and acetophenone were chromatographed on either a 6 ft × 6 mm glass column packed with 10% poly(ethylene glycol) (Carbowax 4000) on 80–100 mesh Gas Chrom Q and operated at 100° on a Barber-Colman 5000 gas chromatograph or a 6 ft × 1.8 mm stainless steel column packed with 10% poly(propylene glycol) (Ucon 50-HB-2000) on 80–100 mesh, silylated Gas Chrom S held at 100°, on a Varian Aerograph 1200 gas chromatograph. Both instruments were equipped with flame ionization detectors.

The enantiomeric composition of the enzymically formed 1-phenylethanol was measured gas chromatographically following diastereomer formation as previously described by Anders and Cooper.[19] For these estimations, each benzene extract was transferred to an evaporator tube (Kontes K-288250) and 20 μl of pyridine and 3 mg of 3-β-acetoxy-Δ⁵-etienic acid chloride were added. The mixture was maintained at 90° in a Kontes tube heater for 30 min; the temperature was then increased to 130° to concentrate the reaction mixture to about 0.1 ml. The residue (5–10 μl) was injected directly into the gas chromatographic column.

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Conformation of Histamine Derivatives. 1. Application of Molecular Orbital Calculations and Nuclear Magnetic Resonance Spectroscopy

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The conformational energies of the histamine dication and of the two tautomeric forms of its monocation are calculated using EHT and CNDO molecular orbital procedures. The relative populations of trans/gauche conformers predicted by the EHT method agree well with nmr-derived aqueous solution data, but the CNDO predictions do not agree. The applicability of EHT calculations is further indicated by comparing predicted with observed (nmr-derived) rotamer populations for the series 2-, 3-, 4-, and *N*-methyl-, *N,N*-dimethyl-, and *N,N,N*-trimethylhistamine dications. The nmr data indicate that the mole fraction of trans rotamers is somewhat greater for the histamine dication (0.54) than for the monocation (0.45) and that monomethylation has but little additional influence. Further methylation at the amino group of histamine increases the trans rotamer mole fraction from 0.57 (for $-N^+H_2Me$) to 0.72 (for $-N^+HMe_2$) to 0.92 (for $-N^+Me_3$).

Conformational analysis of histamine in relation to biological activity is of contemporary interest[1,2] and there have been several recent studies using quantum mechanical (molecular orbital) techniques.[3-6] Following Ash and Schild's suggestion[7] that the pharmacological actions of histamine could be mediated by at least two types of histamine receptor in tissues, Kier proposed[3] that the drug-receptor interactions involved two distinct histamine conformations and adduced evidence from extended Hückel theory (EHT) that the lone gaseous histamine molecule has two preferred minimum energy conformations. Subsequent studies indicated[8] that this might also be the case for histamine in aqueous solution.

Others have taken a different view of the molecular orbital calculation procedure and made different predictions.[4,5] In any case, there is no *a priori* reason why a minimum energy conformation should be the biologically active one and, although the proposition is attractive, there is as yet no corroborative evidence that relates the dual activity of histamine to its conformation. The problem is further complicated by the fact that histamine exists as an equilibrium mixture of different tautomeric and ionic species. Although Kier selected the species that is probably the most prevalent under physiological conditions, there are others which should be considered.

For these reasons it seems necessary to examine the prob-

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Table I. Population of Histamine Species in Solution[a]

Species	Relative populations	
	At pH 7.4	At pH 6.0
Dication	0.025	0.387
Monocation	0.966	0.613
Neutral	0.01	0.0004
Anion	2.5×10^{-6}	10^{-8}

[a] Derived from pK_a values at 37° (Paiva, *et al.*[10]): $pK_{a_1} = 5.80$, $pK_{a_2} = 9.40$, $pK_{a_3} = 14$.

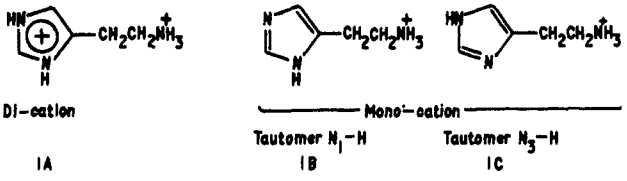

Figure 1 Histamine species.

lem in further detail. In the first place we have compared EHT and CNDO (complete neglect of differential overlap) predictions for different histamine species and related the results to data derived from nuclear magnetic resonance (nmr) studies. Secondly, we have investigated the conformational influence of methyl substituents placed at various positions in the histamine molecule. This approach has two particular merits. It permits an analysis of the chemical properties relative to histamine as reference and so allows one to avoid some of the problems associated with assessing the validity of absolute data. It has an obvious biological relevance since, as recently reported,[9] methyl substitution in histamine can affect profoundly the activity at histamine receptors.

The populations of various possible ionized forms of histamine in solution in the physiological pH range, calculated from the pK_a values,[10] are shown in Table I. At a pH of 7.4 (*i.e.*, at the pH generally taken to be that of the extracellular fluids) the monocation would predominate to the extent of 96%, but since the pH of the site of action is unknown and could be considerably lower, as in the vicinity of some membranes,[11] populations at pH 6.0 have also been considered. At this pH, a substantial proportion, *ca.* 40%, would be present as the dication (IA). The monocation exists also as an equilibrating mixture of the two tautomers, IB and IC (Figure 1). Tautomer IC has previously been associated with one type of histamine activity[12] (at the H_1 receptor) but there is no *a priori* reason to exclude tautomer IB from a possible involvement at other histaminic receptors. One of us‡ has obtained evidence from pK_a studies for the presence in aqueous solution of an appreciable proportion of tautomer IB (*ca.* 20% of the monocation). Thus, the dication and both tautomers of the monocation may be present in the biophase in amounts large enough to be considered likely as active species. In the present work we have considered these three forms.

Theoretical Method. The conformational analysis of histamine is essentially the analysis of a 1,2-disubstituted ethane. Using the numbering system of Figure 2, the conformation can be described by the three torsion angles θ_1, θ_2, and θ_3 which represent rotation about the respective bonds C_5-C_β, C_β-C_α, and C_α-N. In fact, the essential angles are θ_1 and θ_2 since, as has been demonstrated by others for various arylethylamines,[14] the symmetrical

‡C. R. Ganellin, unpublished results.

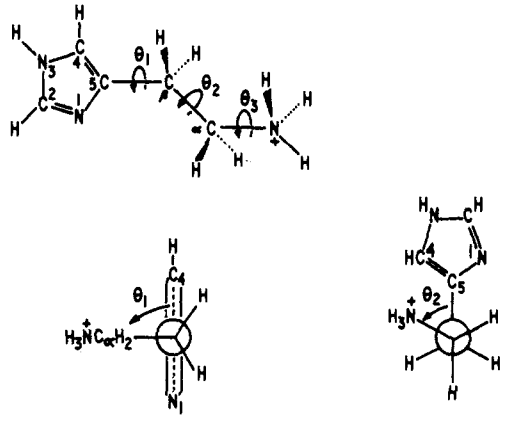

Figure 2. Histamine monocation showing (a) atom numbering and torsion angles; (b) torsion angle θ_1 viewed along C_β-C_5 bond from C_β to C_5; and (c) torsion angle θ_2 viewed along C_α-C_β bond from C_α to C_β.§

Table II. Bond Lengths and Angles Used in Calculations[a]

	Bond lengths, Å			Angles, deg	
	Monocation	Dication		Monocation	Dication
N_1-C_2	1.339	1.336	C_5-N_1-C_2	109.7	108.6
C_2-N_3	1.339	1.311	N_1-C_2-N_3	108.2	108.8
N_3-C_4	1.339	1.383	C_2-N_3-C_4	108.1	108.6
C_4-C_5	1.395	1.346	N_3-C_4-C_5	108.3	107.5
C_5-C_β	1.510	1.510	C_4-C_5-N_1	105.8	106.4
C_β-C_α	1.537	1.49	C_4-C_5-C_β	127.1	131.0
C_α-N_+	1.479	1.49	C_5-N_1-H	125.9	131.0
N_1-H		0.94	N_1-C_2-H	125.9	124.0
C_2-H	1.086	1.04	C_4-N_3-H	130.0	126.0
N_3-H	1.010	0.94	C_5-C_4-H	125.9	128.0
C_4-H	1.086	1.04			
C_β-H	1.073	1.05	(other angles tetrahedral)		
C_α-H	1.073	1.05			
N_+-H	1.032	1.05			

[a] Atom numbering as in Figure 2. The same coordinates were used for each tautomer of the monocation (except, of course, for the mobile proton).

ammonium group may be considered as adopting a staggered position with $\theta_3 = 60$, 180, and 300°. Assuming these values, the total energy of the molecule can then be minimized as a function of the two angles θ_1 and θ_2.

The scope of the problem requires one to make a large number of individual calculations so that in practice one is limited to computationally rapid methods of the semiempirical molecular orbital type such as the extended Hückel method of Hoffman,[15] which is the simplest procedure for including σ and π electrons, or the CNDO/2 method developed by Pople, Santry, and Segal.[16,17] The molecular geometry used as input data (see Table II) is taken from X-ray crystallographic results[18-20] augmented by standard values.[21] The simplifying assumptions have been used throughout that the imidazole ring is rigid and that bond lengths and angles are unaltered by conformational change The angles θ_1 and θ_2 have been separately optimized in terms of calculated molecular energy, initially by taking 60° increments but then, as appropriate, by 10-15° increments

§There is considerable diversity in the naming of histamine derivatives. Our nomenclature is derived from the system customary for histidine[13] in which (a) substituents on the side-chain amino group are assigned for the prefix N-, (b) in the side chain, the carbon atom adjacent to the amino group is called the α position, and (c) the ring-nitrogen atom adjacent to the aminoalkyl side chain is designated position 1. The other ring atoms are numbered serially in a way that assigns the smallest possible number, 3, to the second ring-nitrogen atom.

in order to locate more precisely the global minima.

Theoretical Results. Histamine Dication. The histamine dication is the best reference standard for calculation since it is the only free species of histamine for which a reliable geometry is available from X-ray crystallography.[18] For our calculations, the side-chain parameters have been altered slightly to accommodate the expectation that the side chain would be more symmetric in the lone molecule than in the solid lattice. The results are shown in Tables III and IV. The CNDO calculations give one very deep global minimum, at $\theta_1 = 0°$ and $\theta_2 = 180°$ (Figure 3), corresponding to the form in which all the carbon and nitrogen atoms are coplanar and the ring and ammonium group have a trans arrangement about the C-C bond as in Figure 4a. This method therefore predicts that the dication would exist exclusively in a trans form as, indeed, is found in the crystalline state. In the crystal the nucleus is not coplanar with the side chain but is almost perpendicular to it ($\theta_1 = 82°$); however, the predicted energy difference between these two orientations of the nucleus, *i.e.*, when $\theta_1 = 0$ or 80°, is small, at around 0.4 kcal/mol. The EHT method, on the other hand, predicts the existence of several minimum energy conformations and suggests in particular that the most stable forms are those in which the side-chain atoms are staggered in the usual manner for 1,2-disubstituted ethanes giving a trans ($\theta_2 = 180°$) or one of two gauche ($\theta_2 = 60$ or 300°) rotamers, as in Figure 4. The calculations reveal that the energies of these rotamers also depend on θ_1, the orientation of the nucleus, reflecting the interactions between the side chain and ring. Energy profiles showing the influence of nuclear orientation on the potential energy of the rotamers are given in Figure 5; with the trans form ($\theta_2 = 180°$) the profile is symmetrical about a maximum at $\theta_1 = 180°$ and reaches a minimum value corresponding to the most stable orientation when $\theta_1 = 110°$ (and its enantiomeric equivalent at $\theta_1 = 250°$). With the gauche form $\theta_2 = 300°$, minima occur when $\theta_1 = 110$ and 290°. Two nonequivalent forms arise because the nucleus is not symmetrical when substituted at C_5. They represent conformations in which the nucleus has turned through 180° with the consequence that the ring N_1 atom is further from ($\theta_1 = 110°$) or nearer to ($\theta_1 = 290°$) the side-chain N atom.

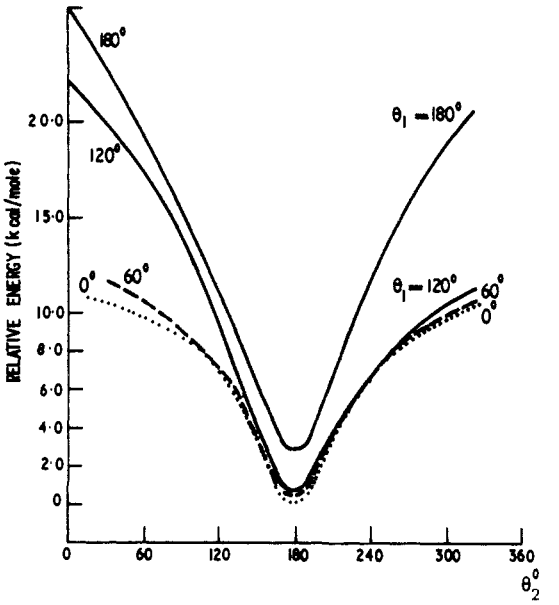

Figure 3. Histamine dication. Relative energies from CNDO showing the influence of side-chain rotation θ_2 for different values of ring orientation θ_1.

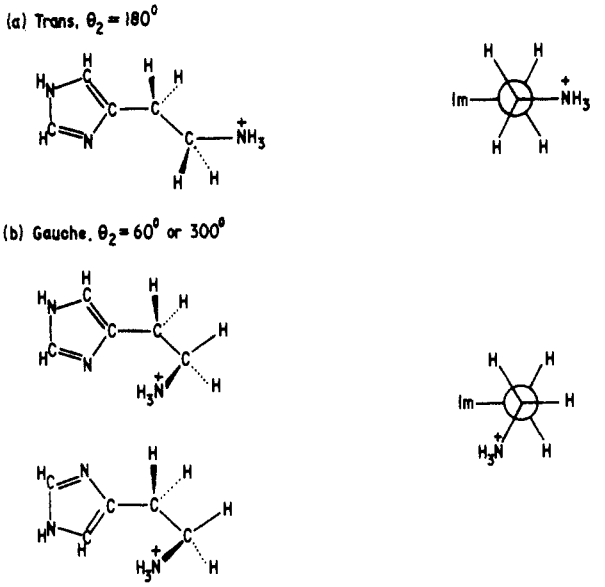

Figure 4. Trans and gauche conformers of histamine.

Table III. Total Energies by CNDO of Minimum Energy Conformations for Different Histamine Species

	Trans ($\theta_2 = 180°$)		Gauche ($\theta_2 = 300°$)	
Species	θ_1, deg	E, au[a]	θ_1, deg	E, au[a]
IA	0	−78.2687[e]	0	−78.2531[b]
IB	0	−77.9065[c]	60	−77.9125[e]
IC	180	−77.9158[d]	240	−77.9257[e]

[a] 1 au = 27.2 eV = 627.5 kcal/mol. [b] Least energy found for a gauche form but not a stable minimum. [c] Energy relative to most stable conformation (kcal/mol) = 3.7. [d] >6. [e] Most stable conformation for each species.

Table IV. Energies by EHT of Minimum Energy Conformations for the Different Histamine Species and Methylhistamine Dications

Molecule	Position of methyl substituent[a]	Trans ($\theta_2 = 180°$)		Gauche ($\theta_2 = 300°$)				
		θ_1, deg	Total energy, eV	θ_1, deg	ΔE^c	θ_1, deg	ΔE^c	$n_t{}^b$
IA		110	−805.980	110	0.5	290	1.3	0.64
II	2	110	−910.223	110	0.5	290	1.3	0.64
III	3	110	−910.567	110	0.5	290	1.3	0.64
IV	4	105	−910.070	120	0.8	280	1.3	0.72
V	N	110	−910.756	110	0.5	290	1.3	0.64
VI	N,N	110	−1014.820	120	0.9	300	1.8	0.78
VII	N,N,N	110	−1117.520	120	>10			>0.99
IB		105	−799.457	110	0.5	280	1.0	0.61
IC		120	−799.600	120	0.3	270	1.0	0.55

[a] Substituent numbering as in Figure 2. [b] Population of trans conformer (mole fraction) at 37°. [c] Energy relative to trans conformation (the most stable) in kilocalories per mole.

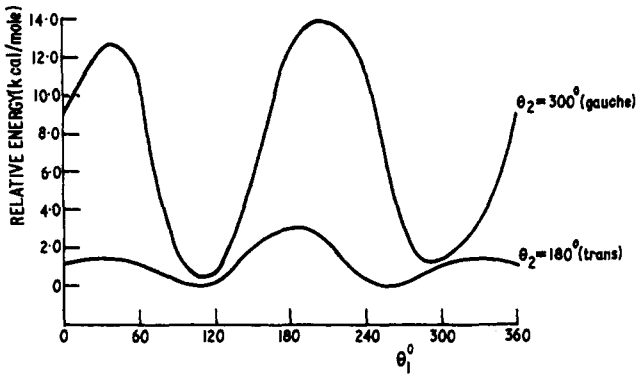

Figure 5. Histamine dication. Relative energies from EHT of the trans and gauche rotamers showing the influence of ring orientation θ_1.

Table V. Calculated and Observed Population Percentages of the Trans Conformer of Histamine by Different Procedures

Procedure	Reference	Dication	Monocation[a]
EHT	*b*	"64"	"55"
	3		"60"
CNDO	*b*	>99	<1
INDO	5		<1
PCILO	4		<1
Nmr	*b*	54	45
		48	
	8		25–45
Crystal	18	100	

[a]Calculations on tautomer IC. [b]This work.

The gauche form $\theta_2 = 60°$ is related enantiomerically so that the equivalent minima occur when $\theta_1 = 250$ and $70°$ (*i.e.*, the conformer $\theta_1 = 110°$, $\theta_2 = 300°$ is an enantiomer of $\theta_1 = 250°$, $\theta_2 = 60°$). The energies of the most stable gauche forms are slightly greater (respectively around 0.5 and 1.3 kcal/mol) than of the most favored trans form. The EHT calculations therefore suggest that a trans conformation which closely resembles the crystalline form is stable but that it is only slightly more stable than the gauche so that it would perhaps prevail to just a limited extent in equilibrium with the gauche conformers. If the above energy differences are assumed to be correct, then an estimate of the conformer populations can be made by using the expression $\Delta G° = \Delta H° - T\Delta S°$ for the free-energy difference between trans and individual gauche conformations; the potential energy difference between conformations is equated to the enthalpy difference $\Delta H°$, and the various conformations are assumed to be equal in entropy.[22] If the respective mole fractions in the three stable conformations, one trans and two gauche, are n_t, n_{g_1}, and n_{g_2}, and the free-energy differences between the trans and each of the gauche conformations are $\Delta G_1°$ and $\Delta G_2°$ then, for the above values of potential energy differences, 0.5 and 1.3 kcal/mol at 37°

$$\frac{n_t}{n_{g_1}} = e^{-\Delta G_1°/RT} = 2.25 \qquad \frac{n_t}{n_{g_2}} = e^{-\Delta G_2°/RT} = 8.3$$

since $n_t + n_{g_1} + n_{g_2} = 1$, it follows that $n_t = 0.64$. That is, 64% of the total molecules present are in the trans conformation at 37°. It is not possible, however, to use EHT calculations with such precision but for the convenience of further discussion we will refer to this value as "64%" with the intention that it should simply reflect the EHT prediction that the gauche and trans conformers are of comparable energy, with the possibility that the latter is slightly more preferred.

Histamine Monocation. For the histamine monocation we have assumed the same geometry for each tautomer, since the imidazole nucleus is known to be geometrically symmetrical.[20] The results are shown in Tables III and IV. The CNDO calculations predict energy minima for trans and gauche forms for each of the tautomers and ascribe considerable stability to particular gauche forms. With tautomer IC, the gauche form $\theta_1 = 240°$, $\theta_2 = 300°$ seems to be especially stable, as if there were an attractive interaction between the ammonium group and the ring N_1 atom such as that proposed by Niemann and Hays.[23] The results of Pullman[4] and Green[5] and their coworkers for tautomer IC similarly suggest an overwhelming stability for a gauche form. With the other tautomer, IB, our calculations indicate $\theta_1 = 60°$, $\theta_2 = 300°$ to be the most stable conformation; thus, in IB the orientation of the nucleus is reversed in comparison to IC and there is no suggestion of a stabilizing interaction specifically involving the ring N_1 atom, in keeping with the fact that it carries a proton. The energy differences between the most stable of the trans and gauche forms of either tautomer, as predicted by the CNDO calculations, are sufficiently large to suggest that more than 99% of histamine monocation would exist in a gauche configuration.

The EHT method also predicts stable trans and gauche conformers for each of the tautomers and indicates that of the two most stable forms, a trans ($\theta_1 = 105$–$120°$, $\theta_2 = 180°$) and a gauche ($\theta_1 = 110$–$120°$, $\theta_2 = 300°$), the former is possibly slightly favored. This agrees with the results published by Kier[3] for tautomer IC. A second stable gauche form ($\theta_1 = 270$–$280°$, $\theta_2 = 300°$) is of higher (about 1 kcal/mol) energy. The predictions for each tautomer (Table IV) are so similar that it is doubtful whether the slight differences have a real significance. Likewise, the differences between the mono- and dications by the EHT method are unlikely to be significant.

In summary, the CNDO calculations predict different conformations to be stable for the different species of histamine and that their relative populations depend on the ionization state. It predicts that the dication would be entirely trans but that the monocation would be overwhelmingly gauche. The EHT procedure, however, predicts the stable conformations and their relative population to be substantially similar for the dication and each of the monocation tautomers and that there would be very little preference between trans and gauche forms. Since these two calculation procedures give entirely different results, it is necessary to consider which is better suited to conformational analysis of histamine as a drug molecule or, indeed, whether either can be considered as adequate. As a first approximation it seems reasonable to regard the conformation in water as a reference model for the situation in the aqueous biophase. It is realized that this may not necessarily be helpful for considering interactions within membranes or with receptors. We have therefore obtained an experimental estimate of the gauche and trans conformer populations, separately for the dication and monocation in water, by analysis of the nmr spectrum. The nmr results of Casy, *et al.*,[8] indicate that histamine monocation in aqueous solution appears to exist as a mixture of trans and both gauche conformers in approximately equal proportions, in agreement with the EHT predictions. Our results, discussed below, agree with this. We also find that the histamine dication in water exists as a mixture of trans and gauche conformers in approximately equal proportions. These findings, summarized in Table V, fully uphold the predictions of the EHT method and are in complete disagreement with those of the CNDO procedure.

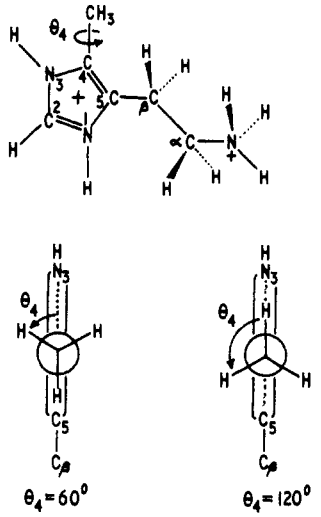

Figure 6. 4-Methylhistamine dication showing torsion angle θ_4 = 60 or 120° and view along the CH_3–C_4 bond, looking from CH_3 to C_4.

Methylhistamine Dications. It is arguable as to whether the EHT calculations on isolated molecules really can reflect the situation in aqueous solution, particularly for an extensively solvated molecule such as histamine. It may be that the apparent agreement simply arises from a fortunate cancellation of errors. Even so, the EHT calculations could be of value for use empirically as a predictive model with substituted histamines if it could first be established that it is applicable. Since we wish to consider the influence of methyl substituents on the conformation of histamine, we have compared the EHT predictions of conformer preference with nmr-derived results for six methylhistamine dications. Three of the compounds (II, III, and IV) have the substituent in the nucleus, *viz.* 2-, 3-, and 4-methylhistamine; the other three form a series in which methyl has been successively introduced at the side-chain ammonium group (V–VII). The minimum energy conformations, as determined by EHT for each of these molecules, are listed in Table IV together with molecular energies of the most stable (i.e., trans) and relative energies of the less stable (*i.e.*, gauche) conformers, and the mole fractions n_t of trans conformer calculated to be present at 37°.

With 2- and 3-methylhistamine dications, the methyl group is remote from the side chain and should have little direct steric influence on conformation, but it will alter charge densities within the nucleus. Additionally in 3-methylhistamine (III) the methyl group will prevent tautomerism. The relative energies of the stable conformations predicted by EHT are found to be identical with those of histamine. The situation is somewhat different with 4-methylhistamine (IV) in which the methyl substituent, being adjacent to the side chain, can have a direct steric influence, especially through interacting with the hydrogen atoms at the α position. The complete conformational description requires an additional torsion angle θ_4 to represent rotation of the methyl group. By setting this group in a staggered configuration with respect to the ring, *i.e.*, in one of the symmetrical forms with θ_4 = 60 or 120° as shown in Figure 6, the problem reduces to the evaluation of conformational stability again as a function of the torsion angles θ_1 and θ_2. The EHT calculation predicts that the stable conformations are staggered (θ_2 = 180 or 300°) and finds the values of θ_1 for minimum energy to be similar to those in the histamine dication. No further differences result from comparing θ_4 = 60 or 120°.

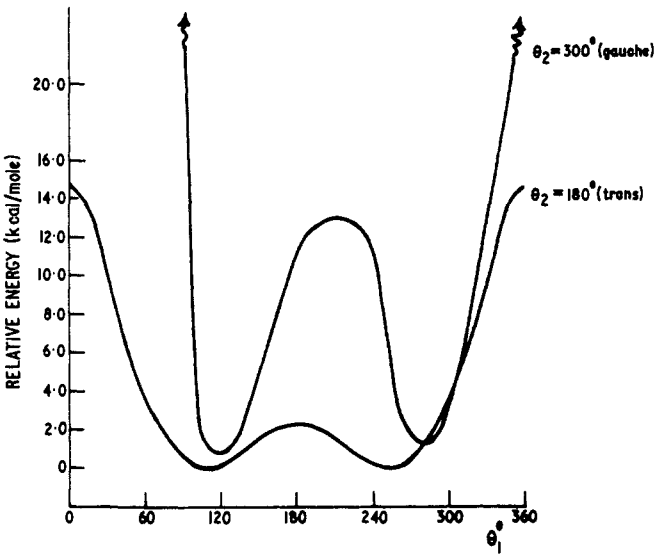

Figure 7. 4-Methylhistamine dication. Relative energies from EHT of the trans and gauche rotamers showing the influence of ring orientation θ_1.

The trans conformation is again predicted to be the most stable but with a suggestion that the trans population might be a few per cent greater than for histamine. Energy profiles, showing the influence of ring orientation on the potential energy of the rotamers, are given in Figure 7; the influence of the methyl group in raising the barriers to rotation is clearly evident.

With the three N-Me compounds the methyl groups are considered to have a staggered arrangement and at least one of the methyl groups is set into a trans configuration with respect to the C_α-N bond (*i.e.*, θ_5 = 180°). For the monomethyl derivative V the problem then reduces to that of histamine and, by way of confirmation, it is found that the same three stable conformations having the same relative energies are predicted as was found for histamine. With *N,N*-dimethylhistamine (VI) one of the methyl groups must be gauche with respect to the C_α-N bond (*i.e.*, θ_5 = 60°). This leads to greater eclipsing within the side chain for the gauche forms than for the trans so that the gauche became relatively higher in energy and less favored. This is reflected in the EHT predictions where the energy of the two gauche forms is found to be around 1–2 kcal/mol greater than for the trans conformer. If so, this would imply that about 80% of the molecules could be in the trans form. For the quaternary trimethylammonium derivative VII the predicted energy difference between the gauche and trans conformations is very large, suggesting that over 99% of the molecules would have a trans conformation.

Nmr Method and Results. The conformation about the CH_2–CH_2 bond of the histamine dication and its methyl-derivatives was determined on 0.4 *M* solutions of dihydrochlorides in D_2O at 40° and 60 MHz on a Varian A-60A spectrometer calibrated with a Muirhead-Wigan D-890-A decade oscillator. Histamine was also examined at pD 7.9 when approximately 96% was present as monocation. The spectrum of the histamine dication showed a doublet ($J_{2,4(5)}$ = 1.4 Hz) at δ 8.72 ppm (relative to sodium 2,2-dimethyl-2-silapentane-5-sulfonate) arising from the imidazole 2-proton, a multiplet at 7.46 ppm (imidazole 4(5) proton), and a multiplet at high field from the CH_2N (3.42 ppm) and CH_2Im (3.24 ppm) groups. There was a long-range coupling of −0.7 Hz between the imidazole 4(5) proton and the side-chain β-methylene group which gave rise to an

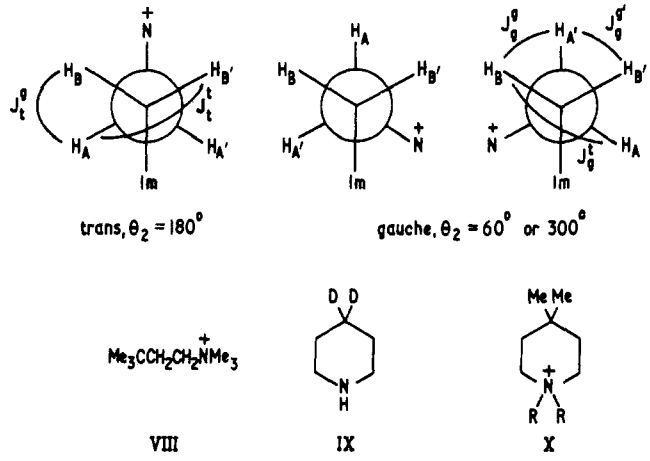

Figure 8. Histamine rotamers showing nmr nomenclature.

AA'BB'X spin system. This led to a slight complication in the analysis but it was still possible to extract the AA' part arising from the α-methylenic protons to provide some initial values for the spectral parameters. These values were used to calculate trial spectra and the input parameters were then refined using an iterative program (LAOCOON III)[24] to obtain a least-squares fit between observed and calculated line positions. Estimates of the vicinal coupling constants, J and J', were obtained in those cases where there was a sufficient number of transitions in the spectrum; the remaining spectra were deceptively simple and it was only possible to derive the sum, $N = J + J'$.

The observed vicinal coupling constants are J, the weighted mean of the couplings between protons H_A and H_B, and J', the corresponding couplings between protons H_A and $H_{B'}$ (*i.e.*, respectively J_{AB} and $J_{AB'}$) in the XCH_2-CH_2N^+ chain of the three rapidly equilibrating staggered rotamers (Figure 8). Using the approach and nomenclature of Abraham and Gatti[25] for 1,2-disubstituted ethanes X·CH_2-CH_2·Y and with the assumptions that dihedral angles are 60° and that the individual vicinal proton-proton couplings depend only on the dihedral angle and the electronegativities of the substituent atoms X and Y

$$J = n_t J_t^g + {}^1/_2 n_g (J_g^t + J_g^{g'}) \quad (1)$$

$$J' = n_t J_t^t + n_g J_g^g \quad (2)$$

$$n_t + n_g = 1 \quad (3)$$

where n_t and n_g are the fractional populations of the trans and gauche rotamers, respectively. Suitable values of the individual vicinal couplings for histamine are those given by Abraham and Gatti[25] derived from the model compounds, 3,3-dimethylbutyltrimethylammonium iodide (VIII, giving $J_t^t = 12.75$ and $J_t^g = 4.62$ Hz) and 4,4-dideuteriopiperidine (IX, giving $J_g^g = 3.77$ and $J_g^t + J_g^{g'} = 15.76$ Hz). Substituting these values and combining eq 1-3 gives

$$n_t = \frac{N - 11.65}{5.72} \quad (4)$$

where $N = J + J'$ and

$$n_t = \frac{J' - 3.77}{8.98} \quad (5)$$

Since the model compound IX for the gauche couplings is uncharged, we examined two other piperidines (X, R = H or Me) to serve as charged models. Unfortunately, the spectra were not sufficiently well resolved, due to interference from N^{14} proton coupling and quadrupolar broadening, and it was only possible to extract an approximate value, 12.0 Hz, for $J + J'$. This compares favorably with the value 11.65 Hz obtained by Lambert, *et al.*,[26] for the uncharged piperidine IX and suggests that a charge on the nitrogen atom would not alter vicinal coupling constants to any marked degree.

Table VI. Nmr Results for Histamine and Methylhistamines

Molecule	$\Delta\nu$,[a] Hz	N,[b,c] Hz	J',[c] Hz	n_t[d]	n_t[e]
Dications[f]					
IA	10.90	14.75	8.15	0.48	0.54
II	13.15	14.75	8.00	0.47	0.54
III	12.20	14.90	8.35	0.51	0.57
IV	11.65	14.90	8.35	0.51	0.57
V	11.80	14.90	8.40	0.51	0.57
VI	14.75	15.75	9.85	0.68	0.72
VII	19.75	16.90	11.75	0.89	0.92
Monocations					
I (B + C)[g]	19.00	14.20			0.45
IV[h]	20.30	14.20			0.45
VII	28.45	16.50			0.85

[a] Observed chemical shift difference between the methylene groups at 60 MHz. [b] The sum of the vicinal coupling constants, $J + J'$. [c] Measured to within 0.05 Hz; an error of ±0.1 Hz would affect n_t by ±0.02. [d] Corresponding populations of trans rotamer derived from J' or [e] N. [f] In the analysis providing J', the difference between the geminal coupling constants $M = J_{AA'} - J_{BB}$ was respectively 2.75 (IA), 2.65 (II, III), 2.50 (IV), 2.85 (V), 2.35 (VI), and 1.65 Hz (VII). The sum of geminal couplings $K = J_{AA'} + J_{BB'}$ was taken as −25 Hz; J_{AX} and $J_{A'X}$ were zero, and J_{BX} and $J_{B'X}$ were −0.7 Hz. [g] Examined at pD 7.9. [h] Examined at pD 8.4.

Our results with histamine and the methyl derivatives are given in Table VI which shows the chemical shift difference $\Delta\nu$ between the methylene groups, the values of N, and, where available, J' extracted from the spectra, and the corresponding estimates of n_t derived from eq 4 and 5. The degree of certainty with which these estimates represent absolute values is unknown but the measurements are sufficiently precise as to provide a good assessment of the rotamer population of each compound relative to histamine. Most compounds afforded spectra with a sufficient number of transitions to permit the full analysis required for the determination of J'. The estimates of n_t derived from J' are not very different from those based on N but they are possibly the more accurate.

With histamine we find that the relative population of the trans conformer (expressed as a percentage of the total molecules) is somewhat greater for the dication (54%) than for the monocation (45%). Similar effects are found with the two derivatives IV and VII. These results are in keeping with the expectation that charge repulsions in the dication would tend to favor the extended (trans) conformation. Monomethylation appears to have little influence on conformational preference but our results show that further methylation at the side-chain nitrogen atom successively increases the percentage of the trans form, from 57% for *N*-methylhistamine (V) to 72% for *N,N*-dimethylhistamine (VI) to 92% for the *N,N,N*-trimethylammonium derivative VII. The trend in these data corresponds extremely well with the EHT predictions (compare n_t values in Tables IV and VI). The EHT calculations, in comparison with the nmr derived populations, suggest a slightly greater proportion of trans conformer but this may be due to the known tendency of EHT to exaggerate energy differences. By either method, the monomethyl derivatives are found to resemble histamine, and additional substitution on the side-chain ammonium group increasingly favors the trans form. It should be noted that the values derived from the nmr mea-

surements depend on the assumption that the dihedral angles of the ethane skeleton are 60° *i.e.*, they refer to rotamers in which the values of θ_2 are either 180 (trans), 60 (gauche), or 300° (gauche). Since the EHT calculations predict that the internal energy of the molecule is minimized at these same values of θ_2, it is permissible to compare directly, at least in an operational manner, the rotamer preferences determined by the two methods.

In conclusion it appears that the EHT-predicted conformer population ratio may reasonably be used to give an indication of the likely situation in aqueous solution for methylhistamines. Its value lies in the possibility for predicting the conformation of molecules, such as side-chain *C*-methyl-substituted histamines, when there are difficulties in deriving the information by experiment.

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Conformation of Histamine Derivatives. 2. Molecular Orbital Calculations of Preferred Conformations in Relation to Dual Receptor Activity[1]

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The EHT calculated trans/gauche conformer population ratios of a series of methylhistamines are compared with their H_1/H_2 histamine receptor agonist activity ratios. Conformer ratios for α- and β-methyl- and *N*,*N*-dimethylhistamines are predicted to be quite different, respectively 0.1, 0.02, and 4; yet none of these compounds show significant biological selectivity. On the other hand, 2- and 4-methylhistamines have very similar trans/gauche conformational preferences but show a dramatic 1000-fold difference in their activity ratios. Thus, side-chain rotamer preference does not appear to determine whether these conformationally mobile derivatives distinguish between H_1 and H_2 receptors. However, an altered rotamer preference is accompanied by reduced activity and the results are consistent with a trans conformer possibly being involved at both types of histamine receptor.

The physiological actions of histamine can be considered to be mediated by at least two distinct classes of receptor (Ash and Schild).[2] One receptor type, designated H_1 by Ash and Schild, occurs in the smooth muscle of guinea-pig ileum and bronchi and is characterized by the ability of the tertiary amine class of antihistamines to antagonize specifically the response of these tissues. A second receptor type (designated H_2)[3,4] mediates histamine action in stimulating gastric acid secretion, in inhibiting contractions in the rat uterus, and in increasing the heart rate in the guinea pig. The differentiation of these two receptor types is reflected in the relative agonist activities of certain histamine analogs (Black, *et al.*[4]). Thus, 4-methylhistamine‡ selectively stimulates H_2 receptors (the activities, relative to histamine, are in the ratio $H_1:H_2 \approx 1:200$) whereas 2-methylhistamine is signifi-

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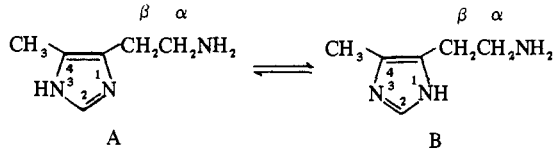
‡Using the nomenclature defined in paper 1,[1] 4-methylhistamine represents both tautomeric structure A and B.



A B