J. Pharm. Pharmac., 1973, **25**, 787–792 *Received February* 26, 1973

The tautomer ratio of histamine

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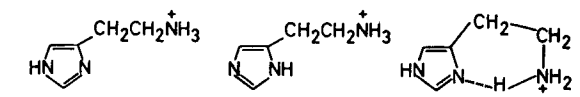
The tautomeric equilibrium constant K_t for the two tautomeric forms (N^τ-H and N^π-H) of histamine mono-cation is determined from the difference between the pK_a values of N^π- and N^τ-methylhistamines, and of N^π- and N^τ-benzylhistamines. The ratio of concentrations of tautomers $[N^\tau\text{-H}]/[N^\pi\text{-H}] = K_t$ is 4·2 indicating that the histamine mono-cation exists in aqueous solution approximately 80% as the N^τ-H-tautomer and 20% as the N^π-H tautomer. From the value of K_t and pK_{a_1} of histamine the individual ionization constants at 25° of the two tautomers are derived. Tautomer N^τ-H (pK_{a_1} 6·16) is a slightly weaker base than is tautomer N^π-H (pK_{a_1} 6·79).

The histamine mono-cation, although generally represented by the formula IA, exists as an equilibrium mixture of two tautomeric forms (IA and B). Niemann & Hays (1942) have suggested that an intramolecular hydrogen bond (e.g. IC) would stabilize tautomer IA and that histamine mono-cation would exist almost entirely in this form, even in aqueous solution. The stability of the proposed H-bond has recently been questioned by Kier (1968) and by Paiva, Tominaga & Paiva (1970) and in the absence of evidence to support Niemann and Hays' proposal the possibility must be considered that a substantial proportion of the mono-cation is in the alternative form IB. This would not deny that the biologically active form of histamine could be



IA (tautomer N^τ-H) IB (tautomer N^π-H) IC

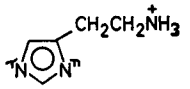
tautomer IA. Indeed, the well-known histamine-like activity of 2-(2-pyridyl)- and 2-(2-thiazolyl)-ethylamines on guinea-pig isolated ileum (Lee & Jones, 1949) emphasises that IA is more likely to be the active form of histamine at the H_1 receptor (Ash & Schild, 1966).

A determination of the proportion of tautomers IA and IB for the histamine mono-cation is now reported.

MATERIALS AND METHODS

Nomenclature

For the analogous ring *N*-methylhistidines the IUPAC-IUB Commission on Biochemical Nomenclature (1972) have recommended that the imidazole N nearer the side-chain residue is designated *pros* (N^π) and the one farther, *tele* (N^τ). We have extended this system to histamine as shown:



Thus, 1,4-methylhistamine (IV, R=Me) (Schayer & Karjala, 1956) for example is designated N^{τ}-methylhistamine and in the present report the two tautomers are respectively, N^{τ}-H (IA) and N^{π}-H (IB).

Materials

N^{π}- and N^{τ}-methyl, and N^{π}- and N^{τ}-benzylhistamine dihydrochlorides were synthesized in these laboratories under the aegis of Drs. G. J. Durant and J. C. Emmett. All compounds had satisfactory elemental analyses (C, H, N and Cl), infrared and nmr spectra. Their respective melting points are given in Table 1. Histamine dihydrochloride was from BDH Ltd.

As standards, 0·05M potassium hydrogen phthalate, 0·01M disodium tetraborate 10 H_2O, and 0·05M phosphate buffers were used to calibrate the pH meter.

Table 1. *Ionization constants and melting points of the substituted histamines.* The values for pK_{a_1} and pK_{a_2} were obtained from the same titration and determined in duplicate using separately prepared solutions at 25°.

Compounds	Structure	[1]m.p. °C	pK_{a_1}	pK_{a_2}
N^{π}-methylhistamine	III, R=Me	269–71[3]	6·63	9·51
			6·63	9·48
N^{τ}-methylhistamine[2]	IV, R=Me	204–6[4]	5·99	9·95
			5·99	9·94
N^{π}-benzylhistamine	III, R=CH_2Ph	222–3	6·21	9·49
			6·24	9·52
N^{τ}-benzylhistamine	IV, R=CH_2Ph	209–11[5]	5·62	9·99
			5·61	9·99
Histamine			6·07	9·81

[1] Corrected m.p. of dihydrochloride.
[2] Reported by Lebermann & Rabin (1960) pK_{a_1} 5·80, pK_{a_2} 9·90.
[3] Reported m.p. 265–6° (Jones & McLaughlin, 1949).
[4] Reported m.p. 204–6° (Pyman, 1911).
[5] Monohydrate.

Potentiometric titrations

The potentiometric titrations were conducted using a Radiometer Model PHM 52 pH meter with a G2222C glass electrode and a K4112 calomel electrode. Solutions of the dihydrochlorides were made up accurately to approximately 0·005M, in 0·1M KCl, using boiled de-ionized water in a nitrogen atmosphere and placed in a polythene titration vessel. Temperature was maintained at 25·0 ± 0·1° by a water jacket. The pH meter was calibrated before and after each set of titrations using the standard values of pH as reported in "Handbook of Chemistry and Physics", The Chemical Rubber Co., 46th Edn, 1965, pp. D-74, (maximum tolerance for variation was 0·005 pH units). Titrations were performed on 10 cc aliquots, with magnetic stirring under a nitrogen atmosphere, by the addition of carbonate-free KOH of known concentration (about 0·1M) from a Radiometer ABU 12 Autoburette with digital display to 0·001cc. Titrations were carried out by adding 0·05 cc increments of titrant except near the equivalence point when 0·005 cc aliquots were added. Equivalence points were obtained graphically by plotting pH against volume increments of titrant.

Results were calculated from the following equations:

$$pKa_1 = pH - \log \frac{[B]}{[BH^+]};\ pKa_2 = pH - \log \frac{[BH^+]}{[BH_2^{2+}]}$$

$$\text{where } \frac{[B]}{[BH^+]} = \frac{V_1N + (V_1 + V_2)[H^+]}{(V_3 - V_1)N - (V_1 + V_2)[H^+]}$$

$$\frac{[BH^+]}{[BH_2^{2+}]} = \frac{(V_1 - V_3)N - (V_1 + V_2)[OH^-]}{(2V_3 - V_1)N + (V_1 + V_2)[OH^-]}$$

V_1 = total volume of titrant added at the measured pH; V_2 = volume of aliquot taken for titration; V_3 = volume of alkali at equivalence (obtained graphically); N = normality of alkali used as titrant; $[H^+]$ = (f. antilog pH)$^{-1}$; $[OH^-]$ = (f. antilog $[pK_w - pH]$)$^{-1}$ for pH $\leqslant$ 9·2; or, $[OH^-]$ = (f. antilog $[pK_w - pH - 0{\cdot}014\,(pH - 9{\cdot}2)]$)$^{-1}$ for pH $>$ 9·2; f = mean ionic activity coefficient, taken as 0·77 for 0·1M KCl at 25°; pK_w = 13·997 at 25°.

RESULTS

The apparent pK_a values of the substituted histamines, determined in duplicate on separately prepared solutions at 25°, are shown in Table 1. The first ionization constant (pK_{a_1}) refers to the acidity of the imidazolium group, the second (pK_{a_2}) to the acidity of the ammonium group. The values for histamine were likewise determined and serve for reference. The values quoted for each determination are the arithmetical means of a set of 8–10 individual readings, (the first or last reading being excluded when the titration increment is <10 or >90% of the total titre for equivalence). The standard deviations from the mean for these sets were not greater than $\pm 0{\cdot}01$ (pK_{a_1}) or $\pm 0{\cdot}02$ (pK_{a_2}). The pK_a values of imidazole (7·05) and *N*-methylimidazole (7·15) were also determined for comparison.

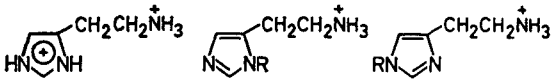
DISCUSSION

The ionization constants of two tautomers are not directly determinable but it is well established that they usually correspond closely to the ionization constants of their respective derivatives where the mobile proton has been substituted by an immobile methyl group (cf. Katritzky & Lagowski 1963). This is supported by the similarity between imidazole itself (pK_a 7·05) and *N*-methylimidazole (pK_a 7·15). Interpretation is complicated by a statistical factor (Grimison, Ridd & Smith, 1960) because the imidazole cation has two ionizable protons whereas the *N*-methyl derivative has but one. The values indicate that the methyl group effectively reduces the basicity of the other nitrogen atom by 0·2 unit probably through decreased solvation of the conjugate acid. Making the reasonable assumption that solvation changes should be similar for substitution on either of the two ring-nitrogen atoms, the acidities of the conjugate acids N^{π}-methyl- and N^{τ}-methyl-histamines, (III and IV respectively, R = Me) should be *proportional* to the acidities of the respective protons in the conjugate acid of the N–H forms. Therefore the ratio of concentrations of the two tautomeric forms will be the ratio of the dissociation constants of the two corresponding methyl isomers (Gallo, Pasqualucci & others, 1964), i.e. the tautometric equilibrium constant

$$K_t = \frac{[IA]}{[IB]} = \frac{K_{IA}}{K_{IB}} \approx \frac{K_{a(IV)}}{K_{a(III)}} = \text{antilog}\,(pK_{a(III)} - pK_{a(IV)})$$

Table 2. *Mean* pK_{a_1} *values of substituted histamines (III and IV), the derived tautomer ratios* K_t *and respective mole fractions.*

	R = Me	$R = CH_2Ph$
Structure III, $pK_{a(III)}$	6·63	6·23
Structure IV, $pK_{a(IV)}$	5·99	5·62
$pK_{a(III)} - pK_{a(IV)}$	0·64 ± 0·01	0·61 ± 0·02
K_t	4·37 ± 0·1	4·07 ± 0·2
Mole fraction of IA $= \frac{K_t}{1 + K_t}$	0·81 ± 0·01	0·80 ± 0·01
Mole fraction of IB $= \frac{1}{1 + K_t}$	0·19 ± 0·01	0·20 ± 0·01



II III IV

In comparing the two alkylated forms, the errors introduced by assuming $K_{IA} \equiv K_{a(IV)}$ etc. should partially cancel. As a check that steric factors are not operating differently for the two derivatives, a second pair of compounds, substituted by the larger benzyl group in place of methyl was examined. Benzyl also has the advantage of being electronically a more neutral group than methyl.

The imidazolium pK_a values (uncorrected for ionic strength) for the di-cations of two pairs of isomeric compounds, viz: N^{π}- and N^{τ}-methylhistamines and N^{π}- and N^{τ}-benzylhistamines (III and IV, R = CH_2Ph), determined under identical conditions, are shown in Table 2 together with the values of K_t and mole fractions of tautomers. The values of K_t from each pair of compounds are in excellent agreement and indicate that approximately 80% of the histamine mono-cation is in the tautomeric form N^{τ}-H (IA) and 20% in the form N^{π}-H (IB). This corresponds to a free energy difference of 0·8 kcal mol^{-1} (3·5 kJ mol^{-1}) at 37° between the two tautomers. In Table 3, the mole fractions at 37° for each of the main species of histamine present in aqueous solution at three pH values are expressed as percentage, of the total.

The greater stability of tautomer N^{τ}-H (IA) can be ascribed to the electron-withdrawing effect of the ammonium-ethyl group which causes the nearer imino-hydrogen of the conjugate acid (II) to be the more acidic and is in accord with the behaviour of

Table 3. *Mole fractions of histamine species at 37° in aqueous solution at pH values of 6·4, 7·4 and 8·4, expressed as a percentage of the total.* The mole fractions are derived from the pK_a values for histamine at 37° of 5·80 and 9·40 [given by Paiva & others (1970)] and K_t of 4·2.

Species	Percentages at pH of 6·4	7·4	8·4
Neutral (combined tautomers)	0·1	1	9
Mono-cation, tautomer N^{τ}-H (IA)	64	77	72·5
Mono-cation, tautomer N^{π}-H (IB)	16	19	18
Di-cation (II)	20	2·5	0·25

4(5)-nitro- and 4(5)-chloro-imidazoles (Grimison & others, 1960; Gallo & others, 1964).

The above method of estimating K_t does not involve the pK_a of histamine itself and thus avoids the problem of the effect *per se* of the *N*-substituent on the ionization constant. It is possible, however, to derive the separate ideal, but experimentally inaccessible, acid dissociation constants of the two tautomers from the K_{a1} of histamine. The overall equation for ionization of the di-cation is:

$$II + H_2O \rightleftharpoons [IA + IB] + H_3O^+$$

and the first ionization constant K_{a1} of histamine di-cation(II) can be expressed as:

$$K_{a1} = \frac{[H_3O^+][IA + IB]}{[II][H_2O]} = \frac{[H_3O^+][IA]}{[II][H_2O]} + \frac{[H_3O^+][IB]}{[II][H_2O]} = K_{IA} + K_{IB}$$

That is, the empirical ionization constant, K_{a1}, of the di-cation is a composite of the separate constants of the two tautomeric species. Substituting K_t

$$K_{IA} = \frac{K_{a1}\,K_t}{1 + K_t} \quad ; \quad K_{IB} = \frac{K_{a1}}{1 + K_t}$$

For histamine at 25° $K_{a1} = 8{\cdot}51 \times 10^{-5}$ (i.e. from $pK_{a1} = 6{\cdot}07$, given in Table 1)
$K_t = 4{\cdot}2$ (average of values from Table 2)

whence, at 25° $K_{IA} = 6{\cdot}87 \times 10^{-5}$ whence $pK_{IA} = 6{\cdot}16$
$K_{IB} = 1{\cdot}64 \times 10^{-5}$ whence $pK_{IB} = 6{\cdot}79$

The corresponding pK values at 25°, representing the acid dissociation constants at either of the ring nitrogen atoms of histamine di-cation, are respectively 6·16 (to tautomer N^τ-H (IA)) and 6·79 (to tautomer N^π-H (IB)), i.e. IA is a slightly weaker base than is IB.

Published data on the pK_a values of the N^π- and N^τ-methylhist*idines* (Hultquist, Moyer & Boyer, 1966; the ring pK_a's reported as 6·4 and 5·7 respectively) suggest that the tautomer proportions of histidine are very similar to those of histamine. The difference of 0·7 pK_a units in the reported values corresponds to $K_t = 5{\cdot}0$, giving a value of 0·83 as the mole fraction of N^τ-H tautomer present in *histidine* mono-cation. A recently published nmr study reports a similar value for neutral histidine (Reynolds, Peat & others, 1973).

Acknowledgements

The author gratefully acknowledges his debt to Mr. M. J. Graham of these laboratories for his excellent experimental work in determining the pK_a values and to Dr. E. S. Pepper for writing the computer program used in calculations.

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