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THE KINETICS OF TRITIUM-HYDROGEN EXCHANGE IN XANTHOSINE, THEOBROMINE AND MONOMETHYLATED DERIVATIVES OF XANTHINE

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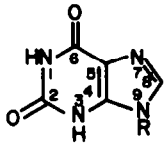
Abstract—Tritium exchange at C-8 of xanthosine, theobromine, 1-, 3- and 7-methylxanthine in water has been studied. The rates of detritiation of these compounds have been determined over a pH range at constant temperature. Several mechanisms of exchange involving various ionic forms of substrate operating at different pH have been suggested.

Hydrogen isotope exchange at the 8-position of purines is a reaction of biological significance and indicates that C-8 is a nucleophilic centre in purine nucleosides. On the other hand studies of an isotope exchange reaction may provide valuable information of synthetic use. For these reasons considerable attention has been focussed upon C(8)H exchange in purines, especially such as adenine, guanine, hypoxanthine and their nucleosides.[1-3] More detailed kinetic studies have indicated the influence of NH group dissociation on the rate of hydrogen exchange from C-8.[1]

The effect of these groups on rates and mechanisms of C(8)H exchange is especially interesting in xanthine derivatives which, in comparison with guanine and hyposanthine, have an additional N(3)H group.

Continuing our previous studies[4,5] we have investigated tritium-hydrogen exchange in 8-position of xanthosine (1), theobromine (2a), 1-, 3- and 7-methylxanthine (2b, c, d)



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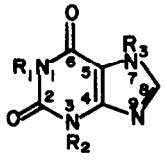
R = β-D-ribofuranosyl



2

2a: $R_1 = H$, $R_2 = R_3 = CH_3$
2b: $R_1 = CH_3$, $R_2 = R_3 = H$
2c: $R_2 = CH_3$, $R_1 = R_3 = H$
2d: $R_3 = CH_3$, $R_1 = R_2 = H$

It has been found however that the mechanism of hydrogen exchange in such compounds depended not only on the number of NH groups and the sequence of their ionisation but on the charge distribution in the molecule as well.

The conclusions emerging from our studies, concerning tautomeric forms and ionic structures of xanthine derivatives will be a subject of a separate paper. In the present paper only the analysis of the rate-pH dependences followed by the mechanistic conclusions is reported.

EXPERIMENTAL

Xanthosine (Lachema) and theobromine (Sigma) were purchased commercially. 1-Methylxanthine was kindly provided by Prof. W. Pfleiderer (Konstanz University), 7-methyl- and 3-methyl-xanthine were synthesized in Department of Biophysics of Warsaw University.

All tritiated compounds were prepared by homogeneous exchange using tritiated water (80 mCi/cm^3). Because of the low solubility of these compounds in neutral pH, the alkaline solutions were prepared adding the appropriate amount of solid KOH. About 30 cm^3 of solution containing 1–2 g of substrate was maintained at 90° for about 4 days. Water was removed by freeze drying, a small amount of H_2O was added to exchange labile hydrogen atoms and the solution was lyophilised again. If necessary, this procedure was repeated several times. Then the product was dissolved in water and the solution was neutralized by HCl. The compound precipitated was washed out and dried in a vacuum.

Kinetics

The methods used to follow the rates of detritiation were as given previously.[5] The kinetic measurements were carried out at 82°. The pH ranges for the compounds studied were limited by their low solubility in acidic solutions. The reaction involving 3-methylxanthine was slow and the pseudo-first-order rate constant (k_{obs}) was obtained as previously,[5] by initial rate method[6] from the slope of the plot of radioactivity of water against time. When the reactions were faster, k_{obs} were determined from the slope of the plot of log $(A_\infty - A_t)$ vs time (A_t—specific activity of water, A_∞—specific activity of the sample of compound taken to exchange).

RESULTS AND DISCUSSION

It was indicated previously[1-3,7] that the C(8)H isotope exchange in purines is catalyzed by the hydroxide ion which attacks the molecule of a substrate in the rate-determining step of reaction. However, the ionic forms of the molecules interacting with the OH^- group were the subject of discussions. Xanthine derivatives form in aqueous solutions an especially large number of tautomers and ions whose structures are not as yet satisfactorily known.[8]

In such a situation the discussion of results obtained becomes difficult and at the present state it seems reasonable to simplify the formal description of the exchange mechanisms. Therefore considering the participation of ionic forms of xanthines with a given resultant charge (protonated form, neutral form, monoanion, dianion) we neglect the charge distribution within the molecule.

In 1-methyl derivative of xanthine just as in nonsubstituted xanthine both N(3)H and N(7)H groups are able

to dissociate in aqueous solution. The similar behaviour of these two compounds was found in spectroscopic studies of their solution.[8] Also the rate-pH profile observed for 1-methylxanthine exchange (Fig. 1) is reminiscent of that previously obtained for xanthine.[5] Hence in both cases we can assume the same mechanism of exchange involving the rate-determining attack by the hydroxide ion on the neutral molecule of the substrate. Such a mechanism was also postulated for C-8 hydrogen exchange at high pH in 9-substituted purine, adenosine, guanine, guanosine, etc.[1] The k_{obs}-pH relation for 1-methylxanthine may be described by eqn (1):†

$$k^{II}_{obs} = \frac{k_2^{II} K_w}{[H^+] + \frac{[H^+]^2}{K_{a1}} + K_{a2} + \frac{K_{a2}K_{a3}}{[H^+]}} \quad (1)$$

where: K_{a1}, K_{a2} and K_{a3} represent successive dissociation constants of substrate (K_{a1} refers to dissociation of protonated form); K_w—dissociation constant of water and k_2''—second-order rate constant of reaction between neutral molecule and OH^-.

For $[H^+] \ll K_{a1}$ we obtain:

$$k^{II}_{obs} = \frac{k_2^{II} K_w}{[H^+] + K_{a2} + \frac{K_{a2}K_{a3}}{[H^+]}}. \quad (2)$$

The calculated curve which was fitted to the experimental points by the nonlinear least-squares treatment‡ is shown by the solid line in Fig. 1.

Because of the low solubility of 1-methylxanthine in acidic solutions the exchange was not studied below pH 6.6. However at acidic pH region, where the dissociation of NH groups is restrained, the difference in behaviour of xanthine derivatives diminishes. Here we can expect for all of them the exchange mechanism involving protonated substrate previously observed in theophylline and caffeine.[5] This assumption is supported by the results obtained for theobromine (Fig. 2) which is a little more soluble than 1-methylxanthine. Moreover the pKa_2 value of theobromine is higher than that of the latter, thus the mechanism involving the neutral molecule is adequately shifted towards higher pH. As a result at the pH range 3–7 we obtain pH-independent rate constant (k_{obs}) accordingly to eqn (3):

$$k^{I}_{obs} = \frac{k_2^{I} K_w}{[H^+] + K_{a1} + \frac{K_{a1}K_{a2}}{[H^+]}} \quad (3)$$

when $K_{a1} \gg [H^+] \gg K_{a1}K_{a2}$

Equation (3) describes the rate-pH relation typical for the mechanism involving a hydroxide ion attack on the protonated molecule of a substrate. The corresponding calculated curve is presented in Fig. 2 by dotted line I. At higher pH the observed rate constant of theobromine increases what is probably connected with the mechanism that involves the neutral substrate and the hydroxide ion. In this case due to the lack of the third dissociation, eqn (2) may be reduced to:

$$k^{II}_{obs} = \frac{k_2^{II} K_w}{[H^+] + K_{a2}} \quad (4)$$

and the corresponding calculated curve is shown in Fig. 2 by dotted line II. At high pH when $[H^+] \ll K_{a2}$, k_{obs} becomes independent of pH. In spite of that, in alkaline solution the exchange rate increases dramatically. This suggests that an additional mechanism, most probably involving the rate determining attack by the hydroxide

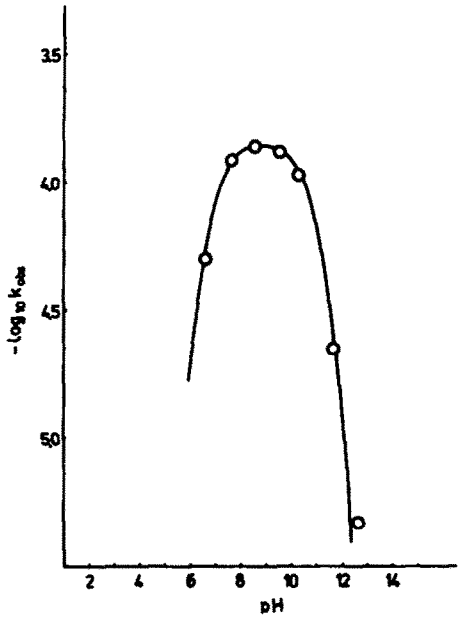


Fig. 1. Rate-pH profile for 8-[^{3}H] 1-methylxanthine. The solid line is drawn using eqn (2).

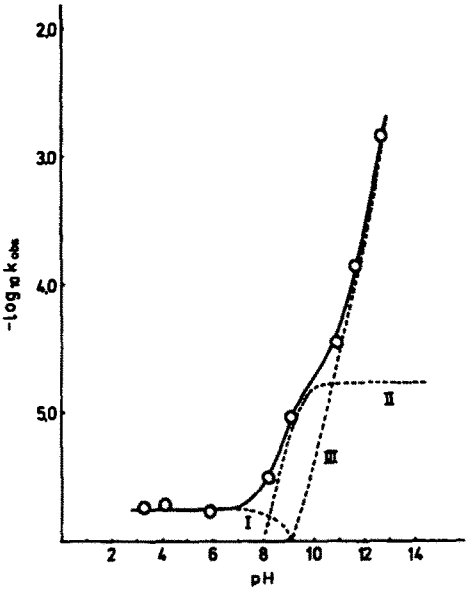


Fig. 2. Rate-pH profile for [8-^{3}H] theobromine. The solid line presents the superposition of partial processes with participation of the following forms of theobromine: (a) protonated form (dotted line I, eqn 3); (b) neutral molecule (dotted line II, eqn 4); (c) monoanion (dotted line III, eqn 5).

†All calculated curves presented in this paper were obtained by least-squares procedure using literature pK_a values[8] as starting points.

‡The notation k^{I}_{obs}, k^{II}_{obs}, k^{III}_{obs}, k^{IV}_{obs}, and k_2^{I}, k_2^{II}, k_2^{III}, k_2^{IV} refers to the mechanisms involving protonated form, neutral form, monoanion, and dianion, respectively.

ion on the monoanion of the substrate comes into operation at high pH. The contribution of this mechanisms to the total k_{obs} may be described by the following equation:

$$k_{obs}^{III} = \frac{k_2^{III} K_w}{[H^+]}. \quad (5)$$

The calculated curve corresponding to the eqn (5) is presented in Fig. 2 by dotted line III.

The similar exchange mechanisms as we observed in theobromine can occur in xanthosine. In this case in pH-range studied the mechanism involving protonated substrate is not observed but may be expected in acidic solutions. The pH-rate profile obtained for xanthosine is presented in Fig. 3. The dotted line I corresponds to the mechanism involving neutral molecule, according to the eqn (2). At higher pH the increase of exchange rate is observed which is probably consistent with a participation of monoanionic form in the rate-determining step of reaction. In the case of xanthosine which possesses one NH group more than theobromine the eqn (5) should be replaced by eqn (6),

$$k_{obs}^{III} = \frac{k_2^{III} K_w}{[H^+] + K_{a3}} \quad (6)$$

and in the strong alkaline solution the k_{obs} becomes independent of pH (the curve represented in Fig. 3 by dotted line III).

Taking into account the similarity between xanthosine and 7-methylxanthine (both compounds have one substituent in the imidazole ring and two free NH groups in the pyrimidine ring) we can expect for them the analogous mechanisms of the exchange. However the rate-pH profile obtained for 7-methylxanthine (Fig. 4) is rather different than for xanthosine. We can distinguish here

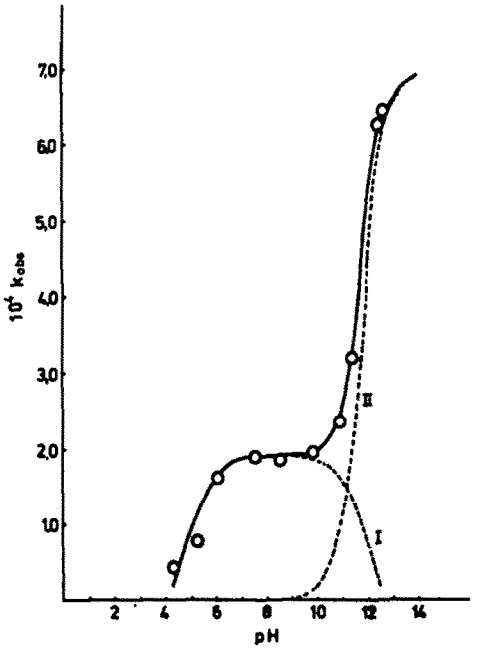


Fig. 3. Rate-pH profile for [8-^{3}H] xanthosine. The solid line presents the superposition of partial processes with participation of the following forms of xanthosine: (a) neutral form (dotted line I, eqn 2); (b) monoanion (dotted line II, eqn 6).

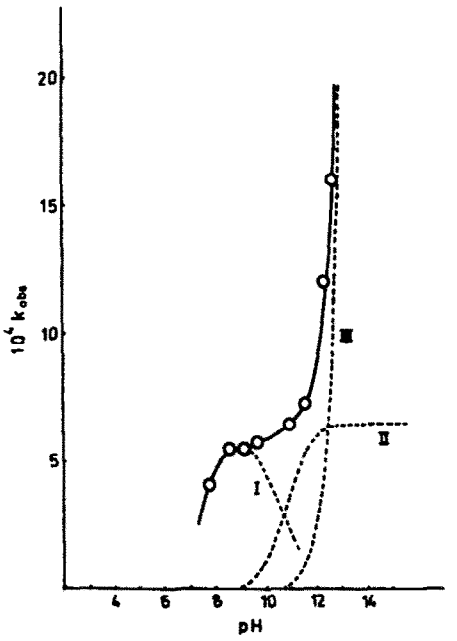


Fig. 4. Rate-pH profile for [8-^{3}H] 7-methylxanthine. The solid line presents the superposition of partial processes with participation of the following forms of 7-methylxanthine: (a) neutral form (dotted line I, eqn 2); (b) monoanion (dotted line II, eqn 6); (c) dianion (dotted line III, eqn 7).

the mechanism involving the neutral molecule (eqn (2), dotted line I) and the monoanionic form (eqn (6), dotted line II) but in alkaline solution instead of the pH-independence of k_{obs} we find an increase of exchange rate. This suggests that at high pH region the hydroxide ion begins to attack the dianionic form of substrate. The relation between k_{obs} and $[H^+]$ may be described as follows:

$$k_{obs}^{IV} = \frac{k_2^{IV} K_w}{[H^+]} \quad (7)$$

and the corresponding calculated curve is presented in Fig. 4 by dotted line III.

The difference in the behaviour of 7-methylxanthine and xanthosine at high pH can be ascribed to the higher pK_{a3} value for the latter.[8]

For all cases discussed presently and described previously[1-5,7] we can draw the following conclusions:

(1) At low pH region in all compounds studied C-8 hydrogen abstraction from a protonated substrate by a hydroxide ion is a rate-determining-step of exchange reaction.

(2) At higher pH values most of these compounds participate in exchange reaction in the neutral form.

(3) Some of compounds which at intermediate pH interact as a neutral molecule with a hydroxide ion, in alkaline solutions interact as a monoanion and even as a dianion.

However this sequence of exchange mechanisms is not always followed. The rate-pH profile obtained for 3-methylxanthine (Fig. 5) shows that this compound is such an exception. Two mechanisms can be distinguished here: the first involving protonated form (eqn (3), curve I) at low pH and the other with a monoanionic form (eqn (6), curve II) at high pH. At intermediate pH, when the neutral molecules dominate in solution the rate

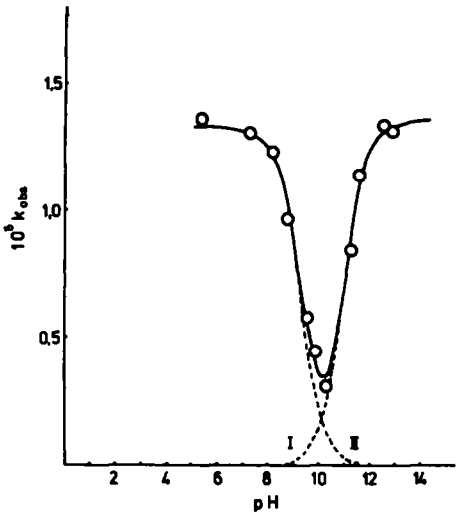


Fig. 5. Rate-pH profile for [8-^{3}H] 3-methylxanthine. The solid line presents the superposition of partial processes with participation of the following forms of 3-methylxanthine: (a) protonated form (dotted line I, eqn 3); (b) monoanion (dotted line II, eqn 6).

of exchange decreases, so we can suppose that such a form of substrate is unable to interact with hydroxide ion. It is possible that the similar effect could have been also observed for theophylline,[5] but because of its decomposition in alkaline solutions, the exchange reaction at high pH was not studied.

The results presented here show that hydroxide ion, catalyzing C-8 hydrogen exchange, may interact with a variety of ionic forms of xanthine derivatives, and even with their anions. On the other hand in some cases not all forms of substrate being presented in solution undergo the attack of OH^- ion. These facts confirm the suggestion mentioned above that the charge distribution in molecule (especially nearby the C-8 position) determines an occurrence of some mechanisms of exchange. Therefore it would be interesting to discuss some conclusions emerging from the data obtained and concerning the tautomeric forms and ionic structures of xanthines. The details of this problem will be presented in the next paper.

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