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ADDITION REACTIONS OF
7, 8-DIHYDROPTERIDINES AND THE BIOSYNTHESIS
OF XANTHOPTERIN

ALEXANDER STUART

AUGUST 1962
ERRATA

(i) Page 25 line 3, for 'observer' read 'observed'.
(ii) Page 43 line 9, for '(CV)' read '2-amino-4,6-dihydroxy-5-nitropyrimidine'.
(iii) Page 56 line 4, for '(XCVIII)' read '(XCIII)'.
(iv) Page 65 line 4, for 'evidence' read 'existence'.
(v) Page 85 line 19, for '292 μμ' read '275 μμ'.
(vi) Page 88 line 6, for '(XClC)' read '(XClX)'.
(vii) Page 126 line 19, for 'C₁₃H₁₉N₅O₅' read 'C₁₃H₁₉N₅O₅ . H₂O'.

A Thesis

submitted to

THE UNIVERSITY OF GLASGOW

in fulfilment of the
requirements for the

DEGREE OF DOCTOR OF PHILOSOPHY

by

ALEXANDER STUART

August, 1962.
ACKNOWLEDGEMENTS

The author takes this opportunity of expressing his sincere gratitude to Dr. H. C. S. Wood for his inspired guidance and supervision of this work. He also wishes to thank Professor P. L. Pauson for laboratory facilities.

The author gratefully acknowledges the award of a maintenance grant from the Department of Scientific and Industrial Research.
OBJECTIVES

Current interest in reduced pteridines and their possible role in pteridine biosynthesis has focused attention on their chemical behaviour. This thesis describes the unambiguous synthesis of a simple 7,8-dihydropteridine, and an investigation of its reaction with a number of simple reagents.

Theoretical considerations dealing with the biosynthesis of xanthopterin have been examined and the chemical feasibility of such hypotheses has been studied.
SUMMARY

The addition reactions undergone by double bonds in certain heterocyclic compounds are reviewed.

The formation of 2,6-diamino-4-hydroxypteridine by reoxidation of 2-amino-4-hydroxy-5,6,7,8-tetrahydropteridine in the presence of ammonia is discussed with reference to two possible dihydropteridine intermediates. These intermediates could be considered to be sufficiently reactive to add ammonia followed by oxidation of the addition product to give 2,6-diamino-4-hydroxypteridine.

The unambiguous synthesis of one of these dihydropteridines, 2-amino-4-hydroxy-7,8-dihydropteridine, is outlined and its reaction with ammonia has been investigated. This revealed the ready addition of ammonia to give a tetrahydropteridine derivative which on oxidation gave 2,6-diamino-4-hydroxypteridine, thus confirming the above theory. The structure of this compound was confirmed by an unambiguous synthesis. This led to a new synthesis of 7,8-dihydroxanthopterin which involved an interesting modification of existing methods.

Spectral studies of the dihydropteridines and their oxidation products, together with comparisons with related pyrimidines, gave rise to a relationship between the structure of a dihydropteridine and its ultraviolet spectra.

The addition reactions undergone by 2-amino-4-hydroxy-
7,8-dihydropteridine and a number of simple reagents have been investigated, and are found to give rise to a variety of 6-substituted pteridines.

The addition of hydrogen cyanide gave a tetrahydropteridine derivative which, on oxidation, gave a dihydropteridine whose ultraviolet spectra indicated the possible existence of a new chromophore in pteridine chemistry. Oxidation of this compound gave 2-amino-4-hydroxypteridine-6-carboxylic acid.

The addition of hydrogen sulphite led to the formation of 2-amino-4-hydroxypteridine-6-sulphonic acid.

The addition of Michael-type reagents proceeded smoothly to give tetrahydropteridine derivatives which, on oxidation, gave 2-amino-4-hydroxypteridine-6-carboxylic acid. Thiols were found to add particularly easily to give tetrahydropteridines.

Alkali added only with difficulty giving xanthopterin after oxidation.

Current theories of the biosynthesis of xanthopterin have suggested that \(1-[2', 5'-diamo-no-6'-hydroxy-4'-pyrimidinyl-amino]-1-deoxy-D-erythropentulose\) is a key intermediate. This material has been synthesised and its conversion via 7,8-dihydroxanthopterin to xanthopterin has been achieved, thus furnishing chemical support for the above theories.
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INTRODUCTION
PART I

ADDITION REACTIONS OF DOUBLE BONDS IN HETEROCYCLIC SYSTEMS
By far the greatest number of additions to heterocyclic double bonds is to be found in that class of compound known as heterocaromatics. This is such the largest and most varied class of heterocyclic compounds and it has been subdivided into $\pi$-electron deficient types (e.g., pyridine) and $\pi$-electron excessive (e.g., pyrrole).

The $\pi$-electron deficient types are those which would be expected to undergo addition reactions more readily because the ring nitrogen atom in such molecules can attract electrons from the $\pi$-double layer. This has the effect of weakening or destroying the aromatic structure leaving the double bonds relatively more reactive. Examples are more common when more than one ring nitrogen atom is present or when the nitrogen atom has its electron-attracting properties increased e.g. by the presence of a nitro-group, or by salt formation. Reactivity can also be induced, especially in the pyrimidines and pteridines, by the presence of substituents in the ring (e.g., hydroxyl-groups) which can undergo tautomerisation involving a ring nitrogen atom, thus upsetting the aromatic system and conferring a relative degree of reactivity on the double bonds in the ring.

The reagents which are involved in additions of this nature are those which add to ethylenic bonds conjugated with
a carbonyl group and to carbonyl groups themselves, i.e. water, hydrogen cyanide, sodium bisulphite, ammonia, alcohols, thiols and carbanions derived from Michael-type reagents e.g., diethylmalonate, ethylacetoacetate, ethlycyanacacetate, acetone and acetylacetone.

Examples of this type of addition reaction in various heterocyclic series are discussed below.

Quinoxalines.

Bergstrom and Ogg, in their studies of quinoxaline (I) chemistry, found that this compound reacted as an 'ammino-glyoxal' in that it added two molecules of sodium bisulphite in the cold to give (II; R = SO$_3$Na), while, on heating for two hours at 20° with hydrogen cyanide in a bomb, it gave (II; R = CN).

In this department, Cresswell, Hill and Wood, during a study of isocalloxazine syntheses, prepared a quinoxaline derivative, methyl-3,4-dihydro-4-methyl-3-oxo-quinoxaline-2-carboxylate (III) which, on treatment with guanidine or urea in
the presence of sodium methoxide, was found to yield a spiro-
quinoxaline (IV; R = O, NH). A possible explanation of this

![Chemical Structures](image)

observation is that the reaction proceeds via an intermediate
(V; R = O, NH). Addition of the terminal amino-group in the
side-chain across the 1,2-double bond of the quinoxaline ring
would give the spiro-quinoxaline (IV). The reactivity of this
double bond would be enhanced by the cyclic amide at positions
3 and 4.

**Acridines.**

Additions of bisulphite and cyanide ion to the acridine
molecule (VI) to give products of the type (VII; R = SO₃Na, CN)
have been described by Albert. It was assumed that addition
takes place across the central ring followed by a mild oxidative step.

More recently, Kröhnke and Honig have reported a number of additions to acridine using Michael-type reagents. Resonance phenomena in acridine can lead to an electrophilic centre at C₆ and a nucleophilic centre at N₁₀ (VIa). Reaction with active-methylene compounds therefore involves the initial formation of a carbanion followed by its incorporation at the 5 position to give a 5,10-dihydro-acridine derivative (VIII). The addition products from nitromethane (VIII; R = H, R' = NO₂), malondinitrile (VIII; R = R' = CN) and desoxybenzoin (VIII; R = C₆H₅, R' = COC₆H₅) were obtained by standing a concentrated solution of acridine in ethanol with the appropriate reagent when the adducts separated in good yields. The products from acetylacetone (VIII; R = R' = COCH₃), ethylacetoacetate (VIII; R = COOC₂H₅, R' = COCH₃), ethylbenzoylacetae (VIII; R = COOC₂H₅, R' = COC₆H₅), ethylcyanooacetate (VIII; R = COOC₂H₅, R' = COC₆H₅),
R' = CN) and diethylmalonate (VIII; R = R' = COOC₂H₅), formed similarly, but were obtained on removing the solvent. The addition products, with the exception of the nitromethane adduct, were found to be colourless, unstable compounds. The nitromethane adduct, however, was yellow and reasonably stable and this was attributed to chelation between the nitro-group and the secondary amino-group at the 10 position. Oxidation of these compounds was carried out using lead tetra-acetate in benzene to yield the fully aromatic compounds (IX), the identity of which was established in each case by condensing 5-chloroacridine (X) with the sodium salt of the appropriate Michael reagent.

![Chemical structures](image)

**Pyrimidines**

Uracil (2,4-dioxo-1,2,3,4-tetrahydropyrimidines, XI) in neutral solution exists predominantly in its cyclic amide form with the consequent degree of reactivity of the 5,6-double bond which assumes the character of a polarised double bond. This reactivity can be enhanced by the introduction of a nitro-group.
and Johnson has described the addition of hypobromous acid to
5-nitro-uracil (XII) to give (XIV). An identical product was
obtained by the addition of nitric acid to 5-bromo-uracil
(XIII). Irradiation experiments on uracil caused it to
add water giving (XV), while a study of the less aromatic
material 1,3-dimethyluracil (XVI) showed that it also underwent
the addition of the elements of water across the 5,6-double
bond when irradiated in aqueous solution, giving (XVII).

**Pyridine Quaternary Salts.**

A number of addition reactions of the coenzyme
diphosphopyridine nucleotide (D.P.N.; XVIII) have been studied
e.g. with alkali, cyanide, bisulphite, dihydroxy-
acetone, sodium dithionite, hydroxylamine, and thiols.
The above reagents are nucleophilic in character and are therefore expected to attack electrophilic centres. In the case of the additions of cyanide and dithionite, it has been established \(^{17\text{a},14}\) that this takes place at the para-position. The addition of a number of carbonyl compounds has also been studied\(^3\), and the results have indicated that the ease of reaction depends on the ability of the carbonyl moiety to form a carbanion in the presence of hydroxyl ions. It was thus generally assumed that reaction took place via the quinonoid structure (XIX) which underwent nucleophilic attack at the para-position to give the addition product (XX).

**Heterocyclic Ethers**

Parham\(^1\) and his co-workers have initiated a study of the reactions of a number of heterocyclic vinyl ethers (XXI - XXIV). In their investigations of the properties of the first member of this series p-oxathione (XXI), they found...
it to undergo additions of unsymmetrical reagents such as water and methanol in the presence of acids. Thus p-oxathione, when cooled to 0° and treated with cold methanol containing a catalytic quantity of dry hydrogen chloride, gave the acetal \((XXV)\) in 87% yield.

![XXV](image)

This type of addition reaction has supplied valuable information regarding the relative ability of oxygen and sulphur to release electrons in the direction of their covalent bonds, and the inference that oxygen possesses a greater ability to do so is in keeping with the conclusions of earlier workers.19

**Pteridines**

In more recent years, a number of pteridines have furnished examples of addition reactions, and, of particular interest, has been their ability to hydrate and the subsequent effects on their physical and spectral properties.
Pteridine.

Pteridine (XXVI), the parent compound of this series, appears to add water covalently. Although it has no ionisable hydrogen, it behaves as a weak acid (pKa 12.2) on titration with alkali and Albert has attributed this to covalent addition of the elements of water across the 3,4-double bond. The anion formed on subsequent loss of a proton would then have structure (XXVII), and would be stabilised by hydrogen bonding of the hydroxyl group and by the resonance arising from distribution of the negative charge between $N_1$ and $N_2$.

Perrin has recently made a detailed study of this hydration phenomenon and has shown by potentiometric and rapid-flow spectrophotometric methods that dissolution of pteridine in water leads to an equilibrium involving covalent hydration.

From spectral studies, it was found that pteridine, when dissolved in water and left for 10 hours at 20°, underwent a reversible addition of water across the 3,4-double bond to form the hydrated species 3,4-dihydro-4-hydroxypteridine (XXVIII) with
an equilibrium ratio of 3.5 to 1 in favour of the anhydrous species. On the other hand, when pteridine was dissolved in 0.01M-hydrochloric acid and neutralised within several minutes, the hydrate was formed quantitatively. This material, when oxidised with hydrogen peroxide or potassium permanganate, gave 4-hydroxypteridine (XXIX) in good yield.

![XXIX](image)

Acidification to pH 2 of a neutral solution of pteridine led to the formation of the cation of the hydrate. Neutralisation of this solution gave the hydrated material and not pteridine. Finally, acidification of pteridine hydrate gave a solution whose spectrum was the same as that of the cation.

Similarly, pteridine, dissolved in 0.5M-sodium hydroxide, underwent spectral changes which were attributed to the formation of the anion (XXVII), already referred to by Albert.

The spectrum of a solution of the cation in 0.01 M-hydrochloric acid was found to change steadily during 2–3 hr, thereafter remaining stable. Neutralisation gave a material which was slowly transformed into a mixture of pteridine and its hydrate. This material contained an aldehyde grouping since
it gave the characteristic colour reaction with benzidine, and this evidence, together with its ready conversion to pteridine, indicated that it was the amidine aldehyde (XXX). In addition,

its ultraviolet spectrum closely resembled that of an authentic sample of the oxime of the aldehyde (XXX).

The fact that hydration is an intermediate stage in the ring opening process is a further indication that the hydrate has structure (XXVIII).

Methylpteridines.

Examination of 2-methyl-(XXXI) and 7-methylpteridines (XXXII) yielded similar results. In the case of 4-methylpteridine (XXXIII), although reversible ring-fission was found
to occur, the hydration equilibrium was found to lie too far on the side of the anhydrous species for the existence of the hydrate to be demonstrated.

**Hydroxypteridines.**

Albert et al. in 1952 referred to an abnormal hysteresis effect observed during titration of 6-hydroxypteridine (XXXIV). Addition of alkali to an aqueous solution of 6-hydroxypteridine gave pH values which were much higher than those found on back-titrating with acid. Albert attributed this phenomenon to tautomeric shifts in the molecule and suggested two possibilities, (XXXIVa) and (XXXIVb). However, only structure (XXXIVb) was considered, since it involved prototropy between oxygen and carbon which was considered to be slow enough to be detected by physical methods. However, the existence of an active methylene grouping at position 7 was questioned, since 6-hydroxypteridine could not be nitrated, nitrosated or brominated, nor could it be condensed with aldehydes.

It was reported however, that the compound obtained from a
solution of 6-hydroxypteridine in aqueous acid, analysed for a monohydrate from which water could not be readily removed, that its ultraviolet spectrum was closely similar to that of 6-hydroxy-7,8-dihydropteroxide (XXXV) and that, on oxidation with hydrogen peroxide, it gave 6,7-dihydroxypteridine (XXXVI).

![XXXV](image1) ![XXXVI](image2) ![XXXVII](image3)

7-Hydroxypteridine (XXXVII) was also studied, but was found to exhibit no abnormal hysteresis effects.

Brown and Mason, continuing this work, made a study of the four possible monohydraxoxypteridines and divided them into two categories on the basis of their ability to hydrate.

2-Hydroxypteridine (XXXVIII) and 6-hydroxypteridine (XXXIV) were found to retain a molecule of water even when subjected to a temperature of 110°. It was suggested that this was water of constitution and that, in fact, it had added across the 3,4- and 7,8-double bonds respectively of the two isomers to give the structures (XXXIX) and (XL). In contrast to the neutral molecules, the sodium salts of these isomers were found to be anhydrous, since
the ultraviolet spectra resembled the spectra of the neutral molecules of the corresponding mono-aminopteridines. The ultraviolet spectra of the neutral molecules however, had an unusually low long-wavelength band attributable to the loss of a double bond as a result of hydration. These compounds also displayed stronger basic properties than the 4-hydroxy-(XLI) and 7-hydroxy-(XXXVII) isomers, which is also consistent with the
hydrated structures. In addition, 2-hydroxypteridine hydrate, on treatment with potassium permanganate at 20°, gave 2,4-dihydroxypteridine (XLIX) in good yield, while 6-hydroxypteridinehydrate yielded 6,7-dihydroxypteridine (XXXVI) under the same conditions, thus further confirming the proposed structures. The 7,8-double bond of 6-hydroxypteridine was found to be generally susceptible to attack. Albert found that with sodium amalgam, 6-hydroxy-7,8-dihydropteridine (XXXV) was formed and, later, that hydroxylamine added across this double bond with subsequent oxidation to produce 7-amino-6-hydroxypteridine (XLIII).

4-Hydroxypteridine (XLI) and 7-hydroxypteridine (XXXVII) on the other hand, exhibited stronger acidic properties and behaved as typical tetra-azanaphthalenes with an hydroxyl group a or γ to a ring nitrogen atom. The neutral molecules possessed a three-banded ultraviolet absorption spectrum with the long-wavelength band just above 300 μμ typical of other bicyclic aromatic compounds. On anion formation, bathochromic shifts of 23 μμ were observed in contrast to 68 μμ for the 2- and 6- isomers. In addition, they were unattacked by potassium permanganate.

Albert and Reich extended the study of 6-hydroxypteridine and further confirmed its hydrated structure by synthesizing 6-hydroxy-7-methylpteridine (XLIV) and showing that hydration
of the 7,8-double bond was hindered sterically by the 7-methyl-group.

\[
\text{XLIV} \quad \text{XLV} \quad \text{XLVI}
\]

In this respect, an analogy existed with xanthopterin (2-amino-4,6-dihydroxypteridine, XLIV), in that a comparison of the ultraviolet absorption spectra of xanthopterin and 7-methylxanthopterin (XLVI) at pH 4, where both substances were present as neutral molecules, showed that the long-wavelength peak of 7-methylxanthopterin at 378 \(\text{nm}\) had exactly twice the height of the corresponding peak of equilibrated xanthopterin (385 \(\text{nm}\)). This appeared to corroborate Schou's \(^{38}\) observation that xanthopterin is, under these conditions, a mixture of equal amounts of two related substances, and Albert \(^{27}\) postulated that the peak at 385 \(\text{nm}\) in equilibrated xanthopterin was due to the anhydrous form (XLIV), while the shoulder at 305 \(\text{nm}\) was attributed to the 7,8-hydrate (XLVII). From ultraviolet spectral studies of the neutral molecule and the cation of 6-hydroxy-7-methylpteridine, it was estimated that the equilibrated neutral
molecule existed with a hydrate in a 1:1 ratio (this compared with 1:100 for 6-hydroxypteridine), and that the existence of a 7-methyl-grouping affected hydration.

From these results, it might be expected that the presence of a 4-methyl-grouping in 2-hydroxypteridine would exhibit a similar steric effect on hydration across the 3,4-double bond, but there is not experimental evidence available as yet. Albert also reported the addition of weak acids other than water to the 7,8-double bond of 6-hydroxypteridine. Earlier work had shown the susceptibility of this double bond to additive attack and it was now suggested that weak acids such as those used in Michael condensations could form carbanions which would attack the electrophilic centres of polarised carbon-nitrogen double bonds. The additions of such reagents have already been discussed with reference to the acridine molecule and pyridine quaternary salts.
6-Hydroxypteridine (XXXIV), in cold alkaline solution, was found to react readily with acetone, diethylmalonate and ethylcyanoacetate, the carbanion adding at C7 and the mobile hydrogen at N6 to give compounds of type (XLVIII). These adducts had

![Image](image)

similar ultraviolet spectra and ionisation constants to 6-hydroxy-7,8-dihydropteridine (XXXV). On heating with Na-sodium hydroxide solution, hydrolysis of the side-chain at the 7-position took place and, on oxidation, 6,7-dihydroxypteridine (XXXVI) was formed.

Brown and Mason also found that the ability of the monohydroxypteridines to hydrate was reflected in their N-methyl derivatives. Those derived from 2-hydroxypteridine and 6-hydroxypteridine retained a molecule of water tenaciously, whereas those derived from 4-hydroxypteridine and 7-hydroxypteridine did not exhibit this property. Thus 1-methyl (XLIX) and 3-methyl (L) derivatives of 2-hydroxypteridine could not be dehydrated without fundamental change and 6,7,8-trimethyl-2-pteridone (LI),

![Images](image)
the closest analogy to an 8-methyl derivative, only became anhydrous at 120° in vacuo with rehydration in air at room temperature.

Their formal structures indicated no acidic groupings or N-H linkages, yet they could be dissolved in alkali and reprecipitated unchanged by acid. In carbon-tetrachloride or chloroform solution, they showed a band in the infrared due to an N-H stretching vibration which persisted when the molecule of water was exchanged for alcohol by crystallisation from that solvent. The hydrates, but not the alcoholates, also showed a weak band in the O-H stretching vibration region. On dehydration, this N-H stretching vibration was much reduced in intensity. Moreover, the ultraviolet spectra of the 1- and 3-methyl isomers showed a striking similarity to that of 2-hydroxypteridine hydrate (XXXIX) and, since chemical tests had excluded structures in which ring fission had occurred, Brown and Mason concluded that, on balance, the hydrated N-methyl derivatives were best represented by (LIII).

For valency reasons, anhydrous 6-hydroxypteridine can form only one N-methyl derivative, the 5-methyl (LIII). The method of preparation involved condensation of 4-amino-5-methylamino-pyrimidine (LIV) with ethylglyoxalate hemiacetal and gave rise to
two isomeric products, one from condensation in neutral solution, the other by carrying out the reaction in dilute mineral acid. Both compounds were acidic, analysed for monohydrates and, on the basis of chemical tests, the possibility of ring-opened structures was excluded. The compound formed under neutral conditions had an ultraviolet spectrum similar to that of 7-hydroxy-5,6-dihydropertidine (IV) and hence was represented by (IVI). The compound formed under acid conditions had, in its neutral and cationic forms, an ultraviolet spectrum almost identical with that of 6-hydroxypteridine hydrate (X), and hence was represented by (LVII). Both compounds, on oxidation with potassium permanganate at 20°, gave 5-methyl-6,7-dioxo-5,6,7,8-tetrahydropertidine (LVIII), prepared by an independent method. The balance of evidence thus indicated that in the 5-methyl derivative of 6-hydroxypteridine, hydration took place across the 7,8-double bond as in the parent 6-hydroxypteridine.

The hydrated structures proposed for 2- and 6-hydroxypteridines and their N-methyl derivatives explain the abnormal hysteresis effects
referred to by Albert and Brown and are consistent with the decrease in acidity usually associated with the fully aromatic structures.

![Chemical structures](image)

**LXVII**  **XXXVIII**  **XXXIX**  

An explanation for the existence of these hydrates can be approached in two ways:

- either (a) the double bond in question can be shown to possess a certain degree of reactivity (i.e. polarisability) which may be due to the medium in which it exists or the influence of neighbouring substituents,

- or (b) the hydrated species may be shown to have a structure stabilised by one or more resonance forms.

In the case of 2-hydroxypteridine (XXXVIII) both these arguments may be invoked. Thus the electron density at C₄ would be expected to be reduced (i) by conjugation with the amide carbonyl group at the 2-position, and (ii) by the proximity of the electron-withdrawing pyrazine ring. An alternative explanation would be that the hydrated structure (XXXIX) is stabilised by a type
of resonance found in urea and its derivatives. Both these effects are inoperative in 4-hydroxypteridine.

The hydration of 6-hydroxypteridine and its derivatives has been suggested by Albert\(^2\) to be due to the 4-aminopyridine type of resonance exhibited by the hydrated structure (XL).

![Chemical structure](attachment:image.png)

\[\text{XL}_a \leftrightarrow \text{XL}_b\]

This transannular effect may explain why 7-hydroxypteridine does not hydrate. The anhydrous molecule (XXXVII) might be expected to possess this type of resonance without hydration across the 5,6-double bond. Thus hydration would produce a less

![Chemical structure](attachment:image.png)

\[\text{XXXVII}_a \leftrightarrow \text{XXXVII}_b\]

stabilised structure, since it would have destroyed the 5,6-double bond which forms part of the transannular conjugated system.

The validity of such transannular effects might be checked by a study of the hydration of dihydroxypteridines with one oxygen
function in a position favourable to hydration and the other
hindering it e.g. 4,6-dihydroxypteridine (LIX), in which the
carbonyl group at position 4 might be expected to inhibit the
formation of the resonance structures on which the stability of

the 6-hydroxypteridine hydrate is thought to depend. Consequently,
if transannular conjugation structures are involved in the stability
of 6-hydroxypteridine hydrate, a carbonyl grouping at C₄ should
decrease or exclude hydration across the 7,8-double bond. Also
2,7-dihydroxypteridine (LIX) might also be expected to remain
predominantly anhydrous since the double bond character of the bond
between N₃ and C₄ is reduced by the transannular conjugation with
the carbonyl grouping at the 7-position.

In the light of the above reasoning, cation formation might
be expected to enhance hydration,

(a) since it could favourably influence the polarisation of a
double bond, and

(b) since it could further promote the transannular conjugation
effects already referred to.
This is borne out by fact. Brown and Mason\textsuperscript{23} postulated the existence of a hydrated cation of 6-hydroxypteridine and its existence was later confirmed by Albert\textsuperscript{27}. In addition, Albert found that while a methyl-grouping in the 7-position was sufficient to sterically hinder hydration in the neutral molecule, nevertheless, the cation of 6-hydroxy-7-methylpteridine was found to exist predominantly in the hydrated form, and a favourable comparison was made between its ultraviolet spectrum and ionisation constants and those of the cation of 6-hydroxypteridine.

Cation formation was suggested by Albert to involve protonation of $\mathbb{N}_3$ in 6-hydroxypteridine which would be expected to enhance the formation of the 4-aminopyridine type of resonance structure.

\begin{center}
\begin{tikzpicture}
\node [label=above:6] (a) at (0,0) {\textbf{IXIIa}};
\node [label=above:6] (b) at (2,0) {\textbf{IXIIb}};
\node [label=above:6] (c) at (1,0.5) {\textbf{IXIIc}};
\node [label=above:6] (d) at (1,-0.5) {\textbf{IXIID}};
\draw (a) -- (b);
\end{tikzpicture}
\end{center}

Quinazolines.

The existence of hydrated cations was extended by Albert\textsuperscript{29} to other heterocyclic systems. It was pointed out that while pyridazine (LXIII) and cinnoline (LXIII) had comparable basic strengths and that of pyrazine (LXIV) and quinoxaline (I) were of the same order, nevertheless, the $pK_a$'s of pyrimidine (LXV, 1.3)
and quinazoline (LXVI, 3.5) were significantly different, i.e., quinazoline appeared to be an anomalously strong base. 4-
Substituted quinazolines, however, compared favourably with 4-substituted pyrimidines. In addition, from a study of the ultraviolet spectra of quinazoline and 4-methyl-quinazoline (LXVII), it was found that in dilute aqueous acid solutions,

\[
\begin{align*}
\text{LXV} & \quad \text{LXVI} & \quad \text{LXVII}
\end{align*}
\]

quinazoline formed a cation radically different from that formed by 4-methyl-quinazoline. This phenomenon had been observed earlier by Short, who had suggested that the anomaly could be explained in terms of the addition of water to the 3,4-double bond of the normal quinazoline cation (LXVIII), to give the abnormal species
(LXXI), which would be stabilised by an amidinium-type resonance.

The abnormality in the ultraviolet spectrum was shown by Albert\textsuperscript{39} to be due to water by determining the spectra in anhydrous dichloroacetic acid. In this solvent, quinazolone gave a normal cation whose spectrum closely resembled that of 4-methylquinazolone cation. This theory was confirmed by Albert\textsuperscript{39}, who observed that oxidation of quinazolone in 2\textsubscript{1}H-sulphuric acid (in which 98\% is present as the abnormal cation) by hydrogen peroxide or chromic acid at 20° gave a high yield of 4-hydroxyquinazolone (LIX).

Moreover, a study of the ultraviolet spectra of a number of 4-substituted quinazolines indicated that these gave mainly normal cations, although electron-withdrawing substituents would have been expected to enhance the possibilities of hydration.
Hence steric factors were inferred to predominate in resistance to hydration and support for the theory of hydration at the 3,4-double bond was strengthened.

Short reported the existence of several 3-methyl-quinazolinium derivatives containing a very firmly-held alcohol molecule which was thought to be covalently bound. Albert also reported the existence of two different hydrochlorides of quinazoline, one formed from the other by absorption of one molecule of water. The hydrated species was the more stable compound and withstood dehydration in vacuo at 60°C. Its infrared spectrum indicated that it contained covalently bound water since it showed extra bands at 1474 and 1240 cm⁻¹ which Albert attributed to CH and OH bonding vibrations of the CHOH group in (LXIX).

By a study of quinazoline in sulphuric acid-water mixtures, it was found that the normal anhydrous cation (LXVIII) of quinazoline could exist, but that the abnormal cation was the energetically-preferred species in dilute aqueous solution.

The position at which hydration took place was not fully established, since a comparison of the ultraviolet spectra of the abnormal cation (LXIX) and 3,4-dihydroquinazoline cation (LXXX) showed a hypsochromic shift of 20 μm on replacing H by OH at position 4 in the quinazoline molecule.
In a later paper, however, Albert showed that a shift of this magnitude was significant in quinazoline derivatives. A study of the ultraviolet spectra of 4-hydroxy-3-methyl-3,4-dihydroquinazoline (LXXII; \( R = \text{CH}_3, \ X = \text{OH} \)) in water and cyclohexane showed no complications apart from a solvent shift of 4 \( \mu \text{m} \). A comparison with 3-methyl-3,4-dihydroquinazoline (LXXII; \( R = \text{CH}_3, \ X = \text{H} \)) showed a hypsochromic shift of 28 \( \mu \text{m} \) on replacement of \( \text{H} \) by \( \text{OH} \) at position 4.

In addition, Albert found, from ultraviolet spectroscopic studies using a rapid-flow technique, that quinazoline formed a short-lived neutral hydrated species (LXXII; \( R = \text{H}, \ X = \text{OH} \)). This species had a half-life of 9 seconds and therefore could not be isolated. A comparison of the ultraviolet spectrum of this short-lived abnormal neutral species with that of 3,4-dihydroquinazoline (LXXII; \( R = X = \text{H} \)) showed a similar shift of 26 \( \mu \text{m} \), which strongly suggested that the compound had structure (LXXII; \( R = \text{H}, \ X = \text{OH} \)).

On the other hand, the product from the addition of alkali to 3-methylquinazolinium iodide was isolated and gave an analysis for a hydrated 3-methylquinazoline (LXXII; \( R = \text{CH}_3, \ X = \text{OH} \)). The ring-fission product (LXXIII; \( R = \text{CH}_3 \)) was ruled out since the infrared spectrum of the compound showed no carbonyl
peak typical of an 2-aminobenzaldehyde derivative. It did, however, give a band at 3570 cm\(^{-1}\) which Albert attributed to unassociated C-H stretching which indicated that the compound did indeed have structure (LXXIII, \(R = \text{CH}_3, X = \text{OH}\)).

\[
\text{LXXIII}
\]

Armarego\textsuperscript{36} earlier this year published the results of a study of the effect on hydration of simple substituents in the benzene ring of quinazoline. For purposes of comparison, the ultraviolet spectrum of the quinazoline cation was taken as typical of an almost completely hydrated cation, while that of 4-methylquinazoline cation was taken as a typically anhydrous cation. Thus, the spectrum of a quinazoline whose cation contained both anhydrous and hydrated species, should show features of both the typical spectra. The ratio of hydrated cations to anhydrous cations was calculated from the extinction coefficients.

Methyl substituents in any of the four positions of the benzene ring produced an inhibiting effect on hydration, since an examination of the spectra of the cations indicated mixtures of
anhydrous and hydrated cations. The four possible chloroquinazoline cations were almost completely hydrated. The methoxy- and hydroxy-derivatives gave mixtures of hydrated and anhydrous cations, except in the cases of 7-methoxy and 7-hydroxyquinazoline cations which were anhydrous. The failure of these derivatives to form hydrated cations was explained in terms of the following mesomeric structures (LXXIV, LXXV; \( R = H \) or \( CH_3 \)).

These effects are inoperative in \( \pi \)-quinonoid systems.

Perrin has made a study of the hysteresis effects already referred to by Albert and Brown and has extended these studies to cover several 2-hydroxy and 6-hydroxypteridine derivatives. In addition, 2-hydroxy-1,3,8-triazanaphthalene (LXXVI) and 1,4,6-
triazanaphthalene (LXXVII, as cation) were studied, and were found to exhibit similar hysteresis curves, thus indicating that hydration phenomena may be a feature of heterocyclic systems other than quinazolines and pteridines.

![Diagram of chemical structures](image-url)
PART II

THE BIOSYNTHESIS OF XANTHOPTERIN
AND LEUCOPTERIN
The structural similarity of the purine (LXXVIII), diamino-
pyrimidine (LXXX) and pteridine (LXXI) molecules has led to the idea
that a biological relationship might exist between these groups of
compounds.

Albert suggested that purines or their derived nucleosides
(LXXVIII; R = glycosyl) were biosynthetic precursors of pteridines.
He suggested that the imidazole ring of a purine could undergo
ring-opening with loss of C₈ as a one-carbon fragment to give a
diaminopyrimidine, and subsequent condensation of the latter compound
with a two-carbon fragment, to give a pteridine. Albert provided
evidence in support of this theory by demonstrating the transformation
in vitro of 2-hydroxypurine (LXXXI) to 2-hydroxypteridine (LXXXII).
In this example, C₈ of the purine is lost as a one-carbon fragment
and C₈ and C₉ of the pteridine are formed from condensation with a
two-carbon fragment. It is possible however, to visualise a simple
variation of this theory, in which a diaminopyrimidine (LXXIX).
R = CH₂COX) might undergo an intramolecular condensation to form a 7,8-dihydropteridine derivative (LXXXIII) which could then oxidise to a fully aromatic pteridine.

Biochemical evidence furnished by Ziegler-Günder⁸⁹ and by Weygand⁴⁰ supported the theory that diaminopyrimidines were possible intermediates in the biosynthesis of pteridines.

Ziegler-Günder injected the larvae of the amphibian Xenopus intradermally with guanine [2⁻¹⁴C] (LXXXIV; R = H).

Ten days later, the skins of the sacrificed larvae were extracted with N-ammonia solution at 20°, and chromatographed on paper. This showed a number of blue fluorescent spots, one of which, on oxidation, was converted to labelled 2-amino-4-hydroxypteridines-
-6-carboxylic acid (LXXXV).

Weigand\textsuperscript{40} administered 2,4,5-triamino-6-hydroxypyrimidine $[2^{-14}C]$ (LXXXVI) to pierid caterpillars in their diet, and later isolated labelled xanthopterin (XLIV) from the wings of the adult butterflies.

\[ \text{XLIV} \]

Information regarding the source of C$_6$ and C$_7$ in xanthopterin and leucopterin (LXXXVII), formed in the wings of the cabbage butterfly (Pieris brassicae), was obtained by Weigand\textsuperscript{42} by injecting 3-4 day old cocoons with glucose $[1^{-14}C]$. It was found that 53% of the radioactivity resided in C$_6$ and C$_7$ of the resulting leucopterin.

\[ \text{LXXXVII} \]

On repeating\textsuperscript{42} the experiment with glucose $[2^{-14}C]$, a label recovery of 78% was reported at positions 6 and 7 in the
leucopterin formed. It was thus postulated that a ribose fragment with a labelled carbon in position 1 could possibly contribute to the radioactivity at C₆ and C₇, since it was known that glucose-6-phosphate easily underwent an oxidative decarboxylation with the loss of C₄ as CO₂. Thus, injection of ribose [l-¹⁴C] produced a label recovery of 64%.

In addition, Weygand has injected, in the early stages of development of the cabbage butterfly, a number of labelled compounds, such as glycine, formate etc., known to be precursors of guanine. He then estimated the label recovery and the activity of specific atoms of the guanine and leucopterin formed in the later stages of development.

On injection with glucose [2-¹⁴C] and glycine [2-¹⁴C], it was found, in both cases, that the specific activities of guanine and leucopterin were fairly similar, while, on injection with formate [¹⁴C], the absolute activity of C₂ in both compounds was almost identical.

These results were considered by Weygand to be indicative of guanine and leucopterin having some common intermediate as opposed to leucopterin being formed by an independent de novo synthesis.

This evidence, together with the knowledge that, early in the biosynthesis of purines, condensation takes place between a
ribose-5-phosphate and the purine precursor, enabled Weygand and Wood independently to postulate a general theory for the biosynthesis of xanthopterin and leucopterin.

It was suggested that ring-opening of the imidazole ring of guanosine occurred to give a 5-amino-4-ribosylaminopyrimidine (LXXXVIII), which then underwent an Amadori rearrangement (typical of glycosylamines) with the formation of a ketose (LXXXIX). Cyclisation of this ketose resulted in the formation of the polyhydroxyalkyltetrahydropteridine derivative (XCI), which could lose its polyhydroxy side chain in the form of glyceraldehyde or its 3-phosphate, and subsequently undergo an oxidative step to xanthopterin. Leucopterin was visualised as arising from covalent hydration across the 7,8-double bond of xanthopterin to produce the hydrate (XCI) which would further oxidise to
leucopterin. The general chemical feasibility of the hydration step in this theory has been dealt with in Part I of this thesis, and specific chemical support for it is to be found in the work of Schou$\textsuperscript{26}$ and the interpretations laid on it by Albert$\textsuperscript{27}$.

To furnish further chemical support for this theory of the biogenesis of xanthopterin is, in part, the purpose of this thesis and our results and their implications are recorded in the appropriate sections.

Some measure of corroboration has already been found in the work of Nawa$\textsuperscript{45}$ who proposed the structure (XCII) for Sepiapterin, a yellow pigment found in the eyes of the fruit-fly Drosophila melanogaster (sepia mutant). Oxidation in borax solution at 35\(^{\circ}\) in the dark for 5 hours produced 7,8-dihydroxanthopterin (XCIII) and lactic acid in a molar ratio of 1:1 with the absorption of 0.5 moles of oxygen. Nawa explained the formation of xanthopterin in terms of the absorption by Sepiapterin of two moles of water to form (XCIV) which would exist as a borate...
complex (typical of polyhydroxycompounds). Absorption of 0.5 moles of oxygen with the elimination of the side-chain as lactic acid would thus lead to the formation of 7,8-dihydroxanthoptorin.
PART I

ADDITION REACTIONS OF 2-AMINO-4-HYDROXY-7,8-DIHYDROPTERIDINE
Our interest in the chemistry of reduced pteridines and their possible role in the biosynthesis of fully-aromatic pteridines, was stimulated by Van Baalen and Forrest's report of the formation of 2,6-diamino-4-hydroxypteridine (XCVI, R = NH₂) from a reduced form of 2-amino-4-hydroxypteridine (XCV, R = H). These workers investigated the hydrogenation of 2-amino-4-hydroxypteridine using platinum and palladium catalysts, when two mol. of hydrogen were absorbed to give 2-amino-4-hydroxy-5,6,7,8-tetrahydropteridine (XCVI). When this product was reoxidised by manganese dioxide in the presence of ammonia without exposure to the air, they isolated, by chromatographic techniques, a yellow solid having a greenish-yellow fluorescence. From its elementary analysis and its quantitative conversion to xanthopterin (2-amino-4,6-dihydroxypteridine, XLV) on treatment with nitrous acid, it was considered to have the structure (XCVI, R = NH₂). When the reoxidation step was carried out in alkali, a small quantity of xanthopterin was detected. In both cases, 2-amino-4-hydroxypteridine was also recovered. Folic
acid (XCV; \( R = \text{CH}_2\text{NHCOH}_3\text{H}_2\text{COO}_{\text{H}} \)),
bioppterin (XCV; \( R = (\text{CHOH})_2\cdot\text{CH}_3 \)), and a number of other pteridines
were found to produce similar results.

The American workers suggested that 2,6-diamino-4-
hydroxypteridine and xanthopterin were formed via a reactive
dihydro derivative of 2-amino-4-hydroxypteridine which could
add ammonia or hydroxyl ions at the 6-position, and which would
subsequently oxidise to the fully aromatic products. Two such
intermediates were postulated, 2-amino-4-hydroxy-7,8-dihydro-
pteridine (XCVII), and 2-amino-4-hydroxy-5,8-dihydropteridine
(XCVIII). Addition of ammonia or water across the 5,6-double
bond of (XCVII) or the 6,7-double bond of (XCVIII) would give
the tetrahydro derivative (XCIX; \( R = \text{NH}_2, \text{OH} \)), which, on
subsequent oxidation, would give rise to the required fully
aromatic products (XCV; \( R = \text{NH}_2, \text{OH} \)).
Van Baalen and Forrest have also reported the isolation of 2,6-diamino-4-hydroxypteridine (XCV, R = NH₂) from Drosophila melanogaster and from the blue-green algae, Anacystis nidulans and Nostoc muscorum G. They suggest that this pteridine may be an artefact arising from a reactive 2-amino-4-hydroxydihydropteridine occurring in these organisms.

On chemical grounds, we believe that addition of nucleophilic reagents to the polarised C=N grouping of 2-amino-4-hydroxy-7,8-dihydropteridine (XCVII) is more likely than similar additions to the C=C grouping in the isomeric 5,6-dihydropteridine (XCVIII). Addition of nucleophilic reagents to a C=N grouping in heterocyclic compounds is well-known, and has been discussed earlier in the Introduction.

In this thesis, we report the unambiguous synthesis of the parent 7,8-dihydropteridine, 2-amino-4-hydroxy-7,8-dihydropteridine (XCVII). We have also investigated the reaction between this compound and ammonia, hydrogen cyanide, and a number of other simple reagents.

The synthesis was based on the method developed by Boon et al. for the unambiguous synthesis of 7,8-dihydropteridines. This consisted in condensing a pyrimidine having a reactive chloro group (C₁ R¹¹ = NO₂, PhN₂) with a suitable amine (CI) to give the pyrimidine (CII). Reduction of the nitro or phenylazo
group gives the unstable diaminopyrimidine (CIII), which then undergoes an intramolecular condensation to give a 7,8-dihydroppteridine (CIV).

2-Amino-4,6-dihydroxypyrimidine (CV) gave 2-amino-4,6-dichloropyrimidine (CVII) on refluxing with phosphoryl chloride, and, on subsequent hydrolysis with alkali, 2-amino-4-chloro-6-hydroxypyrimidine (CVII) was formed.
This compound was nitratèd by dissolving in concentrated sulphuric acid and adding fuming nitric acid at $< 45^\circ$. The product, 2-amino-4-chloro-6-hydroxy-5-nitropyrimidine (CVIII), was precipitated by pouring on to ice. The nitro group serves the dual function of activating the adjacent chloro group, and providing $N_3$ of the pteridine molecule. The nitro-chloropyrimidine was very reactive and condensed smoothly with a number of amines to give pyrimidines of the type (CIX). On treatment with alkali, the product was (CV).

![Chemical Structure](image)

As was expected, the introduction of a nitro grouping produced a bathochromic shift of some 16 nm in the ultraviolet spectrum to give a maximum at 300 nm in 0.1N-hydrochloric acid. The quality of the product in each batch was gauged by ascertaining the yield from its condensation with benzylamine to give the product (CIX; $R = CH_2C_6H_5$).

Condensation of (CVIII) with aminoacetal (aminoacetaldehyde diethylacetal, CX) gave the pyrimidine [CIX; $R = CH_2CH(C_2H_5)_2$]. Hydrolysis of the acetal group with
concentrated hydrochloric acid gave the pyrimidine (CIX, $R = \text{CH}_2\text{CHO}$). This material although colourless and crystalline, could not be readily recrystallised, and discoloured on heating in water. This was attributed to the ready self-condensation to dihydropyrazines (CXI), typical of $\alpha$-aminoketone derivatives. Oxime formation, however, yielded a stable product (CXII), which was readily recrystallised from water. The pyrimidine aldehyde (CIX; $R = \text{CH}_2\text{CHO}$) however, when subjected to paper chromatography and examined in ultraviolet light, showed a single absorption spot and was thus considered pure enough to be used for further work.

2-Amino-4-hydroxy-7,8-dihydropteridine (XCVII).

An aqueous suspension of the aldehyde (CIX; $R = \text{CH}_2\text{CHO}$), on treatment with sodium dithionite gave the unstable 4,5-diaminopyrimidine (CXIII; $R = \text{CH}_2\text{CHO}$), which rapidly underwent intramolecular condensation to form 2-amino-4-hydroxy-7,8-
dihydropteridine, isolated as the sulphite salt (CXIV). This separated as a colourless, non-crystalline powder.

![Chemical structures](image)

It was unstable in boiling water and was insoluble in organic solvents. It was considered to be the sulphite salt of a dihydropteridine and not the sulphite adduct (XCIX; \( \text{R} = \text{SO}_3\text{H} \)), since it absorbed one mol. of hydrogen when hydrogenated with platinum or palladium catalysts, giving 2-amino-4-hydroxy-5,6,7,8-tetrahydropteridine. This view was confirmed by spectral comparison of the sulphite salt and the compound (XCIX; \( \text{R} = \text{SO}_3\text{H} \)), which was found to be extremely unstable in air, and which was prepared by catalytic hydrogenation of (XCV; \( \text{R} = \text{SO}_3\text{H} \)). The synthesis of the latter compounds will be discussed at greater length later in this thesis. Moreover, when the sulphite salt was treated with 2N-sodium hydroxide solution, the sulphite group was easily removed, and, on chilling the solution, a crystalline sodium salt of the pteridine was obtained. This material discoloured rapidly in air, and was analysed in crude form. Its ultraviolet spectra,
however, were identical with those of the sulphite salt.
Similarly, on treatment with acid, sulphur dioxide was evolved on
gentle heating, leaving the free base.

The presence of the sulphite anion stabilised the
material somewhat to oxidation, and it could be kept for
several days in a desiccator before showing signs of discoloration.
Removal of the sulphite grouping by alkali, however, liberated
the free base which proved to be extremely unstable, and rapidly
oxidised, in the presence of air and excess alkali, to the
fully aromatic 2-amino-4-hydroxypteridine (XCV, R = H). The
same product was obtained in good yields by treating alkaline
solutions of the sulphite salt with cold potassium permanganate
solution or powdered manganese dioxide, and proved to be
identical in all respects with an authentic sample.34

Catalytic reduction of the pyrimidine aldehyde (CIX, R = CH₂CHO) with Raney Nickel gave the unstable 4,5-diamino

\[
\text{pyrimidine (CXIII, } R = \text{CH}_2\text{CHO). The ultraviolet spectra in}
\]

0.1N acid and alkali of the reaction mixture at this stage were
closely similar to those obtained for the 4,5-diaminopyrimidine \( [\text{CXIII}; R = \text{CH}_2\text{CH(C}_2\text{H}_5)_2] \), and were assumed to indicate the existence of \( [\text{CXIII}; R = \text{CH}_2\text{CHO}] \). On concentrating the reaction mixture in vacuo, however, ring-closure took place with formation of the dihydropteridine \( [\text{XCIV}] \), which gave ultraviolet spectra in 0.1N acid and alkali identical to those of the sulphite and sodium salts. The cyclised material, however, was unstable and could not be easily purified. Accordingly, it was characterised as the sulphite salt, which was obtained by passage of sulphur dioxide gas through the reaction mixture.

**Tetrahydropteridines.**

**Catalytic hydrogenation of the above dihydropteridine** with platinum and palladium catalysts gave the tetrahydro derivative, 2-amino-4-hydroxy-5,6,7,8-tetrahydropteridine \( [\text{XCIX}; R = \text{H}] \). This material was even more unstable than the dihydro derivative, and, in solution on exposure to air, it oxidised rapidly to give the fully-aromatic 2-amino-4-hydroxypteridine \( [\text{XCV}; R = \text{H}] \). Consequently, no analytical figures
are available and its presence was indicated by its ultraviolet spectra in 0.1N-hydrochloric acid and sodium hydroxide solutions. These showed maxima in the 250-260 nm region, typical of compounds having the chromophoric system of a 4,5-diaminopyrimidine ring not conjugated with any other double bond system. This behaviour is typical of 5,6,7,8-tetrahydropteridines.

The Structure of Dihydropteridines.

The formation of a dihydropteridine has been found to result in the formation of a separate peak of low intensity at longer wavelengths in the ultraviolet spectrum, in addition to the characteristic maximum attributed to the pyrimidine ring. In some cases, this new 'peak' appeared as only a shoulder in the ultraviolet spectrum. On oxidation of a dihydropteridine to the fully aromatic pteridine, the new peak in spectra in alkaline solution, was found to increase in intensity and wavelength to give the typical doublet spectrum of an aromatic bicyclic compound. This was repeated in some acid spectra, whilst in others, single maxima, usually of increased intensity and wavelengths were observed. This examination of the ultraviolet spectra of dihydropteridines and the changes in spectra on oxidation to the aromatic pteridine, together with comparisons between the spectra of dihydropteridines and those of certain pyrimidines, have enabled additional information about the
structure of dihydropteridines to be obtained. The structure of the parent dihydropteridine (XCVII) was determined by the 'unambiguous' method of synthesis. Nevertheless, the possibility of tautomeric shifts of the type (XCVIIa - XCVIII) was not entirely excluded, and therefore, some relation had to be established between the ultraviolet spectra of a dihydropteridine and its chromophoric system. The ultraviolet spectra of the dihydropteridine showed a maximum in acid solution of 256 μm with a low intensity peak or shoulder at 312-314 μm. In alkali, bathochromic shifts in both peaks gave maxima at 284 and 330 μm. The spectra of the fully-aromatic 2-amino-4-hydroxypteridine showed, by comparison, a single peak in acid at 314 μm of increased intensity, while, in alkali, the long-wavelength peak had undergone a bathochromic shift to 358 μm also with an increase in intensity. This shift in the long-wavelength peaks in both acid and alkali on oxidation of the dihydropteridine, indicated that the position and
Intensity of these peaks were related to the degree of conjugation in the pyrazine ring of the pteridine molecule. The spectra of compounds of the type (CIX) can be seen by reference to the Table to be closely similar. Their spectra in acid solution show a single maxima in the region 314–334 μm with a slight bathochromic shift in alkali to the 340 μm region. These spectra compare favourably with the long-wavelength peaks of the spectra of the dihydropteridine and suggest that these compounds have a common chromophoric system. This could only be possible if the dihydropteridine in question had the chromophore of a 7,8-dihydropteridine, in which case, the common chromophoric system would be that inscribed by the broken lines (CIX) and (XCVII). Thus, a 7,8-dihydropteridine, having the same nuclear substituents as the compounds discussed above, would be expected to have an ultraviolet spectrum which would have the following general form: a peak at 250–280 μm together with a low-intensity peak or shoulder at or above 300 μm in acid, and a similar or closely-related spectrum in alkali, with perhaps bathochromic
shifts of both peaks and an increase in their intensities.

In accepting the validity of these theoretical considerations, we believe that the dihydropteridine discussed above, having been synthesised by Boon's method, has undergone no tautomeric changes within the molecule.

Thus it would appear that Boon's synthesis is indeed an unambiguous one. The synthesis of two other dihydropteridines by this method will be discussed later in this section, and an examination of their spectra with reference to the general pattern of spectra of 7,8-dihydropteridines was found to support this view. To form an absolute relationship, however, between the chromophoric system of a 7,8-dihydropteridine and its ultraviolet spectra would involve synthesis of the 7,8-dihydropteridine derivative (CXY) in which tautomeric shifts of the type discussed earlier would be impossible. This is outside the scope of the present thesis but might prove to be an interesting problem.
Before discussing the addition reactions undergone by 2-amino-4-hydroxy-7,8-dihydropteridine, it might be relevant to consider the approach made by Viscontini and Weilenmann to the problem of reactive 2-amino-4-hydroxy-dihydropteridines, since it involves determination of structures of dihydropteridines from consideration of their ultraviolet spectra.

Viscontini prepared 2-amino-4-hydroxy-5,6,7,8-tetrahydropteridine (XCIX; R = H), isolated as the sulphite salt and allowed it to reoxidise in air. Three unstable compounds were isolated as eluates from chromatograms, and Viscontini suggested that these were dihydropteridines, and were intermediates in the reoxidation process. These compounds were allocated structural formulae (XCVII, XCVIII, CXVI) on the basis of a comparison of their ultraviolet spectra with the spectra of three known compounds. Thus, Viscontini claims that the chromophoric systems resemble those of isoxanthopterin (CXVII), the dihydroxanthopterin of O'Dell, which, one must suppose, was considered...
by Viscontini to have a 5,6-dihydro structure (CXVIII), and xanthopterin (XIV) respectively, thus providing a basis for the above structural assignments.

The three dihydro intermediates were found to oxidise to 2-amino-4-hydroxypteridine (XCV, \( R = H \)). However, two other compounds were also isolated. On oxidation of the tetrahydropteridine sulphite salt in air on a cellulose column, a yellow solid was obtained. On the basis of its elementary analysis, Viscontini and Weilenmann claimed that this was 2-amino-4-hydroxypteridine-6-sulphonic acid (XCV, \( R = \text{SO}_3\text{H} \)). Its similarity in chemical properties to 2-amino-4-hydroxypteridine-6-carboxylic acid (XCV, \( R = \text{CO}_2\text{H} \)) supported this view.
On reoxidation of the tetrahydropteridine sulphite salt in ammonia, a dihydroxanthopterin was obtained. This was different from that of O'Dell, and had ultraviolet spectra which were almost identical with those of one of the 2- amino-4-hydroxy-dihydropteridines (CXVI). Thus, it was formulated as a 5,6-dihydroxanthopterin (CXIX; R = OH). Viscontini and Weilenmann suggested a reaction scheme, which visualised the formation of these compounds by nucleophilic addition to a reactive dihydro species, obtained from any or all of the three dihydro intermediates by a tautomeric shift, which was thought to be acid-catalysed. For example, the 5,6-dihydro intermediate (XCVIIIa) was thought to exist in the form (XCVIIIb), which, on protonation would give the reactive species (XCVIIIc, XCVIIIId) having an electrophilic centre at the 6-position. Treatment with the appropriate nucleophile $\mathcal{R}^@$ would give rise to the tetrahydropteridine (XCIX), which, on oxidation, would yield the products (XCV; $\mathcal{R} = \text{SO}_3\mathcal{H}$) and (CXIX; $\mathcal{R} = \text{OH}$) referred to earlier.

The conclusions reached by Viscontini, however, are not entirely valid. For example, the allocation of structures to the dihydro intermediates (XCVII, XCVIII, CXVI) on the basis of spectral comparisons with isoxanthopterin, dihydroxanthopterin, and xanthopterin cannot be accepted. In the
case of isoxanthopterin and xanthopterin, a realistic comparison of spectra in alkaline solution would be impossible, because the oxygen substituents in the pyrazine rings of these pteridines would be in the phenolic form, which must affect the ultraviolet spectra. Moreover, it would be doubtful if spectral comparison could be justified, even with spectra in acid solution, where the oxygen functions would be present as amide carbonyl
groups, which would still be in conjugation with the pyrimidine double bond system. In addition, the dihydroxanthopterin of O'Dell has been synthesised unambiguously by Boon who has formulated it as a 7,8-dihydroxanthopterin (XCVIII).

Two modified Boon syntheses reported by us in the Experimental section of this thesis give support to this structure, and the incorporation of its ultraviolet spectra in the general pattern for 7,8-dihydropteridines outlined earlier, further confirms this view. A full discussion of the syntheses of this dihydropteridine, however, will take place later in this section.

Thus, from an examination of the ultraviolet spectra recorded by Viscontini and Weilenmann for the three dihydro intermediates, it would appear that the 5,6-dihydropteridine (XCVIII) has spectral characteristics of a 7,8-dihydropteridine. Its spectra, however, are not identical with those of 2-amino-4-hydroxy-7,8-dihydropteridine prepared by us. Its spectrum in acid showed a peak at about 270 μm with a shoulder at 312 μm.
while its alkaline spectrum showed little change, except for
an increase in intensity of the low-wavelength peak.

The addition reactions undergone by 2-amino-4-hydroxy-
7,8-dihydropterdidine discussed in this section are thought to
be nucleophilic additions to a polarised carbon-nitrogen
double bond. It was our experience, however, that these
additions are not catalysed by acids, as suggested by Viscontini,
but that they proceed smoothly to completion in alkaline or
neutral solutions. This conforms with previous experience,
where addition of reagents such as hydrogen cyanide, sodium
hydrogen sulphite, etc., to a polarised double bond is initiated
by nucleophilic attack. Finally, we have succeeded in adding
ammonia to 2-amino-4-hydroxy-7,8-dihydropterdidine, which makes
questionable the formation of a dihydroxanthopterin from such
a reaction.

The general conditions leading to the participation of
pteridine derivatives in addition reactions were outlined in
the Introduction to this thesis, with particular reference to
certain monohydroxyppteridines and their derivatives. It was
suggested that tautomeric shifts leading to the disruption of
the aromatic system of double bonds in either the pyrazine or
pyrimidine rings of a pteridine, would lead to an increase in
reactivity (i.e. polarisability) of the remaining double bonds.
Also, the capacity of some pteridines for undergoing addition reactions was explained in terms of increased stability of their adducts, brought about by resonance.

In the case of 2-amino-4-hydroxy-7,8-dihydropteridine (XCVII), the pyrasine ring exists in the dihydro form, conferring a degree of reactivity on the 5,6-double bond. We consider the polarisation of this double bond by solvent or by the electron-withdrawing properties of the adjacent pyrimidine ring, to be the dominant factor in the addition reactions undergone by this compound. Thus, polarisation of the 5,6-double bond of 2-amino-4-hydroxy-7,8-dihydropteridine (XCVIIa) takes place to give an electrophilic centre at C₆ (XCVIIb). Addition of the appropriate nucleophile would give the addition.
product (XCIX) which, on oxidation, would give rise to the fully-aromatic pteridine (XCV).

**Addition Reactions of 2-Amino-4-hydroxy-7,8-dihydropteridine.**

1. **Addition of Ammonia**

2-Amino-4-hydroxy-7,8-dihydropteridine sulphite was dissolved in the minimum of concentrated ammonia solution and left in a tightly-stoppered flask for 2-3 days. This produced a crop of yellow needles, whose elementary analyses indicated that they were a mixture of 2,6-diamino-4-hydroxy-5,6,7,8-tetrahydropteridine (XCIX; R = NH₂), and a dihydro derivative. Oxidation of this material by cold alkaline potassium permanganate or by powdered manganese dioxide, gave a yellow-green-fluorescing solution, which, on neutralisation, yielded a bright orange, non-crystalline solid. This material had ultraviolet spectra, in acid and alkaline solution, identical with those reported by Van Baalen and Forrest⁴⁶ for the compound (XCV; R = NH₂), obtained by oxidation of 2-amino-4-hydroxy-5,6,7,8-tetrahydropteridine (XCIX; R = H) in ammonia. In an attempt to effect a
direct comparison of these compounds, a synthesis of the material was attempted, employing the general conditions laid down by Van Baalen and Forrest. Thus, reoxidation of the tetrahydropteridine (XCIX; R = H) by manganese dioxide, in the presence of ammonia, under nitrogen, gave a yellow solid which proved to be a mixture on examination by paper chromatography. Isolation of the green-fluorescing component of this mixture by formation of its crystalline sodium salt gave a material, which, on chromatography, was identical in Rf values, and in ultraviolet spectra, with the compound formed by oxidation of the addition product. The structure (XCV; R = NH3) formulated by Van Baalen and Forrest for this compound has been confirmed by us by analysis and by the following unambiguous synthesis.

Condensation of 2-amino-4-chloro-6-hydroxy-5-nitropyrimidine (CVIII) with aminoacetonitrile (CXX) gave the pyrimidine (CIX; R = CH2CN). Reduction of the nitro group

\[
\begin{align*}
\text{H}_2\text{NCH}_2\text{CN} & \quad \text{CVIII} \\
\text{Cl} & \quad \text{CIX} \\
\end{align*}
\]

with sodium dithionite gave the unstable 4,5-diaminopyrimidine (CXLIII; R = CH2CN), which underwent ring-closure to give 2,6-diamino-4-hydroxy-7,8-dihydropteridine (CXXI; R = NH2).
The ultraviolet spectrum of the compound in acid was typical of a 7,8-dihydropteridine. It gave a peak at 274 µm, with another of low intensity at 319 µm. In alkaline solution, however, this long-wavelength peak disappeared, with the main peak undergoing a bathochromic shift to 280 µm.

Oxidation of this compound with cold alkaline potassium permanganate or powdered manganese dioxide gave 2,6-diamino-4-hydroxypteridine (XCV, R = NH₂), identical in R₂ values, ultraviolet and infrared spectra with the solid obtained from oxidation of the addition product. As has already been mentioned, pure 2,6-diamino-4-hydroxy-5,6,7,8-tetrahydropteridine (XCVI, R = NH₂) could not be obtained by addition of ammonia to the 7,8-dihydropteridine (XCVII), due to the instability of the adduct, especially in basic media. The ultraviolet spectrum in acid of the freshly-prepared addition product, showed a peak at 274 µm with a shoulder at 306 µm, and a sample left for 6 hr. in alkali gave, on acidification, an ultraviolet spectrum in which the shoulder
at 306 mu had developed into a low-intensity peak at 320 mu. This spectrum was identical with that obtained for 2,6-diamino-4-hydroxy-7,8-dihydropteridine (CXXI; R = NH$_2$), and indicated that oxidation from the tetrahydro species to the fully-aromatic pteridines proceeded via the 7,8-dihydro intermediate.

Hydrogenation experiments with platinum and palladium catalysts indicated that the addition product contained about 40-50% of the dihydro product. The product of hydrogenation, 2,6-diamino-4-hydroxy-5,6,7,8-tetrahydropteridine (XCV; R = NH$_2$), was colourless in solution under hydrogen. On exposure to air, the solution rapidly turned pale yellow, indicative of oxidation. The extreme instability of the product precluded the possibility of obtaining analytical data. However, ultraviolet spectra in acid and alkali were quickly carried out on the reaction mixture. These showed a maximum at 270 mu in acid, with disappearance of the shoulder at 306 mu, present in the material before hydrogenation. The alkali spectrum, having a maximum at 280 mu, showed no significant change. These spectra are characteristic of a tetrahydropteridine.

Viscontini and Piraux, in a paper published in March 1962, questioned the structure (XCV; R = NH$_2$), proposed by van Baalen and Forrest, for the compound obtained from the
reoxidation of 2-amino-4-hydroxy-5,6,7,8-tetrahydropteridine (XCIX; R = H) in ammonia.

In an earlier publication, Viscontini and Weilenmann had reported the isolation of a compound from this reaction, which was formulated as a 5,6-dihydroxanthopterin (CXIX; R = OH), and this has already been discussed, but he now suggested that it was best represented by a 5,8-dihydro structure (CXXII; R = OH). It was further suggested that the analytical data published by Van Baalen and Forrest did not indicate a fully-aromatic pteridine, as had been suggested. Moreover, comparison of analytical figures obtained from a sample of the material which had been isolated from ammonia solution, then recrystallized from water, led Viscontini and Piraux to suggest the existence of two closely-related compounds (CXXII; R = NH$_2$) and (CXXII; R = OH). On the basis of their analytical figures,
they suggested that the compounds were 5,8-dihydro derivatives and that Van Baalen and Forrest had isolated one (CXXII; R = NH₂), whilst they had isolated the other (CXXII; R = OH).

Recrystallisation from the appropriate solvent was sufficient to bring about the transformation. Catalytic hydrogenation over platinum for 10 min. resulted in the absorption of 1 mol. of hydrogen, to give a product which was formulated as a tetrahydropteridine (XCIX; R = NH₂).

This general thesis, however, does not bear critical examination. The ultraviolet spectra of the material obtained by Van Baalen and Forrest cannot be explained by either a 5,6- or 5,8-dihydro structure. (5,8-Dihydropteridines and the general conditions which we believe to be involved in their formation, will be discussed later in this section). Moreover, 2-amino-4,6-dihydroxy-5,8-dihydropteridine (CXVIIIa)
would not be expected to be stable in this tautomeric form, and would revert to 7,8-dihydroxanthopterin (XCIII), which has the stable aminoacetamide (CXXIIia) chromophore. The evidence of a 5,8-dihydro derivative of this type, would infer that aminoacetamide would exist as (CXXIIb).

The analytical data submitted by Forrest do not indicate that the compound is a dihydro derivative, although some discrepancy exists in the calculated and experimental figures for the nitrogen content.

In addition, we have found that catalytic hydrogenation of the compound in question over a platinum catalyst, resulted in the absorption of two mol. of hydrogen. The acid and alkali spectra of the product were characteristic of tetrahydropteridines, and were identical with those of 2,6-diamino-4-hydroxy-5,6,7,8-tetrahydropteridine (XCIX, R = NH₂), whose preparation was discussed earlier. Finally, the hydrogenation product obtained by Viscontini after the absorption of 1 mol. of hydrogen, although formulated as a tetrahydropteridine, had, in fact, ultraviolet spectra similar to the 7,8-dihydroxanthopterin (XCIII) of O'Dell and were identical with the spectra of 2,6-diamino-4-hydroxy-7,8-dihydropteridine (CXXI, R = NH₂) whose synthesis was described above.
We think that this constitutes sufficient evidence that the compound originally obtained by Van Baalen and Forrest has the fully-aromatic structure (XCV; $R = NH_2$). This being the case, hydrolysis of the 6-amino group, which is suggested by Viscontini's analytical figures, seems unlikely. We found that the purification process of the material involved precipitation several times from aqueous solutions, and the presence of xanthopterin could not be detected on paper chromatograms.

Earlier attempts to synthesize 2,6-diamino-4-hydroxy-7,8-dihydropterdine (CXXI; $R = NH_2$), produced an interesting modification of the Boon synthesis. 2-Amino-4-chloro-6-hydroxy-5-nitropyrimidine (CVIII) was condensed with aminoacetamide (CXXIIa) (prepared by condensation of chloroacetamide with ammonia), to give the pyrimidine (CIX; $R = CH_2CONH_2$)
Reduction of the nitro group in this pyrimidine with sodium dithionite gave the unstable diaminopyrimidine (CXXXIII, R = CH₂CONH₂).

It was thought that this compound would then cyclise with elimination of the elements of water to give the required 2,6-diamino-4-hydroxy-7,8-dihydropteridine (CXXI, R = NH₂). However, cyclisation took place with elimination of the elements of ammonia, to give 7,8-dihydroxanthopterin (XCIII) in good yield. This synthesis was supported by the preparation of an authentic sample of 7,8-dihydroxanthopterin as follows.

2-Amino-4-chloro-6-hydroxy-5-nitropyrimidine (CVIII) was condensed with glycine ethyl ester (CXXIV) to give the pyrimidine (CIX, R = CH₂CO₂C₂H₅).

\[ \text{CXXIV} \]

Sodium dithionite reduction of the nitro group gave the diaminopyrimidine (CXXXIII, R = CH₂CO₂C₂H₅), which immediately cyclised to give 7,8-dihydroxanthopterin. This material was identical in ultraviolet spectra with that obtained by O'Dell and later by Boon. It was also identical in ultraviolet and infrared spectra with the above sample.
Treatment with cold alkaline potassium permanganate or powdered manganese dioxide gave xanthopterin good yields. Its ultraviolet spectra in acid and alkali closely resembled those obtained for 2,6-diamino-4-hydroxy-7,8-dihydropteridine (CXXI; \( R = \text{NH}_2 \)) (See Figs. 1, 2), and its spectrum in acid corresponded to that of a typical 7,8-dihydropteridine.

The synthesis of 2,6-diamino-4-hydroxy-7,8-dihydropteridine (CXXI; \( R = \text{NH}_2 \)) by reduction and cyclisation of the pyrimidine (CIX; \( R = \text{CH}_2\text{CN} \)) has already been described. The conversion of this 6-amino series of 7,8-dihydropteridines to the 6-hydroxy series of 7,8-dihydropteridines, was established by hydrolysis of the pyrimidine (CIX; \( R = \text{CH}_2\text{CN} \)) to give the pyrimidine (CIX; \( R = \text{CH}_2\text{CONH}_2 \)).


Forrest and Viscontini have reported, in a joint publication, the isolation of a yellow solid from the reoxidation of 2-amino-4-hydroxy-5,6,7,8-tetrahydropteridine (XCIX; \( R = \text{H} \)) in the presence of potassium cyanide. This compound gave strongly green-fluorescing solutions in alkali, and its ultraviolet spectrum at pH13 showed a long wavelength maximum at almost 400 \( \text{m} \mu \). Analysis indicated an empirical formula corresponding to 2-amino-4-hydroxydihydropteridine-6-carboxamide. Oxidation with platinum oxide in alkaline solution gave 2-amino-4-hydroxypteridine-6-carboxamide (XCV; \( R = \text{CONH}_2 \)).
Catalytic hydrogenation with a platinum catalyst resulted in the absorption of 1 mol. of hydrogen, to give a tetrahydropteridine (XCV, R = CONH₂) which quickly reoxidised in air to give the original compound.

Forrest and Viscontini suggested that the compound was 2-amino-4-hydroxy-5,8-dihydropteridine-6-carboxamide (CXXII, R = CONH₂), and claimed that this structure was supported by the following evidence:

(i) the ultraviolet spectrum was typical of certain para-dihydropyrazine⁶⁰ (CXXV) and dihydropyridine derivatives⁶¹ (CXXVI, CXXVII);

(ii) on oxidation to the fully-aromatic pteridine (XCV; R = CONH₂), the spectra in acid and alkali showed hypsochromic shifts which are unusual in passing from a dihydro derivative
to the fully-aromatic compound. In our studies of addition reactions of 7,8-dihydropteridines, hydrogen cyanide was found to add across the double bond particularly easily. Addition of a cold saturated solution of potassium cyanide to 2-amino-4-hydroxy-7,8-dihydropteridine sulphite, followed by refrigeration for about an hour, gave the adduct, 2-amino-4-hydroxy-6-cyano-5,6,7,8-tetrahydropteridine (XCIX; R = CN), as its colourless, crystalline, potassium salt. However, this material was so unstable that no analytical or satisfactory spectral data could be obtained for it. When treated with roughly the amount of alkaline potassium permanganate required to remove two hydrogen atoms, it gave a yellow microcrystalline solid, having a strong green fluorescence in alkaline solutions. This material was identical in ultraviolet spectra with the compound obtained by Forrest and Viscontini, and its elementary analysis indicated an equivalent empirical formula to that reported by the above workers. Catalytic hydrogenation with a platinum catalyst established that the material was a dihydropteridine, since only one mol. of hydrogen was absorbed. The product,

![Chemical Structure](image)

XCIX
which was non-fluorescent in solution, quickly reoxidised to the dihydro compound on exposure to the air. Oxidation of the yellow solid with an excess of alkaline potassium permanganate, gave 2-amino-4-hydroxypteridine-6-carboxylic acid (XCV; R = CO₂H) in good yield. This was accompanied by a hypsochromic shift in the ultraviolet spectra. From a comparison of the alkaline spectra of 2-amino-4-hydroxypteridine-6-carboxylic acid (XCV; R = CO₂H), and that of our dihydropteridine, it can be seen that the hypsochromic shift on oxidation amounts to about 30 μ." 

On the other hand, the results of spectral studies on 7,8-dihydropteridines and their oxidation products were discussed earlier, when it was shown that oxidation to the fully-aromatic structure involved a bathochromic shift. In addition, the long wavelength absorption of the dihydropteridine at present under discussion, was unusually high compared with that of the 7,8-dihydropteridines which have been studied by us, and its acid and alkali spectra did not conform to the general pattern which we have found to exist for 7,8-dihydropteridines. Accordingly, on the basis of the above evidence, it was decided that this dihydropteridine did not have the chromophore of a 7,8-dihydropteridine. Furthermore, the data submitted by Forrest and Viscontini, drawing attention to the similarity of
the ultraviolet spectra of certain *para*-dihydropyrazine derivatives ⁶⁰ (CXXV) and *para*-dihydropyridines ⁶¹ (CXXVI, CXXVII) to those of the dihydropteridine must be considered as evidence for its having a 5,8-dihydro structure.

The 5,8-dihydro structure (CXXII; R = OH) proposed for a dihydroxanthopterin by Viscontini and Piraux was discussed earlier in this section, and was considered to be improbable, partly because of its expected instability. We suggested that this compound would easily revert to 7,8-dihydroxanthopterin (XCIII), having a stable amino-acetamide type structure (CXXIIIa). In the case of the above dihydropteridine, however, we consider a 5,8-dihydro structure to be a distinct possibility. In this Department, chemical studies ⁶² of compounds of the type (CXXVIIIa) accepted the possibility of an enamine tautomer (CXXVIIIb) as

![Diagram](image)

suggested by the work of Glickman and Cope ⁶³. Subsequent condensations of this compound with dicarbonyl compounds to produce pyrazines, and with one carbon fragments to produce pyrimidines, supported this theory. Thus, it may be argued
that the enamine chromophore exists, and that its incorporation into a ring system to produce a 5,8-dihydropteridine might be sufficient to make it the more stable structure. In addition, we consider the existence of a carboxyl or carbonyl group to be necessary to stabilise the enamine tautomer, and we believe that only dihydropteridines meeting this requirement by having a carboxyl or carbonyl substituent in the 6-position, would have a stable 5,8-dihydro structure.

Finally, evidence submitted by Pyleiderer and Taylor,\textsuperscript{64} has further confirmed this theory. They condensed 2,4-bis-ethylamino-5-aminopyrimidine (CXXXI; $R = H$) with mesoxalic acid, to give the pteridine (CXXXII; $R = H$). Reduction of this compound

\[
\begin{align*}
\text{CXXXI} & \quad \text{CXXXII} \\
\begin{align*}
& \quad \text{CXXXIII} \\
\end{align*}
\end{align*}
\]

with sodium borohydride gave a bright, yellow material, and was accompanied by a bathochromic shift of 48 nm in the ultraviolet spectrum to 404 nm. This compound was formulated as a 5,8-dihydropteridine derivative (CXXXIII). The spectral characteristics of this compound were shown to be due to the presence of the 6-carboxyl group, since decarboxylation was accompanied
by a marked hypsochromic shift. The 5,6-dihydro structure was thought to arise by reduction of the 5,6-double bond, followed by enolisation to the intramolecularly hydrogen-bonded 5,6-dihydro structure (CXXXI). This theory was confirmed by condensing 2-ethylamino-4,5-diaminopyrimidines (CXXXII) with mesoxalic acid to give (CXXXIII), formulated as a hydrogen-bonded structure by analogy with 7-hydroxypteridine-6-carboxylic acid, which is known to exist in the enolised lactim form (CXXXIV) because of the stabilising effect of intramolecular hydrogen bonding. Sodium borohydride reduction of (CXXXIII) was accompanied by a hypsochromic shift, typical of reduction to a dihydropteridine, to give (CXXXV). This compound was already stabilised by intramolecular hydrogen bonding and therefore did not undergo further tautomeric change.
Although these results indicate that hydrogen bonding is a stabilising factor in the formation of 5,8-dihydropteridines, nevertheless, they do not explain the abnormal ultraviolet absorption of 2-amino-4-hydroxy-5,8-dihydropteridine-6-carboxamide, where intramolecular hydrogen bonding of the type discussed above is impossible.

Additional evidence for the 5,8-dihydro structure, however, was obtained by noting that the absorption spectra of 5,8-dihydropteridines were identical, irrespective of the nature of the 2-substituent. Since resonance of type (CXXXa) was known to exist in the fully-aromatic compounds and would depend on the ability of the 2-substituent to take part, it was inferred by Pfleiderer and Taylor that, in the dihydro structure, conjugation of the carboxyl group with the pyrimidine was excluded.

Strong evidence for the 5,8-dihydro structure was also obtained by consideration of a report by Baltrop, Richards and Russell, who catalytically reduced 2-acetyl-3-
methylquinoxaline (CXXXVI) and proved conclusively that the product was (CXXXVII). This material had a maximum in ethanol at 489.5 μm. Similarly, King and Clark-Lewis reported the abnormal longwavelength absorption of the compound (CXXXVIII), having a chromophore identical to that of a 5,8-dihydropteridine.

\[
\text{CXXXVIII} \quad \text{CXXXVII} \quad \text{CXXXII}
\]

Finally, a report by Viscontini and Piraux, dealt with the addition of hydrogen cyanide to xanthopterin (XLIV) and his dihydroxanthopterin (CXXII; R = OH). The additions were carried out in the presence of ammonia and the compounds obtained (CXXXIX; R = NH₂, OH, R' = CONH₂, CN) after oxidation are analogous to those already described by Pfleiderer and Taylor. These compounds were shown to be dihydro derivatives

\[
\text{CXXXIX}
\]

by catalytic hydrogenation, and their ultraviolet absorption at pH13 ranged from 430 to 450 μm. In addition, they had a measure
of stability which, in the light of the results of Pfleiderer
and Taylor, may be attributed to intramolecular hydrogen bonding.

3. **The Addition of Hydrogen Sulphite.**

\[
\text{CXIV} \quad \text{CXV}
\]

The oxidation of 2-amino-4-hydroxy-7,8-dihydropteridine
sulphite (CXIV), in alkaline media, to give 2-amino-4-hydroxy-
pteridine (CXIV; \( R = \text{H} \)) has been discussed earlier in this thesis.
However, on oxidation with potassium permanganate in neutral
solutions, and subjecting the reaction mixture to paper chromato-
tography, it was noted that 2-amino-4-hydroxypteridine was
present only in a small amount. The main product had a lower
\( R^2 \) in the propanol-ammonia solvent system than 2-amino-4-
hydroxypteridine, and was much more strongly acid than this
compound. The product was purified by making use of the fact that
it formed a crystalline sodium salt in solutions of \( 2\text{M} \)-sodium
hydroxide, while 2-amino-4-hydroxypteridine did not. Its general
chemical behaviour was similar to that of 2-amino-4-hydroxy-
pteridine-6-carboxylic acid (CXIV; \( R = \text{CO}_2\text{H} \)) which is discussed
later, and this similarity was reflected in its ultraviolet spectra.
In acid, this showed a single maximum at 325 μm, while in alkali the familiar doublet characteristic of fully-aromatic pteridines was observed at 264 and 369 μm.

These results are in excellent agreement with those published by Viscontini and Weilenmann for the 2-amino-4-hydroxypteridine-6-sulphonic acid (XCV, R = SO₃H), discussed earlier in this thesis. The conditions reported by Viscontini for formation of this compound involved aerial oxidation of 2-amino-4-hydroxy-5,6,7,8-tetrahydropteridine sulphite on a cellulose column, and, as such, do not differ significantly from those described by us.

2-Amino-4-hydroxypteridine-6-sulphonic acid is thought to arise by oxidation of 2-amino-4-hydroxy-5,6,7,8-tetrahydropteridine-6-sulphonic acid (XCVI, R = SO₃H) which, in turn, is formed by addition of the hydrogen sulphite group of the sulphite salt across the 5,6-double bond of the dihydropteridine (XCVII). This type of addition is well-known in heterocyclic chemistry and specific examples of it, with reference to quinoxalines and acridines, have
been described in the Introduction of this thesis.

Catalytic hydrogenation of 2-amino-4-hydroxypteridine-6-sulphonic acid with a platinum catalyst resulted in the absorption of two mols of hydrogen to give (XGIX; R = SO₃H), which is isomeric with 2-amino-4-hydroxy-7,8-dihydropteridine sulphite (CXIV). However, the reduction product possessed the instability typical of tetrahydropteridines and could not be analysed. On exposure to air it oxidised rapidly in alkali with recurrence of the faint blue fluorescence typical of the fully aromatic compound (XCV; R = SO₃H). The ultraviolet spectrum of the reduced product in acid showed a high-intensity peak at 265 μ with a much weaker one at 314 μ. In alkali, it gave a peak at 250 μ while the long-wavelength peak suffered a bathochromic shift to 355 μ. These spectra are characteristic of a 7,8-dihydropteridine, possibly (CXXI; R = SO₃H) formed by aerial oxidation of the tetrahydropteridine (XGIX; R = SO₃H) and contrast with the high absorption of 6-carboxy-dihydropteridines discussed earlier which is thought to be indicative of a 5,8-dihydro structure.

4. The Addition of Michael-type Reagents.

Addition of compounds (CXL), containing active methylene groups, to 2-amino-4-hydroxy-7,8-dihydropteridine (XCVII) was found to take place smoothly in alkaline solution at room temperature.
The additions are thought to take place by formation of the carbanion (CXLa) followed by a nucleophilic attack at the 6-position as described earlier in general terms. The products of this reaction were tetrahydropteridines of general formula (CXL). They were extremely unstable in solution but, as solids, could be kept for 10-12 hr. in a desiccator. They were soluble in water, acetone and ethanol, but oxidised so rapidly in solution that recrystallisation from these solvents was impossible. The compounds were characterised by their ultraviolet spectra, elementary analysis (where this was possible), their failure to take up hydrogen on catalytic hydrogenation, and their oxidation to 2-amino-4-hydroxypteridine-6-carboxylic acid (XCV; R = CO₂H). They were extremely unstable in alkaline solution which removed the side-chain at
the 6-position and facilitated their oxidation. In acid media, they were rather more stable, although, as an examination of their ultraviolet spectra will indicate, oxidation to a dihydropteridine took place fairly quickly in most cases. Moreover, the side-chain at the 6-position appeared to be more stable in acid media, and this factor was used when studying the oxidation of these compounds. Oxidation with cold acid potassium permanganate solution was found to be sufficient to oxidise the tetrahydropteridine (CXLII) to a fully-aromatic form (XCV), the side-chain remaining essentially intact. Oxidation of this material with alkaline potassium permanganate gave 2-amino-4-hydroxypteridine-6-carboxylic acid (XCV; R = CO₂H).

The first compound studied in this series was (CXLIII; R' = R'' = CO₂C₂H₅), obtained by adding diethylmalonate (CXL; R = R'' = CO₂C₂H₅) to a solution of the sodium salt of 2-amino-4-hydroxy-7,8-dihydropteridine (XCVII) in alkaline solution. Reaction is virtually instantaneous, and the adduct separated as a pale yellow powder. Analysis indicated that this was a tetrahydropteridine and a freshly-prepared sample could not be hydrogenated with a platinum catalyst in neutral solution. In acid or alkaline solution, however, oxidation took place and its ultraviolet spectrum in acid showed a peak at 264 μm with a low-intensity one at 300 μm indicative of a 7,8-dihydropteridine. Its alkaline spectrum showed no significant change.
except for a bathochromic shift of these peaks to 286 and 364 μm respectively. Oxidation with cold acid permanganate, and examination of the ultraviolet spectra of the reaction mixture at this stage, showed a shoulder at 246 μm with a high-intensity long-wavelength peak at 320 μm in acid, while the alkaline spectrum had maxima at 256 and 356 μm. These are in excellent agreement with the spectra of 2-amino-4-hydroxypteridine-6-acetic acid (XCV; R = CH₂CO₂H) published by Mowat et al. Further oxidation with alkaline permanganate solution gave 2-amino-4-hydroxypteridine-6-carboxylic acid (XCV; R = CO₂H).

The addition of ethylacetoacetate (CXL; R¹ = COCH₃, R'' = CO₂C₂H₅) gave the adduct (CXL; R¹ = COCH₃, R'' = CO₂C₂H₅) as a bright yellow solid. It could not be hydrogenated by a platinum catalyst in neutral solution. Its ultraviolet spectra, however, showed a peak at 266 μm with a shoulder at 300 μm. A very low-intensity broad maximum was detected at 386 μm. The alkaline spectrum gave a high-intensity maximum at 276 μm with a shoulder at 330 μm. Again, these spectra indicate that
oxidation of the tetrahydropteridine had taken place in solution with the possible formation of a 7,8-dihydropteridine. Oxidation with acid permanganate gave rise to a fully-aromatic pteridine possibly with structure (XCV; R = CH₂COCH₃).

Completion of the oxidation with an alkaline permanganate gave 2-amino-4-hydroxypteridine-6-carboxylic acid (XCV; R = CO₂H).

The addition of acetylacetone (CXL; Rᵢ = Rᵣ = COCH₃) to 2-amino-4-hydroxy-7,8-dihydropteridine gave the adduct (CXL; Rᵢ = Rᵣ = COCH₃) as a bright yellow solid, which, when freshly-prepared, could not be hydrogenated by a platinum catalyst in neutral solution. Its ultraviolet spectra showed a maximum in acid of 260 μ with a very low intensity peak at 386 μ. In alkali the spectrum showed a single maximum at 292 μ. As before, this indicated the formation of a 7,8-dihydropteridine, although this adduct would appear to have a greater measure of stability in alkali, since in this medium its spectrum corresponded to that of a tetrahydropteridine. Oxidation of the adduct with acid potassium permanganate gave the intermediate [XCV; R = CH(COCH₃)₂] which on treatment with alkaline permanganate, gave 2-amino-4-hydroxypteridine-6-carboxylic acid (XCV; R = CO₂H).

Treatment of the dihydropteridine (XCVII) with ethyl cyanoacetate (CXL; Rᵢ = CN, Rᵣ = CO₂C₂H₅) gave the adduct
(CXLII; \( R^1 = \text{CN}, \ R^{11} = \text{CO}_2\text{C}_2\text{H}_5 \)) as a cream solid which could not be hydrogenated. Its ultraviolet spectra did not conform to any recognisable pattern. In acid, its spectrum had a shoulder at 270 \( \mu\text{m} \) with a maximum at 300 \( \mu\text{m} \), while the alkaline spectrum gave maxima at 248, 290 and 364 \( \mu\text{m} \). The intensity of this long wavelength peak at 364 \( \mu\text{m} \) indicated the formation of a more conjugated species by oxidation and suggested that the adduct was unstable in this media.

As before oxidation with potassium permanganate gave eventually 2-amino-4-hydroxypteridine-6-carboxylic acid (XCV; \( R = \text{CO}_2\text{H} \)).

5. The Addition of Thiols.

Thiols (CXLII), which are known to be excellent reagents for adding across polarised double bonds, were found to react smoothly with the sodium salt of 2-amino-4-hydroxy-7,8-dihydropteridine (XCVII) to give adducts of general formula

\[
\begin{align*}
\text{R} & \quad \text{S} \quad \text{H} \\
\text{CXLII} & \\
\text{XCVII} & \\
\text{CXLIII} &
\end{align*}
\]

(CXLIII). Like the additions of active methylene compounds
described above, the thiol additions were found to take place most effectively in basic media. The adducts studied in this series were colourless, crystalline unstable compounds, which, when pure, could not be hydrogenated.

The addition of mercaptoethanol (CXLII; \( R = \text{CH}_2\text{CH}_2\text{OH} \)) to the dihydropteridine gave the adduct (CXLIII; \( R = \text{CH}_2\text{CH}_2\text{OH} \)). This material had an ultraviolet spectrum in acid having a maximum at 256 \( \text{m} \mu \) with a low-intensity peak at 370 \( \text{m} \mu \). In alkali, however, its spectrum was almost identical to that of 2-amino-4-hydroxy-7,8-dihydropteridine, having maxima at 262 and 330 \( \text{m} \mu \) which indicated that oxidation to a 7,8-dihydropteridine had taken place in this medium.

The addition of thioglycollic acid (CXLII; \( R = \text{CH}_2\text{CO}_2\text{H} \)) occurred almost instantaneously, with the separation of the adduct (CXLIII; \( R = \text{CH}_2\text{CO}_2\text{H} \)) as a colourless crystalline solid. Its ultraviolet spectra were characteristic of a tetrahydropteridine. Its acid spectrum showed a single high intensity peak at 282 \( \text{m} \mu \), while the alkaline spectrum had a single maximum at 292 \( \text{m} \mu \). The increase in stability of this compound indicated by its ultraviolet spectra can be explained in terms of a condensation between the hydrogen atom at \( N_6 \) and the functional carboxyl group of the side-chain at the
6-position. This would give rise to a tetrahydropteridine (CXLIV) in which oxidation to a 7,8-dihydropteridine derivative is impossible.

\[ \text{CXLIV} \]

6. **The Addition of Water**

2-Amino-4-hydroxy-7,8-dihydropteridine (as the sulphite or sodium salt) was dissolved in sodium hydroxide solution and set aside for a few days. The solution was then oxidised and the reaction mixture subjected to paper chromatography. Examination of the chromatogram in ultraviolet light showed the presence of three discernible products. The main product was 2-amino-4-hydroxypteridine, which was isolated and identified as described in the Experimental section. A second product, present only in trace quantities, had \( R_f \) values identical with those of xanthopterin (XLV), and was thought to arise by addition of the elements of water across the 5,6-double bond of the dihydropteridine (XCVII) to give tetrahydroxanthopterin (XGIX, \( R = \text{OH} \)), which, on oxidation, would give rise to
xanthopterin. The third product, also present in very small quantities, had a green fluorescence. This compound was thought to be a dimeric pteridine formed by the addition of a carbanion (XCVIIc), derived from the dihydropteridine in alkali, to the electrophilic centre at the 6-position of the dihydropteridine (XCVII), to give the tetrahydropteridine (CXIV), which, on oxidation,

would form the highly-conjugated dimer (CXIV). The formation of dimeric pteridines has already been reported by Albert.26

The formation of xanthopterin and the possible dimer from these reactions, however, was not investigated further.
Oxidation of Tetrahydropteridines.

All the tetrahydropteridines considered in this thesis, with one exception, were found to give 2-amino-4-hydroxypteridine (XCIV, $R = H$), on oxidation in alkaline solution, in addition to the expected addition products. For example, the tetrahydropteridines (XCIV, $R = NH_2$, $SO_3H$), when set aside in alkaline solution for 2 or 3 days and then subjected to paper chromatography, showed two spots. One corresponded to the pteridine (XCIV, $R = NH_2$, $SO_3H$) the other had $R_f$ values identical with those of 2-amino-4-hydroxypteridine (XCIV, $R = H$).
The Michael-type adducts (CXLII) were more unstable in alkali and oxidised in this media to give 2-amino-4-hydroxypteridine which, from an examination by chromatography, appeared to be the main product.

The thiol adducts (CXLIII) were similarly oxidised in alkaline solution to give 2-amino-4-hydroxypteridine.

The tetrahydropteridine (XCIX; R = CN) did not give 2-amino-4-hydroxypteridine on oxidation. Some significance may be attached to the fact that this compound gave a 5,8-dihydro derivative, whilst the others appeared to oxidise via 7,8-dihydropteridines. Thus, it would appear that 2-amino-4-hydroxypteridine would arise via its 7,8-dihydro derivative. This theory was confirmed in the case of the Michael and thiol adducts by allowing them to reoxidise in the presence of ammonia. Examination of the reaction mixtures by chromatography showed the presence of 2,6-diamino-4-hydroxypteridine (XCV; R = NH₂) which is thought to arise by addition of ammonia to the intermediate 2-amino-4-hydroxy-7,8-dihydropteridine (XCVII) formed in these reactions.
PART II

THE BIOSYNTHESIS OF XANTHOPTERIN
As explained at the beginning of Part I of this section, our interest in dihydropteridines lay in the possible role played by these substances in pteridine biosynthesis. In particular, the biosynthesis of xanthopterin (2-amino-4,6-dihydroxypteridine, XIV) held our attention, since the evidence furnished by Weygand and dealt with in the Introduction to this thesis, suggested to us the possibility of a 7,8-dihydropteridine intermediate being involved.

Weygand's hypothesis for the biosynthesis of xanthopterin in the cabbage butterfly (Pieris brassicae) involved ring-opening of the imidazole ring of guanosine (LXXXIV, $R = \text{ribosyl}$) to give the 4,5-diaminopyrimidine derivative

(LXXXVIII). He suggested that this pyrimidine could then
undergo an Amadori rearrangement, which is typical of glycosylamine derivatives, to give the pyrimidine (LXXXIX). This ketose could then ring-close to give the tetrahydropteridine derivative (XC) which, he suggested, might lose its polyhydroxy side-chain possibly as glyceraldehyde or its 3-phosphate and subsequently oxidise to xanthopterin (XIV).

In this Department, Neilson and Wood during their studies of the biosynthesis of riboflavin, prepared the pyrimidine (CXLVII). Raney nickel reduction of the nitro group in this pyrimidine gave a compound which was believed to be (CXLVIII) and which was isolated as its sodium salt. On aerial oxidation in alkaline solution, this compound gave 2,4,6-trihydroxypteridine (CXLIX).
This suggested to us that the 7,8-dihydropteridine (CXLIII) was undergoing addition reactions similar to those described in Part I of this section. Addition of water would give rise to the tetrahydropteridine (CL) which could lose its polyhydroxy side-chain and oxidise to 2,4,6-trihydroxypteridine in the manner outlined by Weygand.

It was decided to test the chemical feasibility of Weygand's hypothesis by synthesising the pyrimidine (LXXXIX) and investigating the products obtained after ring-closure. Initially, however, the model pyrimidine (CLI) was prepared to ascertain the possibilities of such a transformation.

1-Amino-1-deoxy-D-fructose (isoglucoamnine, CLII) was prepared by a modification of the literature method. D-glucose, on treatment with phenylhydrazine in an acetate buffer at 90° gave glucosazone as a yellow crystalline solid. This material was hydrogenated at a pressure of 4 atmospheres in the presence of acetic acid using a palladium oxide-barium
sulphate catalyst. In the acid medium, the product (CLII) was isolated as its acetate. This material, on condensation with (CVIII) gave the pyrimidine (CLIII). This compound was hygroscopic and was characterised as its crystalline oxime. Raney nickel reduction of the nitro group gave the unstable 4,5-diamino-pyrimidine derivative (CLIV). The reaction mixture from the reduction was then made alkaline and was set aside for three days. On neutralisation of the solution, 7,8-dihydroxanthopterin (XCIII) separated, identical in all respects with an authentic sample. Our attention was now directed to the synthesis of (LXXXIX).

The synthesis of L-amino-L-deoxy-D-erythropentulose (CLV, R = H) was achieved as follows. D-arabinose and benzylamine were condensed in ethanol to give N-benzyl-D-arabinosylamine. By employing a modification of conditions reported by Michael and Hageman, this was rearranged in dry dioxan with a molar quantity of anhydrous oxalic acid, to give
l-benzylamino-l-deoxy-D-erythropentulose (CLV; R = CH$_2$Ph) as its oxalate. Catalytic debenzylation was effected using a palladium-charcoal catalyst to give l-amino-l-deoxy-D-erythropentulose oxalate. The oxalate residue was removed by treatment with sodium ethoxide and the free base immediately condensed with (CVIII) to give (CLVI), which was very hygroscopic and was characterised as its oxime. Raney nickel reduction of the nitro group gave the unstable diaminopyrimidine derivative (LXXXIX). Treatment of the reaction mixture with alkali and neutralisation after three days produced no solid. Chromatographic evidence that 7,8-dihydroxanthopterin was the major product of the reaction, was obtained by examination of the reaction mixture in a number of solvents. Potassium permanganate oxidation and chromatography revealed the presence of xanthopterin (XIV) together with a number of blue-fluorescing spots, which were not identified.
The detailed mechanism of this reaction has not been fully elucidated. Two theories are, however, worthy of consideration:

(i) that the product (LXXXIX) of the reduction ring-closes to give the tetrahydropteridine (XC), which then undergoes the transformations already described to give xanthopterin; and

(ii) that dehydration of (XC) occurs after Raney nickel reduction to give a 7,8-dihydropteridine (CLVII) which, on addition of alkali, gives (XC), which then oxidises with loss of its side-chain to give xanthopterin via 7,8-dihydroxanthopterin.

The ultraviolet spectra of the reaction mixture after Raney nickel reduction showed maxima at 258 and 360 μ in acid and 282, 328 μ in alkali. These do not correspond to the spectra of a dianimopyrimidin derivative since Raney nickel reduction of the oxime of (CLVI) showed maxima at 266 μ in acid and 286 μ in alkali, a result characteristic of a 4,5-dianimopyrimidine. On the other hand, they compare favourably with
the spectra of 2-amino-4-hydroxy-6-methyl-7,8-dihydropteridine (GLVIII) reported by Booth et al., which show maxima at 252
and 365 μm in acid and 282 and 325 μm in alkali.

Thus, it would appear that a 7,8-dihydropteridine
species exists which undergoes hydration in alkaline solution
to give the tetrahydropteridine derivative (XC). The oxidation
of tetrahydropteridines in alkaline solution has been dealt with
earlier, and it is suggested that (XC) behaves in an analogous
fashion and gives rise to xanthopterin via its 7,8-dihydro
derivative.

It has been found, however, that addition of ammonia
in place of alkali failed to produce a spot of 2,6-diamino-4-
hydroxypteridine (XCV: R = NH₂) on examination by chromatography.
One would have expected this product had an additive process
been involved. We have no explanation as yet for this
apparent anomaly.
Paper Chromatography

Chromatograms were developed by the ascending technique, the solvents being (a) butan-1-ol-5N-acetic acid (7:3), (b) 3% aqueous ammonium chloride (c) propan-1-ol-water-conc. ammonia (S.G., 0.88) (40:20:1), (d) butan-1-ol-ethanol-water (50:15:35) and (e) methanol-butanol-1-ol-water-benzene (2:1:1:1).

Absorption Spectra

Infrared spectra were determined with Grubb-Persoons and Perkin-Elmer infrared spectrophotometers on nujol mulls and KCl discs.

Ultraviolet spectra were determined with Unicam SP600 and Optika Spectrophotometers on aqueous solutions of standard pH.

Hydrogenations

Values of hydrogen uptake were reduced to N.T.P. in all cases.
2-Amino-4,6-dihydroxypyrimidine (CV).  

Sodium (100 g.) was dissolved in ethanol (1500 ml.), dry powdered guanidine hydrochloride (200 g.) and diethyl malonate (320 g.) were added, and the mixture was refluxed for 2 hr. at 110°. After distilling off the ethanol (90.600 ml.) in vacuo, water (2000 ml.) was added, and the solution was acidified with glacial acetic acid. The precipitate was filtered off and dried in air at 110° to give the pyrimidine (150 g., 56.5%), m.p. > 360°.

2-Amino-4,6-dichloropyrimidine (CVI).  

2-Amino-4,6-dihydroxypyrimidine (50 g.) was refluxed at 100° for 3 hr. with redistilled phosphoryl chloride (200 ml.) and redistilled N,N-diethylaniline (50 ml.). Excess phosphoryl chloride and N,N-diethylaniline were distilled off in vacuo and the remaining dark viscous mass was poured on to crushed ice (600 g.). After stirring till all oily material had dissolved, the brown solid was collected, washed with aqueous 2N-sodium hydroxide solution to remove unchanged phenolic material, and dried. Recrystallisation from ethanol (charcoal) yielded the dichloropyrimidine as colourless needles (20 g., 64.5%), m.p. 221°.

2-Amino-4-chloro-6-hydroxypyrimidine (CVII).  

2-Amino-4,6-dichloropyrimidine (30 g.) was refluxed for 6 hr. with N-sodium hydroxide solution (560 ml.). The
solution was filtered, cooled, and acidified with glacial acetic acid. The precipitate was collected and dried in air at 110°. Crystallisation from aqueous ethanol yielded the pyrimidine as needles (20 g., 86%), m.p. 261°.

2-Amino-4-chloro-6-hydroxy-5-nitropyrimidine. (CVIII).

2-Amino-4-chloro-6-hydroxy-5-nitropyrimidine (5.0 g.) was dissolved in concentrated sulphuric acid (60 ml.) at < 45°. Fuming nitric acid (S.G., 1.5, 5.3 ml.) was cautiously added at < 45° with stirring. After 30 min., the mixture was poured on to ice and the solid which precipitated was collected, washed with water (2 x 20 ml.), ethanol (20 ml.), and ether (20 ml.) to give the 4-chloro-5-nitropyrimidine (5.0 g., 91.5%), m.p. > 360°. Analysis was carried out on a crude sample dried in vacuo over potassium hydroxide pellets without heat. (Found: C₂₃.2%; H₂.2%; N₂.26.4%. C₄H₃N₄O₃Cl.H₂O requires C₂₃.0%; H₂.4%; N₂.26.8%).

2-Amino-4-benzylamino-6-hydroxy-5-nitropyrimidine. (CIX, R=CH₃C₆H₅).

2-Amino-4-chloro-6-hydroxy-5-nitropyrimidine (0.50 g., 1 eq.) was dissolved in ethanol (100 ml.) and benzylamine (0.6 g., 2 eq.) in ethanol (10 ml.) was added to the solution. The mixture was heated on the steam-bath for 20 min. and, on cooling, a colourless microcrystalline solid separated. This was collected, and washed with water, ethanol, and ether and
dried. Recrystallisation from boiling dimethylformamide and ethanol gave 2-amino-4-benzylamino-6-hydroxy-5-nitropyrimidine as a colourless microcrystalline solid (0.6 g., 88%), m.p. 334-335° (dec.). (Found: C, 50.6; H, 4.3; N, 26.6. \( \text{C}_1\text{H}_1\text{H}_3\text{N}_3\text{O}_3 \) requires C, 50.6; H, 4.2; N, 26.8%).

2-Amino-4-(2',2'-diethoxyethylamino)-6-hydroxy-5-nitropyrimidine. \((\text{CIX}_6 \text{ R} = \text{CH}_2\text{CH(C}_2\text{H}_5)_2).\)

2-Amino-4-chloro-6-hydroxy-5-nitropyrimidine (0.50 g., 1 eq.) was dissolved in ethanol (100 ml.) and aminoacetal (aminoacetaldehyde diethylacetal, 0.7 ml., 2 eq.) in ethanol (10 ml.) was added to the solution. The mixture was heated on the steam-bath for 20 min. and, on cooling, a pale yellow solid separated. This was collected and washed with water, ethanol, and ether. Recrystallisation from ethanol gave the diethylacetal (0.5 g., 67%) as microcrystalline needles (Found: C, 41.7; H, 5.8; N, 24.4. \( \text{C}_{10}\text{H}_{17}\text{N}_3\text{O}_5 \) requires C, 41.8; H, 6.0; N, 24.4%).

Raney Nickel Reduction of 2-Amino-4-(2',2'-diethoxyethylamino)-6-hydroxy-5-nitropyrimidine. \((\text{CIX}, \text{ R} = \text{CH}_2\text{CH(C}_2\text{H}_5)_2).\)

2-Amino-4-(2',2'-diethoxyethylamino)-6-hydroxy-5-nitropyrimidine (0.2 g.) in water (50 ml.) and Raney Nickel (1 spatula measure) was added. The suspension was hydrogenated overnight
at room temperature and pressure when 1 mol. of hydrogen was absorbed (43 ml., theoretical, 47 ml.). The product self-condensed fairly rapidly and attempts to isolate pure samples were unsuccessful.

Its ultraviolet spectra, however, were characteristic of a 4,5-diaminopyrimidine (see table).

2-Amino-4-formylmethylamino-6-hydroxy-5-nitropyrimidine

2-Amino-4-(2,2'-diethoxyethylamino)-6-hydroxy-5-nitropyrimidine (0.55 g.), was heated on the steam-bath with concentrated hydrochloric acid (5.0 ml.) for 20 min. On cooling, the aldehyde separated as colourless crystalline needles (0.35 g., 86%) which could not readily be recrystallised. The solid was therefore washed with water, ethanol, and ether, and dried. This material was characterised as its oxime, which was prepared as follows:

A solution of sodium (0.043 g., 2 eq.) in ethanol (4.5 ml.) was added to hydroxylamine hydrochloride (0.13 g., 2 eq.) in water (10 ml.), and after a few minutes, this was added to a suspension of 2-amino-4-formylmethylamino-6-hydroxy-5-nitropyrimidine (0.2 g., 1 eq.) in water (10 ml.), and the mixture was heated on the steam-bath for 20 min. On cooling, a colourless, microcrystalline solid separated, and this was
washed with water, ethanol, and ether. Recrystallisation from water gave the oxime \((0.19 \text{ g.}, 89\%)\), m.p. > 300° (Found: \(C\_31.3; H\_4.0; N\_36.7\). \(C\_6H\_6N\_6O\_4\) requires \(C\_31.6; H\_3.5; N\_36.8\%\)).

**2-Amino-4-hydroxy-7,8-dihydropyridine (XCVII).**

(a) As the sulphite salt.

2-Amino-4-formylmethylamino-6-hydroxy-5-nitropyrimidine (0.25 g.) in water (30 ml.) was heated gently on the steam-bath. Solid sodium dithionite was added until the pyrimidine dissolved and the solution became colourless. On further heating, a white solid separated. After cooling, this was collected and washed with water, ethanol, and ether to give 2-amino-4-hydroxy-7,8-dihydropyridine sulphite (0.22 g., 75%). The product was unstable and could not be recrystallised from water. It was insoluble in organic solvents. Analysis was carried out on the uncrystallised material, and samples were dried in vacuo over potassium hydroxide pellets for 2 hr. without heat. (Found: \(C\_28.3; H\_3.8; N\_27.1; S\_12.6\). \(C\_6H\_7N\_6O\cdotH\_2SO\_3\cdot0.5H\_2O\) requires \(C\_28.2; H\_3.9; N\_27.3; S\_12.5\%).

(b) As the sodium salt.

2-Amino-4-hydroxy-7,8-dihydropyridine sulphite (1.29 g.) was dissolved without heat in 2\(\text{M}\)-sodium hydroxide solution (30 ml.).
10N-Sodium hydroxide solution (1 drop) was added, and the solution was left at 0° for 15 min. Fine, colourless crystalline needles were obtained which were collected and washed with aqueous ethanol, ethanol, and ether to give the sodium salt (0.78 g., 83%). The compound was extremely unstable and rapidly discoloured on exposure to air. Analysis was carried out on freshly-prepared, undried material. (Found: C, 29.1; H, 5.1. C₆H₃NO Na·3.5H₂O requires C, 28.8; H, 5.2%).

(a) As the free acid.

2-Amino-4-formylaminomethyl-6-hydroxy-5-nitropyrimidine (0.60 g.) was suspended in water (40 ml.) and Raney Nickel (2 spatula measures) was added. The suspension was hydrogenated overnight at room temperature and pressure when 3 mols. of hydrogen were absorbed (183 ml., theoretical, 190 ml.). The catalyst was removed by filtration and the filtrate concentrated in vacuo to yield a colourless, non-crystalline powder (0.30 g., 65%). This material was extremely unstable in solution. The powder was dissolved in water (30 ml.), and a stream of sulphur dioxide gas was bubbled through the solution for 2 min. A colourless, microcrystalline solid (0.15 g., 21%) separated almost immediately. This material was identical in ultraviolet and infrared spectra with the sulphite salt obtained from method (a).
2-Amino-4-hydroxypteridine (XCV; \( R = H \))

(a) From 2,4,5-triamino-6-hydroxypyrimidine (CXIII, \( R = H \)).

2,4,5-Triamino-6-hydroxypyrimidine sulphate monohydrate (2.0 g.) in water (60 ml.) was heated on the steam-bath and glyoxal (0.6 g.) in water (3 ml.) was added. A dark, red precipitate formed immediately, the colour faded, and, after 30 min., the precipitate (0.8 g.) was collected. The solid was dissolved in dilute ammonia and boiled with charcoal for 15 min. The solution was filtered, and the ammonia removed by evaporation to precipitate a yellow solid. This was collected and washed with water, ethanol, and ether, to give 2-amino-4-hydroxypteridine (0.7 g., 55%).

(b) From 2-amino-4-hydroxy-7,8-dihydropteridine sulphite.

(i) Aerial Oxidation

2-Amino-4-hydroxy-7,8-dihydropteridine sulphite (1.0 g.) was dissolved in \( \tilde{N} \)-sodium hydroxide solution (30 ml.) and set aside for 2-3 days at room temperature. The solution was then adjusted to \( \text{pH} 7 \) with \( 2\tilde{N} \)-hydrochloric acid to precipitate an orange solid. This was dissolved in \( \tilde{N} \)-sodium hydroxide solution (20 ml.), treated with charcoal, and adjusted to \( \text{pH} 7 \) with \( 2\tilde{N} \)-hydrochloric acid to give 2-amino-4-hydroxypteridine as a yellow solid (0.47 g., 74%). This material was dissolved with warming in \( 10\tilde{N} \)-sodium hydroxide solution (10 ml.), filtered and cooled
to give the sodium salt as colourless needles (0.2 g., 38%).
It was identical in R\textsubscript{f} value, ultraviolet and infrared spectra
with the sodium salt of the material obtained from method (a).

(ii) Manganese Dioxide Oxidation

2-Amino-4-hydroxy-7,8-dihydropteridine sulphite (0.2 g.)
was dissolved in \(\text{NaOH}\) solution (10 ml.) and
manganese dioxide (1 spatula measure) was added. The mixture
was stirred mechanically for 4 hr. and then filtered. The
filtrate was brought to pH7 with 2\(\text{N}\)-hydrochloric acid when an
orange solid precipitated. This was treated with charcoal in
\(\text{NaOH}\) solution to give a yellow solid (0.83 g.,
65%) whose sodium salt agreed in R\textsubscript{f} value, ultraviolet and
infrared spectra with the sodium salt of the material obtained
from method (a).

(iii) Potassium Permanganate Oxidation

2-Amino-4-hydroxy-7,8-dihydropteridine sulphite (0.30 g.)
was dissolved in \(\text{NaOH}\) solution (10 ml.) and
0.02\(\text{M}\)-potassium permanganate solution was added until a permanent
green colouration was obtained. This was destroyed by the
addition of a little sodium dithionite, and the precipitate of
manganese dioxide was filtered off. The filtrate was brought
to pH7 with 2\(\text{N}\)-hydrochloric acid, when a whitish-yellow solid
precipitated. This was collected and washed with water, ethanol,
and ether to give a pale yellow solid (0.11 g., 58%). The sodium salt of this material was identical in R₅ value, ultraviolet and infrared spectra with the sodium salt of 2-amino-4-hydroxypteridine obtained from method (a).

(c) From 2-Amino-4-hydroxy-7,8-dihydropteridine sodium salt.

2-Amino-4-hydroxy-7,8-dihydropteridine sodium salt (0.75 g.) was oxidised with alkaline potassium permanganate as described immediately above. The product (0.45 g., 69%) gave a sodium salt which was identical in R₅ value, ultraviolet and infrared spectra with the sodium salt of 2-amino-4-hydroxypteridine obtained from method (a).

Catalytic Hydrogenation of 2-Amino-4-hydroxy-7,8-dihydropteridine Sulphite:

(a) Using Adam's catalyst

Adam's catalyst (0.20 g.) in water (30 ml.) was pre-reduced until uptake of hydrogen had ceased (46 ml.). 2-Amino-4-hydroxy-7,8-dihydropteridine sulphite (0.20 g.) was added, and the suspension hydrogenated overnight at room temperature and pressure. One mol. of hydrogen was absorbed (16 ml., theoretical, 17.5 ml.). The product was extremely unstable and oxidised readily. Its ultraviolet spectra in decinormal acid and alkali indicated that the material was 2-amino-4-hydroxy-5,6,7,8-
tetrahydropteridine. Attempts to isolate pure samples of this material were unsuccessful.

(b) Using palladium on charcoal.

2-Amino-4-hydroxy-7,8-dihydropteridine sulphite (0.20 g.) was hydrogenated overnight at normal pressure with previously-reduced palladium on charcoal (10% 0.20 g.) in water (30 ml.). One mol. of hydrogen was absorbed (15 ml., theoretical, 17.5 ml.) to give a product which was extremely unstable and which had ultraviolet spectra in acid and alkali characteristic of 2-amino-4-hydroxy-5,6,7,8-tetrahydropteridine. Attempts to isolate pure samples of this material were unsuccessful.

Catalytic Hydrogenation of 2-Amino-4-hydroxy-7,8-dihydropteridine Sodium Salt.

Similar reductions of the sodium salt of 2-amino-4-hydroxy-7,8-dihydropteridine resulted in the uptake of one mol. of hydrogen in each case.

Addition of Ammonia to 2-Amino-4-hydroxy-7,8-dihydropteridine (XCVII).

2-Amino-4-hydroxy-7,8-dihydropteridine sulphite (0.50 g.) was dissolved in concentrated ammonia solution (20 ml.) and left for 2-3 days at room temperature in a tightly-stoppered flask. Pale yellow crystals of crude 2,6-diamino-4-hydroxy-5,6,7,8-
tetrahydropteridine appeared (0.18 g., 49.5%), and these were collected and washed with water, ethanol, and ether. This material was extremely unstable and in alkaline solution was found to undergo rapid aerial oxidation. Recrystallisation proved impossible and analyses were carried out on crude material. (Found: C, 39.4; H, 4.8. \( \text{C}_6\text{H}_{10}\text{N}_6\text{O} \) requires C, 39.6; H, 5.5%). A second sample analysed for a dihydro derivative (Found: C, 39.9; H, 4.5; N, 46.3. \( \text{C}_6\text{H}_8\text{N}_6\text{O} \) requires C, 40.0; H, 4.5; N, 46.7%). Spectroscopic studies (see discussion) and the hydrogenation experiments reported below confirm that this solid material was a mixture of dihydro- and tetrahydropteridines.

**Catalytic Hydrogenation of Crude 2,6-Diamino-4-hydroxy-5,6,7,8-tetrahydropteridine** (XCIII; \( R = \text{NH}_2 \)).

(1) **Using Adam's catalyst.**

Adam's catalyst (0.30 g.) in water (15 ml.) was hydrogenated at room temperature until uptake of hydrogen ceased (55 ml.), and the above addition product (0.30 g.) in \( \text{NaOH} \) solution (15 ml.) was added. The solution was hydrogenated overnight at room temperature and pressure when the uptake of hydrogen was (18 ml., theoretical for dihydropteridine, 37 ml., for tetra-hydropteridine, 0 ml.). This indicated the presence of almost 50% of dihydropteridine in the crude addition product.
(ii) Using Palladium on Charcoal

Palladium on charcoal (10%, 0.10 g.) in water (20 ml.) was hydrogenated until the uptake of hydrogen ceased (7 ml.). The crude addition product (0.10 g.) in N-sodium hydroxide solution (10 ml.) was added, and the solution was hydrogenated overnight at room temperature and pressure when the uptake of hydrogen was (5 ml., theoretical for dihydropteridine, 12.4 ml., for tetrahydropteridine, 0 ml.) This indicated that about 40% of the crude addition product was a dihydropteridine.

The ultraviolet spectral characteristics of the product of hydrogenation were typical of a tetrahydropteridine and are included in the table.

Alkaline solutions of this tetrahydropteridine when left to oxidise in air developed the green-fluorescing solutions characteristic of the orthodox oxidation product 2,6-diamino-4-hydroxypteridine (see below). However, paper chromatography of the reaction mixture indicated the presence of traces of 2-amino-4-hydroxypteridine as well as a third pteridine (both blue-fluorescing) which was not identified.

**Aminocetanitriile**

Glycollonitrile (5.0 g.) was added slowly to a cooled flask containing concentrated ammonia (S.G. 0.88, 120 ml.). The flask was gently shaken, stoppered and left overnight.
The excess ammonia was evaporated off with ethanol, and the residual pale yellow liquid was made up to 100 ml. with water. Yield > 95%.

2-Amino-4-cyanomethy lamino-6-hydroxy-5-nitropyrimid ine

Aminoacetonitrile solution (20% w/w prepared as above) 6 ml., 2 eq.) in ethanol (10 ml.), was added slowly, with stirring, to a solution of 2-amino-4-chloro-6-hydroxy-5-nitropyrimidine (0.50 g.) in ethanol (20 ml.), and the mixture was heated on the steam-bath for 20 min. On cooling, a cream-colored, microcrystalline solid separated. Recrystallisation from dilute ammonia gave the nitrile (0.42 g., 76%) (Found: C, 34.4; H, 3.0; N, 39.7. C_8H_6N_2O_3 requires C, 34.3; H, 2.9; N, 40.0%).

2,6-Diamino-4-hydroxy-7,8-dihydroppteridine (CXXXI; R = NH_2)

2-Amino-4-cyanomethylamino-6-hydroxy-5-nitropyrimidine (6.0 g.) was suspended in water (150 ml.) and heated on the steam-bath, while solid sodium dithionite was added in portions until the pyrimidine had entirely dissolved. The excess sodium dithionite was destroyed by the addition of 2N-hydrochloric acid (10 ml.), when a pinkish solid separated. After cooling, this solid was collected, and washed with water, ethanol, and ether.
Recrystallisation from 0.1N-hydrochloric acid gave the diaminodihydropteridine (4.7 g., 91%). (Found: C, 36.3; H, 5.0; N, 42.3. C₆H₆N₆O₂H₂O requires C, 36.4; H, 5.1; N, 42.4%).

2,6-Diamino-4-hydroxypteridine (XCVII R = NH₂).

(a) From crude 2,6-diamino-4-hydroxy-5,6,7,8-tetrahydropteridine (XCIX; R = NH₂).

(i) Potassium Permanganate Oxidation.

The crude ammonia-addition product (0.50 g.) (see above) was dissolved in N-sodium hydroxide solution (25 ml.) and 0.02M-potassium permanganate solution (20 ml.) was added dropwise with stirring. The solution was left at room temperature for 30 min., and excess permanganate was then destroyed by addition of a little solid sodium dithionite. The coagulated precipitate of manganese dioxide was filtered off, and the precipitate was washed with hot water (10 ml.). The combined filtrate and washings were cooled, and brought to pH7 with 2N-hydrochloric acid. An orange solid (0.24 g.) precipitated and this was collected and washed with water, ethanol, and ether. Paper chromatograms of this material in a number of solvents indicated the presence of a little 2-amino-4-hydroxypteridine. The 2,6-diamino-4-hydroxypteridine was therefore purified by formation of its sodium salt:-
crude solid (0.24 g.) in 2N-sodium hydroxide solution (12 ml.) was heated until dissolution took place. The solution was filtered hot and cooled at 0°. The sodium salt separated as yellow-orange needles (0.165 g., 30% overall). This material was identical in Rf values, ultraviolet and infrared spectra with an authentic sample of the sodium salt of 2,6-diamino-4-hydroxypteridine obtained by method (c) below.

(ii) Manganese Dioxide Oxidation.

The crude addition product (0.20 g.) was dissolved in 2N-sodium hydroxide solution (10 ml.), manganese dioxide (1 spatula measure) was added, and the mixture was stirred mechanically for 4 hr. The manganese dioxide was filtered off, and washed with hot water (5 ml.). The combined filtrate and washings were cooled, and brought to pH7 with 2N-hydrochloric acid. An orange solid (0.09 g.) precipitated, and this was collected, and washed with water, ethanol, and ether.

Paper chromatograms of this material in a number of solvents indicated the presence of 2-amino-4-hydroxypteridine, and the material was therefore purified by formation of its sodium salt (prepared as in (i), 0.06 g., 26% overall).

(b) From 2-amino-4-hydroxypteridine<sub>246</sub> (XCVIII, R = H)

Adam's catalyst (0.20 g.) in 2N-ammonia solution (300 ml.) was hydrogenated until uptake of hydrogen ceased
(45 ml.). 2-Amino-4-hydroxypteridine (0.44 g.) was added and the mixture was hydrogenated overnight, when 2 mols. of hydrogen were absorbed (110 ml., theoretical, 121 ml.). The catalyst was removed by filtration, and manganese dioxide (ca. 0.50 g.) was added. The mixture was stirred mechanically while oxygen-free nitrogen was bubbled through the reaction mixture for 6 hr. 2N-Sodium hydroxide solution (15 ml.) was then added, and the mixture was heated gently on the steam-bath. The manganese dioxide was removed by filtration, washed with hot water (10 ml.), and the combined washings and filtrate were cooled. On adjusting the solution to pH 7 with 5N-hydrochloric acid, a yellow solid (0.20 g.) was precipitated. This was collected and washed with water, ethanol, and ether. Paper chromatograms of this material in a number of solvents indicated the presence of 2-amino-4-hydroxypteridine, together with a green-fluorescing material whose Rf values agreed with that of authentic 2,6-diamino-4-hydroxypteridine (see below). The crude material was dissolved in 2N-sodium hydroxide solution (12 ml.) by heating on the steam-bath. A little charcoal was added, and the solution was heated for a further 15 min. The solution was filtered hot and cooled at 0°. The sodium salt of 2,6-diamino-4-hydroxypteridine separated as yellow needles (0.13 g., 24%
which were collected and washed with aqueous ethanol, ethanol, and ether. This material was identical in \( R_f \) values, ultraviolet and infrared spectra with the sodium salt prepared above.

(\( o \)) From 2,6-diamino-4-hydroxy-7,8-dihydropteridine (CXXI; \( R = \text{NH}_2 \))

(\( i \)) Potassium Permanganate Oxidation.

2,6-Diamino-4-hydroxy-7,8-dihydropteridine (0.40 g.) (see above) was dissolved in \( \text{Na}_2\text{CO}_3 \) sodium hydroxide solution (6.0 ml.) and 0.02M-potassium permanganate (10 ml.) was added. The addition of solid sodium dithionite destroyed the excess permanganate, and the coagulated precipitate of manganese dioxide was filtered off. The precipitate was washed further with hot water (10 ml.) and combined with the filtrate. After cooling, the filtrate was brought to pH7 with 2\( \text{Na}_2\text{HCO}_3 \) hydrochloric acid to precipitate the pteridine as an orange non-crystalline solid (0.29 g., 73%).

This material was then dissolved in 2\( \text{Na}_2\text{CO}_3 \) sodium hydroxide (15 ml.) with gentle heating on the steam-bath. Boiling with charcoal for 15 min., followed by filtration and cooling of the filtrate, gave the sodium salt of 2,6-diamino-4-hydroxypteridine as yellow-orange needles (0.20 g., 45% overall). Analyses samples were dried in vacuum over potassium hydroxide.
without heat (Found: C, 26.7; H, 4.6; N, 30.8. C₆H₆N₆O₅Na₂·4H₂O requires C, 26.5; H, 4.8; N, 30.9%).

(ii) Manganese Dioxide Oxidation.

2,6-Diamino-4-hydroxy-7,8-dihydropteridine (0.50 g.) was dissolved in N-sodium hydroxide solution (10 ml.) and manganese dioxide (2 spatula measures) was added. The mixture was stirred mechanically for 4 hr., the manganese dioxide was removed by filtration, and washed with hot water (6.0 ml.). The filtrate and washings were combined and brought to pH 7 with 2N-hydrochloric acid to precipitate the pteridine (0.35 g., 73%). The sodium salt of this material (yellow-orange needles), (0.20 g., 36% overall) was identical to that obtained by method (c) (i).

Catalytic hydrogenation of 2,6-diamino-4-hydroxypteridine (XCV; R = NH₃).

Adam's catalyst (0.10 g.) in 0.5N-sodium hydroxide solution (30 ml.) was hydrogenated until uptake of hydrogen ceased (24 ml.). 2,6-Diamino-4-hydroxypteridine (0.05 g.) was added and the mixture was hydrogenated overnight at room temperature and pressure when 2 mols. of hydrogen were absorbed (11.5 ml., theoretical, 12.6 ml.) and the green fluorescence had disappeared. The ultraviolet spectra of the product were
typical of a tetrahydropteridine and were identical with that of the tetrahydropteridine obtained by hydrogenation of the crude ammonia product (see above). The product was extremely unstable and could not be isolated.

**Catalytic hydrogenation of the sodium salt of 2,6-diamino-4-hydroxypteridine (XCV, R = NH₂).**

Adam's catalyst (0.10 g. in water 20 ml.) was hydrogenated until uptake of hydrogen ceased (23 ml.). The sodium salt (0.05 g.) was added and the mixture was hydrogenated overnight at room temperature and pressure. Two mols. of hydrogen were absorbed (12.5 ml., theoretical, 11.2 ml.) with disappearance of the green fluorescence. The product, which was extremely unstable, had ultraviolet spectra identical with those obtained from the hydrogenation of 2,6-diamino-4-hydroxypteridine (see above).

**Glycinamide Hydrochloride** (Aminoacetamide hydrochloride, CXXIIIa).

Ammonia (S.G. 0.88, 500 ml.) was saturated with ammonia gas with stirring at 0°. Chloroacetamide (46.8 g.) was added and the mixture was kept at 0° for 3 days when a clear solution was obtained. This was concentrated in vacuo to a damp solid residue which was allowed to dry in air. This material was
dissolved in the minimum of hot water and crystallisation was initiated by addition of ethanol. On cooling overnight, glycinamide hydrochloride was obtained as colourless crystals (29.6 g., 80%), m.p. 203-205° [lit. 203-205°].

2-Amino-4-carboxamidomethylamino-6-hydroxy-5-nitropyrimidine (CIX, R = CH₂CONH₂).

(a) From glycinamide hydrochloride.

Glycinamide hydrochloride (2.20 g., 2 eq.) was dissolved in water (25 ml.) with sodium bicarbonate (1.68 g., 2 eq.), and stirred until effervescence ceased. This solution was added slowly with stirring to a solution of 2-amino-4-chloro-6-hydroxy-5-nitropyrimidine (1.90 g., 1 eq.) in ethanol (100 ml.) and the mixture was heated on the steam-bath for 20 min. On cooling, a cream-coloured solid separated, which was collected, washed with water, ethanol, and ether. This material was purified by dissolving in dilute ammonia, filtering, and reprecipitating with 2N-hydrochloric acid to give the pyrimidine as needles (1.90 g., 83%). (Found: C₃₁.₇₆, H₃.₈₆, N₃₆.₆. C₁₀H₈N₃O₄ requires C₃₁.₆₃, H₃.₆₄, N₃₆.₉%).

(b) From 2-amino-4-cyanomethylamino-6-hydroxy-5-nitropyrimidine (CIX, R = CH₂CN).

2-Amino-4-cyanomethylamino-6-hydroxy-5-nitropyrimidine (0.20 g.) (see above) was suspended in water (10 ml.) and
and hydrogen peroxide solution (30 vol., 3 drops) was added. The mixture was heated on the steam-bath for 30 min. Effer-
vescence took place with dissolution of the pyrimidine. At the same time, a cream-coloured solid separated. After cooling,
this material (0.15 g.) was collected, and washed with water, ethanol, and ether. The ammonium salt was prepared by dissolv-
ing in warm concentrated ammonia, and allowing to cool overnight, when colourless plates (0.08 g., 50%) were obtained.

This material was identical in Rf values, ultraviolet and infrared spectra with the ammonium salt of the material obtained by method (a).

2-Amino-4-ethoxycarbonylmethylamino-6-hydroxy-5-nitropyrimidine (CIX, R = CH₂CO₂C₂H₅).

Glycine ethyl ester hydrochloride (1.50 g., 2 eq.) was dissolved in water (20 ml.) with sodium bicarbonate (0.09 g.,
2 eq.,) and stirred until effervescence ceased. This solution was added slowly, with stirring, to a solution of 2-amino-4-
chloro-6-hydroxy-5-nitropyrimidine (1.0 g., 1 eq.) in ethanol (50 ml.), and the mixture was heated on the steam-bath for
20 min. On cooling, a colourless, crystalline solid separated, which was collected, and washed with water, ethanol, and ether.
Recrystallisation from water gave the ester (1.2 g., 89%).
m.p. 360-370° (deco.). (Found: C, 37.5% H, 4.3% N, 27.6%)

C₆H₁₁N₅O₆ requires C, 37.4% H, 4.3% N, 27.2%.

7,8-dihydroxanthopterin (2-amino-4,6-dihydroxy-7,8-dihydropteridine)(XCIII).

(a) From 2-amino-4-carbamoylmethylamino-6-hydroxy-5-nitropyrimidine (CIX₃ R = CH₂CONH₂).

2-Amino-4-carbamoylmethylamino-6-hydroxy-5-nitropyrimidine (0.50 g.) was suspended in water (20 ml.) and heated on the steam-bath while solid sodium dithionite was added in portions. The pyrimidine dissolved to give an almost colourless solution and, on further addition of dithionite, a cream-coloured solid began to separate. On cooling, this solid quickly crystallised as needles which were collected, and washed with water, ethanol, and ether. Recrystallisation from 0.1N-hydrochloric acid gave 7,8-dihydroxanthopterin (0.35 g., 89%), m.p. > 320° (Found: C, 36.3% H, 4.5% N, 35.7%. Calculated for C₆H₇N₆O₃.H₂O C, 36.2% H, 4.5% N, 35.2%).

2-Amino-4,6-dihydroxy-7,8-dihydropteridine (0.50 g.) was dissolved in the minimum of 2N-sodium hydroxide solution with gentle heating on the steam-bath. The solution was quickly filtered and cooled immediately when the sodium salt of 7,8-dihydroxanthopterin separated as fine white needles (0.54 g., 96%) (Found: C, 32.9% H, 3.5% N, 32.2%. C₆H₈N₆O₄Na.H₂O requires
This material was unstable and rapidly oxidised in air to xanthopterin.

(b) From 2-amino-4-ethoxycarbonylmethylamino-6-hydroxy-5-nitro-
pyrimidine. \((\text{CIX}, R = \text{CH}_2\text{CO}_2\text{C}_2\text{H}_5)\).

2-Amino-4-ethoxycarbonylmethylamino-6-hydroxy-5-nitro-
pyrimidine (0.85 g.) was suspended in water (40 ml.) and treated
with portions of solid sodium dithionite while heating gently
on the steam-bath. The pyrimidine slowly dissolved to give a
colourless solution and, on further addition of dithionite, a
cream-coloured crystalline solid separated.

The solution was cooled and the solid collected, and
washed with water, ethanol, and ether. Recrystallisation
from 0.1N-hydrochloric acid gave 7,8-dihydroxanthopterin
(0.50 g., 83.5%), m.p. > 320°. This material was identical
in R₂ values, ultraviolet and infrared spectra with the
material obtained by method (a).

**Xanthopterin** \(^{77}\) (2-Amino-4,6-dihydroxypyridine, XLI)

(a) From 2,4,5-triamino-6-hydroxypyrimidine (CXIII, \(R = \text{H}\)).

2,4,5-Triamino-6-hydroxypyrimidine sulphate mono-
hydrate (2.4 g.) was dissolved in 80% sulphuric acid (50 ml.).
Ethylglyoxalate hemiacetal \(^{78}\) (1.5 g., \(d, 1.1\)) was added and the
mixture was heated for 20 min. on the steam-bath. The solution
was then cooled, poured on to ice, and the solution adjusted to pH7 with ammonia. The brown solid (1.3 g.) which precipitated was collected, and washed with water, ethanol, and ether. This material was purified by formation of the barium salt as follows:-

Crude xanthopterin (1.3 g.) was dissolved in 2N-ammonium hydroxide (400 ml.) and 0.2N barium hydroxide (200 ml.) was added. The solution was kept at 0° for 3 days when the crystalline barium salt (2.4 g.) separated. This was collected, and washed with water, ethanol, and ether. This material was dissolved in 5N-sulphuric acid (200 ml.) and the barium sulphate which precipitated was allowed to settle. The barium sulphate was then filtered off, washed with water, and the filtrate brought to pH7 with 5N-sodium hydroxide solution when xanthopterin (0.08 g., 48%) separated as a bright yellow solid which was collected, washed with water, ethanol, and ether and dried.

(b) From 7,8-dihydroxanthopterin (XCIII).

(i) Potassium Permanganate Oxidation.

7,8-Dihydroxanthopterin (0.20 g.) was dissolved in N-sodium hydroxide solution (20 ml.) and 0.02M-potassium permanganate solution (5.0 ml.) was added dropwise with stirring. The solution was left at room temperature for 30 min. with occasional
shaking, then excess permanganate was destroyed by addition of 
a little solid sodium dithionite. The precipitate of manganese 
dioxide was filtered and washed with hot water (6.0 ml.). The 
combined washings and filtrate were brought to pH7 with 2N-
hydrochloric acid. The crude xanthopterin (0.13 g., 66%) which 
separated was purified by formation of the barium salt as above 
The purified material was identical in $R_f$ values, ultraviolet 
and infrared spectra with xanthopterin obtained by method (a). 

(ii) Manganese Dioxide Oxidation.

7,8-Dihydroxanthopterin (0.20 g.) was dissolved in N-
sodium hydroxide solution (20 ml.) and manganese dioxide (1 spatula 
measure) was added. The mixture was stirred mechanically for 
4 hr. at room temperature. Manganese dioxide was removed by 
filtration, and the filtrate was brought to pH7 with 2N-hydro-
chloric acid, when a yellow solid was precipitated. This was 
purified via the barium salt to give xanthopterin (0.085 g., 
43%). 

This was identical in $R_f$ values, ultraviolet and 
infrared spectra with xanthopterin obtained by method (a).

2-Amino-4-hydroxy-6-cyano-5,6,7,8-tetrahydropteridine (XCI\text{\textsuperscript{9}, } R = CN).

2-Amino-4-hydroxy-7,8-dihydropteridine sulphite (0.60 g.) was dissolved without heating in a saturated aqueous solution of
potassium cyanide (10 ml.) and the reaction was left in a tightly-stoppered flask for 30 min. at room temperature. On cooling at 0° for a further 30 min., the tetrahydropteridine separated as colourless needles of its potassium salt (0.50 g., 93%). This material was collected, and washed with aqueous ethanol, ethanol, and ether. The tetrahydropteridine was very unstable and discoloured rapidly on exposure to the air.

Analysis proved impossible, and no satisfactory ultraviolet spectra were obtained.

2-Amino-4-hydroxy-5,8-dihydropteridine-6-carboxamide (CXXII; \( R = \text{CONH}_2 \)).

The above addition product (0.50 g.) was dissolved in \( \text{OH} \)-sodium hydroxide solution (20 ml.), and 0.025M-potassium permanganate solution (5.5 ml.) was added dropwise, with stirring. The reaction was left at room temperature for 30 min., and the precipitate of manganese dioxide was removed by filtration. This was washed with hot water (6.0 ml.), and the combined washings and filtrate were then brought to pH6 with 2\( \text{OH} \)-hydrochloric acid, when a yellow, non-crystalline solid separated slowly. After leaving overnight at 0°, the product was collected, and washed with water, ethanol, and ether. Recrystallisation from water gave the 6-carboxamide (0.22 g.,
Analytical samples were dried in vacuo over potassium hydroxide pellets without heat (Found: C, 37.23; H, 4.48; N, 37.0. 
C₇H₉N₆O₂·H₂O requires C, 37.23; H, 4.48; N, 37.2%).

**Catalytic Hydrogenation of 2-Amino-4-hydroxy-5,9-dihydro-6-carboxamide (CXXII; R = CONH₂).**

Adam's catalyst (0.10 g.) in water (30 ml.) was hydrogenated until uptake of hydrogen ceased (23 ml.). 2-Amino-4-hydroxy-5,8-dihydropteridine-6-carboxamide (0.10 g.) and Na-sodium hydroxide solution (4.0 ml.) were added, and the mixture was hydrogenated overnight at room temperature and pressure. One mol. of hydrogen was absorbed (11.5 ml., theoretical, 11.1 ml.) and the green fluorescence of the dihydropteridine disappeared. The product oxidised rapidly in air with the recurrence of the green fluorescence and no satisfactory ultraviolet spectra could be obtained. On leaving for longer periods in alkali, the green fluorescence disappeared to give a solution which gave a blue fluorescence when examined in ultraviolet light, and which was found to be similar in Rφ values and ultraviolet spectra to 2-amino-4-hydroxypteridine-6-carboxylic acid (see below).

**2-Amino-4-hydroxypteridine-6-sulphonic acid (XCIV; R = SO₂H).**

2-Amino-4-hydroxy-7,8-dihydropteridine sulphite (0.50 g.) was suspended in water (50 ml.) and a solution of
0.5M-potassium permanganate (10 ml.) was added dropwise with stirring until a permanent pink colouration persisted. The solution was made alkaline by the addition of 2N-sodium hydroxide solution (3 ml.) and excess potassium permanganate was destroyed by addition of a little solid sodium dithionite. The precipitate of manganese dioxide was filtered off, and the filtrate brought to pH2 with 2N-hydrochloric acid. A white non-crystalline solid (0.45 g., 95%) was precipitated, and this was collected and washed with water, ethanol, and ether. Paper chromatography of this material in a number of solvents indicated the presence of a trace of 2-amino-4-hydroxypteridine. The sulphonic acid was purified by the formation of the sodium salt:

the crude product (3.5 g.) was dissolved in 2N-sodium hydroxide solution (100 ml.) by heating gently on the steam-bath. The solution was boiled with charcoal for a further 15 min., filtered, and cooled at 0°.

The sodium salt separated as pale yellow needles (4.0 g., 93% overall). These were collected and washed with aqueous ethanol, ethanol and ether. A specimen for analysis was prepared by dissolving the sodium salt in water and adjusting the solution to pH4 with 2N hydrochloric acid when 2-amino-4-hydroxypteridine-6-sulphonic acid separated. This procedure was repeated until the material obtained showed only one fluorescing
spot when subjected to chromatography and examined in ultraviolet light. (Found: C, 25.6; H, 2.6; S, 12.2. Calculated for C₆H₅O₄S₂H₂O. C, 25.8; H, 3.2; S, 11.5%. Found: C, 23.9; H, 2.1. C₆H₅O₄SN₄.H₂O requires C, 23.6; H, 1.6%).

Catalytic Hydrogenation of 2-Amino-4-hydroxypteridine-6-sulphonic acid (XCV; R = SO₃H).

Adam's catalyst (0.20 g.) in water (30 ml.) was hydrogenated until uptake of hydrogen ceased (46 ml.). 2-Amino-4-hydroxypteridine-6-sulphonic acid (0.10 g.) and Na-sodium hydroxide solution (2 ml.) were added and the solution hydrogenated overnight at room temperature and pressure. Two mols. of hydrogen were absorbed (16.5 ml., theoretical, 18 ml.).

The product 2-amino-4-hydroxy-5,6,7,8-tetrahydropteridine-6-sulphonic acid was extremely unstable and could not be isolated. Its ultraviolet spectra was characteristic of a 7,8-dihydropteridine formed by oxidation of the tetrahydropteridine. Aerial oxidation of this compound in alkaline solution and examination of the products by chromatography showed the presence of 2-amino-4-hydroxypteridine in addition to 2-amino-4-hydroxypteridine-6-sulphonic acid.
2-Amino-4-hydroxy-6-diethoxycarbonylmethyl-5,6,7,8-tetrahydropteridine (CXLII, \( R^I = R^II = \text{CO}_2\text{C}_2\text{H}_5 \)).

2-Amino-4-hydroxy-7,8-dihydropteridine sodium salt (0.20 g.) was dissolved in water (25 ml.) without heat. Diethylmalonate (2 ml.) was added and 2\( \text{Na} \)-sodium hydroxide solution dropwise until a clear solution was obtained.

The solution was shaken briefly, then left for 1 hr., at room temperature in a tightly-stoppered flask. The tetrahydropteridine was obtained as a yellow, non-crystalline solid (0.17 g., 49%), which was collected and washed with water, ethanol, and ether. This material was extremely unstable in solution and, in alkali, it underwent hydrolysis and oxidation to yield 2-amino-4-hydroxypteridine as the main product. A freshly-prepared sample could not be hydrogenated with a platinum catalyst in aqueous solution. Recrystallisation from water and ethanol did not give pure product, and analyses were carried out on the uncrystallised material which was dried in vacuo over potassium hydroxide pellets for 2 hr., with no heat. (Found: C, 45.3; H, 6.1; N, 20.5. \( \text{C}_{18}\text{H}_{19}\text{N}_{5}\text{O}_{6} \) requires C, 45.5; H, 6.2; N, 20.4%).

2-Amino-4-hydroxy-6-(\( \alpha \)-acetyl-\( \alpha \)-ethoxycarbonylmethyl)-5,6,7,8-tetrahydropteridine (CXLII, \( R^I = \text{COCH}_3, R^II = \text{CO}_2\text{C}_2\text{H}_5 \)).
2-Amino-4-hydroxy-7,8-dihydropteridine sodium salt (0.40 g.) was dissolved without heat in water (30 ml.) and ethylacetocetate (2 ml.) was added. 2N-Sodium hydroxide solution was added dropwise until a clear solution was obtained, the solution was shaken briefly and left for 1 hr. at room temperature in a tightly-stoppered flask. On cooling overnight at 0°, the tetrahydropteridine was deposited as a non-crystalline yellow solid (0.28 g., 44.5%). This was collected, and washed with water, ethanol, and ether. This material was extremely unstable and underwent hydrolysis and oxidation in alkaline solution to give 2-amino-4-hydroxypterdine as the main product. A freshly-prepared sample could not be hydrogenated. Recrystallisation from water and ethanol did not give pure product, and analyses were carried out on the uncrystallised material which was dried in vacuo over potassium hydroxide pellets without heat. (Found: C, 44.6; H, 6.1. C_{12}H_{14}O_{5} requires C, 44.7; H, 6.2%).

2-Amino-4-hydroxy-6-acetylacetonyl-5,6,7,8-tetrahydropteridine (CXLIX, \( R' = R'' = \text{CCCH}_3 \)).

2-Amino-4-hydroxy-7,8-dihydropteridine sodium salt (0.04 g.) was dissolved in water (10 ml.) and acetylacetone (2 ml.) was added. 2N-Sodium hydroxide solution was added
dropwise until a clear solution was obtained. The reaction mixture was worked up as described above to give the tetrahydropteridine (0.21 g., 37%). (Found: C, 46.2; H, 6.2.
C_{11}H_{13}N_{5}O_{3} \cdot H_{2}O requires C, 46.7; H, 6.0%).

2-Amino-4-hydroxy-6-(α-cyano-α-ethoxycarbonylmethyl)-5,6,7,8-
tetrahydropteridine (CXXI; R' = CN, R'' = CO_{2}C_{2}H_{5}).

2-Amino-4-hydroxy-7,8-dihydropteridine sodium salt (0.40 g.) was dissolved in water (30 ml.) without heat and ethylecyanacetate (3 ml.) was added. The reaction mixture was worked up as before and the tetrahydropteridine (0.24 g., 40%) was isolated as above (Found: C, 44.6; H, 5.7. C_{11}H_{14}N_{6}O_{3} \cdot H_{2}O requires C, 44.6; H, 5.5%).

Aerial oxidation in alkali of the four tetrahydropteridines described above gave 2-amino-4-hydroxypteridine as the main product on examination by chromatography. Aerial oxidation in ammonia gave 2,6-diamino-4-hydroxypteridine as one of the products identified by R_{f} and ultraviolet spectra in acid and alkali.

2-Amino-4-hydroxypteridine-6-carboxylic acid (XCV; R = CO_{2}H).
(a) From 2-amino-4-hydroxy-5,8-dihydropteridine-6-carboxamide (CXXII; R = CONH_{2}).

2-Amino-4-hydroxy-5,8-dihydropteridine-6-carboxamide (0.30 g.) was dissolved in Na-sodium hydroxide solution (10 ml.)
0.025M-potassium permanganate solution was added dropwise, with stirring, until a permanent green colouration persisted. This was destroyed by the addition of a little solid sodium dithionite and the precipitate of manganese dioxide was removed by filtration. The precipitate was washed with hot water (4 ml.), the combined washings and filtrate cooled, and adjusted to pH 2 with 2N-hydrochloric acid. A cream-coloured non-crystalline solid (0.24 g., 80%) was precipitated, and this was collected and washed with water, ethanol, and ether. This material was purified by formation of the sodium salt—

The crude product (0.24 g.) was dissolved in the minimum of 2N sodium hydroxide with gentle heating on the steam-bath.

Charcoal was added and the mixture was heated for a further 15 min. The hot solution was filtered and cooled at 0°. The sodium salt crystallised as fine colourless needles (0.29 g., 78%). These were collected and washed with aqueous ethanol, ethanol, and ether. An analysis specimen was prepared by dissolving the sodium salt in water and adjusting the solution to pH 2 with 2N-hydrochloric acid to precipitate the free acid. This procedure was repeated until the material obtained showed a single spot on paper chromatography. (Found: C, 40.8; H, 2.6; N, 35.9. Calculated for C₁₁H₁₉N₆O₈: C, 40.6; H, 2.4; N, 33.9%).
(b) From 2-amino-4-hydroxy-6-diethoxycarbonylmethyl-5,6,7,8-tetrahydropteridine (CXLII; $R' = R'' = CO_2 C_2 H_5$).

2-Amino-4-hydroxy-6-diethoxycarbonylmethyl-5,6,7,8-tetrahydropteridine (0.50 g.) was dissolved in dilute sulphuric acid (20 ml.) without heat and 0.5M-potassium permanganate solution was added dropwise with stirring until a permanent pink colouration prevailed. Paper chromatography at this stage and examination of the chromatogram in ultraviolet light showed the presence of a blue-fluorescing material as the main product together with 2-amino-4-hydroxypteridine as a by-product of the reaction. The solution was now made alkaline with 10N-sodium hydroxide solution (5 ml.) and the mixture was heated on the steam-bath. Dropwise addition of 0.5M-potassium permanganate was continued, with stirring, until a permanent green colouration was obtained. This was destroyed by addition of a little solid sodium dithionite. The precipitate of manganese dioxide was then filtered and washed with hot water (15 ml.). The filtrate was cooled and adjusted to pH 2 with 5N-hydrochloric acid when crude 2-amino-4-hydroxypteridine-6-carboxylic acid was precipitated as a cream-coloured solid (0.07 g.). This was collected and washed with water, ethanol, and ether. Paper chromatography indicated the presence of
some 2-amino-4-hydroxypteridine, and the carboxylic acid was therefore purified by formation of its sodium salt prepared as described in method (a). The purified material was identical in Rf values, ultraviolet and infrared spectra with the carboxylic acid obtained by method (a).

(c) From 2-Amino-4-hydroxy-6-(α-acetyl-α-ethoxycarbonylmethyl)-5,6,7,8-tetrahydropteridine (CXLI; R' = COCH3, R'' = CO2C2H5).

2-Amino-4-hydroxy-6-(α-acetyl-α-ethoxycarbonylmethyl)-5,6,7,8-tetrahydropteridine (0.25 g.) was oxidised with potassium permanganate as described immediately above, and the product purified via the sodium salt to give 2-amino-4-hydroxypteridine-6-carboxylic acid (0.03 g., 17%) identified with authentic material. The crude product contained 2-amino-4-hydroxypteridine.

(d) From 2-Amino-4-hydroxy-6-acetylacetonyl-5,6,7,8-tetrahydropteridine (CXLI; R' = R'' = COCH3).

2-Amino-4-hydroxy-6-acetylacetonyl-5,6,7,8-tetrahydropteridine (0.24 g.) was oxidised as described above to give 2-amino-4-hydroxypteridine-6-carboxylic acid (0.07 g., 37.4%) with small amounts of 2-amino-4-hydroxypteridine.

(e) From 2-Amino-4-hydroxy-6-(α-cyano-α-ethoxycarbonylmethyl)-5,6,7,8-tetrahydropteridine (CXLI0; R' = CN, R'' = CO2C2H5).

2-Amino-4-hydroxy-6-(α-cyano-α-ethoxycarbonylmethyl)-5,6,7,8-tetrahydropteridine (0.22 g.), on oxidation with
potassium permanganate gave the pteridine-6-carboxylic acid (0.04 g., 24.4%), together with 2-amino-4-hydroxypteridine.

2-Amino-4-hydroxy-5,6,7,8-tetrahydropteridine-6-thioglycollic acid (CXLIII, R = CH₂CO₂H).

2-Amino-4-hydroxy-7,8-dihydropteridine sodium salt (0.15 g.) was dissolved in water (20 ml.). 2N-Sodium hydroxide solution (2 drops) was added, then thioglycollic acid (1 ml.). The solution was shaken briefly then set aside in a stoppered flask for 1 hr. at room temperature. The tetrahydropteridine separated as a white solid (0.14 g., 68%) which was collected and washed with water, ethanol, and ether. The material was reasonably stable (see discussion). However, in alkaline solution it underwent hydrolysis and oxidation to give 2-amino-4-hydroxypteridine. Analysis samples were taken from the uncrystallised material and were dried in vacuo over potassium hydroxide pellets for 2 hr., without heat. (Found: C, 36.3; H, 4.0; S, 11.2. C₈H₁₁No₃S·O·5H₂O requires C, 36.1; H, 4.5; S, 12.0%).

2-Amino-4-hydroxy-5,6,7,8-tetrahydropteridine-6-mercaptoethanol (CXLIII, R = CH₂CH₂OH).

2-Amino-4-hydroxy-7,8-dihydropteridine sodium salt (0.10 g.) was dissolved in water (10 ml.). 2N-Sodium hydroxide solution (2 drops) was added followed by mercaptoethanol (1 ml.).
The solution was shaken and left for 1 hr. at room temperature in a stoppered flask. On cooling overnight the tetrahydropteridine separated as colourless plates (0.08 g., 61.5%). This was collected and washed with water, ethanol, and ether. This material had the instability characteristic of tetrahydropteridines and in alkaline solution oxidised to give 2-amino-4-hydroxypteridine as the main product. Analysis samples from the crude product were treated as described above. (Found: C,36.5; H,6.3; N,27.2. C₈H₁₃N₆O₂S.H₂O requires C,36.8; H,5.8; N,26.8%).

Neither of these tetrahydropteridines could be hydrogenated and when oxidised in ammonia gave 2,6-diamino-4-hydroxypteridine in addition to other products which were not identified.
Glucosazone

D-Glucose (20 g.), sodium acetate trihydrate (60 g.), and a saturated solution (50 ml.) of sodium meta-bisulphite were added to a solution of phenylhydrazine hydrochloride (40 g.) in water (400 ml.). The solution was filtered to remove tarry material and heated on a steam-bath until precipitation of the yellow azasone was complete. The solid was collected, washed with warm ethanol, until the washings were colourless and then with ether and dried to give glucosazone (30 g., 75%) m.p. 210° (lit., 210°).

Isoglucosamine Acetate

Glucosazone (20 g.) was made into a paste with a mixture of glacial acetic acid (100 ml.), ethanol (50 ml.) and water (20 ml.). A palladium oxide-barium sulphate catalyst (5.0 g.) was added to the mixture which was shaken overnight under hydrogen at 3-5 atm. The catalyst was removed and the filtrate was concentrated in vacuo to an orange oil. Ethanol (100 ml.) was added and the mixture was left overnight at 0°. A white solid separated, and this was removed and washed with ethanol (2 x 20 ml.). Crystallisation from 90% ethanol gave isoglucosamine acetate as colourless needles (8 g., 60%) m.p. 157° (lit., 157°).
An ethanolic solution of sodium (0.2 g., 2 eqs.) in ethanol (20 ml.) was added to a solution of isoglucosamine acetate (2.0 g., 2 eqs.) in the minimum of water and the mixture was left for 30 min. at room temperature. A suspension of 2-amino-4-chloro-6-hydroxy-5-nitropyrimidine (0.80 g., 1 eq.) in ethanol (200 ml.) was added, and the mixture was heated gently on the steam-bath for 20 min. The pyrimidine dissolved and, on cooling at 0°C, a pale yellow non-crystalline solid (0.80 g., 57%) separated. This material was collected and washed with ethanol, and ether. The material was hygroscopic and was characterised as its oxime:

hydroxylamine hydrochloride (0.085 g., 2 eq.) was dissolved in the minimum of water, sodium (0.028 g., 2 eqs.) in ethanol (2.8 ml.) was added, and the solution was left for 30 min. at room temperature. A solution of 1-[2'-amino-6'-hydroxy-5'-nitro-4'-pyrimidinylamino]-1-deoxy-D-fructose (0.20 g., 1 eq.) in water (7 ml.) was added to the solution and the mixture was heated on the steam-bath for 1 hr. On cooling, a white microcrystalline solid separated (0.20 g.). Recrystallisation from water gave the oxime (0.18 g., 86%). (Found: C, 33.5; H, 5.1;
7,8-Dihydroxanthopterin (2-amino-4,6-dihydroxy-7,8-dihydropteridine) (XCIII).

1-[2'-Amino-6'-hydroxy-5'-nitro-4'-pyrimidinylamine]-1-deoxy-D-fructose (0.70 g.) was dissolved in water (40 ml.) and Raney nickel (2 spatula measures) was added. The mixture was hydrogenated overnight at normal pressure when 3 mls. of hydrogen were absorbed (130 ml. theoretical 141 ml.).

The catalyst was removed by filtration and the solution was made alkaline by addition of 2N sodium hydroxide solution (1 ml.). The solution was set aside for 72 hr. and, on neutralisation of the solution and reducing the volume in vacuo, a brown non-crystalline solid (0.20 g.) separated. Recrystallisation from 0.5N-hydrochloric acid gave 7,8-dihydroxanthopterin as a cream coloured solid (0.14 g., 37%). This material was oxidised quantitatively to xanthopterin, and agreed in Rf values ultraviolet and infrared spectra with authentic material.

N-Benzyl-D-Arabinosylamine (GIW R = CH2C6H5).

D-Arabinose (5 g.) and benzylamine (4 g.) were refluxed together in ethanol (50 ml.) for 15 min. The mixture was left at 0° for 2 days when a white solid (5 g., 60°) separated. This was filtered at the pump and washed with water, ethanol, and ether.
Recrystallisation from ethanol gave the glycosylamine as fine colourless needles, m.p. 118° [Lit. m.p. 117-118°(dec)].

1-Benzylamine-1-deoxy-D-erythropentulose Oxalate

N-Benzyl-D-arabinosylamine (5 g.) was dissolved in dry dioxan (70 ml.) and cooled, and a cold solution of anhydrous oxalic acid (1.88 g.) in dry dioxan (50 ml.) was added. A colourless oil formed which was probably the glycosylamine hemioxalate. The flask was stoppered and the oil dissolved by gentle heating in a water bath with vigorous shaking. The temperature was kept below 50° at which temperature the Amadori rearrangement took place and the solution thickened to a pale yellow jelly. The jelly was set aside for 30 min. and then dissolved by the addition of water (6 ml.). Benzylamine hemioxalate separated as a colourless crystalline solid and was removed by filtration. The filtrate was refrigerated overnight to deposit 1-benzylamine-1-deoxy-D-erythropentulose oxalate as colourless needles (6 g., 80%) m.p. 145° [Lit. 145-146°(dec.)].

1-[2'-Amino-4'-hydroxy-5'-nitro-4'-pyrimidinylamino]-1-deoxy-D-erythropentulose (GIWI).

1-Benzylamine-1-deoxy-D-erythropentulose (3 g.) in methanol (20 ml.) was hydrogenated overnight with a previously reduced suspension of palladium on charcoal catalyst (10%, 1 g.)
in methanol (20 ml.). One mol. of hydrogen was absorbed (200 ml., theoretical, 204 ml.), and the resulting solution of 1-amino-1-deoxy-D-erythropentulose oxalate was used without isolating solid material. The catalyst was removed by filtration and a solution of sodium (0.4 g., 2 eqs.) in ethanol (40 ml.) was added. The sodium oxalate was removed by filtration and the filtrate was added to a solution of 2-amino-4-chloro-6-hydroxy-5-nitropyrimidine (0.325 g., 1 eq.) in ethanol (100 ml.). The mixture was heated for 20 min. on the steam-bath and filtered hot. On cooling at 0° overnight a cream colored solid (0.75 g., 58% separated). This material was hygroscopic and was characterised as its oxime-

hydroxylamine hydrochloride (0.12 g., 2 eqs.) was dissolved in water (4 ml.) and sodium (0.04 g., 2 eqs.) in ethanol (4 ml.) was added. The mixture was set aside for 30 min. then added to a solution of 1-[2′-amino-4′-hydroxy-5′-nitro-4′-pyrimidinylamino]-1-deoxy-D-erythropentulose (0.26 g., 1 eq.) in water (15 ml.) and the mixture was heated on the steam-bath for 1 hr. On cooling a white microcrystalline solid (0.2 g.) separated. Recrystallisation from water gave the oxime (0.15 g., 57%). (Found: C, 33.0; H, 4.3; N, 25.3. C₉H₁₄N₃O₄·0.5H₂O requires C, 33.0; H, 4.6; N, 25.7%).
1-[2′-Amino-4′-hydroxy-5′-nitro-4′-pyrimidinylamino]-
1-deoxy-D-erythro-pentulose (0.20 g.) in water (30 ml.) and
Raney nickel (2 spatula measures) was added. The mixture was
hydrogenated overnight at room temperature and pressure when
3 moles of hydrogen were absorbed (46 ml., theoretical, 45 ml.).
The catalyst was removed by filtration, the solution made
alkaline by addition of 2N-sodium hydroxide solution (1 ml.),
and set aside for 72 hr. Neutralisation of this solution
did not precipitate a solid. The solution, when
subjected to chromatography, was shown to be a mixture of
several compounds. The presence of 7,8-dihydroxanthopterin was
established by Rf comparison with an authentic sample, and,
on oxidation with cold potassium permanganate, the presence of
xanthopterin was observed by comparison of Rf values and
ultraviolet spectra. The remaining compounds were not
identified.
ULTRAVIOLET ABSORPTION SPECTRA

$\lambda_{\text{max}}$(nm) $\varepsilon$ values in parenthesis.

2-Amino-4-chloro-6-hydroxy-5-nitropyrimidine (CVIII), 300 \( \text{pH} \).

5-Nitropyrimidines (CIX).

<table>
<thead>
<tr>
<th>R</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
<th>$\varepsilon$ (M$^{-1}$cm$^{-1}$)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{CH}_2\text{C}_6\text{H}_3$</td>
<td>332</td>
<td></td>
<td>pH1.</td>
</tr>
<tr>
<td>$\text{NHCH}_2\text{CH(OC}_2\text{H}_3)_2$</td>
<td>314(9,900)</td>
<td></td>
<td>pH1.</td>
</tr>
<tr>
<td>$\text{NHCH}_2\text{CHO}$</td>
<td>314(9,200)</td>
<td></td>
<td>pH1.</td>
</tr>
<tr>
<td>$\text{NHCH}_2\text{CH}(:\text{NOH})$</td>
<td>314(11,800)</td>
<td></td>
<td>pH1.</td>
</tr>
<tr>
<td>$\text{NHCH}_2\text{CONH}_2$</td>
<td>332(13,400)</td>
<td></td>
<td>pH1.</td>
</tr>
<tr>
<td>$\text{NHCH}_2\text{CO}_2\text{C}_2\text{H}_3$</td>
<td>344(7,900)</td>
<td></td>
<td>pH1.</td>
</tr>
<tr>
<td>$\text{NHCH}_2\text{CN}$</td>
<td>330(16,300)</td>
<td></td>
<td>pH1.</td>
</tr>
<tr>
<td>$\text{NHCH}_2\text{CO(CHOH)}_3\text{CH}_2\text{OH}$</td>
<td>332</td>
<td></td>
<td>pH1.</td>
</tr>
<tr>
<td>$\text{NHCH}_2\text{C(\text{NOH})(CHOH)}_3\text{CH}_2\text{OH}$</td>
<td>334(16,600)</td>
<td></td>
<td>pH1.</td>
</tr>
</tbody>
</table>
R = NHCH₂ CO(CHOH)₂ CH₂ OH  334(12,200).  pH₁.
       340(15,700).  pH₁₃.
R = NHCH₂ C(=NOH)(CHOH)₂ CH₂ OH  335.  pH₁.
       342.  pH₁₃.

4,5-Diaminopyrimidine Derivatives (CXIII).
R = H  262(14,300).  pH₁.
       280(4,100).  pH₁₃.
R = NHCH₂ CH(OC₂ H₅)₂  270.  pH₁.
       282.  pH₁₃.
R = NHCH₂ CHO  266.  pH₁.
       280.  pH₁₃.
R = NHCH₂ C(=NOH)(CHOH)₂ CH₂ OH  266.  pH₁.
       286.  pH₁₃.

7,8-Dihydropteridines (CXXI).
R = H  256(12,900), 314(5,600).  pH₁.
       284(9,500), 330(7,300).  pH₁₃.
R = NH₂  274(13,600), 319(6,100).  pH₁.
       280(10,800), 310(6,800).  pH₁₃.

2-Amino-4,6-dihydroxy-7,8-dihydropteridine (XCIII).
278(14,000), 306(10,700).  pH₁.
       278(12,300), 310(9,000).  pH₁₃.
### 5,6-Dihydropteridines (CXXII).

| $R$          | $\lambda$ (nm) | $\varepsilon$ (M⁻¹ cm⁻¹) | pH | \(\text{pH}^+\) |
|--------------|----------------|---------------------------|----|----------------| |
| CONH₂        | 280 (5,300), 380 (4,500) |                             | pH1. |                   |
|              | 260 (19,000), 396 (11,300) |                             | pH13. |               |

### Pteridines (XCV).

| $R$          | $\lambda$ (nm) | $\varepsilon$ (M⁻¹ cm⁻¹) | pH | \(\text{pH}^+\) |
|--------------|----------------|---------------------------|----|----------------| |
| H            | 314 (6,000) |                             | pH1. |                   |
|              | 252 (20,600), 358 (7,300) |                             | pH13. |               |
| NH₂          | 271 (21,700), 377 (7,000) |                             | pH1. |                   |
|              | 260 (16,000), 392 (5,200) |                             | pH13. |               |
| SO₃H         | 325 (9,900) |                             | pH1. |                   |
|              | 264 (19,000), 369 (6,900) |                             | pH13. |               |
| CO₂H         | 263 (6,600), 310-20 (5,800) |                             | pH1. |                   |
|              | 266 (18,800), 366 (7,900) |                             | pH13. |               |

### 2-Amino-4,6-dihydroxypteridine (XLV).

| $R$          | $\lambda$ (nm) | $\varepsilon$ (M⁻¹ cm⁻¹) | pH | \(\text{pH}^+\) |
|--------------|----------------|---------------------------|----|----------------| |
| H            | 260 (9,800), 355 (4,900) |                             | pH1. |                   |
|              | 254 (17,000), 390 (6,500) |                             | pH13. |               |

### Tetrahydropteridines (XCIX).

| $R$          | $\lambda$ (nm) | $\varepsilon$ (M⁻¹ cm⁻¹) | pH | \(\text{pH}^+\) |
|--------------|----------------|---------------------------|----|----------------| |
| H            | 268.           |                             | pH1. |                   |
| NH₂          | 270.           |                             | pH1. |                   |
|              | 280.           |                             | pH13. |               |
| SO₃H         | 265,314.       |                             | pH1. |                   |
Tetrahydropteridines (CXLII).

<table>
<thead>
<tr>
<th>R' = R'' = CO₂ C₂ H₅</th>
<th>264(10,900), 300(7,000). pH1.</th>
</tr>
</thead>
<tbody>
<tr>
<td>R' = COCH₃, R'' = CO₂ C₂ H₅</td>
<td>268(9,900), 300(4,100), 386(2,600). pH1.</td>
</tr>
<tr>
<td>R' = R'' = COCH₃</td>
<td>260(10,300). pH13.</td>
</tr>
<tr>
<td></td>
<td>292(24,400).</td>
</tr>
<tr>
<td>R' = CN, R'' = CO₂ C₂ H₅</td>
<td>300(8,000), 270(6,800). pH1.</td>
</tr>
<tr>
<td></td>
<td>248(14,200), 290(9,700), 364(6,700). pH13.</td>
</tr>
</tbody>
</table>

Tetrahydropteridines (CXLIII).

| R = CH₂ CH₂ OH        | 256(17,700), 370(3,700). pH1. |
| R = CH₂ CO₂ H         | 282(18,000). pH1.            |
|                       | 275(13,100). pH13.          |

Shoulders are underlined.
Fig. 1 Wavelength (nm)

Fig. 2 Wavelength (nm)


27. Albert and Reich, J., 1961, 127.
32. Gabriel and Colman, Ber., 1904, 37, 3643.
33. Schopf and Oeschler, Annalen, 1936, 523, 1.
34. Fry, Kendall and Morgan, J., 1960, 5062.


47. Boon, Jones and Ramage, *J.*, 1951, 96.


