Purines, Pyrimidines, and Imidazoles. Part 62.[1] Isotopic Hydrogen Exchange from the C-2 Position in an Imidazole Nucleoside related to Intermediates in Purine Nucleotide *de novo* Biosynthesis

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The rate of hydrogen exchange at H-2 in ethyl 5-amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-imidazole-4-carboxylate has been measured in deuteriated phosphate and glycine buffers over a pH range at 65 °C. The bell-shaped rate curve produced is consistent with rate-determining hydroxide-ion attack on the protonated form of the nucleoside. The assignment of N-3 as the preferred site for protonation has been confirmed by calculations using the MNDO semi-empirical MO method.

The 5-aminoimidazolecarboxylic acid ribotide (**1a**) (CAIR) and the corresponding decarboxylated ribotide (**1b**) (AIR) are central intermediates in the *de novo* biosynthetic pathway leading to purine nucleotides[2] and have also been implicated as intermediates in the biosynthesis of thiamine.[3–6] The latter reactions probably involve alkylation at the C-2 position of the imidazole ribotide prior to ring expansion to produce the 2-substituted pyrimidine unit present in thiamine. We have been interested in the preparation of various (including labelled) 2-substituted 5-aminoimidazoles of these types by various methods including direct alkylation reactions, and as an aid to the formulation of useful syntheses and reaction conditions the proton transfers at C-2 in 5-aminoimidazoles of the above types have been examined.

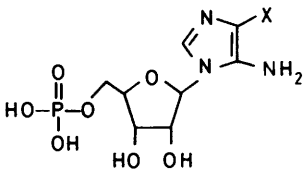
The results of exchange reactions with imidazole and some related purines have been recorded in several publications.[7] In the particular derivatives examined exchange of H-2 has been proposed to take place *via* the N-3 (imidazole) or N-7 (purine) protonated species. In addition, a second exchange reaction involving the neutral species has been postulated for both imidazoles and for purines.

We have earlier commented[8,9] on the likely involvement of similar species in the ready and reversible reaction CAIR $\rightleftharpoons$ AIR and have in addition, determined[10] the ionisation constants of CAIR and several related 5-aminoimidazoles using both spectrophotometric and potentiometric techniques.

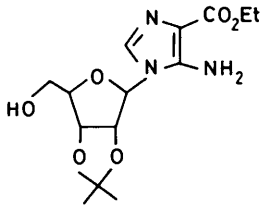
By comparison of our results with those recorded for other imidazoles we were able to assign pK values to the carboxylic acid groups in 5-aminoimidazole-4-carboxylic acid derivatives and to the doubly bonded imidazole nitrogen atom (N-3) in such compounds.

Calculations using the MNDO semi-empirical MO method have now confirmed these assignments and show that in typical 5-aminoimidazole derivatives including N-1-substituted compounds related to CAIR and AIR, N-3 is the preferred site for protonation from thermodynamic argument if the entropies of protonation plus solvation are assumed to be approximately the same irrespective of the protonation site (Table 1).

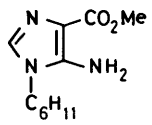
The nucleoside ethyl 5-amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carboxylate[11] (**2**) was chosen for study since among easily available compounds related to CAIR it appeared the most promising candidate for studies involving reactions at the 2-positions. The free 5-aminoimidazole-4-carboxylic acids of course are unstable and very readily undergo decarboxylation. The exchange of H-2 in a 0.3M solution of (**2**) at 65 °C was followed by ^{1}H n.m.r. spectroscopy over a range of pH values in deuteriated phosphate and glycine buffers by measurement of the rate of loss of H-2. The first-order rate constants were obtained from plots of log (H-2/CMe) *versus* time.



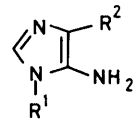
(**1**) **a**; X = CO_2H
b; X = H



(**2**)



(**3**)



(**4**)

Table 1. Heats of formation of some N-1 and N-3 protonated imidazoles*

Compound (4)		ΔH_F/kJ mol^{-1}	
R^1	R^2	N-1-H$^+$	N-3-H$^+$
H	H	929.2	774.6
H	CH_3	938.4	769.5
CH_3	CH_3	887.0	721.5
H	CO_2CH_3	601.9	437.2
CH_3	CO_2CH_3	611.1	434.7

* QCPE-1978, 353 (Modified for the NORD 560).

Results and Discussion

In the pH range examined (1.77—12.0) an aminoimidazole can exist as the monoprotonated $(BH_2)^+$, the neutral (**BH**), and if

$$BH_2^+ \overset{K_a}{\rightleftharpoons} BH + H^+ \quad (1)$$

hydrogen substituted at N-1, the monoanion (B^-) forms, and

$$BH \overset{K_a}{\rightleftharpoons} B^- + H^+ \quad (2)$$

if the mechanism of isotopic exchange is the same as for purine and 1-methylbenzimidazole namely rate-determining hydroxide-ion attack on BH_2^+ then the rate–pH profile should be bell

$$BH_2^+ + OD^- \xrightarrow{k_3} BDH^+ + OH^- \quad (3)$$

shaped. This is borne out by the results of our experiments (Tables 2 and 3 and Figure 1).

An alternative mechanism[12] operates at high pH with 9-substituted purines although not with 1-alkylbenzimidazoles and this probably involves rate-determining attack by the hydroxide ion on the neutral molecule. The rate of proton

$$BH + OD^- \xrightarrow{k_4} BD + OH^- \quad (4)$$

exchange may be expressed as (5) in which the total concentration of base $[BH]_T$ is given by (6). From equations (1), (2), and (6) the neutral species concentration is given by (7) and the

$$\text{Rate} = k_3[BH_2^+][OD^-] + k_4[BH][OD^-] = k_{obs}[BH]_T \quad (5)$$

$$[BH]_T = [BH] + [BH_2^+] + [B^-] \quad (6)$$

protonated species concentration by (8).

Substitution of (7) and (8) into the rate equation (5) gives (9). On the right hand side of this equation the first term is the

Table 2. Rate–pH data at 65 °C for H-2 exchange in ethyl 5-amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carboxylate*

pH	$10^3 k_{obs}$/min^{-1}	Relative rate (R)	log $[R/(1-R)]$	log $[(1-R)/R]$
1.77	2.90	0.278	−0.414	
2.0	4.12	0.395	−0.185	
2.25	5.87	0.563	0.110	
2.51	6.98	0.669	0.306	
2.75	7.58	0.727	0.425	
3.0	8.94	0.857	0.778	
4.0	10.43	1.00		
5.0				
6.0				
7.0				
8.0				
9.0				
10.0	9.93	0.952		−1.300
10.8	7.81	0.747		−0.475
11.75	5.20	0.499		0.002
12.0	4.58	0.439		0.106

* From plots of pH *versus* log $[R/(1-R)]$ and pH *versus* log $[(1-R)/R]$. pK_a evaluated as 2.20 and $pK_{a'}$ evaluated as 11.72.

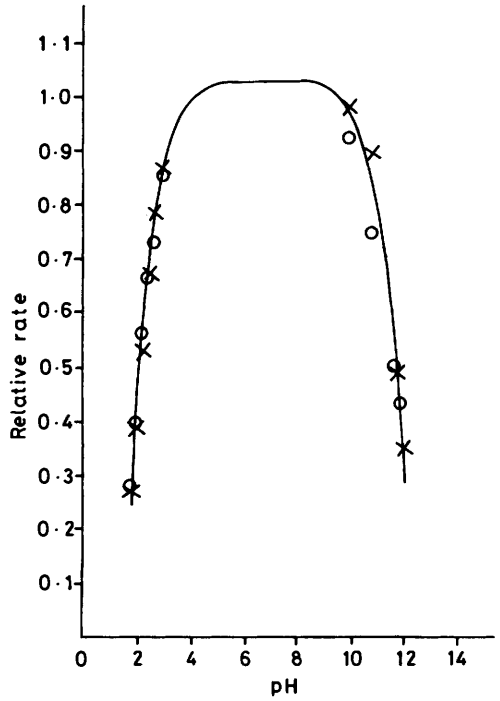


Figure 1. Rate–pH profile at 65 °C. For H-2 exchange in ethyl 5-amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carboxylate. The calculated lines are drawn from data in Table 3: ×, calculated; ○, observed

Table 3. Calculated and observed rate–pH data for H-2 exchange in compound (2)

pH	$[H^+]$	$K_{a'}/[H^+]$[a]	$[H^+]/K_a$[b]	Relative rate (R) Calculated[c]	Relative rate (R) Observed
1.77	1.698×10^{-2}	1.114×10^{-10}	2.664	0.273	0.278
2.0	1×10^{-2}	1.891×10^{-10}	1.569	0.389	0.395
2.25	5.623×10^{-3}	3.364×10^{-10}	8.882×10^{-1}	0.531	0.562
2.51	3.090×10^{-3}	6.121×10^{-10}	4.848×10^{-1}	0.673	0.669
2.75	1.778×10^{-3}	1.064×10^{-9}	2.790×10^{-1}	0.782	0.727
3.0	1×10^{-3}	1.891×10^{-9}	1.569×10^{-1}		
4.0	1×10^{-4}	1.891×10^{-8}	1.569×10^{-2}		
5.0	1×10^{-5}	1.891×10^{-7}	1.569×10^{-3}		
6.0	1×10^{-6}	1.891×10^{-6}	1.569×10^{-4}		
7.0	1×10^{-7}	1.891×10^{-5}	1.569×10^{-5}		
8.0	1×10^{-8}	1.891×10^{-4}	1.569×10^{-6}		
9.0	1×10^{-9}	1.891×10^{-3}	1.569×10^{-7}		
10.0	1×10^{-10}	1.891×10^{-2}	1.569×10^{-8}	0.981	0.952
10.8	1.584×10^{-11}	0.119	2.487×10^{-9}	0.893	0.749
11.75	1.778×10^{-12}	1.064	2.790×10^{-10}	0.485	0.498
12.0	1×10^{-12}	1.891	1.569×10^{-10}	0.346	0.438

[a] $K_{a'}$ 1.891×10^{-12}. [b] K_a 6.374×10^{-3} obtained from plots of log (H-2/CMe). [c] Calculated from equation (16).

$$[BH] = \frac{[BH]_T}{1 + \frac{[H^+]}{K_a} + \frac{K_{a'}}{[H^+]}} \quad (7)$$

$$[BH_2^+] = \frac{[BH]_T}{\frac{K_a}{[H^+]} + 1 + \frac{K_aK_{a'}}{[H^+]^2}} \quad (8)$$

$$k_{obs}[BH]_T = \frac{k_3[BH]_T[OD^-]}{\frac{K_a}{[H^+]} + 1 + \frac{K_aK_{a'}}{[H^+]^2}} + \frac{k_4[BH]_T[OD^-]}{1 + \frac{[H^+]}{K_a} + \frac{K_{a'}}{[H^+]}} \quad (9)$$

rate of proton exchange from the protonated molecule and the second term is that from the neutral molecule.

Rearrangement of (9) gives esxpression (10) for the observed

$$k_{obs} = \frac{k_3K_W + k_4K_a[OD^-]}{[H^+] + K_a + \frac{K_aK_{a'}}{[H^+]}} \quad (10)$$

rate constant k_{obs}. The formation of a B^- species would normally be considered to arise by loss of a proton from N-1 of an unsubstituted imidazole. In such derivatives then the value of $K_{a'}$ becomes important. If the value of $K_{a'}$ is large the concentration of neutral species would be negligible especially at high pH and the second term in equation (9) may be ignored. The observed rate constant for these molecules would therefore take the form (11).

$$k_{obs} = \frac{k_3K_W}{[H^+] + K_a + \frac{K_aK_{a'}}{[H^+]}} \quad (11)$$

This predicts for a plot of k_{obs} *versus* pH a bell-shaped curve illustrating that there is an initial increase in rate due to the increase in $[OD^-]$ in reaction (3) and at high pH a reduction in rate caused by the formation of a B^- species. This is experimentally observed.[7]

When, however, the N-1 position is glycosylated as in our example, the B^- species cannot be formed and $K_{a'} = 0$. Equation (10) then becomes (12). This system predicts an initial

$$k_{obs} = \frac{k_3K_W + k_4K_a[OD^-]}{[H^+] + K_a} \quad (12)$$

rate increase due to reaction (3). However, if K_a is large, $K_a \gg 2[H^+]$ and the neutral species **BH** is unlikely to form until high pH. Again, reaction (4) would not be observed and equation (12) becomes (13). A constant rate would be expected

$$k_{obs} = k_3K_W/K_a \quad (13)$$

between the neutral and high pH range and this is observed.[7]

If K_a is not so large and neutral molecules can be formed in the pH range, then in the region where $K_a \gg [H^+]$ the observed rate constant would be given from equation (12) as (14). A linear

$$k_{obs} = k_3K_W/K_a + k_4[OD^-] \quad (14)$$

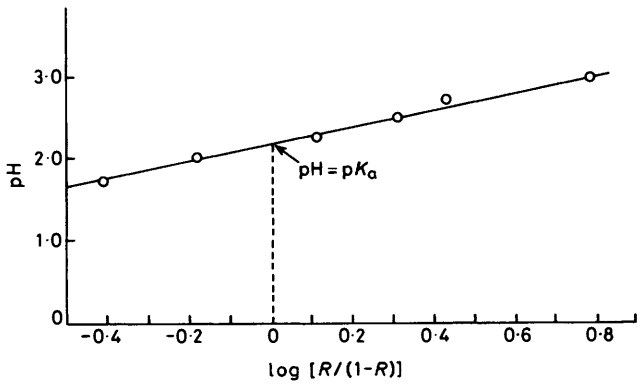


Figure 2. Plot of pH against log $[R/(1 - R)]$

relationship between k_{obs} and $[OD^-]$ is predicted and this is also observed for certain compounds.[7,12]

The relative rate R is defined as the rate divided by k_3K_W/K_a.

Equation (11) can be applied to N-1 hydrogen-substituted species with high $K_{a'}$ values or N-1-alkyl (glycosyl) derivatives at low pH where B^- species are not formed; then dividing equation (11) as indicated gives equations (15) and (16). Thus if $[H^+] \gg K_{a'}$ then we have equations (17)—(19). Thus a plot

$$R = \frac{K_a}{K_a + [H^+] + \frac{K_aK_{a'}}{[H^+]}} \quad (15)$$

$$\frac{1}{R} = 1 + \frac{[H^+]}{K_a} + \frac{K_{a'}}{[H^+]} \quad (16)$$

$$\frac{1}{R} = 1 + \frac{[H^+]}{K_a} \quad (17)$$

$$[H^+] = K_a\left(\frac{1 - R}{R}\right) \quad (18)$$

$$pH = pK_a + \log\left(\frac{R}{1 - R}\right) \quad (19)$$

of pH *versus* log $[R/(1 - R)]$ will be linear (Figure 2) having an intercept on the pH axis equal to pK_a. In bell-shaped profiles equations (18) and (19) indicate at $R = 0.5$ at low pH, $pH = pK_a$ and at high pH, $pH = pK_{a'}$.

Inspection of the rate–pH profile for the nucleoside (**2**) indicates a pK_a value of 2.2 and a $pK_{a'}$ value of 11.72. The pK_a value 2.2 (associated with protonation of the doubly bonded nitrogen) is much less than that assigned to many imidazoles including imidazole (7.03), imidazole 4(5)-carboxylic acid (6.02—6.26), histamine (6.03) or CAIR (**1a**) (6.28) but closer to the reported[10] albeit approximate value obtained for the analogous methyl 5-amino-1-cyclohexylimidazole-4-carboxylate (**3**), determined by spectrophotometry to be 4.8. The low pK_a value obtained for (**2**) may undoubtedly be attributed to the acid strengthening effect of the ethoxycarbonyl group which will be augmented by the $-I$ effect of the ribofuranosyl group. Similar, even greater effects are observed in 3-methyl-4-nitroimidazole (pK 2.13) and 1-methyl-4-nitroimidazole (pK −0.53).[13] The nitro group of course is noteworthy for its powerful acid-strengthening properties by virtue of $-I$ and $-M$ effects.

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