Solution Conformations of Histamine and Some Related Derivatives

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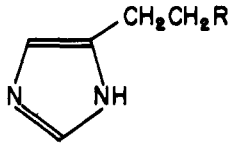
The conformations of histamine and some related compounds of pharmacological interest have been assessed in aqueous solution using high-resolution pmr spectroscopy. At physiological pH, the trans side-chain rotamer of the histamine cation is populated to the extent of 47% compared with 53% for the two equivalent gauche forms, and as the pH is lowered there is a further increase in the population of the trans rotamer. The gastric secretagogue *N,N*-dimethylhistamine shows a higher proportion of the trans rotamer than histamine, as does betazole, 5-iodohistamine, and the *N*-trimethylhistamine quaternary ion. These findings are discussed in relation to the biological activities of the compounds and recent work concerned with the conformational isomerism of histamine and its physiological actions at both H_1 and gastric secretory receptors.

Many pharmacologically active amines have a $-CH_2CH_2N$ side chain as a part of the molecule. With an ethanic fragment there may be appreciable conformational freedom about the central C-C bond, and it is therefore of considerable pharmacological interest to establish the conformational preference of such molecules, both in the solid state and in solution.

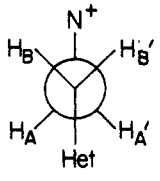
An amine of this type is histamine, 4(5)-(β-aminoethyl)-imidazole (Ia), and this important autacoid has been studied theoretically by Kier[1] using extended Hückel molecular orbital formalism and in the solid state by X-ray diffrac-



I(a): R = $^+NH_3$
I(b): R = $^+NHMe_2$
I(c): R = $^+NMe_3$

I

tion.[2] Of the three possible conformations with respect to the C-C bond of the side chain, namely, II, III, and IV, Kier found that the conformations II (trans) and III, IV (gauche) have very nearly equal total energies. For histamine diphosphate monohydrate in the solid state however, the $CH_2CH_2N^+$ side chain is in the trans form (II).



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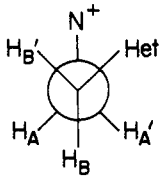

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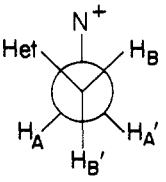


IV

In aqueous solution at physiological pH, on the other hand, the balance between intra- and intermolecular forces may be different from that in the solid state and the predominant solution conformation may not be the same as in the crystal. Our preliminary communication[3] showed that for histamine in solution there is a considerable proportion of the gauche conformers present.

In this paper, proton magnetic resonance (pmr) spectra are used to obtain conformational results for histamine in aqueous solution. Derivatives in which the primary amino group is methylated (Ib,c) and the pyrazole-based analog (V) of histamine are also studied, since these are representative of compounds with significant histaminic activities.[4]

Spectral Analysis

The pmr spectra of the histamine derivatives show two regions of absorption, one around δ 3.3 ppm downfield from TMS for the aliphatic protons of the $CH_2CH_2N^+$ fragment and the other, between δ 7 and 9 ppm, for the protons of the heterocyclic rings.

The chemical shift data for the molecules are listed in Table I. The variation of chemical shift of the histamine peaks was followed through the pH range 3-9 and is shown in Figure 1. Protonation of the imidazole-ring nitrogen occurring between pH 5.5 and 7.5 causes the largest downfield shift for imidazole proton 2 and a smaller shift for proton 5. Of the side-chain protons, the higher field multiplet shifts more in this pH region and is therefore assigned to the CCH_2C set of protons. The side-chain NH_2 group with a pK_a of 9.80 is much more basic than the imidazole ring,[4] so that at physiological pH, histamine and its deriva-

Table I. Chemical Shift Data[a]

Compound	Imidazole protons		Aliphatic protons		
	H_2	H_5	CCH_2C[b]	CCH_2N^+	$^+NCH_3$
Histamine					
0.1 *N* D_2SO_4	8.74	7.48	3.22	3.41	
pH 6.3	8.42	7.37	3.19	3.43	
pH 7	7.95	7.16	3.04	3.36	
4(5)-(β-Dimethylamino-ethyl)imidazole (Ib)					
pH 4	8.71	7.45	3.31	3.56	3.01
pH 6.5	8.29	7.31	3.21	3.51	2.96
pH 8.5	7.85	7.14	3.12	3.47	3.06
4(5)-(β-Trimethylam-moniumethyl)imid-azole (Ic)	7.74	7.10	3.17	3.64	3.20
5-Iodohistamine (VI)	8.86		3.20	3.38	
	Pyrazole protons				
	H_4	H_5			
3-(β-Aminoethyl)-pyrazole (V)					
pH 1	6.76	8.13	3.31	3.47	
pH 7	6.36	7.72	3.09	3.35	

[a] Values in ppm downfield from TMS. [b] The identification of these protons also follows from observation of a long-range coupling with imidazole proton H_5.

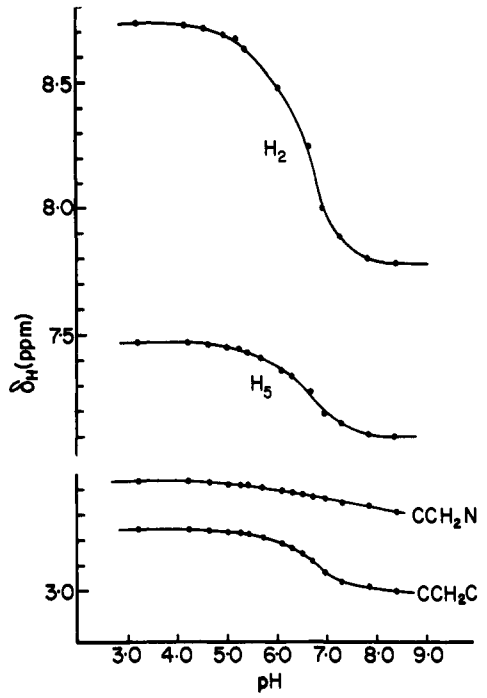


Figure 1. Variation of chemical shifts of histamine protons, in parts per million from TMS, with pH. This titration was performed in H_2O with a histamine concentration of 1 g/ml.

tives are present as the univalent cation with the aliphatic nitrogen protonated, as in Ia.

The conformational information for these molecules comes largely from an analysis of the CH_2-CH_2 methylene proton spectra. With relatively rapid rotation about the CC bond, the methylene spectrum is an average of that of the individual rotamers, II, III, and IV, and is expected to be of the AA'BB' type.[5] Features of this type of spectrum have been discussed by Grant, Hirst, and Gutowsky[6,7] and Garbisch.[8] Trial parameters were obtained by the procedures outlined in those papers and the final parameters obtained by iterative calculations using the program LAOCOON.[9] Besides the difference in chemical shift δ_{AB} between protons A and B, four other parameters are required, *viz.*, *K*, *M*, *N*, and *L*.[6] The latter two are defined by

$$N = J_{AB} + J_{AB'} \quad (1)$$

and

$$L = J_{AB} - J_{AB'} \quad (2)$$

where J_{AB} and $J_{AB'}$ are the vicinal proton-proton coupling constants. *K* and *M* are respectively the sum and difference of the geminal proton-proton coupling constants, $J_{AA'}$ and $J_{BB'}$.

4(5)-(β-Dimethylaminoethyl)imidazole (Ib). At pH 6.5, where the imidazole ring is approximately half protonated, the AA'BB' spectrum at 100 MHz for the side-chain methylene protons of Ib was not symmetrical; decoupling experiments showed that long-range couplings with the imidazole ring protons were responsible for the complexity in the high-field half of the multiplet. Irradiation of the proton at 7.3 ppm removed most of the asymmetry, thus confirming the identification of the upfield methylene protons as those of the CCH_2C group. Double irradiation at 7.3 and 8.29 ppm produced the methylene spectrum shown in Figure 2, with each half showing nine distinct lines. Iterative refinement of the spectrum gave the parameters listed in Table II. These also reproduced the observed 60-MHz spectrum.

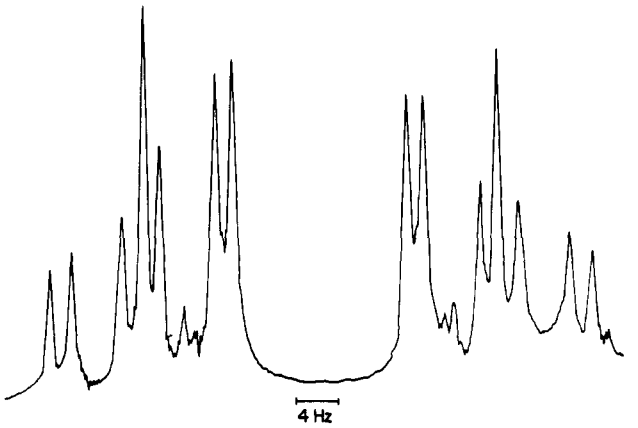


Figure 2. 100-MHz methylene proton spectrum of *N,N*-dimethylhistamine (Ib) in D_2O at pH 6.5 with both imidazole protons decoupled.

Table II. AA'BB' Internal Chemical Shifts (ppm) and Coupling Constants (Hz)

Compound	δ_{AB}	*N*	*L*[a]	*M*[a]	*K*[a]
4(5)-(β-Dimethylaminoethyl)imidazole (Ib)					
pH 6.5	0.30	15.35	3.0	2.32	−28.1
pH 4	0.25	15.75	4.1	2.32	−28.1[b]
pH 8.5		14.8	2	2.32[b]	−28.1[b]
4(5)-(β-Trimethylammoniumethyl)imidazole (Ic)	0.47	16.4	5.4	1.2	−27.7
3-(β-Aminoethyl)pyrazole (V)					
pH 7.3		14.8			
pH 1		15.4			
5-Iodohistamine (VI)		15.4			
Histamine					
pH 7	0.32	14.5	<1	2.3[b]	
pH 6.3	0.24	14.5	<1	2.3[b]	
0.1 *N* D_2SO_4		15.0			

[a]The signs of *M* and *L* are not determined from the spectral analysis. If *N* is assumed to be positive, then *K* is negative in the two cases in which a value can be obtained. [b]Assumed value.

Although it is often not possible to obtain *K* values from AA'BB' spectra,[7] in this case the *L*, *K*, and δ_{AB} values are such that the spectrum is sensitive to *K*. The cross-ring coupling between the imidazole ring protons is 1.0 Hz and if this is assumed positive then calculated spectra show that the long-range coupling (*o*-benzylic type) between proton H_5 and the CCH_2C protons is -0.8 ± 0.1 Hz.

At pH 4, where the divalent cation predominates, irradiation of both imidazole ring protons was again necessary to obtain a symmetrical spectrum. The final parameters obtained are listed in Table II. Because of the different downfield shifts of the methylene protons on protonation of the imidazole ring nitrogen, the chemical shift difference has now dropped to such a value that the *K* value could only be determined to be more negative than about −24 Hz, and the spectrum is quite consistent with the same value of −28.1 Hz observed at pH 6.5.

At pH 8.5 the spectrum of Ib was considerably broader than at lower pH's; also, at this pH the high-field half of the AA'BB' multiplet was under the strong NMe_2 peak. For these two reasons a full analysis of this spectrum was not possible. However, from the eight lines observed in the low-field half of the multiplet, we conclude that, assuming an *M* value of 2.3 Hz, *L* is very close to 2 Hz, and the approximate chemical shift difference for the methylene protons is 0.35 ppm. The *N* value of 14.8 Hz is clearly obtained.

4(5)-(β-Trimethylammoniumethyl)imidazole Iodide (Ic). In the 100-MHz spectrum of this quaternary methiodide, the signal from the $^{+}NMe_3$ protons coincided with part of the upfield half of the AA′BB′ multiplet and again, as with Ib, the clear part of the upfield signal was not a mirror image of the low-field half of the multiplet. In this case, however, decoupling from both the imidazole ring protons did not restore complete symmetry. With a quaternary nitrogen compound another cause of spectral complexity is additional coupling between protons and a ^{14}N nucleus ($I = 1$), which can result in the tripling of the proton signal.[10,11] The $^{+}NCH_\alpha$ coupling is usually very small but for β protons the $^{+}NCCH_\beta$ coupling can range up to 2 Hz.[11,12] The broadening of the upfield half of the decoupled spectrum here is due to such a three-bond coupling between ^{14}N and the β protons of the CCH_2C group.

From the low-field methylene signal, trial parameters for K, L, M, and N were obtained and these were then refined iteratively. With these iterated parameters, values of the AB chemical-shift difference, the *o*-benzylic coupling, and the tripling constant J_{NCCH} were obtained by matching calculated and experimental (decoupled and undecoupled) spectra. These values are $\delta_{AB} = 0.47$ ppm, *o*-benzylic $J_{HCC=CH} = -0.6$ Hz and $J_{HCCN} = 1.15 \pm 0.05$ Hz. Other parameters are given in Table II.

Histamine. Pmr spectra of histamine were recorded at a number of pH values between 1 and 9. The line widths were found to be pH dependent and at a minimum in the pH range 5-6. Two spectra were analyzed; one was the univalent cation at pH 7 and, in the other, the solution was acidified to pH 6.3 until the imidazole ring was approximately 50% protonated. The spectra were essentially the same apart from a decrease in the internal AB chemical shift difference from 0.32 ppm for the cation to 0.24 ppm in the second case.

For both these solutions the AA′BB′ spectrum at 100 MHz was not symmetrical and irradiation at both imidazole proton frequencies was needed to restore the mirror symmetry expected for the AA′BB′ system.

Figure 3 shows the decoupled spectrum for an aqueous solution, pH 6.3. The chemical-shift difference between the A and B protons is 0.24 ppm, which at 100 MHz is close to or slightly less than the estimated value for K. Hence, the spectrum is not expected to yield an accurate value for K.[8]

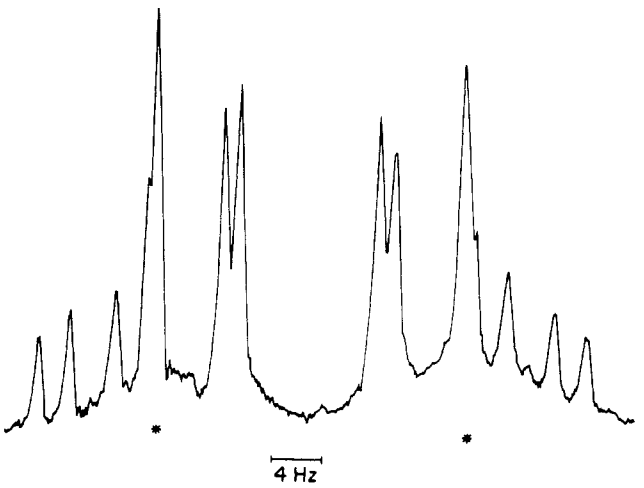


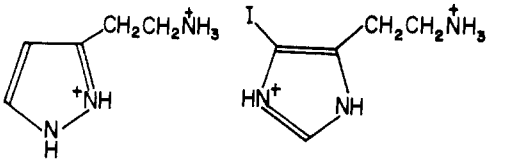
Figure 3. 100-MHz methylene proton spectrum of histamine (Ia) in D_2O at pH 6.3 with both imidazole protons decoupled. The asterisks indicate the lines contained in the M or antisymmetric quartets; see ref 8.

This seven-line spectrum is superficially an example in which L is zero. The decoupled methylene multiplets can be analyzed adequately to give $\nu_A = 3.19$ ppm, $\nu_B = 3.43$ ppm (downfield from TMS), and $N = 14.5$ Hz. With $L = 0$, however, M can have any value. The effect of nonzero M and L values is to broaden and eventually split the lines indicated by the asterisks in Figure 3 into the M, or antisymmetric, quartets.[8] A range of M and L values, which gave spectra indistinguishable from that shown in Figure 3, was obtained by rejecting any calculated spectrum in which the intensity of the broadened M-quartet peak was less than 90% of the labeled peak (*). For M less than 2.3 Hz (the largest value found for Ib or Ic), L is less than 1 Hz. The imidazole protons have a cross-ring coupling of 1.2 Hz and the upfield ring proton has a long-range *o*-benzylic type coupling of −0.8 Hz with the CCH_2C protons.

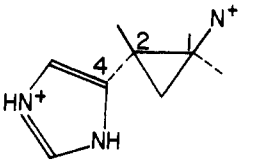
For the histamine dication the line widths were generally poor; however, there was an indication that the N value was larger than for the univalent cation. One sample of the dihydrochloride was therefore studied in 0.1 N D_2SO_4. On irradiating both imidazole protons, the high-field multiplet sharpened sufficiently to show that N had increased to 15 Hz but no further analysis was attempted.

3-(β-Aminoethyl)pyrazole (V) or Betazole. In view of its known action as a gastric secretory agent,[4] V was studied both as the hydrochloride (at pH 7.3) and in more acidic solutions as the dihydrochloride. The AA′BB′ spectrum of the hydrochloride is very similar to that of the histamine cation since it can be analyzed with $L = 0$. The value of N is 14.8 Hz, but in the absence of estimates for M no reliable conclusion as regards L can be made. For the dihydrochloride both halves of the AA′BB′ signal were broad and irradiation of the ring protons sharpened the signal from the CCH_2C protons sufficiently to give the N value of 15.4 Hz.

5-Iodohistamine Dihydrochloride (VI). This molecule with a bulky substituent in the imidazole ring near the CH_2CH_2N side chain gave an AA′BB′ spectrum in which each half consisted of five broad lines. N was estimated to be 15.4 Hz.



V VI



VII

Conformational Analysis

The calculation of rotamer populations from the vicinal coupling constants J_{AB} and $J_{AB'}$ requires some guide as to the values expected in individual rotamers since, for the present molecules, it is not practicable to freeze out any preferred rotamer in aqueous solution.

The earliest treatments of this rotational isomerism problem simplified it by the recognition of only two vicinal proton-proton coupling constants for rotamers II, III, and

IV.[13,14] With the assumptions of classical staggered conformations, these couplings were J_t for two protons with a 180° dihedral angle and J_g for two protons with a 60° dihedral angle. The observed coupling constants are a weighted mean of the couplings in the individual rotamers, so that

$$J_{AB} = n_t J_g + \tfrac{1}{2}(1 - n_t)(J_g + J_t) \quad (3)$$

and

$$J_{AB'} = n_t J_t + (1 - n_t) J_g \quad (4)$$

where n_t is the proportion of trans rotamer II. The spectral parameters N and L then become

$$N = \tfrac{1}{2}\{J_t + 3J_g + n_t(J_t - J_g)\} \quad (5)$$

and

$$L = (\tfrac{1}{2} - \tfrac{3}{2}n_t)(J_t - J_g) \quad (6)$$

Karplus in his treatment of the dihedral-angle dependence of J_{vic} has shown that $J_t - J_g$ is positive,[15] but the individual values for a specific system are difficult to predict.

Abraham and Pachler showed that the experimental quantity $\tfrac{1}{2}N + \tfrac{1}{6}L$ was independent of the rotamer populations[14] since

$$\tfrac{1}{2}N + \tfrac{1}{6}L = \tfrac{1}{3}(J_t + 2J_g) = J_{av} \quad (7)$$

and they also derived the correlation

$$J_{av} = 17.97 - 0.80\Sigma E \quad (8)$$

where ΣE is the sum of the Huggins' electronegativities[16] of the substituent atoms attached to the C-C fragment. However, there are two main objections in adapting this correlation for calculating rotamer populations. Firstly, it is found that as the percentage of trans isomer increases, the observed J_{av} values are greater than those calculated from the correlation.[14] Secondly, the correlation itself ignores the stereospecific effect of electronegative substituents pointed out by Booth,[17] in which a substituent exerts a maximum effect on lowering a vicinal J_{HH} value when it is in a planar trans arrangement with respect to one of the coupled protons. Both of these difficulties represent a breakdown of the assumption of only two vicinal J_{HH} constants and show that it is necessary to recognize different J_t and J_g values for the gauche and trans rotamers.

Nevertheless, this correlation can be used for the less stringent determination of the sign of L. This allows deduction of the most stable rotamer, since eq 6 for L shows that if L is negative then n_t is greater than $\frac{1}{3}$ and the trans rotamer is more stable. For the N-methylated histamine derivatives the calculated value of J_{av} is 6.5 Hz, which is to be compared with observed values, at the various pH's, ranging from 9.1 to 7.7 Hz with $L > 0$ and from 7.3 to 7.1 Hz for $L < 0$. These figures indicate that the trans rotamer is the most stable in these derivatives. For histamine itself no firm conclusion can be drawn by this method as regards the sign of L.

Table III. Proportions (n_t) of Trans Rotamer for Histamine and Derivatives

Compound	N	n_t	L	n_t
NMe_3 histamine (Ic)	16.4	0.76	−5.4	0.77
NMe_2 histamine (Ib)				
pH 4	15.75	0.66	−4.1	0.67
pH 6.5	15.35	0.60	−3.0	0.58
pH 8.5	14.8	0.52	−2.0	0.50
Histamine				
pH 7	14.5	0.47	−1[a]	0.41
0.1 N D_2SO_4	15.0	0.55		
Betazole (V)				
pH 7.3	14.8	0.52		
pH 1	15.4	0.61		
5-Iodohistamine (VI)	15.4	0.61		

[a] This value is an estimate from the spectral analysis, but the negative sign is indicated since $L = 1$ yields an n_t value of 0.25.

In a recent series of papers, Abraham and coworkers[18] have investigated both experimentally and theoretically the effect of various solvents on the energy differences between the rotamers of a number of 1,2-disubstituted ethanes, YCH_2CH_2X. From the values of the rotationally averaged vicinal coupling constants J_{AB} and $J_{AB'}$, they have derived the coupling constants in the individual rotamers,† *viz.*, J_g^T and J_t^T for the trans rotamer II, and for the two gauche rotamers III and IV the constants $\tfrac{1}{2}(J_{g'}^G + J_t^G)$ and J_g^G.

Combining their results with data from the literature, they were able to demonstrate the effects of the substituent electronegativity, E_X and E_Y, on each of the four coupling constants with an average root mean square error between observed and calculated values of 0.3 Hz.

trans rotamer

$$J_g^T = 1.35 + 0.63(E_X + E_Y) \quad (9)$$

$$J_t^T = 18.07 - 0.88(E_X + E_Y) \quad (10)$$

gauche rotamer

$$J_g^G = 8.94 - 0.94(E_X + E_Y) \quad (11)$$

$$J_{g'}^G + J_t^G = 26.92 - 2.03(E_X + E_Y) \quad (12)$$

With the definition of the individual J's, one can derive the following expressions for the spectral parameters N and L.

$$N = n_t J_g^T + \tfrac{1}{2}n_g(J_{g'}^G + J_t^G) + n_t J_t^T + n_g J_g^G \quad (13)$$

$$L = n_t J_g^T + \tfrac{1}{2}n_g(J_{g'}^G + J_t^G) - n_t J_t^T - n_g J_g^G \quad (14)$$

In the present examples the sum of the electronegativities of the substituents (C and N) is 5.65, so that eq 13 and 14 may be combined with eq 9-12 to give the following expressions for the spectral parameters N and L of the histamine derivatives.

$$N = 11.35 + 6.5n_t \quad (15)$$

$$L = 4.1 - 12.3n_t$$

These expressions then allow two determinations of the proportion of trans rotamer present and the n_t values derived in this way are shown in Table III.

Long-Range Proton-Proton Couplings. In histamine and the methyl derivatives Ib and Ic, a four-bond long-range coupling between the imidazole proton H_5 and the CH_2 adjacent to the imidazole ring was observed. The values, ranging between −0.6 and −0.8 Hz, indicate little conformational preference about the ring to side-chain bond.[19]

Discussion

In aqueous solution both the gauche and trans rotamers of the histamine cation are available at physiological pH

†In this connection a number of notations have been used and the present notation, which is different from that of Abraham, *et al.*, preserves the use of the subscript t or g to indicate a dihedral angle of 180 or 60°, respectively, between the coupled protons. The superscript T or G designates the rotamer, described trans or gauche by the disposition of the substituents X and Y. Thus, in II, $J_{AB} = J_g^T$ and $J_{AB'} = J_t^T$ while, in III, $J_{AB} = J_{g'}^G$, $J_{A'B'} = J_t^G$, and $J_{A'B} = J_{AB'} = J_g^G$ and, in IV, $J_{AB} = J_t^G$, $J_{A'B'} = J_{g'}^G$, and $J_{A'B} = J_{AB'} = J_g^G$.

and are freely interconvertible. At low pH (where the histamine divalent cation dominates) there is a larger proportion of the trans rotamer than at pH 7. For Ib, methylation has increased the proportion of trans rotamer as compared with histamine, and this increase is observed for both the singly and doubly charged cations. The *N*-trimethylhistamine iodide, with the bulky trimethyl group on the terminal nitrogen, has the highest proportion of trans rotamer found for these derivatives. Increasing substitution at the terminal-N atom and increasing the positive charge on the cation both favor an increase in the proportion of trans rotamer. In summary, then, the detailed conformational picture here is one of considerable flexibility, with the precise details depending in a subtle way on substituents and charge characteristics.

It is also noteworthy that neither histamine nor any of the derivatives and analogs studied here has been found to be exclusively in the trans conformation in solution. These observations serve to emphasize the importance of intermolecular forces in determining the conformation in the solid state. In the monohydrate with $H_2PO_4^-$ at the anion, the histamine dication is in the trans form but this conformation, involving five NH-O hydrogen bonds, would seem to be an integral part of the three-dimensional network of $H_2PO_4^-$ ions, held together by the NH-O and six OH-O hydrogen bonds.[2] In solution histamine acid phosphate gives a dication pmr spectrum very similar to that of the chloride in 0.1 *N* D_2SO_4.

The pharmacological actions of histamine are divided at least into two categories by the fact that drugs generally recognized as antihistaminic agents only block one group of receptors, *e.g.*, those responsible for contractions of smooth muscle from the gut and bronchi. For these Ash and Schild have used the notation H_1 receptors.[20] On the other hand, the stimulation of gastric secretion by histamine is not blocked by antihistamines, but two recent reports have discovered drugs which are gastric secretory inhibitors.[21,22] The theoretical studies of histamine and the X-ray crystal structure determinations, in which both histamine and the antihistamine methapyrilene‡ had the trans structure in the solid state,[2,23] have led to the association of the trans conformation II with those histaminic actions at H_1 receptors. Similar conclusions have been reached from considerations of the activities and configurations of the 4-amino-1,2-diarylbut-2-ene antihistamines[24] and also from studies of the solution conformations of several antihistamines of the ethylenediamine type.[25]

It may then be argued that histamine and related antagonists interact in the gauche form at non-H_1 receptors as proposed by Kier.[1] However, *N,N*-dimethylhistamine (Ib) in which the trans rotamer is preferred over gauche forms, is significantly more active as a gastric stimulant than histamine itself[26-28] and it has also been suggested that the metabolic formation of this compound may contribute to the secretory effect of histamine.[27,28] This evidence therefore points to the conclusion that histamine agonists act in the trans conformation at gastric receptors. Likewise the weaker potency of Ib compared with histamine in causing gut motility and fall in blood pressure[26] supports non-trans rotamers as the active species at H_1 receptors. Although the very weak activity of *trans*-2-(4-imidazolyl)cyclopropylamine (VII) as a gastric secretagogue[29] could be interpreted to mean that histamine is not acting in its trans conformation at these gastric receptors, this derivative has limitations as a trans model since the $NC^1C^2C^4$ dihedral angle probably deviates significantly from the ideal value of 180° (*cf.* studies of *trans*-2-acetoxycyclopropyltrimethylammonium iodide[30] where the analogous NCCO dihedral angle is 137°). §

If deductions drawn from evidence upon Ib are valid, the corresponding methiodide Ic, with 80% trans conformation, would be expected to be an effective agonist for gastric activity. In fact, the quaternary salt is completely inactive at gastric sites[31,#] but this result may be due to the failure of the completely ionized molecule to be transferred from the blood plasma to the region within the gastric mucosa where the appropriate receptors are located. The difficulty with which quaternary ammonium compounds penetrate lipid barriers is well known.[32] Distribution factors are less critical in the guinea-pig ileum test for H_1 receptors (an *in vitro* procedure) and here the inactivity of the methiodide# supports a non-trans conformer as the active form of H_1 agonists.

Betazole, a pyrazole-based analog of histamine, has a very similar conformational preference to histamine. It is an agonist (albeit weak) for gastric secretion but is ineffective at H_1 sites.[4,26] These findings are difficult to reconcile with the above generalizations, but since the analog is based on pyrazole rather than imidazole its mode of action may differ from that of histamine.

Histamine itself increasingly favors the trans conformation as the pH is lowered to form the dication. This change was predicted on the basis of repulsion between the two positive centers on the dication,[33] and this trend may be relevant to its interaction with the gastric receptors, since diprotonated histamine should predominate at gastric pH.

The present conformational results do not lead, unfortunately, to definite and clear-cut structure-activity conclusions. It seems that factors other than conformational preference have to be considered. Such factors might include drug distribution, the influence of N-methylation upon intrinsic activity and affinity, and the possibility that an energetically unfavored conformation is the pharmacologically active species.[34]

Experimental Section

Melting points were determined on a Thomas-Hoover Unimelt capillary melting point apparatus and are uncorrected. Commercial samples of histamine dihydrochloride were used. The sample of betazole dihydrochloride (V) was kindly provided by Eli Lilly and Co. 5-Iodohistamine dihydrochloride (VI) was prepared by the method of Tominaga and Paiva.[35] The dihydrochloride of Ib was prepared by reductive methylation of histamine by the following procedure.

Histamine dihydrochloride (2.00 g) was dissolved in H_2O (3 ml) before dilution of the solution with EtOH (125 ml). After addition of HCHO (4 ml, 40% aqueous solution) and 10% Pd/C (0.5 g) the mixture was stirred with H_2 at room temperature and atmospheric pressure until gas absorption ceased (1.5 hr). The reaction mixture was filtered through kieselguhr and the filtrate evaporated to dryness under reduced pressure. C_6H_6 (150 ml) was added to the residual syrup and the last remaining traces of moisture were removed by azeotropic distillation using a Dean-Stark head. Removal of C_6H_6

‡ Methapyrilene is 2-[(2-dimethylaminoethyl)-2-thenylamino]-pyridine, and in the hydrochloride the two nitrogen atoms of the $N-CH_2-CH_2-N^+$ chain are in the trans conformation.[23]

§ A referee has pointed out that the low potency of VII may be due to the presence of the methylene substituent in the aminoethyl fragment of the molecule (both α- and β-methylhistamine are very feeble histaminic agonists.[22] If this be the case, the potency differences of VII at H_1 and gastric sites (0.0078 and 0.016 times histamine respectively)[29] may have conformational significance. Pharmacological data upon the cis analog of VII is required to clarify the issue.

#E. C. Kornfeld, personal communication, 1970.

by rotary evaporation left the crude product as an oil, which was dissolved in a few milliliters of hot MeOH and after the addition of EtCOMe the pure dihydrochloride of Ib [1.63 g; 70%; mp 186° (lit.[36] 184°)] separated out. Ic was prepared as follows. The dihydrochloride of Ib (0.42 g) was suspended in Et_2O (50 ml) containing 2 drops of H_2O and after the addition of one pellet of NaOH the mixture, contained in a mortar, was vigorously triturated with a pestle. In this way, the free base of the substrate passed into the Et_2O and NaCl solid began to appear. The ethereal solution was decanted off and dried (Na_2SO_4), and MeI (0.15 ml) was added to the filtered solution. After a few minutes the quaternary methiodide began to separate. After standing at 0°, several collections yielded Ic (0.22 g, 40%), mp 236° (MeOH-Et_2O). *Anal.* ($C_8H_{16}IN_3$) C, H. Samples were dissolved in D_2O and spectra were recorded on Varian A-60 and HA-100 spectrometers, using *tert*-butyl alcohol as internal reference. These chemical shifts were then referred to TMS by the addition of 1.26 ppm. The pH values were determined with a glass electrode and are not corrected to pD values. Generally the line widths were found to be pH dependent and the pH of the solution was adjusted by adding NaOD to give the best line width within the pH region of interest.

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A New Chiral Reagent for the Determination of Enantiomeric Purity and Absolute Configuration† of Certain Substituted β-Arylethylamines

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The synthesis and resolution of α-methyl-α-methoxypentafluorophenylacetic acid are described. The resolved acid has electron-capture properties and is shown to be a useful reagent for the determination of enantiomeric composition of chiral arylethylamines by glc after exposure of racemic amines to biological membranes. It is also shown to be a useful reagent for the prediction of the absolute configuration of certain substituted β-arylethylamines by nmr.

The determination of the enantiomeric composition of chiral substances in recent years has been the subject of considerable investigation both in chemistry and biology. The methods most commonly employed in these studies have been based either on glc or nmr. Early workers[2] demonstrated the utility of glc as a method of estimating enantiomeric composition and the same fundamental approach was utilized by Raban and Mislow,[3a,‡] who showed that enantiomeric composition could also be determined by nmr after conversion to a diastereomeric mixture. Dale, Dull, and Mosher[4a] refined the nmr method by developing the chiral reagent α-methoxy-

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‡For a critical review of the nmr method, see ref 3b.