THE BIOSYNTHESIS OF RIBOFLAVIN AND RELATED PTERIDINES

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A T H E S I S

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OBJECTIVES

It is now an established fact that purines, or the derived nucleosides, are the biogenetic precursors of riboflavin and probably also of other pteridines. This thesis describes the synthesis of possible intermediates in the above transformation, and an investigation of the chemical transformation of these compounds into riboflavin and other simpler pteridines.

A preliminary study of the ring-opening reactions of guanosine was also undertaken since ring-cleavage of the purine is probably the initial step in the above transformation.

I N D E X

SUMMARY

The evidence in the literature relating to the biosynthesis of riboflavin and of other simpler pteridines is reviewed. Possible intermediates in the transformation of purines into pteridines are discussed and the chemistry of some of these compounds has been investigated. The key intermediate appears to be 1-[2',6'-dihydroxy-5'-nitro-4'-pyrimidinylamino]-1-deoxy-D-erythropentulose which could be formed by Amadori rearrangement of a substituted diaminopyrimidine glycoside. This intermediate has been synthesised by the condensation of 4-chloro-2,6-dihydroxy-5-nitropyrimidine with 1-amino-1-deoxy-D-erythropentulose. Stereospecific reduction of the carbonyl group in the side-chain of this pyrimidine was achieved giving 2,6-dihydroxy-5-nitro-4-D-ribitylaminopyrimidine which on subsequent reduction of the 5-nitro group and condensation with the dimer of 3,4-dimethyl-o-benzoquinone gave riboflavin. Proof of the stereospecific reduction was supplied by unambiguous synthesis of both pyrimidine isomers. Model experiments using 1-[2',6'-dihydroxy-5'-nitro-4'-pyrimidinylamino]-1-deoxy-D-fructose are also described. The implications of these reactions in the biosynthesis and synthesis of riboflavin are discussed.

Oxidative self-condensation and condensation with alloxan of 5-amino-2,6-dihydroxy-4-substituted aminopyrimidines gave 2,10-dihydro-2-oxo-10-substituted-4,6,8-trihydroxypyrimido[5,4-g] pteridines.

The conversion of the key intermediate, 1-[2',6'-dihydroxy-5'-nitro-4'-pyrimidinylamino]-1-deoxy-D-erythropentulose to simple pteridines was also investigated. Thus, reduction of the 5-nitro group of the model, 1-[2',6'-dihydroxy-5'-nitro-4'-pyrimidinylamino]-1-deoxy-D-fructose, and intramolecular condensation followed by oxidation of the resulting dihydropteridine, gave the 2-hydroxy analogue of xanthopterin and not the expected 6-polyhydroxyalkylpteridine. Model experiments using 4-acetonylamino-2,6-dihydroxy-5-nitropyrimidine are also described. The hydrazine derivative of this model pyrimidine gave 2,4-dihydroxy-6-methylpteridine directly, but attempts to apply this technique to sugar containing pyrimidine were not successful.

4-Acetonylamino-2,6-dihydroxy-5-nitropyrimidine and 2,6-dihydroxy-4-2'-hydroxyethylamino-5-nitropyrimidine underwent an unusual intramolecular condensation to give imidazo[1,2-c] pyrimidine derivatives.

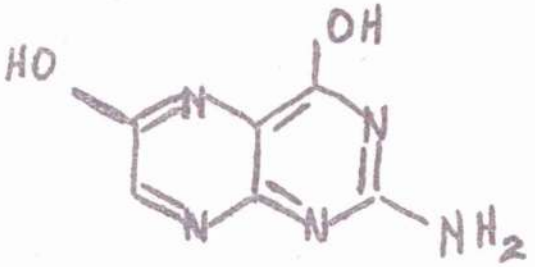
Alkylation of guanosine using benzyl bromide, gave a crystalline quaternary salt which on subsequent treatment with dilute alkali underwent cleavage of the imidazole ring to give a 5-amino-4-D-ribosylaminopyrimidine derivative.

An attempt to synthesise this glycoside directly proved unsuccessful as the product was suspected of undergoing an Amadori rearrangement.

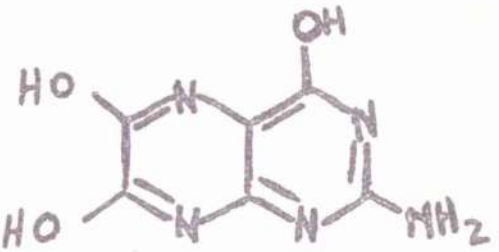
INTRODUCTION

It is only within the last twenty years that a systematic study has been made of the naturally occurring pteridines. Early investigations in this field were carried out, however, by Gowland-Hopkins[1] as long ago as 1890. These compounds are widely distributed throughout the animal and plant kingdom, and they range from simple pigments to highly complex growth factors.

Xanthopterin[2], which is the yellow colouring matter present in wasps, and leucopterin[3], which is the white pigment present in the wings of cabbage butterflies, are two such pigments. It is possible that these pteridines are degradation products of some more highly complex pteridine systems involved in the metabolism or growth of these insects.

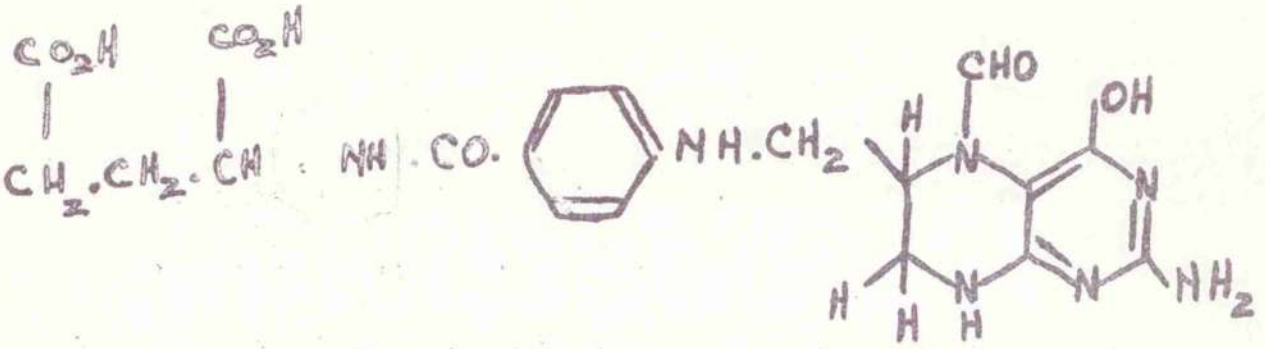


Xanthopterin



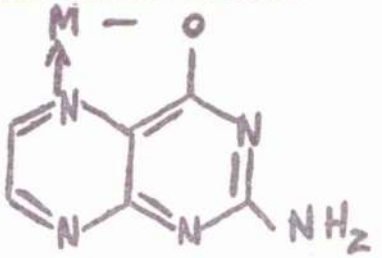
Leucopterin

It is known[4] that all mammalian cells make use of a pteridine which is a derivative of folic acid in order to divide. This substance is known as the "citrovorum factor" and has been the subject of intensive study.



Folic Acid

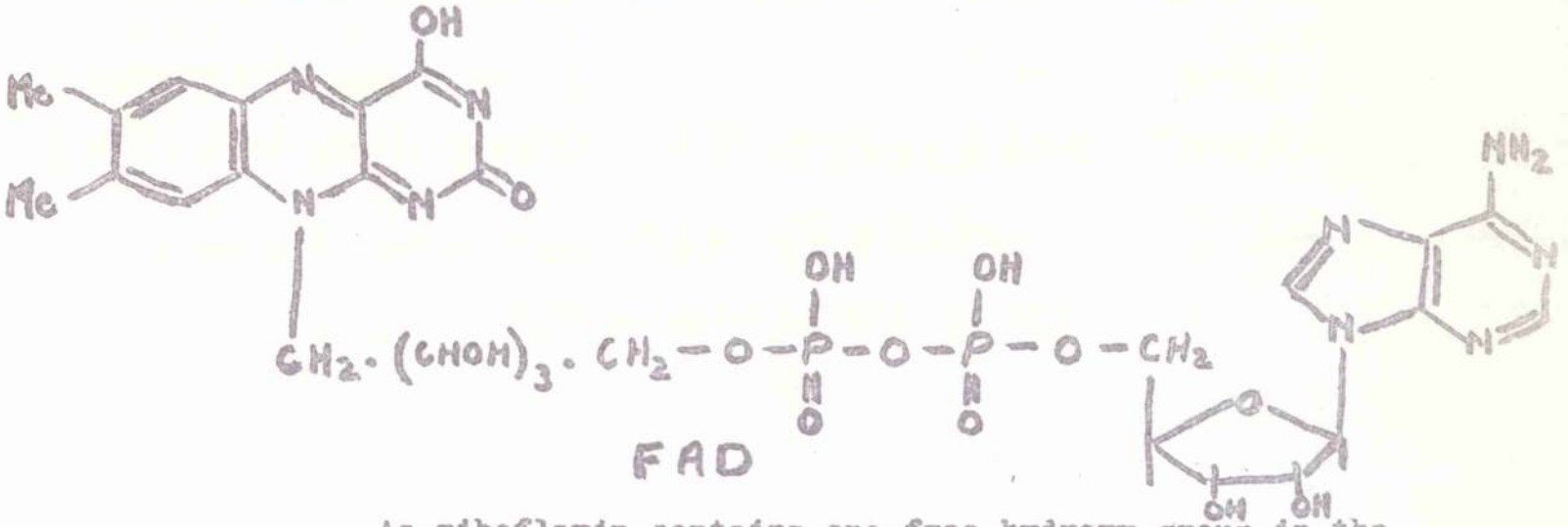
Most naturally occurring pteridines have 2-amino and 4-hydroxy groups attached to the parent nucleus. The latter group chelates readily with bivalent metals and it is probable that many pteridines exist in biological systems as metallic complexes such as that shown below.



Riboflavin[5], which can be regarded as a benzopteridine, is a universally distributed vitamin, probably existing in small amounts in every animal and plant organism, some of which produce it in relatively large quantities.[6,7]

Riboflavin is mainly found in flavin-adenine dinucleotide (FAD), the prosthetic group of a flavo-protein enzyme, where it can readily be reduced to give a leuco[8] compound. This

redox reaction reverses with ease, and hence riboflavin derives its biological activity from the fact that it is a hydrogen carrier in these enzymes.

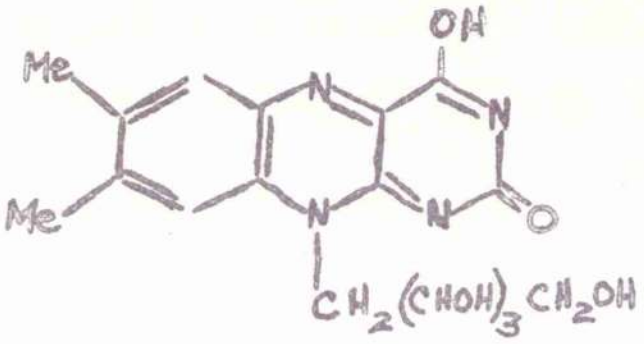


FAD

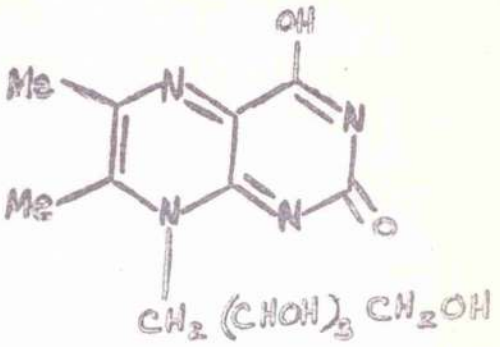
As riboflavin contains one free hydroxy group in the heterocyclic nucleus, it resembles 8-hydroxyquinoline in its ability to chelate with bivalent metallic ions.[9] It is known to chelate with Fe^{++} (1:1)[10] in enzyme systems and such systems function as dehydrogenases.

BIOSYNTHESIS OF PTERIDINES

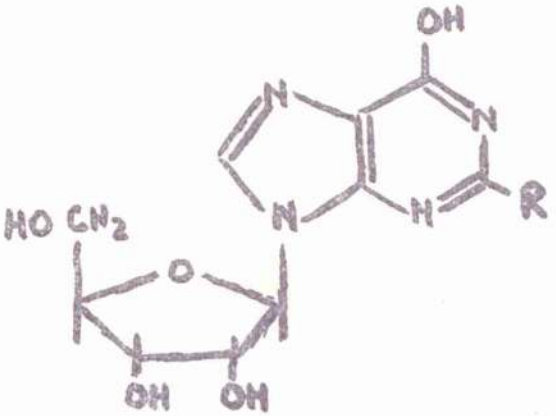
Comparison of the structural formulae of the naturally occurring purines, pteridines, and flavins, shows a striking resemblance particularly as each incorporates in its nucleus the skeleton of a diaminopyrimidine. This is shown clearly in the examples given below. This similarity seems to suggest a biological relationship between these groups of compounds.



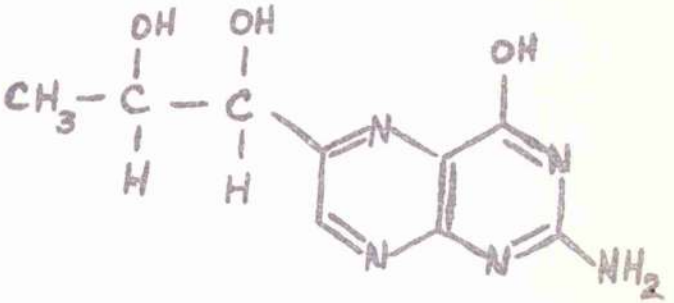
Riboflavin: (I)



G-Compound: (II)



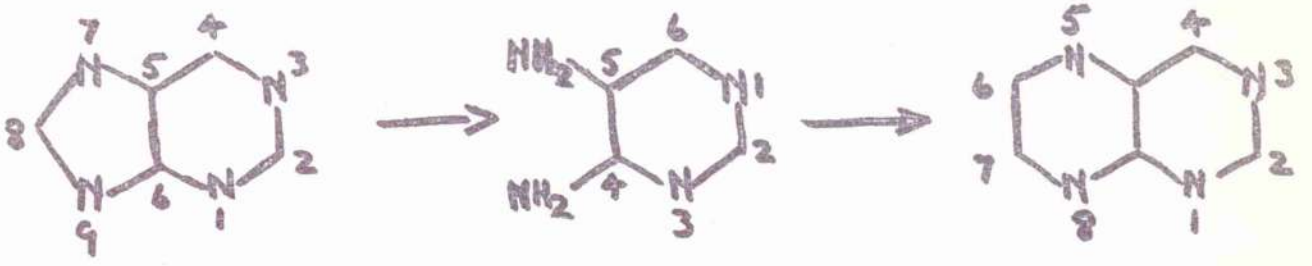
(R = NH_2, Guanosine: (III)
(R = OH, Xanthosine: (IV)



Biopterin: (V)

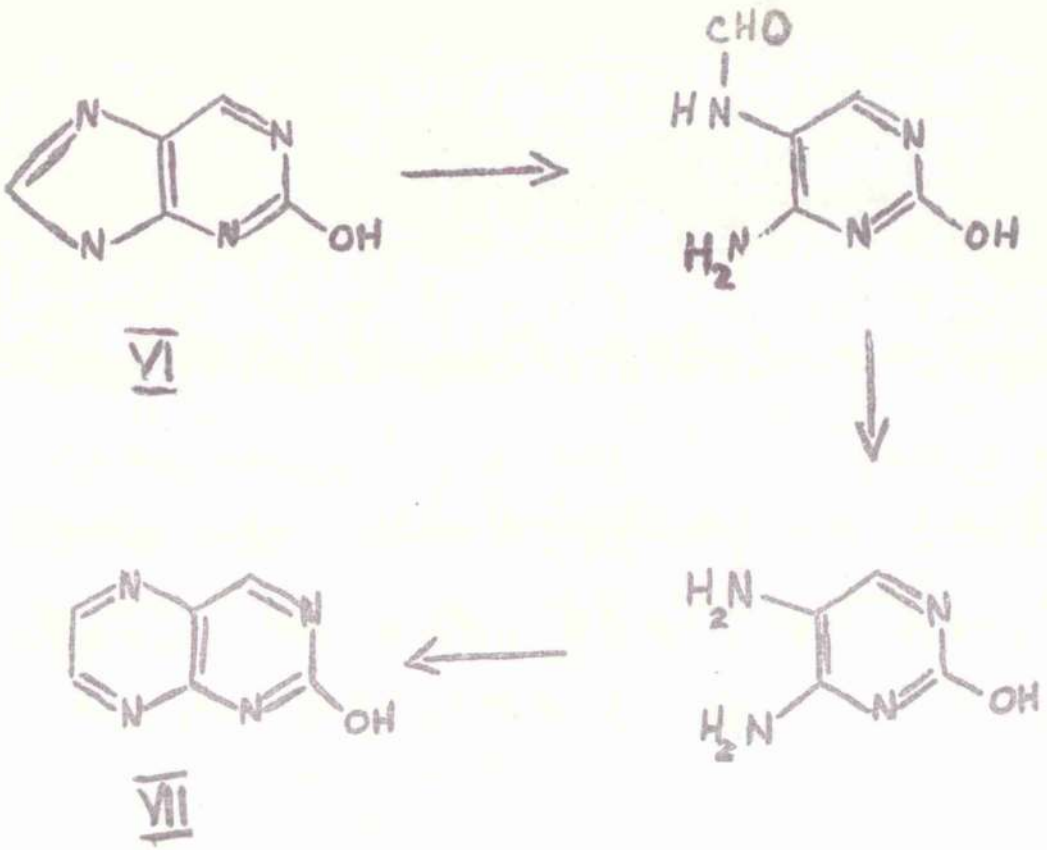
Albert[11] was the first to suggest that the purines, or their derived nucleosides, were the biosynthetic precursors of

pteridines. A similar suggestion relating to the biosynthesis of riboflavin had been made earlier by MacLaren[12] and this will be discussed later in this thesis. Albert suggested that the imidazole ring of the purine opens to give a 4,5-diamino-pyrimidine derivative with loss of C_8, and subsequent condensation with a two carbon fragment to give the pteridine ring structure. This is illustrated below. The numbering of the various ring systems is also shown.



purine pyrimidine pteridine

Albert[11] was able to demonstrate the <u>in vitro</u> transformation of 2-hydroxypurine (VI) to 2-hydroxypteridine (VII). In this case the imidazole ring opens at pH5 and 20° to give 4-amino-5-formamido-2-hydroxypteridine. The latter compound loses its formyl group at pH2 to give a 4,5-diaminopyrimidine. Condensation with glyoxal then gives 2-hydroxypteridine in the usual way.



VI

VII

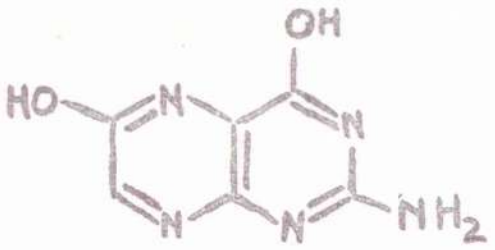
In this transformation C_8 of the purine has been lost as formic acid and C_6 and C_7 of the pteridine have been obtained from the dicarbonyl derivative.

Biochemical evidence for the existence of diaminopyrimidines as possible intermediates, came from the work Ziegler-Gunder et al.[13] who injected the larvae of the amphibian Xenopus intradermally with guanine [2-^{14}C]. Ten days later, the skins of the sacrificed larvae, were extracted with N-ammonia solution at 20°. Paper chromatograms of the extract yielded blue fluorescent spots, one of which, on oxidation, was converted to labelled 2-amino-4-hydroxypteridine-6-carboxylic acid.

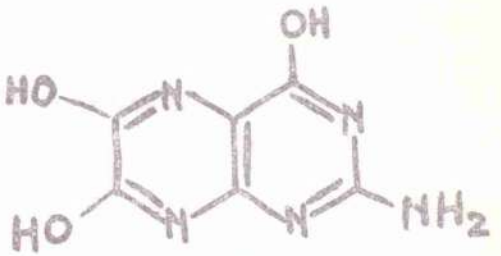
Further biochemical evidence was supplied by Weygand[14] who administered 2,4,5-triamino-6-hydroxypyrimidine [2-^{14}C] to

pierid caterpillars in their diet, and later isolated labelled xanthopterin (VIII) from the wings of the adult butterflies.

Weygand[15] also inoculated 3-4 day old cocoons of Pieris brassicae with glucose [2-^{14}C] and found that a 78% recovery of the label was obtained in carbon atoms 6 and 7 of the resulting leucòpterin (IX). A similar experiment using ribose [1-^{14}C] gave a 64% recovery of the label.

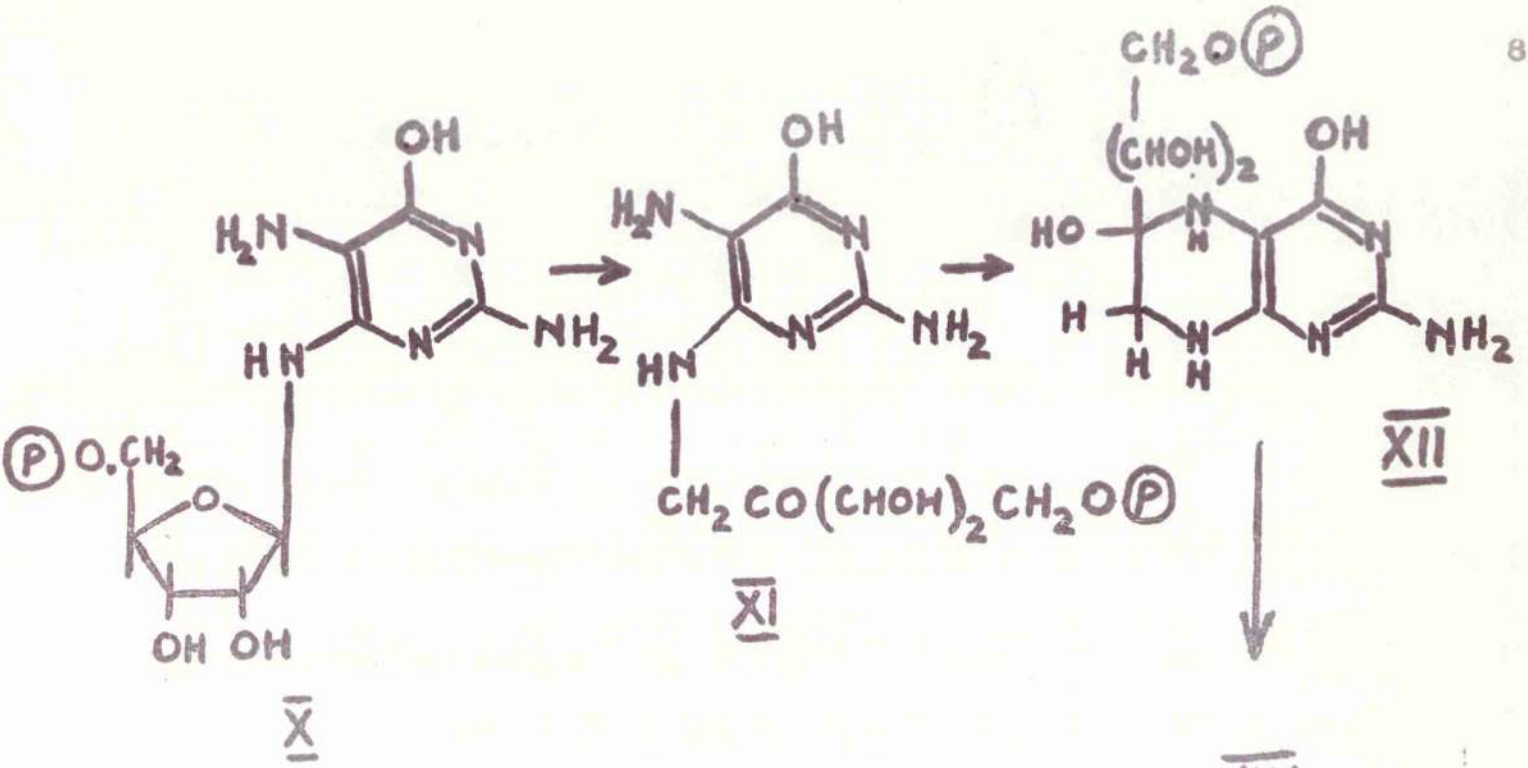


VIII



IX

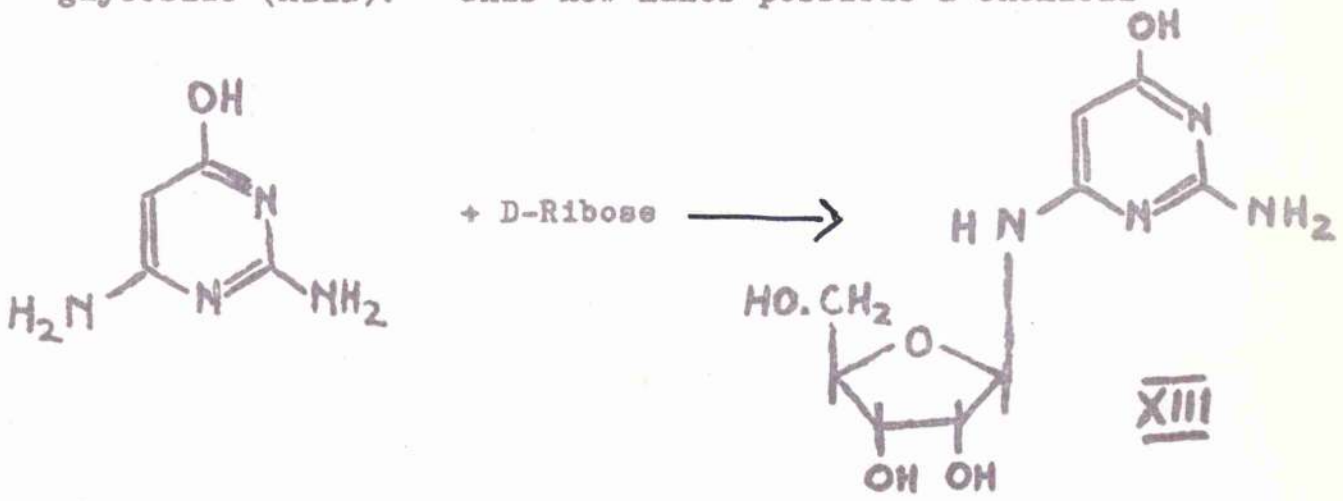
From these two observations, Weygand postulated that, in the above biological system, a pyrimidine glycoside 5'-phosphate (X) could exist, which could undergo an Amadori rearrangement to give the pyrimidine (XI). Intramolecular ring closure would then yield a pteridine (XII). He postulated that subsequent oxidation of this pteridine caused it to lose its polyhydroxyalkyl side chain and give xanthopterin (VIII). Addition of a molecule of water and further oxidation would then give leucopterin (IX).



where Ⓟ = H_2PO_3

16

Recently Remboldt has succeeded in condensing D-ribose with 2,4-diamino-6-hydroxypyrimidine to give the glycoside (XIII). This now makes possible a chemical

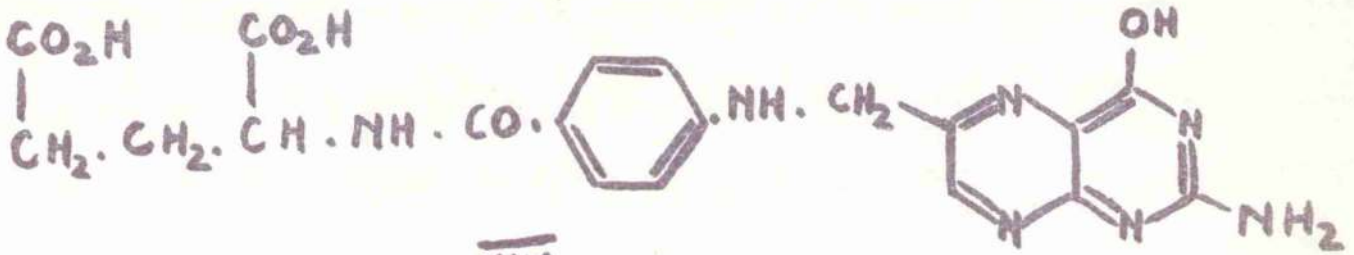


synthesis of the glycoside (X), but this is not discussed further in this thesis.

Details of the biosynthesis of the more important naturally occurring pteridines, will now be summarised.

Folic Acid.[17]

The folic acid (pteroylglutamic acid) molecule (XIV) contains three parts, those of L-glutamic acid, p-aminobenzoic acid and a substituted pteridine.

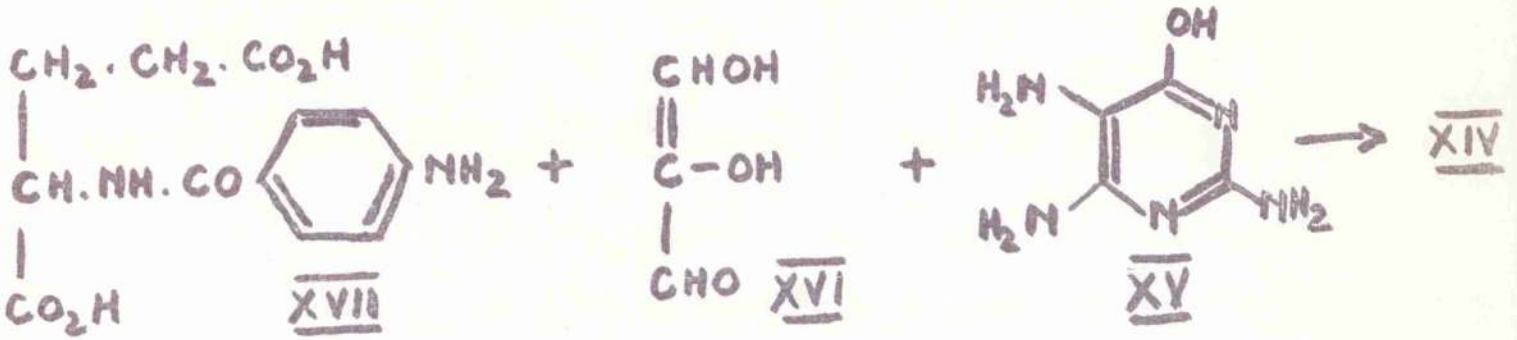


XIV

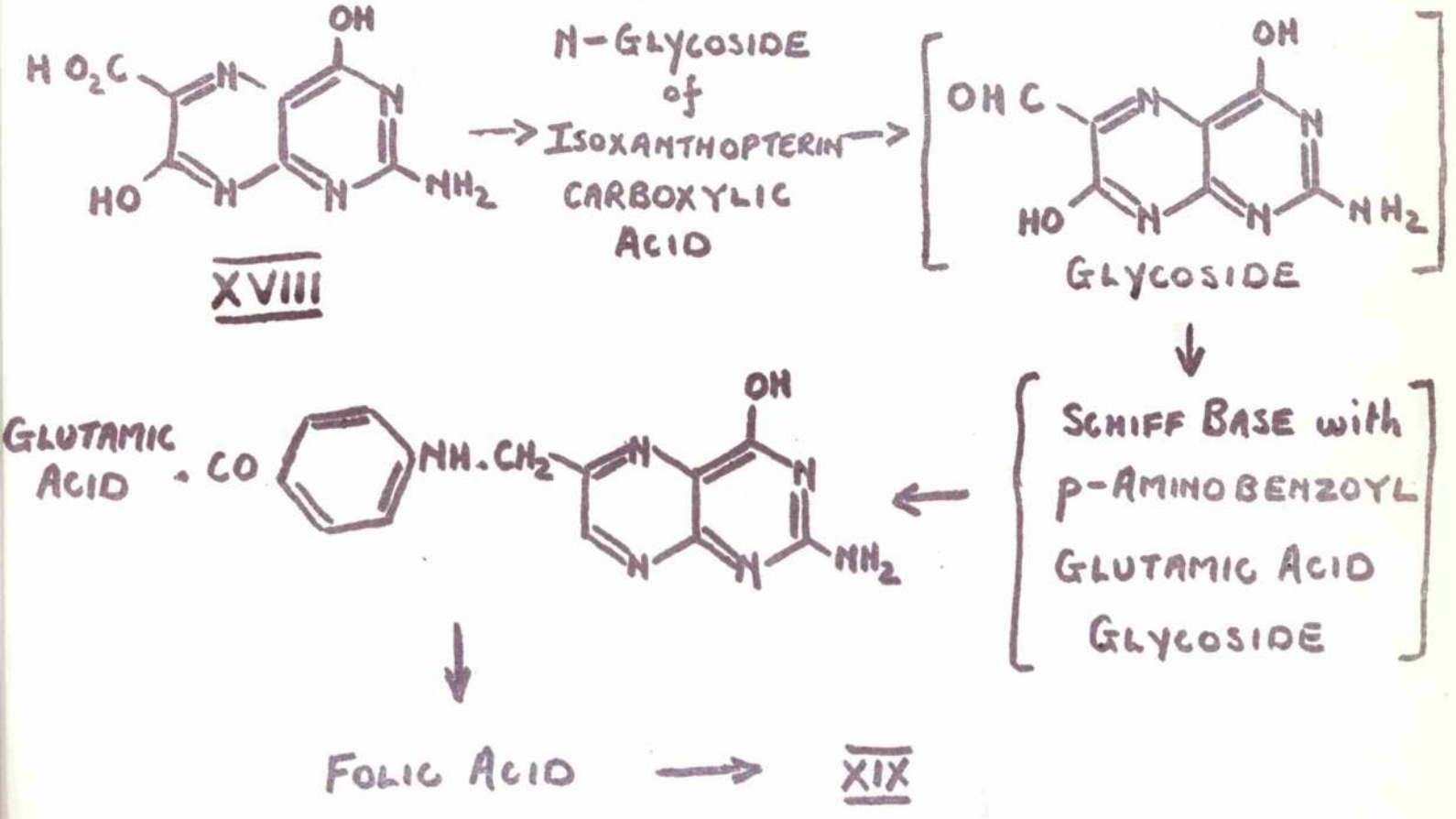
Suggestions for the biosynthesis of this molecule have been based on the hypothesis that the three residues are separately synthesised and then put together. The biologically active form of folic acid contains a reduced pyrazine ring and, although its precise chemical nature is yet unknown, it is thought to be a 5,6,7,8-tetrahydropteridine derivative. This active form is known as coenzyme F (CoF), and it is concerned with the transfer of 1-carbon atom units in vivo.

One of the earliest theories was that folic acid arose from 2,4,5-triamino-6-hydroxypyrimidine (XV), a 3 carbon compound (reductone: XVI), and p-aminobenzoylglutamic acid[18] (XVII). These constituents will combine in the test tube to give folic

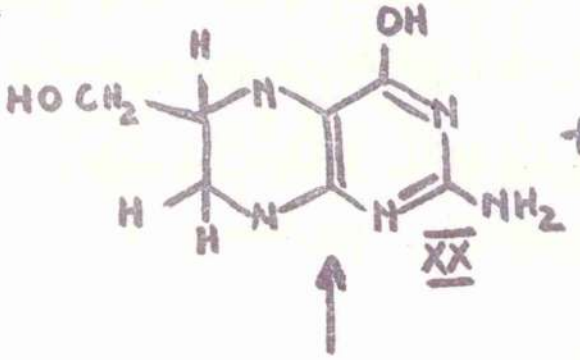
acid, but there is no biological evidence for the validity of this theory.



Tschesche and Korte[19] on the basis of growth experiments with S faecalis presented a scheme shown below, and Korte et al.[20] adduced evidence for this scheme showing that isoxanthopterin carboxylic acid [4-^{14}C] (XVIII) is converted to a compound or compounds which can be degraded to 2-amino-4-hydroxy-6-carboxy-pteridine (XIX).



A simple production of "folic acid-like compounds" has recently been achieved by Shiota[21] who has shown that tetrahydro-2-amino-4-hydroxy-6-hydroxymethylpteridine (XX) is converted by L. arabinosus extracts in the presence of ATP and magnesium, to materials giving a microbiological assay for folic acid.



XX

Biopterin

+ p-Aminobenzoyl Glutamic Acid → Folic Acid

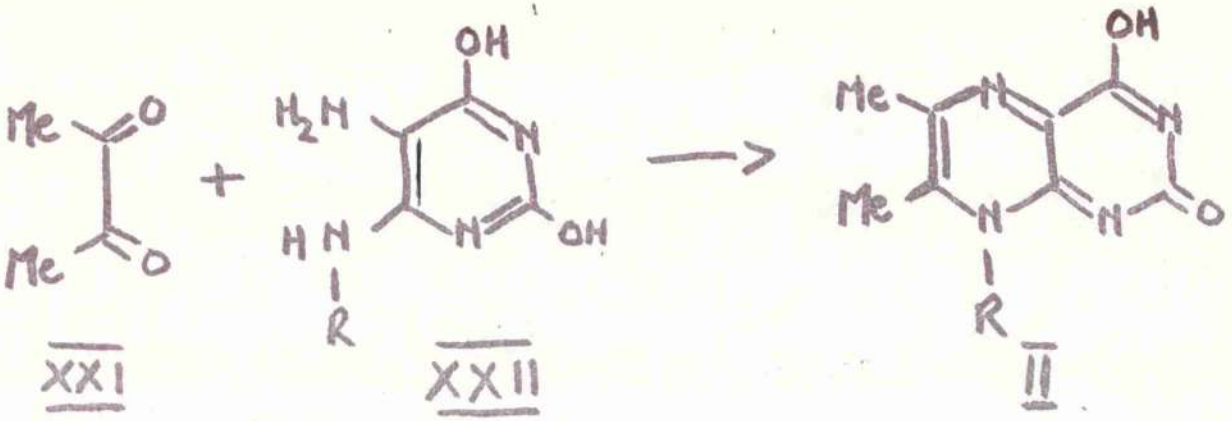
or

p-Aminobenzoic Acid → Like Compounds

It is, however, very interesting that the reduced compound is much more active than the non-reduced material, indicating again that biosynthesis in this organism, at least, may proceed to give reduced compounds.

"G-Compound".

2,8-Dihydro-4-hydroxy-6,7-dimethyl-2-oxo-8-D-ribityl-pteridine (Masuda's "G-compound") (II; R = D-ribityl) was first isolated together with riboflavin from cultures of Eremothecium ashbyii by Masuda,[22] and subsequently by other workers from various organisms. The structure of this compound was confirmed by synthesis[23,24] which involved the condensation of biacetyl (XXI) and 5-amino-4-D-ribitylamino-uracil (XXII; R = D-ribityl).

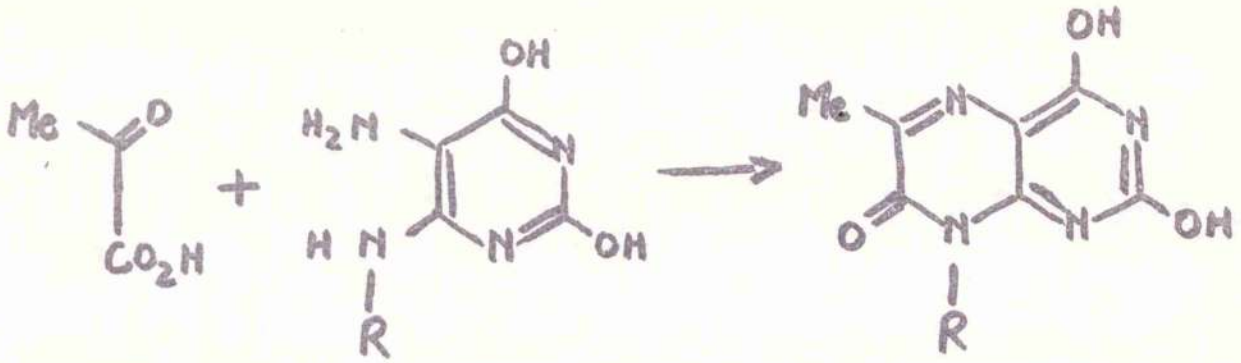


XXI XXII II

From the work to be described in the section dealing with the biosynthesis of riboflavin, it will be apparent that G-compound is formed *in vivo* from a purine precursor by loss of C_8, and subsequent condensation with biacetyl as in the chemical synthesis above.

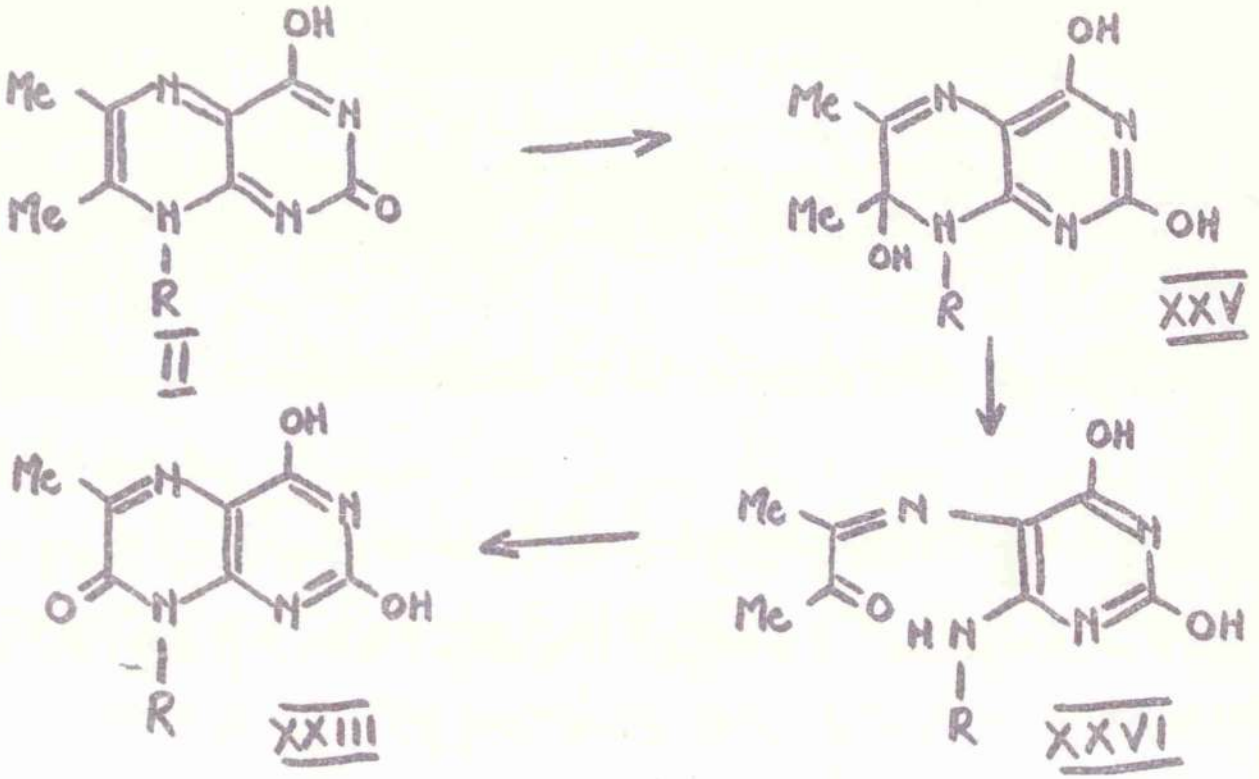
"V-Compound".

7,8-Dihydro-2,4-dihydroxy-6-methyl-7-oxo-8-D-ribityl-pteridine (Masuda's "V-compound") (XXIII; R = D-ribityl) was also isolated from cultures of *Eremothecium ashbyii* by Masuda.[25] Chemical proof of the structure again rests on synthesis[26,27] which was achieved by condensation of pyruvic acid (XXIV) and 5-amino-4-D-ribitylamino-uracil (XXII; R = D-ribityl) as shown below.



XXIV XXII XXIII

Biochemical experiments[28] show that V-compound is formed *in vivo* from G-compound, and this has been attributed to simple oxidation[29] of the methyl group at C_7 in pteridine (II). Recently, Wood *et al.*[30] have suggested a more reasonable method for this transformation. The pteridine (II; R = D-ribityl) became hydrated in alkaline solution to give a dihydropteridine (XXV; R = D-ribityl) which is a carbinolamine and probably exists in equilibrium with the pyrimidine (XXVI; R = D-ribityl). Displacement of the biacetyl residue by pyruvic acid, occurred readily on gentle reflux to give V-compound (XXIII; R = D-ribityl). It seems likely that such a mechanism could operate under biological-type conditions.

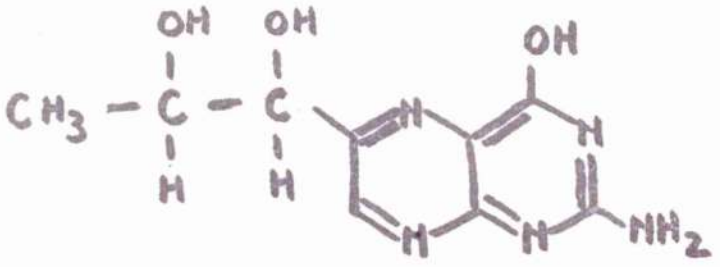


Biopterin.

This polyhydroxyalkylpteridine has been obtained from two natural sources, (a) the eyes of the fruit fly, Drosophila melanogaster, and (b) human urine.

In case (a), biopterin was isolated[31,32] along with two other closely related pteridines by means of chromatography.

In case (b), biopterin was again isolated[33] by careful chromatography, and was characterised as 2-amino-4-hydroxy-6-(1',2'-dihydroxypropyl)-pteridine (V).

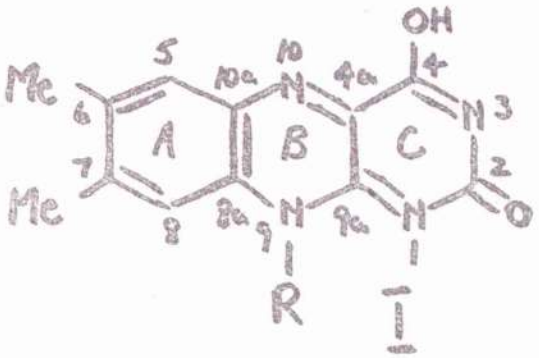


V

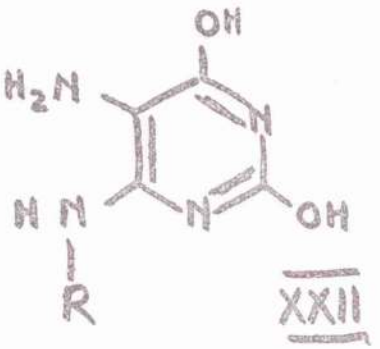
There are no substantiated reports in the literature dealing with the biosynthesis of this pteridine, and this problem will be dealt with in the theoretical section of this thesis.

BIOSYNTHESIS OF RIBOFLAVIN

Recent views on the biosynthesis of riboflavin (I; R = D-ribityl) suggest that it is also formed from purines through an intermediate 4,5-diaminopyrimidine derivative (XXII; R = D-ribityl). This hypothesis, which was first



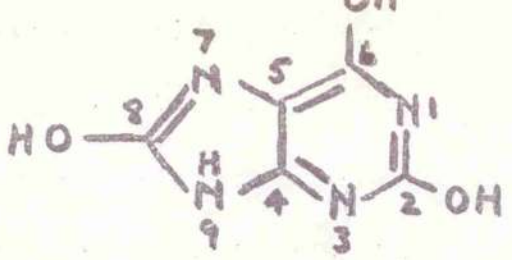
I



XXII

postulated by MacLaren,[12] has been confirmed by many workers, and their results are summarised below.

Results obtained by Plaut,[34] on the incorporation of radioactively labelled compounds into riboflavin using Ashbya gosaypii, were similar to those obtained by Buchanan et al.[35,36] on the incorporation of the same labelled compounds into uric acid (XXVII).



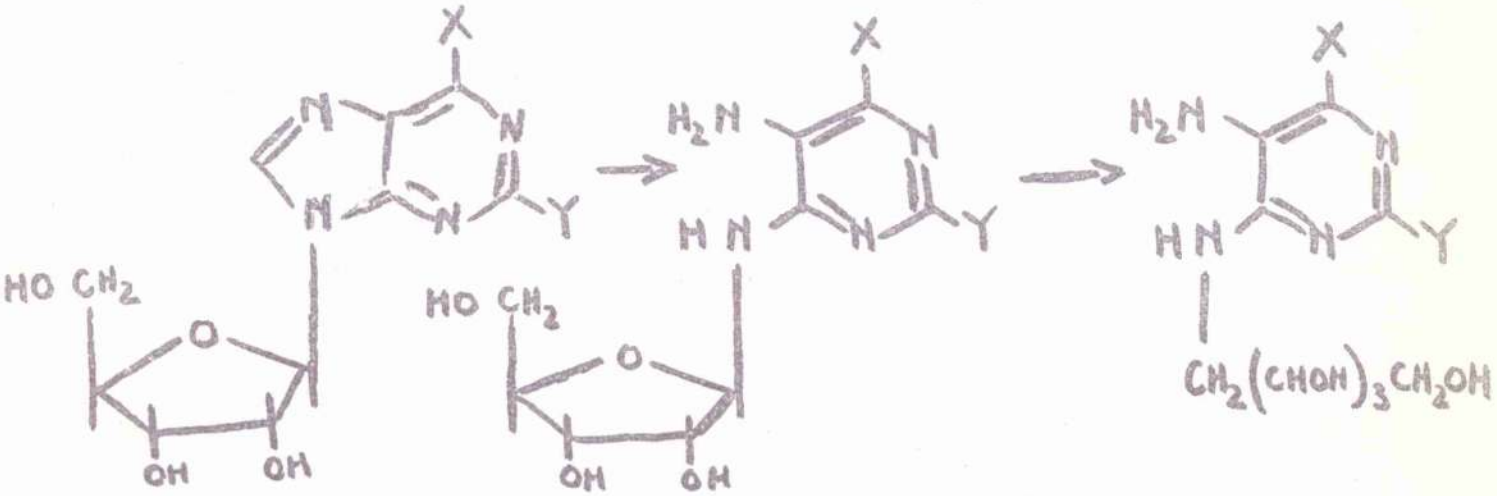
XXVII

Thus, ^{14}C-formate enters position 2 of riboflavin, and positions 2 and 8 of uric acid, ^{14}C-carbon dioxide or $CH_3.^{14}CO_2H$ enters position 4 of riboflavin, and position 6 of uric acid, and the carbon atoms of glycine enter positions 9a and 4a of riboflavin, and positions 4 and 5 of uric acid. The nitrogen of glycine enters position 7 of uric acid, and although no degradation has as yet been worked out that will give the contribution to the individual nitrogen atoms in riboflavin, nitrogens 9 and 10 contain thirty times the amount of label carried by nitrogens 1 and 3. Goodwin and his group supported these incorporation studies by showing that serine stimulated flavinogenesis,[37,38] and that before its incorporation, it was transformed to formate and glycine.[38] Adenine could dilute out the incorporation of serine.[38] Further support came from Klungsyr,[39] who demonstrated the incorporation of labelled formate into the 2 position of riboflavin.

Important evidence for the conversion of purines into riboflavin came from the work of McNutt,[40,41] who, working with

adenine and the mould Eremothecium ashbyii, showed that in the transformation, carbon atom 8 of the purine was "lost". He confirmed this result with 8-^{14}C adenine[40] and with randomly labelled adenine.[41] From his results, he came to the conclusion that the contribution of the purine to riboflavin was an intact pyrimidine ring.

The vital intermediate in the transformation of purines is probably 5-amino-4-D-ribitylaminouracil (XXII; R = D-ribityl), even although this compound has never been isolated or detected in vivo. This failure to detect such an important precursor is, however, not surprising, as it rapidly undergoes self-condensation. This is discussed further in the theoretical section of this thesis. The formation of the ribitylaminopyrimidine from



purine nucleoside ribosylamino-pyrimidine ribitylamino pyrimidine

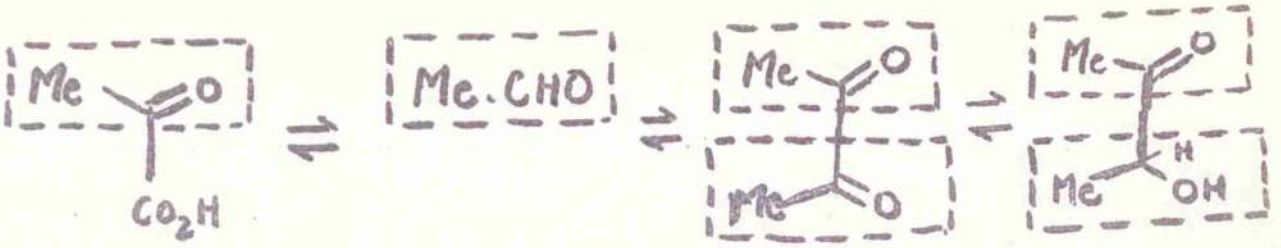
the corresponding ribosylaminopyrimidine which would be expected to be the primary product obtained from the ring-opening of a purine nucleoside, is also discussed in the theoretical section.

Goodwin[42] has established that the purine precursors of riboflavin fall into the following order of decreasing effectiveness; guanine, xanthine, adenine, hypoxanthine, and uric acid. This order is of interest in view of the ring-opening reaction of guanosine, which will be discussed later in this thesis.

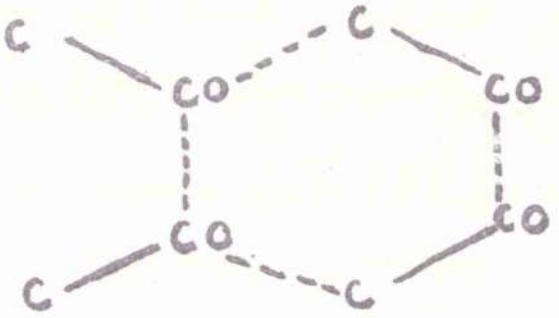
The other portion of the riboflavin nucleus which has still to be accounted for, is the benzene ring, and it is here, that the focus of present controversy is situated. Fortunately, some elegant work[43,44] with tracer compounds has given a clear picture on which to base subsequent work.

Birch[45] was first to suggest the now generally accepted pattern for the formation of the benzene ring skeleton which arises from the head to head and head to tail linkages of four molecules of acetic acid, probably as biacetyl or acetoin, derived from four molecules of pyruvic acid.

Plaut,[43] using incorporation techniques, has been able to demonstrate the following scheme. The label from $CH_3{}^{14}CO_2H$ is

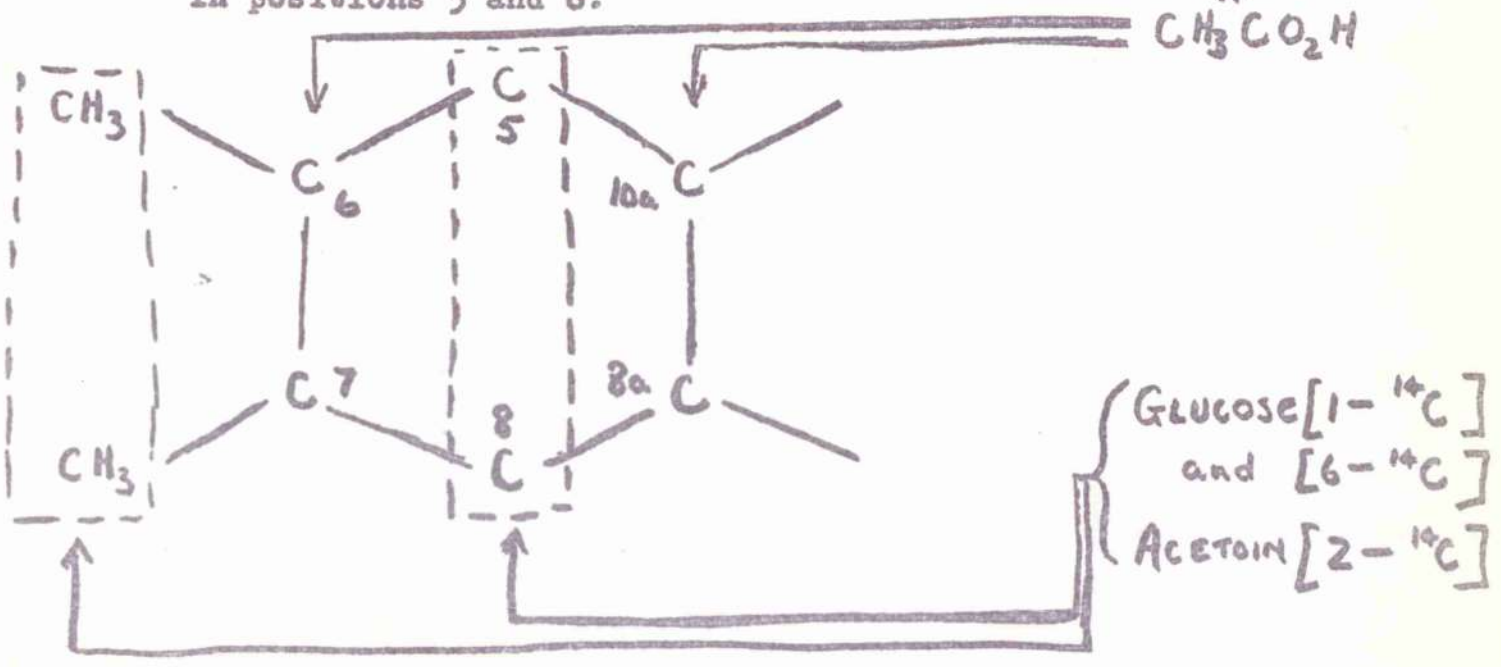


pyruvic acid acetaldehyde



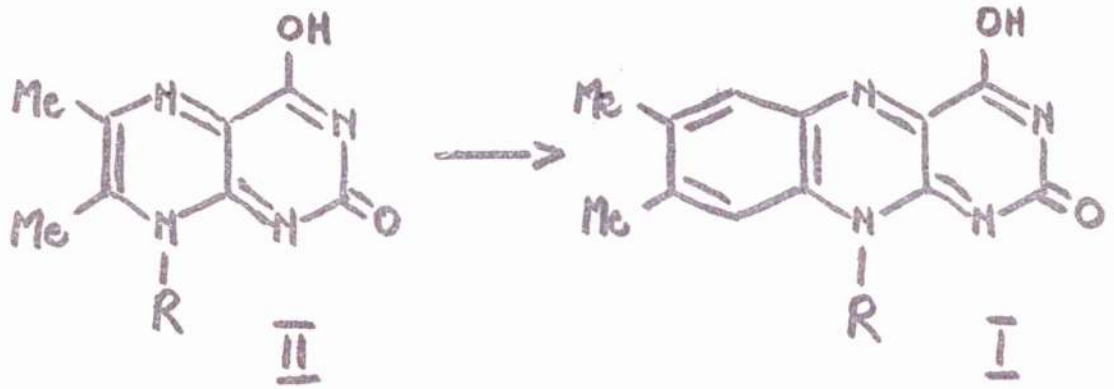
Birch's Picture

incorporated at positions 6 and 7, and positions 8a and 10a. of the riboflavin mucleus, and the label from glucose [1-^{14}C] and glucose [6-^{14}C], which are metabolised to acetate $^{14}CH_3CO_2H$, is incorporated in the methyl groups, and in positions 5 and 8. Goodwin[44] also demonstrated that the label from acetoin, labelled with ^{14}C in one of its methyl groups, is incorporated one half in the methyl groups of riboflavin, and the other half in positions 5 and 8.



The origin of the atoms forming the benzene portion of riboflavin is established, but there is considerable uncertainty as to how the benzene ring precursor presents itself to the diaminopyrimidine.

Masuda[46,47] was first to suggest that biacetyl or acetoin condenses with the ribitylaminopyrimidine (XXII; R = D-ribityl) to give 2,8-dihydro-4-hydroxy-6,7-dimethyl-2-oxo-8-d-ribitylpteridine (II; R = D-ribityl) which he called for convenience G-compound. He then postulates[47] that G-compound reacts with a further mole of biacetyl or acetoin to give riboflavin (I; R = D-ribityl).



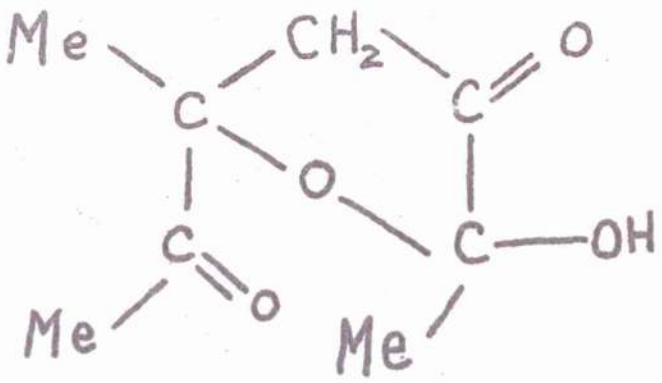
Evidence for this route has gained support from the fact that Masuda[22] and others[24,48] have been able to isolate G-compound from both E.ashbyii and A.gossypii.

The strongest evidence that G-compound is a precursor in the biosynthesis of riboflavin, comes from its transformation

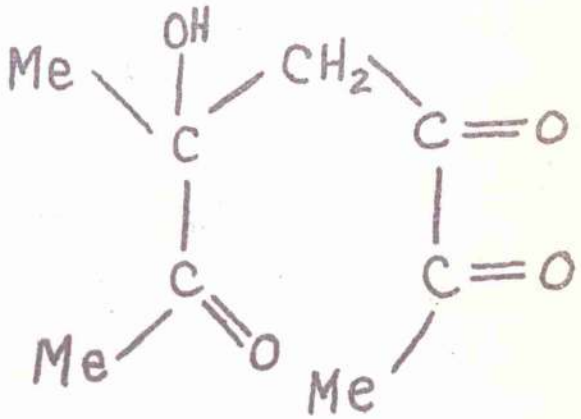
into riboflavin by various biological systems.[49,50,51,52] Masuda[47] claims that G-compound reacts with biacetyl at 120-130° *in vitro* to give riboflavin, but this condensation could not be repeated by Birch.[53]

A critical study of the biological systems used in the transformation of G-compound to riboflavin, suggests several points which indicate that an alternative pathway for the biosynthesis of the benzene ring of riboflavin may operate.

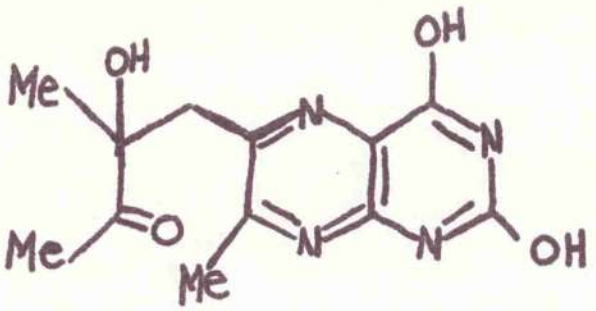
This second hypothesis is based on the chemical studies by Birch and Moye.[45,53] Two moles of biacetyl can be self-condensed to form an aldol of probable structure (XXVIII),[45] which reacts as a hexane trione (XXIX) on condensation with either 4,5-diaminouracil (XXII; R = H) or 5-amino-4-methylamino uracil(XXII; R = Me) to give respectively the pteridines (XXX) and (XXXI; R = Me) or their 7-isomers[45,53]



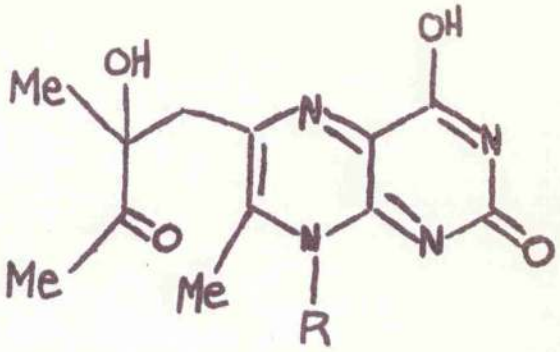
XXVIII



XXIX

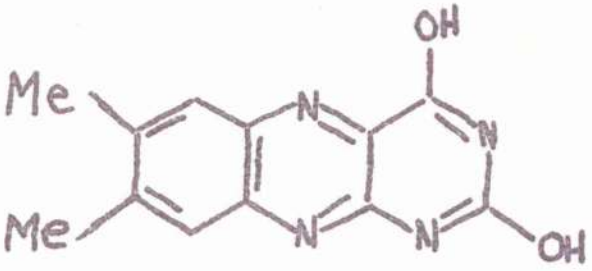


XXX

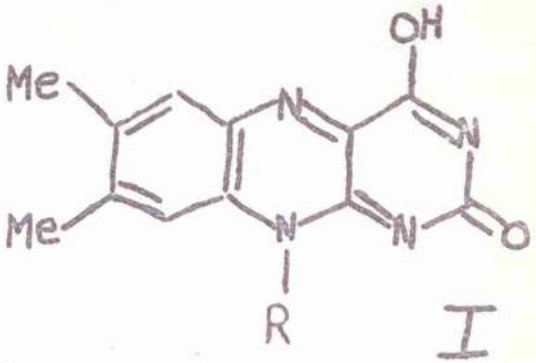


XXXI

Cyclisation[48] of the pteridine (XXX) was carried out by reflux in N-sodium hydroxide solution to give lumichrome (XXXII), while the cyclisation[53] of the 8-methylpteridine was achieved in polyphosphoric acid giving lumiflavin (I; R = Me).

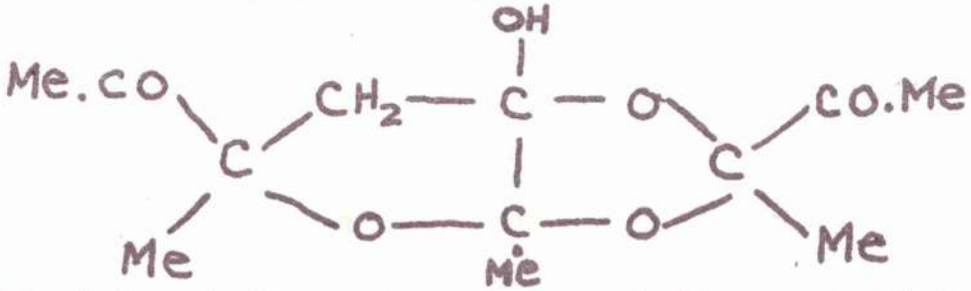


XXXII



I

Recently, Wood and Cresswell[54] have isolated a trimeric form of biacetyl, which appears to have structure (XXXIII).[55]



XXXIII

This trimer behaves in aqueous solution as a mixture of biacetyl and the dimeric aldol (XXVIII) or the related open-chain hexane trione (XXIX). Thus it reacts with

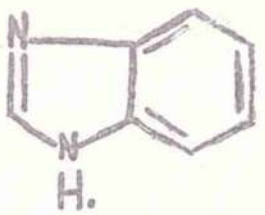
5-amino-4-D-ribitylaminouracil (XXII; R = D-ribityl) to give a mixture of 2,8-dihydro-4-hydroxy-6,7-methyl-2-oxo-8-D--ribitylpteridine (II) and the pteridine (XXXI, R = D-ribityl) or its 7-isomer. The latter pteridine was cyclised readily to riboflavin with polyphosphoric acid. It then appears that the trimeric form of biacetyl could explain the formation, by micro-organisms, of riboflavin together with G-compound (II)

RING CLEAVAGE REACTIONS OF PURINE NUCLEOSIDES AND NUCLEOTIDES.

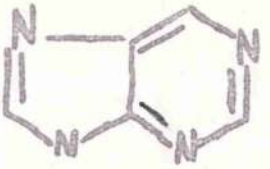
In previous sections of the thesis, hypotheses dealing with the biosynthesis of pteridines and flavins, were discussed In general, the initial step in such biosynthetic paths involved ring-opening of the imidazole ring of a purine or purine nucleoside. Evidence in the literature dealing with ring cleavage in purines and related heterocycles, will now be discussed.

Benzimidazoles[56]

This group of compounds consists of a benzene ring fused to an imidazole or 1,3-diazole ring system, giving the nucleus (XXXIV). It thus bears a formal similarity to the purine nucleus (XXXV).

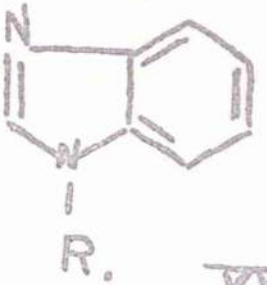


XXXIV

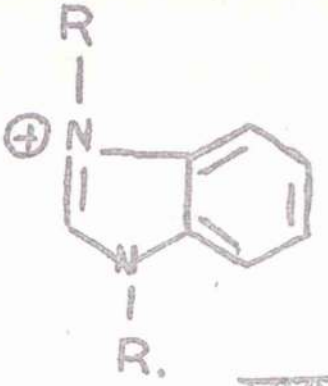


XXXV

Stepwise alkylation of benzimidazole gives first a mono-alkyl derivative (XXXVI) which on further reaction with an alkyl halide, gives a 1,3-dialkylated benzimidazolium salt (XXXVII)



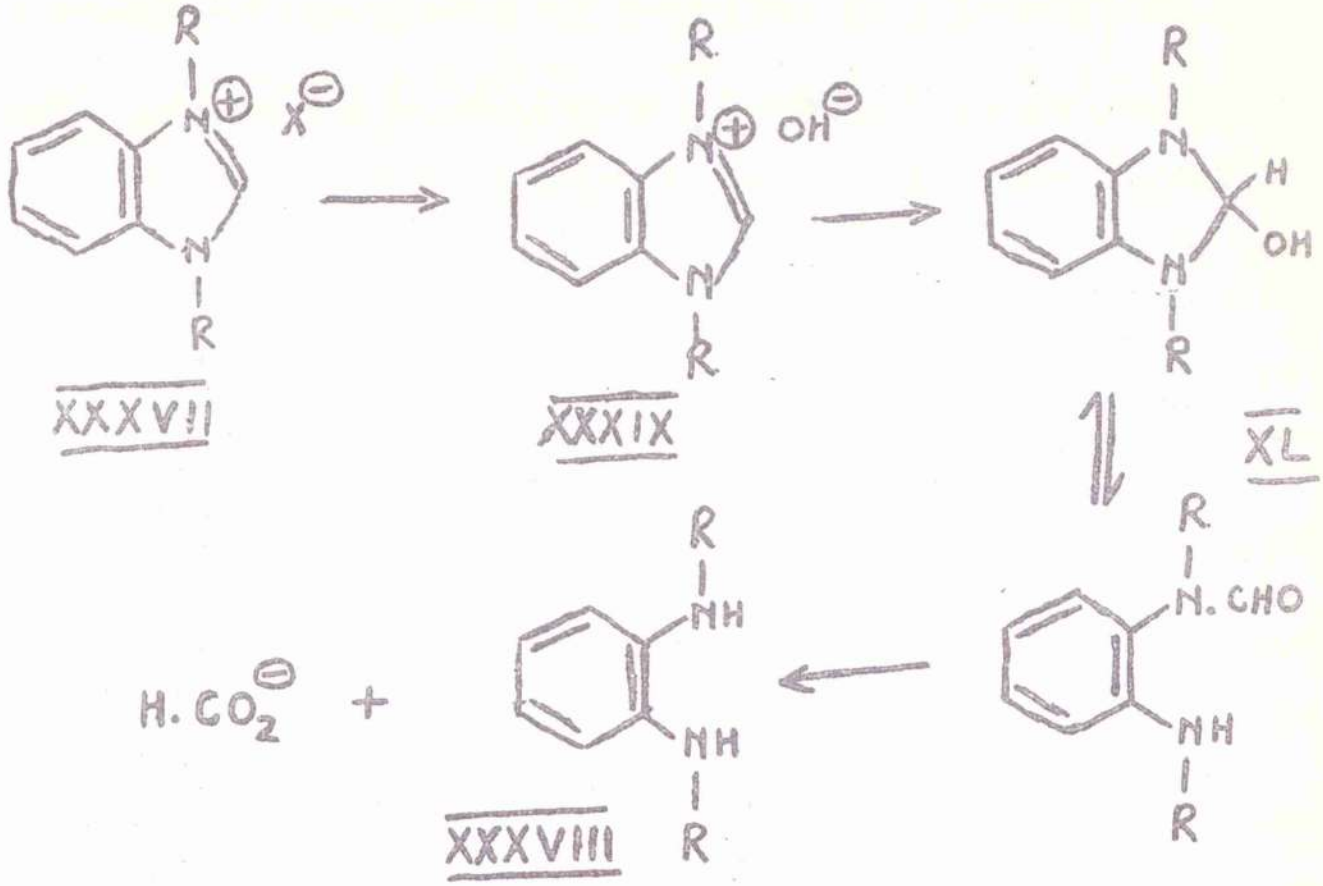
XXXVI



XXXVII

Under the influence of warm alkali, 1,3-dialkylbenzimidazolium salts undergo ring fission with the formation of a molecule of an N,N'-dialkyl-o-phenylenediamine (XXXVIII) and a molecule of formic acid.[86] The transformation of the salt (XXXVII) into the "ammonium base" (XXXIX) initiates the reaction. The proximity of the highly electron-attracting quaternary nitrogen atoms renders the carbon atom at position 2 of the 1,3-dialkylbenzimidazolium ion electron-deficient, and

this carbon atom is consequently attacked by a hydroxyl ion with formation of the "carbinol base" (XL). Futher hydrolysis brings about fragmentation of the molecule with the formation of N,N'-dialkyl-o-phenylenediamine (XXXVIII) and sodium formate.

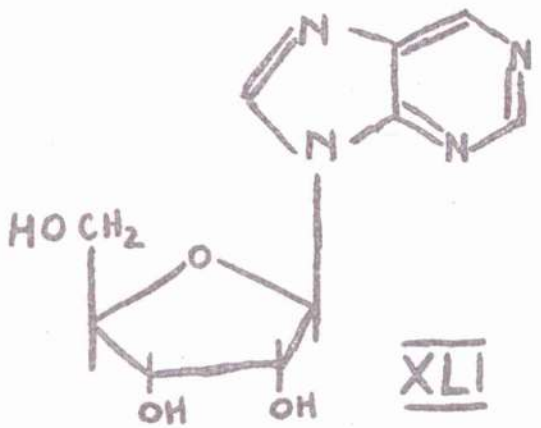


Application to a purine nucleoside of a similar sequence of reactions, is discussed in the Theoretical Section of this thesis.

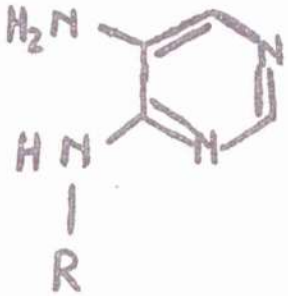
Purine Nucleosides and Nucleotides.

(a) 9-β-D-Ribofuranosylpurine ("Nebularine")

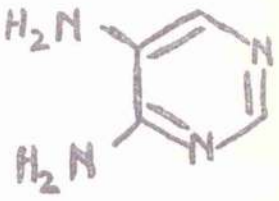
Nebularine,[57] a purine riboside isolated from the mushroom Agaricus nebularis has been shown to have structure (XLI) by comparison with a synthetic specimen prepared by Brown and Weliky.[58] Study of the chemistry of this compound by Brown and his co-workers[59] revealed that it was extremely unstable to aqueous alkali. Three degradation products were obtained: one of these was positively identified as 4,5-diaminopyrimidine (XLII). On the basis of their spectra, various colour reactions, and their behaviour on paper chromatography, the other two products were tentatively identified as glycosides (XLIII; R = D-ribofuranosyl or D-ribopyranosyl) derived from 4,5-diaminopyrimidine.



XLI



XLIII

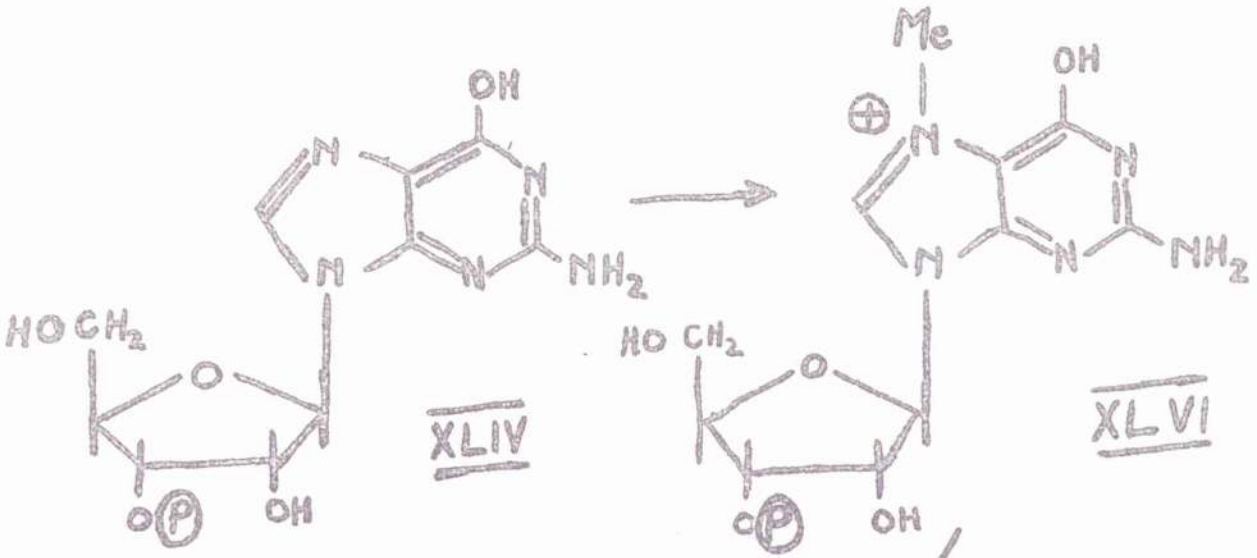


XLII

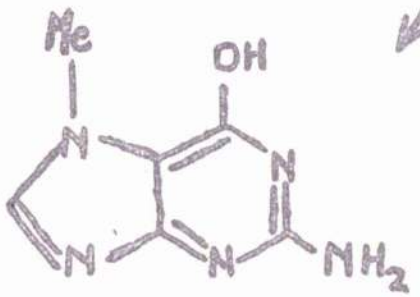
(b) Guanine Nucleosides and Nucleotides.

Lawley and Wallick[60] investigated the alkylation of guanylic acid (XLIV) using dimethyl sulphate at pH7, and

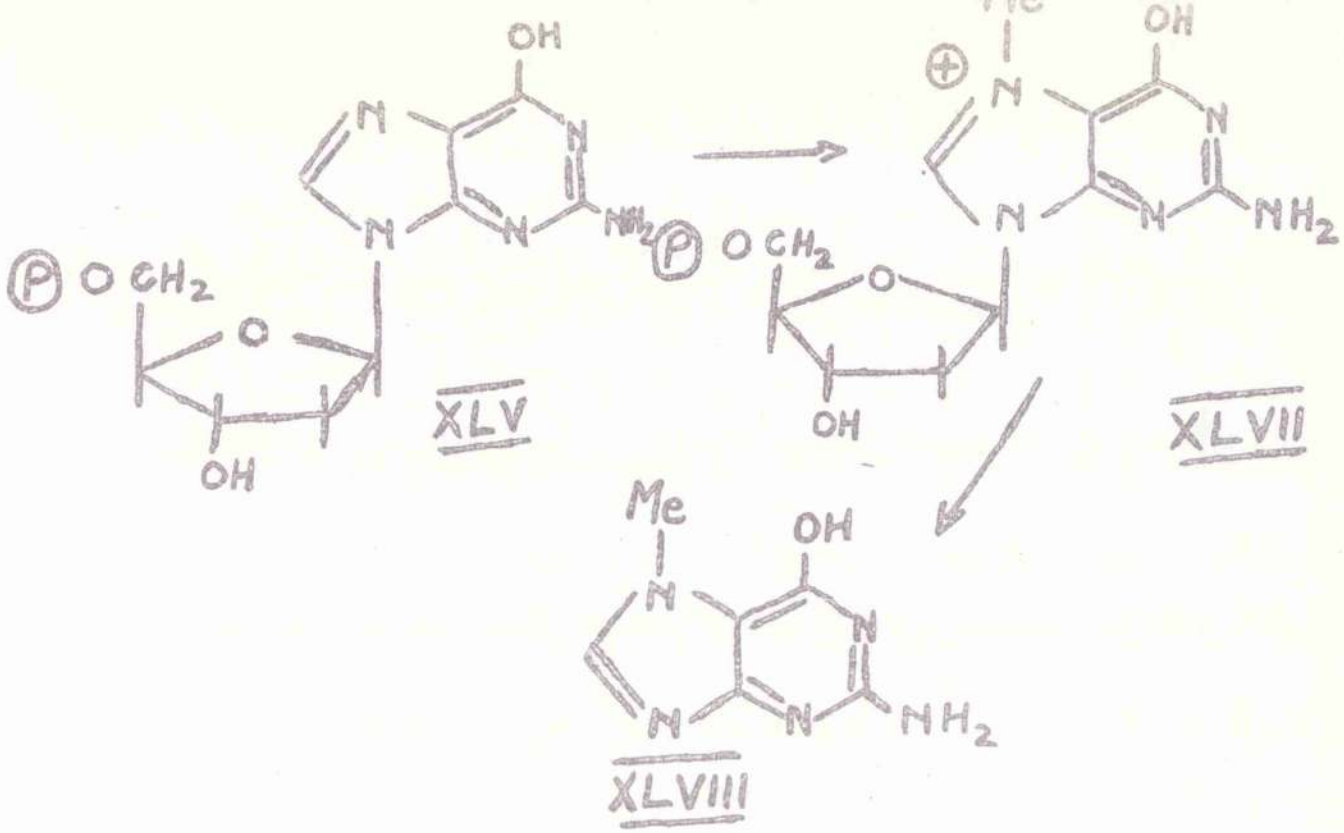
later Lawley[61] reported a similar methylation of deoxyguanylic acid (XLV). Lawley deduced from his spectroscopic studies of these reactions that methylation had taken place in each case at nitrogen-7 of the guanine moiety to give the quaternary salts (XLVI) and (XLVII) respectively. In the case of the deoxyguanylic acid derivative (XLVII), hydrolysis of the sugar residue occurs under the conditions of the experiment to give 7-methyl guanine (XLVIII). Acid hydrolysis of the quaternary salt, derived from guanylic acid similarly gave 7-methyl guanine (XLVIII).



XLIV XLVI



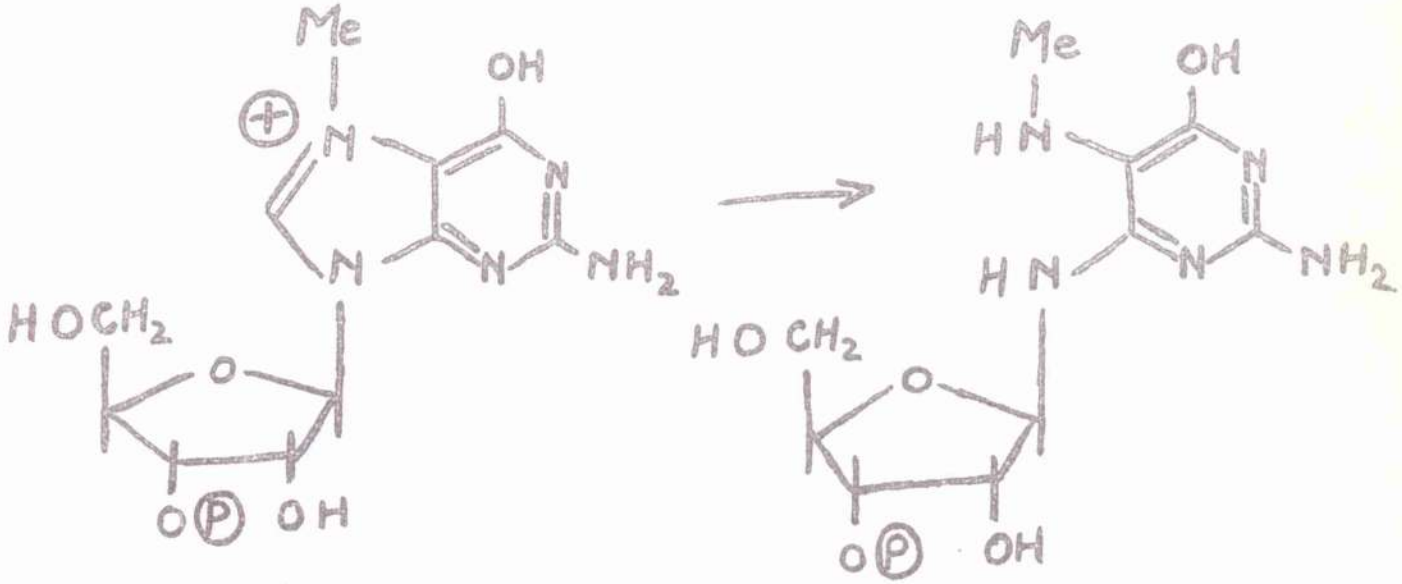
XLVIII



XLV XLVII XLVIII

where (P), in both cases, = H_2PO_3.

The former quaternary salt (XLVI), however, underwent a more fundamental change on treatment with dilute alkali and Lawley and Wallick[60] deduced from spectrophotometric measurements that ring-opening of the purine ring had taken place to give a 4,5-diaminopyrimidine derivative (XLIX).

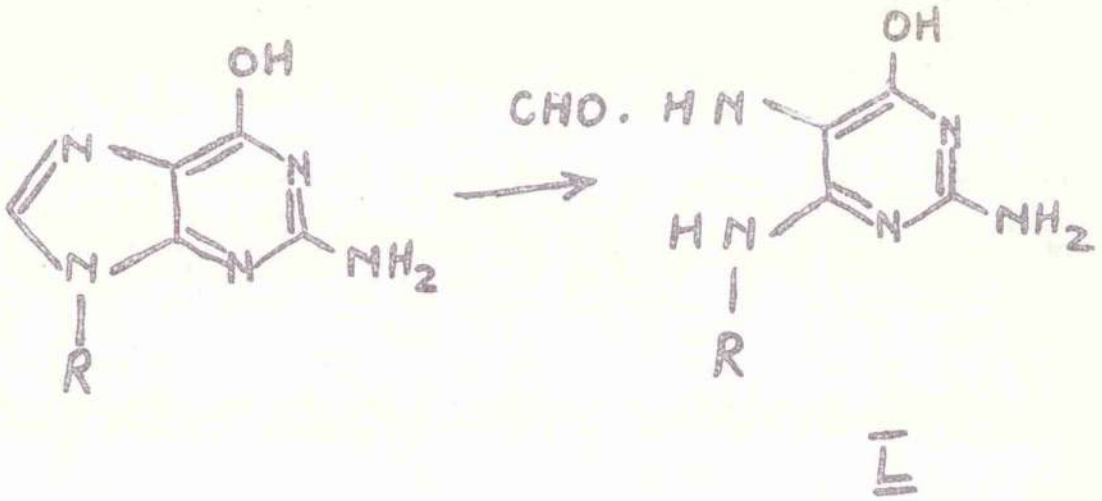


XLVI XLIX

These workers were not able to isolate and characterise either the quaternary salt (XLVI) or the pyrimidine glycoside (XLIX). A further study of this reaction will be reported in the Theoretical Section of this thesis as it obviously has a bearing on the hypotheses already outlined for the biosynthesis of pteridines and riboflavin.

Lawley and Wallick[60] also found that alkylation of deoxyribonucleic acid (D.N.A.) using a nitrogen mustard (di-(2-chloroethyl)methylamine) gave, after acid hydrolysis, a 7-alkylguanine as the major product. This showed that only the guanine moiety of the D.N.A. was susceptible to alkylation under these mild conditions and that the reaction proceeded as described above for the isolated nucleotide (XLV).

Hems[62] has shown that a similar ring-opening of guanosine and guanylic acid can be brought about by the action of ionising radiation on aqueous solutions of the purines. The products of the reaction were again characterised only by their ultraviolet absorption spectra and by their behaviour on paper chromatograms. It seems probable, however, that ring-opening has taken place in the imidazole ring to give pyrimidine glycosides (L).



L

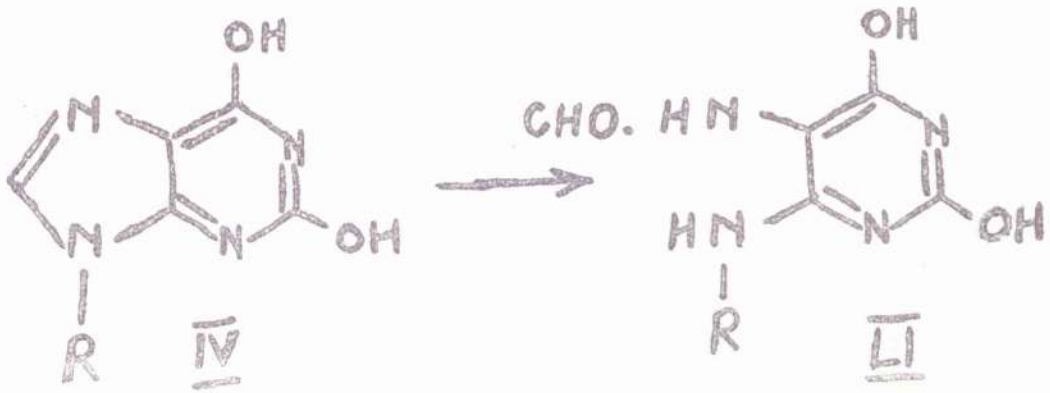
where R = D-ribosyl or D-ribosylphosphate.

A secondary reaction involved cleavage of the sugar moiety and formation of guanine itself. Similar results were obtained[63] by the action of high energy electrons on the intact D.N.A. molecule.

These reactions are very similar to those brought about by the action of alkylating agents and they may go some way to explaining the so-called "radiomimetric" effect of the biological alkylating agents.

(c) Xanthosine.

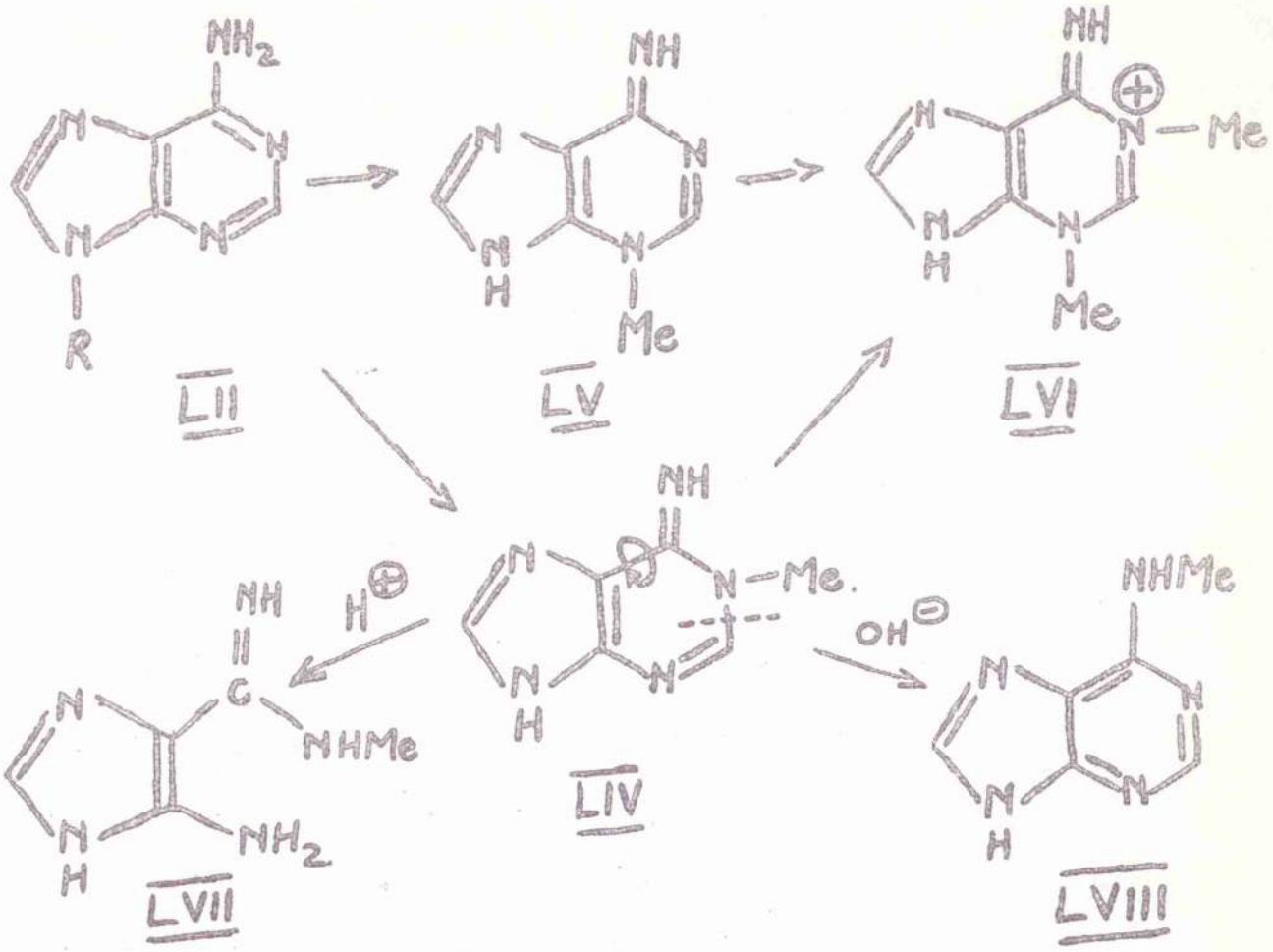
Hems[64] has also described the ring-opening of xanthosine when subjected to ionising radiation. He reported that xanthosine ring-opens in the same way as guanosine giving a 4,5-diaminouracil derivative (LI; R = D-ribosyl).



IV LI

(d) Adenine and Hypoxanthine Nucleosides and Nucleotides.

When the purine derivative has only one substituent in the pyrimidine ring as in the case of adenosine (LII), and inosine (LIII), alkylation appears to take place in this ring, and this is followed by ring-opening reactions which leave the imidazole ring intact. Such results will therefore be discussed only briefly.

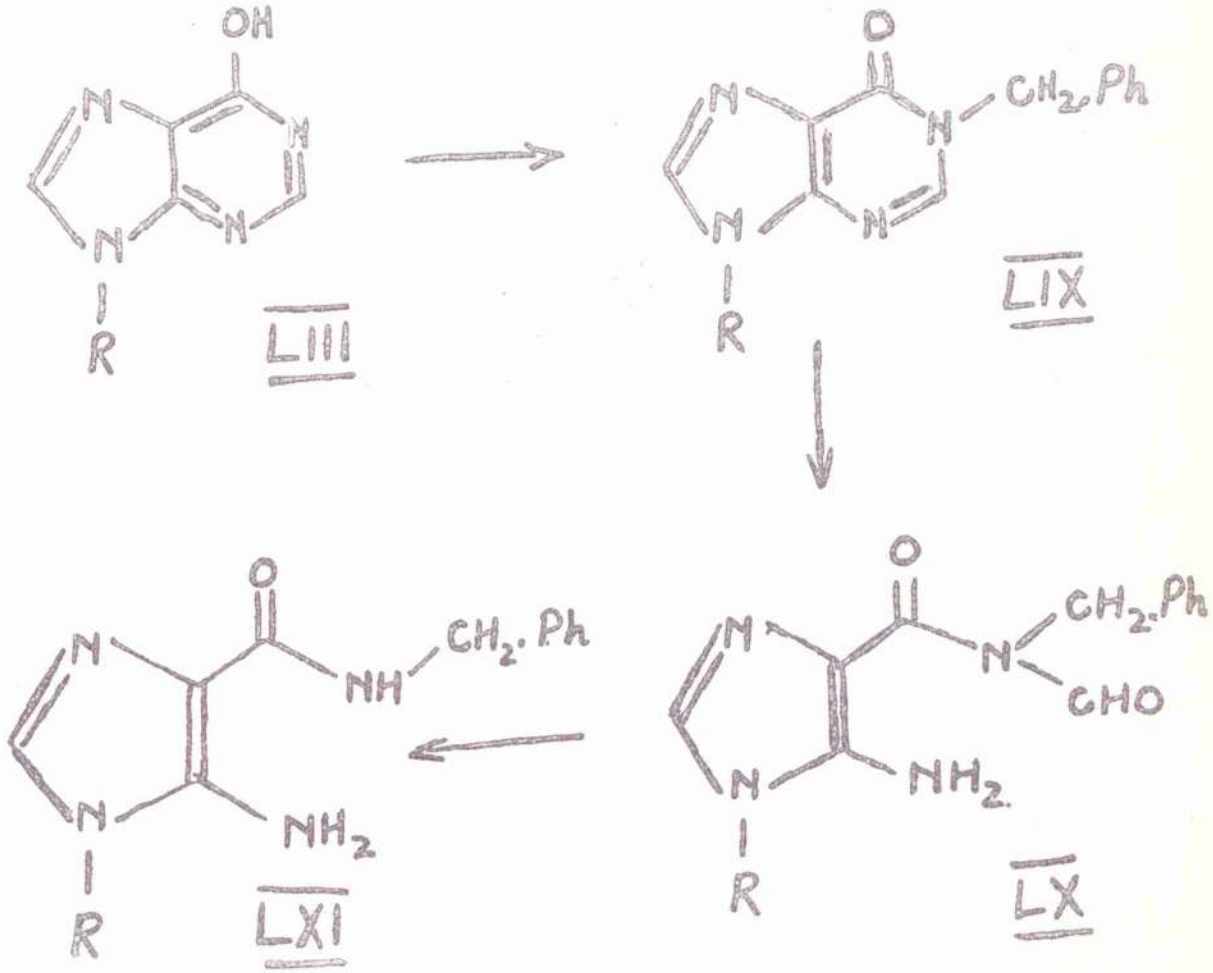


When adenosine (LII; R = D-ribosyl) was heated with methyl sulphate in N,N-dimethylformamide at 100°, followed by acid hydrolysis, four main compounds were isolated by chromatography of the reaction mixture.[65] They were 1-methyl adenine (LIV), 3-methyladenine (LV), a 1,3-dimethyladenine quaternary

salt (LVI) and 5-aminoimidazole-4-N'-methylcarboxyamidine (LVII). Both acid and alkali cleaved the pyrimidine ring of the purine derivative (LIV), but in the case of the alkaline ring-opening, fission of the 1-2 bond resulted in the rotation of bond 5-6 and subsequent reforming of the pyrimidine ring to give a 6-methylaminopurine derivative (LVIII).

Adenylic acid, the 3'-phosphate of adenosine behaves similarly on methylation.[68]

Shaw has described the alkylation of inosine (LIII; R = D-ribosyl) using benzyl bromide. The initial product is



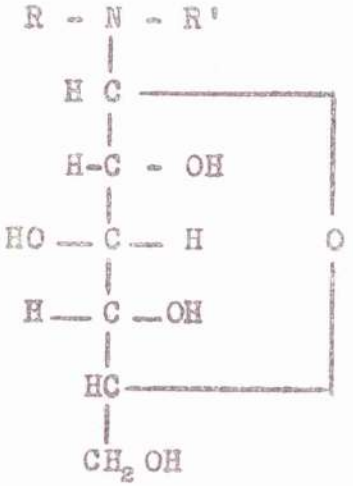
the 1-benzyl derivative (LIX, R = D-ribosyl) which on treatment with alkali undergoes the expected ring-opening to give the imidazole (LX; R = D-ribosyl) and finally compound (LXI; R = D-ribosyl).

An acylation[67] using p-toluene sulphonyl chloride gave similar results.

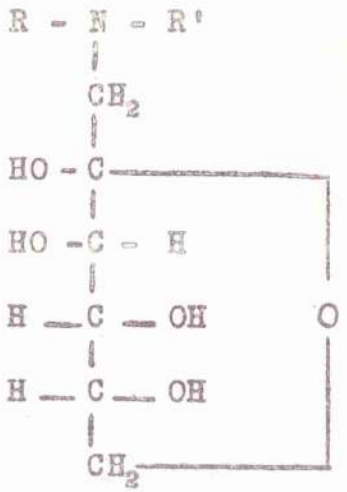
It is interesting to note that Pullmann,[68] using quantum mechanics, has calculated the relative electronegativity of each nitrogen of the natural purines, guanine and adenine. His results agree with the chemical evidence that positions 7 and 1 respectively of these purines, are the centres of most probable electrophilic attack.

AMADORI REARRANGEMENT[69]

The Amadori rearrangement is the irreversible isomerisation of an N-substituted aldosylamine to a 1-substituted amino-1-deoxy-2-ketose in an anhydrous medium by acid catalysis.



(LXII)



(LXIII)

The example above shows the conversion of the acid labile N-glycoside (LXII) into its stabler isomer (LXIII) in the D-glucose series.

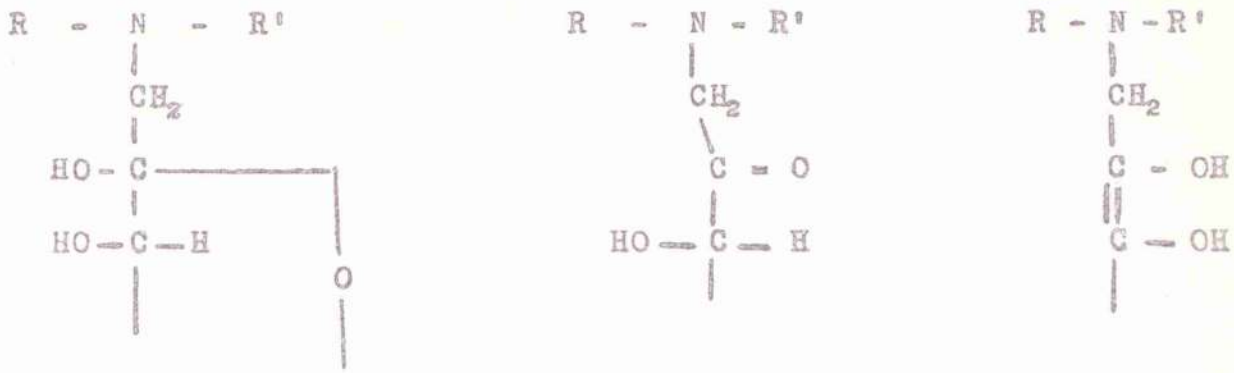
Amadori pioneered this rearrangement, but he did not realise that an aldose-ketose change was taking place. This was later determined by Kuhn and Weygand[70] who showed that the rearrangement isomer could not be hydrolysed by aqueous acid into its sugar and amine components.

Amadori[71] heated an aromatic amine and D-glucose together in the dry state, to give a brown mass from which was isolated the 1-arylamino-1-deoxy-D-fructose. Later workers[72] extended the scope of the reaction to all classes of amines and aldoses. The use of anhydrous solvents and catalytic amounts of weak acids[73] greatly improved the quality and the quantity of the product. Hodge[74,75] used compounds containing active methylene groups as catalysts, but such "catalysts" must be present in relatively larger proportion. The rearrangement is always accompanied by decomposition of the sugar moiety, forming tars which tend to colour the reaction mixture.

The unsubstituted 1-amino-1-deoxy-D-fructose was synthesised by Fischer,[76] and was called isoglucosamine. This compound is usually prepared as a salt, e.g., an acetate, because the free base which is an α-aminoketose, self-condenses

to form a dihydropyrazine derivative.

One characteristic feature of the products of Amadori rearrangements, is that they will readily reduce Redox indicators, such as methylene blue and o-dinitrobenzene[77] in alkaline solution. They do so, because they can be regarded as ammono analogues of ene-diols, e.g.:-



The carbonyl group can be reduced in neutral or alkaline media by Adam's platinum catalyst, usually giving an epimeric mixture of 1-substituted amino-1-deoxy-glycitols.

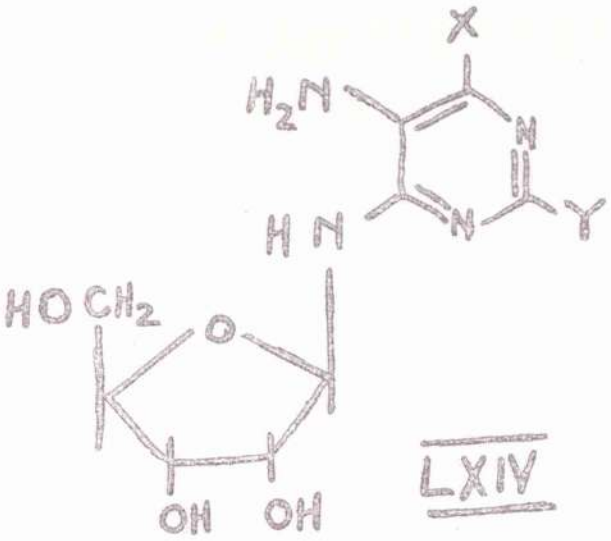
The Amadori rearrangement is becoming increasingly important in chemical synthesis. Weygand has used it in a synthesis of riboflavin.[73] and it has also been used in the preparation of folic acid.[78] It is now suspected that this rearrangement is involved in the non-enzymic browning of food and ripening of fruits, as both these systems seem to involve sugar-amine condensations.

The possible application of this rearrangement in the biosynthesis of pteridines and flavins will be discussed in the Theoretical Section of this thesis.

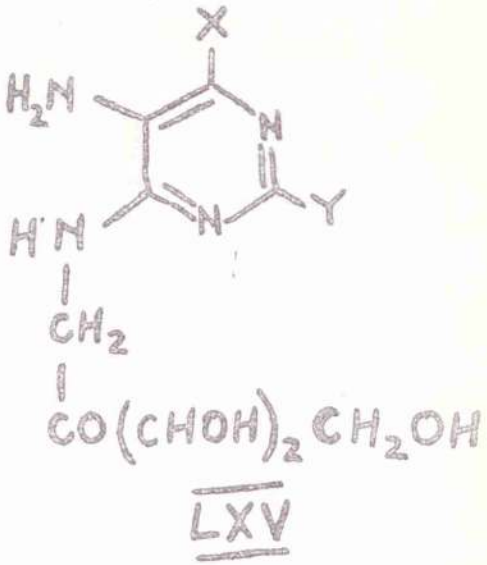
THEORETICAL PART I

Chemical Studies of the Biosynthesis of Riboflavin and Related Pteridines

Weygand's hypothesis[15] for the transformation of purines into pteridines has already been summarised in a previous section of this thesis. He suggested that one step in the sequence of reactions involves the conversion of a pyrimidine glycoside of the type (LXIV) into a pyrimidine (LXV) via an Amadori rearrangement. The final stages of his reaction scheme then involved intramolecular cyclisation of (LXV) to give a dihydropteridine.



LXIV

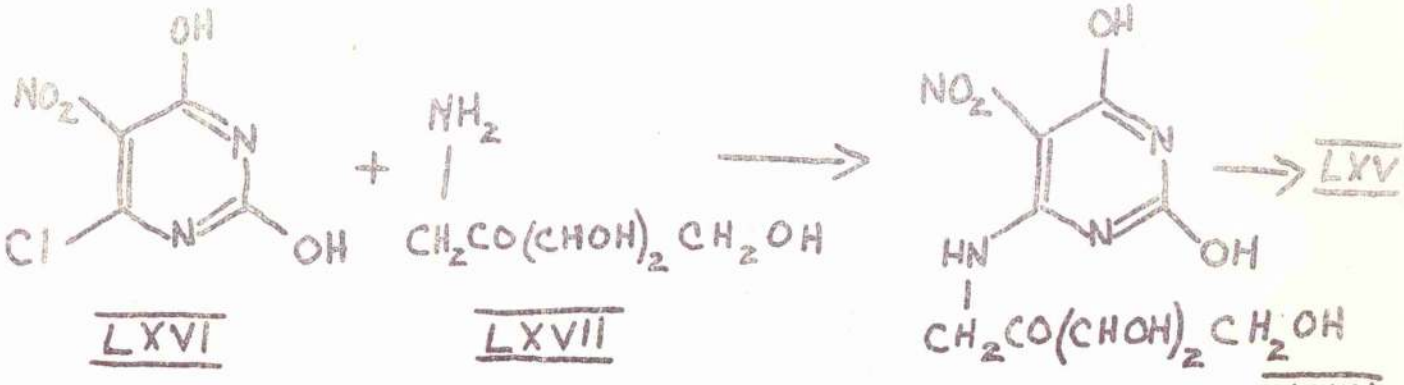


LXV

It is interesting to note that stereospecific reduction of the carbonyl group of pyrimidine (LXV) could give a D-ribityl side chain as found in riboflavin (I). As it has been suggested that riboflavin itself arises from purine precursors, this series of changes may explain the transformation of the ribosyl group of the purine nucleoside to the ribityl group of riboflavin in the biosynthetic pathway, provided that the side-chain remains attached to the heterocyclic moiety throughout the sequence of reactions.

Experiments using randomly labelled guanosine led McNutt and Forrest[79] to suggest that the ribosyl group of guanosine was not incorporated into the riboflavin molecule, since the ribityl side-chain of the latter compound was shown to contain little radioactivity. Such experiments are not conclusive, however, since the ribosyl group of guanosine is, without doubt, in equilibrium with a much larger quantity of D-ribose in the metabolic pool, and the radioactivity would thereby be greatly diluted.

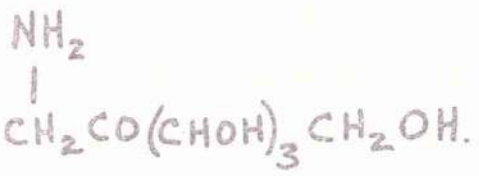
The principal aim of this section is to prepare a pyrimidine (LXV; X = Y = OH) and to study its conversion to riboflavin. The most reasonable approach would seem to involve the condensation of 4-chloro-5-nitrouracil[84] (LXVI) with 1-amino-1-deoxy-D-erythropentulose (LXVII), followed by reduction of the 5-nitro group.



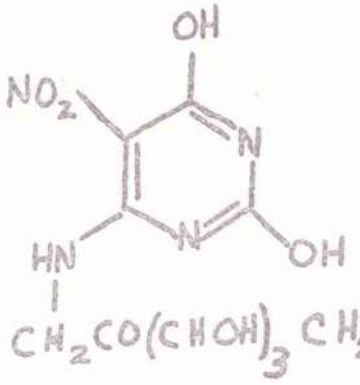
LXVI LXVII LXVIII

The pyrimidine (LXVI) is readily available as the result of recent work in this department,[84] but a search of the literature revealed that only one 1-amino-1-deoxy-D-ketose was

known and that is the D-fructose derivative[76] (LXIX) known as "isoglucosamine". It was therefore decided to carry out initial experiments using this sugar amine and to synthesise the model substance (LXX) by the method outlined above.



LXIX

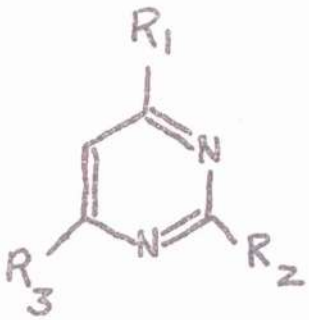


LXX

Two main investigations were undertaken with this model substance.

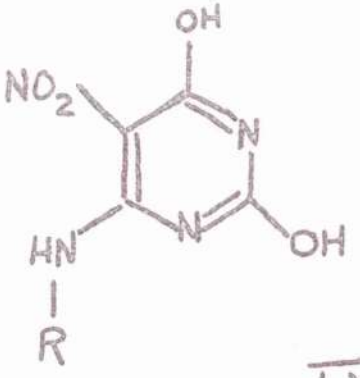
(a) Reduction of the carbonyl group in the side chain. The possibility of a stereospecific reduction was given particular attention. Subsequent reduction of the 5-nitro group and condensation with an o-benzoquinone derivative then gave a C_8-analogue of riboflavin.

(b) Reduction of the 5-nitro group and intramolecular condensation gave a dihydropteridine whose properties proved to be very interesting.



LXXI

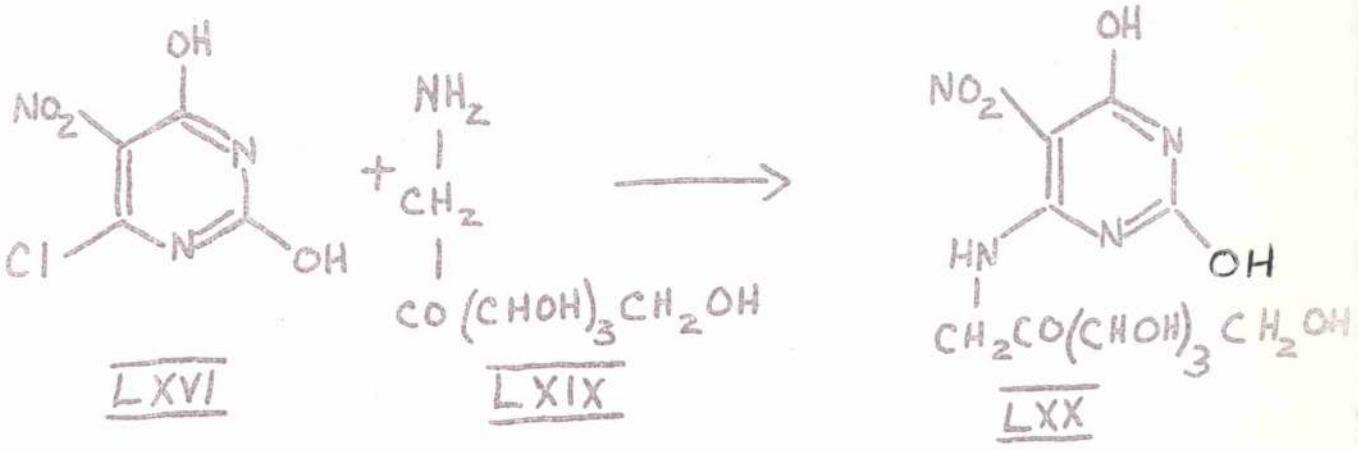
2,4,6-Trihydroxypyrimidine[80] (LXXI, $R_1 = R_2 = R_3 = OH$, "barbituric acid"), was converted[81] to its trichloro derivative (LXXI; $R_1 = R_2 = R_3 = Cl$). Alkaline hydrolysis of this trichloropyrimidine gave 4-chloro-2,6-dihydroxypyrimidine (LXXI; $R_1 = R_2 = OH$, $R_3 = Cl$, "4-chlorouracil"). This one-step conversion was recommended by Langley,[82] and is superior to the two-step literature method. Nitration[54] of 4-chlorouracil using fuming nitric acid with concentrated sulphuric acid readily gave 4-chloro-2,6-dihydroxy-5-nitropyrimidine (LXVI), which was extremely reactive, and which had no definite m.p. Its purity was estimated routinely by means of its ultraviolet absorption spectrum, or more accurately, by reacting it with excess ethanolamine and collecting and weighing the 2,6-dihydroxy-4-2'-hydroxyethylamino-5-nitropyrimidine[54] (LXXII; $R = CH_2.CH_2OH$) which resulted.



LXXII

The other component, 1-amino-1-deoxy-D-fructose (LXIX; "isoglucosamine"), was prepared by a modification of the literature method.[76] Glucosazone was made from D-glucose by

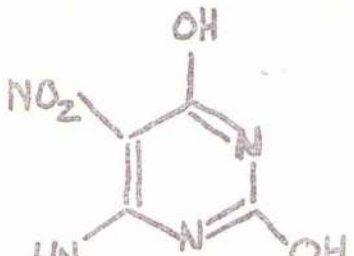
treating with phenylhydrazine in an acetate buffer at 90°. This material was hydrogenated while suspended in an acetic acid-water-ethanol solvent, under four atmospheres of hydrogen and using a palladium-barium sulphate catalyst. The disappearance of the yellow osazone indicated the end-point of the reduction Isoglucosamine (LXIX) was isolated from the reaction mixture as its acetate. Treatment of this acetate with the calculated amount of sodium ethoxide, gave the free base, isoglucosamine, which was immediately condensed with half the molar proportion of pyrimidine (LXVI) added as an ethanolic solution.



1-[2',6'-Dihydroxy-5'-nitro-4'-pyrimidinylamino]-1-deoxy-D-fructose (LXX) was isolated as hydroscopic white powder and was characterised by preparation of its oxime.

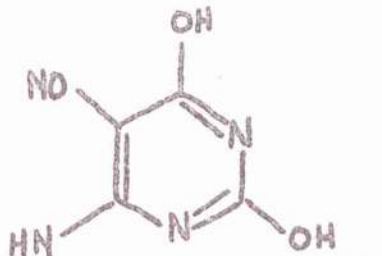
Reduction of the carbonyl group of pyrimidine (LXX) by sodium borohydride was carried out overnight in 0.1 N-sodium hydroxide solution. After destruction of the excess borohydride with formic acid, the reaction mixture was adsorbed on an anion exchange resin at pH10 and the products were eluted using

ammonium formate buffer at various pH values. Buffer at pH4 eluted the major product which on isolation proved to be 2,6-dihydroxy-5-nitro-4-D-sorbitylaminopyrimidine (LXXII; R = D-sorbityl). Proof of the stereochemistry of this product is adduced below.

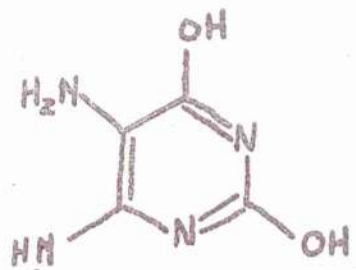


LXXII

Reduction of the 5-nitro group in (LXXII; R = D-sorbityl) was achieved by reduction with sodium dithionite in 0.1N-sodium hydroxide solution. This chemical reduction proceeds through the corresponding 5-nitrosopyrimidine (LXXIII; R = D-sorbityl) which has an intense red colour, giving finally the colourless 5-amino compound (XXII; R = D-sorbityl).



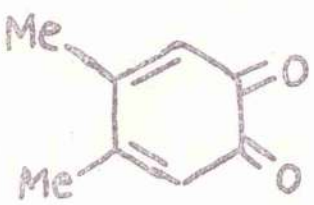
LXXIII



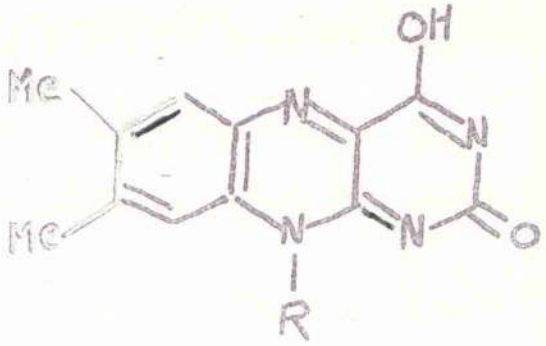
XXII

The pH of the resulting solution was adjusted to 5, an ethanolic solution of the dimer of 3,4-dimethyl-o-benzoquinone[83]

(LXXIV) was added immediately and the orange mixture was warmed for ten minutes. On cooling 6,7-dimethyl-9-D-sorbityliso-alloxazine[84] (I; R = D-sorbityl, "sorboflavin"), separated.

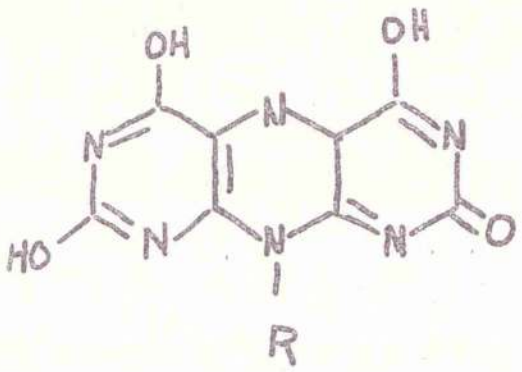


LXXIV

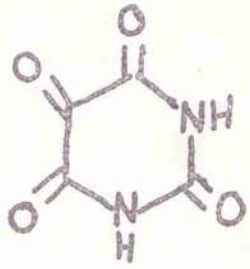


I

Addition of the o-benzoquinone must not be delayed otherwise the 5-amino-4-D-sorbitylaminouracil (XXII; R = D-sorbityl) undergoes an oxidation self-condensation to give a pyrimidopteridine (LXXV; R = D-sorbityl). This compound can also be prepared by addition of alloxan[85] (LXXVI) to the 5-amino pyrimidine (XXII; R = D-sorbityl). Thus, alloxan was added to a similar solution of this pyrimidine at pH5. The mixture became purple, probably due to the 5-oxo group of alloxan condensing with the 5-amino group of the pyrimidine. Subsequent reflux for 2 hr. in N-hydrochloric acid solution gave a yellow solution of 2,10-dihydro-4,6,8-trihydroxy-2-oxo-10-D-sorbityl-pyrimido[5,4-g]pteridine (LXXV; R = D-sorbityl) which separated on cooling. Pyrimidopteridines (LXXV) of this type have a strong light blue fluorescence in aqueous solutions of high dilution.

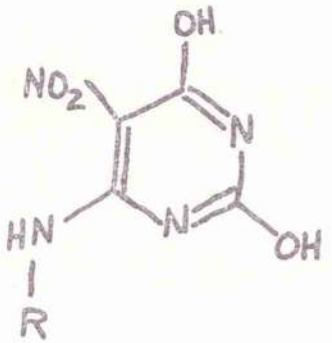


LXXV



LXXVI

The pyrimidopteridine (LXXV; R = D-sorbityl) was dissolved in water and treated with excess sodium periodate solution and left overnight. 2,10-Dihydro-4,6,8-trihydroxy-2-oxo-10-formylmethylpyrimido[5,4-g]pteridine (LXXV; R = $CH_2.CHO$) separated and was reduced in alkaline solution with sodium borohydride to give 2,10-dihydro-4,6,8-trihydroxy-2-oxo-10-2'-hydroxyethylpyrimido[5,4-g]pteridine (LXXV; R = $CH_2.CH_2OH$). This material was identical in infrared and ultraviolet spectra and on paper chromatography in various solvents with an authentic sample made by condensation of alloxan (LXXVI) with 2,6-dihydroxy-4-2'-hydroxyethylamino-5-nitropyrimidine[54] (LXXII; R = $CH_2.CH_2OH$) which had previously been reduced with sodium dithionite in alkaline solution.



LXXII

From the foregoing series of reactions, it is obvious that a polyhydroxyalkyl side-chain must have been present in the pyrimidopteridine (LXXV; R = D-sorbityl) and must have been of structure (LXXVII) as it is oxidised by periodate

—— $CH_2(CHOH)_4CH_2OH$ —— $CH_2 \cdot CHO$ —— $CH_2 \cdot CH_2OH$

(LXXVII) (LXXVIII) (LXXIX)

to the aldehyde (LXXVIII) which is then reduced to a 2'-hydroxyethyl radical (LXXIX) with sodium borohydride. Since the side chain (LXXVII) arose from the side chain (LXXX) of the original pyrimidine (LXX),

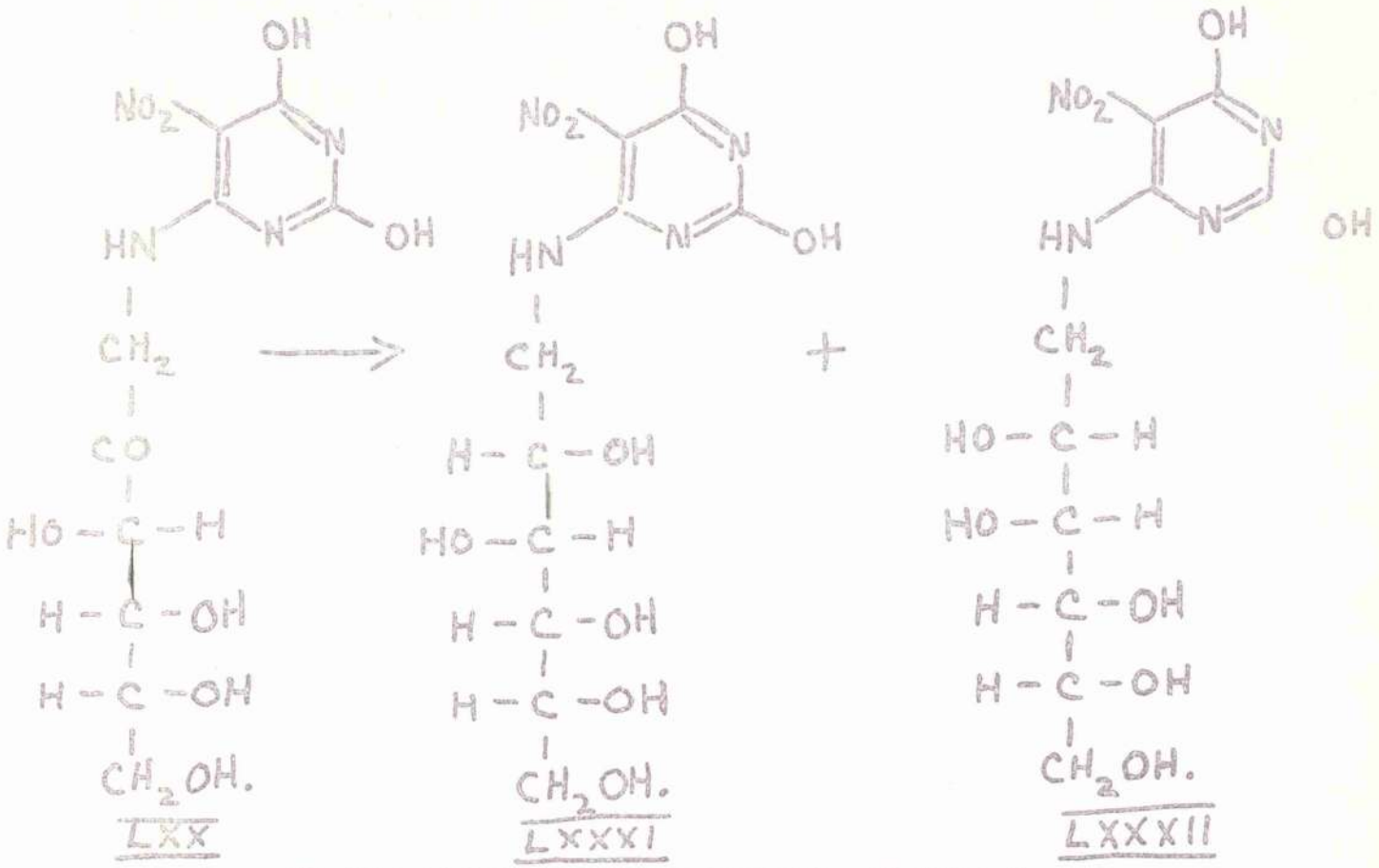
—— $CH_2CO(CHOH)_3CH_2OH$

(LXXX)

only the stereochemistry at C_2 in the side chain (LXXVII) of the reduction product is in doubt.

Theoretically reduction of the carbonyl group of a D-fructose derivative should give an equimolar mixture of the

two epimers, in this case, a D-glucose (D-sorbose) derivative and a D-mannose derivative. Hence reduction of the pyrimidine (LXX) with sodium borohydride should give an epimeric mixture of a D-sorbitylaminopyrimidine (LXXXI) and a D-mannitylamino-pyrimidine (LXXXII).



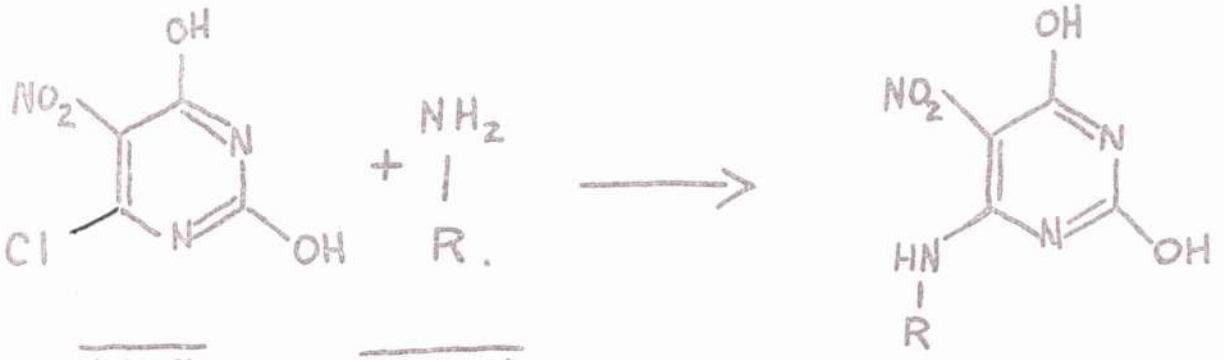
LXX → LXXXI + LXXXII

In the reduction of compounds of this type, an equimolar epimeric mixture is seldom obtained as the pyrimidine ring and the remainder of the asymmetrical sugar moiety exert steric effects: because of this only one epimer is often produced. Weygand[73] made use of this stereospecific reduction in his synthesis of riboflavin from D-arabinose which is described later.

To determine the percentage of each epimer formed in the reduction of 1-[2',6'-dihydroxy-5'-nitro-4'-pyrimidinylamino]-1-deoxy-D-fructose (LXX), the unambiguous synthesis of each isomer was carried out.

D-glucose Series.

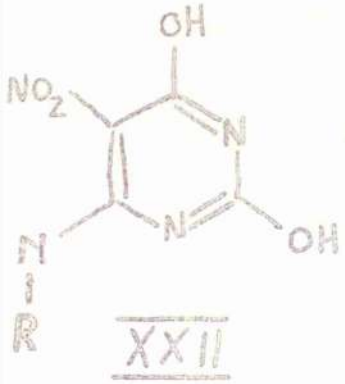
D-glucose oxime[86] was reduced over Adams platinum catalyst under hydrogen to give D-sorbitylamine (LXXXIII; R = D-sorbityl) which was reacted with 4-chloro-2,6-dihydroxy-5-nitropyrimidine (LXVI). A crude solution of resulting 2,6-dihydroxy-5-nitro-4-D-sorbitylaminopyrimidine (LXXII; R = D-sorbityl) was purified on a column of an anion-exchange resin (Amberlite C.G.400-formate form). Ammonium formate buffer at pH4 eluted the pure pyrimidine.



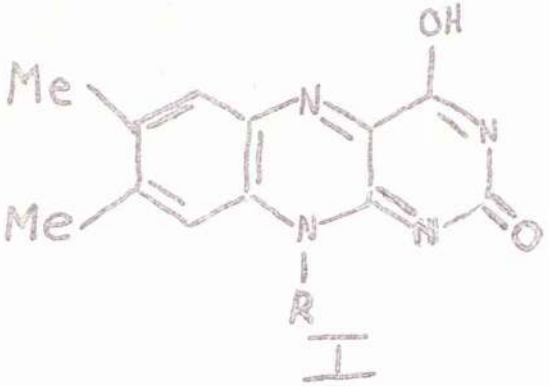
LXVI LXXXIII LXXII

Reduction of the nitropyrimidine (LXXII; R = D-sorbityl) with sodium dithionite and condensation with alloxan gave the D-sorbitylpyrimido[5,4-g]pteridine (LXXV; R = D-sorbityl) which could also be prepared by oxidative self-condensation of the

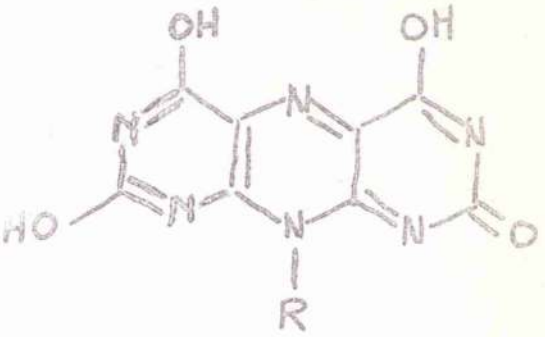
5-aminopyrimidine (XXII; R = D-sorbityl). Similar condensation of the latter compound with the dimer of 3,4-dimethyl-o-benzoquinone[83] gave the D-sorbitylflavin (I, R = D-sorbityl) which had been reported by Karrer.[84] Acetylation of this flavin in pyridine-acetic acid gave a penta-acetate.[84] Karrer did not, however, fully characterise either of these compounds, and the appropriate details are recorded in the experimental section.



XXII



I



LXXV

D-Mannose Series.

D-Mannose oxime[87] was reduced in a similar manner as in the section above. The same reactions were carried out using the resulting D-mannitylamine (LXXXIII; R = D-mannityl) to give the D-mannitylflavin (I; R = D-mannityl) and the pyrimido[5,4-g] pteridine (LXXV; R = D-mannityl). The penta-acetate of the D-mannitylflavin was not prepared.

The following table summarises the physical constants of the products in the two series and compares them with the products formed from the reduced 1-[2',6'-dihydroxy-5'-nitro-4'-pyrimidinyl-

amino]-1-deoxy-D-fructose (LXX).

	Pyrimidines		Pyrimido-Pteridines		Flavins		Flavin Acetate
	m.p.	$[\alpha]_D$	m.p.	$[\alpha]_D$	m.p.	$[\alpha]_D$	m.p.
Reduced D-fructose	225°	+15°	>300°	+61°	274°	-45°	238°
D-glucose	225	+17.5	>300	+60	275	-45	238
D-mannose	247	- 2.5	>300	+17	285	+61	-

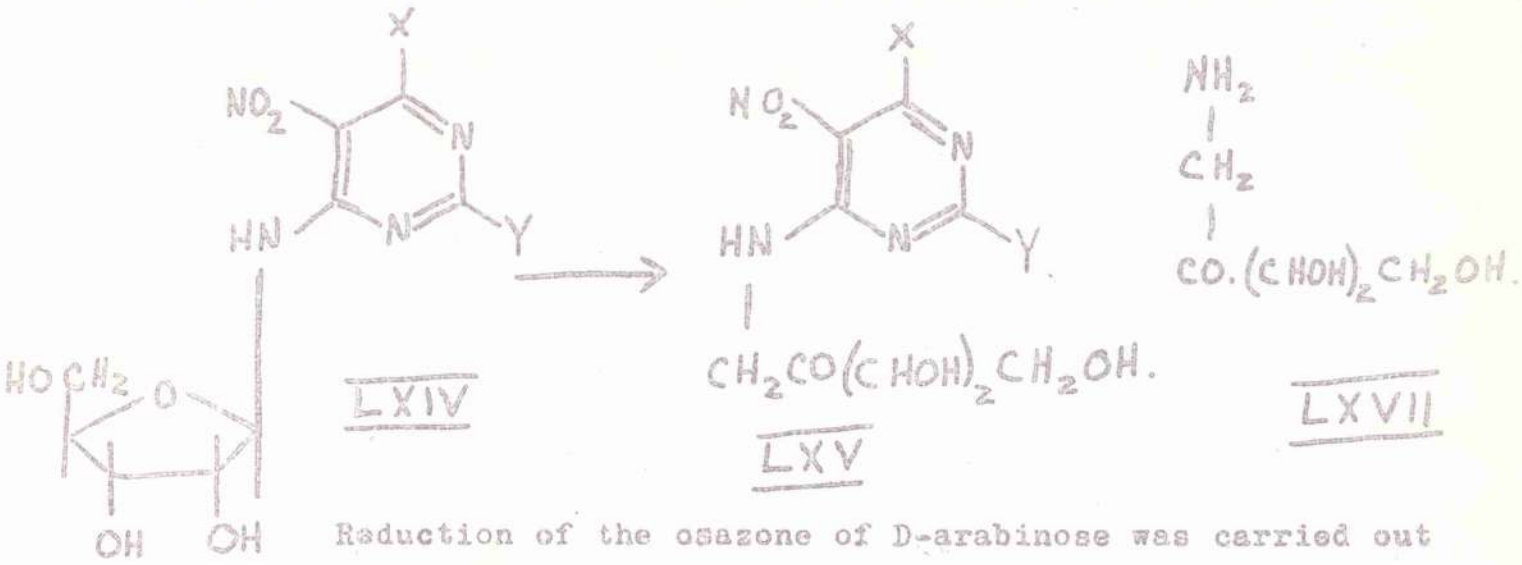
The reduced pyrimidine (LXXII, R = D-sorbityl) was found to be identical in all respects, melting point, specific rotation, ultraviolet spectra and paper chromatography in various solvents, with 2,6-dihydroxy-5-nitro-4-D-sorbitylaminopyrimidine (LXXII, R = D-sorbityl). From the table, it is obvious that the pyrimidopteridines and flavins derived from these pyrimidines are also identical. Their spectra and R_F values were also identical.

Sodium borohydride in alkaline solution has therefore reduced 1-[2',6'-dihydroxy-5'-nitro-4'-D-pyrimidinylamino]-1-deoxy-D-fructose (LXX) stereospecifically to 2,6-dihydroxy-5-nitro-4-D-sorbitylaminopyrimidine (LXXII, R = D-sorbityl).

This <u>in vitro</u> reduction may well indicate that the biosynthetic pathway to riboflavin from purines, does involve a pyrimidine glycoside which undergoes an Amadori rearrangement, followed by a stereospecific reduction. It is interesting to note that the D-glucose derivative is formed in this reaction. If

D-erythro-pentulose, the C_5 analogue of fructose, is used, then, by analogy, we might expect formation of the D-ribose derivative.

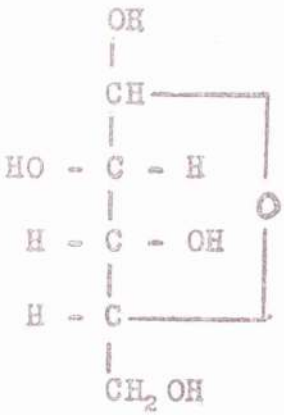
The above model experiments having proved successful, attention was now turned to the synthesis of pyrimidine (LXV; X = Y = OH) which would result from Amadori rearrangement of a pyrimidine glycoside of type (LXIV; X = Y = OH). This necessitated the preparation of 1-amino-1-deoxy-D-erythro-pentulose (LXVII).



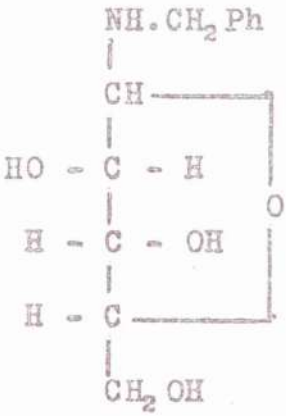
Reduction of the osazone of D-arabinose was carried out using the same conditions[76] as for D-glucose, but no solid could be isolated. On testing a little of the syrup, however, with o-dinitrobenzene[77] in alkali, a positive test for an Amadori one-diol compound was obtained. Treatment of the crude base with 4-chloro-2,6-dihydroxy-5-nitropyrimidine (LXVI) was not attempted as the reaction mixture contained aniline and ammonia which would also react to give a complex mixture of 5-nitropyrimidines. An alternative approach to the sugar amine was therefore attempted.

Recently Micheel and Hagemann[88] have prepared N-benzyl D-xylosamine and have shown that this compound undergoes an Amadori rearrangement when treated with an equimolar amount of anhydrous oxalic acid in dry isopropanol.

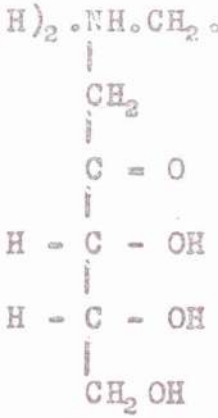
D-arabinose (LXXXIV) and benzylamine were therefore condensed in ethanol to give N-benzyl-D-arabinosylamine (LXXXV) which was rearranged in dry dioxan using a molar quantity of anhydrous oxalic acid to give 1-benzylamino-1-deoxy-D-erythro-pentulose oxalate (LXXXVI) in good yield. Catalytic debenzylation in ethanol using a palladium charcoal catalyst gave 1-amino-1-deoxy-D-erythro-pentulose oxalate (LXXXVII). This compound gave a deep purple colour with o-dinitrobenzene[77] in alkali.



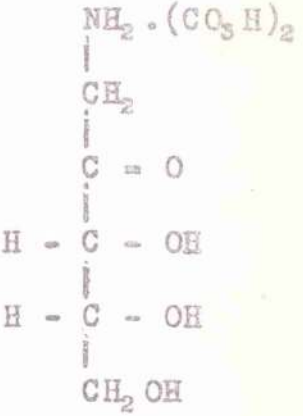
(LXXXIV)



(LXXXV)

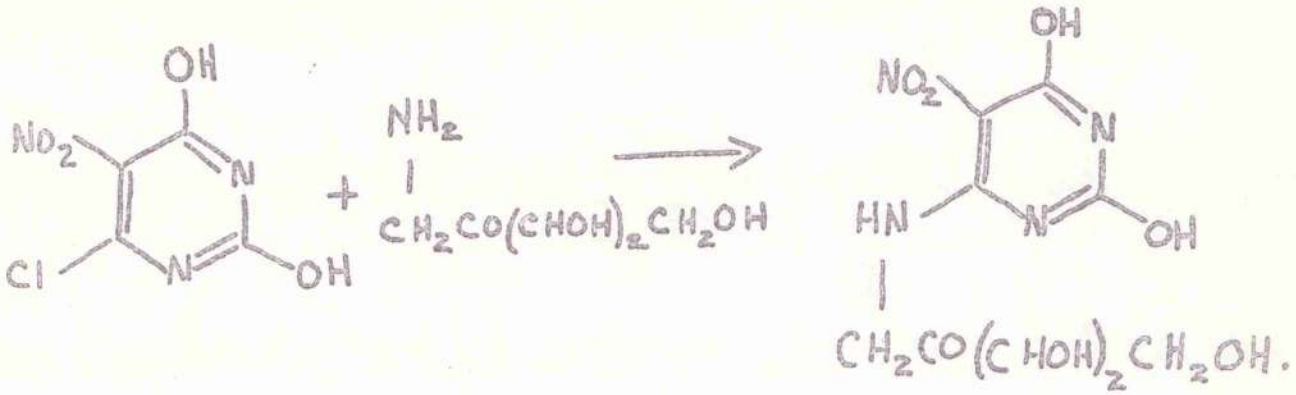


(LXXXVI)



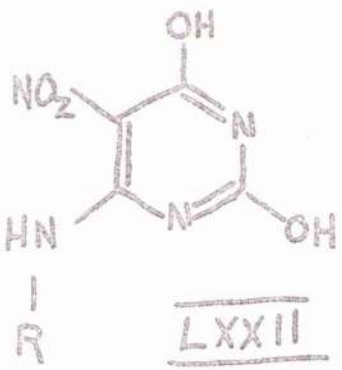
(LXXXVII)

The oxalate residue was removed from the 1-amino-1-deoxy-D-erythro-pentulose (LXVII) by treatment with sodium ethoxide. The free base was immediately condensed with 4-chloro-2,6-dihydroxy-5-nitropyrimidine (LXVI) giving 1-[2',6'-dihydroxy-5'-nitro-4'-pyrimidinylamino]-1-deoxy-D-erythro-pentulose (LXVIII).

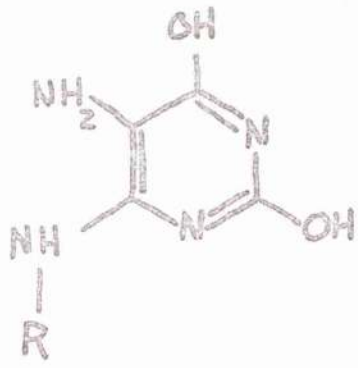


LXVI LXVII LXVIII

Sodium borohydride in alkaline solution reduced the carbonyl group as before to give 2,6-dihydroxy-5-nitro-4-D-ribitylaminopyrimidine (LXXII; R = D-ribityl).

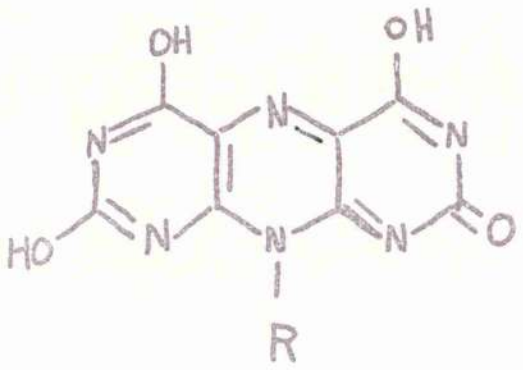


LXXII

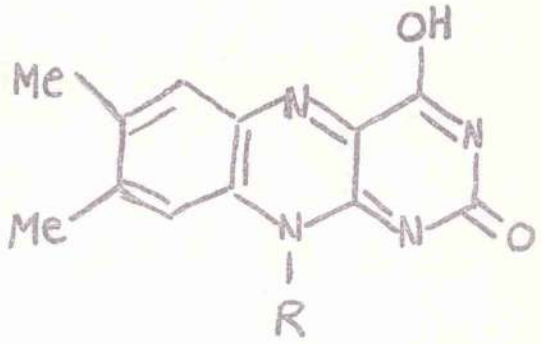


XXII

Sodium dithionite reduction of the nitro group and subsequent condensation at pH6 with alloxan gave 2,10-dihydro-4,6,8-trihydroxy-2-oxo-10-D-ribitylpyrimido[5,4-g]pteridine (LXXV; R = D-ribityl). Similar treatment of the 5-aminopyrimidine (XXII; R = D-ribityl) with the dimer of 3,4-dimethyl-o-benzoquinone[63] gave 6,7-dimethyl-9-D-ribityl-isoalloxazine (I; R = D-ribityl, "Riboflavin").

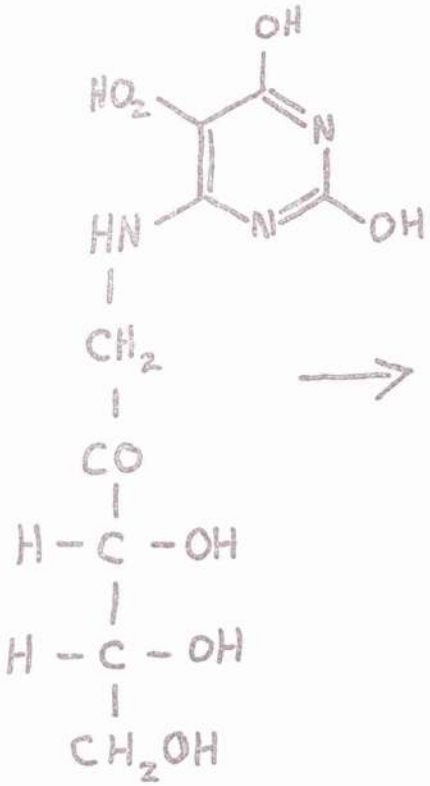


LXXV

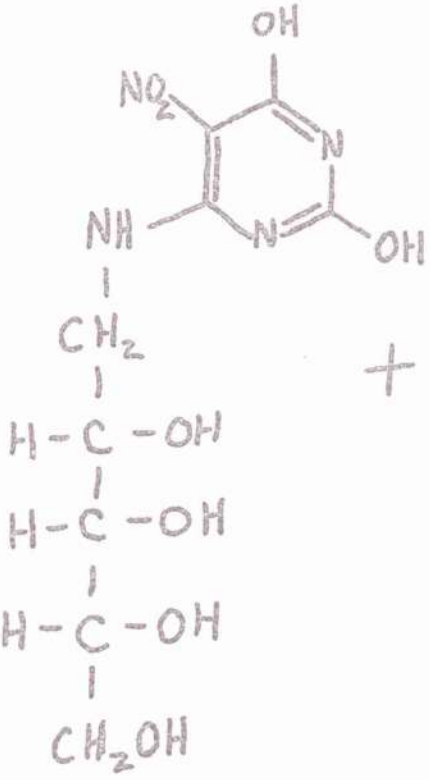


I

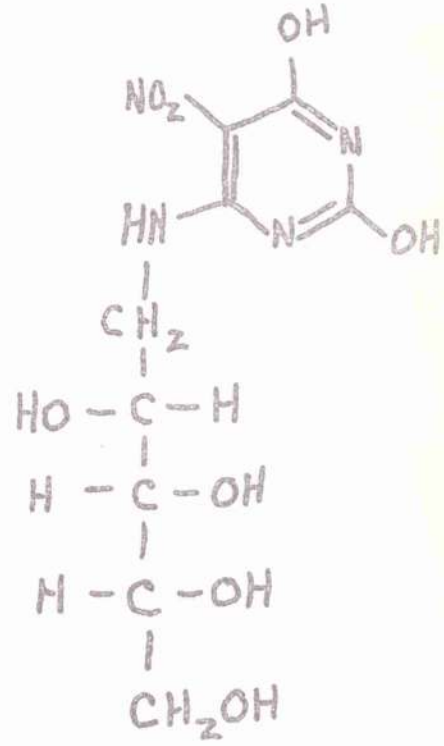
It has been assumed that the epimer obtained from the reduction of pyrimidine (LXVII) was 2,6-dihydroxy-5-nitro-4--D-ribitylpyrimidine (LXXII, R = D-ribityl). Proof that this reduction was stereospecific, was again determined by synthesising both epimers unambiguously.



LXVIII



LXXXVIII



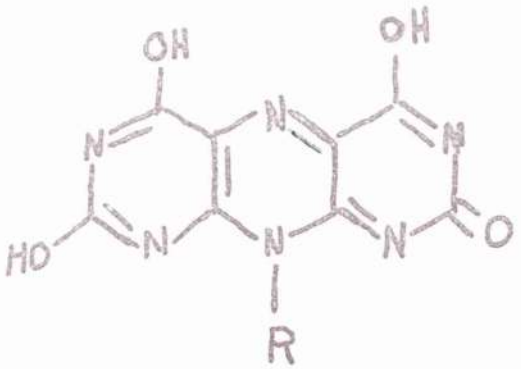
LXXXIX

By analogy with the above model experiments, it was expected that reduction of pyrimidine (LXVIII) with sodium borohydride, would give an epimeric mixture of a D-ribityl-aminopyrimidine (LXXXVIII) and a D-arabitylaminopyrimidine (LXXXIX).

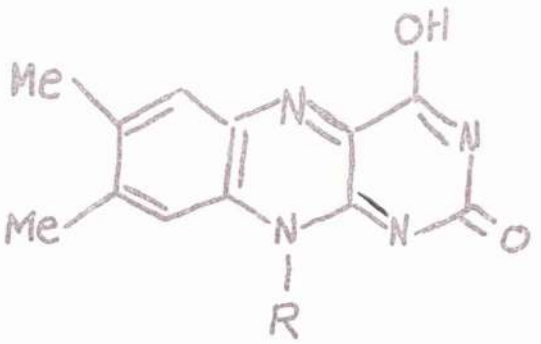
To determine the percentage of each epimer formed in the reduction, the unambiguous synthesis of each isomer was carried out as before.

D-Arabinose Series.

D-Arabinose oxime[89] was reduced as in the D-glucose series. Condensation of the resulting D-arabitylamine with 4-chloro-5-nitrouracil (LXVI) gave pyrimidine (LXXXIX). Reduction of the 5-nitro group as before and condensation with alloxan, gave 10-D-arabityl-2,10-dihydro-4,6,8-trihydroxy-2-oxopyrimido[5,4-g]pteridine (LXXV; R = D-arabityl). D-Arabitylflavin (I; R = D-arabityl) is well characterised in the literature.[90]



LXXV



I

D-Ribose Series

The physical constants of 2,6-dihydroxy-5-nitro-4-D-ribitylpyrimidine (LXXXVIII), 2,10-dihydro-4,6,8-trihydroxy-2--oxo-10-D-ribitylpyrimido[5,4-g]pteridine (LXXV; R = D-ribityl) and riboflavin (I; R = D-ribityl) have all been given by Cresswell and Wood.[54]

The following table summarises the physical constants of the products in the two series and compares them with those of the products formed from the reduced 1-[2',6'-dihydroxy-5'-nitro-4'-pyrimidinylamino]-1-deoxy-D-erythro-pentulose (LXVIII)

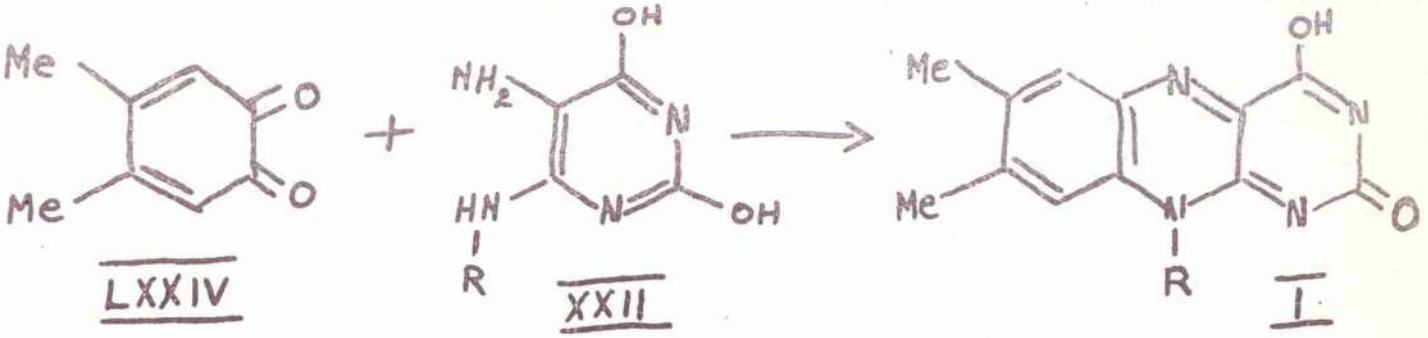
Series	Pyrimidines		Pyrimidopteridines		Flavins	
	m.p.	$[\alpha]_D$	m.p.	$[\alpha]_D$	m.p.	$[\alpha]_D$
Reduced D-erythro pentulose	202°	+5°	> 300°	-33°	288°	-116°
D-ribose	204°	+4.5°	> 300°	-26°	292°	-115°
D-arabinose	185°	-17.5°	> 300°	-76°	303°	+ 67°

The reduced pyrimidine (LXXXVIII) and its derivatives were found to be identical in all respects, melting point, specific rotation, ultraviolet and infrared spectra and paper chromatography in various solvents with 2,6-dihydroxy-5-nitro-4-D-ribitylamino-pyrimidine (LXXII; R = D-ribityl) and its derivatives.

Sodium borohydride in alkaline solution has therefore reduced 1-[2',6'-dihydroxy-5'-nitro-4'-pyrimidinylamino]-1-deoxy-D-

-erythropentulose stereospecifically to 2,6-dihydroxy-5-nitro-4-D-ribitylaminopyrimidine.

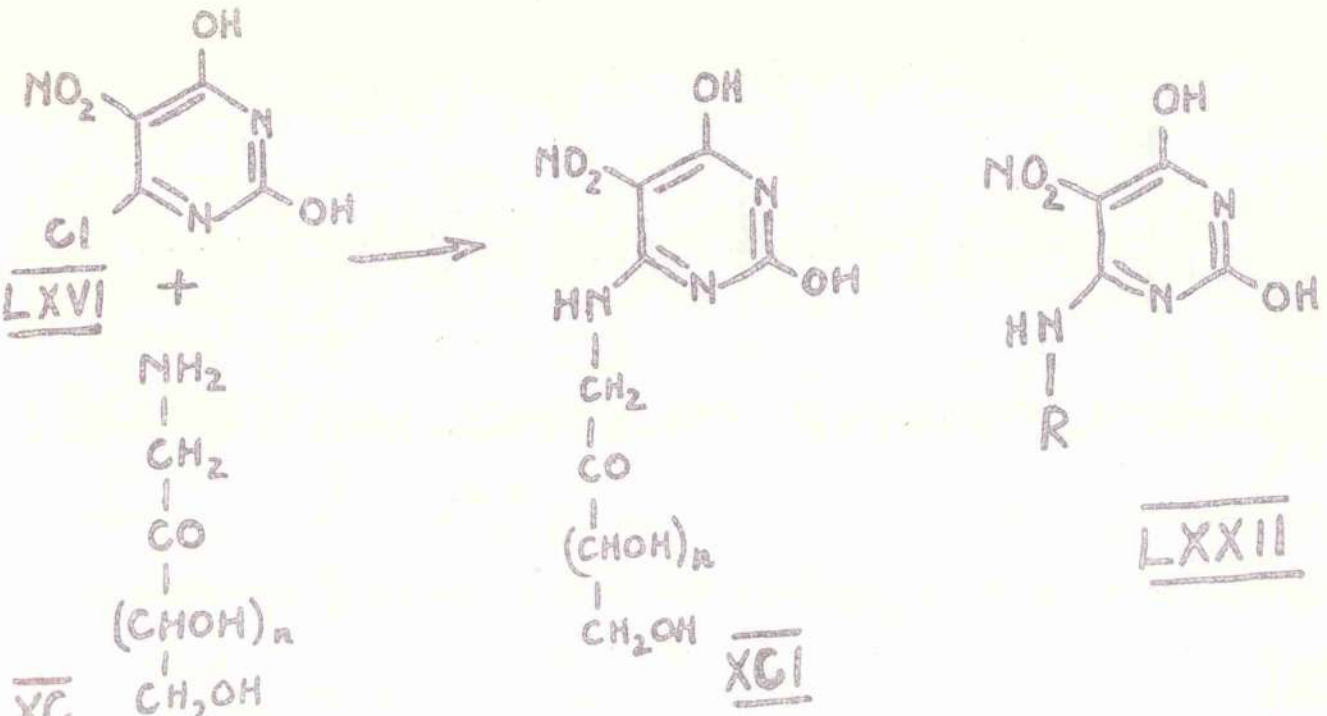
Condensation of 3,4-dimethyl-o-benzoquinone[83] (LXXIV) with 5-amino-4-D-ribitylaminouracil (XXII; R = D-ribityl) constitutes a new synthesis of riboflavin (I; R = D-ribityl). This work has been published recently in a joint paper.[91] The method can be extended to the preparation of all 6,7-dimethylflavins (I) by condensation with the appropriate 5-aminouracil derivative (XXII)



The uracil derivative may be prepared in two ways.

(a) Direct condensation of a sugar amine (LXXXIII) and 4-chloro--5-nitrouracil (LXVI) gave a 5-nitrouracil derivative (LXXII) which could be reduced to the 5-aminouracil derivative (XXII).

(b) Condensation of a 1-amino-1-deoxy-ketose (XC) and 4-chloro-5-nitrouracil (LXVI) gave a 1-pyrimidinylamino-1-deoxy-ketose derivative (XCI). It has been shown above that the carbonyl group in these compounds can be reduced stereospecifically to a 5-nitrouracil derivative (LXXII), followed by reduction of the nitro group to give a 5-aminouracil derivative (XXII).

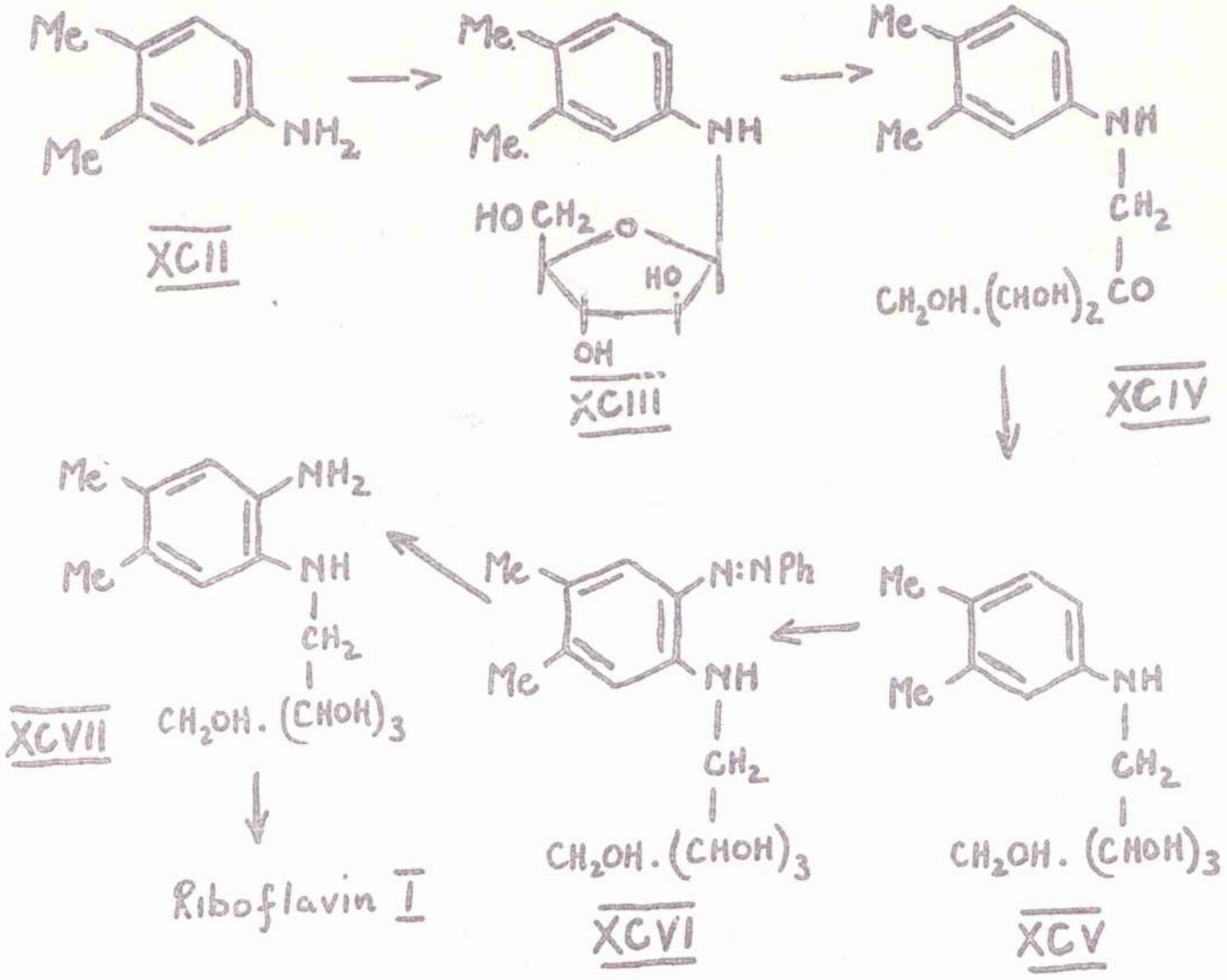


LXVI + XC → XCI → LXXII

Method (a) allows the preparation of all flavins of the 6,7-dimethyl type where as method (b) is limited to exactly half that number. This, of course, assumes that stereospecific reduction of the carbonyl group as described above, will always be possible.

Perhaps the most elegant of previous riboflavin syntheses is that by Weygand[73] which involved the use of the less expensive D-arabinose. This sugar was condensed with 3,4-xylidine (XCII) in the presence of small amounts of acid to give the D-arabinoside (XCIII) which underwent an Amadori rearrangement upon heating to 75° to form the D-erythropentulose derivative (XCIV). Catalytic hydrogenation in alkaline medium converted the ketose to 4,5-dimethyl--1-D-ribitylaminobenzene (XCV). The second amino group was introduced by coupling[92] with a diazonium salt to form an azo-dye (XCVI), which on reduction, either catalytically or chemically,

gave the diamine derivative (XCVII). Condensation[93] with alloxan in the presence of boric acid, gave riboflavin (I; R = D-ribityl).



The weakness of this method resides in the oxygen sensitivity of the required o-phenylenediamine derivative (XCVII) which is so pronounced that this intermediate is never isolated.

Another notable recent synthesis due to Cresswell and Wood[54], was outlined in the historical section. This involved a

novel preparation of 5-amino-4-D-ribitylaminouracil (XXII; R = D-ribityl). Condensation with the aldol of biacetyl gave a pteridine derivative which underwent intramolecular condensation on warming in acid to give riboflavin. The weakness of this method resides in the oxygen-sensitivity of the required diaminouracil derivative and in the use of D-ribose which is expensive.

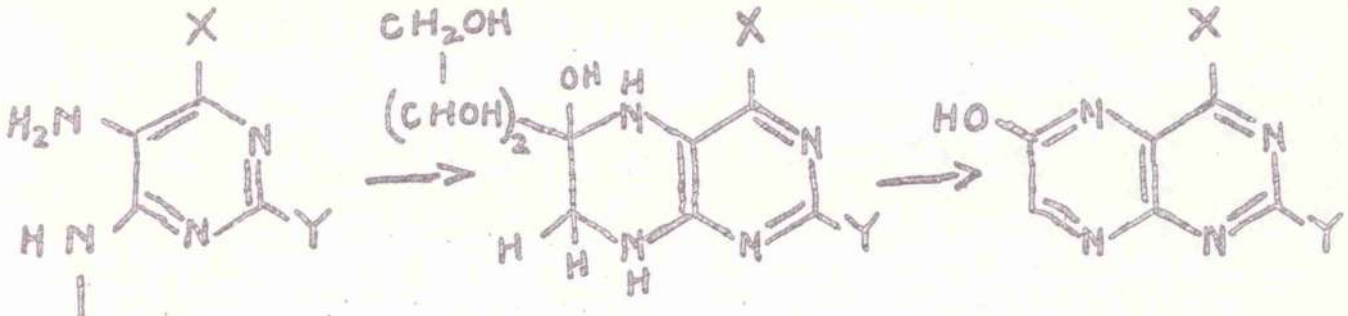
This latest synthesis of riboflavin combines the best points of both these earlier synthesis.

(1) D-Arabinose is used in preference to the more expensive D-ribose.

(2) Condensation between 4-chloro-5-nitrouracil and 1-amino-1-deoxy-D-erythropentulose is very efficient as in Cresswell's synthesis.

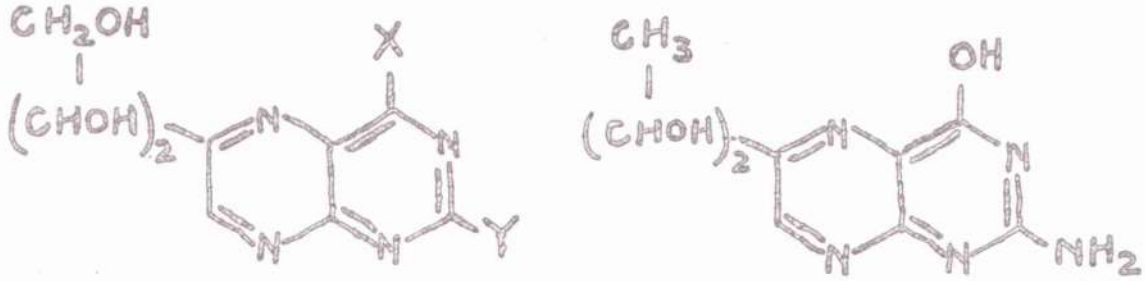
This method also makes use of 3,4-dimethyl-o-benzoquinone which condenses smoothly with the diaminouracil derivative at pH6.

The next step in Weygand's hypothesis[15] for the biosynthesis of xanthopterin involved intramolecular condensation of the rearranged 5-aminopyrimidine derivative (LXV) to give a pteridine derivative (XCVIII) which could then oxidise to a 6-hydroxypteridine (XCIX) with loss of the polyhydroxyalkyl side-chain.



LXV XCVIII XCIX

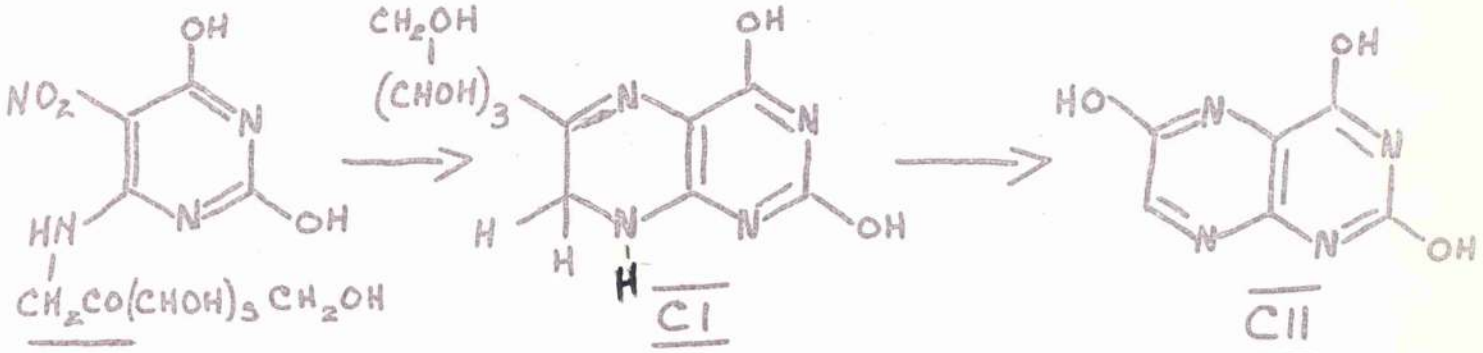
If, however, oxidation of pteridine (XCVIII) removes water instead from the 5-6 bond, then an unambiguous synthesis of biopterin-type compounds (C) from pyrimidine glycosides is possible.



C Biopterin V

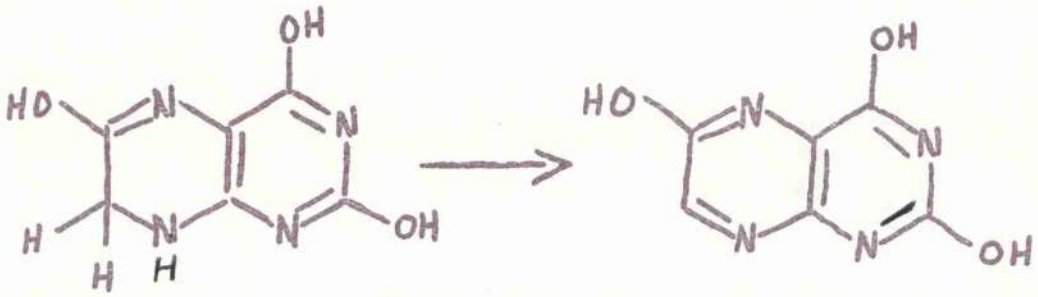
This may well be the biosynthetic route to these pteridines. It appears, therefore, that all the well-known natural pteridine groups - flavins, xanthopterin and its analogues, and biopterin type compounds could arise from a common intermediate, pyrimidine (LXV) which is produced by Amadori rearrangement of a pyrimidine glycoside which in its turn, can be obtained by degradation of a purine nucleoside.

Investigation of these proposed reactions was now attempted using 1-[2',6'-dihydroxy-5'-nitro-4'-pyrimidinylamino]-1-deoxy-D-fructose (LXX). Raney nickel reduction gave the dihydropteridine (CI) which proved unstable in air, but was characterised as its sodium salt. 2,4-Dihydroxy-7,8-dihydro-6-[D-arabo-tetrahydroxybutyl]pteridine (CI) gave 2,4,6-trihydroxypteridine (CII) on aerial oxidation in alkaline solution. This material was identical with an authentic sample of 2,4,6-trihydroxypteridine[94] in ultraviolet and infrared spectra and on paper chromatography in various solvents. No trace of the polyhydroxyalkylpteridine could be detected but other unidentified pteridines were formed in trace quantities.



LXX CI CII

During one experiment the sodium salt of 7,8-dihydro-2,4,6-trihydroxypteridine (CIII) separated from the reaction mixture. Further standing in alkaline solution converted this salt to the fully aromatic 2,4,6-trihydroxypteridine (CII).



CIII CII

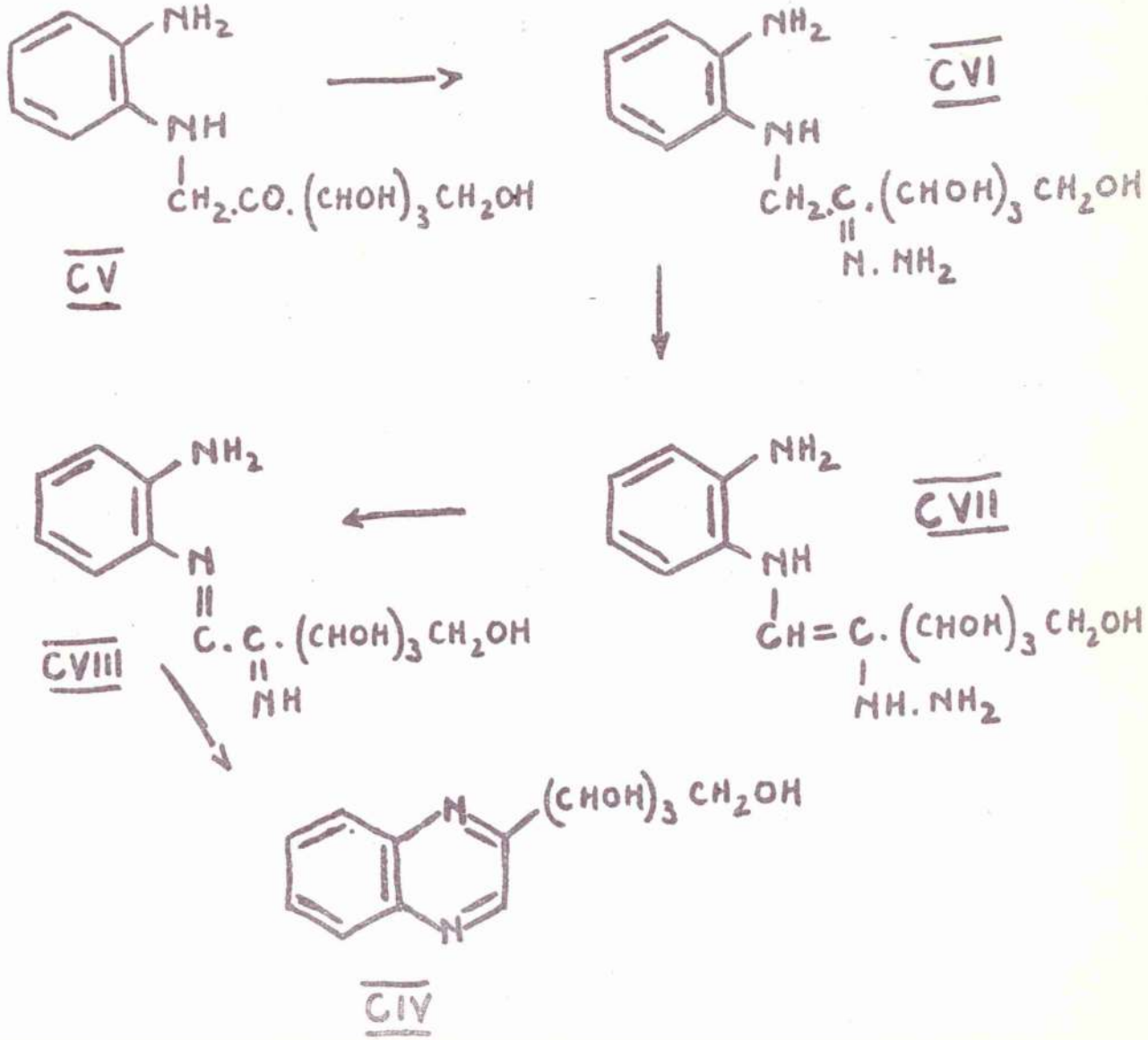
Oxidation of 2,4-dihydroxy-7,8-dihydro-6-[D-arabo-tetrahydroxybutyl]pteridine (CI) to give 2,4,6-trihydroxypteridine (CII) could also be carried out by cold alkaline potassium permanganate.

Although these results were rather disappointing so far as the synthesis of polyhydroxyalkylpteridines was concerned this series of reactions supports Weygand's hypothesis[15] and provides a reasonable biosynthetic route to xanthopterin, since 2,4,6-trihydroxypteridine (CII) is the 2-hydroxy analogue of xanthopterin (VIII).

CII VIII

This problem is at present being investigated in this Department, but will not be considered further in this Thesis.

An alternative approach to 6-polyhydroxyalkylpteridines was suggested by experiments in the literature which lead to the synthesis of quinoxalines. Weygand and Bergmann[98] have synthesised a 2-polyhydroxyalkylquinoxaline derivative (CIV) directly from an o-phenylenediamine derivative (CV) without involving a 1,2-dihydroquinoxaline.

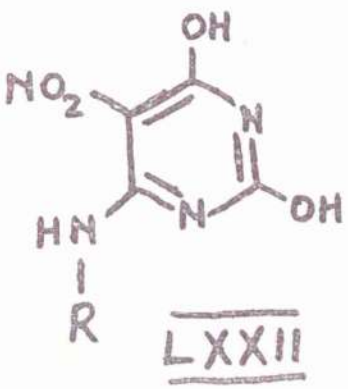


The hydrazone (CVI) rearranged in acetic acid solution to give finally (CVIII) which underwent intramolecular condensation to give a quinoxaline derivative (CIV) directly.

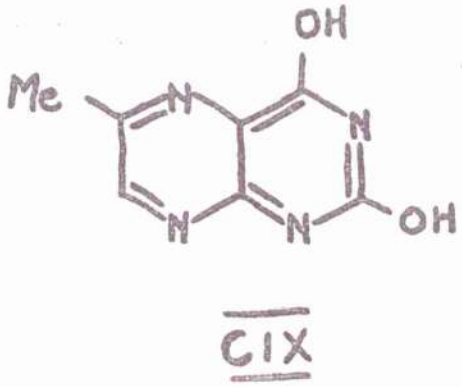
As the dihydropteridine (CI) obtained from the intramolecular condensation of 1-[2',6'-dihydroxy-5'-nitro-4'-pyrimidinyl amino]-1-deoxy-D-fructose (LXX) has been shown to be unstable, a reaction scheme of the above type, suggested itself. This method would by-pass oxidation of the labile dihydropteridine (CI) and should give the biopterin-type compound in one step.

It is interesting to note that rearrangement of the hydrazone derivative as described above, is a very elegant method for oxidation of the side-chain before intramolecular condensation and hence oxidation of the bicyclic product is not required.

To test this rearrangement of the side-chain in pyrimidine-pteridine transformations, it was decided to use a simple model. This involved preparation of 4-acetonylamino-2,6-dihydroxy-5-nitropyrimidine (LXXII; R = $CH_2.CO.CH_3$) which would lead to 2,4-dihydroxy-6-methylpteridine (CIX).

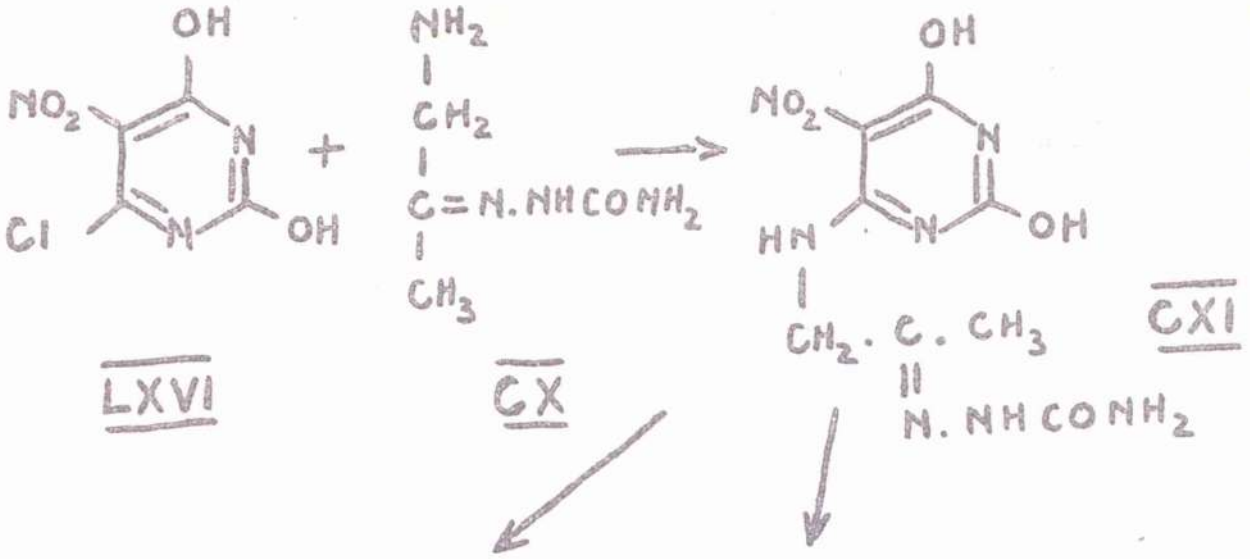


LXXII

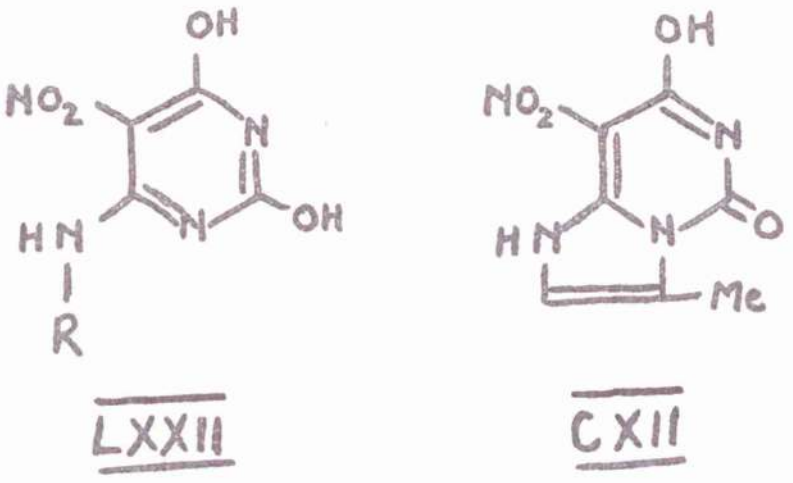


CIX

Condensation of 4-chloro-5-nitrouracil (LXVI) with aminoacetone[96] was unsatisfactory and so the condensation was carried out using the semicarbazone[97] of aminoacetone (CX), giving 4-acetonylamino-5-nitrouracil semicarbazone (CXI). This was hydrolysed to the parent pyrimidine (LXXII; R = $CH_2.CO.CH_3$) with N-hydrochloric acid at 37°. Hydrolysis at reflux temperature gave a different product, 4,5-dihydro-7-hydroxy-3-methyl-8-nitro-5-oxoimidazo[1,2-c]pyrimidine (CXII) whose occurrence will be discussed later in this section.



LXVI CX CXI



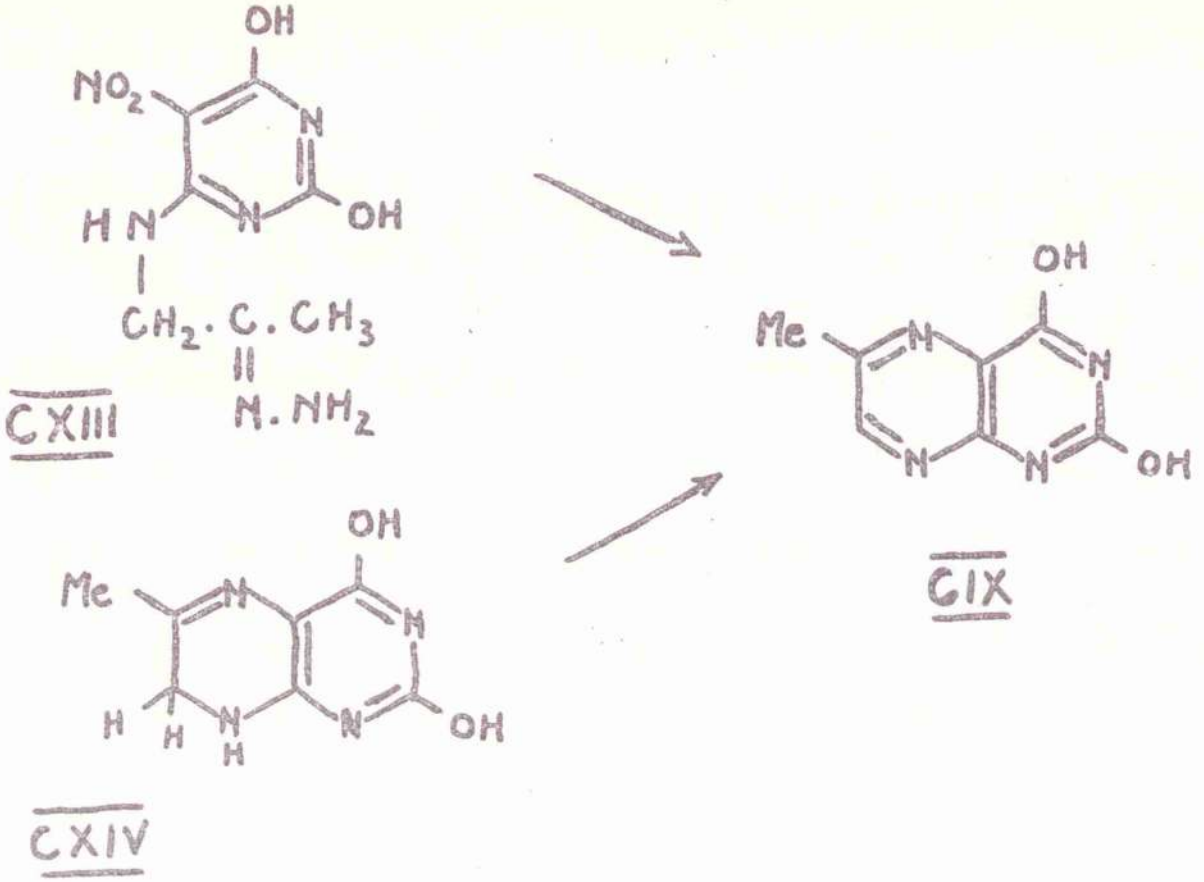
LXXII CXII

Since two products were obtained from acid hydrolysis, it was necessary to determine which one was the free methyl ketone.

The product from hydrolysis at 37° could be converted to the second product by refluxing in acid solution for a short time. An iodoform reaction on the product from hydrolysis at 37° gave 2,6-dihydroxy-5-nitro-4-pyrimidinylaminoacetic acid (LXXII, R = CH_2CO_2H) which on sodium borohydride reduction in alkaline solution gave 2,6-dihydroxy-5-nitro-4-2'-hydroxyethyl-aminopyrimidine (LXXII; R = $CH_2.CH_2OH$).

This latter material was identical in all respects with an authentic sample prepared by condensation between ethanolamine and 4-chloro-5-nitrouracil. It seems therefore that the product from hydrolysis at 37° is 4-acetonylamino-5-nitrouracil (LXXII, R = $CH_2.CO.CH_3$).

4-Acetonylamino-5-nitrouracil formed a hydrazone (CXIII) in the usual way. Reduction of the 5-nitro group of this compound with hydrogen over a Raney nickel catalyst and subsequent reflux with 2N-acetic acid gave 2,4-dihydroxy-6-methylpteridine (CIX), identical with the material made by oxidation of the corresponding 7,8-dihydropteridine (CXIV). This was prepared directly from 4-acetonylamino-5-nitrouracil (LXXII, R = $CH_2.CO.CH_3$) by reduction of the nitro group and subsequent condensation.

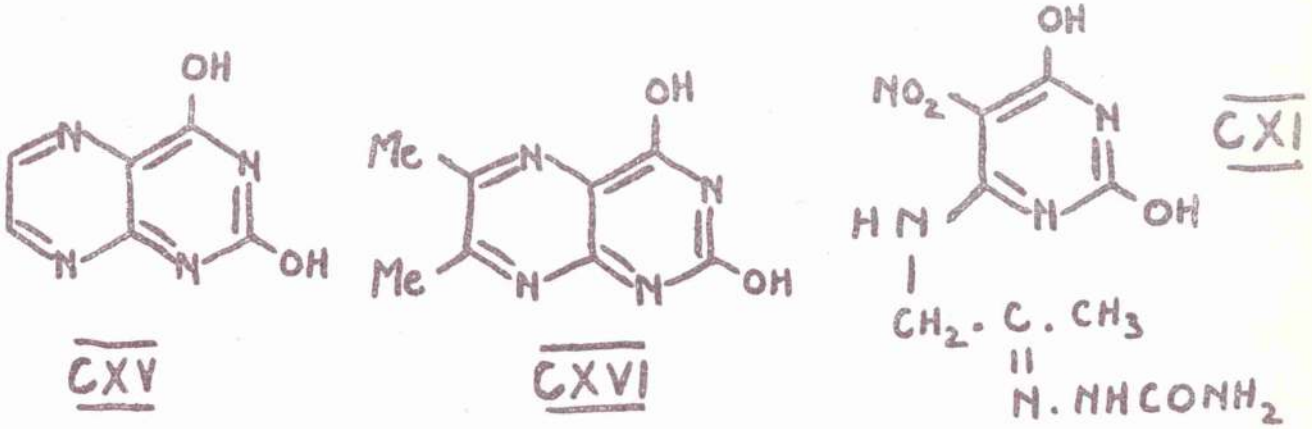


CXIII

CIX

CXIV

The ultra-violet spectrum of 2,4-dihydroxy-6-methylpteridine (CIX) was virtually identical with those of 2,4-dihydroxypteridine[98] (CXV) and its 6,7-dimethyl derivative[99] (CXVI) which were prepared by standard methods.



CXV

CXVI

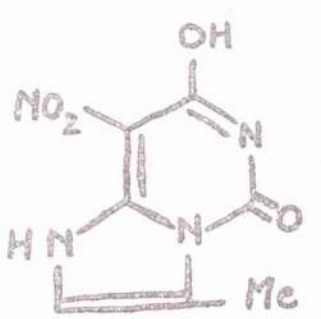
CXI

Reduction of the 5-nitro group of 4-acetonylamino-5-nitrouracil semicarbazone (CXI) with hydrogen over a Raney nickel catalyst and subsequent reflux with 2N-acetic acid gave 7,8-

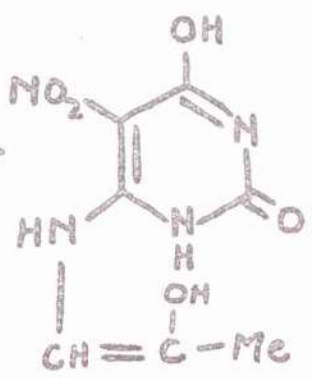
dihydro-2,4-dihydroxy-6-methylpteridine (CXIV), identical with material made from 4-acetonylamino-5-nitrouracil.

It is interesting to note that reduction and subsequent reflux of the semicarbazone gave the dihydropteridine and not the fully aromatic substance. There cannot be any rearrangement within the semicarbazide group such as that described for the hydrazone residue and so acid removes the group intact. The resulting 4-acetonylamino-5-aminouracil then condenses intramolecularly to give the 7,8-dihydropteridine derivative (CXIV).

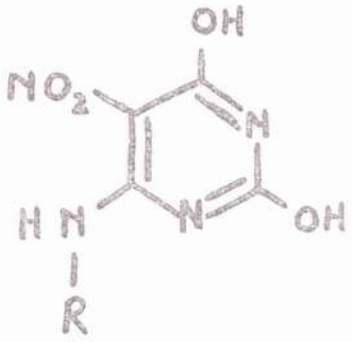
The other product from acid hydrolysis of the semicarbazone has been identified as 4,5-dihydro-7-hydroxy-3-methyl-8-nitro-5-oxoimidazo[1,2-c]pyrimidine (CXII).



CXII



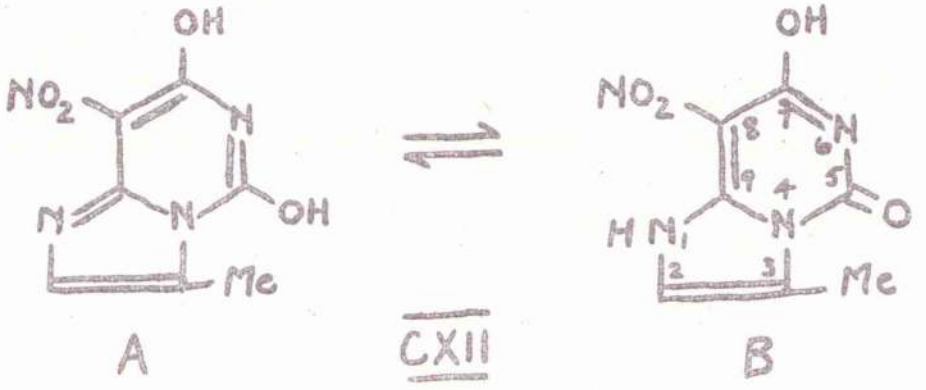
CXVII



LXXII

This is presumably formed by condensation between the carbonyl group of the acetonylamino side chain which can react in its enolic form (CXVII) and the pyrimidine ring nitrogen atom 3 to give a pyrimidine-imidazole fused ring-system. The ultraviolet spectrum of pyrimidine (CXII) was 20 mu higher than

a pyrimidine of type (LXXII) at both pH1 and 13. An increase in conjugation between the pyrimidine and imidazole rings probably accounts for this difference. The bicyclic system may therefore exist in the tautomeric form (A).



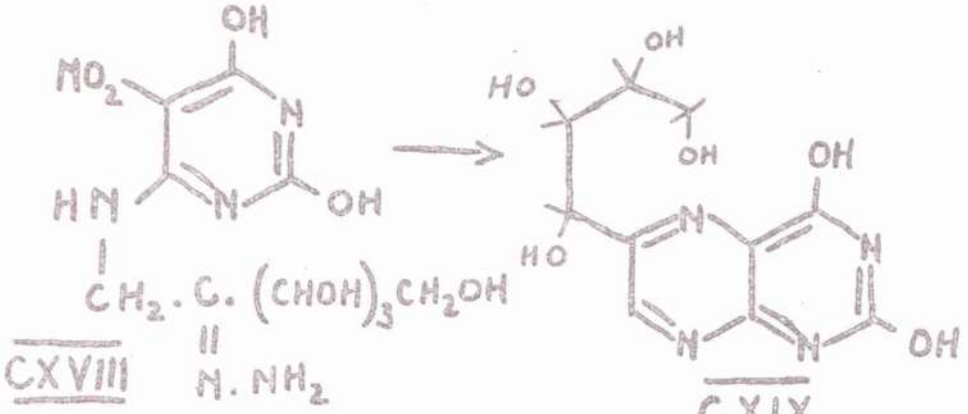
Chemical evidence for this structure (CXII A) came from the fact that when the 8-nitro group was reduced to an 8-amino group, compound (CXII) could not undergo an oxidative self-condensation or condense with alloxan in acid medium. This indicated that nitrogen atom 1 was tertiary.

These model experiments have shown that this approach to fully aromatic pteridines via hydrazone derivatives is reasonable, and synthesis of a 6-polyhydroxyalkylpteridine by this method was now attempted.

The hydrazone (CXVIII) of 1-[2',6'-dihydroxy-5'-nitro-4'-pyrimidinylamino]-1-deoxy-D-fructose was prepared in the usual way. Reduction of the 5'-nitro group and subsequent reflux with 2N-acetic acid gave a reaction mixture containing three compounds. Examination of paper chromatograms showed the main spot to be a fluorescent yellow; the others were present

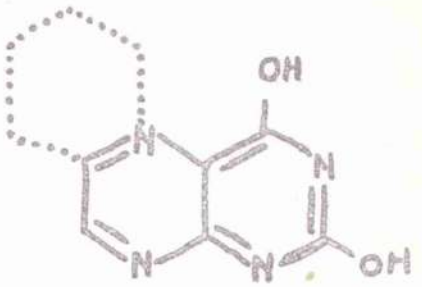
only in trace amounts. An orange solid was obtained from the reaction mixture. This was very soluble in water and had an ultraviolet spectrum which was completely new but bore a slight resemblence to that of the flavins.

The structure of this product has not been fully elucidated. However, if the polyhydroxyalkylpteridine (CXIX) had been formed then the terminal hydroxy group of the side-chain could condense with nitrogen atom 5 of the pteridine ring to give a tricyclic ring system with the skeleton (CXX).



CXVIII

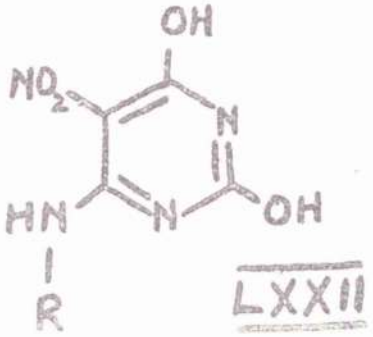
CXIX



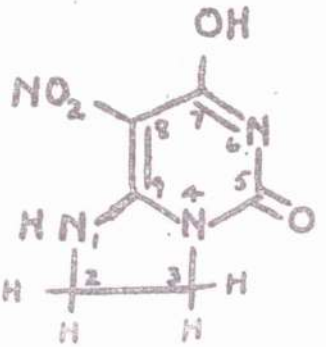
CXX

The feasibility of this type of cyclisation has been demonstrated by Lister[100] and by the following experiment.

On warming 2,6-dihydroxy-4-2'-hydroxyethylamino-5-nitropyrimidine (LXXII; R = $CH_2.CH_2OH$) in N-hydrochloric acid solution, 2,3,4,5-tetrahydro-7-hydroxy-8-nitro-5-oxoimidazo[1,2-a]pyrimidine (CXXI) was formed.



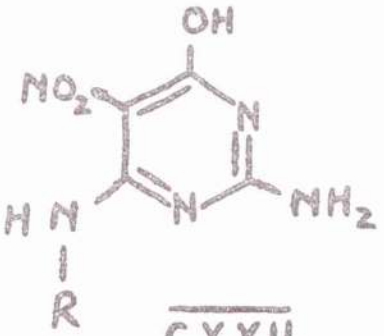
LXXII



CXXI

This is another case of intramolecular condensation between a ring nitrogen atom and an oxygen function of a side-chain. As in the case of the imidazopyrimidine (CXII), the product was a much more stable compound with a higher melting point than that of the parent pyrimidine (LXXII; R = CH_2CH_2OH). The ultraviolet spectrum of the imidazopyrimidine (CXXI) was however, similar to that of its parent. This was expected since this condensation has produced a fully reduced imidazole ring.

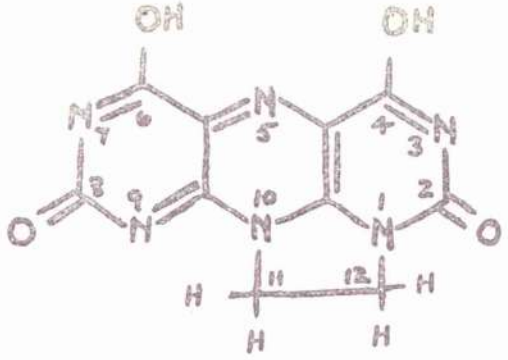
An attempt to bring about this type of condensation in the case of a 2-aminopyrimidine was unsuccessful. Thus, acid reflux of 2-amino-6-hydroxy-4-2'-hydroxyethylamino-5-nitropyrimidine (CXXII; R = CH_2CH_2OH) in N-hydrochloric acid only gave starting material.



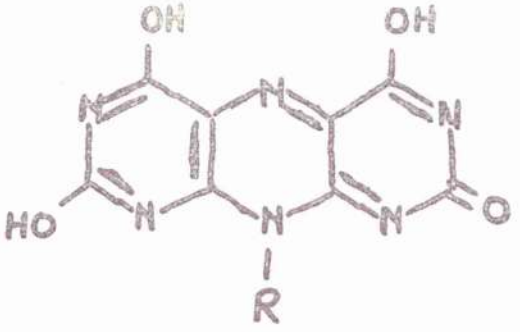
CXXII

This is possibly due to the fact that 2-aminopyrimidines are known[101] to exist in the 'amino' form where there is no proton on the adjacent ring nitrogen, whereas 2-hydroxypyrimidines exist predominantly in the 2-oxo form[102] in which the ring nitrogen does carry a proton:-

Since the imidazole ring of 2,3,4,5-tetrahydro-7-hydroxy-8-nitro-5-oxo-imidazo[1,2-c]pyrimidine (CXXI) is fully reduced, the nitrogen atom at position 1 is secondary, and so oxidative self-condensation and condensation with alloxan should occur on reduction of the 8-nitro group. The products of these reactions were different although both were bright orange crystalline solids The former has not been identified. The product from the alloxan condensation however, appears to have structure (CXXIII) analogous to those discussed earlier in this thesis, since it exhibits ultraviolet absorption characteristics of a pyrimido[5,4-g]pteridine of type (LXXV). Compound (CXXIII) was called 1,2,8,10,11,12-hexahydro-4,6-dihydroxy-2,8-dioxoimidazo[1,2;2,3-ij]pyrimido[5,4-g]pteridine.



CXXIII



LXXV

Table of Ultraviolet Absorption Spectra

5-Nitropyrimidines (LXXII)

R = CH_2CH_2OH	228 (23,000),	324 (13,400)	pH1
	216 (17,900),	333 (11,500)	pH13
R = $CH_2CO(CHOH)_3CH_2OH$	228 (18,300),	323 (11,100)	pH1
	220 (12,000),	337 (10,900)	pH13
R = D-sorbityl	228 (18,500),	322 (11,000)	pH1
	220 (13,700),	336 (14,300)	pH13
R = D-mannityl	228 (19,000),	324 (11,100)	pH1
	220 (13,900),	340 (14,500)	pH13
R = $CH_2CO(CHOH)_2CH_2OH$	228	323	pH1
	219	335	pH13
R = D-ribityl	228 (20,700),	323 (11,200)	pH1
	217 (13,900),	336 (14,000)	pH13
R = D-arabityl	228 (19,000),	322 (10,900)	pH1
	220 (12,100),	335 (13,400)	pH13
R = $CH_2C(Me)=N.NHCONH_2$	228 (18,600),	314 (12,800)	pH1
	224 (18,500),	338 (9,700)	pH13
R = CH_2COMe	228 (21,400),	314 (14,300)	pH1
	220 (11,500),	332 (12,700)	pH13
R = $CH_2C(Me)=N.NH_2$	228 (17,800),	316 (11,600)	pH1
	220 (13,800),	336 (12,400)	pH13
R = $CH_2C(:NOH)(CHOH)_3CH_2OH$	228 (17,800),	319 (12,400)	pH1
	220 (26,500),	337 (15,000)	pH13
R = $CH_2C(:N.NH_2)(CHOH)_3CH_2OH$	228 (14,300),	315 (11,300)	pH1
	224 (14,500),	336 (13,300)	pH13
(CXII)	224 (10,600),	268 (5,500), 360 (11,600)	pH1
	238 (12,000),	394 (9,700)	pH13
(CXXI)	228 (24,000),	322 (13,500)	pH1
	221 (15,800),	334 (16,400)	pH13

Pyrimido[5,4-g]pteridines

(LXXV)				
R = $CH_2.CH_2OH$	228(20,400),	278(9300),	422(20,800)	pH1
	231(29,100),	272(13,100),	436(31,400)	pH13
R = D-sorbityl	224(25,800),	280(9500),	422(18,400)	pH1
	230(32,100),	272(16,000),	438(25,800)	pH13
R = D-mannityl	230(22,100),	280(10,800),	422(22,300)	pH1
	231(26,500),	272(11,600),	438(27,600)	pH13
R = CH_2CHO	221(28,800),	276(15,200),	404(20,300)	pH1
	232(21,300),	272(10,000),	436(23,400)	pH13
R = D-ribityl	229(25,200),	280(9800),	422(22,200)	pH1
	230(23,400),	272(9900),	433(28,000)	pH13
R = D-arabityl	230(26,600),	280(11,900),	421(27,100)	pH1
	231(27,000),	272(11,900),	434(30,400)	pH13
(CXXIII)	226(19,700),	278(9500),	418(19,300)	pH1
	232(28,000),	274(12,000),	436(32,200)	pH13
Self-Condensation of CXXI	224	267	418	
	232	274	436	

Isoalloxazines (I)

R = D-sorbityl	222(27,500),	266(29,000),	374(10,000),	444(11,400)	pH1
	222(25,000),	270(28,100),	356(9900),	449(9700)	pH13
R = D-mannityl	223(34,300),	266(37,000),	374(12,600),	444(13,500)	pH1
	222(23,000),	270(26,700),	356(8900),	445(8500)	pH13
R = D-ribityl	223(35,500),	267(35,500),	376(10,700),	445(11,500)	pH1
	222(26,600),	270(31,700),	356(10,600),	447(10,600)	pH13

Other Pteridines

(CII)	222(9800), 258(8700), 364(4700)	pH1
	234(12,500), 274(10,300), 394(6100)	pH13
(CIX)	205(21,200), 230(17,600), 331(10,500)	pH1
	218(11,700), 254(16,500), 370(6100)	pH13
(CXV)	204(20,000), 228(19,400), 323(13,000)	pH1
	216(8800), 252(14,200), 366(4700)	pH13
(CXVI)	208(19,500), 324(10,800),	pH1
	216(11,100), 240(18,000), 346(9000)	pH13
(CXIV)	228(11,200), 267(15,400), 350(4500)	pH1
	226(23,000), 276(13,000), 318(6500)	pH13.
Cyclisation of (CXVIII)	204, 236, 281, 402	pH1
	219, 270, 410.	pH13

EXPERIMENTAL

PART I

2,4,6-Trihydroxypyrimidine('barbituric acid').[80] - Ethyl malonate (80 g.) and urea (30 g.) in absolute ethanol (250 ml.) were added to sodium ethoxide [sodium (11.5 g.) in absolute ethanol (250 ml.)] and the mixture was refluxed for 7 hr. in an oil bath at 100-110°. Hot water (500 ml. at 50°) was then added followed by 12N-hydrochloric acid solution (47 ml.). After stirring, the solution was filtered and left to stand overnight at 0°. The crystalline product was collected, washed with cold water (50 ml.) and oven-dried at 100-110° for 2 hr. to give off-white crystals (50 g., 78%), m.p. 240° (decomp.) [lit.,[80] m.p. 240°].

2,4,6-Trichloropyrimidine.[81] - 2,4,6-Trihydroxypyrimidine (50 g.) was added portionwise over 10 min. to a mixture of diethylaniline (95 ml.) and phosphoryl chloride (156 ml.), after which the whole was refluxed for 5 min., cooled, and poured on ice (800 g.). The mixture was extracted with ether (750 ml.) and the ether extract was dried over anhydrous sodium sulphate. The ether was removed by evaporation *in vacuo* and the oily residue was subjected to fractional distillation at reduced pressure, the fraction of b.p. 110° being collected to give the trichloropyrimidine (48 g., 66%) as a clear viscous oil with extreme vesicant properties.

4-Chloro-2,6-dihydroxypyrimidine (4-chlorouracil').[82] - 2,4,6-Trichloropyrimidine (48 g.) was refluxed for 16 hr. in a solution of sodium hydroxide (41.8 g.) in water (420 ml.). On

cooling the sodium salt of 4-chloro-2,6-dihydroxypyrimidine separated, and this was converted to the free pyrimidine by addition of 12-N hydrochloric acid solution (50 ml.). The product was collected, washed with water, dried over silica gel, to give the pyrimidine as a white powder (35.2 g.). Crystallisation from water gave needles (32.0 g., 84%), m.p. 300°.

4-Chloro-2,6-dihydroxy-5-nitropyrimidine.[54] - 4-Chloro-2,6-dihydroxypyrimidine (5 g.) was dissolved gradually in concentrated sulphuric acid (6 ml., 36N) at 0°. After cooling the mixture in a carbon dioxide-acetone bath, fuming nitric acid (d,1.5, 5.3 ml.) was added portionwise. The mixture was kept below 0° until solution was complete and then left at room temperature for 30 min. until solid precipitated, after which it was treated with ice (20 g.). The white product was collected, washed with ice-water (2 x 20 ml.), ether (2 x 20 ml.) and dried *in vacuo* over phosphorus pentoxide. Yield 3.5 g., 55%, m.p. 222° [lit.[54] 220-222°].

Glucosazone. - D-Glucose (20 g.), sodium acetate trihydrate (60 g.), and a saturated solution (50 ml.) of sodium metabisulphite were added to a solution of phenylhydrazine hydrochloride (40 g.) in water (400 ml.). The solution was filtered to remove tarry material and heated on a steam-bath until precipitation of the yellow osazone was complete. The solid was collected, washed with warm ethanol, until the washings

were colourless and then with ether and dried to give the product (30 g., 75%) m.p. 210°.

Isoglucosamine Acetate.[76] - Glucosazone (20 g.) was made into a paste with a mixture of glacial acetic acid (100 ml.), ethanol (50 ml.) and water (20 ml.) A palladium-barium sulphate[103] catalyst (5 g.) was added to the mixture which was shaken overnight under hydrogen at 3-5 atm. The catalyst was removed and the filtrate was concentrated in vacuo to an orange oil. Ethanol (100 ml.) was added and the mixture was left overnight at 0°. A white solid separated, and this was removed and washed with ethanol (2 x 20 ml.). Crystallisation from 90% ethanol gave colourless needles (8 g., 60%) m.p. 137° [lit.,[76] 137°].

1-[2',6'-Dihydroxy-5'-nitro-4'-pyrimidinylamino]-1-deoxy-D-fructose. - An ethanolic solution (50 ml.) of sodium (0.47 g., 2 eq.) was added to suspension of isoglucosamine acetate (5 g., 2 eq.) in ethanol (30 ml.) and the mixture was left at room temperature for 1 hr. An ethanolic solution (50 ml.) of 4-chloro-2,6-dihydroxy-5-nitropyrimidine (2 g., 1 eq.) was then added with stirring when a yellow solid precipitated almost immediately. After 30 min. the solid (2 g., 55%) was collected, quickly washed with ethanol (50 ml.) and ether (2 x 30 ml.), and dried over phosphorus pentoxide. This compound proved to be very hygroscopic and was characterised as its oxime, see below.

1-[2',6'-Dihydroxy-5'-nitro-4'-pyrimidinylamino]-1-deoxy-D-fructose oxime. - An ethanolic solution (10 ml.) of sodium (23 mg.) was added to a suspension of hydroxylamine hydrochloride (70 mg.) in ethanol (10 ml.) and the mixture was left for 30 min. An aqueous solution (10 ml.) of 1-[2',6'-dihydroxy-5'-nitro-4'-pyrimidinylamino]-1-deoxy-D-fructose (334 mg.) was then added and the mixture was refluxed for 1 hr. After leaving the solution overnight at 0°, a solid (300 mg.) separated. Concentration of the mother liquors yielded a further 50 mg. Recrystallisation from water gave the oxime as micro-needles (250 mg., 70%) m.p. 291° (Found: C,34.7; H,4.6; N,20.1. $C_{10}H_{15}N_5O_9$ requires C,34.4; H,4.3; N,20.0%) $[\alpha]_D$ - 49.8 (c,0.20 in 0.05N-NaOH).

D-Glucose Oxime[86]. - Hydroxylamine hydrochloride (15.4 g.) was dissolved in hot water (25 ml.). To this was added an equally warm solution of sodium (4.6 g.) in ethanol (60 ml.). On cooling, sodium chloride was filtered off and was washed with ethanol (2 x 20 ml.). Anhydrous D-glucose (36 g.) was dissolved in the filtrate and the solution was kept at 35-40° overnight. After 7 days in the refrigerator micro-needles (20 g. 51%), had separated, m.p. 135°C [lit.[86], 137°].

D-Sorbitylamine. - D-Glucose oxime (4.0 g.) in water (150 ml.) was shaken with platinum oxide (0.5 g.) under hydrogen until the theoretical volume (920 ml.) had been absorbed. The catalyst was removed, and the resulting solution of D-sorbitylamine

was used directly.

2,6-Dihydroxy-5-nitro-4-D-sorbitylaminopyrimidine. - (a) To the above solution of crude D-sorbitylamine was added 4-chloro-2,6-dihydroxy-5-nitropyrimidine (1.6 g.) in ethanol (150 ml.). The mixture was heated on a steam bath for 15 min., then brought to pH5 with 2N-hydrochloric acid solution and chilled overnight. A little 4-amino-2,6-dihydroxy-5-nitropyrimidine[104] separated and was removed. The pH of the filtrate was adjusted to 10 with ammonium formate buffer, and the solution run on to a column of an anion exchange resin (Amberlite CG 400; formate form) which had previously been prepared by washing the resin with ammonium formate buffer (0.1M with respect to formic acid) at pH10. The column was washed thoroughly with ammonium formate buffer at pH10 and then with buffer of pH7 (0.1M with respect to formic acid); a bright yellow pyrimidine was eluted which showed ultraviolet absorption maxima at 218 and 334 mu at pH13 but is as yet unidentified.

Elution with buffer of pH4 (0.1M with respect to ammonia) eluted a second pyrimidine in a narrow band. Concentration *in vacuo* of the eluate gave 2,6-dihydroxy-5-nitro-4-D-sorbitylamino-pyrimidine as a white solid (1.6 g., 55%). Recrystallisation from water gave needles, m.p. 224-225° (Found: C,34.3; H,4.9; N,16.0. $C_{10}H_{16}N_4O_9.H_2O$ requires C,34.0; H,5.1; N,15.8%), $[\alpha]_D$ + 17.5° (c,0.20 in 0.05N-NaOH).

(b) 1-[2',6'-Dihydroxy-5'-nitro-4'-pyrimidinylamino]-1-deoxy-D-fructose (1.2 g.) was dissolved in 0.2N-sodium hydroxide solution and an aqueous solution (10 ml.) of sodium borohydride (0.15 g.) was added with stirring. After the initial reaction was complete, the yellow solution was stirred for a further 2 hr. at room temperature. After neutralisation with formic acid which destroyed the excess borohydride, the pH of the solution was adjusted to 9 with ammonium hydroxide solution and the solution was chromatographed as above. The nitro-pyrimidine was eluted from the column in pH range 4.5-4.0 and these fractions were bulked and concentrated to 20 ml. A white powder (800 mg., 66%) was precipitated by addition of ethanol (60 ml.). Crystallisation from water yielded the pyrimidine as micro-needles, m.p. 225°, $[\alpha]_D$ + 15° (c,0.18 in 0.05 N-NaOH).

Samples from methods (a) and (b) were identical in all respects.

6,7-Dimethyl-9-D-sorbitylisoalloxazine. - 2,6-Dihydroxy--5-nitro-4-D-sorbitylaminopyrimidine (400 mg.) was dissolved in 0.1N-sodium hydroxide solution (25 ml.) and the nitro group was reduced using solid sodium dithionite. When the reduction was complete, the pH of the solution was adjusted to 5 by 2N-hydrochloric acid solution. An alcoholic solution (10 ml.) of the dimer of 3,4-dimethyl-o-benzoquinone[83] (320 mg.) was added. The mixture

was heated for 30 min. at 90° and was finally made strongly acid by the addition of more 2N-hydrochloric acid solution. On cooling, pure 6,7-dimethyl-9-D-sorbitylisoalloxazine separated as orange needles (115 mg., 23%), m.p. 275° [lit.[84], 272°] (Found: C, 52.1; H, 5.5; N, 13.4. $C_{18}H_{22}N_4O_7 . 0.5H_2O$ requires C, 52.1; H, 5.6; N, 13.5%), $[\alpha]_D$ = - 45° (c, 0.18 in 0.05N-NaOH) (lit.[34], - 47.7°).

Samples of this flavin, identical in all respects, were obtained using starting material prepared by either method (a) or (b) above.

6,7-Dimethyl-9-D-sorbitylisoalloxazine penta-acetate. - 6,7-Dimethyl-9-D-sorbitylisoalloxazine (100 mg.) was heated in pyridine (40 ml.) with acetic anhydride (40 ml.) for 5 min. The mixture was cooled and diluted with chloroform (60 ml.). The organic layer was shaken with 2N-hydrochloric acid solution until acidic to Congo Red indicator paper and then washed with water (4 x 50 ml.). The chloroform was removed *in vacuo* leaving an orange residue which crystallised from aqueous ethanol as needles, m.p. 238° (lit.,[84] 237°) (Found: C, 54.5; H, 5.9. $C_{28}H_{32}N_6O_{12} . C_2H_6O$ requires C, 54.4; H, 5.8%).

2,10-Dihydro-4,6,8-trihydroxy-2-oxo-10-D-sorbitylpyrimido [5,4-g]pteridine. - (a) 2,6-Dihydroxy-5-nitro-4-D-sorbitylamino-pyrimidine (400 mg.) was dissolved in 0.1N-sodium hydroxide solution and the nitro group was reduced in the usual way using solid sodium dithionite. When the reduction was complete, the

pH was adjusted to 3.5 by addition of 2N-hydrochloric acid solution. Alloxan (190 mg., 1.1 eq.) was added immediately, the mixture was refluxed for 2 hr. and left overnight. A yellow solid separated (320 mg., 65%). Recrystallisation from N-hydrochloric acid solution gave the pyrimidopteridine as bright yellow needles, m.p. > 325° (Found: C, 37.6; H, 4.4; N, 18.4. $C_{14}H_{16}N_6O_9.2H_2O$ requires C, 37.5; H, 4.5; N, 18.7%), $[\alpha]_D$ + 61° (c, 0.28 in 0.05N-NaOH).

Samples of this pyrimidopteridine, identical in all respects, were obtained using starting material prepared by either method (a) or (b) above

(b) 2,6-Dihydroxy-5-nitro-4-D-sorbitylaminopyrimidine (250 mg.) was reduced in alkaline solution as above. On adjustment of the solution pH to 2, the reaction mixture was refluxed for 2 hr. giving a yellow solution from which separated the pyrimidopteridine as a yellow solid (100 mg., 65%), identical with that prepared in (a) above.

10-Formylmethyl-2,10-dihydro-4,6,8-trihydroxy-2-oxo-pyrimido[5,4-g]pteridine. - 2,10-Dihydro-4,6,8-trihydroxy-2-oxo-10-D-sorbitylpyrimido[5,4-g]pteridine (180 mg.) was dissolved in water (50 ml.) and an aqueous solution (10 ml.) of anhydrous sodium periodate (650 mg.-6 eq.) was added. Yellow crystals separated during 2 days at room temperature. Recrystallisation from water gave the pyrimidopteridine as bright yellow plates,

m.p.>325° (Found: C,38.9; H,2.3; N,27.3. $C_{10}H_6N_6O_5.H_2O$ requires C,38.9; H,2.6; N,27.3%).

2,6-Dihydroxy-4,2'-hydroxyethylamino-5-nitropyrimidine.[54] - (a) To an ethanolic solution (20 ml.) of ethanolamine (250 mg., 2 eq.) was added an ethanolic solution (30 ml.) of 4-chloro-2,6-dihydroxy-5-nitropyrimidine (400 mg., 1 eq.). The mixture was heated on the steam bath for 10 min., then cooled. The white solid which separated was recrystallised from aqueous ethanol as needles (400 mg.,95%) m.p. 219° [lit.[54], 217-219°].

(b) 4-Acetonylamino-2,6-dihydroxy-5-nitropyrimidine (200 mg.) were dissolved in water (5 ml.) and an iodine-potassium iodide solution (1 ml.) (iodine (125 g.) and potassium iodide (250 g.) per litre) was added. N-Sodium hydroxide was added till the reaction mixture became yellow. On warming to 60°, iodoform separated leaving a solution of the sodium salt of 2,6-dihydroxy-5-nitro-4-pyrimidinyl-amino acetic acid which was reduced overnight using sodium boro-hydride (70 mg.). Neutralisation with 2N-hydrochloric acid and concentration *in vacuo* of the reaction mixture gave a white solid (100 mg., 55%). Recrystallisation from aqueous ethanol gave needles, m.p. 219°.

This material was identical in all respects with the material prepared by method (a).

2,10-Dihydro-4,6,8-trihydroxy-10,2'-hydroxyethyl-2-oxo pyrimido[5,4-g]pteridine. - (a) 2,6-Dihydroxy-4,2'-hydroxyethyl-amino-5-nitropyrimidine (216 mg.) was dissolved in 0.1N-sodium

hydroxide solution and the nitro group was reduced using solid sodium dithionite. When the reduction was complete, the pH was adjusted to 3.5 by the addition of 2N-hydrochloric acid solution. Alloxan (182 mg. 1.1 eq.) was added immediately, the mixture was refluxed for 2 hr. and left overnight. A yellow solid separated (200 mg. 56%) and was recrystallised from water to give the pyrimidopteridine as bright yellow needles, m.p. > 325°. (Found: C,38.0; H,3.7; N,26.5. $C_{10}H_8N_6O_5.H_2O$ requires C,38.7; H,3.3; N,27.1%).

(b) 10-Formylmethyl-2,10-dihydro-4,6,8-trihydroxy-2-oxopyrimido [5,4-g]pteridine (60 mg.) was dissolved in 0.2N-sodium hydroxide solution (10 ml.) and an aqueous solution (5 ml.) of sodium borohydride (15 mg.) was added. The mixture was left overnight, and then 2N-hydrochloric acid solution was added to give pH3. A yellow solid separated and was recrystallised from water, to give the pyrimidopteridine (36 mg., 59%) as yellow needles, m.p. > 325°.

This compound proved identical in infrared, ultraviolet spectra and on paper chromatography in various solvents to a sample prepared by method (a).

D-Mannose Oxime.[87] Hydroxylamine hydrochloride (7.1 g.) was dissolved in hot water (3.5 ml.). To this was added an equally warm solution of sodium (2.3 g.) in ethanol (40 ml.). On cooling, sodium chloride was removed and washed with ethanol (2 x 10 ml.). D-Mannose (10 g.) in water (7 ml.) was added to the filtrate and the mixture was heated at 70° for 90 min. On

cooling the oxime separated. Recrystallisation from water gave needles (10 g. 90%) m.p. 182° [lit.[97], 184°].

D-Mannitylamine. - D-Mannose oxime (2.5 g.) in water (150 ml.) was shaken with hydrogen and platinum oxide (0.5 g.) until the theoretical volume (580 ml.) had been absorbed. The catalyst was removed, and the resulting solution of D-mannitylamine was used directly.

2,6-Dihydroxy-5-nitro-4-D-mannitylaminopyrimidine. - To the above solution of crude D-mannitylamine was added 4-chloro-2,6-dihydroxy-5-nitropyrimidine (1.25 g.) in ethanol (100 ml.). On adjustment to pH 5, the solution was left at room temperature for 24 hr. 4-Amino-2,6-dihydroxy-5-nitropyrimidine (100 mg.) separated and was removed. The pH of the filtrate was raised to 10 and put on to an anion exchange resin column (CG 400; formate form) similarly prepared as for the D-sorbitylaminopyrimidine. Buffer eluted 2,6-dihydroxy-5-nitro-4-D-mannitylaminopyrimidine at pH4 and concentration of the eluate gave a white solid. Recrystallisation from water gave needles, m.p. 247° (Found: C,33.1; H,5.4; N,15.3. $C_{10}H_{16}N_4O_9.1.5H_2O$ requires C,33.0; H,5.3; N,15.4%), $[\alpha]_D$ - 2.5° (c,0.22 in 0.05N-NaOH).

6,7-Dimethyl-9-D-mannitylisoalloxazine. - 2,6-Dihydroxy-4-D-mannitylamino-5-nitropyrimidine (400 mg.) was dissolved in 0.1N-sodium hydroxide solution (25 ml.) and was reduced using

solid sodium dithionite. When the reduction was complete, the pH of the solution was adjusted to 5 with 2N-hydrochloric acid solution. An ethanolic solution (10 ml.) of the dimer of 3,4-dimethyl-o-benzoquinone (320 mg.)[83] was added. The mixture was heated for 30 min. at 90° and finally made strongly acid by the addition of more 2N-hydrochloric acid solution. On cooling, 6,7-dimethyl-9-D-mannitylisoalloxazine separated (240 mg., 49%) as orange needles, m.p. 285° (Found: C, 52.4; H, 5.2; N, 13.5. $C_{18}H_{22}N_4O_7.0.5H_2O$ requires C, 52.1; H, 5.6; N, 13.5%), $[\alpha]_D$ + 61° (c, 0.18 in 0.05N-NaOH). This material was quite distinct from the analogous D-sorbityl compound.

2,10-Dihydro-4,6,8-trihydroxy-10-D-mannityl-2-oxopyrimido [5,4-g]pteridine. - 2,6-Dihydroxy-5-nitro-6-D-mannitylamino pyrimidine (400 mg.) was dissolved in 0.1N-sodium hydroxide solution and the nitro group was reduced using solid sodium dithionite. When the reduction was complete, the pH was adjusted to 3.5 by the addition of 2N-hydrochloric acid solution. Alloxan (190 mg. - 1.1 eq.) was immediately added, the mixture refluxed for 2 hr. and left overnight. A yellow solid separated (300 mg. 61%). Recrystallisation from N-hydrochloric acid solution gave the pyrimidopteridine as bright yellow needles. m.p. $>$ 325° (Found: C, 36.4; H, 4.6; N, 17.8. $C_{14}H_{10}N_6O_9.3H_2O$ requires C, 36.0; H, 4.8; N, 18.0%) $[\alpha]_D$ + 17° (c, 0.33 in 0.05'-NaOH).

This material was also quite distinct from the analogues D-sorbityl compound.

N-Benzyl-D-arabinosylamine. - D-Arabinose (5 g.) and benzylamine (4 g.) were refluxed together in ethanol (50 ml.) for 15 min. The mixture was left at 0° for 2 days. A white solid (5 g.; 60%) separated. This was collected by filtration and washed with ethanol (30 ml.) and then with ether (30 ml.). Recrystallisation from ethanol gave the glycosylamine as fine colourless needles, m.p. 118° $[\alpha]_D$ - 4°) (Found: C,60.3; H,6.9; N,5.7. $C_{12}H_{17}NO_4$ requires C,60.3; H,7.1; N,5.9%).

1-Benzylamino-1-deoxy-D-erythropentulose Oxalate. - The above glycosylamine (5 g.) was dissolved in dry dioxan (70 ml.) and cooled, and a cold solution of anhydrous oxalic acid (1.8 g.) in dioxan (50 ml.) was added. A colourless oil formed which was probably the glycosylamine hemioxalate. On gentle heating to 40-50° this oil slowly dissolved and a jelly precipitated causing the whole reaction to become solid. Water (10 ml.) was added and the jelly dissolved on gentle heating. On standing overnight, a white crystalline solid separated which was identified as benzylamine hemi-oxalate[105] by m.p. 180°, mixed m.p. and infrared spectrum. Dioxan was removed from the filtrate *in vacuo* leaving a yellow solid. Recrystallisation from ethanol (50 ml.) gave the

oxalate (3 g., 40%) as needles, m.p. 145° $[\alpha]_D$ + 5.2° (Found: C, 50.8; H, 5.8; N, 4.2. $C_{12}H_{17}NO_6.(CO_2H)_2$ requires C, 51.3; H, 5.8; N, 4.2%).

This compound gave a purple colouration with o-dinitrobenzene[77] in alkaline solution.

1-Amino-1-deoxy-D-erythropentulose Oxalate ('Isoribosamine Oxalate'). - 1-Benzylamino-1-deoxy-D-erythropentulose oxalate (2.8 g.) was dissolved in ethanol (50 ml.) and added to a suspension of previously reduced palladium-charcoal catalyst (1 g.) in ethanol (20 ml.). The mixture was hydrogenated until 1 mole of hydrogen had been absorbed (220 ml.). The catalyst was removed and the filtrate was taken to a colourless gum *in vacuo*. Crystallisation of this gum from methanol-ethanol gave the oxalate (1 g., 50%) as needles, m.p. 70° $[\alpha]_D$ -1° (Found: C, 34.75; H, 5.9; N, 5.9. $C_7H_{15}NO_8$ requires C, 34.9; H, 6.2; N, 5.8%).

This compound gave a purple colouration with o-dinitrobenzene[77] in alkaline solution.

1-[2',6'-Dihydroxy-5'-nitro-4'-pyrimidinylamino]-1-deoxy-D-erythropentulose. - 1-Benzylamino-1-deoxy-D-erythropentulose oxalate (3.0 g.) was dissolved in ethanol-water (50 ml., 4:1) and reduced as above. After removal of the catalyst, an ethanolic solution (40 ml.) of sodium (0.4 g., 2 eq.) was added to the filtrate, which was refiltered to remove sodium oxalate. An ethanolic solution (30 ml.) of 4-chloro-5-nitrouracil (830 mg.-0.5 eq.)

was added and the reaction mixture warmed for 15 min. Ethanol was removed *in vacuo* and the pH of the aqueous residue was raised to 10 and run on to an anion exchange resin (Amberlite CG 400; formate form) which had been prepared in the usual way. Elution with buffer of pH4 gave a pyrimidine as a narrow band. Concentration *in vacuo* of the eluate gave the required *5-nitropyrimidine* as a jelly which was used directly in the experiment below: The amount of pyrimidine (600 mg.) present was determined by ultraviolet intensity. This compound gave a deep red colouration with *o*-dinitrobenzene[77] in alkaline solution.

2,6-Dihydroxy-5-nitro-4-D-ribitylaminopyrimidine. - Crude 1-[2',6'-dihydroxy-5'-nitro-4'-pyrimidinylamino]-1-deoxy-D-*erythro*pentulose (600 mg.) as a jelly from the above experiment was dissolved using 2N-sodium hydroxide (2 ml.). An aqueous solution (5 ml.) of sodium borohydride (0.08 g.) was added, and the mixture stirred for 15 min. and left for 2 hr. After neutralisation with formic acid which destroyed the excess borohydride, the pH of the solution was raised to 10 with ammonium hydroxide. The solution was chromatographed as above. Buffer of pH4 eluted a pyrimidine whose fractions were bulked and concentrated to 10 ml. *in vacuo*. Addition of ethanol (50 ml.) precipitated the nitropyrimidine (300 mg.) as a white solid, m.p. 202° [lit.[54] 204°]. $[\alpha]_D$ + 5° (,0.21 in 0.05N-NaOH). This material was identical in all respects to an authentic sample.

2,10-Dihydro-4,6,8-trihydroxy-2-oxo-10-D-ribitylpyrimido [5,4-g]pteridine. - 2,6-Dihydroxy-5-nitro-4-D-ribitylamino-pyrimidine (400 mg.) was dissolved in 0.1N-sodium hydroxide solution and the nitro group was reduced using solid sodium dithionite. When the reduction was complete, the pH was adjusted to 3.5 by the addition of 2N-hydrochloric acid solution. Alloxan (190 mg. - 1.1 eq.) was immediately added, the mixture refluxed for 2 hr. and left overnight. A yellow solid separated (300 mg. 63%). Recrystallisation from N-hydrochloric acid solution gave the pyrimidopteridine as bright yellow needles, m.p. > 325° [lit.[106], 325°] $[\alpha]_D$ - 33° (c, 0.20 in 0.05N-NaOH). This material was identical in all respects with an authentic sample.

6,7-Dimethyl-9-D-ribitylisoalloxazine ('Riboflavin'). - 2,6-Dihydroxy-5-nitro-4-ribitylaminopyrimidine (200 mg.) was dissolved in 0.1N-sodium hydroxide (20 ml.) and was reduced using solid sodium dithionite. When the reduction was complete, the pH of the solution was adjusted to 5 with 2N-hydrochloric acid solution. An ethanolic solution (10 ml.) of the dimer of 3,4-dimethyl-o-benzoquinone[83] (180 mg.) was added. The mixture was heated for 30 min. at 90° and finally made strongly acid by addition of more 2N-hydrochloric acid solution. On cooling, 6,7-dimethyl-9-D-ribitylisoalloxazine separated (90 mg., 29 %) as orange needles, mp. 288° [lit.[84] 292°] $[\alpha]_D$ -116° (c, 0.53 in 0.10 N-NaOH). This material was identical in all respects with an

authentic sample.

D-Arabinose Oxime.[89] - Sodium ethoxide (1.16 g. of sodium in 25 ml. of ethanol) was added to a solution of dry hydroxylamine hydrochloride (3.6 g.) in water (10 ml.) and the mixture was allowed to stand overnight. After removal, by filtration, of sodium chloride, the filtrate was heated to 70° and D-arabinose (5 g.) was added. The mixture was heated for 30 min. and then allowed to stand overnight at room temperature. On reducing the volume *in vacuo*, D-arabinose oxime separated. Recrystallisation from aqueous ethanol gave white needles (5 g., 90%) m.p. 139° [lit.[89] m.p. 139°].

D-Arabitylamine. - PtO_2 (0.5 g.) was suspended in ethanol (100 ml.) and reduced with hydrogen. A solution of D-arabinose oxime (2 g.) in water (50 ml.) was then added, and the whole hydrogenated until the theoretical uptake of hydrogen was obtained (500 ml.). After removal of the catalyst the solution of D-arabitylamine was used directly as described below.

4-D-Arabitylamino-2,6-dihydroxy-5-nitropyrimidine. - The amine solution from the above experiment was treated with an ethanolic solution (50 ml.) of 4-chloro-2,6-dihydroxy-5-nitropyrimidine (1.15 g.). The resulting mixture was allowed to stand overnight at room temperature and a small amount of 4-amino-2,6-dihydroxy-5-nitropyrimidine[103] (100 mg.) which separated was removed by filtration. Concentration of the filtrate to 20 ml.

in vacuo and addition of ethanol (100 ml.) gave a white precipitate of *nitropyrimidine* (1.5 g., 78%) which crystallised from aqueous ethanol as white needles, m.p. 185° $[\alpha]_D$ - 17.5° [c,0.22 in 0.05 N-NaOH](Found: C,35.2; H,4.5; N,18.0. $C_9H_{14}N_4O_8$ requires C,35.3; H,4.6; N,18.3%).

This material was different from the corresponding D-ribityl pyrimidine.

10-D-*Arabityl*-2,10-*dihydro*-2-*oxo*-4,6,8-*trihydroxypyrimido*[5,4-g]pteridine. - D-Arabityl-2,6-dihydroxy-5-nitropyrimidine (250 mg.) was dissolved in water (10 ml.) and was treated with N-sodium hydroxide solution (5 ml.). Sufficient solid sodium dithionite was added to reduce the nitro group and resulting colourless solution was taken to pH4 with 2N-hydrochloric acid solution. Alloxan (0.13 g.) was added to the cooled solution and the mixture was refluxed for 1 hr. and left overnight. Yellow needles of the *pyrimidopteridine* separated (200 mg., 67%) which were recrystallised from N-hydrochloric acid, m.p. > 325° $[\alpha]_D$ - 76° (c,0.20 in 0.05N-NaOH) (Found: C,41.6; H,3.8; N,21.2. $C_{13}H_{14}N_6O_8.0.5H_2O$ requires C,41.6; H,3.7; N,21.0%).

2,4-*Dihydroxy*-7,8-*dihydro*-6-[D-arabo-*tetrahydroxybutyl*]*pteridine*. - 1-[2',6'-Dihydroxy-5'-nitro-4'-pyrimidinylamino]-1-deoxy-D-fructose (1 g.) was dissolved in water (10 ml.) and Raney nickel sludge (approx. 0.5 g.) was added. The mixture was

hydrogenated overnight when the theoretical volume of hydrogen (200 ml.) was absorbed. A grey-white material separated and this was dissolved using 2N-sodium hydroxide solution (2 ml.). The catalyst was removed and left overnight at 0°. A solid separated (400 mg., 45%) and was purified by warming in water (10 ml.) which contained a trace of sodium dithionite. The compound proved to be the mono-sodium salt of the dihydropteridine (Found: C,39.4; H,4.7. $C_{10}H_{13}N_4O_6Na$ requires C,39.0; H,4.2%). The dihydropteridine itself was unstable in air.

2,4,6-Trihydroxypteridine.[94] - (a) To 5,6-diamino-2,4-dihydroxypyrimidine hemisulphate (6 g.) dissolved in 78% w/w sulphuric acid solution (72 ml.) at 90° was added ethyl glyoxylate hemiacetal[107] (6.6 g.). After 2 min. at 90°, water (300 ml.) was added and the mixture left at 0°. The crystals and potassium carbonate (8.5 g.) were dissolved in boiling water (100 ml.). The potassium salt was filtered from the chilled solution, dissolved in boiling water, brought to pH4 with citric acid and recrystallised from water giving 2,4,6-trihydroxypteridine (50%) m.p.360-380°(dec.).

(b) 1-[2',6'-Dihydroxy-5'-nitro-4'-pyrimidinylamino]-1-deoxy-D-fructose (1 g.) was dissolved in water (10 ml.) and reduced as in the previous experiment. The dihydropteridine was dissolved as before and left at room temperature for 3 days during which oxygen was continuously bubbled through the solution. A yellow colour

formed and gradually intensified and on acidification with 2N-hydrochloric acid solution yielded an orange solid (300 mg.) Recrystallisation and boiling with charcoal gave 2,4,6-trihydroxypteridine which was identical to an authentic sample (see above) in infrared and ultraviolet spectra and on paper chromatography in various solvents.

Phthalimidoacetone.[96] - Potassium phthalimide (100 g.) was refluxed in dry xylene (100 ml.) with chloroacetone (100 ml.) in an oil bath at 120-130°. After 1 hr. a light brown syrup resulted. The xylene and excess chloroacetone were removed in vacuo. The resulting oil solidified on cooling and was crystallised from boiling water (6 l.) to give the product (70 g., 50%), m.p. 117° [lit., 117°].

Aminoacetone Hydrochloride. - Phthalimidoacetone (100 g.) was heated with 20% hydrochloric acid solution (700 ml.) under reflux. After 1 hr. the mixture became homogeneous, and on further boiling phthalic acid began to separate. After 4 hr. the reaction mixture was allowed to cool, the phthalic acid was removed and the filtrate was taken to dryness in vacuo, at 40-45° The resulting syrup was dissolved in twice its volume of warm absolute alcohol. A small amount of ammonium chloride which separated was removed. Excess ether was added and on standing aminoacetone hydrochloride (25 g., 47%) separated as plates, which were washed with ether and dried over phosphorus pentoxide.

Aminoacetone Semicarbazone Hydrochloride.[97] - Aminoacetone hydrochloride (38 g.) was dissolved in ethanol (250 ml.) and an aqueous solution (100 ml.) of semicarbazide hydrochloride (48 g.) was added. After 2 hr. at 0° the white solid (53 g., 95%) which separated was filtered off and was washed with ethanol. Recrystallisation from aqueous ethanol gave needles, m.p. 212° [lit.[97], 212°].

4-Acetonylamino-2,6-dihydroxy-5-nitropyrimidine semicarbazone. - Aminoacetone hydrochloride semicarbazone (4.62 g. - 2 eq.) was suspended in an ethanolic solution (40 ml.) of sodium (0.64 g. - 2eq.) and left at room temperature for 30 min. To this mixture was added an ethanolic solution (60 ml.) of 4-chloro-2,6-dihydroxy-5-nitropyrimidine (2.66 - 1 eq.). Precipitation occurred almost immediately and the mixture was well stirred. Water (20 ml.) was added and the yellow solid was filtered off. Recrystallisation from water gave the semicarbazone as needles (4.0 g., 96%) m.p. > 300° (Found: C, 32.5; H, 4.4; N, 33.6. $C_8H_{11}N_7O_5 . 0.5H_2O$ requires C, 32.6; H, 4.1; N, 33.4%).

4-Acetonylamino-2,6-dihydroxy-5-nitropyrimidine. - 4-Acetonylamino-2,6-dihydroxy-5-nitropyrimidine semicarbazone (1 g.) suspended in N-hydrochloric acid solution (50 ml.) was kept at 37° for 3 days. On cooling overnight at 0°, a solid separated (300 mg.). Concentration of the mother liquors gave a further 300 mg. (Total 76%). Recrystallisation from water gave the

pyrimidine as prisms, m.p. 273° (Found: C,36.8; H,3.7; N,24.2. $C_7H_8N_4O_5$ requires C,36.8; H,3.5; N,24.5%).

4,5-Dihydro-7-hydroxy-3-methyl-8-nitro-5-oxo-imidazo [1,2-c]pyrimidine. - (a) 4-Acetonylamino-2,6-dihydroxy-5-nitropyrimidine semicarbazone (2 g.) was refluxed in 2N-hydrochloric acid solution (50 ml.). On cooling a yellow solid separated. Recrystallisation from water gave the product as fine needles (1.2 g., 80%), m.p. 265° (Found: C,39.8; H,3.2; N,26.8. $C_7H_6N_4O_4$ requires C,40.0; H,2.9; N,26.7%).

(b) 4-Acetonylamino-2,6-dihydroxy-5-nitropyrimidine (100 mg.) was refluxed in 2N-hydrochloric acid solution (10 ml.). On cooling a yellow solid separated which was recrystallised from water as fine needles (60 mg., 64%), m.p. 265°. The product was identical with the material prepared in method (a) in all respects.

Hydrazine Salt of 4-Acetonylamino-2,6-dihydroxy-5-nitropyrimidinehydrazone. - 4-Acetonylamino-2,6-dihydroxy-5-nitropyrimidine (400 mg.) was suspended in ethanol (60 ml.) and excess hydrazine (0.15 ml.) was added. On heating a gum formed which slowly dissolved. On cooling a white solid separated (400 mg.; 83%). Recrystallisation from water gave the hydrazone salt as micro needles. m.p. > 300° (Found: C, 30.5; H,4.8; N,40.2. $C_7H_{10}N_6O_4.N_2H_4$ requires C,30.6; H,5.1; N,40.8%).

7,8-Dihydro-2,4-Dihydroxy-6-methylpteridine. - (a) Acetonylamino-2,6-dihydroxy-5-nitropyrimidine (300 mg.) was suspended in water (25 ml.) and hydrogenated overnight with Raney nickel sludge (approx. 0.5 g.). The theoretical uptake was absorbed (90 ml.). The solution was taken to pH13 with 2N-sodium hydroxide solution and warmed. The catalyst was removed and a crystalline sodium salt separated on cooling the filtrate. Neutralisation with 2N-hydrochloric acid solution gave a jelly which on heating gave a yellow solid. Recrystallisation from water gave the dihydropteridine needles (160 mg., 67%) m.p. > 300° (Found: C,46.8; H,4.5; N,31.0. $C_7H_8N_4O_2$ requires C,46.7; H,4.8; N,31.1%).

(b) 4-Acetonylamino-2,6-dihydroxy-5-nitropyrimidine semicarbazone (400 mg.) was added to a suspension of Raney nickel (approx. 0.5 g.) in water (50 ml.) and the mixture was hydrogenated until 3 moles of hydrogen had been absorbed. The catalyst was removed and the filtrate was made 2N with respect to acetic acid and refluxed for 1 hr. The water and acetic acid were removed *in vacuo* leaving a yellowish oil. Crystallisation from water gave the dihydropteridine as yellow needles (100 mg., 40%). This material was identical with the product obtained from method (a).

2,4-Dihydroxy-6-methylpteridine. - (a) 7,8-Dihydro-2,4-dihydroxy-6-methylpteridine (120 mg.) was dissolved in 0.1N-sodium

hydroxide solution. 0.2M-potassium permanganate solution (2.2 ml.) was added giving a suspension of manganese dioxide in a faint green solution. The manganese dioxide was filtered off and the green colour destroyed by the addition of a trace of sodium dithionite. On acidification a white solid separated which recrystallised from water to give the pteridine as colourless needles (80 mg. 67%), m.p. 300° (Found: C,47.0; H,3.1; N,31.7. $C_7H_6N_4O_2$ requires C,47.2; H,3.4; N,31.5%).

(b) The hydrazine salt of 4-acetonylamino-2,6-dihydroxy-5-nitro-pyrimidine hydrazine (200 mg.) was added to a suspension of Raney nickel (approx. 0.5 g.) in water (25 ml.) and the mixture was hydrogenated until the uptake of hydrogen was complete (24 hr.). The catalyst was removed and the filtrate was made 2N with respect to acetic acid and refluxed for 1 hr. The water and acetic acid were removed *in vacuo* leaving a light brown gum which crystallised from a little aqueous ethanol. Recrystallisation from water gave the pteridine as colourless needles similar in m.p., ultraviolet and infrared spectra, and chromatography in various solvents with the material obtained from method (a).

Hydrazine Salt of 1-[2',6'-Dihydroxy-5'-nitro-4'-pyrimidinylamino]-1-deoxy-D-fructose Hydrazone. - 1-[2',6'-Dihydroxy-5'-nitro-4'-pyrimidinylamino]-1-deoxy-D-fructose (500 mg.) was suspended in ethanol (50 ml.) and excess hydrazine

(0.15 ml.) was added. The mixture was refluxed for 1 hr. and left overnight at room temperature. A yellowish solid (400 mg., 75%) separated which could not be purified by crystallisation. m.p.>300°. Satisfactory analytical data could not be obtained but ultraviolet and infrared spectral analysis indicated that the correct compound had been prepared. Paper chromatography showed one spot in several solvents.

Cyclisation of the Hydrazine Salt of 1-[2',6'-Dihydroxy-5'-nitro-4'-pyrimidinylamino]-1-deoxy-D-fructose Hydrazine. - Hydrazine salt of 1-[2',6'-dihydroxy-5'-nitro-4'-pyrimidinylamino]-1-deoxy-D-fructose hydrazone (400 mg.) was dissolved in water (30 ml.). Raney nickel (approx. 0.5 g.) was added and the mixture hydrogenated until uptake was complete. After the catalyst was removed, the filtrate was made 2N with respect to acetic acid and was refluxed for 30 min. The solution developed an orange colouration and removal of the water and acetic acid in vacuo gave an orange solid (150 mg.). Purification by crystallisation was not possible.

2,3,4,5-Tetrahydro-7-hydroxy-8-nitro-5-oxoimidazo[1,2-c]pyrimidine. - 2,6-Dihydroxy-4-2'-hydroxyethylamino-5-nitropyrimidine (2 g.) was refluxed in N-hydrochloric acid (200 ml.) for 15 min. On chilling, a white solid separated. Recrystallisation from water gave the imidazopyrimidine as needles (1.4 g., 70%) m.p.

275°. (Found: C,33.45; H,3.7; N,25.5. $C_6H_6N_4O_4.H_2O$ requires C,33.3; H,3.7; N,25.9%). This material was entirely different from the parent compound.

1,2,8,10,11,12-Hexahydro-4,6-dihydro-2,8-dioxoimidazo[1,2,3-ij]pyrimido[5,4-g]pteridine. - 2,3,4,5-Tetrahydro-7-hydroxy-8-nitro-5-oxoimidazo-[1,2-c]pyrimidine (500 mg.) was dissolved in 0.1N-sodium hydroxide solution and the nitro group was reduced using solid sodium dithionite. When the reduction was complete, the pH of the solution was adjusted to 3 by the addition of 2N-hydrochloric acid solution. Alloxan (400 mg.) was immediately added, the mixture was refluxed for 1 hr. and left overnight. A yellow solid separated (600 mg., 92%). Recrystallisation from N-hydrochloric acid solution gave yellow needles, m.p. > 325° (Found: C,42.6; H,3.25; N,29.2. $C_{10}H_8N_6O_4.0.5H_2O$ requires C,42.2; H,3.15; N,29.7%).

Oxidative Self-condensation of 8-Amino-2,3,4,5-tetrahydro-7-hydroxy-5-oxoimidazo[1,2-c]pyrimidine. - 2,3,4,5-Tetrahydro-7-hydroxy-8-nitro-5-oxoimidazo[1,2-c]pyrimidine (500 mg.) was dissolved in 0.1N-sodium hydroxide solution and the nitro group was reduced as above. The solution pH was adjusted to 1 with 2N-hydrochloric acid and the mixture refluxed for 2 hr. and left overnight. An orange solid separated (300 mg.) and was recrystallised from water as orange needles, m.p. > 325° (Found: C,39.7;

H,3.5; N,26.25%).

This material was different from the product prepared from the alloxan condensation, but no structure fitted the analytical data.

Paper Chromatography

Chromatograms were developed by the ascending technique the solvents being (A), butan-1-ol-5N acetic acid (7:3), (B), 3% aqueous ammonium chloride, (C) propanol-1-ol-water - conc. ammonia (d,0.88) (40:20:1) and were viewed in ultraviolet light of wave-lengths 254 and 365 mu.

Absorption Spectra

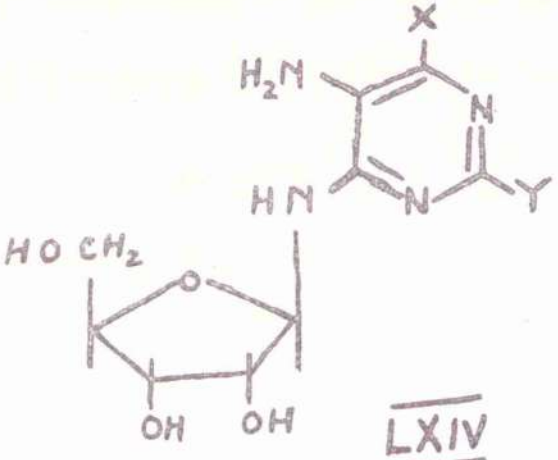
Infrared spectra were determined with a Grubb Parsons infrared spectrophotometer on Nujol mulls and KCl discs.

Ultraviolet spectra were determined with a Unicam SP600 spectrophotometer on aqueous solutions of standard pH.

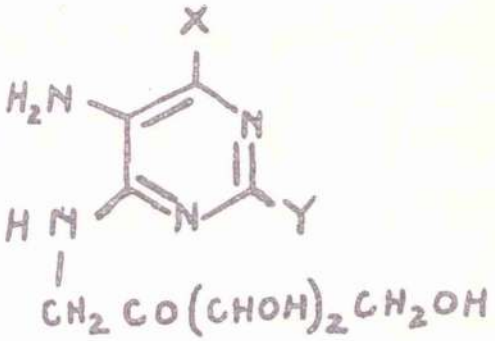
THEORETICAL PART II

Ring Opening of Guanosine

In the previous section of this thesis, the synthesis of 1-[5'-amino-2',6'-dihydroxy-4'-pyrimidinylamino]-1-deoxy-D-erythro pentulose (LXV; X = Y = OH) has been described. This compound corresponds to the Amadori-rearranged product in Weygand's hypothesis[15] for the biosynthesis of pteridines.



LXIV



LXV

Attention was now turned to an investigation of a method for the preparation of the parent pyrimidine glycoside (LXIV).

Two ideas suggest themselves:

(a) Degradation of a suitable purine nucleoside.

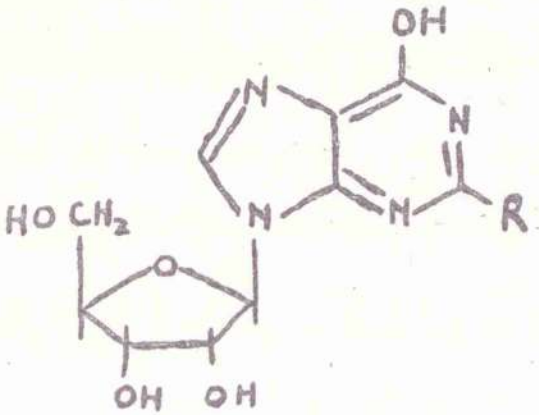
(b) Direct synthesis.

The preparation of the pyrimidine glycoside (LXIV) by ring-opening of a purine nucleoside is of particular interest since purine derivatives have been identified as the starting materials in the biosynthesis of flavins and pteridines.

The first part of this section has been devoted to studies

of ring-opening in the purine nucleoside, guanosine.

The reasons for selecting guanosine instead of xanthosine were threefold.



R = NH_2: Guanosine

R = OH: Xanthosine

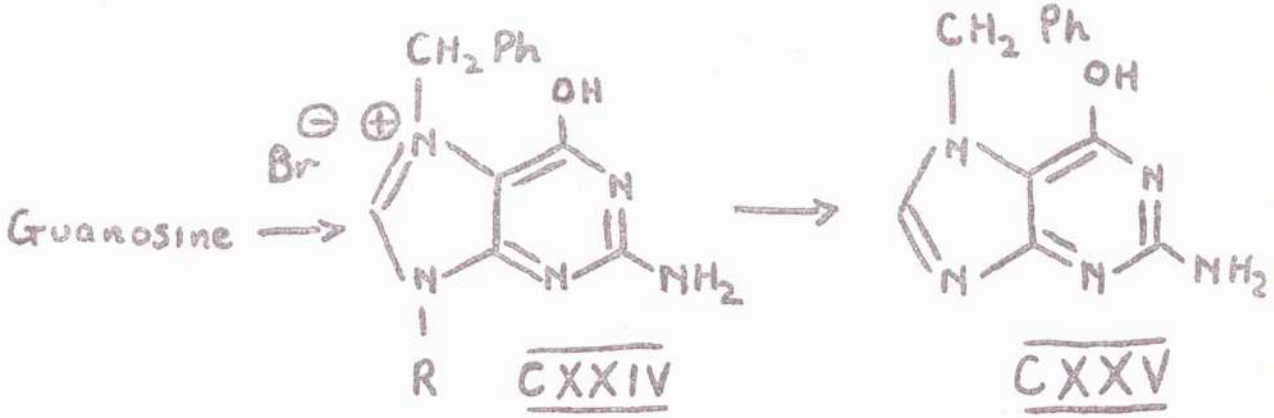
(a) Goodwin[48] has discovered that of the purine nucleosides, guanosine was the most effective precursor of riboflavin.

(b) The amino group of a purine, pyrimidine or flavin can be smoothly converted to a hydroxy group by the action of dilute nitrous acid. An amino group can also be regarded as a biogenetic precursor of a hydroxy group.

(c) As described in the Historical Section, Lawley[60,61] and his coworkers have described the alkylation of guanosine, and on the basis of spectral studies; these workers have suggested that ring-opening of the imidazole ring can occur readily on treatment with alkali.[60]

A re-investigation of the alkylation and ring-opening of guanosine was therefore undertaken using benzyl bromide as the alkylating agent.

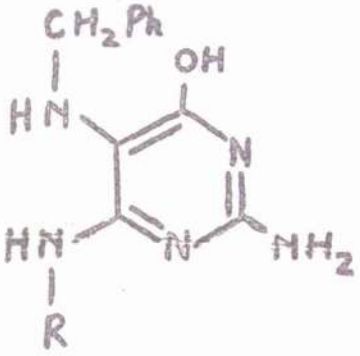
Treatment of guanosine with benzyl bromide under mild conditions gave crystalline 7-benzylguanosinium bromide (CXXIV; R = D-ribosyl) as the principal product. Comparison of the ultraviolet spectra of 7-methyldeoxyguanosinium-5'-phosphate methosulphate[61] and quaternary salt (CXXIV; R = D-ribosyl) in pH7 buffer indicated structural similarity. Acid hydrolysis of the quaternary salt (CXXIV; R = D-ribosyl) gave 7-benzylguanine (CXXV), characterised by analysis and spectral comparison with 7-methylguanine[60,61] (XLVIII). D-Ribose was identified by running a spot of the reaction mixture on a paper chromatogram and spraying with aniline hydrogen phthalate.[106]



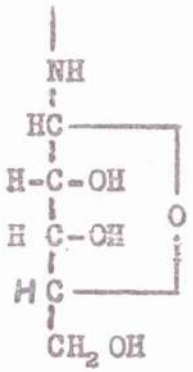
Treatment of the quaternary bromide with dilute sodium hydroxide solution gave 2-amino-5-benzylamino-4-hydroxy-6-D-ribosylaminopyrimidine (CXXVI; R = D-ribosyl) which was partially purified on an anion exchange resin.

Paper chromatography of this solution in various solvents showed that two spots were obtained in acidic solvent systems and

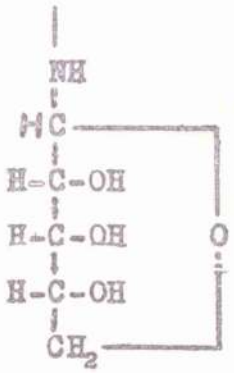
only one in neutral and alkaline systems. Ultraviolet spectra were obtained by eluting the spots from paper chromatograms and these proved to be identical. Since a formyl derivative of pyrimidine (CXXVI; R = D-ribosyl) could never survive the alkaline conditions of the reaction, it was adduced that the sugar moiety in the pyrimidine glycoside (CXXVI; R = D-ribosyl) must exist in two forms - furanosyl (CXXVII) and pyranosyl (CXXVIII) - in acidic conditions. An example of two such isomers occurring together, was mentioned in the Historical Section, pertaining to the alkaline degradation products of Nebularine.[89]



CXXVI



CXXVII



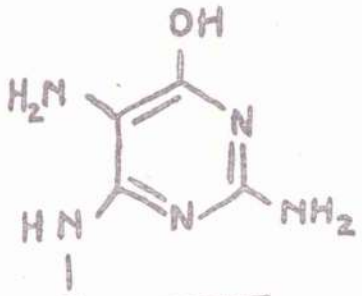
CXXVIII

Acid hydrolysis of the pyrimidine glycoside (CXXVI; R = D-ribosyl) gave 2,6-diamino-5-benzylamino-4-hydroxypyrimidine (CXXVI; R = H), the purification of which yielded only a gum which was characterised as a crystalline picrate derivative. D-Ribose was identified as above.

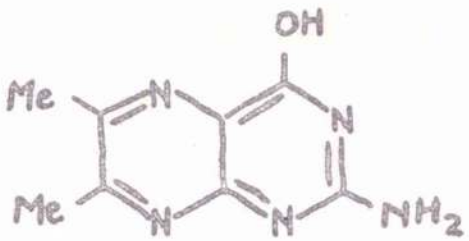
Removal of the benzyl group from pyrimidine glycoside (CXXVI; R = D-ribosyl) was achieved using sodium and liquid

ammonia[66] to give the unstable 2,5-diamino-4-hydroxy-6-D-ribosylaminopyrimidine (CXXIX; R = D-ribosyl). Immediate hydrolysis of this pyrimidine gave 2,4,5-triamino-4-hydroxypyrimidine (CXXIX; R = H) and D-ribose.

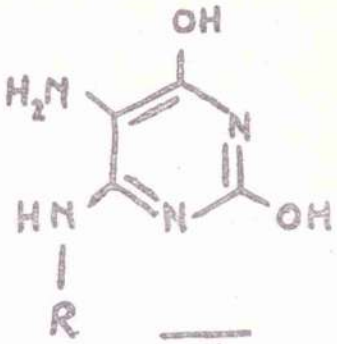
Condensation of this well-characterised pyrimidine (CXXIX; R = H) with biacetyl gave 2-amino-4-hydroxy-6,7-dimethylpteridine (CXXX) which was identical in all respects with an authentic sample.[109]



CXXIX



CXXX



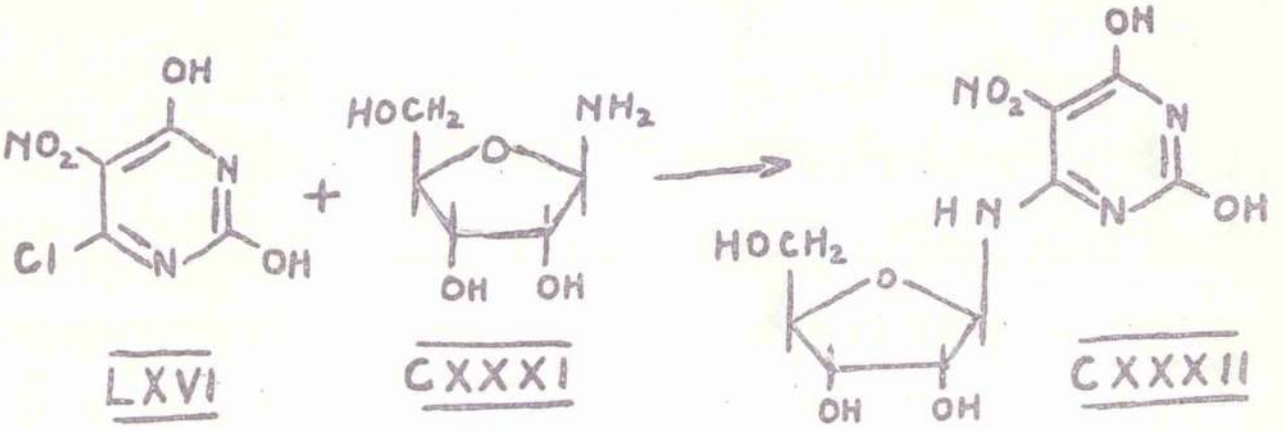
XXII

Pyrimidine glycoside (CXXIX) was acid labile as it underwent an oxidative self-condensation similar to compounds of type (XXII) in the first part of this thesis.

Although some of the intermediates have not been characterised completely, it is obvious that guanosine had been successfully converted to the acid labile 2,5-diamino-4-hydroxy-6-D-ribosylaminopyrimidine (CXXIX; R = D-ribosyl).

In an attempt to make more of this material available for further study, attention was now turned to the direct synthesis of a pyrimidine glycoside of type (LXIV).

The proposed synthesis involved the condensation between 5-nitro-4-chlorouracil (LXVI) and D-ribosylamine (CXXXI) with subsequent reduction of the 5-nitro group of the product (CXXXII).



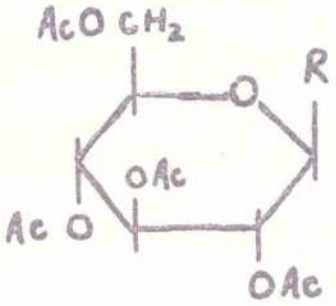
LXVI CXXXI CXXXII

Time has only permitted the investigation of model reactions in this series. Since in the proposed condensation, the production of hydrogen chloride was expected and as glycosylamines are prone to rearrangement and self-condensation under acidic conditions, it was decided to use an acetylated glycosylamine.

The literature offered 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosylamine[110] (CXXXIV, R = NH_2) as the most suitable model sugar amine. This is derived from D-glucose and the preparative details of the various steps in its synthesis are reliable.

D-glucose was acetylated to give 1,2,3,4,6-penta-O-acetyl-D-glucose[111] which was then brominated in position 1 with

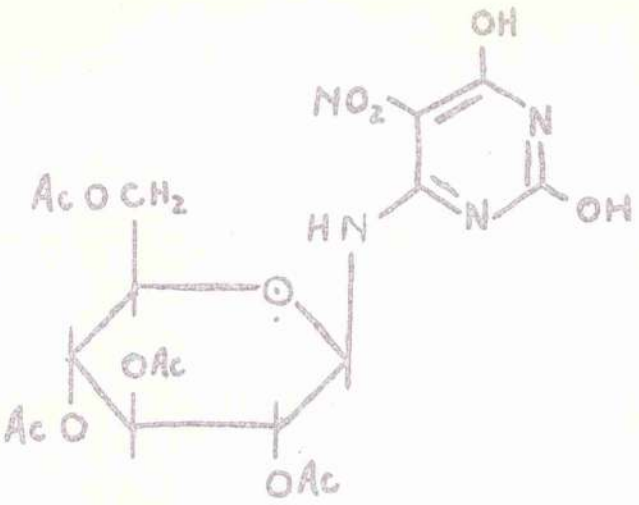
hydrogen bromide to give α-acetobromoglucose,[111] This unstable product was refluxed in methyl cyanide with sodium azide to give the corresponding β-azido[112] compound (CXXXIV; R = N_3) which was then reduced with hydrogen in ethyl acetate over Adams platinum catalyst to give the required β-amino[110] compound (CXXXIV; R = NH_2)



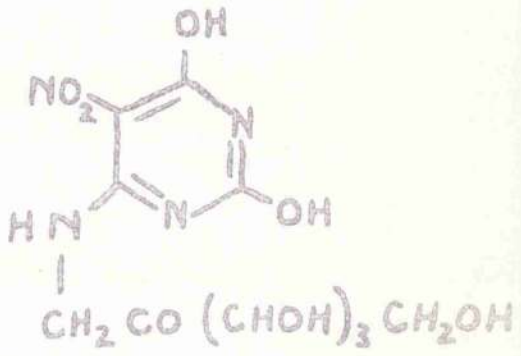
CXXXIV

Condensation between4-chloro-5-nitrouracil (LXVI) and 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosylamine (CXXXIV; R = NH_2) gave 2,6-dihydroxy-4-2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosyl-amino-5-nitropyrimidine (CXXXV) as a yellow oil which could not be properly characterised. Ultraviolet and infrared spectra and paper chromatography indicated, however, that condensation had taken place. A by-product from this reaction was N,N-'α'-Di-(D-glucosyl)amine octaacetate[113] probably formed by condensation of two molecules of 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosylamine hydrochloride.

Acid hydrolysis of pyrimidine (CXXXV) gave 4-amino-2,6-dihydroxy-5-nitropyrimidine[104] and D-glucose. This confirmed that pyrimidine (CXXXV) was a glycoside. Deacetylation of this pyrimidine glycoside in ethanol with liquid ammonia gave a



CXXXV



LXX

crystalline pyrimidine-sugar compound (CXXXVI) from which the sugar could not be removed by acid hydrolysis. Reacetylation of this material gave a crystalline acetate which was different from the acetylated glycoside (CXXXV).

The crystalline compound (CXXXVI) gave colour tests[77] which indicated that glycoside (CXXXV) had undergone an Amadori rearrangement on treatment with liquid ammonia. If this were the case, the pyrimidine (CXXXVI) should be identical with 1-[2',6'-dihydroxy-5'-nitro-4'-pyrimidinylamino]-1-deoxy-D-fructose (LXX) produced by the condensation of 4-chloro-5-nitrouracil and isoglucosamine as in the previous section. It has not been possible, however, to confirm beyond doubt the identity of these two materials or of the acetates prepared from them.

The occurrence of an Amadori rearrangement in liquid ammonia solution is most unusual, although it could be attributed to the presence of ammonium acetate in the reaction mixture functioning as a proton donor. It would seem that a detailed study of this problem is warranted, but it has not been considered further in this thesis.

TABLE OF R_F VALUES AND ULTRAVIOLET ABSORPTION MAXIMA

	R_F					ULTRAVIOLET		
Compound	(A)	(B)	(C)	(D)	(E)	pH1	pH13	pH7
CXXIV	0.5					260	267	259
						284		285
CXXV	-					250	282	
CXXVI; R = D-ribosyl	0.33	0.81	0.78	0.56	0.33	274	266	
	0.44			0.72				
CXXVI; R = H	0.95					272	262	
CXXVI; R = H-picrate	0.74					-	-	
CXXIX; R = D-ribosyl	0.10					272	266	
CXXIX; R = H	0.47					270	263	
CXXX	0.60	0.54				251	249	
						322	358	
D-Ribose	0.25					-	-	
CXXXV	0.80	0.70				228	220	
						320	336	
LXXII; R = H	0.1	0.6				228	220	
						318	336	
CXXXVI	0.1					228	220	
						320	341	
Acetate of CXXXVI	0.6					-	-	
D-Glucose	0.2	0.9				-	-	

EXPERIMENTAL

PART II

7-Benzyl-guanosinium Bromide. - Guanosine (2.5 g.) was dissolved in N,N-dimethylformamide (250 ml.). Sodium bicarbonate (5 g.) and redistilled benzyl bromide (2 ml., 2 eq.) were added and the mixture was left for 20 hr. at 37°, and then for 1 day at 0°. Solid sodium bicarbonate was removed by filtration and the filtrate was taken to dryness in vacuo at 40°. Ethanol (100 ml.) was added to the residue, most of which dissolved, leaving unreacted guanosine which was filtered off. The filtrate was re-evaporated at 40° and the procedure was repeated with another portion of ethanol (100 ml.). Chloroform (150 ml.) was added giving a white hygroscopic solid (3.6 g.) which was collected quickly, washed with chloroform (20 ml.) and dried in vacuo over silica gel. Recrystallisation from methanol-ethyl acetate gave the bromide as needles (1.3 g., 32%) m.p. 171° (dec.), $[\alpha]_D$ -15.6° (water) (Found: C, 45.2; H, 4.4; N, 15.0. $C_{17}H_{20}N_5O_5Br$ requires C, 44.9; H, 4.4; N, 15.5%.

7-Benzylguanine. - 7-Benzylguanosinium bromide (0.3 g.) was refluxed for 1 hr. with N-hydrochloric acid (15 ml.). The reaction mixture on cooling gave 7-benzylguanine hydrochloride (0.14 g.) as needles. These were dissolved in water and the solution pH adjusted to 7 when 7-benzylguanine separated (0.10 g.) m.p. > 300° (Found: C, 59.9; H, 4.6; N, 29.0. $C_{12}H_{11}N_5O$ requires C, 59.7; H, 4.6; N, 29.0%).

2-Amino-5-benzylamino-4-hydroxy-6-D-ribosylaminopyrimidine.- 7-Benzylguanosinium bromide (1.0 g.) was dissolved in 0.1N-sodium hydroxide solution and left for 1 day at room temperature. Two purification techniques were attempted.

(a) The solution was passed down a column of a weakly acidic ion-exchange resin (Amberlite I.R.C.50; H^+ form) till the eluate was free of pentose.[114] The pH of the eluate was adjusted to 8 with dilute ammonia solution and the eluate taken to dryness *in vacuo* at 40° giving a white hygroscopic solid (0.46 g.) which was dried at 100° over phosphorus pentoxide *in vacuo*. Soxhlet extraction with dry acetone was carried out for 20 hr. Evaporation of the extract gave a white solid (0.27 g.).

(b) A similar solution was run on to an Amberlite C.G.400 column (formate form) at pH12. Ammonium formate buffer (pH9) eluted the glycoside fractions which were bulked and evaporated to dryness *in vacuo* at 40°. Ammonium formate was removed by sublimation at 60° *in vacuo* leaving a hygroscopic pink residue which could not be crystallised. Analytical purity was not reached in either case.

Paper chromatography in acidic solvent systems showed two spots, whereas chromatography in neutral or alkaline solution gave only one spot.

2,6-Diamino-5-benzylamino-4-hydroxypyrimidine. - 2-Amino-5-benzylamino-4-hydroxy-6-D-ribosylaminopyrimidine (100 mg.) was dissolved in N-hydrochloric acid (5 ml.) and left overnight at

37°. On neutralisation with 2N-sodium hydroxide, a brown solid separated and was filtered off. Attempted crystallisation from ethanol gave a solid gum m.p. > 300°.

2,6-Diamino-5-benzylamino-4-hydroxypyrimidine picrate. - Crude 2,6-diamino-5-benzylamino-4-hydroxypyrimidine was dissolved in warm 0.1N-hydrochloric acid and a warm aqueous solution of picric acid in approx. equimolar quantity, was added and the mixture left overnight. Yellow needles separated and were recrystallised from water m.p. 267°. This picrate exploded on heating and thus, gave faulty analytical data.

2,5-Diamino-4-hydroxy-6-D-ribosylaminopyrimidine. - 2-Amino-5-benzylamino-4-hydroxy-6-D-ribosylaminopyrimidine (0.5 g.) was dissolved in liquid ammonia (10 ml.) and was added portion-wise over a period of 3 min. to sodium (0.4 g.) dissolved in redistilled liquid ammonia (100 ml.). The mixture was stirred during the addition and for a further 6 min. Addition of solid ammonium chloride (1 g.) terminated the reduction and destroyed the deep blue colouration. The mixture was left overnight at room temperature. The cream residue was dissolved in water (25 ml.) and chromatographed by passing down a column of Amberlite I.R.C as described before. A white solid (0,2 g.) was obtained which contained a little sodium chloride.

2,5,6-Triamino-4-hydroxypyrimidine. - Crude 2,5-diamino-4-hydroxy-6-D-ribosylaminopyrimidine (1 g.) was dissolved in 0.1N-sulphuric acid solution (50 ml.) and warmed on a steam-bath for 30 min. No attempt was made to isolate the free base and the solution was used directly for the next experiment.

2-Amino-6,7-dimethyl-4-hydroxypteridine. - Excess biacetyl (0.1 g.) was added to the solution obtained from the above experiment and the mixture was left overnight at 37°. Neutralisation with 2N-sodium hydroxide solution gave a yellow precipitate which was collected. Crystallisation from aqueous ammonia solution gave off-white needles (300 mg.) m.p. > 300°. This material was identical in all respects with an authentic sample of this pteridine.[109]

D-Ribose. - In each hydrolysis of the three pyrimidine glycosides described above, a spot of the hydrolysate was examined by paper chromatography in solvent (A) and the D-ribose spot developed as a brown stain by spraying with aniline hydrogen phthalate[108] in n-butanol.

Tetra-O-acetyl-β-D-glucopyranosylazide.[112] - α-Acetobromo-glucose[111] (10 g.) and finely powdered sodium azide (5 g.) in methyl cyanide (50 ml.) were heated on a steam-bath for 4 hr. Water vapour was excluded from the reaction. Sodium bromide and excess sodium azide were removed and the filtrate was taken to a gum by evaporation *in vacuo*. Crystallisation from methanol

(40 ml.) gave needles (6 g., 66%) m.p. 130° [lit., 129°].

Tetra-O-acetyl-β-D-glucosopyranosylamine.[110] - Tetra-O-acetyl-β-D-glucosopyranosylazide (5 g.) dissolved in ethyl acetate (150 ml.) was shaken with Adams platinum catalyst (250 mg.) under hydrogen for 2 hr. The catalyst was removed and the filtrate was taken to a clear oil by evaporation *in vacuo*. Crystallisation from ethanol gave needles (4 g., 86%) m.p. 126° [lit., 126°].

The progress of this hydrogenolysis was followed by infrared spectroscopy which showed the gradual disappearance of the azide absorption band.

Attempts to Condense Acetylated Glycosylamines with 4-Chloro-2,6-dihydroxy-5-nitropyrimidine.

2,6-Dihydroxy-5-nitro-6-tetra-O-acetyl-D-glucosylamino-pyrimidine. - 4-Chloro-2,6-dihydroxy-5-nitropyrimidine (500 mg.) was dissolved in ethanol (50 ml.) and added to an ethanolic solution (50 ml.) of 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosylamine[110] (1.8 g., 2 eq.) and heated on a steam-bath for 10 min. On cooling a white solid separated which crystallised from ethanol as needles, m.p. 216°, $[\alpha]_D$ -85° (ethanol) and was identified as N,N-'α'-Di-(D-glucosyl)amine octaacetate [lit.,[113] m.p. 213-216°, $[\alpha]_D$ - 87° (chloroform)]. On concentration of the solution, more octaacetate separated. An oil remained which could not be crystallised. Purification using an anion exchange column

did not give a crystalline product. This material showed ultraviolet absorption, with maxima at 320 mu (pH1) and at 336 mu (pH13), characteristic of a 5-nitrouracil derivative. Paper chromatography showed the presence of a single component with R_F = 0.8 in solvent (A) and R_F = 0.7 in solvent (B). The data suggests that the required compound has been formed.

Hydrolysis of 2,6-Dihydroxy-5-nitro-6-tetra-O-acetyl-D-glucosylaminopyrimidine. - 2,6-Dihydroxy-5-nitro-6-tetra-O-acetyl-D-glucosylaminopyrimidine (0.5 ml.) as the crude oil from above, was refluxed in 0.2N-hydrochloric acid solution (5 ml.) for 3 min. Paper chromatograms in two solvent systems showed that 4-amino-2,6-dihydroxy-5-nitropyrimidine[104] and D-glucose were formed. The sugar was detected by spraying with aniline hydrogen phthalate[108] solution and the pyrimidine by examining the chromatogram with ultraviolet light of wavelength 254 mu.

R_F Solvent System	Oil	Hydrolysate	Base	D-Glucose
(A)	0.8	0.1	0.1	
		0.2		0.2
(B)	0.7	0.6	0.6	
		0.9		0.9

A solid separated (100 mg.) and was identified as 4-amino-2,6-dihydroxy-5-nitropyrimidine by infrared spectrum with

an authentic sample.[104]

Attempted Deacetylation of above Glycoside. - An ethanolic solution of liquid ammonia (1:1, v:v) (50 ml.) was added to the crude oil of 2,6-dihydroxy-5-nitro-6-tetra-O-acetyl-D-glucosylaminopyrimidine made as above from 4-chloro-5-nitrouracil (500 mg.) and the mixture allowed to stand for 3 days at room temperature. A solid separated which was recrystallised from ethanol as needles (500 mg.) m.p. 210° (Found: C, 31.0; H, 5.5; N, 17.7. $C_{10}H_{14}N_4O_{11}.NH_3.2H_2O$ requires C, 31.0; H, 5.5; N, 18.1%). The ultraviolet absorption spectrum showed maxima at 320 mu (pH1) and 341 mu (pH13) indicative of a 5-nitro-6-alkylaminouracil derivative. A faint purple colouration was obtained in alkaline solution with o-dinitrobenzene.[77]

Attempted hydrolysis of this material using hydrochloric acid solution (0.1N or N) brought about no apparent change as judged by paper chromatograms.

Reacetylation of this material (100 mg.) was carried out as follows. The pyrimidine was dissolved in pyridine (6 ml.) and acetic anhydride (6 ml.) and left overnight. As the starting material dissolved, other crystals separated. Excess acetic anhydride was destroyed by addition of ethanol (50 ml.) and the now homogeneous solution was left for 2 hr. The mixture was taken to a gum by distillation in vacuo and taken down twice with ethanol (2 x 10 ml.). Crystallisation from ethanol-petroleum

ether (40-60) gave colourless needles (100 mg.) m.p. 180° (Found: C,46.6; H,4.6%).

Comparison of this acetate with a sample prepared by acetylating 1-[2',6'-dihydroxy-5'-nitro-4'-pyrimidinylamino]-1-deoxy-D-fructose did not provide conclusive proof of the identity of these compounds.

Paper Chromatography

Chromatograms were developed by the ascending technique, the solvents being,(A), butan-1-ol-5N-acetic acid (7:3), (B), 3% aqueous ammonium chloride, (C), propan-1-ol-water-conc. ammonia (d,0.88) (40:20:1),(D). Wyatt's solvent, and (E), butan-1-ol-ethanol-water (50:15:35) and were viewed in ultraviolet light of wavelength 254 and 365 mu.

Absorption Spectra

Infrared spectra were determined with a Grubb-Parsons infrared spectrophotometer on Nujol mulls and KCl discs.

Ultraviolet spectra were determined with a Unicam SP600 spectrophotometer on aqueous solutions of standard pH.

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