Proton Transfer from Heterocyclic Compounds. Part 7.[1] Methylated Guanosine and Inosine Derivatives and the Question of Zwitterionic Involvement

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The rates of detritiation of 1-methyl[8-^{3}H]guanosine, 7-methyl[8-^{3}H]guanosine, and 1-methyl[8-^{3}H]inosine have been measured over a pH range at 85 °C and the results compared with those already available for guanosine and inosine. At low pH where the mechanism involves hydroxide ion attack on the protonated substrate methyl substitution has only a marginal effect on the rate. By contrast the results at high pH show that methyl substitution in the 1-position brings about a large rate retardation (*ca.* 10^3) and leads to the conclusion that in the case of both guanosine and inosine the hydroxide ion reacts with both the neutral and zwitterionic forms.

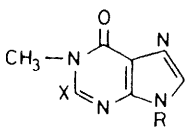
In studies of isotopic hydrogen exchange from the C-2 position of imidazoles and the equivalent C-8 position of purines we have shown [2] that at low pH (<5) the operative mechanism always involves the protonated molecule and the hydroxide ion. For 1-methylbenzimidazole [3] we were able to take 1,3-dimethylbenzimidazolium ion as a model compound and show that the second-order rate constant for hydroxide ion attack was very similar to that obtained for exchange involving the proposed 1-methylbenzimidazolium cation.

At higher pH additional mechanisms [1,2,4] come into play; these again involve the hydroxide ion and various ionised forms of the substrate, most usually the neutral form. The operation of these different mechanisms may be discerned from the form of the rate–pH profiles. This approach is not however possible when two kinetically equivalent forms of the substrate may be involved. Such a situation is highlighted by the work of Tomasz and her co-workers [5] on isotopic hydrogen exchange (at 37 °C) from the C-8 position of guanosine, 1-methylguanosine, adenosine, and quanine residues of DNA. At high pH (>8) they observed a large rate acceleration for guanosine and this result, together with the failure to observe a similar acceleration in the case of 1-methylguanosine, was ascribed to the involvement of the guanosine zwitterion (1) rather than the neutral guanosine molecule (2). Our own studies [6] at a higher temperature (85 °C) and a wider pH range on adenosine (which can not form a zwitterion) showed that at high pH the detritiation rate constant increased dramatically as a result of reaction between the neutral form and hydroxide ions. Nevertheless the idea of zwitterionic participation is an attractive one as protonation at N-7 with only partial neutralisation of the positive charge should facilitate isotopic exchange at the nearby C-8 position. This kind of mechanism is, after all, analogous to that operative at low pH.

Although Maslova *et al.*[7] have assumed that the zwitterionic pathway is important in the hydrogen exchange from the C-8 position of hypoxanthine, guanine, and xanthine, and Lichtenberg and Bergmann [8] have explained the exchange behaviour of various hypoxanthines in similar terms, no quantitative information has been forthcoming. For this reason we undertook the present investigation. The results for the detritiation of 1-methylguanosine (3a) and 1-methylinosine (3b), both of which cannot form zwitterions, can be compared with those available for the parent nucleosides, guanosine and inosine. In addition 7-methylguanosine (4), with its close resemblance to the N-7-protonated guanosine molecule, was also studied. Previous work [9,10] had shown that isotope exchange from the C-8 position of this compound was extremely rapid even under neutral conditions.

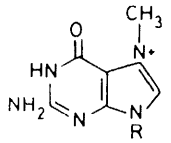
(2) (1)

R = β–D–ribofuranosyl



(3) a; X = NH_2, R = β–D–ribofuranosyl

b; X = H, R = β–D–ribofuranosyl



(4) R = β–D–ribofuranosyl

EXPERIMENTAL

Materials.—The methylated nucleosides were commercially available. 1-Methyl[8-^{3}H]guanosine and 1-methyl[8-^{3}H]inosine were prepared by incubating a mixture of the nucleoside (*ca.* 30 mg) and tritiated water (20 μl, 5 Ci ml^{-1}) at 85 °C for 18 h. The solvent was removed by freeze-drying, a small amount of water was added to exchange very labile tritium and the water removed once again.

7-Methyl[8-^{3}H]guanosine, by virtue of the known lability [9,10] of the C-8 proton under neutral conditions, was prepared by incubating the inactive compound (*ca.* 30 mg) with tritiated water (20 μl, 5 Ci ml^{-1}) at 25 °C for 18 h, after which the pH was lowered and the solvent removed by freeze-drying. The residue was washed with a small amount of water (*ca.* 1 ml), as before, before being freeze-dried again. In this case the low pH used ensured that back exchange of tritium from the C-8 position was kept to a minimum. ^{1}H and ^{3}H N.m.r. spectroscopy of

the substrates served to show that no decomposition had occurred under the tritiation conditions.

Kinetics.—Throughout the work aqueous buffer systems of known pH–temperature dependence were used. Rates of detritiation were measured, as described in detail elsewhere,[3] by following the increase in the radioactivity of the water after separation from the tritiated compound had been achieved by freeze-drying. In all cases good first-order kinetics were obtained. It is pertinent to mention despite the fact that the data are not included here that in the case of 1-methylinosine at pH <2 detritiation from the C-8 position is accompanied by hydrolysis [11,12] to the parent heterocyclic base, 1-methylhypoxanthine, and that this compound also undergoes detritiation under the experimental conditions. In such circumstances the curvature of the first-order plots can be analysed to obtain the hydrolysis rate constant.

RESULTS AND DISCUSSION

The results for 1-methylguanosine and 1-methylinosine (Table 1) give rise to the same kind of rate–pH profile (Figure) that was observed previously for both adenosine [7] and the 9-alkylpurines.[13] If therefore the same mechanisms operate, namely hydroxide ion attack on the protonated substrate (BH^+) at low pH and on the neutral substrate (B) at high pH we have equation (1)

TABLE 1

Detritiation rate constants ($k_{obs.}$) for 1-methyl[8-^{3}H]-guanosine (3a), 1-methyl[8-^{3}H]inosine (3b), and 7-methyl[8-^{3}H]guanosine (4) in aqueous buffers at 85 °C

	$10^5 k_{obs.}/s^{-1}$		
pH (at 85 °C)	(3a)	(3b)	(4)
1.81	8.05		
2.12			70.0
2.13	8.67		
2.36			106
2.50			189
2.69		7.89	278
2.80	16.4		
2.88			405
3.11		8.28	
4.10	19.4	9.21	
6.25	18.1	9.34	
8.70	16.3	8.37	
10.47		32.2	
10.69		43.8	
10.76	23.9		
10.91		58.5	
10.97	26.5		
11.13		88.4	
11.16	32.2		
11.20	34.2		
11.50	44.8	125	
11.80	59.0		
12.00	72.2		
12.03		322	

TABLE 2

Derived acidity and rate constants (l mol^{-1} s^{-1}) for guanosine and inosine and the methyl derivatives at 85 °C

Compound	pK_a	pK_a'	$10^{-6}k$	k'
Guanosine [a]	2.2	8.9	3.1	6.05
1-Methylguanosine	2.2		5.3	2.51×10^{-3}
7-Methylguanosine			16.9	
Inosine [a]	1.2	8.2	15.6	16.8
1-Methylinosine	1.2		18.0	19.0×10^{-3}

[a] Data given in J. A. Elvidge, J. R. Jones, C. O'Brien, E. A. Evans, and H. C. Sheppard, *J.C.S. Perkin II*, 1974, 174.

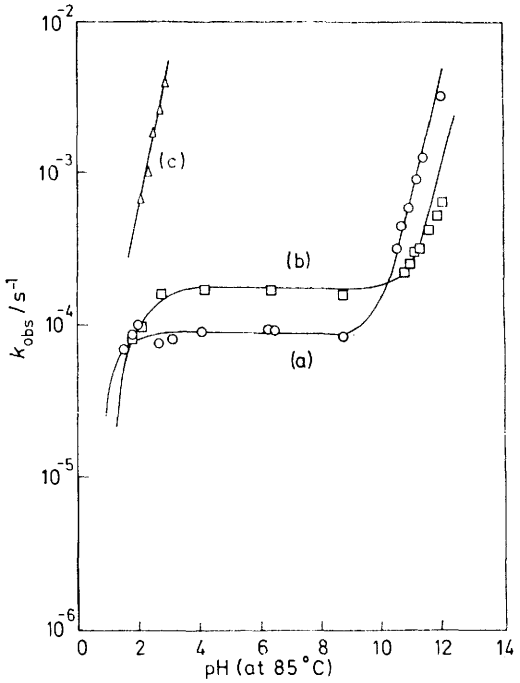
from which equation (2) can be derived.[6] This, in turn, reduces to equation (3) at high pH so that k' values

$$\text{Rate} = k[BH]^+[OH^-] + k'[B][OH^-] \quad (1)$$

$$k_{obs.} = \frac{kK_w}{K_a + [H^+]} + \frac{k'K_a[OH^-]}{K_a + [H^+]} \quad (2)$$

$$k_{obs.} = \frac{kK_w}{K_a} + k'[OH^-] \quad (3)$$

(Table 2) can be obtained from the slope of the plot of $k_{obs.}$ against $[OH^-]$. For 1-methylguanosine such a plot is linear up to a hydroxide ion concentration of 0.1M, and for 1-methylinosine up to 0.04M. The deviations



Rate–pH profiles for the detritiation of (a) 1-methyl[8-^{3}H]-inosine, (b) 1-methyl[8-^{3}H]guanosine, and (c) 7-methyl[8-^{3}H]-guanosine in aqueous buffers at 85 °C. The drawn curves for 1-methylinosine and 1-methylguanosine are computed using equation (2). The line for 7-methylguanosine is of unit slope

that occur at still higher concentrations probably result from the ionisation of the ribose hydroxylic proton (for guanosine the pK_a is 12.33, for inosine the pK_a is 12.36, both at 25 °C).[14] The pK_a values that give the best fit to the kinetic data are 2.2 (1-methylguanosine) and 1.2 (1-methylinosine).

For the model compound 7-methylguanosine, which with its fixed positive charge can only exist in one (protonated) form, equations (4) and (5) apply. The derived value of k (16.9×10^6 l mol^{-1} s^{-1}) is approximately five times the value for guanosine itself and in

$$\text{Rate} = k[BH^+][OH^-] \quad (4)$$

$$k_{obs.} = k[OH^-] \quad (5)$$

line with the proposed mechanism; the 1,3-dimethylbenzimidazolium ion is *ca.* 40 times faster than the

benzimidazolium ion itself.[3] Both the 1-methylguanosine and 1-methylinosine are only marginally faster than the parent nucleosides. It is clear therefore that the methylated compounds are very good models for the reaction between the protonated substrates and hydroxide ion.

For the reaction at high pH guanosine reacts faster than its 1-methyl analogue by a factor of 2.2×10^3; the corresponding value for inosine (9×10^2) is also very large. Taken together the results strongly suggest the involvement of the zwitterionic species (1) in isotopic hydrogen exchange from the C-8 position in both guanosine and inosine. The fact that the 1-methyl nucleosides undergo reaction at high pH as the neutral substrates furthermore suggests that both mechanisms must be operative. In other words the second-order detritiation rate constant (k') is a composite function comprising contributions from the neutral and zwitterionic species.

$$k' = k_0 + K_{zw}k_{\pm} \simeq K_{zw}k_{\pm} \qquad (6)$$

k_0 and $k_{\pm}$ are the second-order rate constants for detritiation from the neutral and zwitterionic species, respectively and K_{zw} is given by the quotient [zwitterion]/[neutral molecule].

Tomasz and her co-workers [5] treated their data on guanosine in this way, having estimated a value of K_{zw} by using the method of Tucker and Irvin.[15] Adopting the same procedure the value of K_{zw} at 85 °C (assuming the Perrin equation holds) becomes 1.7×10^{-4}. The derived value of $k_{\pm}$ (3.6×10^4 l mol^{-1} s^{-1}) is *ca.* 85 times smaller than k, a finding consistent with partial neutralisation of the positive charge in the imidazole ring by the negative charge in the pyrimidine ring.

The present results are consistent with our recently reported findings [4] on the xanthines where reducing the degree of methylation, and therefore increasing the scope for zwitterionic involvement, was accompanied by a large decrease in the ratio k/k' (10^8 for caffeine, 1.8×10^2 for xanthosine).

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