is in the plane of the ring but on the side of $C_4$ at the global minimum of the $N_1$—H tautomer and somewhat above the plane of the ring but still on the side of $C_4$ at the secondary local minimum of this tautomer. Apparently the repulsion between the positively charged ammonium group and the positively charged $N_1$ is responsible for the switching of the ammonium group to the side of $C_4$ in the $N_1$—H tautomer. Although this repulsion does not seem to be strong enough to make the *trans* conformer of the $N_1$—H tautomer the preferred one, there appears in Fig. 9 a local energy minimum for the planar-*trans* form at $\tau_1 = \tau_2 = 180°$, 4 kcal above the global one, and this represents another difference between the $N_1$—H tautomer and the $N_3$—H one, in which the energy gap between the two forms is much higher.

For computation of the conformational energy map of the hydrated species of the $N_1$—H tautomer, the hydration scheme of Fig. 10, implying 4 attached water molecules, was used. The resulting conformational energy map is represented in Fig. 11. Surprisingly, at first sight, it shows a global energy minimum for a *gauche* form with $\tau_1 = -90°$, $\tau_2 = -60°$ (and the symmetrical coordinates $\tau_1 = 90°$, $\tau_2 = 60°$). Examination of the corresponding model indicates that this particular form appears to be stabilized by an interaction of the lone pair of the oxygen atom of one of the water molecules attached to the $N^+H_3$ group with the hydrogen atom of the $N_1$—H bond. Should this somewhat questionable conformation prove incorrect, the results of Fig. 11 would suggest a possible coexistence of other *gauche* conformations ($\tau_1 = 150°$, $\tau_2 = -60°$, and the symmetrical coordinates $\tau_1 = -150°$, $\tau_2 = 60°$) with the *trans* one ($\tau_1 = \tau_2 = 180°$). In contrast to the situation prevailing with the hydrated $N_3$—H tautomer, the *gauche* energy minimum in this case is still 1 kcal/mole more stable than the *trans* one. It thus appears possible that the two tautomers have somewhat different conformational preferences, and this situation must be kept in mind in relation to the pharmacological activity of histamine, especially since the two tautomers also differ in a number of electronic properties. Figure 12 shows the distribution of the electronic charges in the two stable *trans* conformers of the two tautomers (conformation $\tau_1 = 30°$, $\tau_2 = 180°$ for tautomer $N_3$—H; conformation $\tau_1 = \tau_2 = 180°$ for tautomer $N_1$—H). The distinct differences in charge distribution are clearly visible.

### *N-Trimethylhistamine*

NMR results in solution (2, 17) indicate that di- and particularly trimethylation of the amino nitrogen of histamine cations increases substantially the proportion of the *trans* form in the equilibrium mixture, up to about 80% in the trimethyl derivative of the monocation of the $N_3$—H tautomer and to about 90% in the trimethyl derivative of the dication (2). We investigated this phenomenon, using the monocation of the *N*-trimethylated derivatives of the predominant $N_3$—H tautomer. The conformational energy map of this derivative (Fig. 13) indicates a very significant change with respect to the

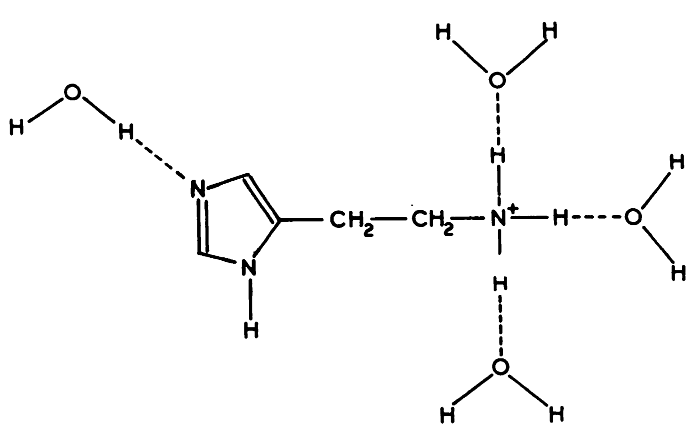

FIGURE. 10. *Hydration sites in rare $N_1$—H tautomer of histamine (schematic diagram)*

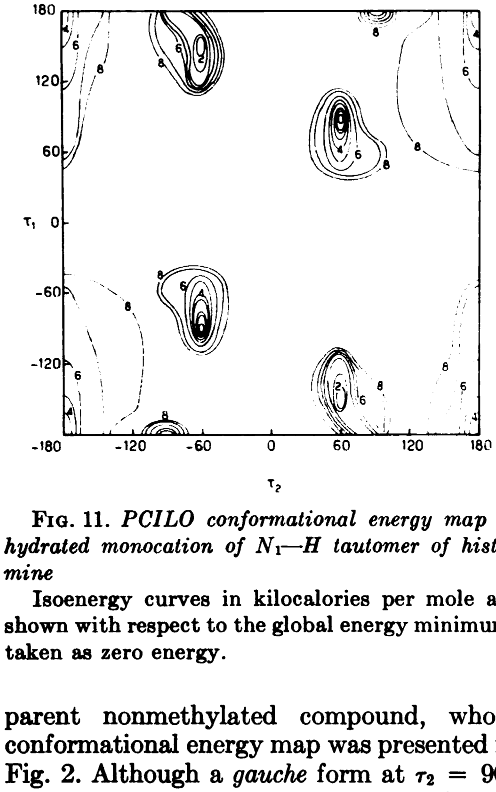

FIG. 11. *PCILO conformational energy map of hydrated monocation of $N_1$—H tautomer of histamine*

Isoenergy curves in kilocalories per mole are shown with respect to the global energy minimum, taken as zero energy.

parent nonmethylated compound, whose conformational energy map was presented in Fig. 2. Although a *gauche* form at $\tau_2 = 90°$ still represents the global energy minimum, it is associated with a different value of $\tau_1$ ($=180°$ instead of $\pm30°$ in the present approach) and moreover is nearly degenerate, with a local energy minimum (0.5 kcal/mole above the global one) corresponding to a *trans* form ($\tau_1 = \tau_2 = 180°$). *N*-Trimethylation thus has the obvious effect of bringing closer the *gauche* and *trans* minima, thereby increasing the relative importance of the *trans* conformer with respect to the situation in the parent cation. The replacement of the amino hydrogen atoms by methyl groups manifestly inhibits the very strong interactions observed in the parent compound between the $N^+$—H bonds of the cationic head and $N_2$ of the histaminic ring. The theoretical results for the isolated molecule, however, still suggest slight predominance of a *gauche* form, whereas the NMR results cited above indicate a strong preponderance of the *trans* form in solution. In order to solve this puzzle, the conformational map was recomputed for the hydrated form of the monocationic trimethylhistamine. In this molecule there is one outstanding hydration site, consisting of atom $N_1$ of the imidazole ring. The calculations were thus performed for the supermolecular species of Fig. 14, and the resulting conformational energy map (Fig. 15) shows a distinct displacement of the global minimum toward $\tau_1 = \pm30°$, $\tau_2 = 180°$, corresponding to a *trans* form. No specific energy minimum appears for any *gauche* form, which would be 2–5 kcal/mole higher in energy than the *trans* form. In this case again the explicit inclusion of the solvent effect by the supermolecular hydrated model brings theory and experiment into close agreement.

## CONCLUSIONS

The principal conclusions which can be drawn from this study are threefold. First, from the methodological point of view the confirmation by the *ab initio* computations of the correctness of the PCILO results for

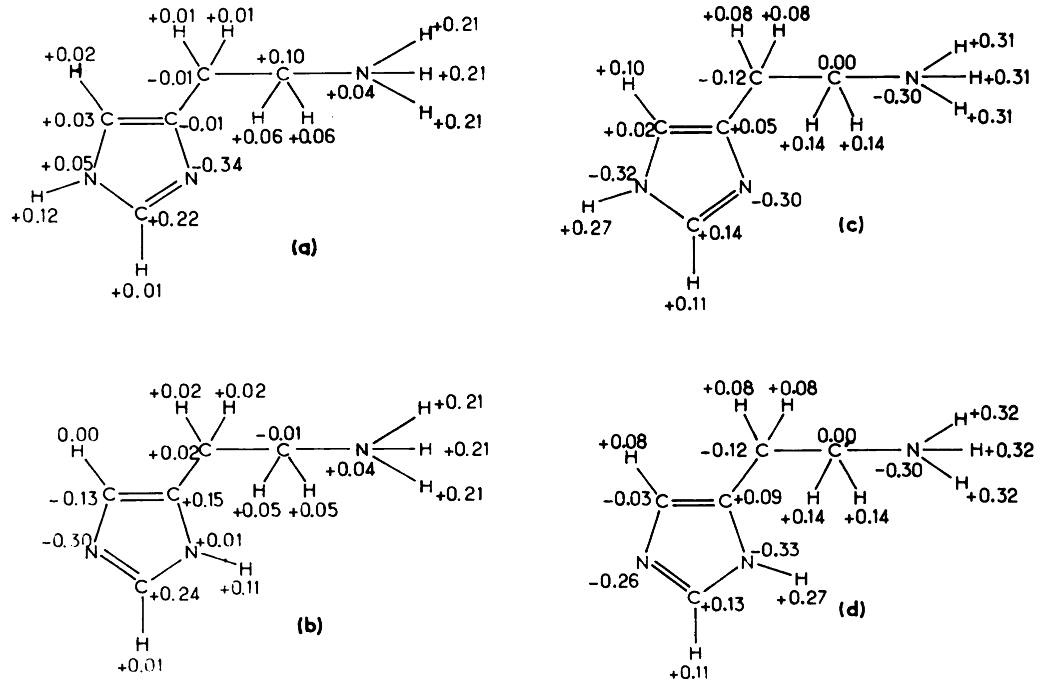

FIG. 12. *Net electronic charges for comparable extended conformations of the two tautomers of histamine monocation (in electron units)* a. PCILO charges, usual tautomer (at $\tau_1 = 0°, \tau_2 = 180°$). b. PCILO charges, rare tautomer (at $\tau_1 = \tau_2 = 180°$). c and d. Corresponding charges *ab initio*.

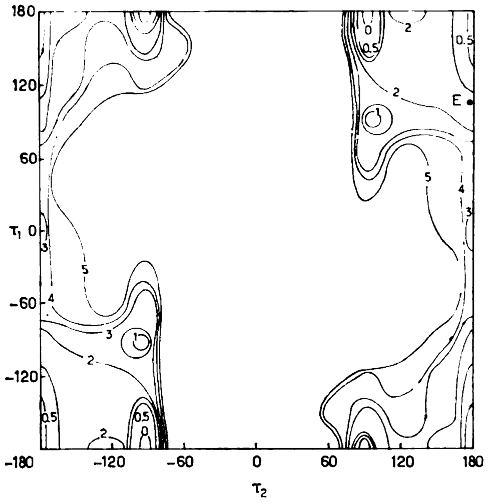

FIG. 13. *PCILO conformational energy map of monocation of N-trimethylhistamine*

Isoenergy curves in kilocalories per mole are shown with respect to the global energy minimum, taken as zero energy. *E* is the global minimum of extended Hückel theory calculations.

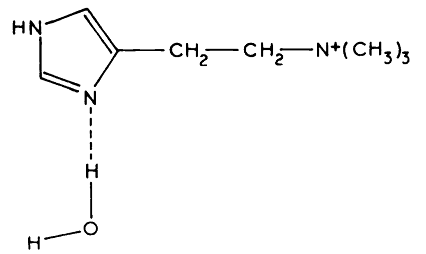

FIG. 14. *Hydration site in predominant $N_3$—H monocation of N-trimethylhistamine (schematic diagram)*

free molecules represents even further demonstration of the precision of this method, which, together with its remarkable efficiency, makes it an excellent tool for the exploration of the conformational aspects of pharmacology. Second, the study demonstrates the importance, at least in this case, of the solvation effect, and shows the possibility of taking account of it by the supermolecular approach. It is clear that, even

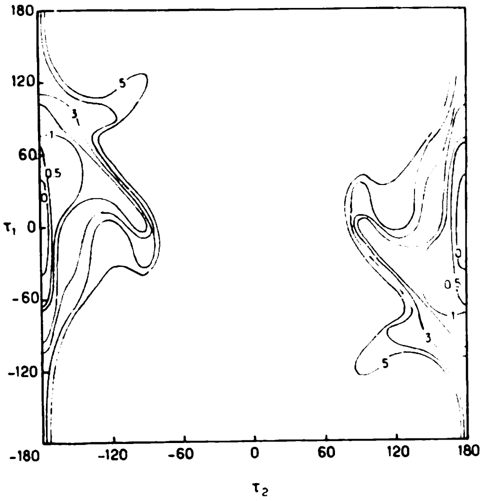

FIG. 15. *PCILO conformational energy map of hydrated monocation of N-trimethylhistamine*

Isoenergy curves in kilocalories per mole are shown with respect to the global energy minimum, taken as zero energy.

limited to the introduction of the first hyhydration shell, careful application of this procedure leads to significant results, which, at least qualitatively seem quite satisfactory. Broader exploration of the potential usefulness of such a procedure is still needed, although it appears promising. Finally, the conformational and electronic differences revealed between the two possible monocationic species of histamine at physiological pH suggest significant probable differences in their steric and chemical interactions with possible cellular targets and demand careful investigation into their selective or differential involvement at the two receptors ($H_1$ and $H_2$) which have been shown to exist for histamine and its derivatives (32, 33).

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