Tetrahedron Letters, Vol.22, No.45, pp 4525 - 4528, 1981
Printed in Great Britain

0040-4039/81/454525-04$02.00/0

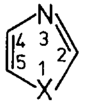
ISOTOPIC HYDROGEN EXCHANGE AT C_8 IN PURINES. EFFECTS OF THE SITE OF PROTONATION.

John R. Jones[a] and Spencer E. Taylor[b]

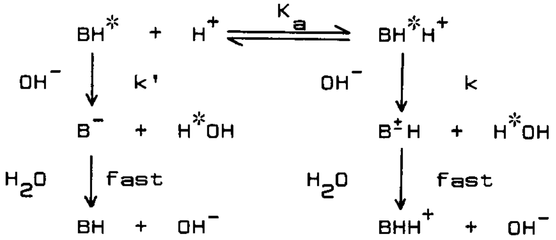
[a]Chemistry Department, University of Surrey, Guildford, Surrey, GU2 5XH, and [b]Inorganic Chemistry Laboratory, University of Oxford, South Parks Road, Oxford, OX1 3QR

Abstract

An analysis of linear-free-energy data for base-catalysed isotopic hydrogen exchange from the C_8 position of protonated purines indicates that N_7 is only partially protonated in aqueous solution.

Extensive studies have been made on the exchange of the C_2 proton in azole systems of the type 

where X = N-R, O, S, and Se.[1] The rate-pH profiles measured in aqueous buffers are, in general, readily explained in terms of rate-determining hydroxide ion attack on (a) the N_3-protonated molecule, BH^*H^+ (H^* represents the C_2 proton), which is operative at low pH (rate constant k), and (b) the neutral molecule, BH^*, operative at high pH (rate constant k'). The respective ylidic and carbanionic intermediates are then reprotonated by the solvent in a rapid step (Scheme). This scheme is expected to become more complex for substrates which are able to exist in more protonic states, and this has been borne out in our studies on the purines.[2]



Scheme

By analogy with the simple azoles (e.g. imidazole), we have assumed that the active purine species at low pH are the N_7-protonated molecules.[2a] However, as has been indicated both theoretically[3] and experimentally,[4] protonation is not restricted to N_7 in the purines, and sites such as N_1 and

N_3 have been implicated. We wish to show that the extensive literature data[2] available for tritium-hydrogen exchange from purine C_8 positions at 85°C are consistent with both of these views, and that it is indeed the N_7-protonated molecule, present to varying extents, that is responsible for exchange.

We have previously shown[1] that a linear relationship exists between log k and pK_a (defined in the Scheme) for a series of azolium ions, in which protonation has occurred α to the exchange site (i.e. at N_3). This is shown as the solid line in Figure 1,[5] the slope of which (-0.72; $r = 0.999$, 6 data points) we believe reflects the unequal substituent field effects on the protonation and detritiation processes. In linear-free-energy relationships (LFER's) of this type, therefore, it would be expected that $|\text{slope}| < 1$ if the exchange site is more remote than the protonation site, and *vice versa*.[6]

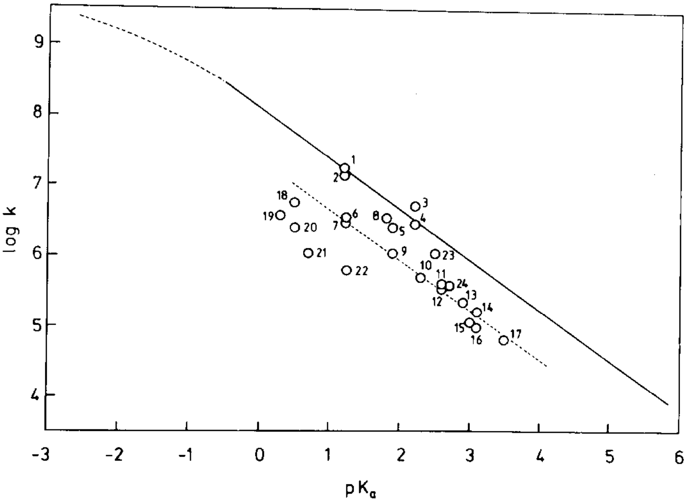


Figure 1. Plot of log k for detritiation from the C_2 position of protonated azoles and the C_8 position of protonated purines against their pK_a's at 85°C. The solid line drawn for the azoles is taken from ref.1. The numbered points refer to the following purines:- 1-methylinosine (1), inosine (2), 1-methylguanosine (3), guanosine (4), 9-methylhypoxanthine (5), 6-mercaptopurine riboside (6), 6-mercaptopurine (7), xanthosine (8), hypoxanthine (9), purine (10), puromycin (11), guanine (12), adenosine (13), adenosine 3'-monophosphate (14), adenosine 5'-monophosphate (15), adenosine 3',5'-cyclic monophosphate (16), adenine (17), paraxanthine (18), theobromine (19), caffeine (20), theophylline (21), xanthine (22), 9-iso-propylpurine (23), and 9-tert-butylpurine (24).

When the corresponding data for the purine derivatives are plotted[2] (Fig. 1), in general they do not appear to fit the same correlation. Only in a few instances do the data fall on, or close to, the drawn line. For the majority of the remaining points, a reasonable correlation exists (dotted line) with approximately the same slope, but is displaced by *ca.* 0.7 log units below the original line. We rationalise these observations in terms of exclusive N_7 protonation in those compounds which fit the LFER, and only partial N_7 protonation in those compounds falling below this line. On electrostatic grounds, contributions to the rate from other than N_7-protonated forms are expected to be negligible. On this assumption, for those compounds which fall on the dotted line, N_7 is protonated to the extent of *ca*. 20%, whereas in the xanthines in which N_7 is methylated, the results suggest that only *ca*. 6% of the N_9-protonated form exists, in line with previous u.v. and ^{1}H n.m.r. spectroscopic data[8] which indicated that O_6 was the major protonation site.

The abnormally low exchange rates encountered above are also reflected in the magnitude of the proton activating factors (**paf**'s) associated with these substrates. In the present examples, the **paf** is defined as the ratio k/k' (Scheme). Stewart and Srinivasan's compilation[9] of **paf** values for different classes of carbon acid illustrates the broad range of values obtained (spanning *ca*. 8 orders of magnitude) and demonstrates that there are many factors to be considered under the general guise of "proton activation". However, as we have previously shown,[10] protonation and ionisation effects on rates of detritiation in purines can be adequately analysed in terms of simple electrostatics, assuming the formation of a product-like transition-state. Within this class of substrate, therefore, it is not unreasonable to expect that, providing common protonation sites exist throughout such a series, the **paf** value should remain constant, i.e. log k = log k' + log(**paf**). Figure 2 is plotted according to this

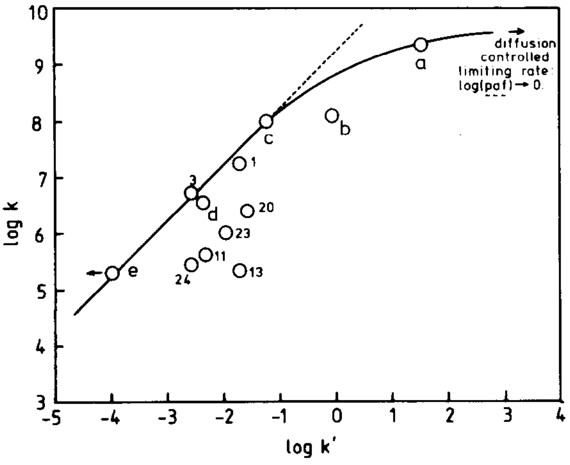


Figure 2. Plot of log k vs. log k' for the detritiation of tetrazole (a), benzoxazole (b), benzothiazole (c), thiazole (d), 1-methylbenzimidazole (e), and several purines (the numbers correspond to those listed in Fig. 1). The azole data is taken from refs. 1 and 11, and the log k' value for 1-methylbenzimidazole constitutes an upper limit. The straight line is of unit slope.

equation for the available literature data; clearly, the limited data for those substrates in which exclusive N_7-protonation (or the equivalent) occurs fit such a correlation (the line is drawn with unit slope and log(**paf**) = 9.20) until, for the most reactive substrate, tetrazole, some deviation occurs due to the advent of a diffusion-limiting value of k. Once again, the purines which do not have N_7 as the sole protonation site are deviant, resulting in a decreased **paf**.

In conclusion, the reactivity of protonated purines towards C_8 proton exchange is generally lower than expected when compared to substrates which protonate exclusively α to the exchange site, which is a result of additional, more remote protonation sites in the former. This is in agreement with other solution studies on purines, which suggest the existence of several protonated species in solution, unlike crystallographic studies which single-out the thermodynamically most stable form. The correlations presented herein also add support to the interpretation that isotopic hydrogen exchange at C_8 in purines proceeds *via* the N_7- (or N_9-) protonated molecule.[2a]

References and Notes

1. See for example J.A. Elvidge, J.R. Jones, R. Salih, M.Y. Shandala and S.E. Taylor, *J. Chem. Res.* (S) 172; (M) 2375 (1980) and refs. therein.
2. (a) J.A. Elvidge, J.R. Jones, C. O'Brien, E.A. Evans and H.C. Sheppard, *J.C.S. Perkin II*, 1889(1973); (b) *idem*., 2138(1973); (c) *idem*., 174(1974); (d) J.R. Jones and S.E. Taylor, *ibid*., 1253(1979); (e) *idem*., 1587(1979); (f) J.A. Elvidge, J.R. Jones and R. Salih, *ibid*., 1590(1979); (g) J.R. Jones and S.E. Taylor, *ibid*., 441(1980).
3. B. Pullman and A. Pullman, "Quantum Biochemistry", Wiley-Interscience, London, 1963, pp 232-233.
4. (a) H.C. Borrensen, *Acta Chem. Scand*., **21**, 2463(1967); (b) A. Bendich, P.J. Russell, jr. and J.J. Fox, *J. Amer. Chem. Soc*., **76**, 6073(1954); J.M. Read, jr. and J.H. Goldstein, *ibid*., **87**, 3440(1965); R.J. Pugmire and D.M. Grant, *ibid*., **93**, 1880(1971); V. Markowski, G.R. Sullivan and J.D. Roberts, *ibid*., **99**, 714(1977).
5. Since the second-order rate constant, k, is calculated from the pseudo-first-order rate constant in the pH-independent region of the rate-pH profile, k_ψ, according to $k_\psi = k\,K_w/K_a$, a plot of log k_ψ *vs*. pK_a will give an equally good correlation with slope 0.28; however, the second-order rate constants convey greater significance, and, hence, are preferred here.
6. We are grateful to Prof. J.A. Zoltewicz, University of Florida, who has drawn our attention to his study[7] on the rates of formation of 3-substituted pyridinium ylides at the 2- and 6-positions. The slopes of the derived log k *vs*. pK_a (for protonation at the 1-position) plots are respectively *ca*. -1.2 and *ca*. -0.65, which are consistent with our present interpretation.
7. J.A. Zoltewicz and R.E. Cross, *J.C.S. Perkin II*, 1363(1974).
8. D. Lichtenberg, F. Bergmann and Z. Neiman, *J. Chem. Soc. (C)*, 1676(1971).
9. R. Stewart and R. Srinivasan, *Acc. Chem. Res*., **11**, 271(1978).
10. S.E. Taylor, *Canad. J. Chem*., **58**, 86(1980).
11. J.A. Elvidge, J.R. Jones, C. O'Brien, E.A. Evans and J.C. Turner, *J.C.S. Perkin II*, 432(1973).

(Received in UK 21 August 1981)