QUARTERLY REVIEWS

THE PTERIDINES

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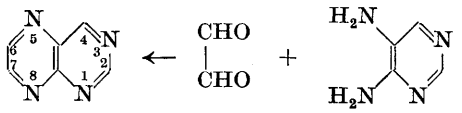
THE pteridines, because of their biological importance, have attracted more attention than any other family of tetra-azanaphthalenes. Previous Reviewers [1, 2, 3, 4, 5] have paid special attention to the history of the discovery of pteridines in Nature, the unravelling of their constitution, and their eventual synthesis. The purpose of the present Review is to discuss the pteridines mainly from the standpoint of pure chemistry (sections 1—13). It will be seen that this reveals a number of interesting correlations between structure and properties, and these correlations should furnish the biochemist and cell-physiologist with new approaches in their investigations of the role of pteridines in vital processes. This Review will conclude with some references to the naturally occurring pteridines and what is already known of their biological significance (sections 14 and 15).



(I) † (II)

Pteridine (I) is a yellow solid, m.p. 139·5°.[6, 7] It is volatile but odourless. It is very soluble in cold water (7 parts), and can be recrystallised from alcohol (5 parts), benzene (10 parts), or light petroleum (300 parts).[6]

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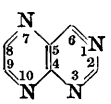
† The following numbering is also encountered, mainly in German literature.



[1] Purrmann, *Fort. Chem. org. Naturstoffe*, 1945, 64.

[2] Elvidge, *Ann. Reports*, 1948, **45**, 226.

[3] Gates, *Chem. Reviews*, 1947, **41**, 63.

[4] Simpson, *Ann. Reports*, 1946, **43**, 250; "Thorpe's Dictionary of Applied Chemistry", 1950, Vol. X, p. 264.

[5] Tschesche, *Arch. Pharm.*, 1950, **283**, 137.

[6] Albert, Brown, and Cheeseman, *J.*, 1951, 474.

[7] *Idem, ibid.*, in the press.

It is distributed between chloroform and water in the ratio of 2 : 1. It is readily destroyed by cold acid or alkali; when a solution is run through a column of commercial chromatographic alumina, 75% is destroyed [6] because of the alkali present.

2 : 4 : 6 : 7-Tetrahydroxypteridine, on the other hand, remains unmelted and chemically unchanged at 350°. One part requires 58,000 parts of cold water to dissolve it (formamide is the only other known solvent, and a poor one). It is remarkably stable to boiling acids and alkalis (Table 4).

Thus, the pteridines are a family covering a wide range of solubility, stability, and melting points (6 : 7-diethylpteridine [7] melts as low as 51°). A more detailed consideration of these properties [7] (sections 2 and 6) has led to two rules for the pteridine series, *viz.*: (*a*) hydrogen-bonding substituents greatly increase the crystal-lattice energy, thus raising melting points and lowering solubilities, and (*b*) electron-donating substituents greatly increase the stability of the molecule.

Pteridine was first synthesised in 1948 and is prepared by the condensation of glyoxal with 4 : 5-diaminopyrimidine (II),[6, 8] a general method which has been used for the synthesis of more pteridines than any other yet devised (section 13). Several reactions are known by which pteridines can be transformed into pyrimidines, pyrazines, and other pteridines (sections 6—12). However, metathetical changes seem to be much more limited than in simpler heterocyclic compounds, and a substituted pteridine is usually best prepared by having the substituent already present in the intermediate.

Highly substituted pteridines have been known since 1891 when Gowland Hopkins discovered the first member in the wings of butterflies. The chemical nature of the natural pteridines remained unknown until 1940.[12]

1. Criteria of Purity.—Because the majority of pteridines are infusible, little use can be made of mixed melting points as tests of identity, or of crystallisation to constant melting point as an index of purity. To fill this gap, various other physical methods have been utilised; of these, paper chromatography has been found particularly useful (see below).

The need for accessory physical measurements is made evident in the pteridine series by difficulties encountered in ultimate analysis. Presumably because of high lattice energy (see section 2), the hydroxy-, amino-, and mercapto-pteridines do not burn readily. Nevertheless, with practice, excellent results for carbon and hydrogen analyses can be obtained, by using Pregl's "universal filling" at dull red heat with subsequent stronger heating. Nitrogen determinations present greater difficulty.[9]

Low values for nitrogen were the principal cause of the acceptance of incorrect empirical formulæ for xanthopterin for many years. In 1933, analytical figures were obtained which led to the adoption [10] of the formula, $C_{19}H_{19}O_7N_{15}$. In 1939, this was modified [11] to $C_{19}H_{18}O_6N_{16}$ and in 1940

[8] Jones, *Nature*, 1948, **162**, 524.
[9] Brancone and Fulmor, *Analyt. Chem.*, 1949, **21**, 1147.
[10] Schöpf and Becker, *Annalen*, 1933, **507**, 266.
[11] Schöpf, Becker, and Reichert, *ibid.*, 1939, **539**, 156.

to the correct $C_6H_5O_2N_5$. The improvement can be ascribed partly to improvements in nitrogen analysis,[12] but partly to the synthesis of trimethyl-leucopterin, the first derivative soluble enough for molecular-weight determination.[13]

X-Ray powder photographs have been used for proving the identity of two specimens obtained from different sources,[14, 15] and some good reproductions of typical photographs have been printed.[16]

Infra-red spectrometry, which is rapidly becoming more accessible, is to be preferred : if identity is not established, some of the structure can be inferred from the spectrum. An example of its use for the establishment of identity was the comparison [17] of three specimens of 7 : 8-dihydro-6-hydroxypteridine, synthesised by different methods : examination of the significant 7—14-μ region disclosed 20 peaks which occurred at identical wave-lengths in all cases and no foreign peaks were found. The entire examination took less than an hour and used only 3 milligrams of each specimen (mulled in paraffin).[18]

Ultra-violet spectroscopy has been much used as a criterion of purity in the pteridine series, *e.g.*, by crystallisation to constant spectrum. Once the pK_a values are known (see below), a spectrum can usually be recorded for several distinct ionic species such as the cation, the neutral molecule, the monoanion, and the dianion.

Many ionisation constants of pteridines have been recorded in the convenient form of pK_a values (see section 3 and Table 2). These values have been used to assess both purity and identity. A pK_a identical ($\pm$ 0·06) at 9 equidistant parts of the titration curve is an indication of purity exceeding 90%, unless the impurity has a closely similar pK_a. Identity is almost certain when samples agree in two pK_a values.

Chromatographic analysis of naturally occurring mixtures of pteridines has been made by running solutions in 0·004N-hydrochloric acid through columns of alumina or frankonite.[19]

The paper-chromatography of pteridines [20, 21] has now been adopted as one of the most convenient aids for following a synthesis and judging when the time has arrived for more exacting tests of purity.[6, 22] For rapid results the ascending method [23] is used, the satisfactory solvents being dimethylformamide : water, 9 : 1, or 3% aqueous ammonium chloride.[22, 24] When time permits, far better differentiation is obtained

[12] Purrmann, *Annalen*, 1940, **546**, 98.
[13] Wieland and Decker, *ibid.*, 1941, **547**, 180.
[14] Schöpf and Becker, *ibid.*, 1936, **524**, 49.
[15] Wieland and Purrmann, *ibid.*, 1939, **539**, 179.
[16] Forrest and Walker, *J.*, 1949, 79.
[17] Albert, Brown, and Cheeseman, *J.*, 1952, 1620.
[18] Dr. L. N. Short, private communication.
[19] Becker and Schöpf, *Annalen*, 1936, **524**, 49, 124.
[20] Crammer, *Nature*, 1948, **161**, 349.
[21] Good and Allen, *ibid.*, 1949, **163**, 31.
[22] Tschesche and Korte, *Chem. Ber.*, 1951, **84**, 641, 801.
[23] Williams and Kirby, *Science*, 1948, **107**, 481.
[24] Renfrew and Piatt, *J. Amer. Pharm. Assoc.*, 1950, **39**, 657.

by the overnight use of a slow-moving solvent, *e.g.*, a mixture of butanol (70 ml.) and 5N-acetic acid (30 ml.).[23] Fig. 1 is typical of the results obtained.

The papers are finally dried over a hot-plate and read in the light of a special type of mercury discharge lamp [25] giving 99% of its radiation at 254 mμ after passage through a suitable filter.[26] At this wave-length, the majority of pteridines, and the intermediates used in preparing them, either absorb (appearing as black spots on the white paper) or fluoresce in characteristic colours. The more familiar type of mercury lamp, emitting Wood's light at 365 mμ, is of little use for this purpose. Occasionally the 254-mμ lamp causes photo-decomposition: 7-hydroxypteridine is seen as a black spot under 254-mμ light and is invisible under 365-mμ light, but after irradiation for a minute at 254 mμ it appears violet under both lamps.[17]

A No. 1 Whatman paper, or its equivalent, is most suitable for the chromatography of pteridines. Occasional batches of paper contain iron and other heavy metals. This defect causes 4-hydroxypteridines to give

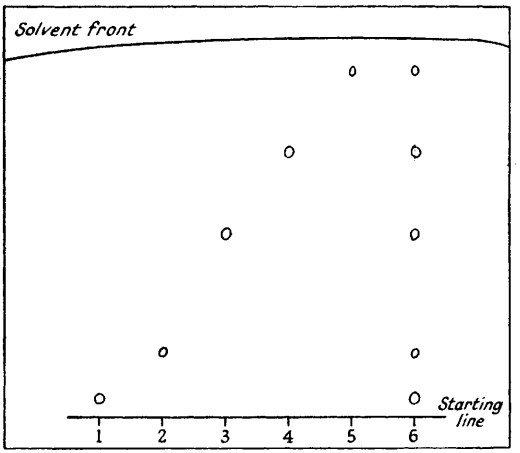


FIG. 1

Descending paper chromatogram (butanol and acetic acid).

	Pteridine	R_F *in butanol–acetic acid*
1,	4 : 6 : 7-*Trihydroxy-* . .	0·05
2,	6 : 7-*Dihydroxy-* . . .	0·35
3,	4-*Hydroxy-*	0·50
4,	4-*Amino-*	0·70
5,	2-*Dimethylamino-* . .	0·90
6,	1 + 2 + 3 + 4 + 5	

one or more supplementary spots, because of their chelating properties (see section 4), unless a small crystal of sodium sulphide is added to the butanol–acetic mixture.[7]

The somewhat more troublesome descending method [27] gives R_F values

[25] The Thermal Syndicate's model T/M5/369E is suitable.

[26] Chance Brothers' OX7/19874 is suitable.

[27] Consden, Gordon, and Martin, *Biochem. J.*, 1944, **38**, 224.

which may be used as constants provided that reference substances are included in each run (4-hydroxy- and 4-amino-pteridine are suitable, see Fig. 1). Any run must be rejected if these markers do not give their proper values.[17] For the descending method, a modified solvent is recommended (butanol 66 ml., 5N-acetic acid 33 ml.).[6]

Tests of identity are best made in several different solvents : two substances which have the same R_F values in two solvents may give different values in a third solvent. Pteridines giving satisfactory analytical figures (C, H and N) are not necessarily chromatographically pure. For example, 2 : 4- dihydroxypteridine prepared according to Kuhn and Cook " in schönen gelben Nädelchen " [135] revealed 5 spots : when purified with alumina until only one spot was given, it became colourless.[6]

Crystallisation to constant solubility and knowledge of the figure thus obtained have proved helpful guides in purification and identification respectively.[6]

The obstinacy with which some pteridines (*e.g.*, 2-hydroxy- and 6-hydroxypteridine) retain water of crystallisation makes it essential to preclude the possibility that they are actually the corresponding pyrazine or pyrimidine intermediates which have failed to undergo ring-closure.

2. Solubility and Melting Point.—As Table 1 indicates, pteridines with no hydrogen-bonding substituent are low-melting and fairly soluble in water, whereas those with —NH_2, —OH (or —SH) [7] groups remain unmelted at 350° (or decompose above 230° without melting). Each addition

TABLE 1. *Solubilities and melting points* [6, 7]

Pteridine	M.p.	Solubility in water :	
		at 20°, 1 part in	at 100°, 1 part in
(Unsubstituted)	140°	7·2	
2-Dimethylamino-	125	2·5	
4-Dimethylamino-	165	60	4
2-Methoxy-	150	80	4
4-Methoxy-	195	80	9
6-Methoxy-	124	85	4
(*N*-Methyl-7-pteridone)	125	50	10
2-Amino-	(dec. 275)	1350	100
4-Amino-	(dec. 305)	1400	80
2-Hydroxy-	(dec. 240)	600	50
4-Hydroxy-	> 350	200	29
6-Hydroxy-	(dec. 240)	3500	230
7-Hydroxy-	(dec. 230)	900	76
2 : 4-Diamino-	(dec. 315)	3000	130
6 : 7-Dihydroxy-	> 350	3500	290
4 : 6 : 7-Trihydroxy-	> 350	27,000	7000
2 : 4 : 6 : 7-Tetrahydroxy-	> 350	58,000	
2-Amino-4 : 6 : 7-trihydroxy- (leucopterin)	> 350	750,000	

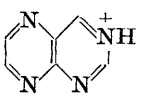
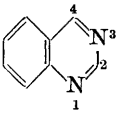
of a hydrogen-bonding substituent usually increases the insolubility, sometimes by a very large amount. Moreover, the combination of an amino-

group and a hydroxy-group in the one molecule produces particularly insoluble substances [7] (cf. leucopterin in Table 1). A comparison of 7-hydroxypteridine with *N*-methyl-7-pteridone (its methylated keto-form) shows that the absence of a bondable hydrogen atom is enough to ensure low melting point and high solubility (Table 1).

Interpretation is helped by the knowledge that the pteridines which are fairly soluble in water are also soluble in alcohol, chloroform, benzene, and the majority of other organic solvents. However, the hydroxy-, (primary) amino-, and mercapto-pteridines are practically insoluble in organic solvents, particularly if two such groups are present. Evidently these hydrogen-bonding groups form stronger bonds to other pteridine molecules than to the molecules of solvents. Apparently this leads to a crystal structure of lattice energy unusual for an organic compound. Even in the closely related purine series, these symptoms of high lattice energy are not quite so pronounced.

3. Ionisation.—The introduction of further ring-nitrogen atoms into the nucleus of pyridine or quinoline usually has a base-weakening effect, especially if these nitrogen atoms occupy positions 1 : 4 to each other.[28] Pteridine is a diaza-derivative of quinazoline and of 1 : 8-naphthyridine (which have pK_a values of 3·5 and 3·4 respectively) ; [28, 6] hence, its strength as a base (pK_a = 4·1, see Table 2) is higher than would be expected—indeed it is not much weaker than quinoline (pK_a = 4·9).

It would be interesting to know which nitrogen in pteridine accepts the first proton. The low pK_a values [28] for pyrazine (0·6) and quinoxaline (0·8) compared with those of pyrimidine (1·3) and quinazoline (3·5) make it likely that either $N_{(1)}$ or $N_{(3)}$ is the most basic nitrogen.

(III)   (IV)

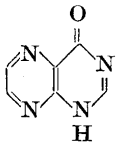
The unexpectedly low pK_a of 4-aminopteridine provides indirect evidence that (III) correctly represents the cation of pteridine. Thus, 4-aminopteridine (Table 2) fails to show the exaltation of strength (over pteridine) which is such a feature of aromatic six-membered rings bearing an amino-group γ to a ring-nitrogen atom.[28] This exaltation arises from the increased resonance which this configuration makes possible in the ion but not in the neutral molecule. This low pK_a becomes intelligible if $N_{(1)}$ is not the most basic nitrogen atom, but the second most basic one. A similar situation is met in quinazoline (IV) where the most basic nitrogen is known, from degradation [29] of the methochloride, to be $N_{(3)}$. 4-Aminoquinazoline shows too little exaltation of basic strength over quinazoline to be γ to the most basic nitrogen atom.[30]

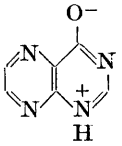
4-Aminopteridine is amphoteric : a new spectrum [6] appears at pH 13,

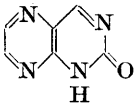
[28] Albert, Goldacre, and Phillips, *J.*, 1948, 2240.
[29] Gabriel and Colman, *Ber.*, 1904, **37**, 3643.
[30] Mr. J. N. Phillips, personal communication.

reverting to that of the neutral molecule at pH 12. The methoxypteridines are all weaker bases than pteridine.

The hydroxypteridines are, as would be expected, all stronger acids than analogous substances with fewer ring-nitrogen atoms. They all exist in tautomeric equilibrium with forms containing doubly bound oxygen, these forms being stabilised by the well-known amide-type resonance (*e.g.*, V*a*, V*b*). A simple method exists for calculating the equilibrium constants [31] from the basic pK_a values in tautomerism of this kind : in this way the ratio of —OH to =O forms in 4-hydroxyquinoline has been determined.[32] Unfortunately, it is not easy to apply this method to substances containing more than one ring-nitrogen atom.


(V*a*)


(V*b*)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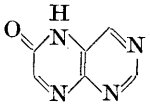
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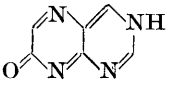

(VIII)

The anions of the hydroxypteridines should be stabilised by resonance involving forms that bear the negative charge either on oxygen or (to a lesser extent) on nitrogen respectively. The isomers with the highest acidic strength (Table 2) may be expected to owe this to a greater excess of resonance in the anion compared with that of the neutral molecule.

Because of the relative positions of $N_{(1)}$, $N_{(3)}$, and $N_{(8)}$ in pteridine, the molecules of 2-, 4-, and 7-hydroxypteridines can each transfer a hydrogen atom to each of these three nitrogen atoms without violating the canons of valency. On the other hand, 6-hydroxypteridine can transfer hydrogen to the 5-position only. Gore and Phillips' principle,[33] if applicable here, would single out (V), (VI), (VII), and (VIII) as the principal forms with doubly bound oxygen of 4-, 2-, 6-, and 7-hydroxypteridines respectively. This assumption throws light on the relative basic-strengths of the hydroxypteridines.[17] Only the 6-isomer has marked basic properties. It is evident, that if (VI), (V), and (VIII) are the best formulæ to represent 2-, 4-, and 7-hydroxypteridines, one of the two most basic centres has received a proton and the other has been reduced in basic strength by a coulombic effect. Nevertheless, 6-hydroxypteridine (VII) should have much the same basic strength as pteridine. Reference to Table 2 shows that these expectations are fulfilled.

[31] Branch and Calvin, " The Theory of Organic Chemistry ", New York, 1941, p. 301.

[32] Tucker and Irvin, *J. Amer. Chem. Soc.*, 1951, **73**, 1923.

[33] Gore and Phillips, *Nature*, 1949, **163**, 690.

TABLE 2. *Ionisation constants of pteridines (water, 20°) (expressed as pK_a values)* [6, 7, 17]

Pteridine	Cationic	Anionic
(Unsubstituted)	4·12	
2-Amino-	4·29	
4-Amino-	3·56	>14
6-Amino-	4·15	
2-Dimethylamino-	3·03	
4-Dimethylamino-	4·33	
6-Dimethylamino-	4·31	
2-Methoxy-	2·13	
4-Methoxy-	<1·5	
6-Methoxy-	3·60	
2-Hydroxy-	<2	11·13
4-Hydroxy-	<1·3	7·89
6-Hydroxy-	3·67	6·7
7-Hydroxy-	1·2; −2·0	6·41
2 : 4-Dihydroxy-	<1·3	7·91
6 : 7-Dihydroxy-	<2·7	6·87; 10·0
2-Mercapto-		9·98
4-Mercapto-		6·81
6-Hydroxy-7 : 8-dihydro-	4·78	10·54
7-Hydroxy-5 : 6-dihydro-	3·36	9·94
2-Amino-4-hydroxy-	2·31	7·92
(*N*-Methyl-7-pteridone)	1·1	

By far the most remarkable property of hydroxypteridines is the hysteresis loop which 6-hydroxypteridine produces on titration with alkali followed by back-titration with acid [17] (Fig. 2) (the basic group of this substance can be titrated with no sign of abnormality). Even such closely

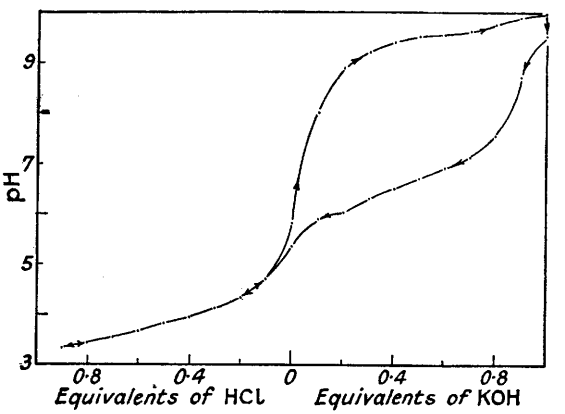


FIG. 2

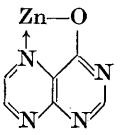
Hysteresis loop produced on titration of 0·002 M-*aqueous* 6-*hydroxypteridine* (250 *ml.*) *with* 1 *equiv.* (5 *ml.*) *of* 0·1 N-*acid and -alkali at* 20°.

related substances as 2-hydroxypyrazine, 2-hydroxyquinoxaline, and 6-aminopteridine do not behave in this way, but xanthopterin (2-amino-

4 : 6-dihydroxypteridine) does.[17, 34] The possible nature of the slow tautomeric change has been discussed.[17]

The pK_a values of monosubstituted pteridines being known, it is easy to assign to relevant groups the pK_a values obtained by titrating more complex examples. For instance, xanthopterin, although poorly soluble, yields two pK_a values (6·25 and 9·23) on back-titration.[6] The 6·25 value can now be assigned to the 6-hydroxy-group (the 2-amino-group must be lower) and the 9·23 value to the 4-hydroxy-group which is inevitably weakened by the coulombic effect when the 6-hydroxy-group is ionised. The pK_a of 8·26 found for pteroylglutamic acid (XVIII*a*) is evidently that of a 4-hydroxy-group, somewhat weakened by the ionisation of two, rather distant, carboxyl groups.

4. Metal-binding Properties.—With the ions of divalent metals, 4-hydroxypteridines form 1 : 1 and 1 : 2 complexes, as exemplified by (IX) and (X).[35, 36] All naturally occurring pteridines possess a 4-hydroxy-group and are usually



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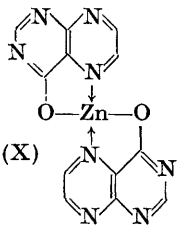


(X)

isolated after treatment of the tissues with alkali. Hence it is likely that many of these substances occur naturally in combination with divalent metals. Indeed some pteridines probably exert their biological function by this chelation process [*e.g.*, the effect of xanthopterin in preventing oxidative formation of melanin (the skin-pigment) [37]].

The constants governing equilibria between these 1 : 2 complexes and free metallic ions have been determined by a potentiometric method [38] and are given in Table 3. Glycine is included for comparison because

TABLE 3. *Stability constants of* 1 : 2 *metal–pteridine complexes (expressed as logarithms)*

	4-Hydroxypteridine	Folic acid	Glycine (for comparison)
Cu^{++}	9·5	7·8	15·4
Ni^{++}	7·8	9·0	11·0
Zn^{++}	*ca.* 6	7·5	9·3
Co^{++}	6·7	8·1	8·9
Cd^{++}	*ca.* 6	6·7	8·1
Fe^{++}	5·9	7·9	7·8
Mn^{++}	4·5	*ca.* 6	5·5
Mg^{++}	< 4	< 6	*ca.* 4

[34] Scou, *Arch. Biochem.*, 1950, **28**, 10. [35] Albert, *Biochem. J.*, 1950, **47**, ix.
[36] Albert and Brown, Proc. 1st Internat. Congr. Biochem., Cambridge, 1949, p. 241.
[37] Isaka, *Nature*, 1952, **169**, 74. [38] Albert, *Biochem. J.*, 1950, **47**, 531.

the order of preference which it exerts for various metals is that commonly exerted by complex-forming reagents (*i.e.*, the Mellor and Maley sequence [39]). Obviously the pteridines do not observe this sequence very closely. Xanthopterin shows a far greater avidity for metals than does 4-hydroxypteridine.[40]

5. Spectroscopic Data.—The majority of pteridines are colourless, but several are yellow and a few are more highly coloured. 4-Mercaptopteridine is reddish-orange and a few red pteridines (erythropterin,[41] pterorhodin and its isomers [42]) are known, but these have an ethylenic bond conjugated with the pteridine ring.

The majority of pteridines have a well-defined ultra-violet spectrum consisting of two main peaks which may exhibit fine structure (as in 6-chloro- and 6-methoxy-pteridine).[17] An incompletely resolved supplementary peak (as in the 235-mμ region of pteridine, Fig. 3, and the anion of 2-hydroxypteridine, Fig. 4) is usually present and sometimes three major peaks occur (as in 6-aminopteridine, Fig. 6, or in the anion of leucopterin [43]).

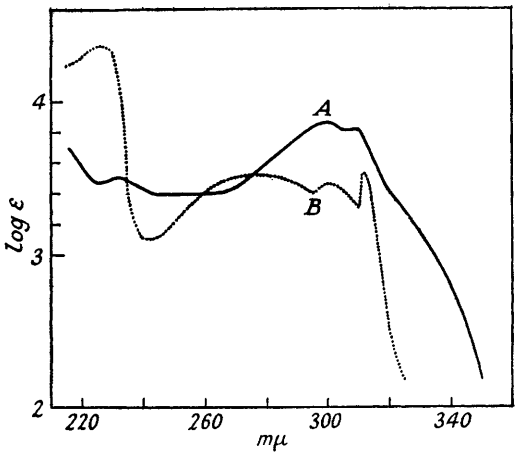


FIG. 3

Absorption spectra in water of the molecules of (*A*) *pteridine* (pH 6·2) *and* (*B*) *quinoline* (pH 6·3).

The spectrum [6] of pteridine is shown in Fig. 3, with that of quinoline. A certain similarity is to be expected because =CH— and =N— are to a large extent optically interchangeable.[44, 44a] As with quinazoline, the short-wave peak of pteridine has been displaced to shorter wave-lengths where it cannot be completely traced.

[39] Mellor and Maley, *Nature*, 1948, **161**, 436.
[40] Albert and Brown, unpublished results.
[41] Tschesche and Korte, *Chem. Ber.*, 1951, **84**, 77; Purrmann and Eulitz, *Annalen*, 1948, **559**, 169.
[42] Russell, Purrmann, Schmitt, and Hitchings, *J. Amer. Chem. Soc.*, 1949, **71**, 3412.
[43] Hüttel and Sprengling, *Annalen*, 1943, **554**, 69.
[44] Braude, *Ann. Reports*, 1945, **42**, 129.
[44a] Radulescu and Ostrogovich, *Ber.*, 1931, **64**, 2233.

The spectra [6, 7, 17] of the monochloropteridines closely resemble that of pteridine but are shifted to slightly longer wave-lengths.

The spectra of the monoaminopteridines (as neutral molecules) resemble those of pteridine but, as expected, are shifted to much longer wave-lengths.[6, 17] The spectra of the three known isomers (as neutral *molecules*) closely resemble the spectra of the *anions* of the corresponding hydroxy-compounds, a relationship that is usual among amines and phenols derived from aromatic hydrocarbons.[45] Fig. 4 exemplifies this effect. This relationship has been used to help to establish that the neutralisation of 6-hydroxypteridine does not cause ring-fission,[17] in spite of the abnormality of the titration curve (Fig. 2).

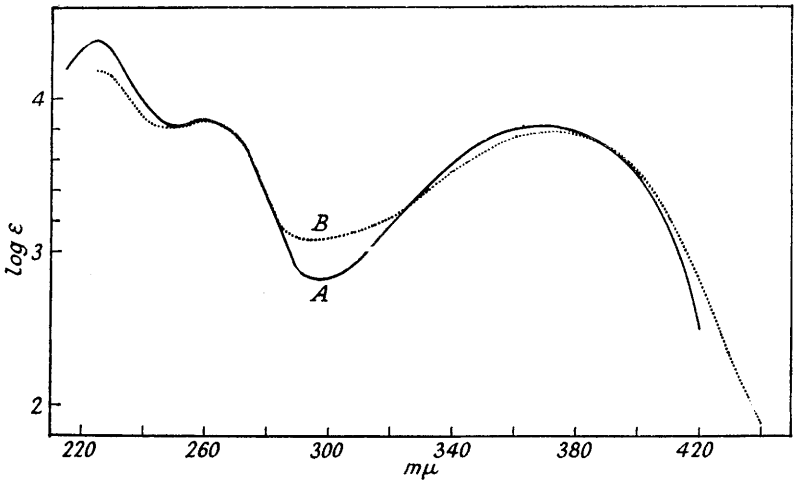
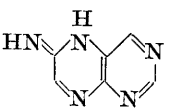


FIG. 4

Absorption spectra in water of (*A*) *the molecule of* 2-*aminopteridine* (pH 7·1) *and* (*B*) *the anion of* 2-*hydroxypteridine* (pH 13).

Ionisation of aminopteridines as cations shifts the peaks to much shorter wave-lengths (cf. Fig. 7) [6, 17] which is the reverse of what usually occurs with heteroaromatic bases (however there seems to be a very slight shift in this direction in 2- and 4-aminoquinoline, alone of the seven amino-quinolines [46]).

The spectra [6, 7, 17] of the neutral molecules of 2-, 4-, and 6-dimethyl-aminopteridine are similar to those of 2-, 4-, and 6-amino-pteridine respectively, but each peak is shifted to longer wave-lengths, as with the analogous pair, aniline and dimethylaniline.[47] Such a pair [7, 17] is shown in Fig. 6. These pairs of spectra make it unlikely that any of the three known aminopteridines exist in an imino-form such as (XI).



(XI)

The long-wave peaks of the monohydroxypteridines (neutral molecules)

[45] Jones, *J. Amer. Chem. Soc.*, 1945, **67**, 2127.
[46] Steck and Ewing, *ibid.*, 1948, **70**, 3397.
[47] Heertjes, Bakker, and van Kerkhof, *Rec. Trav. chim.*, 1943, **62**, 737.

lack the expected bathochromic shift.[45, 48] Each of the four isomerides has this peak [6, 17] in much the same position as pteridine.

Two of the three known monomethoxypteridines (the 2- and the 6-isomer) show the strong bathochromic shift that this group causes when introduced into aromatic hydrocarbons,[45] but 4-methoxypteridine does not.

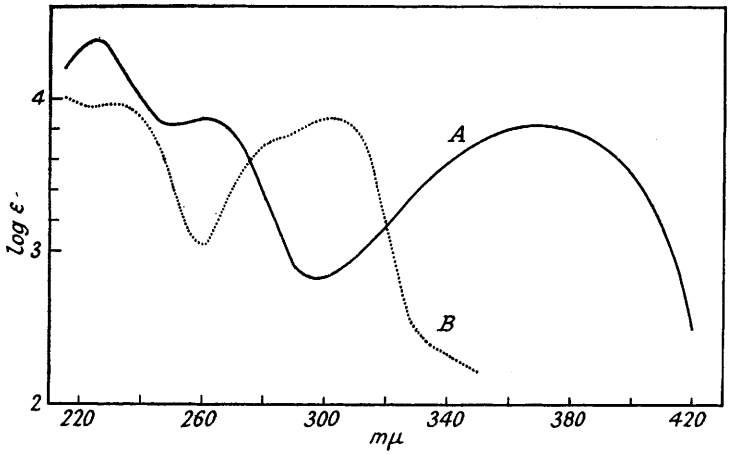


FIG. 5

Absorption spectra in water of (*A*) *the molecule of* 2-*aminopteridine* (pH 7·1) *and* (*B*) *the cation of* 2-*aminopteridine* (pH 2·1).

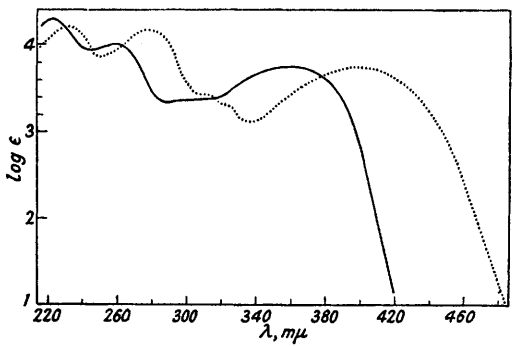


FIG. 6

Absorption spectra in water of the molecules of —— 6-*aminopteridine* (pH 7·0) *and* ..... 6-*dimethylaminopteridine* (pH 7·0).

The lack of correspondence between the spectra of 2- and 6-hydroxypteridine and their methoxy-analogues [7, 17] makes it likely that these are not normal hydroxy-derivatives,[45] but rather resonance hybrids in which forms with doubly bound oxygen, *e.g.*, (VI), play a very important part. 7-Methoxypteridine is not known, but the spectrum of 7-hydroxypteridine

[48] Albert and Short, *J.*, 1945, 760.

is very similar to that of *N*-methyl-7-pteridone (see Fig. 8).[17] It seems that this isomeride may exist largely as a hybrid with doubly bound oxygen. These correlations can only be considered tentative until both *O*- and *N*-methyl derivatives of all the monohydroxypteridines are known.

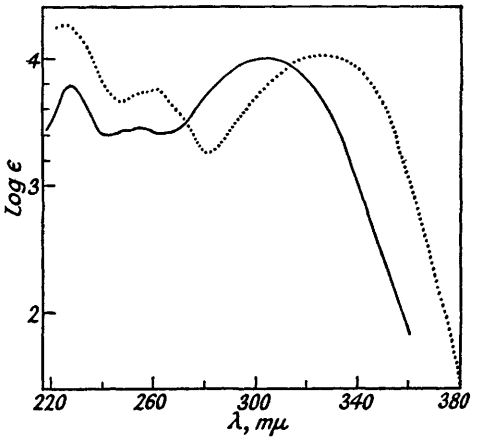


FIG. 7

Absorption spectra in water of —— *the molecule of 7-hydroxypteridine* (pH 4) *and* ..... *the anion of 7-hydroxypteridine* (pH 9).

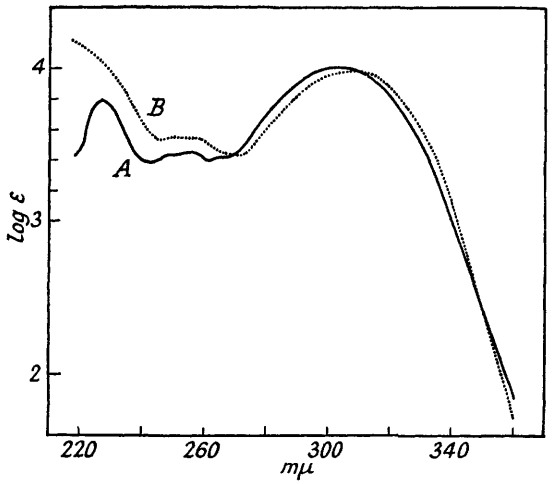


FIG. 8

Absorption spectra in water of the molecules of (*A*) *7-hydroxypteridine* (pH 4·0) *and* (*B*) *N-methyl-7-pteridone* (pH 4·0).

Addition of further hydroxy-groups to a monohydroxypteridine usually causes the long-wave peaks to move to still longer wave-lengths.[6, 17 40]

The long-wave peaks of the spectra of the anions of monohydroxypteridines are displaced to much longer wave-lengths than those of the

neutral molecule. This effect is shown for 7-hydroxypteridine in Fig. 7. The displacement is 23 mμ for the 4- and the 7-isomer and 68 mμ for the 2- and the 6-isomer. In the polyhydroxypteridines, the displacement due to the ionisation of groups other than the first tends to be small.

The substituents in 4-amino-2-hydroxypteridine and 2-amino-4-hydroxypteridine are not mutually bathochromic ; these molecules absorb at shorter wave-lengths than would be expected, presumably because of a complex electronic redistribution.[7]

Critical values for the spectra of xanthopterin (2-amino-4 : 6-dihydroxypteridine) are of special interest because the purity of this substance is evaluated spectrometrically and biochemists have been confused [49] by the discrepancies in the early literature. Xanthopterin monohydrate (at pH 11—13) gives the following $\lambda_{max.}$ and log $\varepsilon_{max.}$ (molecular) values :

255 (4·26) ; 392 (3·85) (cf. Refs. 50—53).

It will be noticed that some different [10, 52, 54] figures have been recorded in the literature. In ref. 10 there are two errors : the graph is drawn in natural logarithms, but labelled " log " and the molecular weight is wrong, xanthopterin being thought in 1933 to be a C_{19} (instead of a C_6) substance. In ref. 51 the concentration was wrongly reported as 1%, instead of 0·001%.[55]

Spectra of 2 : 4-dihydroxy-, 2-amino-4-hydroxy-, and 2 : 4-dihydroxypteridines have been compared with their 6 : 7-dialkyl-,[56, 57] diaryl-,[58] carboxy-,[59] and carbethoxy-derivatives.[59] The following derivatives of 2-amino-4-hydroxypteridine have also been examined : 6- and 7-methyl-, 6- and 7-carboxy-,[60, 61, 62] 7-carboxymethyl-,[60] 6-aldehyde,[63] 7-hydroxy-6-methyl-, 6-hydroxy-7-methyl-, 6-carboxy-7-hydroxy-, and 7-carboxy-6-hydroxy-.[64] Other spectra recorded include : *iso*xanthopterin (2-amino-4 : 7-dihydroxypteridine),[22] 2 : 4 : 6-trihydroxypteridine and several of its derivatives,[22] tetrahydroxypteridine,[65] leucopterin,[43] pteroylglutamic acid,[66, 67] ichthyopterin,[68] erythropterin,[41] and pterorhodin.[42]

[49] *e.g.* Goodwin and Srisukh, *Biochem. J.*, 1951, **49,** 84.
[50] Anderson and Nelson, *J. Amer. Chem. Soc.*, 1949, **71,** 3837.
[51] Elion, Light, and Hitchings, *ibid.*, p. 741.
[52] Totter, *J. Biol. Chem.*, 1944, **154,** 105.
[52a] Albert and Wood, *J. Appl. Chem.*, 1952, in the press.
[53] Rickes, Chaiet and Keresztesy, *J. Amer. Chem. Soc.*, 1947, **69,** 2749.
[54] Karrer, Manuta, and Schwyzer, *Helv. Chim. Acta*, 1948, **31,** 1214.
[55] Dr. George Hitchings, personal communication.
[56] Cain, Mallette, and Taylor, *J. Amer. Chem. Soc.*, 1946, **68,** 1996.
[57] Mallette, Taylor, and Cain, *ibid.*, 1947, **69,** 1814.
[58] Cain, Taylor, and Daniel, *ibid.*, 1949, **71,** 892.
[59] Cain, Mallette, and Taylor, *ibid.*, 1948, **70,** 3026.
[60] Mowat *et al.*, *ibid.*, p. 14.
[61] Karrer and Schwyzer, *Helv. Chim. Acta*, 1949, **32,** 423 ; 1950, **33,** 39.
[62] Backer and Houtman, *Rec. Trav. chim.*, 1951, **70,** 725.
[63] Waller *et al.*, *J. Amer. Chem. Soc.*, 1950, **72,** 4630.
[64] Elion, Hitchings, and Russell, *ibid.*, p. 78.
[65] Bertho and Bentler, *Annalen*, 1950, **570,** 127.
[66] Waller *et al.*, *J. Amer. Chem. Soc.*, 1948, **70,** 19.
[67] Tschesche, Korte, and Petersen, *Chem. Ber.*, 1951, **84,** 579.
[68] Tschesche and Korte, *ibid.*, p. 801.

Only a few infra-red spectra of pteridines have yet been published.[66, 69]

6. Stability to Acid and Alkali.—The pteridines are unstable to acid and alkali unless powerfully electron-donating groups [OH, $N(CH_3)_2$, NH_2, etc.] are present. As a generalisation, it may be said that the greater the number of such groups, the greater the stabilisation achieved [7] (see Table 4). Decomposition, where it occurs, can consist either of ring-opening or polymerisation. Weaker electron-donating groups than OH (*e.g.*, C_2H_5) have a smaller protective influence.[7]

The instability of pteridine appears to be due to the electron-attracting nature of ring-nitrogen atoms. In pteridine four nitrogen atoms are competing for the ten π-electrons. This depletion seriously interferes with the aromatic stabilisation. It would be interesting to investigate pteridine by *X*-ray crystallography in the expectation of finding it non-planar. The electron-donating groups apparently redress this deficit of electrons and restore the aromatic state.[7]

TABLE 4. *Decomposition of pteridines by excess of acid or alkali at* 110°

Pteridine	Decomposed, %, in one hour by	
	N-H_2SO_4	10N-NaOH
2 : 4 : 6 : 7-Tetrahydroxy-	*	6
4 : 6 : 7-Trihydroxy-	*	4
6 : 7-Dihydroxy-	7	12
2 : 4-Dihydroxy-	6	4
2-Hydroxy-	55	89
4-Hydroxy-	60	94
6-Hydroxy-	2	100
7-Hydroxy-	52	76
(unsubstituted)	74	>57

* Apparently unaffected, but their high degree of insolubility in acid prevents a just comparison.

Quinoline, with a ratio of only one nitrogen to seven carbon atoms is fairly stable to concentrated acid and alkali. Pteridine on the other hand is rapidly converted into 2-aminopyrazine-3-aldehyde by concentrated (and, more slowly, by dilute) acids.[112] 2-Hydroxy- and 2-amino-pteridine form coloured substances with both acid and alkali. Refluxing with 10N-sodium hydrozide converts 4-hydroxypteridine into 2-aminopyrazine-3-carboxylic acid in excellent yield : 2 : 4-dihydroxypteridine can also be hydrolysed quantitatively to this acid, but much more severe conditions (autoclaving at 180°) are required. 4-Hydroxypteridine, when refluxed with 2N-sulphuric acid, gives 2-aminopyrazine-3-carboxyamide and the corresponding acid.[7]

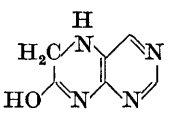
Xanthopterin (2-amino-4 : 6-dihydroxypteridine) is stable to boiling

[69] Hutchings *et al.*, *J. Amer. Chem. Soc.*, 1948, **70**, 10.

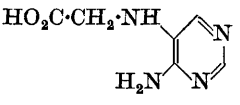
7N-hydrochloric acid,[70] but has been broken down to glycine in 45% * yield [11] by 4N-hydrochloric acid during 5 hours at 200°. Xanthopterin is little affected by being boiled with 1·5N-barium hydroxide for 20 hours.[71] Leucopterin (7-hydroxyxanthopterin) is almost unaffected by concentrated sulphuric acid (2 hours at 150°), but is quantitatively broken down by 10N-hydrochloric acid under more severe conditions (5 hours at 165°) to glycine, carbon monoxide, carbon dioxide, and ammonia in the ratio 1 : 1 : 3 : 4.[72]

From what has been written above, it would be expected that other six-membered heterocyclic rings with high N : C ratios would be unstable unless substituted with strong electron-donating groups. Such a structure would be possessed by 1 : 3 : 5-triazine which has resisted all attempts to prepare it.[73] 2 : 4 : 6-Trimethyl-1 : 3 : 5-triazine is split by 2N-hydrochloric acid to acetic acid and ammonia, even at room temperature ; [73] however the 2 : 4 : 6-triamino- and -trihydroxy-analogues (melamine and cyanuric acid) are very stable.

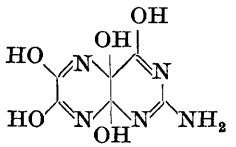
The dihydropteridines are less stable than the pteridines from which they are derived. Dihydroxanthopterin is more easily hydrolysed by acid to glycine than is xanthopterin.[70] 5 : 6-Dihydro-7-hydroxypteridine (XII) is instantly converted into 4-amino-5-carboxymethylaminopyrimidine (XIII) by boiling N-sodium hydroxide ; this reaction is reversed by one hour's refluxing with N-hydrochloric acid.[17]



(XII)



(XIII)



(XIV)

The so-called leucopterin glycol (XIV) is hydrolysed, by boiling dilute acids, to carbon dioxide, ammonium oxalate, and guanidine.[72] Cold alkali gives carbon dioxide, oxalic acid, and two derivatives of hydantoin.[71, 72, 74]

The hydrolysis of aminopteridines to hydroxypteridines is dealt with in section 9.

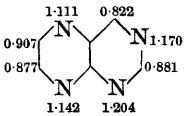
7. Substitution Reactions.—Electrophilic reagents. No example of nitration,[6] nitrosation, sulphonation, or halogenation (of C–H) has been recorded in the pteridine series. The reported coupling of xanthopterin with diazotised 2 : 5-dichloroaniline [10] could not be confirmed [17] when pure xanthopterin was used.

Nucleophilic reagents. Pteridine rapidly reacts with perphthalic acid to give 4-hydroxypteridine [7] (incorrectly reported, originally, as pteridine

[70] Wieland and Schöpf, *Ber.*, 1925, **58**, 2178.
[71] Wieland and Purrmann, *Annalen*, 1940, **544**, 163.
[72] Wieland, Metzger, Schöpf, and Bülow, *ibid.*, 1933, **507**, 226.
[73] Grundmann and Weisse, *Chem. Ber.*, 1951, **84**, 684.
[74] Wieland and Kotzschmar, *Annalen*, 1937, **530**, 152.

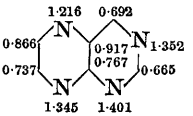
* On the assumption that one molecule of xanthopterin gives only one of glycine alternatively, 22·5% if two molecules are formed.

N-oxide [6]). It is interesting to compare this evidence of electron-deficiency in the 4-position in pteridine with π-electron diagrams prepared by three leading schools (XV*a* and *b*). In (XV*a*) the 4-position is indicated as the most deficient in electrons; in (XV*b*), this property is ascribed to the 2-position, the calculations being made by a different method.



(π-Electron charges, calculated from molecular orbitals [75, 76])

(XV*a*)



(π-Electron charges, calculated by valence-bond method [77])

(XV*b*)

The polarisation of σ-electrons may play a small part in this hydroxylation but is not represented in the diagrams. These diagrams presuppose that the pteridine molecule is flat (see section 6, however).

Hydrogen peroxide converts 6-hydroxypteridine into 6 : 7-dihydroxypteridine in 45% yield,[17] and xanthopterin into leucopterin in 31% yield.[15] 6-Hydroxypteridine and sodamide, when heated in diethylaniline at 150° and then treated with ice, give 6 : 7-dihydroxypteridine in 50% yield. Hydrogen peroxide has very little effect on 7-hydroxypteridine, but cold brown nitric acid (*d* 1·5) converts it into 6 : 7-dihydroxypteridine in 30% yield.[17] 2-Amino-4 : 7-dihydroxypteridine is converted by nitrous acid into tetrahydroxypteridine in 60% yield,[78] but this reagent destroys 7-hydroxypteridine.[17] Attempts to aminate pteridine with sodamide in diethylaniline were unsuccessful.[6]

A colourless peroxide is an intermediate in the above conversion of xanthopterin into leucopterin.[15] This peroxide reverts to xanthopterin when heated with water. Xanthopterin suspended in 2N-acetic acid and shaken with oxygen over platinum for 24 hours gives leucopterin (63% yield).[71, 79] However, 6-hydroxypteridine is unaltered by this treatment even at 100° when completely dissolved.[17]

8. Oxidation and Reduction.—Oxidation. Oxidations in the pteridine series comprise (i) the replacement of H by OH (section 7), (ii) the formation of a glycol at a double bond, and (iii) the removal of hydrogen atoms from a hydropteridine. Items (ii) and (iii) will now be dealt with. Leucopterin, like uric acid, is converted by chlorine water into a glycol (XIV), the junction of the two rings being attacked. Chlorine in methanol gives the corresponding 9 : 10-dimethoxy-derivative.[72, 74]

7 : 8-Dihydro-6-hydroxy-,[17] 5 : 6-dihydro-7-hydroxy-,[17] and 2-amino-7 : 8-dihydro-4 : 6-dihydroxy-pteridine [51] are dehydrogenated in 80% yields

[75] Chalvet and Sandorfy, *Compt. rend.*, 1949, **228**, 566.

[76] Prof. C. A. Coulson independently arrived at similar figures (personal communication).

[77] Dr. B. Pullman, personal communication.

[78] Wieland and Liebig, *Annalen*, 1944, **555**, 146.

[79] O'Dell, Vandenbelt, Bloom, and Pfiffner, *J. Amer. Chem. Soc.*, 1947, **69**, 250.

by cold alkaline potassium permanganate. This appears to be the best general reagent for the purpose even though dihydropteridines with several electron-donating substituents can be oxidised by milder reagents such as oxygen in alkaline solution (uncatalysed),[79] hydrogen peroxide,[80] alkaline silver nitrate, methylene-blue, sodium hypobromite, chloramine-T, benzoquinone, and dichlorophenolindophenol.[52] The (? 5 : 6-)dihydro-derivative of pteroylglutamic acid, which arises during its manufacture, has usually been oxidised to folic acid by disproportionation which is wasteful. According to a patent,[81] much better yields are obtained by the use of iodine, chlorine, ferric chloride, potassium ferricyanide, or sodium dichromate (all at pH 3—4) or of benzoquinone or hydrogen peroxide (both at pH 4—5). These oxidising agents are intended to be present at the start of the condensation, at 40°, of dibromopropaldehyde, 2 : 4 : 5-triamino-6-hydroxypyrimidine, and *p*-aminobenzoylglutamic acid. Mercuric acetate has also been used for this oxidation:[82]

It is interesting to compare the conditions for the oxidation of 7 : 8-dihydroxanthopterin to xanthopterin[83] with oxygen over platinum (30 minutes in 0·3N-sodium hydroxide; yield, 83%) with those given in section 7 for the oxidation by the same reagents of xanthopterin to leucopterin.

An interesting series of pairs of isomeric dihydropteridines has been studied (6 : 7-diaryl-2-ethylthio-4-hydroxy- and 4-amino-6 : 7-diaryl-2-ethylthio-dihydropteridines).[84, 85] The (orange) α-forms are formed by neutral condensation of benzoins and 4 : 5-diaminopyrimidines and are believed to be 5 : 6-dihydro-compounds. The (yellow) β-forms are formed by similar condensation in the presence of acetic acid and are known to be the 7 : 8-dihydro-isomers.[86] An α- can be converted into its β-isomer by being heated with acetic acid. Ferric chloride oxidises both α- and β-isomers to the same pteridine.

The oxidation of methyl groups and of aldehydes is dealt with in section 12.

Reduction. 6- and 7-Hydroxypteridines are the only simple pteridines yet submitted to reduction. 6-Hydroxypteridine, heated with sodium amalgam (4%) at 60°, gives 7 : 8-dihydro-6-hydroxypteridine in 85% yield.[17] 7-Hydroxypteridine, boiled with alkaline sodium hydrosulphite (dithionite), gives a 95% yield of 4-amino-5-carboxymethylaminopyrimidine (XIII) which cyclises to 5 : 6-dihydro-7-hydroxypteridine (XII) when refluxed with N-hydrochloric acid.[17] Hydrogenation of 7-hydroxypteridine in alkali, with a platinum catalyst, gives a similar result. Xanthopterin takes up two atoms of hydrogen in 0·1N-sodium hydroxide or acetic acid (palladium or

[80] Boothe *et al.*, *J. Amer. Chem. Soc.*, 1948, **70**, 27.
[81] Amer. Cyanamid Co., B.P. 646,149/1950.
[82] Uyeo and Mizukami, *Japan. J. Pharm. Chem.*, 1949, **21**, 237; per *Chem. Abs.*, 1952, **46**, 119.
[83] Purrmann, *Annalen*, 1941, **548**, 284.
[84] Polonovski and Pesson, 1st Intern. Congr. Biochem., Cambridge, 1949, p. 231.
[85] Pesson, *Bull. Soc. chim.*, 1948, **15**, 963.
[86] Polonovski, Pesson, and Puister, *Compt. rend.*, 1950, **230**, 2205.

platinum catalysts), but leucopterin and *iso*xanthopterin (2-amino-4 : 7-dihydroxypteridine) could not be hydrogenated.[79] 2-Amino-4-hydroxypteridine-6-carboxylic acid (and its 7-methyl analogue) take up four atoms under similar conditions, whereas folic acid (XVIII*a*, p. 222) takes up four atoms over platinum, but only two over palladium.[79]

Xanthopterin is reduced to dihydroxanthopterin by zinc dust (in acid or in alkali),[70] sodium hydrosulphite (dithionite),[87] or fuming hydriodic acid ; [88] xanthopterin-7-carboxylic acid also gives a dihydro-compound with hydriodic acid.[83] The constitution of dihydroxanthopterin has been proved to be 2-amino-7 : 8-dihydro-4 : 6-dihydroxypteridine by an unambiguous synthesis.[89] *iso*Xanthopterin (2-amino-4 : 7-dihydroxypteridine) is also reduced to a dihydro-compound by fuming hydriodic acid, but, on four-fold dilution with water, the iodine oxidises the dihydro-derivative back to *iso*xanthopterin.[88] Ichthyopterin, which is the corresponding 6-carboxymethyl derivative,[68] behaves similarly.[43] However, 7-hydroxypteridine, when reduced by hydriodic acid, is not re-oxidised on dilution.[17]

Hydrogenation of 4-hydroxy-2 : 6 : 7-trimethylpteridine over nickel gives a yellow (*i.e.*, β-, see above) dihydro-compound which passes on further hydrogenation to a tetrahydro-compound.[84] In air, the tetrahydro-derivative is oxidised to the original dihydro-compound. That these yellow compounds are 7 : 8-dihydropteridines has been shown [86] by the unambiguous synthesis of one of them, 2-ethoxy-7 : 8-dihydro-4-methyl-6 : 7-diphenylpteridine. This was made by condensing 2 : 4-dichloro-6-methyl-5-nitropyrimidine and desylamine (α-benzoylbenzylamine) and reducing the product to 2-chloro-7 : 8-dihydro-4-methyl-6 : 7-diphenylpteridine which was converted into the 2-ethoxy-analogue by sodium ethoxide.

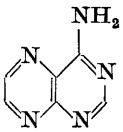
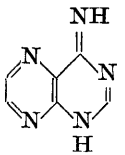
When the orientation of a di- or a tetra-hydropteridine made by alkaline reduction is unknown, it is usual to assume that hydrogenation has occurred exclusively in the pyrazine ring because pyrazines are readily hydrogenated,[90] but pyrimidines only with difficulty,[91, 92] under alkaline conditions. 5-Formyltetrahydrofolic acid (the " citrovorum factor ") is hydrogenated entirely in the pyrazine ring.[93]

The replacement of hydroxy-groups by hydrogen is dealt with in section 10.

9. Reactions of Aminopteridines.—It is becoming increasingly clear that the amino-derivatives of six-membered nitrogenous heteroaromatic rings prefer the normal amino-structure (XVI) to the imino-structure (XVII) which was at one time thought better to represent α- and γ-amino-derivatives. *X*-Ray crystallography has demonstrated that 2-amino-6-chloro-4-methyl-

[87] Koschara, *Z. physiol. Chem.*, 1936, **240,** 127.
[88] Wieland, Tartter, and Purrmann, *Annalen*, 1940, **545,** 209.
[89] Boon and Leigh, *J.*, 1951, 1497.
[90] Krems and Spoerri, *Chem. Reviews*, 1947, **40,** 279.
[91] Lythgoe and Rayner, *J.*, 1951, 2323.
[92] Brown and Johnson, *J. Amer. Chem. Soc.*, 1923, **45,** 2702.
[93] Pohland, Flynn, Jones, and Shive, *ibid.*, 1951, **73,** 3248 ; Allen, Pasternak, and Seaman, *ibid.*, 1952, **74,** 3264.

pyrimidine,[94] 6-aminopurine,[95] and 2 : 4 : 6-triamino-1 : 3 : 5-triazine [96] have NH_2· (but no NH:) groups. These determinations apply only to the solid state. However, fairly convincing spectrographic evidence has been put forward that 2- and 4-aminopyridine have the —NH_2 structure in solution.[97] That the aminopteridines also have this structure is strongly suggested by the spectra of 2-, 4-, and 6-monoaminopteridine which closely resemble those of the corresponding dimethylamino-analogues (see Fig. 6 ; also refs. 6, 7) whose structures present no ambiguity. The spectra of these tertiary amines resemble those of the primary amines in detail but shifted to longer wavelengths as with the pair aniline–dimethylaniline.[47] 7-Aminopteridine is still unknown but some *C*-substituted 7-aminopteridines have recently been prepared.[98]

(XVI)   (XVII)

The amino-group in 4-, 6-, and 7-aminopteridines forms part of an amidine system. Hence it does not behave as an aromatic amino-group (it cannot be diazotised and coupled) but, as would be expected, is fairly easily hydrolysed by acids and alkalis. The 2-amino-group is (as part of a guanidine system) less subject to hydrolysis by acid or alkali, but is often susceptible to hydrolysis by nitrous acid.

Acid hydrolysis. 6-Amino- and 6-dimethylamino-pteridine are rapidly hydrolysed by cold 0·01N-hydrochloric acid, in fact too rapidly for accurate spectra of the cations to be plotted on a photoelectric instrument.[17] 2- and 4-aminopteridine are not readily attacked by N-hydrochloric acid at 20° ; the 2-isomer is radically destroyed at 100° (as in 2-amino-6 : 7-diethylpteridine also) and the 4-isomer is converted at 100° into 4-hydroxypteridine somewhat faster than the acid can destroy the latter.[6, 7]

2 : 4-Diaminopteridine is converted into 2-amino-4-hydroxypteridine (56% yield) and 2 : 4-dihydroxypteridine (63% yield) by refluxing it with 6N-hydrochloric acid for ½ and 30 hours respectively. 4-Amino-2-hydroxypteridine similarly gives 2 : 4-dihydroxypteridine (90% yield) in 20 minutes.[99]

Alkaline hydrolysis. This is apt to be gentler and is often preferred as a preparative method. Refluxing with 0·01N-sodium hydroxide for 3 minutes converts 4-aminopteridine entirely into 4-hydroxypteridine,[6] but 4-amino-6 : 7-diethylpteridine requires longer heating. This is consistent with other evidence that electron-donating groups make the acid and

[94] Clews and Cochran, *Acta Cryst.*, 1948, **1**, 4.
[95] Broomhead, *ibid.*, p. 324.
[96] Hughes, *J. Amer. Chem. Soc.*, 1941, **63**, 1737 ; Knaggs and Lonsdale, *Proc. Roy. Soc.*, 1940, *A*, **177**, 140.
[97] Anderson and Seeger, *J. Amer. Chem. Soc.*, 1949, **71**, 340.
[98] (*a*) Dr. G. M. Timmis and Mr. G. Spickett, personal communication ; (*b*) Prof. H. N. Rydon, personal communication.
[99] Taylor and Cain, *J. Amer. Chem. Soc.*, 1949, **71**, 2538.

alkaline hydrolysis of $—NH_2$ to —OH more difficult in the pteridine series (see section 6 for the stability of the amino-group in xanthopterin and leucopterin which have two and three powerfully electron-donating groups respectively). 2-Aminopteridine is rapidly destroyed by boiling 2·5N-sodium hydroxide, but 2-amino-6 : 7-diethylpteridine is unaffected after two hours.[7]

2 : 4-Diamino-7- and -6-methylpteridine are hydrolysed to the 2-amino-4-hydroxy-analogues in excellent yields by boiling N-sodium hydroxide.[100] Aminopterin (the 4-amino-analogue of folic acid, XVIII*a*) is deaminated to this acid by refluxing it with N-sodium hydroxide for 6 hours (50% yield), the 2-amino-group remaining unaffected. Folic acid had been similarly obtained from its 4-piperidyl- and 4-dimethylamino-analogues.[101]

Thus in ease of hydrolysis by either acid or alkali, $4\text{-}NH_2 > 2\text{-}NH_2$.

Hydrolysis by nitrous acid. The reluctant acid hydrolysis of 2-amino-groups can sometimes be greatly speeded by the addition of sodium nitrite.[99, 102] 2-Amino-4-hydroxypteridine gives 2 : 4-dihydroxypteridine (50% yield) in 5 minutes. The surprising conclusion was drawn from this reaction that the 2-amino-group in pteridine has the $—NH_2$ structure and the 4-amino-group is actually $=NH$.[99] As is pointed out above, the presence of a $=NH$ group is unlikely ; moreover reactions involving covalent bonds seldom shed much light upon structural problems.

Nitrous acid destroys 2- and 4-aminopteridine [6, 40] and xanthopterin,[103] successfully deaminates the 2-amino-group of rhizopterin [104] and leucopterin,[71, 72] and is without effect on 4-amino-2 : 6 : 7-trihydroxy-, 2-amino-4-hydroxy-6 : 7-diphenyl-, and 2 : 4-diamino-pteridine.[99]

Replacement reactions. The 4-amino-group can be replaced by exchange with secondary and tertiary amines. 2 : 4-Diamino-6 : 7-dimethylpteridine gives 2-amino-4-2′-hydroxyethylamino-6 : 7-dimethylpteridine quantitatively when refluxed with 2-hydroxyethylamine. 2 : 4-Diamino-6 : 7-diphenylpteridine behaves similarly with hydroxyethylamine and benzylamine, and 6 : 7-dimethyl-2 : 4-bismethylaminopteridine gives a 64% yield of 4-2′-hydroxyethylamino-6 : 7-dimethyl-2-methylaminopteridine.[105] It has been shown that such transaminations involve ring-opening and reclosure.[105a]

4-Aminopteridine resists conversion into 4-chloropteridine by the action of sodium nitrite in 10N-hydrochloric acid, a method which often succeeds in the purine series ; [106] nor is it converted into 4-mercaptopteridine by hydrogen sulphide at pH 4 or by boiling N-potassium hydrogen sulphide.[6]

2- and 4-Amino-groups are stable to phosphorus oxychloride and pentachloride, provided that electron-donating substituents are present to stabilise the nucleus (for examples see refs. 60, 72, 78).

Acylation. 2- and 4-Aminopteridines are acetylated in good yields by

[100] Seeger, Cosulich, Smith, and Hultquist, *J. Amer. Chem. Soc.*, p. 1753.
[101] Roth, Smith, and Hultquist, *ibid.*, 1950, **72**, 1914.
[102] Tschesche and Korte, *Chem. Ber.*, 1951, **84**, 801.
[103] Schöpf and Kottler, *Annalen*, 1939, **539**, 128.
[104] Wolf *et al.*, *J. Amer. Chem. Soc.*, 1947, **69**, 2753.
[105] Taylor and Cain, *ibid.*, 1951, **73**, 4384.
[105a] Taylor, *ibid.*, 1952, **74**, 1651.
[106] Kögl, Want, and Salemink, *Rec. Trav. chim.*, 1948, **67**, 29.

refluxing them with acetic anhydride for a few minutes.[6] 2 : 4-Diamino-6- and -7-methylpteridine similarly give diacetyl derivatives,[57] but 2 : 4-diamino-6 : 7-diphenylpteridine gives only a monoacetyl compound, of undetermined orientation, after 16 hours' refluxing [107] (addition of sulphuric acid facilitated diacetylation, a 68% yield being obtained after 1 hour at 100°). Leucopterin cannot be acetylated by acetic anhydride, alone or with pyridine; here again sulphuric acid overcomes the difficulty.[72] Hydroxyl groups are not acetylated under these conditions if attached directly to the pteridine skeleton.

Acetamidopteridines are lower-melting and more soluble in all solvents than the amines from which they are derived (*e.g.*, ref. 6). Some of them have been hydrolysed quantitatively by 0·3N-sodium hydroxide at room temperature (36 hours).[62]

Xanthopterin has been *N*-benzoylated by being heated at 200° with benzoic anhydride,[104] and 2-amino-4-hydroxy-7-hydroxymethylpteridine by the Schotten–Baumann method.[108]

N-Alkylation had not yet been attempted.

10. Reactions of Hydroxy-, Methoxy-, and Mercapto-pteridines.—Hydroxypteridines. It has been suggested above that the hydroxypteridines may exist principally in the " pteridone " state (*e.g.*, VII), *i.e.*, as vinylogous acid amides. Nevertheless, their readiness to tautomerise enables them to react also as hydroxy-compounds.

Although phosphorus oxychloride, sulphuryl chloride, and thionyl chloride have been found of no use for conversion of OH into Cl in this series,[17, 72] a mixture of phosphorus oxychloride and pentachloride is successful in the following cases: 6-hydroxypteridine,[17] 2 : 4 : 6 : 7-tetrahydroxypteridine [109] (into tetrachloropteridine), 2-amino-4 : 6 : 7-trihydroxypteridine [72] (into 2-amino-4-chloro-6 : 7-dihydroxypteridine), 4-amino-2 : 6 : 7-trihydroxypteridine [78] (into 4-amino-2-chloro-6 : 7-dihydroxypteridine), 2-amino-4 : 7-dihydroxypteridine-6-carboxylic acid [60] (into the 7-chloro-analogue), 2-amino-4-hydroxy-6 : 7-diphenylpteridine [58] and 2 : 4-dihydroxy-6 : 7-diphenylpteridine [105] (into the 2 : 4-dichloro-analogue) (in 65, 55, 60, ?, 80, 81, and 92% yields respectively). This mixture, however, destroys 2-, 4-, and 7-hydroxy- and 2 : 4-dihydroxy-pteridine. The use of diethylaniline (so valuable in catalysing similar pyrimidine chlorinations) has not yet been found helpful in this series.[40]

The conversion of OH into NH_2 has been little studied. 7-Hydroxypteridine cannot be converted into 7-aminopteridine by heating it with ammonia in *p*-cresol [17] or in alcohol (copper-catalysed) [110] at 200°, but 4-hydroxy-2-mercapto-6 : 7-dimethylpteridine is transformed into the 2 : 4-bismethylamino-analogue (85% yield) by alcoholic methylamine at 190° (18 hours).[105]

Phosphorus pentasulphide destroys 7-hydroxypteridine,[17] and the only

[107] Cain, Taylor, and Daniel, *J. Amer. Chem. Soc.*, 1949, **71**, 892.
[108] Backer and Houtman, *Rec. Trav. chim.*, 1948, **67**, 260.
[109] Schöpf, Reichert, and Riefstahl, *Annalen*, 1941, **548**, 82.
[110] Dr. D. J. Brown, personal communication.

example of replacement of —OH by —SH is indirect: 4-hydroxypteridine can be converted into 4-mercaptopteridine by alkaline hydrolysis to 2-aminopyrazine-3-carboxyamide;[7] this can be transformed into the thioamide which gives 4-mercaptopteridine with ethyl orthoformate.[6]

The hydroxy-group, when attached to the pteridine nucleus, has so far resisted all attempts at acetylation or benzoylation,[6, 17, 72] but hydroxymethyl-groups can be readily acetylated.[62, 108]

Methylation of hydroxypteridines has seldom been attempted. 7-Hydroxypteridine and diazomethane give an *N*-methyl-7-pteridone and only a trace of 7-methoxypteridine.[17] An excess of diazomethane apparently causes *C*-methylation of this pteridone [17], a phenomenon already known in the purine series.[111] Methylation of 2 : 4 : 7-trihydroxypteridine with methyl sulphate (at 35° and pH 8) gave a mono-*N*-methyl derivative of assumed but incompletely proved orientation.[68] Leucopterin with diazomethane gives a mixture of two trimethyl derivatives which are much more soluble in alcohol and water than the starting material.[13] Eight years earlier Wieland and his collaborators stated [72] that leucopterin did not react with diazomethane: this earlier failure is ascribed to absence of water.

The replacement of OH by H has been accomplished in several ways. For example, OH can sometimes be converted into Cl (see above), which is then readily removed with hydrogen iodide (section 11).

Sodium amalgam specifically removes a hydroxyl group from the 7-position of leucopterin (2-amino-4 : 6 : 7-trihydroxypteridine [51, 52]) and also from 6 : 7-dihydroxy- [17] and 4 : 6 : 7-trihydroxy-pteridine.[40] The yields are high, but the reaction seems to depend on the presence of a 6-hydroxy-group because 4 : 7-dihydroxy-6-methylpteridine does not react in this way (it even resists sodium in glycol at 180°).[40, 112] The products of the sodium amalgam reaction are 7 : 8-dihydropteridines which are readily dehydrogenated by potassium permanganate (section 8). This addition of hydrogen to the 7- and the 8-position doubtless occurs after the 7-hydroxy-group has been eliminated, because 6 : 7-dihydroxy- and 6-hydroxy-pteridine are both converted into 7 : 8-dihydro-6-hydroxypteridine by sodium amalgam. It is strange that leucopterin was originally stated not to react with sodium amalgam,[72] whereas it is now the standard practice to prepare xanthopterin from leucopterin by this reaction.

A hydroxy-group can be removed from the 2-position of 2 : 4-dihydroxypteridine by alkaline hydrolysis to 2-aminopyrazine 3-carboxylic acid, which is converted first into the amide and then (by ethyl orthoformate) into 4-hydroxypteridine.[6] The 6-hydroxy-group was removed from 2-amino-4 : 6 : 7-trihydroxypteridine by electrolysis in 75% sulphuric acid with lead electrodes (5 amp. for 5 hours): the yield of *iso*xanthopterin was only 13%.[78]

Methoxypteridines. These are too inaccessible to have found much use as intermediates. 2-, 4-, and 6-Methoxypteridine are hydrolysed to hydroxypteridines by N-sodium hydroxide (2 hours at 20°, or 1 minute at 100°).[7]

[111] Biltz and Sauer, *Ber.*, 1931, **64**, 752.
[112] Albert, Brown, and Wood, unpublished.

7-Methoxypteridine has only been obtained in traces but appears to behave similarly.[17]

Mercaptopteridines. No method is yet known for replacing SH by H. Refluxing with Raney nickel in aqueous ammonia smoothly effects this change in the pyrimidine series,[113] but leaves 4-amino-2-mercaptopteridine unchanged and destroys 2-mercapto- and 4-hydroxy-2-mercapto-pteridine.

Cold alkaline hydrogen peroxide converts SH into OH in the following 2-mercaptopteridines: [114] 4-hydroxy-, 4-hydroxy-6 : 7-diphenyl-, and 6-carboxy-4 : 7-dihydroxy- (yields, about 35%).

Thiols can also be converted into amines. 4-Amino-2-mercapto-6 : 7-dimethylpteridine, heated with alcoholic dimethylamine at 215°, gives 2 : 4-bisdimethylamino-6 : 7-dimethylpteridine (21% yield); again, 4-amino-2-mercapto-6 : 7-diphenylpteridine, heated with 2-hydroxyethylamine or benzylamine, gives 2 : 4-bis-2′-hydroxyethylamino- and 2 : 4-bisbenzylamino-6 : 7-diphenylpteridines (100 and 80% yield respectively).[105]

Two 2-mercaptopteridines have been alkylated: the 4-hydroxy- and 4-hydroxy-6 : 7-diphenyl-derivatives. Ethyl bromide was used, in sodium ethoxide (under reducing conditions). Surprisingly, the alkyl group became attached to sulphur, as was confirmed by direct synthesis.[114] No yields have been recorded.

11. Reactions of Chloropteridines.—Replacement of Cl by H has, so far, always been effected by hydriodic acid (*d* 1·7), for example, at 100° for 5—10 minutes; acetic acid has sometimes been used as a diluent. The most successful example of this method is 2-amino-4-chloro-7 : 8-dihydroxypteridine [88] (85% yield); chlorine has also been removed from the 2- [78] and the 7-position [60] of other pteridines.

2-Chloro-7 : 8-dihydro-6-hydroxypteridine is converted into dihydro-6-hydroxypteridine (90% yield) by hydriodic acid (*d* 1·7) and red phosphorus at 160° (one hour); the 4-chloro-isomer behaves similarly.[115] Hydrogenation with Raney nickel, even in the presence of sodium hydroxide, does not remove the chlorine from these substances.[116]

Chloropteridines are sensitive to hydrolysis. Of the three known monochloropteridines, the 4-isomer is fairly readily hydrolysed (to 4-hydroxypteridine) by cold water, and the 6-isomer is only a little less sensitive; the 2-isomer is readily hydrolysed by boiling, but not cold, water. These reactions seem to be autocatalysed by the hydrogen chloride liberated,[6, 7, 17] but alkaline hydrolyses have also been recorded. 2 : 4 : 6 : 7-Tetrachloropteridine was hydrolysed to (presumed) 2 : 4-dichloro-6 : 7-dihydroxypteridine by warm 0·2N-sodium hydroxide, and to tetrahydroxypteridine by ON-sodium hydroxide when heated at 140° for 6 hours.[109] 2-Amino-4-chloro-6 : 7-dihydroxypteridine is stable to hot, dilute acid and alkali but gives 2-aminotrihydroxypteridine with boiling 6N-sodium hydroxide.[72, 88]

6-Chloropteridine is converted into 6-methoxypteridine by methanolic

[113] *e.g.* Brown, *J. Soc. Chem. Ind.*, 1950, **69**, 353.
[114] Polonovski, Vieillefosse, and Pesson, *Bull. Soc. chim.*, 1945, **12**, 78.
[115] Boon, Jones, and Ramage, *J.*, 1951, 96.
[116] Dr. G. W. H. Cheeseman, personal communication.

sodium methoxide at 20° (53% yield).[17] 2-Chloro-7 : 8-dihydro-4-methyl-7 : 8-diphenylpteridine is converted into the 2-ethoxy-analogue by sodium ethoxide.[86]

6-Chloropteridine reacts with ammonia in benzene at 20°, to give 6-aminopteridine (53% yield) ; [17] one chlorine atom of 2 : 4 : 6 : 7-tetrachloropteridine is converted into NH_2 by ammonia in ether (33% yield).[109] 2 : 4-Dichloro-6 : 7-dimethylpteridine gives the 2 : 4-bisdimethylamino-analogue when heated with dimethylamine at 110° for 8 hours (no yield given), and 2 : 4-dichloro-6 : 7-diphenylpteridine gives the 2 : 4-bisbenzylamino-analogue when heated under reflux with benzylamine for 3 hours (87% yield).[105]

Profound decomposition occurred when the preparation of 6-mercaptopteridine was attempted by warming 6-chloropteridine with thiourea or sodium hydrogen sulphide.[17]

For the reactions of chloromethylpteridines, see the following section.

12. Reactions of Carbon-containing Substituents.—Methyl. Because they are α to ring nitrogen atoms, all the methyl groups in pteridines should be activated, as in quinaldine and lepidine.

Several brominations of 6-methyl(ene) groups are known. 2-Amino-4 : 7-dihydroxy-6-methylpteridine gives the monobromo-derivative when heated at 100° for 2 minutes with bromine in acetic and sulphuric acids (no yield given).[117] 2-Amino-4-hydroxy-6-pteridinylacetic acid, similarly treated at 20°, gives the α-bromo-derivative in 70% yield.[67] 7-Acetonyl-4 : 6-dihydroxy-2-aminopteridine diacetate is brominated at 30° in acetic acid (30% yield).[41] 2-Amino-4-hydroxy-6-methylpteridine, heated with excess of bromine in hydrobromic acid at 100°, gives the dibromo-derivative in 45% yield ; [63] considerable amounts of the monobromo-analogue are formed in this synthesis and also when the same substance is heated in an autoclave at 150° with bromine.[80] This 6-methylpteridine is chlorinated to a small extent with sulphuryl chloride, when benzoyl peroxide is used as catalyst.[80]

Oxidation of 2-amino-4 : 7-dihydroxy-6-methylpteridine to the corresponding 6-aldehyde (67% yield) can be accomplished with selenium dioxide.[22, 117]

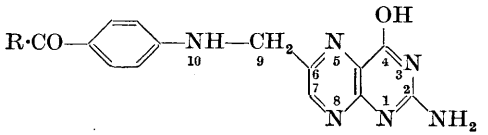
Various 6- and 7-methyl- and 6 : 7-dimethyl-pteridines (bearing either OH or NH_2 groups in the 2- and the 4-position) have been oxidised to the corresponding carboxylic acids by alkaline potassium permanganate at 100° (approx. 80% yields).[59, 60, 118] However, 4 : 7-dihydroxy-6-methylpteridine was half destroyed and the rest left unchanged by this procedure.[40] The latter pteridine did not condense with benzaldehyde in boiling acetic anhydride.[40]

Hydroxymethyl. This group is readily acetylated,[62, 108] halogenated by thionyl chloride, [119] and oxidised to CO_2H at 90° by alkaline potassium permanganate.[62] 2-Amino-4 : 6-dihydroxy-7-(1-hydroxy-2-ketopropyl)-

[117] Tschesche, Köhncke, and Korte, *Z. Naturforsch.*, 1950, **5** *b*, 132.
[118] Wittle, O'Dell, Vandenbelt, and Pfiffner, *J. Amer. Chem. Soc.*, 1947, **69**, 1786.
[119] Roche Products Ltd., B.P. 624,394/1949.

pteridine is oxidised to the 7-aldehyde by periodic acid.[41] The oxidation of longer side-chains (*e.g.*, sugar alcohols) in the 6- or 7-position to CO_2H has been accomplished with hot alkaline permanganate,[16, 120] and to CHO with periodic acid [16, 121] or lead tetra-acetate.[122] A similar periodic acid oxidation once gave rise to a $—CH_2·CHO$ group.[121]

Aminomethyl. No simple example has been studied. Pteroylglutamic acid (XVIII*a*) has been decomposed in various ways: aerobic alkaline hydrolysis [123] or oxidation by chlorine [118] gave 2-amino-4-hydroxypteridine-6-carboxylic acid; cleavage by sulphurous acid gave 2-amino-4-hydroxydihydropteridine-6-aldehyde; [63, 69] anaerobic acid hydrolysis gave 2-amino-4-hydroxy-6-methylpteridine; [123] ultra-violet light produced 2-amino-4-hydroxypteridine-6-aldehyde.[124]



(XVIII)

(*a*) R = $NH·CH(CO_2H)·CH_2·CH_2·CO_2H$
(*b*) R = OH
(*c*) R = $[NH·CH(CO_2H)·CH_2·CH_2·CO]_3·OH$

Halogenomethyl. 2-Amino-6-chloromethyl- [119] and 2-amino-6-bromomethyl-4-hydroxypteridine [117] condense readily with ethyl *p*-aminobenzoate and diethyl *p*-aminobenzoylglutamate, *e.g.*, in ethylene glycol [80, 117, 125] at 100—140° or in cold formic acid.[119] 2-Amino-α-bromo-4-hydroxy-6-pteridylacetic acid readily combines with *p*-aminobenzoylglutamic acid at 18° (40% yield), with loss of carbon dioxide.[67]

2-Amino-7-(1-bromo-2-ketopropyl)-4 : 6-dihydroxypteridine exchanges Br for OAc in sodium acetate solution, and this group is hydrolysed to OH by hot water.[41]

2-Amino-6-dibromomethyl-4-hydroxypteridine is hydrolysed to the corresponding aldehyde by boiling water (70% yield).[63]

Aldehyde. 2-Amino-4-hydroxypteridine-6-aldehyde is oxidised to the corresponding acid at 100° with an excess of alkaline hydrogen peroxide (80% yield); [16] 2-amino-4 : 6-dihydroxypteridine-7-aldehyde gives the corresponding acid with potassium permanganate in very dilute alkali at 20°.[41]

These amino-hydroxy-aldehydes readily give phenylhydrazones [22, 63, 117] and oximes,[63] also anils with *p*-aminobenzoic acid [117] and aniline.[63]

2-Amino-4-hydroxypteridine-6-aldehyde undergoes the Cannizzaro reaction (5 days in 2·5N-sodium hydroxide, 5°), giving 2-amino-4-hydroxypteri-

[120] Forrest and Walker, *J.*, 1949, 2077.
[121] Weygand, Wacker, and Schmied-Kowarzik, *Chem. Ber.*, 1949, **82**, 25.
[122] Petering and Weisblat, *J. Amer. Chem. Soc.*, 1947, **69**, 2566.
[123] Stokstad *et al.*, *ibid.*, 1948, **70**, 5.
[124] Lowry, Bessey, and Crawford, *J. Biol. Chem.*, 1949, **180**, 389.
[125] Tschesche, Köhncke, and Korte, *Chem. Ber.*, 1951, **84**, 485.

dine-6-carboxylic acid and 2-amino-4-hydroxy-6-hydroxymethylpteridine in good yield.[63] In the presence of sodium sulphite, the dihydro-derivative of this aldehyde is formed,[63] and this undergoes alkaline dismutation to a mixture of the above acid and 2-amino-4-hydroxy-6-methylpteridine.[69]

The anil of 2-amino-4-hydroxypteridine-6-aldehyde and *p*-aminobenzoylglutamic acid can be hydrogenated to pteroylglutamic acid.[121, 125]

Carboxylic acids. Hydroxy- and amino-pteridine-7-carboxylic acids (and 6 : 7-dicarboxylic acids) are readily esterified at 20° by hydrogen chloride in methanol (average yields, 75%).[59, 118] 2-Amino-4 : 7-dihydroxypteridine-6-carboxylic acid was converted into its acid chloride by reaction with phosphorus pentachloride at 20° for two days.[126]

Decarboxylation of pteridine-6-carboxylic acids is usually effected by heating the dry substance in a stream of nitrogen at 260° for half an hour.[83, 102] Dihydropteridine-6-carboxylic acids are decarboxylated at lower temperatures.[127] No way of decarboxylating pteridine-7-carboxylic acids has yet been found without previous dihydrogenation followed by oxidation after decarboxylation.[83] Pteridine-6 : 7-dicarboxylic acids readily give the corresponding 7-monocarboxylic acids when heated in boiling quinoline for an hour.[59] 6-Pteridylacetic acids have been decarboxylated at 250—280°,[102, 117, 125] but some of them are decarboxylated completely in boiling dilute mineral acid within a few minutes, notably 4 : 7-dihydroxy-6-pteridylacetic acid [98b] and its 2-amino-derivative [102] (cf. also ref. 64).

2-Amino-4-hydroxy-6-pteridylacetic acid is oxidised by alkaline permanganate at 75° to the 6-carboxylic acid.[60] The bromination of pteridylacetic acids is discussed on p. 221.

Hofmann, Curtius, or Schmidt degradations do not appear to have been attempted in the pteridine series. No acid amides are known.

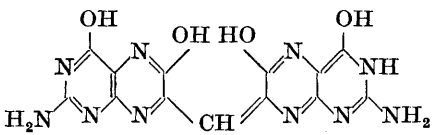
Dipteridylmethines. Oxidation, in air, of crude natural xanthopterin (2-amino-4 : 6-dihydroxypteridine), which contains the 7-methyl homologue as an impurity, produces a red-violet substance named, at first, rhodopterin and later pterorhodin, whose constitution is now known to be (XIX).[42] It can be better prepared by heating xanthopterin, in N-sulphuric acid, with acetaldehyde (or acetone) and hydrogen peroxide.[42] It arises, in traces, when xanthopterin is acetylated with acetic anhydride. Condensation of *iso*xanthopterin with methylxanthopterin or with methyl*iso*xanthopterin gives the two expected isomerides. Similar substances have apparently been prepared by the reaction of glyceraldehyde with 2 : 4 : 5-triamino-6-hydroxypyrimidine and of 2-acetamido-4-hydroxy-6-methylpteridine with 2-acetamido-4-hydroxy-7-methylpteridine.[61]

Pterorhodin is reduced by sodium amalgam to a tetrahydro-derivative, is oxidised by hydrogen peroxide to a mixture of leucopterin and xanthopterin-7-carboxylic acid, and is cleaved by chlorine water to oxaloylguanidine.[128]

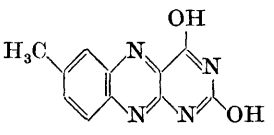
13. The Synthesis of Pteridines.—From what has been written above

[126] Woolley and Pringle, *J. Biol. Chem.*, 1948, **174**, 327.
[127] Elion and Hitchings, *J. Amer. Chem. Soc.*, in the press.
[128] Purrmann and Maas, *Annalen*, 1944, **556**, 186.

(sections 7—12), it will be realised that metathetical reactions cannot always be depended upon to convert an easily synthesised pteridine into one bearing the required substituent(s). For this reason, it is often expedient to effect any necessary exchange of substituents in the pyrazine or pyrimidine intermediates from which pteridines are commonly synthesised.



(XIX)



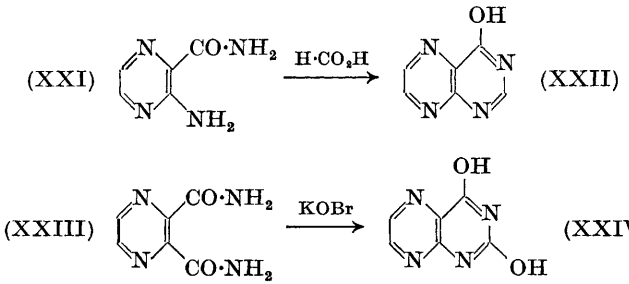
(XX)

Syntheses from more complex substances. " Toluallоxazine " (2 : 4-dihydroxy-2′-methyl-6 : 7-benzopteridine) (XX) was oxidised by alkaline permanganate to 2 : 4-dihydroxypteridine-6 : 7-dicarboxylic acid, which was decarboxylated in two stages to 2 : 4-dihydroxypteridine (XXIV).[129] No yield was mentioned and no other example of this approach is known. It is of special interest as being the first preparation of a pteridine to be described, antedating any other synthesis by 12 years. The " toluallоxazine " was prepared from alloxan and *o*-phenylenediamine.

The removal of 2- and 7-hydroxy-groups from dihydroxypteridines has already been mentioned (section 10).

Syntheses from pyrazines. 4-Hydroxypteridine (XXII) is formed in 75% yield by heating 2-aminopyrazine-3-carboxyamide (XXI) with ethyl orthoformate and acetic anhydride under reflux.[6] Similarly, 2-aminopyrazine-3-carboxythioamide gives 4-mercaptopteridine (84% yield).[6] These syntheses are of interest to biochemists because the 2-position can be labelled isotopically. When formic acid replaces the ester, the yield falls to 65%. The required amides can be prepared by a series of reactions beginning with the oxidation of quinoxaline or, better, the alkaline hydrolysis of 2 : 4-dihydroxypteridine (which is easier to prepare from pyrimidine intermediates than is 4-hydroxypteridine).[6]

The only other known pyrazine synthesis is the preparation of 2 : 4-dihydroxypteridine (XXIV) (in 40% yield) by the action of potassium hypo-



(XXI) (XXII)

(XXIII) (XXIV)

[129] Kühling, *Ber.*, 1895, **28**, 1968.

bromite on pyrazine-2 : 3-dicarboxyamide (XXIII) [130] which is obtained by the oxidation of quinoxaline.[131]

Syntheses from pyrimidines. (a) *The Isay reaction.* The first example of the synthesis of a pteridine by condensing a 4 : 5-diaminopyrimidine with a 1 : 2-dicarbonyl compound was Isay's preparation (in 1906) of 6 : 7-diphenylpteridine from 4 : 5-diaminopyrimidine and benzil.[132] The reaction was more intensively studied by Sachs and Meyerheim [133] in 1908 and has since become the route most frequently used for the preparation of pteridines. All pyrimidines containing primary amino-groups in both the 4- and the 5-position appear to be suitable for use in this reaction. 4 : 5-Diaminopyrimidine itself, although until recently a rare chemical, is now more easily accessible.[134] At the same time, an improved synthesis of formamidine [134] makes more readily available a series of 4 : 5-diaminopyrimidines unsubstituted in the 2-position.

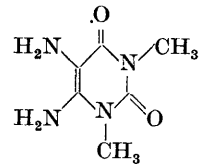
The dicarbonyl compounds which have been used include a dialdehyde (glyoxal),[135] aldehydo-ketones (phenylglyoxal,[135] methylglyoxal,[135] hydroxymethylglyoxal,[62, 108] glucoreductone [16]), diketones (diacetyl [133, 135] dipropionyl,[7] triketopentane,[133] dihydroxytartaric acid,[135] benzil,[56, 57, 58] and substituted benzils,[58] thirteen higher homologues of dipropionyl,[136] dehydroascorbic acid,[41] and glucosone [120]), an aldehydo-acid (glyoxylic acid [137] and the more accessible ethyl glyoxylate hemiacetal [17]), keto-acids (mesoxalic acid,[64, 83, 133] pyruvic acid,[64, 133, 138, 139] ethyl oxalopropionate,[102] ethyl oxalosuccinate,[102] ethyl $\alpha\gamma$-diketovalerate,[41, 102] ethyl oxaloacetate [42, 64, 102, 117, 125]), a dibasic acid (oxalic acid [17, 78, 138–141]), and a potential aldehydo-acid (dichloroacetic acid [12, 133]).

The use of *N*-substituted pyrimidine intermediates such as 4 : 5-diamino-1 : 4-dihydro-2 : 6-diketo-1 : 3-dimethylpyrimidine (XXV) has been much explored [85, 133, 142] for the synthesis of 1- and 3-alkyl-2- and -4-pteridones (*e.g.*, XXVI). Some 8-ethyl-7-pteridones (*e.g.*, XXVII) have been made by heating 2 : 5-diamino-4-ethylamino-6-hydroxypyrimidine with oxalic acid or benzil, and a 8-glycosyl analogue was prepared similarly.[141]

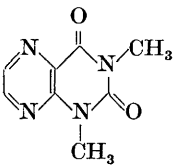
The Isay reaction can be carried out under neutral, acid, or alkaline conditions: neutral condensations usually give the best yields. Glyoxal is used as the commercial 35—50% syrupy solution or as the sodium hydrogen sulphite compound. The latter apparently gives rise to dihydropteridine-

[130] Gabriel and Sonn, *Ber.*, 1907, **40**, 4857.
[131] Jones and McLaughlin, *Org. Synth.*, 1950, **30**, 86.
[132] Isay, *Ber.*, 1906, **39**, 250.
[133] Sachs and Meyerheim, *ibid.*, 1908, **41**, 3957.
[134] Brown, *J. Appl. Chem.*, 1952, **2**, 239, 202.
[135] Kuhn and Cook, *Ber.*, 1937, **70**, 761.
[136] Campbell, Dunsmuir, and Fitzgerald, *J.*, 1950, 2743.
[137] Koschara, *Z. physiol. Chem.*, 1943, **277**, 159.
[138] Elion and Hitchings, *J. Amer. Chem. Soc.*, 1947, **69**, 2553.
[139] Gal, *ibid.*, 1950, **72**, 3532, 5315.
[140] Purrmann, *Annalen*, 1940, **544**, 182; Bertho and Bentler, *ibid.*, 1950, **570**, 127.
[141] Forrest, Hull, Rodda, and Todd, *J.*, 1951, 3.
[142] von Euler, Brandt, and Neumüller, *Biochem. Z.*, 1935, **281**, 206; Roth *et al.*, *J. Amer. Chem. Soc.*, 1951, **73**, 2864.

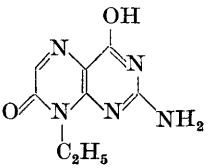
sulphonic acids which have to be decomposed to pteridines with strong acid or alkali.[6] When highly sensitive products are expected (pteridine, chloropteridines, methoxypteridines) it is best to carry out the condensation in methanol or ethanol using the polymerised glyoxal which is found as a deposit in old bottles of the syrup.[6, 7] A fairly satisfactory alternative is syrupy glyoxal in phosphate buffer (1·5M; pH 7).[7]



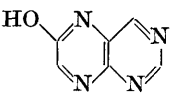
(XXV)



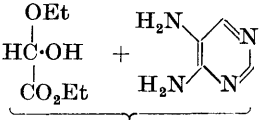
(XXVI)



(XXVII)



(XXVIII)



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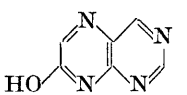


(XXX)

When the dicarbonyl compound, in Isay's reaction, is not symmetrical, two isomers can arise. This leads to difficulties in separation and identification. It is known that when the dicarbonyl compound is an acid (or ester) highly acidic conditions tend to favour the formation of 6-hydroxypteridines, whereas mildly acid or neutral conditions tend to favour that of 7-hydroxypteridines.[17, 64, 83] For example, 4 : 5-diaminopyrimidine and the hemiacetal of ethyl glyoxalate (XXIX) give a mixture of 6- (XXVIII) and 7-hydroxypteridine (XXX) in a 3 : 1 ratio when condensed in 2N-sulphuric acid (pH = − 0·25). However, at pH 6 the ratio was reversed to 1 : 3. The separation, in this example, depended on the higher basic strength of

TABLE 5. *Dependence of yields of isomeric hydroxypteridines on acidity* [64]

Pyrimidine	Reagent	pH 5 (acetate buffer)		pH − 0·25 (2N-sulphuric acid)	
		6-OH	7-OH	6-OH	7-OH
Triamino-6-hydroxy-	$CH_3{\cdot}CO{\cdot}CO_2H$	42%	10%	76%	0%
,,	$CO_2Et{\cdot}CO{\cdot}CH_2{\cdot}CO_2Et$	(trace)	40	69	11
,,	$CO(CO_2Et)_2$	0	85	42	29
Tetra-amino- . .	$CH_3{\cdot}CO{\cdot}CO_2H$	13	37	92	3
,, . . .	$CO_2Et{\cdot}CO{\cdot}CH_2{\cdot}CO_2Et$	0	66	49	22
,, . . .	$CO(CO_2Et)_2$	0	87	0	90

6-hydroxypteridine.[6] Some other relevant figures are given in Table 5. In this Table, pyruvic acid and ethyl oxaloacetate are shown as giving the same product; actually ethyl oxaloacetate gives the ethyl esters of hydroxy-

pteridylacetic acids which become hydrolysed and decarboxylated under vigorous conditions. However, it is possible to isolate 7-hydroxy-6-pteridylacetic acids (or their esters) under milder conditions.[66, 117]

It is obvious from Table 5 that the orientation depends on electronic influences exerted by both the pyrimidines and the dicarbonyl compounds.

It is legitimate to infer that an isomer possesses a 7-hydroxy-group if its yield is considerably decreased (and that of the other isomer increased) by carrying out the condensation in sulphuric acid instead of acetate buffer. But it is not legitimate to ascribe the 7-orientation to the major product of a reaction at pH 5—7. For example, 4 : 5-diamino-6-hydroxypyrimidine and ethyl glyoxylate hemiacetal give 4 : 6-dihydroxypteridine almost quantitatively at pH 7, 5, or 0 [40] (note also the first example of Table 5).

The reason for the orientating effect of acidity in these condensations has been attributed [64] to the known higher basic strength of a 4- compared with a 5-aminopyrimidine.[28] Thus the 4-amino-group of a 4 : 5-diaminopyrimidine accepts a proton in acetate buffer and hence the 5-amino-group is in a better state for acylation.[132, 143–146] It is further assumed [64] that in 2N-sulphuric acid both groups accept protons, but this does not explain why acylation still takes place, even if in a different sense. A comprehensive explanation has yet to be put forward which will deal also with the behaviour of the aldehyde (or ketone) group. Clearly, high acidity is no barrier to anil formation in this series because glyoxal condenses as readily with 4 : 5-diaminopyrimidines in 2N-sulphuric acid [56] as in water. It had been commonly assumed,[83, 147] without experimental backing, that aldehydes condense preferentially with the 5-amino-group of 4 : 5-diaminopyrimidines. However, recent evidence points to greater preference for the 4-amino-group.[148] It would be interesting to know how changes in pH affect this preference, possibly by influencing the uptake of a proton by the aldehyde or ketone group, which can be followed spectroscopically. Anils from 4 : 5-diaminopyrimidines with *two* molecules of glyoxal have been isolated.[127] They are rapidly cyclised to pteridines in alkaline solution.

When the asymmetrical dicarbonyl compound is neither an acid nor an ester, the most successful attempts to influence orientation have involved the use of aldehyde- and ketone-binding reagents (such as hydrazine and sodium hydrogen sulphite), the use of which tends to force an alkyl group into the 6-position. For example, tetra-aminopyrimidine and methylglyoxal give 2 : 4-diamino-7-methylpteridine (63% yield ; only product isolated) in 0·25N-hydrochloric acid ; but, in aqueous sodium sulphite and sodium bisulphite, only a trace of this material was produced (5% yield), and the 6-methyl isomer (65% yield) predominated.[100] Again triamino-6-hydroxypyrimidine and methylglyoxal give 2-amino-4-hydroxy-6-methylpteridine if the methylglyoxal is first allowed to react with hydrazine in an

[143] Wilson, *J.*, 1948, 1157.
[144] Traube, *Ber.*, 1900, **33**, 3035.
[145] Levene and Senior, *J. Biol. Chem.*, 1916, **25**, 617.
[146] Hitchings and Elion, *J. Amer. Chem. Soc.*, 1949, **71**, 467.
[147] Traube and Nithack, *Ber.*, 1906, **39**, 227.
[148] King and Spensley, *Nature*, 1949, **164**, 574 ; *J.*, 1952, 2144.

acetate buffer,[120] but the 7-methyl isomer is obtained (in 4N-hydrochloric acid) if hydrazine is omitted.[60, 120] Similarly, this pyrimidine and glucosone give the 6- or the 7-tetrahydroxybutyl analogue depending on whether hydrazine is present or absent, both experiments being conducted in an acetate buffer.[16] Other examples where the 7-isomer is obtained in the absence of carbonyl-binding reagents are the preparation of 2 : 4-dihydroxy-7-methylpteridine from 4 : 5-diamino-2 : 6-dihydroxypyrimidine and methylglyoxal, and of 2-amino-4-hydroxy-7-hydroxymethylpteridine from triamino-6-hydroxypyrimidine and hydroxymethylglyoxal (both reactions were done in neutral solution).[149] It will be gathered from the above notes on conditions, that pH plays no important part in orientating these condensations.

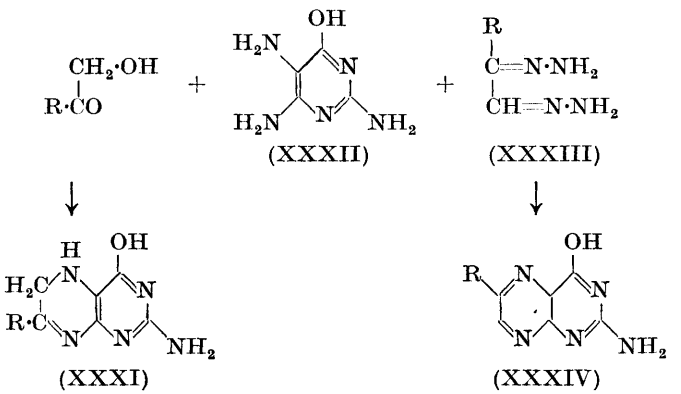
1 : 1-Dichloroacetone (which may be regarded as a simple derivative of a 1 : 2-aldehydo-ketone) and triamino-6-hydroxypyrimidine gave 2-amino-4-hydroxy-6-methylpteridine in an acetate buffer (53% yield). $\omega\omega$-Dichloroacetophenone gave the 6-phenyl analogue (60% yield).[148]

Isomers of unknown orientation, produced by the above or by the following reaction, are identified either by alkaline degradation to a substituted 2-aminopyrazine-3-carboxylic acid [7, 59, 120, 149] or by reactions, described under (c) below, which give products of unambiguous orientation.[115, 150, 151, 152] A hydroxy-group that can be eliminated by sodium amalgam may safely be assumed to be in the 7-position (section 10), and a carboxylic acid which is decarboxylated below 300° may be assumed to be in the 6-acid (section 12) : but in neither case does failure to react necessarily signify the alternative orientation.

(b) *Syntheses from α-substituted carbonyl compounds.* Aldehydo- and keto-alcohols react with triamino-6-hydroxypyrimidine (XXXII) (but not, so far as is known, with monosubstituted derivatives of 4 : 5-diaminopyrimidine). The nature of the products was the subject of much controversy in 1948—49 ; [120] the following summarises the present position. The most common reaction products are 7-alkyl-2-amino-5 : 6-dihydro-4-hydroxypteridines (XXXI) which are spontaneously oxidised, in air, to the corresponding pteridines. In this way, (XXXII) gives 2-amino-4-hydroxy-7-methylpteridine with hydroxyacetone (acetol) [120] and also with dihydroxyacetone. In the latter case loss of the elements of water takes the place of dehydrogenation.[120] Glucose and fructose react simultaneously by both mechanisms, each giving a mixture of the same 2-amino-4-hydroxy-7-tri- and -tetra-hydroxybutylpteridine.[16, 120] In the presence of hydrazine, glucose and fructose give the 6-tetrahydroxybutyl isomer,[16] and this has been attributed to intermediate formation of an osazone (XXXIII), which enables dehydrogenation to precede formation of the pteridine ring.[16] Acetol, incubated with hydrazine, gives a mixture of the 6- and the 7-methyl

[149] Weijlard, Tishler, and Erickson, *J. Amer. Chem. Soc.*, 1945, **67**, 802.
[150] Timmis, *Nature*, 1949, **164**, 139.
[151] Timmis and Spickett, Abs. 12th Internat. Congr. Pure Appl. Chem., 1951, p. 423.
[152] Boon and Jones, *J.*, 1951, 591.

analogue; dihydroxyacetone gives a mixture of the 6- (principally) and the 7-hydroxymethyl analogue and a trace of a methyl analogue.[62, 120] All the reactions mentioned in this paragraph were conducted in an acetate buffer



Aromatic keto-alcohols (*e.g.*, benzoin) give pairs of stable isomeric dihydropteridines: 7 : 8-dihydropteridines are formed in the presence of acetic acid and different (apparently 5 : 6-)dihydropteridines in its absence (see section 8).[84—86]

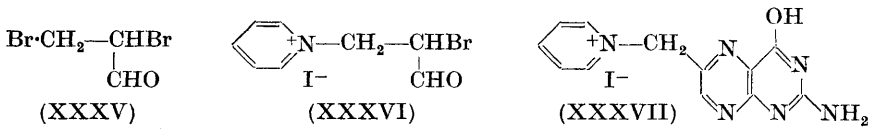
The dehydrating (as opposed to dehydrogenating) type of closure, as exemplified above by dihydroxyacetone, has its parallel in the liberation of hydrogen chloride (or bromide) when triamino-6-hydroxypyrimidine reacts with 1 : 3-dichloroacetone, $\alpha\beta$-dichloropropaldehyde, and α-bromotetronic acid. Similarly glyceraldehyde eliminates water: in each case 2-amino-4-hydroxy-7-methylpteridine is formed.[153] Diacetoxyacetone, diaminoacetone, 1 : 3-di-(*p*-carboxyformanilido)acetone and *p*-tolyl-D-*iso*glucosamine all take part in similar elimination reactions in hydrazine-free condensations.[120, 121, 154]

Neither the Isay nor the aldehydo alcohol synthesis has proved suitable for the preparation of 2-amino-6-chloromethyl-4-hydroxypteridine, which is a desirable intermediate for the manufacture of pteroylglutamic acid (XVIII*a*). One of the most successful syntheses [155] of this acid depends on the simultaneous condensation at pH 4 of *p*-aminobenzoylglutamic acid, triamino-6-hydroxypyrimidine, and $\alpha\beta$-dibromopropaldehyde (XXXV). Apparently one molecule of *p*-aminobenzoylglutamic acid condenses with the aldehyde group, thus favouring condensation of the 2-bromo-atom with the 5-amino-group of the pyrimidine (*i.e.*, like other aldehyde-binding agents, it favours the formation of 6-substituted pteridines). A further molecule of the acid combines with the 3-bromo-atom, and at some stage two hydrogen atoms are lost, apparently by disproportionation. The yield is poor (15%) but can be improved if an oxidising agent is present throughout.[81, 82] If

[153] Angier *et al.*, *J. Amer. Chem. Soc.*, 1948, **70**, 3029.
[154] Weygand, Wacker, and Schmied-Kowarzik, *Experientia*, 1948, **4**, 427.
[155] Waller *et al.*, *J. Amer. Chem. Soc.*, 1948, **70**, 19.

the three reactants are not present simultaneously, 7-substituted pteridines are sometimes obtained.[155]

1-(2-Bromo-2-formylethyl)pyridinium iodide (XXXVI), which is readily obtained from (XXXV) and pyridine, reacts with (XXXII) to give 1-(2-amino-4-hydroxy-6-pteridylmethyl)pyridinium iodide (XXXVII). The latter condenses with *p*-aminobenzoylglutamic acid at 140° to give pteroylglutamic acid.[156]



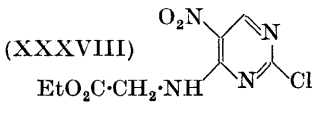
Reductone (2 : 3-dihydroxyacraldehyde) condenses with methyl *p*-aminobenzoate to give methyl *p*-(2 : 3-dihydroxyprop-2-enylideneamino)benzoate which combines with (XXXII) to give pteroic acid [157] (diethyl *p*-aminobenzoylglutamate similarly gives pteroylglutamic acid [158]). One school of thought believes that the synthesis of pteroylglutamic acid in Nature proceeds along these lines [157, 159] (for an alternative view, see Tschesche *et al.*[160]).

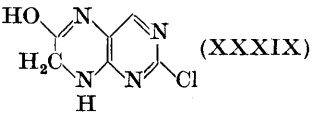
An attempt has been made to use chloroacetic acid in condensations of this kind: however, with triamino-6-hydroxypyrimidine it gave 2 : 4-diamino-6-hydroxy-*p*-oxazinopyrimidine[127] formerly (but mistakenly) known as "*β*-dihydroxanthopterin".[146]

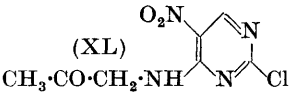
(c) *Unambiguous syntheses.* 2 : 4-Dichloro-5-nitropyrimidine condenses with the ethyl ester of glycine to give ethyl 2-chloro-5-nitro-4-pyrimidylaminoacetate (XXXVIII). This may be catalytically reduced and then cyclised by boiling water, to give 2-chloro-7 : 8-dihydro-6-hydroxypteridine (XXXIX) in an overall yield of 30%.[115, 161] 2 : 4-Dichloro-6-methyl-5-nitro-, 4 : 6-dichloro-5-nitro-, 4 : 6-dichloro-2-methyl-5-nitro-, 2- and 4-amino-6-chloro-5-nitro-, and 2-amino-4-chloro-6-methyl-5-nitro-pyrimidine react similarly,[115, 161] and so does 2-amino-4-chloro-6-hydroxy-5-phenylazopyrimidine.[89] Chlorine atoms at the (XXXVIII) stage can be hydrolysed to hydroxyl groups (by an acetate buffer) or exchanged for amino-groups (primary or substituted). Chlorine atoms in the dihydropteridines can be easily removed by hot hydriodic acid and phosphorus ; [115] dehydrogenation to pteridines is readily effected with cold alkaline potassium permanganate (80% yield).[17]

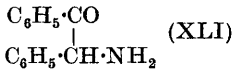
Because of the flexibility of this reaction, it is likely to be used extensively for the preparation of various 6-hydroxypteridines and for determining the orientation of 6-hydroxypteridines prepared by Isay's reaction.

[156] Hultquist *et al.*, *J. Amer. Chem. Soc.*, 1948, **70**, 23.
[157] Forrest and Walker, *J.*, 1949, 2002.
[158] Angier *et al.*, *J. Amer. Chem. Soc.*, 1948, **70**, 25.
[159] O'Meara, McNally, and Nelson, *Lancet*, 1947, II, 747.
[160] Tschesche, Korte and Korte, *Z. Naturforsch.*, 1951, **6**, *b*, 304.
[161] Polonovski and Jérôme, *Compt. rend.*, 1950, **230**, 392.





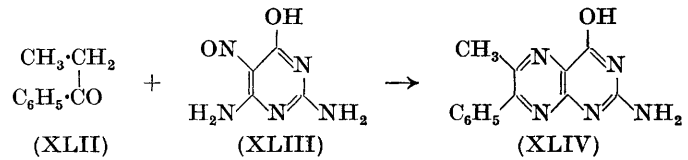




4-Chloro-5-nitropyrimidines similarly react with aminoacetone to give 5-nitro-4-pyrimidylaminoacetones (*e.g.*, XL), some of which can be converted into 7 : 8-dihydro-6-methylpteridines by hydrogenation over Raney nickel.[152] Desylamine (XLI) [86] and ω-aminoacetophenone [152] react similarly. 2-Amino-4-chloro-6-hydroxy-5-phenylazopyrimidine is similarly transformed into 2-amino-7 : 8-dihydro-4-hydroxy-6-methylpteridine.[89]

4-Amino-5-nitrosopyrimidines (*e.g.*, XLIII) condense readily with ketones containing active, adjacent methylene and carbonyl groups [such as ethyl phenyl ketone (XLII) and benzyl phenyl ketone], to give pteridines such as (XLIV).[150] At present, the scope of this reaction is limited by the lack of monosubstituted (and unsubstituted) 4-amino-5-nitrosopyrimidines.

Somewhat similarly, two molecules of benzoin can be condensed with one of a 4-amino-5-nitrosopyrimidine to give a 6 : 7-diphenylpteridine.[151] An intermediate in this reaction may be the $N_{(5)}$-oxide of the pteridine, which is reduced by the second molecule of benzoin.



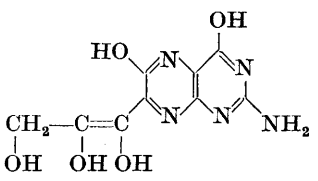
Benzyl cyanide (and its derivatives) condense with (XLIII), giving 2 : 7-diamino-4-hydroxy-6-phenylpteridines in about 50% yields.[98a] Phenylacetyl chloride and its derivatives similarly give 2-amino-4 : 7-dihydroxy-6-phenylpteridines.

14. The Naturally Occurring Pteridines.—The first naturally occurring pteridine whose constitution was established was leucopterin. It was found, in 1940, by degradation and synthesis,[140] to be 2-amino-4 : 6 : 7-trihydroxypteridine. The structures of xanthopterin (2-amino-4 : 6-dihydroxypteridine) [12] and *iso*xanthopterin (2-amino-4 : 7-dihydroxypteridine) [83] were determined in the same year; that of pterorhodin (XIX) [41, 42] in 1944, then erythropterin (XLV),[41] chrysopterin (7-methylxanthopterin),[162] and ichthyopterin (6-carboxymethyl*iso*xanthopterin),[102] all in 1951. A provisional formula (XLVI) has been put forward for urothion.[163]

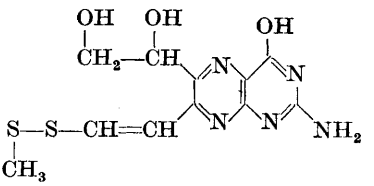
The name " folic acid " had been coined in 1944 to designate a substance, present in leaves and mammalian organs, which was able to stimulate the

[162] Tschesche and Korte, *Chem. Ber.*, 1951, **84**, 641.
[163] Koschara, *Chemie*, 1943, **56**, 195; *Z. physiol. Chem.*, 1943, **279**, 44.

growth of the bacterium, *Streptococcus fæcalis* R (*S. lactis*).[163a] In 1946 a substance of similar biological properties was isolated from liver and was shown by degradation and synthesis to be pteroylglutamic acid (XVIII*a*) [164] [the name pteroic acid was devised at this time as a convenient expression for 2-amino-6-(*p*-carboxyanilinomethyl)-4-hydroxypteridine (XVIII*b*)]. Syntheses of pteroic acid and pteroylglutamic acid have been described in sections 12 and 13. Pteroylglutamic acid proved to be the first of a series of naturally occurring pteridines with 6-*p*-carboxyanilinomethyl side chains.



(XLV)



(XLVI)

Certain species of the bacterium *Corynebacterium* were found to produce a substance termed " the fermentation *L. casei* factor " which could be hydrolysed to pteroylglutamic acid and was subsequently shown to be pteroyl-γ-L-glutamyl-γ-L-glutamyl-L-glutamic acid (XVIII*c*).[165–167] It differs from pteroylglutamic acid in having very little growth-promoting effect on *Streptococcus fæcalis* R. It has been prepared synthetically in quantity and used in medicine under the name " Teropterin ". A substance obtained from yeast, termed " vitamin B_c conjugate ", and having almost no bacteria-stimulating activity, was shown to be hexa-L-glutamylpteroyl-L-glutamic acid.[168] It is not known for certain that the γ-orientation exists throughout this polypeptide side chain.

A substance known as rhizopterin was obtained from *Rhizopus nigrans* and found to be a growth-stimulant for *Strept. fæcalis* R, but not for *L. casei*. It was shown to be 10-formylpteroic acid (XLVII).[169]

It was found in 1948 that the bacterium *Leuconostoc citrovorum* would grow on a synthetic medium containing liver extract.[170] The liver extract could be replaced by an optically active substance obtained by hydrogenating 10-formylpteroylglutamic acid over platinum and autoclaving the product.[171] It was later shown that the autoclaving caused a migration of the formyl

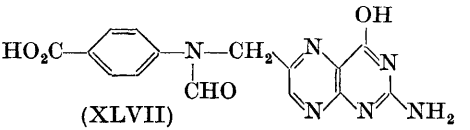
[163a] Mitchell, Snell, and Williams, *J. Amer. Chem. Soc.*, 1944, **66**, 267.
[164] Angier *et al.*, *Science*, 1946, **103**, 667.
[165] Boothe *et al.*, *J. Amer. Chem. Soc.*, 1948, **70**, 1099; *Trans. N.Y. Acad. Sci.*, 1948, **10**, 70.
[166] Boothe *et al.*, *J. Amer. Chem. Soc.*, 1949, **71**, 2304.
[167] Angier *et al.*, *ibid.*, 1950, **72**, 74.
[168] Pfiffner, Calkins, Bloom, and O'Dell, *ibid.*, 1946, **68**, 1392.
[169] Wolf *et al.*, *ibid.*, 1947, **69**, 2753.
[170] Sauberlich and Baumann, *J. Biol. Chem.*, 1948, **176**, 165.
[171] Shive, Bardos, Bond, and Rogers, *J. Amer. Chem. Soc.*, 1950, **72**, 2817.
[172] Polonovski, Busnel, and Pesson, *Helv. Chim. Acta*, 1946, **29**, 1328.
[173] Raven, *Z. physiol. Chem.*, 1951, **288**, 10.
[174] Cosulich *et al.*, *J. Amer. Chem. Soc.*, 1951, **73**, 5006.

TABLE 6. *Glossary of synonyms*

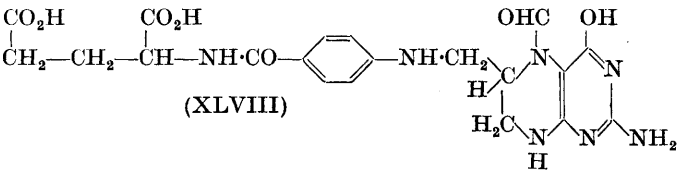
Name	Nature	Ref.
Amethopterin †	10-Methyl derivative of aminopterin	100
Aminoanfol †	4-Amino-analogue of pteroylaspartic acid, *i.e.*, *N*-(*p*-2 : 4-diamino-6-pteridylmethyl-aminobenzoyl)aspartic acid	
Aminopterin †	4-Amino-analogue of pteroyl-L-glutamic acid	100
Anhydroleucopterin *	*iso*Xanthopterin	83
Aporhizopterin	Pteroic acid (XVIII*b*)	169
Chrysopterin	7-Methylxanthopterin	162
Citrovorum factor	(XLVIII)	93, 174
Desiminoleucopterin *	2 : 4 : 6 : 7-Tetrahydroxypteridine	
Desiminoxanthopterin *	2 : 4 : 6-Trihydroxypteridine	
6-Desoxyleucopterin *	2-Amino-6 : 7-dihydroxypteridine	
8-Desoxyleucopterin *	*iso*Xanthopterin	
β-Dihydroxanthopterin	2 : 4-Diamino-6-hydroxy-*p*-oxazino-pyrimidine	127
Diopterin †	Pteroyl-α-L-glutamyl-L-glutamic acid	165—167
Erythropterin	(XLV)	41
Fermentation *L. casei* factor	Pteroyl-γ-L-glutamyl-γ-L-glutamyl-L-glutamic acid	165—167
Fluorescyanine	Apparently the same as ichthyopterin	172
Folic acid	Most frequently used as a synonym for pteroylglutamic acid but originally applied to a naturally occurring mixture of analogous substances. The plural is sometimes used as a group name for naturally occurring derivatives of pteroic acid	155
Folinic acid SF	Citrovorum factor (XLVIII)	93, 174
Folvite †	An unrefined pteroyl-L-glutamic acid	
Guanopterin *	*iso*Guanine (a purine)	
Ichthyopterin	2-Amino-6-carboxymethyl-4 : 6-dihydroxy-pteridine	102
Lepidoporphyrin *	Pterorhodin (XIX)	
Leucopterin	2-Amino-4 : 6 : 7-trihydroxypteridine	13, 140
*iso*Leucopterin *	4-Amino-2 : 6 : 7-trihydroxypteridine ; name formerly used for a fraction of crude leucopterin, now known to be ordinary leucopterin	72, 78
Leucovorin †	(XLVIII)	93, 174
Liver *L. casei* factor	Pteroyl-L-glutamic acid (XVIII*a*)	60, 66
Lumazine	2 : 4-Dihydroxypteridine	135
Mesopterin	*iso*Xanthopterin	
Methylpteridine red	A mixture	61
Norite-eluate factor	Pteroyl-L-glutamic acid	123
Pteridoxamine	2-Amino-4-hydroxypteridine	173
Pterins	Naturally occurring pteridines	
Pteroic acid	(XVIII*b*)	66
Pterorhodin	(XIX)	42, 128
Pteroylglutamic acid	Pteroyl-L-glutamic acid (XVIII*a*)	66
Rhizopterin	10-Formylpteroic acid (XLVII)	169
Rhodopterin *	Pterorhodin (XIX)	42, 128
Teropterin †	Pteroyl-γ-L-glutamyl-γ-L-glutamyl-L-glutamic acid	165—167
Uropterin *	Xanthopterin	
Urothion	(XLVI)	163
Vitamin B_c	Pteroyl-L-glutamic acid (XVIII*a*)	118
Vitamin B_c conjugate	Hexa-(L-glutamyl)pteroyl-L-glutamic acid	168
Xanthopterin	2-Amino-4 : 6-dihydroxypteridine	12
*iso*Xanthopterin	2-Amino-4 : 7-dihydroxypteridine	83

* These names are passing out of use. † Trademarks.

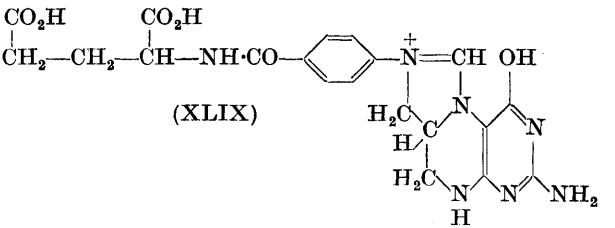
group from the 10- to the 5-position and that the structure of the final product is apparently (XLVIII).[93] This substance is known as the " citrovorum factor," leucovorin, or folinic acid SF (pteroyl-L-glutamic acid is only faintly stimulating to this *Leuconostoc*). The citrovorum factor is stable in 0·1N-sodium hydroxide (6 hours at 90°),[93] but in acid solution (2 hours at pH 1) it gives the pteridinoglyoxalinium compound (XLIX), a reaction which is reversed in alkaline solution.[174, 175]



(XLVII)



(XLVIII)



(XLIX)

It will be noted that all known naturally occurring pteridines have an amino-group in the 2-position and a hydroxy-group in the 4-position. This combination produces a high degree of insolubility (see section 2). The presence of the 4-hydroxy-group confers a high avidity for bivalent metals (see section 4). Metal-containing pteridines should accordingly be sought in Nature : the usual techniques of isolation (extraction with strong acid or alkali) are such as would break up metallic complexes. The citrovorum factor, alone of the known natural pteridines, has the 5-position blocked and hence is not capable of chelation.

Table 6 is a glossary of synonyms of pteridines, both natural and synthetic. A number of synonyms which are now seldom used have been included to assist the study of literature before 1941 : these are marked with an asterisk.

15. The Biological Importance of Pteridines.—Growth promotion. Jacobson's discovery that the cells of mammals make use of the citrovorum factor (XLVIII) for purposes of division [176, 177] has brought to light the most

[175] May *et al.*, *J. Amer. Chem. Soc.*, 1951, **73**, 3067.

[176] Jacobson, Intern. Congr. Cell Biology, New Haven, U.S.A., 1950 ; Trans. 13th Conf. on Problems of Ageing, New York, 1951.

[177] *Idem*, *J. Path. Bact.*, 1952, **64**, 245.

important known function of pteridines in Nature. Every kind of mammalian cell so far investigated behaves in this way. Apparently the cells of mammals acquire pre-formed pteroylglutamic acid (from food and intestinal bacteria) and convert it into the citrovorum factor by reductive formylation.[178] This process can be blocked by bringing the cells into contact with a minute amount of aminopterin (the 4-amino-analogue of pteroylglutamic acid) and it was by using this antagonist that the role of the citrovorum factor was discovered.[176, 177] As Fig. 9 shows, aminopterin brings cell division to an end in the second stage of the mitotic cycle, with the result that all the chromosomes are arrested in metaphase and are unable to undergo normal splitting (anaphase). This arrest in mitosis can be reversed by supplying the cells with a slight excess of the citrovorum factor, but

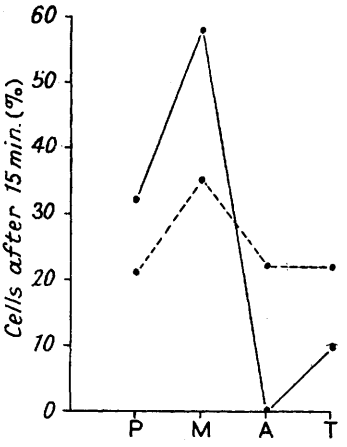


FIG. 9

Effect of aminopterin in depriving bone-marrow cells of folic acid essential for the anaphase stage of division (Jacobson [176]).

– – – *Control* ——— *Aminopterin*, 0·05%
P = *Prophase* *M* = *Metaphase*
A = *Anaphase* *T* = *Telephase*

pteroylglutamic acid is ineffective.[177] This work is likely to advance knowledge of the chemical basis of growth and reproduction. Already evidence has been obtained that the citrovorum factor causes nucleoprotein to be shed into the cytoplasm and this initiates the metaphase–anaphase transformation.[179]

Xanthopterin, which is the yellow colouring matter present in the bands of wasps, is by no means confined to the insect world. There are indications that it may be the natural organiser responsible for the development of primitive cells into the typical cells of the kidney.[180] When mammals (various species) are injected with xanthopterin, the kidneys enlarge to many times their normal size, although they remain normal in function. It

[178] Nichol and Welch, *Proc. Soc. Exp. Biol. N.Y.*, 1950, **74**, 52, 403.
[179] Jacobson and Webb, *Exp. Cell Research*, 1952, **3**, 163.
[180] Haddow, *Brit. Med. Bull.*, 1947, **4**, 338.

has been found that xanthopterin directly stimulates mitosis and cell division in the epithelium of the renal tubules. When the injections are stopped, the kidneys slowly revert to normal size. 2-Amino-4-hydroxypteridine has a very similar effect, but further simplification of the xanthopterin molecule produces relatively inert substances.[181]

Minute doses of pteroylglutamic acid rapidly cure the anæmia of pregnancy,[182] and the macrocytic anæmia caused by certain kinds of surgical interference with the small intestine.[183] In these two conditions the cobalt-containing substance known as vitamin B_{12} is ineffective. Pteroylglutamic acid is also highly effective in pernicious anæmia, but not so completely curative as vitamin B_{12}. The citrovorum factor is no more effective than pteroylglutamic acid in pernicious anæmia.[184]

Large quantities of pteridines have been found in the wings of the *Pierid* butterflies. For example, leucopterin forms 4% of the weight of the cabbage butterfly's wings. These pteridines are probably transformation products of other pteridines concerned in cell division, and the large amounts found correspond to the exceedingly rapid growth of these insects which must lack the normal mechanism for breaking down the pteridine nucleus.[185]

Many natural biochemical reactions seem to be facilitated by pteridines.[186] Injection of formic acid (marked with ^{14}C) into rats deprived of pteroylglutamic acid led to formation of only one-tenth as much liver protein as in normal rats similarly treated with formic acid. Amino-acids, isolated from this and other proteins of the treated rats, have the specific radioactivity concentrated principally in the β-carbon atom of serine, $HO{\cdot}CH_2{\cdot}CH(NH_2){\cdot}CO_2H$.[187]

There are already some indications that different pteridines are required by different species of organism. For example, the citrovorum factor is only half as active as pteroylglutamic acid for *Streptococcus fæcalis*. It is surprising that the growth of such familiar pathogens as *E. coli* and *Staph. aureus* and *pneumococci*, when suppressed by sulphonamides, can be restored competitively by *p*-aminobenzoic acid but not at all (or at all events only competitively) by pteroylglutamic acid [188] or by the citrovorum factor.[189]

Growth inhibition. Many pteridines have been found to inhibit growth by acting as analogues of other pteridines concerned in normal metabolism. The subject of folic acid antagonists as antibacterials and anti-cancer agents was reviewed in 1950.[190]

[181] Dr. A. Haddow and Dr. E. Horning, personal communication.
[182] Ungley and Thompson, *Brit. Med. J.*, 1950, I, 919.
[183] Watson and Witts, *ibid.*, 1952, I, 13.
[184] Cartwright, Wintrobe, Broquist, and Jukes, *Proc. Soc. Exp. Biol. N.Y.*, 1951, **78,** 563.
[185] Dr. W. Jacobson, personal communication.
[186] Williams, *J. Biol. Chem.*, 1951, **191,** 123; Sakami, *ibid.*, 1948, **176,** 995; Holland and Meinke, *ibid.*, 1949, **178,** 7; Rodney, Swendseid, and Swanson, *ibid.*, 1949, **179,** 19.
[187] Plaut, Betheil, and Lardy, *ibid.*, 1950, **184,** 795.
[188] Woods, *Ann. New York Acad. Sci.*, 1950, **52,** Art. 8, 1199.
[189] Dr. D. D. Woods, personal communication.
[190] Snell and Wright, *Ann. Rev. Biochem.*, 1950, **19,** 277.

In leukæmia (cancer of the bone-marrow) the citrovorum factor content of the leucocytes is abnormally high.[191] Even before this was known, the use of pteridines in the treatment of acute leukæmia in children was introduced [192] and these are still among the most effective substances in prolonging the life of the patient. The most commonly used substances are aminopterin and amethopterin (see Table 6). Although these very poisonous analogues of pteroylglutamic acid can temporarily arrest the pathological process, the marrow-cells usually become resistant after a few months.[193] It seems likely that resistance follows the accumulation of enzymes capable of changing NH_2 to OH in the 4-position and effecting demethylation in the 10-position, thus producing pteroylglutamic acid.[194]

Only a few of the simpler pteridines exhibit significant growth-retarding properties.[175, 195, 196] These they owe to competition with the folic acids or their precursors. For example, 2 : 4-diamino-6 : 7-diphenylpteridine suppresses the growth of the malarial parasite in chicks infected with *P. gallinaceum*, an effect potentiated by sulphonamides and significantly inhibited by pteroylglutamic acid.[195]

Enzyme inhibition. It has been known since 1944 that xanthine oxidase readily oxidises xanthopterin to leucopterin.[78, 197] Xanthine oxidase is inhibited by pteraldehyde (2-amino-4-hydroxypteridine-6-aldehyde) at such a great dilution (10^{-9}M) [198] as to lead to the hypothesis that this aldehyde may be a natural regulator of the enzyme in the living cell. The corresponding alcohol is no less active.[199] Commercial grades of pteroylglutamic acid ("folic acid") inhibit xanthine oxidase strongly,[200] but not when the pteraldehyde, which is a contaminant, is removed.[197]

[191] Swendseid, Bethell, and Bird, *Cancer Res.*, 1951, **11**, 864.

[192] Farber, Diamond, Mercer, Sylvester, and Wolff, *New England J. Med.*, 1948, **238**, 787.

[193] Burchenal, Johnson, and Waring, *Proc. Soc. Exp. Biol. Med.*, 1951, **78**, 348.

[194] Burchenal, Waring, and Hutchison, *ibid.*, p. 311 ; Kidder, Dewey, and Parks, *ibid.*, p. 88.

[195] Greenberg, *J. Pharmacol.*, 1949, **97**, 484.

[196] Hitchings, Elion, Falco, Russell and Van der Werff, *Ann. N.Y. Acad. Sci.*, 1950, **52**, Art. 8, 1330 ; Daniel, Norris, Scott, and Heuser, *J. Biol. Chem.*, 1947, **169**, 689 ; Daniel and Norris, *ibid.*, 1947, **170**, 747 ; Collier and Waterhouse, *Ann. Trop. Med. Parasit.*, 1950, **44**, 156 ; Collier, Hall, and Waterhouse, *ibid.*, p. 161.

[197] Kalckar, Kjelgaard, and Klenow, *Biochem. Biophys. Acta*, 1950, **5**, 575, 586.

[198] Lowry, Bessey, and Crawford, *J. Biol. Chem.*, 1949, **180**, 389, 399.

[199] Petering and Schmitt, *J. Amer. Chem. Soc.*, 1950, **72**, 2995.

[200] Kalckar and Klenow, *J. Biol. Chem.*, 1948, **172**, 349.