298. *Pteridine Studies. Part II.* 6- and 7-Hydroxypteridines and their Derivatives.*

By Adrien Albert, D. J. Brown, and Gordon Cheeseman.

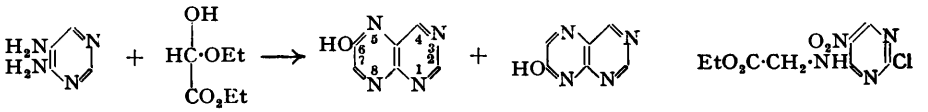
Syntheses of a number of monosubstituted pteridines and dihydropteridines are reported, together with their ionization constants, R_F values, and ultra-violet spectra. All possible mono-hydroxypteridines are now known : the 6-isomeride is distinguished by giving a hysteresis loop when titrated, which is ascribed to a slow tautomerism.

The syntheses of monosubstituted pteridines, commenced in Part I,* have been continued by the introduction of various groups in the 6-position and of the hydroxy-group in the 7-position.

When 4 : 5-diaminopyrimidine (I) was heated with ethyl glyoxylate hemiacetal (II) in water at 100°, two isomeric monohydroxypteridines were obtained. The less basic isomeride, which predominated (65% yield) when the reaction was carried out at pH 6,

* Part I, *J.*, 1951, 474.

was separated from the more basic isomeride (25% yield) at pH 2 and freed from traces of the latter by recrystallization, as the sodium salt, from boiling N-sodium hydroxide. The more basic isomeride, which predominated when the reaction was carried out at pH —0·2 was freed from the less basic isomeride (20% yield) by filtration at pH 2 and was liberated in 65% yield when the pH was raised to 5·5. It proved to be rapidly destroyed by boiling N-sodium hydroxide.



(I) (II) (III) (IV) (V)

In the synthesis of more complex pteridines, highly acid conditions favour the hydroxy-group entering the 6-position, and less acid conditions may direct it to the 7-position (Purrmann, *Annalen*, 1941, **548**, 284; Elion, Hitchings, and Russell, *J. Amer. Chem. Soc.*, 1950, **72**, 78). Hence the less and the more basic isomeride produced in the above reaction should be 7- and 6-hydroxypteridine, respectively. This was confirmed by the synthesis of 6-hydroxypteridine (III) by an unambiguous method, *viz.*, the oxidation of 7 : 8-dihydro-6-hydroxypteridine, prepared by the reduction, ring closure, and dehalogenation of 4-carbethoxymethylamino-2-chloro-5-nitropyrimidine (V) (Boon, Jones, and Ramage, *J.*, 1951, 96). The preceding route, however, gave a better yield as well as a small amount of (IV) as a valuable by-product.

6-*Hydroxypteridine and its Derivatives.*—When 6-hydroxypteridine was gently warmed with sodium amalgam, 85% of 7 : 8-dihydro-6-hydroxypteridine was obtained. It was similarly obtained from 6 : 7-dihydroxypteridine (made by heating 4 : 5-diaminopyrimidine with oxalic acid). The identity of the specimens of 7 : 8-dihydro-6-hydroxypteridine prepared by these three routes was confirmed by measurements of solubility, ionization constants, ultra-violet and infra-red spectroscopy, and by paper chromatography in four different solvents. This reduction of 6 : 7-dihydroxypteridine is analogous to the transformation of leucopterin (2-amino-4 : 6 : 7-trihydroxypteridine) to dihydroxanthopterin (2-amino-7 : 8-dihydro-4 : 6-dihydroxypteridine) under similar conditions (Totter, *J. Biol. Chem.*, 1944, **154**, 105; Boon and Leigh, *J.*, 1951, 1497).

All three specimens of 7 : 8-dihydro-6-hydroxypteridine gave 6-hydroxypteridine by oxidation with potassium permanganate. Thus no analogue has been produced of the so-called β-form of dihydroxanthopterin, which resists oxidation to xanthopterin by this reagent (Hitchings and Elion, *J. Amer. Chem. Soc.*, 1949, **71**, 467).

6-Hydroxypterdine differed from 2-, 4-, and 7-hydroxypteridines in resisting destruction of the pteridinei ring by phosphorus halides. Phosphorus oxychloride alone had no effect on 6-hydroxypteridine but, in combination with phosphorus pentachloride, produced 6-chloropteridine. 6-Chloropteridine was converted into 6-amino-, 6-dimethylamino- and 6-methoxy-pteridines in the usual way. The spectra of 6-amino- and 6-dimethylamino-pteridines are given in Fig. 5 (p. 1626). As with the pair aniline–dimethylaniline, the tertiary amine absorbs at longer wave-lengths. However, the two spectra are so similar as to suggest that 6-aminopteridine cannot have the isomeric "imino"-structure. 6-Amino- and 6-dimethylamino-pteridines, more sensitive to acids than their 2- and 4-isomerides, are fairly rapidly hydrolysed to 6-hydroxypteridine at pH 2 (20°). As with the other amino-pteridines, the cations absorb at much lower wave-lengths than the neutral molecules.

Profound decomposition took place when the preparation of 6-mercaptopteridine was attempted by the reaction of 6-chloropteridine with thiourea or sodium hydrogen sulphide, or by the reaction of 6-hydroxypteridine with phosphorus pentasulphide.

6-Hydroxypteridine gave no colour with diazotized amines (aniline, *p*-aminodimethyl-aniline, and 2 : 5-dichloroaniline) under the conditions which were said to give an orange-brown colour from the last-named and xanthopterin (Schöpf and Becker, *Annalen*, 1933, **507**, 266). Xanthopterin had not been obtained pure in 1933, but pure xanthopterin (prepared by the method of Elion, Light, and Hitchings, *J. Amer. Chem. Soc.*, 1949, **71**, 741) gave no decisive colour change. 6-Hydroxypteridine could not be benzoylated.

6-Hydroxypteridine was oxidized to 6 : 7-dihydroxypteridine by hydrogen peroxide; by this method xanthopterin had been oxidized to leucopterin (Wieland and Purrmann, *Annalen*, 1939, **539**, 179), but in that case the 7-position was the only one open to substitution. The same authors (*Annalen*, 1940, **544**, 163) converted xanthopterin into leucopterin by shaking a suspension in dilute acetic acid with oxygen in the presence of Adams's platinum catalyst; however we found 6-hydroxypteridine unaltered by this treatment, even at 100°, when completely dissolved in glacial acetic acid. Sodamide in diethylaniline at 150°, followed by treatment with ice, gave 6 : 7-dihydroxypteridine. These reactions suggest that there is a considerable deficiency of electrons in the 7-position of 6-hydroxypteridine.

By far the most remarkable property of 6-hydroxypteridine is the hysteresis loop which is produced on titration (Fig. 1). Running alkali into an aqueous solution of 6-hydroxypteridine gave pH values which are much higher than those found on back-titration with acid. Moreover, the pH tends to fall during a pause in the addition of alkali and to rise during the addition of acid, equilibrium being obtained in approximately 2 hours (the loop was retraced when the solution was re-titrated). On the other hand, the basic group of this substance can be titrated, and back-titrated, without suggestion of abnormality (Fig. 1).

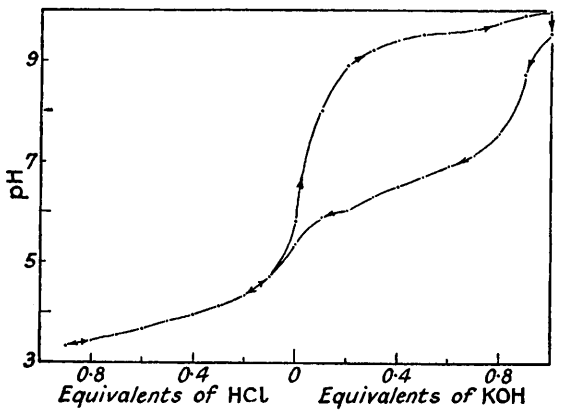


FIG. 1. *Hysteresis loop produced on titrating 250 ml. of 0·002M-aqueous 6-hydroxypteridine with 1 equiv. (5 ml.) of 0·1N-acid and -alkali at 20°.*

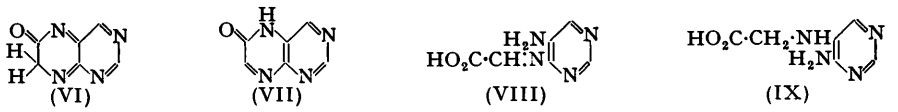
It has been suggested, on spectrographic grounds, that xanthopterin (2-amino-4 : 6-dihydroxypteridine) undergoes slow tautomerism in alkaline solution (Schou, *Arch. Biochem.*, 1950, **28**, 10). We found that a potentiometric back-titration produced pH drifts as with 6-hydroxypteridine. This effect in xanthopterin must be ascribed to the 6-hydroxy-group because neither 2-amino- nor 4-hydroxy-pteridine forms hysteresis loops upon titration; in fact we never observed this phenomenon in any hydroxy-heterocyclic substance that was not a derivative of 6-hydroxypteridine : even such closely related substances as 2-hydroxypyrazine * and 2-hydroxyquinoxaline * did not behave in this way.

It is not easy to decide whether this effect involves a change from (III) to (VI) or to (VII). Of these two types of tautomerism, prototropy between carbon and oxygen is commonly slow enough to be detected by physical means. On the other hand, prototropy between nitrogen and oxygen is usually considered to be instantaneous, although the independent existence of both ketimine and enamine tautomers of the neutral molecules of 2- and 4-hydroxyacridines has been demonstrated spectroscopically (Albert and Short, *J.*, 1945, 760).

That (VI) may be involved is suggested by two pieces of evidence. First, 7 : 8-dihydro-6-hydroxypteridine does not give this effect and could not do so because it cannot take another hydrogen in the 7-position. 6 : 7-Dihydroxypteridine also does not give the effect, and this may be attributed partly to steric hindrance by the 7-hydroxy-group and par-

* pK_a 8·23 ± 0·02 and 9·08 ± 0·03 respectively at 20°.

ticularly to the well-known favouring of enolization by electron-attracting substituents [Meyer, *Ber.*, 1912, **45**, 2843; a hydroxy-group would be electron-attracting in the 7-position of (VI)]. However, this is only negative evidence and, if structure (VI) were involved, it



should be possible to demonstrate a reactive methylene group in the 7-position of 6-hydroxypteridine, similar to that in the 5-position of barbituric acid (2 : 4 : 6-trihydroxypyrimidine). However, unlike barbituric acid, 6-hydroxypteridine could not be nitrated, with or without the addition of sulphuric or acetic acid; neither could it be nitrosated, brominated, or caused to react with aldehydes. Moreover barbituric acid did not show slow tautomerism on potentiometric titration.

It is unlikely that the slow tautomerism involves ring fission by alkali to the pyrimidine (VIII). The ultra-violet spectrum of the 6-hydroxypteridine anion (Fig. 2) became steady

FIG. 2. *6-Hydroxypteridine (absorption spectra in water).*

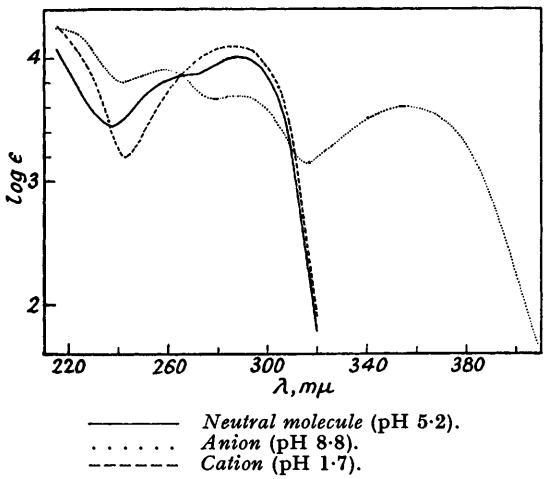
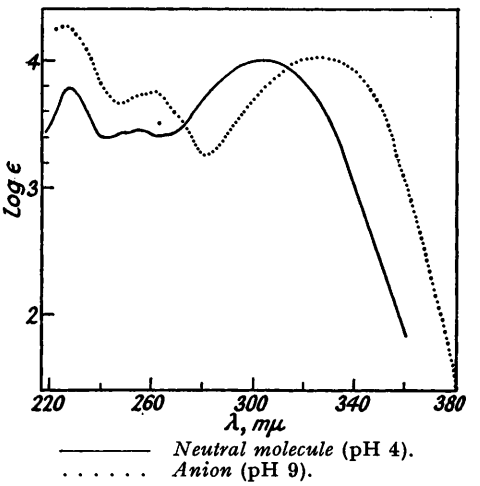


FIG. 3. *7-Hydroxypteridine (absorption spectra in water).*



n 2 hours at pH 8·8 and remained so for at least 8 hours; it was similar in outline to the anionic spectra of 2- and 7-hydroxypteridine and the long-wave peak of the 6-isomeride fell between those of these isomerides (cf. Figs. 2, 3, and Part I). Moreover, it overlaps much of the spectrum of 6-aminopteridine (neutral molecule), a relation to be expected from the comparable behaviour of the 2- and 4-hydroxy- and -amino-isomerides (see Part I). The spectrum of the neutral molecule of 6-hydroxypteridine (stable for 48 hours at pH 5·2) is similar in shape to that of the 2-isomeride but shifted a little to shorter wavelengths.

Furthermore, the infra-red spectra of 6-hydroxypteridine and its sodium salt (as solids) closely resembled one another in the 1400—1700-cm.$^{-1}$ region. Had the ring opened to give (VIII), bands characteristic of NH_2, C=N, and pyrimidine-ring absorption should have appeared in this region. The intensity of the band at 1700 cm.$^{-1}$, which is almost certainly a carbonyl bond-stretching vibration, was very much reduced after salt formation, as would be expected.

6-Amino- and 6-dimethylamino-pteridines showed no hysteresis on titration.

7-Hydroxypteridine and its Derivatives.—These were outstanding among pteridines for their ready tendency to crystallize quickly and in large crystals.

7-Hydroxypteridine was broken down by phosphorus oxychloride, pentachloride, or

pentasulphide; hence 7-chloro- and 7-mercapto-pteridines could not be obtained in this way. 7-Hydroxypteridine was unaffected by ammonia in *p*-cresol at 200°.

Methylation of 7-hydroxypteridine by diazomethane in ether gave an *N*-methyl-7-pteridone. The spectrum (Table 1) resembles that of 7-hydroxypteridine. An excess of diazomethane produced a substance, analysis of which showed addition of CH_2 to the molecule; this may be a *C*-methylation, cf. the action of methyl sulphate on N^9-methylxanthine to give C^8 : N^9-dimethylxanthine (Biltz and Sauer, *Ber.*, 1931, **64**, 752). Only a trace of a substance with a property suggestive of 7-methoxypteridine (hydrolysis by boiling with N-sodium hydroxide for 1 minute, as with the 2-, 4-, and 6-isomers) could be obtained by alkylation under various conditions.

When 7-hydroxypteridine was hydrogenated over platinum in 0·1N-sodium hydroxide or boiled with alkaline sodium dithionite (hydrosulphite), an amphoteric white substance,

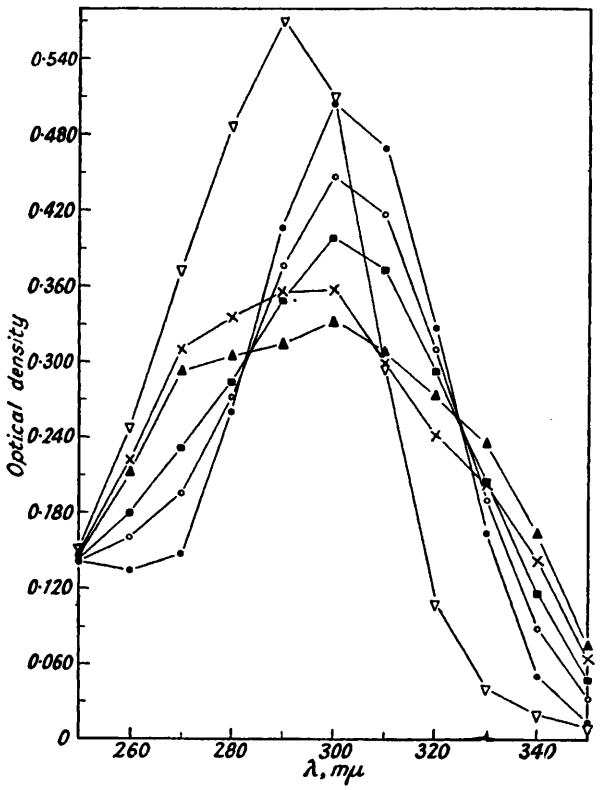


FIG. 4. *7-Hydroxypteridine (optical density of 5·0 × 10^{-5}M-aqueous solution).*

● pH 4·11.
○ pH 1·47.
■ pH 1·11.
▲ pH 0·24.
× ~pH −1·0 (5N-H_2SO_4).
▽ ~pH −5 (23N H_2SO_4; 70% w/w).

$C_6H_8O_2N_4$, was isolated on acidification to pH 5 at, or below, 20°. Acidification at a higher temperature gave also a dihydro-7-hydroxypteridine ($C_6H_6ON_4$). The H_8 substance could be completely converted by refluxing N-hydrochloric acid during 1 hour into the H_6 substance and then regenerated by boiling N-sodium hydroxide in one minute. These reactions, and the fact that the two substances differ from one another by the elements of water, suggested that ring-opening and -closing were involved. That the H_8 substance was a carboxylic acid was confirmed by its ready esterification in methanolic hydrogen chloride. The strikingly different R_F values of these three substances in paper chromatography made it easy to follow these reactions.

In the case of the dihydro-7-hydroxypteridine, reduction is assumed to have taken place exclusively in the pyrazine ring because pyrazines are readily hydrogenated under alkaline conditions (Krems and Spoerri, *Chem. Reviews*, 1947, **40**, 279), but pyrimidines only with difficulty (Lythgoe and Rayner, *J.*, 1951, 2323; Brown and Johnson, *J. Amer. Chem. Soc.*, 1923, **45**, 2702). Moreover, all known reductions in the pteridine series have occurred

exclusively at the pyrazine ring, *e.g.*, 6-hydroxypteridine and leucopterin (see above), folic acid (Pohland, Flynn, Jones, and Shive, *J. Amer. Chem. Soc.*, 1951, **73**, 3248), and 2-ethoxy-4-methyl-6 : 7-diphenylpteridine (Polonovski, Pesson, and Pinster, *Compt. rend.*, 1950, **230**, 2205).

The dihydro-7-hydroxypteridine is amphoteric (Table 1) and hence could not be the 7 : 8-dihydro-derivative which would not be acidic. Again, its $\lambda_{max.}$ value exceeds that of 7-hydroxypteridine and hence it could not be the 5 : 8-dihydro-derivative which has an isolated double bond and a short conjugated pathway. Hence it is considered to be 5 : 6-dihydro-7-hydroxypteridine. It was oxidized to 7-hydroxypteridine by potassium permanganate. The carboxylic acid, which would accordingly be 4-amino-5-carboxymethylaminopyrimidine (IX), was destroyed when oxidation was attempted under the same conditions.

Nitrous acid destroyed 7-hydroxypteridine, under the conditions which enabled Wieland and Liebig to insert a 6-hydroxyl-group into *iso*xanthopterin (2-amino-4 : 7-dihydroxy-

TABLE 1. *Physical properties of pteridines.*

Pteridine derivative	pK_a (in water) and concn. at which determined (20°)	R_F [2]	Spectrography in water: $\lambda_{max.}$ (mμ)	log $\varepsilon_{max.}$ (mol.)	pH
6-Hydroxy (+1H_2O)	—	0·30—0·70 [3]	<215; 289	>4·10; 4·00	5·2
(anion)	6·7 M/500	—	<215; 258; 289; 356	>4·25; 3·90; 3·69; 3·60	8·8
(cation)	3·67 (± 0·04) M/500	—	<215; 287	>4·27; 4·09	1·7
7-Hydroxy	—	0·75 [4]	227; 248 + 256 [8]; 303	3·79; 3·44 + 3·45; 4·00	4·0
(anion)	6·41 (± 0·02) M/200	—	226; 260; 326	4·27; 3·76; 4·04	9·0
(monocation)	1·2 (± 0·2) [1]	—	(See Fig. 4)	—	—
(dication)	−2·0 (± 0·5) [1]	—	(See Fig. 4)	—	—
6 : 7-Dihydroxy	—	0·35 [5]	<220; 249; 301	>3·98; 3·71; 4·18	4·0
(monoanion)	6·87 (± 0·03) M/500	—	227; 268; 319	4·03; 3·71; 4·29	8·4
(dianion)	10·0 (± 0·08) M/500	—	<220; 240; 324 + 338	>4·47; 4·13; 4·30 + 4·25	12
(cation)	<2·7 M/500	—	—	—	—
7 : 8-Dihydro-6-hydroxy	—	0·50 [6]	<215; 293	>4·09; 3·94	7·4
(anion)	10·54 (± 0·02) M/500	—	—	—	—
(cation)	4·78 (± 0·03) M/500	—	<220; 292	>4·20; 4·01	2·4
5 : 6-Dihydro-7-hydroxy	—	0·75 [7]	<215; 271; 319	>4·42; 3·58; 3·70	6·0
(anion)	9·94 (± 0·05) M/400	—	—	—	—
(cation)	3·36 (± 0·01) M/400	—	223; 284; 352	4·47; 3·74; 3·71	1·02
6-Amino	—	0·50	223; 258; 362	4·30; 4·01; 3·75	7·0
(cation)	4·15 (± 0·02) M/200	—	(See text)	—	
6-Dimethylamino	—	—	231; 279; 399	4·21; 4·17; 3·74	7·0
(cation)	4·31 (± 0·03) M/200	—	(See text)	—	—
6-Chloro	—	—	(220; 303 + 310 + 315 [9]) [10]	4·46; 3·84 + 3·92 + 3·86	—
6-Methoxy	—	—	(<215; 303 + 309 + 316 + 323 + 331 [9]) [10]	>4·36; 3·83 + 3·84 + 4·01 + 3·84 + 3·97	—
(cation)	3·60 (± 0·03) M/100	—	—	—	—
(*N*-Methyl-7-pteridone)	—	—	<220; 250 + 257; 306	>4·13; 3·55 + 3·54 3·97	4·0
(cation)	1·1 (± 0·1) M/30	—	<220; 295	>4·19; 3·97	−2·0
(4-Amino-5-carboxymethylaminopyrimidine)	—	0·30 [6]	<220; 282	>4·04; 3·93	4·84
(anion)	3·02 (± 0·03) M/100	—	—	—	—
(cation)	6·67 (± 0·02) M/100	—	<220; 288	>4·05; 3·94	1·04

[1] Determined spectroscopically. [2] In butanol–acetic acid, 4-aminopteridine (R_F = 0·70) and 4-hydroxypteridine (R_F = 0·50) being run as controls. [3] Long streak, because of slow tautomerism in this solvent, but a well-resolved spot (R_F 0·90) is given in a mixture of 9 parts of dimethylformamide (azeotrope containing 6% of formic acid) and 1 part of water. [4] This spot is invisible under Wood's light (360 mμ) unless the paper has been exposed to strong daylight; a dark spot is given under light of 254 mμ which changes to bright blue after 1 minute's irradiation and is then bright violet under Wood's light (photo-oxidation effect). [5] Bright violet spot. [6] Dark spot. [7] Bright violet spot under Wood's light without prior irradiation. [8] Not completely resolved. [9] Fine structure. [10] In *cyclo*hexane because of ready hydrolysis by water; pteridine gives almost the same spectrum in both solvents.

pteridine) (*Annalen*, 1944, **555**, 146). However, cold brown nitric acid (*d* 1·5) converted 7-hydroxypteridine into 6 : 7-dihydroxypteridine, thus providing yet a third synthesis of this new compound. Hydrogen peroxide (used as in the oxidation of 6-hydroxypteridine) produced only a trace of the dihydroxy-compound; the latter was identified as a product of the action of diffuse sunlight and of ultra-violet light (principally λ 254 mμ) on 7-hydroxypteridine.

7-Hydroxypteridine could not be benzoylated. Both 6- and 7-hydroxypteridine were reduced by cold hydriodic acid (*d* 1·94 or 1·7). The colour of the liberated iodine persisted to the same extent after dilution with water; in contrast, several derivatives of 7-hydroxypteridine (*e.g.*, *iso*xanthopterin) decolorize the liberated iodine on dilution although xanthopterin (a 6-hydroxypteridine) does not (Wieland, Tartter, and Purrmann, *Annalen*, 1940, **545**, 209). 7-Hydroxypteridine did not couple with diazotized 2 : 5-dichloroaniline.

The spectra of 7-hydroxypteridine given in Fig. 3 somewhat resemble those of 4-hydroxypteridine. Fig. 4 shows changes in the spectra in solutions of increasing acid

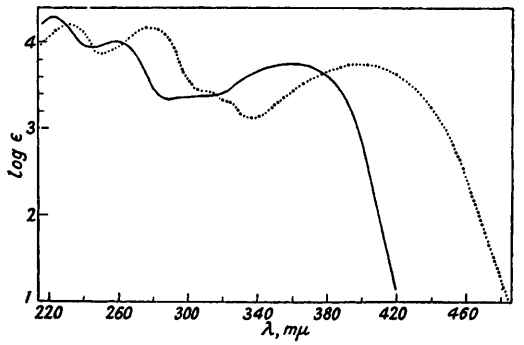


FIG. 5. *Absorption spectra in water.*

——— 6-*Aminopteridine* (*neutral molecule*, pH 7·0).
. . . . . 6-*Dimethylaminopteridine* (*neutral molecule*, pH 7·0).

strength : these changes (which are reversed when the pH is increased) reveal the formation of two cations.

Physical Constants.—A number of ionization constants are recorded in Table 1. 6- and 7-Hydroxypteridine are stronger acids than their isomerides (cf. Part I). Of all the monohydroxypteridines, only the 6-isomeride has marked basic properties.

Xanthopterin was found in Part I to have two acidic pK_a values (6·25 and 9·23) : the 6·25 value can now be definitely assigned to the 6-hydroxy-group, because of the relative strength of 6-hydroxypteridine (cf. 4-hydroxypteridine, pK_a 7·89). As would be expected, once the 6-hydroxy-group in xanthopterin has ionized, the ionization of the 4-hydroxy-group is depressed by the coulombic effect.

TABLE 2. *Fluorescences in dilute aqueous solution observed by the light of a Wood's lamp (principally 360 mμ).*

Pteridine derivative	Water	0·05N-NaOH	0·05N-H_2SO_4
4-Hydroxy (for comparison with Part I)	Violet ++	Blue +	Little or none
6-Hydroxy	None	None	None
7-Hydroxy	Violet ++	None	Little or none
6 : 7-Dihydroxy	Violet +	Little or none	Violet +
7 : 8-Dihydro-6-hydroxy	None	Violet +	None
5 : 6-Dihydro-7-hydroxy	Violet +	Violet ++	Violet ++
6-Amino	Violet ++	Violet ++	Blue ++
6-Dimethylamino	Yellow +	Yellow +	Violet +

Table 1 also summarizes the spectrographic and chromatographic data. We wish to withdraw the R_F of 0·30 recorded for pteridine in Part I as being due to photo-decomposition of this unstable substance.

The new substances described in this paper differ from xanthopterin by not fluorescing to any extent in daylight. The colours seen in ultra-violet light are given in Table 2.

Experimental

(M. p.s are uncorrected. Microanalyses were by Mr. A. Bennett and Mr. R. Baker, Beckenham.)

Physical Measurements.—The absorption spectra, potentiometric titrations, and paper chromatography were carried out as described in Part I.

In order to choose two suitable conditions of acidity to give maximal yields of 6- and 7-hydroxypteridines, the pK_a values of 4 : 5-diaminopyrimidine were determined and found to be 6·03 ± 0·04 (M/100) and <0 (M/1), both at 20°. The higher value corresponds to the ionization of 4-aminopyrimidine (pK_a, 5·71; Albert, Goldacre, and Phillips, *J.*, 1948, 2240), and the latter to that of 5-aminopyrimidine (2·83), after allowance for depression due to coulombic repulsion of the second proton after the first ionization.

Ethyl glyoxylate hemiacetal was prepared from ethyl tartrate and sodium bismuthate by Rigby's method (*J.*, 1950, 1912).

6-*Hydroxypteridine and its derivatives.*

6-*Hydroxypteridine.*—(*a*) *Direct synthesis.* 4 : 5-Diaminopyrimidine (4·4 g., 0·04 mole) was dissolved in 2N-sulphuric acid (80 ml.) at 30°. Ethyl glyoxylate hemiacetal (9 g., 0·06 mole) was added. The mixture was shaken until clear (5 minutes) and kept at about 20° for 18 hours. It was then gently refluxed for 15 minutes, clarified with carbon, and taken to pH 2·0 (metanil-yellow) with 6N-sodium hydroxide (about 16 ml.). After an hour at 20° the 7-hydroxypteridine (see below) was filtered off (20% yield). The filtrate was taken to pH 5·5 (bromocresol-green) with sodium citrate (10 g.) and 2·5N-sodium hydroxide (about 25 ml.) and chilled overnight. The white crystals were recrystallized from 230 parts of water, giving 4·3 g. (65%) of 6-*hydroxypteridine monohydrate* (Found, for material dried at 120°/0·1 mm.: C, 43·7; H, 3·45; N, 33·7. $C_6H_4ON_4,H_2O$ requires C, 43·4; H, 3·65; N, 33·75%). 6-Hydroxypteridine decomposes progressively on heating above 240°. It is soluble in 35 parts of boiling dimethylformamide (acid-free, or formic acid azeotrope) and the solution does not deposit solid at 20°. It dissolves in 50 parts of boiling acetic acid but is almost insoluble in pyridine, butanol, acetone, toluene, and light petroleum. It is decomposed by 0·1N-sodium hydroxide (almost instantaneously at 100°): the product is less soluble than 1 : 2000 in boiling water and very soluble in cold N-sodium hydroxide and appears to be a polymer. 6-Hydroxypteridine hydrochloride crystallizes from a 15% solution in hot N-hydrochloric acid. Unlike 2-hydroxypteridine, it is unchanged by 10N-hydrochloric acid at 20°.

(*b*) *From its* 7 : 8-*dihydro-derivative.* 7 : 8-Dihydro-6-hydroxypteridine (0·4 g.) was dissolved in a boiling aqueous sodium hydroxide (0·2 g. in 25 ml.) and quickly cooled to 20°. Potassium permanganate (17 ml.; 0·1M) was added during 10 minutes, with agitation. The suspension was centrifuged, the remaining solid was extracted with boiling water (12 ml.), and the combined extracts were brought to pH 5·5 and refrigerated overnight. The 6-hydroxypteridine (80% yield) was filtered off and recrystallized from water.

7 : 8-*Dihydro-6-hydroxypteridine.*—Powdered sodium amalgam (48 g., 4%) was added with gentle shaking to a suspension of 6-hydroxypteridine monohydrate (3·3 g., 0·02 mole) in water (24 ml.), at such a rate that the temperature remained between 45° and 50°. The mercury was run off. (At this stage when 6 : 7-dihydroxypteridine was the starting material, the residual solution was acidified with hydrochloric acid to pH 3·3 and kept at 20° for an hour; charcoal was then added and the mixture filtered from unchanged dihydroxypteridine.) The filtrate was adjusted to pH 8. After refrigeration overnight, the 7 : 8-dihydro-6-hydroxypteridine was filtered off and dried at 110°. It was purified by recrystallization from 400 parts of boiling water (white crystals, 85% yield). It decomposes between 310° and 340° without melting. It also recrystallizes well from (neutral) dimethylformamide and is slightly more soluble in organic solvents than 6-hydroxypteridine (Found: C, 48·0; H, 3·8; N, 37·7. Calc. for $C_6H_6ON_4$: C, 48·0; H, 4·0; N, 37·3%.) It is not oxidizable by iodine or by silver oxide (in contrast to dihydroxanthopterin, Totter, *loc. cit.*).

6 : 7-*Dihydroxypteridine. Direct Synthesis.*—Oxalic acid dihydrate (8·8 g., 7 equivs.) and 4 : 5-diaminopyrimidine (1·1 g.) were heated, under a slight vacuum (10—15 cm. of mercury), to 160° during 30 minutes and kept at 160—170° for 2 hours. The product was extracted with boiling water (125 ml.) containing enough sodium hydroxide for the mixture to give the full red colour with phenolphthalein. The filtrate was brought to pH 4 with acetic acid, cooled, and filtered after 3 hours. Recrystallization of the precipitate from 290 parts of boiling water gave large white plates of 6 : 7-*dihydroxypteridine* (1·45 g., 88%) (Found: C, 44·0; H, 2·3; N, 34·5.

$C_6H_4O_2N_4$ requires C, 43·9; H, 2·45; N, 34·2%). It is unchanged when heated to 350° or boiled with 10N-hydrochloric acid. It is almost insoluble in dilute acids and in common organic solvents, including butanol and pyridine; however it is very soluble in formamide and 4-formylmorpholine. Purification through the potassium salt was found to be as effective as recrystallization from water : 6 : 7-dihydroxypteridine (1·64 g.) and potassium carbonate (2 g., 3 equivs.) were dissolved in boiling water (32 ml.). The solution was stirred with charcoal and filtered. Next day, the potassium salt was filtered off, dissolved in boiling water (25 ml.) with the help of a little sodium hydroxide, and brought to pH 4 with acetic acid (87% recovery).

6-*Chloropteridine.*—A mixture of dried and finely ground 6-hydroxypteridine monohydrate (1 g.), phosphorus pentachloride (5 g.), and freshly distilled phosphorus oxychloride (50 ml.) was refluxed for 2 hours in a bath at 115—120°. The phosphorus oxychloride was removed by vacuum distillation at 60° and the residue was cautiously decomposed by stirring with ice, neutralized with 10N-sodium hydroxide solution, and filtered. The filtrate was exhaustively extracted with ice-cold chloroform. During the extraction the aqueous layer, which tended to become acid, was kept at pH 7. The chloroform extracts were dried (Na_2SO_4 + a little K_2CO_3), and the solvent was evaporated in a vacuum, leaving 6-*chloropteridine* (0·65 g., 65%). A smaller percentage yield was obtained when 8 g. of starting material were used. For analysis, it was crystallized from 250 parts of light petroleum (b. p. 60—80°), then from 20 parts of alcohol, giving pale yellow crystals, decomp. 146—147° (Found : Cl, 21·2. $C_6H_3N_4Cl$ requires Cl, 21·3%).

6-*Aminopteridine.*—Dry ammonia was passed into a filtered solution of crude 6-chloropteridine (0·85 g.) in benzene (85 ml.) at 20° for an hour. The precipitate was recrystallized from boiling water (80 ml.) containing carbon (0·3 g.). A further quantity was obtained from the mother-liquor. On recrystallization from water 6-*aminopteridine* was thus obtained as 1-cm. long yellow needles (53% yield) which decompose rapidly when heated above 300° and give green-fluorescing solutions (Found : C, 48·9; H, 3·4; N, 47·65. $C_6H_5N_5$ requires C, 49·0; H, 3·4; N, 47·6%). It was largely destroyed when heated under reflux with acetic anhydride for 30 minutes.

6-*Dimethylaminopteridine.*—Methanolic dimethylamine (50% w/v; 18 ml.) was added to a solution of crude 6-chloropteridine (1·5 g.) in methanol (50 ml.). After 4 hours at 20°, the solution was concentrated to *ca.* 10 ml. and treated with charcoal. The cooled filtrate deposited 6-dimethylaminopteridine in two crops, m. p. 210—211° (total yield, 78%). After dissolution in methanol (30 parts, containing charcoal), the filtrate was concentrated, to yield long yellow crystals of 6-*dimethylaminopteridine*, m. p. 212° (Found : C, 55·0; H, 4·85; N, 40·0. $C_8H_9N_5$ requires C, 54·8; H, 5·2; N, 40·0%).

6-*Methoxypteridine.*—A solution of sodium methoxide (9·2 ml., 1 equiv.), prepared from sodium (0·5 g.) and methanol (50 ml.), was added to an ice-cooled solution of crude 6-chloropteridine (0·66 g.) in methanol (25 ml.). The reaction mixture was kept at 20° for 30 minutes and then neutralized with a rapid stream of carbon dioxide. The residue obtained after removal of solvent in a vacuum was repeatedly extracted with light petroleum (b. p. 60—80°; total 300 ml.) until constant in weight. It was ground between each extraction. The combined extracts were boiled with charcoal, filtered, and concentrated to 50 ml., depositing 6-methoxypteridine in two crops (m. p. 122—125°) (total yield, 53%). Recrystallization from light petroleum (b. p. 60—80°; 120 parts) gave pure 6-*methoxypteridine* as slightly yellow needles, m. p. 124—125° (80% recovery) (Found : C, 52·1; H, 3·3; N, 34·3. $C_7H_6ON_4$ requires C, 51·85; H, 3·7; N, 34·55%).

Oxidation of 6-*Hydroxypteridine to* 6 : 7-*Dihydroxypteridine.*—6-Hydroxypteridine monohydrate (1 g.) was dissolved in boiling acetic acid (50 ml.). Hydrogen peroxide (15 ml.; 30%) was added and the mixture kept at 20° for 3 days. The precipitate was filtered off, ground under hydrogen peroxide (20 ml.; 30%), and kept at 20° for 4 days. The crystals were filtered off and boiled with water (50 ml.). The suspension was taken to pH 2·0 (metanil-yellow) with sulphuric acid and refrigerated. The crystals which were deposited were recrystallized from water, giving 6 : 7-dihydroxypteridine (45% yield).

7-*Hydroxypteridine and its derivatives.*

7-*Hydroxypteridine.*—4 : 5-Diaminopyrimidine (4·4 g., 0·04 mole), 5N-acetic acid (10 ml.), and sodium acetate trihydrate (10 g.) were dissolved in boiling water (40 ml.). Ethyl glyoxylate ethyl hemiacetal (9 g., 0·06 mole) was added and the mixture heated in a boiling water-bath, with occasional shaking, for 1 hour during which the pH fell from 6·5 to 5·5. The mixture was taken to pH 2·0 (metanil-yellow) with 5N-sulphuric acid (about 18 ml.), boiled for 5 minutes,

and chilled overnight. The crystals were filtered off and dissolved in boiling N-sodium hydroxide (70 ml.). The sodium salt of 7-hydroxypteridine crystallized in 2-cm. long, white needles which were collected after an hour at 20°, and dissolved in boiling water (70 ml.) containing N-sodium hydroxide (2 ml.). The boiling solution was brought to pH 2·5 (metanil-yellow) with 5N-sulphuric acid and chilled overnight. The *7-hydroxypteridine* was filtered off and recrystallized from 76 parts of boiling water. The yield was 65% of 0·5-cm. long, white plates, decomposing slowly at >230°. It is soluble in approx. 12 parts of boiling (and 70 parts at 20° dry pyridine, in 10 parts of boiling dimethylformamide (and in 6 parts of dimethylformamide azeotrope containing 6% of formic acid; in both cases 90% of the pteridine crystallizes on cooling), in 15 parts of boiling glacial acetic acid, and in 100 parts of boiling *n*-butanol. It is practically insoluble in acetone, ethanol, toluene, or light petroleum (Found: C, 48·6; H, 2·6; N, 37·4. $C_6H_4ON_4$ requires C, 48·65; H, 2·7; N, 37·8%.) Unlike 2-hydroxypteridine, it gave no colour with 10N-hydrochloric acid (1 day at 20°); it was destroyed on long boiling with N-sulphuric acid.

The above filtrate, at pH 2, was clarified with carbon and taken to pH 5·5 (bromocresol-green) with sodium citrate (4 g.) and 6N-sodium hydroxide, and refrigerated overnight. The crystals were recrystallized from the minimum of boiling water (about 390 ml.), giving 6-hydroxypteridine (see p. 1627) in 26% yield.

N-*Methyl-7-pteridone*.—A dried ethereal solution of diazomethane (0·026 mole in 20 ml.) was cooled to −70° and added portionwise, during 1 hour, to a stirred, ice-cold suspension of 7-hydroxypteridine (1·8 g., 0·012 mole) and its sodium salt (0·2 g.) in dry methanol (200 ml.). Stirring was continued for 2 hours and the reaction mixture left at 0° overnight. Undissolved solid (0·15 g.) was removed by filtration and the filtrate taken to dryness in a vacuum. The residue was extracted with boiling light petroleum (b. p. 60—80°; 100 + 50 ml.), leaving a large residue. The extracts were treated with charcoal and concentrated to 50 ml. On cooling of the solution, colourless crystals (0·8 g.; m. p. 114—119°) separated. These were dissolved in boiling light petroleum (b. p. 60—80°; 120 ml.) and this solution on cooling deposited 0·64 g. of presumed N-*methyl-7-pteridone* as white plates, m. p. 124·5—125·5° (32%) (Found: C, 52·0; H, 3·8; N, 35·1. $C_7H_6ON_4$ requires C, 51·85; H, 3·75; N, 34·55%). The combined mother-liquors, after concentration and cooling to 0°, deposited solid (0·29 g.; m. p. 93—103°). A sample (0·094 g.) of this material was heated under reflux with N-sodium hydroxide (2 ml.) for 5 minutes. On cooling of the solution, the sodium salt of 7-hydroxypteridine separated as white needles. These were collected and 0·014 g. of 7-hydroxypteridine was regenerated from them. This suggests that a trace of 7-methoxypteridine was formed, probably not more then 1% based on the 7-hydroxypteridine.

The Higher-melting Compound obtained from 7-Hydroxypteridine by Diazomethane.—(a) *Direct method*. A dried ethereal solution of diazomethane (25 ml., 0·03 mole) was added to a stirred ice-cooled suspension of 7-hydroxypteridine (1·5 g., 0·01 mole) in dry methanol (15 ml.). After 1 hour, evolution of nitrogen had ceased and a further portion of diazomethane (25 ml., 0·03 mole) was added. Stirring was continued for 2 hours and the pinkish-white crystalline product (0·95 g.; m. p. 144—145·5°) filtered off and washed with a little ice-cold methanol. A further crop (0·15 g.; m. p. 143—145°) was obtained by vacuum-concentration of the mother-liquor. Crystallization from methanol (15 parts) and treatment with charcoal gave white needles of presumed CN-*dimethyl-7-pteridone*, m. p. 146—147° (75% recovery) (Found: C, 54·30; H, 4·35; N, 31·9. $C_8H_8ON_4$ requires C, 54·55; H, 4·6; N, 31·8%).

(b) *By methylation of* N-*methyl-7-pteridone*. A dried ethereal solution of diazomethane (15 ml., 0·02 mole) was added in one portion to an ice-cooled suspension of *N*-methyl-7-pteridone (0·32 g., 0·002 mole) in dry methanol (5 ml.). Nitrogen was evolved and the reaction mixture was kept at 0° overnight. Ether and excess of diazomethane were then removed in a vacuum and the separated solid (0·27 g.; m. p. 141·5—143·5°) was collected by filtration. Sublimation at 130°/0·5 mm. gave white needles, m. p. 146—147°, not depressed on admixture with the compound described above.

Reduction of 7-Hydroxypteridine.—7-Hydroxypteridine (1·48 g., 0·01 mole) and sodium hydroxide (1·6 g., 0·04 mole) were dissolved in boiling water (120 ml.). Sodium dithionite dihydrate (4·2 g., 0·02 mole) was added during 3 minutes while the solution was vigorously boiled. The solution was rapidly concentrated to 60 ml. (pH 8), cooled in ice, and then brought to pH 4·8 with acetic acid (approx. 2·5 ml.), giving 1·6 g. (95%) of colourless crystals of *4-amino-5-carboxymethylaminopyrimidine* (IX), chromatographically homogeneous. For analysis, it was recrystallized from 19 parts of water (85% recovery). It decomposes about 310°, is very soluble in cold N-sodium hydroxide, and almost insoluble in all organic solvents (Found, for

material dried at 100°/0·1 mm. : C, 42·4; H, 4·6; N, 33·6. $C_6H_8O_2N_4$ requires C, 42·8; H, 4·8; N, 33·3%). The hydrochloride is sparingly soluble in cold N-hydrochloric acid.

4-Amino-5-carbomethoxymethylaminopyrimidine Dihydrochloride.—The above acid (0·34 g., 0·002 mole) was shaken for 4 hours with methanol (15 ml.) containing hydrogen chloride (2 g.). Air was blown through the solution until the volume fell to 2 ml. Anhydrous ether (5 ml.) was added and the chromatographically homogeneous, colourless crystals (0·37 g.) of the *ester dihydrochloride* were filtered off and dried in a vacuum at 20° (Found : C, 33·0; H, 4·55; Cl, 27·6. $C_7H_{10}O_2N_4,2HCl$ requires C, 33·0; H, 4·7; Cl, 27·8%). At pH 4·8, $\lambda_{max.}$ was 290 mμ; there was no absorption at 352 mμ (absence of 5 : 6-dihydro-7-hydroxypteridine). The R_F was 0·55 (dark spot); no spot characteristic of the free acid was seen, but a mixture of the acid and ester showed both spots in the correct positions.

5 : 6-Dihydro-7-hydroxypteridine was obtained in 70% yield by refluxing 4-amino-5-carboxymethylaminopyrimidine with 18 volumes (3 equiv.) of N-hydrochloric acid for 1 hour, and adjusting the pH of the hot solution to 5·5 with sodium citrate and sodium hydroxide. The chromatographically homogeneous, colourless crystals were recrystallized from 175 parts of boiling water (Found : C, 48·2; H, 3·9; N, 37·5. $C_6H_6ON_4$ requires C, 48·0; H, 4·0; N, 37·3%). The solubilities of this substance in organic solvents are very similar to those of 7-hydroxypteridine, but unlike the latter it is very soluble in cold 2N-sodium hydroxide and cold N-hydrochloric acid. Oxidation to 7-hydroxypteridine was carried out as in the case of 7 : 8-dihydro-6-hydroxypteridine (above); because 7-hydroxypteridine is much more soluble than the 6-isomeride, the mixed filtrates were evaporated to small bulk before being acidified.

Oxidation of 7-Hydroxypteridine to 6 : 7-Dihydroxypteridine.—7-Hydroxypteridine (1·2 g.) and yellow nitric acid (2·4 ml.; *d* 1·5) were mixed with cooling, and set aside at about 20° overnight. Water (24 ml.) was added. The pale yellow solution was boiled for 2 minutes and quickly cooled. Sodium citrate (1 g.) was dissolved in the solution, which was then brought to pH 4·5 with 6N-sodium hydroxide (about 8 ml.). The precipitate (0·6 g.) was purified through the potassium salt as described under 6 : 7-dihydroxypteridine (direct preparation) above. The yield was 30%.

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