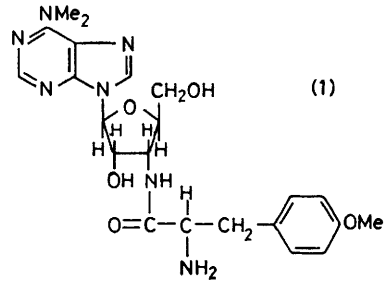
# Proton Transfer from Heterocyclic Compounds. Part 8.[1] Purine-containing Drugs

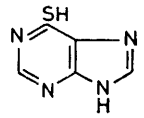
By **John A. Elvidge, John R. Jones,*** and **Rawa Salih,** Chemistry Department, University of Surrey, Guildford GU2 5XH

Rates of detritiation from the C-8 position of puromycin, mercaptopurine, and mercaptopurine riboside have been measured over a pH range at 85 °C. For the first two compounds two mechanisms, one involving the protonated molecule and hydroxide ion at low pH, the other the neutral molecule and the hydroxide ion at high pH, are operative. In the case of mercaptopurine riboside an additional mechanism which has previously only been observed for [8-$^3$H]-theobromine and -paraxanthine, is invoked; this involves hydroxide ion attack on the monoanionic form of the substrate.

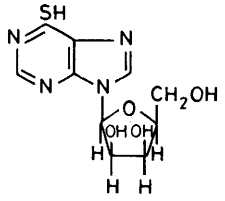
In recent years the criteria for acceptance of new drugs for pharmacological use have become more demanding. Knowledge of their metabolism, which is normally required before acceptance and usage is permitted, can frequently be obtained through the use of the appropriately labelled compound. Tritium labelling of drugs can, in general, be readily effected, obviating special synthesis, which materially reduces costs. The specificity of labelling can now be directly observed by $^3$H n.m.r. spectroscopy [2] which also enables possible trivial exchange reactions [3] to be readily detected. Thus the possible disadvantages in the use of tritium-labelled drugs are now overcome or minimised. Detailed studies of the rates of isotopic exchange define more closely the conditions under which the compounds may be safely employed; they can also provide valuable information concerning the reaction mechanism(s).[4] The present study reports findings on the kinetics of detritiation from the C-8 position of three purine-containing drugs,




(1)




(2)




(3)

puromycin (1) a well known antibiotic, 6-mercaptopurine (2) which has been widely used in the treatment of acute leukemia in children, and 6-mercaptopurine riboside (3) which is thought to be still more effective in this respect.

## EXPERIMENTAL

*Materials.*—The drugs were commercially available and were purified by standard methods. [8-$^3$H]Puromycin was prepared by dissolving the dihydrochloride (40 mg) in tritiated water (0.1 ml, 5 Ci ml$^{-1}$) and the solution kept in a sealed tube for 48 h at 60 °C. The tritiated water was then lyophilised, a small amount (1—2 ml) of water was added, and the solution neutralised with dilute alkali. On further lyophilisation the required product was obtained.

6-Mercapto[8-$^3$H]purine was prepared by first allowing a solution of 6-chloropurine (60 mg) in dioxan (0.5 ml) and tritiated water (10 μl, 50 Ci ml$^{-1}$) in a sealed tube to be kept for 12 h at 60 °C. Thiourea (35 mg) was then added and the reaction mixture warmed to 50 °C for 5 min before being left overnight at room temperature. Water (0.5 ml) was then added and the solvent lyophilised. The crude product was twice recrystallised from water–dioxan.

6-Mercapto[8-$^3$H]purine-9β-D-ribofuranoside was prepared by keeping a solution of the substrate (10 mg) in dioxan (0.02 ml) and tritiated water (0.05 ml, 5 Ci ml$^{-1}$) in a sealed tube for four days at 50 °C. At the end of this period the solvent was lyophilised and the solid washed twice with water to exchange labile hydrogens before final lyophilisation.

*Kinetics.*—Aqueous buffer solutions of known pH–temperature dependence were used and rates of detritiation were followed by measuring the increase in the radioactivity of the water; details of the procedure have been given.[5,6] Separate studies [7-9] have shown that all three drugs undergo decomposition when kept in dilute acid or alkali. For this reason it was customary to monitor (by u.v. spectroscopy) the reaction solutions. In all cases decomposition was negligible over the time interval in which the detritiation was studied.

## RESULTS AND DISCUSSION

The pseudo-first order detritiation rate constants for [8-$^3$H]puromycin (Table) when plotted (Figure) in the form of a rate–pH profile show three distinct regions: (1) at low pH (<4) where the rate decreases with increasing acidity, (2) at intermediate pH (4—9) where the rate is independent of pH, and (3) at high pH (>10) where the rate increases dramatically. This behaviour is reminiscent of that witnessed for 9-alkylpurines [6] and leads us to suggest that two mechanisms are operative. One at low pH involves the *N*-7 protonated molecule and the hydroxide ion (no buffer base catalysis was

TABLE

Detritiation rate constants ($k_{obs.}$) for puromycin (1), mercaptopurine (2), and mercaptopurine riboside (3) in aqueous buffers at 85 °C

| (1) | | (2) | | (3) | |
|---|---|---|---|---|---|
| pH (at 85 °C) | $10^5 k_{obs.}$ / $s^{-1}$ | pH (at 85 °C) | $10^5 k_{obs.}$ / $s^{-1}$ | pH (at 85 °C) | $10^5 k_{obs.}$ / $s^{-1}$ |
| 2.02 | 0.58 | 0.01 | 0.14 | 0.80 | 1.85 |
| 3.00 | 1.77 | 0.31 | 0.18 | 1.79 | 1.88 |
| 3.57 | 3.59 | 1.01 | 0.59 | 2.26 | 1.82 |
| 3.77 | 5.39 | 2.31 | 1.76 | 2.77 | 4.33 |
| 4.00 | 5.35 | 3.64 | 1.55 | 4.00 | 7.75 |
| 4.40 | 5.33 | 5.39 | 2.52 | 5.17 | 12.1 |
| 7.01 | 5.17 | 6.35 | 4.39 | 6.09 | 30.4 |
| 9.95 | 5.37 | 6.72 | 5.46 | 6.56 | 55.2 |
| 10.65 | 12.8 | 7.06 | 6.85 | 6.87 | 66.8 |
| 10.90 | 18.6 | 7.52 | 9.59 | 7.10 | 73.6 |
| 11.20 | 29.5 | 8.12 | 9.77 | 7.86 | 82.4 |
| 11.38 | 41.2 | 9.02 | 9.68 | 8.95 | 78.9 |
| 11.50 | 52.4 | 9.65 | 9.36 | 10.52 | 93.8 |
| | | | | 11.68 | 128 |
| | | | | 12.00 | 165 |
| | | | | 12.22 | 202 |
| | | | | 12.50 | 344 |

observed) and the other at high pH, involves reaction between the neutral molecule and hydroxide ion so that the rate is given by (1). With the total puromycin

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

concentration being expressed by $[B]_T = [BH^+] + [B]$ and $K_a = [B][H^+]/[BH^+]$, equation (2) can be derived; this simplifies to equation (3) at high pH so that a plot of $k_{obs.}$ against $[OH^-]$ should be linear (slope = $k'$). This is

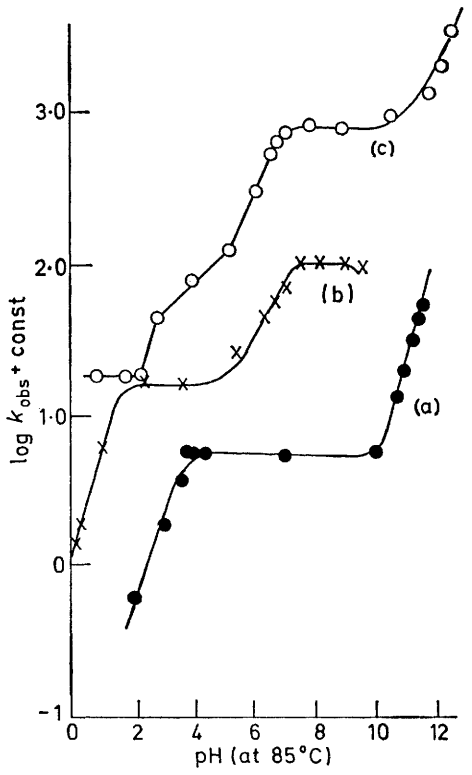
$$k_{obs.} = \frac{kK_w}{K_a + [H^+]} + \frac{k'K_a[OH^-]}{K_a + [H^+]} \quad (2)$$

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

indeed the case and by using the following values, $k' = 4.64 \times 10^{-3}$ l mol$^{-1}$ s$^{-1}$, $pK_a = 2.6$ (chosen by a trial and error procedure), and $k$ ($4.25 \times 10^5$ l mol$^{-1}$ s$^{-1}$) from $k_{obs.}$ ($5.35 \times 10^{-5}$ s$^{-1}$) = $kK_w/K_a$ in equation (2) a theoretical rate–pH profile can be constructed and found to be in good agreement with the experimental results, thereby supporting the proposed mechanism.

The rate–pH profile (Figure) for 6-mercapto[8-$^3$H]-purine is similar to that observed for guanine.[10] Both compounds have three $pK_a$ values in the pH range 0—14, protonation at N-7 ($K_a$) and ionisation at N-9 ($K_a''$) being common to both. If therefore we can assume that ionisation at S-6 ($K_a'$) is equivalent to ionisation at $N$-1 the derived rate expression (4), assuming that equation (1) is again valid, is the same as for guanine. The $pK_a$ values at 25 °C are as follows:[11] $pK_a < 2$, $pK_a' = 8.67$, and $pK_a'' = 11.9$. The best fit to the experimental data is obtained by using $pK_a = 1.2$, $pK_a' = 6.6$ and $pK_a'' = 9.7$, together with values of $k$($3.1 \times 10^6$ l mol$^{-1}$ s$^{-1}$) and $k'$ (77 l mol$^{-1}$ s$^{-1}$).

The insertion of the β-ribofuranosyl group in 6-mercaptopurine riboside effectively blocks ionisation at N-9. The very large rate increase (Figure) at high pH (>11) can therefore only come about as a result of hydroxide ion attack on the mono-anionic species. Such a mechanism has not been reported previously although experiments on [8-$^3$H]-theobromine and -para-




Rate–pH profile for the detritiation of (a) [8-$^3$H]puromycin; ●, experimental points, — calculated using equation (2); (b) [8-$^3$H]mercaptopurine; × experimental points, — calculated using equation (4); (c) [8-$^3$H]mercaptopyrine riboside; ○ experimental points, — calculated using equation (6). In the case of puromycin log $k_{obs.}$ is displaced by 1 unit

$$k_{obs.} = \frac{kK_w}{[H^+] + K_a + \frac{K_aK_a'}{[H^+]} + \frac{K_aK_a'K_a''}{[H^+]^2}} + \frac{k'k_w}{[H^+] + \frac{[H^+]^2}{K_a} + K'_a + \frac{K'_aK_a''}{[H^+]}} \quad (4)$$

xanthine in our laboratory [12] have led to similar conclusions. In the pH range 2—10 the rate-dependence is similar to the two other compounds studied. We therefore have a three-term rate equation (5).

$$\text{Rate} = k[BH_2^+][OH^-] + k'[BH][OH^-] + k''[B^-][OH^-] \quad (5)$$

which leads to (6).

$$k_{obs.} = \frac{kK_w}{[H^+] + K_a + \frac{K_aK_a'}{[H^+]}} + \frac{k'K_w}{[H^+] + \frac{[H^+]^2}{K_a} + K_a'} + \frac{k''[OH^-]}{1 + \frac{[H^+]}{K_a} + \frac{[H^+]^2}{K_aK_a'}} \quad (6)$$

At high pH (>10) equation (6) reduces to (7). The plot of $k_{obs.}$ against $[OH^-]$ is linear (slope = $k'' = 2.74 \times 10^{-3}$ l mol$^{-1}$s$^{-1}$). A good fit to the experimental data can

$$k_{obs.} = \frac{k'K_w}{K_a'} + k''[OH^-] \quad (7)$$

be obtained using this value as well as $pK_a = 1.2$. $pK_a' = 6.2$, $k = 3.7 \times 10^6$ l mol$^{-1}$ s$^{-1}$ and $k' = 1\,600$ l mol$^{-1}$ s$^{-1}$. The magnitude of the respective rate constants illustrate the importance of electrostatic factors in these reactions.

The results of these rate–pH profiles allow one to appreciate more clearly the factors that can influence the rates of isotopic hydrogen exchange; possible dangers of misinterpretation arising from the use of these labelled compounds are therefore minimised and optimum conditions for labelling (pH 9—11) and storage (pH 0—2 revealed.

[9/090 *Received*, 19*th January*, 1979]

REFERENCES

Part 7, J. R. Jones and S. E. Taylor, preceding paper.
[2] V. M. A. Chambers, E. A. Evans, J. A. Elvidge, and J. R. Jones, 'Tritium Nuclear Magnetic Resonance (tnmr) Spectroscopy,' Review 19, The Radiochemical Centre, Amersham, 1978.
[3] J. A. Elvidge, D. K. Jaiswal, J. R. Jones, and R. Thomas, *J.C.S. Perkin II*, 1977, 1080.
[4] J. A. Elvidge, J. R. Jones, C. O'Brien, E. A. Evans, and H. C. Sheppard, *Adv. Heterocyclic Chem.*, 1974, **16**, 1.
[5] J. A. Elvidge, J. R. Jones, C. O'Brien, E. A. Evans, and J. C. Turner, *J.C.S. Perkin II*, 1973, 432.
[6] J. A. Elvidge, J. R. Jones, C. O'Brien, E. A. Evans, and H. C. Sheppard, *J.C.S. Perkin II*, 1973, 1889.
[7] C. W. Waller, P. W. Fryth, B. L. Hutchings, and J. H. Williams, *J. Amer. Chem. Soc.*, 1953, **75**, 2025.
[8] P. W. Fryth, C. W. Waller, B. L. Hutchings, and J. H. Williams, *J. Amer. Chem. Soc.*, 1958, **80**, 2736.
[9] J. J. Fox, I. Wempen, A. Hampton, and I. L. Doer, *J. Amer. Chem. Soc.*, 1958, **80**, 1669.
[10] J. A. Elvidge, J. R. Jones, C. O'Brien, E. A. Evans, and H. C. Sheppard, *J.C.S. Perkin II*, 1974, 174.
[11] G. E. Cheney, H. Freiser, and Q. Fernando, *J. Amer. Chem. Soc.*, 1959, **81**, 2611.
[12] J. R. Jones and S. E. Taylor, *J.C.S. Perkin II*, 1979, 1253.