"REDUCTION OF PTERIDINES"

a Thesis
submitted for the

Degree of Doctor of Philosophy

in the

Australian National University

by

Sadao Matsuura

Department of Medical Chemistry
John Curtin School of Medical Research
Institute of Advanced Studies
Australian National University.

The work described in this Thesis was carried out by the candidate at the Australian National University. In the few instances where the work of others has been used, proper acknowledgment is made.

S. Matsunaga
ACKNOWLEDGMENTS.

The author wishes to express his appreciation to Professor Adrien Albert, F.A.A., Head of the Department of Medical Chemistry, for his continuous help and encouragement, and to Dr. D.J. Brown for helpful suggestions and discussions.

He also wishes to express his thanks to the Australian National University for a scholarship during the period March 1958 to March 1961 while on study leave from Nagoya University.
Numbering of the Pteridine Ring.

The numbering of the pteridine ring in this thesis accords with the international rules of nomenclature.

\[
\begin{array}{c}
\text{N} & 5 & 4 \\
6 & \text{N} & 3 \\
7 & \text{N} & 2 \\
8 & \text{N} & 1 \\
\text{(I)}
\end{array}
\]

Recent studies, using physical methods, indicate that hydroxypteridines exist (in the cases studied) in the lactam form, for example (II).

\[
\begin{array}{c}
\text{N} & \text{O} \\
\text{C} & \text{N} & \text{H} \\
\text{N} & \text{N} & \text{H} \\
\text{O} & \text{N} & \text{H} \\
\text{OH} & \text{N} & \text{N} \\
\text{HO} & \text{N} & \text{N} \\
\text{(II)} & \text{(III)}
\end{array}
\]

However, for ease in reference, in this thesis hydroxypteridines are named, in accordance with tradition, i.e. as if they have the lactim form (III). Thus (III) is named 2,4,7-trihydroxypteridine, and not 1,2,3,4,7,8-hexahydro-2,4,7-trioxopteridine.
Summary

Reduction: The fifteen possible hydroxy- and polyhydroxypteridines were reduced, chemically and catalytically. Potassium borohydride, sodium dithionite, and potassium (or sodium) amalgam were used as the chemical reducing agents. Hydrogenation over palladium, platinum, and Raney-nickel were used for catalytic reductions. Except 2,4,7-trihydroxypteridine all these were successfully reduced by at least one of the above methods. Hydroxypteridines lacking a hydroxy group in the pyrazine ring gave tetrahydropteridines and, in some cases, a dihydro-compound as well. Thus 2-hydroxypteridine gave 5,6,7,8-tetrahydro-2-hydroxypteridine and also 3,4-dihydro-2-hydroxypteridine. The latter is the first example of the reduction of a pteridine in the pyrimidine ring.

On the other hand, hydroxypteridines having a hydroxy group in the pyrazine ring gave only dihydro-compounds on reduction. Thus 6-hydroxypteridines gave 7,8-dihydro-6-hydroxypteridines, and 7-hydroxypteridines gave their 5,6-dihydro-derivatives.

Synthesis: The structures of the products, often indicated by ionization constants and ultraviolet spectra, were confirmed by synthesis in most cases.

A synthetic route to the hitherto inaccessible 5,6-dihydropteridines was discovered. This involved reduction
of the Schiff's base of a 4,5-diaminopyrimidine. In addition a method was devised for preparing 5,6,7,8-tetrahydro-4-hydroxypteridine which could not be synthesized by Lister and Ramage's method (cyclization of a 5-amino-4-(benzyl-ß-hydroxyethylamino)-pyrimidine). It is a well-known defect of this method that it cannot be used to make tetrahydrohydroxypteridines. For, if a hydroxy-group is present in the pyrimidine ring before the cyclization, an alternative reaction occurs and gives a glyoxalinopyrimidine in place of the desired tetrahydropteridine. The alternative reaction succeeds because of the tautomeric possibilities of the hydroxy-group. Hence the problem was solved by introducing an ethoxy group at the pyrimidine stage. The ethoxy-group, under the conditions of cyclization, was hydrolysed to a hydroxy-group and gave the desired tetrahydrohydroxypteridine.

Some 7,8-dihydropteridines were required lacking a substituent in the 6-position. But no method for producing such substances has been recorded. However it seemed that 7,8-dihydropteridines, lacking a substituent in the 6-position, could be prepared by condensing aminoacetal and a 4-chloro-5-nitropyrimidine. In practice, a difficulty arose in the last step (cyclization), but this was solved by hydrolysis of the acetal group before the reduction of the
nitro-group. 7,8-Dihydro-2-hydroxypteridine was prepared in this way.

In all thirty six new compounds were prepared and are listed at the end of this summary.

Ionization constants: Ionization constants were measured in order to record precise spectra of single ionic species. Sometimes these constants gave useful clues to structure, e.g. the significant rise of basic strength in 7,8-dihydropteridines compared to the parent pteridines. This rise is undoubtedly caused by extra resonance in the cation exactly as in simple nitrogen heterocyclic compounds having an amino-group in the γ-position (e.g. 4-aminopyridine).

Spectra: Close similarity of the ultraviolet spectra of 5,6,7,8-tetrahydropteridines and of the corresponding 4,5-diaminopyrimidines furnished a convenient confirmation of the structure of tetrahydro-2-hydroxypteridine. A similar relationship confirmed the structure of the alkaline hydrolysis product of dihydro-7-hydroxypteridine, as 4-amino-5-carboxymethylaminopyrimidine. The similarity in ultraviolet spectra of x,y-dihydro-2-hydroxypteridine and of 2-hydroxypteridine (known to be covalently hydrated across the 3,4-bond) confirmed the structure of the former as 3,4-dihydro-2-hydroxypteridine.
New Substances (All analysed)

Pteridine

2-Hydroxy-6-methylpteridine

Dihydropterdines

7,8-Dihydro-4,6-dimethylpteridine
3,4-Dihydro-2-hydroxypteridine
7,8-Dihydro-2-hydroxypteridine
3,4-Dihydro-2-hydroxy-6-methylpteridine
3,4-Dihydro-2-hydroxy-6-methylpteridine
7,8-Dihydro-2-hydroxy-6-methylpteridine
x,y-Dihydro-4-hydroxypteridine
5,6-Dihydro-4-hydroxypteridine
x,y-Dihydro-2,7-dihydroxypteridine
5,6-Dihydro-4,7-dihydroxypteridine

Tetrahydropterdines

8-Benzyl-4-chloro-5,6,7,8-tetrahydropteridine
8-Benzyl-5,6,7,8-tetrahydro-4-hydroxypteridine
5,6,7,8-Tetrahydro-2-hydroxypteridine
5,6,7,8-Tetrahydro-4-hydroxypteridine
5,6,7,8-Tetrahydro-2,4-dihydroxypteridine
Formyltetrahydro-2,4-dihydroxypteridine

Sulphur Containing Products

Sodium tetrahydro-2,4-dihydroxypteridine sulphonate
Sodium tetrahydro-2,7-dihydropterdine sulphonate
Sodium dihydro-2,7-dihydroxypteridine sulphonate
Sodium dihydro-2-hydroxypteridine sulphonate
**Pyrimidines**

4-Acetonylamino-2-hydroxy-5-nitropyrimidine
4-Acetonylamino-6-hydroxy-5-nitropyrimidine
5-Amino-4-(benzyl-β-hydroxyethy lamino)-6-benzyloxypyrimidine hydrochloride
5-Amino-4-(benzyl-β-hydroxyethylamino)-6-chloropyrimidine hydrochloride
5-Amino-4-(benzyl-β-hydroxyethylamino)-6-hydroxypyrimidine
4-Amino-5-cyanomethylaminopyrimidine
4-Amino-5-ethoxycarbonylmethylamino-6-hydroxypyrimidine
4-Amino-5-β-diethoxyethylamino-2-hydroxypyrimidine
4-Amino-5-β-diethoxyethylamino-6-hydroxypyrimidine
5-Amino-4-β-diethoxyethylamino-2-hydroxypyrimidine
5-Bromo-4-phthaloylglycylaminopyrimidine
4-β-Diethoxyethylamino-2-hydroxy-5-nitropyrimidine
4-β-Diethoxyethylamino-6-hydroxy-5-nitropyrimidine
2,4-Diis-β-diethoxyethylamino-5-nitropyrimidine
4-Formylmethylamino-2-hydroxy-5-nitropyrimidine

**Pyrazine**

2-Amino-3-hydroxymethylpyrazine.
CONTENTS

Certificate of originality page
Acknowledgments
Numbering of the pteridine ring
Summary

Section 1 : Introduction
   a. Opening remarks 1
   b. Historical outline 3
   c. Scope of the present work 19

Section 2 : Methods
   a. Methods available for reduction 21
   b. Methods available for diagnosing the positions where hydrogen atoms have entered the molecule 26
   c. Likely positions of hydrogen atoms in reduced hydroxypteridines 35
   d. Method available for syntheses of hydrogenated hydroxypteridines 38

Section 3 : Results and discussions
   a. Reductions 41
   b. Syntheses 67
   c. Ionization constants 98
   d. Spectra 109
Section 4: Experimental

a. Reductions 122

b. Syntheses of starting materials 141

c. Syntheses of hydrogenated pteridines 147

d. Ionization constants 177

e. Spectra 182

Appendix: Ultraviolet and infrared spectra 183

Bibliography 210
SECTION 1.

INTRODUCTION.

a. Opening Remarks.

Many pteridine derivatives such as xanthopterin, leucopterin, isoxanthopterin, erythropterin, chrysopterin, ichthyopterin, biopterin, drosopterin and the folic acid group have been found in nature and their structures have been investigated. In the course of those studies it was found that some of these derivatives have relatively low oxidation reduction potentials and a number of reversible reductions were demonstrated. Some of the reductions are now known to be of great importance for the reproduction of living cells.

The biologically active substance "folic acid" was isolated from liver and yeast (Pfiffner et al., 1943, 1947; Stokstad 1943; Stokstad, Hutchings and Subba Row, 1948) and its structure was shown to be a pteridine derivative (1a-I) (Angier et al., 1945, 1946).
The biological function of folic acid was then investigated, and it was eventually found that it acted as a coenzyme after reduction to 5,6,7,8-tetrahydrofolic acid. In some enzyme systems this functioned in loose combination with formaldehyde giving the so-called "active formaldehyde". In other enzymes it is combined with formic acid ("active formate"). These pteridine coenzymes bring about the transfer of single carbon atom fragments in the biosynthesis of purines, pyrimidines and some amino acids. Careful work has established the metabolic route from folic acid to the 5,6,7,8-tetrahydrofolic acid via a dihydro derivative. The same dihydrofolic acid was also prepared by chemical reduction (O'Dell, Vandenbelt, Bloom and Pfiffner, 1947; Futtermann, 1957) and it is now considered to be the 7,8-dihydro derivative from somewhat indirect chemical analogies (O'Dell et al., 1947) and by enzymic evidence
based on lack of a new centre of asymmetry (Osborn and Huennekens, 1958).

Simpler pteridines have been little investigated from the viewpoint of reduction. By studying the reduction of hydroxypteridines, the present work seeks partly to remedy this state of affairs.

b. Historical Outline.

The earliest work on the reduction of the pteridine ring arose during structural investigations of the natural pigments; xanthopterin (lb-I), isoxanthopterin (lb-II), and leucopterin (lb-III), folic acid (la-I), and other naturally occurring pteridines.
Although the structure of xanthopterin was not known until 1940, it was found to be reduced with zinc dust in acidic or in alkaline solution in 1927 (Wieland and Schöpf, 1925). Fuming hydriodic acid (Wieland, Tartter and Purrmann, 1940), hydrogen sulphide, sodium dithionite and sodium sulphite (Koschara, 1936, 1937) were all found to reduce xanthopterin to a leuco-compound, which was reoxidized to the starting material by shaking in the air. Xanthopterin was also reduced catalytically, and it was reported that three moles of hydrogen were taken up if the formula was $\text{C}_{10}\text{H}_{18}\text{N}_{6}\text{O}_{6}$ and two moles if the formula was $\text{C}_{13}\text{H}_{11}\text{N}_{11}\text{O}_{4}$ (Koschara, 1937). (The uncertainty arose from difficulties in micro-combustion, and the formula was proved later to be $\text{C}_{6}\text{H}_{5}\text{N}_{5}\text{O}_{2}$).

Isoxanthopterin (lb-II) was reduced with hydriodic acid (Wieland, Tartter and Purrmann, 1940), but reduction was assumed only on the evidence of liberated iodine and no reduced product was isolated. Leucopterin could not be reduced by these reagents. During 1940-1, the structures of xanthopterin (Purrmann, 1940a), isoxanthopterin (Purrmann, 1941), and leucopterin (Purrmann, 1940b) were finally established. In 1944, leucopterin was reduced with sodium amalgam to a dihydroxanthopterin which was identical with the
product obtained by the reduction of xanthopterin with zinc, fuming hydriodic acid etc., (Totter, 1944).

About 1943, a biologically active substance, "folic acid", was isolated, and research was concentrated on the determination of its structure. In 1947, O'Dell et al., hydrogenated folic acid in acidic and in alkaline solution over palladium or Adams' catalyst. They found that folic acid absorbed two atoms of hydrogen in alkaline solution using a palladium catalyst, but four atoms of hydrogen in acidic solution over Adams' catalyst. The reduction of folic acid has since been carried out with sodium dithionite, and dihydrofolic acid was obtained (Futtermann, 1957; Blakley, 1960).

Some other hydrogenated pteridines were made by reduction, and some synthetic work was attempted (as in Pesson's synthesis, described below). However, it was not until 1950 that any method was discovered which gave hydropteridines in which the positions of the hydrogen atoms were known beyond doubt. Pesson (1948) condensed 4,5-diamino-2-ethylthio-6-hydroxypyrimidine (lb-IV) with benzoin and obtained two isomeric dihydro compounds (X and Y), which were oxidized to the same pteridine (lb-V).
A general synthetic route from chloropyrimidines to 7,8-dihydropteridines was developed by Boon, Ramage and their collaborators and independently by Polonovski and his collaborators. This was a notable advance because it gave rise to dihydropteridines which were indubitably 7,8-dihydro-derivatives. Up to the inception of the work described in the present thesis, no similar method for producing dihydropteridines with the hydrogen atoms in other positions had been found.

Thus 7,8-dihydro-6-hydroxypteridines (lb-VIIIa) (Boon, Jones and Ramage, 1951; Polonovski and Jerome, 1950) and 7,8-dihydro-6-alkylpteridines (lb-VIIIb) (Boon and Jones, 1951; Polonovski, Pesson and Puister, 1950) were unambiguously synthesized by the route (lb-VI → lb-VIII).
A number of 7,8-dihydro-hydroxypteridines were synthesized by this method. For example, 7,8-dihydro-6-hydroxypteridine (lb-XIb) was synthesized from 2,4-dichloro-5-nitropyrimidine (lb-IXa) (Boon, Jones and Ramage, 1951). Condensation of glycine ethyl ester with 2,4-dichloro-5-nitropyrimidine (lb-IXa) gave 2-chloro-4-ethoxycarbonylmethylamino-5-nitropyrimidine (lb-Xa) which, on hydrogenation over Raney nickel, gave 2-chloro-7,8-dihydro-6-hydroxypteridine (lb-XIa). 7,8-Dihydro-6-hydroxypteridine (lb-XIb) was obtained by reductive removal of the 2-chloro-group of (lb-XIa) with hydriodic acid.
7,8-Dihydro-2,6-dihydroxypteridine (lb-XIc) was synthesized from the same pyrimidine (lb-IXa) via (lb-Xa) and (lb-Xb) (Boon, Jones and Ramage, 1951). 7,8-Dihydro-4,6-dihydroxypteridine (lb-XId) was synthesized from 4,6-dichloro-5-nitropyrimidine (lb-IXb) (Boon, Jones and Ramage, 1951) by a similar synthesis to that used for 7,8-dihydro-2,6-dihydroxypteridine.
7,8-Dihydro-2,4,6-trihydroxypteridine (lb-XV) was synthesized as shown (Boon and Leigh, 1952):

\[
\begin{array}{c}
\text{OH} \\
\text{Cl} \\
\text{N} \\
\text{N} \\
\text{OH}
\end{array}
\rightarrow
\begin{array}{c}
\text{OH} \\
\text{Cl} \\
\text{N} \\
\text{N} \\
\text{OH}
\end{array}
\rightarrow
\begin{array}{c}
\text{HN} \\
\text{N} \\
\text{O} \\
\text{N} \\
\text{OH}
\end{array}
\]

(lb-XII) (lb-XIII) (lb-XIV)

\[
\begin{array}{c}
\text{HO} \\
\text{N} \\
\text{H} \\
\text{H} \\
\text{N}
\end{array}
\rightarrow
\begin{array}{c}
\text{HO} \\
\text{N} \\
\text{H} \\
\text{H} \\
\text{N}
\end{array}
\]

(lb-XV)

4-Chloro-2,6-dihydroxypyrimidine (lb-XII) was condensed with p-toluenediazonium salt to give (lb-XIII), and this was then condensed with glycine methyl ester to give (lb-XIV) which, on reduction, gave 7,8-dihydro-2,4,6-trihydroxypteridine (lb-XV).

The structure of dihydroxanthopterin was finally
established as 2-amino-7,8-dihydro-4,6-dihydroxypteridine (lb-XVI) by a slight and obvious modification of this synthesis (Boon and Leigh, 1951, 1952).

7,8-Dihydro-2-hydroxy-4,6-dimethylpteridine (lb-XX) was synthesized from 2,4-dichloro-6-methyl-5-nitropyrimidine (lb-XVII) by the following route (Lister and Ramage, 1953):
However, before the present work was undertaken, no method existed for preparing 7,8-dihydropteridines lacking a hydroxy- or alkyl-group in the 6-position.

7,8-Dihydropteridines, which have no hydroxy group in the 6-position, were found to be readily hydrogenated to the corresponding tetrahydropteridines (Polonovski, Pesson and Puister, 1950; Lister and Ramage, 1953; Lister, Ramage and Coates, 1954). (All of these had an alkyl-group in the 6-position). Because these tetrahydropteridines had ultraviolet absorption spectra similar to the corresponding 4,5-diaminopyrimidines, the 5,6,7,8-tetrahydro structure was assigned to them. For example the 7,8-dihydro-2-hydroxy-4,6-dimethylpteridine (lb-XX) was reduced to a tetrahydro compound which had ultraviolet absorption spectra similar to those of 4,5-diamino-2-hydroxy-6-methylpyrimidine and it was therefore assigned the structure 5,6,7,8-tetrahydro-2-hydroxy-4,6-dimethylpteridine (lb-XXI) (Lister and Ramage, 1953).

Another unambiguous synthetic route to 5,6,7,8-tetrahydropteridines had been worked out (Brook and Ramage, 1955, 1957), and 5,6,7,8-tetrahydropteridines with no other substituent in the 6-positions are also available by this method. However the synthesis of 5,6,7,8-tetrahydro-hydroxypteridines with no substituent in the 8-position could
not be accomplished. 8-Benzyl-5,6,7,8-tetrahydro-2-hydroxy-4-methylpteridine (lb-XXVI) was prepared unambiguously as follows, but unfortunately it resisted debenzylolation (Brook and Ramage, 1955).

\[
\begin{align*}
\text{(lb-XXII)}
\quad & R = \text{CH}_3 \\
\quad & a,
\quad & R = \text{H} \\
\text{(lb-XXIII)}
\quad & R = \text{CH}_3 \\
\quad & a
\quad & R = \text{H} \\
\text{(lb-XXIV)}
\quad & a, R = \text{CH}_3 \\
\quad & b, R = \text{H} \\
\text{(lb-XXV)}
\quad & \text{R} \\
\text{(lb-XXVI)}
\quad & \text{R} = \text{CH}_3
\end{align*}
\]
Condensation of 2,4-dichloro-6-methyl-5-nitropyrimidine (lb-XXII) with benzyl-β-hydroxyethylamine gave (lb-XXIII). 8-Benzyl-2-chloro-5,6,7,8-tetrahydro-4-methylpteridine (lb-XXIV) was obtained from (lb-XXIII) by reduction and then by treatment with phosphorus trichloride. Hydrolysis of (lb-XXIV) with 5N-hydrochloric acid gave 8-benzyl-5,6,7,8-tetrahydro-2-hydroxy-4-methylpteridine (lb-XXVI) but attempt at replacement of the 8-benzyl-group with hydrogen by treatment with sodium in liquid ammonia gave an unstable product. 5,6,7,8-Tetrahydropteridine (lb-XXV) was synthesized from (lb-XXIV) by reduction with sodium in liquid ammonia, a process which achieved both debenzylation and dechlorination simultaneously (Brook and Ramage, 1957). The disadvantage of this method for preparing tetrahydropteridines is that an alternative cyclization to glyoxalino-pyrimidines occurs if a tautomizable group (e.g. -OH or -NH₂) is present at stage (lb-XXIII) (Ramage and Trappe, 1952).

A little work has been done on the reduction of amino- and ethoxy-pteridines. However, as the present research work is concerned with hydroxypteridines, the rest of this historical outline will be confined to an account of work on the reduction of pteridine and hydroxypteridines, and will include proofs of structures.

Pteridine was first prepared by the condensation of 4,5-diaminopyrimidine with glyoxal (Jones, 1943) and was
later reduced to the tetrahydro derivative with lithium aluminium hydride (Taylor and Sherman, 1959). The same tetrahydropteridine was also prepared by catalytic hydrogenation of 2,4-dichloro-5,6,7,8-tetrahydropteridine (Taylor and Sherman, 1959) and was identified as 5,6,7,8-tetrahydropteridine by comparison with a sample which was unambiguously prepared by Brook and Ramage (1957), as described above.

All the possible mono- and poly- hydroxypteridines are known (Table 1, see p. 17) but, prior to the present work few had been reduced. Reduction of 6-hydroxy-, and 6,7-dihydroxy-, pteridines with sodium amalgam always gave the corresponding 7,8-dihydro-6-hydroxy derivatives. Thus for example, both 6-hydroxy-, and 6,7-dihydroxy-, pteridine gave 7,8-dihydro-6-hydroxypteridine (lb-XIb) identical with that obtained by Doon's synthesis (Albert, Brown and Cheeseman, 1952). Similarly, 4,6,7-trihydroxypteridine gave the known 7,8-dihydro-4,6-dihydroxypteridine (lb-XId) (Albert and Brown, 1953). 2,4,6,7-Tetrahydroxypteridine was reduced with sodium amalgam to what was apparently 7,8-dihydro-2,4,6-trihydroxy-pteridine (Albert, Lister and Pedersen, 1956) because it was oxidized to 2,4,6-trihydroxypteridine. The same dihydro-compound was obtained by the reduction of 2,4-dichloro-6,7-dihydroxypteridine with sodium amalgam (Taylor and Sherman, 1959). Similarly sodium amalgam reduction of 4,6,7-trihydroxy-N(1)-methyl-2-oxopteridine produced 7,8-dihydro-4,6-dihydroxy-N(1)-methyl-2-oxopteridine (Pfleiderer, 1957).
The use of two other reducing agents is exemplified in the reduction of 4-hydroxy-6-methylpteridine by sodium borohydride to a tetrahydro-4-hydroxy-6-methylpteridine (Blakley, 1959), and in the reductive cleavage of 7-hydroxypteridine (lb-XXVII) by sodium dithionite to give 4-amino-5-carboxymethylaminopyrimidine (lb-XXVIII) (Albert, Brown and Cheeseman, 1952). The same compound (lb-XXVIII) was also obtained by a catalytic hydrogenation over Adams' catalyst in 0.1 N-sodium hydroxide solution (Albert, Brown and Cheeseman, 1952).

Evidence for the ring opening structure (lb-XXVIII) was obtained from analytical figures and from the presence of a carboxyl group. This compound gave an ester hydrochloride, as do amino acids, by treatment with hydrogen chloride in methanol, but conclusive evidence for the position of the introduced hydrogen was not presented. The compound
(lb-XXVIII) easily cyclized to a dihydro-7-
hydroxypteridine, believed to be (lb-XXIX), by refluxing
in N-hydrochloric acid and it was regenerated by boiling

All hydroxypteridines which had been reduced to
products of confirmed orientation, prior to the present work
are shown in Table 2 (see p. 18).
Table 1

List of known hydroxypteridines

<table>
<thead>
<tr>
<th>Hydroxypteridine</th>
<th>Reference</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Hydroxypteridine</td>
<td>Albert, Brown and Cheeseman</td>
<td>1951</td>
</tr>
<tr>
<td>4-Hydroxypteridine</td>
<td>Albert, Brown and Cheeseman</td>
<td>1951</td>
</tr>
<tr>
<td>6-Hydroxypteridine</td>
<td>Albert, Brown and Cheeseman</td>
<td>1952</td>
</tr>
<tr>
<td>7-Hydroxypteridine</td>
<td>Albert, Brown and Cheeseman</td>
<td>1952</td>
</tr>
<tr>
<td>2,4-Dihydroxypteridine</td>
<td>Kuhn and Cook</td>
<td>1937</td>
</tr>
<tr>
<td>2,6-Dihydroxypteridine</td>
<td>Albert, Lister and Pedersen</td>
<td>1956</td>
</tr>
<tr>
<td>2,7-Dihydroxypteridine</td>
<td>Albert, Lister and Pedersen</td>
<td>1956</td>
</tr>
<tr>
<td>4,6-Dihydroxypteridine</td>
<td>Albert and Brown</td>
<td>1953</td>
</tr>
<tr>
<td>4,7-Dihydroxypteridine</td>
<td>Albert and Brown</td>
<td>1953</td>
</tr>
<tr>
<td>6,7-Dihydroxypteridine</td>
<td>Albert, Brown and Cheeseman</td>
<td>1952</td>
</tr>
<tr>
<td>2,4,6-Trihydroxypteridine</td>
<td>Wieland and Liebig</td>
<td>1944</td>
</tr>
<tr>
<td>2,4,7-Trihydroxypteridine</td>
<td>Tschesche and Korte</td>
<td>1951</td>
</tr>
<tr>
<td>2,6,7-Trihydroxypteridine</td>
<td>Albert, Lister and Pedersen</td>
<td>1956</td>
</tr>
<tr>
<td>4,6,7-Trihydroxypteridine</td>
<td>Albert and Brown</td>
<td>1953</td>
</tr>
<tr>
<td>2,4,6,7-Tetrahydroxypteridine</td>
<td>Schöpf, Reichert and Riefstahl</td>
<td>1941</td>
</tr>
</tbody>
</table>
Table 2.
Hydroxypteridines where the positions of the hydrogen atoms are unambiguously known.

<table>
<thead>
<tr>
<th>Pteridines which have been reduced and the product unambiguously identified</th>
<th>Reducing agent</th>
<th>Reference (Reduction)</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-Hydroxypteridine</td>
<td>Na-Hg</td>
<td>1)</td>
<td><img src="image1.png" alt="Image" /></td>
</tr>
<tr>
<td>6,7-Dihydroxypteridine</td>
<td>Na-Hg</td>
<td>1)</td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>4,6,7-Trihydroxypteridine</td>
<td>Na-Hg</td>
<td>3)</td>
<td><img src="image3.png" alt="Image" /></td>
</tr>
<tr>
<td>2,4,6,7-Tetrahydroxypteridine</td>
<td>Na-Hg</td>
<td>4)</td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
</tbody>
</table>

This substance has been synthesized, but not obtained by reduction.

1) Albert, Brown and Cheeseman, 1952
2) Boon, Jones and Ramage, 1951
3) Albert and Brown, 1953
4) Albert, Lister and Pedersen, 1956
5) Boon and Leigh, 1952
The structures of the reduction products were mainly confirmed by comparison with samples prepared unambiguously. The first synthetic routes to 7,8-dihydro-, 5,6-dihydro-, and 5,6,7,8-tetrahydro-, hydroxypteridines having no substituent in the 6-, and 7-positions, were worked out.

Ionization constants of the hydrogenated pteridines were determined by potentiometric titration or by spectroscopic methods.

The ultraviolet absorption spectra, and the infrared absorption spectra of the reduced pteridines were also determined for comparison of structure.
SECTION 2.
METHODS.


As indicated in the historical outline, many methods have been used for the reduction of pteridine derivatives. They include physical, chemical and biochemical procedures. For systematic investigation of the reduction of the pteridine ring, it is important to select a proper combination of reducing agent and the substance to be reduced. Many such combinations are possible, but for simplicity and in order to achieve practical result, attention has been confined to selected variations in (a) the reducing agent and (b) the ionic species of the substance to be reduced.

As all hydroxypteridines have acidic and basic functions, the pH of the solution determines the ionic species of the hydroxypteridine in the reaction mixture. It is clearly desirable, therefore to restrict each reduction of a hydroxypteridine to one ionic species in any one experiment. For example, 7-hydroxypteridine has three pKa values of 6.41 (acidic), 1.2 (basic) and -2.0 (basic). Hence, if the 7-hydroxypteridine is reduced in a solution of pH 9, the reaction occurs almost entirely between the 7-hydroxypteridine anion and the reducing agent, but at pH 4 the reaction occurs almost entirely between the neutral molecule and the reducing agent. The pKa values of hydroxypteridines are shown in Table 3, p. 25. In some
cases the number of ionic species available for the reduction was limited on account of insolubility of one or other of the species.

The most important variable in the reduction of pteridines is the choice of a reducing agent. Classical theory suggests that reduction using chemical reagents is initiated by the attack of a proton to give an intermediate cation. The cation then takes up two electrons from the reducing agent which converts it into an anion, and the reduction is then completed by abstraction of a second proton from the surrounding medium. However, it is now commonly accepted that other mechanisms of reduction are evoked by particular classes of reagents. For example, dissolving metals reduce the substance by a hydrogen anion \((2\, e^+ + H^+\) mechanism. (Baker, 1952; Ingold, 1953). Complex metal hydrides also reduce a substance by a hydrogen anion mechanism, in some cases with the formation of a transient addition complex. The reduction of pyridinenucleotides by sodium dithionite, also proceeds through an addition complex, but it is of another type (Yarmolinsky and Colowick, 1956). Finally hydrogenations using metal catalysts are considered to be reductions by free hydrogen atoms (Conant and Cutter, 1922; Laidler, 1951).

The following reagents were selected for the present work: potassium borohydride (hydrogen anion type), sodium
amalgam (dissolving metal type), sodium dithionite (reduction through an addition compound), hydrogenation over platinum, palladium and Raney nickel (free hydrogen atoms).

Potassium borohydride is one of the best of the complex hydrides because it can be used in aqueous solution. This is a great advantage for the reduction of hydroxypteridines because most hydroxypteridines are quite insoluble in organic solvents. For this reason lithium aluminium hydride (a more active complex metal hydride than potassium borohydride) has been used only for the reduction of lipo-soluble pteridines, e.g. pteridine, a chloropteridine (Taylor and Sherman, 1959) and an alkylpteridine (Brook and Ramage, 1957). All hydroxypteridines in the present work, were treated with potassium borohydride.

Because potassium (or sodium) amalgam is used in alkaline conditions, all hydroxypteridines can be treated with this reagent. (Some hydroxypteridines, for example 7-hydroxypteridine, give an insoluble sodium salt and in such cases potassium amalgam is superior to sodium amalgam). Reduction of some 6,7-dihydroxypteridines by this reagent is unusual in that elimination of the 7-hydroxy group occurs, giving the corresponding 7,8-dihydro-6-hydroxypteridines. Some reductions of hydroxypteridines by this reagent have been described in the historical outline (p.14).

Sodium dithionite was chosen as an example of the type
of reducing agent which makes an addition compound with the substance to be reduced. This compound is usually hydrolysed below pH 8. This reagent is used in an alkaline solution and when this is made less alkaline to decompose the addition complex, sulphur dioxide is produced. In practice, this restricts the application of the method, because sulphite ion combines with some reduced hydroxypteridines to give sulphur-containing products (as will be described later).

All catalytic hydrogenations in the present work were carried out at room temperature and atmospheric pressure. Because of the poor solubility of polyhydroxypteridines in alcohol only the monohydroxypteridines were available for the reduction over Raney-nickel which is deactivated if used in alcohol more dilute than 85%. The reduction over palladium catalyst in 0.1N-sodium (or potassium) hydroxide solution was employed for all hydroxypteridines, and in a number of cases platinum catalyst was also used. Because catalytic hydrogenation may be followed quantitatively, it can give information on the facility and the extent to which a substance is being reduced.

Biochemical and electrolytic reductions were not investigated in the present work.
Table 3.

$\text{pK}_a$ values of hydroxypteridines.

<table>
<thead>
<tr>
<th>Hydroxypteridines</th>
<th>$\text{pK}_a$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>basic</td>
<td>acidic</td>
</tr>
<tr>
<td>2-Hydroxypteridine</td>
<td>2</td>
<td>11.13</td>
</tr>
<tr>
<td>4-Hydroxypteridine</td>
<td>-0.17</td>
<td>7.89</td>
</tr>
<tr>
<td>6-Hydroxypteridine</td>
<td>3.67</td>
<td>6.7</td>
</tr>
<tr>
<td>7-Hydroxypteridine</td>
<td>-2.0, 1.2</td>
<td>6.41</td>
</tr>
<tr>
<td>2,4-Dihydroxypteridine</td>
<td>1.0</td>
<td>7.91</td>
</tr>
<tr>
<td>2,6-Dihydroxypteridine</td>
<td>2.7</td>
<td>6.7*</td>
</tr>
<tr>
<td>2,7-Dihydroxypteridine</td>
<td></td>
<td>5.83, 10.07</td>
</tr>
<tr>
<td>4,6-Dihydroxypteridine</td>
<td></td>
<td>6.08, 9.73</td>
</tr>
<tr>
<td>4,7-Dihydroxypteridine</td>
<td></td>
<td>6.08, 9.62</td>
</tr>
<tr>
<td>6,7-Dihydroxypteridine</td>
<td></td>
<td>6.9, 10</td>
</tr>
<tr>
<td>2,4,6-Trihydroxypteridine</td>
<td></td>
<td>5.73, 9.41</td>
</tr>
<tr>
<td>2,4,7-Trihydroxypteridine</td>
<td></td>
<td>3.61</td>
</tr>
</tbody>
</table>

*for anhydrous species.

1) Albert, Brown and Cheeseman, 1951
2) Brown and Mason, 1956
3) Albert, Brown and Cheeseman, 1952
4) Albert, Lister and Pedersen, 1956
5) Albert and Brown, 1953
b. Methods Available for Diagnosing the Positions where Hydrogen Atoms have Entered the Molecule.

Chemical, physical, biochemical methods as well as combinations of these methods have been used for determining the positions at which external hydrogens are introduced during reduction of heteroaromatic substances.

The two chemical methods for determining molecular structures consist of the synthetic and the degradative approaches. The synthetic method gives conclusive evidence as long as any shift of a double bond is avoided during the synthesis. Much use has been made, in this thesis, of the synthetic method, and this will be discussed in 3b (p.67).

In the degradative method it is likewise important that neither the original molecule nor the resulting smaller molecules are altered by the process. An example of this method is seen in the fission of double bonds by ozonolysis. Thus, the decomposition of 3,5-diethoxycarbonyl-2,6-dimethyl-4-phenyldihydropyridine by ozonolysis yielded phenylacetic acid (2b-II). Since bond rearrangements were unlikely, this suggested the 1,4-dihydro structure (2b-I) for this pyridine (Kuss and Karrer, 1957).
This method however cannot be used to determine the structure of reduced hydroxypteridines, because the type of reduced hydroxypteridines with which this thesis is concerned have two hydrogen atoms on the relevant carbon atom. Such reduced pteridines are too readily oxidized to the original pteridines for the ozonolysis technique to be practicable.

Providing the introduced hydrogen atoms do not migrate from the original positions, and providing that a suitable reaction for removing hydrogen atoms from definite positions in the reduced molecule is available, a tracer method may be used for the determining where the hydrogen atoms were originally introduced. For example, the structure of reduced diphosphopyridine nucleotide was established as a 1,4-dihydro configuration by using deuterium as a tracer (Pullman, San Pietro and Colowick, 1954). The principle of the method is shown below.
Diphosphopyridine nucleotide (DPN) (2b-III), when reduced in heavy water with sodium dithionite, took up one atom of deuterium to form a "dihydro"-compound (2b-IV?). This was reoxidized to DPN (2b-V) which retained half an atom of deuterium per mole of DPN. The deuterated DPN (2b-V) was hydrolysed to a nicotinamide and then methylated to N(1)-methylnicotinamide (2b-V, R = CH₃). The nicotamide was then oxidized with alkaline ferricyanide to a mixture of the corresponding 2-, and 6-, pyridones (2b-VIa and 2b-VIb).

By examining the deuterium content of (2b-V, R = CH₃),
(2b-VIa) and (2b-VIb), the position of the introduced deuterium could be established as follows. If one of the final products (2b-VIa) contained (the equivalent of) half an atom of deuterium and the other contained none, the first dihydro compound would have to be the 1,6-dihydro derivative of DPN. Conversely, if (2b-VIb) contained half an atom of deuterium and (2b-VIa) contained none, the first reduction product would have to be a 1,2-dihydro structure. If (2b-VIa) and (2b-VIb) both contained half an atom of deuterium, the first dihydro compound would have to be a 1,4-dihydro derivative of DPN. Finally if DPN was reduced to a 1,2- and a 1,6-dihydro compound in equal amount, then both (2b-VIa) and (2b-VIb) would have the same deuterium content. However, in this case the deuterium content of each compound would be reduced to half of that of (2b-V) at the stage of ferricyanide oxidation. The structure of the reduced diphosphopyridine nucleotide was in fact confirmed as the 1,4-dihydro derivative, from the deuterium contents of (2b-V), (2b-VIa) and (2b-VIb). This method can theoretically be used to distinguish the 3,4-dihydro-2-hydroxypteridine from other possible dihydro-2-hydroxypteridines if the following reactions could be done in practice.
The reduction of (2b-VII) to a dihydro derivative (as 2b-VIII) is described in the present work, and the oxidation of (2b-IX) → (2b-X) (non-deuterated) was described by Brown and Mason (1956). Unfortunately, a trial experiment showed that oxidation of the dihydro-2-hydroxypteridine (2b-VIII) to 2-hydroxypteridine could not be done satisfactorily, even with cold dilute potassium permanganate. Therefore, this method could not be used for determining the structure of x,y-dihydro-2-hydroxypteridine.

If a new asymmetric centre is introduced by the reduction of a molecule at a certain position, it is possible to limit the possible structures of the product. For example, dihydrofolic acid had three possible structures, (2b-XI),
(2b-XII) and (2b-XIII). Structure (2b-XIII) has an asymmetric centre. The two dihydrofolic acids which were prepared respectively by biological and chemical methods, showed no difference in biological activity. This means that the dihydrofolic acid prepared by the chemical method had no asymmetric centre in the molecule, and the possibility of the structure (2b-XIII) was therefore eliminated (Osborn and Huennekens, 1958).

However, since no asymmetric centre is introduced by reduction of simple hydroxypteridines, the method is inapplicable to such cases.

Physical methods are also important tools for the structural investigation of reduced pteridines. The identity of two specimens (of a single substance) which have been prepared by different method can readily be established by comparison of their physical constants. Also, very important fundamental information about the structure of the molecule can be obtained from such physical constants as the pKa values, ultraviolet absorption spectra,
infrared absorption spectra and nuclear magnetic resonance spectra. In some cases the information obtained yields its meaning only after comparison with results from substances of known constitution. In the present work such standards were often lacking and had to be prepared. Electronic absorption is caused by a transition of electrons from one electronic energy level to another. The main factors that determine the electronic absorption are the electronic structure and polarizability of the molecule. Compounds which have a similar electronic structure have similar ultraviolet spectra.

Thus, there is a close similarity between the ultraviolet absorption spectra of the neutral molecule of 4,5-diaminopyrimidine (2b-XIV) and of the anion of 4-amino-5-carboxymethylaminopyrimidine (2b-XV), and also between the spectra of 4,5-diaminopyrimidine as cation (2b-XVI) and the neutral molecule of 4-amino-5-carboxymethylaminopyrimidine (2b-XVII). These facts follow from the principle that the main factor which contributes to the
ultraviolet absorption is the nature of the conjugated system. In the above examples, the carbonyl groups in (2b-XV) and (2b-XVII) are separated from the conjugated system of the pyrimidine ring by a \(-\text{CH}_2\) group and do not contribute to the ultraviolet absorption. So, (2b-XV) has the same conjugated system as (2b-IV), and (2b-XVII) the same conjugated system as (2b-XVI). No conclusive evidence on the orientation of hydrogen atoms in reduced hydroxy-pteridines could be obtained from infrared spectra, because the spectra of these compounds can only be measured in the solid state, where there is intense intra-molecular hydrogen-bonding. Infrared spectra are, however, of immense value in ascertaining the identity of two specimens.

Nuclear magnetic resonance (NMR) spectra give direct information on the orientation of hydrogen atoms in a molecule. Conclusive evidence for the location of hydrogen atoms can often be obtained by this method because the structure of the molecule is entirely unaltered during measurement. Thus any possibility of misunderstanding caused by an unusual reaction or by the shift of a proton is excluded. The structures of dihydro-\(N(1)\)-methylnicotinamide (Hutton and Westheimer, 1958; Dubb, Sanders and Wang, 1958) and dihydro-3,5-diethoxycarbonyl-1,2,6-trimethylpyridine (Smith, 1958) were confirmed respectively as 1,4-dihydro-structures, (2b-XVIII) and (2b-XIX). (These results are in agreement
with the results obtained by the isotope tracer method).

\[ \text{H}_2\text{CONH}_2 \quad \text{EtO}_2\text{C} \quad \text{CO}_2\text{Et} \]

\[ \text{CH}_3 \quad \text{CH}_3 \quad \text{CH}_3 \]

(2b-XVIII) (2b-XIX)

It first seemed that the NMR method could be used for the determination of the structures of xy-dihydro-2-hydroxypteridine and xy-dihydro-4-hydroxypteridine. It soon appeared, however, that difficulty in the application of the method to these dihydropteridines might be encountered through the insolubility of these hydroxypteridine compounds, and in fact the nuclear magnetic resonance measurements, when made, did not give sufficiently intense spectra for this very reason (These measurements were kindly carried out at the Institute of the Atomic Power Centre of Japan by Mr. H. Hayakawa).

Biochemical methods were not used during the present work because no suitable enzymes are known.
c. **Likely Positions of Hydrogen Atoms in Reduced Hydroxypteridines.**

There are many possible reduced forms of the pteridine ring, but the reduction of pteridines should yield the more stable isomers, that is to say the final products will retain as much conjugation as possible. Therefore, it is unlikely to find a reduction product with such a structure as (2c-I), because of the greatly reduced conjugation (it should be noted that (2c-I) does not contain even one fully-conjugated ring).

![Diagram of 2c-I]

(2c-I)

On these grounds it seems reasonable that the di- and tetra-hydropteridines likely to be obtained by reduction will be represented by the following formulae only (or a selection of these):
In these structures the two pairs of compounds, (B) and (C), and (F) and (G) each have a common cation so that interchange to the more stable form of each pair would instantly occur on protonation. Which is the more stable form will be decided by substituents in the ring (an example will be discussed later in the case of x,y-dihydro-4-hydroxypteridine, see p. 48).

This contention is supported by the fact that all attempts to synthesise a related substance, 1,4-dihydroquinazoline (2c-II), gave 3,4-dihydroquinazoline (2c-III) (Dr. W.L.F. Armarego, personal communication).
A further exclusion of some of the remaining eight reduced structures (A, C, D, E, F, G, H and I) can be made on the following grounds.

Previous work had indicated that hydroxypteridines having a hydroxy group in the 6- and 7-position were always reduced in the pyrazine ring (no exceptions were found to this rule in the present work). Hence, 2-hydroxy-, and 4-hydroxy-pteridine are the only hydroxypteridines likely to give respectively a 3,4-dihydro-, and 1,2-dihydro-structure on reduction. It has also been indicated that the only examples of the elimination of a hydroxy group from the pyrazine ring during reduction are losses of a 7-hydroxy-group from 6,7-dihydroxypteridines when sodium amalgam is used. In such cases, 7,8-dihydro-6-hydroxypteridines were obtained (see p. 14). No elimination of a hydroxy-group from the pyrimidine ring has been recorded, and no 1,2,3,4-tetrahydropteridines have ever been obtained by reduction.

A, C, D, E, F, G and H are therefore the structures most likely to be obtained by reduction of simple hydroxypteridines.
d. Method Available for Syntheses of Hydrogenated Hydroxypteridines.

Before the present work only 7,8-dihydro- and 5,6,7,8-tetrahydro-pteridines have been made by synthesis.

The synthetic route from chloronitropyrimidines to 7,8-dihydropteridines has been well developed by Boon, Ramage and their collaborators and by Polonovski and collaborators (see p. 6). Unfortunately, this method is suitable only for preparing those 7,8-dihydropteridines which have an alkyl-, or hydroxy-group in the 6-position. In the present work, the syntheses of 7,8-dihydropteridines having no substituent in the 6-position was desired, and for this purpose considerable modification of the above method was necessary. The other synthetic method for 7,8-dihydropteridines consists of a condensation of 4-alkylamino-5-aminopyrimidines (2d-I) with benzoin (Forrest, Hull, Rodda and Todd, 1951; Fidler and Wood, 1957). But all hydropteridines (2d-II) which were prepared by this method had alkyl (or aryl) groups in 6-, 7-, and 8-positions.
The essential process of this method consists of an orientation of the incoming double bond to the 5,6-position by an alkyl group attached to the 4-amino group of the pyrimidine (2d-I) ring. Therefore, no modification of this method to prepare unsubstituted dihydropteridines is possible.

5,6,7,8-Tetrahydropteridines have been prepared by two ways. The first method is a reduction of 7,8-dihydropteridines (see p.11) and the second one is an unambiguous synthesis (see p.11). These are general methods and are applicable for the present purpose after some modification.

No general method has been developed for the syntheses of 1,2-, 3,4-, 5,6-, and 5,8-dihydropteridines. However, for the present work, some syntheses of these ring systems were desired, and new methods had to be thought out.
A method for the preparation of 7-amino-5,6-dihydro-1,3-dimethyl-2,4-dioxopteridine (2d-V) by cyanomethylation of the corresponding 4,5-diaminopyrimidine (2d-IV) (Blicke and Godt, 1954), is an isolated instance of a method which is not general.
SECTION 3

RESULTS AND DISCUSSIONS

a. Reduction

In the present study of reduction of hydroxypteridines, there are four variables:
(i) the positions of the hydroxy groups attached to the pteridine ring, (ii) the presence of groups other than hydroxy, (iii) the ionic species of the pteridine, and (iv) the reducing agent.

In practice it was found that the position of the hydroxy group was the chief controlling influence on the position of the entering hydrogen atoms.

i) Position of the hydroxy group.

2-Hydroxypteridine was reduced in alkaline solution with potassium borohydride to a small amount of a tetrahydro-2-hydroxypteridine and a fairly good yield of a dihydro-2-hydroxypteridine. Hydrogenation in alkaline solution over Adams' platinum oxide, palladium on carbon, or Raney-nickel catalyst, gave the same two products as above. On the other hand, hydrogenation over Adams' catalyst in neutral solution led to the absorption of four moles of hydrogen, indicating that a complete hydrogenation (or hydrogenolysis) of the pteridine ring had occurred.
Sodium dithionite reduced 2-hydroxypteridine to the same \( x,y \)-dihydro-2-hydroxypteridine and a highly insoluble product, presumably a polymerized compound. Sodium amalgam reduced 2-hydroxypteridine to an unstable compound which rapidly changed to a resin in air.

The tetrahydro-2-hydroxypteridine was shown to be \( 5,6,7,8 \)-tetrahydro-2-hydroxypteridine by comparison of its ultraviolet absorption and Rf values with an authentic sample prepared by synthesis (see p. 71)

<table>
<thead>
<tr>
<th></th>
<th>The reduction product of 2-hydroxypteridine</th>
<th>( 5,6,7,8 )-tetrahydro-2-hydroxypteridine obtained by synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rf. ( \text{NH}_4 \text{Cl} )</td>
<td>0.74 2B/3G</td>
<td>0.74 2B/3G</td>
</tr>
<tr>
<td>( \text{Bu}/\text{Ac} )</td>
<td>0.22 2/3 GB</td>
<td>0.22 2/3 GB</td>
</tr>
<tr>
<td>UV-absorption</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \lambda_{\text{max (m}\mu)} )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 7</td>
<td>230, 305</td>
<td>232, 306</td>
</tr>
<tr>
<td>1.0</td>
<td>327</td>
<td>327</td>
</tr>
</tbody>
</table>

Although the structure of the \( x,y \)-dihydro-2-hydroxypteridine is still not beyond doubt, it seems likely from the following evidence to be \( 3,4 \)-dihydro-2-hydroxypteridine.

\( x,y \)-Dihydro-2-hydroxypteridine is not identical with \( 7,8 \)-dihydro-2-hydroxypteridine which was obtained by synthesis (see p. 70). Also, it is spectroscopically and chromatographically different from synthetic "5,6-dihydro-2-
hydroxypteridine", (although an analytically pure sample of this substance was not obtained) (see 3b and 3d). The remaining probable structures for this compound are 3,4-dihydro-2-hydroxypteridine (3a-I) and 5,8-dihydro-2-hydroxypteridine (3a-II).

**Dihydro-2-hydroxypteridines**

<table>
<thead>
<tr>
<th></th>
<th>x,y-dihydro-</th>
<th>7,8-dihydro-</th>
<th>&quot;5,6-dihydro-&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rf. (NH₄Cl)</td>
<td>0.69 2B/3V</td>
<td>0.61 2/3B</td>
<td>0.76 2B/3SB</td>
</tr>
<tr>
<td>(Bu/Ac)</td>
<td>0.50 2B/3V</td>
<td>0.32 2/3Y</td>
<td></td>
</tr>
<tr>
<td>pKa basic</td>
<td>0</td>
<td>3.50 ± 0.02</td>
<td>0.4</td>
</tr>
<tr>
<td>acidic</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UV-spectra</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(λ_max μm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>o</td>
<td>248, 317</td>
<td>223, 298</td>
<td>296 (pH 7)</td>
</tr>
<tr>
<td>+</td>
<td>254, 337</td>
<td>229, 312</td>
<td>282 (pH 0)</td>
</tr>
<tr>
<td>-</td>
<td>281, 343</td>
<td>224, 308 (pH12)</td>
<td>298 (pH 12)</td>
</tr>
</tbody>
</table>

![Chemical Structures](image)

(3a-I)  
(3a-II)

Attempts to synthesize (3a-I) were unsuccessful (see p. 75), and no synthetic route to (3a-II) could be

* o: neutral molecule,  + : cation,  - : anion.
found.

Infrared spectra, and tracer methods could not be used for reasons described earlier (see 2b ). Nuclear magnetic resonance spectra were looked for (by kindness of Mr. N. Hayakawa), but the insolubility of the substance prevented strong enough signals being obtained.

The first evidence in favour of the 3,4-dihydro-structure was obtained, however, from studying the reduction of the x,y-dihydro-2-hydroxypteridine and 2-hydroxypteridine. x,y-Dihydro-2-hydroxypteridine could not be further reduced in alkaline solution; but in neutral solution it absorbed hydrogen (eventually three moles) over Adams' catalyst, yet no 5,6,7,8-tetrahydro-2-hydroxypteridine was detected during any stage of the hydrogenation. This suggests that the 3,4- is more likely than the 5,8- orientation for the original hydrogenation.

The second piece of evidence was obtained from the reduction of 2-hydroxypteridine. It has been established (Brown and Mason, 1956) that 2-hydroxypteridine exists as an anhydrous anion (3a-III), but in neutral solution it forms a more stable hydrated neutral molecule. These authors assigned the structure (3a-IV) to this hydrated molecule from comparison of its ultraviolet spectrum with that of its 1-methyl-, and 3-methyl-derivatives.
It would be reasonable to obtain 3,4-dihydro-2-hydroxypteridine from (3a-III), but not from (3a-IV). It will be recalled that the x,y-dihydro-2-hydroxypteridine was the main product of the reduction of 2-hydroxypteridine anion (3a-III) in alkaline solution with potassium borohydride or hydrogenation over palladium but no such dihydro structure was obtained from hydrogenation of (3a-IV) in neutral solution.

A few 5,8-dihydropteridines are known, the best established being 8-ethyl-2-ethylamino-5,8-dihydro-7-hydroxypteridine-6-carboxylic acid (3a-V) (Pfleiderer and Taylor, 1960; see also Forrest, Baalen, Viscontini and Firaux, 1960). The acid (3a-V) shows a strong bathochromic shift (54 μm) compared to its decarboxylation product, which is apparently a 5,6-dihydropteridine (3a-VI). Thus the 5,8-dihydro-structure, which has diminished conjugation, is believed (Pfleiderer and Taylor) only to be stabilized by special groups e.g. the hydrogen bond ring of (3a-V).
x,y-Dihydro-2-hydroxypteridine neutral molecule showed only a small bathochromic shift (10 \( \mu \)) when compared to the hydrated 2-hydroxypteridine neutral molecule (Fig. 1, p.186). A similar small bathochromic shift (Albert, Brown and Cheeseman, 1952) also exists between the 3,4-dihydro-6-hydroxypteridine neutral molecule and hydrated neutral molecule of 6-hydroxypteridine (Fig. 2, p.186). This evidence strongly supports the 3,4-dihydro-structure (3a-I) for x,y-dihydro-2-hydroxypteridine (see also p. 112).

The fact that pyrimidines are more difficult to reduce than pyrazines suggested that pteridines should be reduced preferentially in the pyrazine ring (Albert, 1952; Taylor and Sherman, 1959), and no examples to the contrary had been found prior to the present work. However it is relevant that in the quinazoline series the pyrimidine ring can be easily reduced. For example, quinazoline was reduced easily to 3,4-dihydroquinazoline by hydrogenation over Dams' catalyst (Marr and Bogert, 1935), and 4-chloroquinazoline gave the same 3,4-dihydroquinazoline by
catalytic hydrogenation over palladium on CaCO₃. (Elderfield, Williamson, Gensler and Kremer, 1947).

All the above considerations point to 3,4-dihydro-2-hydroxypteridine (3a-I) as the most likely structure for x,y-dihydro-2-hydroxypteridine. 4-Hydroxypteridine was reduced with potassium borohydride to give a tetrahydro-4-hydroxypteridine and a dihydro-4-hydroxypteridine (roughly in a 1:1 ratio). Sodium amalgam reduction gave a product unstable to aerial oxidation and a small amount of the same tetrahydro-4-hydroxypteridine. Catalytic hydrogenation over Raney-nickel in neutral conditions gave only this tetrahydro-4-hydroxypteridine. On the other hand, hydrogenation over palladium catalyst in 0.1N-sodium hydroxide solution absorbed 60% of the calculated hydrogen for 4H, and gave the same tetrahydro-4-hydroxypteridine along with a product unstable to aerial oxidation.

The structure of the tetrahydro-4-hydroxypteridine was established as 5,6,7,8-tetrahydro-4-hydroxypteridine by unambiguous synthesis (see p. 84).
Tetrahydro-4-hydroxy-pteridine obtained by reduction

<table>
<thead>
<tr>
<th>m.p.</th>
<th>230° (decomp.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pKₐ</td>
<td></td>
</tr>
<tr>
<td>basic</td>
<td>3.86 ± 0.02</td>
</tr>
<tr>
<td>acidic</td>
<td>10.13 ± 0.03</td>
</tr>
<tr>
<td>UV-spectra</td>
<td></td>
</tr>
<tr>
<td>λ max. (mμ)</td>
<td>log ε</td>
</tr>
<tr>
<td>0</td>
<td>289</td>
</tr>
<tr>
<td>+</td>
<td>259</td>
</tr>
<tr>
<td>-</td>
<td>284</td>
</tr>
</tbody>
</table>

5,6,7,8-Tetrahydro-4-hydroxypteridine obtained by synthesis

<table>
<thead>
<tr>
<th>m.p.</th>
<th>230-238° (decomp.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pKₐ</td>
<td></td>
</tr>
<tr>
<td>basic</td>
<td>3.87 ± 0.02</td>
</tr>
<tr>
<td>acidic</td>
<td>10.10 ± 0.03</td>
</tr>
<tr>
<td>UV-spectra</td>
<td></td>
</tr>
<tr>
<td>λ max. (mμ)</td>
<td>log ε</td>
</tr>
<tr>
<td>0</td>
<td>289</td>
</tr>
<tr>
<td>+</td>
<td>259</td>
</tr>
<tr>
<td>-</td>
<td>284</td>
</tr>
</tbody>
</table>

The structure of the x,y-dihydro-4-hydroxypteridine is still uncertain because it could not be synthesised (see, 3b). However, it was not identical with 5,6-dihydro-4-hydroxypteridine which was prepared synthetically (see, p.84), and the 1,2-dihydro structure was excluded because it was reduced further to 5,6,7,8-tetrahydro-4-hydroxypteridine both by sodium borohydride, and by hydrogenation over Raney-nickel catalyst. Three possible structures, 5,8-dihydro-, 7,8-dihydro-, and the transprotonated 3,7-dihydro-, structures (see p. 36 (G) therefore remain. The NMR method was attempted on this compound, but unfortunately no spectrum was obtained because it was not sufficiently soluble. The ultraviolet spectrum did not give conclusive information but was quite unlike that of any other 7,8-dihydropteridine of which many are known (Table 15, p.120). However, the IR-spectra of this compound has a strong C = O stretching absorption at 1675 cm⁻¹ which precludes the possibility of a 3,7-dihydro-structure in the solid state. (Fig.38, p.206).
6-Hydroxypteridine was reduced with potassium borohydride, or with sodium dithionite to a dihydro compound. This was identical with the product obtained by the reduction of 6-hydroxypteridine with sodium amalgam (Albert, Brown and Cheeseman, 1952). Hydrogenation of 6-hydroxypteridine in 0.1N-sodium hydroxide over palladium catalyst gave the same dihydro-6-hydroxypteridine as above. The structure of this compound follows from its identity with the 7,8-dihydro-6-hydroxypteridine (3a-VII) unambiguously synthesized by Boon, Jones and Ramage (1951) (see p. 7).

![Chemical structure](image)

(3a-VII)

<table>
<thead>
<tr>
<th>Compound</th>
<th>pH Medium</th>
<th>λ_{max} (μm)</th>
<th>log ε</th>
</tr>
</thead>
<tbody>
<tr>
<td>7,8-Dihydro-6-hydroxypteridine*</td>
<td>0.1N-HCl</td>
<td>293</td>
<td>4.02</td>
</tr>
<tr>
<td></td>
<td>0.1N-NaOH</td>
<td>307</td>
<td>4.05</td>
</tr>
<tr>
<td>Dihydro-6-hydroxypteridine</td>
<td>0.01N-HCl</td>
<td>292</td>
<td>3.97</td>
</tr>
<tr>
<td></td>
<td>0.1N-KOH</td>
<td>308</td>
<td>4.05</td>
</tr>
</tbody>
</table>

*Boon, Jones and Ramage, 1951.*
7-Hydroxypteridine was reduced with potassium borohydride to a dihydro-7-hydroxypteridine. The same compound was obtained by the reduction of 7-hydroxypteridine with cold potassium amalgam.

On the other hand, catalytic hydrogenation over palladium catalyst in 0.1N-potassium hydroxide solution gave the same ring-opened product as that obtained by the reduction of this pteridine with sodium dithionite or by the hydrogenation over Adams' catalyst in 0.1N-sodium hydroxide solution (Albert, Brown and Cheeseman, 1952). The structure 4-amino-5-carboxymethylaminopyrimidine was assigned to this product on its analytical figures and the formation of the methyl ester with methanolic hydrogen chloride (Albert, Brown and Cheeseman, 1952).

However, this evidence did not completely exclude the possibility of the reduced ring structure (3a-XIX) for the ring opened product. The spectroscopic data of the present work do however exclude any possibility of the structure (3a-XIX) (see p.111), and hence the structure of the product
is confirmed as 4-amino-5-carboxymethylaminopyrimidine (3a-VIII). Since the reversible conversion between x,y-dihydro-7-hydroxopteridine and 4-amino-5-carboxymethylaminopyrimidine (3a-VII) by heating with alkali or with acid has been established (Albert, Brown and Cheeseman, 1952), the structure of the x,y-dihydro-7-hydroxopteridine is confirmed as 5,6-dihydro-7-hydroxopteridine (3a-XX).

\[
\begin{array}{c}
\text{H} \\
\text{H} \\
\text{N} \\
\text{N} \\
\text{N} \\
\text{N} \\
\text{HO} \\
\end{array}
\]

(3a-XX)

2,4-Dihydroxopteridine absorbed two moles of hydrogen over Adams' catalyst in acidic solution and gave a product very unstable to oxidation. The same product was also obtained by hydrogenation over palladium or Raney-nickel in acidic or in alkaline solution, and by reduction with sodium amalgam under nitrogen. The product gave one spot on paper chromatography (developed and dried in a nitrogen atmosphere) and was reoxidized to 2,4-dihydroxopteridine by absorbing one mole of oxygen in alkaline solution* (see

*In the reoxidation mixture a small amount of 2,4,6-trihydroxopteridine was detected by paper chromatography.
Fig. 3). Thus this compound is a tetrahydro-2,4-dihydroxypteridine.

Because of its instability, the correct analysis of this compound was obtained only after using a little 2,3-dimercaptopropanol as an antioxidant in the water used for its purification. Acetic formic anhydride gave the more stable formyl tetrahydro derivative. The only possible tetrahydro structure for this compound is 5,6,7,8-tetrahydro-2,4-dihydroxypteridine, and its ultraviolet spectra and ionization constant also support this structure.
Because both substances are quite unstable to oxidation, correct log $\varepsilon$ and $pK_a$ values were not obtained.

Although all other known hydroxypteridines are reduced similarly by either potassium borohydride or catalytic hydrogenation, 2,4-dihydroxypteridine was unaffected by potassium borohydride reduction.

Sodium dithionite reduced 2,4-dihydroxypteridine to the same tetrahydro compound as was obtained by catalytic hydrogenation, but also produced a sulphur containing product. The structure of the latter has not finally been established, but the analytical figures, $C_6H_9N_4NaO_6S$, suggest the following possible structures (or their isomers).

\[
\text{(3a-XXI)} \quad \begin{array}{c}
\text{NaO}_3S \\
\text{H} \\
\text{H} \\
\text{H} \\
\text{H} \\
\text{N} \\
\text{N} \\
\text{H} \\
\text{H} \\
\text{H} \\
\text{OH} \\
\end{array}
\quad \begin{array}{c}
\text{OH} \\
\text{H}_2N \\
\text{N} \\
\text{N} \\
\text{H} \\
\text{H} \\
\text{H} \\
\text{H} \\
\text{H} \\
\text{O}_H \\
\end{array}
\]

\[
\text{(3a-XXII)} \quad \begin{array}{c}
\text{H}_2N \\
\text{N} \\
\text{N} \\
\text{OH} \\
\text{CH}_2\text{CH}_2\text{SO}_3\text{Na} \\
\text{OH} \\
\end{array}
\]
The following analogy suggests that formula (3a-XXI) is correct, i.e. that a sulphonic group is introduced into the 6-position of a pteridine nucleus. 2-Amino-4-hydroxypteridine (Viscontini and Weilenman, 1959; Baalen and Forrest, 1959) has been reduced to an unstable tetrahydro derivative which was easily oxidized. The reoxidation of the tetrahydro-2-amino-4-hydroxypteridine in the presence of such anions as, \( \text{OH}^- \), \( \text{SO}_3\text{H}^- \), or \( \text{CN}^- \), or in ammonia leads to an introduction of various groups into the 6-position of the ring (Baalen and Forrest, 1959; Viscontini and Weilenman, 1959; Forrest, Baalen, Viscontini and Piraux, 1960).
Thus they obtained 2-amino-4,6-dihydroxypteridine (xanthopterin) (3a-XXVI, $R = \text{OH}$), 2,6-diamino-4-hydroxypteridine (3a-XXVI, $R = \text{NH}_2$) and the well known 2-amino-4-hydroxypteridine-6-carboxylic acid (3a-XXVI, $R = \text{CO}_2\text{H}$). The same sort of reaction can be expected with the sulphite ion, and they also isolated what they believed to be (3a-XXVI, $R = \text{SO}_3\text{H}$). But they seem to be mistaken as to the level of oxidation because their analytical figures suggest the tetrahydro-analogue (3a-XXVIII, $\text{H}_2\text{O}$, $R = \text{SO}_3\text{H}$).

2-Amino-4-hydroxypteridine-6-carboxamide

Forrest, Baalen, Viscontini and Firaux.


![Structural formulae](image)

A: $\text{C}_7\text{H}_6\text{N}_4\text{O}_2$  
B: $\text{C}_7\text{H}_{10}\text{N}_4\text{O}_2$

**Found:**  
C, 40.02  
H, 4.69  
N, 40.22

**Calc. for $\text{C}_7\text{H}_6\text{N}_4\text{O}_2$:**  
C, 40.8  
H, 2.9  
N, 40.8

**Calc. for $\text{C}_7\text{H}_{10}\text{N}_4\text{O}_2$:**  
C, 40.0  
H, 4.8  
N, 40.0
2-Amino-4-hydroxypteridine-6-sulphonic acid

![Chemical structures](image)

**G, C₆H₅N₅O₄S**

**D, C₆H₉N₅O₄S**

**Found:**

C, 27.15  H, 4.54  N, 25.8  S, 11.4

**Calc. for C₆H₅N₅O₄S·H₂O:**

C, 27.6  H, 2.7  N, 26.8  S, 12.3

**Calc. for C₆H₉N₅O₄S·H₂O:**

C, 27.05  H, 4.0  N, 25.5  S, 11.65

Regarding the mechanism of the reduction, the same authors suggest that addition of the anion occurs through a 5,8-dihydro-intermediate (3a-XXIV), but they offer no evidence for this hypothesis. It seems more likely that such an addition would take place through a 7,8-dihydro structure, because the C,N-double bond is more easily polarized than is the C,O-double bond, and would lead an attack of the anion to the positive carbon atom. Such additions of anions to carbon atoms of heterocyclic compounds are well known. For example, CN⁻, SO₃H⁻, OH⁻ or N₂H₃⁻ groups easily attack the 4-position of quinazoline (3a-XXVII) with the formation of addition compounds (3a-XXVIII) (R = CN, SO₃H, OH, or N₂H₃⁻) (H. Higashino, 1960).
2,6-Dihydroxypteridine and 4,6-Dihydropteridine were both reduced with potassium borohydride to a respective 2- and 4-hydroxy-7,8-dihydro-6-hydroxypteridines. These structures were confirmed by comparing with the corresponding authentic samples unambiguously synthesized by the method of Boon and Ramage (see p. 8; 94).

<table>
<thead>
<tr>
<th></th>
<th>Dihydro-2,6-dihydroxypteridine obtained by reduction</th>
<th>7,8-Dihydro-2,6-dihydropteridine obtained by synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rf. (NH₄Cl)</td>
<td>0.52 2B/3V</td>
<td>0.52 2B/3V</td>
</tr>
<tr>
<td>(Bu/Ac)</td>
<td>0.08 2B/3X</td>
<td>0.08 2B/3X</td>
</tr>
<tr>
<td>pKₐ (basic)</td>
<td>2.80 ± 0.02</td>
<td>2.82 ± 0.02</td>
</tr>
<tr>
<td>(acidic)</td>
<td>10.23 ± 0.02</td>
<td>10.26 ± 0.03</td>
</tr>
<tr>
<td>UV-spectra</td>
<td>λ max. (µ) log Ε</td>
<td>λ max. (µ) log Ε</td>
</tr>
<tr>
<td>0</td>
<td>237, 265* 3.95, 3.46</td>
<td>237, 265* 3.95, 3.46</td>
</tr>
<tr>
<td>+</td>
<td>257, 317 3.94, 3.85</td>
<td>257, 318 3.94, 3.85</td>
</tr>
<tr>
<td>-</td>
<td>279 4.01</td>
<td>279 4.01</td>
</tr>
<tr>
<td>IR spectra</td>
<td>see Fig. 40, p. 207</td>
<td>see Fig. 39, p. 207</td>
</tr>
</tbody>
</table>

*Shoulder
Catalytic hydrogenation over palladium catalyst in 0.1N-potassium hydroxide solution gave the same results as above. Although potassium amalgam reduction of 4,6-dihydroxypteridine gave 7,8-dihydro-4,6-dihydroxypteridine, 2,6-dihydroxypteridine was not reduced successfully with potassium amalgam and 7,8-dihydro-2,6-dihydroxypteridine could not be detected in the reaction mixture. This is due to the instability of the product under these conditions. 7,8-Dihydro-2,6-dihydroxypteridine also decomposed with potassium amalgam treatment and the product could not be detected by paper chromatography.
2,7-Dihydroxypteridine and 4,7-dihydroxypteridine were reduced with potassium borohydride, or with potassium amalgam, to the corresponding dihydro-compounds. Catalytic hydrogenation over palladium catalyst in 0.1N-potassium hydroxide solution gave the same results.

The structure of the dihydro-4,7-dihydroxypteridine was shown to be 5,6-dihydro-4,7-dihydroxypteridine by synthesis (see p.95). 5,6-Dihydro-2,7-dihydroxypteridine was not obtained by synthesis because of technical difficulties (see p.94), and two possible structures, 5,6-dihydro- and 3,4-dihydro-2,7-dihydroxypteridine, remain for this compound.

<table>
<thead>
<tr>
<th></th>
<th>Dihydro-4,7-dihydroxypteridine obtained by reduction</th>
<th>5,6-Dihydro-4,7-dihydroxypteridine obtained by synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rf. (NH₄Cl)</strong></td>
<td>0.54 2D/3V*</td>
<td>0.54 2D/3V*</td>
</tr>
<tr>
<td>(Bu/Ac)</td>
<td>0.08 2D/3V</td>
<td>0.08 2D/3V</td>
</tr>
<tr>
<td><strong>pKa (acidic)</strong></td>
<td>8.45 ± 0.02</td>
<td>8.48 ± 0.05</td>
</tr>
<tr>
<td><strong>UV-spectra</strong></td>
<td>( \lambda_{\text{max.}}(\text{m}_{\mu}) ) \ log ( \varepsilon )</td>
<td>( \lambda_{\text{max.}}(\text{m}_{\mu}) ) \ log ( \varepsilon )</td>
</tr>
<tr>
<td>o</td>
<td>276, 328 3.78, 3.70</td>
<td>276, 328 3.78, 3.71</td>
</tr>
<tr>
<td><strong>IR-spectra</strong></td>
<td>See Fig.44, p.209</td>
<td>See Fig.43, p.209</td>
</tr>
</tbody>
</table>

* Violet fluorescence appeared after exposure to 245 m\( \mu \) lamp.
Sodium dithionite reduction of 2,7-dihydroxypteridine gave a sulphur containing product, \( \text{C}_6\text{H}_7\text{N}_4\text{Na}_2\text{O}_5\text{S} \), whose structure was not investigated further. 2,7-Dihydroxypteridine also gave an addition compound, \( \text{C}_6\text{H}_4\text{N}_4\text{Na}_2\text{O}_5\text{S}\cdot\text{H}_2\text{O} \), with sodium hydrogen sulphite.

6,7-Dihydroxypteridine was not reduced with potassium borohydride, sodium dithionite, or by hydrogenation over platinum or palladium catalyst. The reduction of this pteridine with sodium amalgam leads to elimination of the 7-hydroxy-group, giving 7,8-dihydro-6-hydroxypteridine (Albert, Brown and Cheeseman, 1952).

2,4,6-Trihydroxypteridine was reduced with potassium borohydride to a dihydro-compound. The only possible dihydro-2,4,6-trihydroxypteridine is known to be 7,8-dihydro-2,4,6-trihydroxypteridine. Catalytic hydrogenation of 2,4,6-trihydroxypteridine over palladium on carbon gave the same dihydro-compound. 7,8-Dihydro-2,4,6-trihydroxypteridine has been synthesized before (Boon and Leigh, 1952).

2,4,7-Trihydroxypteridine was not affected by reducing agents such as potassium borohydride, potassium amalgam, or by hydrogenation over palladium catalyst in 0.1N-potassium hydroxide solution.

2,5,7-Trihydroxypteridine was not affected by potassium borohydride or by hydrogenation over palladium catalyst. This compound behaved unusually towards potassium amalgam.
reduction, and 7,8-dihydro-2,6-dihydroxypteridine was not detected in the reduction mixture, presumably because 7,8-dihydro-2,6-dihydroxypteridine is unstable to potassium amalgam (for the same reason as in the case of 2,6-dihydroxypteridine, see above).

**4,6,7-Trihydroxypteridine** was not affected by potassium borohydride or by hydrogenation over palladium catalyst. However, this compound has been reduced by sodium amalgam with elimination of the 7-hydroxy-group, giving 7,8-dihydro-4,6-dihydroxypteridine (Albert and Brown, 1953).

**2,4,6,7-Tetrahydroxypteridine** was not affected by potassium borohydride or by hydrogenation over palladium catalyst. This compound has been reduced by sodium amalgam with elimination of the 7-hydroxy group to 7,8-dihydro-2,4,6-trihydroxypteridine (Albert, Lister and Pedersen, 1956).

ii) **The presence of groups other than hydroxy group.**

Some hydroxypteridines having other substituents were also reduced in order to examine briefly the effect of such substituents on the reduction.

**2-Amino-4,6-dihydroxypteridine** (xanthopterin) was reduced to the known 2-amino-7,8-dihydro-4,6-dihydroxypteridine with potassium borohydride or sodium amalgam. This indicates a parallel between the effects of a 2-hydroxy- and a 2-amino-group on the course of reduction. This is of
interest in connection with naturally-occurring pteridines, almost all of which have an amino-group in the 2-position.

2-Hydroxy-6-methylpteridine (like 2-hydroxypteridine) was reduced with potassium borohydride to two compounds, one was an x,y-dihydro-2-hydroxy-6-methylpteridine and had quite similar properties (ultraviolet spectra and pKa) to 3,4-dihydro-2-hydroxypteridine. This suggests the 3,4-dihydro-structure for this compound. [The other product also had quite similar Rf values and fluorescence to 5,6,7,8-tetrahydro-2-hydroxypteridine]. The methyl group in the 6-position has therefore no appreciable effect on the reduction. This information is relevant to the folic acid series which always has a substituted methyl group in the 6-position.

<table>
<thead>
<tr>
<th></th>
<th>3,4-Dihydro-2-hydroxy-pteridine</th>
<th>3,4-Dihydro-2-hydroxy-6-methylpteridine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rf. (NH₄Cl) (fu/Ac)</td>
<td>0.60 2B/3V</td>
<td>0.58 2B/3V</td>
</tr>
<tr>
<td>pKa (basic)</td>
<td>0</td>
<td>0.20 ± 0.06</td>
</tr>
<tr>
<td>pKa (acidic)</td>
<td>12.5</td>
<td>13.05 ± 0.02</td>
</tr>
<tr>
<td>UV-spectra</td>
<td>λ max. (μν) log ε</td>
<td>λ max. (μν) log ε</td>
</tr>
<tr>
<td>o</td>
<td>248, 317 3.96, 3.88</td>
<td>248, 321 3.83, 3.92</td>
</tr>
<tr>
<td>-</td>
<td>261, 343 3.94, 3.82</td>
<td>282, 350 4.01, 3.84</td>
</tr>
</tbody>
</table>
iii) Ionic species.

A clear case of ionic species affecting reduction is that of the hydrogenation of 2-hydroxypteridine (see p.44). This effect arises from the fact that the neutral molecule (unlike the anion) is hydrated across the 3,4-double bond (Brown and Mason, 1956), and is therefore already a dihydropteridine.

iv) Reducing agent.

Close similarity was shown by the two reducing agents, potassium borohydride and hydrogenation over palladium catalyst in alkaline solution. There was however one exception. 2,4-Dihydroxypteridined was successfully reduced over palladium catalyst but was not affected by potassium borohydride.

Palladium on carbon catalyst was more powerful than Adams' platinum oxide catalyst in alkaline solution, and this is seen in the rate of reduction by these two catalysts (see Table 4).
Table 4.

Reduction period of hydroxypteridines in 0.1N-potassium hydroxide solution.

<table>
<thead>
<tr>
<th>Pteridines</th>
<th>Catalyst</th>
<th>10% Palladium on Carbon</th>
<th>Adams' Platinum Oxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Hydroxy-a)</td>
<td></td>
<td>20 min.</td>
<td></td>
</tr>
<tr>
<td>4-Hydroxy-</td>
<td></td>
<td>30 min.</td>
<td></td>
</tr>
<tr>
<td>6-Hydroxy-</td>
<td></td>
<td>20 min.</td>
<td></td>
</tr>
<tr>
<td>7-Hydroxy-b)</td>
<td></td>
<td>40 min.</td>
<td></td>
</tr>
<tr>
<td>2,4-Dihydroxy-</td>
<td></td>
<td>40 min.</td>
<td></td>
</tr>
<tr>
<td>2,6-Dihydroxy-</td>
<td></td>
<td>10 min.</td>
<td>7 hr. *</td>
</tr>
<tr>
<td>2,7-Dihydroxy-</td>
<td></td>
<td>70 min.</td>
<td>40 hr. *</td>
</tr>
<tr>
<td>4,6-Dihydroxy-</td>
<td></td>
<td>70 min.</td>
<td></td>
</tr>
<tr>
<td>4,7-Dihydroxy-c)</td>
<td></td>
<td>17 hr.</td>
<td></td>
</tr>
<tr>
<td>2,4,6-Trihydroxy-</td>
<td></td>
<td>10 min.</td>
<td></td>
</tr>
</tbody>
</table>

a) mono-hydroxypteridine (1 m mole) in 1.5 eq. 0.1N-KOH

b) di-hydroxypteridine  (1 m mole) in 2 x 1.5 eq.

c) tri-hydroxypteridine  (1 m mole) in 3 x 1.5 eq.

were reduced over 50 mg. 10% palladium on carbon catalyst.

* 5 mg. Adams' catalyst.
Sodium amalgam reduced all 6-hydroxypteridines to the corresponding 7,8-dihydro-6-hydroxypteridines, even when it required the elimination of a 7-hydroxy-group as in the case of 6,7-dihydroxypteridines. Two apparent exceptions were noted (the reductions of 2,6-dihydroxy- and 2,6,7-trihydroxy-pteridines) due to the instability of the product to this reagent.

Sodium dithionite produced a sulphur containing product (see e.g. 2,4-dihydroxypteridine, p.53 ) in the reduction of several polyhydroxypteridines.

v) Positions and numbers of hydroxy groups.

A systematic correlation between the facility of hydrogenation and the number and positions of hydroxy groups can be seen in Table 4, p. 64. It can be said generally that 6-hydroxypteridines are more easily reduced than the 7-isomers, and 2-hydroxypteridines are more easily reduced than the 4-isomers. The results which were obtained by reduction of hydroxypteridines are summarized in Table 5, p. 66.
### Table 5.
Reduction products of hydroxypteridines by various reducing agents.

<table>
<thead>
<tr>
<th>2-Hydroxypteridine</th>
<th>Potassium borohydride</th>
<th>Potassium amalgam</th>
<th>Sodium dithionite</th>
<th>Hydrogenation over Pd/C</th>
<th>5,6,7,8-TH-2</th>
<th>5,6,7,8-TH-2</th>
<th>5,6,7,8-TH-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,4-DH-2</td>
<td>UI</td>
<td>3,4-DH-2</td>
<td>UI</td>
<td>UI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5,6,7,8-TH-2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-Hydroxypteridine</td>
<td>x,y-DH-4</td>
<td>5,6,7,8-TH-4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5,6,7,8-TH-4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-Hydroxypteridine</td>
<td>7,8-DH-6</td>
<td>7,8-DH-6 (1)</td>
<td>7,8-DH-6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7,8-DH-6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-Hydroxypteridine</td>
<td>5,6-DH-7</td>
<td>5,6-DH-7</td>
<td>5,6-DH-7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5,6-DH-7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,4-Dihydroxypteridine</td>
<td>No</td>
<td>5,6,7,8-TH-4</td>
<td>5,6,7,8-TH-4</td>
<td></td>
<td>5,6,7,8-TH-4</td>
<td>5,6,7,8-TH-4</td>
<td>5,6,7,8-TH-4</td>
</tr>
<tr>
<td>2,6-Dihydroxypteridine</td>
<td>7,8-DH-2,6</td>
<td>D</td>
<td></td>
<td></td>
<td>7,8-DH-2,6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,7-Dihydroxypteridine</td>
<td>x,y-DH-2,7</td>
<td>S</td>
<td></td>
<td></td>
<td>x,y-DH-2,7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4,6-Dihydroxypteridine</td>
<td>7,8-DH-4,6</td>
<td>7,8-DH-4,6 (2)</td>
<td></td>
<td></td>
<td>5,6-DH-4,6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4,7-Dihydroxypteridine</td>
<td>5,6-DH-4,7</td>
<td>5,6-DH-4,7</td>
<td></td>
<td></td>
<td>5,6-DH-4,7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6,7-Dihydroxypteridine</td>
<td>No</td>
<td>7,8-DH-6 (1)</td>
<td>No</td>
<td></td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,4,6-Trihydroxypteridine</td>
<td>7,8-DH-2,4,6</td>
<td>7,8-DH-2,4,6</td>
<td></td>
<td></td>
<td>7,8-DH-2,4,6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,4,7-Trihydroxypteridine</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>2,6,7-Trihydroxypteridine</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>4,6,7-Trihydroxypteridine</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>2,4,6,7-Trihydroxypteridine</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

(1) Albert, Brown and Cheeseman, 1952
(2) Albert and Brown, 1953
(3) Albert, Lister and Pedersen, 1956

**Key to Symbols**
- x,y-DH-4 = x,y-Dihydro-4-hydroxypteridine
- 5,6,7,8-TH-2 = 5,6,7,8-Tetrahydro-2-hydroxypteridine
- UI = Unidentified unstable product
- D = Decomposition
- No = No reduction
- RO = Ring opened product
- S = Sulphur containing product.
b. Syntheses.

Syntheses Directed Towards Hydrogenated 2-Hydroxypteridines.

7,8-Dihydro-2-hydroxypteridine (and its 6-methyl-derivative).

The syntheses of 7,8-dihydropteridines have been well developed, and 7,8-dihydro-2-hydroxy-4,6-dimethylpteridine has been prepared (Lister and Ramage, 1953) (see p. 10). In the present work, 7,8-dihydro-2-hydroxy-6-methylpteridine was synthesized by a similar method, in order to compare the reduction behaviour of 2-hydroxypteridine and its 6-methyl derivative. 2-Hydroxy-6-methylpteridine was prepared from the 7,8-dihydro-derivative by oxidation with potassium permanganate, and reduced with potassium borohydride (see p. 62). The 6-methyl-isomers, 2-hydroxy-6-methylpteridine, 7,8-dihydro-2-hydroxy-6-methylpteridine and x,y-dihydro-2-hydroxy-6-methylpteridine, had quite similar properties, pKa values, ultraviolet spectra and Rf. values to the corresponding 2-hydroxypteridines (see Table 6, p. 104 and Table 15, p. 120). Although no 7,8-dihydropteridines unsubstituted in the 6-position have been prepared, it was hoped that 7,8-dihydroxy-2-hydroxypteridine could be synthesized by using amino acetal in place of amino acetone in the Lister-Ramage synthesis (see p. 6, 10). Although some difficulties were encountered (e.g. the tendency of aminoacetaldehydes to dimerize to pyrazines), this new synthetic method was successful.
Condensation of 2,4-dichloro-5-nitropyrimidine (3b-I) with amino acetal has been attempted before (Boon and Jones, 1950), but no product was isolated. In the present work, all attempts to isolate (3b-II) from its mixture with the 2,4-disubstituted analogues were unsuccessful. Accordingly, the crude condensation product was hydrolyzed directly with
sodium hydroxide to give 4-ß-diethoxyethylamino-2-hydroxy-5-nitropyrimidine (3b-III). At this stage the disubstituted derivative, 2,4-bis-ß-diethoxyethylamino-5-nitropyrimidine, was easily removed because of its insolubility in N-sodium hydroxide solution. Hydrogenation of (3b-III) gave the 5-amino derivative (3b-IV). Attempted brief hydrolysis of the acetal group of (3b-IV) with 0.1N-hydrochloric acid gave a product showing three spots on paper chromatography. Various conditions of pH and reaction time were examined to separate three products. The first substance corresponded to (3b-VII), and the second substance was later confirmed as 7,8-dihydro-2-hydroxypteridine (3b-VI) by the synthesis described below, but an analytically pure sample could not be isolated from this reaction. The third substance was the acid decomposition product of 7,8-dihydro-2-hydroxypteridine.

![Structure of 3b-VII]

A successful route was found in which (3b-III) was hydrolysed by acid before the hydrogenation. Thus, 2-hydroxy-4-formylmethylamino-5-nitropyrimidine (3b-V) was obtained from (3b-III) by refluxing with N-hydrochloric acid.
The aldehyde was quite unstable in alkali, and it was found necessary to stop the neutralization of the above reaction mixture at pH 4; neutralization even to pH 7 caused partial decomposition of the product. The aldehyde (3b-V) was successfully hydrogenated over Raney-nickel in methanol (but not in ethanol) to give 7,8-dihydro-2-hydroxypteridine (3b-VI). This 7,8-dihydro-2-hydroxypteridine (see p. 43) had a high basic pKa (see Table 9, p.107) compared to 2-hydroxypteridine.

5,6,7,8-Tetrahydro-2-hydroxypteridine. One attempt to synthesize 5,6,7,8-tetrahydro-2-hydroxypteridine through 8-benzyl-5,6,7,8-tetrahydro-2-hydroxypteridine has been reported, but it was unsuccessful because of difficulty in the debenzylation (Brook and Ramage, 1955) (see, p. 12). On the other hand it has been known that 7,8-dihydropteridines can be hydrogenated to 5,6,7,8-tetrahydropteridines (Lister and Ramage, 1953). (This method is a little ambiguous because of the possible hydrogen migration by the metal catalyst). 5,6,7,8-Tetrahydro-2-hydroxypteridine was synthesized from the above 7,8-dihydro-2-hydroxypteridine by hydrogenation over Adams' catalyst in neutral aqueous solution (hydrogenation in water gave better results than in acidic conditions). The structure was confirmed as 5,6,7,8-tetrahydro-2-hydroxypteridine by comparison of its pKa values, and ultraviolet spectra with those of 4,5-diamino-2-
hydroxypyrimidine (see Table 8, p.106, and Table 13, p.118).

The structure of the minor product obtained by reduction of 2-hydroxypteridine with potassium borohydride, and by hydrogenation over palladium, Raney-nickel, or Adams' catalyst in alkaline solution, was shown to be 5,6,7,8-tetrahydro-2-hydroxypteridine by comparison of its ultraviolet spectra and Rf values with the above (see p.42).

5,6-Dihydro-2-hydroxypteridine. No attempts to prepare 5,6-dihydropteridines had previously been made. The two following routes are possibilities for the preparation of 5,6-dihydropteridines (3b-X):

a) by cyclization of 4,5-diaminopyrimidines substituted on the 4-amino group (e.g. 3b-VIII)

b) by cyclization of 4,5-diaminopyrimidines substituted on the 5-amino group (e.g. 3b-IX)
Method (a) could not be used for the synthesis of 5,6-dihydro-2-hydroxypteridine because of difficulties in the preparation of intermediates of the type (3b-VIII). The only difficulty in method (b) is the preparation of 5-alkylaminopyrimidines. Direct alkylation leads to quaternization. However, two methods for indirect alkylations are possible. One is reduction of 5-formylaminopyrimidines with lithium aluminium hydride (Baker, Schaub and Joseph, 1954), and the other is

(Where \( R_1, R_2 \) and \( Y = \text{OH or H; } \)

\( X = \text{halogen; } \)

\( Z = -\text{CHO, -COOH or any groups which can be changed to these groups} \)
reductive alkylation (or reduction of the Schiff's base) of 5-aminopyrimidines. It is known that 5-aminopyrimidines condense with benzaldehyde to give Schiff's bases (Traube and Nithak, 1906; Shirakawa, 1953), but with aliphatic aldehydes only resinous products are produced (Shirakawa, 1953). The sole example of reductive alkylation of aminopyrimidine is the conversion of 5-amino-4-methylpyrimidine (3b-XI) to 5-ethylamino-4-methylpyrimidine (3b-XII) using a palladium catalyst and an excess of acetaldehyde (Overberger, Kogon and Einstman, 1954).

![Chemical structures](image)

In the present work the following route was used to prepare 5,6-dihydro-2-hydroxypteridine (3b-XVI)
4,5-Diamino-2-hydroxypteridine (3b-XIII) was condensed with glyoxal monodiethyl acetal (this was prepared from acrolein, Witzemann, Evans, Hass and Schroeder, 1943 b,a,c; Fischer and Baer, 1935), and although no product could be isolated paper chromatograms showed that the reaction had taken place. The reaction mixture was then hydrogenated over Raney-nickel catalyst, and 4-amino-5-ß-diethoxyethylamino-2-hydroxy-pyrimidine (3b-XV) was isolated. The structure of (3b-XV) was confirmed by its non-identity with 5-amino-4-ß-diethoxyethylamino-2-hydroxypyrimidine (3b-IV), prepared by an unambiguous synthesis (see, p.58). The pyrimidine gave a yellow amorphous product on treatment with N-hydrochloric acid. This substance is believed to be 5,6-dihydro-2-hydroxypteridine because pure 5,6-dihydro-4-hydroxypteridine was made from 4,5-diamino-6-hydroxy-
pteridine in the same way (see below). Although a good analysis could not be obtained for this compound because of partial decomposition during purification, paper chromatography indicated its essential homogeneity and that it was not \(x,y\)-dihydro-2-hydroxypteridine.

**3,4-Dihydro-2-hydroxypteridine.** No attempts to prepare 3,4-dihydropteridines have been previously made. However, an example of the synthesis of a fused 3,4-dihydropyrimidine ring is seen in the preparation of 3,4-dihydroquinazoline (3b-XVIII) from the \(o\)-aminobenzylamine derivative (3b-XVII) (Gabriel and Jansen, 1890).

The following routes were considered for the synthesis of 3,4-dihydro-2-hydroxypteridine (3b-XXI):
2-Amino-3-formylpyrazine oxime (3b-XIX) was synthesized by a known method (Albert, Brown and Wood, 1956). Reduction of the oxime (3b-XIX) to 2-amino-3-aminomethylpyrazine (3b-XX) under several sets of conditions was examined (hydrogenation over Adams', palladium, or Raney-nickel catalysts, and reduction with lithium aluminium hydride, sodium amalgam (under neutral conditions) and sodium hydrosulphite). The catalytic hydrogenation of the corresponding nitrile was also unsuccessful.
The most promising result was the hydrogenation over Adams' catalyst where the oxime absorbed 2.5 moles of hydrogen (theoretical amount is 2 moles) and gave a green oil. Chromatography showed that the product had one main spot, but no solid material could be isolated from the reaction mixture, and treatment of the crude product with urea, cyanic acid, ethyl chloroformate, or with urethane gave a complex mixture in which no product identical with the \( x, y \)-dihydro-2-hydroxypteridine could be seen when compared on a paper chromatogram.

Another synthetic route through 2-amino-3-carboxy-pyrazine (3b-XXII) was examined:

\[
\begin{align*}
(3b-\text{XXII}) & \xrightarrow{\text{LiAlH}_4} (3b-\text{XXIII}) & \xrightarrow{\text{H}_2, \text{HBr}, \text{urea}, \text{KCN}} (3b-\text{XXIV}) \\
& \xrightarrow{} (3b-\text{XXV})
\end{align*}
\]
(3b-XXII) was prepared from 2,4-dihydroxypteridine (Weijlard, Tischler and Erickson, 1945), but direct reduction of the acid (3b-XXIII) with aluminium amalgam failed. However, the methyl ester (3b-XXIII) was easily reduced with lithium aluminium hydride to 2-amino-3-hydroxymethylpyrazine (3b-XXIV). However, the attempted replacement of the hydroxy group with bromine by heating with hydrobromic acid led only to decomposition. Other attempts to utilize this intermediate (3b-XXIV), such as fusion with urea, or reaction with cyanic acid, gave no better results (such reactions had been successfully carried out in the quinazoline field, Söderbaum and Widman, 1889; Gabriel and Stelzner, 1896).

Another approach to 3,4-dihydro-2-hydroxypteridine was planned, starting from the hydrogenated pyrimidine, (3b-XXVI) → (3b-XXI). In hydrochloric acid 4,5-diamino-2-hydroxypyrimidine (3b-XXVI) absorbed one mole of hydrogen over palladium catalyst. The product, however, did not correspond to C₄H₇N₄O-HCl, 4,5-diamino-dihydro-2-hydroxypteridine (3b-XXVII), but analysed as C₄H₇N₃O₂·HCl·H₂O. These figures show that one amino group of (3b-XXVII) was replaced by a hydroxyl group and the compound could therefore not be used as an intermediate for dihydro-2-hydroxypteridine (3b-XXI).
Syntheses Directed Towards Hydrogenated 4-Hydroxypteridines

5,6,7,8-Tetrahydro-4-hydroxypteridine. An unambiguous route to 5,6,7,8-tetrahydropteridines has been described by Brook and Ramage (1955, 1957) (see p. 11). In using this method it is essential to protect all groups (such as NH$_2$ or OH) which have hydrogen atoms tautomizerable with the ring nitrogen atoms. This protection, which could be afforded by O-alkylation (-OME) or N-benzylation (-NHCH$_2$Ph), is required to prevent alternative ring closure to pyrimidinoimidazoles (Ramage and Trappe, 1952; Brook and Ramage, 1955, 1957).

Failure to afford such protection to groups other than those forming the pyrazine ring has, in practice, limited
the number of known 5,6,7,8-tetrahydropteridines to those having a hydrogen-, methyl-, or chloro-group in the 2-, or the 4-position. The solitary exception is 5,6,7,8-tetrahydro-2-hydroxy-4-methyl-8-benzylpteridine (lb-XXVI) which was prepared by hydrolysis of the corresponding 2-chloro derivative (see p. 12).

In the present work, this method was attempted for the 4-hydroxy-isomer but found unsuccessful, although, as will be shown later, the desired substance was eventually obtained by modification of the route:

\[
\begin{align*}
\text{Cl} & \quad \text{Cl} \\
\text{O}_2\text{N} & \quad \text{O}_2\text{N} \\
\text{Cl} & \quad \text{H}_2\text{Ni} \\
\text{HOCH}_2\text{CH}_2\text{N} & \quad \text{HOCH}_2\text{CH}_2\text{N} \\
\text{CH}_2\text{Ph} & \quad \text{CH}_2\text{Ph} \\
\end{align*}
\]

\((3b-\text{XXVIII}) \quad \rightarrow \quad (3b-\text{XXIX}) \quad \rightarrow \quad (3b-\text{XXX})\)

\[
\begin{align*}
\text{Cl} & \quad \text{Cl} \\
\text{H} & \quad \text{H} \\
\text{H} & \quad \text{H} \\
\text{H} & \quad \text{H} \\
\text{Cl} & \quad \text{Na}/\text{NH}_3 \\
\text{CH}_2\text{Ph} & \quad \text{CH}_2\text{Ph} \\
\end{align*}
\]

\((3b-\text{XXXI}) \quad \rightarrow \quad (3b-\text{XXXII})\)
4,6-Dichloro-5-nitopyrimidine (3b-XXVIII) was condensed with β-benzylaminoethanol in chloroform to give (3b-XXIX) but the product could not be isolated. The product was hydrogenated over Raney-nickel and 5-amino-4-(benzyl-β-hydroxyethylamino)-6-chloropyrimidine hydrochloride (3b-XXX) was isolated. (This hydrochloride, $C_{13}H_{16}Cl_2N_4O$, was isomerized when boiled in ethanol to an unidentified compound, $C_{13}H_{16}Cl_2N_4O$, which did not cyclize on treatment with phosphorus trichloride). The 5-aminopyrimidine hydrochloride (3b-XXX) was cyclized directly with phosphorus trichloride to 8-benzyl-4-chloro-5,6,7,8-tetrahydropteridine (3b-XXXI) the structure of which was confirmed by reduction with sodium in liquid ammonia to the known 5,6,7,8-tetrahydropteridine (3b-XXXII). However, the 4-chloro group could not be replaced with a hydroxyl group by such treatment as refluxing for three hours with (a) pH 5 buffer, (b) glacial acetic acid plus sodium acetate, (c) 6N-hydrochloric acid, (d) 12N-hydrochloric acid, (e) $N$-sodium hydroxide, or (f) methanolic sodium methoxide. Heating in triethylene glycol at 120° with sodium hydroxide decomposed the material.

Because of the difficulty of replacement of the 4-chloro group in the above tetrahydropteridine, it seems reasonable to introduce other types of group into the 4-position. It is well known, that the benzyl group can be reduced to a hydroxy group by catalytic hydrogenolysis using palladium on carbon. The following route was therefore
Attempts to isolate the product from each of the steps (l., and 2) were unsuccessful, but (3b-XXXIII) was finally isolated as the hydrochloride in low yield. Treatment of (3b-XXXIII) with phosphorus trichloride gave the pyrimidine (3b-XXXIV) instead of the desired tetrahydropteridine, and the method was therefore abandoned.

However, the facile hydrolysis of the 4-benzyloxy group suggested a new synthetic route in which ethyl replaced benzyl.
4-Chloro-6-ethoxy-5-nitropyrimidine (3b-XXXV) (Boon and Jones, 1951) was condensed with β-benzylaminoethanol and the condensation product (3b-XXXVI) was hydrogenated over Raney-nickel. The aminopyrimidine (3b-XXXVII) could not be isolated, but was treated with phosphorus trichloride at room temperature to cyclise it. Not only cyclisation but also simultaneously slow hydrolysis of the 4-ethoxy group occurred. Paper chromatography showed that the hydrolysis was complete after two days at room temperature, and from the reaction mixture 8-benzyl-5,6,7,8-tetrahydro-4-hydroxypteridine (3b-XXXVIII) was isolated. Debenzylation of (3b-XXXVIII) was
accomplished with sodium in liquid ammonia, and 5,6,7,8-tetrahydro-4-hydroxypteridine (3b-XXXIX) was obtained. By comparison of their pKa values, ultraviolet and infrared spectra, the 5,6,7,8-tetrahydro-4-hydroxypteridine was shown to be identical with the product obtained by reduction of 4-hydroxypteridine (see p.48).

5,6-Dihydro-4-hydroxypteridine (3b-XLII) was synthesized by essentially the same method as was used in the preparation of the 2-hydroxy-isomer (see p.74).

4,5-Diamino-6-hydroxypyrimidine (3b-XL), hydrogenated over Raney-nickel in ethanol containing glyoxal monoacetal, gave 4-amino-5-β-diethoxyethylamino-6-hydroxypyrimidine (3b-XLI). This pyrimidine, when boiled with N-hydrochloric acid for one minute, gave 5,6-dihydro-4-hydroxypteridine
(3b-XLII). The compound was not identical with the \( x,y \)-dihydro-4-hydroxypteridine, formed by reduction, which must therefore be an isomer.

<table>
<thead>
<tr>
<th></th>
<th>( x,y )-Dihydro-4-hydroxypteridine obtained by reduction</th>
<th>5,6-Dihydro-4-hydroxypteridine obtained by synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>colour</td>
<td>yellow</td>
<td>colourless</td>
</tr>
<tr>
<td>m.p.</td>
<td>263-265 (decomp.)</td>
<td>230 (decomp.)</td>
</tr>
<tr>
<td>pKa basic</td>
<td>0.32 ± 0.05</td>
<td>2.94 ± 0.04</td>
</tr>
<tr>
<td>acidic</td>
<td>12.13 ± 0.03</td>
<td>10.24 ± 0.1</td>
</tr>
</tbody>
</table>

7,8-Dihydro-4-hydroxypteridine (and 7,8-dihydro-4-hydroxy-6-methylpteridine). A method similar to the one used for the synthesis of 7,8-dihydro-2-hydroxypteridine (3b-VI) (see p.68) was attempted for the preparation of 7,8-dihydro-4-hydroxypteridine, but it failed to yield a 7,8-dihydro compound.

3,7-Dihydro-4-hydroxypteridine. No suitable method could be found to prepare 3,7-dihydro-4-hydroxypteridine. However, 3,7-dihydro-4-hydroxypteridine and its 7,8-dihydro-isomer have a common cation. If the 3,7-isomer is more stable than the 7,8-dihydro-isomer, this compound would be obtained by any syntheses suitable for the 7,8-dihydro-isomer. Unfortunately, the synthesis of 7,8-dihydro-4-hydroxypteridine was also unsuccessful.
4,6-Dichloro-5-nitropyrimidine (3b-XXVIII) was condensed with aminoacetal and although the intermediate (3b-XLIII) could not be isolated, the condensed product was hydrolysed by heating with N-sodium hydroxide to give 4-ß-diethoxyethylamino-6-hydroxy-5-nitropyrimidine (3b-XLIV). This absorbed five moles of hydrogen (cf. three moles for a nitro group) over Raney-nickel and none of the desired aminopyrimidine (3b-XLV) could be isolated. Reduction of (3b-XLIV) with zinc dust in acetic acid also gave a mixture from which (3b-XLV) could not
be isolated. Hydrolysis of (3b-XLIV) with N-hydrochloric acid gave a product analysing for $\text{C}_{12}\text{H}_8\text{N}_8\text{O}_6\cdot\frac{1}{2}\text{H}_2\text{O}$, and this compound absorbed ten moles of hydrogen over Raney-nickel (calculated on the above formula) and gave a dark resinous product. No 7,8-dihydro-4-hydroxypteridine was obtained by this method.

Because of the difficulty of synthesizing the 6-unsubstituted derivative, the synthesis of 7,8-dihydro-4-hydroxy-6-methylpteridine was attempted because it should have similar pKa values and spectra. The following route was used:

\[
\begin{align*}
\text{Cl} & \quad \text{Cl} \\
\text{O}_2\text{N} & \quad \text{O}_2\text{N} \\
\text{Cl} & \quad \text{HN} \\
\text{N} & \quad \text{N} \\
\text{N} & \quad \text{N} \\
\text{N} & \quad \text{N} \\
\text{N} & \quad \text{N} \\
\end{align*}
\]

(3b-XXVIII) \hspace{2cm} \text{Cl} \hspace{2cm} \text{OH}

\[
\begin{align*}
\text{NH}_2\text{CH}_2\text{COCH}_3 & \quad \text{CH}_2\text{COCH}_3 \\
\text{O}_2\text{N} & \quad \text{O}_2\text{N} \\
\text{HN} & \quad \text{HN} \\
\text{N} & \quad \text{N} \\
\text{N} & \quad \text{N} \\
\end{align*}
\]

(3b-XLVII) \hspace{2cm} (3b-XL VIII)

4,6-Dichloro-5-nitropyrimidine was condensed with aminoacetone by Boon's method (Boon and Jones, 1951), and the product (presumably 3b-XLVII) was hydrolyzed with aqueous ethanol to 4-acetonylamino-6-hydroxy-5-nitropyrimidine (3b-XL VIII). However, in the hydrogenation of the pyrimidine (3b-XL VIII) over Raney-nickel, a side-reaction took place similar to that
which happened in the reduction of the 4-ß-diethoxyethylamino-
isomer (3b-XXIV) (see p. 86). No 7,8-dihydro-4-hydroxy-6-
 methylpteridine was obtained.

1,2-Dihydro-4-hydroxypteridine. The 1,2-dihydro-
structure is improbable for the reduction product of 4-hydroxy-
pteridine, because it was found possible to reduce the x,y-
dihydro-4-hydroxypteridine further with potassium borohydride or
by hydrogenation over Raney-nickel catalyst to give 5,6,7,8-
 tetrahydro-4-hydroxypteridine.

No attempts have been previously made to prepare 1,2-
dihydropteridines. An example of the synthesis of a fused
1,2-dihydropyrimidine ring is seen in the preparation of
1-phenyl-1,2-dihydro-4-quinazoline (3b-L) from
2-aminobenzanilide (3b-XLIX) and formaldehyde (Feldman and
Wagner, 1942).

\[
\begin{align*}
\text{CONHPh} & \quad \text{HCHO} \\
\text{NH}_{2} & \quad \text{(3b-XLIX)} \quad \text{O} \\
\text{O} & \quad \text{Ph} \\
\text{NH} & \quad \text{H} \\
\text{N} & \quad \text{H} \\
\text{H} & \quad \text{H} \\
\text{(3b-L)}
\end{align*}
\]

Because this reaction seemed to offer an easy opportunity to
obtain a 1,2-dihydropteridine, 2-amino-3-carbamoylpyrazine
3b-II) was warmed with formaldehyde but no reaction occurred
under the described conditions.
Synthesis Directed Towards Hydrogenated 7-Hydroxypteridine.

5,6-Dihydro-7-hydroxypteridine. As it seemed likely that the reduction product of 7-hydroxypteridine had this constitution, its synthesis was attempted by the following three routes:

a) The reduction of a Schiff's base (3b-LXVI) of ethyl glyoxylate hemiacetal and 4,5-diamino-6-hydroxypyrimidine gave the corresponding 5-alkylaminopyrimidine (3b-LXVII) which cyclized to 5,6-dihydro-4,7-dihydroxypteridine (see, p.95). Unfortunately this promising method was not successful with 4,5-diaminopyrimidine (3b-LIII). Thus 4,5-diaminopyrimidine (3b-LIII) showed no tendency to condense with ethyl glyoxylate hemiacetal (3b-LIV) in ethanol, and although it did so in aqueous ethanol, there followed spontaneous cyclization to 7-hydroxypteridine (3b-LVI) and no Schiff's base (3b-LV) could be isolated.
b) By analogy with a derivative of 5,6-dihydro-7-amino-pteridine (2d-V) which has been prepared through the 5-cyanomethylaminopyrimidine (2d-IV) (Blicke and Godt, 1954) (see p. 40), 4,5-diaminopyrimidine (3b-LIII) was condensed with formaldehyde and hydrogen cyanide to 4-amino-5-cyanomethylaminopyrimidine (3b-LVII). However, this compound (3b-LVII) was quite unstable to acid and alkali (hydrolysis) and it decomposed to 4,5-diaminopyrimidine (3b-LVI) and hydrogen cyanide on attempted cyclization.
c) An attempted preparation of 5,6-dihydro-7-hydroxypteridine through a 4-substituted aminopyrimidine (see p. 72) is shown below, but it was unsuccessful:

Condensation of 4-amino-5-bromopyrimidine (3b-LVIII) with phthaloylglycyl chloride gave 5-bromo-4-phthaloylglycylamino-pyrimidine (3b-LIX). However, the amide linkage between
the phthaloylglycyl group and the 4-aminopyrimidine was so labile that 2-amino-5-bromopyrimidine (3b-LVIII) was formed by hydrolysis of (3b-LIX) with hydrazine hydrate or with acid, and none of the desired (3b-LX) was obtained. These three routes are therefore abandoned.

As synthetic methods were unsuccessful, conversion of the ring opened product obtained by reduction of 7-hydroxypteridine (believed to be* 4-amino-5-carboxymethylaminopyrimidine; 3b-LXI) to 4-amino-5-cyanomethylaminopyrimidine (3b-LVII) was attempted. The methyl ester (3b-LXII) gave x,y-dihydro-7-hydroxypteridine on treatment with aqueous or methanolic ammonia. Heating the ammonium salt of the acid (3b-LXII) gave the same x,y-dihydro-7-hydroxypteridine, but ammonolysis of this (3b-LXIII) was unsuccessful.

---

*This constitution was later established, see p.111.
Later, confirmation of the constitution of (3b-LXI) established that of \( x,y \)-dihydro-7-hydroxypteridine as 5,6-dihydro-7-hydroxypteridine. Hence other attempts to synthesize (3b-LXIII) were dropped.

**Syntheses Directed Towards Hydrogenated 2,4-Dihydroxypteridine.**

Because of the instability of tetrahydro-2,4-dihydroxypteridine to oxidation (see p.51.), a route through 7,8-dihydro-2,4-dihydroxypteridine was sought. This involved condensation of 2,4,6-trichloro-5-nitropyrimidine with
aminocetal. A complex mixture of products resulted, but none of the desired substance was isolated. This synthesis was not further pursued as the constitution of the tetrhydro-compound is not in doubt.

Syntheses Directed Towards Hydrogenated 2,6-Dihydroxypteridine.

7,8-Dihydro-2,6-dihydroxypteridine was synthesized according to the method of Boon, Jones and Ramage (1951) (see p. 8). The 7,8-dihydro-2,6-dihydroxypteridine was identical with the sole product obtained by reduction of 2,6-dihydroxypteridine (see p. 57).

Syntheses Directed Towards Hydrogenated 4,6-Dihydroxypteridine.

7,8-Dihydro-4,6-dihydroxypteridine was synthesized according to the method of Boon, Jones and Ramage (1951) (see p. 8). The 7,8-dihydro-4,6-dihydroxypteridine was identical with the sole product obtained by reduction of 4,6-dihydroxypteridine (see p. 58).

Syntheses Directed Towards Hydrogenated 2,7-Dihydroxypteridine.

5,6-Dihydro-2,7-dihydroxypteridine. The synthesis of this compound by the same method used for the preparation of 5,6-dihydro-4,7-dihydroxypteridine (see p. 95), proved unsuccessful. Because condensation of 4,5-diamino-2-hydroxy- pyrimidine and ethyl glyoxylate hemiacetal gave 2,7-dihydroxypteridine in place of the desired Schiff's base. Proceeding by another route 4,5-diamino-2-hydroxypteridine did not give a cyanomethyl-derivative as did 4,5-diaminopyrimidine.
Synthesis Directed Towards Hydrogenated 4,7-Dihydroxypteridine.

5,6-Dihydro-4,7-dihydroxypteridine (3b-LXVIII) was synthesized by the following route (see also p.71), which is an entirely new method:

\[
\begin{align*}
\text{OH} & \quad \text{EtO}_2\text{CCH}=\text{N} \\
\text{H}_2\text{N} & \quad \text{EtO}_2\text{CHCO}_2\text{Et} \\
\text{H}_2\text{N} & \quad \text{EtO}_2\text{C}-\text{CH}_2\text{NH} \\
\text{Ni/H}_2 & \quad \text{HCl}
\end{align*}
\]

(3b-LXV)  
(3b-LXVI)  
(3b-LXVII)  
(3b-LXVIII)

The reduction of a Schiff's base is a well known method of preparing a secondary amine (Emerson, 1948). 4-Amino-5-ethoxycarbonylmethylenamino-6-hydroxypyrimidine (3b-LXVI) (Pfleiderer, 1959) absorbed one mole of hydrogen over Raney-nickel and gave 4-amino-5-ethoxycarbonylamino-6-hydroxypyrimidine (3b-LXVII). On treatment with hydrochloric acid, this pyrimidine gave 5,6-dihydro-4,7-dihydroxypteridine (3b-LXVIII). This substance was shown to be identical with the dihydro-4,7-dihydroxypteridine obtained as sole product by reduction of 4,7-dihydroxypteridine (see p.59).
Synthesis of 7,8-Dihydro-4,6-dimethylpteridine. The synthesis of unsubstituted 7,8-dihydropteridine was desired in order to lay a foundation for correlating the basic $pK_a$ values of 7,8-dihydropteridines and of the corresponding pteridines. However, no suitable method was found to prepare 7,8-dihydropteridine. Fortunately, however, it was found possible to prepare a simple methyl derivative, 7,8-dihydro-4,6-dimethylpteridine, by the following route:

$$\text{O}_2\text{N}$$
$$\text{Cl}$$
$$\text{N}$$
$$\text{N}$$
$$\text{Cl}$$
$$\text{CH}_3$$

(3b-LXIX)

$$\rightarrow$$

$$\text{O}_2\text{N}$$
$$\text{HN}$$
$$\text{CH}_2\text{COCH}_3$$
$$\text{Cl}$$
$$\text{N}$$
$$\text{N}$$

(3b-LXX)

$$\text{H}_2/\text{Ni}$$

(3b-LXXI)

$$\rightarrow$$

$$\text{H}_2/\text{Pd}$$

(3b-LXXII)

2-Chloro-7,8-dihydro-4,6-dimethylpteridine (3b-LXXI) has been prepared by Lister and Ramage (1953) from 2,4-dichloro-6-methyl-5-nitropyrimidine (3b-LXIX) via 4-acetonylamino-2-chloro-
6-methyl-5-nitropyrimidine (3b-LXX). Replacement of a 2-chloro-group by hydrogen had not been attempted before. Reduction with hydriodic acid was unsuccessful in this case, as the dihydropteridine was unstable to such conditions. However, 7,8-dihydro-4,6-dimethylpteridine was obtained by catalytic hydrogenation of 2-chloro-7,8-dihydro-4,6-dimethylpteridine over palladium on carbon catalyst.
c. Ionization Constants*.

Ionization constants are very useful to help in identification of substances which have no melting points and have been used several times for this purpose in the preceding pages (results summarized in Table 7). But apart from this, ionization constants yield two further types of information, both of them important.

First, an ionization constant can indicate which of the ionic species of the molecule is present in the solution at a particular pH. This information is essential when correlation of ultraviolet spectra and structure is attempted.

Second, information can be obtained from ionization constants which concerns directly the molecular structure. Although it is difficult to discuss the molecular structure of an isolated compound with only the information supplied by its ionization constants, one can do so when such information is available about a series of related compounds. In such a case, a good deal of important information on the structure of a new compound in a series can be obtained from a study of the ionization constants of

*The ionization constants of several hydrogenated hydroxypteridines, obtained by synthesis and by reduction, are shown in Table 5 (p.104) and 7. They are expressed as pKa values; which are negative logarithms of the constants. The higher the pKa value the stronger the base, and the weaker the acid.
the series.

In the present work, ionization constants of a number of (mainly hydrogenated) pteridines were determined for the above reasons, and the values are summarized in Table 6, p. 104.

The following paragraphs exemplify how knowledge of ionization constants was used in the present work to throw light on structures.

(a) When one looks at the structure of 5,6,7,8-tetrahydropteridine (3c-II), it is evident that this substance may be thought of as 4,5-diaminopyrimidine (3c-I), in which the two primary amino groups are alkylated by ethylene. Hence the two substances should have similar ionization constants. This turns out to be the case, for 4,5-diaminopyrimidine has $pK_a$ 6.03 and 5,6,7,8-tetrahydropteridine has $pK_a$ 6.63 (see Table 8).

![Diagram](image)

(3c-I) (3c-II)

The charge of the cation is shared between $N(1)$, $N(3)$ and the 4-amino group. The 5-amino group has almost no basic properties. It is known that acylation of $N(8)$ strikingly reduces the basic strength of this molecule.
In the present work, it was seen that 5,6,7,8-tetrahydro-4-hydroxypteridine (pKₐ, 3.86, 10.13) is related in this way to 4,5-diamino-6-hydroxypyrimidine (pKₐ 3.57, 9.86). Hence the pKₐ of the tetrahydro-2-hydroxypteridine (obtained by reducing 7,8-dihydro-2-hydroxypteridine, and suspected to be the 5,6,7,8-tetrahydro derivative) was examined to see if it had a pKₐ similar to that of 4,5-diamino-2-hydroxypteridine. The tetrahydro-2-hydroxypteridine had pKₐ values (4.35 and 12.5) similar to those of 4,5-diamino-2-hydroxypyrimidine (4.37 and 11.45) and the structure was suggested to be 5,6,7,8-tetrahydro-2-hydroxypteridine.

(b) Usually, 7,8-dihydropteridine have much higher pKₐ values (both acidic and basic) than have the corresponding pteridines (Table 9, p. 107). This difference does not exist if hydrated forms of the non-hydrogenated pteridines are used for comparison (only 2-, and 6-, hydroxypteridines have stable hydrated forms). The hydrated form of 6-hydroxypteridine, which is actually 7,8-dihydro-6,7-dihydroxypteridine (3c-IV) (Brown and Mason, 1956) has a molecular structure similar to 7,8-dihydro-6-hydroxypteridine, and shows the same base-strengthening and acid-weakening effect (Table 9, p. 107), when compared with the anhydrous form of 6-hydroxypteridine. This base strengthening affect is considered to be due to resonance stabilization of the cations of the 7,8-dihydro structure, (3c-V) ←→ (3c-VI), (R = H, or OH).
This kind of base strengthening effect has been shown (Albert, Goldacre and Phillips, 1948) to be general in those nitrogen heterocyclic compounds having a $\sigma$-amino structure which permits a resonance stabilization of the cation as above (Table 10, p.108). The base strengthening effect due to the $\sigma$-amino structure is much less marked than that due to the $\pi$-amino structure (Table 10, p.108), and $m$-amino groups show only the expected small inductive effect.

The constitution of $x,y$-dihydro-2-hydroxypteridine is also illuminated by comparison with the hydrated form of 2-hydroxypteridine, neither of which has any basic pKa value higher than 2, and both of which have very high acidic pK's (12.6 and 11.1 respectively). It has been known that 2-hydroxypteridine is hydrated at the 3,4-position (Brown and
Mason, 1956; see p. 44). Again, x,y-dihydro-2-hydroxy-6-methylpteridine has pKa values (both basic and acidic) somewhat similar to those of the hydrated 2-hydroxy-6-methylpteridine (Table 9, p. 107). For this reason, and because the ultraviolet spectra of the neutral molecules of hydrated 2-hydroxypteridines and their x,y-dihydro compounds are similar (see p.113), it is suggested that x,y-dihydro-2-hydroxypteridine has the 3,4-dihydro structure. (The close similarity of 2-hydroxypteridine and its 6-methyl derivative suggest that these two compounds are hydrated at the same positions). It is unfortunate that it proved impossible to synthesize 3,4-dihydro-2-hydroxypteridine (see p. 75, for attempts).

c) x,y-Dihydro-4-hydroxypteridine is not an appreciably stronger base than 4-hydroxypteridine (see Table 9, p.107). This may seem to support the 3,7-dihydro-structure (3c-V) for this compound, because it was found that 7,8-dihydropteridines are much stronger bases than the corresponding pteridines.

\[
\text{(3c-V)}
\]

However, the other two possible dihydro-4-hydroxypteridines would exist mainly in the lactam forms (3c-VI) and (3c-VII), and such structures can not take part in the above base strengthening resonance.
The acidic pKa of \( x,y \)-dihydro-4-hydroxypteridine is much higher than that of 4-hydroxypteridine. This acid weakening effect might be expected from the above three formulae but it does not help in the choice of any one of these structures.

In conclusion, no effective information on the structure of \( x,y \)-dihydro-4-hydroxypteridine can be obtained from ionization constants at the present stage.
<table>
<thead>
<tr>
<th>Pteridines</th>
<th>basic</th>
<th>acidic</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Hydroxy-6-methylpteridine</td>
<td>0.2</td>
<td>8.0*(anhydrous)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11.0*(hydrated)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dihydropteridines</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>7,8-Dihydro-4,6-dimethylpteridine</td>
<td>6.00 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>7,8-Dihydro-2-hydroxypteridine</td>
<td>3.50 ± 0.02</td>
<td>***</td>
</tr>
<tr>
<td>3,4-Dihydro-2-hydroxypteridine</td>
<td>0</td>
<td>12.6</td>
</tr>
<tr>
<td>7,8-Dihydro-2-hydroxy-6-methylpteridine</td>
<td>3.42 ± 0.06</td>
<td>11.85 ± 0.02</td>
</tr>
<tr>
<td>3,4-Dihydro-2-hydroxy-6-methylpteridine</td>
<td>0.20 ± 0.06</td>
<td>13.05 ± 0.02</td>
</tr>
<tr>
<td>7,8-Dihydro-2-hydroxy-4,6-dimethylpteridine</td>
<td>3.39 ± 0.02</td>
<td>12.50 ± 0.04</td>
</tr>
<tr>
<td>5,6-Dihydro-4-hydroxypteridine</td>
<td>2.94 ± 0.04</td>
<td>10.24 ± 0.1</td>
</tr>
<tr>
<td>x,y-Dihydro-4-hydroxypteridine</td>
<td>0.32 ± 0.05</td>
<td>12.13 ± 0.03</td>
</tr>
<tr>
<td>7,8-Dihydro-6-hydroxypteridine</td>
<td>4.53 ± 0.03**</td>
<td>10.54 ± 0.02**</td>
</tr>
<tr>
<td>5,6-Dihydro-7-hydroxypteridine</td>
<td>3.35 ± 0.01**</td>
<td>9.94 ± 0.05**</td>
</tr>
<tr>
<td>7,8-Dihydro-2,5-dihydroxypteridine</td>
<td>2.80 ± 0.02</td>
<td>10.22 ± 0.01</td>
</tr>
<tr>
<td>x,y-Dihydro-2,7-dihydroxypteridine</td>
<td>5.84 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>7,8-Dihydro-4,6-dihydroxypteridine</td>
<td>9.14 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>5,6-Dihydro-4,7-dihydroxypteridine</td>
<td>8.55 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>7,8-Dihydro-2,4,6-trihydroxypteridine</td>
<td>7.07 ± 0.03</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tetrahydropteridines</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>5,6,7,8-Tetrahydro-2-hydroxypteridine</td>
<td>4.35 ± 0.05</td>
<td>12.5</td>
</tr>
<tr>
<td>5,6,7,8-Tetrahydro-4-hydroxypteridine</td>
<td>3.86 ± 0.02</td>
<td>10.13 ± 0.03</td>
</tr>
</tbody>
</table>

* Determined by Dr. D.D. Perrin using rapid titration method.
** Albert, Brown and Cheeseman, 1952.
*** Because of its instability in strong alkali, no exact pKₐ value was obtained.
Table 7

Use of ionization constants to establish identity.

$pK_a$ values of hydrogenated pteridines prepared by synthesis and by reduction.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$pK_a (20^\circ)$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(basic)</td>
</tr>
<tr>
<td>7,8-Dihydro-2,6-dihydroxypteridine</td>
<td>2.82 ± 0.02</td>
</tr>
<tr>
<td>(authentic)</td>
<td></td>
</tr>
<tr>
<td>Dihydro-2,6-dihydroxypteridine</td>
<td>2.80 ± 0.02</td>
</tr>
<tr>
<td>(by reduction)</td>
<td></td>
</tr>
<tr>
<td>7,8-Dihydro-4,6-dihydroxypteridine</td>
<td></td>
</tr>
<tr>
<td>(authentic)</td>
<td></td>
</tr>
<tr>
<td>Dihydro-4,6-dihydroxypteridine</td>
<td></td>
</tr>
<tr>
<td>(by reduction)</td>
<td></td>
</tr>
<tr>
<td>5,6-Dihydro-4,7-dihydroxypteridine</td>
<td></td>
</tr>
<tr>
<td>(authentic)</td>
<td></td>
</tr>
<tr>
<td>Dihydro-4,7-dihydroxypteridine</td>
<td></td>
</tr>
<tr>
<td>(by reduction)</td>
<td></td>
</tr>
<tr>
<td>5,6,7,8-Tetrahydro-4-hydroxypteridine</td>
<td>3.87 ± 0.02</td>
</tr>
<tr>
<td>(authentic)</td>
<td></td>
</tr>
<tr>
<td>Tetrahydro-4-hydroxypteridine</td>
<td>3.86 ± 0.02</td>
</tr>
<tr>
<td>(by reduction)</td>
<td></td>
</tr>
</tbody>
</table>
Table 8.

$pK_a$ values of tetrahydropteridines and the corresponding 4,5-diaminopyrimidines.

<table>
<thead>
<tr>
<th></th>
<th>$pK_a (20^\circ)$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>basic</td>
</tr>
<tr>
<td>5,6,7,8-Tetrahydropteridine</td>
<td>6.63*</td>
</tr>
<tr>
<td>4,5-Diaminopyrimidine</td>
<td>6.03**</td>
</tr>
<tr>
<td>5,6,7,8-Tetrahydro-4-hydroxypteridine</td>
<td>3.86</td>
</tr>
<tr>
<td>4,5-Diamino-6-hydroxypyrimidine</td>
<td>3.57**</td>
</tr>
<tr>
<td>5,6,7,8-Tetrahydro-2-hydroxypteridine</td>
<td>4.35</td>
</tr>
<tr>
<td>4,5-Diamino-2-hydroxypyrimidine</td>
<td>4.37**</td>
</tr>
</tbody>
</table>

* Brook and Ramage, 1957.

** Mason, 1954.
<table>
<thead>
<tr>
<th>Pteridines</th>
<th>Anhydrous (basic)</th>
<th>Hydrated (basic)</th>
<th>7,8-Dihydropteridines (basic)</th>
<th>x,y-Dihydropteridines (basic)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(acidic)</td>
<td>(acidic)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4,6-Dimethylpteridine</td>
<td>7.72)</td>
<td>-1)</td>
<td>6.00</td>
<td>2</td>
</tr>
<tr>
<td>2-Hydroxypteridine</td>
<td>8.03)</td>
<td>-0.4</td>
<td>3.50</td>
<td>0.2</td>
</tr>
<tr>
<td>2-Hydroxy-6-methylpteridine</td>
<td>8.03)</td>
<td>-0.4</td>
<td>3.42</td>
<td>0.2</td>
</tr>
<tr>
<td>2-Hydroxy-4,6-dimethylpteridine</td>
<td>Substance unknown</td>
<td>-1)</td>
<td>3.99</td>
<td>0.2</td>
</tr>
<tr>
<td>4-Hydroxypteridine</td>
<td>-0.17</td>
<td>7.892)</td>
<td>4.78</td>
<td>0.32</td>
</tr>
<tr>
<td>6-Hydroxypteridine</td>
<td>6.452)</td>
<td>3.674)</td>
<td>4.78</td>
<td>0.32</td>
</tr>
<tr>
<td>2,6-Dihydroxypteridine</td>
<td>5.99,9.662)</td>
<td>8.84,10.292)</td>
<td>2.80</td>
<td>10.22</td>
</tr>
<tr>
<td>4,6-Dihydroxypteridine</td>
<td>6.05,9.512)</td>
<td>8.34,10.082)</td>
<td>9.14</td>
<td></td>
</tr>
</tbody>
</table>

1) This compound is not known, but the $pK_a$ values of similar methyl derivatives (respectively 2.94 and 2.93 for 4-methyl- and 6,7-dimethyl-pteridines) suggest that the $pK_a$ value of this compound is about 3.

2) Perrin and Inoue, 1960.

3) Kindly measured by Dr. D.D. Perrin by a special rapid technique.


5) This compound has an acidic $pK_a$ near 12, but because of its instability in strong alkali a good value was not obtained.

d. Spectra
i) Ultraviolet Absorption Spectra

Ultraviolet spectra are important in molecular structure determination, not only in identification of specimens prepared by different routes, but also for the fundamental information that they can contribute (see p. 31).

In the present work, ultraviolet spectra of hydrogenated pteridines were used for both reasons. The spectra were determined on each ionic species of the substances, carefully isolated from other species by using buffers whose pH values were indicated by knowledge of the appropriate pKa values. These spectra are summarized in Table 11, p. 116.

The following cases exemplify the use of ultraviolet spectra in determining the structure of hydrogenated pteridines.

a) It has been reported that 5,6,7,3-tetrahydropteridines have ultraviolet absorptions similar to those of the corresponding 4,5-diaminopyrimidines, and that a 15-18 μ bathochromic shift is observed in the spectra of tetrahydropteridines when compared to the corresponding 4,5-diaminopyrimidines (Lister and Ramage, 1953; Taylor and Sherman, 1959) (Table 12, p. 117).

In the present work the synthesis of 5,6,7,8-tetrahydro-2-hydroxypteridine involved the catalytic hydrogenation of 7,8-dihydro-2-hydroxypteridine (see p. 70). The result of this reaction is rather ambiguous due to the
possibility of migration of hydrogen atoms in the presence of a metal catalyst. Evidence for the 5,6,7,8-tetrahydro structure, however, was obtained by comparing the ultraviolet spectra of the substance (neutral molecule, cation and anion) with those of 4,5-diamino-2-hydroxypyrimidine (Table 13, p. 118, and Fig. 4,5 and 6). It will be noticed that each ionic species of tetrahydro-2-hydroxypteridine has an absorption curve closely similar to that of the corresponding species of 4,5-diamino-2-hydroxypyrimidine, but shifted to longer wavelengths by approximately 12-22 mp. These facts are in accord with the results obtained by Lister and Ramage (1953), and by Taylor and Sherman (1959), and it may therefore be reasonably concluded that the hydrogen atoms in tetrahydro-2-hydroxypteridine occupy the 5-, 6-, 7-, and 8-, positions. Further, 5,6,7,8-tetrahydro-4-hydroxypteridine, prepared unambiguously, also has ultraviolet spectra (of neutral molecule, cation and anion) closely similar to those of the corresponding ionic species of 4,5-diamino-6-hydroxypyrimidine (Table 13, and Fig. 7,8 and 9).

b) As already stated (p.15) the product obtained by alkaline hydrolysis of dihydro-7-hydroxypteridine seemed to be 4-amino-5-carboxymethylaminopyrimidine (3d-I) on reasonable chemical evidence (Albert, Brown and Cheeseman, 1952). However, the possibility of its being 4-amino-5-
carboxymethylenaminodihydropyrimidine (3d-II) was not completely excluded. The structure (3d-I) has a conjugated system similar to 4,5-diaminopyrimidine (3d-III), and therefore the ultraviolet spectra of (3d-I) should be similar to those of 4,5-diaminopyrimidine (3d-III). On the other hand, the conjugated system of the dihydropyrimidine (3d-II) is dissimilar to that of (3d-III). It would be possible to distinguish these two structures, 3d-I and 3d-II, by comparing their ultraviolet spectra with those of 4,5-diaminopyrimidine (3d-III).
However, 4-amino-5-carboxymethylaminopyrimidine exists mainly as a zwitterion (3d-IV), as described before (p.32), and therefore care was taken to compare the ultraviolet spectrum of the acid's neutral molecule with the spectrum of the 4,5-diaminopyrimidine cation, and similarly the spectrum of the acid's anion with that of the neutral molecule of 4,5-diaminopyrimidine. The ultraviolet spectra of these two pairs have indeed a close similarity (Table 14, and Fig. 10, 11), and this eliminates the possibility of the dihydropyrimidine structure (3d-II), thus the product obtained by alkaline hydrolysis of dihydro-7-hydroxypteridine is 4-amino-5-carboxymethylaminopyrimidine (3d-I). Accordingly, the parent x,y-dihydro-7-hydroxypteridine is 5,6-dihydro-7-hydroxypteridine.

c) The structure of x,y-dihydro-2-hydroxypteridine could not be confirmed by synthesis, but it had pKa values somewhat similar to those of hydrated 2-hydroxypteridine (3d-V) (see p. 101). This hydrated 2-hydroxypteridine (3d-V) has the same conjugated system as had 3,4-dihydro-2-hydroxypteridine (3a-VI), thus the pair of the compounds would be expected to have similar ultraviolet spectra*. Marked similarity of ultraviolet

*As a basis for this expectation, correlations between the spectra of hydrated pteridines and hydrogenated pteridines which have the same conjugated systems have been observed in some 7,8-hydrated 6-hydroxypteridines (3d-VII) and the corresponding 7,8-dihydro derivatives (3d-VIII) (Fig.2,12,13).
spectra is seen between the \(x,y\)-dihydro-2-hydroxypteridine and hydrated 2-hydroxypteridine (Fig. 1). Since the structure of hydrated 2-hydroxypteridine has been established as 3,4-dihydro-2,4-dihydroxypteridine (3d-V) (Brown and Mason, 1956), the above spectroscopic evidence supports the 3,4-dihydro structure for \(x,y\)-dihydro-2-hydroxypteridine.

\[
\text{(3d-V)}
\]

\[
\text{(3d-VI)}
\]

\[
\text{(3d-VII)}
\]

\[
\text{(3d-VIII)}
\]

d) As described before (p. 48, 85 and 102) the \(x,y\)-dihydro-4-hydroxypteridine obtained by reduction of 4-hydroxypteridine with potassium borohydride could reasonably be 5,8-, 3,7- or 7,8-, dihydro-4-hydroxypteridine. The \(x,y\)-dihydro-4-hydroxypteridine shows a strong bathochromic shift (57 \(\text{m}_{\text{u}}\)) in
its ultraviolet absorption when compared with 4-hydroxypteridine (see Table 15, p. 120). Such a strong bathochromic shift has never been observed between pteridines and their 7,8-dihydro derivatives (Table 15, p. 120). However, an unusually strong bathochromic shift (54 μm) was recently found between a pteridine and its 5,8-dihydro derivative (Pfleiderer and Taylor, 1960, see p. 45) who explained this shift by the stabilization, by the external ring formation, of a 5,8-dihydro structure. In the present case, two possible structures, 5,8-dihydro-, and 3,7-dihydro-, 4-hydroxypteridine remain. But no decision can be reached on the constitution of this minor product of the reduction of 4-hydroxypteridine.

ii) Infrared Absorption Spectra.

Because of the insolubility of hydrogenated pteridines in organic solvents, all infrared absorption spectra were determined in the solid state (potassium bromide disk method). This prevented an intensive discussion about the structure of the compounds as revealed by the infrared spectra, because solid state measurements present a bewildering variety of peaks arising from interactions between the molecules. For
this reason infrared spectra were used in the present work, mainly for testing identity.

iii) **Nuclear Magnetic Resonance Spectra.**

The nuclear magnetic resonance spectra of solids can only be determined in solution. For this reason, in spite of its great value in the determination of the position of hydrogen atoms in a molecule, this method could not be used in the present work. Several attempts to use the method are described (see p. 33), but in no case could a sufficiently concentrated solution be obtained to give clear signals.
Table 11.

Ultraviolet spectra of hydrogenated pteridines (obtained in the present work).
Values underlined refer to shoulders or inflexions.

<table>
<thead>
<tr>
<th>Hydropteridines</th>
<th>116</th>
<th>(measured) species</th>
<th>λ_max. (μm)</th>
<th>log ε</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,6-Dihydro-4,6-dimethylpteridine</td>
<td>3.00 ± 0.04</td>
<td>3.0</td>
<td>219; 293</td>
<td>4.27; 3.73</td>
</tr>
<tr>
<td>2,4-Dihydroxy-2-hydroxypteridine</td>
<td>7.0</td>
<td>-2</td>
<td>240; 317</td>
<td>3.72; 3.58</td>
</tr>
<tr>
<td>2,8-Dihydroxy-6-methylpteridine</td>
<td>14.15</td>
<td>3.0</td>
<td>260; 343</td>
<td>3.50; 3.04</td>
</tr>
<tr>
<td>2,6-Dihydro-2-hydroxypteridine</td>
<td>7.0</td>
<td>+</td>
<td>225; 262; 290; 310</td>
<td>3.05; 3.75; 3.75; 2.7</td>
</tr>
<tr>
<td>2,4-Dihydroxy-2-hydroxy-6-methylpteridine</td>
<td>7.0</td>
<td>+</td>
<td>240; 321</td>
<td>3.05; 3.22</td>
</tr>
<tr>
<td>2,6-Dihydro-2-hydroxy-6-methylpteridine</td>
<td>7.0</td>
<td>+</td>
<td>237; 347</td>
<td>4.01; 3.94</td>
</tr>
<tr>
<td>2,6-Dihydro-2-hydroxy-4,6-dimethylpteridine</td>
<td>7.0</td>
<td>+</td>
<td>279; 327; 310</td>
<td>4.05; 4.04; 3.05</td>
</tr>
<tr>
<td>2,6-Dihydro-2-hydroxy-4,6-dimethylpteridine</td>
<td>7.0</td>
<td>+</td>
<td>225; 262; 290; 310</td>
<td>4.33; 4.04</td>
</tr>
<tr>
<td>2,6-Dihydro-4-hydroxypteridine</td>
<td>7.0</td>
<td>+</td>
<td>257; 304</td>
<td>4.37; 4.10</td>
</tr>
</tbody>
</table>

HCO
<table>
<thead>
<tr>
<th>Compound</th>
<th>m/z</th>
<th>Error</th>
<th>dertz</th>
<th>wertz</th>
<th>Error</th>
<th>dertz</th>
<th>wertz</th>
</tr>
</thead>
<tbody>
<tr>
<td>x,y-Dihydro-4-hydroxypteridine</td>
<td>7.0</td>
<td>0</td>
<td>268; 367</td>
<td>3.86; 3.63</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.32 ± 0.05</td>
<td>-2.0 b)</td>
<td>+</td>
<td>257; 374</td>
<td>3.07; 3.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12.13 ± 0.03</td>
<td>14.15</td>
<td>-</td>
<td>253; 354</td>
<td>3.27; 3.70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7,8-Dihydro-5-hydroxypteridine 1</td>
<td>7.0</td>
<td>0</td>
<td>225</td>
<td>3.03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.53 ± 0.03</td>
<td>2.0</td>
<td>+</td>
<td>222</td>
<td>4.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.54 ± 0.02</td>
<td>13.0</td>
<td>-</td>
<td>227; 302</td>
<td>4.02; 4.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5,6-Dihydro-7-hydroxypteridine 1</td>
<td>6.0</td>
<td>0</td>
<td>271; 319</td>
<td>3.50; 3.70</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.36 ± 0.01</td>
<td>1.02</td>
<td>+</td>
<td>223; 284; 352</td>
<td>4.47; 3.71; 3.72</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.94 ± 0.05</td>
<td>1.02</td>
<td>-</td>
<td>270; 315</td>
<td>4.47; 3.71; 3.72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7,8-Dihydro-2,6-dihydroxypteridine</td>
<td>6.0</td>
<td>0</td>
<td>237; 255; 280</td>
<td>4.11; 3.03; 3.46</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.80 ± 0.02</td>
<td>0</td>
<td>+</td>
<td>237; 317</td>
<td>4.01; 3.36</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.22 ± 0.01</td>
<td>12.3</td>
<td>-</td>
<td>270; 315</td>
<td>4.01; 3.72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7,8-Dihydro-4,6-dihydroxypteridine</td>
<td>6.5</td>
<td>0</td>
<td>274; 313</td>
<td>3.80; 3.63</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.07 ± 0.05</td>
<td>12.0</td>
<td>-</td>
<td>273; 300</td>
<td>3.83; 2.57</td>
<td></td>
<td></td>
</tr>
<tr>
<td>x,y-Dihydro-2,7-dihydroxypteridine</td>
<td>3.52</td>
<td>0</td>
<td>252; 324</td>
<td>3.59; 3.97</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.84 ± 0.02</td>
<td>7.93</td>
<td>-</td>
<td>253; 340</td>
<td>3.42; 4.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5,6-Dihydro-4,7-dihydroxypteridine</td>
<td>5.0</td>
<td>0</td>
<td>217; 275; 323</td>
<td>4.23; 3.72; 3.72</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.45 ± 0.02</td>
<td>10.90</td>
<td>-</td>
<td>254; 310</td>
<td>3.72; 3.52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7,8-Dihydro-2,4,6-trihydroxy-</td>
<td>4.78</td>
<td>0</td>
<td>267; 305</td>
<td>3.69; 4.03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pteridine</td>
<td>7.07 ± 0.03</td>
<td>9.14</td>
<td>-</td>
<td>223; 272; 305</td>
<td>4.34; 4.05; 3.82</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Ultraviolet spectra of 5,6,7,8-tetrahydropyridines and the corresponding 4,5-diaminopyrimidines (from literature). ('neutral molecule' for those done in alcohol, and 'cation' for those done in acid*).

(N.B. The 4- and 6-positions in pyrimidine are equivalent).

<table>
<thead>
<tr>
<th>5,6,7,8-Tetrahydropyridine</th>
<th>4,5-Diaminopyrimidine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\lambda_{\text{max.}}$ (m(\mu))</td>
</tr>
<tr>
<td>Unsubstituted</td>
<td>208; 304(^{1})</td>
</tr>
<tr>
<td>2,4-Dichloro-</td>
<td>318 (^{3})</td>
</tr>
<tr>
<td>4,6-Dimethyl-</td>
<td>213; 306(^{4})</td>
</tr>
<tr>
<td>2-Chloro-4,6-dimethyl-</td>
<td>221; 310(^{4})</td>
</tr>
<tr>
<td>2-Hydroxy-4,6-dimethyl-</td>
<td>230; 325(^{4})</td>
</tr>
</tbody>
</table>

1) cation; Brook and Ramage, 1957.
2) cation; Mason, 1954.
3) neutral molecule (in ethanol); Taylor and Sherman, 1959.
4) cation; Lister and Ramage, 1953.

*Many of the above measurements were not made on isolated ionic species of the substance. However, the spectra which were determined in 0.1M-hydrochloric acid are considered to be largely those of cations and those determined in ethanol are considered to be largely those of neutral molecules.
Comparison of ultraviolet spectra of isolated species of tetrahydropteridines and the corresponding 4,5-diaminopyrimidines. (N.B. The 4- and 6-positions are equivalent in pyrimidine).

<table>
<thead>
<tr>
<th>ionic species***</th>
<th>(\lambda_{\text{max.}}) ((\mu))</th>
<th>(\log \varepsilon)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5,6,7,8-Tetrahydro-2-hydroxypteridine*</td>
<td>0</td>
<td>306</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>327</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>315</td>
</tr>
<tr>
<td>4,5-Diamino-2-hydroxypyrimidine**</td>
<td>0</td>
<td>292</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>305</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>303</td>
</tr>
<tr>
<td>5,6,7,8-Tetrahydro-4-hydroxypteridine*</td>
<td>0</td>
<td>289</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>259</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>284</td>
</tr>
<tr>
<td>4,5-Diamino-6-hydroxypyrimidine**</td>
<td>0</td>
<td>278</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>258</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>272</td>
</tr>
</tbody>
</table>

* Present work.
** Mason, 1954.
*** 0: neutral molecule, +: cation, -: anion.
Table 14

Ultraviolet spectra of 4,5-diaminopyrimidine and of the product obtained by alkaline hydrolysis of dihydro-7-hydroxypteridine.

<table>
<thead>
<tr>
<th>ionic species</th>
<th>pH</th>
<th>$\lambda_{\text{max}}$ (m\textmu)</th>
<th>log $\varepsilon$</th>
</tr>
</thead>
<tbody>
<tr>
<td>4,5-Diaminopyrimidine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>neutral molecule</td>
<td>8.05</td>
<td>289</td>
<td>3.86$^1)$</td>
</tr>
<tr>
<td>cation</td>
<td>3.25</td>
<td>284</td>
<td>3.94$^1)$</td>
</tr>
<tr>
<td>Alkaline hydrolysis product of dihydro-7-hydroxypteridine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cation</td>
<td>3.0</td>
<td>288</td>
<td>3.94$^2)$</td>
</tr>
<tr>
<td>zwitterion</td>
<td>6.67</td>
<td>282</td>
<td>3.93$^2)$</td>
</tr>
</tbody>
</table>

1) Mason, 1954.

### Table 15

Ultraviolet spectra of pteridines and their 7,8-dihydro-derivatives. (Values underlined refer to shoulders or inflexions).

<table>
<thead>
<tr>
<th></th>
<th>Pteridine $\lambda_{\text{max.}}$ (µ)</th>
<th>7,8-Dihydro- $\lambda_{\text{max.}}$ (µ)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aminopteridine</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(both in 0.1N-HCl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Amino-6-methylpteridine</td>
<td>235; 305</td>
<td>290</td>
</tr>
<tr>
<td>2-Amino-6,7-diphenylpteridine</td>
<td>275; 335</td>
<td>232; 335</td>
</tr>
<tr>
<td>2-Diethylamino-6,7-diphenylpteridine</td>
<td>230; 273; 335</td>
<td>232; 280; 337</td>
</tr>
<tr>
<td>4-Amino-6,7-diphenylpteridine</td>
<td>278; 375</td>
<td>257; 370</td>
</tr>
<tr>
<td>4-Dimethylamino-6,7-diphenylpteridine</td>
<td>395</td>
<td>230; 265; 310; 385</td>
</tr>
<tr>
<td><strong>Hydroxypteridine</strong></td>
<td><strong>Ionic species</strong></td>
<td></td>
</tr>
<tr>
<td>2-Hydroxypteridine</td>
<td>0</td>
<td>230; 307</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>233; 308</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>260; 375</td>
</tr>
<tr>
<td>2-Hydroxy-6-methylpteridine</td>
<td>0</td>
<td>235; 315</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>240; 310; 335</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>261; 377</td>
</tr>
<tr>
<td>2-Hydroxy-4,6-dimethylpteridine</td>
<td>0</td>
<td>222; 289</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>283; 312</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>222; 304</td>
</tr>
<tr>
<td>4-Hydroxypteridine</td>
<td>0</td>
<td>230; 310</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>257; 303</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>242; 333</td>
</tr>
</tbody>
</table>

1) Boon and Jones, 1951.
2) Brown and Mason, 1956.

* Present work.
SECTION 4.

EXPERIMENTAL

Microanalyses were kindly carried out by Dr. J.E. Fildes and her staff in the Department of Medical Chemistry in this university. Samples submitted for analyses were dried at 106\(^{\circ}\)/0.1 mm., unless otherwise indicated. In the experimental part, the analytical values between 0.07 and 0.13\% are recorded as 0.1\% and those between 0.14 and 0.16\% as 0.15\%.

All melting points recorded below were taken in soda glass capillaries and are uncorrected.

All paper chromatographic runs mentioned in this thesis were carried out by the "ascending front" method using Whatman No 1 or No 4 paper. Both (a) 3\% aqueous ammonium chloride (NH\(_4\)Cl) and (b) n-butanol (7 volumes) + 5 N-acetic acid (3 volumes) (Bu/Ac) were used as solvents. The paper chromatograms were examined under ultraviolet light at two wavelengths, (a) principally 365 mu, with a mercury vaper lamp and a Wood's glass filter, and (b) principally 254 mu, with a mercury resonance lamp and a Chance Brother's 0X7/19874 filter. The colours of observed fluorescences are recorded in the experimental part of this thesis as follows: Y = yellow, G = green, W = white, SB = sky blue, B = blue, V = violet. Two further symbols mean: D = dark (i.e. absorption occurs), X = neither absorption
nor fluorescence occurs. Thus 0.75 2D/3B indicated a dark spot visible in the light of the 254 μm lamp, and blue fluorescence in the light of the 365 μm lamp, and 0.75 is the Rf value. Similarly 2/3B indicates a blue fluorescence under both the 254 μm and the 265 μm lamps.

When two substances had to be compared for identity, they were always run simultaneously.

All new compounds can be distinguished from previously reported compounds by the convention of The Chemical Society, namely that the ideal analytical figures for a new compound are cited as ".......requires.......", and for a previously known compound as ".......calc. for.......". The names of new compounds are underlined at their first mention in the experimental part. (As in the Journal of the Chemical Society, names which are paragraph headings are also underlined but this does not indicate that the compound is new).

a. Reduction.

Reduction of 2-hydroxypteridine.

i) with potassium borohydride. Potassium borohydride (330 mg.) was added to a solution of 2-hydroxypteridine monohydrate (1.0 g.) in 0.1N-sodium hydroxide (100 ml.), and the mixture was kept under nitrogen overnight at room temperature. Neutralization with phosphoric acid gave a precipitate which on recrystallization from water gave 3,4-dihydro-2-hydroxypteridine (610 mg., 55%), it
darkens at 250° (Found: C, 48.1; H, 4.1; N, 36.9.
C₆H₆N₄O requires C, 48.0; H, 4.0; N, 37.0%). From the
filtrate of the reaction mixture, 5,6,7,8-tetrahydro-2-
hydroxypteridine was detected by paper chromatography.

<table>
<thead>
<tr>
<th>NH₄Cl</th>
<th>Bu/Ac.</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,4-dihydro-2-hydroxypteridine</td>
<td>0.69 2B/3V 0.502B/3V</td>
</tr>
<tr>
<td>the filtrate</td>
<td>0.74 2SE/3G 0.22 2/3G</td>
</tr>
<tr>
<td>5,6,7,8-tetrahydro-2-hydroxypteridine*</td>
<td>0.74 2SE/3G 0.22 2/3G</td>
</tr>
</tbody>
</table>

ii) with sodium dithionite. Sodium dithionite (4.8 g.)
was added to a boiling solution of 2-hydroxypteridine
monohydrate (1.0 g.) in N-sodium carbonate (30 ml.). After
chilling overnight under carbon dioxide, the precipitate
was extracted with hot water (20 + 20 ml.), the second
extract was concentrated to 5 ml. and added to the first.
Chilling gave 3,4-dihydro-2-hydroxypteridine (230 mg., 25%),
decomposing at 250°C without melting (Found for material
dried at 20°C: C, 47.9; H, 4.05; N, 37.1. Calc. for C₆H₆N₄O:
C, 48.0; H, 4.0; N, 37.3%). A product (65 mg.) insoluble
in boiling water, remained on recrystallization.

General procedure for catalytic hydrogenation.

The hydrogenation of hydroxypteridines was carried
out using a semimicro hydrogenation apparatus at room
temperature (20-22°C) and atmospheric pressure (about 710-
720 mm.). After absorption of hydrogen by the catalyst had

* Obtained by synthesis, see p. 152.
ceased, the sample was introduced and further absorption of hydrogen was recorded. The following is a typical example of the hydrogenation over palladium on carbon catalyst, and the results of all such hydrogenations are summarized in Table 11. Only exceptional cases are detailed in the experimental part.

**Hydrogenation of 2-hydroxypteridine.**

i) **over palladium catalyst in 0.1N-sodium hydroxide solution.** 2-Hydroxypteridine monohydrate (1.0 g., 0.6 m mole) in 0.1N-sodium hydroxide (100 ml.) was hydrogenated over palladium on carbon (10% Pd; 500 mg.), and 120 ml. of hydrogen (80% of theoretical for 2H) was absorbed in 20 min., when the rate of absorption fell sharply. After filtering off the catalyst, the filtrate was adjusted to pH 6, giving 3,4-dihydro-2-hydroxypteridine (600 mg., 67%), which was crystallized from water and identified by paper chromatography. Rf. 0.69 2B/3V (NH₄Cl), 0.50 2B/3V (Bu/Ac).

ii) **over Raney-nickel.** (a) 2-Hydroxypteridine (1.0 g., 6 m mole) in methanol (500 ml.) was hydrogenated over Raney-nickel, and the absorption of hydrogen stopped after 20 ml. was absorbed (13% of theoretical for 2H).

(b) 2-Hydroxypteridine (1.0 g., 6 m mole) in methanol (500 ml.) containing N-sodium hydroxide (6 ml.) was hydrogenated over Raney-nickel, and 170 ml. of hydrogen (120% of theoretical for 2H) was absorbed in 4 hr. After removal of the catalyst, the filtrate was evaporated to
dryness (in vacuo), and recrystallization of the residue from water gave 3,4-dihydro-2-hydroxypteridine (311 mg. 42%), it darkens at 250° (Found: C, 47.9; H, 4.1; N, 37.2.
Calc. for C₆H₆N₄O : C, 48.0; H, 4.0; N, 37.3%).

The filtrate from the recrystallization was taken to dryness and extracted with water (1.5 ml.). The extract was evaporated to dryness in desiccator, and reextracted with methanol. The methanol solution was examined by paper chromatography and by ultraviolet spectra. It gave Rf. values 0.74 2SB/3G (NH₄Cl), 0.22 2/3G. (Authentic 5,6,7,8-tetrahydro-2-hydroxypteridine gave 0.74 2SB/3G (NH₄Cl), 0.22 2/3G), and λ_{max.} 325 μm at pH 1, and 304 μm at pH 7* (authentic sample gives λ_{max.} 327 μm at pH 1.0, and 306 μm at pH 7.16).

iii) over Adams' platinum oxide catalyst. (a) 2-Hydroxypteridine in formic acid or in dimethylformamide did not absorb hydrogen over Adams' catalyst.

(b) 2-Hydroxypteridine (166 mg., 1 m mole) in water (300 ml.) over Adams' catalyst absorbed 100 ml. of hydrogen (this corresponded to 8H). The solution was concentrated, but neither absorption nor fluorescence was detected on paper chromatography. The product is very soluble in water and further investigation was abandoned because it was either a fully hydrogenated product or its decomposition fragments.

*As there was not enough material to purify, the correct extinction could not be determined.
Reduction of 3,4-dihydro-2-hydroxypteridine over Adams' catalyst. When 3,4-dihydro-2-hydroxypteridine (150 mg., 0.3 m mole) was hydrogenated in water (150 ml.), it absorbed 80 ml. of hydrogen (6 H) in 40 hr. As in the above case, neither absorption nor fluorescence was detected in the reduction mixture and no further investigation of the product was undertaken.

Reduction of 4-hydroxypteridine.

1) with potassium borohydride. Potassium borohydride (500 mg., 8 H) was added to a solution of 4-hydroxypteridine (740 mg., 5 m mole) in N-potassium carbonate (10 ml.), and kept overnight at room temperature. The mixture was adjusted to pH 5 with hydrochloric acid to decompose the excess of borohydride, and then adjusted to pH 10 with potassium hydroxide. The solution was evaporated to dryness in vacuo with "Hyflo-supercel" (2 g.) and extracted with ethyl acetate using a Soxhlet extractor (for 40 hr.). The extract gave a mixture of di- and tetra-hydro-4-hydroxypteridine (224 mg.) whereas the unchanged 4-hydroxypteridine remained in the thimble. The mixture of hydro-derivatives was extracted with warm ethanol (20 ml.), and the residue, crystallized from water (2.5 ml.), gave x,y-dihydro-4-hydroxypteridine as yellow needles (56 mg., 7.5%), m.p. 263-265° (decomp.) (Found: C, 48.2; H, 4.05; N, 36.95. C6H6N4O requires C, 48.0; H, 4.0; N, 37.3%).
The ethanol extract, on concentration and recrystallization from ethanol (2 ml.), gave 5,6,7,8-tetrahydro-4-hydroxypteridine as buff prisms (66 mg., 8.3%), m.p. 230° (decomp.). Found: C, 47.35; H, 5.5. C₆H₆N₄O requires C, 47.35; H, 5.3%. (See p. 48 for establishment of its identity with synthetic material; see below for material obtained by hydrogenation).

ii) with sodium amalgam. Sodium amalgam (4%, 4 g.) was added with stirring to a suspension of 4-hydroxypteridine (146 mg., 1 m mole). The mixture changed first to yellow, then brown and finally it became colourless. After removal of the mercury, the solution (on adjustment to pH 7) gave a white precipitate, which in air changed to a brown substance, Rf. 0.41 2/3V (NH₄Cl) 0.05 2V/3Y. Paper chromatography of the filtrate detected 5,6,7,8-tetrahydro-4-hydroxypteridine, Rf. 0.63 2B/3V (NH₄Cl), 0.26 2B/3V (Bu/Ac).

Hydrogenation of 4-Hydroxypteridine.

i) over Raney-nickel. 4-Hydroxypteridine (2.25 g., 15 m mole) in ethanol (300 ml.) was hydrogenated over Raney-nickel (20 g.) and 470 ml. of hydrogen was absorbed in 40 min. The filtrate from the catalyst was concentrated to dryness and the residue crystallized from ethanol, giving 5,6,7,8-tetrahydro-4-hydroxypteridine as pale buff prisms.
(1.38 g., 60%), m.p. 228-230° (Found C, 47.5; H, 5.45; N, 36.95. Calc. for C₁₆H₁₆N₂O: C, 47.35; H, 5.3; N, 36.8%).

ii) over palladium on carbon. 4-Hydroxypteridine (148 mg., 1 m mole) in 0.1N-potassium hydroxide (10 ml.) was hydrogenated over palladium on carbon. Absorption of hydrogen ceased after 40 min. and 32.2 ml. (60% for 4H) of hydrogen was absorbed. No crystallizable substance was isolated from the reaction mixture but an unstable product (20 mg.) was obtained, which oxidized to a brown substance in air. In the filtrate 5,6,7,8-tetrahydro-4-hydroxypteridine was detected by paper chromatography, Rf. 0.68 2B/3V (NH₄Cl), 0.26 2B/3V (Bu/Ac), (5,6,7,8-tetrahydro-4-hydroxypteridine gave Rf. 0.68 2B/3V (NH₄Cl), 0.26 2B/3V (Bu/Ac)).

Reduction of 6-hydroxypteridine.

1) with potassium borohydride. Reduction of 6-hydroxypteridine is one of the most typical examples of the reduction of hydroxypteridines with potassium borohydride. The results of all such reactions are summarized in Table 17, p.140, and only exceptional cases are detailed in the experimental part.

Potassium borohydride (55 mg.) was added to 6-hydroxypteridine monohydrate (166 mg., 1 m mole) in 0.1N-potassium
hydroxide (10 ml.), and kept overnight at room temperature. The mixture was adjusted to pH 6 with phosphoric acid. The precipitate of 7,8-dihydro-6-hydroxypteridine (143 mg., 86%), crystallized from water, decomposed at 310° without melting (Found: C, 47.6; H, 4.0; N, 36.5. Calc. for C₆H₆N₄O: C, 48.0; H, 4.0; N, 37.3%).

ii) with sodium dithionite. Sodium dithionite (300 mg.) was added to a hot solution of 6-hydroxypteridine monohydrate (200 mg., 1.2 m mole) in N-sodium carbonate (6 ml.), boiled for one min., and cooled to room temperature. Neutralization of the mixture with phosphoric acid gave a crude product, which was boiled with N-hydrochloric acid and then adjusted to pH 7 with sodium hydroxide. Recrystallization of the precipitate from water gave 7,8-dihydro-6-hydroxypteridine as colourless micro needles (132 mg., 65.7%), decomposing at 310° without melting (Found: C, 48.25; H, 4.2; N, 36.6. Calc. for C₆H₆N₄O: C, 48.0; H, 4.0; N, 37.3%).

Hydrogenation of 6-hydroxypteridine over palladium on carbon. This gave 7,8-dihydro-6-hydroxypteridine in 63% yield (see Table 16, p.139).

Reduction of 7-hydroxypteridine.

i) with potassium borohydride. This gave 5,6-dihydro-7-hydroxypteridine in 80% yield (see Table 17).
ii) with potassium amalgam. 4% Potassium amalgam (5 g., a 150% excess for 2 H) was added with stirring to an ice cooled suspension of 7-hydroxypteridine (148 mg., 1 m mole) in water (10 ml.). After 10 min. the supernatant solution was adjusted to pH 7 with hydrochloric acid, recrystallization of the precipitate gave 5,6-dihydro-7-hydroxypteridine as colourless needles (63 mg., 43%), decomposing at 235° without melting (Found: C, 48.25; H, 3.9; N, 36.86. Calc. for C_{6}H_{6}N_{4}O: C, 48.00; H, 4.0; N, 37.3%).

Hydrogenation of 7-hydroxypteridine over palladium catalyst. This gave 4-amino-5-carboxymethylaminopyrimidine in 63% yield (see Table 16).

Hydrogenation of 2,4-dihydroxypteridine.

i) over Adams' catalyst. When 2,4-dihydroxypteridine (164 mg., 1 m mole) in formic acid (12 ml.) was hydrogenated over Adams' catalyst, it absorbed 48 ml. (4 H) of hydrogen and gave the same spot on paper chromatography as that obtained by the reduction of 2,4-dihydroxypteridine with sodium amalgam.

ii) over palladium on carbon. When 2,4-dihydroxypteridine (164 mg., 0.1 mole) in 0.1N-sodium hydroxide was hydrogenated over palladium on carbon, it absorbed 50 ml. of hydrogen (4 H). Paper chromatography indicated that the
product is the same 5,6,7,8-tetrahydro-2,4-dihydroxypteridine obtained by reduction of the pteridine with sodium amalgam.

**Reduction of 2,4-dihydroxypteridine.**

**i) with sodium amalgam.** (a) 2,4-Dihydroxypteridine (500 mg., 3 m mole) was reduced with 4%-sodium amalgam (9 g.) under a nitrogen atmosphere. 2,3-Dimercaptopropanol (1 x 10⁻³ M solution, 3 ml.) was added to the mixture as an antioxidant and then the mercury was separated by decantation. The solution was quickly adjusted to pH 5 with acetic acid and the precipitate centrifuged, washed with 1 x 10⁻⁴ M dimercaptopropanol solution (3 x 50 ml.), and dried at room temperature under nitrogen at 10⁻¹ mm. This gave 5,6,7,8-tetrahydro-2,4-dihydroxypteridine as a pale buff powder (317 mg., 63%). This sample quickly changed to a brown substance in air and no accurate decomposition point was determined. (Found for substance dried at 20°, 10⁻¹ mm: C, 42.2; H, 4.1; N, 32.2, adjusted for presence of 5% sodium acetate* to C, 42.95; H, 4.2; N, 33.3).

*As the substance precipitated as a slime, and had to be centrifuged, some sodium acetate remained in spite of repeated washing. This explains the trace of ash in the combusted sample. Further washing caused decomposition of the substance due to oxidation.
$C_6H_8O_2N_4$ requires $C$, 42.85; $H$, 4.8; $N$, 33.3%. It gave $R_f$ 0.69 2D/3B (NH$_4$Cl)**.

(b) 2,4-Dihydroxypterididine (500 mg.) was reduced with sodium amalgam as above, and the solution was added to 10% acetic acid containing $1 \times 10^{-3}$ mole dimercaptopropanol (20 ml.). The precipitate was centrifuged and dried under nitrogen at $10^{-1}$ mm. over $P_2O_5$. Formic acid (50 ml.) and acetic formic anhydride (5 ml.) were added to the product and kept at room temperature overnight. The small amount of precipitate was discarded and the filtrate was evaporated to dryness under reduced pressure. Crystallization of the residue from water gave N-formyl-5,6,7,8-tetrahydro-2,4-dihydroxypterididine (140 mg., 28%), m.p. ca 300° (decomp.) (Found: $C$, 40.55; $H$, 4.1; $N$, 27.2. $C_7H_8N_4O_3.H_2O$ requires $C$, 41.0; $H$, 4.4; $N$, 27.05%).

(c) Reoxidation. 2,4-Dihydroxypteridine (164 mg., 1 m mole) was reduced with sodium amalgam exactly as above, and the solution was reoxidized with air using a semimicro-hydrogenation apparatus, and 20 ml. of oxygen was absorbed (80% calc. for $O_2$).

ii) With sodium dithionite. Sodium dithionite (4.8 g.) was added to a hot solution of 2,4-dihydroxypteridine (1.64 g., 0.01 mole) in 0.1N-sodium hydroxide (100 ml.) and boiled

*All processes of paper chromatography for tetrahydro-2,4-dihydroxypteridine, i.e. spotting, development and drying, were undertaken under nitrogen.
for two min. The mixture was refrigerated overnight under a nitrogen atmosphere and the white precipitate centrifuged. The precipitate was washed with 30%, 45% and 60% ethanol and when dried under nitrogen at 10⁻¹ mm, gave a pale buff powder (900 mg., 54%). This product changed to brown in air as tetrahydro-2,4-dihydroxypteridine did, and gave the same Rf. 0.59 2D/3B (NH₄Cl under nitrogen).

The supernatant solution from the centrifuge was concentrated to about 50 ml. under reduced pressure at room temperature and ethanol (50 ml.) was added. After removal of the precipitate, the filtrate was concentrated to 30 ml. and 60 ml. ethanol was added, and the precipitate was recrystallized from 30% ethanol, giving sodium tetrahydro-2,4-dihydroxypteridine sulphonate as a white powder, slowly decomposing at 80° (Found for substance dried at room temperature 10⁻¹ mm. C, 24.75; H, 3.1; N, 19.15; S, 10.65. C₆H₇N₄NaO₅S.H₂O requires C, 25.0; H, 3.15; N, 19.4; S, 11.1%).

Reduction of 2,6-dihydroxypteridine.

1) with potassium borohydride. This gave 7,8-dihydro-2,6-dihydroxypteridine in 80% yield (see Table 17) (Found: C, 43.3; H, 3.75; N, 33.7. Calc. for C₆H₆N₄O₂: C, 43.35; H, 3.65; N, 33.7%).

2) with potassium amalgam. 2,6-Dihydroxypteridine monohydrate (182 mg., 1 m mole) was reduced with 3% potassium amalgam (5 g., 100% excess for 2 H). This method
gave no insoluble product and it was abandoned when it was found that potassium amalgam decomposed 7,8-dihydro-2,6-dihydropteridine to a similar compound.

**Hydrogenation of 2,6-dihydropteridine.**

i) over palladium on carbon. This gave 7,8-dihydro-2,6-dihydropteridine in 73% yield (see Table 16).

ii) over Adams' catalyst. When 2,6-dihydroxypteridine monohydrate (91 mg., 0.5 m mole) in 0.1N-potassium hydroxide (10 ml.) was hydrogenated over Adams' catalyst (10 mg.), 13 ml. of hydrogen (=2 H) was absorbed in 7 hr. After removal of the catalyst the filtrate was dropped into boiling N-sodium acetate (50 ml.), giving 7,8-dihydro-2,6-dihydroxypteridine (71 mg., 85%).

**Reduction of 4,6-dihydroxypteridine.**

i) with potassium borohydride. This gave 7,8-dihydro-4,6-dihydroxypteridine in 96% yield (see Table 17) (Found: C, 43.15; H, 3.6; N, 33.5. Calc. for C₆H₆N₄O₂: C, 43.4; H, 3.65; N, 33.7%).

ii) with potassium amalgam. 4,6-Dihydroxypteridine (182 mg., 1 m mole) was reduced with 3% potassium amalgam (5 g. 4 H). The solution, on adjustment to pH 6 with hydrochloric acid, gave 7,8-dihydro-4,6-dihydroxypteridine, which was recrystallized from water (120 mg., 72%).

**Hydrogenation of 4,6-dihydroxypteridine over palladium on carbon.** This gave 7,8-dihydro-4,6-dihydroxypteridine in
79% yield (see Table 16).

Reduction of 2,7-dihydroxypteridine.

i) with potassium borohydride. This gave \( x,y \)-dihydro-2,7-dihydroxypteridine in 84\% yield (see Table 17) (Found: C, 43.45; H, 3.75; N, 33.65. \( \text{C}_6\text{H}_6\text{N}_4\text{O}_2 \) requires C, 43.4; H, 3.65; N, 33.7\%).

ii) with potassium amalgam. 3\%-Potassium amalgam (5g., 4 H) was added to a suspension of 4,7-dihydroxypteridine monohydrate (182 mg., 1 m mole) in water (5 ml.). After removal of the mercury the solution was adjusted to pH 6 with acetic acid. Recrystallization of the precipitate from water gave \( x,y \)-dihydro-2,7-dihydroxypteridine as colourless needles (96 mg., 64\%).

iii) with sodium dithionite. Sodium dithionite (600 mg.) was added to a hot solution of 2,7-dihydroxypteridine monohydrate (182 mg., 1 m mole) in 0.5 N-potassium hydroxide (6 ml.) and boiled for 1 min. The mixture was cooled to room temperature, adjusted to pH 0 with hydrochloric acid, and refrigerated overnight. This gave sodium tetrahydro-2,7-dihydroxypteridine sulphonate as yellow needles (160 mg., 60\% calc. for \( \text{C}_6\text{H}_7\text{N}_4\text{NaO}_5\text{S} \)), which was reprecipitated from cold water (2 ml.) with hydrochloric acid (pH 0) as yellow needles, decomposing at 245\°C without melting (Found for material dried at 65\°C/10\^-1 mm.: C, 27.05; H, 2.7; S, 11.10; 10\% ash. \( \text{C}_6\text{H}_7\text{N}_4\text{NaO}_5\text{S} \) requires C, 26.7; H, 2.6; S, 11.85\%).
Sodium dihydro-2,7-dihydroxypteridine sulphonate.

Sodium metabisulphite (600 mg. in 0.5 ml. water) was added to a solution of 2,7-dihydroxypteridine (323 mg.) in N-sodium hydroxide (1.5 ml.) and kept at room temperature for 5 hr., and refrigerated overnight. The product, recrystallized from water, gave sodium dihydro-2,7-dihydroxypteridine sulphonate as colourless needles (147 mg.), m.p. 310° (Found after drying at 60°/10-1 mm.: C, 23.3; H, 2.4; N, 18.3; S, 10.9. C₆H₅N₄Na₂O₅S·H₂O requires C, 23.4; H, 2.0; N, 18.1; S, 10.4%).

Sodium dihydro-2-hydroxypteridine sulphonate. Sodium metabisulphite (500 mg.) was added to a solution of 2-hydroxypteridine monohydrate (164 mg.) in N-sodium hydroxide (1 ml.). After refrigeration overnight the precipitate on crystallization from water gave sodium dihydro-2-hydroxypteridine sulphonate as colourless needles (90 mg.), m.p. 310° (decomp.) (Found: C, 27.85; H, 2.4; N, 21.45; S, 12.25. C₆H₅N₄Na₂O₅S·H₂O requires C, 27.5; H, 2.3; N, 21.45; S, 12.3%).

Reduction of 4,7-dihydroxypteridine with potassium borohydride. This gave 5,6-dihydro-4,7-dihydroxypteridine in 85% yield, m.p. 300° (decomp.) (Found: C, 43.5; H, 3.9; N, 33.4. C₆H₆N₄O₂ requires C, 43.4; H, 3.65; N, 33.7%) (see table 17).

Hydrogenation of 4,7-dihydroxypteridine over palladium on carbon. This gave 5,6-dihydro-4,7-dihydroxypteridine
in 67% yield (see Table 16).

Reduction of 2,4,6-trihydroxypteridine.

i) with potassium borohydride. This gave 7,8-dihydro-2,4,6-trihydroxypteridine in 77% yield (see Table 17).

ii) with potassium amalgam. 2,4,6-Trihydroxypteridine (198 mg., 1 m mole) was reduced with 3% potassium amalgam (5 g., 4 H). The solution, on adjustment to pH 6 with hydrochloric acid, gave 7,8-dihydro-2,4,6-trihydroxypteridine, which was crystallized from water (1 l.) (120 mg., 65%).

Hydrogenation of 2,4,6-trihydroxypteridine over palladium on carbon. This gave 7,8-dihydro-2,4,6-trihydroxypteridine in 75% yield (see Table 16).

Reduction of 2-amino-4,6-dihydroxypteridine (xanthopterin)

i) with potassium borohydride. This gave 2-amino-7,8-dihydro-4,6-dihydroxypteridine in 90% yield (see Table 17).

ii) with sodium amalgam. 2-Amino-4,6-dihydroxypteridine (1 g.) was reduced with 4% sodium amalgam (10 g., 3.4 H) to 2-amino-7,8-dihydro-4,6-dihydroxypteridine (752 mg., 75%).

Reduction of 2-hydroxy-6-methylpteridine with potassium borohydride. 2-Hydroxy-6-methylpteridine (360 mg.) (see p. 148) was reduced by the method used for 2-hydroxypteridine and gave 3,4-dihydro-2-hydroxy-6-methylpteridine (96 mg., 30%), darkening above 265° but unmelted even at 300° (Found: C, 51.3; H, 4.95; N, 34.0. C₇H₆N₄O requires
C, 51.2; H, 4.9; N, 34.1%). Chromatography indicated that this product was not identical with 7,8-dihydro-2-hydroxy-6-methylpteridine prepared unambiguously. In the reduction mixture a trace of a product, possibly 5,6,7,8-tetrahydro-2-hydroxy-6-methylpteridine was detected by paper chromatography, Rf: 0.75 2SB/3G (NH₄Cl), 0.23 2/3 GB (Bu/Ac).
Hydrogenation of hydroxypteridines over 10% palladium on carbon

<table>
<thead>
<tr>
<th>Pteridine</th>
<th>solvent (ml.)</th>
<th>period (min.)</th>
<th>hydrogen</th>
<th>Product</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Hydroxy-</td>
<td>0.1N-KOH</td>
<td>10</td>
<td>20</td>
<td>80 for 4H 3,4-Dihydro- + 5,6,7,8-Tetrahydro-</td>
<td>67 (3,4-Dihydro-)</td>
</tr>
<tr>
<td>4-Hydroxy-</td>
<td></td>
<td>30</td>
<td>60</td>
<td>5,6,7,8-Tetrahydro- + unstable substance</td>
<td>-</td>
</tr>
<tr>
<td>6-Hydroxy-</td>
<td></td>
<td>20</td>
<td>2H</td>
<td>7,8-Dihydro-**</td>
<td>63</td>
</tr>
<tr>
<td>7-Hydroxy-</td>
<td></td>
<td>40</td>
<td>2H</td>
<td>4-Amino-5-carboxymethylaminopyrimidine***</td>
<td>64</td>
</tr>
<tr>
<td>2,4-Dihydroxy-</td>
<td></td>
<td>20</td>
<td>4H</td>
<td>5,6,7,8-Tetrahydro-</td>
<td>-</td>
</tr>
<tr>
<td>2,5-Dihydroxy-</td>
<td></td>
<td>10</td>
<td>2H</td>
<td>7,8-Dihydro-</td>
<td>73</td>
</tr>
<tr>
<td>2,6-Dihydroxy-</td>
<td></td>
<td>70</td>
<td>2H</td>
<td>7,8-Dihydro-</td>
<td>73</td>
</tr>
<tr>
<td>2,7-Dihydroxy-</td>
<td></td>
<td>70</td>
<td>2H</td>
<td>7,8-Dihydro-</td>
<td>92</td>
</tr>
<tr>
<td>4,7-Dihydroxy-</td>
<td></td>
<td>17 hr.</td>
<td>2H</td>
<td>5,6-Dihydro-</td>
<td>67</td>
</tr>
<tr>
<td>6-7-Dihydroxy-</td>
<td></td>
<td>-</td>
<td>0</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>2,4,6-Trihydroxy-</td>
<td>0.2N-KOH</td>
<td>15</td>
<td>15</td>
<td>2H 7,8-Dihydro-</td>
<td>75</td>
</tr>
<tr>
<td>2,4,7-Trihydroxy-</td>
<td></td>
<td>-</td>
<td>0</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>2,5,7-Trihydroxy-</td>
<td></td>
<td>-</td>
<td>0</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>4,5,7-Trihydroxy-</td>
<td></td>
<td>-</td>
<td>0</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>2,4,6,7-Tetrahydroxy-</td>
<td>0.5N-KOH</td>
<td>10</td>
<td>-</td>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>

In all experiments 1 m. mole of hydroxypteridines were reduced over 10% palladium on carbon catalyst (50 mg.).

**Found: C, 48.4; H, 4.2; N, 37.8. Calc. for C_{6}H_{6}O_{2}N_{4} C, 48.0; H, 4.0; N, 37.3.**

**Found: C, 42.7; H, 4.8; N, 33.3. Calc. for C_{6}H_{6}O_{2}N_{4} C, 42.85; H, 4.8; N, 33.3.**

**This product was detected by paper chromatography.**
Table 17

Reduction of hydroxypteridines with potassium borohydride

<table>
<thead>
<tr>
<th>Starting material (m.Mole)</th>
<th>Solution (ml.)</th>
<th>HN₄H₄O₄ (mg.)</th>
<th>Yield (%)</th>
<th>Found:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>C</td>
<td>H</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Hydroxy-</td>
<td>6</td>
<td>0.1N-KOH</td>
<td>100</td>
<td>330</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-Hydroxy-</td>
<td>5</td>
<td>0.1N-KOH</td>
<td>100</td>
<td>330</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-Hydroxy-</td>
<td>1</td>
<td>0.1N-KOH</td>
<td>100</td>
<td>330</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-Hydroxy-</td>
<td>1</td>
<td>0.1N-KOH</td>
<td>100</td>
<td>330</td>
</tr>
<tr>
<td>2,4-Dihydroxy-</td>
<td>1</td>
<td>0.1N-KOH</td>
<td>100</td>
<td>330</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,6-Dihydroxy-</td>
<td>1</td>
<td>0.1N-KOH</td>
<td>100</td>
<td>330</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4,6-Dihydroxy-</td>
<td>1</td>
<td>0.1N-KOH</td>
<td>100</td>
<td>330</td>
</tr>
<tr>
<td>2,7-Dihydroxy-</td>
<td>1</td>
<td>0.1N-KOH</td>
<td>100</td>
<td>330</td>
</tr>
<tr>
<td>4,7-Dihydroxy-</td>
<td>1</td>
<td>0.1N-KOH</td>
<td>100</td>
<td>330</td>
</tr>
<tr>
<td>5,7-Dihydroxy-</td>
<td>1</td>
<td>0.1N-KOH</td>
<td>100</td>
<td>330</td>
</tr>
<tr>
<td>2,4,5-Trihydroxy-</td>
<td>0.75</td>
<td>0.3N-KOH</td>
<td>100</td>
<td>330</td>
</tr>
<tr>
<td>2,4,7-Trihydroxy-</td>
<td>0.5</td>
<td>0.2N-KOH</td>
<td>100</td>
<td>330</td>
</tr>
<tr>
<td>2,6,7-Trihydroxy-</td>
<td>0.5</td>
<td>0.2N-KOH</td>
<td>100</td>
<td>330</td>
</tr>
<tr>
<td>2,4,6,7-Tetrahydroxy-</td>
<td>0.5</td>
<td>0.2N-KOH</td>
<td>100</td>
<td>330</td>
</tr>
<tr>
<td>2-Amino-4,6-dihydroxy-</td>
<td>1 H₂O</td>
<td>5</td>
<td>80</td>
<td>7,8-Dihydro-</td>
</tr>
</tbody>
</table>
b. Syntheses of Starting Materials

1) Chloronitropyrimidines.

2,4-Dichloro-5-nitropyrimidine

2,4-Dihydroxypyrimidine (uracil) was prepared (89% yield) from 4-hydroxy-2-mercaptoypyrimidine (thiouracil) according to Brown (1952). 2,4-Dihydroxy-5-nitropyrimidine was prepared (85% yield) from 2,4-dihydroxypyrimidine by nitration according to Brown (1952). 2,4-Dichloro-5-nitropyrimidine was prepared (77% yield) from 2,4-dihydroxy-5-nitropyrimidine according to Whittaker (1951). It distilled at 130°/10 mm. (lit. 138-139°/14 mm.).

4,6-Dichloro-5-nitropyrimidine.

4,6-Dihydroxypyrimidine was prepared (61% yield) from malondiamide and ethyl formate according to Hull (1951). The product was identified by paper chromatography with an authentic sample and it had m.p. > 300° (Hull gives 56% yield, m.p. > 300°). 4,6-Dihydroxy-5-nitropyrimidine was prepared (93% yield) by nitration of 4,6-dihydroxypyrimidine according to Boon, Jones and Ramage (1951). The product was identified by paper chromatography with an authentic sample. 4,6-Dichloro-5-nitropyrimidine was prepared (77% yield) from 4,6-dihydroxy-5-nitropyrimidine by the action of phosphorus oxychloride and diethylaniline according to Boon, Jones and Ramage. It had m.p. 103° (no m.p. in lit.).
2,4-Dichloro-6-methyl-5-nitropyrimidine was prepared (69% yield) by chlorination of 2,4-dihydroxy-5-methyl-5-nitropyrimidine according to Albert, Brown and Wood (1954). It had m.p. 53° (lit. 53-54°).

2,4,6-Trichloro-5-nitropyrimidine.

2,4,6-Trihydroxypyrimidine (barbituric acid) was prepared (66% yield) by condensation of urea and diethyl malonate according to Dickey and Gray (1943). 5-Nitro-2,4,6-trihydroxypyrimidine was prepared (85% yield) by nitration of 2,4,6-trihydroxypyrimidine according to Hartman and Sheppard (1943). It had m.p. 187° (decomp.) (lit. 181-183° (decomp.)). 2,4,6-Trichloro-5-nitropyrimidine was prepared (23% yield) by chlorination of 2,4,6-trihydroxy-5-nitropyrimidine according to Robins, Dille and Christensen (1954). It had m.p. 57° (lit. m.p. 57-58°).

2) 4,5-Diaminopyrimidines

4,5-Diaminopyrimidine

4-Amino-2-chloro-5-nitropyrimidine was prepared (79% yield) from 2,4-dihydroxy-5-nitropyrimidine by the action of phosphorus oxychloride and then ammonia according to Brown (1952). The product after two recrystallization from ethanol had m.p. 216° (lit. 215-217°). 4,5-Diamino-2-mercaptopyrimidine was prepared (54% yield, lit. 42%)
from 4-amino-2-chloro-5-nitropyrimidine by the action of sodium hydrogen sulphide according to Brown (1952). The product gave the same Rf. values as the authentic sample. 4,5-Diaminopyrimidine was prepared (70% yield) from 4,5-diamino-2-mercapto.pyrimidine by the action of Raney-nickel according to Brown (1952). The product had m.p. 203-204.5° (lit. 65% yield and m.p. 201°).

4,5-Diamino-2-hydroxypyrimidine.

2,4-Dimercaptopyrimidine was prepared (65% yield) from 4-hydroxy-2-mercapto.pyrimidine by the action of phosphorus pentasulphide according to Brown (1950). The product darkened at 240° and decomposed at 255°, and was used for the next reaction without further purification as it gave only one spot on paper chromatography (Rf: 0.50 2D/3Y (NH₄Cl), 0.74 2D/3Y (Bu/AC) (lit. m.p. 265-270° (decomp.)).

4-Amino-2-mercapto.pyrimidine (thiocytosine) was prepared (75% yield) from 2,4-dimercaptopyrimidine by the action of ammonia, according to Brown (1950), it had m.p. 266° (decomp.) (lit. 85% yield, m.p. 265° for crude and 273° for pure material). The crude material was used for the next synthesis without further purification. 4-Amino-2-hydroxypyrimidine (cytosine) was prepared (95% yield) by hydrolysis of 4-amino-2-mercapto.pyrimidine according to Brown (1950). It had m.p. 306° (decomp.) (lit. 75%
yield and m.p. 305-308° (decomp.). 4-Amino-2-hydroxy-5-nitropyrimidine was prepared (89% yield) by nitration of 4-amino-2-hydroxypyrimidine according to Johns (1911). The product was identified by paper chromatography with an authentic sample. 4,5-Diamino-2-hydroxypyrimidine was prepared (76% yield) by reduction of 4-amino-2-hydroxy-5-nitropyrimidine according to Brown (1957), m.p. 270-275° (decomp.) (Found: C, 38.3; H, 4.85; N, 44.35. Calc. for C₆H₆N₄O : C, 38.1; H, 4.83; N, 44.4%).

4,5-Diamino-6-hydroxypyrimidine

4-Amino-6-hydroxy-2-mercaptopyrimidine was prepared (79% yield) from urea and ethyl cyanoacetate according to Traube (1904). 4,5-Diamino-6-hydroxy-2-mercaptopyrimidine was prepared (89% yield) from 4-amino-6-hydroxy-2-mercapto-

pyrimidine by nitrosation and then reduction according to Albert, Brown and Cheeseman (1951). The product was identified with an authentic sample by paper chromatography. 4,5-Diamino-6-hydroxypyrimidine was prepared (46% yield) from 4,5-diamino-6-hydroxy-2-mercaptopyrimidine by the action of Raney-nickel according to Albert, Brown and Cheeseman (1951). It had m.p. 238° (decomp.) (lit. 239°).

4,5-Diamino-2,6-dihydroxypyrimidine

4-Amino-2,6-dihydroxypyrimidine was prepared from urea and ethyl cyanoacetate according to Cain, Mallette and Taylor (1946). 4,5-Diamino-2,6-dihydroxypyrimidine was prepared from 4-amino-2,6-dihydroxypyrimidine by nitrosation and
subsequent reduction with sodium dithionite according to Cain et al. (1946). The overall yield from ethyl cyanoacetate was 27%. The product was identified with an authentic sample by paper chromatography.

2,4,5-Triamino-6-hydroxypyrimidine bisulphite, was prepared (70% yield) from guanidine hydrochloride and ethyl cyanoacetate according to Cain, Mallette and Taylor (1946). The product was identified with an authentic sample by paper chromatography.

3) Pteridines.

All pteridines in Table 18 were prepared in one step by known methods from the 4,5-diaminopyrimidines described above. Most pteridines have no melting points and usually have no sharp decomposition points. Hence all such pteridines described in this experimental part were examined for their identity and purity by paper chromatography. These results are summarized in Table 18.
<table>
<thead>
<tr>
<th>Pteridine</th>
<th>Yield Ref. (%)</th>
<th>m.p.</th>
<th>Rf. NH₄Cl</th>
<th>Bu/Ac</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unsubstituted</td>
<td>1 70</td>
<td>138-139</td>
<td>0.78 2DB/3D</td>
<td>0.71 2X/3B</td>
</tr>
<tr>
<td>2-Hydroxy-</td>
<td>1 87</td>
<td>240(d)*</td>
<td>0.74 2WB/3X</td>
<td>0.80 2W/3B</td>
</tr>
<tr>
<td>4-Hydroxy-</td>
<td>2 60</td>
<td>&gt;300(d)</td>
<td>0.68 2D/3X</td>
<td>0.26 2D/3X</td>
</tr>
<tr>
<td>6-Hydroxy-</td>
<td>3 85</td>
<td>240(d)</td>
<td>0.66 2D/3X</td>
<td></td>
</tr>
<tr>
<td>7-Hydroxy-</td>
<td>4 70</td>
<td>&gt;300(d)</td>
<td>0.64 2E/3V</td>
<td>0.54 2X/3B</td>
</tr>
<tr>
<td>2,4-Dihydroxy-</td>
<td>1 51</td>
<td>250(d)</td>
<td>0.65 2D/3B</td>
<td>0.23 2W/3B</td>
</tr>
<tr>
<td>2,6-Dihydroxy-</td>
<td>4 61</td>
<td>&gt;280(d)</td>
<td>0.67 2/3B</td>
<td>0.13 2/3B</td>
</tr>
<tr>
<td>2,7-Dihydroxy-</td>
<td>4 35</td>
<td>&gt;320(d)</td>
<td>0.50 2DB/3V</td>
<td>0.16 2D/3V</td>
</tr>
<tr>
<td>4,6-Dihydroxy-</td>
<td>5 50</td>
<td>&gt;300(d)</td>
<td>0.63 2/3B</td>
<td>0.32 2/3B</td>
</tr>
<tr>
<td>4,7-Dihydroxy-</td>
<td>6 20</td>
<td>&gt;300(d)</td>
<td>0.61 2DB/3BV</td>
<td>0.14 2X/3B</td>
</tr>
<tr>
<td>6,7-Dihydroxy-</td>
<td>7 88</td>
<td>&gt;300(d)</td>
<td>0.60 2L/3BV</td>
<td>0.25 2B/3V</td>
</tr>
<tr>
<td>2,4,6-Trihydroxy-</td>
<td>4 50</td>
<td>&gt;300(d)</td>
<td>0.50 2/3SB</td>
<td>0.25 2/3SB</td>
</tr>
<tr>
<td>2,4,7-Trihydroxy-</td>
<td>8</td>
<td>&gt;300(d)</td>
<td>0.43 2/3B</td>
<td>0.05 2B/3VB</td>
</tr>
<tr>
<td>2,5,7-Trihydroxy-</td>
<td>4 95</td>
<td>&gt;300(d)</td>
<td>0.63 2/3B**</td>
<td>0.06 2WB/3B</td>
</tr>
<tr>
<td>4,6,7-Trihydroxy-</td>
<td>5 90</td>
<td>&gt;300(d)</td>
<td>0.37 2WB/3B</td>
<td>0.04 2W/3X</td>
</tr>
<tr>
<td>2,4,5,7-Tetrahydroxy-</td>
<td>4 45</td>
<td>&gt;300(d)</td>
<td>0.30 2WB/3B</td>
<td>0.02 2D/3X</td>
</tr>
<tr>
<td>2-Amino-4,6-dihydroxy-</td>
<td>9 45</td>
<td>&gt;300(d)</td>
<td>0.36 2/3Y</td>
<td>0.15 2/3Y</td>
</tr>
</tbody>
</table>

1 Albert, Brown and Cheeseman, 1951
2 Albert, Brown and Wood, 1956
3 Albert, 1955
4 Albert, Lister and Pedersen, 1956
5 Albert and Brown, 1953
6 Pfleiderer, 1959
7 Albert, Brown and Cheeseman, 1952
8 Pfleiderer, 1957
9 Korte, 1954

*Decomposed without melting.
**In aqueous solution, this compound present as an equilibrium mixture of two species, Albert, Lister and Pedersen (1954).
c. **Syntheses of Hydrogenated Pteridines.**

7,8-Dihydro-2-hydroxy-6-methylpteridine.

**Phthalimidoacetone** was prepared as follows according to Dr. F. Reich (personal communication): Bromoacetone (60 g., 0.43 mole) was added to potassium phthalimide (92 g., 0.5 mole) in dimethylformamide at 60-70° and kept at this temperature for 1 hr. Water (450 ml.) was added to the reaction mixture and then extracted with chloroform (3 x 150 ml.). The chloroform layer was washed with 0.5 N sodium hydroxide and with water. Removal of the chloroform gave a solid which melted at 122° (Ellinger and Goldberg, 1949 give m.p. 111-120°). Aminoacetone hydrochloride was prepared (74% yield) by hydrolysis of phthalimidoacetone according to Ellinger and Goldberg (1949). It had m.p. 81°. 4-Acetonylamino-2-chloro-5-nitropyrimidine was prepared (62% yield) from 2,4-dichloro-5-nitropyrimidine (see p.141) and aminoacetone hydrochloride according to Doon and Jones (1951). It had m.p. 129-130° (lit. 131°). The chloro-substituent was changed into a hydroxy group as follows: 2-chloro-4-acetonylamino-5-nitropyrimidine (6.9 g., 0.03 mole) and sodium acetate trihydrate (6 g., 0.054 mole) in glacial acetic acid (100 ml.) were refluxed for 80 min. Recrystallization of the product from water gave 4-acetonylamino-2-hydroxy-5-nitropyrimidine as colourless prisms (2.3 g., 36%), m.p. 186° (decomp.) (Found: C, 39.55; H, 3.9. C<sub>7</sub>H<sub>8</sub>N<sub>4</sub>O<sub>4</sub> requires C, 39.6; H, 3.8%).

This product was reduced and cyclized simultaneously as follows:
4-acetonyl-2-hydroxy-5-nitopyrimidine (2.12 g., 10 m mole) in 90% ethanol (500 ml.) was hydrogenated at 50°C over Raney-nickel (5 g). When absorption of hydrogen ceased the mixture was refrigerated overnight, and the catalyst was filtered off. Extraction of the catalyst with boiling water (250 ml.) gave a product, which was recrystallized from water. The 7,8-dihydro-2-hydroxy-6-methylpteridine, micro prisms (0.866 g., 53%, decomposed at 280° but did not melt below 320° (Found, after drying at 135°/10⁻¹ mm.: C, 50.95; H, 4.8; N, 34.0. C₇H₆N₄O requires C, 51.2; H, 4.9; N, 34.1%).

2-Hydroxy-6-methylpteridine. 0.1 M - Potassium permanganate (20 ml.) was added dropwise with stirring to 7,8-dihydro-2-hydroxy-6-methylpteridine (520 mg.) in 0.1 N - potassium hydroxide at 0°. The manganese dioxide was filtered off and washed with hot water (10 ml.). The combined filtrate and washings were adjusted to pH 6 with acetic acid and refrigerated overnight. Crystallization of the product from water gave 2-hydroxy-6-methylpteridine as colourless needles (380 mg., 67%). It decomposed at 245° but did not melt below 300° (Found: C, 46.95; H, 4.55; N, 31.3. C₇H₆N₄O.H₂O requires C, 46.65; H, 4.5; N, 31.15%).

7,8-Dihydro-2-hydroxypteridine

Aminoacetal was prepared by a modification of the method of Allen and Clark (1944). Bromoacetal (110 g.) was heated in an autoclave with liquid ammonia (450 g.) at 110° for 4 hr.
After removal of the ammonia, 6 N - sodium hydroxide was added to the residue and the solution was continuously extracted with benzene. The extract, after drying over sodium sulphate, was fractionated, and the product distilled at 65°/17 mm (55.2 g., 75%). (Allen et al. give 32-39% yield). Woodward and Doering (1945) record 72.5% and b.p. 99-103°/100 mm., but such a high yield has not been obtained here by following their directions which evidently lack some essential detail.

4-β-Diethoxyethylamino-2-hydroxy-5-nitropyrimidine.

Aminoacetal (135 g., 0.1 mole) in water (100 ml.) was adjusted to pH 7.5 with acetic acid and sodium bicarbonate (12 g.) was added. The solution was added dropwise, with shaking, to 2,4-dichloro-5-nitropyrimidine (20 g., 0.101 mole) in chloroform (100 ml.). Shaking was continued for a further 3 hr. until evolution of carbon dioxide had ceased. The chloroform layer was distilled with N - sodium hydroxide (500 ml.) on a steam bath while bubbling nitrogen through the solution for about 20 min. (During this time the 2-chloro-substituent was replaced by a hydroxy group). The mixture was extracted with benzene (3 x 30 ml.) and the aqueous solution, after adjustment to pH 6, was refrigerated overnight. The product crystallized from ethanol giving 4-β-diethoxyethylamino-2-hydroxy-5-nitropyrimidine as colourless leaflets (14.4 g., 53%), m.p. 157° (Found:
C, 44.15; H, 5.9.  \( \text{C}_{16}\text{H}_{29}\text{N}_{5}\text{O}_{6} \) requires C, 44.1; H, 5.9%.

The benzene extract was evaporated to dryness, and the residue recrystallized from aqueous ethanol giving 2,4-bis-\( \beta \)-diethoxyethylamino-5-nitropyrimidine as colourless needles, m.p. 99-100° (Found: C, 49.8; H, 7.3.  
\( \text{C}_{16}\text{H}_{29}\text{N}_{5}\text{O}_{6} \) requires C, 49.6; H, 7.55%).

**5-Amino-4-\( \beta \)-diethoxyethylamino-2-hydroxypyrindine.**

4-\( \beta \)-Diethoxyethylamino-2-hydroxy-5-nitropyrimidine (2.53 g.) in ethanol (300 ml.) was hydrogenated over Raney-nickel. After the absorption had ceased (640 ml. of hydrogen, 89% for 6 h) the catalyst was filtered off and the filtrate was evaporated to dryness at room temperature in a nitrogen atmosphere. The residue crystallized from ethanol-ethyl acetate (1:9) to give 5-amino-4-\( \beta \)-diethoxyethylamino-2-hydroxypyrindine as a pale green powder (1.2 g., 53%), m.p. 174-175° (Found for substance dried at 20°/10⁻¹ mm.: C, 47.0; H, 7.9; N, 22.1.  \( \text{C}_{16}\text{H}_{29}\text{N}_{5}\text{O}_{6} \cdot 3/4 \text{H}_{2}\text{O} \) requires C, 46.95; H, 7.7; N, 21.95%).

**Attempted cyclization of 5-amino-4-\( \beta \)-diethoxyethylamino-2-hydroxypyrindine (a)** The 5-aminopyrimidine (154 mg.) in 0.1 N hydrochloric acid was refluxed under nitrogen for 15 min., and adjusted to pH 6 with sodium hydroxide. The precipitate was filtered off and reprecipitated from 0.1 N hydrochloric acid solution with N-sodium hydroxide giving a pale buff powder (22 mg.) which decomposed at 220-230°.
without melting (Found: C, 42.7; H, 4.65; N, 32.95.
Calc. for C₆H₆N₄O,H₂O : C, 42.85; H, 4.8; N, 33.3%).
Rf. 0.61 2/3B (NH₄Cl), 0.07 (Bu/Ac). (7,8-Dihydro-2-
hydroxypteridine prepared by a different method (see, p.152)
had Rf. 0.61 2/3 B (NH₄Cl)).

The filtrate was evaporated to dryness under nitrogen
and the residue was extracted with hot ethanol (6 ml.) and
evaporated to dryness. The residue was crystallized from
ethanol-ethyl acetate (1:1) giving a pale buff powder. (It
behaved like (3b-VII) see p. 69), m.p. 170-175° (decomp.)
(Found: C, 41.1; H, 5.65; N, 23.1. C₈H₁₄N₄O₃·H₂O requires
C, 41.4; H, 6.9; N, 24.1%). Rf. 0.83 2/3 B (NH₄Cl), 0.35
2/3 B (Bu/Ac). Although paper chromatography showed that
it contained a small amount of the former compound (Rf.
0.61 2/3 B), further purification of this compound was
abandoned because during the purification it changed to
the former compound.

(b) The 5-amino-4-ß-diethoxyethylaminopyrimidine, when
refluxed with N-hydrochloric acid for 45 min. under nitrogen,
gave a buff powder, Rf. 0.29 2D/3E (NH₄Cl), 0.08 2/3B
(Bu/Ac). The same compound was also obtained from the above
two compounds by heating with N- hydrochloric acid.

4-Formylmethy lamino-2-hydroxy-5-nitropyrimidine. 4-ß-
Diethoxyethylamino-2-hydroxy-5-nitropyrimidine (14 g.) was
refluxed with N- hydrochloric acid (100 ml.) for 20 min.
Adjustment of the mixture to pH 4 with sodium bicarbonate gave 4-formylmethylamino-2-hydroxy-5-nitropyrimidin as colourless needles (8.4 g., 82%) which were recrystallized from water. It darkened at 220-225° without melting (Found: C, 36.5; H, 3.15. C$_6$H$_6$N$_4$O$_4$ requires C, 36.4; H, 3.05%).

7,8-Dihydro-2-hydroxypteridine. 4-Formylmethylamino-2-hydroxy-5-nitropyrimidin (1.98 g.) was hydrogenated over Raney-nickel. The catalyst was collected and extracted with boiling water (80 ml.) giving 7,8-dihydro-2-hydroxypteridine (352 mg., 23%) which recrystallized from water as micro-prisms, decomposing at 190° without melting (Found, after drying at 135°/10^{-1} mm.: C, 47.0; H, 4.1; N, 36.6. C$_6$H$_6$N$_4$O$_{1/4}$H$_2$O requires C, 46.6; H, 4.25; N, 36.2%). Rf. 0.61 2/3 (NH$_4$Cl): in Bu/Ac it decomposed. The methanolic filtrate on concentration, gave more of the crude dihydro-compound which had the same Rf. values as above, but this did not satisfactorily recrystallize from water, probably due to impurity.

5,6,7,8-Tetrahydro-2-hydroxypteridine.

7,8-Dihydro-2-hydroxypteridine in water (100 ml.) was hydrogenated over Adams’ catalyst. After removal of the catalyst the filtrate was evaporated to dryness. The residue, on crystallization from water (5 ml.), gave 5,6,7,8-tetrahydro-2-hydroxypteridine as pale buff needles,
decomposing at 220° without melting (Found, after drying at \(106^\circ/10^{-1}\) mm.: C, 47.5; H, 5.9; N, 36.4. \(\text{C}_6\text{H}_8\text{O}_4\) requires C, 47.35; H, 5.3; N, 36.8%). The anhydrous material was unusually hygroscopic and absorbed one mole of water from air in 20 min.

5,6-Dihydro-2-hydroxypteridine

\(\beta\)-Chloropropionaldehyde acetal was prepared (19% yield) from acrolein by the action of ethanolic hydrogen chloride according to Witzemann, Evans, Hass and Schroeder (1943). It had b.p. 65-70\(^\circ/15\) mm. (lit. 53-62\(^\circ/3\) mm.). It was converted to acrolein acetal (in 78% yield) by the action of potassium hydroxide (same reference). It boiled at 120-121\(^\circ/720\) mm. (lit. 122-126\(^\circ\)). Glyceraldehyde acetal was prepared (50% yield) from acrolein acetal by oxidation with potassium permanganate (same reference). It boiled at 79\(^\circ/1\) mm. (lit. 120-121\(^\circ/3\) mm.). Glyoxal monoacetal was prepared (46% yield) from glyceraldehyde acetal according to Fischer and Daer (1935). It boiled at 50-60\(^\circ/18\) mm. (lit. 42-43\(^\circ/12-13\) mm.).

4-Amino-5-\(\beta\)-diethoxyethylamino-2-hydroxypyrimidine.

4,5-Diamino-2-hydroxypyrimidine (530 mg., 5 mmole) and glyoxal monoacetal (850 mg., 5.2 mmole) in water (25 ml.) were heated for 10 min. on a steam bath. The mixture was evaporated to dryness under reduced pressure and the residue was triturated with acetone. The solid, in 97% ethanol (50 ml.),
was hydrogenated over Raney-nickel. Removal of the catalyst, and concentration of the filtrate gave 4-amino-5-β-diethoxyethylamino-2-hydroxypyrimidine (117 mg., 10%) as needles which crystallized from ethanol, m.p. 198-200° (Found: C, 49.1; H, 7.4; N, 22.7. C10H18N4O3 requires C, 49.6; H, 7.5; N, 23.1%).

**Attempt to prepare 3,4-dihydro-2-hydroxypteridine.**

2-Aminopyrazine-3-aldehyde oxime was prepared (54% yield) from pteridine by the action of hydroxylamine hydrochloride in 2N-sodium carbonate followed by heating with N-acetic acid according to Albert, Brown and Wood (1956). It had m.p. 200° (lit. 199-200°).

**Reduction of 2-aminopyrazine-3-aldehyde oxime.** Various conditions (see Table 19) were used for the reduction of the oxime, and hydrogenation over Adams' catalyst in N-acetic acid gave the best result. Although the reduction mixture exhibited one main spot on paper chromatography, 0.70 2/3 V (NH₄Cl) and 0.20 2/3 V (Bu/Ac), no pure compound could be isolated and it slowly changed in air to a dark resin. Treatment of the crude product with urea (190°, 45 min.), urethane (180°, 1 hr.) or ethyl chloroformate (reflux for 3 hr.) gave no product corresponding to 3,4-dihydro-2-hydroxypteridine.
Table 19

Reduction of 2-aminopyrazine-3-aldehyde oxime

<table>
<thead>
<tr>
<th>Reductant</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na$_2$S$_2$O$_4$</td>
<td>Many spots on paper chromatography.</td>
</tr>
<tr>
<td>Na-Hg</td>
<td>Ammonia evolved.</td>
</tr>
<tr>
<td>LiAlH$_4$</td>
<td>Many spots on paper chromatography.</td>
</tr>
<tr>
<td>H$_2$/PtO$_2$ in HCl</td>
<td>Went brown, half of theoretical H$_2$ absorbed.</td>
</tr>
<tr>
<td>H$_2$/PtO$_2$ in aqueous NH$_4$OH</td>
<td>No reduction.</td>
</tr>
<tr>
<td>H$_2$/PtO$_2$ in 0.1N-acetic acid</td>
<td>5 H were absorbed (4 H for theoretical) and one main spot on paper chromatography.</td>
</tr>
</tbody>
</table>

Reduction of 2-amino-3-cyanopyrazine over Adams' catalyst gave results similar to the reduction of the oxime described above.

2-Amino-3-carboxypyrazine was prepared (79% yield) by heating the ammonium salt of 2,4-dihydroxypteridine with 2.5N-sodium hydroxide according to Weijlard, Tishler and Erickson (1945). It decomposed at 203° (lit. 201°).

2-Amino-3-methoxycarbonylpyrazine was prepared (70% yield) from 2-amino-3-carboxypyrazine by the action of methanolic sulphuric acid according to Ellingson, Henry and McDonald (1945). It had m.p. 172-173° (lit. 172°).
2-Amino-3-hydroxymethylpyrazine. Lithium aluminium hydride (80 mg.) was added to a solution of 2-amino-3-carboxymethylpyrazine (1.53 g.) in tetrahydrofuran (200 ml.) and kept at room temperature for 2 hr. The complex was decomposed with water (3 ml.) and the inorganic salt was filtered off. The filtrate was evaporated to dryness and extracted with ether. After removal of the ether, crystallization of the residue from amyl acetate gave 2-amino-3-hydroxymethylpyrazine as colourless needles (574 mg., 46%), m.p. 118-119.5° (Found, after drying at 65°/10⁻¹ mm.: C, 47.65; H, 5.7; N, 33.1. C₅H₇N₃O requires C, 48.0; H, 5.65; N, 33.6%).

Attempts at the synthesis of 3,4-dihydro-2-hydroxypteridine from 2-amino-3-hydroxymethylpyrazine (a) The pyrazine (100 mg.) in hydrobromic acid (sp. gr. 1.48, 1 ml.) was kept overnight at room temperature. Only the starting material was detected by paper chromatography.

(b) The pyrazine (100 mg.) was refluxed with hydrobromic acid (sp. gr. 1.48, 2.5 ml.). Soon after boiling the mixture became a brown resin and no product was obtained from it.

(c) The pyrazine (100 mg.) was mixed with urea and heated at 170° for 45 min. Paper chromatography of the product indicated that no reaction had occurred.

(d) The pyrazine (100 mg.) and urethane (0.5 g.) was heated at 190° for 1 hr. Paper chromatography of the product indicated
that no reaction had occurred.

(e) The pyrazine (63 mg.) and potassium cyanate (31 mg.) in N-hydrochloric acid (6.5 ml.) were refluxed for 45 min. Paper chromatography of the reaction mixture indicated that no reaction had occurred.

**Hydrogenation of 4,5-diamino-2-hydroxypyrimidine.** When 4,5-diamino-2-hydroxypyrimidine (1.26 g., 10 mmole) in 0.5N-hydrochloric acid (40 ml., 2 eq.) was hydrogenated over 10% palladium on carbon, 250 ml. of hydrogen (2 H) was absorbed. After removal of the catalyst, the filtrate was concentrated to 3 ml. and the product was precipitated with methanol (3 ml.). Recrystallization from 90% methanol (50 ml.) gave the product as colourless needles, m.p. 242-248° (decomp.) (Found, after drying at 62°/10⁻¹ mm.: C, 27.7; H, 5.2; N, 23.85. C₄H₇N₃O₂·HCl·½H₂O requires C, 27.5; H, 5.15; N, 24.1%). The analysis and properties indicate the product is an amino-dihydro-dihydroxy-pyrimidine hydrochloride hemihydrate.

5,6,7,8-Tetrahydro-4-hydroxypteridine.

Benzyilaminoethanol was prepared from benzylchloride and ethanolamine according to Dr. D.J. Brown (personal communication) with slight modification. Benzylchloride (250 g., 2 mole) and ethanolamine (500 g., 8 mole) was heated on a steam bath for 2 hr. with gentle stirring.
After cooling, 2N-sodium hydroxide (1 l.) was added to the mixture and extracted with ether. The extract was washed with water and dried over potassium carbonate. After removal of the ether, the residue was fractionated under vacuum, benzylaminoethanol distilled at 94-96°/0.02 mm. (121 g., 41%) (lit. 148-149/13 mm.).

5-Amino-4-(benzyl-ß-hydroxyethylamino)-6-chloropyrimidine hydrochloride. A solution of benzylaminoethanol (15 g., 0.1 mole) in chloroform (40 ml.) was added dropwise with shaking to a mixture of 4,6-dichloro-5-nitropyrimidine (19.5 g., 0.1 mole) in chloroform (150 ml.) and sodium bicarbonate (8.4 g., 0.1 mole) in water (30 ml.). Shaking was continued until the evolution of carbon dioxide ceased. The chloroform layer was washed with water (50 ml.) dried over sodium sulphate, and evaporated under reduced pressure to give an oil (37 g.). The oil, on hydrogenation over Raney-nickel, absorbed 6.6 l. of hydrogen (6 l.). After removal of the catalyst the filtrate was concentrated to 100 ml., adjusted to about pH 1 with ethanolic hydrogen chloride. Refrigeration of the solution gave 5-amino-4-(benzyl-ß-hydroxyethylamino)-6-chloropyrimidine hydrochloride as colourless prisms (9.3 g., 34%). For analysis it was rapidly recrystallized from ethanol below 70° giving colourless prisms, m.p. 130° (Found, after drying at
62°/ 10^{-1} \text{ mm.}: \ C, 49.6; \ H, 5.2; \ Cl, 22.5; \ N, 17.8. 

C_{13}H_{16}Cl_{2}N_4O \text{ requires } C, 49.5; \ H, 5.1; \ Cl, 22.5; \ N, 17.8\%.

Rf.: 0.62 2D/3X (NH_4Cl), 0.94 2D/3X (Bu/Ac). When boiled in ethanol for 5 min. the above pyrimidine rearranged to an isomer, m.p. 202-203° (Found, after drying at 62°/10^{-1} \text{ mm.}: \ C, 49.65; \ H, 5.2; \ Cl, 22.6; \ N, 18.0. \ C_{13}H_{16}Cl_{2}N_4O \text{ requires } C, 49.5; \ H, 5.1; \ Cl, 22.5; \ N, 17.8\%.

Rf.: 0.77 2D/3X (NH_4Cl), 0.66 2D/3X (Bu/Ac). This compound on treatment with phosphorus trichloride did not give a tetrahydropteridine.

8-Benzyl-4-chloro-5,6,7,8-tetrahydropteridine . 5-Amino-4-(benzyl-\beta-hydroxyethylamino)-6-chloropyrimidine (1.5 g.) was added at 10° G. to phosphorus trichloride and kept at room temperature overnight. The excess of phosphorus trichloride was distilled off at room temperature and the residue was dissolved in water (30 ml.). The aqueous solution was extracted with chloroform and then adjusted to pH 7 with sodium bicarbonate. The solution was extracted with chloroform. Removal of the chloroform gave 8-benzyl-4-chloro-5,6,7,8-tetrahydropteridine (975 mg., 79\%) which was recrystallized from ethanol (4.5 ml.) as colourless needles, m.p. 127-128° (Found, after drying at 74°/10^{-1} \text{ mm.}: \ C, 59.9; \ H, 5.0; \ Cl, 13.95; \ N, 21.3. \ C_{13}H_{13}ClN_4 \text{ requires } C, 59.9; \ H, 5.0; \ Cl, 13.6; \ N, 21.45\%).
5,6,7,8-Tetrahydropteridine. Metallic sodium was added in small portions to a solution of 8-benzyl-4-chloro-5,6,7,8-tetrahydropteridine in liquid ammonia (130 ml.) until a permanent blue colour remained, and the colour was retained for 50 min. by addition of sodium. Ammonium chloride was added until the blue colour disappeared and the ammonia was evaporated. Water (3 ml.) was added to the residue which was then extracted with chloroform (5 x 10 ml.). The chloroform extract was evaporated to dryness and the residue was purified by sublimation. 5,6,7,8-Tetrahydropteridine (66 mg., 14%) had m.p. 147° (Brook and Ramage (1957) give m.p. 146-7°).

Attempted hydrolysis of 8-benzyl-4-chloro-5,6,7,8-tetrahydropteridine. A typical method is shown below: 8-Benzyl-4-chloro-5,6,7,8-tetrahydropteridine (120 mg.) was refluxed with Na-sodium hydroxide (50 ml.) and ethanol (10 ml.) under nitrogen for 3 hr. The mixture was adjusted to pH 7 and evaporated to dryness. The residue was dissolved in ethanol (50 ml.), and water (50 ml.) was added dropwise to the boiling ethanol solution. This gave colourless needles (100 mg., m.p. 126.5-127°) which had the same Rf. values as the starting material and showed no melting point depression with it. The results of the reactions under various conditions are summarized below.
### Reaction conditions

<table>
<thead>
<tr>
<th>Reaction conditions</th>
<th>Starting material (mg.)</th>
<th>Recovery of the starting material (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(refluxed for 3 hr.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetate buffer (pH 5)</td>
<td>95</td>
<td>86</td>
</tr>
<tr>
<td>glacial acetic acid + sodium acetate</td>
<td>120</td>
<td>100</td>
</tr>
<tr>
<td>6N-HCl</td>
<td>120</td>
<td>95</td>
</tr>
<tr>
<td>12N-HCl</td>
<td>120</td>
<td>95</td>
</tr>
<tr>
<td>N-NaOH</td>
<td>120</td>
<td>100</td>
</tr>
<tr>
<td>2% sodium methoxide</td>
<td>120</td>
<td>96</td>
</tr>
<tr>
<td>4% sodium hydroxide</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>in tri-(ethylenglycol)</td>
<td>120</td>
<td>*</td>
</tr>
</tbody>
</table>

*No product was isolated. No spot corresponding to the 4-hydroxy derivative was detected.

5-Amino-4-(benzyl-β-hydroxyethylamino)-6-benzyloxy-pyrimidine. 4,6-Dichloro-5-nitropyrimidine (10 g.) and benzylaminoethanol (7.8 g.) was condensed by the method described before, and the chloroform layer was washed with water, dried over sodium sulphate, and the chloroform removed. Benzene (15 ml.) was added to the residual oil and then distilled off under vacuum at 30°C, this treatment was repeated to remove the water and chloroform completely. Sodium benzylooxide (sodium, 2.5 g. in benzylalcohol, 70 ml.) was added to the residue and heated on a steam bath for 10 min. Dry-ice was added to the cooled mixture to decompose the alcoholate and
then water (50 ml.) was added. The mixture was adjusted to pH 6 with acetic acid and then the benzyl alcohol was removed by steam distillation (4 hr.). The residual solution was extracted with benzene, and the benzene layer was dried over sodium sulphate. After removal of the benzene a light brown oil (21 g.) remained. The oil (21 g.) in ethanol over Raney-nickel absorbed 2.6 l. of hydrogen (this corresponds to 13.3 g. of the product). After removal of the catalyst, the filtrate was concentrated to about 40 ml. 10% Ethanolic hydrogen chloride (10 ml.) was added to the concentrate and refrigerated for two days, giving needles (604 mg., 3%). The product after crystallization from ethanol gave 5-amino-4-(benzyl-β-hydroxyethylamino)-6-benzyloxypyrimidine as colourless needles, m.p. 203° (Found: C, 62.5; H, 6.0; N, 14.35. C20H22N4O2.HCl requires C, 62.1; H, 6.0; N, 14.5%).

Attempted cyclization of 5-amino-4-(benzyl-β-hydroxyethylamino)-6-benzyloxypyrimidine. 5-Amino-6-benzyloxy-4-(benzyl-β-hydroxyethylamino)-pyrimidine (600 mg.) was added to phosphorus trichloride (10 ml.) and kept at room temperature overnight. Excess phosphorus trichloride was distilled off under reduced pressure at room temperature and crushed ice (5 g.) was added to the residue. The solution was extracted with benzene and adjustment of the aqueous solution
to pH 6 gave 5-amino-4-(benzyl-β-hydroxyethylamino)-6-hydroxypyrimidine hydrochloride (218 mg., 48%) which was recrystallized from water, m.p. 246-247° (decomp.). The free base was obtained by recrystallization of the hydrochloride at pH 9.7, m.p. 140-142° (Found: C, 59.7; H, 6.3; N, 21.0. \( \text{C}_{13}\text{H}_{16}\text{N}_4\text{O}_2 \) requires C, 60.0; H, 6.2; N, 21.5%).

4-Chloro-6-ethoxy-5-nitropyrimidine was prepared (88%) from 4,6-dichloro-5-nitropyrimidine and sodium ethoxide according to Boon and Jones (1951). The product had b.p. 74°/1 mm. and m.p. 45.5-46.5° (lit. m.p. 42°).

8-Benzyl-5,6,7,8-tetrahydro-4-hydroxypteridine.

Benzylaminoethanol (3.8 g. in 10 ml. methanol, 26 m. mole) was added to 4-chloro-6-ethoxy-5-nitropyrimidine (2.47 g. in 15 ml. methanol, 12 m. mole) at 0°, and kept at room temperature overnight. Excess methanol was removed and water (50 ml.) was added to the residual oil. The mixture was extracted with benzene (2 x 25 ml.) and the benzene layer was washed with water (5 x 50 ml.) to remove benzylaminoethanol.

After removal of the benzene under reduced pressure the residual oil was hydrogenated in ethanol over Raney-nickel, and 390 ml. of hydrogen was absorbed. After removal of the catalyst, the filtrate was concentrated and the excess ethanol was completely removed by distillation with benzene.

Phosphorus trichloride (15 g. in 30 ml. benzene) was added to the residual oil (in 30 ml. benzene) at 0°, and the
mixture kept at room temperature for 2.5 days. Paper chromatography indicated that cyclization and simultaneous hydrolysis occurred during this period. The benzene layer was discarded, and crushed ice (50 g.) was added to the residue. The mixture was extracted with benzene, and the aqueous solution was adjusted to pH 7 with sodium bicarbonate. Crystallization of the product from ethanol gave 8-benzyl-5,6,7,8-tetrahydro-4-hydroxypteridine as colourless needles (747 mg., 26%), m.p. 170-171° (Found: C, 64.15; H, 5.75; N, 23.1. C₁₃H₁₄N₄O requires C, 64.45; H, 5.8; N, 23.1%).

5,6,7,8-Tetrahydro-4-hydroxypteridine. Finely grounded 8-benzyl-5,6,7,8-tetrahydro-4-hydroxypteridine (1.0 g.) was added to liquid ammonia (130 ml.) followed by sodium until the solution became deep blue, and this blue colour was retained for 30 min. by the addition of sodium (about 350 mg. was necessary). Ammonium chloride was added until the blue colour disappeared, and the excess of ammonia was evaporated under nitrogen. Water was added to the residue and the solution was adjusted to pH 6 with N-hydrochloric acid. The solution was extracted with chloroform and the aqueous layer was evaporated to dryness under reduced pressure. The residue was extracted with hot ethanol and the extract was evaporated to dryness. Crystallization of the residue from water gave 5,6,7,8-tetrahydro-4-hydroxypteridine as pale buff needles (348 mg., 54%), m.p. 234-238° (decomp.) (Found, after drying at 135°C/10⁻¹mm:...
C, 47.25; H, 5.6; N, 36.35 Calc. for C₆H₈N₄O : C, 47.35; H, 5.3; N, 36.8%).

5,6-Dihydro-4-hydroxypteridine

4-Amino-5-ß-diethoxyethylamino-6-hydroxypyrimidine. 4,5-Diamino-6-hydroxypyrimidine (1.26 g., 10 m mole) and glyoxal monoacetal (1.7 g., 13 m mole) in 90% ethanol (200 ml.) were hydrogenated over Raney-nickel. After removal of the catalyst, the filtrate was evaporated to dryness. Crystallization of the residue from acetone gave 4-amino-5-ß-diethoxyethylamino-6-hydroxypyrimidine (837 mg., 35%) as colourless needles. It decomposed at 142° (Found, after drying at 65°/10⁻¹ mm.: C, 49.1; H, 7.6; N, 23.15. C₁₀H₁₈N₄O₃ requires C, 49.55; H, 7.5; N, 23.15%).

5,6-Dihydro-4-hydroxypteridine. 4-Amino-5-ß-diethoxyethylamino-6-hydroxypyrimidine (839 mg.) was boiled with 0.5N-hydrochloric acid (10 ml.) for 1 min. and cooled immediately. The solution was adjusted to pH 5 with sodium bicarbonate and refrigerated overnight. The precipitate on crystallization from water gave 5,6-dihydro-4-hydroxypteridine as colourless needles 486 mg., 88%. It decomposed at 230° (Found, after drying at 20°/10⁻¹ mm.: C, 46.4; H, 4.35; N, 36.5. C₆H₆ON₄.1/4 H₂O requires C, 46.6; H, 4.25; N, 36.2%). Although the product retained 1/4 molecule of water when dried at 20°/10⁻¹ mm., no isolated carbonyl group could be detected by an infrared spectrum. This indicates that the product has entirely the ring-closed structure.
Attempted syntheses for 7,8-dihydro-4-hydroxypteridine.

4-β-Diethoxyethylamino-6-hydroxy-5-nitropyrimidine. Amino acetal (5.4 g.) in water (50 ml.) was adjusted to pH 8 with acetic acid and then sodium bicarbonate (6.0 g.) was added to the solution. The solution was added dropwise with shaking to a cold solution of 4,6-dichloro-5-nitropyrimidine (7.9 g.) in chloroform (50 ml.). Shaking was continued for a further 2 hr. at room temperature. The chloroform layer was distilled with N-sodium hydroxide (200 ml.) on a steam bath while bubbling nitrogen through the solution for about 20 min. (During this time the 4-chloro-substituent was replaced by a hydroxy group). The residual solution was extracted with benzene and the aqueous solution was adjusted to pH 6 with acetic acid. The precipitate on crystallization from ethanol gave 4-β-diethoxyethylamino-6-hydroxy-5-nitropyrimidine (2.99 g., 27%), m.p. 118-120°. (Found: C, 42.35; H, 6.1.

C₁₀H₁₆N₄O₅·H₂O requires C, 42.75; H, 6.1%).

Reduction of 4-β-diethoxyethylamino-6-hydroxy-5-nitropyrimidine (a) When 4-β-diethoxyethylamino-6-hydroxy-5-nitropyrimidine (140 mg., 0.5 m mole) was hydrogenated over Raney-nickel, 120 ml. of hydrogen (=10 H, 6 H for NO₂ group) was absorbed, and the reduction mixture turned brown. Concentration of the filtrate gave a brown oil which on heating with 0.1 N-hydrochloric acid for 15 min. gave a dark resin, and no product could be isolated. (b) The nitropyrimidine (700 mg.) in acetic acid was reduced with zinc
dust (2.1 g.). After removal of the excess zinc the filtrate
was concentrated to dryness, and the residue extracted with
methanol. The extract gave a spot similar to the above
reduction product (a) on paper chromatography, but no product
could be isolated.

Hydrolysis of 4-β-diethoxyethylamino-6-hydroxy-5-
nitropyrimidine. 4-β-Diethoxyethylamino-6-hydroxy-5-
nitropyrimidine (1.5 g., 5 m mole) in N-hydrochloric acid
(25 ml.) was refluxed for 10 min. The crystals were
filtered off and recrystallized from water giving colourless
needles (967 mg., 89%). The substance decomposed slowly at
230° without melting (Found: C, 39.25; H, 2.6.
C₁₂H₈N₈O₆.₅H₂O requires C, 39.05; H, 2.45%). The product
absorbed 5 molecules of hydrogen over Raney-nickel and gave
a dark resinous product.

Attempted syntheses for 7,8-dihydro-4-hydroxy-6-methylpteridine.

4-Acetonylamino-6-chloro-5-nitropyrimidine was prepared
(47% yield) from 4,6-dichloro-5-nitropyrimidine and aminoacetone
hydrochloride according to Boon and Jones (1951). It had
m.p. 84° (lit. 86°).

4-Acetonylamino-6-hydroxy-5-nitropyrimidine. 4-
Acetonylamino-6-chloro-5-nitropyrimidine (3.6 g.) was dissolved
in 50% aqueous ethanol and kept at room temperature overnight.
The precipitate, crystallized from ethanol, gave 4-
acetonylamino-6-hydroxy-5-nitropyrimidine as needles, m.p.
207° (decomp.) (Found: C, 39.1; H, 4.3. C₇H₆N₄O₄ requires C, 39.6, H, 3.8%). Repeated crystallization of the product caused loss of one molecule of water from the molecule and gave a product which decomposed at 230° without melting (Found: C, 43.2; H, 3.5. C₇H₆N₄O₃ requires C, 43.4; H, 3.1%).

Hydrogenation of 4-acetonylamino-6-hydroxy-5-nitropyrimidine. The nitropyrimidine absorbed 3 mole of hydrogen over Raney-nickel, but the reduction mixture turned brown. No pure product could be isolated from the mixture, and no spot similar to that of x,y-dihydro-4-hydroxypteridine was detected on paper chromatography.

Attempted synthesis for 1,2-dihydro-4-hydroxypteridine (a) 2-Amino-3-carbamoylpyrazine (30 mg.) in (5 ml.) water was heated with 30% formaldehyde (0.2 ml.) on a steam bath for 20 min. No product other than the starting material was detected in the reaction mixture. (b) The pyrazine (50 mg.) in ethanol (10 ml.) was refluxed for 45 min. with 30% formaldehyde (0.1 ml.). No product other than the starting material was detected by paper chromatography.

Attempted syntheses of 5,6-dihydro-7-hydroxypteridine.

Attempted preparation of the Schiff's base of 4,5-diaminopyrimidine and ethyl glyoxylate. (a) Ethyl glyoxylate hemiacetal (Rigby, 1950) (145 mg., 1 m mole) was added to a solution of 4,5-diaminopyrimidine (110 mg., 1 m mole) and
kept at room temperature for 10 min. Only 7-hydroxypteridine was detected in the reaction mixture, and no spot corresponding to the Schiff's base was detected. (b) Ethyl glyoxylate hemiacetal (15 mg., 0.1 m mole) was added to 4,5-diamino- pyrimidine (10 mg., 0.1 m mole) in methanol (10 ml.). The solution had the same ultraviolet spectrum as 4,5-diamino- pyrimidine. When the solution was diluted with water, it gave the same ultraviolet spectrum as 7-hydroxypteridine, and no Schiff's base formation was detected.

4-Amino-5-cyanomethylaminopyrimidine. Potassium cyanide (70 mg., in 2 ml. water) was added to 4,5-diaminopyrimidine (1.1 g.) in methanol (10 ml.) and the mixture was adjusted to pH 7.5 with hydrochloric acid. 30% Formaldehyde solution (0.9 ml.) was added to the solution and the mixture was kept at 40-45° for 45 min. The crystals were filtered off and washed with cold water. The 4-amino-5-cyanomethylaminopyrimidine decomposed at 223° (Found*, after drying at 20°/10⁻¹ mm.: C, 47.05; H, 5.4; N, 46.35. C₅H₇N₅ requires C 48.3; H, 4.7; N, 46.95%). This compound gave 4,5-diaminopyrimidine on hydrolysis in acidic or alkaline condition.

Phthaloylglycine was prepared (87% yield) by heating a mixture of glycine and phthalic anhydride at 200° for 20 min.

*This compound was unstable to hydrolysis and partially decomposed on recrystallization from water; hence the poor analytical figures.
It had m.p. 189-193° (Reese (1887) gives m.p. 192°).

Phthaloylglycyl chloride was prepared (80% yield) from phthaloyl glycine by the action of thionyl chloride. It boiled at 145-150°/2 - 3 mm. and had m.p. 85-86° (Gabriel (1907) gives m.p. 84-85°).

5-Bromo-4-hydroxypyrimidine was prepared (80% yield) by bromination of 4-hydroxypteridine according to Chesterfield, McOmie and Sayer (1955). It had m.p. 198-200° (lit. 199-200°). 4-Amino-5-bromopyrimidine was prepared (57% yield) from 5-bromo-4-hydroxypyrimidine by the action of phosphorus oxychloride and then ammonia according to Chesterfield, McOmie and Sayer (1955). It had m.p. 209-211° (lit. 208-210°).

5-Bromo-4-phthaloylglycylaminopyrimidine. 4-Amino-5-bromopyrimidine (1.0 g.) and phthaloylglycyl chloride (1.3 g.) in pyridine (5 ml.) was refluxed for 1 hr. After removal of the pyridine, water (20 ml.) was added to the residue. The precipitate was triturated with sodium bicarbonate solution (1 g. NaHCO₃ in 10 ml. water) and then crystallized from ethanol giving 5-bromo-4-phthaloylglycylaminopyrimidine as colourless needles, m.p. 223-225° (Found: C, 46.2; H, 2.6; N, 15.3. C₁₄H₉BrN₄O₃ requires C, 46.55; H, 2.5; N, 15.5%).

Hydrazinolysis of 5-bromo-4-phthaloylglycylaminopyrimidine. Hydrazine hydrate (0.12 ml.) was added to 5-bromo-4-phthaloylglycylaminopyrimidine (723 mg.) in methanol (30 ml.), and the solution was kept at room temperature overnight. After
removal of the crystals* (224 mg.), the filtrate was evaporated to dryness and the residue recrystallized from 50% ethanol. The colourless needles (252 mg., 73%), m.p. 212-213°, were identified as 4-amino-5-bromopyrimidine from the Rf. value (0.56: 2D/3X), and mixed melting point with an authentic sample.

4-Amino-5-methoxycarbonylmethylaminopyrimidine was prepared from 4-amino-5-carboxymethylaminopyrimidine (see p.130) by the action of methanolic hydrogen chloride according to Albert, Brown and Cheeseman (1952). It decomposed at 188-189° (Found: N, 21.8; Cl, 27.5. Calc. for C7H10N4O2·2HCl: N, 21.95; Cl, 27.8%).

Attempts at syntheses for 4-amino-5-carbamoylmethylaminopyrimidine (a) 4-Amino-5-methoxycarbonylmethylaminopyrimidine dihydrochloride (100 mg.) was added to methanolic ammonia (5%, 2 ml.) and kept at room temperature overnight. Only 5,6-dihydro-7-hydroxypteridine was detected in the reaction mixture by paper chromatography. (b) 4-Amino-5-carboxymethylaminopyrimidine (163 mg.) was dissolved in aqueous ammonia and evaporated to dryness. The residual ammonium salt was heated at 170° for 10 min. The product was identified as 5,6-dihydro-7-hydroxypteridine and none of the desired amide was produced.

*This compound had a low nitrogen content (6.9%) and corresponds to α-carboxybenzoylglycine (C10H9O5N; N, 6.3%).
Attempted synthesis of 7,8-dihydro-2,4-dihydroxypteridine. 2,4,6-Trichloro-5-nitropyrimidine was condensed with aminoacetal by the same method as described before (see p. 149). Paper chromatography of the reaction mixture showed many spots, and no product was isolated from the reaction mixture after hydrolysis with N-sodium hydroxide.

7,8-Dihydro-2,6-dihydroxypteridine.

2-Chloro-4-ethoxycarbonylmethylamino-5-nitropyrimidine was prepared (73% yield) from 2,4-dichloro-5-nitropyrimidine and ethyl aminoacetate hydrochloride according to Boon, Jones and Ramage (1951). It had m.p. 101-103° (lit. 101-102°).

4-Ethoxycarbonylmethylamino-2-hydroxy-5-nitropyrimidine was prepared (67% yield) by hydrolysis of 2-chloro-4-ethoxycarbonylmethylamino-5-nitropyrimidine according to Boon, Jones and Ramage (1951). It decomposed at 232° (lit. 230-232°).

7,8-Dihydro-2,6-dihydroxypteridine was prepared (61% yield) from 4-ethoxycarbonylamino-2-hydroxy-5-nitropyrimidine by reduction followed by hydrolysis according to Boon, Jones and Ramage (1951). It had m.p. >300° (Found: C, 43.0; H, 3.85; N, 33.55. Calc. for C₆H₅N₄O₂: C, 43.35; H, 3.65; N, 33.7%).

7,8-Dihydro-4,6-dihydroxypteridine.

6-Chloro-4-ethoxycarbonylmethylamino-5-nitropyrimidine was prepared (62% yield) from 4,6-dichloro-5-nitropyrimidine and ethyl aminoacetate hydrochloride according to Boon, Jones and Ramage (1951). It had m.p. 90-92° (lit. 93-94°).
4-ethoxycarbonylmethylamino-6-hydroxy-5-nitropyrimidine was prepared (41% yield) by hydrolysis of 6-chloro-4-ethoxy-carbonylmethylamino-5-nitropyrimidine according to Boon, Jones and Ramage (1951). It had m.p. 213-214° (lit. 214°).

7,8-Dihydro-4,6-dihydroxypteridine was prepared from 4-ethoxycarbonyl-6-hydroxy-5-nitropyrimidine by reduction followed by hydrolysis according to Boon, Jones and Ramage (1951). It decomposed >300° without melting (Found: C, 43.3; H, 3.8; N, 35.6. Calc. for C\textsubscript{6}H\textsubscript{6}N\textsubscript{4}O\textsubscript{2}: C, 43.4; H, 3.65; N, 33.7%).

**Attempted syntheses for 5,6-dihydro-2,7-dihydroxypteridine.**

(a) Ethyl glyoxylate hemiacetal (2.2 g.) was added to 4,5-diamino-2-hydroxypteridine in water (20 ml.) and the solution was kept at room temperature for 1 hr. and then refrigerated. The precipitate crystallized from ethanol as leaflets (2.2 g., 90%) which gave the same Rf. values as 2,7-dihydroxypteridine on paper chromatography. (b) Ethyl glyoxylate hemiacetal (140 mg.) was added to 4,5-diamino-2-hydroxypteridine in ethanol (10 ml.) and the mixture was examined spectroscopically. No reaction took place under anhydrous condition. When water (10 ml.) was added to the mixture, 2,7-dihydroxypteridine was produced, but no evidence of Schiff's base formation was obtained. (c) 4,5-Diamino-2-hydroxypteridine (631 mg.) was dissolved in N-hydrochloric acid (10 ml.) and then adjusted to pH 7.5 with N-sodium hydroxide (5 ml.). Potassium cyanide
(350 mg.) and 37° formaldehyde solution (0.45 ml.) was added to the solution and the solution was kept at room temperature for 2 hr. and refrigerated. The crystals (252 mg.) had the same Rf. values as 4,5-diamino-2-hydroxypyrimidine. Only 4,5-diamino-2-hydroxypyrimidine was detected by paper chromatography in the filtrate.

5,6-Dihydro-4,7-dihydroxysteridine.

4-Amino-5-ethoxycarbonylmethyleneamino-6-hydroxy-
pyrimidine was prepared (68% yield) from 4,5-diamino-6-
hydroxypyrimidine and ethyl glyoxylate hemiacetal according to Pfleiderer (1959). It decomposed at 195-196° (lit. m.p. 196°).

4-Amino-5-ethoxycarbonylmethyleneamino-6-hydroxy-
pyrimidine (1.05 g. 5 m mole) in ethanol (250 ml.) was hydrogenated over Raney-nickel, and 130 ml. of hydrogen (2H) was absorbed during 70 min. After removal of the catalyst the filtrate was concentrated to dryness. The residue, crystallized from ethanol and gave 4-amino-5-ethoxycarbonylmethyleneamino-6-
hydroxypyrimidine as colourless needles (555 mg. 52%). It decomposed at 138-139° (Found, after drying at 65°/10⁻¹ mm: C, 45.2; H, 5.8; N, 26.35. C₆H₁₂N₄O₃ requires C, 45.3; H, 5.7; N, 26.4%).

5,6-Dihydro-4,7-dihydroxysteridine. 4-Amino-5-
ethoxycarbonylmethyleneamino-6-hydroxypyrimidine (307 mg.) in
N-hydrochloric acid (4 ml.) was heated on a steam bath for 1 hr. under nitrogen. The starting material dissolved, and then colourless needles separated. The product on recrystallization from water gave 5,6-dihydro-4,7-dihydroxypteridine as pale yellow needles (192 mg., 80%), m.p. > 300° (Found: C, 43.2; H, 3.7; N, 33.7. Calc. for C₆H₆N₄O₂: C, 43.4; H, 3.65; N, 33.7%).

**7,8-Dihydro-4,6-dimethylpteridine.**

4-Acetonylamino-2-chloro-6-methyl-5-nitropyrimidine was prepared (43% yield) from 2,4-dichloro-6-methyl-5-nitropyrimidine and aminoacetone hydrochloride according to Boon and Jones (1951). It had m.p. 106-108° (lit. 108°). 2-Chloro-7,8-dihydro-4,6-dimethylpteridine was prepared (59%) by reduction of 4-acetonylamino-2-chloro-6-methyl-5-nitropyrimidine according to Lister and Ramage (1953). It decomposed at 195° (lit. 215°) (Found: C, 48.4; H, 4.75; N, 28.05 calc. for C₈H₇ClN₄: C, 48.85; H, 4.6; N, 28.5%).

**7,8-Dihydro-4,6-dimethylpteridine.** (a) 2-Chloro-7,8-dihydro-4,6-dimethylpteridine and red phosphorus (100 mg.) was refluxed with hydriodic acid (d 1.7, 2 ml.) for 1 hr. After removal of the red phosphorus, the filtrate was evaporated to dryness in a vacuum desiccator over potassium hydroxide. The residual oil was dissolved in water, adjusted to pH 10 with N-potassium hydroxide and extracted with chloroform. No product was obtained from the chloroform extract.
(b) 2-Chloro-7,8-dihydro-4,6-dimethylpteridine (920 mg.) was hydrogenated in 50% ethanol over palladium on carbon catalyst, in the presence of magnesium oxide (800 mg.); and 125 ml. of hydrogen (122 ml., 2 H) was absorbed. After removal of the catalyst the filtrate was evaporated to dryness. The residue was dissolved in water (15 ml.) and extracted with chloroform (15 x 8 ml.). The chloroform layer, after drying over calcium chloride, was evaporated to dryness. The crystalline residue (631 mg., 90%) was recrystallized twice from benzene and then sublimed at 90-100°/10⁻¹ mm. The 7,8-dihydro-4,6-dimethylpteridine had m.p. 135-140° (Found, for substance purified by sublimation: C, 59.2; H, 6.15; N, 34.0. C₈H₁₀N₄ requires C, 59.25; H, 6.2; N, 34.55%).
c. Ionization Constants.

Two good general methods are available for the determination of ionization constants, one is by potentiometric titration and the other is by the spectroscopic method (Albert and Phillips, 1956). Potentiometric titration is far less laborious than the latter, but has two limitations: (a) the solubility of the substance should be higher than $10^{-3}$ M, because $1 \times 10^{-3}$ M is usually the lower limit of accuracy. (b) The $pK_a$ values of the substance should lie between 2.7 and 11 (at least in the present work where a 0.005 M solution had mostly to be used because of poor solubility) because potentiometry is inaccurate when the $pK_a$ is less than the logarithm of the dilution; also it is not very accurate in the high alkaline region. The spectroscopic method can be used when the solubility of the substance is less than $10^{-3}$ M, or the $pK_a$ values were lower than 2.7 or higher than 11. However, in this method any small errors, in determining the extinction values of each pure species, can cause a large effect on the $pK_a$ values. This method can only be used for a substance whose two species have different ultraviolet absorption spectra. Both methods need special apparatus and techniques if they have to be used for a substance which has an unstable species.

i) **Potentiometric Titration.**

The dried specimen (0.00005 mole) was dissolved in $\mathbf{C}^{02-}$-free water (10 ml., the concentration of this solution was M/200)
and titrated under nitrogen. A Cambridge Bench pH Meter was used with glass and calomel electrodes (standardized to pH 4.00 with 0.05 M-potassium hydrogen phthalate and 9.23 with 0.05 M-borax at 20°). Only when agreement on restandardization was within ± 0.02 pH unit, without further adjustment, this instrument was considered ready for titration. After the solution was titrated, the meter was checked against that one of the two buffers whose pH was nearer to the pK. Failure to agree within ± 0.02 pH unit, made it necessary to reject the titration. 0.9 Equivalent of 0.1 N-hydrochloric acid (or carbon dioxide-free 0.1 N-potassium hydroxide) was added in nine equal portions and the pH was recorded after each addition. The nine pH values, one for each pH reading, were calculated from the formula (1) when a base was titrated.

$$pK_a = pH - \log \left( \frac{[B] + [H^+]}{[BH^+] - [H^+]} \right)$$  

where [BH+] and [B] are the stoichiometric concentrations of the cation and neutral molecule respectively. When an acid was titrated, formula (2) was used for the calculation of pK_a values.

$$pK_a = pH + \log \left( \frac{[AH] + [OH^-]}{[A^-] - [OH^-]} \right)$$  

where [AH] and [A^-] are the stoichiometric concentrations of the neutral molecule and anion respectively. The results are negative logarithms of concentration ionization constants (pK_a (conc.)) and are valid for the concentration and temperature given. At the concentration used, they would
differ little from the thermodynamic ionization constants \((\text{p}K_a \text{ (therm.)})\). The two constants are related, approximately by equations (3) and (4):

\[
\text{p}K_a \text{ (therm.)} = \text{p}K_a \text{ (conc.)} + 0.5 \sqrt{I} \text{ (for acids), (3)}
\]

\[
\text{p}K_a \text{ (therm.)} = \text{p}K_a \text{ (conc.)} - 0.5 \sqrt{I} \text{ (for bases), (4)}
\]

where \(I\) is the ionic strength at half-neutralization. For example at 0.005 M, the values of \(\text{p}K_a \text{ (conc.)}\) would be only 0.02 too high for bases and 0.02 too low for acids compared to \(\text{p}K_a \text{ (therm.)}\). In the present Thesis all ionization constants were recorded as \(\text{p}K_a \text{ (conc.)}\). For a few substances having unstable species, the \(\text{p}K_a\) values were determined by a rapid titration method using a self-recorder in which the whole titration was carried out within 3 min.

ii) Spectroscopic Determinations of \(\text{p}K_a\).

Solutions were made in a series of buffers, standardized with a glass electrode. This series was decreased in pH down to values where the change in spectrum, corresponding to the step of ionization under study, ceased; and conversely it was increased towards the alkaline direction, until the change in spectrum ceased. The buffers (0.01 M) used were of low ultraviolet absorption: glycine (pH 2.1 - 2.7), formate (pH 3 - 4.2), acetate (pH 4.2 - 5.7), phosphate (pH 6.0 - 7.9), borate (pH 8.3 - 10.2), and ethylamine (pH 10.2 - 11.0). For the low
pH region, the acidity function solutions (sulphuric acid of various strengths) of Hammett (1940) and Eascombe and Bell (1959) were used. For the high pH region, potassium hydroxide solutions of known concentration were used. Measurements were made in the Hilger "Uvispec" Quartz Spectrophotometer using 1 or 4 cm. cells. Buffer solutions of the same strengths were used in the compensating cell. The wavelength was chosen where a marked difference in spectra were observed between the two species. Extinction coefficients at the selected wavelength were then determined for different pH values corresponding to the range from 15 to 85% protonation in 3 equal steps. The $pK_a$ values were determined from formula (5):

$$pK_a = pH - \log \left[ \frac{(\varepsilon_c - \varepsilon)}{(\varepsilon - \varepsilon_M)} \right]$$

(5)

where $\varepsilon_M$ and $\varepsilon_c$ are the extinction coefficients of the pure neutral molecule and of the pure cation respectively. $\varepsilon$ is the extinction coefficient of the sum of the two species at the pH value where measurement was carried out. A similar method and formula (6) was used for the equilibrium involving the neutral molecule and anion:

$$pK_a = pH - \log \left[ \frac{(\varepsilon_M - \varepsilon)}{\varepsilon - \varepsilon_A} \right]$$

(6)

where $\varepsilon_M$, $\varepsilon_A$ and $\varepsilon$ are extinction coefficients of the neutral molecule, the anion and of the equilibrium mixture of the two species respectively. No hydrolysis correction for $[H^+]$ or
[OH⁻] is required in this method. The pKₐ values obtained from the above formulae, (5) and (6), are pKₐ (conc.) values, because, although no corrections for ionic strength of the substance is necessary at the low dilutions, some correction would be required for the 0.01 M buffer solutions if pKₐ (therm.) were required. The nature of these corrections would be to make both acid and bases about 0.03 pKₐ unit weaker.

Approximate pKₐ values of substances very unstable to air oxidation (i.e. tetrahydro-2,4-dihydroxypteridine) were determined as follows: 2,4-Dihydroxypteridine (164 mg., 1 mmole) was hydrogenated in 0.1 N-sodium hydroxide (10 ml.) over H₂-catalyst as described before (p. 130). 2,3-Dimercaptopropanol solution (N/1000; 1 ml.) was added to the reduction mixture and the catalyst filtered off. The filtrate was adjusted to pH 4 and diluted to 100 ml. in a measuring flask with N/10000 - 2,3-dimercaptopropanol. The absorption coefficients of the solutions, buffered for 3.0, 4.2, 5.0 and 7.6, were determined at wavelength 300 μm. From these values, the cationic pKₐ value was calculated by formula (5).
d. Spectra

1) Ultraviolet absorption spectra of the various species were obtained using the self-recording Perkin Elmer "Spectracord" 4000A and the extinction coefficients at the maximum wavelength were rechecked manually on a Hilger "Uvispek" Quartz Spectrophotometer. The solutions were buffered (with the same series of buffers as used in spectrophotometric determinations of $pK_a$), and at least 2 pH units away from the previously determined $pK_a$ value, thus ensuring that at least 99% of the required species (and that no more than 1% of any unwanted species) were present.

2) Infrared absorption spectra of the various specimens were determined by the Perkin Elmer Model 21 instrument fitted with a NaCl prism. As all hydroxypteridines are insoluble in most organic solvents, the potassium bromide disc method had to be used for the determination of these spectra. Of the dried sample, 1 mg. was ground well, mixed with potassium bromide (200 mg.) and pressed to a disc.
<table>
<thead>
<tr>
<th>Fig.</th>
<th>Ultraviolet absorption spectra used for structure determination.</th>
<th>page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(x,y)-Dihydro-2-hydroxypteridine (neutral molecule) and 2-hydroxypteridine (hydrated neutral molecule)</td>
<td>186</td>
</tr>
<tr>
<td>2</td>
<td>7,8-Dihydro-6-hydroxypteridine (neutral molecule) and 6-hydroxypteridine (hydrated neutral molecule)</td>
<td>186</td>
</tr>
<tr>
<td>4</td>
<td>Neutral molecule of tetrahydro-2-hydroxypteridine and 4,5-diamino-2-hydroxy pyrimidine</td>
<td>188</td>
</tr>
<tr>
<td>5</td>
<td>Cation of tetrahydro-2-hydroxypteridine and 4,5-diamino-2-hydroxy pyrimidine</td>
<td>188</td>
</tr>
<tr>
<td>6</td>
<td>Anion of tetrahydro-2-hydroxypteridine and 4,5-diamino-2-hydroxy pyrimidine</td>
<td>189</td>
</tr>
<tr>
<td>7</td>
<td>Neutral molecule of 5,6,7,8-tetrahydro-4-hydroxypteridine and 4,5-diamino-6-hydroxy pyrimidine</td>
<td>189</td>
</tr>
<tr>
<td>8</td>
<td>Cation of 5,6,7,8-tetrahydro-4-hydroxypteridine and 4,5-diamino-6-hydroxy pyrimidine</td>
<td>190</td>
</tr>
<tr>
<td>9</td>
<td>Anion of 5,6,7,8-tetrahydro-4-hydroxypteridine and 4,5-diamino-6-hydroxy pyrimidine</td>
<td>190</td>
</tr>
<tr>
<td>10</td>
<td>Hydrolysis-product of dihydro-7-hydroxypteridine with alkali (neutral molecule) and 4,5-diamino pyrimidine (cation)</td>
<td>191</td>
</tr>
<tr>
<td>11</td>
<td>Hydrolysis-product of dihydro-7-hydroxypteridine with alkali (anion) and 4,5-diaminopyrimidine (neutral molecule)</td>
<td>191</td>
</tr>
<tr>
<td></td>
<td>7,8-Dihydro-2,6-dihydroxypteridine (neutral molecule) and 2,6-dihydroxypteridine (hydrated neutral molecule)</td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>----------------------------------------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>192</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>7,8-Dihydro-4,6-dihydroxypteridine (neutral molecule) and 4,6-dihydroxypteridine (hydrated neutral molecule)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>192</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fig.</th>
<th>Ultraviolet absorption spectra of pteridines and pyrimidines.</th>
<th>page</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>2-Hydroxy-6-methylpteridine</td>
<td>193</td>
</tr>
<tr>
<td>15</td>
<td>7,8-Dihydro-4,6-dimethylpteridine</td>
<td>194</td>
</tr>
<tr>
<td>16</td>
<td>3,4-Dihydro-2-hydroxypteridine</td>
<td>194</td>
</tr>
<tr>
<td>17</td>
<td>7,8-Dihydro-2-hydroxypteridine</td>
<td>195</td>
</tr>
<tr>
<td>18</td>
<td>3,4-Dihydro-2-hydroxy-6-methylpteridine</td>
<td>195</td>
</tr>
<tr>
<td>19</td>
<td>7,8-Dihydro-2-hydroxy-6-methylpteridine</td>
<td>196</td>
</tr>
<tr>
<td>20</td>
<td>5,6-Dihydro-4-hydroxypteridine</td>
<td>196</td>
</tr>
<tr>
<td>21</td>
<td>x,y-Dihydro-4-hydroxypteridine</td>
<td>197</td>
</tr>
<tr>
<td>22</td>
<td>7,8-Dihydro-6-hydroxypteridine</td>
<td>198</td>
</tr>
<tr>
<td>23</td>
<td>5,6-Dihydro-7-hydroxypteridine</td>
<td>198</td>
</tr>
<tr>
<td>24</td>
<td>7,8-Dihydro-2,6-dihydroxypteridine</td>
<td>199</td>
</tr>
<tr>
<td>25</td>
<td>x,y-Dihydro-2,7-dihydroxypteridine</td>
<td>199</td>
</tr>
<tr>
<td>26</td>
<td>7,8-Dihydro-4,6-dihydroxypteridine</td>
<td>200</td>
</tr>
<tr>
<td>27</td>
<td>5,6-Dihydro-4,7-dihydroxypteridine</td>
<td>200</td>
</tr>
<tr>
<td>28</td>
<td>7,8-Dihydro-2,4,6-trihydroxypteridine</td>
<td>201</td>
</tr>
<tr>
<td>29</td>
<td>5,6,7,8-Tetrahydro-2-hydroxypteridine</td>
<td>201</td>
</tr>
<tr>
<td>30</td>
<td>5,6,7,8-Tetrahydro-4-hydroxypteridine</td>
<td>202</td>
</tr>
<tr>
<td>31</td>
<td>4,5-Diaminopyrimidine</td>
<td>302</td>
</tr>
<tr>
<td>32</td>
<td>4-Amino-5-carboxymethylaminopyrimidine</td>
<td>203</td>
</tr>
<tr>
<td>33</td>
<td>4,5-Diamino-2-hydroxypyrimidine</td>
<td>203</td>
</tr>
<tr>
<td>34</td>
<td>4,5-Diamino-6-hydroxypyrimidine</td>
<td>204</td>
</tr>
<tr>
<td>Fig.</td>
<td>Infrared absorption spectra of pteridines</td>
<td>page</td>
</tr>
<tr>
<td>------</td>
<td>------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>35</td>
<td>5,6,7,8-Tetrahydro-4-hydroxypteridine obtained by synthesis</td>
<td>205</td>
</tr>
<tr>
<td>36</td>
<td>Tetrahydro-4-hydroxypteridine obtained by reduction</td>
<td>205</td>
</tr>
<tr>
<td>37</td>
<td>5,6-Dihydro-4-hydroxypteridine</td>
<td>206</td>
</tr>
<tr>
<td>38</td>
<td>x,y-Dihydro-4-hydroxypteridine</td>
<td>206</td>
</tr>
<tr>
<td>39</td>
<td>7,8-Dihydro-2,6-dihydroxypteridine obtained by synthesis</td>
<td>207</td>
</tr>
<tr>
<td>40</td>
<td>Dihydro-2,6-dihydroxypteridine obtained by reduction</td>
<td>207</td>
</tr>
<tr>
<td>41</td>
<td>7,8-Dihydro-4,6-dihydroxypteridine obtained by synthesis</td>
<td>208</td>
</tr>
<tr>
<td>42</td>
<td>Dihydro-4,6-dihydroxypteridine obtained by reduction</td>
<td>208</td>
</tr>
<tr>
<td>43</td>
<td>5,6-Dihydro-4,7-dihydroxypteridine obtained by synthesis</td>
<td>209</td>
</tr>
<tr>
<td>44</td>
<td>Dihydro-4,7-dihydroxypteridine obtained by reduction</td>
<td>209</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fig.</th>
<th>page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Absorption of oxygen by tetrahydro-2,4-dihydroxypteridine</td>
</tr>
</tbody>
</table>

Fig. 3: Absorption of oxygen by tetrahydro-2,4-dihydroxypteridine

Pages: 205, 206, 207, 208, 209, 187
Fig. 1.  
- x,y-Dihydro-2-hydroxypteridine (neutral molecule)  
- 2-Hydroxypteridine (hydrated neutral molecule)  

Fig. 2.  
- 7,8-Dihydro-6-hydroxypteridine (neutral molecule)  
- 6-Hydroxypteridine (hydrated neutral molecule)
Fig. 3.

O₂ absorption (mole)

0 50 100 150

time (min.)

- pH 13
- pH 12.5
- pH 6.0
- pH 5.5
- pH 4.0

absorption of oxygen by tetrahydro-2,4-dihydroxypteridine
Fig. 4. neutral molecule
- Tetrahydro-2-hydroxypteridine
- 4,5-Diamino-2-hydroxy pyrimidine

Fig. 5. cation
- Tetrahydro-2-hydroxypteridine
- 4,5-Diamino-2-hydroxy pyrimidine
Fig. 6. Anion

- Tetrahydro-2-hydroxypteridine
- 4,5-Diamino-2-hydroxypyrimidine

Fig. 7. Neutral molecule

- 5,6,7,8-Tetrahydro-4-hydroxypteridine
- 4,5-Diamino-6-hydroxypyrimidine
Fig. 8. cation

5,6,7,8-Tetrahydro-4-hydroxypteridine
4,5-Diamino-6-hydroxypteridine

Fig. 9. anion

5,6,7,8-Tetrahydro-4-hydroxypteridine
4,5-Diamino-6-hydroxypteridine
Fig. 10.  

Hydrolysis-product of dihydro-7-hydroxyptridine with alkali (neutral molecule)  
4,5-Diaminopyrimidine (cation)  

Fig. 11.  

Hydrolysis-product of dihydro-7-hydroxypteridine with alkali (anion)  
4,5-Diaminopyrimidine (neutral molecule)
Fig. 12.  
7,8-Dihydro-2,6-dihydroxypteridine (neutral molecule)  
2,6-Dihydroxypteridine (hydrated neutral molecule)

Fig. 13.  
7,8-Dihydro-4,6-dihydroxypteridine (neutral molecule)  
4,6-Dihydroxypteridine (hydrated neutral molecule)
Fig. 14. 2-Hydroxy-6-methylpteridine

- solid line: neutral molecule
- dashed line: cation
- dotted line: anion
Fig. 15. 7,8-Dihydro-4,6-dimethylpteridine

- neutral molecule
- cation

Fig. 16. 3,4-Dihydro-2-hydroxypteridine

- neutral molecule
- cation
- anion
Fig. 17. 7,8-Dihydro-2-hydroxypteridine
- neutral molecule
- cation
- anion

Fig. 18. 3,4-Dihydro-2-hydroxy-6-methylpteridine
- neutral molecule
- cation
- anion
Fig. 9. 7,8-Dihydro-2-hydroxy-6-methylpteridine

Fig. 20. 5,6-Dihydro-4-hydroxypteridine
Fig. 22. 7,8-Dihydro-6-hydroxypteridine

- neutral molecule
- cation
- anion

Fig. 23. 5,6-Dihydro-7-hydroxypteridine

- neutral molecule
- cation
- anion
Fig. 24. 7,8-Dihydro-2,6-dihydroxypteridine
- neutral molecule
- cation
- anion

Fig. 25. x,y-Dihydro-2,7-dihydroxypteridine
- neutral molecule
- anion
Fig. 26. 7,8-Dihydro-4,6-dihydroxypteridine

- neutral molecule
- anion

Fig. 27. 5,6-Dihydro-4,7-dihydroxypteridine

- neutral molecule
- anion
Fig. 28. 7,8-Dihydro-2,4,6-trihydroxypteridine

- neutral molecule
- anion

Fig. 29. 5,6,7,8-Tetrahydro-2-hydroxypteridine

- neutral molecule
- cation
- anion
Fig. 30. 5,6,7,8-Tetrahydro-4-hydroxypteridine

- neutral molecule
- cation
- anion

Fig. 31. 4,5-Diaminopyrimidine

- neutral molecule
- cation
**Fig. 32.** 4-Amino-5-carboxymethylaminopyrimidine
- neutral molecule
- ion

**Fig. 33.** 4,5-Diamino-2-hydroxypyrimidine
- neutral molecule
- cation
- anion
Fig. 34. 4,5-Diamino-6-hydroxypyrimidine

- neutral molecule
- cation
- anion
Fig. 35. 5,6,7,8-Tetrahydro-4-hydroxypteridine obtained by synthesis

Fig. 36. Tetrahydro-4-hydroxypteridine obtained by reduction
Fig. 37. 5,6-Dihydro-4-hydroxypteridine

Fig. 38. x,y-Dihydro-4-hydroxypteridine
Fig. 39. 7,8-Dihydro-2,6-dihydroxypteridine obtained by synthesis

Fig. 40. Dihydro-2,6-dihydroxypteridine obtained by reduction
**Fig. 41.** 7,8-Dihydro-4,6-dihydroxypteridine obtained by synthesis

**Fig. 42.** Dihydro-4,6-dihydroxypteridine obtained by reduction
Fig. 43. 5,6-Dihydro-4,7-dihydroxypteridine obtained by synthesis

Fig. 44. Dihydro-4,7-dihydroxypteridine obtained by reduction
BIBLIOGRAPHY.

Albert (1952), Quart. Rev., 6, 197.
Angier, Boothe, Hutchings, Mowat, Semb, Stokstad, SubbaRow, Waller, Cosulich, Fahrenbach, Hultquist, Kuh, Northey, Seeger, Sickels and Smith (1945), Science, 102, 227.
Angier, Boothe, Hutchings, Mowat, Semb, Stokstad, SubbaRow, Waller, Cosulich, Fahrenbach, Hultquist, Kuh, Northey, Seeger, Sickels and Smith (1946), Science, 103, 667.
Elakley (1959), Biochem. J., 72, 707.
Boon, Jones (1950), Brit. Pat., 635,582.
Boon and Leigh (1952), Brit. Pat., 677,342.
Futterman (1957), J. Biol. Chem., 228, 1031.
Gabriel and Jansen (1890), Ber., 23, 2807.
Gabriel and Stelzner (1896), Ber., 29, 1300.
Gabriel (1907), Ber., 40, 2647.
Hutton and Westheimer (1958), Tetrahedron, 3, 73.
Johns (1911), Amer. Chem. J., 45, 79.
Jones (1948), Nature, 162, 524.
Korte (1954), Ber., 87, 1062.
Kuhn and Cook (1937), Ber., 70, 761.


Pfleiderer (1957), *Ber.*, 90, 2604.

Pfleiderer (1959), *Ber.*, 92, 3190.


Purrmann (1940a), *Annalen*, 546, 98.
Purrmann (1940b), *Annalen*, 544, 182.
Purrmann (1941), *Annalen*, 548, 284.
Reese (1887), *Annalen*, 242, 1.
Schöpf, Reichert and Riefstahl (1941), *Annalen*, 548, 82.
Söderbaum and Widman (1889), *Ber.*, 22, 1665.
Totter (1944), *J. Biol. Chem.*, 154, 105.
Traube (1904), *Annalen*, 331, 64.
Traube and Nithak (1906), *Ber.*, 39, 227.
Tschesche and Korte (1951), *Ber.*, 84, 801.

Wieland and Schönf (1925), *Ber.*, 58B, 2178.

Wieland, Tartter and Purrmann (1940), *Annalen*, 545, 209.

Wieland and Liebig (1944), *Annalen*, 555, 146.

