24. *Pteridine Studies. Part XIII.*[1] *Addition to 6-Hydroxypteridines.*

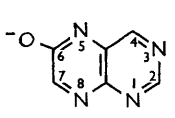
By Adrien Albert and Friedrich Reich.

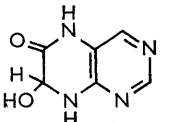
The methyl group in 6-hydroxy-7-methylpteridine had been found to hinder the characteristic addition of water across the 7,8-double bond of 6-hydroxypteridine. The electronic influences in such covalent hydrations are discussed, also why the 7,8-position is favoured and how the 7-methyl group opposes hydration. Similar influences are demonstrated in two natural products, xanthopterin and 7-methylxanthopterin.

Syntheses of six new hydroxy-methylpteridines are described. 6-Hydroxypteridine is shown to undergo Michael-like additions to the 7,8-double bond with ethyl malonate, ethyl cyanoacetate, and acetone.

6-Hydroxypteridine was the first heterocyclic substance recognized as undergoing reversible covalent hydration. The anion of 6-hydroxypteridine (I) is normal in that the ultraviolet spectrum agrees closely [2] with that of the neutral molecule of 6-aminopteridine, as is widely observed in aromatic chemistry; [3] also the sodium salt is anhydrous.[4]

The neutral molecule, however, rapidly undergoes (reversible) covalent hydration,[4] evidence for which is (*a*) the hysteresis loop traced during titration and back-titration [2] (pK 9·8 for the hydrated and 6·5 for the anhydrous form), (*b*) the long-wavelength ultraviolet absorption peak which is 67 mμ lower than that of the anion,[2] suggesting loss of a


(I)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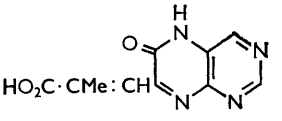

(III)

double bond, (*c*) proof that neither ring-opening nor a 6,7-keto–enol shift occurred,[5] (*d*) identity of the ultraviolet spectra of the neutral molecule with that of 7,8-dihydro-6-hydroxypteridine,[6] and (*e*) the ease with which oxidation to 6,7-dihydroxypteridine can be effected.[6] It is therefore the 7,8-hydrate (II). The cation of 6-hydroxypteridine was shown to be hydrated in the same position.[6]

[1] Part XII, Brown and Jacobsen, *J.*, 1960, 1978.
[2] Albert, Brown, and Cheeseman, *J.*, 1952, 1620.
[3] Jones, *J. Amer. Chem. Soc.*, 1945, **67**, 2127.
[4] Albert, *J.*, 1955, 2690.
[5] Albert, "Ciba Symposium on the Chemistry and Biology of Pteridines," Churchill, London, 1954, p. 210.
[6] Brown and Mason, *J.*, 1956, 3443.

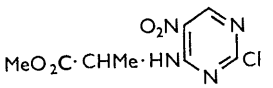
To test our hypothesis, that a 7-methyl group could sterically hinder covalent hydration, the synthesis of 6-hydroxy-7-methylpteridine was attempted. It was decided also to try to add, across the 7,8-double bond, weak acids other than water, particularly those commonly used in Michael condensations.

4,5-Diaminopyrimidine readily condenses with ethyl glyoxylate (or its hemiacetal), to give exclusively 6- or 7-hydroxypteridine in cold 2N-sulphuric acid [4] or boiling 2N-sodium carbonate [7] respectively. Hence it was surprising to find that 4,5-diaminopyrimidine gave no pteridine with ethyl pyruvate (or its acetal, or the free acid) under these conditions. Boiling 2N-sulphuric acid was also ineffective although it gives a 76% yield of 2-amino-4,6-dihydroxy-7-methyl- and 92% of 2,4-diamino-6-hydroxy-7-methyl-pteridine from pyruvic acid and the appropriate 4,5-diaminopyrimidine.[8] However, condensation in cold 5N-sulphuric acid gave us a 21% yield of the required 6-hydroxy-7-methylpteridine. This yield was increased to 60% in 5N-hydrochloric acid, the reaction being accelerated by the insolubility of the product.

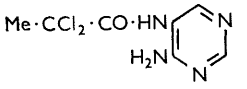
The reluctance of pyruvic acid to add electrophilically to 4,5-diaminopyrimidine is seen as the sum of two unfavourable inductive effects, the ester and the 5-amino-groups being insufficiently electron-attracting and -repelling respectively. Attempted condensation in 30N-sulphuric acid gave 7-(2-carboxyprop-1-enyl)-6-hydroxypteridine (III), which arose from dimerized pyruvic acid (other carboxypropenylpteridines have been isolated from pyruvic acid condensations [9]).

The identity of the 6-hydroxy-7-methylpteridine was confirmed by reduction to 7,8-dihydro-6-hydroxy-7-methylpteridine which was unambiguously synthesized as follows. 2,4-Dichloro-5-nitropyrimidine with alanine methyl ester gave methyl α-(2-chloro-5-nitro-4-pyrimidinylamino)propionate (IV); the nitro-group was then reduced, the ring closed, and the chlorine replaced by hydrogen. Surprisingly this dihydro-compound could not be used for preparing 6-hydroxy-7-methylpteridine as it resisted dehydrogenation and was destroyed on attempted oxidation owing largely to the liability of a 7-methyl group in pteridine.[10]

Attempts to prepare 6-hydroxy-7-methylpteridine by the cyclization of 4-amino-5-αα-dichloropropionamidopyrimidine (V) produced only trivial yields. Thus repeated evaporation of an aqueous solution, found suitable for a dichloroacetamido-analogue,[11] left the amide (V) unchanged, whereas the substance was destroyed when its silver derivative was refluxed with aqueous silver carbonate.[12] The amide (V), together with a little 4,5-bis-αα-dichloropropionamidopyrimidine, was readily formed from αα-dichloropropionyl chloride and 4,5-diaminopyrimidine.



(IV)



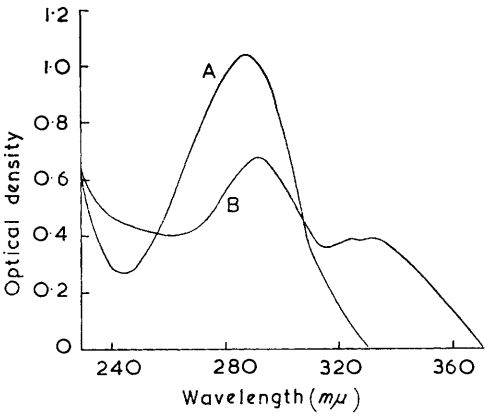
(V)

Two other attempts to prepare 6-hydroxy-7-methylpteridine were unsuccessful. When 4,5-diaminopyrimidine was condensed with ethyl ethoxalylacetate under acid conditions from pH 3·5 to −1, only 6-ethoxycarbonylmethyl-7-hydroxypteridine was produced, and this gave 7-hydroxy-6-methylpteridine (see p. 132) on hydrolysis and decarboxylation.[13] Again, 4,5-diaminopyrimidine and ethyl 2,4-dioxopentanoate in 10N-sulphuric acid gave mainly (and at pH 5, entirely) 6-acetonyl-7-hydroxypteridine which was degraded to 7-hydroxy-6-methylpteridine.

[7] Albert, Lister, and Pedersen, *J.*, 1956, 4621.
[8] Elion, Hitchings, and Russell, *J. Amer. Chem. Soc.*, 1950, **72**, 78.
[9] Pfleiderer, *Chem. Ber.*, 1956, **89**, 641; Russell, Purrmann, Schmitt, and Hitchings, *J. Amer. Chem. Soc.*, 1949, **71**, 3412.
[10] Albert, Brown, and Wood, *J.*, 1954, 3832.
[11] Sachs and Meyerheim, *Ber.*, 1908, **41**, 3957.
[12] Purrmann, *Annalen*, 1940, **546**, 98.
[13] Albert and Brown, *J.*, 1953, 74.

Properties of 6-Hydroxy-7-methylpteridine.—The neutral molecule was anhydrous when dried at 60°. A monosodium derivative, formed in N-sodium hydroxide, was anhydrous at 20°. Potentiometric titration of the anion differed from that of 6-hydroxypteridine in that no rapid drift of potential was encountered. The acid ionization (pK 7·17) corresponds to the anhydrous forms of 6-hydroxypteridine (see Table 1).

Whereas the stable neutral molecule of 6-hydroxypteridine has (at equilibrium) about 100 hydrated molecules to each anhydrous one,[6] the height of the 292 mμ peak for the equilibrated solution of the neutral molecule of 6-hydroxy-7-methylpteridine suggests that this is hydrated in a ratio of about 1 : 1 (see Figure, curve B). In addition there is a new peak of longer wavelength, evidence of extra conjugation. Further this peak, only 18 mμ below that of its anion, now lies within the normal range. Thus the hypothesis that a 7-methyl group would hinder the covalent hydration of 6-hydroxypteridine is substantiated. A less stable (hydrated) form of the neutral molecule of 6-hydroxy-7-methylpteridine, with a spectrum almost identical with that of the stable (hydrated) form of 6-hydroxypteridine, can be demonstrated spectroscopically (see Figure) by quickly



Ultraviolet spectra (in 1 cm. cells) of 6-hydroxy-7-methylpteridine (neutral molecules, 10^{-4}M). (A) *Hydrated form [solution of hydrated cation in 0·02N-hydrochloric acid adjusted to* pH 6 *(buffer) and measured at once].* (B) *Equilibrium mixture of hydrated and anhydrous forms.*

adjusting a 10^{-4}M-solution of the anhydrous form in 0·02N-hydrochloric acid to pH 6. It was stable for about 5 minutes (less stable at pH 7). This experiment also confirms the view that the cation of 6-hydroxy-7-methylpteridine is mainly in the hydrated form. The spectrum of the cation (see Table 1) does not change between 3 minutes (the time taken for dissolution and measurement) and 24 hours. Its spectrum and pK are almost identical with those of the cation of 6-hydroxypteridine; that these two pK's (3·7) are those of hydrates is seen by comparing them with those of 4- and 7-hydroxypteridine (—0·17 and 1·2 respectively [6]) which are not hydrated. The extra basic strength of the hydrates comes from the 4-aminopyridine type [14] of base-strengthening resonance (VI).

A 0·5N-solution of 6-hydroxy-7-methylpteridine in N-sulphuric acid was stable for at least 2 days at 20°, but a new substance (substance H) was formed when this solution was heated at 98° for 30 min. Analysis indicated that this was a hydrate of 6-hydroxy-7-methylpteridine. The substances have different R_F values (Table 2); also the spectrum in alkaline solution is not quite identical with that of 6-hydroxy-7-methylpteridine but slowly reverts to it. Cryoscopic measurements in N-sulphuric acid indicate that substance H is monomeric (Dr. D. D. Perrin, personal communication); it may be the 3,4-hydrate.

6-Hydroxy-7-methylpteridine gives substance H and two apparently dimeric by-products (one orange and one colourless) when boiled with water for 10 minutes. Whereas 6-hydroxypteridine is disproportionated by boiling N-sodium hydroxide, 6-hydroxy-7-methylpteridine is unaffected. The latter slowly dissolves in boiling methanol, giving a

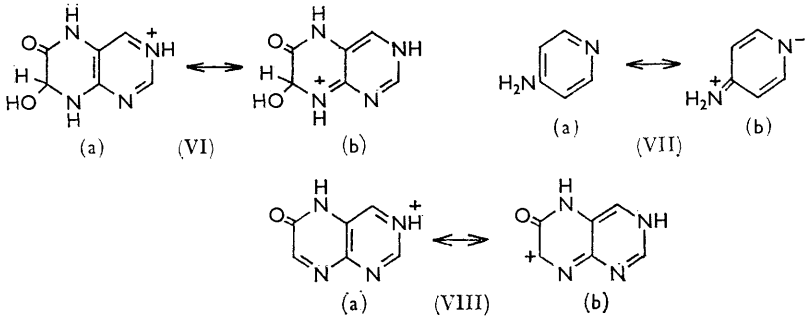
[14] Albert, Goldacre, and Phillips, *J.*, 1948, 2240.

TABLE 1. *Physical properties of pteridines.*

	Ionization (H_2O; 20°) [a]			Spectroscopy in water [i]		
Pteridine	pK_a	spread (±)	concn. [b] (M)	$\lambda_{max.}$ (mμ)	log ε	pH
6-Hydroxy (hydrate, stable) ...	—	—	—	289 [f]	4·00	5·2
anion (anhydr., stable)	6·5 [c]	0·1	0·002	224, 256, 356 [f]	4·29, 3·97, 3·84	13·0
cation (hydrate, stable)	3·67 [d]	—	—	287 [d]	4·09	1·7
6-Hydroxy-7-methyl						
(hydrate, unstable)	—	—	—	288	4·02	6·0
(anhydr., stable)	—	—	—	292, 325 + 331	3·83, 3·58 + 3·58	5·5
anion (anhydr., stable)	7·17	0·03	0·007	225, 257, 349	4·43, 3·91, 3·96	12·0
cation (hydrate, stable)	3·72	0·05	0·007	288	4·09	1·0
6-Hydroxy-2-methyl						
(hydrate, stable)	—	—	—	*267*, 290	*3·90*, 4·04	6·5
anion (hydrate, unstable) ...	9·5 [e]	— [e]	0·004	—	—	—
anion (anhydr., stable)	6·31	0·02	0·004	222, 254, 364	4·40, 4·04, 3·86	12·1
cation (hydrate, stable)	4·67	0·04	0·004	285	4·09	2·0
6-Hydroxy-4-methyl						
(hydrate, stable)	—	—	—	285	4·02	5·25
anion (hydrate, unstable).....	9·5 [e]	— [e]	0·001	—	—	—
anion (anhydr., stable)	6·40	0·04	0·001	223, 259, 359	4·29, 3·94, 3·84	12·0
cation (hydrate, stable)	4·09	0·05	0·001	288	4·13	2·0
7,8-Dihydro-6-hydroxy.....	—	—	—	293 [d]	3·93	7·4
anion	10·54 [f]	—	—	305 [f]	4·07	13·0
cation	4·78	—	—	292 [d]	4·01	2·4
7,8-Dihydro-6-hydroxy-7-methyl	—	—	—	209, 294	4·52, 3·95	7·0
anion	10·89	0·05	0·002	223, 306	4·20, 4·06	13·0
cation	4·80	0·04	0·002	210, 292	4·37, 4·04	1·8
7-Acetonyl-7,8-dihydro-6-						
hydroxy	—	—	—	298	3·98	7·5
anion	10·79	0·05	0·005	311	4·05	12·0
cation	4·73	0·01	0·005	210, 293	4·38, 4·04	1·0
7-Di(ethoxycarbonyl)methyl-						
7,8-dihydro-6-hydroxy	—	—	—	210, 293	4·48, 3·96	7·0
anion	10·17	0·05	0·005	—	—	—
cation	4·12	0·04	0·003	210, 292	4·32, 4·02	1·0
7-Cyanomethyl-7,8-dihydro-6-						
hydroxy	—	—	—	209, 293	4·48, 3·96	7·0
anion	9·89	0·06	0·003	214, 304	4·51, 4·08	12·0
cation	4·18	0·03	0·003	209, 291	4·42, 4·05	1·2
7-(2-Carboxyprop-1-enyl)-6-hydr-						
oxypteridine (III)						
anion	2·93 [g]	0·05	0·003	224, *255*, 383	4·28, *4·05*, 4·07	8·0
cation	5·69 [g]	0·04	0·003	287	4·14	0
7-Hydroxy	—	—	—	227, 248 + 256, 303 [d]	3·79, 3·44 + 3·45, 4·00	4·0
anion	6·41 [d]	—	—	226, 260, 326 [d]	4·27, 3·76, 4·04	9·0
cation	1·2 [d]	—	—	—	—	—
7-Hydroxy-6-methyl.....	—	—	—	257, 299	3·54, 4·09	4·7
anion	6·97	0·03	0·02	*225*, 261, 324	*4·19*, 3·66, 4·12	9·2
7-Hydroxy-2-methyl.....	—	—	—	*259*, 305	*3·52*, 4·09	4·5
anion	6·68	0·03	0·005	213. 258, 327	4·40, 3·69, 4·10	9·0
cation	1·71 [h]	0·04	0·01	210, 294	4·40, 4·04	−0·8
7-Hydroxy-4-methyl.....	—	—	—	253 + 260, 304	3·46 + 3·46, 4·00	4·0
anion	6·79	0·05	0·005	228, *256*, 327	4·37, *3·74*, 4·03	9·3
cation	<2	—	0·01	—	—	—

[a] Determined potentiometrically. [b] Where no figure appears in this column, the results have been quoted, from the literature, for comparison. [c] More accurate figures than previously published (see Experimental section). [d] Albert, Brown, and Cheeseman, *J.*, 1951, 1620. [e] Determined in a single potentiometric titration and, owing to concomitant dehydration, may be low by about 0·5. [f] Brown and Mason, *J.*, 1956, 3443. [g] No hysteresis detected. [h] Each reading, significantly different from values obtained by titrating water under the same conditions, was corrected for mean ionic activity (Davies, *J.*, 1938, 2093). [i] 1 and 4 cm. cells; shoulders in italics. [j] Decomposes at pH 12.

1 : 1 addition product which is rapidly reconverted into 6-hydroxy-7-methylpteridine in 0·1N-sodium hydroxide.



It is remarkable that 6-hydroxypteridine has so strong a tendency to hydration whereas 7-hydroxypteridine has none. The hydrated form of the cation of 6-hydroxypteridine appears to be stabilized mainly by the energy of the resonance hybrid (VI), referred to above, and the hydrated neutral molecule by a similar but smaller resonance of the type (VII) which is well known in the neutral molecule of 4-aminopyridine where it was demonstrated by dipole-moment measurements.[15] No other hydroxypteridines can gain these types of resonance stabilization by hydration. 6-Hydroxy-4-methylpteridine (Table 1) closely resembles 6-hydroxypteridine in having a highly stable hydrate as neutral molecule.

Next, it is necessary to consider how a 7-methyl group reduces the tendency of 6-hydroxypteridine to hydration. The known mechanism of the hydration of C:C links [16] indicates that position 7 in 6-hydroxypteridine should be positively charged to invite nucleophilic addition of a water molecule. However, this position in the neutral molecule is negatively charged both from the inductive effect of $N_{(8)}$ and the mesomeric influence of the carbonyl group. Thus the cation (VIII), particularly a mesomeric form (VIIIb), is the species most likely to undergo hydration. A 7-methyl group would exercise a small unfavourable inductive effect on hydration at position 7, but its main effect is to offer steric hindrance to the approach of the water molecule. Thus, the rate of dehydration (of neutral hydrated species at pH 8·6 and 20°) is comparable with that of 6-hydroxypteridine (Dr. D. D. Perrin, personal communication). Discrepancies in the literature concerning the reported water content of xanthopterin (2-amino-4,6-dihydroxypteridine) may have a similar origin. This substance was first reported as a hemihydrate,[17] then in an anhydrous form,[18] but later, in the same laboratory, xanthopterin could not be obtained with less water than the monohydrate.[19] Here also, the insertion of a 7-methyl group considerably lessens the tendency to hydration. We find that at pH 4, where both substances are present only as neutral molecules, the 378 mμ peak of 7-methylxanthopterin has exactly twice the height of the corresponding peak of equilibrated xanthopterin (385 mμ). This agrees with Schou's demonstration [20] that xanthopterin is, under these conditions, a mixture of equal amounts of two related substances: we believe that here the 385 mμ peak refers to the anhydrous form, and the 305 mμ shoulder to the 7,8-hydrate, and that 7-methylxanthopterin, which shows no equilibration phenomena, is substantially anhydrous.

Michael-type Additions.—Additions, other than of water, to the 7,8-double bond in 6-hydroxypteridine have been demonstrated for hydroxylamine, ammonia, a second

[15] Angyal and Angyal, *J.*, 1952, 1461.
[16] Ingold, " Structure and Mechanism in Organic Chemistry," Bell, London, 1953, p. 695.
[17] Purrmann, *Annalen*, 1941, **548**, 291.
[18] Korte, *Chem. Ber.*, 1954, **87**, 1062.
[19] Korte and Barkemeyer, *Chem. Ber.*, 1956, **89**, 2400.
[20] Schou, *Arch. Biochem.*, 1950, **28**, 10.

molecule of 6-hydroxypteridine, and 7,8-dihydro-6-hydroxypteridine.[4] This encouraged us to use the reagents active in Michael condensations, *e.g.*, ethyl malonate. The C:N group reacts with these in benzylideneaniline, but not in heteroaromatic nuclei (*e.g.*, pyridine) unless more pronounced electron-withdrawing influences are provided (as in nicotinamide

TABLE 2. R_F *values* (paper chromatography).

Substances were dissolved in cold 0·1N-sodium hydroxide, immediately before application to the paper (Whatman No. 1), which was rapidly transferred to the solvent jar.

Substance	In 3% aq. NH_4Cl		In butanol–5N-acetic acid (7 : 3)	
Picric acid (for comparison)	0·55	D	0·55	D
6-Hydroxy-7-methylpteridine	0·70	D [a]	0·60	V [a]
Substance H (p. 129)	0·50—0·60	D	0·30	D
7-Hydroxy-6-methylpteridine	0·70	D [b] ⟶ F	0·65	D [b] ⟶ F
7,8-Dihydro-6-hydroxy-7-methylpteridine	0·55	D	0·50	D
7-Carboxypropenyl-6-hydroxypteridine	0·85	D	0·70	D

The dried paper was read in ultraviolet light of (principally) 254 mμ. Interpretation: D, dark (absorption) spot. F, blue fluorescent spot. D ⟶ F, dark spot which changes to a blue fluorescence after irradiation for 1 min. V, violet absorption spot. [a] Same in light of 360 mμ. [b] Invisible in light of 360 mμ.

methochloride [21] or acridine [22]). Thus it appeared that Michael-like additions to the C:N bond are similar to the addition of water and should yield more stable products, in as much as acridine shows no tendency to add water.

We found that 6-hydroxypteridine, in cold alkaline solution, readily reacted with acetone, diethyl malonate, and ethyl cyanoacetate, the carbanion adding to $C_{(7)}$ and the mobile hydrogen to $N_{(8)}$. The acetone adduct was characterized as its semicarbazone, and the ethyl cyanoacetate adduct was degraded to 7-cyanomethyl-7,8-dihydro-6-hydroxypteridine. All the adducts gave 6,7-dihydroxypteridine [2] when heated with N-sodium hydroxide (in air), thus establishing the orientation. The resemblance of the spectra and ionization constants of 7,8-dihydro-6-hydroxypteridine and these adducts is noteworthy (Table 1).

7-Hydroxy- and 6-hydroxy-7-methyl-pteridine did not react with acetone under these conditions, and 6-hydroxypteridine did not react at pH 2 or in acetone alone.

7-Hydroxypteridines.—Contrary to expectation, very little 7-hydroxy-6-methylpteridine was formed from ethyl pyruvate and 4,5-diaminopyrimidine in water in the range pH 5·5—14 * between 20° and 100°. However, in cold aqueous solution (at pH 4) these substances slowly gave the required pteridine. The new isomer was distinguished from 6-hydroxy-7-methylpteridine by the fluorescence after photoreduction [23] on paper (Table 2). Its orientation was confirmed by methylation to the known, sharply melting, 5,6-dihydro-6,8-dimethyl-7-oxopteridine.[24] It was easily converted into 7-chloro-6-methylpteridine, but resisted reduction with potassium borohydride.

7-Hydroxy-2(and 4)-methylpteridine were prepared without difficulty from ethyl glyoxylate hemiacetal and 2- and 6-methyl-4,5-diaminopyrimidine respectively in 2N-sodium carbonate.

EXPERIMENTAL

Elementary analyses were carried out by the Analytical Section of this Department, under Dr. J. E. Fildes. Yields are based on the stage in purification when the substances first gave a single spot in paper chromatography, but further purification was carried out before analysis.

* Between pH 1 and 3, neither 6-hydroxy-7- nor 7-hydroxy-6-methylpteridine could be isolated, but a small amount of a new substance with a double spot in paper chromatography.

[21] Eys and Kaplan, *J. Biol. Chem.*, 1957, **228**, 305.
[22] Kröhnke and Honig, *Annalen*, 1959, **624**, 97.
[23] Albert, *Nature*, 1956, **178**, 1072.
[24] Albert, Brown, and Wood, *J.*, 1956, 2066.

Ultraviolet spectra were measured on a Perkin–Elmer "Spectrocord" recording spectrophotometer, and the $\lambda_{max.}$ and extinction values rechecked on a Hilger "Uvispek" manual instrument (1 cm. cells). Ionization constants were determined as in earlier work from this Department.

Acid Ionization Constant of 6-Hydroxypteridine.—Five portions (each 0·04150 g.) were separately dissolved in boiled-out water (125 ml.). Each was titrated with two successive portions of 0·1N-potassium hydroxide (each portion: 0·1 equiv.). This allowed the upper curve of the hysteresis loop [2] to be traced more accurately than before (see Table 1).

6-Hydroxy-7-methylpteridine.—4,5-Diaminopyrimidine [25] (2·2 g., 0·02 mole) was suspended in water (10 ml.) and dissolved by the dropwise addition of 10N-hydrochloric acid. Ethyl pyruvate [26] (3·4 g., 1·5 equiv.) and 10N-hydrochloric acid (10 ml. altogether) were added, quickly, in that order and the mixture was shaken for 6 hr. at about 20°, then refrigerated overnight and filtered. The precipitated *hydrochloride* was washed with N-hydrochloric acid and filtered off (Found, for a sample washed with alcohol and dried at 20°: Cl, 21·2; N, 26·9. $C_7H_6N_4O,1·25HCl$ requires Cl, 21·3; N, 26·95%). The mixed hydrochlorides were shaken for an hour in M-sodium citrate (20 ml.), being adjusted to pH 5, if necessary, after the first ½ hr. The suspension was refrigerated, filtered, and washed free from chloride, giving 60% of colourless 6-hydroxy-7-methylpteridine, which was purified, as the *sodium salt*, by dissolution of 0·9 g. in 10N-sodium hydroxide (1 ml.) and boiled-out water (9 ml.). The yellow crystals were washed with N-sodium hydroxide, then with alcohol and dried at 20°/0·01 mm. over P_2O_5 (Found: C, 45·2; H, 2·9; N, 30·2. $C_7H_5N_4NaO$ requires C, 45·05; H, 2·7; N, 30·4%). The aqueous solution, adjusted to pH 5, gave pure *6-hydroxy-7-methylpteridine* (Found, for material dried at 60°/0·01 mm.: C, 51·4; H, 4·1; N, 34·2. $C_7H_6N_4O$ requires C, 51·85; H, 3·7; N, 34·6%). Drying in air at 100° produces an intense orange colour.

This substance (0·1 g.) was boiled with methanol (50 ml.) for 10 min. The mixture was filtered and concentrated to 2 ml., giving *7,8-dihydro-6-hydroxy-7-methoxy-7-methylpteridine* (60%) (Found, for material dried at 20°: C, 49·3; H, 5·55; N, 29·5. $C_8H_{10}N_4O_2$ requires C, 49·5; H, 5·2; N, 28·9%). It readily decomposes to 6-hydroxy-7-methylpteridine in cold, dilute alkali.

7-(2-Carboxyprop-1-enyl)-6-hydroxypteridine (III).—4,5-Diaminopyrimidine (1·1 g.), ethyl pyruvate (2·2 g.), and 30N-sulphuric acid (4 ml., 10 equiv.) were set aside at 20° for a week, then adjusted to pH 4 with sodium hydroxide. The colourless *7-(2-carboxyprop-1-enyl)-6-hydroxypteridine* (27%) was filtered off and recrystallized from 200 parts of boiling water. It gives the iodoform test, decolorizes bromine water, and chars at 240° (Found, for material dried at 20°: C, 51·9; H, 3·55; N, 24·4. $C_{10}H_8N_4O_3$ requires C, 51·7; H, 3·5; N, 24·1%).

7,8-Dihydro-6-hydroxy-7-methylpteridine.—(a) *By the reduction of 6-hydroxy-7-methylpteridine.* Anhydrous 6-hydroxy-7-methylpteridine (0·16 g.), 0·1N-sodium hydroxide (11 ml.), and potassium borohydride (0·027 g.), set aside at 20° for 11 hr. and then brought to pH 7 with phosphoric acid gave a precipitate (90%) of 7,8-dihydro-6-hydroxy-7-methylpteridine. This was recrystallized from 200 parts of water and found by infrared and ultraviolet spectroscopy to be identical with the substance synthesized as below.

(b) *Direct synthesis.* Alanine methyl ester (5 g.) was added at 0° to a stirred solution of 2,4-dichloro-5-nitropyrimidine [25] (5 g.) in ether emulsified in a suspension of sodium hydrogen carbonate (8 g.) in water (25 ml.). Stirring was continued for 30 min. at 0° and 15 min. at 20°. The precipitate was filtered off, dried, and recrystallized from light petroleum (b. p. 100—120°), giving *methyl α-(2'-chloro-5'-nitro-4'-pyrimidinylamino)propionate* (IV), m. p. 105° (88%) (Found: C, 36·8; H, 3·55; Cl, 13·65. $C_8H_9ClN_4O_4$ requires C, 36·8; H, 3·5; Cl, 13·6%). This ester (5 g.) in methanol (350 ml.) was hydrogenated at room temperature and pressure over Raney nickel and calcium carbonate. After the nitro-group had been reduced, the mixture was filtered and refluxed with water (20 ml.) for 30 min. to effect ring-closure. Water (150 ml.) was added, and the mixture concentrated to 200 ml., and later to 50 ml., giving two crops (total 80%) of *2-chloro-7,8-dihydro-6-hydroxy-7-methylpteridine*, decomp. about 260° (Found: C, 42·2; H, 3·6; N, 28·05. $C_7H_7ClN_4O$ requires C, 42·3; H, 3·55; N, 28·2%). This substance (1 g.), red phosphorus (1 g.), and hydriodic acid (6 ml.; *d* 1·7) were heated at 160° for 1 hr. Water (3 ml.) was added, and the filtrate adjusted to pH 7·5 with "Tris" buffer, giving

[25] Brown, *J. Appl. Chem.*, 1952, **2**, 239.
[26] Böeseken and Felix, *Ber.*, 1929, **62**, 1315.

7,8-*dihydro*-6-*hydroxy*-7-*methylpteridine* (85%), which recrystallized from 200 parts of water (Found: C, 51·3; H, 5·1; N, 33·4. $C_7H_8N_4O$ requires C, 51·2; H, 5·0; N, 34·1%). It has a sparingly soluble potassium salt.

4-*Amino*-5-αα-*dichloropropionamidopyrimidine* (V).—αα-Dichloropropionyl chloride [27] (2·9 g.) was added to a stirred solution of 4,5-diaminopyrimidine (1·1 g., 0·5 equiv.) in dried pyridine (70 ml.) at 100°. After 30 min. at 100°, the pyridine was recovered and water (10 ml.) was added to the warm residue. The solid which was deposited on cooling recrystallized from 50 parts of light petroleum (b. p. 60—70°), giving 0·1 g. of 4,5-*bis*-αα-*dichloropropionamidopyrimidine*, m. p. 137—139° (Found: C, 33·2; H, 2·8; N, 15·7; Cl, 39·5. $C_{10}H_{10}Cl_4N_4O_2$ requires C, 33·4; H, 2·8; N, 15·6; Cl, 39·4%). It is insoluble in N-acetic or boiling 0·1N-hydrochloric acid, hence it is not the 5,5-diacyl isomer which would be highly basic.

The filtrate (pH 3) from the diacyl derivative was adjusted to pH 7 with 3N-sodium hydroxide (about 8 ml.). The solid deposited was recrystallized from 30 parts of water, and then 120 parts of benzene, giving 4-*amino*-5-αα-*dichloropropionamidopyrimidine* (0·8 g.), m. p. 144° (slight decomp.), soluble in cold N-acetic acid (Found: C, 35·7; H, 3·5; N, 23·8. $C_7H_8Cl_2N_4O$ requires C, 35·8; H, 3·4; N, 23·8%). It was unchanged by sodium iodide in refluxing acetone.

Substance H (see p. 129).—6-Hydroxy-7-methylpteridine (0·5 g.) and N-sulphuric acid (6 ml., 2 equiv.) were heated at 98° in a sealed tube for 30 min. and cooled. N-Sodium citrate was added, then enough N-sodium hydroxide to give pH 5·5. The precipitate was shaken with 20-ml. portions of cold water until chloride-free, and then with cold water (500 ml.) for an hour, filtered, and concentrated to 25 ml. below 40°. The pale crystals of *substance H* are soluble in 1200 parts of cold water (Found, for material dried at 20°: C, 46·8; H, 4·7; N, 30·5. $C_7H_8N_4O_2$ requires C, 46·7; H, 4·5; N, 31·1%).

6-*Hydroxy*-2- *and* -4-*methylpteridine*.—4,5-Diamino-2-methylpyrimidine [10] (1·24 g., 0·01 mole), ethyl glyoxylate hemiacetal (2 g.), and 2N-sulphuric acid (18 ml.) were set aside for 5 days at 37°, then brought to pH 6 with sodium citrate (1 g.) and 10N-sodium hydroxide. The precipitate of 6-*hydroxy*-2-*methylpteridine* when recrystallized from water (130 parts; 85% yield) began to char above 200° (Found, for material dried at 160°/0·01 mm.: C, 46·6; H, 4·8; N, 30·9. $C_7H_6N_4O,H_2O$ requires C, 46·7; H, 4·5; N, 31·1%).

6-*Hydroxy*-4-*methylpteridine* was similarly obtained from 4,5-diamino-6-methylpyrimidine [10] in 75% yield. It becomes brown at about 230° (Found, for material dried at 160°/0·01 mm.: C, 47·2; H, 4·4; N, 31·1%). Both isomers give a very elongated spot, similar to that of 6-hydroxypteridine on paper chromatography in butanol–acetic acid. None of the 7-hydroxy-isomers (see below) was formed in these reactions.

6-*Hydroxypteridine and Acetone*.—6-Hydroxypteridine hydrate (0·66 g., 0·004 mole), 0·1N-sodium hydroxide (80 ml.), and acetone (10 ml., 40 equiv.) were left at 20° for 2 days. The solution was adjusted to pH 6 (sodium citrate + 5N-sulphuric acid), concentrated to 40 ml. (charcoal), and chilled. The product, recrystallized from 60 parts of water, gave 80% of 7-*acetonyl*-7,8-*dihydro*-6-*hydroxypteridine*, decomp. about 250° (Found: C, 52·2; H, 4·9; N, 26·6. $C_9H_{10}N_4O_2$ requires C, 52·4; H, 4·9; N, 27·2%). This gave a positive iodoform test, and was unchanged by refluxing with an excess of 5N-hydrochloric acid for 6 hr., but boiling N-sodium hydroxide (1 hr.) produced 50% of 6,7-dihydroxypteridine by oxidative hydrolysis. The acetonyl compound resisted oxidation by boiling ferric chloride solution, by hot air at 110° (60 hr.), and by potassium dichromate in boiling N-sulphuric acid. Cold alkaline potassium permanganate solution caused gross destruction. The *semicarbazone* (90% yield) formed colourless crystals from 350 parts of water, decomp. 235° (Found: C, 45·2; H, 5·25. $C_{10}H_{13}N_7O_2$ requires C, 45·6; H, 5·0%).

6-*Hydroxypteridine and Ethyl Malonate*.—6-Hydroxypteridine hydrate (1·66 g.), in hot N-sodium carbonate (30 ml.), was shaken with ethyl malonate (6·4 g.) at 20° for 15 hr. The separated solid was recrystallized from 80 parts of water, giving 60% of 7-*diethoxycarbonylmethyl*-7,8-*dihydro*-6-*hydroxypteridine*, m. p. 184—188° (decomp.) (Found: C, 50·6; H, 5·2; N, 18·3. $C_{13}H_{16}N_4O_5$ requires C, 50·6; H, 5·2; N, 18·2%). This gave a *picrate*, m. p. 217—219° (from water) (Found: C, 42·85; H, 3·45; N, 18·25. $C_{19}H_{19}N_7O_{12}$ requires C, 42·5; H, 3·6; N, 18·25%). Refluxing in 0·1N-hydrochloric acid for 5 hr. produced no change. Attempted dehydrogenations with potassium permanganate, ferric chloride, and boiling nitrobenzene were unsuccessful.

[27] Leimer, *Ber.*, 1937, **70**, 1050.

6-Hydroxypteridine and Ethyl Cyanoacetate.—6-Hydroxypteridine hydrate (0·33 g., 0·002 mole), N-sodium hydroxide (6 ml.), and ethyl cyanoacetate (0·45 g., 2 equiv.) were set aside overnight at 20°, then brought to pH 7 with phosphoric acid. The crystals of the sodium salt of 7-(α-cyano-α-ethoxycarbonylmethyl)-7,8-dihydro-6-hydroxypteridine (R_F 0·65) were boiled with water (10 ml.) for 5 min., giving the sodium salt of 7-(α-cyano-α-carboxymethyl)-7,8-dihydro-6-hydroxypteridine (R_F 0·10), which was precipitated (as the free acid) at pH 2. This acid and water (5 ml.), refluxed for 2 hr., deposited colourless *7-cyanomethyl-7,8-dihydro-6-hydroxypteridine* (R_F 0·50; all R_F's refer to butanol–5N-acetic acid, 7 : 3) (yield, 77% based on 6-hydroxypteridine) (Found, for material dried at 20°: C, 50·8; H, 3·95; N, 36·9. $C_8H_7N_5O$ requires C, 50·8; H, 3·7; N, 37·0%).

7-Hydroxy-6-methylpteridine and its Methylation.—4,5-Diaminopyrimidine (3·3 g.), ethyl pyruvate (4 ml.), and water (75 ml.) were set aside at 20° for 4 days (final pH, 3·5—4). 10N-Sodium hydroxide (5 ml.) was added and the suspension boiled for 3 min. and adjusted to pH 5. The precipitate was extracted with boiling water (110 ml.), giving (on chilling) *7-hydroxy-6-methylpteridine* (50%), soluble in 35 parts of boiling water and in cold N-hydrochloric acid (Found: C, 51·95; H, 3·5; N, 34·6. $C_7H_6N_4O$ requires C, 51·85; H, 3·7; N, 34·6%). The sodium salt crystallized (90% recovery) from 2 equivalents of boiling N-sodium hydroxide. Methyl sulphate (0·12 ml., 1·2 equiv.) was shaken with 7-hydroxy-6-methylpteridine (0·16 g.) in N-potassium hydroxide (1 ml.) at 20° for 30 min. while the pH was kept at 8 by adding N-potassium hydroxide. Extraction with chloroform (4 + 3 ml.) gave 7,8-dihydro-6,8-dimethyl-7-oxopteridine (70%) (from benzene),[24] m. p. and mixed m. p. 146—147°.

7-Chloro-6-methylpteridine.—7-Hydroxy-6-methylpteridine (0·5 g.), phosphorus trichloride (25 ml.), and phosphorus pentachloride (2·5 g.) were refluxed for 6 hr. or until clear. The volatile materials were removed at 100 mm. The residual solid was powdered in a chilled mortar, and ice (10 g.) added in one portion. The slurry was adjusted to pH 7—8 with potassium carbonate, clarified, and extracted with twice its volume of chloroform. The lower layer was dried (Na_2SO_4) and the solvent removed below 40° to prevent dimerization. The product was extracted with cold benzene (5 ml.). The extract, on concentration, deposited *7-chloro-6-methylpteridine*, m. p. 103° (Found: C, 46·0; H, 2·7. $C_7H_5ClN_4$ requires C, 46·6; H, 2·8%). The yield, 50%, fell to 30% when 1 g. of the hydroxypteridine was used. Omission of phosphorus trichloride caused much charring.

7-Hydroxy-2(and 4)-methylpteridine.—4,5-Diamino-2-methylpyrimidine [10] (1·24 g.), 2N-sodium carbonate (10 ml.), and ethyl glyoxylate hemiacetal (2 g.) were refluxed vigorously for 1 hr., then cooled and adjusted to pH 5 with 5N-sulphuric acid. The precipitate recrystallized from 80 parts of water, giving *7-hydroxy-2-methylpteridine* (85%), m. p. 265° (decomp.) (Found: C, 52·0; H, 3·7; N, 34·6. $C_7H_6N_4O$ requires C, 51·85; H, 3·7; N, 34·6%). 4,5-Diamino-6-methylpyrimidine [10] similarly gave *7-hydroxy-4-methylpteridine* (80%) (colourless crystals from 40 parts of water). This, unlike the 2- and the 6-methyl isomer, rapidly became orange when warmed in air, and was prepared under nitrogen (Found: C 51·5; H 3·6; N, 34·2%).

6-Acetonyl-7-hydroxypteridine.—Ethyl 2,4-dioxopentanoate (0·24 g.) was heated at 98° for 15 min. with 4,5-diaminopyrimidine (0·11 g.) and N-acetate buffer (pH 5; 3·5 ml.). The yellow leaflets which were deposited on cooling were filtered off and the filtrate was reheated with more ester (0·24 g.). The total yield of *6-acetonyl-7-hydroxypteridine*, decomp. about 280°, was 35% (Found, for material recrystallized from water: C, 53·0; H, 3·9; N, 27·4. $C_9H_8N_4O_2$ requires C, 52·9; H, 3·95; N, 27·4%). When heated with N-sodium hydroxide at 98° for 30 min., it gave about equal parts of 7-hydroxy-6-methyl- and 6,7-dihydroxy-pteridine.

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