Ionization of Histamine, N-Acetylhistamine, and Their Iodinated Derivatives

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pK values for the dissociation of the imidazolium, ammonium, and imino groups of histamine, 5-iodohistamine, 2,5-diiodohistamine, N-acetylhistamine, N-acetyl-5-iodohistamine, and N-acetyl-2,5-diiodohistamine were determined by electrometric and spectrophotometric titrations. $\Delta H°$ and $\Delta S°$ values were estimated from the temperature dependence of the dissociation constants of histamine and its mono- and diiodo derivatives. The results did not support the hypothesis of an intramolecular NH–N hydrogen bond between the side-chain amino group and an imidazole nitrogen in aqueous solutions of histamine.

Niemann and Hays,[1] based mainly on the histamine-like activity of β-(2-pyridyl)ethylamine, proposed that the biological activity of histamine depends on the existence of an intramolecular H bond between the protonated amino group and the "pyridine" nitrogen of the imidazole ring. This hypothesis was strengthened by Lee and Jones'[2] analysis of the structural requirements for histamine-like activity in a number of compounds. Although the stability of the proposed H bond in aqueous solution is open to question,[3] its existence has been widely accepted by pharmacologists.[4] This is true not only in the case of histamine but also for other imidazole derivatives bearing a side chain with a functional group. The main evidence for such H bonds comes from ir absorption spectra of N-acetylhistamine and methyl dihydrourocanate in the solid phase and in $CHCl_3$,[5] as well as from the titration of mono- and diiodohistidines in aqueous solution.[6]

If the intramolecular H bond occurs in aqueous solutions of histamine this substance should ionize according to Scheme I, analogous to the one proposed by Brunings[6] for histidine. In this scheme, the enthalpy and entropy changes accompanying the first dissociation should include the contribution from H bonding and ring closure. The thermodynamic quantities for the other two ionizations should also have different values from those observed with amino and imidazole compounds that do not have the possibility of intramolecular hydrogen bonding.

In order to obtain experimental data that may be useful for the knowledge of histamine conformation in aqueous solution we have determined the effect of temperature on the ionization constants of histamine, 5-iodohistamine (monoiodohistamine), and 2,5-diiodohistamine (diiodohistamine). We have also determined the ionization constants of N-acetylhistamine, N-acetyl-5-iodohistamine, and N-acetyl-2,5-diiodohistamine.

Titration curves obtained at 25° for histamine and its iodo derivatives are shown in Figure 1. Table I shows the average pK values, obtained for each ionization, from all the titrations performed. In all cases the plot of pK *vs.* the reciprocal of the absolute temperature was linear and estimates of $\Delta H°$, obtained from the slope of the least-square lines, are shown in Table I, as well as the $\Delta S°$ values, calculated for 25°.

The pK values for histamine at 25°, shown in Table I, are the averages of 28 titrations, in which we observed standard deviations of 0.02 pH units for the pK of the imidazolium (pK_1) and of 0.07 pH units for that of the ammonium group (pK_2). For the other values shown in Table I, estimates of the standard deviations cannot be made because they are averages of only three to five determinations. In all these cases, however, the deviations from the average did not exceed 0.05 pH units. The pK of the imidazole imino group ionization in histamine (pK_3) was too high to be determined accurately by the electrometric method, but its value at 25° was estimated spectrophotometrically. Although no absorption maximum occurs in histamine above 220 mμ, a considerable rise in absorbance at 235 mμ was observed when the imidazole group was converted into base, and this was used for measuring ionization ratios, as it has been done before for other imidazole derivatives.[7] Table II shows the results of one of the spectrophotometric titrations that were made, from which a pK_3 value of 14.36 was obtained. Several such experiments yielded an average value of 14.35 with a standard deviation of 0.08. The effect of temperature on the pK_3 of histamine was not studied because no data are available for the H_- acidity scale for temperatures other than 25°.

The titrations of N-acetylhistamine and its mono- and diiodo derivatives were made only at 25° with the electrometric method and the results of one titration of each compound are shown in Figure 2. The pK values found for the imidazolium dissociations were 6.99 for N-acetylhistamine, 4.73 for N-acetyl-5-iodohistamine, and 2.99 for N-acetyl-2,5-diiodohistamine. The pK for the dissociation of the imino group was found to be 11.53 for N-acetylmonoiodohistamine but it was not determined for the two other compounds because of the low solubility of N-acetyldiiodohistamine at alkaline pH and because the pK for N-acetylhistamine was too high for accurate measurement with the electrometric method.

Discussion

The pK values for the dissociation of the imidazolium and ammonium groups of histamine found by us are within the limits of those reported in the litera-

(1) C. Niemann and J. T. Hays, *J. Amer. Chem. Soc.*, **64**, 2288 (1942).
(2) H. M. Lee and R. G. Jones, *J. Pharmacol. Exp. Ther.*, **95**, 71 (1949).
(3) K. Hofmann, "Imidazole and Its Derivatives," Interscience, New York, N. Y., 1953, p 168.
(4) M. Rocha e Silva in "Handbuch der Experimentellen Pharmakologie," Vol. 18/1, O. Eichler and A. Farah, Ed., Springer, Berlin, 1966, p 233.
(5) A. Lukton, *Nature (London)*, **192**, 422 (1961).
(6) K. J. Brunings, *J. Amer. Chem. Soc.*, **69**, 205 (1947).
(7) (a) H. Walba and R. W. Isensee, *ibid.*, **77**, 5488 (1955); (b) H. Walba and R. W. Isensee, *J. Org. Chem.*, **21**, 702 (1956); (c) H. Walba and R. W. Isensee, *ibid.*, **26**, 2789 (1961); (d) G. Yagil, *Tetrahedron*, **23**, 2855 (1967).

TABLE I

pK VALUES FOR THE DISSOCIATIONS OF HISTAMINE AND ITS IODINATED DERIVATIVES AT SEVERAL TEMPERATURES, REDUCED TO ZERO IONIC STRENGTH

		pK — Temperature, °C							$\Delta H°$[a] kcal/mole	$\Delta S°$ (25°)[a] e.u. mole
Compd	Group	12	15	20	25	30	35	38		
Histamine	$=NH^+$		6.23	6.14	6.04	5.94	5.84		8.0 ± 0.2	−0.9 ± 0.6
	$-NH_3^+$		10.03	9.93	9.75	9.61	9.47		11.7 ± 0.5	−5.4 ± 1.7
Monoiodo-histamine	$=NH^+$	4.21	4.17	4.11	4.06	3.99		3.89	4.9 ± 0.1	−2.0 ± 0.5
	$-NH_3^+$	9.62	9.52	9.37	9.20	9.08		8.86	11.9 ± 0.2	−2.2 ± 0.7
	$=NH$		12.32	12.11	11.88	11.72	11.60	11.38	15.8 ± 0.9	−2.3 ± 3.0
Diiodo-histamine	$=NH^+$	2.51	2.44	2.38	2.31	2.25		2.12	5.8 ± 0.2	+9.0 ± 0.8
	$-NH_3^+$	8.52	8.45	8.29	8.20	8.08		7.93	9.3 ± 0.3	−6.3 ± 1.1
	$=NH$	10.58	10.47	10.28	10.11	9.97		9.72	13.4 ± 0.2	−1.3 ± 0.8

[a] The limits shown for $\Delta H°$ and $\Delta S°$ values correspond to ± one standard deviation.

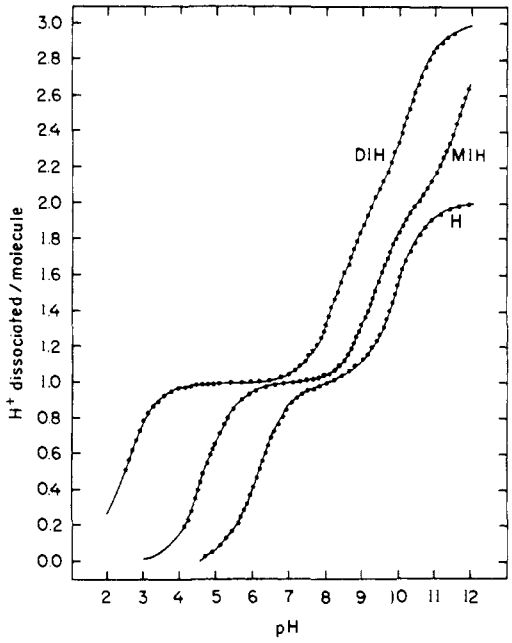

Figure 1.—Titration curves for histamine (H), monoiodohistamine (MIH), and diiodohistamine (DIH) at 25°, 0.1 *M* KCl solution. The points shown were obtained experimentally, in one titration of each compound, and the lines are the theoretical curves calculated with the p*K* values shown in Table I.

ture.[8] They are about one pH unit lower than the respective p*K* values in imidazole (7.0) and $EtNH_2$ (10.8), and this has been taken as evidence of the intramolecular hydrogen bonds between the imidazole ring and the ammonium group.[9] However, if the imidazolium p*K* values of the 4(5)-substituted imidazoles are plotted against Taft's σ^* substitution constants (Figure 3) it is seen that all the available data are fitted to eq 1.

$$pK = 7.50 - 2.05\sigma^* \pm 0.47 \quad (1)$$

(8) D. D. Perrin, "Dissociation Constants of Organic Bases in Aqueous Solution," Butterworths, London, 1965, p 191.

(9) L. Schutte, P. Provó Kluit, and E. Havinga, *Tetrahedron Suppl.*, **7**, 295 (1966).

SCHEME I

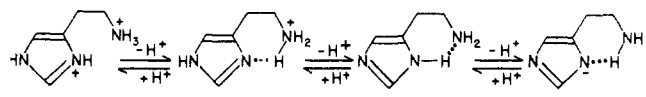

TABLE II

SPECTROPHOTOMETRIC DETERMINATION OF THE p*K* OF THE IMIDAZOLE GROUP OF HISTAMINE[a]

M_{OH^-}	A	A_-	r	c_{OH^-}	H_-	p*K*
8.334	0.545	0.545				
5.557	0.540	0.540				
4.167	0.537	0.537				
2.778	0.410	0.534	2.960	2.763	14.77	14.30
2.381	0.375	0.533	2.101	2.367	14.64	14.32
1.923	0.325	0.532	1.362	1.909	14.48	14.35
1.389	0.255	0.531	0.768	1.376	14.28	14.39
0.833	0.179	0.530	0.388	0.821	14.07	14.42
0.556	0.140	0.529	0.249	0.544	13.72	14.37
0.417	0.111	0.529	0.163	0.405	13.61	14.40
0.042	0.043					

[a] For the meaning of symbols on column headings see eq 5–7 in the Experimental Section.

Since the p*K* value for histamine falls within the limits predicted by this equation the inductive effect of the ethylammonium side chain might be the only factor responsible for the difference in pK_1 between histamine and imidazole. On the other hand, the formation of the postulated intramolecular H bond should not greatly affect the pK_1 of histamine because the negative enthalpy change of H bonding might be approximately balanced by the entropy term due to the ring closure that would accompany it. For a p*K* lowering of 0.5 pH units the calculated equilibrium constants for the formation of the H bond would be about 2. Following the treatment described by Laskowski and Scheraga,[10] if we assume an entropy change of −5 e.u. for each bond "frozen" and an enthalpy change of 3.5 kcal/mole or less for the NH–NH bonding, the

(10) M. Laskowski and H. A. Scheraga, *J. Amer. Chem. Soc.*, **76**, 6305 (1954).

formation of such a bond on imidazolium dissociation would not result in a significant decrease in the pK value.

Comparison of the $\Delta H°$ and $\Delta S°$ for the ionization of the imidazolium group of histamine (Table I) with those of imidazole ($\Delta H°$ = 7.7 kcal/mol; $\Delta S°$ = −6 cal/deg mol)[10] do not show the negative contributions to both $\Delta H°$ and $\Delta S°$ values that would be expected if H bonding occurred upon the dissociation of the imidazolium group. Our values for the $\Delta H°$ and $\Delta S°$ of imidazolium dissociation in histamine are not in agreement with those previously reported by Nicholas and Fernelius,[11] who found a significantly higher $\Delta H°$ (10.1 kcal/mol) and, consequently, a positive value for $\Delta S°$ (+7 cal/deg mol). Our value for the enthalpy of imidazolium dissociation in histamine is not significantly different from those reported for imidazole.[12,13] The lower pK value observed in histamine is apparently due to a less negative entropy of dissociation indicating that the effect of the ethylammonium side chain on the dissociation of the imidazolium group may be the result of differences in solvent–solute interactions.

The absence of significant differences between the values of the thermodynamic functions for the dissociation of the ammonium group of histamine (Table I) and those of other simple primary amines[14] is also evidence against Scheme I, in which pK_2 is seen to really correspond to the removal of a proton from the imidazole ring.

No previous determination of the pK_3 of histamine has been reported. The value of 14.36 (Table II) is very close to those reported for imidazole and histidine.[7b-d] This, again, is evidence against the proposed hydrogen bond (Scheme I).

The large shifts of pK_2 values to the acid side, which we observed in the mono- and diiodo derivatives of histamine and N-acetylhistamine, were comparable with those found for mono- and diiodohistidine[6] and were an expected result of the substitution of iodine on the imidazole ring. A fine interpretation of the $\Delta H°$ and $\Delta S°$ data shown in Table I is precluded by the large errors inherent in the method employed for their estimation, as witnessed by the standard deviations of the entropy values. It is possible to note, however, that the introduction of the first I atom, in position 5 of the imidazole ring in monoiodohistamine,[15] favors imidazolium dissociation by producing a decrease of its endothermicity, while the substitution of a second I, in position 2, further increases the acidity by an entropic effect. It seems probable that this effect may be due to a greater localization of positive charge in one of the imidazolium nitrogens in diiodohistamine, resulting in greater orientation of neighboring water molecules than in the case of histamine or monoiodohistamine, where the charge would be more evenly distributed.

Also comparable with what was previously observed in the case of histidine were the rather marked shifts in the pK values for the ammonium dissociation, which were thought by Brunings[6] to be too large if the effect of the I substituted in the imidazole ring is assumed to be transmitted through the side chain. This, however, does not seem to be good evidence for the occurrence of the postulated NH–N hydrogen bond. It is known, for instance, that the pK of the ammonium group in 3,5-diiodotyrosine, where no such H bond could exist, is 1.3 pH units lower than that of tyrosine.[16]

We conclude that the occurrence of the intramolecular NH–N hydrogen bond in aqueous solutions of histamine is not supported by the available experimental data, in agreement with the theoretical results obtained by Kier[17] from molecular orbital calculations.

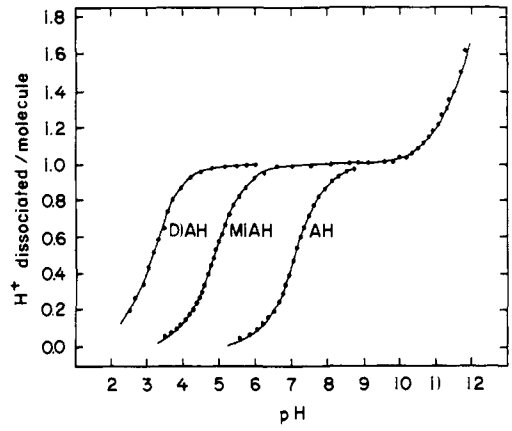

Figure 2.—Titration curves for N-acetylhistamine (AH), N-acetyl-5-iodohistamine (MIAH), and N-acetyl-2,5-diiodohistamine (DIAH) in 0.1 M KCl solution at 25°. The points shown were obtained experimentally, in one titration of each compound, and the lines are the theoretical curves calculated with the pK values reported in the text.

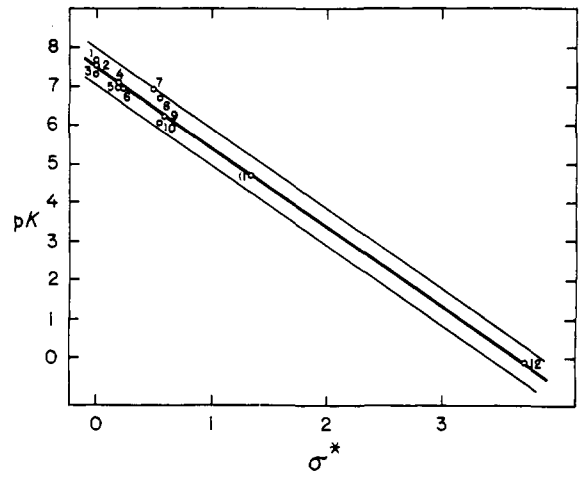

Figure 3.—pK of the imidazolium groups of 4(5)-substituted imidazoles *vs.* Taft's polar substituent constants. The heavy line corresponds to the equation pK = 7.50 − 2.05σ* and the lighter ones to the 95% fiducial limits (±0.47). σ* values were taken from C. D. Ritchie and W. F. Lager, *Progr. Phys. Org. Chem.*, **2**, 323 (1964). The pK values were obtained from Perrin,[8] except for the compounds for which other references are given: **1**, 4-carboxypropylimidazole; **2**, 4-methylimidazole; **3**, 4-methoxycarbonylpropylimidazole; **4**, 4-hydroxyethylimidazole (K. D. Kopple and Nitecki, *J. Amer. Chem. Soc.*, **84**, 4457 (1962); **5**, N-acetylhistamine (this paper); **6**, 4-acetoxyethylimidazole; **7**, imidazole; **8**, 4-hydroxymethylimidazole; **9**, 4-phenylimidazole; **10**, histamine; **11**, 4-aminomethylimidazole; **12**, 4-nitroimidazole.

(11) W. C. Nicholas and W. C. Fernelius, *J. Phys. Chem.*, **65**, 1047 (1961).
(12) C. Tanford and M. L. Wagner, *J. Amer. Chem. Soc.*, **75**, 434 (1953).
(13) Y. Nozaki, F. R. N. Gurd, R. F. Chen, and J. T. Edsall, *ibid.*, **79**, 2123 (1957).
(14) J. W. Smith, "The Chemistry of Amino Groups," S. Patai, Ed., Interscience, New York, N. Y., 1969, p 161.
(15) M. Tominaga and A. C. M. Paiva, *J. Med. Chem.*, **12**, 693 (1969).
(16) J. B. Dalton, P. L. Kirk, and C. L. A. Schmidt, *J. Biol. Chem.*, **88**, 589 (1930).
(17) L. B. Kier, *J. Med. Chem.*, **11**, 441 (1968).

Experimental Section

Materials.—Histamine·2HCl was from the California Corporation for Biochemical Research; 5-iodohistamine·2HCl, 2,5-diiodohistamine, *N*-acetylhistamine, *N*-acetyl-5-iodohistamine, and *N*-acetyl-2,5-diiodohistamine were prepared as previously described.[15] The standard buffers used for calibration of the pH-meter were 0.05 *M* K acid phthalate, 0.025 *M* phosphate, and 0.01 *M* Na borate solutions prepared according to the recomnendations of the National Bureau of Standards.[18]

Electrometric Titrations.—The electrometric titrations were done on a Radiometer Model 4c pH meter with a G202B glass electrode and a K100 saturated calomel electrode. The solutions were placed in a jacketed glass chamber equipped with a magnetic stirrer and shielded from electrostatic disturbances by a Faraday cage. The temperature desired inside the titration chamber was maintained within ±0.1° by the circulation, through the jacket, of water from a Forma-Temp bath. To assure temperature equilibration the electrodes were kept at the proper temperature for at least 16 hr before use. Both before and after each titration the pH-meter was calibrated with the phosphate buffer and the pH of the phthalate and borax buffers were checked. The maximum tolerance for discrepancies between the pH measurements of the standard buffers was of 0.03 pH units, and that for variations during the period of titration was of 0.015 pH units. During the standardization and titration procedures the solutions were flushed by a stream of N_2. The solutions to be titrated contained 80–120 μmol of material dissolved in 25 ml of 0.1 *M* KCl and they were titrated by the addition of either HCl or KOH solutions of known concentrations, about 1 *N*. The acid and base solutions were added from calibrated Agla syringes driven by Shardlow micrometers graduated in 0.01 mm, so as to change the pH by about 0.05–0.1 unit after each addition. Solvent blanks were titrated in exactly the same manner at each temperature, and the calculation of the experimental titration curve was done by using the apparent activity coefficients calculated from eq 2 and 3.

$$\mathrm{pH} = -\log [\mathrm{H^+}] - \log \gamma_{\mathrm{H}}' \quad (2)$$

$$\mathrm{p}K_w - \mathrm{pH} = \mathrm{pOH} = -\log [\mathrm{OH^-}] - \log \gamma_{\mathrm{OH}}' \quad (3)$$

The apparent activity coefficients, γ_{H}' and γ_{OH}' include any errors due to the glass electrode response and liquid junction potential.[19] The p*K* values were determined from theoretical curves containing Henderson–Hasselbalch terms that best fit the experimental points. The reported p*K* values were reduced to zero ionic strength by means of a simplified Debye equation (eq 4).

$$\mathrm{p}K_{\mu=0} = \mathrm{p}K_{\mathrm{obsd}} - \frac{0.5\,\mu^{1/2}}{1 + \mu^{1/2}} \quad (4)$$

Spectrophotometric Titrations.—For the determination of the pK_3 of histamine a spectrophotometric method previously employed for other imidazole derivatives[7] was used.

Histamine·HCl was dissolved in KOH solutions of known concentrations, ranging from 0.2103 to 8.567 *M*. The histamine concentration was about 5×10^{-3} *M*, and the absorbance of the neutral species (A°) was determined in the pH region between 11.5 and 12.0. A small linear dependence of absorbance on KOH concentration, between 5 and 8 *M*, was considered to be due to medium effect, and this dependence was extrapolated to obtain a corrected estimate for the absorbance due to the anionic form of histamine (A^-) at the lower KOH concentrations. The ionization ratio (r) in each of these solutions was calculated from its absorbance (A) as in eq 5. The concentration of free KOH

$$r = \frac{A - A^\circ}{A^- - A} \quad (5)$$

(c_{OH^-}) was obtained from

$$c_{\mathrm{OH}^-} = M_{\mathrm{OH}^-} - \left(2 + \frac{1}{r+1}\right) c_X \quad (6)$$

where M_{OH^-} is the stoichiometric concentration of KOH and c_X is the concentration of histamine·2HCl. For each solution a p*K* value was calculated by the relation

$$\mathrm{p}K = H_- - \log r \quad (7)$$

where H_- values were derived from c_{OH^-} values according to the acidity scale obtained by Yagil[20] based on indole derivatives as indicators.

The absorbances were determined in a Beckman DB spectrophotometer with 1-cm path-length cells, thermostated at 25 ± 0.1°.

Acknowledgments.—This work was supported by grants from the Fundacão de Amparo a Pesquisa do Estado de São Paulo, and the Brazilian National Research Council (CNPq).

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