103. *Pteridine Studies. Part I. Pteridine, and 2- and 4-Amino- and 2- and 4-Hydroxy-pteridines.*

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

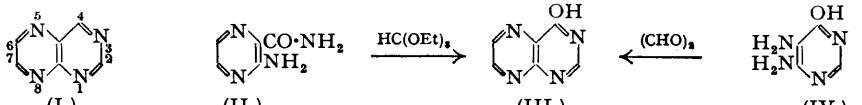
Syntheses of pteridine and a number of mono-substituted pteridines are reported, including a new route to the pteridine series from pyrazine intermediates. Solubilities, ionization constants, and ultra-violet spectra are recorded and discussed from the point of view of comparative heterocyclic chemistry.

The value of paper chromatography as well as ultra-violet spectroscopy for following the purification of non-melting pteridines is emphasized. 4-Hydroxypteridine has marked chelating affinity for the cations of heavy metals, a finding that is likely to be of significance in the living cell. The relative chemical stability of individual pteridines is discussed.

Syntheses of a number of pyrazines and pyrimidines, required as intermediates, are described.

Pteridine derivatives were found in Nature as long ago as 1889, but their constitution remained baffling until Purrmann in 1940 showed that xanthopterin and leucopterin are amino-polyhydroxy-derivatives of the (then) unknown substance pyrimidino-4′ : 5′-2 : 3-pyrazine (I) to which Wieland then gave the name pteridine. More pteridines have since been recognized in Nature, notably folic acid which plays an important part in hæmatopoiesis and cell-division.

All natural pteridines have at least one amino- *and* one hydroxy-substituent. As no monohydroxy- or monoamino-pteridine was known, it was decided to synthesize all of them and to investigate the connexions between structure, physical properties, and biological activity in the pteridine series. This approach was suggested by the success achieved in revealing the chemical basis of antisepsis by aminoacridines and by hydroxyquinolines through similar exploration of all possible mono-isomerides (Albert, Rubbo, Goldacre, Davey, and Stone, *Brit. J. Exper. Path.*, 1945, **26**, 160; Albert, Rubbo, Goldacre, and Balfour, *ibid.*, 1947, **28**, 69). The present paper is mainly concerned with pteridines substituted in the pyrimidine ring.



(I.) (II.) (III.) (IV.)

Syntheses.—Hitherto, with one exception, all the known pteridines have been prepared by condensing 4 : 5-diaminopyrimidines (*e.g.*, IV) with carbonyl compounds or their derivatives. The exception was the synthesis of 2 : 4-dihydroxypteridine in 40% yield by the action of potassium hypobromite on pyrazine-2 : 3-dicarboxyamide (Gabriel and Sohn, *Ber.*, 1907, **40**, 4857). Some new syntheses, using pyrazine intermediates, are now described. 2-Amino-

pyrazine-3-carboxyamide (II) when refluxed with ethyl orthoformate gave 4-hydroxypteridine (III) in 75% yield, and (III) was also obtained from (II) by acetic anhydride–formic acid and from the formyl derivative of (II) by sodium ethoxide. Likewise 2-aminopyrazine-3-carboxythioamide and ethyl orthoformate gave 4-mercaptopteridine in excellent yield. Some unsuccessful attempts to devise other syntheses of pteridines from pyrazines are recorded in the Experimental section.

Pteridine and the following new pteridines were obtained by condensing 4 : 5-diaminopyrimidines (*e.g.*, IV), with glyoxal or glyoxal sodium bisulphite : 2-amino-, 2-dimethylamino-, 4-amino-, 2-hydroxy-, 4-hydroxy-, and 2-chloro-pteridine; 4-hydroxy-6 : 7-dimethylpteridine was similarly obtained by use of diacetyl. Glyoxal sodium bisulphite appears to give dihydropteridinesulphonic acids analogous to the dihydroquinoxalinesulphonic acids which are similarly formed (Bergstrom and Ogg, *J. Amer. Chem. Soc.*, 1931, **53**, 245) and these have to be broken down to pteridines with acid or alkali.

The only recorded synthesis of pteridine involves the reaction of 4 : 5-diaminopyrimidine with glyoxal sodium bisulphite in aqueous solution (Jones, *Nature*, 1948, **162**, 524; no details or yield given). In our experience, no higher yield than 15% could be obtained in this way; this was increased to 50% when the condensation was carried out in alcohol using commercial syrupy glyoxal and to 63% by using the polyglyoxal that had deposited in an old bottle of the syrup.

4-Hydroxypteridine was prepared in yet a third way : by the alkaline hydrolysis of 4-aminopteridine.

Purity.—Because most pteridines decompose at high temperatures without melting sharply, special reliance has been placed upon three criteria : purification (i) to constant solubility (in water), (ii) to constant spectral properties, and (iii) until only one spot appears on paper-chromatography. For the last, a mixture of butanol and aqueous acetic acid has been found most discriminatory (see Experimental section) and the R_F values thus obtained are listed in Table I. As an example of the value of (iii), the paper chromatogram of 2 : 4-dihydroxypteridine, prepared according to Kuhn and Cook (*Ber.*, 1937, **70**, 761) " in schönen gelben Nädelchen," or according to Cain, Mallette, and Taylor (*J. Amer. Chem. Soc.*, 1946, **68**, 1996), initially revealed 5 spots. We purified this substance by repeated crystallization after boiling it with relatively large amounts of alumina until only one spot remained on paper chromatography. The substance then consisted of white crystals, 2 mm. long, giving correct analytical figures, as the yellow specimen of Kuhn and Cook had done. Hence it is doubtful if this substance had previously been obtained pure : Polonovski, Vieillefosse, and Pesson (*Bull. Soc. chim.*, 1945, **12**, 78) describe their specimen as pale yellow, and the extinction coefficients recorded by Cain, Mallette, and Taylor (*loc. cit.*) are only 72—76% of those given by the present material.

Paper chromatography is very useful in this series for establishing identity by running the doubtful material alongside (or even mixed with) an authentic specimen and comparing the R_F values and the colours of the spots under ultra-violet light of different wave-lengths.

Physical Properties.—Solubility. Introduction of even one hydroxy-, mercapto-, or primary amino-group into pteridine greatly lowers the solubility (roughly one-hundredfold) (see Table I). The solubility of acridine is also lowered by the insertion of amino- or hydroxy-groups, but to a lesser degree (Albert, " The Acridines, their Preparation, Properties and Uses," London, Edward Arnold, 1951, p. 152). This effect is probably more common among hetero-aromatic bases than has been realized. It is unlikely that amino- and hydroxy-groups cease to exert their well-known water-attracting properties, but that they exert an even stronger effect on the negatively charged ring-nitrogen atoms of neighbouring molecules, an attraction which makes a far stronger crystal lattice. That this effect depends more on hydrogen-bonding than on the attraction of oppositely charged dipoles is shown by 2-dimethylaminopteridine which is 540 times as soluble in water as 2-aminopteridine. The hydroxy- and primary amino-pteridines are almost insoluble in boiling alcohol, benzene, or pyridine, whereas pteridine, 2-chloro-, and 2-dimethylamino-pteridine are highly soluble in all these solvents, even in the cold. The solubilities of the acetamidopteridines lie between those of the primary aminopteridines and of pteridine. The fact that all the known hydroxy-, (primary) amino-, and aminohydroxypteridines decompose above 240° without melting is another expression of the ability of these substituent groups to strengthen the crystal lattice. Pteridine and 2-dimethylaminopteridine, on the other hand, melt at 140° and 126° respectively, without decomposition.

Ionizing properties. Introduction of further ring-nitrogen atoms into a hetero-aromatic base usually has a base-weakening effect (Albert, Goldacre, and Phillips, *J.*, 1948, 2240). In so far as pteridine is the diaza-derivative of quinoxaline, of 1 : 8-naphthyridine, and of quinazoline

(which have pK_a values of 0·8, 3·4, and 3·5 respectively), its tendency to ionize was found unexpectedly high ($pK_a = 4·1$, see Table I; cf. also quinoline in Table II). Evidently a complex electronic interplay takes place between the several nitrogen atoms. The 2- and

TABLE I.

Physical properties of pteridines.

Pteridine.	Solubility (approx.) in H_2O (20—25°), 1 in :	pK_a (in water) and concentration at which determined (20°).		R_P.[3]	Spectrography in water. $\lambda_{max.}$ (mμ.).	log $\epsilon_{max.}$ (mol.).	pH.
(Unsubstituted)	7·2	—	—	0·25	<220; 298 + 309 [5]	>3·83; 3·87; 3·83	6·2
cation	—	4·12 (± 0·05)	M/20	—	<220; 300	>3·84; 3·92	1·1 or 2·1
2-Amino-	1350	—	—	0·70	225; 370	4·38; 3·82	7·1
cation	—	4·29 (±0·03)	M/100	—	<210; 302	>4·04; 3·87	2·1
4-Amino-	1400	—	—	0·70	244; 335	4·20; 3·82	7·3
cation	—	3·56 (±0·08)	M/200	—	229; 324	4·10; 3·99	1·1
anion	—	>14	—	—	(see graph)	—	—
2-Dimethylamino-	2·5	—	—	0·90	236; 281; 410	4·37; 4·02; 3·82	7·1
cation	—	3·03 (±0·02)	M/100	—	237; 305	4·16; 3·90	1·0
2-Hydroxy- (+1H_2O)	600	—	—	0·80	230; 307	3·88; 3·83	7·1
anion	—	11·13 (±0·05)	M/100	—	260; 375	3·85; 3·78	13
cation	—	<2	M/100	—	—	—	—
4-Hydroxy-	200	—	—	0·50 [4]	230; 265; 310	3·98; 3·54; 3·82	5·6
anion	—	7·89 (±0·03)	M/100	—	242; 333	4·23; 3·79	10·0
cation	—	<1·5	M/100	—	—	—	—
4-Hydroxy-6 : 7-dimethyl-	1100	—	—	0·60	230; 270; 313	4·03; 3·67; 3·93	6·2
anion	—	8·39 (±0·03)	M/200	—	—	—	—
2 : 4-Dihydroxy- (lumazine)	800	—	—	0·50	230; 324	4·00; 3·84	5·9
anion (mono-)	—	7·91 (±0·07)	M/100	—	235; 270; 347	4·02; 3·95; 3·69	10·1
anion (di-)	—	—	—	—	252; 365	4·23; 3·78	13
cation	—	<1·0	M/20	—	—	—	—
2-Amino-4-hydroxy-	57,000	—	—	—	—	—	—
anion	—	(approx. 8·0) [1]	—	—	252; 359	4·30; 3·83	13
2-Amino-4 : 6-dihydroxy (xanthopterin) (+1H_2O)	40,000	—	—	—	—	—	—
anion (mono; 6-position)	—	6·25	M/1000 [2]	—	—	—	—
anion (di; 4- and 6-positions)	—	9·23 (±0·04)	M/1000 [2]	—	255; 392	4·27; 3·85	13
2-Amino-4 : 6 : 7-trihydroxy- (leucopterin)	750,000	—	—	—	—	—	—
anion	—	—	—	—	240; 285; 340	4·20; 3·84; 4·02	13
2-Mercapto (+1H_2O)	1400	—	—	—	<215; 270; 315	>3·74; 4·24; 4·22	7·1
anion	—	9·98 (±0·05)	M/200	—	—	—	—
4-Mercapto-	3600	—	—	0·70	256; 390	4·13; 4·00	4·1
anion	—	6·81 (±0·02)	M/600	—	265; 408	4·22; 3·93	13

[1] Stokstad *et al.*, *J. Amer. Chem. Soc.*, 1948, **70**, 5. [2] Back-titrated. [3] Solvent : BuOH–AcOH. [4] A small extra spot at 0·30 is seen on paper giving an ash rich in iron and copper; this is not seen if 8-hydroxyquinoline is allowed to travel ahead of the 4-hydroxypteridine. [5] Fine structure.

4-aminopteridines (Table I) are bases of approximately the same strength as pteridine. Thus they do not show the exaltation of basic strength through ionic resonance which is such a feature of 2- and 4-aminoquinolines, but which was found to decline in intensity with the addition of a further ring-nitrogen atom to give aminoquinazolines (Albert, Goldacre, and Phillips, *loc. cit.*).

When a concentrated (colourless) solution of 4-aminopteridine is added to an excess of 0·2N-sodium hydroxide, a yellow colour appears (see Fig. 4). When the solution is neutralized the spectrum of the neutral molecule is restored. The colour appears to reach a maximum in 5N-sodium hydroxide, but hydrolysis to 4-hydroxypteridine is then rapid. The colour change clearly indicates that 4-aminopteridine can form an anion; no similar phenomenon has ever been reported for the aminoquinolines or their analogues containing more ring-nitrogen atoms.

4-Hydroxypteridine and its derivatives are, as would be expected, stronger acids (pK *ca.* 8) than 4-hydroxyquinoline and 4-hydroxyquinazoline (pK_a = 12·4 and 10·0 in 50% alcohol; Keneford, Morley, Simpson, and Wright, *J.*, 1949, 1356). A pK_a of 8·26 ± 0·06 found for folic acid (M/500) is apparently that of the 4-hydroxy-group in the pteridine nucleus. 2-Hydroxypteridine is a weaker acid than its 4-isomeride but no figures are available for the corresponding quinoline and quinazoline derivatives. 2- and 4-Mercaptopteridines are stronger than their hydroxy-analogues by about 1 unit of pK. Potentiometric titration of the most concentrated solutions possible failed to disclose any basic properties in 2- and 4-hydroxypteridines, down to pK_a 1·5. This is an outstanding example of the lowering of base-strength by the insertion of a hydroxy-group in such a position as to form what may be regarded as a cyclic amide. For example, 4-hydroxyquinoline is 2·65 units of pK weaker than quinoline (Table II), 2-hydroxypyridine is 4·0 units weaker than pyridine (Stiller, Keresztesy, and Stevens, *J. Amer. Chem. Soc.*, 1939, **61**, 1237), and 4-hydroxyquinazoline is considerably weaker than quinazoline (Keneford *et al.*, *loc. cit.*). However, this effect cannot be universal, for it is not evident in 2- and 4-hydroxypyrimidines (Table II).

TABLE II.

Physical properties of some related heterocyclic substances.

Substance.	pK_a (in water) and concn. at which determined (20°).		Spectrography in water. $\lambda_{max.}$ (mμ).	log $\epsilon_{max.}$ (mol.).	pH.
Quinoline	—	—	226 275 + 299 + 312 [2]	4·36 3·51; 3·46; 3·52	6·3 —
cation	4·94 [1]	M/60	233; 313	4·50; 3·80	1·1
Quinoxaline	—	—	234; 316	4·47; 3·79	7·1
cation	0·8 [1]	M/10	242; 331	4·44; 3·93	−2·0 [6]
Quinazoline	3·51 [1]	M/15	<220 270 + 310 [3]	>4·6 3·4; 3·3	— —
1 : 8-Naphthyridine	—	—	<225 260 + 301 + 309	<4·1 3·62; 3·80; 3·81	6·3 —
cation	3·39 (±0·01)	M/100	<215 302 + 309	>4·1 4·02; 4·01	1·0 —
4-Hydroxyquinoline	—	—	—	—	—
cation	2·29 (±0·05)	M/10	—	—	—
Pyrimidine	—	—	243	3·5; 2·5	—
cation	1·30 [1]	M/15	—	—	—
2-Hydroxypyrimidine	—	—	215; 299	4·00; 3·66	6·1
cation	2·24 (±0·04)	M/10	<210; 309	>3·9; 3·75	0·3
anion	9·17 (±0·06)	M/100	<230; 290	>4·1; 3·66	13
4-Hydroxypyrimidine	—	—	225	3·83	6·3
cation	1·85 (±0·04)	M/10	225	4·00	0·3
anion	8·59 (±0·02)	M/30	229; 265	4·07; 3·68	13
Pyrazine	0·6 [1]	M/10	256 + 260 311 + 316 [5]	3·68; 3·72 2·76; 2·75	— —

[1] Albert, Goldacre, and Phillips, *J.*, 1948, 2240. [2] Fine structure. [3] Neutral molecule in alcohol; Elderfield, Williamson, Gensler, and Kremer, *J. Org. Chem.*, 1947, **12**, 405. [4] Heyroth and Loofbourow, *J. Amer. Chem. Soc.*, 1934, **56**, 1728. [5] In alcohol; Barany, Braude, and Pianka, *J.*, 1949, 1898. [6] 7·76N-H_2SO_4.

4-Hydroxypteridine and its derivatives form stable chelate compounds with cupric, nickel, cobaltous, zinc, ferrous (but not ferric), cadmium, and manganous ions (Albert, *Biochem. J.*, 1950, **47**, ix). All naturally-occurring pteridines contain a 4-hydroxy-group; hence this affinity for the ions of heavy metals probably has a biological significance.

Spectra. The spectrum of pteridine (in water) is shown in Fig. 1, with that of quinoline. A certain similarity is to be expected because =CH− and =N− are to a large extent optically interchangeable (Braude, *Ann. Reports*, 1945, **42**, 129). For example, the spectra of anthracene, acridine, and phenazine (Radulescu and Ostrogovich, *Ber.*, 1931, **64**, 2233) are almost identical, but each nitrogen atom causes a partial loss of detail. Consistently, the spectra (Table II) of quinazoline, quinoxaline, and 1 : 8-naphthyridine are similar. As with quinazoline, the short-wave absorption of pteridine has been displaced to shorter wave-lengths where it cannot be completely traced. In *cyclo*hexane, the spectrum of pteridine reveals no additional fine structure.

When quinoline is converted into the cation, both absorption regions are intensified and shifted slightly to longer wave-lengths. The changes occurring when pteridine ionizes are similar but less (Fig. 2).

FIG. 1.

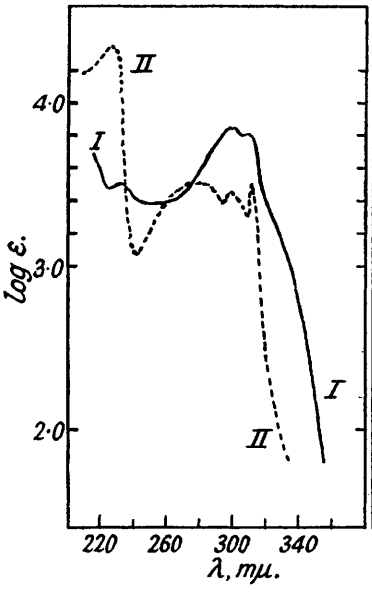
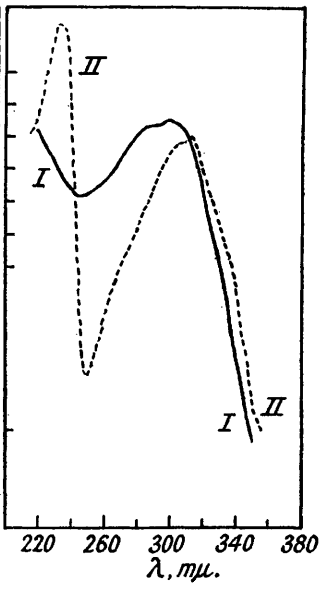


FIG. 2.



Absorption spectra in water of

I. Pteridine (pH 6·2).
II. Quinoline (pH 6·3).

I. The cation of pteridine (pH 2·1).
II. The cation of quinoline (pH 1·1).

FIG. 3.

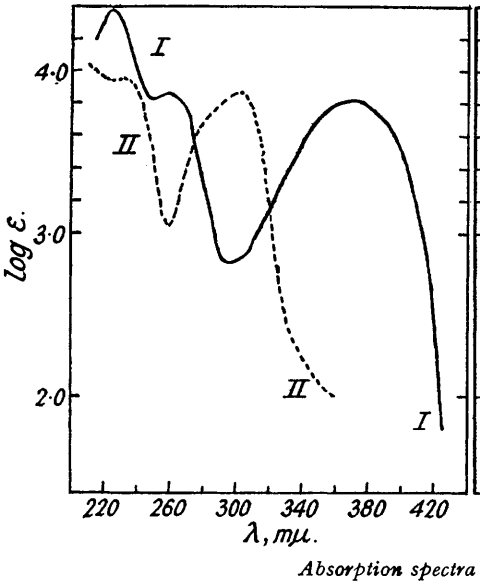
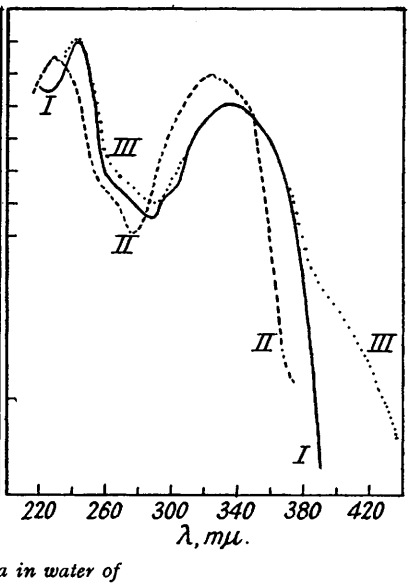


FIG. 4.



Absorption spectra in water of

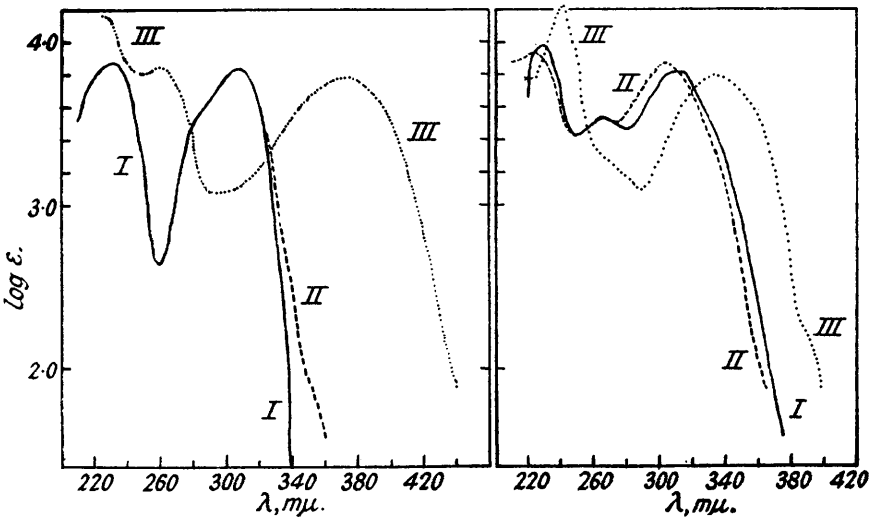
I. 2-Aminopteridine (pH 7·1).
II. The cation of 2-aminopteridine (pH 2·1).

I. 4-Aminopteridine (pH 7·3).
II. The cation of 4-aminopteridine (pH 1·1).
III. The anion of 4-aminopteridine (0·2N-NaOH). *This curve merges with that of the neutral molecule where not shown separately.*

The spectra of 2- and 4-amino- and 2- and 4-hydroxy-pteridines (as neutral molecules) closely resemble that of pteridine itself. The principal difference is that one or both of the absorption regions is shifted to longer wave-lengths, as is usual for amino- and hydroxy-derivatives. The spectrum of the parent substance is less disturbed by the hydroxy- than by the amino-group, but the spectra of the anions of the hydroxy-compounds closely resemble the spectra of the neutral molecules of the corresponding amines. This is usual among the amines and phenols derived from aromatic hydrocarbons (Jones, *J. Amer. Chem. Soc.*, 1945, **67**, 2127), yet 2- and 4-hydroxyquinolines show relatively little change on ionization as anions (Ewing and Steck, *J. Amer. Chem. Soc.*, 1946, **68**, 2181). The spectrum of 4-hydroxypteridine in 93% v/v alcohol was almost identical with that in water; apparently the lowering of dielectric constant did not bring about the ketamine–enimine tautomerism so readily effected in 2- and 4-hydroxy-acridines (Albert and Short, *J.*, 1945, 760). A small shoulder in the spectrum of pteridine at 235 mμ. is apparently the source of a similar area in 2-amino- and 4-hydroxy-pteridines (cf. Figs. 1, 3, and 6).

Ionization as cations causes a shift of the absorption of 2- and 4-aminopteridines to much

FIG. 5. FIG. 6.



Absorption spectra in water of

I. 2-*Hydroxypteridine* (pH 7·1).
II. 2-*Hydroxypteridine at* pH 0·3.
III. *The anion of* 2-*hydroxypteridine* (pH 13).

I. 4-*Hydroxypteridine* (pH 5·6).
II. 4-*Hydroxypteridine at* pH 0·3.
III. *The anion of* 4-*hydroxypteridine* (pH 10·0).

shorter wave-lengths. The reverse happens for 2- and 4-aminopyrimidines (Stimson, *J. Amer. Chem. Soc.*, 1949, **71**, 1470; Williams, Ruehle, and Finkelstein, *ibid.*, 1937, **59**, 526), and no similar case has been recorded for a hetero-aromatic base although there is a hint of it in the spectra of 2- and 4-aminoquinolines, alone of the seven aminoquinolines (Steck and Ewing, *J. Amer. Chem. Soc.*, 1948, **70**, 3397). Such hypsochromy is typical of true aromatic amines (*e.g.*, the naphthylamines), but the aminopteridines cannot be diazotized and coupled.

The effect on the spectrum of the anion of 4-hydroxypteridine when further hydroxy- or amino-groups are added is shown in Table I to be bathochromic (least so in the case of leucopterin).

As with 2- and 4-mercaptoquinolines (Hannan, Lieblich, and Renfrew, *J. Amer. Chem. Soc.*, 1949, **71**, 3733) the absorption maxima of 2- and 4-mercaptopteridines (Table I) are at longer wave-lengths than those of the corresponding hydroxy-derivatives.

Pyrazine, pyrimidine, and 2- and 4-hydroxypyrimidines may be thought of as constituent parts of the hydroxpteridine molecule. However, their spectra (Table II) shed little light on those of pteridine and the hydroxypteridines.

Fluorescence. Unlike xanthopterin, pteridines substituted only in the pyrimidine ring do

not, for the most part, fluoresce in daylight. The colours seen in ultra-violet light are given in Table III.

TABLE III.

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

Pteridine.	Water.	0·05N-NaOH.	0·05N-H_2SO_4.
(Unsubstituted)	Violet ++	Blue ++	Yellow +
2-Amino-	Green ++	Green ++	Blue ++
4-Amino-	Violet +	Violet ++	Yellow ++
2-Hydroxy-	Violet ++	Blue ++	Blue +++
4-Hydroxy-	Violet ++	Blue +	Little or none
2 : 4-Dihydroxy-	Blue +++	Blue +++	Little or none
2-Amino-4-hydroxy-	Violet +++	Violet +++	Yellowish-green +
Xanthopterin	Yellowish-green ++++	Green ++++	Yellow ++

Stability of Pteridine Nucleus.—Pteridines are unstable unless substituted elsewhere than in the 2-position. Pteridine, 2-hydroxy-, 2-amino-, 2-dimethylamino-, and 2-mercaptopteridines gave blue or violet colours in cold 10N-hydrochloric acid, even in the absence of oxygen; the original substance could not be recovered after neutralization. This colour change was most rapid with 2-hydroxy- and 2-amino-pteridines (2 minutes) but in concentrated sulphuric acid the change was almost instantaneous for all these substances. Pteridine was completely destroyed by boiling it with 2M-oxalic acid for 5 minutes or with N-potassium hydroxide for an hour (ammonia was liberated). When 2-hydroxy-, 2-amino-, or 2-dimethylamino-pteridines were boiled for a minute with N-sodium hydroxide, a deep blue or green colour developed. 2-Hydroxypteridine, added to aqueous sodium dithionite, gave an orange solution. Pteridine is sensitive to light, becoming purple; an aqueous solution of 2-aminopteridine begins to deposit an orange precipitate when exposed to sunlight, and an apparently similar decomposition occurs on prolonged drying in air at 125°. Pteridine was strongly adsorbed on a column of alumina * from a 1% benzene solution. Elution with 1 : 1 benzene–ether rapidly removed 25% of the pteridine, but the remainder had been converted into a red methanol-soluble oil.

4-Aminopteridine was completely converted into 4-hydroxypteridine by boiling it with 0·01N-sodium hydroxide for 3 minutes (4-hydroxypteridine withstands boiling 6N-sodium hydroxide). 4-Aminopteridine was partly hydrolysed, by boiling 5N-hydrochloric acid, to 4-hydroxypteridine which was itself partly decomposed to a violet substance under these conditions. When 4-hydroxypteridine was kept at pH 1 (20°) for a week, spectrography then indicated that about 10% had been destroyed.

Just as the stability of the pteridine nucleus is increased by substitution in the 4-position, so it is further increased by substitution in both the 2- and the 4-position. For example, 2 : 4-dihydroxypteridine was unchanged by boiling 10N-hydrochloric acid or 6N-sodium hydroxide.

Specific Chemical Reactions.—Pteridine readily reacted with perphthalic acid to give a *N*-oxide. Pteridine was recovered unchanged after treatment with one equivalent of nitric acid in glacial acetic acid at 20°. 4-Hydroxypteridine was destroyed when nitration was attempted in acetic acid; 4-aminopteridine survived this treatment unchanged but was entirely destroyed on dissolution in a mixture of sulphuric and fuming nitric acids. Attempts to aminate pteridine by sodamide in diethylaniline were unsuccessful. 2-Chloropteridine was easily hydrolysed to 2-hydroxypteridine by boiling water (autocatalysis by the hydrogen chloride liberated).

2- and 4-Aminopteridines were acetylated normally, but the hydroxypteridines resisted acetylation and benzoylation, even in the presence of pyridine or perchloric acid, or as the sodium salt. 2-Aminopteridine was destroyed on attempted diazotization by a method which successfully converted 2-amino-4-hydroxy- into 2 : 4-dihydroxy-pteridine (Taylor and Cain, *J. Amer. Chem. Soc.*, 1949, **71**, 2538). 4-Aminopteridine did not give 4-chloropteridine on diazotization in 10N-hydrochloric acid, a method that succeeds in the purine series (Kögl, Want, and Salemink *Rec. Trav. chim.*, 1948, **67**, 29). Boiling phosphorus oxychloride completely destroyed 2- and 4-hydroxy- and 2 : 4-dihydroxy-pteridine, although 2-amino-4-hydroxy-6 : 7-diphenylpteridine can be converted into its 4-chloro-analogue by this method in 81% yield (Cain, Taylor, and Daniel, *ibid.*, 1949, **71**, 892).

2- and 4-Hydroxypteridines gave ammonia, but no pteridine, when distilled over zinc

* Aluminium oxide " for chromatographic adsorption analysis," British Drug Houses Ltd. This is a highly alkaline preparation (cf. Cahn and Phipers, *Nature*, 1937, **139**, 717).

dust. Likewise 4-hydroxypteridine was destroyed by 2% sodium amalgam in water at 100° or by sodium in boiling amyl alcohol.

2- and 4-Hydroxypteridines were destroyed by phosphorus pentasulphide in boiling xylene. 4-Amino-2-mercaptopteridine was not changed by refluxing it with Raney nickel in aqueous ammonia, whereas 2-mercapto- and 4-hydroxy-2-mercapto-pteridines were destroyed; yet this method smoothly replaces $-SH$ by $-H$ in the pyrimidine series (Brown, *J. Soc. Chem. Ind.*, 1950, in the press). Finally 4-aminopteridine was not converted into 4-mercaptopteridine by boiling N-potassium hydrogen sulphide or by hydrogen sulphide at pH 4—5, methods similar to those found useful in the acridine series (Asquith, Hammick, and Williams, *J.*, 1948, 1181).

These results emphasize that substitutents are best introduced (for the simpler pteridines at east) *before* ring-closure.

Experimental.

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

Absorption Spectra.—These were measured in the Hilger "Uvispek" photoelectric spectrophotometer (with 4-cm. cells and dilutions of from 10^{-5} to 4×10^{-5}M. in water distilled in glass apparatus) at 5-mμ. intervals, except in the neighbourhood of peaks where 1-mμ. intervals were used.

Potentiometric Titrations.—A glass electrode with a shielded lead, and a calomel electrode having a sintered-glass diaphragm, were used with a pH set (Cambridge Instrument Company) standardized against 0·05M-potassium hydrogen phthalate (pH 3·97) and against boric acid (throughout its titratable range). Acidic groups were titrated with 0·1N-potassium hydroxide (carbonate-free) and basic groups with 0·1N-hydrochloric acid (N-hydrochloric acid, delivered from an "Agla" micrometer microburette, for the hydroxypyrimidines and 4-hydroxyquinoline, to minimize volume changes). Each pK_a value quoted is calculated from seven equidistant portions of the titration curve. The figure (*e.g.*, +0·03) following the pK_a gives the magnitude of the greatest divergence of any reading from this average.

Paper Chromatograms.—The chromatogram, prepared in *n*-butanol (2 volumes)–5N-acetic acid (1 volume), was viewed by the light of a mercury discharge lamp (Thermal Syndicate's T/M5/369E), passed through a filter (Chance Brothers, OX7/19874) so that 99% of the radiation was at 254 mμ. In most cases dark spots on a pale fluorescent background were obtained.

Pyrazine Intermediates.

2-Aminopyrazine-3-carboxyamide.—2-Aminopyrazine-3-carboxylic acid was prepared by heating the ammonium salt of lumazine (2 : 4-dihydroxypteridine) with sodium hydroxide according to Weijlard, Tishler, and Erickson (*J. Amer. Chem. Soc.*, 1945, **67**, 802). We find that lumazine, when prepared according to these authors, is actually in the form of its ammonium salt and that the yield of aminopyrazinecarboxylic acid is small if free lumazine is used.

The acid (22·2 g.) was added to a warm (40°) mixture of sulphuric acid (26 ml.; *d* 1·84) and methanol (90 ml.). After being kept for 24 hours at 20°, the mixture was warmed to 40° and stirred until a clear solution was obtained (about 5 minutes). After a further 48 hours at room temperature, the solution was gradually added to aqueous ammonia (280 ml.; *d* 0·88; stirred and cooled). The mixture was set aside at 20° for a day and filtered. The precipitate, recrystallized from boiling water (1200 ml.), had m. p. 232—235° (15·4 g., 70%) (cf. 48—52% obtained by Ellingson, Henry, and McDonald, *J. Amer. Chem. Soc.*, 1945, **67**, 1711, by a somewhat similar method). On recrystallization from anhydrous pyridine (15 parts) or water (70 parts), pale yellow crystals, m. p. 234—235° (238—239°, corr.), were obtained.

2-Aminopyrazine-3-carboxyhydrazide.—Methyl 2-aminopyrazine-3-carboxylate (1·53 g.) and hydrazine hydrate (2·8 ml. of 90% = 5 equivs.) were refluxed at 120° for 4 hours, cooled, diluted with water (5 ml.), refrigerated, and filtered, and the product was dried at 110°. The *2-aminopyrazine-3-carboxyhydrazide* (93%; m. p. 205°), recrystallized from 40 parts of boiling water, gave cream-coloured crystals, m. p. 207—209°, almost insoluble in alcohol or benzene (Found: C, 39·25; H, 4·55; N, 45·6. $C_5H_7ON_5$ requires C, 39·2; H, 4·6; N, 45·7%). When an aqueous solution of this hydrazide was added to ammoniacal potassium ferricyanide at 60°, 2-aminopyrazine-3-carboxyamide was obtained in 70% yield instead of the expected 2-aminopyrazine-3-carboxyaldehyde.

The above hydrazide (1·53 g.) was shaken with dried pyridine (10 ml.) for 5 minutes, then toluene-*p*-sulphonyl chloride (3·1 g.) was added during 30 minutes and the whole shaken for an hour. The pyridine was distilled off in steam. The 2-aminopyrazine-3-carboxy-(*N'*-toluene-*p*-sulphonyl)hydrazide which separated on cooling was obtained from alcohol as a pale yellow solid (90%), m. p. 200—201° (if dried without heat); this did not give the expected 2-amino-3-formylpyrazine when heated with sodium carbonate in glycol (McFadyen and Stevens's method).

2-Aminopyrazine-3-carboxythioamide.—A slow stream of hydrogen sulphide was passed through a solution of 2-amino-3-cyanopyrazine (6·4 g.; Ellingson *et al.*, *loc. cit.*) and triethanolamine (2 g.) in ethanol (900 ml.) at 50—55° for 12 hours, a clear solution being formed. This was refrigerated overnight, and the crystalline precipitate filtered off (4·3 g.) and recrystallized from water (450 ml.) giving 3·1 g. of thioamide (m. p. 167—171°). The original filtrate was evaporated to dryness on the water-bath and the residue extracted with boiling water (400 ml.). The extract, treated with charcoal (2 g.) and cooled, gave a further crop (total yield, 5·8 g., 71%). *2-Aminopyrazine-3-carboxythioamide* formed soft, bright yellow plates [from boiling water (100 parts)], m. p. 168—170°, readily soluble in dilute alkali (colourless solution) (Found: N, 36·4; S, 20·9. $C_5H_6N_4S$ requires N, 36·35; S, 20·8%).

Pyrimidine Intermediates.

4 : 5-*Diamino*-6-*hydroxypyrimidine*-2-*thiol*.—The ammonium salt of 4-amino-6-hydroxy-5-nitrosopyrimidine-2-thiol (55 g.; Traube, *Annalen*, 1904, **331**, 71) was dissolved in N-sodium hydroxide (1250 ml.). To the cooled solution (20°), sodium dithionite (hydrosulphite) (200 g.) was added during 5 minutes, with stirring and addition of ice so that the temperature did not exceed 50°. After 15 minutes, acetic acid was added (75 ml., or enough to give pH 5). The mixture was cooled to 5° and the pale diamine was filtered off, washed with a little water, ethanol, and ether, and dried in warm air (yield 87%).

4 : 5-*Diamino*-6-*hydroxypyrimidine*.—To the above thiol (21 g.), dissolved in boiling water (400 ml.) and ammonia (25 ml.; *d* 0·88), Raney nickel catalyst (60 g., wet with water) was added in portions of 5—10 g., with shaking after each addition. The mixture was then refluxed for 2 hours and filtered. The nickel was washed with boiling water (200 ml.) and the washings were set aside. The main filtrate was refrigerated overnight and then filtered, giving 6·3 g. of diaminohydroxypyrimidine, m. p. 239°. Evaporation of the combined mother-liquor and nickel-washings gave further crops, one of which had to be recrystallized from water. The total yield was 76%.

4 : 5 : 6-*Triaminopyrimidine*.—This was similarly prepared from 4 : 5 : 6-triaminopyrimidine-2-thiol, by the method of Cavalieri, Tinker, and Bendich (*J. Amer. Chem. Soc.*, 1949, **71**, 533).

2 : 4-*Diamino*-5-*nitropyrimidine*.—2 : 4-Dihydroxy-5-nitropyrimidine (nitrouracil) was converted into 2 : 4-dichloro-5-nitropyrimidine by refluxing it with phosphorus oxychloride and diethylaniline (Whittaker, *J.*, in the press). The yield was 67% of material boiling sharply at 135°/17 mm. (Isay, *Ber.*, 1906, **39**, 250). 2 : 4-Dichloro-5-nitropyrimidine (103 g.) and phenol (820 g.) were heated under reflux at 140° (bath) while a stream of ammonia was passed into the mixture for 4 hours. The phenol was distilled off in a current of steam (10 l. of water), and the residual suspension was cooled and filtered. The crystals of 2 : 4-diamino-5-nitropyrimidine were washed with water, then with ethanol, and dried at 110°. The yield was 91%, and the m. p. 345—350°. On one occasion when a dark product was obtained, purification was readily effected by dissolution in 2N-hydrochloric acid (1 l.), treatment with carbon, filtration, and precipitation with ammonia (90% recovery). This diaminonitropyrimidine was also obtained (85%) by treating 4-amino-2-chloro-5-nitropyrimidine (see below) in the same way. These methods are more convenient than that of Isay (*loc. cit.*) which requires a sealed tube.

2 : 4 : 5-*Triaminopyrimidine*.—Finely powdered 2 : 4-diamino-5-nitropyrimidine (75 g.) was heated to 80° with water (1500 ml.), mechanically stirred. Sodium dithionite (375 g.) was added during 3—4 minutes. The resulting solution was stirred until it had cooled to 60°, then powdered anhydrous sodium carbonate (550 g.) was added as fast as frothing (controlled by amyl alcohol) permitted. The thick suspension was taken to dryness in an open basin on the water-bath. The solid was machine-ground and extracted for 20 minutes by stirred boiling ethanol (2·2 l.), which was filtered while hot. The filtrate was taken to dryness and the product re-extracted with ethanol (750 ml.) to remove sodium carbonate. The filtrate, taken to dryness, gave 2 : 4 : 5-triaminopyrimidine, m. p. 173—175° (77%). No suitable solvent for recrystallization was found and vacuum-distillation was too destructive.

4 : 5-*Diaminopyrimidine*.—4-Amino-2-chloro-5-nitropyrimidine was prepared from 2 : 4-dichloro-5-nitropyrimidine by the method of Isay (*loc. cit.*) except that the amination was effected by grinding the dichloro-compound with ice-cold aqueous ammonia. 4 : 5-Diaminopyrimidine-2-thiol was prepared from 4-amino-2-chloro-5-nitropyrimidine by sodium hydrogen sulphide (Elion and Hitchings, *J. Amer. Chem. Soc.*, 1947, **69**, 2553). This thiol (33 g.) was dissolved in water (800 ml.) containing aqueous ammonia (33 ml.; *d* 0·88) and the whole refluxed vigorously with Raney nickel catalyst (140 g., weighed wet with water) for an hour. The mixture was filtered and the filtrate was boiled again with nickel (60 g.) and ammonia (5 ml.) for 30 minutes. The mixture was filtered and the pale yellow filtrate was taken to dryness on a water-bath. The crystalline residue was extracted with boiling alcohol (275 ml.), and the extract was concentrated to 150 ml. 4 : 5-Diaminopyrimidine, m. p. 200—201° (softening at 195°), crystallized on cooling, and a second crop was obtained by concentration to 30 ml., the total yield being 66%.

4 : 5-*Diamino*-2-*chloropyrimidine*.—4-Amino-2-chloro-5-nitropyrimidine (14·1 g.) was suspended in methanol (1400 ml.) and shaken with Raney nickel (25 g., weighed wet) in an atmosphere of hydrogen until 5930 ml. of gas had been absorbed (18° and 769 mm.). The catalyst was filtered off and extracted with boiling methanol. The washings were combined with the filtrate, and the solvent recovered. The residue was dissolved in boiling water (350 ml.). This solution, treated with charcoal and filtered, deposited 4 : 5-diamino-2-chloropyrimidine (8·6 g.). A second crop (1·2 g.) was obtained by concentration. The total yield was 86% of colourless needles, decomposing around 220° when heated rapidly from 200°, and giving a single spot in paper chromatography (Jones, *loc. cit.*, gives m. p. 232°) (Found: N, 38·2. Calc. for $C_4H_5N_4Cl$: N, 38·8%).

4 : 5-*Diamino*-2-*dimethylaminopyrimidine*.—A mixture of 4-amino-2-chloro-5-nitropyrimidine (6·2 g.) and dimethylamine (50% methanolic; 17 ml.) was heated in methanol (50 ml.) for 1 hour at 100°. After cooling to 0°, the solid (6·3 g., 97%), m. p. 205—208°, was filtered off. A sample was purified by dissolution in dilute acid followed by reprecipitation with alkali, and subsequently recrystallized from ethanol (180 pts.) to give colourless 4-*amino*-2-*dimethylamino*-5-*nitropyrimidine*, m. p. 210—211° (Found: C, 39·4; H, 4·6. $C_6H_9O_2N_5$ requires C, 39·3; H, 4·95%).

This substance (6 g.; finely ground) was suspended in water (150 ml.) at 80° and sodium dithionite (45 g.) was added with mechanical stirring during 2 minutes. While the mixture was still hot, sodium carbonate (75 g., anhydrous) was added with stirring. After evaporation to dryness the purple residue was extracted with boiling ethanol (250 ml.). The filtrate, taken to dryness, gave 4 : 5-diamino-2-dimethylaminopyrimidine (3·5 g., 70%), m. p. 115—121°. Its colour was dark and because the solubilities had poor temperature gradients no attempt was made to recrystallize the material.

Pteridines from Pyrazine Intermediates.

4-*Hydroxypteridine*.—2-Aminopyrazine-3-carboxyamide (1·38 g., 0·01 mole), acetic anhydride (20 ml.), and ethyl orthoformate (20 ml.) were refluxed for 2 hours at 150° (bath). The mixture was taken to dryness in a vacuum and the residue was boiled for 3 minutes with N-sodium hydroxide (15 ml., or enough to give pH 10), cooled quickly to 20°, and filtered after 15 minutes. The filtrate was brought to pH 6 with 10N-hydrochloric acid (about 1 ml.) and filtered from a trace of slime which retarded crystallization. Deposition of crystals began and was complete after 2 days, affording cream-coloured (0·5-cm. crystals) 4-*hydroxypteridine* (75%), unchanged at 350°. This gave only one spot in paper chromatography and was recrystallized from the minimum (33 parts) of boiling water (80% recovery). It was almost insoluble in boiling alcohol, amyl alcohol, xylene, anisole, nitrobenzene, pyridine, or acetonitrile, but crystallized well from formamide or diethylformamide. The colourless aqueous solution has a faintly bitter taste (cf. also Table I) (Found: C, 48·7; H, 2·7; N, 37·7. $C_6H_4ON_4$ requires C, 48·65; H, 2·7; N, 37·8%). This compound gave no colour with sulphuric acid or hydrochloric acid (10N.) and formed no picrate in aqueous solution. The sodium salt is soluble in 3 parts of water at 20°. Other chemical properties have been given in the introduction.

2-Aminopyrazine-3-carboxyamide (2 g.) and formic acid (4·5 ml.) were heated in an open vessel for 1 hour at 115° and then for 1 hour at 140°. The amide dissolved and was re-deposited, partly formylated, when the solvent evaporated. It was dried at 110° [Found: N, 35·9. $(C_6H_6O_2N_4)_2,C_5H_6ON_4$ requires N, 36·0. Calc. for $C_6H_6O_2N_4$: N, 33·7%]. The degree of formylation was not increased by the use of anhydrous sodium formate or slowly distilling toluene. When heated to 225°, in a dry state, this partly formylated product gave 4-hydroxypteridine (III) in 43% yield; when it was boiled for 10 minutes with N-sodium ethoxide (alcoholic) a 60% yield was obtained, but refluxing it for 2 hours with acetic anhydride gave only 44% (these yields are based on the unformylated amine).

When 2-aminopyrazine-3-carboxyamide (1·38 g.) was refluxed with acetic anhydride (20 ml.) and formic acid (20 ml.) for 2 hours, 65% of 4-hydroxypteridine was obtained.

4-*Mercaptopteridine*.—2-Aminopyrazine-3-carboxythioamide (5·8 g.), ethyl orthoformate (100 ml.) and acetic anhydride (100 ml.) were heated under reflux at 145—150° for 2 hours. After refrigeration the yellow solid was filtered off and washed thoroughly with acetone. After being dried, it (5·2 g., 84%) was recrystallized from water (4 l.) containing charcoal (2 g.) with 70% recovery. 4-*Mercaptopteridine* formed yellow crystals (decomp. 290°) from 800 pts. of boiling water, or from a very large volume of amyl alcohol or pyridine (Found: C, 44·0; H, 2·5; S, 19·5. $C_6H_4N_4S$ requires C, 43·9; H, 2·5; S, 19·5%).

Unsuccessful Attempts.—Failures to synthesize pteridines from pyrazine intermediates were: (i) 4-hydroxypteridine, by heating 2-aminopyrazine-3-carboxylic acid with formamide at 120° or 160°; (ii) 2 : 4-dihydroxypteridine, by refluxing 2-aminopyrazine-3-carboxyamide (II) with ethyl chloroformate, dioxan, and potassium carbonate, or by heating this amide or the corresponding acid with urea at 150° or 200°, or by dissolving the acid in aqueous potassium cyanate or by heating the acid with urethane; (iii) 4-aminopteridine, by heating 2-aminopyrazine-3-carboxyamide (or the corresponding nitrile) with formamide, even at 215°; (iv) pteridine, by heating 2-aminopyrazine-3-carboxyamide with aqueous formaldehyde in acid solution, or with aqueous hexamine, or with trioxymethylene and formic acid in alcohol, or by refluxing methyl 2-aminopyrazine-3-carboxylate with aqueous hexamine; (v) 4-hydroxy-2-mercaptopteridine, by refluxing 2-aminopyrazine-3-carboxyamide with carbon disulphide in pyridine. Sodium dithioformate did not react with (II).

Pteridines from Pyrimidine Intermediates.

Pteridine.—4 : 5-Diaminopyrimidine (16·2 g.), polyglyoxal (9·8 g., 1·1 equivs.), and alcohol (320 ml.) were refluxed for 30 minutes. The solvent was removed under reduced pressure and the residue extracted with benzene (3 × 100 ml.). The combined benzene extracts were boiled with two successive portions of charcoal, then concentrated to 100 ml. On cooling, pteridine crystallized out as yellow plates (10·2 g., dried in a vacuum at 20°). Further concentration to 10 ml. gave 2·0 g. more (total yield 63%). The m. p. was 137—138·5° (Found: C, 54·65; H, 3·1; N, 42·5. Calc. for $C_6H_4N_4$: C, 54·5; H, 3·0; N, 42·4%). Pteridine sublimes almost quantitatively at 125—130°/20 mm., giving denser yellow crystals, m. p. 139·5—140°, which appear to be another crystalline form because recrystallization from benzene, from 5 parts of alcohol (80% recovery), or from 300 parts of light petroleum (b. p. 60—80°; 80% recovery) gave the yellow plates, m. p. 138—138·5°. Pteridine is volatile in steam to only a small degree, and it was shown spectrographically that 0·1 g. yielded only 0·5 mg. in 15 ml. of distillate. The partition coefficient between chloroform and water is 1·98, whereas benzene and ether extract much less (coefficients: 0·21 and 0·05 respectively). The crystals provoke sneezing. Other physical properties of pteridine are given in Table I. No pteridine could be isolated when water replaced alcohol in the above synthesis.

4 : 5-Diaminopyrimidine (1·6 g.), glyoxal sodium bisulphite (4·5 g.; 1·1 equivs.), and water (14 ml.) were refluxed for 2 hours and then taken to dryness. The yellow residue was sublimed for 30 hours at 160—180°/20 mm. The reaction was completed only during this sublimation and gave a 15% yield of pteridine.

Pteridine N-*Oxide*.—To pteridine (0·5 g.) in chloroform (2 ml.) was added an ethereal solution of perphthalic acid (1·1 equivs.). The pale orange precipitate (1·35 g.) was dissolved in boiling methanol (330 ml.). From this solution, after treatment with charcoal, filtering, and cooling, a N-*oxide* crystallized (0·41 g., 72%). Recrystallization from methanol gave white crystals, decomp. *ca.* 350°, giving a single spot in paper chromatography (Found: C, 48·5; H, 2·55. $C_6H_4ON_4$ requires C, 48·65; H, 2·7%).

2-*Aminopteridine*.—To unpurified 2 : 4 : 5-triaminopyrimidine (20 g.) in water (140 ml.) was added a solution of glyoxal sodium bisulphite (50 g.) in warm water (350 ml.). The mixture was refluxed for 80 minutes, filtered by gravity, and refrigerated overnight. The precipitate (a sulphonic acid) was

filtered off, washed with a little ethanol, and dried at 110°. It was converted into 2-*aminopteridine* by 30 minutes' mechanical shaking at 20° with N-sodium hydroxide (250 ml.). The solid was filtered off, washed with water and alcohol, and dried at 105° (6·9 g.). A further 4·3 g. was obtained by treating the filtrate from the addition product with sodium hydroxide (10N.; 25 ml.) at 0° for 2 hours. The combined material (11·2 g.) was recrystallized 3 times from 110 parts of boiling water containing 0·2 part of charcoal. The crystals were washed with a little ethanol and dried at 105° for 10 minutes only. The final yield was 4·8 g. of yellow crystals, decomposing at about 275°. The substance was virtually insoluble in boiling alcohol, pyridine, or other common organic solvents (Found: C, 49·3; H, 3·15; N, 47·3. $C_6H_5N_5$ requires C, 49·0; H, 3·4; N, 47·6%).

2-*Dimethylaminopteridine.*—A warm solution of glyoxal sodium bisulphite (8 g.) in water (32 ml.) was added to a solution of crude 4 : 5-diamino-2-dimethylaminopyrimidine (3·2 g.) in water (16 ml.). After gently refluxing for 30 minutes, the solution was rapidly filtered from a little slime (heated funnel) and cooled to 0°. The bisulphite complex was filtered off, washed with a little cold water, and dissolved in 2·5N-sodium carbonate (45 ml.). The yellow solution was taken to dryness at 40° under reduced pressure. The residue was extracted with boiling ethanol (200 ml.), and the filtrate taken to dryness. The orange-coloured solid was dissolved in hot *cyclo*hexane (600 ml.), the solution filtered, and set aside at room temperature for 2 hours. The red flocculent precipitate was rejected and the solution concentrated to about 40 ml. and then cooled, and the yellow crystals were filtered off. Further concentration to 10 ml. gave another crop (total yield: 1·6 g., 43%). 2-*Dimethylaminopteridine* formed yellow fern-like crystals (m. p. 125—126°) from *cyclo*hexane. It was readily soluble in cold alcohol and benzene, also in boiling light petroleum and *cyclo*hexane (Found: C, 55·0; H, 5·2; N, 39·9. $C_8H_9N_5$ requires C, 54·85; H, 5·2; N, 40·0%).

4-*Aminopteridine.*—Unpurified 4 : 5 : 6-triaminopyrimidine (9·4 g.; m. p. 235—245°) in hot water (80 ml.) was added to a solution of glyoxal sodium bisulphite (22 g.) in hot water (160 ml.). The mixture was refluxed for 1 hour, cooled to 5°, and stirred with 10N-sodium hydroxide (15 ml.) to decompose the addition product. The mixture was then kept in ice for 30 minutes (no longer) and filtered. The precipitate of 4-*aminopteridine* was washed with a little cold water, then with alcohol, and dried at 110° (yield, 70%). It was twice recrystallized from boiling water (100 parts) containing charcoal (0·2 part) and dried at 110°, giving white plates (5 g.) decomposing at about 305° and almost insoluble in pyridine and other common organic solvents (Found: C, 49·0; H, 3·2; N, 47·5%).

2- *and* 4-*Acetamidopteridines.*—The aminopteridine (0·74 g.; 0·005M.) and acetic anhydride (5 ml.) were refluxed for 30 minutes and chilled overnight. The crystals were filtered off, washed with benzene, then with a little methanol, then shaken at 20° with 5N-ammonia for an hour, collected, and dried at 120°. 2-*Acetamidopteridine* formed pale buff-coloured crystals, m. p. 229—231° (decomp.) (70%), from 30 parts of boiling water; it was very soluble in boiling pyridine (Found: C, 51·0; H, 3·9; N, 36·4. $C_8H_7ON_5$ requires C, 50·8; H, 3·7; N, 37·0%). 4-*Acetamidopteridine* formed pale buff-coloured crystals, m. p. 191—192°, from 2 parts of boiling water or 30 parts of toluene (Found: C, 51·2; H, 3·6; N, 36·1%).

2-*Hydroxypteridine.*—Glyoxal (28 g. of the commercial 50% aqueous solution) was added in one portion to a solution of 4 : 5-diamino-2-hydroxypyrimidine (14 g.; Johns, *Amer. Chem. J.*, 1911, **45**, 79) in boiling water (200 ml.). After 15 minutes' refluxing, the suspension was cooled and refrigerated overnight. The solid was filtered off and, without being dried, recrystallized from the minimal amount of water (*ca.* 750 ml.), containing charcoal (3 g.), and filtered through a heated funnel. The pale orange filtrate at once deposited cream-coloured needles (14·6 g., 80%). After two similar recrystallizations from 50 parts of water, 10 g. of white needles of 2-*hydroxypteridine monohydrate* (decomp. 240°) were obtained. It is almost insoluble in all common organic solvents (Found, for material dried at 120°/0·1 mm. for 30 minutes: C, 43·55; H, 3·65; N, 33·8. $C_6H_4ON_4,H_2O$ requires C, 43·4; H, 3·65; N, 33·75%). Boiling for 30 minutes with 0·1N(or for 1 minute with 5N)-sodium hydroxide or -hydrochloric acid produces a blue colour.

2-Hydroxypteridine was also prepared by the hydrolysis of 2-chloropteridine (0·2 g.) in water (7 ml.) at 100°. The pH rapidly decreased and on reaching *ca.* 1—2 was raised with 5% sodium hydrogen carbonate solution to *ca.* 6. This adjustment was continued until no further fall occurred (¾ hour), by which time 95% of the theoretical amount of alkali had been added. The orange solution, boiled with charcoal, filtered, and refrigerated, gave 2-hydroxypteridine monohydrate (0·1 g.).

4-*Hydroxypteridine.*—4 : 5-Diamino-6-hydroxypyrimidine (13 g.) was added to a solution of glyoxal sodium bisulphite (31·2 g.) in warm water (390 ml.), and the whole refluxed for 1 hour. The solution was cooled to 80° and 10N-hydrochloric acid (25 ml.) added. The solution was boiled vigorously for 10 minutes to expel sulphur dioxide, adjusted to pH 3—4 with 2·5N-sodium hydroxide (*ca.* 20 ml.), cooled to 20°, filtered from a dark slime, and then refrigerated for a day. The buff-coloured crystals were dried at 110° and recrystallized once from 30 parts of water containing 0·4 part of charcoal (yield: 10·4 g., 68%) (Found: C, 49·1; H, 2·7; N, 38·0. $C_6H_4ON_4$ requires C, 48·65; H, 2·7; N, 37·8%). Traces of coloured impurity were removed by filtering off the first few crystals which were found to adsorb it strongly. The properties have been given above.

4-Hydroxypteridine was also prepared from 4-aminopteridine (5 g.) dissolved in boiling water (450 ml.). Sodium hydroxide (50 ml.; 10N.) was added to initiate the hydrolysis and after 5 minutes' refluxing the solution was brought to pH 4—5 with 10N-hydrochloric acid and refrigerated for a day. 4-*Hydroxypteridine* was obtained as fine white needles in 80% yield (including a second crop got by concentration to 150 ml.). After one recrystallization, the material was chromatographically and spectrographically pure (Found: C, 48·55; H, 2·65; N, 37·9%).

4-*Hydroxy*-6 : 7-*dimethylpteridine.*—Commercial diacetyl was fractionated through a 60-cm. Vigreux column fitted with a total condensation head. A reflux ratio of 7 : 1 was maintained and the fraction boiling at 89·5—90° collected. Pure diacetyl (9 ml.) was added, with shaking, to a solution of 4 : 5-di-

amino-6-hydroxypyrimidine (12·5 g.) in water (100 ml.) at 75°. After being heated on the water-bath for 20 minutes the mixture was refrigerated overnight. The precipitate was filtered off, washed with cold water (20 ml.), and dried at 120° (14·3 g., 81%). The product was recrystallized from 100 parts of boiling water with carbon (3 g.), giving 12·3 g. of white needles, 1 cm. long, of *4-hydroxy-6 : 7-dimethylpteridine* (decomp. 355—360°) (Found : C, 54·6; H, 4·35; N, 31·6. $C_8H_8ON_4$ requires C, 54·55; H, 4·55; N, 31·8%). Unlike 4-hydroxypteridine it dissolved in boiling amyl alcohol.

2 : 4-*Dihydroxypteridine.*—Glyoxal sodium bisulphite (70 g.) in warm water (750 ml.) was added to a suspension of 4 : 5-diamino-2 : 6-dihydroxypyrimidine sulphate (50 g.; Bogert and Davidson, *J. Amer. Chem. Soc.*, 1933, **55**, 1667) in 1·2N-hydrochloric acid (570 ml.). The mixture was refluxed for an hour, cooled to 10°, and filtered by gravity. The filtrate, refrigerated for 3 days, deposited brown crystals (30 g.) which were boiled with water (1350 ml.) containing charcoal (15 g.) for 5 minutes and filtered. The filtrate was boiled with chromatographic alumina (15 g.) and re-filtered. The second filtrate was allowed to crystallize overnight and the crystals were treated three times more in the same way, using proportional amounts of adsorbents. Perfectly white crystals, 2 mm. long, decomp. 335—338°, giving a colourless saturated solution in boiling water, were obtained (Found, for material dried at 140° : C, 44·05; H, 2·45; N, 34·9. Calc. for $C_6H_4O_2N_4$: C, 43·9; H, 2·45; N, 34·2%). This highly purified substance is much less fluorescent than the crude material which is known as " lumazine " (Kuhn and Cook, *loc. cit.*). An easier, and almost as satisfactory, purification for small amounts was percolation of 100 ml. of a 1% solution of the ammonium salt of 2 : 4-dihydroxypteridine through a column (10 × 3 cm.) of chromatographic alumina, followed by elution with water and acidification of the eluate.

By isolating their product in the presence of ammonia, some previous workers (*e.g.*, Weijlard, Tishler, and Erickson, *loc. cit.*) have unwittingly obtained the sparingly soluble ammonium salt, ammonia being lost on drying for analysis.

2-*Chloropteridine.*—4 : 5-Diamino-2-chloropyrimidine (3·5 g.) and dry polyglyoxal (2 g.) were refluxed in dry methanol (35 ml.) for 10 minutes. The solution was evaporated *in vacuo* at 30° and the residual thick red gum was extracted first with light petroleum (150 ml.; b. p. 60—80°) and then with light petroleum (150 ml.; b. p. 80—100°). After refrigeration, the extract yielded 1·0 g. (25%) of yellow crystals, m. p. 98—101°. Several recrystallizations from light petroleum (250 parts; b. p. 60—80°) gave slightly yellow needles of *2-chloropteridine*, m. p. 106—107° (decomp.) (Found : N, 33·65; Cl, 21·1. $C_6H_3N_4Cl$ requires N, 33·65; Cl, 21·3%). This is readily hydrolysed to 2-hydroxypteridine in mildly acid solution, but stronger acids disrupt the ring with the production of a violet colour.

2-Mercaptopteridine was synthesized according to Elion and Hitchings (*loc. cit.*); 2-amino-4-hydroxypteridine according to Cain, Mallette, and Taylor (*J. Amer. Chem. Soc.*, 1946, **68**, 1996); leucopterin according to Totter (*J. Biol. Chem.*, 1944, **154**, 105). Xanthopterin was synthesized according to Elion, Light, and Hitchings (*J. Amer. Chem. Soc.*, 1949, **71**, 741) and further purified through its barium salt (Schöpf and Becker, *Annalen*, 1933, **507**, 266) (Found, for material dried at 20°/20 mm. over calcium chloride : C, 36·3; H, 3·4; N, 35·4. Calc. for $C_6H_5O_2N_5,H_2O$: C, 36·5; H, 3·6; N, 35·5%).

The Medical Research Council is thanked for a personal grant (to D. J. B.) which enabled this work to be carried on between October 1948 and March 1949. The authors thank Dr. Derek Phillips for advice on chromatographic matters and Mr. E. W. Rogers for skilled assistance with the spectrography and potentiometry.

Department of Medical Chemistry,
The Australian National University,
183 Euston Road, London, N.W.1.

[*Received, August 24th*, 1950.]