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REVERSIBLE HYDRATION ACROSS C=N BOND IN POLYAZANAPHTHALENES AND THEIR DERIVATIVES

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Abstract—Reversible hydration to a number of substances named in the title is acid-base catalyzed. The details of the manner of the catalysis are discussed. Because of the reversible nature, the relationship between the rates and equilibria involved in these reactions has been demonstrated for a series of compounds. Reversible hydration of 2,6-dihydroxypteridine in aqueous solutions can give rise to two different water-adducts, the 3,4-adduct and the 7,8-adduct. Although the thermodynamically less stable product, the 3,4-adduct, is formed more rapidly in the solution, thereafter, isomerization occurs, leading to an equilibrium mixture of the two isomers, the 7,8-adduct being the major species at equilibrium. The kinetics and equilibrium studies of these reactions have led to definition of the appropriate conditions under which high yields of the less stable reaction product may be obtained.

INTRODUCTION

UNTIL recently, little has been known concerning the ease and manner of addition of water across a C=N bond in an organic molecule, although the addition of water across C=C and C=O bonds has been examined in detail.

The first example of reversible water-addition across a C=N bond was due to the observation by Albert *et al.*[1] of the anomalous physical properties of 6-hydroxypteridine, and the location of the water molecule across $C_{(7)}$-$N_{(8)}$ was demonstrated by Brown and Mason.[2] Subsequently, a number of heteroaromatic compounds are found to show similar hydration effects: 2-Hydroxypteridine and its methyl derivatives hydrate across the 3,4-position in the neutral molecule.[2] Although the hydration reaction is not detectable in quinoline, isoquinoline and their derivatives, among the diazanaphthalenes it is common in the quinazoline series.[3–6] Quinazoline and many of its derivatives exist predominantly as the hydrated form in the cation while the stable neutral molecule is the anhydrous species. Hydration has also been observed among some of the triazanaphthalenes,[7,8] 1,4,5,8-tetraazanaphthalene,[9] and 1,3,5,8-tetraazanaphthalene (= pteridine) and its methyl derivatives.[10,11]

The quantitative investigation of the equilibria involved in reversible hydration of C=N bonds has now been completed, so that the present paper deals with the theoretical aspects of the problem of thermodynamics and kinetics of the process.

[1] A. Albert, D. J. Brown, and G. Cheeseman, *J. Chem. Soc.* 1620 (1952).
[2] D. J. Brown and S. F. Mason, *J. Chem. Soc.* 3443 (1956).
[3] A. Albert, *J. Chem. Soc.* 2690 (1955).
[4] A. Albert, W. L. F. Armarego, and E. Spinner, *J. Chem. Soc.* 2689 (1961).
[5] A. Albert, W. L. F. Armarego, and E. Spinner, *J. Chem. Soc.* 5267 (1961).
[6] W. L. F. Armarego, *J. Chem. Soc.* 561 (1962).
[7] A. Albert and C. Perdersen, *J. Chem. Soc.* 4683 (1956).
[8] W. L. F. Armarego, *J. Chem. Soc.* 4094 (1962).
[9] Personal communication from Dr. W. L. F. Armarego.
[10] A. Albert, D. J. Brown, and H. C. S. Wood, *J. Chem. Soc.* 2066 (1956).
[11] D. D. Perrin, *J. Chem. Soc.* 645 (1962).

EQUILIBRIUM IN HYDRATION REACTIONS

One of the most interesting effects is the production of a hysteresis loop in the course of carrying out a rapid titration of 6-hydroxypteridine in water with alkali, followed by back-titration with acid. On the other hand, if the titration was carried out sufficiently slowly, only one curve (intermediate between the other two) was obtained. This phenomenon is illustrated in Fig. 1.

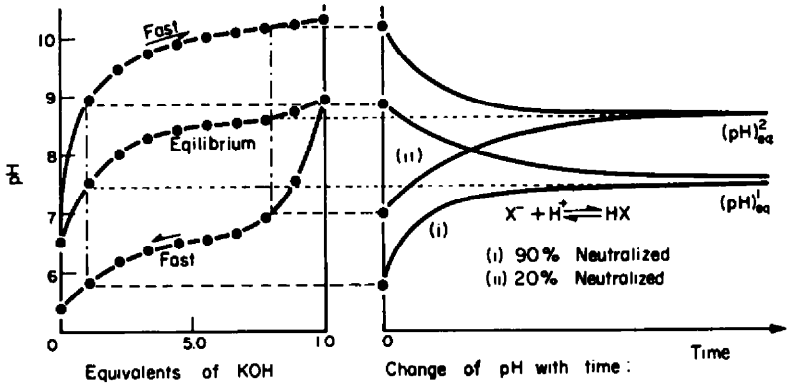
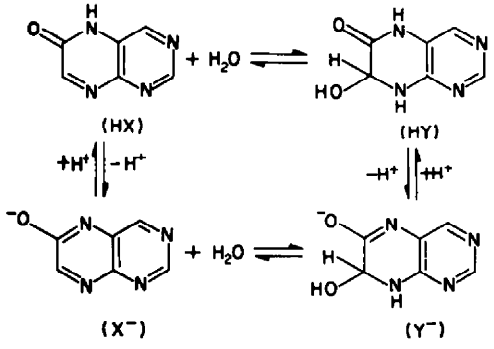


FIG. 1. Hysteresis loop in titration of 10^{-3}M aqueous 6-hydroxypteridine solution with 0·1 N-alkali and -acid.

This hysteresis loop in the titration of 6-hydroxypteridine[12] appeared to be due to the relatively slow, reversible, covalent water-addition reaction, in accordance with the following reaction scheme:



The pK_a value of 6·45 (at 20°) obtained by rapid titration of the anion with acid is practically that of the anhydrous species, while the pK_a value of 9·90 by titration of the neutral molecule with alkali is that of the hydrated form. The marked change in pK_a may be attributed to resonance interaction of the aromatic ring.

The measured dissociation constants, K_a^x and K_a^y ("X" and "Y" denote "anhydrous" and "hydrated", respectively), may be expressed by

$$K_a^x = [H^+][X^-]/[HX] \quad \text{and} \quad K_a^y = [H^+][Y^-]/[HY].$$

[12] Brown and Mason[8] concluded from ionization and spectroscopic data (both UV and IR spectra) that all the monohydroxypteridines exist mainly in the —CO—NH— form both in solid state and in aqueous solution.

Where a monobasic acid, HX, and its anion, X^-, exist in equilibrium with the corresponding hydrated species, HY and Y^-, the measured, equilibrium proton-dissociation constant, K_a^{eq}, is

$$K_a^{eq} = [H^+]([X^-] + [Y^-])/([HX] + [HY]).$$

The equilibrium quotient, K_1, for the reversible hydration of a monobasic acid, HX, to give the hydrated species, HY, is defined as

$$K_1 = [HY]_{eq}/[HX]_{eq} = (K_a^x - K_a^{eq})/(K_a^{eq} - K_a^y). \quad (1)$$

Similarly, for the equilibrium, $X^- + H_2O \rightleftharpoons Y^-$,

$$K_2 = [Y^-]_{eq}/[X^-]_{eq} = K_1K_a^y/K_a^x. \quad (2)$$

The result can be generalized. For the reversible hydration of the multibasic acid, H_nX to H_nY, the corresponding equilibrium quotients can be written as

$$K_{i+1} = K_iK_{a_i}^y/K_{a_i}^x \ (i = 1, 2, 3, ---, n)$$

$$= K_1 \prod_{i=1}^{n} K_{a_i}^y / \prod_{i=1}^{n} K_{a_i}^x = [H_{n-i}Y^{-i}]_{eq}/[H_{n-i}X^{-i}]_{eq}. \quad (3)$$

From an adequate analysis of a hysteresis loop as shown in Fig. 1, K_a^x, K_a^y and K_a^{eq} can be obtained. Thus, in many cases, the upper and the lower curves in this loop represent the titration curves of almost pure hydrated and anhydrous species. This is because, in these cases, in neutral and alkaline solutions the equilibria lie far over towards the hydrated and anhydrous species, respectively. Hence, the two curves obtained from the rapid titrations can be used to afford directly the pK_a values for the hydrated and anhydrous molecules.

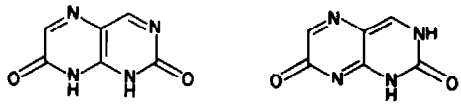
In a similar manner, the intermediate curve in the hysteresis loop leads to an accurate value of pK_a^{eq}. Thus, using these pK_a values obtained in this way, the values of the equilibrium quotients, K_1 and K_2, can be calculated. Some representative results are summarized in Table 1.

The reversible water-addition reaction across C=N bonds in heteroaromatic compounds is by no means universal in occurrence, and no unifying explanation has yet been given of the conditions under which it is found. Nevertheless, qualitatively, resonance stabilization of the water-adduct appears to be important.[13,14] On this hypothesis, the known failure of 7-hydroxypteridine to add water to the 5:6-double bond results from the lack, in the hypothetical hydrated neutral molecule of 7-hydroxypteridine (5,6-dihydro-6,7-dihydroxypteridine), of any of the four types of resonance stabilization, viz., the amidine-type, the guanidine-type, the urea-type, and the 4-aminopyridine-type. However, this hypothesis fails to explain why 2,7-dihydroxypteridine does not form an addition product with water.[15] A useful approach is to consider the structure of 2,7-dihydroxypteridine. If in an aqueous solution this compound exists in the structure (B) instead of (A), the $C_{(4)}$-$N_{(3)}$ bond approximates to a single-bond in character and water-addition will not occur.

[13] A. Albert and F. Reich, *J. Chem. Soc.* 127 (1961).

[14] A. Albert, *Proceedings of the Third International Pteridine Symposium* (1962, Stuttgart), Pergamon, Oxford (1963).

[15] Y. Inoue and D. D. Perrin, *J. Chem. Soc.* 2600 (1962).



(A) (B)

By analogy with 7-hydroxypteridine, water-addition across the $C_{(6)}=N_{(5)}$ bond of (B) would not be expected. That 2,7-dihydroxypteridine has the structure, (B), is not unreasonable because of the part resonance can play in its stabilization: it has the same urea-type resonance[16] as is found in hydrated 2-hydroxypteridine.

In general, anhydrous species are stronger acids and weaker bases than the corresponding hydrated species.[11,15,17,18] In some cases, unless the rapid-titration technique or rapid-flow spectrophotometric method was applied, only a composite pK_a could be obtained, which lies between the pK_a of the anhydrous species and that of the hydrated species. Its magnitude depends on the equilibrium quotients, K_1 and K_2, and sometimes the rate of titration. Thus, for example, the earlier reported

TABLE 1. DISSOCIATION CONSTANTS AND EQUILIBRIUM QUOTIENTS OF "HYDRATED" AND "ANHYDROUS" POLYAZANAPHTHALENE DERIVATIVES

Compound	Temp	pK_a^x	pK_a^y	pK_a^{eq}	K_1	K_2
1,3,5-Triazanaphthalene	20°	—	6·46*	4·11*	0·0045	
2-Hydroxy-1,3,8-triaza-naphthalene	20°	9·1	11·25	10·05	9	0·063
3-Hydroxy-1,4,6-triaza-naphthalene	20°	7·32	10·76	7·48	0·45	0·00016
2-Hydroxypteridine	20°	7·7	11·05	10·15	321	0·143
	40°	7·81	10·27	9·66	92·7	0·321
2-Mercaptopteridine	20°	6·52	9·72	9·00	376	0·236
6-Hydroxypteridine	20°	6·45	9·90	8·55	131	0·047
	40°	6·47	9·13	7·96	30·4	0·066
6-Hydroxy-2-methyl-pteridine	20°	6·53	10·05	8·50	95·2	0·029
	40°	6·60	9·24	8·09	32·2	0·074
6-Hydroxy-4-methyl-pteridine	20°	6·3	10·0	8·20	79·7	0·016
	40°	6·41	9·27	7·77	22·4	0·031
6-Hydroxy-7-methyl-pteridine	20°	7·09	10·02	7·46	1·29	0·0015
	40°	6·88	8·95	7·03	0·31	0·0028
4,6-Dihydroxypteridine	20°	$pK_{a_1}^x = 6·05$	$pK_{a_1}^y = 8·34$	$pK_{a_1}^{eq} = 6·40$	$K_1 = 1·24$	
		$pK_{a_2}^x = 9·51$	$pK_{a_2}^y = 10·08$	$pK_{a_2}^{eq} = 9·51$	$K_2 = 0·0063$	
					$K_3 = 0·0017$	
	40°	$pK_{a_1}^x = 6·06$	$pK_{a_1}^y = 8·31$	$pK_{a_1}^{eq} = 6·34$	$K_1 = 0·916$	
		$pK_{a_2}^x = 9·25$	$pK_{a_2}^y = 9·48$	$pK_{a_2}^{eq} = 9·25$	$K_2 = 0·00515$	
					$K_3 = 0·0030$	
2-Amino-4,6-dihydroxy-pteridine	20°	$pK_{a_1}^x = 6·59$	$pK_{a_1}^y = 8·65$	$pK_{a_1}^{eq} = 6·88$	$K_1 = 1·01$	
		$pK_{a_2}^x = 9·31$	$pK_{a_2}^y = 9·99$	$pK_{a_2}^{eq} = 9·32$	$K_2 = 0·0087$	
	40°	$pK_{a_1}^x = 6·58$	$pK_{a_1}^y = 8·61$	$pK_{a_1}^{eq} = 7·1$	$K_3 = 0·0019$	
					$K_1 = 2·39$	
		$pK_{a_2}^x = 9·15$	$pK_{a_2}^y = 9·41$	$pK_{a_2}^{eq} = 9·22$	$K_2 = 0·0024$	
					$K_3 = 0·0123$	

* Basic pK_a.

[16] W. Kutzelnigg, R. Mecke, B. Schrader, F. Nerdel, and G. Kreese, *Z. Elektrochem.* 65, 10) (1961).
[17] W. L. F. Armarego, *J. Chem. Soc.* 4094 (1962).
[18] D. D. Perrin and Y. Inoue, *Proc. Chem. Soc.* 342 (1960).

pK_a values of quinazoline[19,20] and pteridine[21,22] are composite ones. The ratio, K_a^x/K_a^y, is $2{\cdot}7 \times 10^3$ for 6-hydroxypteridine and $2{\cdot}24 \times 10^3$ for 2-hydroxypteridine at 20°. Much of this large difference is probably due to resonance stabilization which is much greater in the anhydrous anion than in the neutral molecule. This difference results in a "decrease" in the proton affinity[23] of the anion, X^-, so that K_a^x is larger than would be expected from the proton mobility of HX.

The "relative" stability of the hydrated over the anhydrous species in 2-hydroxy-1,3,8-triazanaphthalene and 3-hydroxy-1,4,6-triazanaphthalene can be interpreted on the basis of their structural similarities to 2-hydroxy- and 6-hydroxypteridine, respectively. In general, the relative stability of the hydrated to anhydrous species, is more favourable for 2-hydroxypteridine than for 6-hydroxypteridine. From the structural similarity, the value of K_1 would be expected to be greater for 2-hydroxy-1,3,8-triazanaphthalene (= 5-deaza-2-hydroxypteridine) than 3-hydroxy-1,4,6-triazanaphthalene (= 1-deaza-6-hydroxypteridine). The smaller the number of ring nitrogens, the less the relative stability of the hydrated products.

In the process, $HX + H_2O \overset{K_1}{\rightleftharpoons} HY$, HY is energetically more favoured than $HX + H_2O$, so that for 2-hydroxy- and 6-hydroxypteridine and related species ΔH is positive. Hence the temperature coefficient of K_1, $d(\ln K_1)/dT$, should be negative. Conversely, for the process, $X^- + H_2O \rightleftharpoons Y^-$, because the more stable form is X^-, a positive temperature coefficient is to be expected. This conclusion, which has been confirmed experimentally, is important for the discussion of the results of the kinetic studies.

KINETICS OF REVERSIBLE HYDRATION REACTIONS

At constant pH, changes of optical density of solutions with time showed all the reactions to obey first order rate equations, so that the plots of $\log(D - D_e)$ against

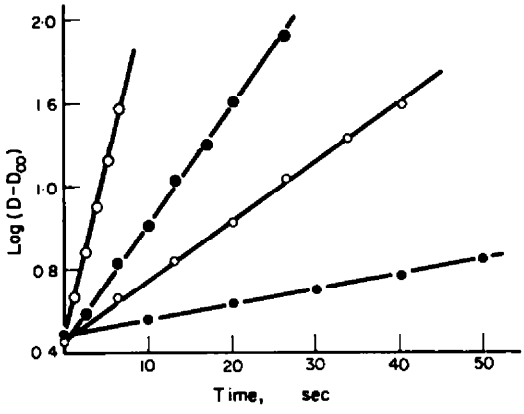


FIG. 2. Plot of $-\log(D - D_\infty)$ against time for 2-mercaptopteridine at different pH (μ = 0·10; Temp = 20°);
—○—○—○—○— pH = 5·26; —●—●—●—●— pH = 5·78;
—○—○—○—○— pH = 6·20; —●—●—●—●— pH = 6·84.

[19] R. C. Elderfield, T. A. Williamson, W. J. Gensler, and C. B. Kremer, *J. Org. Chem.* **12**, 405 (1947).
[20] A. Albert, R. Goldacre, and J. Phillips, *J. Chem. Soc.* 2240 (1948).
[21] A. Albert, D. J. Brown, and G. Cheeseman, *J. Chem. Soc.* 4747 (1951).
[22] D. F. DeTar, C. K. Cain, and B. S. Meeks, *J. Amer. Chem. Soc.* **75**, 5118 (1953).
[23] F. G. Arndt, *Organic Analysis* Vol. 1, pp. 197.

time was a straight line. Results in Fig. 2 and 3 for water-addition to 2-mercaptopteridine (followed neutralization of a solution of anhydrous anion) and 4,6-dihydroxypteridine are typical.

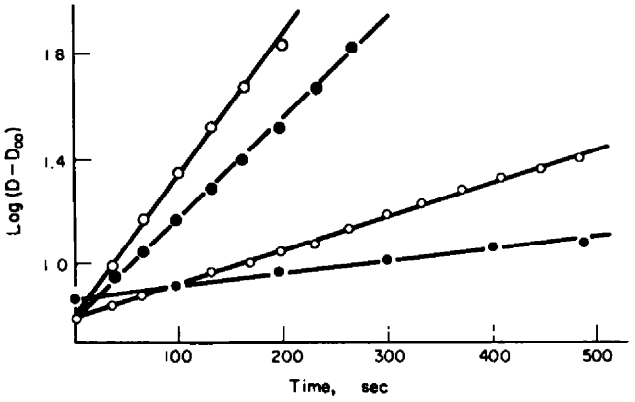
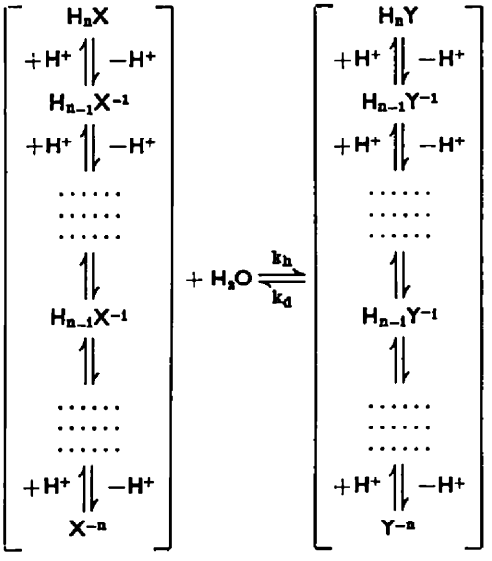


FIG. 3. Plot of $-\log (D - D_\infty)$ against time for 4,6-dihydroxypteridine at different pH ($\mu = 0{\cdot}10$; Temp = 20°):
—○—○—○—○— pH = 4·39; —●—●—●—●— pH = 4·58;
—○—○—○—○— pH = 5·03; —●—●—●—●— pH = 5·78.

The rate constant, k_{obs}, defined by the equation

$$-\frac{1}{D - D_e} \cdot \frac{dD}{dt} = k_{obs} = 2{\cdot}303 \times \frac{1}{t} \cdot \log \frac{D_0 - D_e}{D - D_e}$$

is composite, as may be seen by considering the scheme:



in which k_h and k_d are the overall rate constants for the hydration and dehydration processes. At all times after the preparation of such a solution, under conditions of constant temperature and pH (for example by adding an equilibrated alkaline

solution to a neutral buffer, or *vice versa*), the following equations apply:

$$\sum_{i=0}^{n} \varepsilon_{H_{n-i}X^{-i}}[H_{n-i}X^{-i}] + \sum_{i=0}^{n} \varepsilon_{H_{n-i}Y^{-i}}[H_{n-i}Y^{-i}] = D,$$

$$\sum_{i=0}^{n} [H_{n-i}X^{-i}] + \sum_{i=0}^{n} [H_{n-i}Y^{-i}] = \text{Total conc.},$$

$$K_{a_i}{}^{x}[H_{n-i+1}X^{-i+1}] - [H^+][H_{n-i}X^{-i}] = 0, \quad (i = 1, 2, 3, \cdots, n)$$

$$K_{a_i}{}^{y}[H_{n-i+1}Y^{-i+1}] - [H^+][H_{n-i}Y^{-i}] = 0, \quad (i = 1, 2, 3, \cdots, n)$$

$$-\frac{d}{dt}\sum_{i=0}^{n}[H_{n-i}X^{-i}] = k_h\sum_{i=0}^{n}[H_{n-i}X^{-i}] - k_d\sum_{i=0}^{n}[H_{n-i}Y^{-i}].$$

From these relations, the rate equation can be integrated to yield

$$k_h = \frac{k_{obs}\cdot K_1\left\{\sum_{i=0}^{n}\left([H^+]^i\prod_{j=0}^{n-i}K_{a_j}{}^{y}\right)\right\}}{\sum_{i=0}^{n}\left([H^+]^i\prod_{j=0}^{n-i}K_{a_j}{}^{x}\right) + K_1\left\{\sum_{i=0}^{n}\left([H^+]^i\prod_{j=0}^{n-i}K_{a_j}{}^{y}\right)\right\}} \tag{5}$$

and

$$k_d = \frac{k_{obs}\left\{\sum_{i=0}^{n}\left([H^+]^i\prod_{j=0}^{n-i}K_{a_j}{}^{x}\right)\right\}}{\sum_{i=0}^{n}\left([H^+]^i\prod_{j=0}^{n-i}K_{a_j}{}^{x}\right) + K_1\left\{\sum_{i=0}^{n}\left([H^+]^i\prod_{j=0}^{n-i}K_{a_j}{}^{y}\right)\right\}} \tag{6}$$

where $K_1 = [H_nY]_e/[H_nX]_e$, and $k_{obs} = k_h + k_d$.

Substances able to lose protons may exist as molecules or anions depending on the pH of the solution. In such cases, the various forms (neutral molecules and anions)

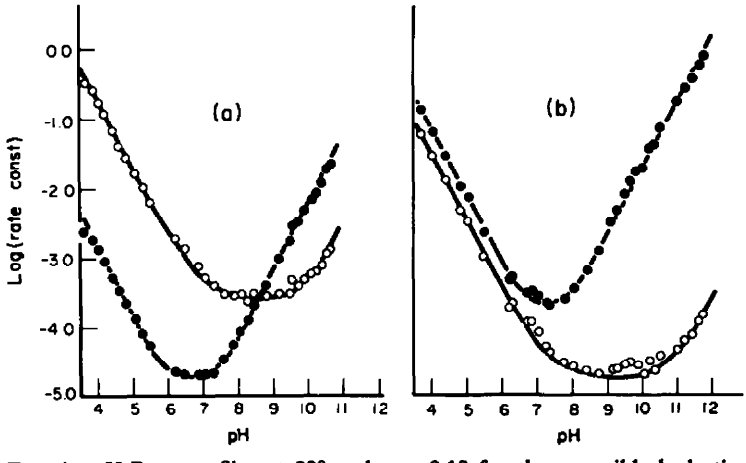


FIG. 4. pH-Rate profiles at 20° and $\mu = 0{\cdot}10$ for the reversible hydration of: (a) 6-hydroxypteridine; (b) 3-hydroxy-1,4,6-triazanaphthalene

—○—○—○—○—○— log k_h *vs* pH

—●—●—●—●—●— log k_d *vs* pH

might be expected to react at different rates.[24,25] In Fig. 4, complete pH-rate profiles for the reversible hydration of some monobasic acids are shown.

Hydration and dehydration are catalyzed strongly by hydrogen ion at lower pH values. There is also, at higher pH values, appreciable catalysis by hydroxyl ion. The reversible hydration of HX type of compounds probably take place through six simultaneous reversible reactions in which the rate constants for reactions *b* and *d*, and *c* and *e*, are not experimentally separable although mechanistically they are different.

$$\begin{aligned}
HX + H_2O + H_3O^+ &\underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} HY + H_3O^+ \\
HX + H_2O + H_2O &\underset{k_{-b}}{\overset{k_b}{\rightleftharpoons}} HY + H_2O \\
HX + H_2O + OH^- &\underset{k_{-c}}{\overset{k_c}{\rightleftharpoons}} HY + OH^- \\
X^- + H_2O + H_3O^+ &\underset{k_{-d}}{\overset{k_d}{\rightleftharpoons}} Y^- + H_3O^+ \\
X^- + H_2O + H_2O &\underset{k_{-e}}{\overset{k_e}{\rightleftharpoons}} Y^- + H_2O \\
X^- + H_2O + OH^- &\underset{k_{-f}}{\overset{k_f}{\rightleftharpoons}} Y^- + OH^-
\end{aligned} \tag{7}$$

Then, the overall rate constants of concurrent forward and reverse reactions can be expressed in either of two equivalent expressions:

$$K_{obs} = \frac{1}{(K_a^x + [H^+])(K_a^y + [H^+])} \times \frac{1}{K_1}\{[H^+](k_a[H^+] + k_b[H_2O] + k_c[OH^-]) + K_a^x(k_d[H^+] + k_e[H_2O] + k_f[OH^-])\}\{K_1(K_a^y + [H^+]) + K_a^x + [H^+]\} \tag{8}$$

and

$$k_{obs} = \frac{1}{(K_a^x + [H^+])(K_a^y + [H^+])} \times \frac{1}{K_1}\{[H^+](k_{-a}[H^+] + k_{-b}[H_2O] + k_{-c}[OH^-]) + K_a^y(k_{-d}[H^+] + k_{-e}[H_2O] + k_{-f}[OH^-])\}\{K_1(K_a^y + [H^+]) + K_a^x + [H^+]\} \tag{9}$$

In the particular case of reversible hydration of a monobasic acid (i.e., n = 1 in Eq. 5, 6), the equations become:

$$k_h = \frac{k_{obs}K_1([H^+] + K_a^y)}{K_1(K_a^y + [H^+]) + K_a^x + [H^+]} \tag{10}$$

and

$$k_d = \frac{k_{obs}([H^+] + K_a^x)}{K_1(K_a^y + [H^+]) + K_a^x + [H^+]}. \tag{11}$$

Then, a pH-rate profile can be obtained from a combination of the six catalytic constants:

$$k_h(K_a^x + [H^+]) = [H^+](k_a[H^+] + k_b[H_2O] + k_c[OH^-]) + K_a^x(k_d[H^+] + k_e[H_2O] + k_f[OH^-]) \tag{12}$$

and

$$k_d(K_a^y + [H^+]) = [H^+](k_{-a}[H^+] + k_{-b}[H_2O] + k_{-c}[OH^-]) + K_a^y(k_{-d}[H^+] + k_{-e}[H_2O] + k_{-f}[OH^-]). \tag{13}$$

[24] L. J. Edwards, *Trans. Faraday Soc.* **46**, 723 (1960).
[25] A. Ågren, U. Hedsten, and B. Jonsson, *Acta Chim. Scand.* **15**, 1532 (1961).

Thus, the pH at which a minimum is observed in the rate profile depends mainly on the values of K_a^x and K_a^y. In the pH-rate profile for hydration of 6-hydroxypteridine (Fig. 4), the linear part in the left may be represented by

$$\frac{1{\cdot}72 \times 10^3[H^+]^2}{K_a^x + [H^+]} \quad (\because K_a^x = 3{\cdot}55 \times 10^{-7}) \tag{14}$$

Similarly, a linear portion in the right part of log k_d *vs* pH profile in Fig. 4 is expressed by

$$\frac{91{\cdot}2K_wK_a^y}{[H^+](K_a^y + [H^+])} \quad (\because K_a^y = 1.26 \times 10^{-10}) \tag{15}$$

where K_w is the ionic product of water. Therefore, above pH 10·5, the pH-rate profile for the hydration reaction should be expressed by

$$\frac{4{\cdot}29K_wK_a^x}{[H^+](K_a^x + [H^+])} \quad (\because K_a^x = 3{\cdot}55 \times 10^{-7}) \tag{16}$$

(14) and (16) correspond to the reactions between HX and H_2O, and X^- and H_2O catalyzed by hydrogen ion and hydroxyl ion, respectively.

A next, necessary, analytical consequence is the determination of $k_b[H_2O] + k_dK_a^x$ and $k_ck_w + k_e[H_2O]K_a^x$. In order to determine these two unknown quantities, the two simultaneous equations have to be solved at two suitable different pHs.

Subtraction of the total contribution of the two reactions *a* and *f*, to the overall rate constant of hydration, k_h, from the observed constant at a certain pH($6 \leqslant$ pH $\leqslant 9$) leaves a difference which may be made up of a combination of some or all of the possible reactions, *b*, *c*, *d*, and *e*. In this way, results for 6-hydroxypteridine yield

$$7{\cdot}83 \times 10^{-4} \leqslant x \leqslant 1{\cdot}24 \times 10^{-3}$$
$$5{\cdot}17 \times 10^{-11} \leqslant y \leqslant 2{\cdot}12 \times 10^{-10}$$

where $x = k_b[H_2O] + k_dK_a^x$, and $y = k_cK_w + k_e[H_2O]K_a^x$, and the complete curve should be represented by:

$$k_h(K_a^x + [H^+]) = 1{\cdot}72 \times 10^3[H^+]^2 + x[H^+] + y + 1{\cdot}03 \times 10^{-20}/[H^+] \tag{17}$$

Similarly, for the dehydration reaction, the following equation was obtained:

$$k_d(K_a^y + [H^+]) = 13{\cdot}1[H^+]^2 + \alpha[H^+] + \beta + 7{\cdot}77 \times 10^{-23}/[H^+] \tag{18}$$

where $\alpha = k_{-b}[H_2O] + k_{-d}K_a^y$ and $\beta = k_{-c}K_w + k_{-e}[H_2O]K_a^y$
and

$$1{\cdot}20 \times 10^{-6} \leqslant \alpha \leqslant 1{\cdot}89 \times 10^{-6}$$
$$7{\cdot}90 \times 10^{-14} \leqslant \beta \leqslant 3{\cdot}24 \times 10^{-13}.$$

The deviations between pH 9·0 and 10·0 can be ascribed to general acid and/or base catalysis of the observed rate, in a region where the catalytic effects of H_3O^+ and OH^- are no longer of overriding importance. Outside this pH region, acid-base catalysis by species other than H_3O^+ and OH^- may be very small.

Evidence for such general acid and base catalysis has been obtained in the study of hydration-dehydration of pteridine and its methyl derivatives[26] and 2-hydroxypteridine.[27]

[26] Y. Inoue and D. D. Perrin, *J. Chem. Soc.* 2648 (1963).
[27] Y. Inoue and D. D. Perrin, *J. Phys. Chem.* **66**, 1689 (1962).

By appropriate analysis of the rate data, equations corresponding to Eq. (17) and (18) can be formulated for the other compounds.[28–30] These equations for some typical compounds are as follows (at 20°):

6-Hydroxypteridine:

$$k_h([H^+] + K_a^x) = 1{\cdot}72 \times 10^3[H^+]^2 + 9{\cdot}81 \times 10^{-4}[H^+] + 1{\cdot}13 \times 10^{-10} + 1{\cdot}03 \times 10^{-20}/[H^+]$$

$$k_d([H^+] + K_a^y) = 1{\cdot}38 \times 10[H^+]^2 + 7{\cdot}49 \times 10^{-6}[H^+] + 8{\cdot}63 \times 10^{-13} + 7{\cdot}77 \times 10^{-23}/[H^+]$$

6-Hydroxy-2-methylpteridine:

$$k_h([H^+] + K_a^x) = 3{\cdot}45 \times 10^3[H^+]^2 + 8{\cdot}83 \times 10^{-4}[H^+] + 4{\cdot}16 \times 10^{-11} + 4{\cdot}59 \times 10^{-21}/[H^+]$$

$$k_d([H^+] + K_a^y) = 3{\cdot}63 \times 10[H^+]^2 + 9{\cdot}28 \times 10^{-6}[H^+] + 4{\cdot}37 \times 10^{-13} + 4{\cdot}78 \times 10^{-23}/[H^+]$$

6-Hydroxy-4-methylpteridine:

$$k_h([H^+] + K_a^x) = 1{\cdot}96 \times 10^3[H^+]^2 + 1{\cdot}19 \times 10^{-3}[H^+] + 6{\cdot}49 \times 10^{-11} + 5{\cdot}79 \times 10^{-21}/[H^+]$$

$$k_d([H^+] + K_a^y) = 2{\cdot}46 \times 10[H^+]^2 + 1{\cdot}49 \times 10^{-5}[H^+] + 8{\cdot}14 \times 10^{-13} + 7{\cdot}10 \times 10^{-23}/[H^+]$$

6-Hydroxy-7-methylpteridine:

$$k_h([H^+] + K_a^x) = 3{\cdot}99 \times 10^2[H^+]^2 + 8{\cdot}80 \times 10^{-5}[H^+] + 3{\cdot}15 \times 10^{-12} + 3{\cdot}06 \times 10^{-22}/[H^+]$$

$$k_d([H^+] + K_a^y) = 3{\cdot}09 \times 10^2[H^+]^2 + 6{\cdot}82 \times 10^{-5}[H^+] + 2{\cdot}44 \times 10^{-12} + 2{\cdot}40 \times 10^{-22}/[H^+]$$

2-Hydroxypteridine:

$$k_h([H^+] + K_a^x) = 6{\cdot}3 \times 10^4[H^+]^2 + 1{\cdot}1 \times 10^{-2}[H^+] + 1{\cdot}0 \times 10^{-11} + 3{\cdot}0 \times 10^{-25}/[H^+]$$

$$k_d([H^+] + K_a^y) = 1{\cdot}96 \times 10^2[H^+]^2 + 3{\cdot}42 \times 10^{-5}[H^+] + 3{\cdot}11 \times 10^{-14} + 9{\cdot}34 \times 10^{-23}/[H^+]$$

2-Mercaptopteridine:

$$k_h([H^+] + K_a^x) = 6{\cdot}92 \times 10^4[H^+]^2 + 3{\cdot}49 \times 10^{-2}[H^+] + 4{\cdot}09 \times 10^{-11} + 2{\cdot}41 \times 10^{-21}/[H^+]$$

$$k_d([H^+] + K_a^y) = 1{\cdot}84 \times 10^2[H^+]^2 + 9{\cdot}28 \times 10^{-5}[H^+] + 1{\cdot}09 \times 10^{-13} + 6{\cdot}43 \times 10^{-24}/[H^+]$$

2-Hydroxy-1,3,8-triazanaphthalene:

$$k_h([H^+] + K_a^x) = 8{\cdot}59 \times 10^4[H^+]^2 + 8{\cdot}42 \times 10^{-4}[H^+] + 8{\cdot}29 \times 10^{-13} + 4{\cdot}77 \times 10^{-24}/[H^+]$$

$$k_d([H^+] + K_a^y) = 9{\cdot}55 \times 10^3[H^+]^2 + 9{\cdot}35 \times 10^{-5}[H^+] + 9{\cdot}21 \times 10^{-14} + 5{\cdot}36 \times 10^{-25}/[H^+]$$

[28] Y. Inoue and D. D. Perrin, *J. Chem. Soc.* 3936 (1963).
[29] Y. Inoue and D. D. Perrin, *J. Chem. Soc.* in press.
[30] Y. Inoue and D. D. Perrin, *J. Chem. Soc.* in press.

3-Hydroxy-1,4,6-triazanaphthalene:

$$k_h([H^+] + K_a^x) = 3{\cdot}26 \times 10^2[H^+]^2 + 6{\cdot}17 \times 10^{-5}[H^+] + 9{\cdot}72 \times 10^{-13} + 1{\cdot}49 \times 10^{-23}/[H^+]$$

$$k_d([H^+] + K_a^y) = 7{\cdot}24 \times 10^2[H^+]^2 + 1{\cdot}37 \times 10^{-4}[H^+] + 2{\cdot}16 \times 10^{-12} + 3{\cdot}39 \times 10^{-23}/[H^+]$$

The pH-rate profiles can be analyzed in the same way as for H_2X type of compounds, except that 18 possible catalytic rate constants must be included:

$$k_h([H^+]^2 + [H^+]K_{a_1}^x + K_{a_1}^xK_{a_2}^x) = k_a[H^+]^3 + (k_b[H_2O] + k_dK_{a_1}^x)[H^+]^2 + (k_cK_w + k_e[H_2O]K_{a_1}^x + k_gK_{a_1}^xK_{a_2}^x)[H^+] + (k_fK_wK_{a_1}^x + k_h[H_2O]K_{a_1}^xK_{a_2}^x) + k_iK_wK_{a_1}^xK_{a_2}^x/[H^+] \quad (19)$$

and

$$k_d([H^+]^2 + [H^+]K_{a_1}^y + K_{a_1}^yK_{a_2}^y) = k_{-a}[H^+]^3 + (k_{-b}[H_2O] + k_{-d}K_{a_1}^y)[H^+]^2 + (k_{-c}K_w + k_{-e}[H_2O]K_{a_1}^y + k_{-g}K_{a_1}^yK_{a_2}^y)[H^+] + (k_{-f}K_wK_{a_1}^y + k_{-h}[H_2O]K_{a_1}^yK_{a_2}^y) + k_{-i}K_wK_{a_1}^yK_{a_2}^y/[H^+] \quad (20)$$

where k_a, k_b, k_c, k_d, k_e, k_f, k_g, k_h, k_i, k_{-a}, k_{-b}, k_{-c}, k_{-d}, k_{-e}, k_{-f}, k_{-g}, k_{-h}, and k_{-i} are defined in the similar manner as for reversible hydration of HX.

As has already been seen in the analysis of the rate processes of HX compounds, the kinetics do not permit the separate evaluation of the nine possible processes. That is to say, the rate constants for the reactions *b* and *d*, *c*, *e*, and *g*, and *f* and *h*, cannot be individually obtained.

From an analysis of the experimental results, the following catalytic rate constants at 20° were obtained for 4,6-dihydroxypteridine:

$$k_a = 1{\cdot}92 \times 10^2;\ k_{-a} = 1{\cdot}55 \times 10^2;\ k_i = 5{\cdot}01 \times 10^{-1};\ k_{-i} = 2{\cdot}95 \times 10^2;$$

$$k_b[H_2O] + k_dK_{a_1}^x = \sim\leq 1{\cdot}02 \times 10^{-4};\ k_{-b}[H_2O] + k_{-d}K_{a_1}^y = \sim\leq 8{\cdot}26 \times 10^{-5};$$

$$k_cK_w + k_e[H_2O]K_{a_1}^x + k_gK_{a_1}^xK_{a_2}^x = \sim\geq 3{\cdot}34 \times 10^{-12};$$

$$k_{-c}K_w + k_{-e}[H_2O]K_{a_1}^y + k_{-g}K_{a_1}^yK_{a_2}^y = \sim\geq 2{\cdot}69 \times 10^{-12};$$

$$k_fK_wK_{a_1}^x + k_h[H_2O]K_{a_1}^xK_{a_2}^x = \sim\leq 6{\cdot}83 \times 10^{-21};$$

and

$$k_{-f}K_wK_{a_1}^y + k_{-h}[H_2O]K_{a_1}^yK_{a_2}^y = \sim\leq 5{\cdot}51 \times 10^{-21}.$$

Similarly, for 2-amino-4,6-dihydroxypteridine (= xanthopterin) the corresponding constants are:

$$k_a = 4{\cdot}40 \times 10^2;\ k_{-a} = 4{\cdot}37 \times 10^2;\ k_i = 3{\cdot}38 \times 10^{-1};\ k_{-i} = 1{\cdot}78 \times 10^2;$$

$$k_b[H_2O] + k_dK_{a_1}^x = \sim\geq 4{\cdot}36 \times 10^{-5};\ k_{-b}[H_2O] + k_{-d}K_{a_1}^y = \sim\geq 4{\cdot}33 \times 10^{-5};$$

$$k_cK_w + k_e[H_2O]K_{a_1}^x + k_gK_{a_1}^xK_{a_2}^x = \sim\leq 1{\cdot}47 \times 10^{-12};$$

$$k_{-c}K_w + k_{-e}[H_2O]K_{a_1}^y + k_{-g}K_{a_1}^yK_{a_2}^y = \sim\leq 1{\cdot}46 \times 10^{-12};$$

$$k_fK_wK_{a_1}^x + k_h[H_2O]K_{a_1}^xK_{a_2}^x = \sim\text{negligible } (\sim 10^{-22});$$

and

$$k_{-f}K_wK_{a_1}^y + k_{-h}[H_2O]K_{a_1}^yK_{a_2}^y = \sim\text{negligible } (\sim 10^{-22}).$$

The pH-rate profile can be reproduced by combining all nine catalytic terms for the forward or the reverse processes. Although 2,6-dihydroxypteridine belongs to this group of hydrating substances, its reversible hydration is not simple; the kinetic results obtained with this compound are discussed separately.

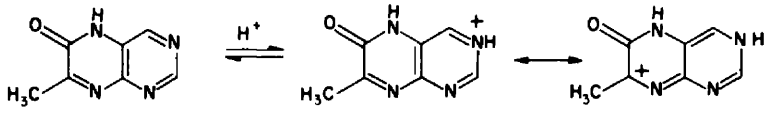
The catalytic coefficients for hydrogen ions have been found to be much greater for the hydration reactions of a series of 2-hydroxypteridines than those for the same reactions of pteridine and its methyl derivatives.[26] Also, the catalytic effect of hydrogen ions on the dehydration of neutral species are comparable or somewhat smaller for a series of 2-hydroxypteridines than for the correspondingly substituted pteridines. Thus, introduction of a hydroxyl group to position 2 in pteridine leads to considerable changes in the relative stabilities of the reactants, anhydrous species, and the products, hydrated species.

The stability of hydrated pteridine can be attributed mainly to resonance effects, which partly offsets the unfavourable energy situation arising from partial loss of aromaticity. However, for the hydrated 2-hydroxypteridine series, the latter factor is much less important (because the anhydrous species is already in the lactam form) so that the degree of stability of the hydrated species depends largely on steric factors. This is because, in the hydrated molecule, the atoms attached to the nitrogen are forced out of the plane of the cyclic amide group so that some steric inhibition of amide resonance should result.[31,32]

The catalytic power of hydrogen ions for the hydration reactions of neutral molecules of 6-hydroxypteridine and its methyl derivatives has been found to be larger than that for pteridine and its methyl derivatives, but for the dehydration of the neutral species the catalytic coefficient for hydrogen ion is far smaller than for either the pteridine or the 2-hydroxypteridine series.

In 6-hydroxypteridine, the stability of the hydrated product involves steric considerations which enable the kinetic and equilibrium results to be interpreted satisfactorily.

It is likely that the acid-catalyzed hydration reaction for 6-hydroxy-7-methylpteridine involves rapid protonation[33] as the initial step, probably at position 3.



Attack on the protonated species by nucleophilic reagents is facilitated by the partial positive charge on $C_{(7)}$, so that it is convenient to discuss reactions in terms of carbonium ion formation. The electron-releasing effect of the methyl group, by reducing the positive charge on $C_{(7)}$ will exert a retarding effect on the reaction: this is also due, in part, to the greater stability of a carbonium ion derived from tertiary, than from secondary carbon atoms. Consistent with this, the rate constant, k_h, for the hydration of 6-hydroxy-7-methylpteridine is much less than that for 6-hydroxypteridine, the ratio being about 0·207 over the pH range from 3·69 to 4·80. The electron-releasing effect of this methyl group also makes 6-hydroxy-7-methylpteridine only about 0·23 times as strong an acid as 6-hydroxypteridine.

In hydrated 6-hydroxypteridine the hydroxy group may be equatorial (or quasi equatorial), whereas in its 7-methyl derivative it may be axial (or quasi axial). This

[31] L. Pauling and R. B. Corey, *Proc. Natl. Acad. Sci. U.S.* **37**, 251 (1951).
[32] R. W. Holley, *Science* **117**, 23 (1953).
[33] The position at which the proton adds to this conjugated pteridine system is unknown. However, it has been suggested, *a priori*, that position-3 is the most likely one.[34]
[34] A. Albert and F. Reich, *J. Chem. Soc.* 127 (1961).

conclusion is a consequence of the general preference for the bulkier substituent to be located in an equatorial rather than an axial orientation, and is based on the interpretation of kinetic measurements.

From the principle of microscopic reversibility, the above leads to increased rate constants for the 7-methyl derivative in the dehydration reaction. Thus, over the pH range from 3·69 to 4·80, the value of k_d for 7,8-dihydro-6,7-dihydroxy-7-methylpteridine was 21 times as much as for the parent compound. From the ratio (4·82) of the rate constants of hydration of 6-hydroxypteridine to those of 6-hydroxy-7-methylpteridine and the ratio (7·4) of the equilibrium quotient, K_1, for these two compounds, the acid-catalyzed reversible hydration can be represented schematically by the following reaction coordinate (Fig. 5).

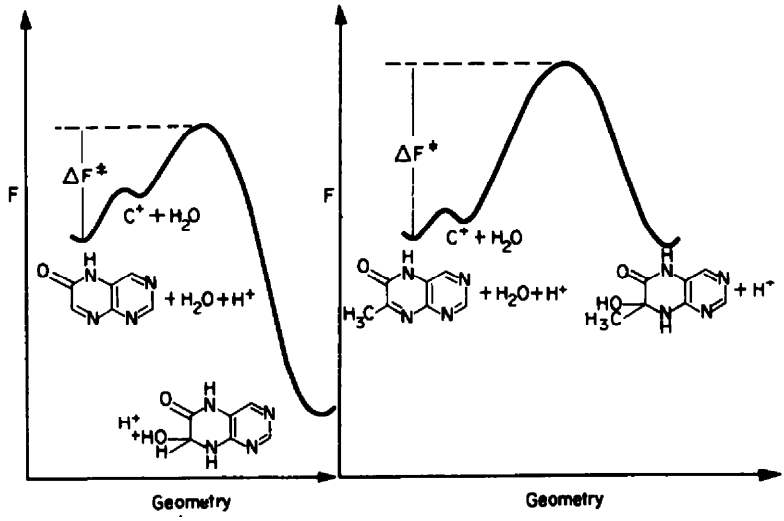


FIG. 5. Schematic energy diagrams for the reversible hydration of 6-hydroxypteridine and its 7-methyl derivative.

For both the acid-catalyzed hydration of neutral molecules and the base-catalyzed hydration of anions of a series of 6-hydroxypteridines (including 4,6-dihydroxypteridine and 2-amino-4,6-dihydroxypteridine), it was noticed that rates varied linearly with equilibrium quotients on their logarithmic scales: this has also been observed in some other reversible systems and the implications of this relationship have been discussed by Leffler.[35]

$$\log k = \alpha \log K + C$$

where k is a rate constant and K is an equilibrium quotient. This relationship can only be significant when the value of α is between 0 and 1. This corresponds to the class *c* in Polanyi's classification.[36] The approximate value of α for the acid-catalyzed hydration of 6-hydroxypteridine derivatives is 0·35, and for base-catalyzed hydration of anion of 6-hydroxypteridine derivatives α is 0·75. The former value indicates that the transition states resemble the anhydrous species more closely than they do the products in the acid-catalyzed hydration of neutral molecules, the value of α, as

[35] J. E. Leffler, *Science* **117**, 340 (1953).
[36] M. Polanyi, *J. Chem. Soc.* 629 (1937).

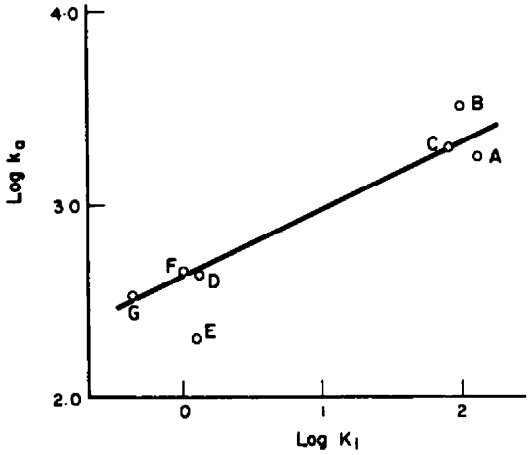


FIG. 6. Linear relationship between log k_a and log K_1 for 6-hydroxypteridine and related compounds at 20°:
A: 6-Hydroxypteridine; B: 6-Hydroxy-2-methylpteridine;
C: 6-Hydroxy-4-methylpteridine; D: 6-Hydroxy-7-methylpteridine;
E: 4,6-Dihydroxypteridine; F: 2-Amino-4,6-dihydroxypteridine;
G: 3-Hydroxy-1,4,6-triazanaphthalene.

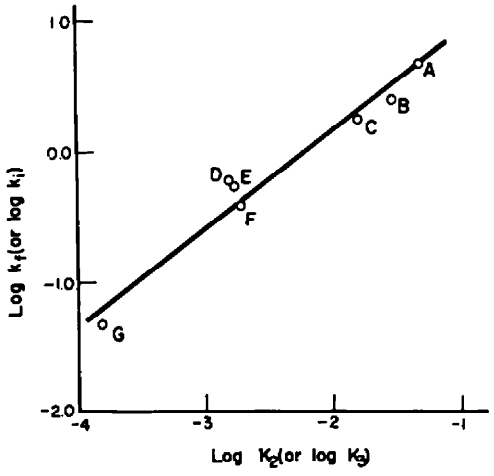


FIG. 7. Linear relationship between log k_f(or log k_1) and log K_2(or log K_3) for 6-Hydroxypteridine and related compounds at 20°:
A: 6-Hydroxypteridine; B: 6-Hydroxy-2-methylpteridine;
C: 6-Hydroxy-4-methylpteridine; D: 6-Hydroxy-7-methylpteridine;
E: 4,6-Dihydroxypteridine; F: 2-Amino-4,6-dihydroxypteridine;
G: 3-Hydroxy-1,4,6-triazanaphthalene.

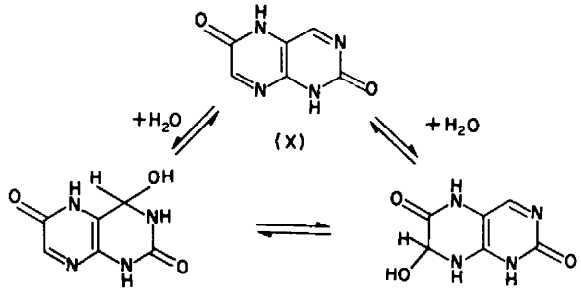
expected,[37] was less than 0·5, because the reactions are highly exothermic. On the other hand, a value of α for the base-catalyzed hydration of the anions of 6-hydroxypteridine series was 0·75, indicating that these reactions are endothermic.

DUAL REACTIVITY OF 2,6-DIHYDROXYPTERIDINE

From the structural similarities of 2,6-dihydroxypteridine to 2-hydroxy- and 6-hydroxypteridine, it was expected that 2,6-dihydroxypteridine would undergo

[37] G. S. Hammond, *J. Amer. Chem. Soc.* 77, 334 (1955).

reversible hydration. From the analysis of the experimental results, it is concluded that hydration of 2,6-dihydroxypteridine (X) takes place *initially* across 3:4-double bond to form (A). Under the same conditions, an isomerization, by an extended allylic type of rearrangement, then occurs, leading to an equilibrium mixture of two species (A) and (B) in which the latter isomer is the main one.



(A) (B)

Evidence for this assignment of the products of the dual reaction of 2,6-dihydroxypteridine may be seen in the preceding paper.[38]

It is known that, if a methyl group is bonded to the carbon of the C=N group across which water-addition occurs, the extent of hydration is greatly reduced. At equilibrium the spectra of solutions of 2,6-dihydroxypteridine and 2,6-dihydroxy-4-methylpteridine are very similar. Under these conditions, (B) and 4-methyl-(B), respectively, are the major species present. Their spectra are also closely similar to that of 7,8-dihydro-2,6-dihydroxypteridine.[39] These results strongly suggest that, in substance (B), there is a molecule of water across $C_{(7)}$ and $N_{(8)}$, but not across $C_{(4)}$ and $N_{(3)}$. Using, in most cases, rapid spectrophotometric techniques, acid dissociation constants were obtained for some of the species of interest in this study.

TABLE 2. ACID DISSOCIATION CONSTANTS OF 2,6-DIHYDROXYPTERIDINE AND RELATED COMPOUNDS AT 20°

Compound	pK_{a_1}	pK_{a_2}
2,6-dihydroxypteridine (X)	5·58	8·64
3,4-water-adduct (A)	8·19*	12·65*
7,8-water-adduct (B)	9·44	11·50

* Calculated as $pK_{a_1} = pK_a^x$(6-hydroxypteridine) + pK_{a_1}(B) − pK_a^x(2-hydroxypteridine), and $pK_{a_2} = pK_a^y$(2-hydroxypteridine) + pK_{a_2}(B) − pK_a^y(6-hydroxypteridine).

As is well known, the thermodynamically stable isomer is not always the main product of a chemical reaction: often a less stable isomer is formed first.[40-43]

This phenomenon appears to operate in the present instance, with the 7:8-adduct (B) being thermodynamically more stable than the 3:4-adduct (A). Conversion,

[38] A. Albert, Y. Inoue, and D. D. Perrin, *J. Chem. Soc.* in press.

[39] A. Albert and S. Matsuura, *J. Chem. Soc.* 2162 (1962).

[40] C. K. Ingold, *Structure and Mechanism in Organic Chemistry* pp. 565, 566. Cornell University Press, Ithaca, N.Y. (1953).

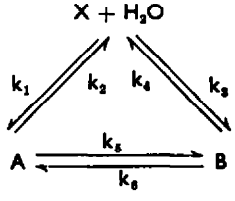
[41] J. Hine, *Physical Organic Chemistry* p. 178. McGraw-Hill, New York (1956).

[42] R. Kuhn and H. Fischer, *Chem. Ber.* 93, 2285 (1960).

[43] J. A. Berson, *Tetrahedron Letters* No. 16, 17 (1960).

initially, into the 3:4-adduct proceeds rapidly, indicating that the free energy of activation is low compared with the corresponding value for the reaction to the 7:8-adduct. This difference can be explained using idea due to Ingold[40] and Hammond.[37] Thus, conversion of the planar carbonium ion into the 3:4-adduct (A) requires a smaller change in geometry and hence a lower activation energy than is involved in forming the 7:8-adduct (B). (This is a consequence of the "principle of least motion."[44–46]) This is because the proximity of the junction double bond (C=C) to the hydroxyl group forces the latter into a quasi-equatorial position in approximately the same plane and this is not the case for (B). However, the thermodynamically controlled reaction conditions lead to the ultimate proportions of the 3:4- and 7:8-adducts being governed only by thermodynamic stabilities of the two products.[47] That (B) is energetically the more stable is probably due to the absence of the constraint towards linearity of the equatorial hydroxyl group.

The kinetics of the hydration reactions of 2,6-dihydroxypteridine is now discussed. The reaction scheme can be written as:



where X, A, and B are 2,6-dihydroxypteridine, its 3:4- and 7:8-water adducts, respectively, and each species may be present as its neutral molecule, mono- or dianion. Beginning with a solution containing only the species, X, of concentration I, we have, after time t, [X] = I − a − b, [A] = a, [B] = b, so that the following rate equations can be obtained:

$$da/dt = k_1(I - a - b) + k_6 b - k_2 a - k_5 a \tag{21}$$

$$db/dt = k_3(I - a - b) + k_5 a - k_4 b - k_6 b \tag{22}$$

therefore,

$$d^2a/dt^2 = -(k_1 + k_2 + k_3)da/dt - (k_1 - k_6)db/dt \tag{23}$$

Substituting db/dt in Eq. (23) by the right-hand side of Eq. (22), and remembering Eq. (21),

$$d^2a/dt^2 + (k_1 + k_2 + k_3 + k_4 + k_5 + k_6)da/dt + (k_1k_4 + k_1k_5 + k_1k_6 + k_2k_3 + k_2k_4 + k_2k_6 + k_3k_6 + k_4k_5)a = k_1k_4 + k_1k_6 + k_3k_6)I \tag{24}$$

Integration of Eq. (24) gives

$$a = \frac{1}{K_1 - K_2}\left\{\left(k_1 + K_2\frac{p}{n}\right)\exp(K_1t) - \left(k_1 + K_1\frac{p}{n}\right)\exp(K_2t)\right\} + \frac{Ip}{n} \tag{25}$$

Similarly,

$$b = \frac{1}{K_1 - K_2}\left\{\left(k_3 + K_2\frac{q}{n}\right)\exp(K_1t) - \left(k_3 + K_1\frac{q}{n}\right)\exp(K_2t)\right\} + \frac{Iq}{n} \tag{26}$$

[44] F. O. Rice and E. Teller, *J. Chem. Phys.* 6, 489 (1938).
[45] E. Peytral, *Bull. Soc. Chim., Fr.* 27, 34 (1920).
[46] W. Hückel, *Theoretical Principles of Organic Chemistry* Vol. I, pp. 19, 241 etc., Vol. II, p. 217. Elsevier (1958).
[47] A. G. Catchpole, E. D. Hughes, and C. K. Ingold, *J. Chem. Soc.* 11 (1948).

where

$$n = k_1k_4 + k_1k_5 + k_1k_6 + k_2k_3 + k_2k_4 + k_3k_5 + k_3k_6 + k_4k_5$$
$$p = k_1k_4 + k_1k_6 + k_3k_6$$
$$q = k_1k_5 + k_2k_3 + k_3k_5$$
$$K_1 = -(m - \sqrt{m^2 - 4n})/2$$
$$K_2 = -(m + \sqrt{m^2 - 4n})/2$$
$$m = k_1 + k_2 + k_3 + k_4 + k_5 + k_6$$

In solutions of 2,6-dihydroxypteridine the spectrum between 330 and 368 mμ rises, with time, to a maximum, followed an exponential decay. Fig. 8 shows a typical trace obtained at a constant wavelength (340 mμ).

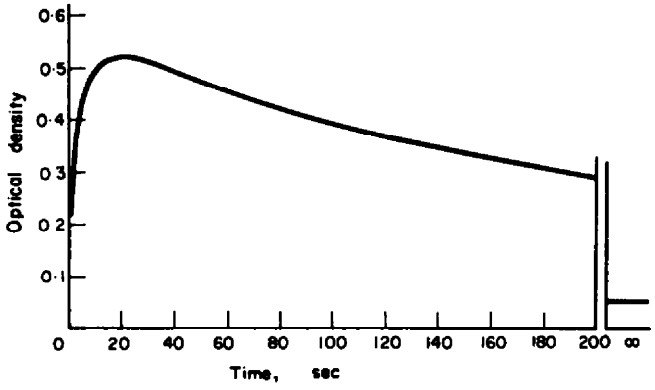


FIG. 8. Spectrophotometrically followed hydration and isomerization starting with anhydrous 2,6-dihydroxypteridine: wavelength = 340 mμ; pH = 5·48; Temp = 20°.

The time (t_{max}) and magnitude of maximum absorption are measurable quantities and can be used to test the validity of some assumptions to be made in the actual calculations. At a particular wavelength, let the molar absorptivities of X, A, and B be ε_X, ε_A, and ε_B. Then the optical density, D may be expressed by:

$$D = \varepsilon_X(I - a - b) + \varepsilon_A a + \varepsilon_B b \tag{27}$$

Differentiation of Eq. (27) gives

$$dD/dt = (\varepsilon_A - \varepsilon_X)da/dt + (\varepsilon_B - \varepsilon_X)db/dt \tag{28}$$

At equilibrium, dD/dt equals to zero, so that

$$\frac{(da/dt)_{t_{max}}}{(db/dt)_{t_{max}}} = \frac{\varepsilon_X - \varepsilon_B}{\varepsilon_A - \varepsilon_X} = S \tag{29}$$

Differentiating Eqs. (25) and (26) with respect to time and inserting these resulting equations into Eq. (29), under the condition that $t = t_{max}$, gives

$$t_{max} = 2{\cdot}303 \times \frac{\log\{n(k_1 - k_3S) + K_2(p - qS)\}|K_1| - \log\{n(k_1 - k_3S) + K_1(p - qS)\}|K_2|}{K_2 - K_1} \tag{30}$$

Application of Guggenheim's method to the initial part of the absorbance-time curve makes it possible to obtain the overall rate constant for the process $X + H_2O \rightleftharpoons A$. Similarly, from the part of a true exponential decay of the maximum, the overall rate constant for the isomerization process $A \rightleftharpoons B$ is determined. The rate constant $(k_3 + k_4)$ for the process $X + H_2O \rightleftharpoons B$ could not be observed directly. However, it is known, from results in the 2-hydroxy- and 6-hydroxypteridine series, that a methyl group does not greatly affect rates so long as it is located in a position remote from the point at which water-addition occurs. For this reason, it was considered reasonable to use the experimental rate constant for the hydration of 2,6-dihydroxy-4-methylpteridine. The following figures were obtained experimentally from changes in optical density:

pH	$k_1 + k_2$	$k_3 + k_4$	$k_5 + k_6$
5·26	0·274	0·00363	0·00419
5·48	0·229	0·00240	0·00344
5·78	0·140	0·00129	0·00237
6·20	0·0566	0·000661	0·00132
6·84	0·0152	0·000224	0·000826
7·06	0·00862	0·000160	0·000410

To analyze the system in further detail, values had to be assumed for pK_{a_1} and pK_{a_2} of the 3:4-adduct: they could not be obtained directly by experiment. The assumption was therefore made that the first pK_a value of the 3:4-adduct differed as much from the corresponding value of anhydrous 2-hydroxypteridine as did the value for the 7:8-adduct from that of anhydrous 6-hydroxypteridine. Similarly, for the second constant, the value could be calculated (Table 2).

Using a spectrophotometric method, the approximate equilibrium pK values were determined:

$$-\log \frac{[H^+]([HX^-]_{eq} + [HB^-]_{eq})}{[H_2X]_{eq} + [H_2B]_{eq}} = 8{\cdot}55$$

and

$$-\log \frac{[H^+]([X^{--}]_{eq} + [B^{--}]_{eq})}{[HX^-]_{eq} + [HB^-]_{eq}} = 9{\cdot}69$$

The equilibrium quotient, $[H_2B]_{eq}/[H_2X]_{eq} = 1100$, could then be calculated. From an examination of absorption spectra at the time of maximum formation of A, and again at equilibrium, the ratio of $[H_2B]_{eq}/[H_2A]_{eq} = 13$ was obtained. Hence, also, from these two quotients, $[H_2A]_{eq}/[H_2X]_{eq} = 85$.

With these additional information and the values in Table 2, the rate constants for each of the forward and reverse processes could be calculated from

$$k_h = \frac{k_{obs} \cdot K_1([H^+]^2 + [H^+]K_{a_1}{}^y + K_{a_1}{}^y K_{a_2}{}^y)}{[H^+]^2 + [H^+]K_{a_1}{}^x + K_{a_1}{}^x K_{a_2}{}^x + K_1([H^+]^2 + [H^+]K_{a_1}{}^y + K_{a_1}{}^y K_{a_2}{}^y)} \quad (31)$$

$$k_d = \frac{k_{obs}([H^+]^2 + [H^+]K_{a_1}{}^x + K_{a_1}{}^x K_{a_2}{}^x)}{[H^+]^2 + [H^+]K_{a_1}{}^x + K_{a_1}{}^x K_{a_2}{}^x + K_1([H^+]^2 + [H^+]K_{a_1}{}^y + K_{a_1}{}^y K_{a_2}{}^y)} \quad (32)$$

which correspond to Eqs. (5) and (6) with $n = 2$. The results are given in Table 3.

TABLE 3. RATE CONSTANTS OF REVERSIBLE HYDRATION OF 2,6-DIHYDROXYPTERIDINE AND THOSE OF ISOMERIZATION OF THE WATER ADDUCTS AT 20°

pH	$k_1 \times 10^4$	$k_2 \times 10^4$	$k_3 \times 10^4$	$k_4 \times 10^4$	$k_5 \times 10^4$	$k_6 \times 10^4$
5·26	2690	46·8	36·2	0·0487	38·9	3·00
5·48	2240	47·3	24·0	0·0391	31·9	2·46
5·78	1360	31·3	12·9	0·0310	22·0	1·70
6·20	534	32·2	6·58	0·0310	12·3	0·951
6·84	125	27·3	2·20	0·0389	7·65	0·613
7·06	63·8	22·4	1·55	0·0450	3·79	0·312

At pH = 7·06, from Eq. (30), the figures in Table 3 give t_{max} = 349 seconds. Experimentally, t_{max} was found to lie between 350 and 360 seconds. The calculated value is thus in extremely good agreement with the observed value. This strongly suggests that the assumptions made in the course of the calculation are reasonable and that they could validly be used in similar calculations of t_{max} values under different experimental conditions.

For the system studied, values of the concentrations of X, A and B have been calculated as a function of time, using Eqs. (25) and (26). These values are plotted in Fig. 9.

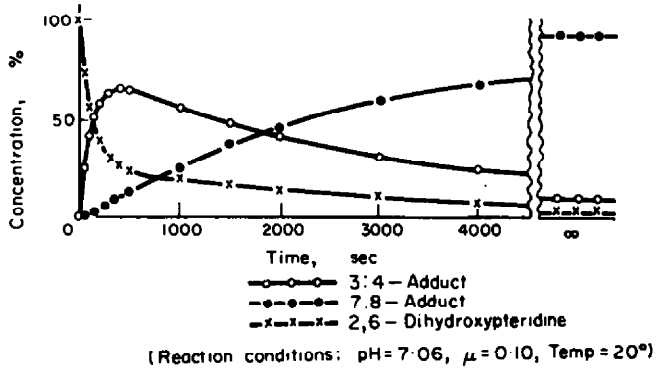


FIG. 9. Changes in concentrations of X, A, and B during the course of the reaction under the conditions where pH = 7·06, μ = 0·10, and Temp = 20°.

As can be seen from Fig. 9, if the reaction is stopped completely 6 or 7 minutes after mixing a solution of the anhydrous dianion of 2,6-dihydroxypteridine and a neutral buffer solution (pH = 7·06 for the final solution), the thermodynamically less stable water adduct should be the major product:

2,6-dihydroxypteridine——26 %
3:4-adduct ——64 %
7:8-adduct ——10 %

However, if the reaction system is left for a long time (e.g., more than 2 or 3 hours) under the same conditions, the composition of the product becomes

2,6-dihydroxypteridine——0·4 %
3:4-adduct ——7·6 %
7:8-adduct ——92·0 %

"Dual reactivity", in the sense discussed for 2,6-dihydroxypteridine, is most likely to be seen when the species contains two reactive centres, connected by a conjugated system.[48,49] Definition of the appropriate conditions under which high yields of a less stable reaction product can be obtained is an important function of physical organic chemistry. The foregoing discussion leads to the conclusion that the following conditions must be met:

1. The reactant must be a highly reactive unstable species so that the reaction is exothermic;
2. In the case of a reversible reaction, the reaction must be stopped at some optimum time;
3. If the reaction is acid-base catalyzed, the pH of solution must not lie such a region that the catalyzed isomerization of the less stable product to the more stable product proceeds rapidly;
4. Similarly, the temperature of the system must be such that the reactions proceed at a convenient rate.

EXPERIMENTAL

Potentiometric measurements. For all potentiometric titrations a "Vibron" Model 33B Electrometer (Electronic Instrument Ltd.), fitted with an internally shielded glass electrode and a saturated calomel electrode, was used. The pH meter was calibrated with the 0·05 M-sodium borate ("Analar") and 0·05 M-potassium hydrogen phthalate ("Analar") for which accurate pH values at different

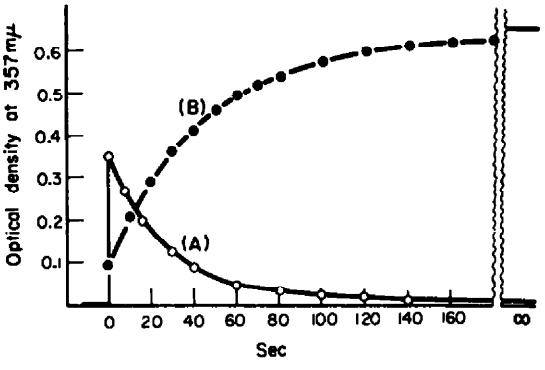


FIG. 10. Time-dependent optical density changes in the reversible hydration of 6-hydroxypteridine. Measurements were made by the stopped-flow method using.
(A) Equilibrated alkaline solution into near-neutral buffer (pH = 5·26)
(B) Equilibrated neutral solution into alkaline buffer (pH = 10·50)
(Ionic strength = 0·10; Temp = 20°, Concentration = $9·02 \times 10^{-5}$ M)

temp are known.[50] In a typical potentiometric titration, 50 ml of an 0·001 M solution in carbonate-free, glass-distilled water, was titrated rapidly with standard 0·1M HCl or an essentially carbonate-free KOH solution using an "Agla" micrometer all-glass syringe, additions being made in steps of one-tenth equivalent. A complete titration took somewhat less than 3 min.

Spectrophotometric measurements (kinetic measurements). Measurements were made on a Shimadzu Model RS-27 Spectrophotometer. Because of a catalytic effect, hydration-dehydration reactions proceed quite rapidly when the pH of the reaction system lies further from the neutral pH than about ±2 pH units (see pH-rate profiles). By using the rapid reaction apparatus, in the

[48] A. N. Nesmeyanov and M. I. Kabachnik, *Experientia* Suppl., No. 2, 49 (1955).
[49] N. Kornblum, R. A. Smiley, R. K. Blackwood and D. C. Iffland, *J. Amer. Chem. Soc.* 77, 6269 (1955).
[50] U.S. National Bureau of Standards, Letter Circular L.C., 993, p. 6.

present work, rate constants could be obtained within $\pm 5\%$ provided the half-time, $t_{1/2}$ was not less than 5 sec. The apparatus is attached to the autorecording spectrophotometer which has a maximum scanning rate of about four inches in 20 sec.

Constancy of temp was maintained by the use of laboratory "Thermomix" (B. Braun Melsungen Apparatebau Thermomix II), water circulating continuously through metal blocks which surrounded both the reaction vessel (quartz cell) and the reactant reservoirs. Buffer solutions of a constant ionic strength were prepared from "Analar" reagents, which permitted the control of pH within $\pm 0{\cdot}02$ pH units over the pH range 3·4 to 12. Sodium chloride was added to solutions to maintain a constant ionic strength. Due to a loss (or reformation) of a double bond on hydration (or dehydration), the compounds studied here showed striking difference in their UV and/or visible spectra in passing from the starting materials to final products.[15,26] This marked difference in spectra, between the more conjugated and the less conjugated systems, permitted the application, at suitably chosen wavelengths, of spectrophotometric method for kinetic studies. An example of the use of the stopped flow system in the recording of the optical density change with time is provided in Fig. 10.

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