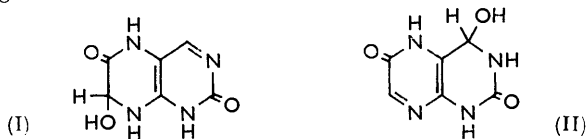


**987. Pteridine Studies. Part XXIV.<sup>1</sup> Competitive Covalent Hydration of 2,6-Dihydroxypteridine: Kinetics and Equilibria.**

By A. ALBERT, Y. INOUE, and D. D. PERRIN.

Covalent hydration of 2,6-dihydroxypteridine in aqueous solutions gives 7,8-dihydro-2,6,7-trihydroxy- (I) and 3,4-dihydro-2,4,6-trihydroxy-pteridine (II). Although substance (I) is thermodynamically the more stable, substance (II) is formed more rapidly in solution, so that, with time, the concentration of the latter rises to a maximum and then falls until, at equilibrium, substance (I) is the major neutral species. Insertion of a 4-methyl group greatly reduces the extent of 3,4 water-addition, so that hydration of 2,6-dihydroxy-4-methylpteridine gives only 7,8-dihydro-2,6,7-trihydroxy-4-methylpteridine: the reaction is approximately of the first order throughout. The anions of 2,6-dihydroxypteridine and its 4-methyl derivatives are essentially "anhydrous."

BECAUSE of its structural similarity to 2- and 6-hydroxypteridine, 2,6-dihydroxypteridine was expected to undergo reversible covalent hydration in aqueous solution. As a 6-hydroxy-derivative, it might add water across the 7,8-double bond,<sup>2</sup> giving compound (I), as a 2-hydroxy-derivative it might do so across the 3,4-double bond, giving compound (II), or both additions might take place to give 3,4,7,8-tetrahydro-2,4,6,7-tetrahydroxypteridine.<sup>2</sup> Evidence that covalent hydration of 2,6-dihydroxypteridine occurs came from large shifts in absorption maxima on anion formation and from well-defined spectroscopical hysteresis effects when the pH of a solution of the anion or the neutral molecule was changed.<sup>2</sup>



The present results indicate that only one molecule of water is added covalently to 2,6-dihydroxypteridine and that, whereas addition takes place more rapidly across the 3,4-position, the 7,8-adduct is thermodynamically the more stable, so that, with time, the concentration of the 3,4-adduct passes through a maximum.

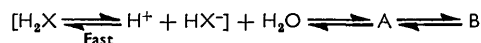
<sup>1</sup> Part XXIII, *J.*, 1963, 4803.

<sup>2</sup> Albert, Lister, and Pedersen, *J.*, 1956, 4621.

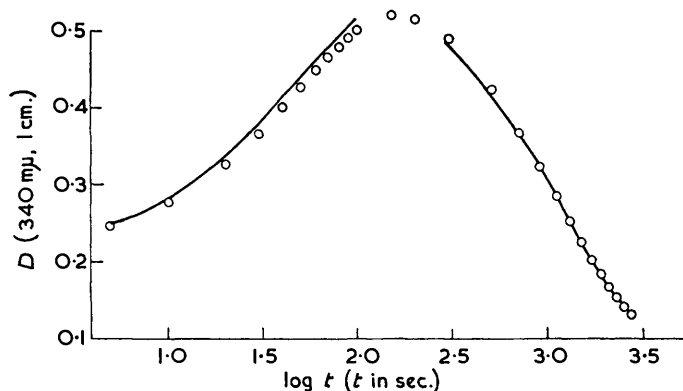
*Experimental.*—Materials and methods were similar to those described in Part XXII.<sup>3</sup>

*2,6-Dihydroxy-4-methylpteridine.*—4,5-Diamino-2-hydroxy-4-methylpyrimidine monohydrate<sup>4</sup> (0.79 g., 0.005 mole), ethyl glyoxylate hemiacetal (1 g.), and 2*N*-sulphuric acid (9 ml.) were set aside for 4 days at about 24°. The solution was adjusted to pH 4.5 with sodium citrate and sodium hydroxide. The insoluble precipitate, for which no crystallising solvent could be found, was filtered off and boiled with water (50 ml., rejected). The residue, when triturated with 0.5*M*-potassium hydroxide (7.5 ml.), left a non-basic impurity undissolved. The filtrate, when adjusted to pH 5 with acetic and sulphuric acid and filtered at 90°, gave chromatographically homogeneous *2,6-dihydroxy-4-methylpteridine* (0.15 g.) which darkened at 300° without melting (Found, for material dried at 20°/20 mm.; C, 42.9; H, 4.1; N, 28.6; loss of wt. at 150°/0.01 mm., 8.2. C<sub>7</sub>H<sub>6</sub>N<sub>4</sub>O<sub>2</sub>·H<sub>2</sub>O requires C, 42.9; H, 4.1; N, 28.55, H<sub>2</sub>O, 9.2%).

*Discussion.*—When an alkaline solution of 2,6-dihydroxypteridine was mixed with a neutral buffer, the optical density of the solution, measured between 300 and 368 mμ, rose, with time, to a maximum and then fell exponentially. Subsequently, the solution was made alkaline and the original spectrum of the dianion of 2,6-dihydroxypteridine was obtained. As shown in the Figure, both the rising and the falling portion of the curve of optical density against time could be adequately fitted by first-order rate equations, calculated by Guggenheim's<sup>5</sup> method. It shows that the reactions were reversible and probably consecutive, so that they could be represented by the scheme



where the portion in brackets denotes anhydrous 2,6-dihydroxypteridine and its monoanion which are present at comparable concentrations and in dynamic equilibrium, and A and B are the species formed.



Change of optical density at 340 mμ with time, following addition of  $2 \times 10^{-4}$ *M*-2,6-dihydroxypteridine in alkaline solution to a buffer of pH 6.64. Lines are theoretical curves for first-order reactions with  $k_{\text{obs}} = 2.20 \times 10^{-2}$  and  $8.09 \times 10^{-4}$  sec.<sup>-1</sup>, respectively.

The approximate absorption spectrum of species A was obtained, by using a recording spectrophotometer, from measurements on solutions at the time of the observed optical density maximum (see Figure). Similar measurements at final equilibrium afforded the approximate absorption spectrum of species B. The results are summarised in Table 1, together with absorption maxima of related compounds. The spectra of 7,8-dihydro-6-hydroxy- and 7,8-dihydro-6,7-dihydroxy-pteridine are an example of the common phenomenon that insertion of a secondary alcoholic group affects the electronic absorption

<sup>3</sup> Inoue and Perrin, *J.*, 1963, 3936.

<sup>4</sup> Robins, Dille, Willits, and Christensen, *J. Amer. Chem. Soc.*, 1953, **75**, 263.

<sup>5</sup> Guggenheim, *Phil. Mag.*, 1926, **2**, 538.

TABLE 1.

Absorption spectra ( $\lambda$  in  $m\mu$ ) of 2,6-dihydroxypteridine and related substances.

Substance	$\lambda_{max}$	$\log \epsilon$	Species	pH
7,8-Dihydro-6-hydroxypteridine .....	293 *	3.93	NM	7.4
	305 *	4.07	MA	13
7,8-Dihydro-6,7-dihydroxypteridine .....	288	3.99	NM	6.0
	294 †	4.03	MA	11.3
2,6-Dihydroxypteridine (anhyd.) .....	245, 406 †	4.21, 3.91	NM + MA ‡	5.5
	246, 282, 415	4.24, 3.62, 3.87	DA	11.1
2,6-Dihydroxy-4-methylpteridine (anhyd.)	399 †	3.60	NM	5.0
	417	3.65	DA	13
7,8-Dihydro-2,6-dihydroxypteridine .....	237, 265, 290 *	4.11, 3.90, 3.46	NM	6
Substance B § .....	235, 260, 300	4.27, 4.00, 3.80	NM	5.5
	273, 313 †	4.07, 3.90	DA	12.4
4-Methyl-B ¶ .....	231, 260, 302	4.17, 3.91, 3.82	NM	6.3
	275, 313 †	4.00, 3.85	DA	12
Substance A    .....	245, 320 **	4.15, 3.56	NM	5.5

NM = neutral molecule, MA = monoanion, DA = dianion. Italics indicate shoulders.

\* Albert and Matsuura, *J.*, 1962, 2162. † Obtained by a rapid-flow technique. ‡ Hydration too rapid at lower pH for spectrum of pure NM to be obtained. § Equilibrated neutral solution of (hydrated) 2,6-dihydroxypteridine. ¶ Equilibrated neutral solution of (hydrated) 2,6-dihydroxy-4-methylpteridine. || Spectrum recorded at time of maximum optical density in Figure. \*\* Broad band.

spectrum only slightly. In this case there is a hypsochromic shift of 11  $m\mu$  in the long-wavelength band. The spectra of 7,8-dihydro-2,6-dihydroxypteridine and of substance B are very similar, except for a hypsochromic shift of 10  $m\mu$  in the long-wavelength band of the former, which suggests that substance B is 7,8-dihydro-2,6,7-trihydroxypteridine, formed from 2,6-dihydroxypteridine by addition of a molecule of water across C-7 and N-8.

Supporting evidence is provided by the equilibrium spectrum of 2,6-dihydroxy-4-methylpteridine. That a methyl group, attached to the carbon atom of the C=N group across which water-addition occurs, greatly reduces the extent of hydration has been shown in the pteridine,<sup>6</sup> 2- and 6-hydroxypteridine,<sup>7</sup> and quinazoline<sup>8</sup> series, and has been suggested as a diagnostic tool in identifying the reaction site.<sup>9</sup> The equilibrium spectra of aqueous solutions of 2,6-dihydroxypteridine and its 4-methyl derivative are similar (Table 1), which confirms the view that in substance B water is not bound across C-4 and N-3. These observations also exclude the possibility that substance B is the "dihydrated" form, 3,4,7,8-tetrahydro-2,4,6,7-tetrahydroxypteridine, in which water would be covalently bonded across both the 3,4- and the 7,8-double bond.

The ionization constants of 2,6-dihydroxypteridine and its hydrated species, given in Table 2, were determined by rapid-reaction spectrophotometry to avoid changes, during the measurements, in the degree of hydration of the substances concerned. These new values are more reliable than those reported earlier<sup>7</sup> from potentiometric titrations, the

TABLE 2.

Acid dissociation constants of 2,6-dihydroxypteridine and related compounds at 20°.

Substance	$pK_{a_1}$	$pK_{a_2}$	Substance	$pK_{a_1}$	$pK_{a_2}$
2-Hydroxypteridine (anhyd.) .....	7.7		Substance B † .....	9.44	11.50
6-Hydroxypteridine (anhyd.) .....	6.45		3,4-Dihydro-2,4-dihydroxypteridine	11.05	—
2,6-Dihydroxypteridine (anhyd.) ...	5.58	8.64	7,8-Dihydro-6,7-dihydroxypteridine	9.90	—
(equil.) ...	8.55	9.69	7,8-Dihydro-6-hydroxypteridine ...	10.56 ‡	—
7,8-Dihydro-2,6-dihydroxypteridine	10.22 *	—			

\* Albert and Matsuura, *J.*, 1962, 2162. † Believed to be 7,8-dihydro-2,6,7-trihydroxypteridine.  
‡ Brown and Mason, *J.*, 1956, 3443.

<sup>6</sup> Inoue and Perrin, *J.*, 1963, 2648.

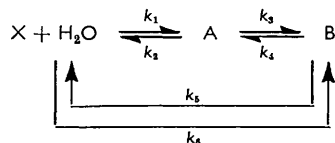
<sup>7</sup> Inoue and Perrin, *J.*, 1962, 2600.

<sup>8</sup> Armarego, *J.*, 1962, 561.

<sup>9</sup> Albert, Armarego, and Spinner, *J.*, 1961, 5267.

biggest differences being in  $pK_{a_1}^X$ ,  $pK_{a_1}^B$ , and  $pK_{a_2}^B$ . Table 2 also lists  $pK_a$  values for some related substances to show that the acid dissociation constants of species B are consistent with its presumed structure. Thus, in hydroxypteridines, water-addition to the ring greatly weakens the acidity of the hydroxyl group. For example, whereas the  $pK_a$  of 2-hydroxypteridine is 7.7, for 3,4-dihydro-2,4-dihydroxypteridine ("hydrated 2-hydroxypteridine") the  $pK_a$  is 11.05. A similar, but rather smaller, effect is produced if the molecule is hydrogenated instead of being covalently hydrated: the  $pK_a$  of 7,8-dihydro-6-hydroxypteridine is 10.56, whereas for 7,8-dihydro-6,7-dihydroxypteridine ("hydrated 6-hydroxypteridine") it is 9.90. On this basis, the difference of 0.78 between the  $pK_a$  values of 7,8-dihydro-2,6-dihydroxypteridine and substance B is consistent with the suggested structure (I) of the latter.

Evidence concerning the identity of substance A is less direct because the physical properties of 3,4-dihydro-2,6-dihydroxypteridine are unknown. Conclusions are based on differences in the spectral changes observed in solutions of (initially "anhydrous") 2,6-dihydroxypteridine and its 4-methyl derivative. Although the final spectra are very similar, spectra recorded during the reactions are different. Unlike 2,6-dihydroxypteridine, 2,6-dihydroxy-4-methylpteridine solutions showed no evidence of any species other than the initial and the final substance. The optical-density changes followed, throughout, a first-order rate equation. These results are readily interpreted in terms of the typical "blocking" effect of the 4-methyl group towards water-addition across the 3,4-position,<sup>1</sup> which occurs without seriously influencing water-addition across the 7,8-double bond. Thus, the complex kinetics of water-addition to 2,6-dihydroxypteridine and its 4-methyl derivative are readily explained if, initially, a molecule of water adds across the 3,4-position of 2,6-dihydroxypteridine to give 3,4-dihydro-2,4,6-trihydroxypteridine (II), which slowly isomerises to the more stable substance B (I). It is possible that in this isomerisation the "dihydrated" form is an unstable intermediate. Rates for the hydration of 2,6-dihydroxy-4-methylpteridine to its 7,8 water-adduct indicate that substance B can also be formed from 2,6-dihydroxypteridine, but more slowly than substance A, so that the complete reaction scheme can be written as:



where X, A, and B are 2,6-dihydroxypteridine and its 3,4 and 7,8 water-adduct, respectively. Each species may be present as its neutral molecule and mono- and di-anion, although, at equilibrium, the neutral molecules exist predominantly as the water-adducts.

The facility with which water can add to either the 3,4- or the 7,8-position of 2,6-dihydroxypteridine is suggested by an inspection of the carbonium ion (IIIa and b), which is the likely reactant in the acid-catalysed reaction. The difference in the rates of formation



of the water-adducts indicates that the free energy of activation for this reaction is lower for 3,4- than for 7,8-addition. We believe that this difference is explicable on steric grounds. Conversion of the planar carbonium ion (III) into the 3,4-adduct requires a smaller change in geometry than is involved in forming the 7,8-adduct. In the 3,4-adduct, approximate planarity is preserved because the proximity to the junction carbon-carbon double bond

forces the hydroxyl group into a quasiequatorial position. Conversely, this constraint towards linearity renders the 3,4-adduct energetically less stable than the 7,8-adduct.

At a wavelength where substances X and B, but not A, have roughly equal extinction coefficients, the observed rate constant,  $k_{\text{obs}}$ , obtained from initial optical density changes, approximates to  $(k_1 + k_2)$ . Similarly, the rate constant from measurements at times exceeding  $5t_{\text{max}}$ , where  $t_{\text{max}}$  is the time needed for maximum formation of substance A, is roughly equal to  $(k_3 + k_4)$ . Measured rate constants for both of these reactions are given in Table 3. The sum of the constants  $k_5$  and  $k_6$  cannot be obtained directly, but, by analogy

TABLE 3.

Rate constants (sec.<sup>-1</sup>) for the initial covalent hydration of 2,6-dihydroxypteridine and for the isomerisation of the initial product, at 20° and  $I = 0.1$ .

pH	$10^3 k_{\text{obs}}$ (3,4-hydrn.)	$10^4 k_{\text{obs}}$ (isomern.)	pH	$10^3 k_{\text{obs}}$ (3,4-hydrn.)	$10^4 k_{\text{obs}}$ (isomern.)	pH	$10^3 k_{\text{obs}}$ (3,4-hydrn.)	$10^4 k_{\text{obs}}$ (isomern.)
5.03	379	57.6	6.64	22.0	8.09	8.43	1.14	1.41
5.26	274	41.9	6.84	15.2	8.26	8.67	1.07	1.95
5.48	229	34.4	7.06	8.62	4.10	8.95	1.04	1.38
5.78	140	23.7	7.28	7.06	3.80	9.25	1.25	1.29
6.20	56.6	13.2	7.58	3.90	3.10	9.46	1.20	—
6.30	56.1	17.5	7.84	2.47	1.70	9.90	1.23	0.805
6.46	39.9	15.1	8.09	1.74	1.49			

TABLE 4.

Rate constants (sec.<sup>-1</sup>) for the reversible hydration of 2,6-dihydroxy-4-methylpteridine at 20° and  $I = 0.1$ .

pH .....	5.26	5.48	5.78	6.20	6.46	6.84	7.06	10.19
$10^3 k_{\text{obs}}$ .....	3.63	2.40	1.29	0.661	0.363	0.224	0.160	1.69
pH .....	10.50	11.00	11.24	11.43	11.63	11.79	12.50	
$10^3 k_{\text{obs}}$ .....	2.07	4.51	9.18	15.7	23.0	35.2	160	

with 6-hydroxypteridine and its 4-methyl derivative (for which the observed rate constants for reversible hydration agree within 10%<sup>1</sup>), they can be assumed to be approximately equal to the observed rate constants for the hydration of 2,6-dihydroxy-4-methylpteridine. These constants are given in Table 4.

From the ionisation constants of the individual species and the equilibrium  $pK_a$  value, the equilibrium ratio of the neutral molecules is obtained as  $[B]_{\text{eq.}}/[X]_{\text{eq.}} = 1100$ . For the monoanions the corresponding ratio is 0.15, and for the dianions 0.015. Also, an analysis of the absorption spectra at  $t_{\text{max}}$  and at final equilibrium gives  $[B]_{\text{eq.}}/[A]_{\text{eq.}} = 13$ .

TABLE 5.

Changes in concentrations of 2,6-dihydroxypteridine (X), its 3,4-water-adduct (A), and its 7,8-water-adduct (B), following rapid neutralisation of an alkaline solution. (Final pH = 7.06,  $I = 0.1$ , 20°.)

Time (sec.)	[A] (%)	[B] (%)	[X] (%)	Time (sec.)	[A] (%)	[B] (%)	[X] (%)	Time (sec.)	[A] (%)	[B] (%)	[X] (%)
0	0.0	0.0	100.0	250	61.0	6.1	32.9	1500	47.5	36.2	16.3
20	11.7	0.3	88.0	300	62.9	7.5	29.6	2000	41.2	45.1	13.7
50	25.6	0.9	73.5	350	63.8	8.9	27.3	3000	31.3	58.9	9.8
100	41.6	2.1	56.3	400	63.9	10.3	25.8	5000	19.4	75.5	5.1
150	51.5	3.4	45.1	500	63.2	13.0	23.8	10,000	9.7	89.1	1.2
200	57.6	4.0	38.4	1000	55.1	25.6	19.3	∞	7.6	92.0	0.4

This additional information enables the individual rate constants to be evaluated. Thus, at pH 7.06 and 20°, the above analysis yields  $k_1 = 6.4 \times 10^{-3}$ ,  $k_2 = 2.2 \times 10^{-3}$ ,  $k_3 = 3.8 \times 10^{-4}$ ,  $k_4 = 3.1 \times 10^{-5}$ ,  $k_5 = 4.5 \times 10^{-6}$ ,  $k_6 = 1.6 \times 10^{-4}$ .

A check on the validity of the analysis is provided by the calculation, from the rate constants, of  $t_{\max}$ . It can be shown that

$$(K_2 - K_1)t_{\max} = 2.303\{\log (K_2[n(k_1 - k_6S) - K_1(p - qS)]) - \log (K_1[n(k_1 - k_6S) - K_2(p - qS)])\},$$

where

$$\begin{aligned} n &= p + q + k_2k_5 + k_3k_5, & p &= k_1k_4 + k_1k_5 + k_4k_6, \\ q &= k_1k_3 + k_2k_6 + k_3k_6, & m &= k_1 + k_2 + k_3 + k_4 + k_5 + k_6, \\ 2K_1 &= m - (m^2 - 4n)^{\frac{1}{2}}, & 2K_2 &= m + (m^2 - 4n)^{\frac{1}{2}}, \end{aligned}$$

and  $S = (\varepsilon_X - \varepsilon_B)/(\varepsilon_A - \varepsilon_X)$ .

Insertion of the above quantities gave  $t_{\max} = 349$  sec. Experimentally,  $t_{\max}$  lay between 350 and 360 sec. The agreement warrants the use of these values to obtain, for the system studied, approximate concentrations of X, A, and B at different times. Values are listed in Table 5.

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