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THE KINETICS OF TRITIUM–HYDROGEN EXCHANGE BETWEEN XANTHINE, THEOPHYLLINE, CAFFEINE AND WATER

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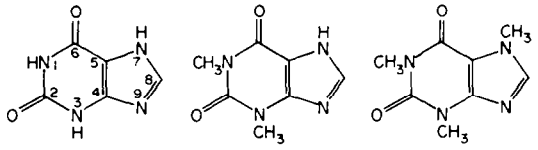
Abstract—Isotopic hydrogen exchange at C-8 of xanthine, theophylline and caffeine in water has been studied at several temperatures and constant pH. The rates of detritiation of these compounds have been determined over a pH range at constant temperature. The rate–pH profiles for theophylline and caffeine are interpreted in terms of rate determining attack by hydroxide ion on protonated substrate. For caffeine and xanthine at high pH an additional mechanism involving hydroxide catalysed exchange of the neutral compound is suggested.

The isotopic hydrogen exchange reactions in purines have applications in preparation of labelled compounds for use in biochemical studies. Data on the exchange rate and the influence of substituents on the lability of C-8 proton provide information on the structure of these compounds. The development of nucleic acid chemistry has stimulated studies on the exchange reaction of hydrogen in purines such as adenine, guanine and their nucleosides and polynucleotides.[1–6] In this work the rate of hydrogen exchange in xanthine **1**, theophylline **2** and caffeine **3** and mechanism of the reaction has been studied.



1 **2** **3**

EXPERIMENTAL

Materials. Xanthine labelling with tritium was described earlier.[7] Theophylline (Sigma) and caffeine (Fluka, AG), easily soluble in water, were labelled by warming with HTO (concentration of theophylline and caffeine 0.4 M and 0.8 M, respectively, time of exchange 40–50 h, temp. 90°C, specific activity of water *ca.* 80 mCi/cm³). After the exchange had been completed the water was removed by distillation, a small portion of H_2O was added to exchange the labile hydrogen atoms and distillation was repeated. The specific activities of theophylline and caffeine were 30 mCi/mmol and 50 mCi/mmole, respectively, 98–99% of activity originated from tritium attached to C-8.

Buffer solutions. For kinetics measurements acetate and ammonia buffers were used. The inoic strength of solutions was equal to 1 M and was kept constant be adding the proper amount of KCl. The pH measurements were carried out at 20°C using digital pH meter (E 500 type). It was assumed that pH of these solutions was independent of temperature. Hydrochloric acid and potassium hydroxide were used to prepare solutions of low and high pH, respectively. The pH values was calculated taking into account the proper values of activity coefficients of these solutes. The value of pK_w at 82°C was taken to be equal to 12.56.[8]

Kinetics. The procedure for determination of detritiation kinetics was as follows. The weighed sample of labelled compound (m = 0.03–0.04 g) was dissolved in buffer (V = 10 cm³) in a vessel equipped with a syringe and kept at constant temperature in a thermostat. At suitable intervals of time a small sample of solution had been taken and water was separated by vacuum distillation. The activity of water was determined in scintillation spectrometer Packard Model 3380. The liquid scintillator of PPO + POPOP with ethoxyethanol in toluene was used.[9]

The reaction was slow and the measurements had to be carried out at elevated temperature. However, the degree of exchange has never exceeded 4–5% and the rate constant of reaction could be calculated from the relation $k_{obs} = A_t/A_\infty t$, where A_t and A_∞ are activities of water at time t of reaction and in equilibrium ($t = \infty$), respectively. A_∞ was assumed to be equal to activity of the sample of the compound taken to exchange.

RESULTS

The plots of relative k_{obs} vs pH for given compounds are presented in Figs. 1–3. The values of k_{obs} were determined for various temperatures and at constant pH to calculate the apparent energy of activation. The data are given in Table 1.

DISCUSSION

The results of kinetic measurements of hydrogen C-8 isotopic exchange in purines are usually interpreted using

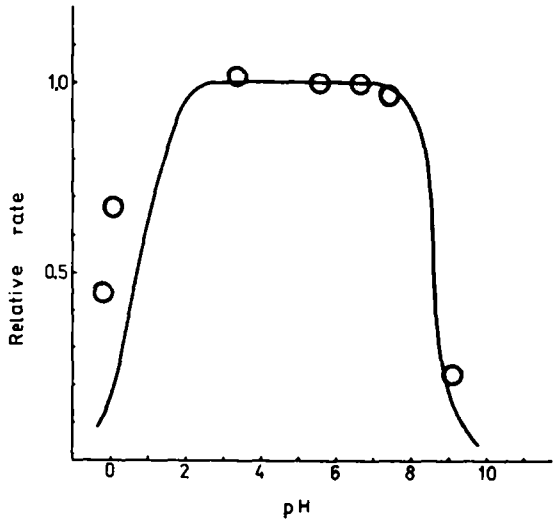


Fig. 1. The α-pH profile for [8-³H] theophylline at 82°C O—the values observed experimentally. The solid line is drawn using Eqn (3).

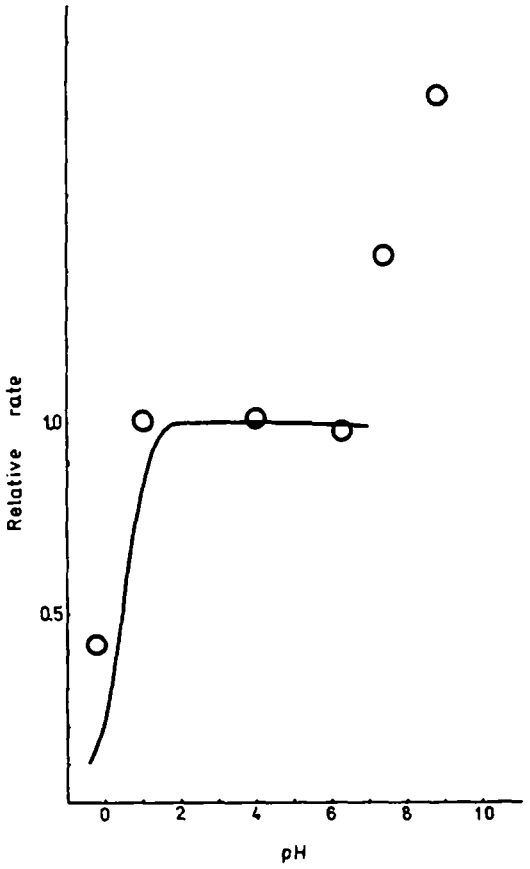


Fig. 2. The α-pH profile for [8-^{3}H] caffeine at 82°C O—the values observed experimentally. The solid line is drawn using Eqn (4).

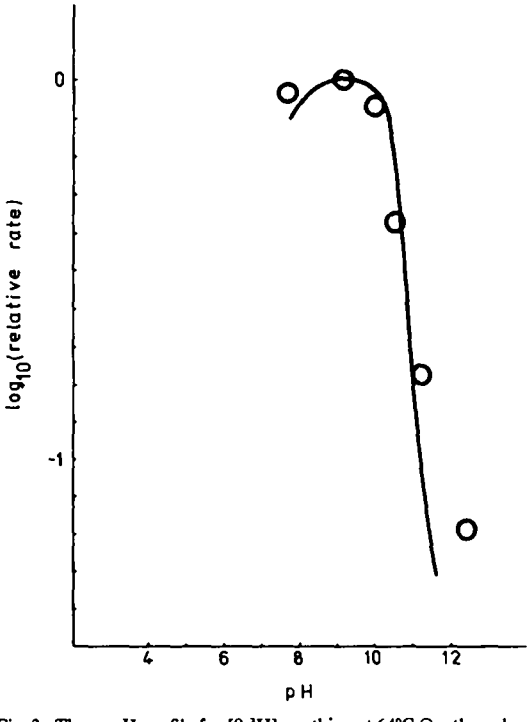


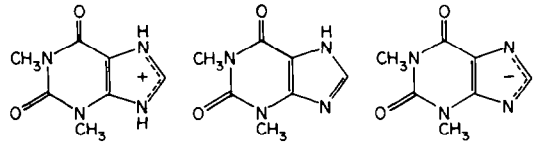
Fig. 3. The α-pH profile for [8-^{3}H] xanthine at 64°C O—the values observed experimentally. The solid line is drawn using Eqn (6).

Table 1. Observed rates of detritiation of [8-^{3}H] theophylline, [8-^{3}H] caffeine and [8-^{3}H] xanthine in H_2O at various temperatures

	theophylline pH = 5.7	caffeine pH = 7.0	xanthine pH = 12.6
T, °K		$10^7 k_{obs}$, s^{-1}	
337			7.5
342		5.8	
346	4.9		18.7
348		10.4	
355	11.0	19.1	37.2
360		34.4	
364	28.5		92.6
		$E_{a\ obs}$, kcal mol^{-1}	
	23.3	25.2	23.8

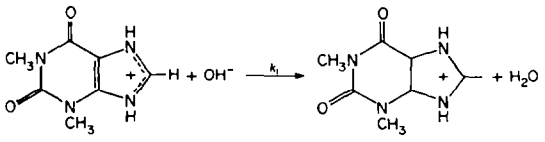
imidazole or benzimidazole as a simple model system (owing to purines C-8 and imidazole C-2 analogy).[2,10-12]

Among the xanthine derivatives, theophylline, which has no free NH groups in the pyrimidine ring, is the most similar to this model. The presence of three different forms of this compound in aqueous solution suggest the

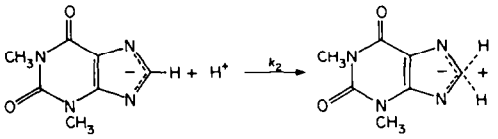


2a **2b** **2c**

possibilities of different mechanism of hydrogen exchange. The rate determining step may be the attack of H^+ ion on C-8 atom of anionic form or neutral molecule, or proton abstraction from C-8 atom of protonated form or neutral molecule, by OH^- ion. From the rate –pH profile (Fig. 1) follows that the mechanism with neutral molecule may be excluded but it is not possible to decide whether the rate determining step is the attack by hydroxide ion on protonated substrate **2a**:



or by hydrogen ion on anionic form **2c**:



In the first case the rate of reaction:

$$v_1 = k_1[TH_2^+][OH^-] = k_{obs}[T].$$

Since the total concentration of theophylline is equal to:

$$[T]_{tot} = [T^-] + [TH] + [TH_2^+],$$

$$K_1 = [TH][H^+]/[TH_2^+]$$

and

$$K_2 = [T^-][H^+]/[TH],$$

the rate of exchange can be written as follows:

$$v_1 = k_1[T][OH^-]/\{1 + K_1/[H^+] + K_1K_2/[H^+]^2\} = k_{obs}[T]$$

and

$$k_{obs} = k_1K_w/\{[H^+] + K_1 + K_1K_2/[H^+]\}. \quad (1)$$

Similarly, for the second case, $v_2 = k_2[T^-][H^+]$ and:

$$k_{obs} = k_2K_1K_2/\{[H^+] + K_1 + K_1K_2/[H^+]\}. \quad (1a)$$

As follows from Eqns 1 or 1a the rate constant has a maximum for $[H^+] = (K_1K_2)^{1/2}$. Since $K_1 \gg K_2$

$$k_{obs}^{max} = k_1K_w/\{2(K_1K_2)^{1/2} + K_1\} = k_1K_w/K_1 \quad (2)$$

or

$$k_{obs}^{max} = k_2K_1K_2/\{2(K_1K_2)^{1/2} + K_1\} = k_2K_2. \quad (2a)$$

The relative rate, defined as a fraction k_{obs}/k_{obs}^{max} may be calculated from the relation:

$$\alpha = k_{obs}/k_{obs}^{max} = K_1[H^+]/\{[H^+]^2 + K_1[H^+] + K_1K_2\}. \quad (3)$$

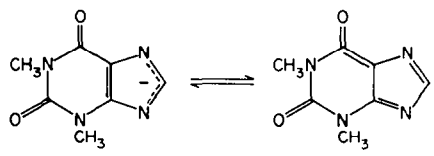
From this relation follows that it is not possible to decide which step of the reaction is rate determining because the α vs pH plot is the same for both mechanisms. In the Fig. 1 this plot is represented by the solid line. The pK values for theophylline at 70°C are known ($pK_1 = 0.7$, $pK_2 = 8.5$)[13] and according to the semi-empirical Perrin equation,[14] at 82°C they are equal to 0.7 and 8.2, respectively.

The mechanism of exchange is more unequivocal for caffeine detritiation. In this case owing to the lack of anionic form the mechanism by the electrophilic attack of H^+ ion is excluded. Assuming the rate determining step as a proton abstraction from C-8 by hydroxide ion we obtain:

$$k_{obs} = k_3K_w/\{[H^+] + K_1\}. \quad (4)$$

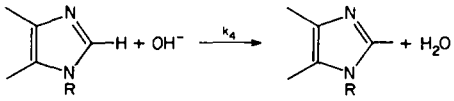
The relative rate constant −pH profile is presented in Fig. 2 as a solid line. The pK value for caffeine is assumed to be equal to 0.5.[13,14] The comparison of profiles for theophylline and caffeine detritiation indicates that proton abstraction by OH^- ion rather than electrophilic attack of H^+ ion is the rate determining step in both cases. The lower values of k_{obs} for theophylline are probably caused by the occurrence of negative charge on imidazole ring owing to the dissociation of N(7)–H group. The spectroscopic data show that anion of theophylline may exist in two forms **2c** and **2c'**.[13,15] It was suggested by Cavalieri and Fox[15] that form **2c'** was predominant but recent data of IR spectra[16] indicates that the anion exists mainly in the form **2c**.

For pH > 7 the increase of the rate constant of exchange for caffeine is observed what points out that



2c **2c'**

another mechanism should be taken into account. The similar effects were observed previously for heterocyclic compounds substituted in imidazole ring.[2,3,4,11,17] It was suggested that it is due to the attack of hydroxide ions on neutral form of compound:



Hence

$$v_4 = k_4[C][OH^-] = k_4[C]_{tot}[OH^-]/\{1 + [H^+]/K_1\}$$

and

$$k_{obs} = k_4K_w/\{[H^+] + [H^+]^2/K_1\}. \quad (5)$$

It is possible that similar mechanism is true for detritiation of caffeine as well. The decomposition of caffeine precludes the investigation at higher pH than 11 (at pH 10, 90° and 4 hr of reaction the decomposition is not significant). The similar mechanism for theophylline and some other compounds with unsubstituted imidazole ring is not observed[10,11] apparently due to the appearence of negative charge owing to the dissociation of N(7)—H group.

The kinetics of xanthine detritiation is more complicated because there are several dissociable groups. The pK values for dissociation of N(7)–H and N(3)–H groups at 70° are equal to 7.5 and 11.0[13] (in the pH range 5–14 xanthine forms only a dianion[13,18]), but the sequence of the dissociation of these groups is still controversial.[13,15] If the N(7)–H group dissociates first the situation would be similar to theophylline, and the rate determining step should be the reaction with protonated form. However, if the dissociation of N(7)–H follows the dissociation of N(3)–H, the likely mechanism would involve rate determining attack by the hydroxide ion on the neutral molecule and the observed rate constants should be written as follows:

$$k_{obs} = k_5K_w/\{[H^+] + [H^+]^2/K_1 + K_2 + K_2K_3/[H^+]\}. \quad (6)$$

The low solubility of xanthine in water does not allow to study the kinetics at pH below 7.5, but the experimental data are in agreement with the relative rate of reaction vs pH plot represented by Eqn (6) (a solid line in Fig. 3). So the attack by the hydroxide ion on the neutral molecule is the rate determining step, at least for high values of pH.

The obtained values of observed activation energy (Table 1) are typical for the proton transfer reactions in water solutions. Similar values E_{obs} are given for adenine[3] and hypoxanthines.[19] Once the mechanism of exchange is chosen, it is also possible to find the values of second-order rate constants of reaction between substrate and hydroxide ion. The rate constants of reaction with

Table 2. Calculated second-order rate constants of detritiation of [8-^{3}H] theophylline and [8-^{3}H] caffeine in H_2O at various temperatures

T, °K	10^{14} K_w	10^{-5} k_1, l mol^{-1}s^{-1}	10^{-5} k_3, l mol^{-1}s^{-1}
342	15.0		12.4
346	18.2	5.4	
348	20.0		16.6
355	27.0	8.2	22.6
360	33.8		32.6
364	40.4	14.1	

protonated substrate in rate determining step for theophylline and caffeine are given in Table 2.

The activation energies obtained from plots of k_1 and k_3 vs 1/T are equal to 13.5 kcal/mol and 12.8 kcal/mol for theophylline and caffeine respectively. The activation entropy for both theophylline and caffeine is about +5 cal/mol K. The similar value of activation entropy obtained for adenine $\Delta S = +7 \pm 1$ cal/mol K[3] was shown to be typical for the reactions between hydroxide ion and protonated substrate.

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REFERENCES

[1]K. R. Shelton and J. M. Clark, *Biochemistry* **6**, 2735 (1967).
[2]M. Tomasz, J. Olson and C. M. Mercado, *Ibid.* **11**, 1235 (1972).
[3]J. A. Elvidge, J. R. Jones, C. O. Brien, E. A. Evans and H. C. Sheppard, *J. Chem. Soc. Perkin II* 2138 (1973).
[4]J. A. Elvidge, J. R. Jones, C. O. Brien, E. A. Evans and H. C. Sheppard, *J. Chem. Soc. Perkin II* 174 (1974).
[5]J. Livramento, G. J. Thomas, *J. Am. Chem. Soc.* **96**, 6529 (1974).
[6]G. V. Sidorov and N. F. Miasoyedov, *Radiochimiya* **16**, 922 (1974).
[7]J. Szydłowski and M. Jelińska, *Radiochem. Radioanal. Letters* **5–6**, 355 (1974).
[8]H. L. Clever, *J. Chem. Educ.* **45**, 231 (1968).
[9]D. White, *Intern. J. Appl. Rad. Isotop.* **19**, 49 (1968).
[10]J. A. Elvidge, J. R. Jones, C. O. Brien, E. A. Evans and J. C. Turner, *J. Chem. Soc. Perkin II* 432 (1973).
[11]J. A. Elvidge, J. R. Jones, C. O. Brien, E. A. Evans and H. C. Speppard, *Ibid.* 1889 (1973).
[12]J. D. Vaughan, Z. Mughrabi and E. C. Wu, *J. Org. Chem.* **35**, 1141 (1970).
[13]D. Lichtenberg, F. Bergmann and Z. Neiman, *J. Chem. Soc.* 1676 (1971).
[14]D. D. Perrin, *Austral. J. Chem.* **17**, 484 (1964).
[15]L. F. Cavalieri, J. J. Fox, A. Stone and N. Chang, *J. Am. Chem. Soc.* **76**, 1119 (1954).
[16]A. Psoda, unpublished data.
[17]J. L. Wong and J. H. Keck, *J. Org. Chem.* **39**, 2398 (1974).
[18]W. Pfleiderer and G. Nubel, *Ann.* **647**, 155 (1961).
[19]D. Lichtenberg and F. Bergmann, *J. Chem. Soc. Perkin I* 789 (1973).