Proton Transfer from Heterocyclic Compounds. Part 9.[1,2] Effect of Transition-metal Ion Co-ordination on Isotopic Hydrogen Exchange in Purines

By **John R. Jones** * and **Spencer E. Taylor**, Chemistry Department, University of Surrey, Guildford GU2 5XH

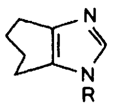
Rates of detritiation from the C-8 position of 1-methyl[8-^{3}H]guanosine and 1-methyl[8-^{3}H]inosine at 85 °C in the presence of various metal ions are reported. Analysis of the data shows that the metal-complexed species are more reactive than the neutral species by factors of 10^4—10^6 but less reactive than the protonated substrates. Detritiation studies on the *cis*[(1,2-diaminoethane)(guanosine)$_2$]platinum di-iodo-complex are consistent with this viewpoint.

THE rates of ionisation of carbon acids[3] have been extensively investigated both as a function of acid strength and the basicity of the medium. Other aspects of the ionisation process, *e.g.* the possibility of intramolecular catalysis,[4] have also been studied. In contrast very little attention has been paid to the way in which metal ions can influence the rates although there are many other kinds of reactions, *e.g.* ester hydrolysis and hydration reactions, where quite startling rate accelerations have been reported. Recent studies by Kluger[5] and Cox[6] not only draw attention to this lack of data but also show that by suitable choice of substrate large rate accelerations can also be observed for ionisation reactions.

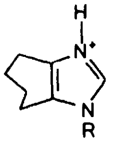
Heterocyclic carbon acids such as the purines form a convenient starting point for investigating metal-ion effects since, first such compounds are known to complex very readily with a number of metal ions, and secondly because isotopic hydrogen exchange (from the C-8 position) can proceed by different mechanisms, the most common being hydroxide ion attack on the neutral (1) and the protonated form (2). Rate constants for the latter are usually 10^8—10^9 times greater than those found for the corresponding neutral molecules,[7] demonstrating the influence of the positive charge α to the site of exchange (the so-called 'proton activation factor').[8] In the presence of added metal ions species such as (2) will be replaced by metal ion co-ordinated species (3) and the rate constant for isotopic exchange *via* this new mechanism would be expected to be considerably higher than for that involving the neutral form.

In the only previous kinetic investigation involving the effect of metal ions on isotopic hydrogen exchange in heterocyclic carbon acids, Olofson *et al.*[9] demonstrated that σ complexation between Cu$^{\text{II}}$ and Zn$^{\text{II}}$ ions and 1-methyl[5-^{2}H]tetrazole led to a large rate enhancement. However, at low pH, where the tetrazole ring is protonated, addition of metal ions had little effect. Under these conditions tetrazole, unlike the purines, is susceptible to exchange *via* an acid-catalysed mechanism so that the effect of metal ions need not necessarily be similar.

More qualitatively, Tobias *et al.*,[10–12] in a laser Raman study of complex formation between heavy metal ions {chiefly MeHg$^+$ and [Pt(en)(H$_2$O)$_2$]$^{2+}$} and purine nucleosides and nucleotides, observed a decrease with time in the intensity of the Raman frequency corresponding to the C-8 hydrogen of the complexed purine on standing in D$_2$O at room temperature. N-7 was deemed to be the site of complex formation, in agreement with the findings of Simpson[13] on methylmercury complex formation. Such labilisation of the C-8 hydrogen could well explain the reported mercuriation of the C-8 position of purine nucleotides with Hg(OAc)$_2$ under mild conditions.[14]



(1)



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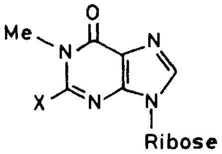
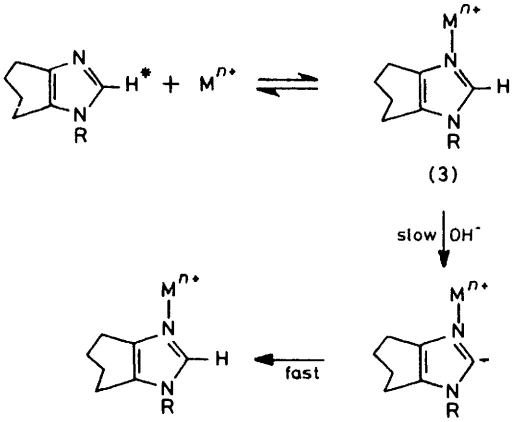


(4) X = NH$_2$

(5) X = H



The effects of metal ions on rates of isotopic hydrogen exchange in purines can, in principle, be studied in two different ways. Firstly, rates of exchange can be studied in the presence of metal ions; in this way the neutral, protonated, and metal-complexed species can all contribute to the overall rate. Secondly, the appropriately labelled complex can be prepared and isolated, and its rate of exchange studied directly. Examples of each method are given in the present study. In the first case rates of detritiation of 1-methyl[8-^{3}H]guanosine (4) in the presence of Cu^{II} ions and of 1-methyl[8-^{3}H]inosine (5) in the presence of Cu^{II}, Zn^{II}, and Ag^{I} ions have been obtained. These two substrates were chosen because they only have one site (N-7) for complexation. Secondly, the detritiation of the complex cation *cis*-[Pt(1,2-diaminoethane)(guanosine)$_2$]$^{2+}$ has been investigated.

EXPERIMENTAL

Materials.—The preparation of the tritiated nucleoside has been described.[1] Copper(II) solutions were prepared using AnalaR $CuSO_4 \cdot 5H_2O$ and $Cu(NO_3)_2 \cdot 3H_2O$ and doubly distilled deionised water, and standardised by EDTA titration. Weighed stoicheiometric amounts of $AgClO_4$ and $ZnSO_4 \cdot 7H_2O$ were dissolved in doubly distilled deionised water.

The complex *cis*-[(1,2-diaminoethane)(guanosine)$_2$]platinum di-iodide was prepared by the method of Kong and Theophanides.[15] cis-[Pt(1,2-diaminoethane)I$_2$] was first prepared by treating an aqueous solution of K_2PtCl_4 with KI, yielding K_2PtI_4 which was then mixed with an equimolar quantity of 1,2-diaminoethane (en). *cis*-[Pt(en)I$_2$] was obtained as a yellow precipitate which was filtered off and washed with water; the compound (0.2 mmol), and guanosine (0.4 mmol) in water (*ca.* 10 ml) were stirred overnight at 55 °C. The resulting solution was filtered and the filtrate evaporated to half its original volume. On refrigeration the excess of guanosine precipitated out and was filtered off and the remaining solution lyophilised. The white solid so obtained analysed as [Pt(en)(guanosine)$_2$]I$_2$.

Tritiation of the complex (*ca.* 10 mg) was effected by dissolution in tritiated water (10 μl, 5Ci ml^{-1}) and incubation at 25 °C for 6 h. The reaction was quenched by lowering the pH and removing the solvent by lyophilisation; any labile tritium was removed by adding a small amount of water (20 μl) followed by a further lyophilisation.

Kinetics.—The procedures employed have been described in detail,[16,17] the only difference now being that in the competitive studies the ionic strength of the medium was kept constant at 0.40M by the addition of $NaClO_4$. The metal-ion solutions were unbuffered in order to exclude possible effects due to complexation with buffer materials; the recorded pH was therefore the ambient pH of the solutions. For the platinum complex phthalate and acetate buffers were used. The experimental accuracy of the observed rate constants is ±3—5%.

RESULTS AND DISCUSSION

The rate–pH profile for 1-methylguanosine recently reported[1] shows that the plateau region extends from pH 2—9. At pH 3.68, therefore, the operative mechanism is that between the protonated species and hydroxide ions. The addition of Cu^{II} ions causes a marked decrease (Table 1 and Figure 1) in the detritiation rate contants both at 85 and 40 °C, and these results can be interpreted in the following manner. In a particular solution the total concentration of 1-methylguanosine $[B]_T$ is given by equation (1) where BH$^+$, B, and BM^{n+} refer to the protonated, neutral and metal-complexed forms, respectively; only 1 : 1 complexing is assumed.

$$[B]_T = [BH^+] + [B] + [BM^{n+}] \qquad (1)$$

Similarly, the total metal-ion concentration is given by

$$[M]_T = [M^{n+}] + [BM^{n+}] \simeq [M^{n+}] \qquad (2)$$

equation (2) as $[M]_T \gg [B_T]$. If therefore we can assume that the hydroxide ion reacts only with the protonated and metal ion-complexed species equation (3) holds:

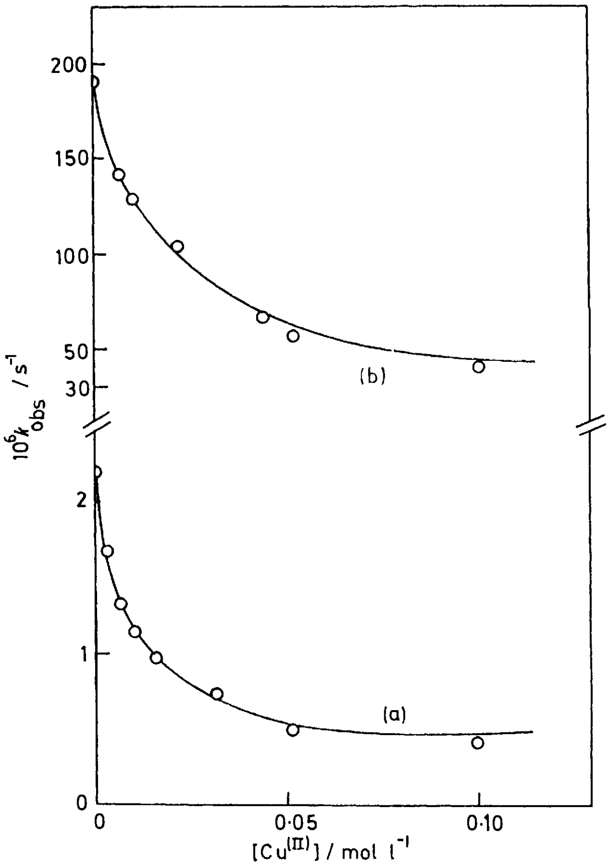


FIGURE 1 Detritiation rate constants for 1-methyl[8-^{3}H]-guanosine in the presence of Cu^{II} ions at (a) 40 °C and (b) 85 °C. $I = 0.40$M, pH = 3.68. The lines are computed using equation (7) and values given in the text

$$\text{Rate} = k_{obs.}[B]_T = k[BH^+][OH^-] + k_M[BM^{n+}][OH^-] \qquad (3)$$

TABLE 1

Detritiation rate constants ($k_{obs.}$) for 1-methyl[8-^{3}H] guanosine in the presence of Cu^{II} ions at 40 and 85 °C (pH = 3.68, I = 0.40M)

10^2[Cu^{2+}]/ mol l^{-1}	$10^6 k_{obs.}$/s^{-1} 40.0 °C	85.0 °C
0	2.21	192
0.315 [a]	1.69	
0.631 [a]	1.33	143
1.03 [b]	1.15	131
1.58 [a]	0.98	
2.17 [a]		105
3.15 [a]	0.73	
4.35 [a]		68
5.15 [b]	0.51	58
10.0	0.41	
10.3		41

[a] $CuSO_4$ solutions. [b] $Cu(NO_3)_2$ solutions.

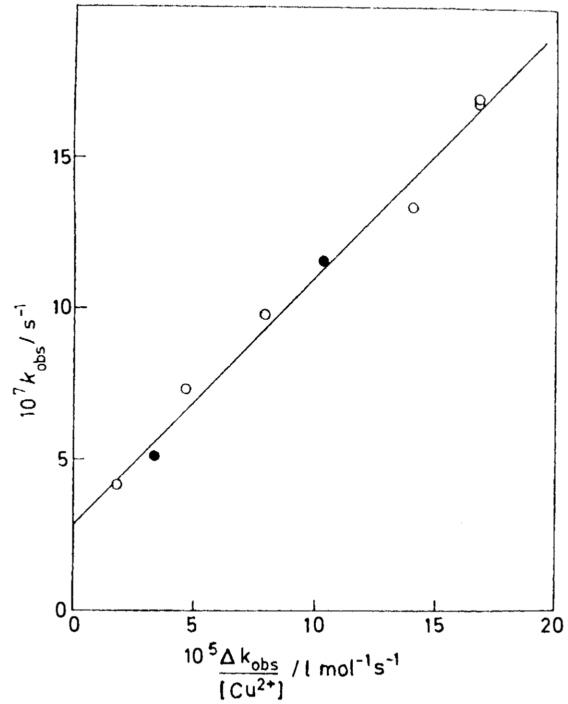


FIGURE 2 Plot of $k_{obs.}$ against $\Delta k_{obs.}/[Cu^{2+}]$ for the detritiation of 1-methyl[8-^{3}H]guanosine at 40 °C

If the stability constant (K') of the metal ion-purine complex is defined by $K' = [M^{n+}][B]/[BM^{n+}]$ and the acid dissociation constant of the protonated purine molecule is given by $K_a = [H^+][B]/[BH^+]$, it can be shown that equations (4) and (5) hold.

$$[BH^+] = \frac{K'[H^+][B]_T}{K'[H^+] + K_aK' + K_a[M^{n+}]} \tag{4}$$

$$[BM^{n+}] = \frac{K_a[M^{n+}][B]_T}{K'[H^+] + K_aK' + K_a[M^{n+}]} \tag{5}$$

Substitution in equation (3) gives (6), which simplifies to (7) as $K_a \gg [H^+]$ under the experimental conditions.

$$k_{obs.} = \frac{kK_wK' + k_MK_a[M^{n+}][OH^-]}{K'[H^+] + K_aK' + K_a[M^{n+}]} \tag{6}$$

$$k_{obs.} = \frac{kK_wK' + k_MK_a[M^{n+}][OH^-]}{K_a(K' + [M^{n+}])} \tag{7}$$

$$k_{obs.} = \frac{K'\Delta k_{obs.}}{[M^{n+}]} + k_M[OH^-] \tag{8}$$

Rearranging equation (7) leads to (8) so that a plot of $k_{obs.}$ against $\Delta k_{obs.}/[M^{n+}]$, where $\Delta k_{obs.}$ is the magnitude of the rate reduction in the presence of metal ions, should be linear with slope (K') and intercept ($k_M[OH^-]$). Figure 2 shows an example of such a plot for the results at 40° from which k_{Cu} (1.7×10^3 l mol^{-1} s^{-1}) and pK' (2.07) values are obtained; taken together with the values of k (3.83×10^5 l mol^{-1} s^{-1}), pK_a (2.28), and pK_w (13.535) they provide the calculated curve in Figure 1 in good agreement with experiment. No pK' values for the 1-methylguanosine–Cu^{II} complex are available; how-

TABLE 2

Detritiation rate constants ($k_{obs.}$) for 1-methyl[8-^{3}H]inosine in the presence of Cu^{II}, Zn^{II}, and Ag^I ions at 85 °C

Metal ion	pH	$10^2[M^{n+}]$/ mol l^{-1}	$10^5 k_{obs.}/s^{-1}$
Cu^{II}	3.68	0	9.02
		1.03 [a]	7.93
		2.17 [b]	7.06
		4.35 [b]	6.27
		5.16 [a]	5.80
		6.31 [b]	5.83
		11.4 [b]	4.67
Zn^{II}	4.16	3.01	8.75
		6.11	9.02
		9.66	8.83
		11.7	8.79
Ag^I	4.70	0.494	7.62
		1.21	6.47
		2.04	5.19
		3.58	3.98
		5.51	3.46
		9.65	2.18

[a] CuSO$_4$ solutions. [b] Cu(NO$_3$)$_2$ solutions.

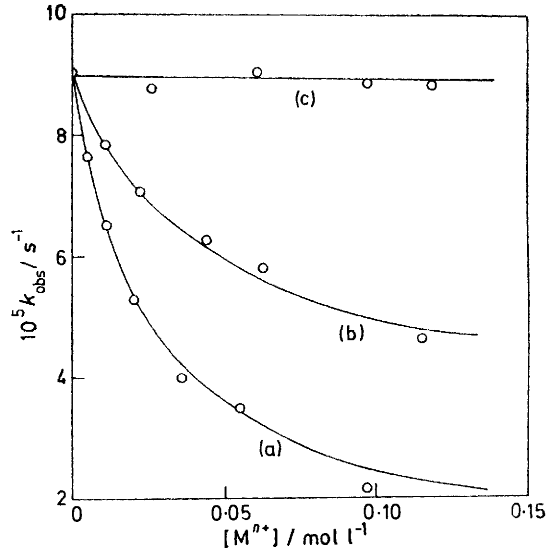


FIGURE 3 Detritiation rate constants for 1-methyl[8-^{3}H]inosine in the presence of (a) Ag^I, (b) Cu^{II}, and (c) Zn^{II} ions at 85 °C. The lines for Ag^I and Cu^{II} are computed using equation (7) and values given in the text

TABLE 3

Derived stability constants (pK_M) and detritiation rate constants at 85 °C

Substrate	pK_a	Metal ion	pK_M	Rate constants (l mol^{-1} s^{-1}) k	k' [a]	k_M
1-Methylguanosine	2.2	Cu^{II}	1.78	5.3×10^6	2.5×10^{-3}	1.4×10^4
1-Methylinosine	1.2	Cu^{II}	1.36	18.0×10^6	19×10^{-3}	2.0×10^4
		Ag^I	1.6			4×10^2

[a] Ref. 7.

ever, for the structurally similar deoxyguanosine a value of 2.15 at 25 °C has been reported.[13] The derived rate and stability constants at 85 °C are given in Table 3.

In the case of 1-methyl[8-^{3}H]inosine (Table 2) the range of metal ions has been extended to include, as well as Cu^{II}, Zn^{II} and Ag^{I}. Marked differences in behaviour are observed; Zn^{II} ions have virtually no affect on the rate, whereas in the case of Ag^{I} ions the rate retardation is even more marked than for the Cu^{II} ions (Figure 3). A similar treatment to that used for 1-methylguanosine gives the results shown in Table 3. The absence of any rate perturbation with Zn^{II} is consistent with the view that this metal does not form stable complexes with nitrogen-donor ligands.

For the platinum complex, which contains no ionisable protons in the pH range studied, the relevant rate equation is (9). The second order rate constant for

$$\text{Rate} = k_{\text{obs.}}[B]_T = k_M[BM^{n+}][OH^-] \qquad (9)$$

detritiation of the complex is obtained by plotting $k_{\text{obs.}}$ (Table 4) against hydroxide ion concentration. The resulting value ($2.7 \pm 0.2 \times 10^5$ l mol^{-1} s^{-1}) can be compared with a value [1] of 6.07×10^5 l mol^{-1} s^{-1} obtained for 7-methylguanosine, where again the positive charge is located at the N-7 position.

A comparison of the respective rate constants k and k_M (Table 3) shows that although none of the metal ions are as effective as a proton in accelerating the rates of detritiation (hence the rate decrease when metal ions are present), if we compare the k' and k_M values it is clear that rate accelerations of the order of 10^4—10^6 have taken place with Cu^{II} being most effective and that the studies on the platinum complex confirm this. In theory these studies could be extended to investigations at high pH where direct comparison of the reactivity of the neutral and metal-complexed species could be made, but precipitation of metals from solution rather limits this approach.

These results, as well as providing a rationale for the observation [10-12] that co-ordination of heavy metal ions to the N-7 position cause a rapid disappearance of the H-8 signal in the n.m.r. spectra of inosine and guanosine derivatives in D_2O, also explain the absence of rate accelerations [19] when studies are carried out at low pH. These would only come about if the second term in equation (8) greatly exceeds the first. Finally, the large magnitude of k_M/k' (metal activation factor) affords the opportunity of investigating metal-ion effects in compounds where the site of complex formation is not adjacent to the exchanging C-8 position.

TABLE 4

Detritiation rate constants ($k_{\text{obs.}}$) for *cis*-[Pt(en)([8-^{3}H]-guanosine)$_2$]I$_2$ in aqueous buffer solutions at 25 °C

pH	$10^{10}[OH^-]$/ mol l^{-1}	$10^5 k_{\text{obs.}}$/s^{-1}	$10^{-5}k_2$/ l mol^{-1} s^{-1}
4.01	1.01	3.09	3.06
4.63	4.27	11.1	2.60
4.80	6.31	18.3	2.90
5.09	12.3	30.4	2.47
5.37	23.5	59.2	2.52
6.02	105	296	2.85

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