8-SUBSTITUTED PTERIDINES AND RELATED TOPICS.

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A THESIS

submitted to

THE UNIVERSITY OF GLASGOW

in fulfilment of the

requirements for the

DEGREE OF DOCTOR OF PHILOSOPHY

by

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September, 1956.

ACKNOWLEDGEMENTS.

The author wishes to thank Professor F.S. Spring, D.Sc.,F.R.S., for facilities provided, and for his interest in the work described in this thesis. He also wishes to record his gratitude to Dr. H.C.S.Wood for his excellent supervision and enthusiastic guidance throughout the course of this work.

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HISTORICAL

Naturally Occurring Pteridines.

Pteridines were first isolated from natural sources, such as the wings of lepidoptera, by Hopkins[1,2,3] in the 1890's. Synthetic compounds having the pteridine nucleus were synthesised first about the same time by Kuhling[4]. The relationship was not recognised until 1940[5,6] when Wieland and his collaborators[7] showed that the wing pigments of butterflies investigated by Hopkins were derived from the pteridine nucleus(I).

Since that time a large number of pteridines have been isolated from a wide variety of natural sources[8,9]. Among the most important of these are the pteridines which constitute the folic acid series.

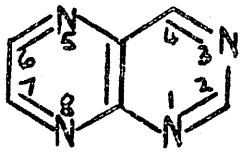
The Folic Acid Series.

(a) Chemical Nature: The members of the group of biologically active compounds called the Folic acids are very similar chemically. All contain the 2-amino and 4-hydroxy groups common to naturally occurring pteridines and all contain a p-aminobenzoic acid residue. Each compound provides the minimal requirement for growth, within the group, for one or another organism which converts its particular active member of the group to the co-enzyme form of the Folic acids. The chemical nature of

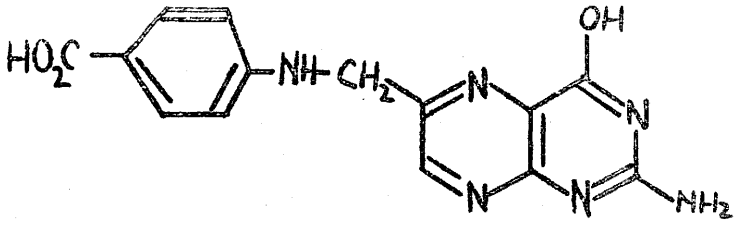
the co-enzyme form, co-enzyme F, is unknown. Leucovorin appears to be closest to it chemically, for leucovorin is a growth factor for Leuconostic citrovorum, for which the other folic acids are either not active or much less so, while leucovorin is as active as the other forms of folic acid for the less exacting organisms.

The first of the group to be characterised chemically was pteroylglutamic acid which was shown to have the full biological activity of a factor, occurring naturally, for the growth of Lactobacillus casei[10].

The relationship between the various members of the group is not yet clear. It is established that none lie on the normal metabolic pathway to the co-enzyme form[11,12]. Present evidence shows that all the biological functions of p-aminobenzoic acid are exerted through the ultimate co-enzyme form of the folic acids, each of which contains a p-aminobenzoic acid residue. Co-enzyme F may have slightly different forms[13,14,15] and conjecture has given rise to a largely hypothetical metabolic pathway from p-aminobenzoic acid to co-enzyme F. There are obviously a number of steps between pteroylglutamic acid and the co-enzyme (Scheme 1).



I



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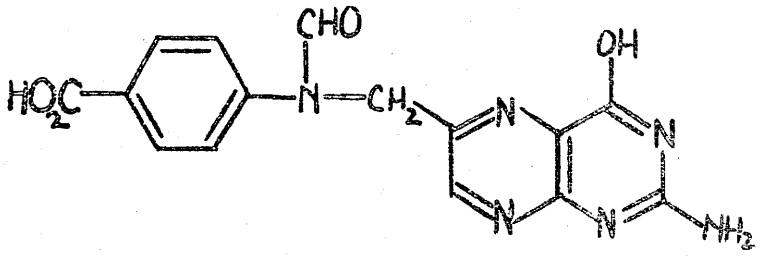

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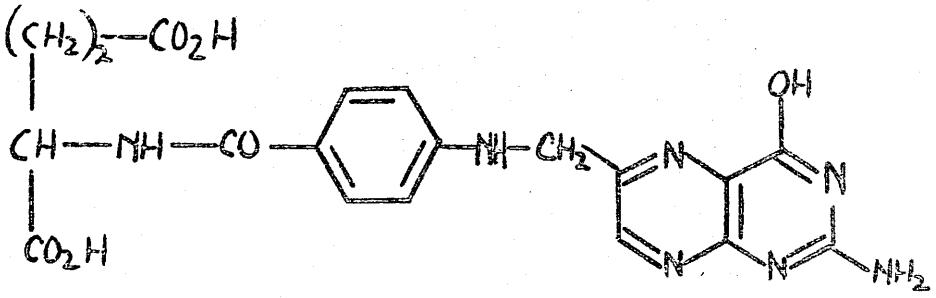

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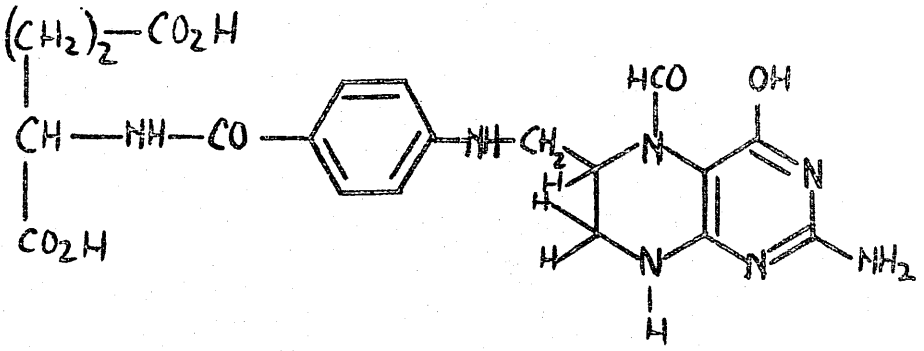


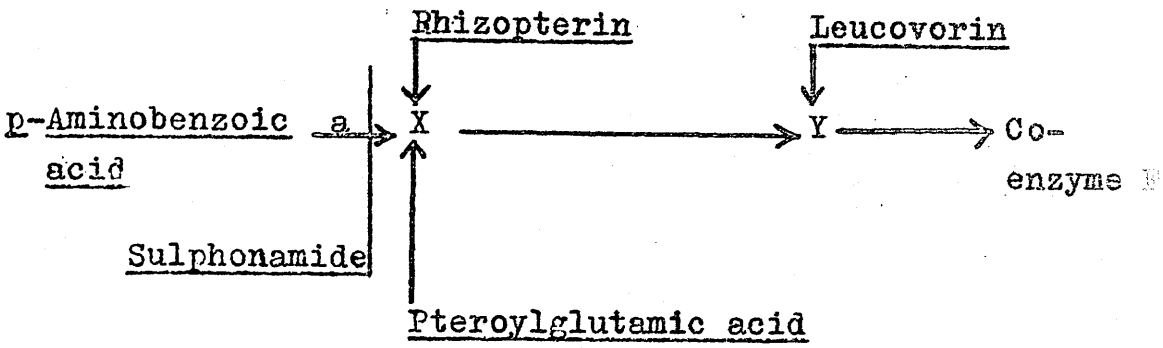
V

II Pteroic acid.

III Rhizopterin.

IV Pteroylglutamic acid.

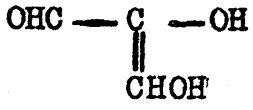
V Leucovorin (Folinic acid).



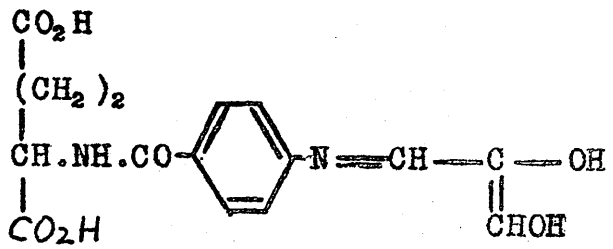
Scheme 1.

X and Y represent pteroylglutamic acid and leucovorin respectively as they exist in the cell. X is formed through utilisation of pteroylglutamic acid by some organisms and through biosynthesis from p-aminobenzoic acid by others (pathway a). The latter route is blocked competitively by sulphonamides. Y is formed by utilisation of leucovorin or metabolically from X[16]. Y may be a reduced, and or, formylated derivative of X or p-aminobenzoic may combine with a precursor of the pteridine moiety, perhaps a triaminopyrimidine, before ring closure occurs.

Forrest and Walker[17] postulate the condensation of reductone (VI) with p-aminobenzoylglutamic acid to give (VII) which then condenses with triamino-4-hydroxypyrimidine, a substance not yet demonstrated in nature.



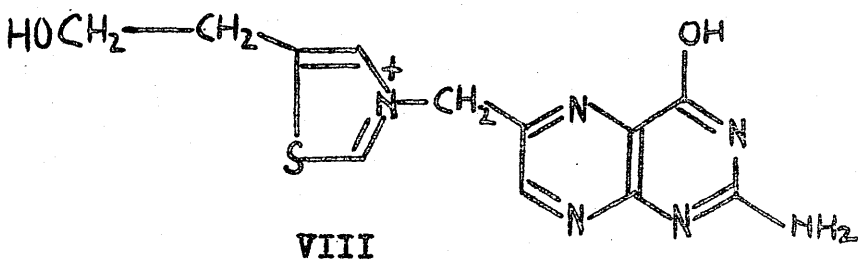
VI.



VII.

Tschesche and his co-workers[18] consider that 2-amino--4:7-dihydroxypteridine-6-aldehyde condenses with p--aminobenzoylglutamic acid (this compound has no growth promoting properties for micro-organisms) and that the product is reduced to pteroylglutamic acid. These workers have shown that this 6-aldehyde (not yet found in nature) as well as the 7-hydroxy derivatives of pteroylglutamic acid strongly promote the growth of S.faecalis.

Woolley believes that pterithiamine (VIII) is acted upon by thiaminase in the presence of p-aminobenzoyl-glutamic acid to give pteroylglutamic acid. Pterithi-amine is not known to occur in nature[19].



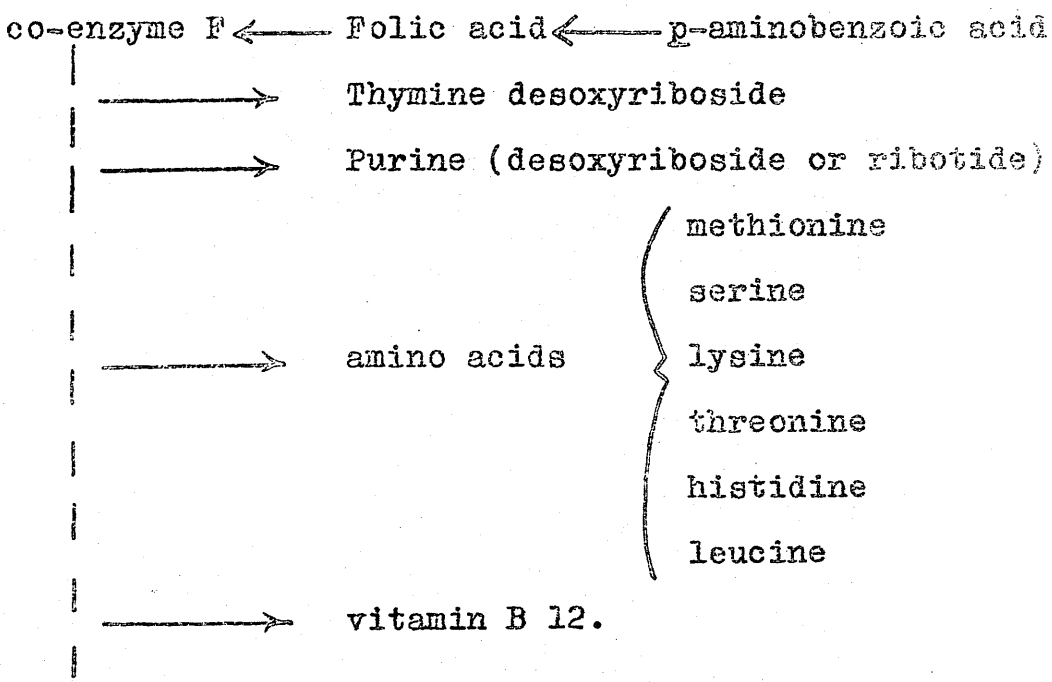
VIII

X and Y may contain some other chemical residue, possibly a pentose or pentose phosphate on N_8[20] which is present in co-enzyme F but not in the known forms of folic acid. In this connection, the pteridine derivatives which bear an alkyl group on N_8, described later in this thesis are of some interest. Greenberg[22] has shown that yeast extract contains a compound active in the ring closure of 5-amino-4-imidazolecarboxamide ribotide to inosine. The active compound, not yet isolated, may be made by incubation of leucovorin, or formate and tetrahydropteroylglutamic acid, with adenosine triphosphate and certain enzymes. Greenberg suggests that this compound is co-enzyme F. This co-enzyme F may contain an adenosine triphosphate residue or a portion of this molecule, or adenosine triphosphate may function merely as a source of phosphate and/or energy.

(b) Metabolic Functions: Folic acid is an absolute requirement for the growth of micro-organisms. These organisms which do not require an exogenous source synthesise folic acid from p-aminobenzoic acid.

The ability of various chemically unrelated compounds, purines, thymine, some amino acids, vitamin B_{12}, to sustain growth in a medium deficient in folic acid

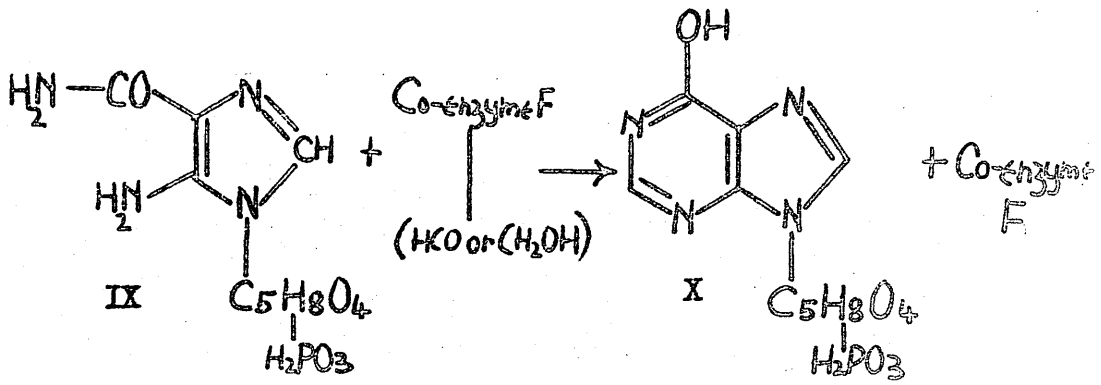
suggests that folic acid is concerned at some stage in the biosynthesis of these compounds[22]. It is now established that the function of folic acid is in the transfer of one carbon residues. The extent of folic acid implication in metabolism is summarised below (Scheme 2).



Scheme 2.

The role of folic acid in purine metabolism was first illuminated by the isolation and characterisation of 5-amino-4-imidazolecarboxamide which was found to accumulate in cultures of Bact. coli deficient in folic

acid[23,24]. This carboxamide requires only one carbon atom to complete ring closure to a purine. Leucovorin is active in the ring closure of 5-amino-4-imidazole-carboxamide ribotide 5'-phosphate (IX) to inosinic acid (X)[25].



It is probable that folic acid is concerned in the provision of the other ureido carbon in the purine skeleton for both have a common origin in formate, the methylene carbon of glycine or the ß-carbon of serine[26,27,28]. Further, in folic acid deficient rats incorporation of C^{14} formate into liver purines is reduced in both positions. Separate enzymes may be responsible for ring closure at the two positions with co-enzyme F a co-enzyme for both.

Pyrimidines have a similar ureido carbon atom to that of purines, there is, however, no evidence that folic acid has a role in ring closure of pyrimidines.

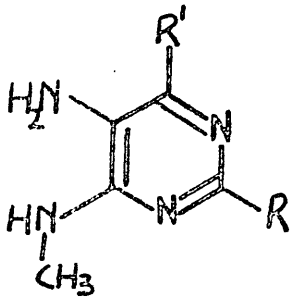
Studies with folic acid antagonists have shown that at the histological level the principal role of folic acid is in cell division. Folic acid antagonists prevent the cells of a leukaemic bone marrow from completing mitosis[29], and produce stunting of the chick embryo[30].

THEORETICAL

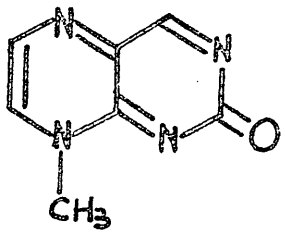
PART I

1. Bisdihydropurinyls.

The condensation of glyoxal with 5-amino-2-hydroxy--4-methylaminopyrimidine (XI; R = OH; R' = H) did not give the expected product 8-methyl-2-pteridone (XII), but gave a pale yellow insoluble compound in good yield. The properties of this compound were markedly different from those of N-alkyl pteridones[31]. It was insoluble in all organic solvents and decomposed without melting at a high temperature. It was soluble in acid and alkali and could be precipitated unchanged from either by neutralisation. Pteridones, on the other hand, are usually soluble in organic solvents, melt below 200° ., and are degraded by alkali[32]. Elementary analysis of the insoluble compound permitted the empirical formula $C_6H_7ON_4$. Insolubility prevented determination of the molecular weight. Solubility in alkali indicated the continued existence of the pyrimidine hydroxyl group as such. Attempts to methylate this group, in order to increase solubility, were made and the following reagents were tried without success: diazomethane; dimethyl sulphate and alkali; methyl iodide and potassium carbonate.



XI

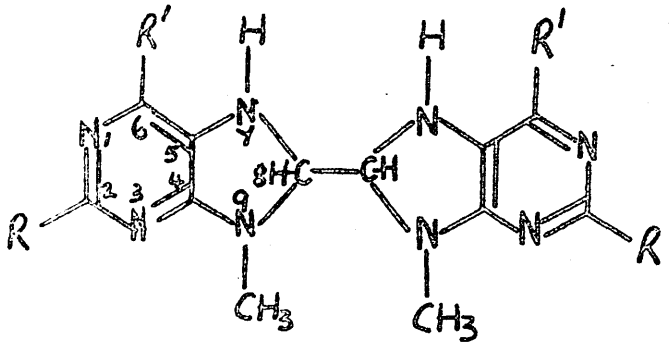


XII

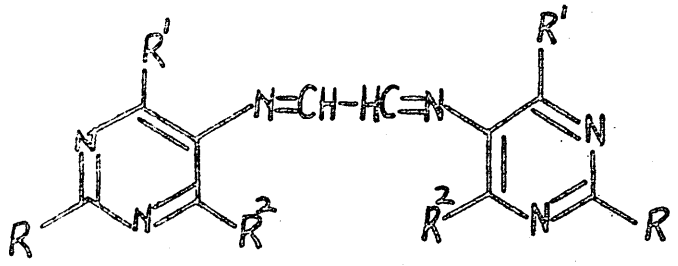
Analogous compounds were synthesised by the condensation of glyoxal with 5-amino-4-methylamino-pyrimidine (XI; R = R' = H), 5-amino-4:6-bismethylamino-pyrimidine (XI; R = H, R' = $NHCH_3$), or 5-amino-4-chloro--6-methylaminopyrimidine (XI; R = H, R' = Cl). The chloro compound was sufficiently soluble in camphor to permit of determination of the molecular weight by a modified Rast procedure. The molecular weight indicated that two pyrimidine nuclei had combined with one glyoxal molecule.

Two possible structures (XIII; and XIV; R^2 = $NHCH_3$) satisfied these requirements. The known lability of azomethines to acid and the stability of the compounds under investigation to acid, militated against formulation (XIV), but further evidence, upon which to base a conclusion, was required. Accordingly, several authentic azomethines were synthesised by the condensation of glyoxal with 5-amino-2:4-bisdimethylamino-

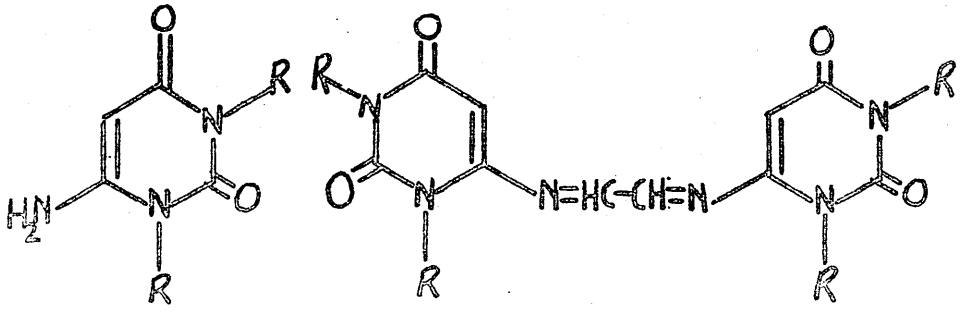
pyrimidine to give the bright red azomethine (XIV; $R = R^1 = N(CH_3)_2$, $R^2 = H$), with 4-amino-2:6-dihydroxy-pyrimidine (XV; R = H) to give the colourless azomethine (XVI; R = H), and with 6-amino-1:2:3:4-tetrahydro-1:3--dimethyl-2:4-dioxopyrimidine (XV; $R = CH_3$) to give the colourless azomethine (XVI; $R = CH_3$).



XIII



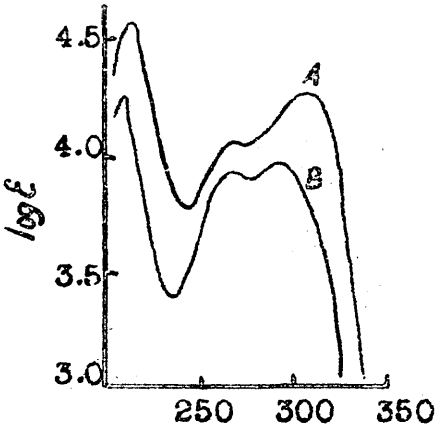
XIV



XV XVI

All three authentic azomethines were acid labile, reversion to the parent pyrimidine taking place on treatment with dilute acid. The ultra-violet absorption spectra of the three azomethines bore no resemblance to those of their parent pyrimidines (Fig.2). This is to be expected, since the conjugation between the two pyrimidine nuclei provided by the glyoxylidene moiety furnishes a new light absorbing system. The ultra-violet absorption spectrum of each insoluble compound, on the other hand, was very similar to the spectrum of its parent pyrimidine (Table I; Fig. 1).

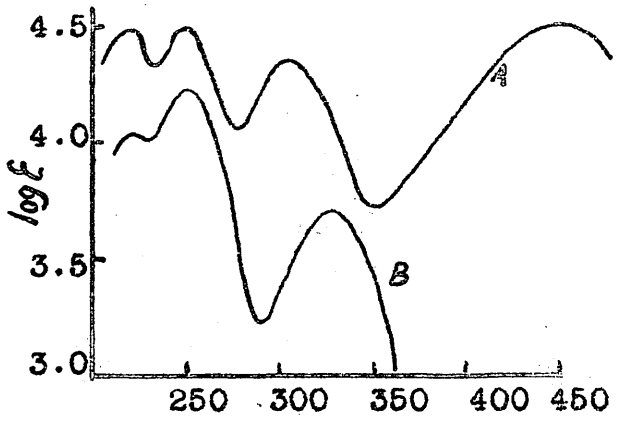
<u>Fig.1</u>. Absorption spectra in ethanol.



A, Bis-(6-Chloro-8:9--dihydro-9-methyl-purin-8-yl).

B, 5-Amino-6-chloro-4--methylaminopyrimidine.

<u>Fig.2</u>. Absorption spectra in ethanol.



A, Glyoxylidenebis-(5-amino-2:4--bisdimethylaminopyrimidine.

B, 5-Amino-2:4-bisdimethyl-aminopyrimidine.

Each insoluble compound showed an increase in the wavelengths of absorption in comparison with those of its parent pyrimidine, and also showed an increase in the intensities of absorption by a factor of two, both effects being attributable to the greater molecular weight of the insoluble compounds. (Lister and Ramage[33] investigated the synthesis and properties of some 5:6:7:8--tetrahydropteridines. They observed that the ultra--violet absorption spectra of these tetrahydropteridines closely resembled those of the corresponding 4:5-di-aminopyrimidines. The spectra of the pteridines showed a slight bathochromic shift due to increased molecular weight.). There was therefore no conjugation between the pyrimidine moieties in the insoluble compounds and the azomethine formulation (XIV) was discarded. The original insoluble compound is consequently formulated as bis-(8:9-dihydro-2-hydroxy-9-methylpurin-8-yl) (XIII; R = OH, R^1 = H) and the analogous compounds are formulated similarly (XIII; R = R^1 = H.XIII; R = H, R^1 = $NHCH_3$.XIII; R = H, R^1 = Cl).

The difference in colour observed in the three azomethines synthesised, one bright red and the others colourless, is due to lack of full aromaticity in the

Table 1.

Absorption spectra (in water).

Compound.	λmax(mu)	ε	pH
5-Amino-2-hydroxy-4--methylaminopyrimidine	222;294	8,850; 5,280	1
Bis-(8:9-dihydro-2--hydroxy-9-methylpurin--8-yl)	233;330	20,150;11,000	1
5-Amino-4-methylamino-pyrimidine	205;288	14,300; 8,700	7
Bis-(8:9-dihydro-9--methylpurin-8-yl)	209;305	29,500;15,300	7
5-Amino-4:6-bismethyl-aminopyrimidine	224;286[a]	25,100;10,250	-
Bis-(8:9-dihydro-9--methyl-6-methylamino-purin-8-yl)	227;292[a]	46,400;21,400	-
Azomethine (XXII)	<220;230; 258;410	>17,300;17,340 15,340;33,340	13
4-Amino-2:6-dihydroxy-pyrimidine	220;266	1,600 ; 3,580	13
Glyoxylidenebis-(4--amino-2:6-dihydroxy-pyrimidine)	222;245; 274	15,590;12,160; 12,800	13
6-Amino-1:2:3:4-tetra-hydro-1:3-dimethyl-2:4--dioxopyrimidine	<220;266	>2,890;20,950	7
Glyoxylidenebis-(6--amino-1:2:3:4-tetra-hydro-1:3-dimethyl-2:4--dioxopyrimidine)	<220;240; 274	>16,100;13,980; 18,980	7

[a] In ethanol.

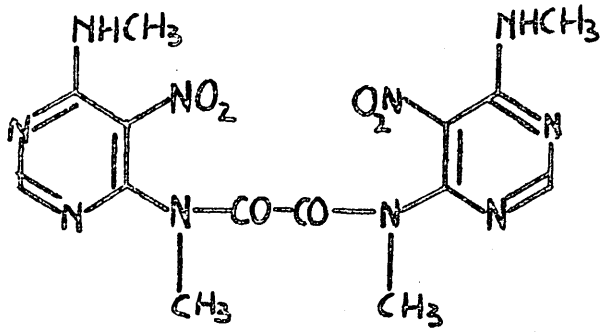
case of the two tetraoxo azomethines. That this is so in the case of the tetrahydroxy azomethine (XVI; R = H) is illustrated by the close resemblance in ultra-violet absorption spectrum of the tetrahydroxy azomethine (XVI; R = H) and of its 1:3:1':3'-tetramethyl homologue (XVI; R = CH_3) (Table 1).

In attempts to synthesise authentic azomethines it was observed that neither 5-aminopyrimidine nor 5-amino--4:6-dichloropyrimidine react with glyoxal under conditions similar to those producing bisdihydropurinyls. This is in agreement with the results of Whittaker[34] who condensed p-nitrobenzaldehyde with 5-aminopyrimidine only under drastic conditions. The ready condensation of p-nitrobenzaldehyde with 5-amino-4-chloro-6-methylaminopyrimidine to give an azomethine indicates that the presence of at least one electron releasing group, in addition to the 5-amino group, is essential for such reactions.

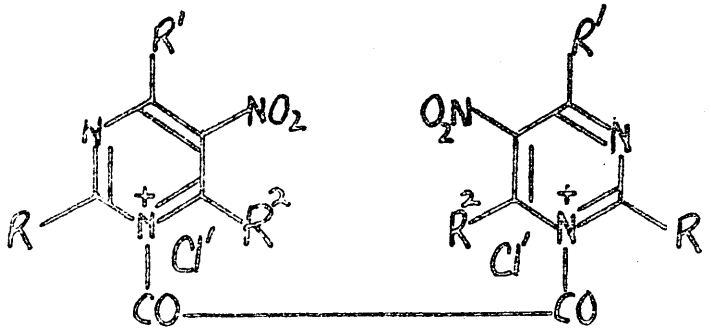
Attempts were made to synthesise a bisdihydropurinyl by another route. Oxalyl chloride was to be reacted with 4:6-bismethylamino-5-nitropyrimidine to give the amide (XVII) which on reduction of the nitro group and cyclisation would have given a bispurin-8-yl derivative.

The reaction product from the oxalyl chloride reaction, however, while analysing for the dihydrochloride of (XVII) was hydrolysed to the original pyrimidine by treatment with boiling water or with cold dilute sodium hydrogen carbonate solution. Hydrogenation using Raney nickel or platinum catalyst gave 5-amino-4:6-bismethylaminopyrimidine. A compound with similar properties and presumably with similar structure was obtained by reaction of oxalyl chloride with 2:4-bisdimethylamino--5-nitropyrimidine. The quaternary salt structures (XVIII; R = H, $R^1 = R^2 = NHCH_3$. and XVIII; $R = R^1 =$ $= N(CH_3)_2$, $R^2 = H$) are accordingly proposed for these compounds, although in the case of the 2:4-bisdimethylamino-5-nitropyrimidine product the possibility of quaternisation on N_3 in the pyrimidine ring or on an exocyclic nitrogen atom cannot be excluded. The case for quaternisation on a ring nitrogen atom in preference to amide formation, is strengthened by theoretical considerations. The potentially tautomeric heterocyclic amines are cyclic amidines or vinylogous amidines and will show to some extent the properties of amidines. As in all amidines cation formation occurs by proton addition to the doubly bonded nitrogen atom, that is,

to the ring nitrogen atom in an aminopyrimidine, for 2- and 4-aminopyrimidines are known to exist in the amino form[35,36,37].



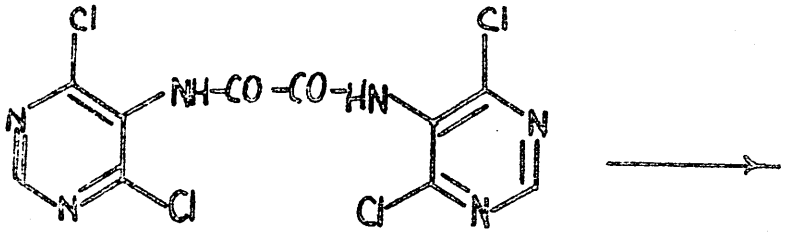
XVII



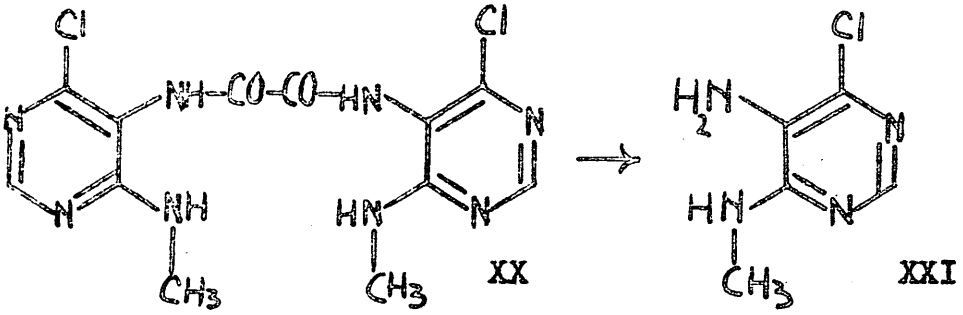
XVIII

A further attempt was made to synthesise a bis-dihydropurinyl by formation of the diamide (XIX) of oxalic acid and 5-amino-4:6-dichloropyrimidine by reaction of oxalyl chloride with this pyrimidine. Treatment of the diamide (XIX) with methylamine did not give the expected bismethylamino diamide (XX). Substitution of one chloro group per pyrimidine nucleus by methylamino took place but was followed by ammonolysis of the amide to give 5-amino-4-chloro-6-methylaminopyrimidine (XXI). That the reaction to the methylaminopyrimidine (XXI)

proceeds through the methylamino amide (XX) was shown when methylamine did not react with 5-amino-4:6-dichloropyrimidine under the conditions used for reaction with the diamide (XIX). It appears that the chloro groups are activated in the amide (XIX) by the unsaturated group at position 5 in the pyrimidine ring, due to the amide existing in the imidol form[38,39].



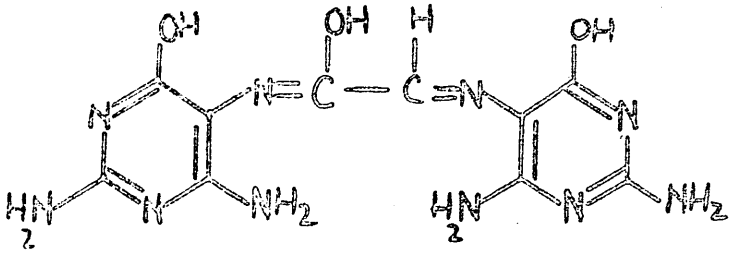
XIX



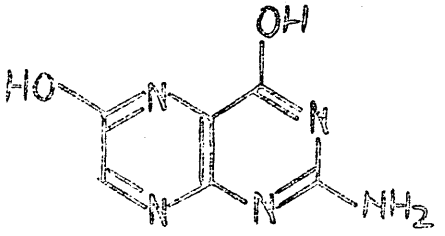
XX XXI

Purrmann[40] has reported the only previously known case of a diketone condensing with two moles of pyrimidine. He condensed chloral with 2:4:5-triamino-6-hydroxypyrimidine to give a compound $C_{10}H_{12}O_3N_{10}$, of unknown structure. This reaction was further investigated by Wood[68], who assigned an azomethine structure

(XXII) to the compound on grounds of spectroscopic comparison with the azomethine (XIV; R = R^1 = $N(CH_3)_2$, R^2 = H), and converted the azomethine (XXII) to xanthopterin (XXIII) by treatment with acid.



XXII



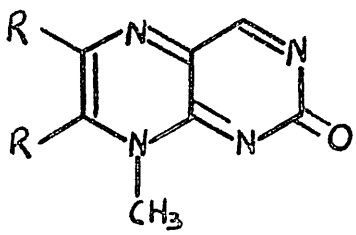
XXIII

2. 2-Pteridones.

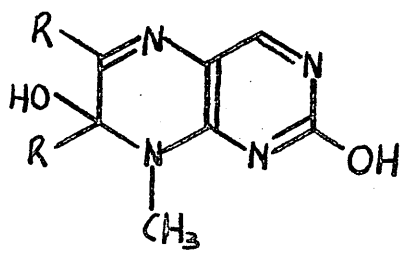
The condensation of glyoxal with 5-amino-2-hydroxy--4-methylaminopyrimidine (XI; R = OH, R^1 = H) has been shown to give rise to bisdihydropurinyls; the reaction of α-diketones with this pyrimidine was next investigated. Condensation of benzil with 5-amino-2-hydroxy-4-methylaminopyrimidine (XI; R = OH, R^1 = H) gave 8-methyl-6:7--diphenyl-2-pteridone (XXIV; R = Ph) and with diacetyl this pyrimidine gave the corresponding trimethyl-2--pteridone (XXIV; R = CH_3). The last named compound has also been prepared by Brown and Mason[41], who investigated the synthesis of all the possible monomethyl derivatives of the four monohydroxy pteridines. The author of this thesis here wishes to thank Dr. D. J. Brown for providing a copy of his paper in advance of publication. The paper was received subsequent to the work hereinunder reported and the conclusions of Brown and Mason are summarised later in this section.

The 2-pteridones (XXIV; R = Ph. XXIV; R = CH_3) are soluble in organic solvents, have melting points, and their absorption spectra are quite distinct from those of the bisdihydropurinyls. These 2-pteridones, however, exhibited peculiar physical and chemical properties

which placed their formulation as the pteridones (XXIV; R = Ph, XXIV; R = CH_3) in doubt. Both compounds have a strong affinity for 1 mole of water, which, in the case of the trimethyl pteridone (XXIV; R = CH_3) can be driven off by heating under vacuum. Hydration re-occurs when this compound is exposed to the atmosphere. In the case of the diphenyl analogue (XXIV; R = Ph) the mole of water cannot be removed. Both compounds are readily soluble in alkali and not at all in water. This property indicates the existence within the molecule of an acidic or phenolic group. The 8-alkyl-2-pteridones also show remarkable stability to alkali; relative to other N-alkyl pteridones. The diphenyl analogue (XXIV; R = Ph), when refluxed with 5 N-alkali for $6\frac{1}{2}$ hours degrades to the extent of only $\frac{2}{3}$rds. by weight. 1:3-Dimethyl-2:4-pteridione, on the other hand, hydrolyses to a pyrazine derivative on refluxing with 1 N-NaOH solution for 1 minute[42]. Both acid and alkaline degradation of the diphenyl pteridone (XXIV; R = Ph) have been investigated and a feasible route to the degradation products postulated.



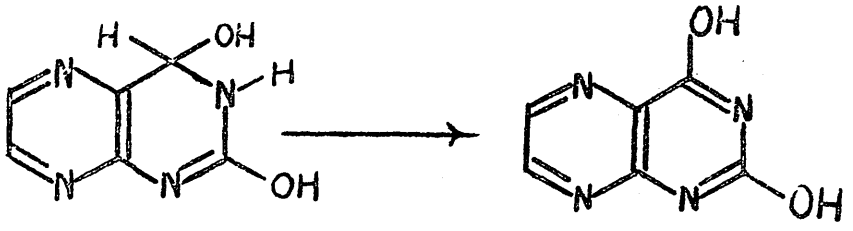
XXIV



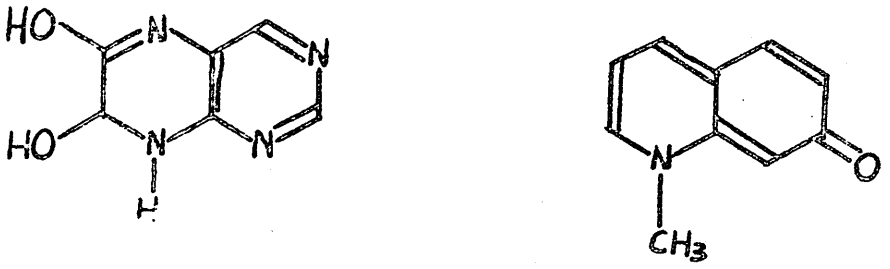
XXV

Alkaline solubility of, and retention of 1 mole of water by the 8-methyl-2-pteridones (XXIV; R = Ph. XXIV; R = CH_3) is most readily explained on the basis of the 2:7-dihydroxy compound (XXV), which may arise by incomplete condensation; this is equivalent to hydration of the pteridone. Brown and Mason favour this theory. They suggest that in the case of 2- and 6-hydroxypteridine and their N-Me derivatives the strongly bound molecule of water is actually constituted in the molecule by addition across the 3:4 and 7:8 double bonds as in XXVI and XXVIII respectively and in 6:7:8--trimethyl-2-pteridone (XXIV; R = CH_3) the water is regarded as adding a hydrogen atom at the 2-oxo function and a hydroxyl group at C_7 as in (XXV; R = CH_3).

The evidence led for this theory by Brown and Mason is that of spectral comparison of the pteridines with selected models. In all cases but that of



XXVI XXVII



XXVIII XXIX

6:7:8-trimethyl-2-pteridone (XXIV; R = CH_3) this evidence is consistent and commanding. Further, 2--hydroxypteridine, postulated with one mole of water as (XXVI) was oxidised to 2:4-dihydroxypteridine (XXVII). No such oxidation could be carried out on 6:7:8-trimethyl--2-pteridone where the hydroxyl function at C_7 would be tertiary (as in XXV). The spectrum of the 2-pteridone (XXIV; R = CH_3), however, more closely resembles that of N-methyl-7-quinolone (XXIX) than that of any other model proposed by these authors. Solubility in alkali of the 2-pteridone (XXIV; R = CH_3) is advanced by Brown and Mason as indicative of the presence of a 2-hydroxyl

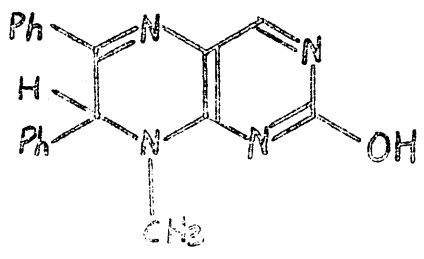
group and consequently of a mole of water constituted in the intact molecule, but this solubility can equally well be explained by partial hydrolysis of the 2--pteridone (XXIV; R = CH_3) by attack of hydroxyl at C_7 to give the anion of (XXV) (see page 32). They also found that the mole of water in the 2-pteridone (XXIV; R = CH_3) can be replaced by ethanol on recrystallisation from that solvent. While water may hydrate across a double bond by ionisation, it is unlikely that ethanol will.

Critical consideration of their evidence thus favours the transannular structure (XXIV; R = CH_3) for 6:7:8-trimethyl-2-pteridone and this is supported by the following additional information which largely relates to the 6:7-diphenyl analogue (XXIV; R = Ph).

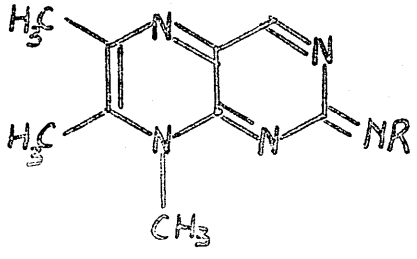
(1) Spectroscopic evidence: (a) Infra-red. - The diphenyl 2-pteridone (XXIV; R = Ph) shows an absorption band in the amide region at 1639 $cm.^{-1}$. No absorption band due to hydroxyl was observed, but this could be due to strong hydrogen bonding.

(b) Ultra-violet. - (1) Synthesis of the 7:8--dihydro analogue (XXX) of the diphenyl pteridone (XXIV; R = Ph) by condensation of benzoin with 5-amino-

-2-hydroxy-4-methylaminopyrimidine permitted a spectrographic comparison between them to be made. The 7:8-dihydropteridine (XXX) exists in the solid state with $\frac{1}{2}$ mole of water and no sinister construction can be placed upon the presence of water in this case. The absorption spectrum of the 2-pteridone (XXIV; R = Ph) is similar in wavelength of absorption to that of the 7:8-dihydropteridine (XXX) but the intensity of absorption of the pteridone (XXIV; R = Ph) is almost double that of the dihydropteridine (XXX). A similar correlation was observed between 2:8-dihydro-4-hydroxy-2-imino-8-methyl-6:7-diphenylpteridine (XCI: A transannular form) and 2-amino-7:8-dihydro-4-hydroxy-8-methyl-6:7-diphenylpteridine (XCII: A 7:8-dihydro form) (see Table 2).



XXX



XXXI

(2) 8-Benzyl-6:7-dimethyl-2-pteridone (LXVIII; R = O, R^1 = CH_3, R^2 = CH_2Ph), investigated in another context (page 50), analyses anhydrous, is not hygroscopic, and has absorption spectra both in ethanol and alkali closely resembling those of the other 2-pteridones (XXIV; R = CH_3. XXIV; R = Ph) (see Table 2).

(3) 2:8-Dihydro-6:7:8-trimethyl-2-methyliminopteridine (XXXI; R = CH_3) (page 57) is anhydrous in the solid state. It has an ultra-violet absorption spectrum in ethanol closely resembling those of the 8-alkyl-2--pteridones. The spectrum is not changed when the compound is examined in solution in dioxan when the conditions have been rigourously controlled to exclude moisture (see Table 2). This compound, therefore, exists in solution in the non-hydrated form, and its spectrum, closely resembling those of the 2-pteridones (XXIV; R = CH_3. XXIV; R = Ph) and of the 2-imino compound (XXXI; R = H), may be taken as a function of the transannular bond system (as in XXIV; XXXI) (Table 2).

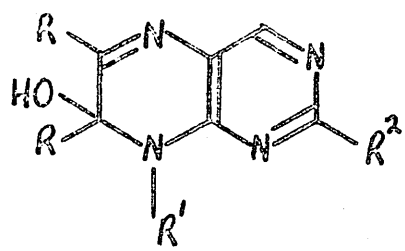
2. Synthetic evidence:- The synthesis of 8--substituted 2-oxo (or imino) pteridines almost certainly goes via a pseudo-base intermediate (XXXII). This route is exemplified in the synthesis of the pseudo-base

Table 2.

Compound	λmax.(mu)	ε	
8-Methyl-6:7-diphenyl--2-pteridone	240;316 234;331	23,200;15,600 22,400;12,400	1 2
6:7:8-trimethyl-2--pteridone	239;327 240;344	21,900;17,800 17,000;15,500	4 2
7:8-dihydro-2-hydroxy--8-methyl-6:7-diphenylpteridine	242;315 234;327	13,500; 8,500 17,200; 9,500	1 2
2:8-dihydro-6:7:8-trimethyl-2-methyliminopteridine	<220;248;352	>17,000;23,000;17,400	1
	<220;247;354	>17,000;21,200;17,300	2
8-Benzyl-6:7-dimethyl--2-pteridone	<220;248;328	>9,000;20,600;18,200	1
	<220;234;334-344	>18,000;18,100;14,700	2
2:8-dihydro-4-hydroxy--2-imino-8-methyl-6:7--diphenylpteridine	270;382;	22,200;13,900	2
2-Amino-7:8-dihydro-4--hydroxy-8-methyl-6:7--diphenylpteridine	260;398	9,700; 6,700	2
2:8-dihydro-2-imino--6:7:8-trimethylpteridine	238;340	18,900;12,200	4

1 = Ethanol. 2 = N/10 NaOH. 3 = dry dioxan. 4 = water

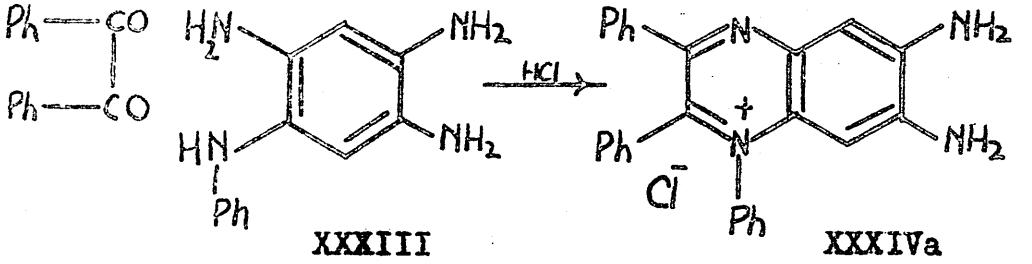
(XXXII; R = Ph, R^1 = CH_3, R^2 = H) by the condensation of benzil with 5-amino-4-methylaminopyrimidine (XI; R = R^1 = H). This pseudo-base is only synthesised in solutions containing hydrogen ion and then in only small yield. In conditions similar to those giving the 2-pteridones in good yield no pseudo-base (XXXII; R = Ph, R^1 = CH_3, R^2 = H) is formed. These observations indicate that in the 2-pteridone syntheses the condensation is not simply one of pseudo-base formation to give a 2-hydroxy pteridine (XXXII; R = Ph or CH_3, R^1 = CH_3, R^2 = OH) and that further reaction, probably to the 2-pteridone (XXIV; R = Ph or CH_3) takes place.



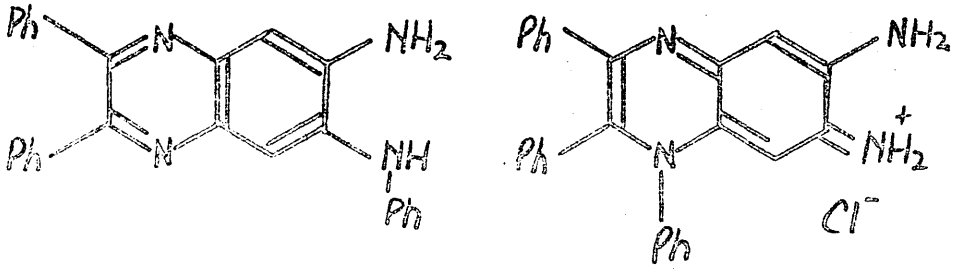
XXXII

The condensation of 2:4:5-triaminodiphenylamine (XXXIII) with benzil in acid solution gives almost exclusively the quaternary salt (XXXIV) and only a little of the fully aromatic quinoxaline (XXXV). The stability of the cation (XXXIV) would thus appear to be greater

than that of the cation of (XXXV)[50b,c]. The seat of the positive charge in the quaternary salt (XXXIV) is almost certainly on an exocyclic nitrogen atom (XXXIVb), for amino groups kept in the imino form by alkylation of a ring nitrogen atom are stronger bases than a heterocyclic N atom[56]. The stability of the transannular bond system in N-alkylated quinoxalines such as (XXXIVb) thus appears to be greater than that of the fully aromatic system. By analogy therefore the stability of N_8-alkylated-2-imino (or oxo) pteridines such as (XXIV.XXXI) should compare favourably in stability with that of fully aromatic pteridines.

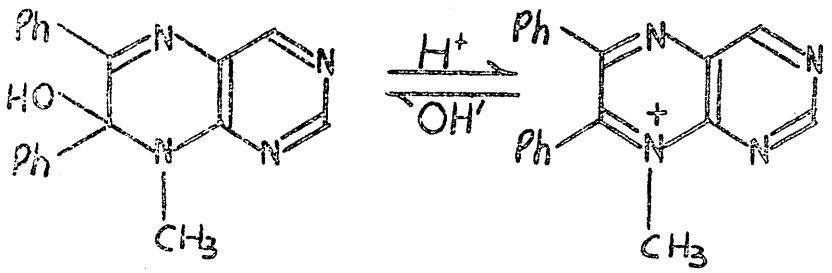


XXXIII XXXIVa



XXXV XXXIVb

The pseudo-base (XXXVI) is soluble in dilute acid and is regenerated from its cation (XXXVII) by neutralisation. The spectrum in acid solution of the pseudo-base (XXXVI) resembles the spectrum of the cation of pteridine. The formation of fully aromatic cations from heterocyclic pseudo-bases is well substantiated[45]. The cation (XXXVII) is formed by hydrogen ion attachment on N_8 of the pseudo-base (XXXVI) followed by elimination of water,



XXXVI XXXVII

3. Alkaline solubility:- The structure of the 8-substituted 2-pteridones being accepted as (XXIV), the solubility of these compounds in alkali must be explained. Three structures for the anion can be postulated. (i) A ring-opened structure (XXXVIII) is unlikely because (a) acid readily regenerates the pteridone (XXIV; R = Ph), (b) no definite absorption band for a carbonyl group is given in the infra-red absorption spectrum of the sodium salt of the diphenyl

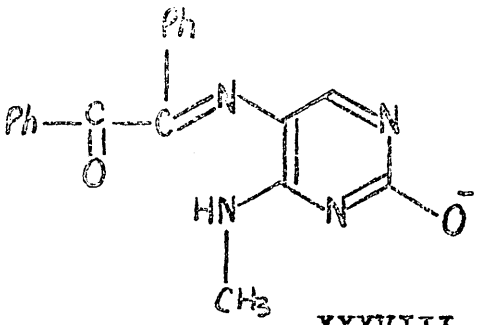
2-pteridone (XXIV; R = Ph), (c) further degradation of the anion (XXXVIII) with alkali would be easy, this has been shown not to be so (see page 35).

(ii) A structure in which a proton is eliminated, only one anion of this type is possible (XXXIX). Further degradation of this anion (XXXIX) to benzil and 5-amino-2-hydroxy-4-methylaminopyrimidine cannot be readily explained and this structure is also eliminated.

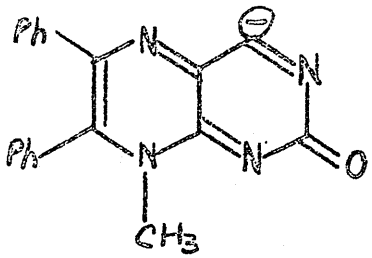
(iii) An anion formed by the addition of a hydroxyl group to the diphenyl pteridone (XXIV; R = Ph). This structure (XL) is the only feasible one and is presumably formed by attack of hydroxyl ion at C_7, a centre rendered susceptible to nucleophilic attack due to induction of electrons by N_8 and by the aromatic ring on C_7. Tautomerism then occurs changing the seat of the negative charge to the oxo function on C_2 to give the anion (XL).

Reactions of 8-Methyl-6:7-diphenyl-2-pteridone with acid and alkali.

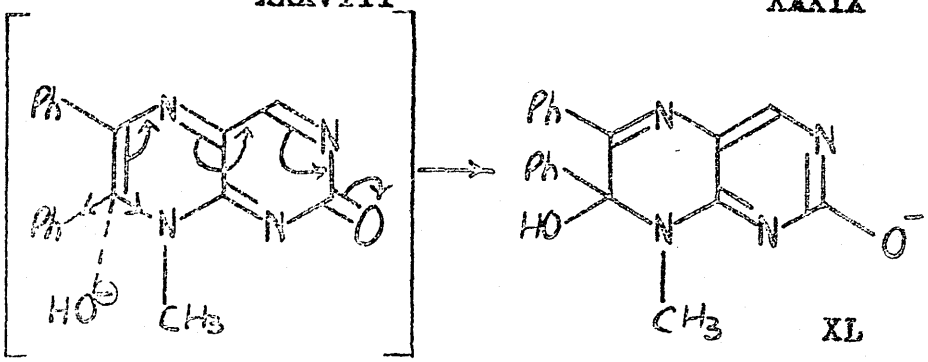
The peculiar stability to hydrolysis by alkali of the 8-methyl-2-pteridones, already referred to, led to an investigation of the degradation of the diphenyl



XXXVIII



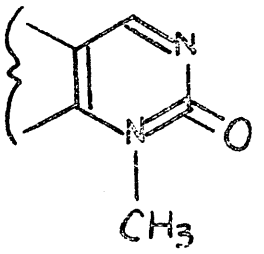
XXXIX



XL

pteridone (XXIV; R = Ph). Most N-alkylated pteridones so far degraded[62] have been alkylated on the ring nitrogen atom adjacent to the carbon atom carrying the oxo function, that is, an N-methyl cyclic amide like (XLI) and fission of the amide linkage has usually occurred[44]. In the case of the N_8-methyl 2-pteridones, however, no such amide linkage exists and the products of degradation were not predictable with certainty. Both acid and alkaline hydrolysis were found to degrade 8-methyl-6:7-diphenyl-2-pteridone (XXIV; R = Ph) to benzil and 5-amino-2-hydroxy-4-methylaminopyrimidine. The stability of the pteridone (XXIV; R = Ph) to alkaline hydrolysis is ascribed to the formation of the

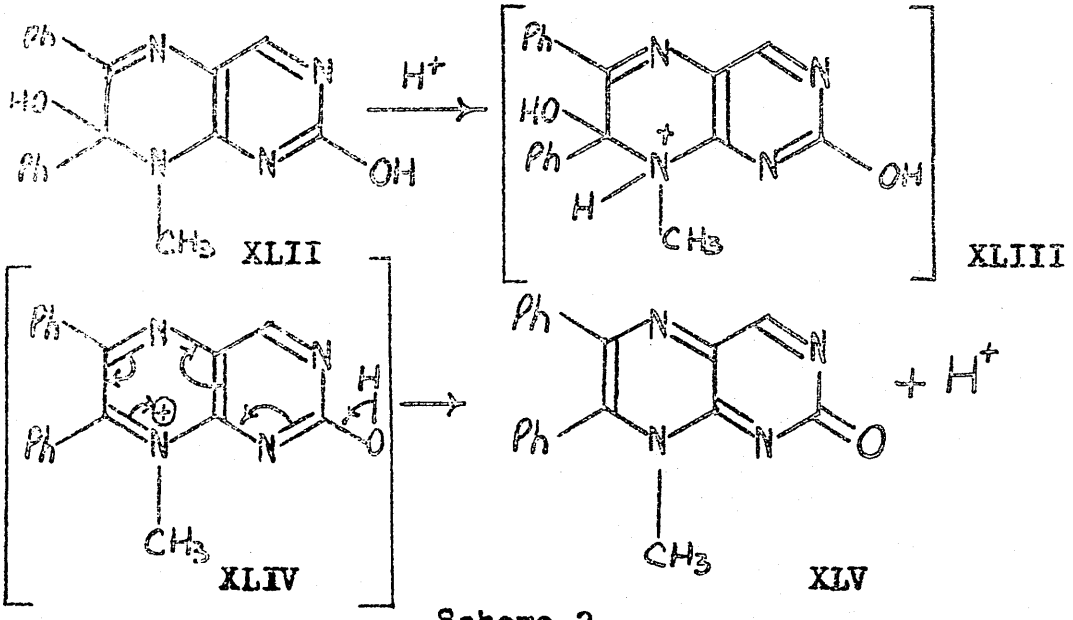
anion (XL), the negative charge of which makes the approach of other hydroxyl ions more difficult. No such stabilised ion occurs in the degradation by acid, which went smoothly and to completion. In both degradations the weight of the pyrimidine fragment was determined by making up the aqueous phase, after chloroform extraction to remove benzil, to a known volume and comparing the absorption intensity of the solution with that of a standard solution of 5-amino-2-hydroxy-4--methylaminopyrimidine at 294 mu. The chloroform extract on evaporation gave benzil, which was weighed.



XLI

The anion (XL) was isolable as its sodium salt by the addition of strong NaOH solution to a solution of the pteridone in alkali. The pteridone (XLV) could be regenerated by acidification of a solution of its sodium salt in water. This takes place by the pseudo-base change in an acid solution to a fully

aromatic system (Scheme 3) shown by the 7:8-dihydropteridine pseudo-base (XXXVI) already described. Initial reaction takes place on the anion (XL) by neutralisation to give the conjugate acid (XLII) of the base (XL). Further hydrogen ion attacks the basic nitrogen atom N_8 to give an intermediate (XLIII) from which water splits off to give the cation (XLIV). Extrusion of hydrogen ion gives the diphenyl pteridone (XLV , i.e. XXIV; R = Ph) on tautomerisation.



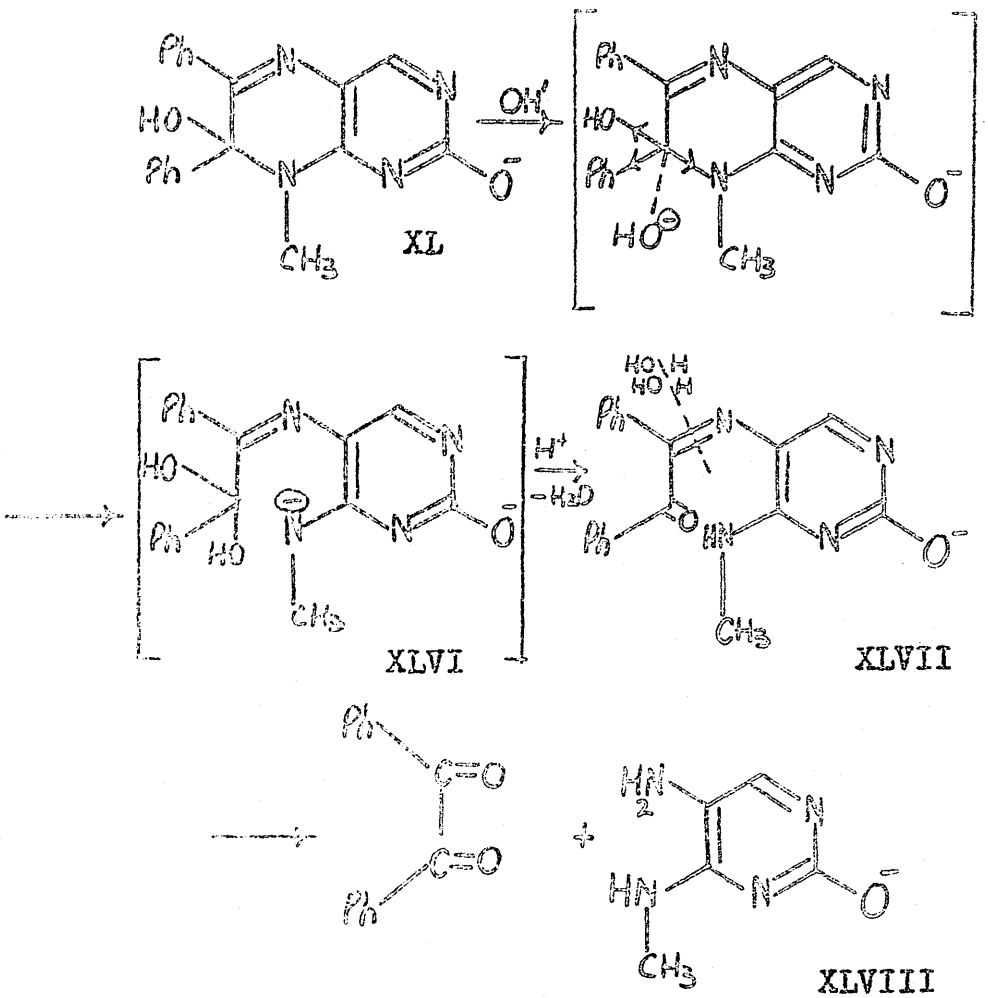
Scheme 3

Degradations: The following schemes for the degradation of 8-methyl-6:7-diphenyl-2-pteridone are probable.

(a) Alkaline. - Alkaline degradation of the 2-

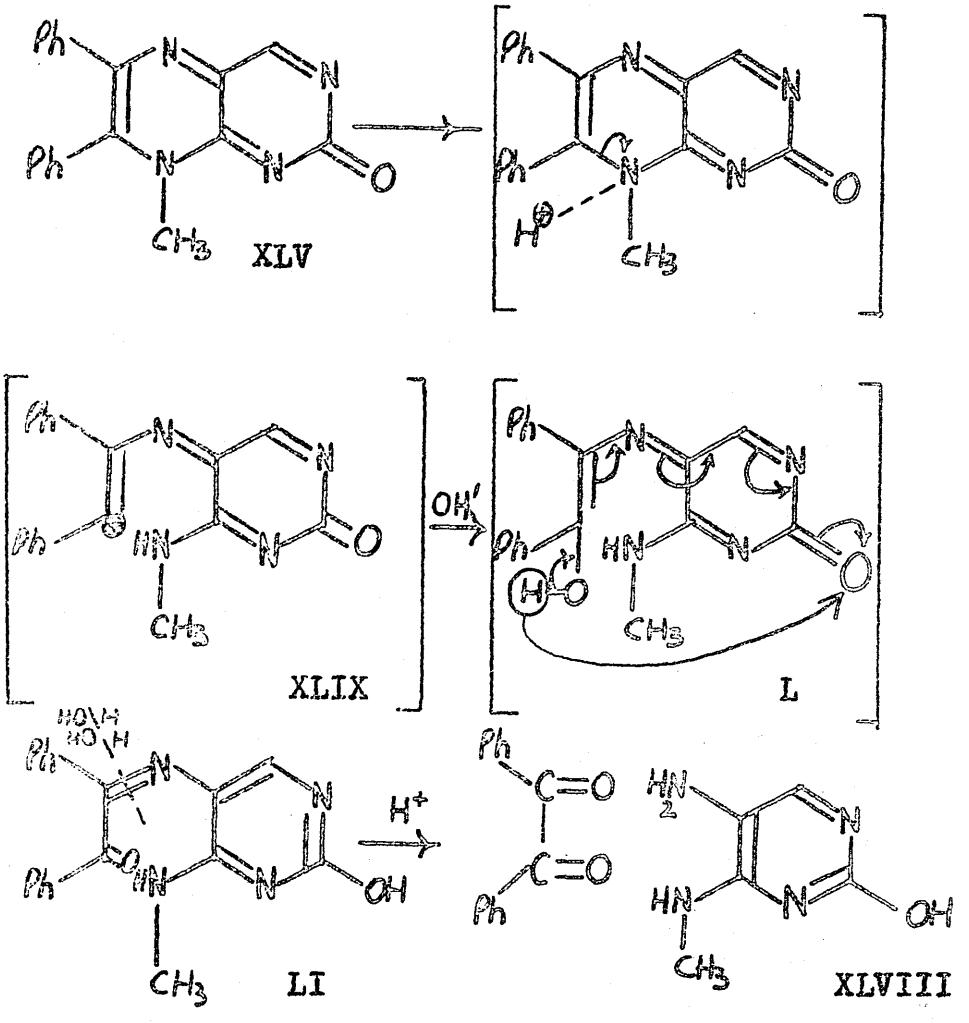
-pteridone (XLV) proceeds through the anion (XL) by further attack of hydroxyl ion at C_7. This is a slow step due to the repulsion of two similarly charged particles. Concurrent with the strengthening of the new hydroxyl-carbon atom bond is a weakening of the C_7-N_8 bond resulting in fission of this bond to give the intermediate (XLVI). Hydrogen ion quickly attacks the double negatively charged ion (XLVI) in a fast ionic reaction to give the azomethine anion (XLVII) which undergoes hydrolysis of the azomethine double bond to give benzil and 5-amino-2-hydroxy-4-methylamino-pyrimidine (XLVIII). Alkaline hydrolysis is slow because each of three steps involving hydroxyl ion participation is hindered by a negative charge on both reacting species. The 2-oxo function is, however, in equilibrium between anion and neutral molecule due to hydrolysis of the salt of a weak acid and a strong base, the hydroxyl ion may then more readily attack the small amount of neutral molecule.

(b) Acid. - Electrophilic attack commences at N_8 by a slow rate determining reaction to give the intermediate (XLIX) which is attacked by hydroxyl ion in a fast ionic reaction to give the intermediate (L).



This intermediate isomerises to the Schiff's base (LI) which is then degraded in the normal manner of acid hydrolysis of an azomethine to give benzil and 5-amino--2-hydroxy-4-methylaminopyrimidine (XLVIII). Acid degradation proceeds more readily than does alkaline degradation because in the former there is no stabilising intermediate ion to hinder attack by

hydrogen ion except after the azomethine (LI) stage when the methylamino group may accept a proton.

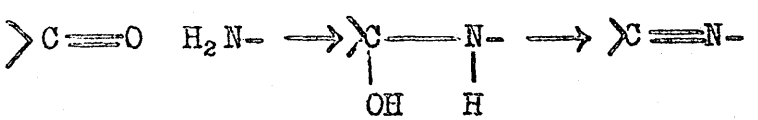


3. Theory of Condensation of Dicarbonyl Compounds with 5-Amino-4-alkyl (or aryl)aminopyrimidines.

Summary: Two factors are operative in the formation of the type of product obtained from the condensation of a carbonyl compound with a 5-amino-4-alkyl(or aryl)

aminopyrimidine. One factor is the molecular size of the carbonyl compound. The other is the nucleophilic reactivity of the 4-alkyl(or aryl)amino nitrogen atom relative to that of the 5-amino nitrogen atom.

The carbonyl function has a tendency to polarise thus $\overset{\delta+}{>}C \rightarrow O^{\delta-}$, rendering the carbon atom more electrophilic. The greater the electrophilic reactivity of the carbon atom the greater will be its tendency to condense with amines. The nitrogen atom of the amino group has a slight negative charge due to electron donation from its substituents. The greater the nucleophilic reactivity of the nitrogen atom the more readily it will condense with a carbonyl group. The first intermediate in the condensation is the formation of an aldehyde-ammonia which dehydrates to form the Schiff's base.



The theory of condensation must reconcile the following experimental observations. Glyoxal condenses with 5-amino-4-methylaminopyrimidines to give a bisdihydropurinyl structure and with 4:5-diaminopyrimidines to give a pteridine structure. Diacetyl and

benzil react with both types of pyrimidine to give a pteridine structure.

At least two factors are operating to give the anomalous results, one is attributable to the pyrimidine reactant, the other to the carbonyl reactant. For, if only one factor operated then either, each pyrimidine would give the same type of condensation product with every diketone, or each diketone would give the same type of product with every type of pyrimidine.

Consideration of the pyrimidine factor.

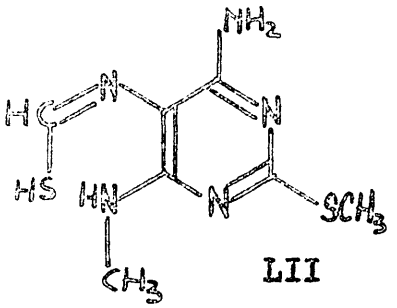
The site of initial attack of the carbonyl compound is of paramount importance in determining the nature of the product. A controlling factor in this is the relative nucleophilic reactivities of the nitrogen atoms on the 5 and 4 positions of the pyrimidine ring. A measure of the relative nucleophilic reactivities of the nitrogen atoms of the C_4 substituents used, may be gained by comparison of the basic strengths of the free bases of some selected substituents (see Table 3).

This correlation permits the arrangement of the 4-substituents in order of decreasing nucleophilic reactivity as follows:- methylamino, amino, benzylamino, anilino. Synthetic work by Todd and co-workers[45]

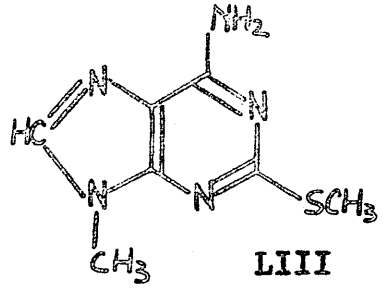
Table 3.

Free base of 4-substituent	C(propnl.to K)
methylamine	5.16
ammonia	1.8
benzylamine	1.04
aniline	0.17

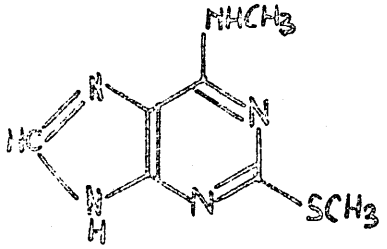
on purine synthesis substantiates, in part, this order. These workers found that 4-amino-6-methylamino-2-methylthio-5-thioformamidopyrimidine (LII) underwent ring closure to give 9-methyl-2-methylthioadenine (LIII) exclusively, and no 4-methylamino-2-methylthiopurine (LIV) was given. Todd anticipated that the reaction would take this path, but on false theoretical assumptions. He considered that a methylamino group would be less likely to tautomerise to the imino form than would an amino group. It has since been shown that both amino and methylamino groups exist as such[57], and do not tautomerise in pyrimidines. The choice of product has here been determined by the relative nucleophilic reactivity of the competing groups.



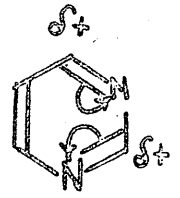
LII



LIII



LIV



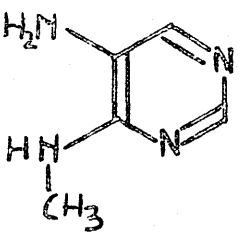
LV

Positions 2 and 4 (4 = 6) of the pyrimidine ring are deactivated by an electromeric effect (LV). Position 5 is deactivated merely by the general inductive electron pull of the aromatic ring and of the ring nitrogen atoms[88,89]. The low electron density of the three positions 2,4, and 6 is transmitted to any attached group. Thus the 5--amino group is the least denuded of electrons of any aminopyrimidine and because of this is the most nucleophilic.

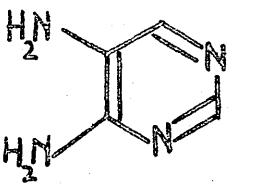
It must now be determined whether the 5-amino group is more reactive than the 4-methylamino group of a pyrimidine. The following experimental observations

are the only evidence which can be led to show that the 4-methylamino group is the more reactive. Synthesis of a bisdihydropurinyl by condensation of glyoxal with a 5-amino-4-methylaminopyrimidine goes more readily (two minutes at 50° in one case) than does condensation of glyoxal with a 4:5-diaminopyrimidine to give a pteridine. This can only be due to greater reactivity of the 4-methylamino group, since the 5-amino group is unaffected by a methyl group on the nitrogen atom at position 4. Thus the methylamino group must be reacting first in a 5-amino-4-methylaminopyrimidine.

Glyoxal will therefore condense first with a 4--methylamino group, then with a 5-amino group followed by a 4-amino, 4-benzylamino, 4-anilino group.



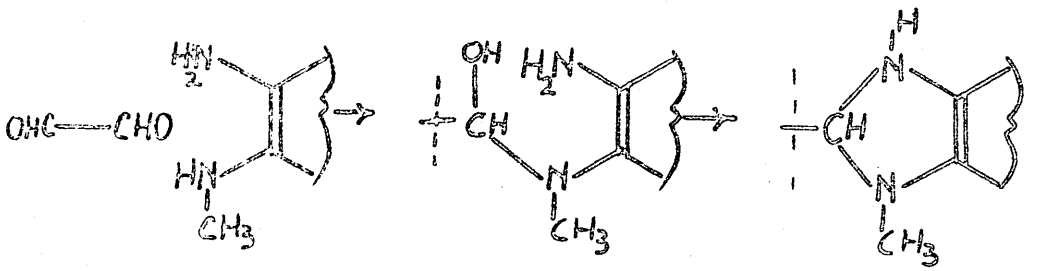
LVI



LVII

Case 1. - With pyrimidine type (LVI) one half of the glyoxal molecule reacts with the 4-methylamino group first, to give the aldehyde-ammonia (LVIII). No Schiff's

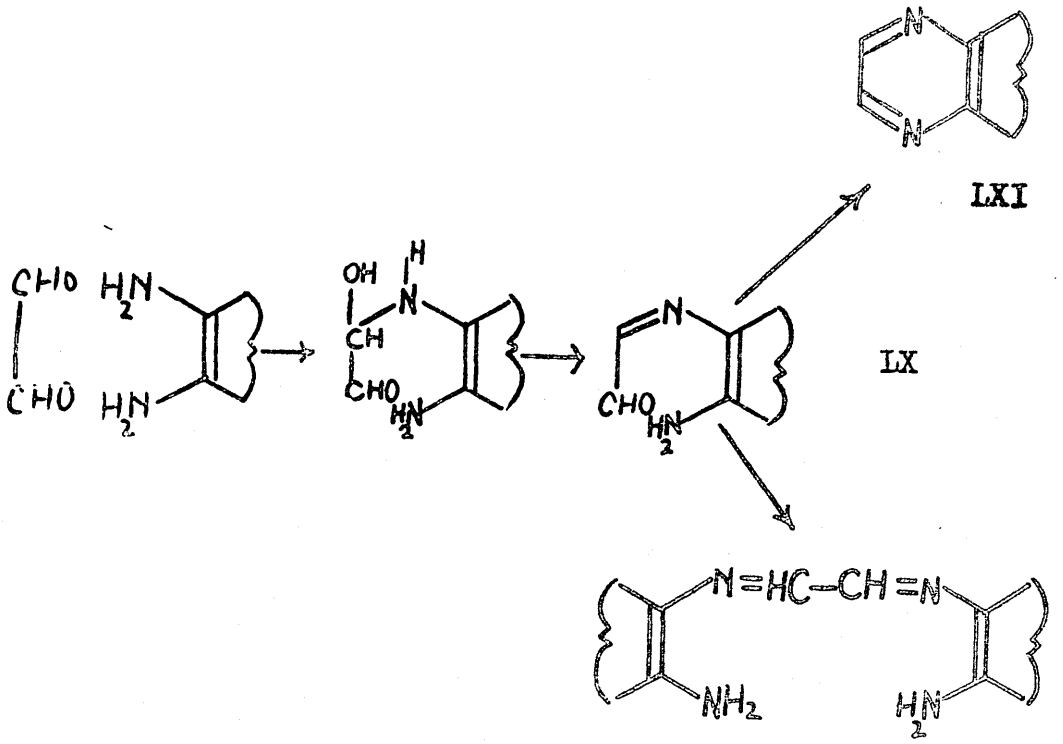
base formation is possible and subsequent ring closure takes place t' give the 5 membered ring of the dihydropurine (LIX). Similar reaction takes place between the other half of the glyoxal molecule and another pyrimidine molecule.



LVIII LIX

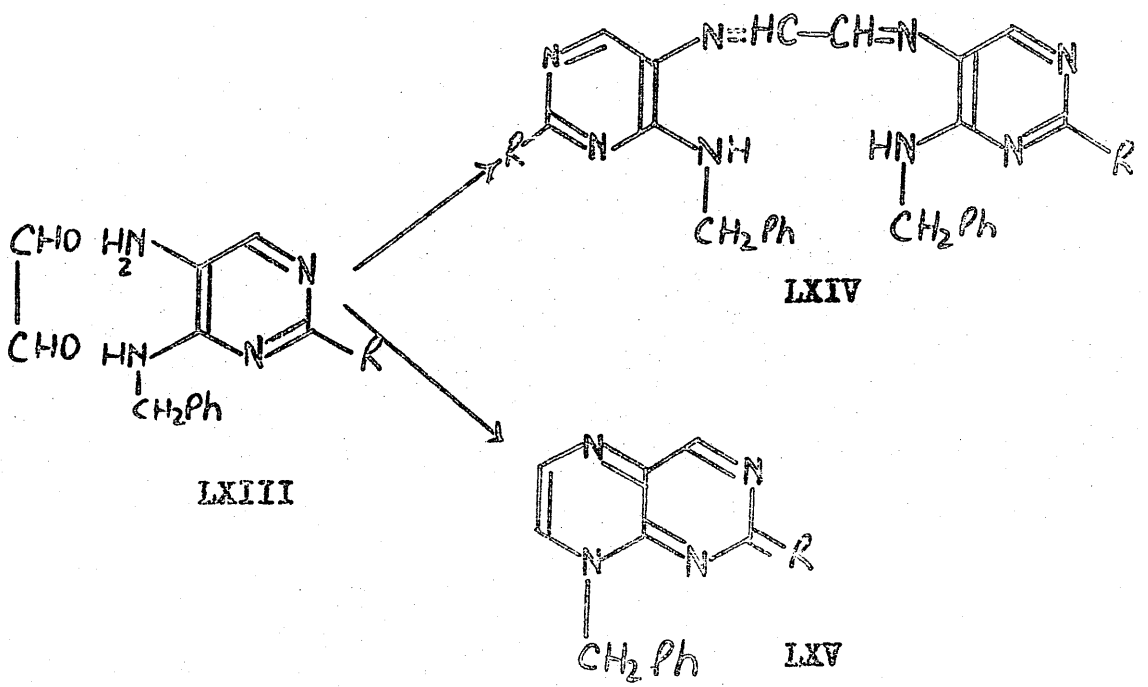
Case 2. - With pyrimidine type (LVII) initial reaction takes place at either amino group, the 5-amino group predominating, to give the aldehyde-ammonia as before, followed by dehydration across the new bond to give the Schiff's base (LX) which then undergoes cyclisation to give the pteridine (LXI). Formation of the pteridine rather than the azomethine (LXII) is favoured for statistical collision reasons.

Consideration of the pyrimidine factor, found to be the relative nucleophilic reactivity of the amino groups, accounts satisfactorily for the behaviour of glyoxal with 5-amino-4-methylaminopyrimidines. This



theory was tested experimentally by condensing glyoxal with 5-amino-4-benzylaminopyrimidines (LXIII). Benzylamine is approximately of the same basic strength as ammonia (Table 3) and the relative nucleophilic reactivity of the 5- and the 4-nitrogen atoms should be close to that of 4:5-diaminopyrimidine (LVII). Thus glyoxal should react first with the 5-amino group to give the aldehyde-ammonia, then the Schiff's base as in

case 2. Cyclisation would then take place to give the pteridine system. Azomethine formation is more likely here however, due to the low nucleophilic reactivity of the benzylamino group, making competition by the 5-amino group of another pyrimidine more marked. Experimentally it was found that condensation of glyoxal with 5-amino-2:4-bisbenzylaminopyrimidine (LXIII; R = $NHCH_2Ph$) gave the iminopteridine (LXV; R = NCH_2Ph) and a trace of the azomethine (LXIV; R = $NHCH_2Ph$). Similarly, glyoxal on condensation with 5-amino-4--benzylamino-2-hydroxypyrimidine (LXIII; R = OH) gave the pteridone (LXV; R = O) and the azomethine (LXIV; R = OH) (see page 50).



LXIII

LXIV

LXV

The theory fails to account for the reactions of benzil and diacetyl which give pteridines with all the pyrimidines discussed here.

Consideration of the α-diketone factor.

The three diketones give varying products depending upon the site of initial attack. Glyoxal attacks the methylamino group first in a 5-amino-4-methylamino-pyrimidine and attacks the 5-amino group first in any other diaminopyrimidine. Diacetyl and benzil attack the 5-amino first in every case. Some correlation must be found which divides the three diketones into two groups, one containing glyoxal, the other containing benzil and diacetyl.

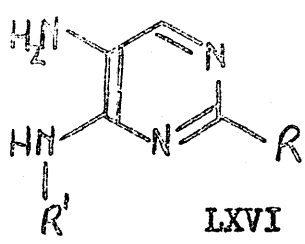
Electronic correlation. - The carbonyl carbon atoms in diacetyl receive electron donation from the methyl groups, rendering them less electrophilic than the carbonyl C atoms of glyoxal which receive electron donation from only a hydrogen atom. Benzil carbonyl C atoms, on the other hand, suffer electron withdrawal due to the aromatic ring. Glyoxal is therefore intermediate in the electronic character of its carbonyl C atoms between the other two diketones. Another operative factor may be a steric one.

Steric correlation:- The glyoxal molecule is the smallest of the three diketones and the 4-methylamino nitrogen atom is sterically hindered. This steric hindrance is illustrated by the formation of an azomethine (that is, initial condensation has taken place on the 5-amino group) of a 5-amino-4-methylaminopyrimidine with an aldehyde, p-nitrobenzaldehyde (page 16), in contradistinction to the action of the dialdehyde, glyoxal. The 4-methylamino group is more nucleophilic than the 5-amino and a carbonyl compound would react first with the former were it possible. The glyoxal molecule is sufficiently small to do this and consequently to give a bisdihydropurinyl. Diacetyl, benzil and p-nitrobenzaldehyde are not and are constrained to react with the 5-amino group first always, with the formation of a Schiff's base followed by cyclisation to the pteridine, where applicable.

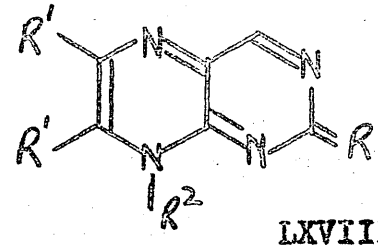
4. Synthesis of 8-Substituted Pteridines by Condensation of Glyoxal with 5-Amino-4-arylaminopyrimidines.

Condensation of glyoxal with 5-amino-2:4-dianilinopyrimidine (LXVI; R = NHPh, R^1 = Ph) gave the azomethine (LXVII; R = R^1 = NHPh) in small amount and an intractable red gum. The gum was very soluble and the ultra-violet

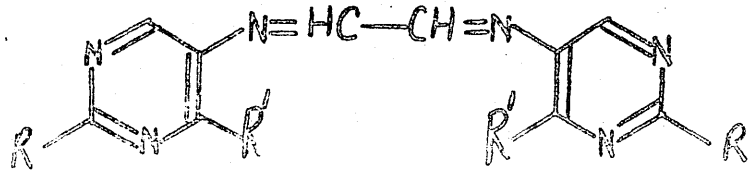
absorption spectrum showed that it was not composed of a bisdihydropurinyl. With 5-amino-2:4-bisbenzylaminopyrimidine (LXVI; R = $NHCH_2Ph$, R^1 = CH_2Ph) glyoxal condensation gave the iminopteridine (LXVIII; R = NCH_2Ph, R^1 = H, R^2 = CH_2Ph) identified as such by elementary analysis and by ultra-violet absorption spectrum, which, allowing for the bathochromic influence of the methyl groups, closely resembled that of the corresponding 6:7-dimethyl-2-iminobenzyl pteridine (LXVIII; R = NCH_2Ph, R^1 = CH_3, R^2 = CH_2Ph) (Table 4). The azomethines were all identified as such by their ready degradation with mineral acid.



LXVI

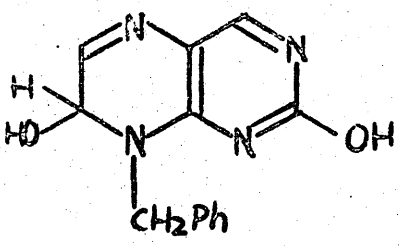


LXVIII

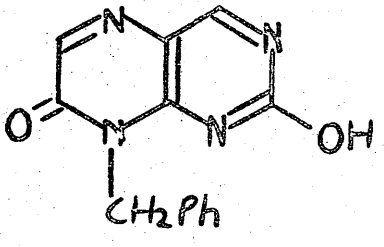


LXVII

Condensation of glyoxal with 5-amino-4-benzylamino-2-hydroxypyrimidine (LXVI; R = OH, R^1 = CH_2Ph) gave the azomethine (LXVII; R = OH, R^1 = $NHCH_2Ph$) and the pteridone (LXVIII; R = O, R^1 = H, R^2 = CH_2Ph). This pteridone was identified as such by elementary analysis, the compound analysed with one mole of water, and by comparison of the ultra-violet absorption spectrum with that of the corresponding 6:7-dimethyl-2-pteridone (LXVIII; R = O, R^1 = CH_3, R^2 = CH_2Ph) in alkali (Table 4). The pteridone (LXVIII; R = O, R^1 = H, R^2 = CH_2Ph) had an ultra-violet absorption spectrum similar to that of the pyrimidine (LXVI; R = OH, R^1 = CH_2Ph), both in ethanol (Table 4). This could be explained by the existence of this pteridone with a mole of water constituted in the molecule as (LXIX).



LXIX



LXX

Oxidation of the pteridone (LXVIII; R = O, R^1 = H, R^2 = CH_2Ph or LXIX) was attempted in the hope that if it were the hydrated compound (LXIX) it would oxidise

Table 4.

Compound	λmax.(mu)	ε	
8-Benzyl-6:7-dimethyl-	< 220;248;	> 9,000;20,600;	1
-2-pteridone	328	18,200	
	< 220;234;	> 18,000;18,100;	2
	334-344	14,700	
8-Benzyl-2-pteridone	< 220;224;	> 23,000;23,400;	2
	308	13,450	
	< 220;230;	> 16,000;23,800;	1
	290	8,050	
5-Amino-4-benzylamino-	220;298	16,350; 6,000	2
-2-hydroxypyrimidine	230;298	15,200; 5,100	1

1 = Ethanol. 2 = N/10 NaOH.

to the corresponding 7-pteridone (LXX). The compound, however, consumed twice the theoretical requirement of potassium permanganate for this reaction and no product could be isolated from the reaction mixture.

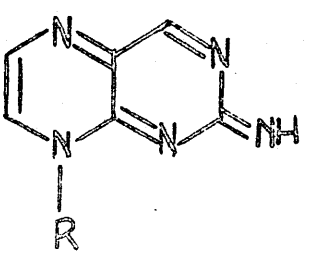
Attempts to synthesise 5-amino-4-benzylamino-2-hydroxypyrimidine (LXVI; R = OH, $R^1 = CH_2Ph$) by nitration, or coupling with p-chlorobenzendiazonium chloride, of 4-benzylamino-2-hydroxypyrimidine, followed by reduction, failed. The required pyrimidine was synthesised by reaction of benzylamine with 2:4-dichloro-5-nitropyrimidine to give a mixture of 4-benzylamino-2-chloro-5-nitropyrimidine and 2:4-bisbenzylamino-5-nitropyrimidine. Treatment of the mixture with hot NaOH solution gave a solution of 4-benzylamino-2-hydroxy-5-nitropyrimidine together with insoluble 2:4-bis-benzylamino-5-nitropyrimidine which was removed by filtration. Acidification of the filtrate gave 4-benzylamino-2-hydroxy-5-nitropyrimidine, catalytic reduction of which gave 5-amino-4-benzylamino-2-hydroxypyrimidine (LXVI; R = OH, $R^1 = CH_2Ph$).

PART II.

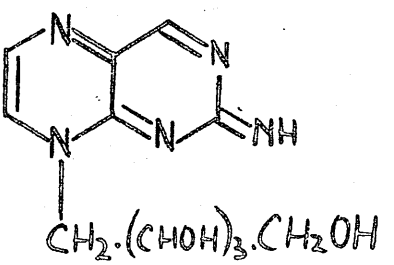
1. Synthesis of 2:8-Dihydro-2-imino-8-substituted Pteridines.

It has been suggested that 2-imino-8-substituted pteridines containing the transannular double-bond system of (LXXI) exist naturally[46]. Since no 2-imino-8--substituted pteridines had at that time been synthesised these suggestions were purely speculative.

(1) An introduction to this series of compounds was made by the synthesis of 2:8-dihydro-2-imino-6:7:8--trimethylpteridine (LXXVII). This compound was synthesised because of structural simplicity and because it would furnish a model for the pteridine moiety of the pigment luciferesceine isolated by Strehler[47] from Photinus pyralis and postulated by Albert[46] to be the imino ribityl pteridine (LXXII).



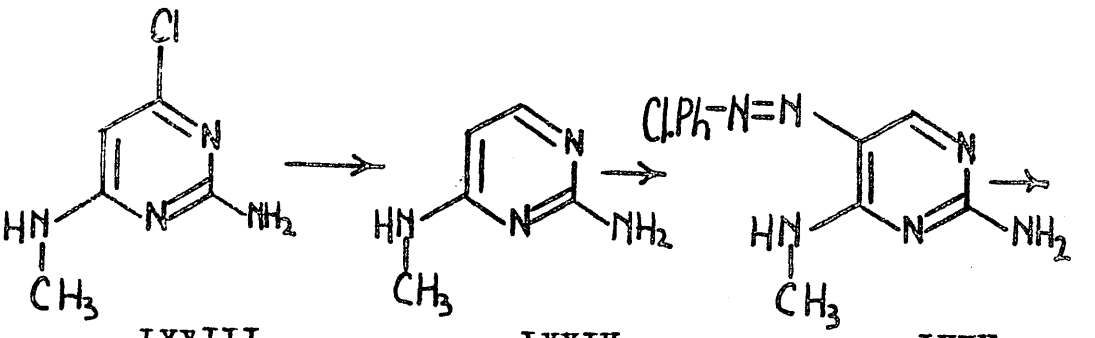
LXXI



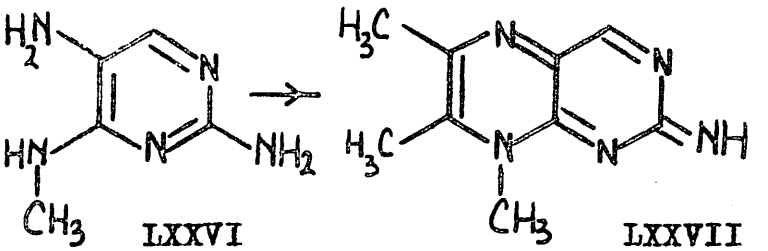
LXXII

The pteridine (LXXVII) was synthesised by condensation of diacetyl with 2:5-diamino-4-methylaminopyrimidine (LXXVI). The route to this pyrimidine (LXXVI) led from the known 2-amino-4-chloro-6-methylaminopyrimidine (LXXIII) which was dechlorinated by catalytic hydrogenation. The resulting 2-amino-4-methylaminopyrimidine (LXXIV) was coupled with p-chlorobenzenediazonium chloride to give 2-amino-5-p-chlorobenzeneazo--4-methylaminopyrimidine (LXXV). The azo compound (LXXV) was hydrogenated over Raney nickel to give the diaminopyrimidine (LXXVI). The diamino pyrimidine (LXXVI), in common with other diaminopyrimidine precursors of the imino and substituted imino pteridines, was too unstable to isolate and the solution from the reduction of the azo compound was used directly for condensation with the appropriate diketone.

The ultra-violet absorption spectrum of the neutral molecule of the 2-iminopteridine (LXXVII) closely resembles those of the 8-alkyl-2-pteridones (see Table 2). Unlike the 2-pteridones the imino compound (LXXVII) has no tendency to hydrate in the solid state. The pKa value of 5·6 is higher than any previously recorded for a simple amino pteridine, the corresponding value for 2-aminopteridine is 4.2. This observation is in



LXXIII LXXIV LXXV

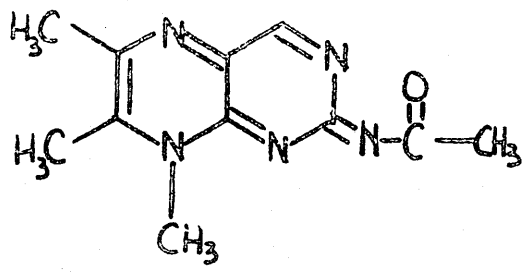


LXXVI LXXVII

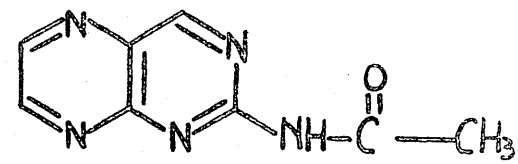
agreement with the findings of Angyal and Angyal[35] who observed that amino groups kept in the imino form by alkylation of a ring nitrogen atom have a high pKa value. The compound when heated with KOH solution liberates ammonia; this test is not, however, specific for imino groups as some compounds containing amino groups also liberate ammonia under similar conditions[35].

The imino-pteridine could not be acetylated under the conditions used by Forrest and Mitchell[48] for the acetylation of the yellow pigment from Drosophila melanogaster, that is, by solution of the pigment in acetic anhydride in the cold with the addition of a drop

of perchloric acid. Acetylation was achieved by heating the iminopteridine (LXXVII) in solution in acetic anhydride on the water bath for 1 hour. The incorporation of the acetyl group increases solubility and decreases stability. The ultra-violet absorption spectrum of the acetylimino pteridine (LXXVIII) differs from that of the parent iminopteridine (LXXVII); the acetylimino pteridine absorbs at longer wavelength. This is contrary to previous observations on the ultra-violet absorption spectra of acetylated amino pteridines where acetylation decreases the wavelength of absorption[49]. The anomaly is due to increase in conjugation provided by the acetyl group of the acetylimino pteridine (LXXVIII). In an acetylamino pteridine (LXXIX) no such increase in conjugation is conferred by the acetyl group.



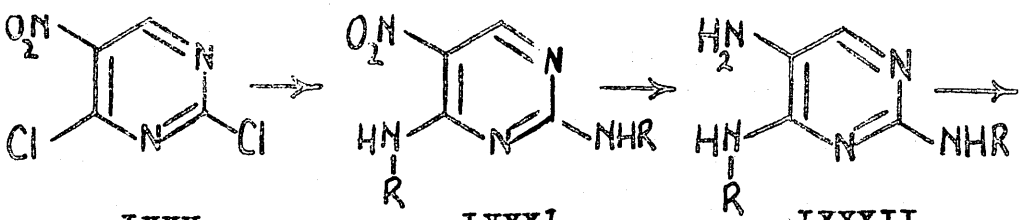
LXXVIII



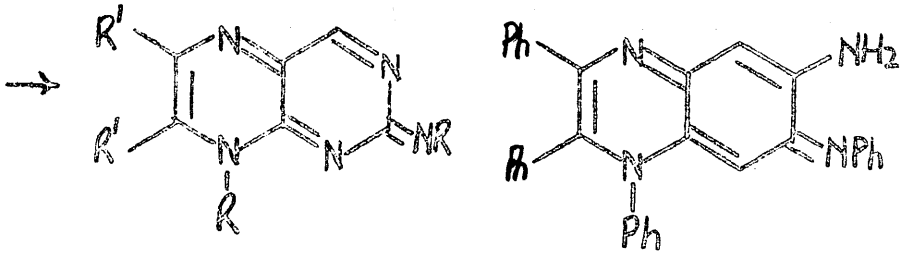
LXXIX

(2). A series of 2-alkylimino and 2-arylimino-pteridines were synthesised by a common method. The

general intermediate was 2:4-dichloro-5-nitropyrimidine (LXXX). The dichloropyrimidine (LXXX) was reacted with methylamine, aniline and benzylamine to give the corresponding 2:4-dialkyl(or diaryl)amino-5-nitropyrimidine (LXXXI; R = CH_3, Ph and CH_2Ph). Catalytic hydrogenation of these nitropyrimidines gave the corresponding 5--aminopyrimidines (LXXXII; R = CH_3. LXXXII; R = Ph. LXXXII; R = CH_2Ph) none of which could be isolated due to instability. The solutions from the reductions were used directly for reaction with the dicarbonyl compounds to give the following five 5:8-dihydro-2-iminopteridines (LXXXIII; R = R^1 = CH_3. LXXXIII; R = Ph, R^1 = CH_3. LXXXIII; R = R^1 = Ph. LXXXIII; R = CH_2Ph, R^1 = CH_3. LXXXIII; R = CH_2Ph, R^1 = H).



LXXX LXXXI LXXXII

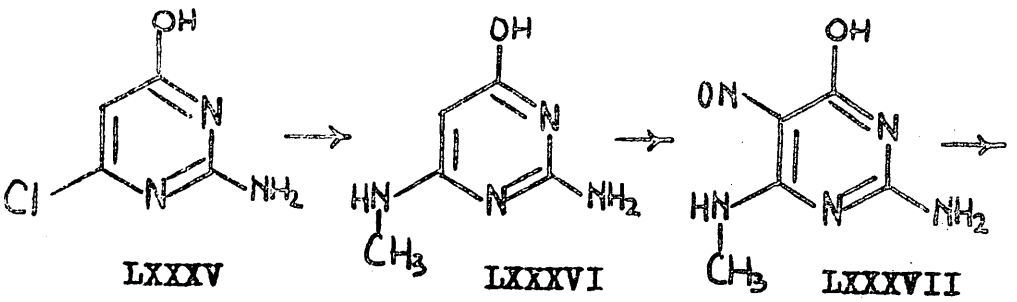


LXXXIII LXXXIV

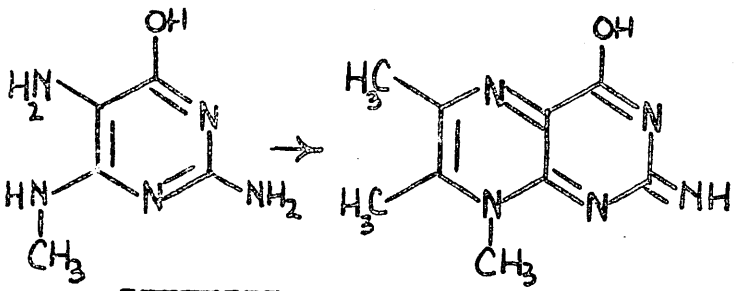
The iminopteridine (LXXXIII; R = R' = Ph) gives a deep green colour with concentrated sulphuric acid as does the corresponding quinoxaline (LXXXIV) [50a]. This halochromic effect is not given by any other pteridine of the series. The pKa value of the iminopteridine (LXXXIII; R = R^1 = CH_3) is 6.1, 0.3 units greater than that for the iminopteridine (LXXVII). This is in agreement with the observation that methylation of an amino group raises the pKa value by about 0.3 units[61]. The methylimino-pteridine (LXXXIII; R = R^1 = CH_3) liberates methylamine when refluxed with KOH solution.

(3). 2:8-Dihydro-4-hydroxy-2-imino-6:7:8-trimethylpteridine (LXXXIX) contains the postulated pteridine nucleus of a pigment isolated from the fruit fly Drosophila melanogaster. It was synthesised in poor yield by condensation of diacetyl with 2:5-diamino-4--hydroxy-6-methylaminopyrimidine (LXXXVIII). This pyrimidine (LXXXVIII) was prepared by the following route. 2-Amino-4-chloro-6-hydroxypyrimidine (LXXXV) was heated with methylamine in a sealed tube at 120° to give 2-amino-4-hydroxy-6-methylaminopyrimidine (LXXXVI). Nitrosation of this compound gave 2-amino-4-hydroxy-6-

-methylamino-5-nitrosopyrimidine (LXXXVII). Reduction of the nitroso compound (LXXXVII) with sodium dithionite gave the diaminopyrimidine (LXXXVIII) which, though unstable, could be isolated.



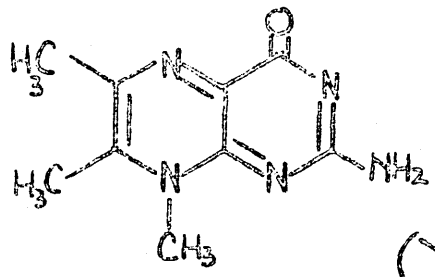
LXXXV LXXXVI LXXXVII



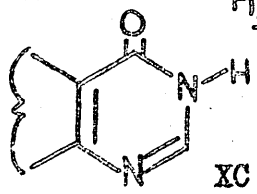
LXXXVIII LXXXIXa

The hydroxyiminopteridine (LXXXIX) is unstable, hygroscopic, bright yellow in colour and fluoresces with a brilliant blue colour under ultra-violet light of 254 mu. That it exists largely in the imino form (LXXXIXa,c) and not in the amino form (LXXXIXb) is indicated by the high pKa value of 5.85, this value being higher than for the iminopteridine (LXXVII; pKa 5·6) where no such tautomerism is possible. The other pKa value of about 8.9 appears to refer to the hydroxyl

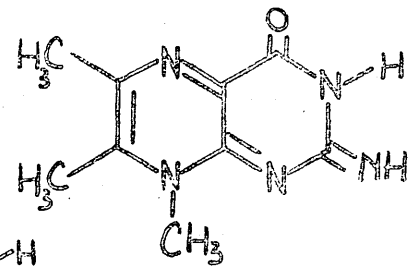
group. The hydroxyiminopteridine (LXXXIX) is probably best represented by structure (LXXXIXc), for hydroxy groups in pyrimidines[52] and the hydroxy groups of some monohydroxypteridines are known to exist in the oxo dihydro form, that is in the cyclic amide form (XC)[53].



LXXXIXb

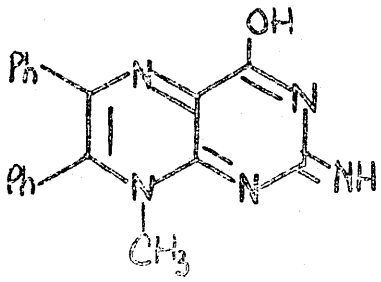


XC

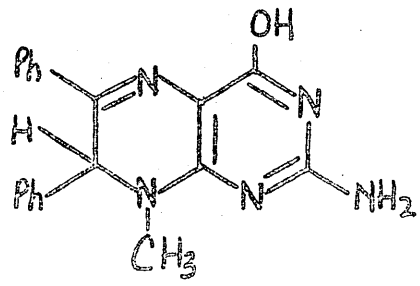


LXXXIXc

The corresponding 6:7-diphenyl analogue (XCI) of the trimethyliminopteridine (LXXXIX) was synthesised by condensation of the diaminopyrimidine (LXXXVIII) with benzil. The 7:8-dihydro-6:7-diphenylpteridine (XCII) was synthesised similarly by condensation of the diaminopyrimidine (LXXXVIII) with benzoin.



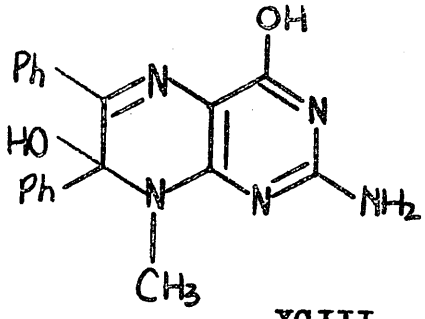
XCI



XCII

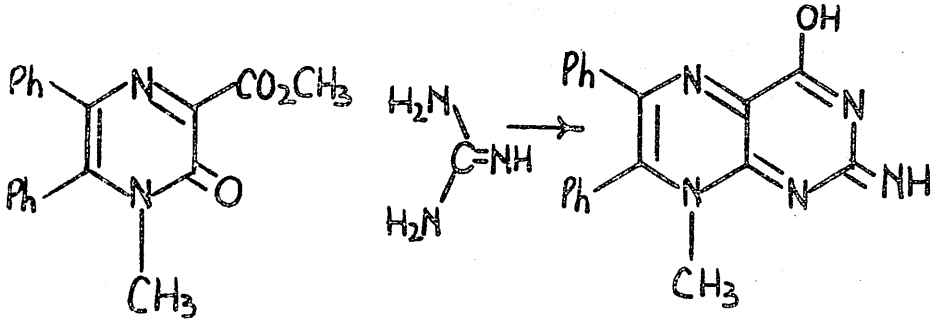
Both diphenylpteridines (XCI.XCII) were bright yellow in colour and fluoresced weakly under ultra--violet light. The 7:8-dihydropteridine (XCII) absorbs at longer wavelength in the ultra-violet region than does the iminopteridine (XCI). This is contrary to expectation, as the 6:7-dimethyl analogue (LXXXIX) absorbs at a longer wavelength than any 7:8-dihydro-pteridine. The anomaly is attributable to the effect of the phenyl groups in conjugation with the pteridine light absorbing system. The diphenyliminopteridine (XCI) analysed with one mole of water. The mole of water is again adventitious as in the case of the 8--alkyl-2-pteridones (see Part I. 2) and is not inherent in the molecule as in (XCIII), because the spectrum of the iminopteridine (XCI) is quite different from that of the 7:8-dihydropteridine (XCII) (see Table 2). The same correlation is observed in variation in intensity of absorption of the two diphenylpteridines (XCI.XCII) as in the absorption maxima of the corresponding pair of compounds 8-methyl-6:7-diphenyl-2--pteridone (XXIV; R = Ph) and 7:8-dihydro-2-hydroxy-8--methyl-6:7-diphenylpteridine (XXX) (see page 26 Table 2).

The iminopteridine (XCI) is identical with the compound prepared by fusion of a pyrazine ester (XCIV)



XCIII

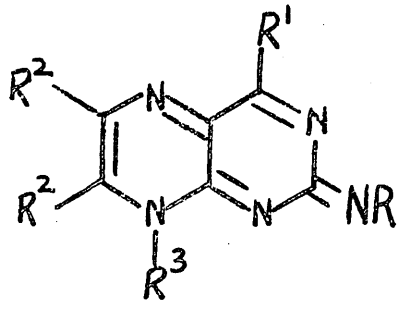
with guanidine carbonate (XCV). This novel route to an iminopteridine was developed by Mr. G.P.G. Dick of this department.



XCIV XCV XCI

(4). 4-Ethoxy-2:8-dihydro-2-imino-6:7:8-trimethylpteridine (C) was synthesised by condensation of 2:5-diamino-4-ethoxy-6-methylaminopyrimidine (XCIX) with diacetyl. It was hoped that hydrolysis of the 4-ethoxyl group might furnish a better route to the 4-hydroxyiminopteridine (LXXXIX). Neither acid nor alkaline hydrolysis conditions, however, effected this conversion

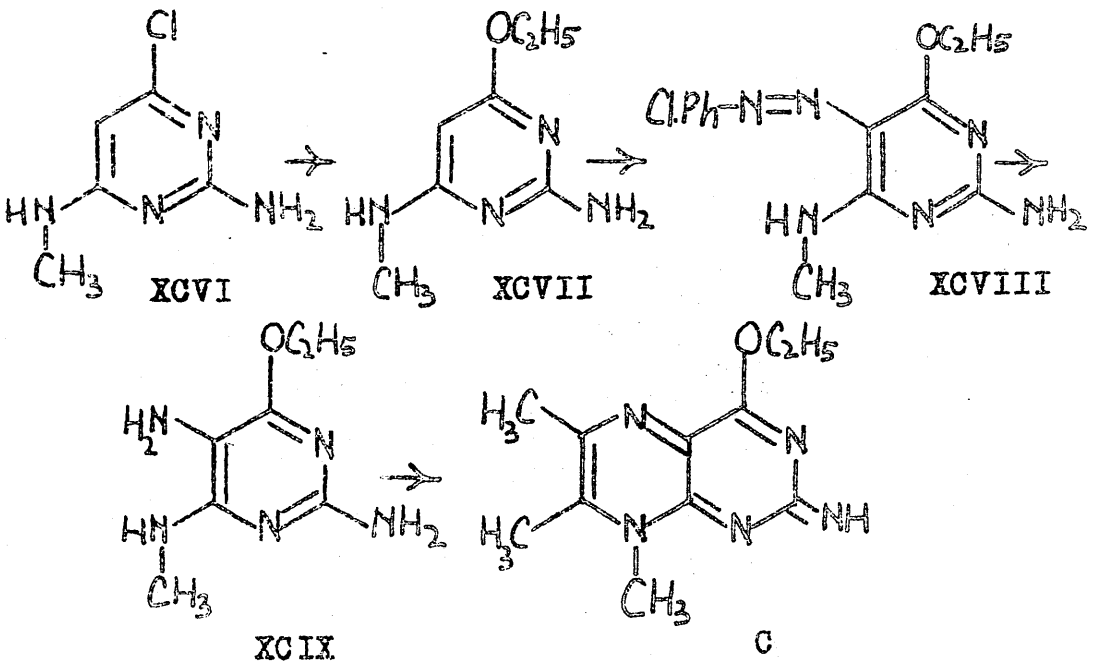
Table 5.



Compound.				λmax.(mu)	ε	
R	R^1	R^2	R^3			
H	H	CH_3	CH_3	<220;235;328	>17,000;20,400;9,900	1
				238;340.	18,900;12,200	2
CH_3	H	CH_3	CH_3	<220;248;352	>17,000;23,000;17,350	3
Ph	H	CH_3	Ph	245;269;370	15,700;18,850;16,150	3
Ph	H	Ph	Ph	<220;360	>30,000;23,200	3
CH_2Ph	H	CH_3	CH_2Ph	<220;251;350	>25,000;22,800;16,800	3
CH_2Ph	H	H	CH_2Ph	<220;232;325	>25,000;15,100;11,800	3
H	OH	CH_3	CH_3	223;268;306;365	18,500; 8,950;11,000; 6,800	4
				<220;254;284;395	>12,000;12,100 13,150;12,500	1
H	OEt	CH_3	CH_3	<220;255;405	>10,000;17,600;13,000	1
				234;301;361	24,350;14,100;10,200	3

1 = N/10 HCl. 2 = water. 3 = ethanol. 4 = N/10 NaOH.

The synthesis of the diaminopyrimidine (XCIX) commenced by treatment of 2-amino-4-chloro-6-methylaminopyrimidine (XCVI) with sodium ethoxide in dry ethanol in a sealed tube to give 2-amino-4-ethoxy-6--methylaminopyrimidine (XCVII). The ethoxypyrimidine (XCVII) was coupled with p-chlorobenzenediazonium chloride to give the 5-p-chlorobenzeneazo compound (XCVIII). The azo compound (XCVIII) on catalytic hydrogenation gave the unstable diaminopyrimidine (XCIX) which could not be isolated. The solution from the reduction reaction was used directly for condensation with diacetyl to give the iminopteridine (C).

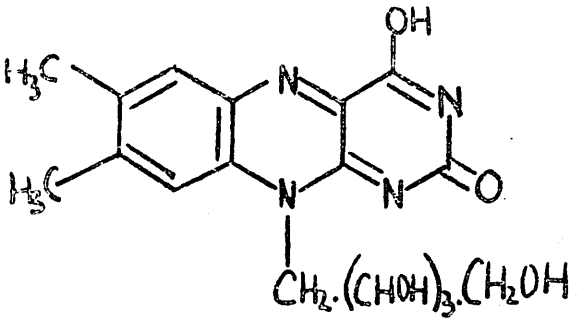


The ethoxyiminopteridine (C) in acid solution absorbs at very long wavelength for a pteridine (Table 5). The longer wavelength absorption in acid solution relative to that for the corresponding 4-hydroxyiminopteridine (LXXXIX) may be explained by the true p--quinonoid structure of the ethoxy compound (C) while the hydroxyiminopteridine exists in the cyclic amide form (LXXXIXc).

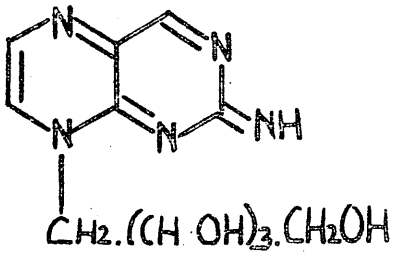
Comparison of Physical Properties of iminopteridines with those of some naturally occurring pteridines.

(a) Luciferesceine, a cream coloured substance isolated by Strehler[47] from Photinus pyralis has the empirical formula $C_{11}H_{15}O_4N_5$, and a pKa of about 8. At pH2 the absorption spectrum has peaks at 291 and 340 mu., at pH 11 peaks appear at 257, 282, 355 mu. Treatment with hot potassium hydroxide liberates ammonia. Albert suggests that luciferesceine may be an imino-ribityl pteridine (CII) and the analogy with riboflavine (CI) is made. This postulated formulation has in essence the light absorbing system of the iminopteridine (LXXVII) and so valid spectroscopic comparison may be made between the two compounds. There is found to be no points of similarity, spectroscopically, be-

tween thisiminopteridine (Table 5) and luciferesceine. The pKa of the iminopteridine (LXXVII), 5.8, is very much lower than that quoted for luciferesceine. The liberation of ammonia by luciferesceine when heated with KOH does not necessarily indicate the presence of an imino group[35].



CI



CII

(b). The yellow pigment from the fruit fly Drosophila melanogaster is discussed in the following section.

2. Pteridines from Drosophila melanogaster.

The study of the fruit fly Drosophila melanogaster has been of great interest to geneticists and much light has been thrown on the problem of genes by study of mutations of this fly. The eye pigments of the fly vary with mutation and the chemical nature of these pigments has excited great interest. The pigments of

the wild type Drosophila are very complex, but a study of the pigments of the sepia mutant by Forrest and Mitchell[48,54,55] has resulted in the identification and characterisation of five pteridines.

1. Isoxanthopterin, which may have a metabolic function in the fly for it has been shown to be implicated in melanogenetic reactions[56].

2. 2-Amino-4-hydroxypteridine, which may be an intermediate in the biosynthetic pathways to, or a degradation product of, the eye pigments.

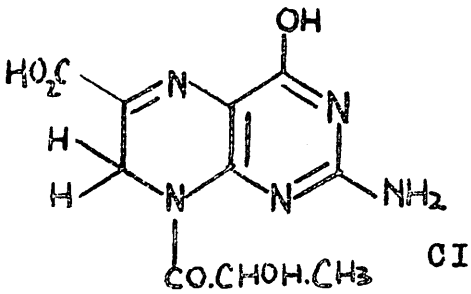
3. 2-Amino-4-hydroxypteridine-6-carboxylic acid (CIV), this compound may again be an intermediate or a degradation product.

4. 2-Amino-4-hydroxy-6-(1',2'-dihydroxypropyl) pteridine (CV), which has been shown to have growth promoting properties for Crithidia fasciculata, replacing pteroylglutamic acid. It has also been isolated from human urine and named biopterin[57].

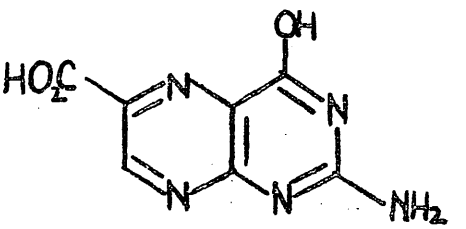
The isolation and characterisation of these four pteridines from Drosophila melanogaster has been substantiated by the independent work of a Swiss group[58] who worked on wild type Drosophila.

5. A yellow pigment giving the analytical results: C,41.7; H,4.3; N,24.1%, which permit the empirical formula $C_{10}H_{11}O_6N_5 \cdot \frac{1}{2}H_2O$. Forrest and Mitchell[48]

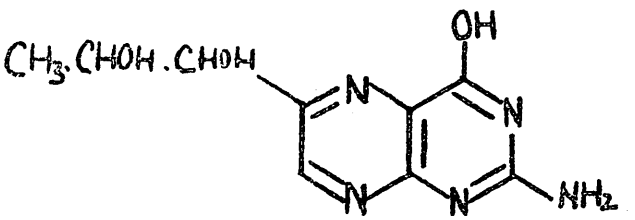
assign the structure 2-amino-6-carboxy-8-lactyl-4--hydroxy-7:8-dihydropteridine (CIII) to the pigment.



CIII



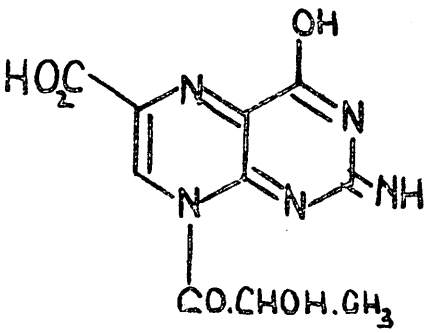
CIV



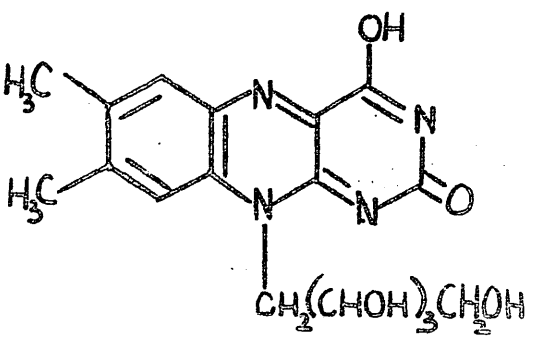
CV

Objections were made to formulation (CIII) initially because of the spectroscopic properties of the pigment which showed absorption in the ultra-violet region at longer wavelengths (440 mu., at pH 13) than that shown by 7:8-dihydropteridines, which normally absorb at about 360 mu[59]. The alternative formulation (CVI) was proposed by Wood[60], when the point was made that this structure (CVI), which resembles a phenazine dye, could have the spectroscopic properties of the yellow pigment. The similarity between the spectrum of the yellow pigment and of that of riboflavine (CVII),

commented upon by Forrest and Mitchell, finds a readier explanation in the transannular structure (CVI) for the pigment.



CVI



CVII

The experimental evidence upon which the formulation (CIII) rests is as follows:

(i). The pigment decomposes in alkaline, neutral or acid solution, in sunlight, to give 2-amino-4-hydroxy-pteridine-6-carboxylic acid (CIV), an autoxidation being involved.

(ii). It forms an oxime and a 2:4-dinitrophenyl-hydrazone.

(iii). It gives a monoacetate.

(iv). The pigment is reduced with the uptake of 2 moles of hydrogen by the reduction presumably of the ketonic group and the 5:6-double bond to give a tetra-hydropteridine. No product was isolated from this re-duction.

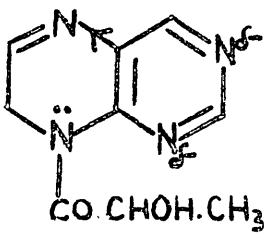
(v). Periodate oxidation of the pigment utilises 2.4 moles to give acetaldehyde and the pteridine (CIV).

(vi). The presence of lactic acid was detected by paper chromatography.

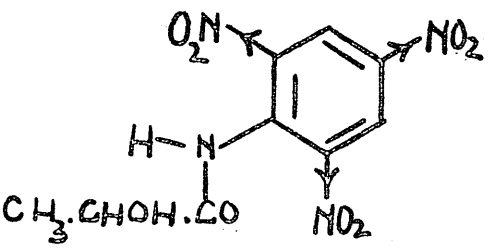
This evidence is now re-examined.

The carbonyl group which gives rise to the oxime and the 2:4-dinitrophenylhydrazone is considered by Forrest and Mitchell to be that attached to N_8 as part of the lactyl group. This carbonyl function is, however, part of an amide and as such does not form normal carbonyl derivatives. It is conceivable that a lactyl group in this environment might form such derivatives either by (a) oxidation of the >CHOH group as in osazone formation or (b) the electron withdrawing properties of the pyrimidine ring exerting sufficient influence to overcome the effect of the amide nitrogen atom, which normally inhibits the carbonyl reactions of an amide carbonyl group. These unlikely possibilities were rejected when attempts to form an oxime or 2:4-dinitrophenylhydrazone of N:N-dimethylactamide and of the 2:4:6-trinitroanilide of lactic acid (CIX) failed. The latter compound approximates closely to the dihydropteridine (CIII, CVIII) in its electronic distribution.

N:N-dimethylactamide reacted with hydroxylamine to give the corresponding hydroxamic acid.



CVIII

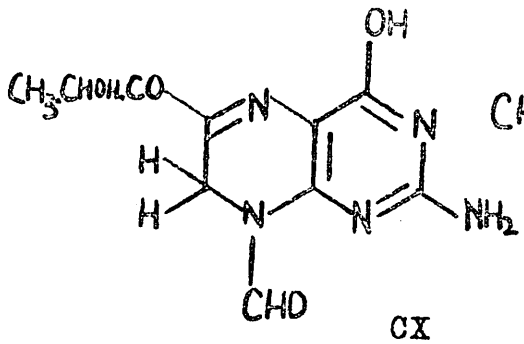


CIX

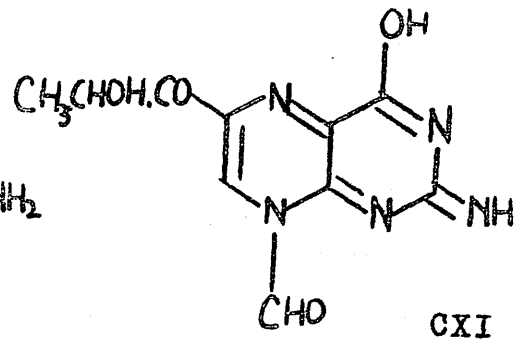
Forrest and Mitchell claim that the formation of a mono-oxime and a mono-2:4-dinitrophenylhydrazone is evidence that only one carbonyl function exists in the molecule, but since the carbonyl function which they assume condenses to give these derivatives is part of an amide, then another ketonic group must be present. Alkaline hydrolysis of either derivative gives the 6--carboxylic acid (CIV) and they assume that the carboxyl group exists as such, but 6-aldehydo pteridines are known to undergo disproportionation in alkali to give the corresponding acid, together with the more soluble hydroxymethyl compound[61]. Having shown to their satisfaction that the intact pigment contains a carboxyl group at C_6, Forrest and Mitchell next consider the site of attachment of the lactyl group. Position 7 was discarded because no degradation products substituted

on C_7 were found. Thus only positions 5 and 8 were possibilities and position 8 was chosen because of the analogy to riboflavine. It has been shown above that the 6-carboxyl group need not exist as such in the pigment and this carboxyl group in the hydrolysis product (CIV) could be generated from either a formyl or a lactyl group, so that possible structures for the pigment are the following.- A 7:8-dihydropteridine (CX) and a 2:8-dihydropteridine (CXI) both having a formyl group on N_8 and a lactyl group on C_6; a 7:8-dihydropteridine (CXII) and a 2:8-dihydropteridine (CXIII) both having a lactyl group on N_8 and a formyl group on C_6. The first two structures are favoured because of the analogy to biopterin (CV) which has a 1',2'-dihydroxypropyl group on C_3. Biopterin could be converted into either of these two compounds (CX; CXI) by formylation, oxidation and reduction, all of which are normal biochemical processes.

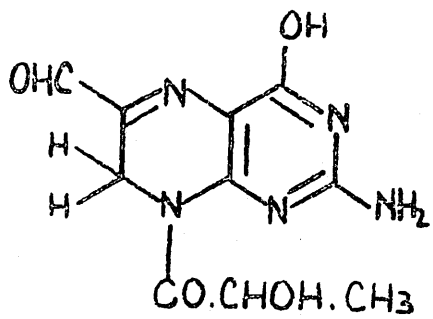
While the 7:8-dihydropteridines (CIII; CX) would be relatively unstable, being prone to hydrolysis followed by oxidation to give a fully aromatic ring structure, the ease of degradation of the yellow pigment suggests that a formulation involving a 2:8-dihydro-2-iminopteridine would be more likely, for only a simple



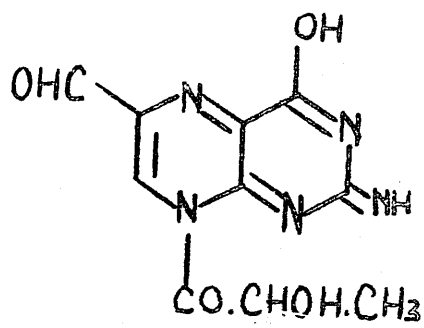
CX



CXI



CXII

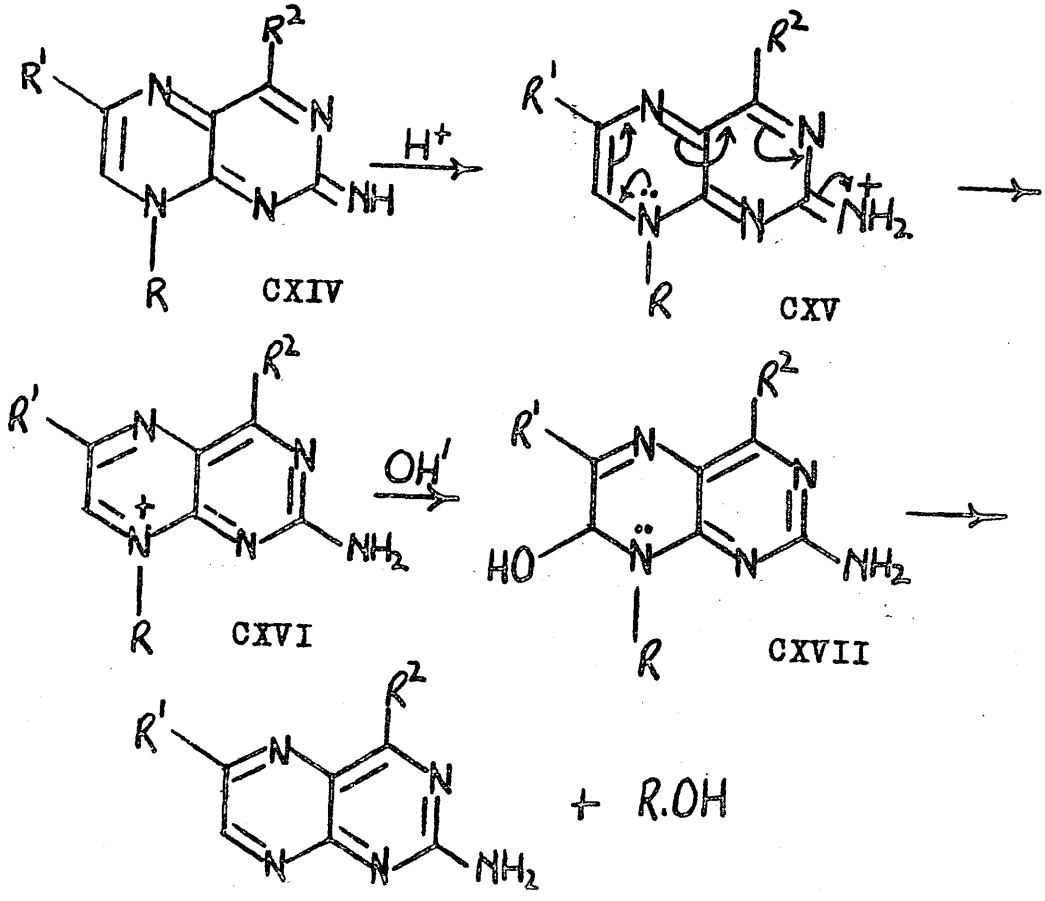


CXIII

hydrolysis mechanism (scheme 4) need be invoked in the latter case to give a fully aromatic pteridine. No such simple mechanism can be advanced for the 7:8-dihydro formulae (CIII,CX,CXII) where an oxidation is required.

The initial attack by hydrogen ion takes place on the doubly bonded nitrogen atom of the cyclic amidine (CXIV)[55]. The cation (CXV) tautomerises to give the cation (CXVI) of a pseudo-base (CXVII). The substituent R is considered to be an acyl group so that the corresponding acid R.OH may split out to give the fully aro-

matic pteridine (CXVIII)

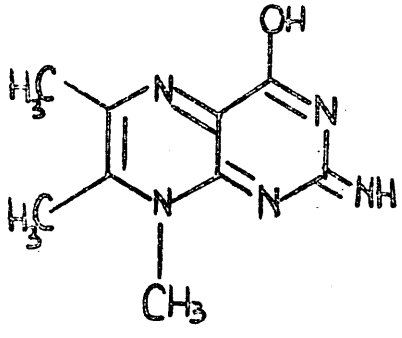


Scheme 4.

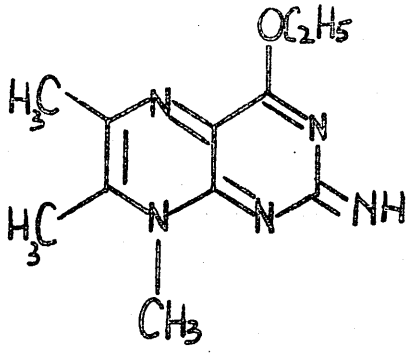
The yellow pigment resembles the 2:8-dihydro-iminopteridine (CXIX) in colour, both are bright yellow; both decompose without melting, are somewhat unstable, are hygroscopic and fluoresce under ultra-violet light.

Spectroscopic evidence:- The absorption spectrum of the yellow pigment is so unusual in its long wave-

length absorption (maxima at 409 mu in acid and 440 mu in alkali) that any relevant factor which increases the wavelength of absorption of a synthetic pteridine must be counted as being of significance in determining the structure of the pigment. While the spectrum of the 2:8-dihydroiminopteridine (CXIX) has no formal resemblance to that of the pigment it has a similar long wavelength absorption (395 mu in acid). Since few pteridines absorb at such very long wavelengths, the exceptions being mercapto pteridines, this is an indication that some similarity in structure may exist between the pigment and the 2:8-dihydro iminopteridine (CXIX) which has the transannular bond system of riboflavine (CVII).



CXIX

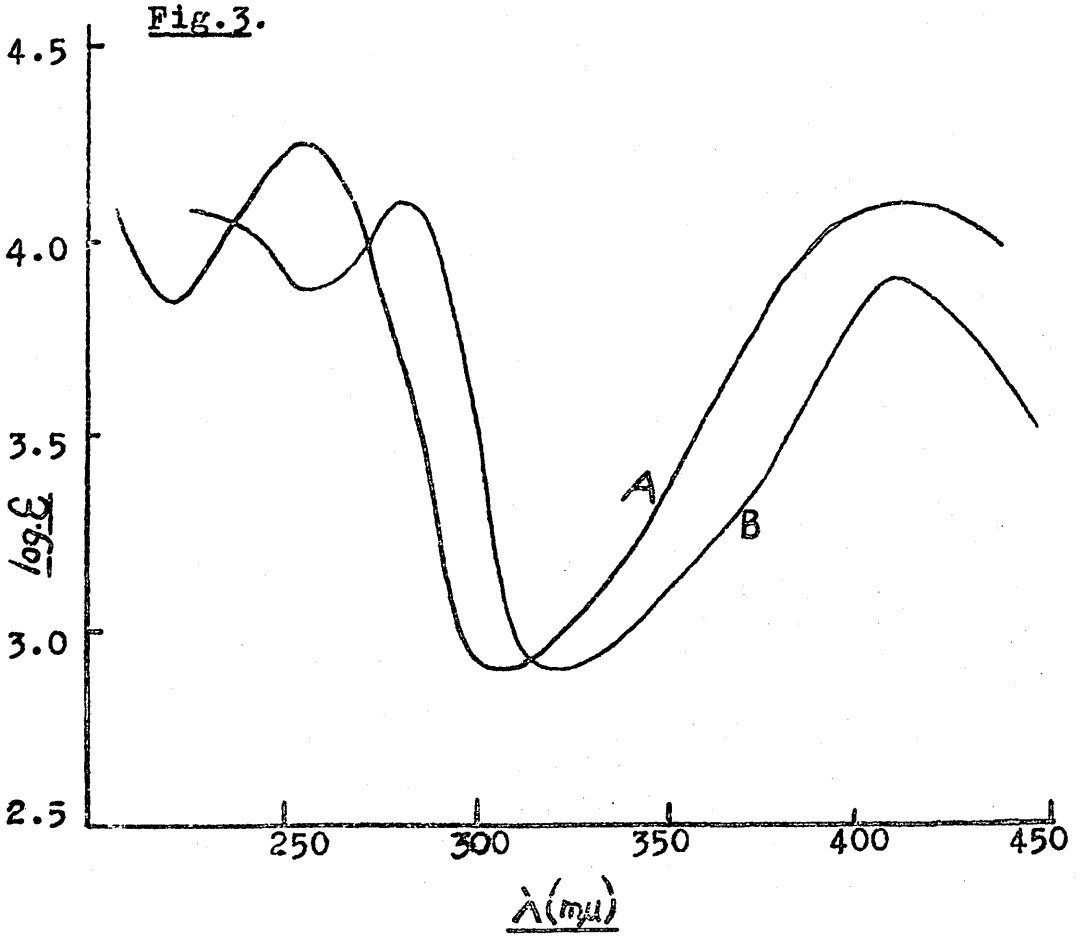


CXX

The Drosophila pigment has an ultra-violet absorption spectrum so remarkably akin to that of the 4--ethoxypteridine (CXX), that a close structural re-

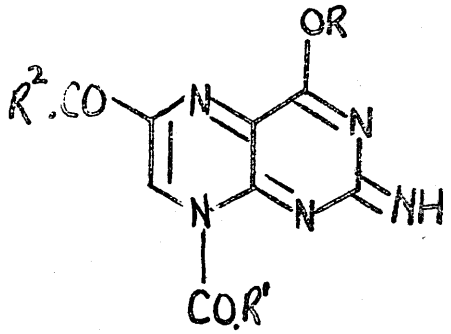
semblance, in the pteridine light absorbing moiety, can be inferred. The spectra (Fig. 3) are not only similar generally, but both differ from other 2:8--dihydro iminopteridines in the very long wavelength absorption above 400 mu., and in the very low intensity trough between the longest and next longest absorption bands.

Fig.3.



A. 4-Ethoxy-2-imino-2:8-dihydro-6:7:8-trimethylpteridine (CXX).
B. Drosophila pigment (CXXI).

The stability of the Drosophila pigment is such that various degradation products may have been involved in the reactions described by Forrest and Mitchell, for it is difficult to postulate one formula which would satisfy all the experimental facts. A pteridine moiety (CXXI) is proposed to accomodate the inferred structural resemblance to the 4-ethoxypteridine (CXX). Substituents R, R^1, and R^2 must account between them for $C_2H_7O_2$.



CXXI

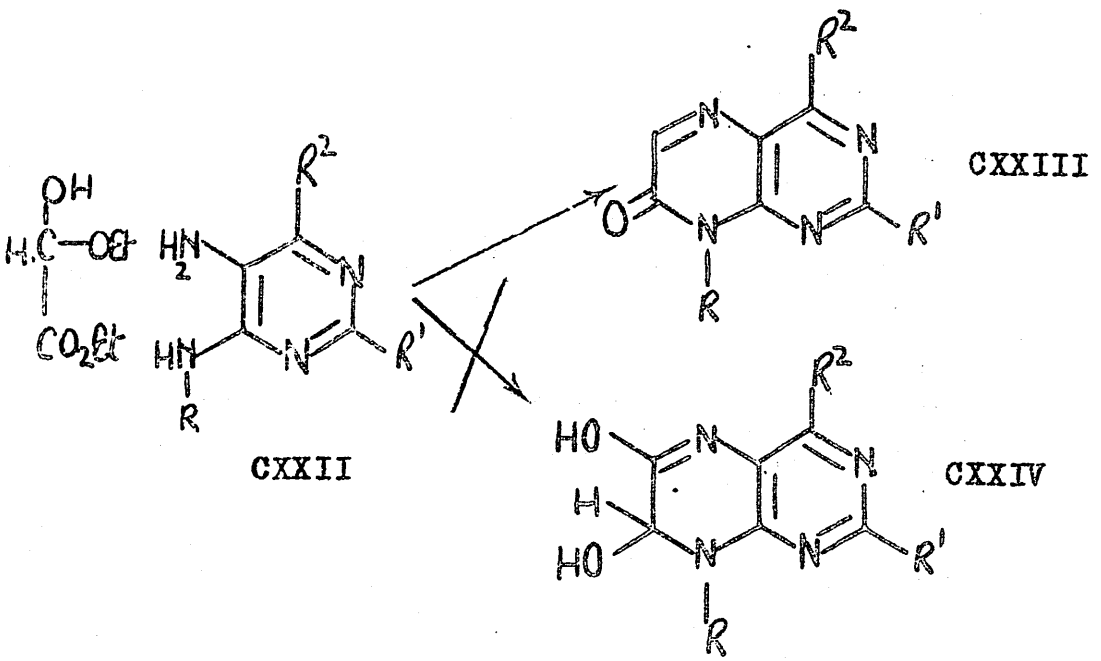
PART III.

The 8-alkyl-7-pteridone (CXXIII) series was investigated to determine whether reduction of the cyclic amide group could be effected to give an 8-substituted 5:6:7:8-tetrahydropteridine (as CXXVI). It is known that 5:6:7:8-tetrahydropteridines are important in metabolism and it is believed that 8-substituted tetrahydropteridines may also fulfill a significant biological function (see historical section).

Several 8-substituted 7-pteridones were known before this study was commenced. Elion and Hitchings[62] synthesised 2-amino-4-hydroxy-8-ß-hydroxyethyl-7-oxo--7:8-dihydropteridine by decarboxylation of the corresponding 6-carboxylic acid. Another group[63] synthesised 8-methyl-7-pteridone (CXXIII; R = CH_3, R^1 = R^2 = H) by condensation of ethyl glyoxylate hemiacetal with 5-amino--4-methylaminopyrimidine (CXXII; R^1 = R^2 = H, R = CH_3).

A number of 8-substituted 7-pteridones (CXXIII) were synthesised by the author using the latter method, and their properties, both chemical and physical, examined. In no case was a 6:7-dihydroxy-7:8-dihydropteridine of type (CXXIV) given, although this type of compound is a possibility, being given by the condensation of the reactants in the reverse juxtaposition.

The non-appearance of a product of this type is probably due to steric effects, the large hemiacetal group being constrained to react with the 5-amino group.



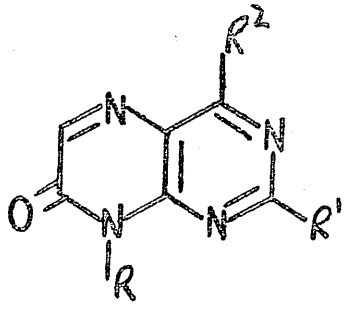
CXXII

CXXIII

CXXIV

The ultra-violet absorption spectra of the 7--pteridones showed a general similarity (see Table 6), both within the group and to the 2-quinoxalones[64].

Table 6.

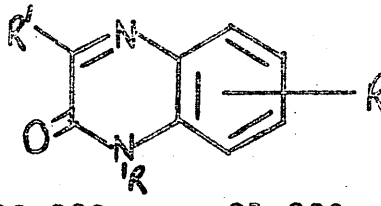


CXXIII

Compound

pteridone CXXIII.

R	R^1	R^2	λmax.(mu)	ε	
CH_3	H	Cl	226;312	19,600; 9,300	1
CH_3	$NHCH_3$	H	220;sh.236; 296;359	23,700;11,100 5,100;15,680	2
CH_3	H	$NHCH_3$	226;240; 258;297; 352	17,850;12,000; 10,700; 4,500; 9,500	2
CH_3	NH_2	H	216;288; 343	29,500; 4,900; 15,700	2
Ph	NHPh	H	268;315; 375	18,000; 9,800 16,000	1
CH_3	NH_2	OH	<220;262; sh.280;378	>18,000;12,900; 6,800;17,100	1



CXXV

quinoxalone CXXV[64]

R	R^1	R^2			
CH_3	CH_3	H	229;280; 336	21,200; 5,600; 6,700	1
CH_3	CH_3	6-Cl	236;278; 342	31,000; 5,200; 5,200	1
CH_3	CH_3	7-Cl	232;281; 385	24,800; 6,200 6,600	1

1 = Ethanol. 2 = water. sh. = shoulder.

4-Chloro-8-methyl-7-pteridone (CXXIII; R = CH_3, R^1 = H, R^2 = Cl) was chosen as the model compound for reduction so that the effect, upon the chloro group, of lithium aluminium hydride might also be investigated. The product was found to be not the expected tetrahydropteridine (CXXVI) but a 7-hydroxy tetrahydropteridine (CXXVII) was given, the amide group being reduced to the corresponding carbinol-amine. The chloro group was untouched but the yield was indifferent (67%) and ionic halogen was found in the mother liquors. Longer treatment with the reducing agent resulted only in a poorer yield of the hydroxy compound (CXXVII)[x]. The relative

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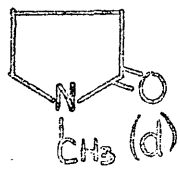
[x] Other anomalous reductions using lithium aluminium hydride have been reported. Brucine gives a reduction product dehydrobrucine in which reduction of the amide group (a) to the carbinol-amine (b) was followed by dehydration to give the unsaturated compound (c)[66].

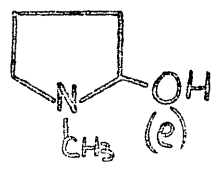
$$\text{>N.CO.CH}_2\text{-} \longrightarrow \underset{\text{OLiAlH}_3}{\text{>N.CH.CH}_2\text{-}} \longrightarrow \text{>N.CH = CH-}$$

(a) (b) (c)

The reaction of 1 mole of lithium aluminium hydride on the lactam (d) gave the carbinol-amine (e)[67]. On the other hand, reduction of the quinoxaline (CXXV; R = $C_2H_4N(C_2H_5)_2$, R^1 = Ph, R^2 = H[85]) gave the corresponding 1:2:3:4-tetrahydroquinoxaline.

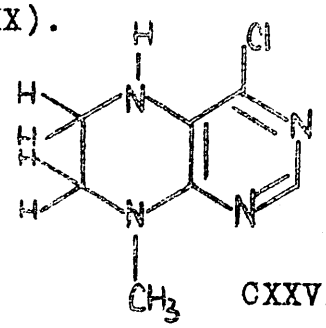


(d)

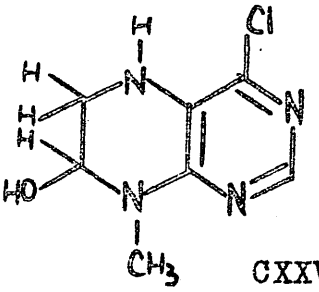


(e)

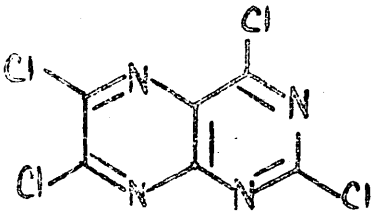
immunity of the chloro group is in agreement with the findings of an American group[65] who found that lithium aluminium hydride reduction of tetrachloropteridine (CXXVIII) gave 2:4-dichloro-5:6:7:8-tetrahydropteridine (CXXIX).



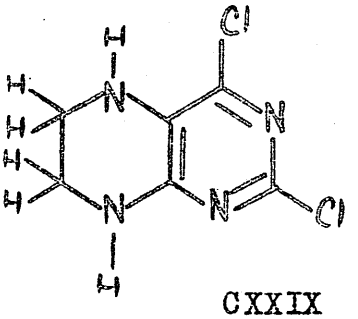
CXXVI



CXXVII



CXXVIII



CXXIX

The 7-hydroxy tetrahydropteridine (CXXVII) is stable, melts without decomposition and has an ultra-violet absorption spectrum (λ max.; 204; sh.294; 324 mu in N/10 HCl) closely resembling that of its parent pyrimidine (CXXII; R = CH_3, R^1 = H, R^2 = Cl) (λ max.; 210; 268; 295 mu in N/10 HCl), allowing for the bathochromic influence due to the greater molecular weight of the pteridine. This is to be expected since the same absorbing

system exists in both compounds and is in agreement with the findings of Lister and Ramage[33] who compared the spectra of diaminopyrimidines and of the tetrahydropteridines derivable from them.

The 4-chloro pteridone (CXXIII; R = CH_3, R^1 = H, R^2 = Cl) is readily converted into the corresponding 4-methylaminopteridine by treatment with methylamine. The 4-chloro group is very reactive because of the unsaturated system at position 5 which is exocyclic to, and in conjugation with, the pyrimidine ring.

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EXPERIMENTAL

Experimental

Yields of substances that have no definite m.p. refer to the stage when they appeared homogeneous in paper chromatography in butanol-5N acetic acid (7:3) on being viewed in ultra-violet light of wave-lengths 365 and 254 mu.

Absorption Spectra:- These were measured in the Unicam SP500 photoelectric spectrophotometer (with 1 cm. cells and concentrations of from 10^{-5} to 10^{-4} M) at 5 mu intervals except in the neighbourhood of peaks where 2 mu intervals were observed.

Potentiometric Titrations:- Weighed quantities of the pteridines (approx. M/1000; 5 mgm.) were dissolved in 25.0 ml. of distilled water and titrated with 0.1 M HCl or 0.1 M NaOH. The reference electrode was a calomel half-cell immersed in the solution with a glass electrode; pH was measured with a Cambridge pH meter. The solution was stirred by a current of nitrogen and the titrant added from an "Agla" micrometer syringe, about fifteen additions being made. The pKa values were calculated according to the equation $pKa = pH - \log \frac{([B] + [H^+])}{([BH^+] - [H^+])}$ where $[B]$, $[BH^+]$, and $[H^+]$ are the calculated concentrations of the base, conjugate acid,

and hydrogen ion respectively, corrected for the dilution caused by addition of acid or alkali.

"Hydrogenation in the usual way" means catalytic hydrogenation in ethanol for from 2-4 hours at 4 atmospheres pressure using Raney nickel catalyst. The catalyst is removed by vacuum filtration.

Dithiouracil[69]. - Thiouracil (140 g.) and powdered phosphorous pentasulphide (420 g.) were suspended in xylene (technical; 2 l.) contained in a 5 l. flask which was fitted with a stirrer and reflux air condenser. The mixture was heated slowly, with stirring, until it boiled, refluxed with stirring for 8 hours (bath temp. 157-162°) and refrigerated overnight. The xylene was removed by filtration. The filter-cake was well pressed, washed with benzene (100 ml.) and sucked fairly dry. The solid was then added to a hot, stirred mixture of ammonia (d, 0.88; 300 ml.) and water (1 l.). The solution was brought to 90° and the solid dissolved completely by the addition of more ammonia, Charcoal (30 ml.) was added and after stirring had continued for a few minutes longer most of the oily impurities were removed by filtration through a 15 cm. Buchner funnel having a layer of moistened sand on the paper. The filtrate was then filtered by gravity through a fluted paper. To the hot solution was added acetic acid (100 ml.) which reduced the pH to about 7 and precipitated the crude dithiol. The suspension was cooled to 5°, filtered off and washed by stirring with cold water (500 ml.). After filtering and drying at 120° the yield was 130 g. of a yellow powder decomposing at 245°.

2-Mercapto-4-methylaminopyrimidine[70]. - Dithiouracil (125 g.) was heated in sealed glass tubes in a Carius furnace at 100° for 3½ hours with methylamine (25-30%; 800 ml.). The reaction mixture was taken to dryness on the water-bath to give a dark brown solid which was taken up in hot water (1.5 l.) and charcoal (80 ml.) added. The mixture was filtered and the filtrate taken down to dryness on the water-bath to give a light brown powder (103 g.). A sample recrystallised twice from water gave the pyrimidine as colourless prisms m.p. 236-239° (Found: C,42.6; H,4.6. Calc. for $C_5H_7N_3S$: C, 42.55; H, 5.0%).

2-Hydroxy-4-methylaminopyrimidine[71]. - 2-Mercapto--4-methylaminopyrimidine (93 g.) and chloracetic acid (100 g.) were suspended in water (730 ml.) and the mixture refluxed for 40 minutes. Concentrated hydrochloric acid (680 ml.) was added and refluxing continued for a further 2 hours. The solution was evaporated to dryness in a porcelain basin on the water-bath. The solid was dissolved in warm water (520 ml.), the solution filtered, and the filtrate adjusted to pH 8-9 with ammonia (d, 0.88; 150 ml.). The solution was cooled to -20° and the white precipitate (28 g.) collected. The solution was concentrated *in vacuo* to 450 ml. and ammonia (d, 0.88;150 ml.)

added. Cooling to -20° gave a second crop of the pyrimidine (18 g., total yield 46 g.), m.p. 263-270°.

2-Hydroxy-4-methylamino-5-nitropyrimidine[72]. - 2-Hydroxy-4-methylaminopyrimidine (4 g.) was dissolved in concentrated sulphuric acid (10 ml.) at room temperature. Nitric acid (d, 1.5; 3.4 ml.) was added dropwise, the temperature of the solution being kept below 60°. The mixture was allowed to stand for 20 minutes at 40°, then poured into ice and water (100 ml.), and neutralised with ammonia (d, 0.88; 25 ml.). The mixture was filtered to give a pale yellow solid (2.3 g.). Recrystallisation from water gave the nitropyrimidine as almost colourless needles, decomp. above 270°. (Found: C,35.6; H,3.3. Calc. for $C_5H_6N_4O_3$: C, 35.3; H, 3.55%).

5-Amino-2-hydroxy-4-methylaminopyrimidine[71]. - 2-Hydroxy-4-methylamino-5-nitropyrimidine (5 g.) was suspended in ethanol (50 ml.) and hydrogenated in the usual way (see page 85). The catalyst was filtered off and washed with hot ethanol (50 ml.). The combined filtrates were evaporated to dryness to give the aminopyrimidine (3 g.) as a buff residue m.p. 215° (decomp.). Further purification was unnecessary.

4:6-Dihydroxy-5-nitropyrimidine. - 4:6-Dihydroxypyrimidine (22 g.)[73] was added slowly to nitric acid (d, 1.5; 72 ml.). The solution was stirred at 20° for 20 minutes and was then poured into ice and water (100 ml.). The near colourless product (15 g.) which precipitated was collected and dried at 100°, m.p. >300°.

4:6-Dichloro-5-nitropyrimidine[74]. - Diethylaniline (5 g.) was added to a suspension of 4:6-dihydroxy-5-nitropyrimidine (5 g.) in phosphorous oxychloride (32 g.). The mixture was refluxed gently for 1 hour (bath temp. 130-140°). The excess phosphorous oxychloride was removed under reduced pressure at 90° (bath). The residue was poured into ice (50 g.) and stirred for 20 minutes. The mixture was filtered and the filtrate extracted with ether (3 x 100 ml.). The combined extracts were washed with water (100 ml.), dried over anhydrous sodium sulphate and evaporated to dryness in vacuo. The residue was recrystallised from petrol (b.p. 60-80°) as needles (3 g.), m.p. 79-80°.

5-Amino-4:6-dichloropyrimidine[75]. - Hydrated barium hydroxide (30 g.), dissolved in hot water (100 ml.), was added to a solution of hydrated ferrous sulphate (25.5 g.) in water (205 ml.) at 75°. The mixture was stirred vigourously for 20 minutes. Finely ground

4:6-dichloro-5-nitropyrimidine (2 g.) was added and the temperature raised to 95° for 20 minutes. The suspension was filtered and the residue washed with hot water (100 ml.). The combined filtrates were extracted with chloroform (4 x 50 ml.). The combined chloroform extracts were washed with water (2 x 50 ml.), dried over anhydrous sodium sulphate and evaporated to dryness in vacuo. The residue was recrystallised from water (30 ml.) to give the pyrimidine as colourless needles (1.35 g.), m.p. 142-145°.

5-Amino-4-chloro-6-methylaminopyrimidine[75]. - 5--Amino-4:6-dichloropyrimidine (5 g.) was heated in a sealed tube with ethanolic methylamine (10% w./w. ; 36.5 ml.) for 6 hours at 125-130°. The solution was evaporated to dryness in vacuo and the dry residue extracted with boiling benzene (2 x 200 ml.). The extracts were cooled and the crystalline solid collected; evaporation of the combined filtrates to 100 ml. gave a second crop (overall yield 3.3 g.), Recrystallisation from water gave the pyrimidine as colourless needles, m.p. 160-162°.

5-Amino-4-methylaminopyrimidine[75]. - 5-Amino-4--chloro-6-methylaminopyrimidine (4.9 g.) was dissolved in hot water (120 ml.) and hydrogenated (25°/1 atmos.)

over freshly hydrogenated palladium catalyst (2.4 g.; 2.5% of Pd., initially as $PdCl_2$, on charcoal) in the presence of magnesium oxide (3.6 g.). Hydrogen uptake of 340 ml. took place in 5 hours. The mixture was filtered and sodium carbonate solution (10%; 30 ml.) was added to the filtrate, which was taken to dryness *in vacuo*. The residue was extracted with boiling iso-butyl methyl ketone (3 x 50 ml.). The combined extracts were chilled and then concentrated to give a total of 2.3 g. of the pyrimidine as stout prisms, m.p. 202-207°.

Bis-(8:9-dihydro-2-hydroxy-9-methylpurin-8-yl). - 5-Amino-2-hydroxy-4-methylaminopyrimidine (2.67 g.) was dissolved in water (55 ml.) at 50°, and glyoxal (50% aqueous solution; 2.67 g.) in water (27 ml.) was added. A bright yellow precipitate formed almost immediately; the colour rapidly changed to buff. The mixture was heated at 50° for 15 minutes, and the product (2.3 g.) was collected, washed with warm water, and dried. A sample was purified by dissolution in dilute hydrochloric acid, filtration of the solution and addition of dilute sodium hydroxide to the filtrate to pH 9-10 when bis-(8:9-dihydro-2-hydroxy-9-methylpurin-8-yl) was precipitated as pale yellow micro-

-needles, m.p. >300°. (Found: C,47.9; H,5.0; N,36.8. $C_{12}H_{14}O_2N_8$ requires: C,47.7; H,4.7; N,37.1%).

The bisdihydropurinyl was soluble in dilute alkali.

Attempted methylation of bis-(8:9-dihydro-2-hydroxy--9-methylpurin-8-yl). - (a) The bisdihydropurinyl (1 g.) was suspended in methanol (25 ml.) and water (5 ml.). Diazomethane (1 g.) in ether (50 ml.), from 8 g. of p-tolylsulphonylmethylnitrosamide, was added and the mixture left overnight. Starting material was recovered unchanged.

(b) The bisdihydropurinyl (0.7 g.) was dissolved in warm potassium hydroxide solution (0.4 g. KOH in 8 ml. water). Hydrochloric acid was added to reduce the pH to 8.5. Dimethyl sulphate (0.6 ml.) was added over 30 minutes with the temperature kept at 35-40° and the pH kept above 8.5 by the addition of potassium hydroxide solution (33% w./w.; 5 ml.). Starting material was recovered unchanged.

(c). The bisdihydropurinyl (0.5 g.), methyl iodide (4.5 ml.), and acetone (15 ml.) were refluxed with potassium carbonate (5 g.) for 24 hours. The solid was filtered off, washed with hot water to remove the potassium carbonate, and dried. Starting material was recovered unchanged.

Bis-(8:9-dihydro-9-methylpurin-8-yl). - 5-Amino--4-methylaminopyrimidine (0.69 g.) was dissolved in boiling water (3 ml.), and a solution of polyglyoxal (0.161 g.) in boiling water (4 ml.) was added. The solution was refluxed for 1 hour, and chilled overnight. The product (0.58 g.) was collected and recrystallised from aqueous ethanol to give bis-(8:9-dihydro-9-methylpurin-8-yl) as colourless needles, m.p. ca. 270° (decomp.). (Found: C,53.2; H,5.0; N,41.2. $C_{12}H_{14}N_8$ requires: C,53.3; H, 5.2; N, 41.5%).

Bis-(6-chloro-8:9-dihydro-9-methylpurin-8-yl). - 5-Amino-4-chloro-6-methylaminopyrimidine was condensed with polyglyoxal as above, to give bis-(6-chloro-8:9--dihydro-9-methylpurin-8-yl) as colourless needles from aqueous ethanol, m.p. ca. 270° (decomp.). (Found: C,42.5; H,3.8; N, 32.9; Cl, 20.8%. M.W., 320, 340. $C_{12}H_{12}N_8Cl_2$ requires: C,42.5; H,3.6; N,33.0; Cl,20.9%; M.W. 339).

Determination of molecular weight of bischlorodihydropurinyl. - About 10 mgm. of the bischlorodihydropurinyl was accurately weighed out into a dry chromic acid cleaned test-tube (3" x $\frac{1}{4}$"). Into the tube was accurately weighed about 250 mgm. of camphor (micro-

-analytical grade). The tube was sealed so as to form a narrow rod of about 4" long on the end. The camphor was melted by immersion of the tube in an oil-bath heated at about 10° above the melting point of camphor. The compound was dissolved and dispersed homogeneously in the camphor by rapid rotation of the rod between forefinger and thumb. The melt was allowed to solidify, and about 10 mgm. of the solid was transferred to a melting-point tube 3 mm. wide using usual molecular weight determination techniques. The melting-point tube was sealed. A similar procedure was carried out with camphor alone and the melting points of both samples determined concurrently.

The above practice was carried out on acetanilide as a control and twice on the chloro compound to give molecular weights for the latter of 320 and 340 (theory 339).

4:6-Bismethylamino-5-nitropyrimidine[75]. - Ethanolic methylamine (22%; 16 ml.) was added to a stirred solution of 4:6-dichloro-5-nitropyrimidine (3.8 g.) in methanol (40 ml.) over 10 minutes at 10°. Stirring was continued for a further 30 minutes. The solid (2.75 g.) was collected and recrystallised from ethanol to give the pyrimidine as pale yellow plates, m.p.192-5°.

5-Amino-4:6-bismethylaminopyrimidine. - 4:6-bis-methylamino-5-nitropyrimidine (0.45 g.) in ethanol (50 ml.) was hydrogenated over Raney nickel catalyst at room temperature and pressure. The catalyst was filtered off and washed with hot ethanol. The combined filtrates were taken to dryness *in vacuo*, and the residue sublimed (110°/0.0005 m.m.) to give 5-amino-4:6-bismethylamino-pyrimidine, m.p. 150° (decomp.). (Found: C,47.3; H,7.2; N, 45.5. $C_6H_{11}N_5$ requires: C,47.0; H,7.2; N,45.7%).

Bis-(8:9-dihydro-9-methyl-6-methylaminopurin-8-yl). - (a) To the above ethanolic solution of 5-amino-4:6--bismethylaminopyrimidine was added polyglyoxal (0.075 g.) in water (10 ml.), and the mixture was heated on the water-bath for 10 minutes. The solution was concentrated *in vacuo* to about 5 ml., and chilled overnight to give bis-(8:9-dihydro-9-methyl-6-methylaminopurin-8-yl) as colourless needles (from ethanol), m.p. 260° (decomp.). (Found: C,50.9; H,5.5; N, 43.2. $C_{14}H_{20}N_{10}$ requires: C, 51.2; H, 6.1; N, 42.7%).

(b) Bis-(6-chloro-8:9-dihydro-9-methylpurin-8-yl) (0.05 g.) and ethanolic methylamine (33%; 1 ml.) were heated together in a sealed tube for 5 hours at 125°. The solution was cooled, the solid collected and recrystallised from ethanol to give the bisdihydromethylaminopurinyl,

m.p. 258° (decomp.).

2:4-Bisdimethylamino-5-nitropyrimidine. - 2:4--Dichloro-5-nitropyrimidine (1 g.)[76] was dissolved in ethanol (20 ml.) and ethanolic dimethylamine (33%; 3 ml.) was added dropwise to the solution. The solution was stirred for 30 minutes, filtered, and the filtrate taken to dryness in vacuo. The residue was sublimed and recrystallised from light petroleum (b.p. 60-80°) to give 2:4-bisdimethylamino-5-nitropyrimidine (1 g.) as yellow needles, m.p. 88-92°. (Found: C,45.7; H,5.5; N,33.7. $C_8H_{13}O_2N_5$ requires: C,45.5; H,6.2; N,33.2%).

5-Amino-2:4-bisdimethylaminopyrimidine. - The above nitro compound (0.25 g.) was hydrogenated in the usual way. Removal of the catalyst and evaporation of the filtrate in vacuo gave 5-amino-2:4-bisdimethylaminopyrimidine as white needles, m.p. 92-96°, after sublimation at 90° (bath)/0.01 m.m. (Found: C,53.8; H,7.9. $C_8H_{15}N_5$ requires: C,53.0; H,8.3%).

Mixed m.p. with 2:4-bisdimethylamino-5-nitropyrimidine was 66-94°.

Azomethine of glyoxal and 5-amino-2:4-bisdimethylaminopyrimidine. - 5-Amino-2:4-bisdimethylaminopyr-

imidine (0.02 g.) was dissolved in a mixture of hot water (10 ml.) and ethanol (3 ml.), and a solution of polyglyoxal (0.038 g.) in hot water (5 ml.) added, whereupon an immediate precipitate was formed. The mixture was heated on the water-bath for 2 minutes and cooled. The product was collected and recrystallised from aqueous ethanol to give glyoxylidenebis-(5-amino--2:4-bisdimethylaminopyrimidine) as bright red prisms, m.p. 195-199°. (Found: C,56.7; H,6.7; N,36.7. $C_{18}H_{20}N_{10}$ requires:C,56.2; H,7.3; N,36.7%).

Treatment of the azomethine with cold N-hydrochloric acid effected immediate hydrolysis.

Azomethine of glyoxal and 4-amino-2:6-dihydroxypyrimidine. - Polyglyoxal (0.23 g.) in warm water (20 ml.) was added to a solution of 4-amino-2:6-dihydroxypyrimidine (1 g.)[77] in boiling water (200 ml.), whereupon a precipitate formed almost immediately. The mixture was refluxed for 1 hour and the solid (0.35 g.) was collected while the mixture was still hot. The product was purified by dissolution in hot sodium carbonate solution (2 N; 20 ml.) from which glyoxylidenebis--(4-amino-2:6-dihydroxypyrimidine), m.p. $>360°$, was precipitated by the addition of dilute hydrochloric acid.

(Found: C,43.7; H,2.9; N,30.0. $C_{10}H_8O_4N_6$ requires: C,43.5; H,2.9; N,30.4%).

Azomethine of glyoxal and 6-amino-1:2:3:4-tetrahydro-1:3-dimethyl-2:4-dioxopyrimidine. - Similar condensation to the above of glyoxal with 6-amino-1:2:3:4-tetrahydro-1:3-dimethyl-2:4-dioxopyrimidine[78] gave glyoxylidenebis-(6-amino-1:2:3:4-tetrahydro-1:3-dimethyl-2:4-dioxopyrimidine) as colourless needles (68%) from water, m.p. >300° (Found: C,50.6; H,4.4; N,25.3. $C_{14}H_{16}O_4N_6$ requires: C,50.6; H,4.9; N,25.3%).

5-Aminopyrimidine. - Hydrogenation of 5-amino-4:6-dichloropyrimidine, in the presence of magnesium oxide using palladium-charcoal catalyst gave 5-aminopyrimidine, m.p. 169-171°. Whittaker[34] obtained this compound in a similar manner from 5-amino-2:4-dichloropyrimidine.

Neither 5-aminopyrimidine nor 5-amino-4:6-dichloropyrimidine reacted when refluxed with glyoxal for several hours in aqueous or ethanolic solution.

Oxalylbis-1-(4:6-bismethylamino-5-nitropyrimidinium)-dichloride. - To a solution of 4:6-bismethylamino-5-nitropyrimidine (0.5 g.) in boiling dry benzene (200 ml.) was added a solution of re-distilled oxalyl chloride

(0.11 ml.) in dry benzene (10 ml.). The solution was refluxed for 30 minutes during which a precipitate gradually formed. Pale yellow crystals of oxalylbis--1-(4:6-bismethylamino-5-nitropyrimidinium)-dichloride (0.43 g.) was collected and dried in vacuo, m.p. 255° (decomp.). (Found: C,34.5; H,4.5; N,28.5; Cl,14.7. $C_{14}H_{16}O_6N_{10}Cl_2$ requires: C,34.1; H,3.7; N,28.4; Cl, 14.4%).

4:6-bismethylamino-5-nitropyrimidine did not react when fused with oxalic acid for 30 minutes at 160°. The dichloride reacted vigourously with sodium hydrogen carbonate solution in the cold to give 4:6-bismethylamino-5-nitropyrimidine.

Oxalylbis-1(or 3)-(2:4-bisdimethylamino-5-nitropyrimidinium)-dichloride. - 2:4-Bisdimethylamino-5--nitropyrimidine was reacted in ether solution with oxalyl chloride as above to give oxalylbis-1(or 3)--(2:4-bisdimethylamino-5-nitropyrimidinium)-dichloride as yellow crystals, m.p. 154-158°. (Found: C,39.2; H,5.0; N,25.2. $C_{18}H_{26}O_6N_{10}Cl_2$ requires: C,39.3; H, 4.8; N,25.5%).

The pyrimidinium dichloride reacted vigourously with sodium hydrogen carbonate solution in the cold to

give 2:4-bisdimethylamino-5-nitropyrimidine.

N:N'-di-(4:6-dichloro-5-pyrimidyl)-oxamide. - 5--Amino-4:6-dichloropyrimidine (2g.) was heated in dry refluxing benzene (100 ml.) until a solution was given, oxalyl chloride (1.15 ml.) was added, and the solution refluxed for 45 minutes. Brown solid crystallised out of solution after 5 minutes refluxing. On cooling, light brown plates were deposited and collected by filtration. The product (2 g.) was insoluble in most organic solvents and was purified by precipitation from an alkaline solution (2 N sodium carbonate) by acidification with dilute hydrochloric acid to give N:N'-di-(4:6-dichloro-5-pyrimidyl)-oxamide, decomp. 275-285°. (Found: C,31.96; H,1.3; Cl,37.18. $C_{10}H_4O_2N_6Cl_4$ requires: C,31.43; H,1.06; Cl,37.13%).

Absorption spectrum in N/10 NaOH λmax.; 218;258; sh.310; sh.330 mu. εmax.; 7,780; 12,700; 3,600; 3,000.

Methylamination of the above diamide. - The diamide (2 g.) was suspended in ethanolic methylamine (33%; 5 ml.). The suspension was mechanically shaken for 5 hours when the diamide was found to be in solution. The solution was evaporated to dryness at 20° in vacuo, and the residue extracted with ethyl acetate. The

extract was taken down to dryness and the residue extracted with petrol. The petrol extract was taken to dryness and the residue recrystallised from chloroform to give 5-amino-4-chloro-6-methylaminopyrimidine, m.p. and m.p. when mixed with an authentic specimen, 160-162°.

8-Methyl-6:7-diphenyl-2-pteridone. - 5-Amino-2--hydroxy-4-methylaminopyrimidine (0.64 g.) was dissolved in boiling water (2 ml.). Benzil (1.07 g.) in ethanol (2 ml.) was added to the hot solution which was then heated on the water-bath for 15 minutes. The brei was chilled and the product collected and recrystallised from ethanol to give 8-methyl-6:7-diphenyl-2-pteridone as pale yellow prisms (1.19 g.) decomp. 180°. (Found: C,68.84; H,4.34; N,17.2. $C_{19}H_{14}ON_4.H_2O$ requires: C,68.66; H,4.85; N,16.9%).

6:7:8-Trimethyl-2-pteridone[41]. - 5-Amino-2-hydroxy--4-methylaminopyrimidine (1.4 g.) was dissolved in boiling water (4 ml.) and treated with diacetyl (1.0 ml.). The resulting brei was warmed on the water-bath for 15 minutes. The product was filtered off and washed with water (4 ml.) and ethanol (1 ml.). Recrystallisation from ethanol (150 ml.) gave the pteridone (0.5 g.) as colourless needles, m.p. 253-257°. (Found: N,29.7;29.0: Calc. for $C_9H_{10}ON_4$: N,29.45%).

Sodium salt of 8-methyl-6:7-diphenyl-2-pteridone. - 8-Methyl-6:7-diphenyl-2-pteridone (0.1 g.) was finely ground and added to hot caustic soda solution (10 N; 10 ml.). The mixture was heated on the water bath for 5 minutes, and warm water (10 ml.) added to effect complete solution. The solution was filtered through a fluted filter paper, heated on the water-bath and strong caustic soda solution (10 N; 10 ml.) added, whereupon a flocculent white precipitate was given. The mixture was cooled, the product collected, and washed with ethanol and acetone. The sodium salt was stored in a desiccator.

The sodium salt was hygroscopic and could not be analysed.

Degradation of 8-methyl-6:7-diphenyl-2-pteridone. - (a) Acid degradation. - The pteridone (0.5 g.) was treated with refluxing dilute hydrochloric acid (1 N; 50 ml.) for 12 hours. The mixture was allowed to cool and extracted with chloroform (3 x 25 ml.). The combined chloroform extracts were washed with water, dried over anhydrous sodium sulphate and taken to dryness to give benzil (0.3126 g.; theory 0.3155 g.), m.p. and mixed m.p. with an authentic sample 93-96°. The aqueous layer and the washings from the chloroform

layer were combined and made up to 250 ml. with hydrochloric acid (0.1 N) and this solution diluted 1 in 25 with hydrochloric acid (0.1 N). Determination of the intensity of absorption at 294 mu in the ultra-violet absorption spectrophotometer gave the weight of 5-amino-2-hydroxy-4-methylaminopyrimidine as 0.227 g. (theory, 0.223 g.).

(b). Alkaline degradation. - The pteridone (0.2g.) was treated with refluxing NaOH solution (5 N; 25 ml.) for 6½ hours. Complete solution after 2 hours was followed by deposition of feathery needles of the sodium salt. The solution was cooled, poured into warm water (200 ml.) and filtered. The filtrate was acidified with concentrated HCl (19 ml.) and allowed to stand overnight. The precipitate of unchanged pteridone was filtered off and the filtrate extracted with chloroform. The extract was worked up as before to give 0.092 g. of benzil (68.5% of theory). The aqueous phase was treated as above and calculation from the extinction value showed that 0.054 g. of 5-amino-2-hydroxy-4-methylaminopyrimidine was present (65.8% of theory).

7:8-Dihydro-2-hydroxy-8-methyl-6:7-diphenylpteridine--2-Hydroxy-4-methylamino-5-nitropyrimidine (0.3 g.) was

suspended in ethanol (40 ml.) and hydrogenated in the usual way to give 5-amino-2-hydroxy-4-methylaminopyrimidine. The mixture was boiled and the catalyst filtered off. To the filtrate was added benzoin (0.4 g.) in ethanol (5 ml.), acetic acid (2 ml.), and boiling water (5 ml.). The whole was refluxed for 2 hours and chilled overnight. The solid was collected and boiled with chloroform (50 ml.). The residue on filtration was leached with hot NaOH (5 N; 10 ml.) and the solution cooled, neutralised with acetic acid, and chilled overnight. 7:8-Dihydro-2-hydroxy-8-methyl-6:7--diphenylpteridine was collected as a colourless powder, m.p. $>$ 300°. (Found: C,70.0: H,4.6; N,17.7. $C_{19}H_{16}ON_4 . \frac{1}{2}H_2O$ requires: C,70.0; H,5.2; N,17.25%).

4-Benzylamino-2-mercaptopyrimidine.- 2:4-Dimercaptopyrimidine (10 g.) was heated with benzylamine (50 ml.) on the water-bath for 72 hours and the white crystalline product (13.5 g.) collected. Recrystallisation from ethanol gave 4-benzylamino-2-mercaptopyrimidine as colourless needles, m.p. 249-253° (decomp.). (Found: C,60.8; H,5.3; N,19.28. $C_{11}H_{11}N_3S$ requires: C,60.75; H,5.08; N,19.35%).

4-Benzylamino-2-hydroxypyrimidine. - 4-Benzylamino--2-mercaptopyrimidine (13 g.) and chloracetic acid (10 g.) were heated together in refluxing water (71.5 ml.) for 40 minutes. Hydrochloric acid (concentrated; 66.5 ml.) was added to the solution and refluxing continued for a further 3 hours. Evaporation of the solution on the water-bath in a porcelain basin gave a dark brown solid which was dissolved in water (70 ml.). The solution was adjusted to pH 8-9 with ammonia and the mixture was stirred for 30 minutes. The white precipitate was collected, washed with water and acetone, and recrystallised from ethanol (400 ml.) to give 4-benzylamino-2-hydroxypyrimidine as colourless needles, m.p. 213-217° (decomp.). (Found: C,65.92; H,5.39; N,21.1. $C_{11}H_{11}ON_3$ requires: C,65.7; H,5.47; N,20.9%).

The hydroxypyrimidine could not be coupled with p--chlorobenzenediazonium chloride, nitration gave a mixture of di- and trinitro derivatives.

4-Benzylamino-2-hydroxy-5-nitropyrimidine. - 2:4--Dichloro-5-nitropyrimidine (2 g.)[79] was dissolved in acetone (10 ml.) and the solution added dropwise to a stirred mixture of ice and water (200 ml.) over 10 minutes. To the stirred mixture was added a solution of

benzylamine (3 ml.) in iced water (10 ml.) over half an hour. The solid was collected, washed with water (50 ml.), heated to boiling with NaOH solution (2 N; 50 ml.) and the mixture filtered. The residue was 2:4--bisbenzylamino-5-nitropyrimidine, m.p. 178-180°. The filtrate was cooled and acidified with acetic acid and the bright yellow product collected. Recrystallisation from ethanol (150 ml.) gave 4-benzylamino-2-hydroxy-5--nitropyrimidine (1.3 g.) as pale yellow needles, m.p. 225-228°. (Found: C,54.05; H,3.7; N,23.16. $C_{11}H_{10}O_3N_4$ requires: C,53.7; H,4.07; N,22.8%).

5-Amino-4-benzylamino-2-hydroxypyrimidine. - 4--Benzylamino-2-hydroxy-5-nitropyrimidine (1.1 g.) in hot ethanol (200 ml.) was hydrogenated in the usual way. The mixture was boiled and the catalyst filtered off. The filtrate was concentrated to 5 ml. and chilled. The pale brown solid was collected and recrystallised from aqueous ethanol to give 5-amino-4-benzylamino-2-hydroxypyrimidine as golden prisms, m.p. 218-223° (decomp.). (Found: C,60.7; H,4.9; N,26.1. $C_{11}H_{12}ON_4$ requires: C,61.1; H,5.55; N,25.9%).

Absorption spectrum in N/10 NaOH; λmax.; 220; 298 mu. εmax.; 16,400; 6,000.

8-Benzyl-6:7-dimethyl-2-pteridone. - 5-Amino-4--benzylamino-2-hydroxypyrimidine (0.1 g.) was dissolved in hot ethanol (10 ml.). To the boiling solution was added diacetyl (0.1 ml.) and the resulting solution heated for 20 minutes on the water-bath and cooled. The product was collected and recrystallised from ethanol (20 ml.) to give 8-benzyl-6:7-dimethyl-2-pteridone (0.06 g.) as golden needles, decomp. above 240°. (Found: C,68.0; H,5.0; N,21.3. $C_{15}H_{14}ON_4$ requires C,67.65; H,5.26; N,21.1%).

The pteridone was soluble in dilute NaOH solution.

8-Benzyl-2-pteridone. - 5-Amino-4-benzylamino-2--hydroxypyrimidine (0.2 g.) was dissolved in hot ethanol (50 ml.) and added over half an hour to a boiling solution of polyglyoxal (0.12 g.; 2 x theoretical) in ethanol (20 ml.). The mixture was heated on the water-bath for a further half-hour during which a yellow solid separated out. The precipitate was collected and the filtrate evaporated *in vacuo* to small volume with the addition of hot water (4 ml.). The solution was chilled overnight to give the product (0.06 g.). Recrystallisation from ethanol and water gave 8-benzyl-2-pteridone as pale brown crystals, m.p. 240° (decomp.) (Found: C,60.7; H,4.8; N,21.2. $C_{13}H_{10}ON_4.H_2O$ requires C,60.9; H,4.65; N,21.9%).

The pteridone was soluble in dilute NaOH.

The precipitate (0.027 g.) was glyoxylidenebis-(5--amino-4-benzylamino-2-hydroxypyrimidine) and was purified by solution in hot 2 N-NaOH/ethanol (1:1) and precipitation by careful neutralisation. (Found: N,25.1. $C_{24}H_{22}O_2N_8$ requires: N,24.7%).

Treatment of the azomethine with dilute mineral acid effected hydrolysis to give 5-amino-4-benzylamino-2-hydroxy-pyrimidine.

4-Chloro-6-methylamino-5-p-nitrobenzilideneamino-pyrimidine. - 5-Amino-4-chloro-6-methylaminopyrimidine (0.4 g.) was dissolved in hot ethanol (20 ml.). To the hot solution was added p-nitrobenzaldehyde (0.44 g.) in hot ethanol (10 ml.). The solution was heated on the water-bath for 30 minutes and chilled. The product (0.6 g.) was collected and the filtrate concentrated to give a second crop (0.16 g.). Recrystallisation from ethyl acetate (30 ml.) gave 4-chloro-6-methylamino-5-p-nitro-benzilideneaminopyrimidine as red prisms, m.p. 198-200°. (Found: C,49.2; H,3.05; N,24.3. $C_{12}H_{10}O_2N_5Cl$ requires: C,49.4; H,3.44; N,24.1%).

Treatment of the azomethine with mineral acid effected immediate hydrolysis. Ultra-violet absorption spectrum

in ethanol: λmax.; 207; 269; 386 mu. εmax.; 23,700; 22,600; 5,100.

7:8-Dihydro-7-hydroxy-8-methyl-6:7-diphenylpteridine. - 5-Amino-4-methylaminopyrimidine (0.19 g.) was dissolved in boiling water (3 ml.) and benzil (0.24 g.) in solution in n-propanol (3 ml.) added. Glacial acetic acid (0.05 ml.) was added to the solution and the whole refluxed for 2 hours. The product (0.24 g.) separated out on cooling. Recrystallisation from ethanol/water gave 7:8-dihydro-7-hydroxy-8-methyl-6:7--diphenylpteridine as colourless blades, m.p. 185-190° (decomp.). (Found: C,72.32; H,4.52; N,18.0. $C_{19}H_{16}ON_4$ requires: C,72.11; H,5.09; N,17.7%).

The pseudo base is soluble in dilute mineral acid.

Ultra-violet absorption spectrum (a) ethanol: λmax.; <220; 348. mu. εmax.; >25,000; 10,000. (b) 1NHCl: λmax.; <220; 266 mu. εmax.; >20,000; 22,150.

5-Benzamido-4-methylaminopyrimidine. - To a solution of 5-amino-4-methylaminopyrimidine (0.5 g.) in boiling dry pyridine (4.7 ml.) was added benzoyl chloride (0.565 g.; 1 equiv.) dropwise. The solution was refluxed for 5 minutes and cooled. Sodium hydrogen carbonate (0.34 g.) and water (2 ml.) were added

to the solution and the whole taken to dryness *in vacuo* on the water-bath. Extraction of the residue with boiling water (2 x 50 ml.) and cooling of the extracts gave the product as colourless needles (0.36 g.). Recrystallisation from water (300 parts) gave 5-benzamido--4-methylaminopyrimidine as colourless needles, m.p. 229-233°. (Found: C,63.0; H,5.0; N,24.7. $C_{12}H_{12}ON_4$ requires: C,63.2; H,5.26; N,24.6%).

9-Methyl-8-phenylpurine. - 5-Benzamido-4-methylaminopyrimidine (0.13 g.) was heated in a test-tube at 240° (bath) for 20 minutes. The melt was cooled, extracted with ethanol, the extract charcoaled and the solution evaporated to dryness. The residue was sublimed at 140°/0.1 m.m. to give colourless crystalline 9-methyl-8-phenylpurine, m.p. 156-160°. (Found: C,68.8; H,4.4; N,27.1. $C_{12}H_{10}N_4$ requires: C,68.5; H,4.76; N,26.7%).

Ultra-violet absorption spectrum in ethanol: λmax.; 230; 282 mu. εmax.; 14,200; 19,800.

2:4:6-Trichloropyrimidine. - The pyrimidine was synthesised by chlorination of barbituric acid (2:4:6--trihydroxypyrimidine) according to the method of

Baddiley and Topham[84]. The yield was increased from 46% to 90% by the use of diethylaniline instead of dimethylaniline.

2-Amino-4:6-dichloropyrimidine. - (a) Treatment of 2:4:6-trichloropyrimidine with ammonia according to the method of Buttner[80] gave a mixture of 2-amino-4:6--dichloropyrimidine and 4-amino-2:6-dichloropyrimidine. Separation of the isomers was effected by soxhlet extraction of the mixture with petrol (b.p. 60-80°) followed by recrystallisation of the extracted solid from ethanol to give 2-amino-4:6-dichloropyrimidine.

(b). 2-Amino-4:6-dihydroxypyrimidine was chlorinated according to the method of Langerman and Banks[81].

2-Amino-4-chloro-6-methylaminopyrimidine[82]. - 2-Amino-4:6-dichloropyrimidine (3 g.) was heated with alcoholic methylamine (15% w./v.; 12 ml.) in a sealed tube at 100° for 3 hours. The mixture was taken to dryness in vacuo and extracted with boiling water (40 ml.) from which the product (1.8 g.) crystallised in needles, m.p. 162-164°.

2-Amino-4-methylaminopyrimidine. - 2-Amino-4--chloro-6-methylaminopyrimidine (1.24 g.) was dissolved in hot water (100 ml.). To the solution was added

freshly reduced palladium catalyst (0.6 g.; originally as 2.5% $PdCl_2$ on charcoal) and magnesium oxide (0.9 g.). The mixture was hydrogenated at room temperature and pressure and the required hydrogen uptake was absorbed in 12 hours. The mixture was heated, filtered, and the residue washed with acetone. To the combined filtrates was added sodium carbonate solution (2 N; 6 ml.) and the mixture evaporated to dryness in vacuo on the water--bath. The residue was extracted with methyl isobutyl ketone (3 x 50 ml.) from which 2-amino-4-methylamino-pyrimidine (0.94 g.) crystallised in colourless needles, m.p. 161-163°, the pyrimidine sublimes at 120°/0.0005 m.m. (Found: C,48.25; H,6.49; N,45.0. $C_5H_8N_4$ requires: C,48.4; H,6.45; N,45.2%).

Mixed melt with starting material depresses 30°. Ultra-violet absorption spectrum in ethanol: λ max.; 212; 238; 284 mu. ε max.; 21,000; 10,600; 7,000.

2-Amino-5-p-chlorobenzeneazo-4-methylaminopyrimidine. - p-Chloroaniline (1.4 g.) was dissolved in dilute hydrochloric acid (1 N; 28 ml.) and diazotised at 0° by the slow addition of sodium nitrite (0.81 g.). The diazo solution was added to a suspension of 2-amino-4--methylaminopyrimidine (0.94 g.) in water (11 ml.) at 0°.

The mixture was allowed to stand for 5 minutes when complete solution had taken place. To the solution was added sodium carbonate (3.5 g.) whereupon coupling took place. The mixture was allowed to stand for 2 hours. The product (2.25 g.) was filtered off and dried in a vacuum desiccator. Recrystallisation from ethanol (30 parts) gave 2-amino-5-p-chlorobenzeneazo--4-methylaminopyrimidine as deep red twisted needles, m.p. 227-229°. (Found: C,50.0; H,4.28; N,31.9. $C_{11}H_{11}N_6Cl$ requires: C,50.3; H,4.2; N,32.1%).

2:5-Diamino-4-methylaminopyrimidine. - 2-Amino--5-p-chlorobenzeneazo-4-methylaminopyrimidine (3 g.) was dissolved in hot methanol (100 ml.) and hydrogenated in the usual way. The catalyst was removed and the crude diamino solution used immediately for condensation, because the diaminopyrimidine was too unstable to isolate.

2:8-Dihydro-2-Imino-6:7:8-trimethylpteridine. - To the above crude methanolic solution of 2:5-diamino--4-methylaminopyrimidine was added diacetyl (1.0 ml.). The solution was refluxed for 30 minutes and reduced *in vacuo* to 5 ml. The solution was chilled and the product (0.5 g.) collected. Recrystallisation from methanol (10 parts) gave 2:8-dihydro-2-imino-6:7:8-

-trimethylpteridine as colourless crystals, m.p. 235-240° (decomp.) pKa 5.6 ± 0.1. (Found: C,56.8; H,5.42; N,37.5. $C_9H_{11}N_5$ requires: C,57.15; H,5.8; N,37.1%).

Ammonia was liberated when the iminopteridine was heated in a strong KOH solution.

2-Acetylamino-4-hydroxy-6:7-diphenylpteridine. - To a suspension of 2-amino-4-hydroxy-6:7-diphenylpteridine (0.05 g.) in acetic anhydride (2 ml.) was added concentrated sulphuric acid (3 drops) and the solution heated on the water-bath for 1 hour. The solution was poured into water (15 ml.) and allowed to stand for 2 hours when a white precipitate had formed. The solid (0.037 g.) was collected, washed with sodium hydrogen carbonate solution, water, and acetone, and dried at 80°. Recrystallisation from aqueous ethanol gave 2-acetylamino-4-hydroxy-6:7-diphenylpteridine as pale yellow blades, m.p. 235-238°. (Found: C,67.1; H,4.1; N,19.7. $C_{20}H_{15}O_2N_5$ requires: C,67.3; H,4.2; N,19.7%).

2-Acetylimino-2:8-dihydro-6:7:8-trimethylpteridine. - 2:8-Dihydro-2-imino-6:7:8-trimethylpteridine (0.10 g.) was dissolved in acetic anhydride (3 ml.) and the solution heated on the water-bath for 1 hour. The sol-

ution was poured into water, allowed to stand for 1 hour and neutralised with sodium hydrogen carbonate solution. The suspension was extracted with chloroform (3 x 50 ml.). The combined extracts were washed with water (3 x 50 ml.), dried over anhydrous sodium sulphate and taken down to dryness in vacuo. The crystalline residue (0.07 g.) was dissolved in methanol (20 ml.), charcoaled, and recrystallised from methanol/ethyl acetate to give 2-acetylimino-2:8-dihydro-6:7:8--trimethylpteridine as colourless needles, m.p. 165-170°. (Found: N,30.7. $C_{11}H_{13}ON_5$ requires: N,30.3%).

Ultra-violet absorption spectrum in ethanol: λmax.; 226; sh.250; 318; 364 mu. εmax.; 25,500; 9,400; 7,150; 6,100.

2:4-Bismethylamino-5-nitropyrimidine. - This pyrimidine was synthesised in the same way as for 4:6--bismethylamino-5-nitropyrimidine (see page 94).

5-Amino-2:4-bismethylaminopyrimidine. - 2:4--Bismethylamino-5-nitropyrimidine was hydrogenated in the usual way. 5-Amino-2:4-bismethylaminopyrimidine was not isolable due to its instability and the filtrate from the reduction was used directly for condensation.

2:8-Dihydro-6:7:8-trimethyl-2-methyliminopteridine. - 2:4-Bismethylamino-5-nitropyrimidine (0.6 g.) was reduced as above to give the 5-aminopyrimidine. To the filtrate was added diacetyl (0.5 ml.). The solution was refluxed for 10 minutes and concentrated to 2 ml. *in vacuo*. The solution was chilled and the product (0.4 g.) collected and recrystallised from methanol/water (10:1; 10 parts) to give 2:8-dihydro-6:7:8-trimethyl-2-methyliminopteridine as pale brown needles, m.p. 197-198° (decomp.), pKa 6.1 ± 0.2. (Found: C,58.9; H,6.2; N,34.3. $C_{10}H_{15}N_5$ requires: C,59.2; H,6.4; N,34.5%).

Methylamine was liberated when the methyliminopteridine was heated with strong KOH solution.

2:4-Dianilino-5-nitropyrimidine. - 2:4-Dichloro--5-nitropyrimidine (0.5 g.) was dissolved in dry benzene (10 ml.) and the solution added dropwise to a vigorously stirred solution of aniline (4 ml.) in benzene (10 ml.). The mixture was stirred for 30 minutes, the bright yellow product (1.3 g.) collected, washed with ethanol, and dried. Recrystallisation from ethanol (200 parts) gave 2:4-dianilino-5-nitropyrimidine as yellow needles m.p. 198-202°. (Found: C,63.0; H,3.99; N,23.2. $C_{16}H_{13}O_2N_5$ requires: C,62.6; H,4.25; N,22.8%).

5-Amino-2:4-dianilinopyrimidine. - 2:4-Dianilino--5-nitropyrimidine (0.3 g.) in ethanol (200 ml.) was hydrogenated in the usual way. The 5-aminopyrimidine was too unstable to isolate and the filtrate from the reduction was used for condensation immediately.

6:7:8-Triphenyl-2-phenyliminopteridine. - To the above solution of 5-amino-2:4-dianilinopyrimidine was added benzil (0.3 g.), acetic acid (6 drops), and water (3 ml.). The solution was heated on the water--bath for 20 minutes and concentrated *in vacuo* to 10 ml. The solution was chilled overnight and the product (0.25 g.) which separated out was collected. Recrystallisation from ethanol (100 parts) gave 2:8-dihydro--6:7:8-triphenyl-2-phenyliminopteridine as pale green plates, m.p. 225-227° (decomp.). (Found: C,78.1; H,4.9; N,15.3. $C_{30}H_{21}N_5$ requires: C,78.1; H,4.8; N,14.95%).

The phenyliminopteridine gave a deep green colour in concentrated sulphuric acid.

2:8-Dihydro-6:7-dimethyl-8-phenyl-2-phenylimino-pteridine. - 2:4-dianilino-5-nitropyrimidine (0.4 g.) in ethanol (100 ml.) was reduced as above. To the filtrate was added acetic acid (3 drops) and diacetyl

(0.3 ml.) and the solution refluxed for 20 minutes, concentrated *in vacuo* to 30 ml., and chilled. The product (0.15 g.) was collected and recrystallised from ethanol to give 2:8-dihydro-6:7-dimethyl-8-phenyl-2--phenyliminopteridine as pale pink needles, m.p. 241--242° (decomp.). (Found: C,73.9; H,5.3; N,21.8. $C_{20}H_{17}N_5$ requires: C,73.4; H,5.2; N,21.45%).

The dimethyliminopteridine gave no colour in concentrated sulphuric acid.

2:4-Bisbenzylamino-5-nitropyrimidine. - 2:4--Dichloro-5-nitropyrimidine (0.55 g.) was dissolved in warm benzene (30 ml.). Benzylamine (2 ml.) was run in slowly to the stirred solution to give an immediate yellow precipitate. The mixture was stirred for a further 20 minutes and the product (1.55 g.) collected. Recrystallisation from ethanol (200 parts) gave 2:4--bisbenzylamino-5-nitropyrimidine as pale yellow plates, m.p. 179-182°. (Found: C,64.46; H,4.33; N,21.0. $C_{18}H_{17}O_2N_5$ requires: C,64.5; H,5.07; N,20.9%).

5-Amino-2:4-bisbenzylaminopyrimidine. - 2:4--Bisbenzylamino-5-nitropyrimidine (0.3 g.) in ethanol (100 ml.) was hydrogenated in the usual way. The 5--aminopyrimidine could not be isolated due to instability

and the filtered solution was used for condensation immediately.

8-Benzyl-2-benzylimino-2:8-dihydro-6:7-dimethylpteridine. - To the above solution of 5-amino-2:4--bisbenzylaminopyrimidine was added diacetyl (0.2 ml.) and the solution heated for 20 minutes on the water--bath. The volume of the solution was reduced *in vacuo* to 5 ml. and the solution chilled. The product (0.2 g.) separated out and was collected. Recrystallisation from ethanol gave 8-benzyl-2-benzylimino-2:8-dihydro--6:7-dimethylpteridine as yellow needles, m.p. 181-185°, (decomp.). (Found: C,74.8; H,5.75; N,19.8. $C_{22}H_{21}N_5$ requires: C,74.4; H,5.95; N,19.7%).

8-Benzyl-2-benzylimino-2:8-dihydropteridine. - 2:4-Bisbenzylamino-5-nitropyrimidine (0.2 g.) in ethanol (70 ml.) was reduced in the usual way. To the filtrate was added acetic acid (0.2 ml.), and polyglyoxal (0.04 g.) in ethanol (20 ml.). The solution was refluxed for 10 minutes and a saturated solution of potassium hydrogen carbonate (2 ml.) in water (70 ml.) added. Ethanol (40 ml.) was distilled *in vacuo* from the solution, and the cooled residual solution was extracted with chloroform (3 x 50 ml.). The combined chloroform extracts

were washed with water (3 x 50 ml.), dried over anhydrous sodium sulphate and taken to dryness *in vacuo* to give 8-*benzyl*-2-*benzylimino*-2:8-*dihydropteridine* as a non-crystallisable light-brown solid. (Found: C,71.1, 71.1; H,4.7, 4.6; N,20.2. $C_{20}H_{17}N_5 \cdot \frac{1}{2}H_2O$ requires: C,71.4; H,5.36; N,20.85%).

2-*Amino*-4-*chloro*-6-*hydroxypyrimidine*[83]. - 2--Amino-4:6-dichloropyrimidine (10 g.) was refluxed for 4 hours with NaOH solution (1 N; 130 ml.). The solution was cooled, acidified with acetic acid and the precipitated product (8.5 g.) collected, m.p. 260-262°.

2-*Amino*-4-*hydroxy*-6-*methylaminopyrimidine*. - 2--Amino-4-chloro-6-hydroxypyrimidine (8.5 g.) was heated in a sealed tube with ethanolic methylamine (33% w./w.; 25 ml.) at 120° for 4 hours. The solution was cooled and the product collected, dissolved in hot, dilute HCl (1 N; 30 ml.), charcoaled, and precipitated with sodium hydrogen carbonate. Recrystallisation from ethanol (60 parts) gave 2-*amino*-4-*hydroxy*-6-*methylaminopyrimidine* (3.7 g.) as light-brown plates, m.p. 255-257°. (Found: C,43.07; H,5.6;N,39.6: $C_5H_8ON_4$ requires: C,42.85; H,5.75; N,40.0%).

2-Amino-4-hydroxy-6-methylamino-5-nitroso-pyrimidine. - 2-Amino-4-hydroxy-6-methylamino-pyrimidine (2.5 g.) was dissolved in dilute HCl (3 N; 20 ml. + 25 ml. of water) at 0°. Sodium nitrite (2.0 g.) in water (25 ml.) was added to the solution and the whole kept overnight at 0°. The precipitated product (2.3 g.) was collected, a second crop was obtained by neutralisation of the mother liquors with sodium hydrogen carbonate and concentration of the solution. Recrystallisation from water (160 parts) gave 2-amino-4-hydroxy-6-methylamino-5-nitrosopyrimidine as red needles, m.p. $>300°$. (Found: C,35.58; H,4.1; N,41.1. $C_5H_7O_2N_5$ requires: C,35.5; H,4.17; N,41.4%).

2:5-Diamino-4-hydroxy-6-methylaminopyrimidine. - 2-Amino-4-hydroxy-6-methylamino-5-nitrosopyrimidine (0.5 g.) was dissolved in hot NaOH solution (0.72 g. NaOH in 8 ml. water). The solution was heated to 70-80° on the water-bath and nitrogen bubbled through vigorously. Sodium hydrosulphite (2 g.) was added over 5 minutes whereupon the colour of the solution changed from deep red to straw yellow. Heating under nitrogen was continued for a further 20 minutes and the solution was chilled. The pH was adjusted to

9.5 with concentrated HCl and the solution chilled for 2 hours. The precipitated product (0.35 g.) was collected. Recrystallisation of 2:5-diamino-4--hydroxy-6-methylaminopyrimidine could not be effected due to the instability of the compound, m.p. 204-210° (decomp.). (Found: C,38.0; H,6.09. $C_5H_9ON_5$ requires: C,38.75; H,5.8%).

2:8-Dihydro-4-hydroxy-2-imino-6:7:8-trimethylpteridine. - 2:5-Diamino-4-hydroxy-6-methylaminopyrimidine (0.31 g.), in acetic acid (3 ml.) and water, (20 ml.) was warmed under nitrogen to effect solution. Diacetyl (0.16 g.) was added and the solution heated at 60-70° for 30 minutes. The solution was chilled and neutralised with potassium hydrogen carbonate solution. The mixture was chilled overnight and the bright yellow product (0.05 g.) collected, and washed with water. Recrystallisation from ethanol was effected, although the compound was unstable, to give 2:8-dihydro-4-hydroxy-2-imino-6:7:8-trimethylpteridine as needles, decomp. above 120°. pKa values 5.85 ± 0.2, 8.9. (Found: C,53.18; H,5.01; N,34.4. $C_9H_{11}ON_5$ requires: C,52.75; H,5.37; N,34.2%).

The hydroxyiminopteridine was slightly hydroscopic.

2:8-Dihydro-4-hydroxy-2-imino-8-methyl-6:7--diphenylpteridine. - 2:5-Diamino-4-hydroxy-6-methylaminopyrimidine (0.3 g.) was dissolved in water (20 ml.) and acetic acid (3 ml.). To the warm solution was added benzil (0.42 g.) in ethanol (12 ml.) and the solution refluxed for 8 hours during which a bright yellow precipitate was deposited. The product (0.040 g.) was collected while the solution was still hot. Recrystallisation from N:N-dimethylformamide (7 ml.) gave 2:8-dihydro-4-hydroxy-2-imino-8-methyl-6:7-diphenylpteridine as yellow prisms, m.p. >300°. (Found: C,66.0, 65.3; H,4.3,4.0: $C_{19}H_{15}ON_5.H_2O$ requires: C,65.7; H,4.9%).

2-Amino-7:8-dihydro-4-hydroxy-8-methyl-6:7-diphenylpteridine. - 2:5-Diamino-4-hydroxy-6-methylaminopyrimidine (0.25 g.) and benzoin (0.4 g.) were dissolved together in ethanol (3 ml.) and acetic acid (2 ml.). The solution was refluxed for 2 hours during which a heavy yellow precipitate settled out. The product (0.29 g.) was collected after chilling the mixture overnight. Recrystallisation from N:N-dimethylformamide gave 2-amino-7:8-dihydro-4-hydroxy-8-methyl--6:7-diphenylpteridine as yellow plates, m.p. >300°

(Found: C,68.2; H,5.2; N,21.15. $C_{19}H_{17}ON_5$ requires: C,68.8; H,5.1; N,21.15%).

2-Amino-4-ethoxy-6-methylaminopyrimidine. - 2--Amino-4-chloro-6-methylaminopyrimidine (6.7 g.) was heated with ethanolic sodium ethoxide (1.05 g. sodium in 60 ml. dry ethanol) in an autoclave at 130° for 3 hours. The solution was evaporated to dryness in vacuo and the residue recrystallised from water (60 ml.) to give 2-amino-4-ethoxy-6-methylaminopyrimidine as colourless prisms, m.p. 123-126°, sublimes 140°/0.0002m.m. (Found: C,49.9; H,7.16; N,33.76. $C_7H_{12}ON_4$ requires: C,50.0; H,7.19; N,33.3%).

2-Amino-5-p-chlorobenzeneazo-4-ethoxy-6-methyl-aminopyrimidine. - 2-Amino-4-ethoxy-6-methylaminopyr-imidine (0.6 g.) was dissolved in water (8 ml.). To the solution at 0° was added a solution of diazotised p--chloroaniline (1.1 g.) in HCl (1 N; 20 ml.). The mixed solutions were kept at 0° for 5 minutes and sodium carbonate (3.5 g.) added slowly. The product separated out and was collected after stirring the mixture for 1 hour. Recrystallisation from aqueous acetone gave 2--amino-5-p-chlorobenzeneazo-4-ethoxy-6-methylamino-pyrimidine (0.8 g.) as orange needles, m.p. 169-172°. (Found: C,50.9; H,4.5; N,27.4. $C_{13}H_{13}ON_5Cl$ requires:

C,50.9; H,4.9; N,27.4%).

2:5-Diamino-4-ethoxy-6-methylaminopyrimidine. - 2-Amino-5-p-chlorobenzeneazo-4-ethoxy-6-methylaminopyrimidine (0.6 g.) was dissolved in ethanol (50 ml.) and hydrogenated in the usual way. The diaminopyrimidine was too unstable to be isolated and the filtered solution of the diaminopyrimidine was used for condensation immediately.

4-Ethoxy-2:8-dihydro-2-imino-6:7:8-trimethylpteridine. - To the above filtered solution was added acetic acid (2 drops) and diacetyl (0.3 ml.). The solution was refluxed for 20 minutes, concentrated *in vacuo* to 5 ml., cooled, and neutralised with saturated potassium hydrogen carbonate solution. The product was collected and recrystallised from aqueous ethanol (charcoal) to give 4-ethoxy-2:8-dihydro-2-imino-6:7:8-trimethylpteridine as red needles, m.p. 178-180°. (Found: C,56.7; H,6.28; N,30.4. $C_{11}H_{15}ON_5$ requires: C,56.6; H,6.45; N,30.1%).

The ethoxy pteridine could not be hydrolysed either with acid or alkali to the corresponding hydroxy pteridine.

4-Chloro-8-methyl-7-pteridone. - 5-Amino-4-chloro--6-methylaminopyrimidine (0.3 g.) and ethyl glyoxylate

hemiacetal (0.4 ml.) were heated together in refluxing water (4 ml.) for 40 minutes and the solution chilled overnight. The crystalline product (0.22 g.) was collected and recrystallised from petrol to give 4--chloro-8-methyl-7-pteridone as colourless needles, m.p. 173-174°. (Found: C,43.48; H,2.59; N,28.3; Cl,18.04. $C_7H_5ON_4Cl$ requires: C,42.8; H,2.54; N,28.5; Cl,18.05%).

4-Chloro-5:6:7:8-tetrahydro-7-hydroxy-8-methyl-pteridine. - 4-Chloro-8-methyl-7-pteridone (0.5 g.) was dissolved in dry ether (70 ml.) and lithium aluminium hydride (0.25 g.) was added in solution in dry ether (70 ml.). The mixture was refluxed with stirring for 24 hours and cooled. Water (4 ml.) was carefully added, followed by the addition of dilute sulphuric acid (1 N; 50 ml.). The acid solution was extracted with ether. This extract was taken to dryness *in vacuo* to give no residue. The aqueous layer was made alkaline with sodium carbonate solution (2N; 38 ml.) and extracted with chloroform (3x50ml.). The combined chloroform extracts were washed with water, dried over anhydrous sodium sulphate and taken to dryness *in vacuo*. The residue (0.34g.) was dissolved in acetone (50 ml.) and filtered through

a bed of alumina. The solution was concentrated and cooled to give 4-chloro-5:6:7:8-tetrahydro-7-hydroxy-8--methylpteridine as pale yellow blades, m.p. 148-153° (decomp.). (Found: C,41.73; H,4.65; N,27.75; Cl,17.65. $C_7H_9ON_4Cl$ requires: C,41.8; H,4.48; N,27.95; Cl,17.73%).

Ultra-violet absorption spectrum in ethanol: λmax.; $\leqslant$220; sh.294; 324 mu. ε max.; $\geqslant$12,000; 6,900; 10,000.

8-Methyl-4-methylamino-7-pteridone. - (a) 4:6--Bismethylamino-5-nitropyrimidine (0.8 g.) in ethanol (100 ml.) was hydrogenated in the usual way. Ethyl glyoxylate hemiacetal (0.3 ml.) was added to the filtrate and the solution heated for 15 minutes on the water--bath, followed by concentration in vacuo to 5 ml., and chilling overnight. The product (0.4 g.) was collected and recrystallised from ethanol/benzene to give 8-methyl-4-methylamino-7-pteridone as colourless needles, m.p. 194-196° (decomp.), sublimes 120°/0.01 m.m. (Found: C,50.1; H,4.73; N,36.7. $C_8H_9ON_5$ requires: C,50.25; H,4.7; N,36.3%).

(b). - 4-Chloro-8-methyl-7-pteridone (0.2 g.) was heated in a sealed tube with ethanolic methylamine (33% w./w.; 0.4 ml.) in ethanol (2 ml.) at 100° for 6 hours. The mixture was taken to dryness in vacuo and

the residual solid recrystallised from ethanol (25 ml.) (Charcoal) to give the methylamino pteridone (0.15 g.) as feathery needles, m.p. and mixed m.p. with authentic specimen, 194-196° (decomp.).

8-Methyl-2-methylamino-7-pteridone. - 2:4-Bis-methylamino-5-nitropyrimidine (0.5 g.) was reduced in the usual way and condensed with ethyl glyoxylate hemiacetal as above to give, on recrystallisation from ethanol, 8-methyl-2-methylamino-7-pteridone (0.26 g.) m.p. 196-200° (decomp.). (Found: C,50.9; H,4.9; N,36.5%).

The 2-methylamino pteridone was unstable to lengthy boiling in ethanol.

2-Amino-8-methyl-7-pteridone. - 2-Amino-5-p--chlorobenzeneazo-4-methylaminopyrimidine (1.0 g.) was reduced in the usual way. To the filtrate was added ethyl glyoxylate hemiacetal (0.3 ml.) and the solution refluxed for 20 minutes. The solution was concentrated to 15 ml. and chilled. The product (0.31 g.) was deposited and collected. Recrystallisation from ethanol gave 2-amino-8-methyl-7-pteridone as pale brown crystals, m.p. 290-294°. (Found: C,47.9; H,3.7; N,39.5. $C_7H_7ON_5$ requires: C,47.5; H,3.96; N,39.6%).

2-Anilino-8-phenyl-7-pteridone. - 2:4-Dianilino--5-nitropyrimidine (0.3 g.) was reduced in the usual way and condensed with ethyl glyoxylate hemiacetal as above. Recrystallisation of the product (0.2 g.) from ethanol gave 2-anilino-8-phenyl-7-pteridone as red prisms, m.p. 197-200°. (Found: C,69.13; H,4.36; N,22.74. $C_{18}H_{12}ON_6$ requires: C,68.9; H,3.8; N,22.3%).

2-Amino-4-hydroxy-8-methyl-7-pteridone. - 2--Amino-4-hydroxy-6-methylamino-5-nitrosopyrimidine (0.4 g.) was suspended in ethanol (70 ml.) and hydrogenated in the usual way. The mixture was boiled under nitrogen and the catalyst filtered off. To the filtrate was added ethyl glyoxylate hemiacetal (0.4 ml.) and the solution heated on the water-bath for 10 minutes. Hot water (1 ml.) was added and the solution heated for a further 10 minutes. The solution was filtered through keiselguhr, concentrated in vacuo to 10 ml., and chilled overnight. The product (0.16 g.) was collected and recrystallised from ethanol (60 parts) to give 2-amino--4-hydroxy-8-methyl-7-pteridone as lustrous yellow plates, m.p. 239-240° (decomp.). (Found: C,43.7; H,4.5: $C_7H_7O_2N_5$ requires: C,43.52; H,3.65%).

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