compounds (II and IV) which do show a dramatic selectivity of action still have a rotamer composition similar to that of histamine. Many factors may affect activity in this series of compounds. These results suggest that trans/gauche rotamer preference, as predicted by EHT calculation, does not appear to be of major importance in determining whether these conformationally flexible molecules distinguish between H_1 and H_2 histamine receptors.

Since the side-chain substituted compounds differ in conformational preference, one can examine whether they show a consistent relationship between activity and rotamer preference at either of the histamine receptors. In fact, a qualitative pattern is apparent since the two *N*-methyl compounds (V and VI) which resemble histamine in rotamer preference are nearly as active as histamine, whereas the two *C*-methyl compounds (VIII and IX) for which the trans rotamer is no longer preferred are much weaker in activity. Their reduced activity would be due to a variety of causes, one of which could be a reduced preference for the trans conformation. Insofar as there is a self-consistency in these observations it suggests that a trans conformer of the agonist may be associated with activity at both types, H_1 and H_2, of histamine receptor. This does not prove that the trans conformer is required and it would be premature to conclude that the gauche form of histamine is not involved in receptor interaction.

Acknowledgments. We gratefully acknowledge our debt to Dr. J. W. Black for his guidance during many stimulating discussions and thank him for permission to quote results.

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Conformation of Histamine Derivatives. 3. A Relationship between Conformation and Pharmacological Activity

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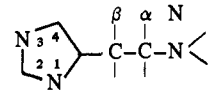
The methyl substituent of 4-methylhistamine affects the conformation of the molecule by influencing the orientation of the imidazole ring with respect to the side chain and introducing a measure of rigidity through restricting rotation. Implications are discussed regarding biological activity at the H_1 and H_2 histamine receptors. The dramatic receptor selectivity of 4-methylhistamine is accounted for in the difference between its conformational properties and those of histamine. It is proposed that for histamine a conformation essential to productive interaction with the H_1 receptor is one in which the side chain is fully extended (trans form) and all carbon and nitrogen atoms are coplanar with the imidazole ring. In this conformation the separation between the ring N_1 and ammonium nitrogen atoms is at the maximum distance of 5.1 Å.

The marked effect on biological activity caused by methylation at different positions in the histamine molecule[1,2] (compounds II–IX in Table I) poses an intriguing medicinal chemical problem. Depending on its position, the presence of the methyl group can result in as much as 1000-fold reduction in histamine-like stimulant activity or be almost without influence. It can also lead to a selectivity such that a molecule appears to be less active at one type of histamine receptor than at another, as, for example, with 2-methylhistamine (II) which is significantly less active as a stimulant of H_2 receptors than of H_1 receptors (the activities, relative to histamine, are in the ratio H_1:$H_2 \approx 4:1$) and 4-methylhistamine (IV) which, conversely, is considerably less active at H_1 than at H_2 receptors (H_1:$H_2 \approx 1:200$).[2] Indeed, the very dramatic influence of the substituent in 4-methylhistamine makes this molecule of especial interest. There is every reason to suppose that for these compounds the differences in activities are on the whole due to differences in drug-receptor interactions,[1,2] so that one may reasonably argue that the presence of the methyl substituent somehow affects the chemistry of the interaction process. In the absence of any molecular description of the receptor one is left to examine the drug molecule, initially to define the chemical consequence of changing the drug structure and subsequently to establish which changes in chemical properties affect biological activity. It could be argued that the methyl substituent would either affect the chemical reactivity of the drug molecule or act sterically to hinder drug-receptor interaction, but then one should adduce supporting evidence and also identify more specifically the manner in which this could occur. To this end we have examined whether the methyl group might alter, *inter alia*, the conformation of the drug molecule.

In a previous paper[1] we reported our findings and considered whether there was evidence among a series of conformationally mobile methylhistamines that the side-chain conformation might determine their ability to stimulate H_1 and H_2 receptors. There was no indication from our results that alteration of rotamer preference affected selectivity but there was a suggestion that it might lead to reduced activity. Conversely, the compounds that showed selectivity did not differ from histamine in their side-chain rotamer preference. It therefore appears that properties other than rotamer preference in the side chain distinguish activity at the two receptor types. The possibility remains that activity is influenced by other conformational effects. In the present paper we examine whether the pronounced selectivity of 4-methylhistamine could be due to a conformational influence of the methyl substituent and, as a corollary, whether one may therefore infer an active conformation for histamine. It must be noted that *we cannot by this means assert that conformation definitely determines biological activity, but we can indicate whether the observed changes in activity accompany-*

Table I. Influence of Methyl Substituents on Proposed H_1 Receptor "Essential" Conformation XV Compared with Agonist Activity



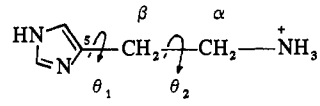
Compd	Position of Me substituents[a]	Steric influence on conformation	Expected H_1 activity	Measured[b] H_1 activity
II	2	Partially occludes ring-N_1 atom	Somewhat reduced	17
III	3	None	Unaffected	0.4
IV	4	Hinders coplanarity with the ring	Weak	0.2
V	N	None	Unaffected	72
VI	N,N	None	Unaffected	44
VIII	β	Partially occludes ring-N_1 atom; greatly reduces trans preference	Weak	0.8
IX	α	Reduces trans preference and hinders coplanarity with the ring	Weak	0.4

[a]Substituent numbering as in the previous paper.[1] [b]Relative to histamine = 100. See Table I of previous paper for details.[1]

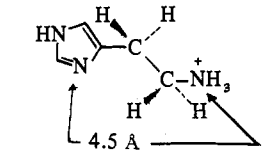
ing structural modification would be consistent with such a view. As in paper 2[1] we shall assume that the biologically active species of histamine is the N_3-H tautomer of the monocation depicted in formula I. The conformations of the various histamine derivatives are described, as previously,[3] by the torsion angles θ_1 and θ_2 which represent rotation about the respective bonds C_5-C_β and C_β-C_α.

Kier[4] related the trans histamine conformation to action at the H_1 receptor by comparing the interatomic distance between the side-chain ammonium group and the ring N_1-nitrogen atom of the trans (XA) and gauche (XB) conformers (having set θ_1, the orientation of the imidazole ring, at 120°) with the internitrogen distance in triprolidine (XI), a potent H_1 receptor antagonist. This approach presumed, however, that the nitrogen atoms of the agonist and antagonist molecules could be directly interrelated. Experimental evidence suggests that this premise was not well founded. The work of van den Brink[5] in examining agonist and antagonist activities of 2-pyridylethylamine derivatives and related compounds indicates that the nitrogen atoms should not be compared in this way. Furthermore, the pyridyl nitrogen atom of triprolidine cannot be considered an essential structural requirement for antagonist activity and therefore cannot be taken as a reference point for interatomic distance since, as recently shown by Ison and Casy,[6] the diphenyl analog XII as as active. Indeed there are some well-known clinically useful antihistamines which possess only one heteroatom.[7] It is therefore necessary to seek some other experimental means of correlating conformation with activity.

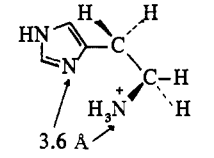
To identify the biologically active conformation of an agonist poses considerable problems. One way is to use conformationally rigid drug molecules, as exemplified by the work of Burger, *et al.*,[8] with the *trans*-imidazolylcyclopropylamine analog XIII of histamine. If this compound, having a structure that approximates to the histamine trans conformation, were to behave in accordance with Kier's suggestion, it ought to be active at H_1 but not at H_2 receptors. Actually, there does not appear to be much difference between its H_1 and H_2 receptor activities;† *i.e.*, it does not seem to be so constituted as to be more favored for action at one receptor type than at another. On the other hand, this compound has a trans configuration and yet is much less active than histamine, apparently in contradiction to our previous interpretation[1] that activity may be associated with trans forms. It could, however, be argued that this is an inadequate test



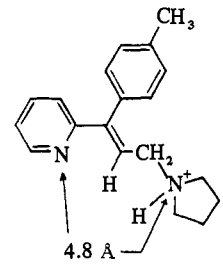
I



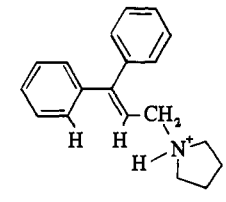
XA ($\theta_1 = 120°, \theta_2 = 180°$)



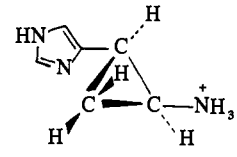
XB ($\theta_1 = 120°, \theta_2 = 300°$)



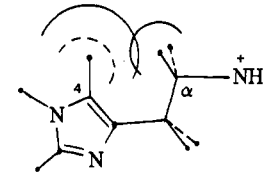
XI



XII



XIII



XIV ($\theta_1 = 0°, \theta_2 = 180°$)

model since the cyclopropane ring might interfere with drug-receptor interaction. It is difficult to devise molecules of a given conformation without also changing some other physicochemical property but the use of rigid analogs has an added disadvantage in that rigidity may deny a molecule the opportunity of undergoing a necessary functional conformational change during its interaction with the receptor as, for example, might occur in the situation recognized by Portoghese.[9]

With 4-methylhistamine conformational mobility is maintained but, as was noted in the previous paper,[1] EHT calculations indicate that the methyl group has a marked effect on θ_1, the orientation of the imidazole ring relative to the side chain, and predict the existence of a high energy barrier to rotation in the region $60° > \theta_1 > 300°$. This influence of the methyl group is also apparent on examination of Corey-Pauling-Koltun (CPK) space-filling molecular models. These

†Although the published figures appear to suggest that this compound is twice as potent at H_2 than at H_1 receptors, it must be remembered that the difference has not been validated statistically.

models, having been designed to represent accurately the dimensions of histidine in peptide residues, are well suited to the study of histamine. It is therefore of considerable interest to find that for 4-methylhistamine they indicate a spatial region $\theta_1 = 0 \pm 60°$, where the steric interaction between the protons of the methyl- and α-methylene groups is so severe that it would lead to a distorted molecule. This holds for all values of θ_2 and is in agreement with the EHT predictions. When $\theta_1 = 0°$ there is substantial overlap of the van der Waals spheres. Formula XIV shows the coplanar ($\theta_1 = 0°$), trans ($\theta_2 = 180°$) conformation of 4-methylhistamine drawn to scale with intersecting arcs (unbroken lines) to represent the overlap of the van der Waals zones for the 4-methyl group and α-methylenic protons (the dashed arc, representing the van der Waals circumference for the ring 4-proton in histamine, is included for comparison to illustrate that there is no overlap of zones in histamine). Thus, it is extremely unlikely that 4-methylhistamine would assume a conformation where θ_1 approaches 0°. In considering whether observed differences in biological activity could be due to changes in drug conformation, *inter alia,* we can relate the above observation to the dramatic effect that the methyl group has on activity, where 4-methylhistamine is approximately half as active as histamine as a stimulant of H_2 receptors and yet only $^1/_{500}$ as active at H_1 receptors. We may draw two conclusions. (1) Quite definitely, since 4-methylhistamine is an effective H_2 receptor stimulant, conformations in which θ_1 approaches 0° cannot be associated with activity at H_2 receptors. (2) The inability of 4-methylhistamine to function properly as an H_1 receptor stimulant can be viewed as a consequence of restricted rotation or of its inability to assume a necessary conformation. Thus, conformations unfavorable for 4-methylhistamine, such as those in which θ_1 approaches 0°, may be associated with effective drug interaction at H_1 receptors. Either the ring is required to rotate through the position defined by $\theta_1 = 0 \pm 60°$ or the molecule has to adopt a conformation within this range of values of θ_1 during receptor activation. We can then infer that the interaction of histamine at H_1 receptors may also involve a conformation in which θ_1 approaches 0° and, by varying θ_2 for histamine, we can investigate whether it is possible to define other conformational aspects. According to the EHT predictions[1] when $\theta_1 = 0$–60°, gauche forms ($\theta_2 = 300°$) of histamine monocation are unstable and energetically very unfavorable (to the extent of *ca.* 10 kcal/mol) in comparison with the preferred trans forms ($\theta_2 = 180°$). This is also evident from an examination of the CPK model of histamine monocation where, except for the trans conformation ($\theta_2 = 180°$), variation of θ_2 when $\theta_1 = 0°$ leads to marked steric interactions between the imidazole 4-proton and the side-chain α-methylenic protons. Thus, when $\theta_1 = 0°$ the preferred form of histamine monocation as indicated by EHT calculations and by the absence of bond distortion in CPK models is the trans rotamer, in which $\theta_2 = 180°$. *These arguments define for histamine a conformation that,* for want of a better term, *may be called "H_1-essential," i.e., one that is essential to drug activity and has to be adopted by the agonist molecule at some stage during productive interaction at the H_1 receptor site.* It may be only one of several forms involved during receptor stimulation or, indeed, it may be involved in only a transient manner while the agonist undergoes a required conformational change. The present data suggest that activity is associated with values of θ_1 within a range of +60 to −60°; very likely there would be a corresponding range of values for θ_2 that define completely the conformational requirements. Although not defining the conformation precisely, these arguments focus attention on a form which is consistent with the data and intuitively attractive. *This is the fully extended trans conformation (XV), where $\theta_1 = 0°$ and $\theta_2 = 180°$, in which the carbon and nitrogen atoms are coplanar with the ring* (illustrated in Figures 1 and 2), *and there is a maximum separation (interatomic distance of 5.1 Å) between the charged ammonium group and the ring N_1-nitrogen atom.* Furthermore, in this conformation any effect from the side chain in obscuring the lone electron pair at N_1 is minimal. This would be a very satisfactory situation if the nitrogen atom were involved in donating its electron pair during productive drug-receptor interaction. It is pertinent to recall that tautomer I is probably the H_1 receptor active form of histamine[10] and that all the known active histamine-like stimulants (H_1-type) have the structural fragment (XVI) in common,[11] although possession of this feature does not guarantee that a compound will have histamine activity. The role of the basic nitrogen-containing aromatic nucleus is unknown but, if one can assume that the nitrogen atom is involved in some definite

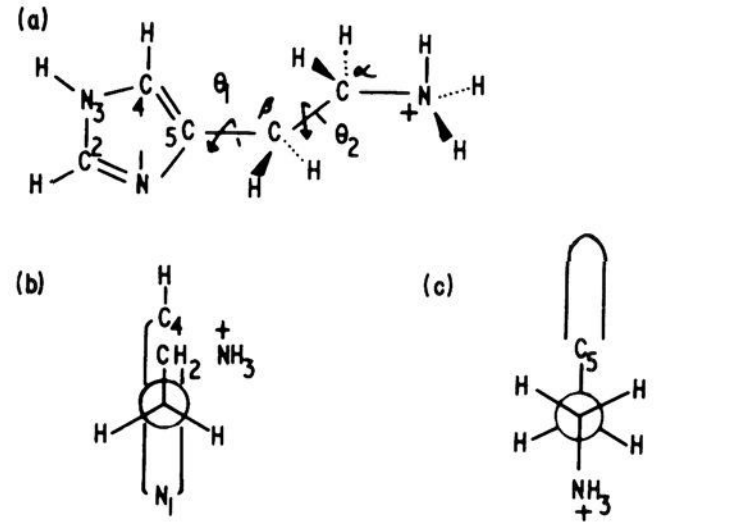


Figure 1. Proposed H_1 receptor "essential" conformation of histamine showing (a) coplanar diagram with nomenclature; (b) $\theta_1 = 0°$ in the Newman projection viewed along the C_β–C_5 bond from C_β to C_5; (c) $\theta_2 = 180°$ in the Newman projection viewed along the C_α–C_β bond from C_α to C_β.

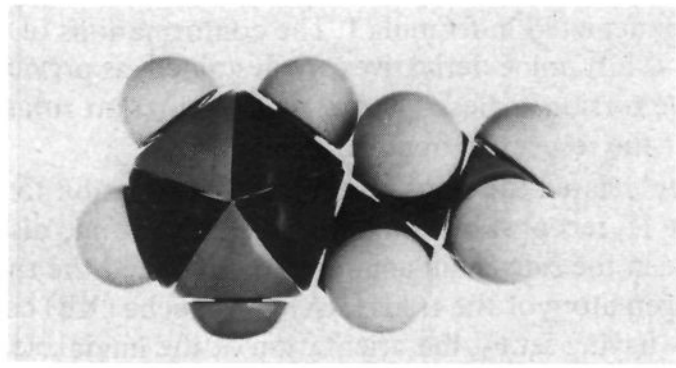
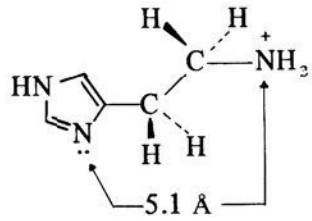
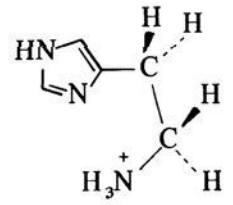


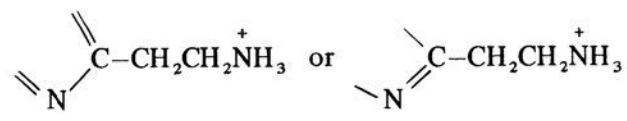
Figure 2. CPK model of histamine in the proposed H_1 receptor "essential" conformation.



XV ($\theta_1 = 0°$, $\theta_2 = 180°$)



XVII ($\theta_1 = 180°$, $\theta_2 = 0°$)



XVI

capacity, then a possible function as an electron pair donor seems very plausible.

The above proposition, although unproven, is suggested by the experimental evidence. It contrasts with that proposed many years ago by Niemann and Hays[10] (XVII), in which the torsion angles approach $\theta_1 = 180°$ and $\theta_2 = 0°$, or the more recent postulate of Kier[4] (XA) in which $\theta_1 = 120°$ and $\theta_2 = 180°$. With neither of these other conformations can one account for the weak H_1 receptor activity of 4-methylhistamine from arguments based on conformational analysis. Thus, insofar as there is evidence that agonist conformation may distinguish between the receptors, the differentiating feature is not θ_2, rotation within the side chain (as was suggested by Kier), but θ_1, rotation of the imidazole ring.

The described "H_1-essential" conformation is not a minimum energy form. Indeed, for histamine, the trans rotamer ($\theta_2 = 180°$) is predicted by EHT calculations to be at a minimum energy when $\theta_1 = 120°$, whereas the "H_1-essential" conformation at $\theta_1 = 0°$ is calculated to have an energy about 3 kcal/mol above this (although the value may be an overestimate). This leads one to speculate that histamine may be required to undergo a conformational change during H_1 receptor stimulation. One can envisage that a histamine molecule would arrive in the neighborhood of the site of action in one of its most probable forms, *i.e.*, in one of the minimum energy conformations as was suggested by Kier,[4] and that perhaps it might then either interact with the receptor and undergo a change which involves the "H_1-essential" conformation or, under a perturbing influence of the receptor, it might adopt the "H_1-essential" conformation prior to forming a drug-receptor complex. In which case, this would be an excellent candidate for the model proposed by Portoghese.[9]

The suggestion of an "H_1-essential" conformation implies that the activity of a molecule will be impaired unless it can adopt this conformation and carries the further connotation that activity could be related to the extent that a molecule is able to achieve this. Using this as a working hypothesis we can analyze the other methylhistamines with reference to the proposed "H_1-essential" conformation to see whether the structure-activity relationships support, or at least do not contradict, the proposal. Inspection of the CPK model of α-methylhistamine (IX) reveals that severe steric interactions occur between the imidazole 4-proton and the methyl group when the nucleus is coplanar ($\theta_1 = 0°$) with the side chain and the latter is in the trans conformation. This strongly suggests that α-methylhistamine would have great difficulty in assuming the proposed "H_1-essential" form. In the CPK model of β-methylhistamine (VIII), the methyl substituent is seen to be partially obscuring the imidazole-N_1 lone electron pair when $\theta_1 = 0°$; this observation taken with the EHT prediction[1] that the methyl group considerably reduces the preference for trans rotamers implies that the ability of β-methylhistamine to function effectively in the proposed "H_1-essential" conformation would be considerably impaired. The analysis for each of these compounds is therefore in accordance with their (experimentally observed) weak activity. For 2-methylhistamine (II), the methyl group could influence access to the imidazole-N_1 lone electron pair but, otherwise, no conformational consequence is expected; in keeping with these observations this molecule is found to be reasonably active (*ca.* $^1/_6$ active as histamine) at H_1 receptors. *N*-Methyl- and *N,N*-dimethylhistamines (V and VI) where the methyl groups should not interfere with formation of the proposed "H_1-essential" conformation are, indeed, almost as active as histamine. Finally, for 3-methylhistamine (III) which has only very weak activity one must conclude that the influence of the methyl group is other than conformational. These observations are enumerated in Table I.

In conclusion, it appears that differences in the conformational properties of histamine and 4-methylhistamine could account for the dramatic receptor selectivity of the latter. The very weak H_1 receptor activity of 4-methylhistamine could result from the influence of the methyl group in introducing a measure of conformational rigidity by restricting rotation within the molecule or in preventing the adoption of an essential conformation. The available evidence is consistent with the proposition that, at H_1 receptors, histamine is required to adopt the conformation XV at some time during the process of receptor stimulation. It seems probable that the H_1 and H_2 receptors differ in the conformational restrictions imposed on agonist molecules.

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