## 120. *Pteridine Studies. Part XVI.*[1] *Equilibria in Aqueous Solutions of Pteridine.*

By D. D. Perrin.

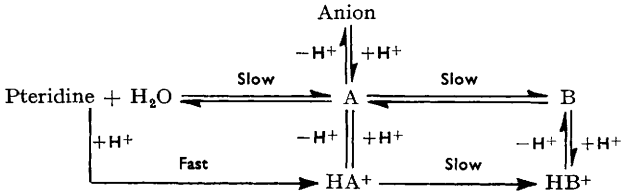
In aqueous solution, pteridine undergoes reversible addition of water across the 3,4-double bond to form 3,4-dihydro-4-hydroxypteridine: at 20° the equilibrium ratio is 3·5 : 1 in favour of the "anhydrous" pteridine. When pteridine is added to acid or alkali, the cation and anion of 3,4-dihydro-4-hydroxypteridine are rapidly and quantitatively formed. This cation slowly undergoes ring fission to the cation of 2-aminomethyleneamino-3-formylpyrazine. On neutralization, ring-closure takes place slowly to give the equilibrium mixture of pteridine and 3,4-dihydro-4-hydroxypteridine.

Both 2- and 7-methylpteridine behave similarly. Although reversible ring fission also occurs in 4-methylpteridine, with the formation of 3-acetyl-2-aminomethyleneaminopyrazine, production of 3,4-dihydro-4-hydroxy-4-methylpteridine could not be demonstrated.

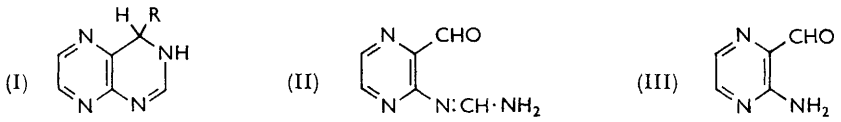
Although it has no ionisable hydrogen, pteridine behaves on titration with alkali as a weak acid with p$K_a$ 12·2.[2] This anomaly has been attributed to formation and ionisation of 3,4-dihydro-4-hydroxypteridine (I; R = OH), resulting from covalent addition of a molecule of water across the 3,4-double bond of pteridine.[3] Such an addition, if reversible, should lead to hysteresis during acid–base titrations similar to that reported for a number of other nitrogen heterocycles, including 2- and 6-hydroxypteridines.[4] Potentiometric and rapid-flow spectrophotometric methods have now shown that dissolution of pteridine in water leads to an equilibrium involving covalent hydration. Evidence, to be presented

[1] Part XV, Albert and Matsuura, *J.*, 1961, 5131.
[2] Albert, Brown, and Wood, *J.*, 1956, 2066.
[3] Brown and Mason, unpublished work, quoted in ref. 2.
[4] Perrin and Inoue, *Proc. Chem. Soc.*, 1960, 342.

in this paper, supports the annexed scheme and suggests that compound A is 3,4-dihydro-4-hydroxypteridine (I; R = OH) and B is 2-aminomethyleneamino-3-formylpyrazine (II).




In acid or alkaline solution the aldehyde (II) slowly decomposes into the amino-aldehyde (III).




*The Equilibrium,* Pteridine + $H_2O \rightleftharpoons$ A.—The ultraviolet spectrum of pteridine in anhydrous ethyl ether had an absorption maximum at 301 mμ (log ε 3·87) with shoulders on this peak near 308, 297, and 291 mμ, a minimum at 264 mμ, and a weak shoulder around 245 mμ. The spectrum of pteridine in cyclohexane [5] is closely similar. Solid, anhydrous pteridine, freshly dissolved in water, gave the spectrum shown in Fig. 1, with log ε 3·875 at 298 mμ (maximum). Except that the shoulder at 308 mμ in ether had become a small peak in water, the change of solvent made little difference to the spectrum, so that there seems no doubt that anhydrous pteridine was initially present, as such, in these solutions. However, when the aqueous solution was kept at 20° the spectrum changed steadily for about 10 hours, but thereafter remained constant: the intensity of the peak at 298 mμ decreased slightly and the " wings " of the band spread out. On the other hand, when pteridine was dissolved in 0·01M-hydrochloric acid and, within several minutes, the solution was neutralised, a new species (A) was formed, whose spectrum is shown in Fig. 1. This species gradually reverted to the same equilibrium mixture as was obtained earlier. The existence of five isosbestic points (223, 237, 247, 281, and 306 mμ) in the ultraviolet spectra of pteridine and species A, and their persistence throughout the changes of either to the equilibrium mixture, strongly suggested that the new product A was a pure species.

From the spectra of pteridine, species A, and the final mixture, an equilibrium ratio of [Pteridine]/[A] = 3·5 at 20° was calculated. [From points taken between 255 and 280 mμ, the average value was 3·4 (range 3·1—3·9), and from points between 315 and 345 mμ, 3·55 (range 3·2—3·7).] Measurements of rate of spectral change gave times to half-equilibrium for the reaction, pteridine + $H_2O \rightleftharpoons$ A, of ~75 minutes in borate buffer of pH 7·4 and ~3 minutes in borate buffer of pH 9·2. These results, and others in weakly acid or more strongly alkaline solutions, indicated the reaction to be acid–base-catalysed.

*The Equilibrium* · $HA^+ \rightleftharpoons H^+ +$ A.—Introduction of further nitrogen atoms into the ring skeletons of quinoline and isoquinoline is base-weakening.[6] Thus, whereas the p$K_a$ value of quinoline is 4·9,[6] for 1,5-, 1,6-, 1,7-, and 1,8-naphthyridine it is 2·9,[7] 3·8,[8] 3·6,[8] and 3·4,[9] respectively, and for 1,4,5- and 1,4,6-triazanaphthalene it is 1·20 [7] and 3·05.[4] An even lower p$K_a$ value would be predicted for pteridine but this value has not yet been obtained: acidification to pH 2 of a neutral pteridine solution led, in less than two seconds,

[5] Mason, *J.*, 1955, 2336.
[6] Albert, Goldacre, and Phillips, *J.*, 1948, 2240.
[7] Albert and Phillips, *J.*, 1956, 1294.
[8] Albert, *J.*, 1960, 1790.
[9] Albert, Brown, and Cheeseman, *J.*, 1951, 474.

to the formation of the cation, $HA^+$, the spectrum of which is shown in Fig. 2. The identity of the species formed was based on the observations that neutralisation of the solution gave material A and not pteridine, and that acidification of material A gave a solution with the same spectrum.

The formation of the cation of A was attended by a hypsochromic shift (18 mμ) of its two main absorption peaks. Although such a shift (which is common in aromatic amines) is unusual in π-deficient *N*-heteroaromatics,[10] it is found, for example, in 3,4-dihydroquinazoline (11 mμ) and its 2-methyl (14 mμ) and 3-methyl (20 mμ) derivative[11] which, for this comparison, might be expected to serve as models for 3,4-dihydro-4-hydroxypteridine.

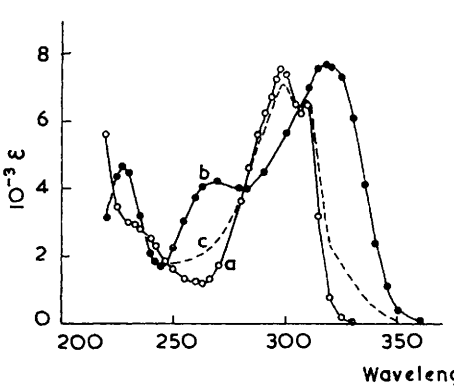



FIG. 1.

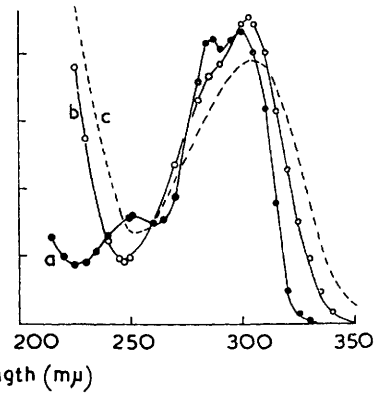



FIG. 2.

FIG. 1. Absorption spectra in water of (a) pteridine, (b) its water-adduct (A), and (c) their equilibrium mixture at 20°.

FIG. 2. Absorption spectra in aqueous 0·01M-HCl of: (a) the cation $HA^+$, of the pteridine water-adduct A; (b) the cation $HB^+$, into which $HA^+$ changes slowly; and (c) the cation of 2-aminomethyleneamino-3-formylpyrazine oxime. The spectrum of $HA^+$ was obtained by the rapid-flow technique.

When freshly prepared solutions were used which were acidified and then buffered to known pH values, the spectrophotometric $pK_a$ of material A was obtained as 4·79 (4·77—4·83 for buffers of pH 4·00—5·60; analytical wavelength 325 mμ). The previous[9] potentiometrically determined $pK_a$ of 4·12 for " pteridine " is now seen to be a composite constant, namely,

$$K_a = [H^+]([A] + [\text{Pteridine}])/[HA^+]$$

determined under experimental conditions where it is likely that species A and pteridine would be in equilibrium. If so, the true $pK_a$ of species A can be obtained from this value be substituting [Pteridine] = 3·5[A]. The value obtained, 4·81, is in good agreement with the direct determination.

*The Species*, B *and* $HB^+$.—The spectrum of a solution of the ion $HA^+$ in 0·01M-hydrochloric acid changed steadily during 2—3 hours, but thereafter remained stable. The formation of a new species, $HB^+$ (Fig. 2), was indicated, and the time of half-conversion at pH 2 was ~35 minutes. Neutralisation of the solution gave a material B, with the spectrum shown in Fig. 3. This species was slowly transformed into an equilibrium mixture of pteridine and species A: these observations were based on spectral changes and the possibility cannot be excluded that a small amount of B also persisted unchanged. The spectra of B and $HB^+$ did not differ sufficiently for an accurate spectrophotometric

[10] Albert, " Heterocyclic Chemistry," Athlone Press, London, 1959, p. 303.
[11] Albert, Armarego, and Spinner, *J.*, 1961, 2689.

p$K_a$ to be determined, but from measurements at 280 mμ in suitable buffers a value of about 5·3 was calculated: subsequently a p$K_a$ value of 5·17 ± 0·06 was obtained by direct titration of a solution of $HB^+$ with alkali.

*The Structures of* A *and* B.—The mildness of the conditions needed to obtain species A and B from pteridine and the ease with which pteridine can be re-formed from them indicate that only minor structural changes are involved. Reversible addition of water, possibly leading to ring-opening,[12] has been shown to give hysteresis effects in acid–base titrations under similar conditions with 1,4,6-triazanaphthalene (1-deazapteridine [4,12]). Addition of water across the 3,4-double bond of pteridine, with or without ring opening, is suggested by the isolation of the oxime of the aldehyde (II) after hydrolysis of pteridine at 20° in 2N-sodium carbonate containing hydroxylamine,[2] and by the isolation of the amine (III) from pteridine solutions heated in N-sulphuric acid for 5 minutes at 120° and

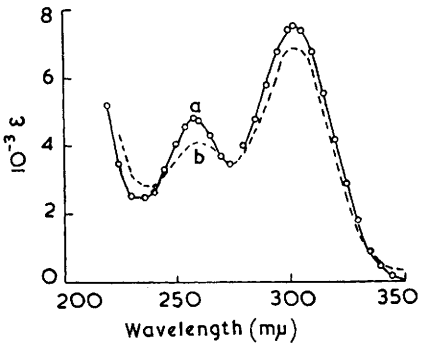



FIG. 3. Absorption spectra in water of (a) the neutral molecule of B from pteridine, and (b) aminomethylene-amino-3-formylpyrazine oxime.

then neutralised with potassium hydroxide.[2] Very slow formation of the amine (III) in pteridine solutions at pH 2 was also demonstrated in the present work by observing during several days the steady development of its characteristic absorption band ($\lambda_{max.}$ 371 mμ).

Brown and Mason [13] found that 2- and 6- (but not 4- or 7-)hydroxypteridine were readily oxidised by cold dilute alkaline permanganate to 2,4- and 6,7-dihydroxypteridine. The reason given for this selectivity is that only 2- and 6-monohydroxypteridine have a molecule of water covalently attached (across the 3,4- and 7,8-double bond, respectively). Similarly, pteridine gave 4-hydroxypteridine.[13] This has been confirmed in the present work: the precipitate obtained by oxidation of pteridine with hydrogen peroxide at pH 9·0 was identified (paper chromatography, infrared spectra, and elementary analysis) as almost pure 4-hydroxypteridine. These oxidations strongly suggest that pteridine in aqueous solution is in equilibrium with species that have water added across the 3,4-double bond.

That material B, but not A, contained an aldehyde group was shown by the rates of colour development when solutions in 0·01M-hydrochloric acid were added to an equal volume of glacial acetic acid containing benzidine. Whereas B, like pyridine-3-aldehyde (run as control), gave almost complete colour development within 40 seconds, compound A and pteridine needed about 6 hours for half-completion of the reaction. Because, in control experiments, half-conversion of $HA^+$ into $HB^+$ took about 10 hours, the limiting factor in the reaction between A and benzidine was probably the time needed to convert it into B, and apparently A, itself, was unable to form a Schiff's base.

The two properties of substance B (its aldehyde group and the ease with which it can re-form pteridine), together with the proneness to attack of the 3,4-double bond of pteridine, indicated that material B is the amidine-aldehyde (II). This was confirmed by the very close similarities of the ultraviolet spectra (Figs. 2 and 3) of B and the oxime

[12] Albert and Pedersen, *J.*, 1956, 4683.
[13] Brown and Mason, *J.*, 1956, 3443.

of compound (II), both as neutral molecules and as cations in 0·01M-hydrochloric acid, because the absorption spectra of oximes generally resemble those of their parent carbonyl compound.[14]

From the evidence discussed above, and the requirement that compound A is an intermediate in the formation of (II) from pteridine, it is concluded that it is the base (I; R = OH). The lower distribution ratio (found to be 0·2 : 1) of compound A between chloroform and water than of pteridine (2 : 1) was also consistent with the presence of a hydroxyl group in the molecule.

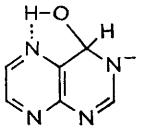
Confirmation of these structures was sought by preparing the neutral and acid oxalates of compound A, and the chloroplatinate, hydrochloride, and picrate of compound B. Assignment of these salts to A or B was based on the ultraviolet spectra of fresh solutions in buffers of pH 7, together with the subsequent spectral changes towards the equilibrium mixture of pteridine and A. (For the picrate, differential spectrophotometry was necessary because of the strong absorption by picric acid.) The infrared spectra (KBr or AgCl discs) of the hydrochloride and the chloroplatinate of B and of 2-aminomethyleneamino-3-formylpyrazine oxime showed a general similarity between 1450 and 4000 cm.$^{-1}$ (including a very strong band at 1545—1555 and a strong band at 1635—1645 cm.$^{-1}$), but the spectrum of the neutral oxalate of A was different. The unexpected absence of any carbonyl-stretching band in the spectra of the B salts was attributed to the addition of water to the aldehyde group to form the stable hydrate (*i.e.*, a *gem*-diol): elementary analysis of the chloroplatinate and picrate confirmed this extra molecule of water. Stabilisation of the hydrated form of the aldehyde in the B cation was due to the electron-withdrawing effect of the positively charged aminomethyleneamino-group [cf. 1-methyl-3- and -4-piperidone in which the carbonyl group is hydrated in the hydrochloride (with loss of the infrared carbonyl-stretching band), but not in the neutral molecule [15]].

Passage of dry hydrogen chloride into a solution of pteridine in anhydrous ether led to opalescence, probably due to true pteridine hydrochloride, with no change in ultraviolet absorption maximum. Clarification of the solution by addition of magnesium-dried methanol gave a spectrum (maximum at 287 mμ and shoulder near 302 mμ) which changed within a few minutes to one with a maximum at 308 mμ and a weak shoulder near 280 mμ. These reactions were probably analogous to the formation of $HA^+$ and $HB^+$. When cyclohexanol was used, reaction did not proceed beyond the first stage and the product, which was isolated, gave an analysis consistent with its being the hydrochloride of the cyclohexanol adduct (I; R = $OC_6H_{11}$).

*Anion Formation.*—Although pteridine cannot form an anion, the ultraviolet spectrum of pteridine solutions is changed by strong alkali,[16] and an apparent p$K_a$ of 12·2 has been obtained by direct titration.[2] In the present work, with suitable buffers and an analytical wavelength of 320 mμ, a p$K_a$ of 11·86 ± 0·02 was found. This is clearly a composite constant, with $K_a = [H^+]/[\text{Anion}]/([\text{Pteridine}] + [A] + [B])$. Although pyridine-2-aldehyde has p$K_a$ 12·80,[17] anion formation in the present instance is due to A because, when pteridine in 0·5M-sodium hydroxide was rapidly neutralised the spectrum appeared to be that of a mixture containing only pteridine and A (attainment, on standing, of the equilibrium spectrum of Fig. 1c showed that there had been no significant decomposition). If anion formation involved B, the true p$K_a$ would be very much less than 11·86, by an amount, log $(1 + x)$, where $x$ is the equilibrium concentration ratio $([\text{Pteridine}] + [A])/[B]$, which would be large because (B) is, at most, only a minor component of neutral equilibrated pteridine solutions. In such a case, use of rapid-flow techniques to run solutions containing $HB^+$ into buffers of, say, pH 10—11 should have led initially to significant anion formation, the concentration then falling with time. This was not

[14] Gillam and Stern, " Electronic Absorption Spectroscopy," Arnold, London, 2nd edn., 1957, p. 56.
[15] Lyle, Adel, and Lyle, *J. Org. Chem.*, 1959, **24**, 342.
[16] Lister, Ramage, and Coates, *J.*, 1954, 4109.
[17] Nakamoto and Martell, *J. Amer. Chem. Soc.*, 1959, **81**, 5827.

found, even with times of mixing below one second, although the postulated reaction should have taken longer than this to approach equilibrium. Although not conclusive, the fact that the position of the absorption maximum in the anion resembles that of A rather than that of B, also favours A as the structure concerned.


(IV)

The true p$K_a$ of the anion derived from A is thus found to be 11·21 by using the equilibrium ratio [Pteridine]/[A] given above.

This anion has been formulated as the resonance-stabilised hydrate (IV).[3]

2- *and* 7-*Methylpteridine*.—Changes in the ultraviolet spectra of 2- and 7-methylpteridine in aqueous solution paralleled those for pteridine, as also did the absorption spectra of the various species (see Table). Similarly, 2-methylpteridine and its A-type

Physical properties of some pteridine species in water at 20°.

| Species | p$K_a$ | $\lambda_{max.}$ | log ε | pH |
|---|---|---|---|---|
| Pteridine | (Not known) | *233*, 298 + 308 | *3·47*, 3·875 + 3·82 | 7·4 |
| A,[a] neutral molecule | 4·79, 11·21 | 228, 269, 318 | 3·66, 3·69, 3·89 | 7·4 |
| cation | | 251, 287 + 300 | 3·50, 3·92 + 3·94 | 2·0 |
| anion | | 325 | 3·97 | 13·9 |
| B,[b] neutral molecule | 5·17 | 258, 302 | 3·68, 3·87 | 7·4 |
| cation | | *285*, 303 | *3·86*, 3·95 | 2·3 |
| 2-Aminomethyleneamino-3-formylpyrazine oxime | | | | |
| neutral molecule | | 259, 303 | 3·61, 3·84 | 7·4 |
| cation | | 305 | 3·92 | 2·0 |
| 2-Amino-3-formylpyrazine | | | | |
| neutral molecule | | 263, 371 | 3·85, 3·83 | 7·4 |
| 2-Methylpteridine | (Not known) | ~*235*, 305 + 317 | *3·54*, 3·93 + 3·89 | 7·4 |
| A,[c] neutral molecule | 5·45, 11·96 | 227, 269, 319 | 3·70, 3·74, 3·89 | 7·4 |
| cation | | 247, 284 + 300 | 3·59, 3·88 + 3·90 | 2 |
| anion | | 249 + 270 (very flat), 326 | 3·71 + 3·70, 3·93 | (2M-KOH) |
| 4-Methylpteridine | 2·94 [f] | 300 + 312 | 3·92 + 3·86 | 7·4 |
| B,[d] from 4-methylpteridine | | | | |
| neutral molecule | 5·51 | 258, 298 | 3·61, 3·86 | 7·4 |
| cation | | 305 | 4·00 | 2·0 |
| 7-Methylpteridine | (Not known) | ~*235*, 298 + 310 | *3·51*, 3·98 + 3·94 | 7·4 |
| A,[e] neutral molecule | 4·91, 11·76 | 232, *270*, 322 | 3·63, 3·57, 3·92 | 8·4 |
| cation | | *251*, 303 | *3·39*, 3·98 | 2 |
| anion | | 328 | 3·97 | (2M-KOH) |

[a] Apparently 3,4-dihydro-4-hydroxypteridine. [b] Apparently 2-aminomethyleneamino-3-formylpyrazine. [c] Apparently 3,4-dihydro-4-hydroxy-2-methylpteridine. [d] Apparently 3-acetyl-2-aminomethyleneaminopyrazine. [e] Apparently 3,4-dihydro-4-hydroxy-7-methylpteridine. [f] Albert, Brown, and Wood, *J.*, 1954, 3832.

compound had five isosbestic points (at 227, 236, 244, 288, and 313 mμ). There seems no doubt that the equilibria involved corresponded to those for pteridine, and that the methyl derivatives of A and B were formed.

An equilibrium ratio of [2-methylpteridine]/[2-methyl-A] = 2·8 at 20° was calculated from measurements at 266 mμ in buffers of pH 7·4 and pH 9. For 7-methylpteridine, however, the ratio was 25 (analytical wavelength, 330 mμ). In studies on quinazoline cations, a similar reduction in hydration following 7-, but not 2-, methylation was observed.[18] These ratios enabled a p$K_a$ of 5·45 for (2-methyl-A) and 4·91 for (7-methyl-A) to be obtained from the composite constants previously reported[19] for " 2-methylpteridine " (4·87) and " 7-methylpteridine " (3·49).

The steady changes in the spectra of acid solutions of 2- and 7-methylpteridine were qualitatively similar to those shown by pteridine: they were attributed to the formation of the corresponding B cations. The equilibria were not studied quantitatively. From reversible changes in the ultraviolet spectra of 2- and 7-methylpteridine on addition of

[18] Armarego, *J.*, 1962, 561.
[19] Albert, Brown, and Wood, *J.*, 1954, 3832.

strong alkali, apparent p$K_a$ values of 12·54 and 13·18, respectively, were obtained (analytical wavelength in each case, 330 mμ). The equilibrium ratios given above lead to p$K_a$ 11·96 for (2-methyl-A) and 11·76 for (7-methyl-A).

*4-Methylpteridine.*—Although the ultraviolet spectrum of a neutral aqueous solution of 4-methylpteridine did not change with time, at pH 3 (or more rapidly at pH 2) the absorption maximum shifted steadily during about an hour (from 300 to 305 mμ) and the intensity increased slightly; the small peak at 312 mμ disappeared. Reaction was half-complete in about 7 minutes. The new spectrum was almost identical with that of the ion $HB^+$ from pteridine. Neutralisation of the solution gave a spectrum initially similar to that of the neutral species (II) but changing gradually and quantitatively into that of 4-methylpteridine.

These results are readily interpreted by the same reaction scheme as was given for pteridine, except that for 4-methylpteridine the equilibrium for the reaction, 4-methylpteridine + $H_2O \rightleftharpoons$ 4-methyl-A, must lie well to the left. This is as expected from results for 6-hydroxy- and 6-hydroxy-7-methyl-pteridine,[4] 2-hydroxy- and 2-hydroxy-4-methyl-pteridine,[4] and the cations of quinazoline and 4-methylquinazoline:[18] in all cases the presence of a methyl group instead of a hydrogen atom on the carbon doubly bound to nitrogen considerably reduced the extent of covalent addition of water. A consequence of the very low equilibrium concentration of 4-methyl-A was that much less of its cation was present in weakly acid solutions, so that the slow formation of the cation of 4-methyl-B was the only reaction observed spectroscopically. The potentiometric p$K_a$ value of 5·51 ± 0·03 for 4-methyl-B was obtained by rapid alkaline titration of the equilibrated acid solution. This value was slightly greater than the p$K_a$ 5·17 for B, as expected from the smaller base-weakening effect of the acetyl than of the formyl group, and it supported the conclusion that the stable cation was that of 3-acetyl-2-aminomethyleneaminopyrazine. The present kinetic measurements suggest that the reported[13] p$K_a$ of 2·94, obtained by direct acid titration of 4-methylpteridine, is the p$K$ of true 4-methylpteridine. This result, taken with the difference of 0·34 between p$K_a$ values for B and 4-methyl-B, suggests that the p$K$ of pteridine itself is about 2·6.

The absence of significant amounts of the hydrated species, 3,4-dihydro-4-hydroxy-4-methylpteridine, explains why no spectroscopic evidence of anion formation could be found on using 4-methylpteridine even in 0·1M-potassium hydroxide.

*The Effect of Methyl Substituents.*—From the foregoing results it is concluded that, among pteridines with alkyl substituents in one or more of the 2-, 6-, and 7-positions (including the unknown 6-methylpteridine), covalent addition of water occurs to a limited extent in neutral aqueous solutions, but in acid solutions the cations present are predominantly those of the corresponding 4-hydroxy-3,4-dihydro-derivatives. The appearance of maxima at 260 and 255 mμ in the ultraviolet spectra of acid solutions of 6,7-dimethyl- and 2,6,7-trimethyl-pteridine, respectively, and their absence from neutral solutions,[19] are consistent with this interpretation, as, also, are results for 7-methoxy-pteridine.[19] Covalent hydration of neutral quinazoline has not yet been demonstrated but (except when it is 4-substituted) the quinazoline cation adds water avidly.[18] This behaviour, the ability of pteridines (including 4-methylpteridine) to undergo reversible ring fission in acid solution, the speed with which pteridine adds water, even in weakly acid solution, and the avidity of its oxalate and hydrochloride for water or alcohol, all suggest that in aqueous solution the pyrimidine portion of the pteridine nucleus has little aromatic character and seem to imply considerable charge localisation (" double-bond character ") in the bond joining $N_{(3)}$ and $C_{(4)}$.

The increase in base strength of 2-methyl-A (p$K_a$ 5·45) and 7-methyl-A (p$K_a$ 4·91) over that of A (p$K_a$ 4·79) can be attributed to the effect of the methyl group. The apparent base-weakening effect of a 4- and a 7-methyl group in pteridine[19] is now explicable. The previous values, obtained by slow potentiometric titration, are composite constants (except for 4-methylpteridine) which involve both hydrated and anhydrous species.

Essentially the same reaction scheme, but without identification of the species present, has been proposed independently (Dr. J. Komenda, personal communication) to explain the polarographic behaviour of aqueous solutions of pteridine and 2-aminopteridine.

## Experimental

Analyses are by Dr. J. E. Fildes and her staff. Samples for analysis were dried *in vacuo* at 20° unless otherwise specified.

*Oxidation of Pteridine to 4-Hydroxypteridine.*—Hydrogen peroxide (30%; 0·1 ml.) was added to pteridine (50 mg.) dissolved in borate buffer (pH 9; 0·4 ml.) at 20°. After 30 min. the copious precipitate was recrystallised from boiling water (2 ml.). The material was identical (chromatography and infrared spectra) with 4-hydroxypteridine (Found: C, 47·4; H, 3·0; N, 36·4. Calc. for $C_6H_4N_4O,0{\cdot}25H_2O$: C, 47·2; H, 3·0; N, 36·7%).

*4-Hydroxy-3,4-dihydropteridine Oxalates.*—Solutions of sublimed oxalic acid (0·18 g.) and pteridine (0·26 g.) in 1 : 4 ether–benzene (50 ml.) were mixed, kept in the cold for 3 hr., and filtered. *4-Hydroxy-3,4-dihydropteridine hydrogen oxalate* was washed with ether–benzene, and the excess of solvent was removed in a stream of air before the product was dried *in vacuo* over paraffin (Found: C, 39·7; H, 3·4. $C_8H_8N_4O_5$ requires C, 40·0; H, 3·4%).

0·5M-Aqueous oxalic acid (20 ml.) and pteridine (0·1 g. in 2 ml.) were set aside in the cold for 1 hr., then filtered. The residue was washed with water and dried (Found: C, 37·3; H, 3·9; N, 21·5. $C_8H_8N_4O_5,H_2O$ requires C, 37·2; H, 3·9; N, 21·7%). This *monohydrate* is probably the " pteridine oxalate " $C_8H_6N_4O_4,2H_2O$, reported by Jones.[20]

Pteridine (0·13 g.), dissolved in water (2 ml.), was added to an oxalic acid–potassium oxalate buffer of pH 2 (0·2M in oxalate; 10 ml.). After 15 min. the precipitate of (neutral) *4-hydroxy-3,4-dihydropteridine oxalate* was washed with water, then ethanol, and dried (Found: C, 43·5; H, 3·2. $C_{14}H_{14}N_8O_6$ requires C, 43·1; H, 3·6%). On prolonged washing with water (30 min.) the precipitate was partially converted into the hydrated acid oxalate.

All the above oxalates decomposed above 125° without melting and, as freshly prepared solutions in neutral buffers, gave the characteristic spectrum of neutral 4-hydroxy-3,4-dihydropteridine (Fig. 1).

*2-Aminomethyleneamino-3-formylpyrazine Tetrachloroplatinate.*—M-Hydrochloric acid (0·45 ml.) was added to pteridine (60 mg.) and the solution was left for 16 hr. in the dark at 20°, then mixed with hydrogen chloroplatinate (0·145 g. in 1 ml. water) and set aside with cooling. The *chloroplatinate* was filtered off and dried [Found, for material sampled in a dry box: C, 19·0; H, 2·55; N, 14·8; Cl, 29·0; Pt, 26·75. $(C_6H_8N_4O_2)_2,H_2PtCl_6$ requires C, 19·3; H, 2·2; N, 15·0; Cl, 28·2; Pt, 26·2%].

*2-Aminomethyleneamino-3-formylpyrazine Salts.*—Pteridine (50 mg.) was dissolved in N-hydrochloric acid (0·375 ml.). The solution was evaporated at 20° during several hours under reduced pressure, leaving a glass which was identified as the aminomethyleneamino-formylpyrazine hydrochloride from its ultraviolet spectra in acid and neutral solution. Pteridine (0·13 g.) in water (1 ml.) was added to saturated aqueous picric acid (20 ml.) and set aside at 20°. After 1 hr. (slight precipitation), N-hydrochloric acid (1 ml.) was added and the mixture was warmed at 85° to redissolve the precipitate. The solution was cooled and left overnight at 20°. The picrate, filtered off, washed with water, and dried, had m. p. 116·5° (Jones [20] reports " pteridine picrate," $C_{12}H_{11}N_7O_9$, m. p. 117·5°) (Found: C, 35·2; H, 3·0. Calc. for $C_{12}H_{11}N_7O_9,0{\cdot}5H_2O$: C, 35·4; H, 3·0%).

*Cyclohexanol Adduct of Pteridine.*—The following manipulation was done in a dry box protected from light. Pteridine (58 mg.) was dissolved in warm redistilled cyclohexanol (3·5 ml.) and the pale greenish-yellow solution was diluted with sodium-dried " AnalaR " ether (5 ml.). Dry hydrogen chloride was passed in until the solution was saturated and the colour disappeared. The mixture was filtered after 30 min. and the filtrate diluted with ether (sodium-dried) until precipitation of the hydrochloride was complete. The solid was filtered off, thoroughly washed with anhydrous ether, and dried, giving an extremely hygroscopic product, believed to be 4-cyclohexyloxy-3,4-dihydropteridine hydrochloride (Found: C, 51·2; H, 6·3; N, 20·1. Calc. for $C_{12}H_{17}ClN_4O$, containing 3·7% of water: C, 51·6; H, 6·55; N, 20·1%).

*Physical Measurements.*—Ultraviolet spectra were obtained on a Perkin-Elmer " Spectracord " instrument, the cell compartment of which had been adapted slightly to accommodate a

[20] Jones, *Nature*, 1948, **162**, 524.

1 cm. cell attached to a modified Chance [21] rapid-reaction apparatus. In this apparatus, which could be used both for stopped-flow and continuous-flow studies, the time taken between mixing of the reactant solutions in the mixing chamber and placing them in the cell was ~1 second. Changes of optical density at selected wavelengths were recorded by applying the electronic output of the " Spectracord," after amplification, to a Rectiriter recording milliameter (Texas Instrument Co.). Where species were relatively stable, their extinction coefficients and maxima were checked on a Hilger " Uvispek " spectrophotometer.

Potentiometric titrations were carried out in a magnetically stirred titration apparatus and recording pH-meter assembly, as described previously.[22]

Freezing-point depressions were used to confirm that the 2-aminomethyleneamino-3-formylpyrazine hydrochloride formed by pteridine in hydrochloric acid solutions was monomeric. The apparatus, which required upwards of 0·5 ml. of solution, consisted essentially of a small-scale Beckman-type unit with a thermistor in a Wheatstone bridge as the temperature-measuring device.

I thank Professor A. Albert for supplying the pteridines and the 2-aminomethyleneamino-3-formylpyrazine oxime used in this work and for stimulating and helpful discussion, Dr. W. L. F. Armarego for preparing the cyclohexanol adduct of pteridine, Dr. R. F. Evans for preparing the chloroplatinate, and Mr. D. T. Light for the infrared spectra.

Department of Medical Chemistry, Australian National University,
Canberra, Australia. [*Received, August 8th,* 1961.]

[21] Chance, in " Rates and Mechanisms of Reactions," Technique of Organic Chemistry," Vol. VIII, ed. Friess and Weissberger, Interscience Publ., Inc., New York, 1953, p. 690.
[22] Perrin, *J.*, 1960, 3189.