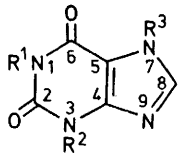
Proton Transfer from Heterocyclic Compounds. Part 6.[1,2] Detritiation Rates of Various Xanthines

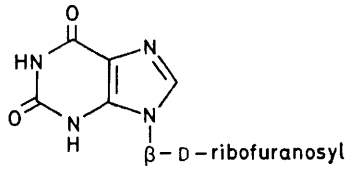
By John R. Jones * and Spencer E. Taylor, Chemistry Department, University of Surrey, Guildford GU2 5XH

Rates of detritiation from the C-8 position of xanthine, xanthosine, and a series of methylated xanthines (caffeine, theophylline, theobromine, and paraxanthine) have been measured over a pH range at 85°. In all cases the results can be interpreted in terms of rate-determining hydroxide ion attack on one or more of the different ionised species present in solution; the less the degree of methylation the greater the number of ionisable forms and the larger the number of potential mechanisms. In the case of theobromine and paraxanthine three forms (protonated, neutral, and monoanionic) are involved and in the case of xanthosine a further form (dianionic) makes a contribution to the overall rate. The possibility of zwitterionic contributions are also discussed.

In previous work [3,4] on rates of detritiation from the C-2 position of the imidazole ring system or the equivalent C-8 position in purines we have varied the number of ionisable groups in a systematic manner and found that in all cases the reaction proceeds by one of two mechanisms, both of which involve overwhelming hydroxide ion catalysis. At low pH, reaction involves the protonated substrate whilst in some cases at high pH the neutral compound also undergoes reaction. The anionic form, produced at high pH as a result of the ionisation of the NH group adjacent to the site of exchange, has always been unreactive. This need not always be the case however if the negative charge is developed at a site well removed from the exchanging CH group. Our results for guanosine, inosine, and 9-methylhypoxanthine [5] seemed to suggest that had the work been extended to pH >11.5 a contribution from hydroxide ion attack on the anionic form might have been observed. To make this mechanism more likely one could therefore employ compounds structurally similar to those mentioned but

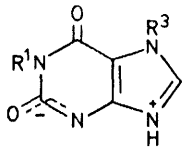


(1)



(2)

a; $R^1 = R^2 = R^3 = H$
b; $R^1 = R^2 = CH_3, R^3 = H$
c; $R^1 = R^2 = R^3 = CH_3$
d; $R^1 = H, R^2 = R^3 = CH_3$
e; $R^1 = R^3 = CH_3, R^2 = H$



(3)

with lower pK_a values; theobromine (1d) and paraxanthine (1e) fall into this category. Alternatively a compound could be chosen with an additional ionisable group in the pyrimidine ring; its pK_a should be intermediate to that of the N(1)–H and N(9)-β-D-ribofuranosyl groups. Xanthosine (2) fulfils these requirements and the ready availability of a number of methylated xanthines allows one to quantify the effect various methyl groups have on the rates of isotopic hydrogen exchange.

Although there are many examples in the literature where xanthines specifically labelled in the C-8 position have been used, very little work has been reported on the rates of isotopic exchange. Maslova and her co-workers [6] investigated [8-^{3}H]xanthine (1a) and obtained a pH–rate profile very similar to that found for guanine. More recently Jelinska and Sobkowski [7] reported on the detritiation of the same compound as well as theophylline (1b) and caffeine (1c). The substrates were of low specific activity and the need for relatively high concentrations led, in the case of xanthine, to solubility difficulties at low pH (<7). Theophylline exhibited a bell-shaped pH–rate profile similar to that observed previously for other heterocycles, *e.g.* benzimidazole,[3] purine,[4] and adenine.[8] The results for caffeine were interpreted in terms of hydroxide-ion attack on the protonated form at low pH followed by reaction between the same catalyst and the neutral caffeine molecule at high pH.

Ionisation constants have been obtained (by Bergmann and his co-workers [9]) for xanthine and its *N*-methyl derivatives as part of their detailed investigations into identifying the possible substrate species involved in the mode of action of xanthine oxidase.

EXPERIMENTAL

Materials.—The xanthines were commercially available. [8-^{3}H]Theophylline was prepared by incubating a mixture of theophylline (*ca.* 50 mg) and tritiated water (20 μl; 5 Ci ml^{-1}) at 85° for 24 h. The solvent was removed by freeze-drying, a small amount of water was added to exchange labile tritium, and the water removed once again.

[8-^{3}H]Caffeine was prepared by incubating a mixture of caffeine (*ca.* 40 mg), dioxan (0.5 ml), and tritiated water (20 μl; 5 Ci ml^{-1}) at 85° for 24 h. The solvent was then removed by freeze-drying and the solid redissolved in dioxan–water to exchange labile tritium.

[8-^{3}H]Xanthine, [8-^{3}H]xanthosine, [8-^{3}H]theobromine, and [8-^{3}H]paraxanthine were prepared by first converting

the inactive compounds into the sodium salts; *ca.* 50 mg of the latter together with tritiated water (20 μl; 5 Ci ml^{-1}) were kept at 85° for 24 h. After removing labile tritium in the usual way the sodium salts were neutralised with dilute hydrochloric acid. The neutral compounds, on precipitation, were filtered off, washed with distilled water, and dried *in vacuo*. Spectral (n.m.r. and u.v.) monitoring of the products were used to ensure that no decomposition had occurred under the tritiation conditions.

Kinetics.—Throughout the work buffer systems of known pH–temperature dependence were employed. Rates of detritiation were measured in the customary manner by following the increase in the radioactivity of the water after separation from the tritiated heterocyclic compound had been achieved by freeze-drying. A detailed account of the procedures has been given previously.[3,4]

Good first-order kinetics were obtained in all cases except for xanthosine at very low pH. Under these conditions as well as detritiation from the C-8 position, hydrolysis to the parent heterocyclic base, xanthine, occurs. The kinetic data could be analysed in terms of two parallel first-order plots, the subsequent detritiation from the C-8 position of xanthine being considerably slower than either process.

RESULTS AND DISCUSSION

The results for the most highly methylated of the compounds, caffeine (1c), give a rate–pH profile (Figure 1) similar to the previously studied 9-alkylpurines [4] and adenosine.[8] In the latter compounds the data was explained in terms of rate-determining hydroxide ion attack on the protonated and neutral molecules at low and high pH, respectively, and it is reasonable to assume that the same mechanisms are in operation for caffeine. Equation (1) leads to (2) which was used to

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

construct the calculated curve. Equation (2) reduces to

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

(3) at high pH; the value of k' obtained from the plot of

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

$k_{obs.}$ against $[OH^-]$ is 2.72×10^{-2} l mol^{-1} s^{-1} and the value of k obtained from equation (4), to which equation (3)

$$k_{obs.} = kK_w/(K_a + [H^+]) \quad (4)$$

reduces to at intermediate pH, is 2.52×10^6 l mol^{-1} s^{-1}. The reported [9] pK_a for protonation at N-7 is 0.5 at 25°. For such low values the Perrin equation [10] shows that the temperature dependence is extremely small and in the absence of any evidence to the contrary we have assumed that there is no change in going from 25 to 85°.

Theophylline (1b) exhibits the simplest kind of pH–rate profile (Figure 1), the detritiation rate constant being virtually unaffected by changes in pH over >4 units. At both very low and high pH rate retardations occur and the results, which are similar to those previously reported for benzimidazole,[3] purine,[4] and adenine, [8] can be rationalised in the same way, namely rate-determining hydroxide ion attack on the protonated molecule. Equation (6), which can be derived from (5), was used to construct the theoretical curve. At high

$$\text{Rate} = k[BH^+][OH^-] \quad (5)$$

$$k_{obs.} = \frac{kK_w}{K_a + \frac{K_aK_a'}{[H^+]} + [H^+]} \quad (6)$$

pH, when $K_a \gg K_a' \gg [H^+]$ holds, equation (6) reduces to (7). Expressing the rates in relative terms, *i.e.*

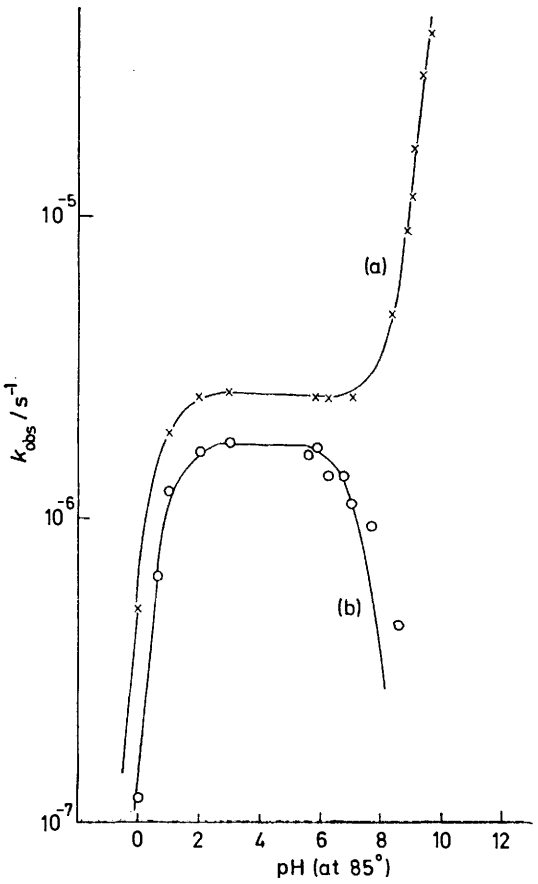


FIGURE 1 Rate–pH profile for the detritiation of (a) [8-^{3}H]-caffeine in aqueous buffers at 85°, × experimental points, — constructed using equation (2); (b) [8-^{3}H]theophylline, ○ experimental points, — constructed using equation (6)

relative to the pH independent region ($k_{obs.} = kK_w/K_a$) we have (8). When $r = 0.5$, pH = pK_a' and for theophylline the value is 7.9 compared with a literature value [9] of 8.5 at 25°. It is assumed that the pK_a for protonation (0.7 at 25°) remains constant over the 60° interval. The value of k used in constructing the theoretical curve was therefore 1.14×10^6 l mol^{-1} s^{-1}.

$$k_{obs.} = kK_w[H^+]/K_a([H^+] + K_a') \quad (7)$$

$$\text{Relative rate } (r) = [H^+]/([H^+] + K_a') \quad (8)$$

The pH–rate profile for theobromine (1e) shows a more complex behaviour (Figure 2) than was witnessed for the isomeric theophylline. The results suggest rate-determining hydroxide ion attack on (1) the N-7 proton-

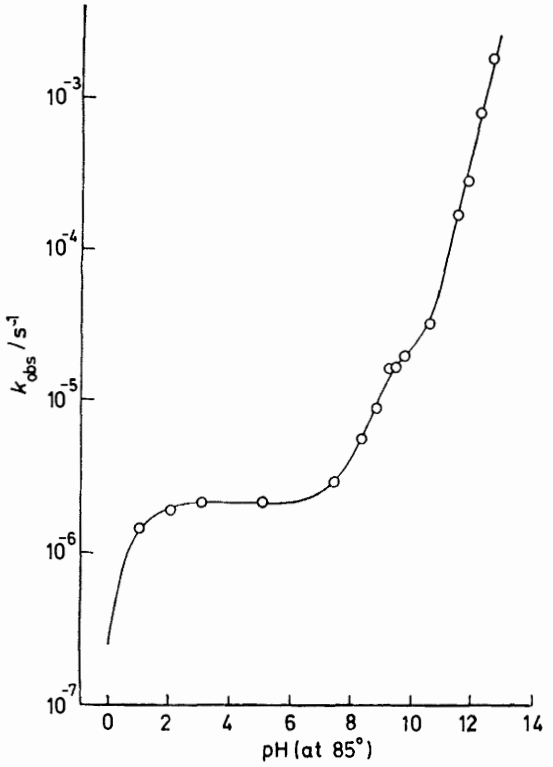


FIGURE 2 Rate–pH profile for the detritiation of [8-^{3}H]theobromine in aqueous buffers at 85°, ○ experimental points, — constructed using equation (11)

ated theobromine molecule, BH_2^+, occurring at low pH, (2) the neutral theobromine molecule, BH, at intermediate pH, and (3) the monoanionic theobromine molecule, B^-, at high pH. The total concentration of

$$\text{Rate} = k_{obs.}[B]_T = k[BH_2^+][OH^-] + k'[BH][OH^-] + k''[B^-][OH^-] \quad (9)$$

theobromine in a particular solution is given by equation (10) and the relevant dissociation constants by $K_a =$

$$[B]_T = [BH_2^+] + [BH] + [B^-] \quad (10)$$

$[BH][H^+]/[BH_2^+]$ and $K_a' = [B^-][H^+]/[BH]$. Substitution for BH_2^+, BH, and B^- in terms of $[B]_T$ in equation (9) leads to (11). At high pH equation (11) reduces to

$$k_{obs.} = \frac{kK_w}{[H^+] + K_a + \frac{K_aK_a'}{[H^+]}} + \frac{k'K_w}{[H^+] + \frac{[H^+]^2}{K_a} + K_a'} + \frac{k''[OH^-]}{\frac{[H^+]^2}{K_aK_a'} + \frac{[H^+]}{K_a} + 1} \quad (11)$$

(12) so that a plot of $k_{obs.}$ against $[OH^-]$ should be linear of slope k'' and of intercept $k'K_w/K_a'$. This is in fact

$$k_{obs.} = k'K_w/K_a' + k''[OH^-] \quad (12)$$

found to be the case, the value of k'' being 1.95×10^{-3} l mol^{-1} s^{-1}. In constructing the theoretical rate–pH profile the literature pK_a [9] value (0.3 at 25°) and the pK_a' value (9.0) determined as for theophylline were used; the latter can be compared with the reported value [9] of 11.0 at 25°. The rate constants k and k' were 3.76×10^6 and 6.34×10^{-2} l mol^{-1} s^{-1} respectively.

The good agreement between the theoretical and experimental rate–pH profiles lends support to the proposed mechanisms. Further evidence comes from the results for paraxanthine (1e). This compound was expected to behave in a similar manner to theobromine and this was found to be the case (Figure 3), the only difference being that the inflection points occur at different pH values. The calculated curve was constructed as for theobromine using the following values: k (5.50×10^6 l mol^{-1} s^{-1}), k' (98.4 l mol^{-1} s^{-1}), k'' (*ca.* 5×10^{-4} l mol^{-1} s^{-1}), pK_a 0.5 (lit.,[9] 0.5 at 25°), and pK_a' 7.6 (lit.,[9] 8.6 at 25°). Although the onset of the additional mechanism at high pH is not as striking as for theobromine there can be no doubt that it makes a real contribution to the overall rate (Table 1).

The rate–pH profile for xanthine (1a) (Figure 4) bears a striking resemblance to that observed previously for guanine, implying that two mechanisms are operative, hydroxide ion attack on the protonated and neutral species [equation (1)]. In principle xanthine can undergo protonation at N-9 as well as ionisation at N-7, -1, and -3 but in view of the above mentioned similarity we shall assume that in the pH range under study only N-7, -9, and -3 (or N-1) are involved. N-3 and not N-1 is assumed to be the position of ionisation since *X*-ray crystallographyl' [11] has shown the absence of a proton at

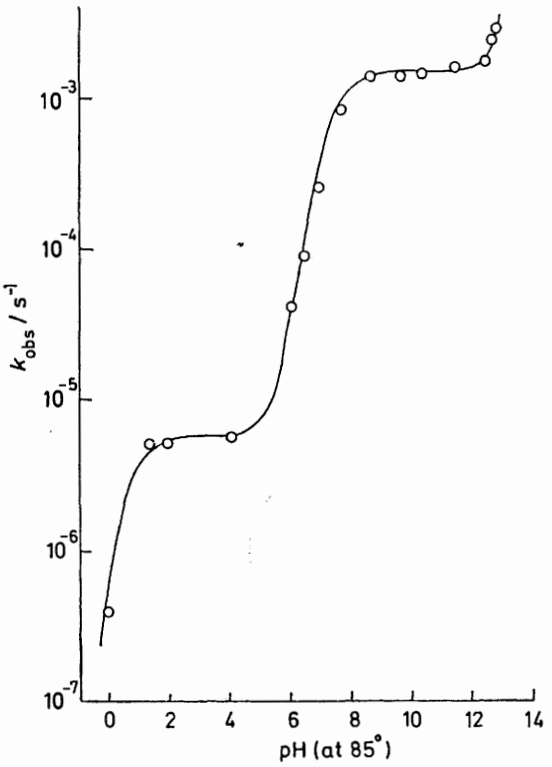


FIGURE 3 Rate–pH profile for the detritiation of [8-^{3}H]paraxanthine in aqueous buffers at 85°, ○ experimental points, — constructed using equation (11)

TABLE 1

Rate–pH data for [8-^{3}H]theophylline (1b), [8-^{3}H]caffeine (1c), [8-^{3}H]theobromine (1d), and [8-^{3}H]paraxanthine (1e)

pH (at 85°)	$10^6 k_{obs.}/s^{-1}$ (1b)	(1c)	(1d)	(1e)
−0.029	0.122			
0.00		0.50		0.388
0.61	0.644			
1.00	1.19	1.91	1.56	
1.40				5.03
2.00	1.66	2.50	2.06	5.12
3.00	1.78	2.58	2.36	
4.10				5.55
5.02			2.37	
5.60	1.64			
5.83	1.71			
5.84		2.52		
6.10				39.8
6.25	1.38	2.52		
6.48				87.4
6.72	1.39			
7.01				245
7.03	1.11	2.53		
7.41			3.10	
7.72				767
7.77	0.947			
8.36		4.70	6.17	
8.58	0.444			
8.70				1 260
8.81			9.22	
8.83		8.97		
9.02		11.6		
9.21		16.4		
9.27			16.9	
9.48		28.5		
9.49			18.1	
9.59		39.1		
9.69			21.5	1 310
10.37				1 390
10.50			34.7	
11.10		1 210		
11.50			190	1 530
11.79			318	
12.20			897	
12.50			1 970	1 700
12.78				2 280
12.90				2 810

N-3 in the xanthine monoanion. In this case the derived rate equation is the same as that obtained for guanine [5] [equation (13)]. In the pH region 2—5

$$k_{obs.} = \frac{kK_w}{[H^+] + K_a + \frac{K_aK_a'}{[H^+]} + \frac{K_aK_a'K_a''}{[H^+]^2}} + \frac{k'K_w}{[H^+] + \frac{[H^+]^2}{K_a} + K_a' + \frac{K_aK_a''}{[H^+]}} \quad (13)$$

equation (13) reduces to $k_{obs.} = kK_w/K_a$ and by using the reported [9] pK_a value (1.2 at 25°) and $k_{obs.}$ (3.33×10^{-6} s^{-1}) we obtain k (6.31×10^5 l mol^{-1} s^{-1}). In the second pH–rate independent region (8—10) equation (13) reduces to $k_{obs.} = k'K_w/K_a'$. The values of $k_{obs.}$ (1.05×10^{-4} s^{-1}) and pK_a' (6.5, *cf.* a reported value [9] of 7.5 at 25°), the latter having been obtained from the experimental data in the usual way, gives k' (92 l mol^{-1} s^{-1}). A value of 10.7 for pK_a'' was also obtained from the experimental data, and the computed curve derived using equation (13).

The final example, xanthosine (2) shows the most complicated behaviour of all (Figure 4). At low pH detritiation is accompanied by hydrolysis (to xanthine) with the result that the conventional first-order plots showed signs of curvature. It was however possible to calculate the detritiation rate constant over the initial part of the reaction; in addition estimates of the half-life of the hydrolysis were made at various pH values and the hydrolytic rate constants are plotted in Figure 4. The drawn line has a slope of −1 and the second-order rate constant at 85°, k_{hyd}, found to be 0.10 ± 0.03 l mol^{-1} s^{-1}. This observation agrees well with the mechanism

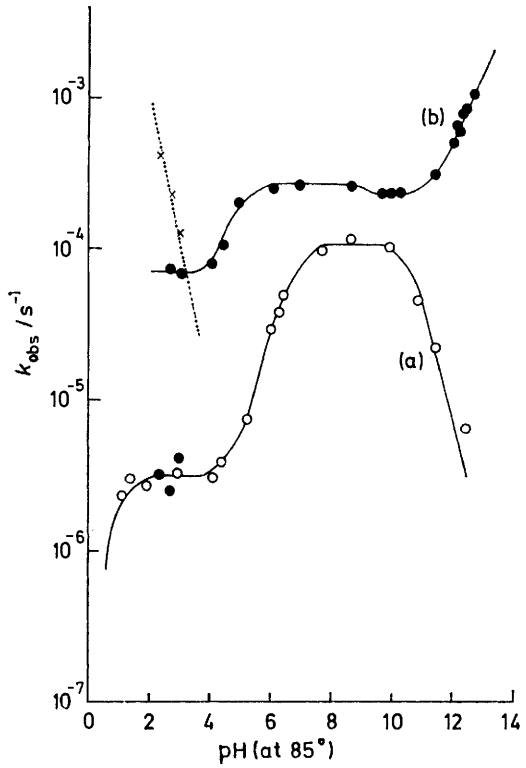


FIGURE 4 Rate–pH profile for the detritiation of (a) [8-^{3}H]-xanthine in aqueous buffers at 85°, ○ experimental points, — constructed using equation (13); (b) [8-^{3}H] xanthosine, ● experimental points, — constructed using equation (15). The crosses represent the rates of hydrolysis of xanthine (see text)

proposed by Zoltewicz *et al.*[12,13] It was also possible to allow the hydrolysis to proceed to completion, remove the tritiated water formed, and study the detritiation of the product. These post-hydrolysis values of the detritiation rate constants fall on the experimental rate–pH profile obtained for xanthine.

Over the pH range 2—8 the rate profiles for xanthosine and xanthine follow the same trend. At still higher pH (>9) they diverge with the detritiation rate constant for xanthosine increasing dramatically. This can only mean that the dianionic species which is the predominant form present in solution must be undergoing exchange. Although the 5′-hydroxy proton is also ionised previous studies [8] on adenosine showed that there was no need to take this into account. If the above interpretation is correct the relevant rate equation

TABLE 2

Rate–pH data for [8-^{3}H]xanthine (1a) and [8-^{3}H]-xanthosine (2)

pH (at 85°)	$10^6 k_{obs.}/s^{-1}$ (1a)	$10^6 k_{obs.}/s^{-1}$ (2) *
1.13	2.35	
1.40	3.02	
1.95	2.66	
2.31		(3.31)
2.72		74.7
		(2.50)
2.95	3.27	
3.03		67.9
		(4.08)
4.10	3.11	79.4
4.36	3.84	
4.46		108
5.00		180
5.24	7.31	
6.07	28.4	
6.10		249
6.31	37.5	
6.44	48.3	
7.01		257
7.72	97.4	
8.70	116	259
9.75		232
9.92	104	
10.01		232
10.30		239
10 92	46.0	
11.50	22.1	310
12.12		487
12.18		638
12.26		588
12.41		761
12.50	6.54	826
12.80		1 056

* Values in parentheses refer to post-hydrolysis results.

is (14). Using the respective dissociation constants $K_a = [BH_2][H^+]/[BH_3^+]$, $K_a' = [BH^-][H^+]/[BH_2]$, and

$$\text{Rate} = k_{obs.}[B]_T = k[BH_3^+][OH^-] + k'[BH_2][OH^-] + k''[BH^-][OH^-] + k'''[B^{2-}][OH^-] \quad (14)$$

$K_a'' = [B^{2-}][H^+]/[BH^-]$ it can be shown that equation (15) applies. At high pH equation (15) reduces to (16) so that a plot of $k_{obs.}$ against $[OH^-]$ should be linear with slope k'''; this is found to be so, the value of k''' being 6.06×10^{-4} l mol^{-1} s^{-1}. The pK_a values used to construct the theoretical pH–rate profile were obtained as described previously and had the following values pK_a 1.8 (derived from the literature pK_a value of 2.0 for 9-methylxanthine at 25°), pK_a' 4.6 (lit.,[14] 5.58 at 25°),

$$k_{obs.} = \frac{kK_w}{[H^+] + K_a + \frac{K_aK_a'}{[H^+]} + \frac{K_aK_a'K_a''}{[H^+]^2}} + \frac{k'K_w}{[H^+] + K_a' + \frac{[H^+]^2}{K_a} + \frac{K_a'K_a''}{[H^+]}} + \frac{k''K_w}{[H^+] + K_a'' + \frac{[H^+]^2}{K_a'} + \frac{[H^+]^3}{K_aK_a'}} + \frac{k'''[OH^-]}{1 + \frac{[H^+]}{K_a''} + \frac{[H^+]^2}{K_a'K_a''} + \frac{[H^+]^3}{K_aK_a'K_a''}} \quad (15)$$

pK_a'' 9.1 (lit.,[14] 9.87 at 25°). The resulting second-order rate constants were k 3.59×10^6, k' 2.05×10^4, and k'' 5.84×10^{-1} l mol^{-1} s^{-1}. With these values good

$$k_{obs.} = k''K_w/K_a'' + k'''[OH^-] \quad (16)$$

agreement with experiment was observed over the whole of the pH range studied.

The substitution of one or more methyl groups should have little effect on the rate constant for the reaction between the protonated species and hydroxide ion and this is borne out by the summarised data (Table 3) showing less than a five-fold difference in the values for xanthine and caffeine. The effect of additional methyl groups however is not strictly additive and it is para-xanthine that is the most reactive. Even in this case there is less than a ten-fold difference in rate. By analogy one might have expected a similarly small effect for the corresponding reaction between the neutral species and the hydroxide ion but this is clearly not the case as the values of k' vary by a factor close to 7.5×10^5. More informative possibly is the variation in the ratio k/k'—this is a maximum for caffeine (10^8), falling to 1.8×10^2 for xanthosine, *i.e.* decreases as the number of methyl groups is reduced. In previous studies, *e.g.* adenosine,[8] 9-alkylpurines,[4] we have shown that the

TABLE 3

Kinetic (l mol^{-1} s^{-1}) and equilibrium data derived from the rate–pH profiles together with known acidity constants

		Compound (1a)	(2)	(1b)	(1c)	(1d)	(1e)
pK_a	lit, 25° [a]	1.2	2.0 [b]	0.7	0.5	0.3	0.5
	kin, 85°	1.2	1.8	0.7	0.5	0.3	0.5
pK_a'	lit, 25° [a]	7.5	5.58 [c]	8.5		11.0	8.6
	kin, 85°	6.5	4.6	7.9		9.0	7.6
pK_a''	lit, 25° [a]		9.87 [b]				
	kin, 85°	10.7	9.1				
	$10^{-6}k$	0.63	3.59	1.14	2.52	3.76	5.50
	k'	92.0	2.05×10^4		2.72×10^{-2}	6.34×10^{-2}	98.4
	k''		0.58			1.95×10^{-3}	*ca.* 5×10^{-4}
	$10^4k'''$		6.06				

[a] Literature acidity constant data is taken from D. Lichtenberg, F. Bergmann, and Z. Neiman, *J. Chem. Soc.* (*C*), 1971, 1676, except where indicated. [b] Value for 9-methylxanthine. [c] P. R. Reddy, K. V. Reddy, and M. M. Taqui Khan, *J. Inorg Nuclear Chem.*, 1976, **38**, 1923.

ratio k/k' is of the order of 10^7—10^8 and can be ascribed to the effect of having a positive charge located [at N-7(9)] close to the C–H group undergoing exchange. The results for caffeine and theobromine are therefore consistent with these findings. As far as the other compounds are concerned we are led to the conclusion that in addition to the neutral compound another kinetically equivalent species [a zwitterion (3)] is involved and that this makes an increasingly important contribution in the order paraxanthine < xanthine < xanthosine. If this interpretation is correct the observed second-order rate constant k' is a composite of two terms, k_0 and $k_\pm$ [equation (17)], which refer to the

$$k' = k_0 + K_{zw}k_\pm \qquad (17)$$

neutral molecule and zwitterion, respectively; K_{zw} = [zwitterion]/[neutral molecule]. It seems significant that the smallest value of k' is for caffeine, the only compound which undergoes this second mechanism and which cannot form a zwitterion. The involvement of zwitterionic species in isotope exchange reactions of purine-containing compounds was previously invoked by Tomasz [15] and the currently observed variations in the ratio k/k' provide firmer evidence in favour of this viewpoint.

For the three compounds, theobromine, paraxanthine, and xanthosine which undergo reaction as the anion the rate constants k'' are considerably lower than the k' values. However the negative charge is located well away from the exchanging C–H position so that the magnitude of the effect in a negative sense is less than that arising from protonation of the nitrogen atom at position 7. For xanthosine the dianionic form is less reactive than the anionic form by a factor close to 10^3.

Finally mention must be made of the fact that in the present investigation on the kinetics of ionisation of various xanthines we have relied heavily on the use of rate–pH profiles to identify the nature of the reactive species. For the corresponding equilibrium ionisation processes u.v. and n.m.r. spectroscopy are the most frequently employed techniques. It follows therefore that in circumstances where the latter are unable to provide unambiguous evidence consideration may be given to the use of rate–pH profiles. This possibility is illustrated by the results for xanthine: from their u.v. data Bergmann and his co-workers [9] were unable to assign the site of ionisation that gave a pK_a of 11.0. The rate–pH profiles show that this value can be associated with the N-7(H) position.

One of us (S. E. T.) is grateful to the S.R.C. for financial support.

[8/1903 *Received*, 1*st November*, 1978]

REFERENCES

[1] Part 5, J. A. Elvidge, J. R. Jones, C. O'Brien, E. A. Evans, and H. C. Sheppard, *Adv. Heterocyclic Chem.*, 1974, **16**, 1.
[2] Presented in part at the Organic Reaction Mechanisms Discussion Group meeting, Cardiff, 1977.
[3] J. A. Elvidge, J. R. Jones, C. O'Brien, E. A. Evans, and J. C. Turner, *J.C.S. Perkin II*, 1973, 432.
[4] J. A. Elvidge, J. R. Jones, C. O'Brien, E. A. Evans, and H. C. Sheppard, *J.C.S. Perkin II*, 1973, 1889.
[5] J. A. Elvidge, J. R. Jones, C. O'Brien, E. A. Evans, and H. C. Sheppard, *J.C.S. Perkin II*, 1974, 174.
[6] R. N. Maslova, E. A. Lesnik, and Ya. M. Varshavsky, *FEBS Letters*, 1974, **49**, 181.
[7] M. Jelinska and J. Sobkowski, *Tetrahedron*, 1977, **33**, 803.
[8] J. A. Elvidge, J. R. Jones, C. O'Brien, E. A. Evans, and H. C. Sheppard, *J.C.S. Perkin II*, 1973, 2138.
[9] D. Lichtenberg, F. Bergmann, and Z. Neiman, *J. Chem. Soc.* (*C*), 1971, 1676.
[10] D. D. Perrin, *J. Chem. Soc.*, 1965, 5590.
[11] H. Mizuno, T. Fujiwara, and K. Tomita, *Bull. Chem. Soc. Japan*, 1969, **42**, 3099.
[12] J. A. Zoltewicz, D. F. Clark, T. W. Sharpless, and G. Grahe, *J. Amer. Chem. Soc.*, 1970, **92**, 1741.
[13] J. A. Zoltewicz and D. F. Clark, *J. Org. Chem.*, 1972, **37**, 1193.
[14] P. R. Reddy, K. V. Reddy, and M. M. Taqui Khan, *J. Inorg. Nuclear Chem.*, 1976, **38**, 1923.
[15] M. Tomasz, J. Olsen, and C. M. Mercado, *Biochemistry*, 1972, **11**, 1235.