747. *Pteridine Studies. Part XXII.*[1] *Kinetics of the Reversible Hydration of Substituted 2-Hydroxypteridines.*

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Reversible hydration across the $C_{(4)}=N_{(3)}$ double bond to form the corresponding 3,4-dihydro-4-hydroxy-compounds has been studied by rapid-reaction spectrophotometric methods, for 2-hydroxy-6- and -7-methyl-, 2-hydroxy-6,7-dimethyl-, 2-hydroxy-6,7-diethyl-, and 2-mercapto-pteridine. First-order rate constants for the reactions, which are acid–base-catalysed, have been obtained. Effects of substituents on rates and equilibria are discussed.

Recently kinetic studies have been made of the reversible, acid–base-catalysed hydration in aqueous solution of pteridine,[1] its methyl derivatives,[1] and 2-hydroxypteridine [2] across the $C_{(4)}=N_{(3)}$ double bond to form the corresponding 3,4-dihydro-4-hydroxy-compounds. These studies have now been extended to include 2-mercaptopteridine and some alkyl derivatives of 2-hydroxypteridine which behave in this way and for which equilibrium ratios of " hydrated " and " anhydrous " species have recently been reported.[3] (The " hydrated " form is strongly favoured in the neutral species, whereas anion formation leads to a preponderance of the " anhydrous " form.)

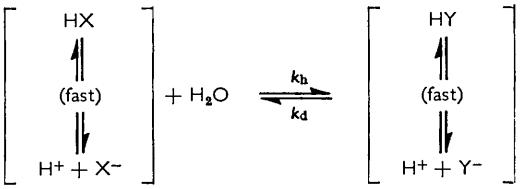
Experimental

Materials.—We are indebted to Professor A. Albert for providing all the pteridine derivatives used in this study. Buffer solutions in the pH range 3·4—6·2 were prepared by mixing 0·05M-sodium borate and 0·05M-succinic acid solutions. In the ranges pH 6·3—9·2 and 9·4—10·6, 0·05M-sodium borate was added to 0·1M-potassium dihydrogen phosphate and 0·1M-sodium carbonate, respectively. Above pH 10·6, 0·1M-sodium hydroxide was added to 0·1M-disodium hydrogen phosphate. Final solutions were adjusted to a constant ionic strength of 0·1 by addition of sodium chloride.

Methods.—Potentiometric titrations and spectrophotometric measurements were carried out with the apparatus and by methods described previously.[2] When solutions containing the anion of the organic species were added to nearly neutral buffer solutions, or when solutions containing the neutral molecule were made alkaline, the absorption spectrum of the final solution changed steadily with time until equilibrium was reached. At constant wavelength and constant pH, optical density changes with time gave first-order rate constants agreeing, on replication, within $\pm 5\%$.

[1] Part XXI, Inoue and Perrin, *J.*, 1963, 2648.
[2] Inoue and Perrin, *J. Phys. Chem.*, 1962, **66**, 1689.
[3] Inoue and Perrin, *J.*, 1962, 2600.

Equilibria in the systems studied can be summarised by the scheme



where HX is the anhydrous 2-hydroxypteridine derivative, HY is its covalently hydrated form, and k_h and k_d are first-order rate constants for hydration and dehydration in the equation,

$$-d([HX] + [X^-])/dt = k_h([HX] + [X^-]) - k_d([HY] + [Y^-]).$$

(As with 2-hydroxypteridine,[2] lactam–lactim tautomerism, which involves only intramolecular proton-transfer between a nitrogen and an oxygen atom, is assumed to be too fast to be detected by the methods used in this work.) The constants, k_h and k_d, are related to k_{obs}, obtained from the observed rate of change of optical density at constant wavelength and constant pH,

$$-dD/dt = k_{obs}(D - D_{eqm}),$$

by the equations [2]

$$k_h = k_{obs}\frac{K_2(a_{H^+}) + K_1K_a^X}{(K_2 + 1)(a_{H^+}) + (K_1 + 1)K_a^X}$$

and

$$k_d = k_{obs} - k_h,$$

where $K_1 = [Y^-]_{eqm}/[X^-]_{eqm}$, $K_2 = [HY]_{eqm}/[HX]_{eqm}$, and $K_a^X = (a_{H^+})[X^-]/[HX]$.

Results and Discussion

First-order rate constants for the reversible hydration at 20° of some substituted 2-hydroxypteridines are given in Table 1. The complete set of experimental values is given for 2-hydroxy-6-methylpteridine. For the other substances, examined at approximately the same pH intervals, only representative values (about one-quarter of the total) are listed. Values used in calculating k_h and k_d from k_{obs} are given in Table 2. The pH–rate profiles for k_h and k_d are V-shaped because of the catalysis by hydronium and hydroxyl ions. For each of the substances studied, the rate constants, k_h, for the hydration can be expressed by equations of the form:

$$k_h[K_a^X + (a_{H^+})] = k_1(a_{H^+})^2 + (k_2[H_2O] + k_3K_a^X)(a_{H^+}) + (k_4K_w + k_5K_a^X[H_2O]) + k_6K_a^XK_w/(a_{H^+}), \quad (1)$$

where k_1, k_2, and k_4 are the individual rate constants for the hydration of HX, as catalysed by hydronium ions, water molecules, and hydroxyl ions, respectively. The constants k_3, k_5, and k_6 are for the corresponding reactions for the anion, X^-. The $[H_2O]$ terms enter the equation, so that the individual rate constants can be used to compare the relative catalytic effects of H^+, H_2O, and OH^-. They are necessary because $[H_2O] = 55{\cdot}5$M whereas, by convention, $a_{H_2O} = 1$. This equation can be derived as follows:

$$\begin{aligned}-d([X^-] + [HX])/dt &= k_h([X^-] + [HX]) - k_d([Y^-] + [HY]) \\ &= k_1[HX](a_{H^+}) + k_2[HX][H_2O] + k_4[HX](a_{OH^-}) + k_3[X^-](a_{H^+}) + \\ &\quad k_5[X^-][H_2O] + k_6[X^-](a_{OH^-}) - (\text{corresponding terms for dehydration of } [Y^-] \text{ and } [HY]) \\ &= -\left(\frac{[K_a^X + (a_{H^+})]}{(a_{H^+})}\right)\frac{d[HX]}{dt},\end{aligned}$$

TABLE 1.

First-order rate constants (in sec.$^{-1}$) for the hydration and dehydration of some substituted 2-hydroxypteridines at 20° and $I = 0{\cdot}1$.

pH	10^3k_{obs}	10^3k_h	10^3k_d	pH	10^3k_{obs}	10^3k_h	10^3k_d	pH	10^3k_{obs}	10^3k_h	10^3k_d
				2-Hydroxy-6-methylpteridine							
4·58	3120	3092	28·1	7·28	7·38	7·30	0·0805	10·05	2·40	1·18	1·22
4·80	3080	3052	27·7	7·58	4·61	4·55	0·0590	10·37	2·50	0·853	1·65
5·03	1190	1179	10·6	7·84	3·14	3·09	0·0498	10·50	2·26	0·653	1·61
5·26	1290	1278	11·6	8·28	2·28	2·22	0·0632	10·60	2·06	0·526	1·53
5·48	294	291	2·66	8·54	2·35	2·25	0·0998	10·62	2·10	0·504	1·60
5·78	178	176	1·61	8·67	1·91	1·80	0·102	11·00	3·43	0·562	2·19
6·20	67·9	67·3	0·624	8·82	2·07	1·92	0·146	11·24	4·68	0·628	4·05
6·30	49·7	49·2	0·457	8·95	1·68	1·53	0·152	11·43	8·22	0·975	7·24
6·64	24·6	24·4	0·232	9·12	1·84	1·65	0·229	11·63	10·2	1·10	9·10
6·84	17·5	17·3	0·169	9·25	1·62	1·37	0·256	11·79	12·7	1·30	11·4
7·06	10·7	10·6	0·109	9·46	2·30	1·78	0·521	12·55	40·6	3·73	36·9
				2-Hydroxy-7-methylpteridine							
4·39	3450	3354	96·4	7·84	2·92	2·79	0·127	11·00	4·41	0·395	4·01
5·03	601	584	16·8	8·67	2·08	1·82	0·260	11·79	12·6	0·771	11·8
6·20	50·4	49·0	14·3	10·19	2·14	0·638	1·50	12·9	64·6	3·55	61·0
6·84	14·6	14·2	4·33								
				2-Hydroxy-6,7-dimethylpteridine							
4·39	2180	2150	29·5	8·09	1·53	1·48	0·482	11·00	8·51	0·849	7·66
5·03	514	507	6·95	9·25	1·22	0·948	0·272	11·79	28·8	1·55	27·2
6·20	42·5	41·9	0·585	10·37	2·41	0·592	1·81	12·9	110	4·94	105
7·06	7·40	7·29	0·113								
				2-Hydroxy-6,7-diethylpteridine							
4·80	885	864	20·8	8·09	1·71	1·63	0·0832	10·50	3·08	0·511	2·57
5·48	222	217	5·25	8·54	1·48	1·35	0·134	11·24	10·1	0·753	9·35
6·46	34·9	34·1	0·841	9·68	1·88	0·931	0·948	11·79	23·7	1·38	22·3
7·28	6·68	6·50	0·184								
				2-Mercaptopteridine							
4·39	2130	2124	5·64	7·84	2·22	2·10	0·120	10·50	2·40	0·533	1·87
5·03	524	523	1·43	8·67	0·887	0·660	0·227	11·43	10·3	2·03	8·30
6·20	64·3	64·1	0·249	9·46	0·857	0·347	0·510	12·5	103	24·9	78·1
6·84	16·1	16·0	0·130								

TABLE 2.

Acid dissociation constants of " hydrated " (Y) and " anhydrous " (X) hydroxypteridines, at 20°.

Pteridine derivative	pK_a^X	pK_a^Y	Pteridine derivative	pK_a^X	pK_a^Y
2-OH-6-Me	7·95 *	11·00 *	2-OH-6,7-Et_2	8·04 †	10·92 †
2-OH-7-Me	8·07 *	10·85 *	2-SH	6·52 *	9·72 *
2-OH-6,7-Me_2	7·95 *	11·15 *			

* From ref. 3. † $pK_a^{eq} = 9{\cdot}65$, giving $[HY]_{eqm}/[HX]_{eqm} = 41{\cdot}5$, $[Y^-]_{eqm}/[X^-]_{eqm} = 0{\cdot}055$.

because the pairs, HX and X^-, HY and Y^-, exist in dynamic equilibrium, and $K_a^X = (a_{H^+})[X^-]/[HX]$. Eliminating $[X^-]$ by making this substitution, and equating the coefficients for the hydration, we obtain eqn. (1). The equation for k_d is of the same form, but K_a^Y replaces K_a^X, and each individual rate constant, k_x, is replaced by the constant for the corresponding back-reaction, k_{-x}. Although they are mechanistically distinct, the rate constants k_2 and k_3, k_4 and k_5, cannot be evaluated separately because of the dynamic equilibrium between neutral species and their anions. For the species studied, the constants that give best fits of the rates of hydration and dehydration to eqn. (1) are summarised in Table 3.

The acid–base catalysed hydration of pteridine and 2-hydroxypteridine across their 3,4-double bonds may involve the formation of cyclic activated complexes in which a

TABLE 3.

Individual rate constants from eqn. (1) to fit the pH–rate profiles at 20° for the hydration and dehydration of 2-hydroxypteridines.

(i) Hydration

Pteridine derivative	$10^{-4}k_1$	$10^3(k_2[H_2O] + k_3K_a^X)$	$10^{12}(k_4K_w + k_5K_a^X[H_2O])$	k_6
2-OH-6-Me	11·5	3·34	3·54	0·355
2-OH-7-Me	6·49	2·66	1·85	0·182
2-OH-6,7-Me_2	5·41	1·99	3·73	0·441
2-OH-6,7-Et_2	6·73	2·55	2·95	0·369
2-SH	6·92	34·9	40·9	1·18
2-OH (ref. 2)	6·3	11	10	0·222

(ii) Dehydration

Pteridine derivative	k_{-1}	$10^5(k_{-2}[H_2O] + k_{-3}K_a^Y)$	$10^{14}(k_{-4}K_w + k_{-5}K_a^X[H_2O])$	k_{-6}
2-OH-6-Me	1050	3·04	3·22	3·55
2-OH-7-Me	1860	7·63	5·32	3·16
2-OH-6,7-Me_2	741	2·73	5·11	9·55
2-OH-6,7-Et_2	1620	6·14	7·12	6·76
2-SH	184	9·28	10·9	4·98
2-OH (ref. 2)	196	3·42	3·11	1·55

proton is bonded to N-3 while, at the same time, an oxygen atom of a water molecule or a hydroxyl ion is attached to C-4.[2] The resulting structures would be analogous to those postulated [4] for the acid–base-catalysed hydrolysis of esters and amides. The closeness of the values of k_1 and k_{-1} for 2-hydroxy- and 2-mercapto-pteridine supports the suggestion [2] that the oxygen atom in 2-hydroxypteridine is not directly involved in the formation of the activated complex in the water-addition. If the suggested structures are correct, the ease of formation of the activated complex from the anhydrous species would depend, not only on the proton affinity of N-3 (and hence on the basic pK_a value of the pteridine), but also on the net positive charge on C-4, whereas formation of activated species from hydrated species would depend on the abilities of the hydroxyl group on C-4 and the proton on N-3 to form hydrogen bonds to the same water molecule or to a hydroxyl ion. (The site of protonation in anhydrous 2-hydroxypteridine is believed to be N-3 for the following reasons. Urea-type resonance stabilisation, similar to that postulated for neutral hydrated 2-hydroxypteridine,[5] is possible if protonation occurs on N-3 because anhydrous 2-hydroxypteridine should, like other hydroxypteridines, exist mainly in the lactam form: in this case, N-1 would be the nitrogen atom concerned in the cyclic amide grouping, $-NH{\cdot}CO-$.[5] The weakness of amides as bases makes N-1 an unlikely site for proton addition. The bond localisation on $C_{(4)}=N_{(3)}$ would also favour N-3 rather than one of the nitrogen atoms of the pyrazine ring.)

According to this interpretation, the effect of substituents on individual rate constants is not easily predictable, nor would the generalisations developed, for example, for the neutral molecules be expected to apply to the anions of the same species. Results in Table 3 show that, in mixed constants such as $k_2[H_2O] + k_3K_a^X$, there is no obvious correlation with k_1 or K_a^X which might be used empirically to obtain k_2 or k_3. From eqn. (1), the only rate constants that are separately determinable are k_1, k_{-1}, k_6, and k_{-6} for the reactions

$$HX + H_2O + H_3O^+ \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} HY + H_3O^+$$

$$X^- + H_2O + OH^- \underset{k_{-6}}{\overset{k_6}{\rightleftharpoons}} Y^- + OH^-$$

Except for 2-hydroxy-4-methylpteridine which was unstable in solution (so that no accurate rate constants could be obtained for its hydration), the rate constants, k_1 and

[4] Laidler and Landskroener, *Trans. Faraday Soc.*, 1956, **52**, 200.
[5] Albert and Howell, *J.*, 1962, 1591.

k_6, of all the alkyl-substituted 2-hydroxypteridines studied did not vary by more than a factor of 2·5. Bigger differences (see Table 3) were found for the dehydrations, where, in all cases, alkyl-substitution in either or both of the positions 6 and 7 increased the rates. The maximum effect was a 9-fold increase in k_{-1} (for 2-hydroxy-7-methylpteridine) and a 6-fold increase in k_{-6} (for 2-hydroxy-6,7-dimethylpteridine). If, as seems reasonable, methylation of the molecule at sites remote from the reaction centre does not significantly alter the entropies of activation of the hydration–dehydration, the decrease in the equilibrium ratio of [HY]/[HX] when an alkyl group is attached to 2-hydroxypteridine in position 6 or 7 can thus be ascribed, mainly, to a decrease in the activation energy for the dehydration, so that the water-adduct reverts more quickly to the " anhydrous " species. (In support of this interpretation, the entropies of activation of hydration at pH 4, over the temperature range, 4·5—40°, are −36·8 and −36·2 cal. deg.$^{-1}$ mole^{-1} for 6-hydroxy- and 6-hydroxy-2-methyl-pteridine, respectively (Y. Inoue, unpublished results).

7-Methyl-substitution in pteridine itself decreases the rate of hydration to between one-half and one-third, while at the same time increasing three-fold the rate of dehydration.[1] The overall effect in the pteridine and 2-hydroxypteridine systems is therefore similar. However, the catalytic coefficients for hydrogen ions are much greater for hydration in the 2-hydroxypteridine series than they are for pteridine and its methyl derivatives, whereas for dehydration of the neutral species they are comparable. In consequence, 2-hydroxypteridine and its derivatives differ considerably from pteridine and the corresponding pteridine derivatives in the equilibrium ratios of hydrated to anhydrous species. This difference can be explained energetically in terms of conjugative effects [3] and urea-type resonance [5] favouring the hydrated neutral molecule of 2-hydroxypteridine, and of the loss of benzene-type aromaticity which must occur on hydration of neutral pteridine (but not of 2-hydroxypteridine, which is present mainly as the lactam tautomer).

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