918. *Pteridine Studies. Part XXIII.*[1] *Kinetics of the Reversible Hydration of 6-Hydroxypteridine and Some Derivatives.*

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Kinetic studies show that, at equilibrium, the neutral molecule of 6-hydroxypteridine is mainly the " hydrated " species, 7,8-dihydro-6,7-dihydroxypteridine, while the anion is mainly " anhydrous." Studies were extended to the 2-, 4-, and 7-methyl-, the 4-hydroxy-, and the 2-amino-4-hydroxy-derivatives. The reaction is acid–base-catalysed, follows a first-order rate equation, and resembles the corresponding hydration across the $C_{(4)},N_{(3)}$-bond of 2-hydroxypteridine. The " blocking " effect of a methyl group attached to the carbon atom involved in water-addition is exerted by decreasing the rate of hydration and increasing the rate of dehydration.

REVERSIBLE covalent hydration occurs across the $C_{(4)},N_{(3)}$-double bond of 2-hydroxypteridine and the $C_{(7)},N_{(8)}$-double bond of 6-hydroxypteridine, so that the neutral molecules are mainly " hydrated " whereas the anions are mainly " anhydrous." Equilibrium ratios of " hydrated " to " anhydrous " species for these hydroxypteridines and some of their derivatives are known.[2] However, although the kinetics of hydration of 2-hydroxypteridine [3] and some of its derivatives [1] have been examined, no comparable study of 6-hydroxypteridine or its derivatives has previously been reported.

In the present work, materials, methods, and calculations followed closely those described in Part XXII.[1]

Results.—On rapid addition of solutions of neutral molecules of 6-hydroxypteridine and its derivatives to alkaline buffers, or of solutions of their anions to near-neutral buffers, time-dependent spectral changes were observed. (For measurements involving the hydrated species derived from 6-hydroxy-7-methylpteridine, acid solutions were added to neutral or alkaline buffers. This was done because the cation of 6-hydroxy-7-methylpteridine is almost completely hydrated whereas, at equilibrium, the neutral species is only partly hydrated.) The optical-density changes at suitable wavelengths followed

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[1] Part XXII, Inoue and Perrin, *J.*, 1963, 3936.
[2] Inoue and Perrin, *J.*, 1962, 2600.
[3] Inoue and Perrin, *J. Phys. Chem.*, 1962, **66**, 1689.

TABLE 1.

First-order rate constants (sec.$^{-1}$) for hydration and dehydration of 6-hydroxypteridine and some of its derivatives, at 20° and $I = 0{\cdot}1$.

pH	10^4k_{obs}	10^4k_{h}	10^4k_{d}	pH	10^4k_{obs}	10^4k_{h}	10^4k_{d}	pH	10^4k_{obs}	10^4k_{h}	10^4k_{d}
6-*Hydroxypteridine* (analytical wavelength = 357 mμ)											
3·69	3190	3166	24·4	7·06	5·35	5·15	0·200	9·50	34·2	4·79	29·4
4·39	695	690	5·32	8·04	3·78	2·90	0·877	10·05	77·5	5·69	71·8
5·26	98·9	98·1	0·796	8·77	6·71	2·70	4·01	10·62	248	13·0	235
6·20	19·0	18·8	0·224								
6-*Hydroxy*-2-*methylpteridine* (364 mμ)											
3·69	5110	5057	53·2	7·58	3·17	2·81	0·36	10·50	157	5·9	151
4·39	1430	1415	15·0	8·67	4·42	1·84	2·58	11·43	1470	42·8	1427
5·26	199	197	2·17	9·46	13·4	1·66	11·7	12·55	16,400	459	15,940
6·30	17·6	17·3	0·289								
6-*Hydroxy*-4-*methylpteridine* (359 mμ)											
3·69	3480	3437	43·0	7·35	1·99	1·73	0·264	10·50	198	4·08	194
4·39	775	765	9·73	8·54	3·62	1·16	2·46	11·43	2060	32·8	2027
5·26	103	102	1·39	9·49	32·2	1·99	30·2	12·5	24,300	363	23,900
6·30	12·9	12·6	0·316								
6-*Hydroxy*-7-*methylpteridine* (350 mμ; 293 mμ above pH 9)											
3·69	1190	673	517	7·06	1·92	0·773	1·15	9·30	55·3	0·515	54·8
4·39	291	130	161	7·79	2·92	0·515	2·40	10·05	247	0·721	246
5·26	52·4	29·3	23·1	8·58	11·0	0·443	10·6	10·62	798	1·51	796
6·30	6·03	3·18	2·85								
4,6-*Dihydroxypteridine* (360 mμ)											
4·19	205	113	92·3	7·79	3·76	0·102	3·66	10·50	671	1·17	670
5·03	30·9	16·4	14·5	8·58	14·1	0·127	14·0	11·63	5720	7·41	5713
5·78	5·39	2·41	2·98	9·30	64·9	0·313	64·6	12·5	15,700	19·9	15,680
6·84	1·74	0·264	1·48								
2-*Amino*-4,6-*dihydroxypteridine* (390 mμ; 395 mμ above pH 10)											
3·69	1810	908	902	7·35	1·96	0·209	1·75	10·19	193	0·514	192
4·58	254	127	127	8·22	4·82	0·137	4·68	11·00	995	1·95	993
5·48	26·7	12·8	13·9	9·30	35·3	0·222	35·1	11·79	4970	9·16	4961
6·35	4·86	1·95	2·91								

first-order rate equations, and Table 1 lists representative experimental values for the rate constant, k_{obs}, obtained from these measurements at 20° and an ionic strength of 0·1.

As discussed earlier,[1,3] the composite constants, k_{h} and k_{d}, for hydration and dehydration have been calculated from k_{obs} by using published [2] values of the acid dissociation constants, $K_{\text{a}}{}^{\text{X}}$,$K_{\text{a}}{}^{\text{Y}}$, of the "anhydrous" (X) and "hydrated" (Y) species. The hydration–dehydration rates are strongly pH-dependent, indicating acid–base catalysis, so that k_{h} and k_{d} can be further resolved into the rate constants for reactions involving catalysis by hydronium ion, water molecules, and hydroxyl ions. These constants, defined as in Part XXII,[1] are listed in Table 2 for 6-hydroxypteridine and its methyl derivatives. For 4,6-dihydroxypteridine and its 2-amino-derivative (xanthopterin) the pH–rate profile can be analysed in the same way as for 2- and 6-hydroxypteridine, except that 18 possible catalytic rate constants must be included to take account of mono- and di-anion formation in the hydrated and anhydrous species. The rate equations can be written as:

$$k_{\text{h}}\{(a_{\text{H}^+})^2 + K_{\text{a}_1}{}^{\text{X}}(a_{\text{H}^+}) + K_{a_1}{}^{\text{X}}K_{a_2}{}^{\text{X}}\} = k_1(a_{\text{H}^+})^3 + f_1(a_{\text{H}^+})^2 + f_2(a_{\text{H}^+}) + f_3 + k_9K_{\text{w}}K_{a_1}{}^{\text{X}}K_{a_2}{}^{\text{X}}/(a_{\text{H}^+}),$$

$$k_{\text{d}}\{(a_{\text{H}^+})^2 + K_{\text{a}_1}{}^{\text{Y}}(a_{\text{H}^+}) + K_{a_1}{}^{\text{Y}}K_{a_2}{}^{\text{Y}}\} = k_{-1}(a_{\text{H}^+})^3 + f_{-1}(a_{\text{H}^+})^2 + f_{-2}(a_{\text{H}^+}) + f_{-3} + k_{-9}K_{\text{w}}K_{\text{a}_1}{}^{\text{Y}}K_{\text{a}_2}{}^{\text{Y}}/(a_{\text{H}^+}),$$

where each f is a term involving two or more rate constants.

Comparison of the rate constants for 6-hydroxypteridine with those for pteridine and 2-hydroxypteridine shows that for the reaction

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

the sequence is, for k_1, 2-hydroxypteridine > 6-hydroxypteridine > pteridine in the approximate ratio 180 : 5 : 1; and, for k_{-1}, pteridine > 2-hydroxypteridine > 6-hydroxypteridine, in the ratio 90 : 14 : 1. Thus, although the equilibrium constant [HY]/[HX] for 2-hydroxypteridine is roughly only three times as great as for 6-hydroxypteridine, the corresponding rates of hydration and dehydration differ considerably, 2-hydroxypteridine reacting much more rapidly in the acid-catalysed reactions. On the other hand, in the reaction

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

6-hydroxypteridine is hydrated about twenty times faster, and dehydrated about sixty times faster, than 2-hydroxypteridine.

TABLE 2.

Individual rate constants * to fit the pH–rate profiles at 20° for hydration and dehydration of 6-hydroxypteridine and its derivatives.

(i) *Hydration*

Pteridine	k_1	$10^5(k_2[H_2O] + k_3K_a^X)$	$10^{11}(k_4K_w + k_5K_a^X[H_2O])$	k_6
6-OH	1810	98·1	1·13	4·29
6-OH-2-Me	3450	88·3	4·16	2·30
6-OH-4-Me	1960	119	6·49	1·71
6-OH-7-Me	399	8·80	0·315	0·557
4,6-$(OH)_2$	192			(0·501) †
4,6-$(OH)_2$-2-NH_2	440			(0·338) †

(ii) *Dehydration*

	k_{-1}	$10^6(k_{-2}[H_2O] + k_{-3}K_a^X)$	$10^{13}(k_{-4}K_w + k_{-5}K_a^X[H_2O])$	k_{-6}
6-OH	13·8	7·49	8·63	91·2
6-OH-2-Me	36·3	9·28	4·37	79·4
6-OH-4-Me	24·6	14·9	8·14	105
6-OH-7-Me	309	68·2	24·4	372
4,6-$(OH)_2$	155			(295) ‡
4,6-$(OH)_2$-2-NH_2	437			(178) ‡

* k_1, k_2, and k_4 refer to hydration of HX catalysed by hydronium ions, water molecules, and hydroxyl ions, respectively. k_3, k_5, and k_6 are for the corresponding reactions for the anion, X^-. † k_9, for the reaction $X^{2-} + H_2O + OH^- \longrightarrow Y^{2-} + OH^-$. ‡ k_{-9}, for the reverse reaction.

Cyclic activated complexes have been suggested as intermediates in the acid–base catalysed hydration of pteridine [4] and 2-hydroxypteridine.[3] Similar complexes are to be expected in the 6-hydroxypteridine series, except that, because hydration occurs across the 7,8-, and not the 3,4-, double bond, they would be formed as a result of nucleophilic attack by a water molecule or a hydroxyl ion on C-7 and subsequent rapid addition of a proton to N-8. As discussed previously,[1] this type of complex formation makes it difficult to predict the effects of substituents on rate constants. In the 6-hydroxypteridine series, a 2- or a 4-methyl group has little effect on the individual rate constants, k_1, k_{-1}, k_6, k_{-6}, but insertion of a 7-methyl group considerably reduces the rate of hydration while at the same time increasing the rate of dehydration. These effects are ascribed, in part, to the electron-releasing effect of the methyl group which, by reducing the positive charge on C-7, retards nucleophilic attack. Steric factors are probably also important. The even greater effect on hydration and dehydration rates of a 4-hydroxyl group in 6-hydroxypteridine may be due, largely, to loss of benzene-type aromaticity in the pyrimidine ring if this compound is present mainly in the tautomeric lactam form.

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[4] Inoue and Perrin, *J.*, 1963, 2648.

It has been suggested that " hydrated " species resist oxidation by xanthine oxidase, and a dependence of the rates of oxidation of mono- and di-hydroxypteridines on the equilibrium ratios of hydrated to anhydrous species has been noted.[2] A controlling factor in such systems would be the rate of dehydration of the hydrated species. Results for 2- and 6-hydroxypteridine confirm the conclusion that at 20° and pH 8 they are quite slow, values of $t_{\frac{1}{2}}$ being about 1 hour [3] and 0·5 hour, respectively.

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