CHEMICAL STUDIES OF 8 - SUBSTITUTED PTERIDINES

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SUMMARY OF THE THESIS

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THE UNIVERSITY OF GLASGOW

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by

THOMAS ROWAN

September, 1963.

CHEMICAL STUDIES OF 8 - SUBSTITUTED PTERIDINES.

An account is given of the methods which are at present available for the synthesis of 8 - substituted pteridines and the closely allied 9 - substituted isoalloxazine derivatives. The theories reported in the literature relating to the biosynthesis of riboflavin, which may be considered to be a benzopteridine with a substituent in the 8 - position of the pteridine moiety, are reviewed.

The chemical feasibility of the biosynthetic routes to riboflavin, which have been suggested in the literature, is investigated. It is found that 6,7 - dimethyl - 8 - D - ribityllumazine ("G - compound"), which is an intermediate in the biosynthesis of the vitamin, can be readily converted chemically into 7,8 - dihydro - 2,6 - dihydroxy - 6 - methyl - 7 - oxo - 8 - D - ribitylpteridine ("V - compound"), which is a known by-product in the biosynthetic process. This conversion is shown to occur by oxidation or, in an inert atmosphere, by displacement of the diacetyl residue of the lumazine derivative by pyruvate. The hydration in alkaline solution of 8 - substituted "quinonoid" pteridines, such as "G - compound", is described and the significance of this in relation to the biosynthetic pathway leading to riboflavin and to the biological conversion of "G - compound" into "V - compound", is discussed. A new synthesis of riboflavin from "G - compound" is reported, the salient feature being its simplicity. Thus, when the lumazine is refluxed in a buffer solution of pH 7.3, in the absence of any enzyme or other source of carbon, riboflavin is produced in good yield. This reaction

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is applied successfully to the synthesis of other isoalloxazines and to isoalloxazine 2 - imines and a possible mechanism for the reaction is suggested.

Attempts to synthesise pteridine 8 - glycosides analogous to the purine nucleosides are described, the two main approaches to this problem being (a) direct alkylation of pteridines and (b) synthesis from 4,5 - diaminopyrimidine precursors. Model reactions with 5 - amino - 4 - alkylaminopyrimidines are reported in which 8 - alkyl - pteridines are readily formed by the well-known "Isay Reaction". It is found, however, that when the corresponding 5 - amino - 4 - glucosylamino pyrimidine is reacted with dicarbonyl compounds such as diacetyl or glyoxal, surprisingly only the intermediate Schiff's base, which cannot be cyclised to the expected pteridine 8 - glucoside, is formed. This is in contrast to the reaction of the pyrimidine glucoside with ethyl pyruvate or with ethyl glyoxylate hemiacetal which readily gives the appropriate 7 - oxo - pteridine 8 - glucoside.

Other attempts to synthesise these elusive glycosides are described in detail.

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A C K N O W L E D G E M E N T S.

The author wishes to record his sincere gratitude to Dr. H.C.S. Wood for his supervision and guidance throughout the course of this work and he is indebted to Professor P.L. Pauson for the laboratory facilities provided. He also takes this opportunity of expressing his heartfelt thanks to his wife for innumerable painstaking hours spent at the typewriter.

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OBJECTIVES.

Theoretical considerations relating to the biosynthesis of riboflavin have been examined and the feasibility of the chemical processes involved has been studied.

Interest in 8 - substituted pteridines has been aroused due to their structural similarity to the biologically important purine nucleosides. Syntheses of 8 - substituted pteridines and their chemical behaviour have been described in addition to attempted preparations of pteridine - 8 - glycosides.

SUMMARY.

An account is given of the methods which are at present available for the synthesis of 8 - substituted pteridines and the closely allied 9 - substituted isoalloxazine derivatives. The theories reported in the literature relating to the biosynthesis of riboflavin, which may be considered to be a benzopteridine with a substituent in the 8 - position of the pteridine moiety, are reviewed.

The chemical feasibility of the biosynthetic routes to riboflavin, which have been suggested in the literature, is investigated. It is found that 6,7 - dimethyl - 8 - D - ribityllumazine ("G - compound"), which is an intermediate in the biosynthesis of the vitamin, can be readily converted chemically into 7,8 - dihydro - 2,6 - dihydroxy - 6 - methyl - 7 - oxo - 8 - D - ribitylpteridine ("V - compound"), which is a known by - product in the biosynthetic process. This conversion is shown to occur by oxidation or, in an inert atmosphere, by displacement of the diacetyl residue of the lumazine derivative by pyruvate. The hydration in alkaline solution of 8 - substituted "quinonoid" pteridines, such as "G - compound", is described and the significance of this in relation to the biosynthetic pathway leading to riboflavin and to the biological conversion of "G - compound" into "V - compound", is discussed. A new synthesis of riboflavin from "G - compound" is reported, the salient feature being its simplicity. Thus, when the lumazine is refluxed in a buffer solution of pH 7.3, in the absence of any enzyme or other source of carbon, riboflavin is produced in good

yield. This reaction is applied successfully to the synthesis of other isoalloxazines and to isoalloxazine 2 - imines and a possible mechanism for the reaction is suggested.

Attempts to synthesise pteridine-8-glycosides analogous to the purine nucleosides are described, the two main approaches to this problem being (a) direct alkylation of pteridines and (b) synthesis from 4,5 - diaminopyrimidine precursors. Model reactions with 5 - amino - 4 - alkylaminopyrimidines are reported in which 8 - alkyl - pteridines are readily formed by the well-known "Isay Reaction". It is found, however, that when the corresponding 5 - amino - 4 - glucosylaminopyrimidine is reacted with dicarbonyl compounds such as diacetyl or glyoxal, surprisingly only the intermediate Schiff's base, which cannot be cyclised to the expected pteridine - 8 - glucoside, is formed. This is in contrast to the reaction of the pyrimidine glucoside with ethyl pyruvate or with ethyl glyoxylate hemiacetal which readily gives the appropriate 7 - oxo - pteridine - 8 - glucoside.

Other attempts to synthesise these elusive glycosides are described in detail.

CONTENTS.

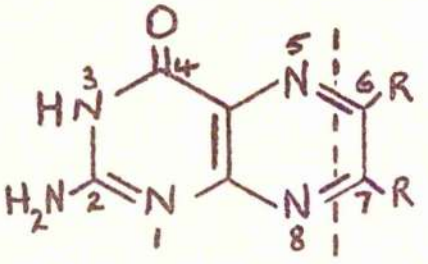
HISTORICAL

INTRODUCTION

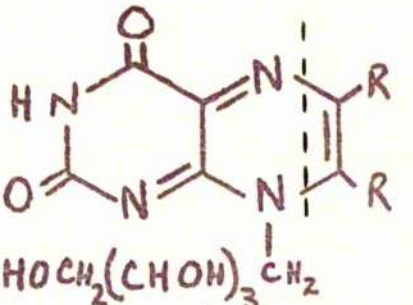
Interest in 8-substituted pteridine derivatives has been stimulated in recent years by the realisation that pteridine-8-glycosides might occur in Nature. The structural relationships existing among the natural pteridines, the purine nucleosides, and the flavins, exemplified by structures (1) to (4), have long since been noted[1,2] and reiterated in the literature. There is a marked similarity in the diaminopyrimidine portion of these molecules as shown on the left hand side of the dotted lines.

The 8-position of the pteridine ring corresponds to the 9-position in the purines and in the isoalloxazines and it would therefore be expected that the most probable substituent to be found in natural 8-subtituted pteridines would be a sugar such as ribose.

Although pteridine glycosides have never been isolated from natural sources, there is a strong possibility that pteridines might be carried through their metabolic pathways with a sugar attached. Glycosides in the pyrimidine and purine series are known and, as these are of considerable biological significance, it would be interesting to know if pteridine glycosides would act as biological antagonists to these nucleosides or to the flavins.



(1)



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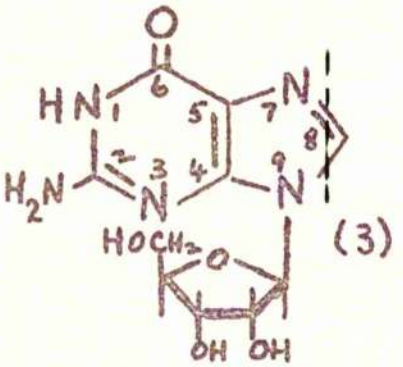

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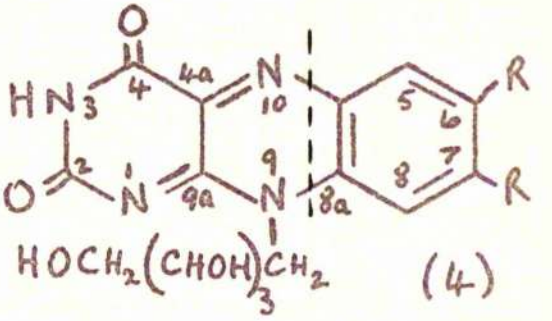


(4)

The conversion of purines into pteridines has been carried out *in vitro* by Albert[3] and *in vivo* by Ziegler-Günder *et al.*[4] Thus, an appropriate transformation of purine nucleosides into the corresponding pteridine glycosides would provide a method of solubilising the extremely insoluble pteridines.

8-Ribitylpteridines[5,6,7] have been isolated from cultures of the Ascomycetes, *Ashbya gossypii* (now known as *Nematospora gossypii*, but the former name will be used throughout this thesis to avoid confusion), and *Eremothecium ashbyii* and these pteridines are, at least in theory, reduction products of pteridine-8-ribosides.

Riboflavin (vitamin B_2) (4; R=CH_3), which belongs to the water-soluble group of vitamins, may be regarded as a benzopteridine with

the D-ribityl substituent in position 8 of the pteridine moiety of the molecule and can, in fact, be simply synthesised from an 8-D-ribityl pteridine (2; R=CH_3) as described in Part I of this thesis. Many organisms are known to synthesise riboflavin in small amounts (e.g. Peltier and Borchers[8] list no fewer than 240 flavin-producing species) but there are three which synthesise it and excrete it into the culture medium in very large amounts. These are Candida spp.[9], E. ashbyii[10], and A. gossypii[11], the last two being the most closely studied of the flavinogenic organisms.

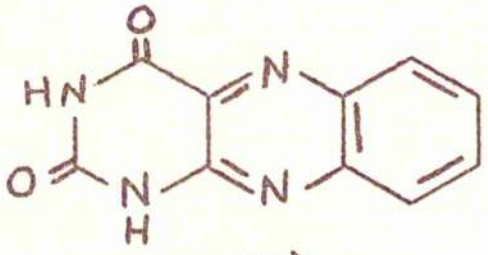
The vitamin properties of riboflavin are apparently dependant to some extent on the ribose constituent as the compound obtained on substituting arabinose for ribose is much less active.[12] Riboflavin takes part in a number of different enzyme systems in tissues and forms part of the prosthetic group which constitutes the coenzyme. There are two riboflavin-containing coenzymes; namely, a mononucleotide (F.M.N.) which is a riboflavin-5-phosphate, and a dinucleotide (F.A.D.) which has the constitution of a riboflavin-adenine dinucleotide. Riboflavin acts in enzyme systems by the reversible acceptance and donation of two atoms of hydrogen, the reduction being accomplished by the addition of hydrogen to the 1- and 10- positions to form dihydro- or leuco- riboflavin which is readily reoxidised by oxygen.[13]

SYNTHESIS OF ISOALLOXAZINES AND 2-IMINO-ISOALLOXAZINES

The syntheses of isoalloxazines and their derivatives can best be classified into three distinct groups according to the starting materials required; namely, o-phenylene diamine derivatives, 4,5-diaminopyrimidines, and quinoxaline derivatives.

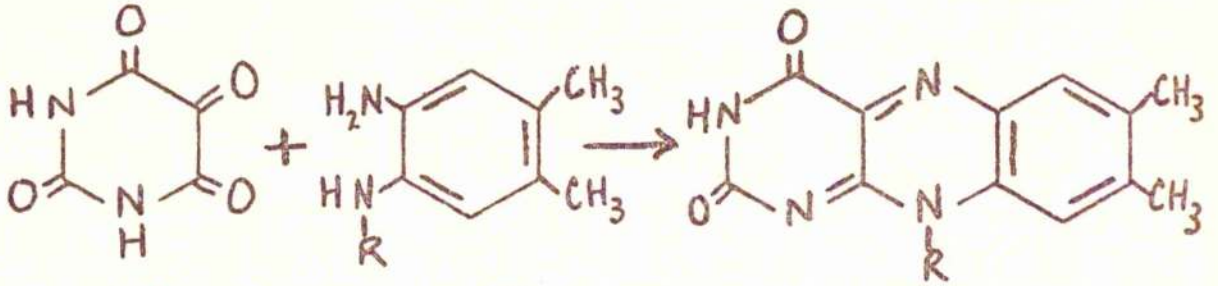
(1) From o-Phenylene Diamine Derivatives.

(a) The first syntheses of isoalloxazines involved the condensation of o-phenylene diamines with alloxan in acid solution after the manner of Kuhn and Reinemund.[14]



(5)

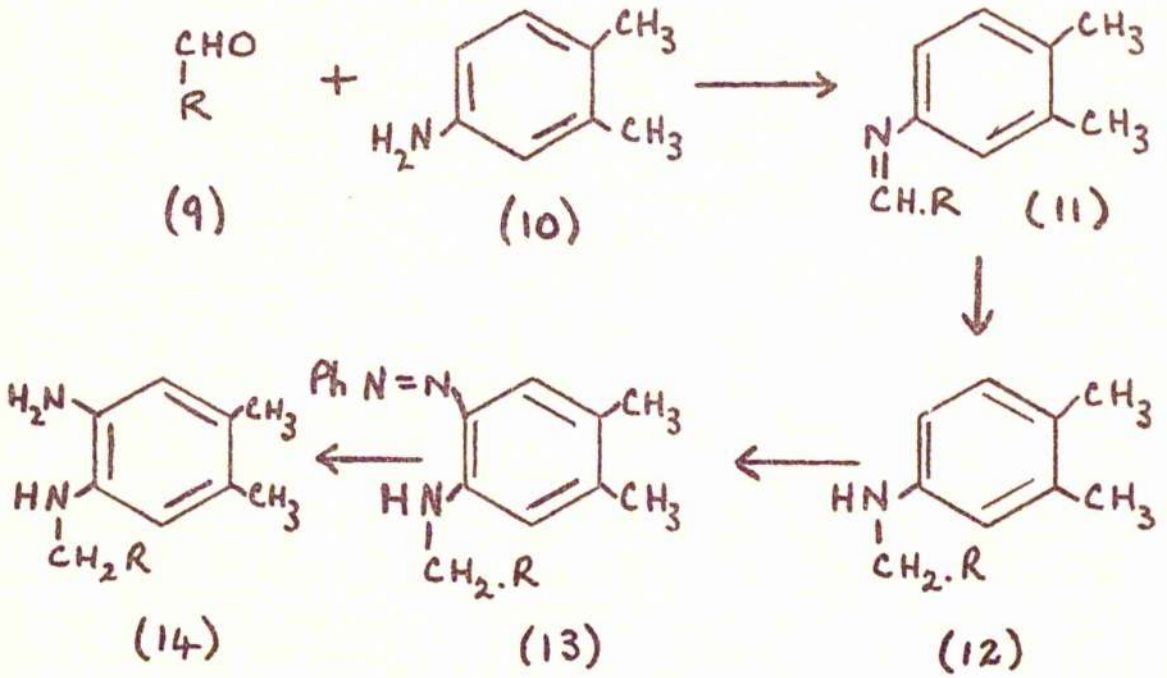
Condensation with unsubstituted o-phenylene diamine yields alloxazine [15] (5). When one of the nitrogen atoms in the diamine is tertiary, simple condensation between the unsubstituted amino group and the 5-keto group in alloxan occurs,[16] but when one nitrogen is secondary, ring closure to an isoalloxazine may occur as in the condensation of 1-amino-4,5-dimethyl-1-D-ribitylamino-benzene (7; R=D-ribityl) with alloxan to give riboflavin (8; R=D-ribityl).[17]



(6) (7) (8)

Kuhn and Weygand[17] carried out the reaction in acetic acid in the presence of boric acid which increased the yield considerably.

The main difficulty in the above method lies in the preparation of the o-phenylene diamine derivatives (7) which are oxygen-labile and are best not isolated. Their synthesis has been achieved in diverse ways with varying success, four of the main routes to the o-phenylene diamine intermediate being briefly described below.

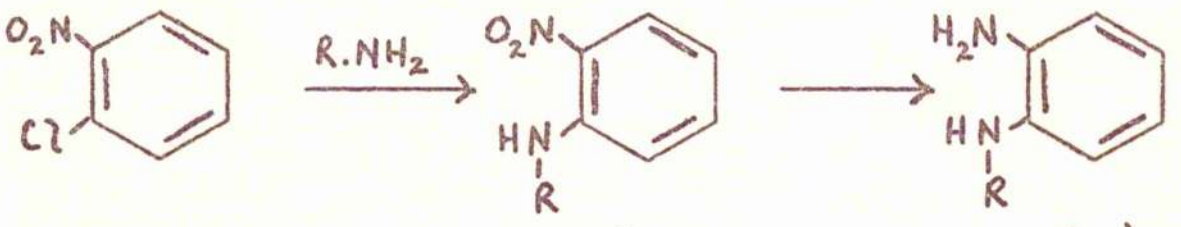


(9) (10) (11) (12) (13) (14)

(i) Condensation of D-ribose (9; R= $-(CHOH)_3.CH_2OH$) with 3,4-xylidine[18] yields the riboside (11) which on catalytic reduction and subsequent coupling with a diasonium salt gives the azo dye (13). The desired diamine (14; R= $-(CHOH)_3.CH_2OH$) [19] is readily obtained by chemical or catalytic reduction. The yield (38%) of riboflavin is very high in comparison with most of the other available methods of preparation <u>via</u> the o-phenylene diamine intermediate and alloxan.

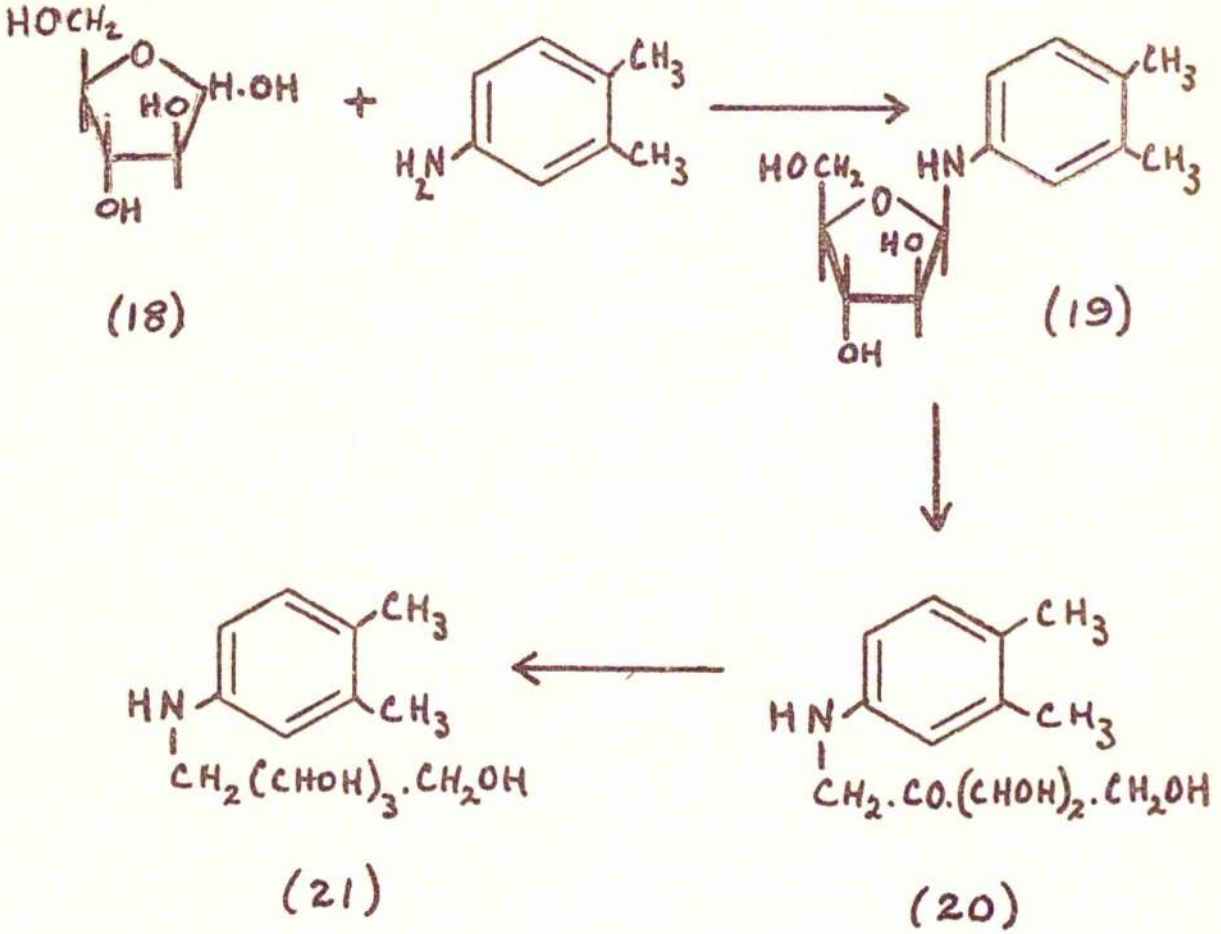
(ii) In this method the second nitrogen function is introduced into the benzene ring in the form of a nitro group before condensation with the sugar. Catalytic reduction then yields the diamine (14) which condenses with alloxan to give riboflavin in 16% yield.[20,21]

(iii) o-Nitro-chlorobenzenes (15) are condensed with amino-sugars and the reaction product is hydrogenated to the diamine[22] (17). This method gives satisfactory yields when the sugar contains two or three hydroxyl groups but poor yields with sugars containing four or five hydroxyl groups as in the case of riboflavin.

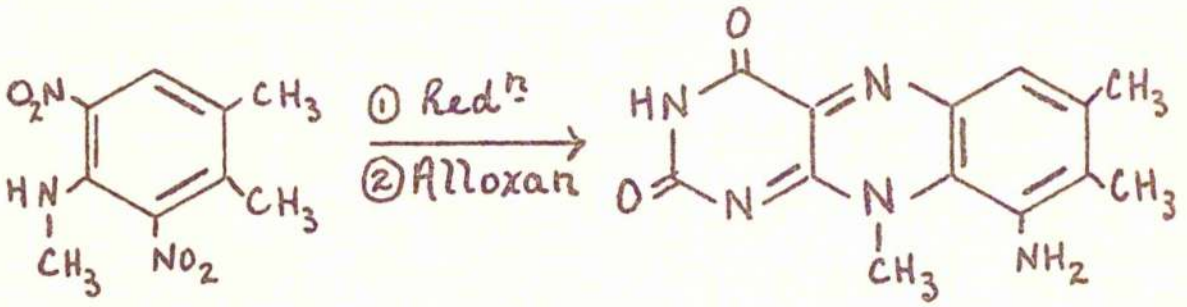


(15) (16) (17)

(iv.) D-arabinose, which is less expensive than D-ribose, has been used in a novel procedure by Weygand[23] to synthesise the dimethyl-D-ribitylaminobenzene (21). 3,4-Xylidine is condensed with D-arabinose (18) in the presence of a trace of acid. The resulting D-arabinoside (19) is made to undergo an Amadori rearrangement by heating to 75°C. and the ketose (20) is catalytically hydrogenated in alkaline solution whence the D-ribitylamino compound (21) is obtained in about 13% yield. Coupling of this product with a diazonium salt and subsequent hydrogenation leads to the required diamine as in method (i).



(b) Hemmerich[24] used a variation of the above methods for synthesising interesting isoalloxazines with substituents in the benzene ring. For example, 8-amino-lumiflavin (23) was obtained from N-methyl-2,6-dinitro-3,4-xylidine (22).

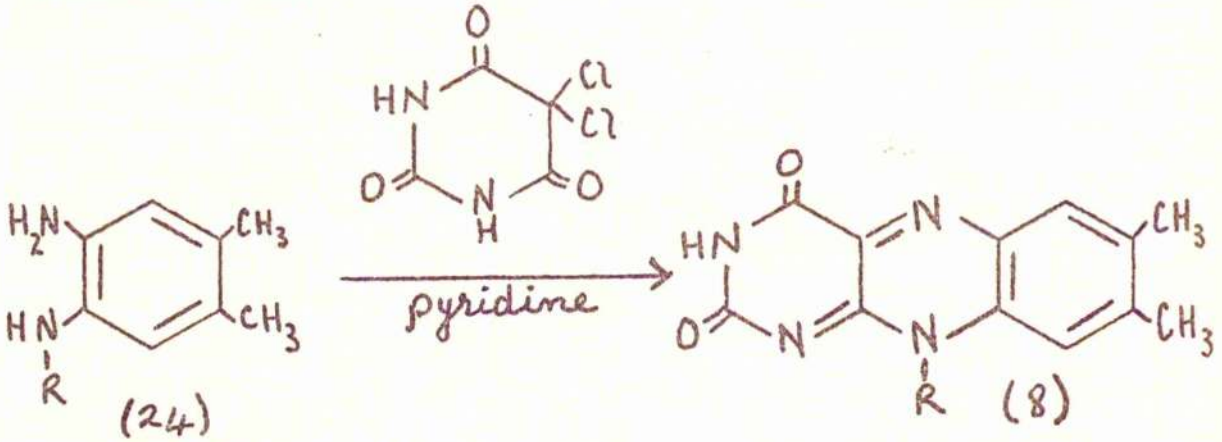


(22) (23)

(c) Two other methods in this group have been developed by Tishler and his co-workers[25,26] who employed barbituric acid or its derivatives in place of alloxan.

(i) "Ladenburg Synthesis."[25] Riboflavin is formed in excellent yields from the o-phenylene diamine derivative (24; R= D-ribityl) and 5,5 - dichlorobarbituric acid using pyridine as solvent. However, if the dihalobarbituric acid is replaced by alloxan, no riboflavin is formed and the brilliant yellow product, which was originally thought to be the "anil" (25; R= D - ribityl), could not be cyclised to riboflavin by heating with tertiary amines, or organic or inorganic acids including hydrofluoric acid. Thus, compounds of this type are not intermediates in the synthesis of isoalloxazines from o-phenylene diamines and alloxan. On prolonged alkaline hydrolysis of this "anil", the elements of urea are lost

and cyclisation to a dihydro - quinoxaline derivative occurs. King and Clark - Lewis[27] subsequently discarded the anil structure (25) in favour of the quinoxalone - 3 - carboxyureide formulation (26).



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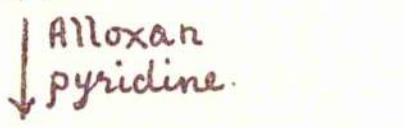

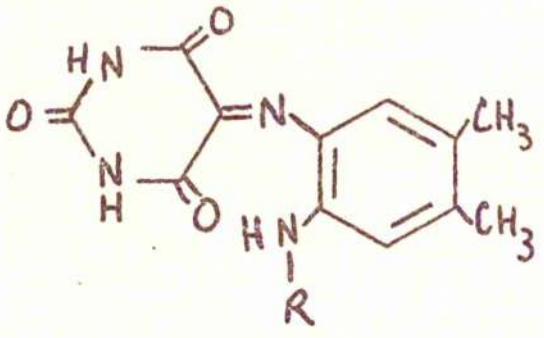

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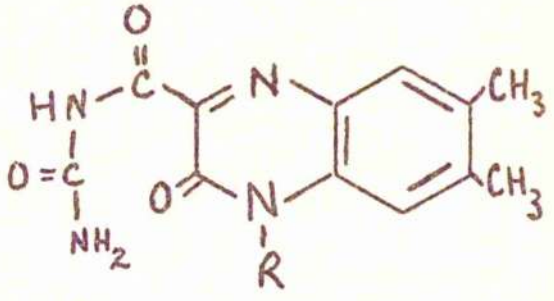
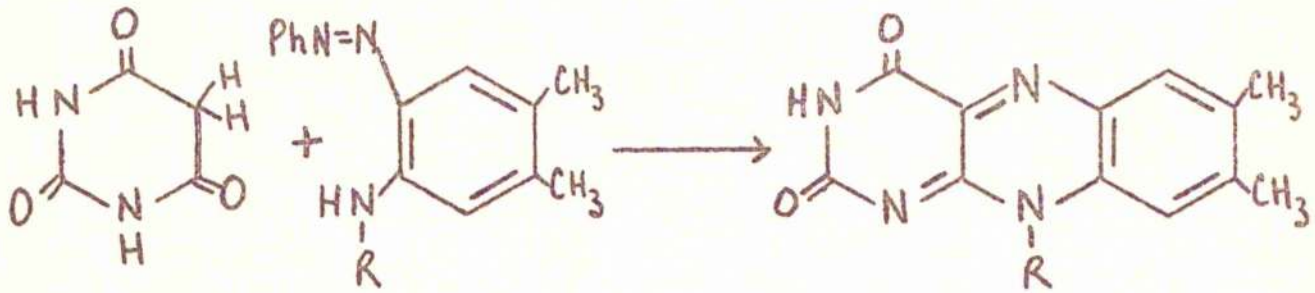


(26)

(ii) " Tishler Condensation " . This second method of Tishler et al.[26] is particularly suitable for the preparation of riboflavin from barbituric acid itself and is based on the Crippa[28] synthesis of quinoxalines from o - amino - azo compounds and ketones. In this reaction, barbituric acid (27) is heated in acid solution with 6 - phenylazo - N - D - ribityl - 3,4 - xylidine (28; R=D - ribityl) to furnish riboflavin in 70% yield. The optimum yields

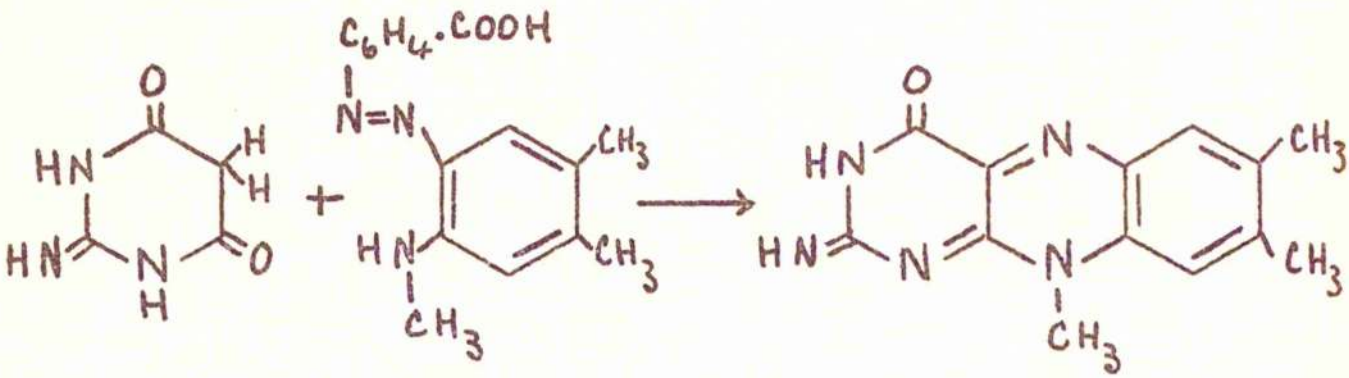
are obtained with glacial acetic acid or with acetic acid - dioxan, the presence of mineral acids not only inhibiting the reaction but also rapidly destroying the azo compound.



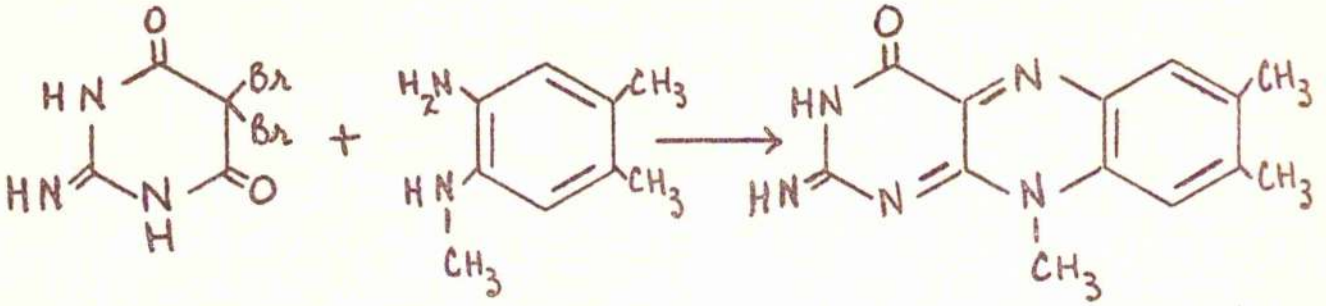
(27) (28) (8)

(d) Some of the procedures outlined above have been extended by Hemmerich et al.[24, 29, 30] to obtain isoalloxazine - 2 - imines in the following ways.

(i) The condensation of imino - barbituric acid (29) with 6 - (p - carboxyphenylazo) - N - methyl - 3,4 - xylidine (30) yields 2 - lumiflavimine (31)[29].



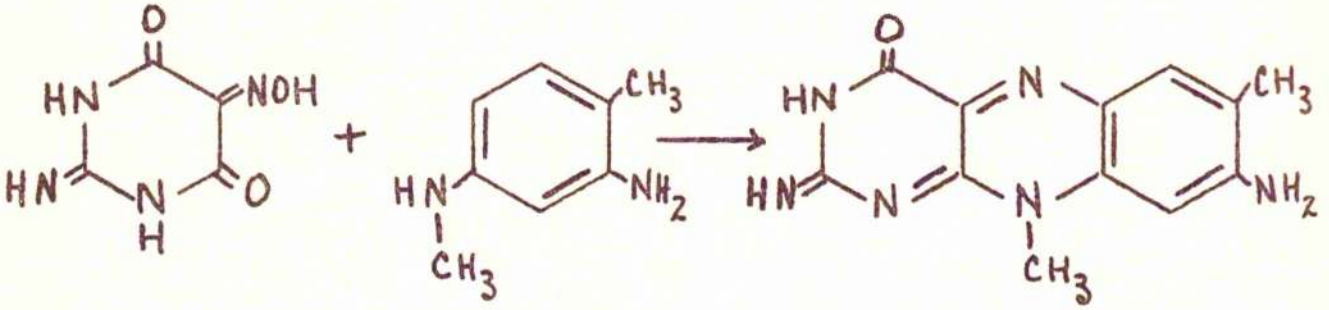
(29) (30) (31)



(32) (33) (31)

(ii) By a modification of the " Ladenburg Synthesis " [25] 2 - lumiflavimine (31) was also produced[24] from 5,5 - dibromo-barbituric acid - 2 - imine (32) and the appropriate diamine (33).

(iii) A novel modification of the isoalloxazine synthesis was achieved by Hemmerich and his collaborators[30] when they obtained 8 - nor - 8 - amino - 2 - lumiflavimine (36) by catalytic reduction of 2 - nitro - 4 - methylamino - toluene to the corresponding 2 - amino compound (35) followed by condensation with violuric acid 2 - imine (34).



(34) (35) (36)

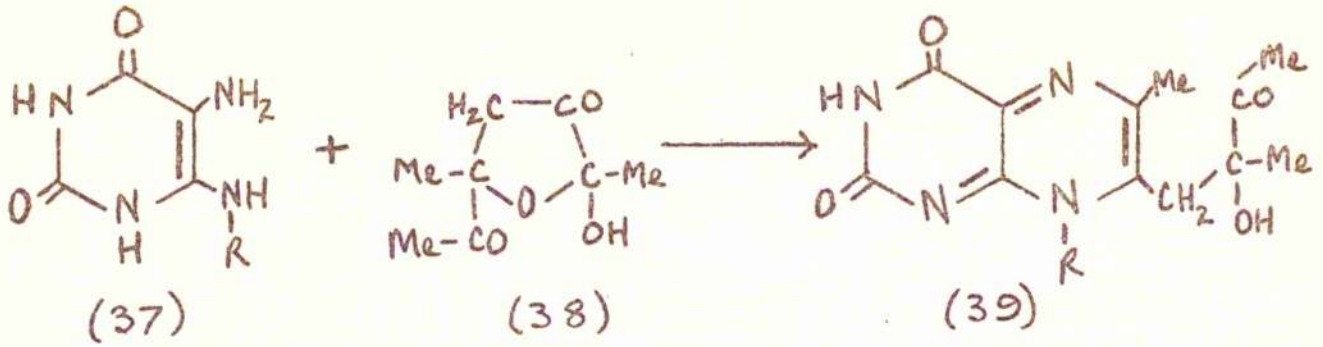
(2) <u>From 4,5 - Diaminopyrimidines.</u>

(a) The preparation of a dimer of diacetyl (38) by Birch and Moye[31] led to rather a unique synthesis of the dimethyl -

benzene ring of lumiflavin (40; R=CH_3)[32]. This was extended by Cresswell and Wood[33] to the synthesis of riboflavin (40; R= D - ribityl) and the corresponding 9 -(2'- hydroxyethyl) compound (40; R= CH_2CH_2OH) via an intermediate of the type (39).

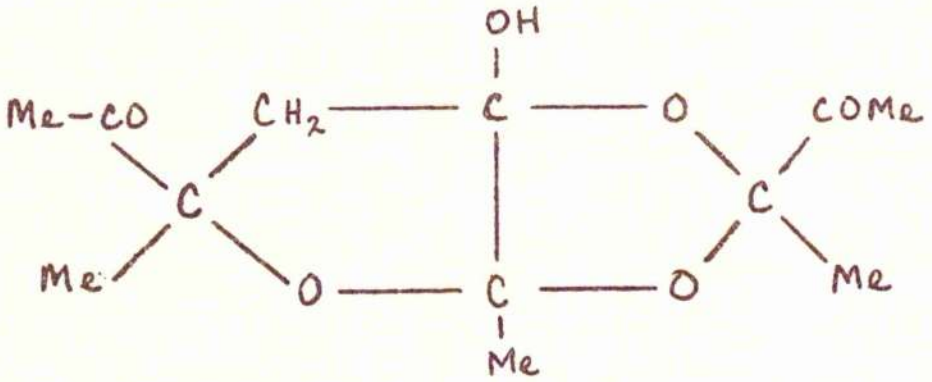
(b) Cresswell and Wood[34] later observed that a trimeric form of diacetyl (42), which differs from that previously described by Diels and Jost,[35] separates from some bottles of diacetyl which have been stored for long periods and that an identical compound can be obtained by keeping diacetyl in contact with an anion - exchange resin (Amberlite CG - 400, OH form) for 4 to 5 days. These authors showed that in aqueous solution with 4,5 -diamino - pyrimidine derivatives, this trimer behaves like a mixture of diacetyl and the aldol (38). Thus, it condensed readily with 5 - amino - 4 - D - ribitylamino - uracil (37; R= D - ribityl) at room temperature to give a mixture of 6, 7 - dimethyl - 8 - D - ribityllumazine (41; R = D - ribityl) and the pteridine (39; R = D - ribityl). Treatment of this mixture with 0.1N - sodium hydroxide or with acid of pH 1 to 6 induced cyclisation of the pteridine (39; R = D - ribityl) to give riboflavin (40; R = D-ribotyl) which was easily separated from the unchanged lumazine derivative (41; R = D - ribityl).

The biochemical significance of the above reactions is discussed elsewhere in this thesis.



(37) (38) (39)

(41) (40)

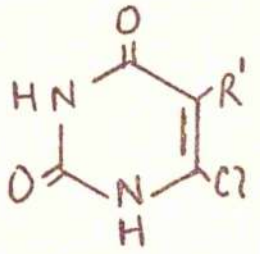


(42)

The 4 - alkylamino - 5 - aminopyrimidines which are required as starting materials have been synthesised in the following ways.

(i) Langley and Walpole[36] have reported the conversion of 4 - chlorouracil (43; R' = H) into 4 - alkylaminouracils (44; R = alkyl, R' = H). This method has been used to form glycityl - aminopyrimidines (44; R = glycityl, R' = H) which were converted

by nitrosation and reduction into the corresponding 5 - amino - compounds (45; R = Glycityl).[37-41].



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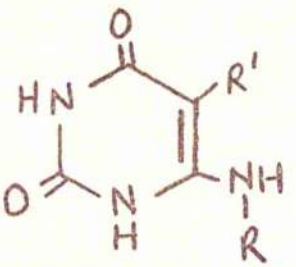

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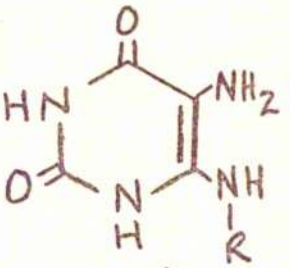

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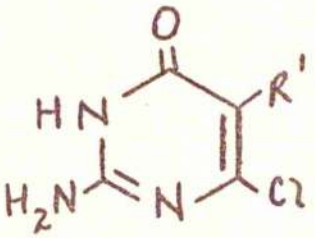

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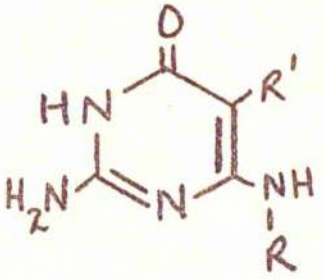


(47)

(ii) 2 - Amino - 4 - alkylaminopyrimidines (47; R^0 = H) have been prepared[1,41,42] from similar precursors (46; R^0 = H) but the conditions required are more drastic, temperatures of 120° to 140° being required.

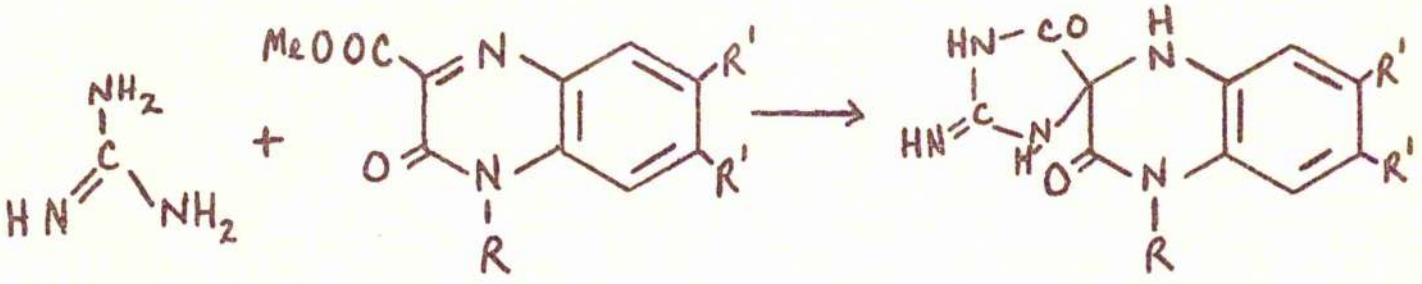
(iii) A much more efficient procedure for the synthesis of the 4,5 - diamino derivatives of uracil and of 2 - amino - 6 - hydroxy-pyrimidine is to first introduce an electron - withdrawing group into the 5 - position of the chloropyrimidines (43 and 46; R^0 = H) to activate the chloro group which then readily condenses with alkylamines under relatively mild conditions. Thus, potential 5 - amino groups have been provided by a phenylazo group[43-45] or by a

nitro group.[33,41]

(c) The use of 4,5 - dimethyl - 1,2 - benzoquinone dimer for the synthesis of benzo [g] pteridines[46] made possible another approach to isoalloxazine derivatives and lumiflavin 2 - imine (50; R = R' = CH_3) [47] was obtained from an appropriate methyl-aminopyrimidine (47; R = CH_3; R' = NH_2) and the dimeric quinone. Later, Cresswell, Neilson and Wood[48], and Davoll and Evans[41] extended this procedure to provide a new general method for the synthesis of riboflavin and other isoalloxazines.

(3) From Quinoxaline Derivatives.

2 - Imino - isoalloxazines (50) have also been obtained by Wood et al.[47] from methyl - 4 - alkyl - 3, 4 - dihydro - 3 - oxoquinoxaline - 2 - carboxylates (48; R= D - ribityl, R' = CH_3; R = CH_3, R' = H) and guanidine. Intermediate compounds formed in this reaction have been identified as octahydroglyoxaline - 4 - spiro - 2' - quinoxalines (49).



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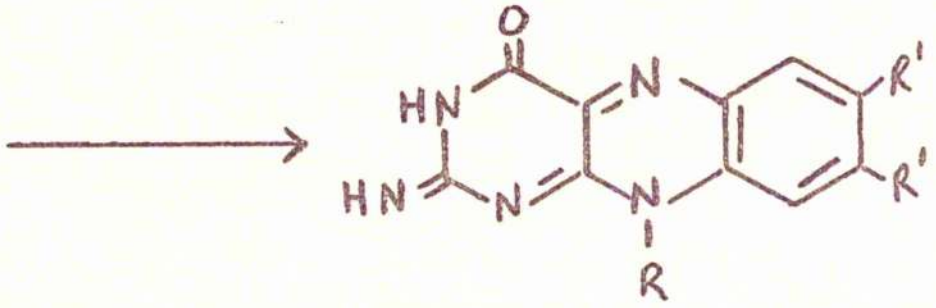


(50)

This new route to isoalloxazine derivatives is based on a pteridine synthesis reported by the same school.[49] The isolation in good yield of the products (50), however, proved difficult due to their facile degradation in particular by alkali, the 9 - alkyl substituent being readily cleaved.

BIOSYNTHESIS OF RIBOFLAVIN.

Although it has been known for many years that riboflavin is a normal constituent of all living organisms[50] and that it acts as an electron carrier in metabolism,[13] it is only in the last decade or so that a detailed study has been made of the biogenesis of the vitamin. Our knowledge concerning the mechanism of biosynthesis of riboflavin is by no means complete but the combined results obtained from (i) nutritional studies, (ii) isotope experiments, (iii) the isolation and characterisation of metabolic intermediates, and (iv) chemical studies in vitro of the reactions involved, have provided a considerable amount of information about the overall nature of the biosynthetic process.

In an attempt to clarify the existing information on the roles played by the diverse precursor compounds, a brief survey is given below.

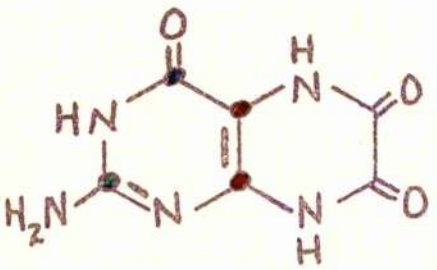
(a) Role of Amino Acids and Simple Organic Compounds.

Similarity in the labelling pattern in analogous portions of purine and flavin molecules was demonstrated when cells of A. gossypii and E. ashbyii were grown with ^{14}C - formate, ^{14}C - carbon dioxide, ^{14}C - glycine and glycine - ^{15}N. 51 - 53.

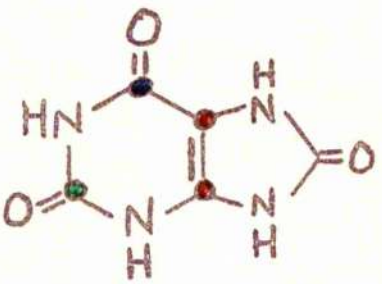
Experiments of this type have also been carried out on pteridines by Weygand and Waldschmidt[54] who reported incorporation of radio-

active formate and glycine into leucopterin by the butterfly Pieris brassicae, and by others who described the distribution of label in certain ribitylpteridines (see (C) below).

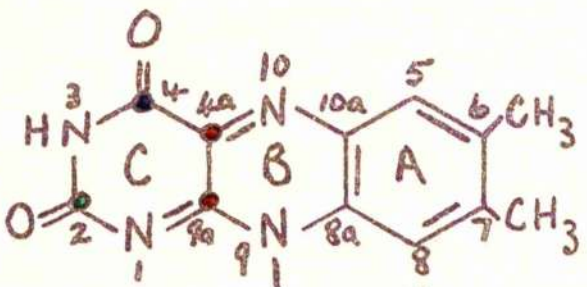
The incorporation of various labelled substances into riboflavin, purines and pteridines is shown below.



Leucopterin.



Uric acid.



Ribityl Riboflavin.

• = $\underline{C}O_2$

• = H.$\underline{C}$OOH

• = $H_2N\underline{C}H_2\underline{C}OOH$

Thus, it is observed[55] that in the case of riboflavin, C_2 is derived from formic acid, C_4 from carbon dioxide, and C_{4a} and C_{9a} from the methylene and carboxyl carbon atoms of glycine respectively, the glycine nitrogen atom being incorporated into position 9 or 10. These results find support in the work of Buchanan et al.[56] on the incorporation of the same labelled compounds into uric acid. (See

above). Furthermore, Klungsøyr[57] demonstrated the incorporation of labelled formate into position 2 of riboflavin and Goodwin showed that serine stimulated flavinogenesis,[58,59] being transformed into formate and glycine before its incorporation.

(b) Role of Purines as Precursors of Rings B and C.

Strong stimulation of riboflavin formation by the addition of purines to the growth medium has been noted with flavinogenic organisms.[59 - 62] Among the first investigations on the biosynthesis of riboflavin were the nutritional studies of MacLaren.[60]

Observing the structural similarities among the purines, diaminopyrimidines and riboflavin, he supplemented the culture medium of E. ashbyii with various purine and pyrimidine bases in order to study their effects on flavinogenesis. The results clearly showed that a definite increase in riboflavin production was obtained, without influencing growth, on the addition of xanthine, adenine, guanine and uric acid; pyrimidines such as uracil, on the other hand, inhibited flavinogenesis. It has also been observed[51,63] that ^{14}C - orotic acid is not incorporated into riboflavin.

Definite evidence for a precursor - product relationship between purines and flavins was produced by the isotope experiments of McNutt[62,64] who found that the carbon atoms from uniformly

^{14}C - labelled adenine were incorporated into the pyrimidine moiety of the isoalloxazine, but there was little incorporation from adenine - 8 - ^{14}C. This implies that the portion of the purine ring system exclusive of carbon 8 is utilised directly for rings B and C without further degradation. Recently Smith et al.[65] suggested that the C_8 which is "lost" from adenine can be re-incorporated at C_2 of riboflavin via the formate pool. McNutt[66] further demonstrated, by degradation of the riboflavin produced by E. ashbyii in the presence of uniformly ^{15}N - labelled adenine, that the nitrogen atoms of the purine were also transferred intact to the vitamin. In addition, 8 - azapurines inhibit flavinogenesis as reported by Brown et al.[61,67] This is to be expected from the above discussion.

Brown et al.[61] established that the purine precursors of riboflavin fall into the following order of decreasing effectiveness: guanine, xanthine, adenine, hypoxanthine and uric acid. The greater effectiveness of guanine compared with xanthine and the fact that most of the pteridines isolated from natural sources have a 2 - amino group, raised the possibility that 2 - iminoriboflavin may be the immediate precursor of riboflavin. However, it has been demonstrated that 2 - iminoriboflavin neither promotes nor inhibits flavinogenesis.[68]

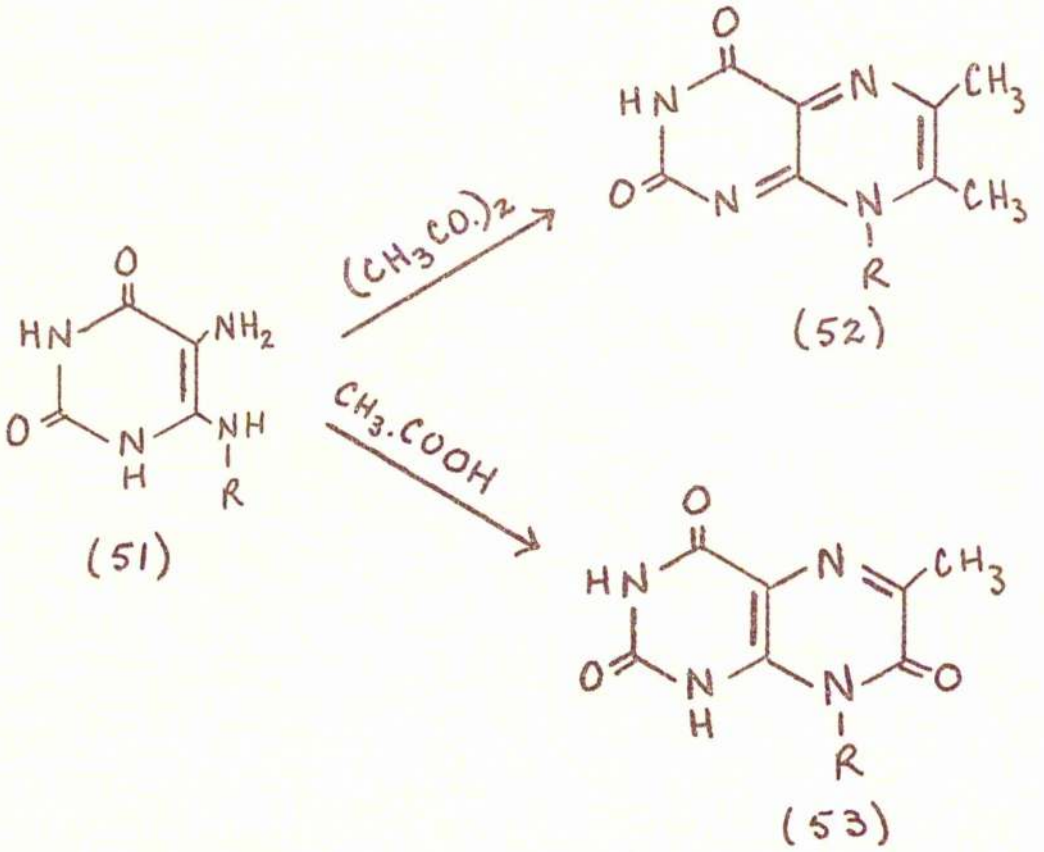
(c) Role of 8 - Substituted Pteridines.

It has been realised in recent years that certain 8 - substituted pteridines play an important part in the production

of riboflavin in microorganisms.

(i) Isolation and Synthesis.

A significant advance was made by Masuda and his collaborators when they isolated a new metabolic intermediate,[69 - 71] which they referred to as " G - compound," due to its green fluorescence, from the mycelium of E. ashbyii. It was ultimately identified[71] as 6,7 - dimethyl - 8 - D - ribityllumazine (52; R = D - ribityl) on the basis of spectrophotometric analysis and chemical degradation.



(51) (52) (53)

The same compound was subsequently isolated from cultures of A. gossypii by Maley and Plaut[38] who noted further that during growth of the organism in the presence of ^{14}C - labelled glycine, the specific radioactivity of the lumazine derivative was higher than that of the vitamin in the early stages of incubation. This

suggested the possibility of a precursor relationship.

Besides riboflavin and " G - compound," Masuda isolated considerable amounts of F.A.D.[72] and another compound which exhibited a violet fluorescence (" V - compound "; q.v. (iii) below). This compound was subsequently shown to be another 8 - D - ribitylpteridine, namely, 7 - hydroxy - 6 - methyl - 8 - D - ribityllumazine. (53; R = D - ribityl).

Consideration of the results of the experiments with labelled purines (described in (b) earlier), leads to the conclusion that the pyrazine ring (Ring B) of riboflavin is formed by elimination of the carbon atom in position 8 of the purine, yielding a diamino-pyrimidine derivative. The lumazine derivatives (52 and 53; R = D - ribityl) could feasibly be formed by condensation of the diaminopyrimidine (51; R = D - ribityl) with a 1,2 - dicarbonyl compound, such as diacetyl or acetoin, and pyruvic acid respectively, the latter compounds being formed from acetate.

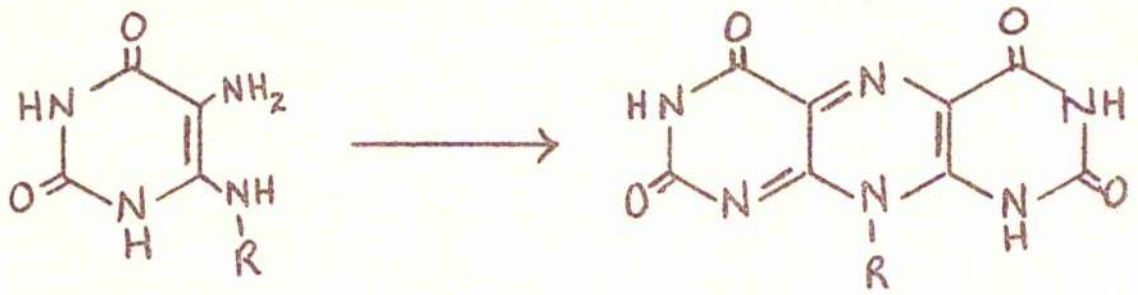
Such reactions were first postulated by Masuda.[69] The pteridines (52 and 53; R = D - ribityl) have been synthesised in this way *in vitro* by several investigators[34,39,41,71,73,74]. but there is not yet any direct experimental evidence for the formation of the hypothetical diaminopyrimidine (51; R = D - ribityl) in the cultures of microorganisms.

Brown *et al.*[67] tested the effect of 4,5 - diaminouracil (51; R = H) on flavin production using *E. ashbyii* but they failed

to find stimulation of riboflavin formation. However, Goodwin and Treble[75] reported that the addition of glyoxal to growing cultures of E. ashbyii led to formation of lumazine, indicating the presence of 4,5 - diaminouracil (51; R = H) in the medium. It should perhaps be emphasised at this stage that inactivity of diaminopyrimidines does not exclude the possibility that substituted aminopyrimidines are the actual intermediates. It may well be that the ribitylamino derivative (51; R = D - ribityl) is the intermediate involved.

Masuda's theory is supported by Kishi et al.[76] and by Katagiri et al.[73,77] who showed that the formation of " G - Compound " (52; R = D - ribityl) from the pyrimidine (51; R = D - ribityl) and acetoin required the presence of an extract from certain microorganisms. The enzyme preparation appears to effect the oxidation of acetoin to diacetyl.[78] The latter condenses readily with the diaminopyrimidine derivative and this reaction is non-enzymic.

It is perhaps not surprising that the required intermediate (51; R = D - ribityl) has not been demonstrated in organisms as it readily self-condenses[33] to give the pyrimidopteridine (54; R = D-ribityl). However, the answer is more complex than this would suggest as this self-condensation product has not been isolated from cultures of the organisms in question and Goodwin and Treble[75] recovered lumazine and not 8 - D - ribityllumazine upon addition of glyoxal to the medium of E. ashbyii.



(51) (54)

(ii) Metabolic Studies of 6,7 - Dimethyl - 8 - D - ribityl - lumazine. (" G - compound ").

That "G - compound" is probably a precursor of riboflavin is suggested by the following factors: (a) its similarity in chemical configuration to the vitamin, (b) A. gossypii incorporates radioactivity from ^{14}C - formate into analogous positions (C_2) in both compounds,[39,55] (c) chemical condensation of the lumazine with diacetyl is claimed by Mazuda[69] to give riboflavin, (d) Plaut[78] showed that riboflavin is produced in incubation mixtures containing only " G - compound," a buffer and an enzyme. Additional evidence is given in the Theoretical Section of Part I.

Furthermore, the molar specific radioactivity of 6,7 - dimethyl - 8 - D - ribityllumazine is always greater than that of the vitamin in the early periods of incubation,[38,39] indicating that the lumazine derivative is an intermediate in riboflavin biosynthesis and not likely to arise by degradation of the latter. Many experiments[77,79 - 82] using a number of microorganisms have

confirmed this conclusion, increased formation of riboflavin having been found on addition of " G - compound." Maley and Plaut[81] have also shown that on incubation of extracts of A. gossypii with 6,7 - dimethyl - 8 - D - ribityllumazine - 2 - ^{14}C, the molar specific radioactivity of riboflavin formed is of the same magnitude as that of the added lumazine derivative which must therefore be utilised for the production of the vitamin and not merely facilitate the catalytic conversion of some other constituent in the extract to the flavin.

Lactobacillus casei, which requires riboflavin for growth, cannot convert " G - compound " into riboflavin,[38,82,83] while microorganisms which can grow in media without added riboflavin do possess this conversion activity. This is consistent with the view that "G - compound " is an intermediate in the biosynthesis of the vitamin. On the other hand, Katagiri et al.[83] observed that beef liver extracts are also capable of effecting this transformation and this observation has found a measure of support from the work of Kuwada et al.[84] who found conversion activity with extracts of bovine liver but not when the same technique was applied to the liver of rats or rabbits.

Korte and his collaborators[63,82] questioned the validity of the route to the flavin via " G - compound ". They reported that the labelled lumazine isolated from a batch of E. ashbyii which had been inoculated with guanine - 5 - ^{14}C could not be converted into riboflavin when inoculated into yet another batch

of E. ashbyii. However, there are doubts as to what conclusions can be drawn from these results as many cases are known in which an added compound has not been utilised by an intact cell but has nevertheless been proved to be an intermediate of a metabolic pathway within the cell.[85]

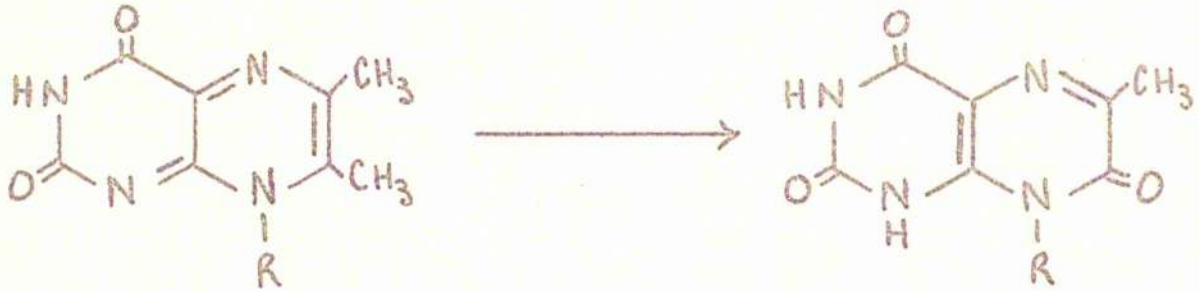
(iii) 7,8 - Dihydro - 2,6 - dihydroxy - 6 - methyl - 7 - oxo - 8 - D - ribitylpteridine. (" V - compound ").

This interesting compound was isolated from cultures of E. ashbyii,[6,86] A. gossypii[7,87] and Clostridium acetobutylicum[88], and was given the trivial name " V - compound " by Masuda[6] due to its violet fluorescence in solution. After some uncertainty there is now general agreement on its structure. (53; R = D - ribityl).[37,40,89]

" V - compound " has been shown[40,87,90] to be related metabolically to " G - compound " and riboflavin by using cultures of E. ashbyii and A. gossypii. Extensive transfer of radioactivity was observed from ^{14}C - labelled adenine and guanine into the pyrimidine portion of the molecules and from ^{14}C - formate into carbon 2.

Masuda suggested[89] that " V - compound " was formed by reaction of pyruvate with 5 - amino - 4 - D - ribitylaminouracil as in its chemical synthesis[7,87] and to support this mechanism reported[91] that the addition of large quantities of pyruvate to cultures of E. ashbyii enhanced the formation of " V - compound "

and reduced the quantity of riboflavin produced. Masuda interpreted this as a competition of pyruvate and acetoin for available 5 - amino - 4 - D - ribitylaminouracil, increased amounts of pyruvate leading to the production of more of the 7 - oxopteridine and less " G - compound ", and hence less riboflavin. However, an alternative explanation is that " V - compound " (53; R = D - ribityl) is formed by displacement of the diacetyl residue of " G - compound " (52; R = D - ribityl) by pyruvate.



(52) (53)

There is also strong evidence[39,63,82] to suggest that this transformation may be an oxidative one and this is discussed in greater detail in the Theoretical Section of Part I.

It is generally agreed[7,86,90,91] that " V - compound " is an end-product of metabolism and is not an intermediate in flavinogenesis.

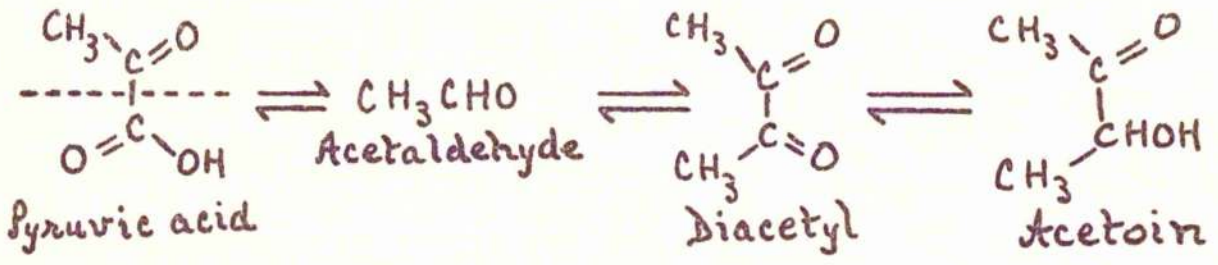
(iv) The Formation of the o - Xylene Ring of Riboflavin.

The o - xylene ring (Ring A) of riboflavin presents rather a novel problem of aromatic biosynthesis.

Goodwin and Pendlington[59] showed that flavin production was stimulated by threonine in E. ashbyii and they explained this by postulating that the o - xylene moiety of riboflavin was formed by condensation of 2 molecules of threonine. This hypothesis was later shown by the same school[92] to be untenable as the label from uniformly ^{14}C - labelled threonine was fixed only into positions 4a and 9a of riboflavin, i.e. into the carbon atoms known to be contributed by glycine.[55] In this case, glycine is probably formed from threonine by way of the enzyme threonine aldolase:

$$CH_3CHOH.\ CH(NH_2).\ COOH \longrightarrow CH_3CHO + CH_2(NH_2).\ COOH.$$

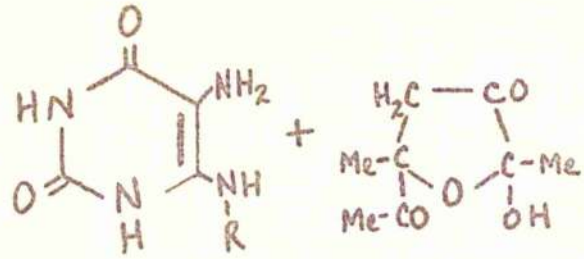
Birch and Moye[31] suggest that the dimethylbenzene ring is formed by head to head and head to tail linkages of four molecules of acetic acid, probably as diacetyl or acetoin derived from four molecules of pyruvic acid.



This suggestion has been supported by the isotope experiments of Plaut.[93] Furthermore, radioactivity from acetoin - 1 - ^{14}C when added to E. ashbyii was incorporated exclusively into Ring A of riboflavin,[94] one half of the radioactivity being in the methyl groups, none in carbons 6 and 7, and the remainder presumably in carbons 5 and 8 by analogy with previous experiments[93] with acetate - ^{14}C and glucose - ^{14}C. The labelled acetoin was incorporated more efficiently[94] into Ring A than acetate - 2 - ^{14}C.

The origin of the atoms forming the o - xylene portion of riboflavin thus seems to be established but there has been some controversy over the manner in which these precursors of Ring A present themselves to the diaminopyrimidine (55; R = D - ribityl).

An interesting hypothesis came from Birch and Moye[31,32] who suggested that Ring A could arise from two molecules of diacetyl by aldol condensation. In fact they were able to synthesise an aldol-type dimer of diacetyl (56) which they condensed with the diaminopyrimidine (55; R = Me) to give lumiflavin (58; R = Me). Later, Cresswell and Wood[33] condensed the dimeric diacetyl of Birch and Moye and also a new trimeric form of diacetyl (60) with the base (55; R = D - ribityl) yielding riboflavin (58; R = D - ribityl) in each case via the intermediate (57; R = D - ribityl).



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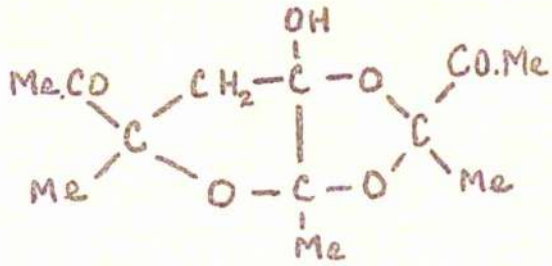

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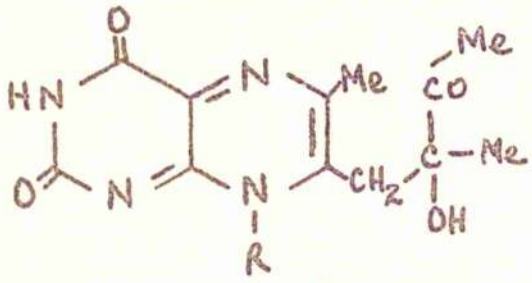

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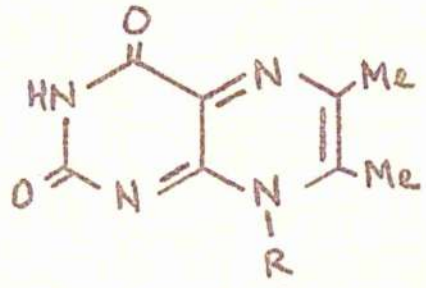

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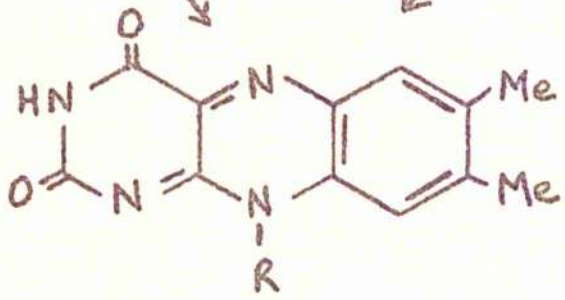


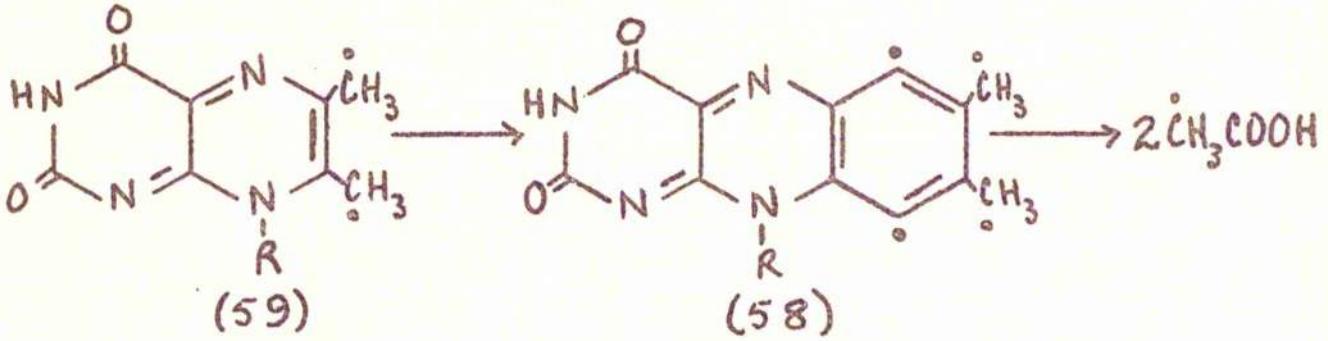
(58)

Masuda[69] postulated evidence that " G - compound " could be chemically converted into riboflavin in presence of acetoin or diacetyl and he suggested that this reaction occurs biologically. Masuda concluded from his results that diacetyl molecules add

successively to the base (55; R = D - ribityl) to give riboflavin (58; R = D - ribityl), via the intermediate " G - compound " (59; R = D - ribityl). However, Birch and Moye[32] could detect no lumiflavin (58; R = Me) when 6,7,8 - trimethyllumazine (59; R = Me) was treated with diacetyl. Furthermore, diacetyl does not appear to fulfil the biological role proposed by Masuda[69] since Katagiri et al.[80] found that the transformation of the ribityllumazine into riboflavin was not stimulated by diacetyl using Clostridium acetobutylicum and Escherichia coli neapolitanus.

Perhaps the most important step towards elucidating the origin of the o-xylene ring of riboflavin was provided by Plaut[78] who reported that incubation mixtures containing only 6,7 - dimethyl - 8 - D - ribityllumazine (59; R = D - ribityl), a suitable buffer and an enzyme were required to obtain formation of riboflavin. Stimulation of activity was not observed on the addition of various substances including acetoin, diacetyl, pyruvate and acetate, and radioactivity from acetate - ^{14}C or glucose - ^{14}C was not incorporated into the vitamin. The implication of these results can only be that " G - compound " supplies not only the ribitylpteridine portion of riboflavin but also the remaining four carbon atoms to complete the o - xylene ring. This is supported by the chemical studies detailed in Part I of this thesis.

If the ribityllumazine (59; R = D - ribityl) condenses with four carbon atoms from an extraneous source, one molecule of the lumazine derivative would be consumed for each molecule of flavin formed in the reaction; on the other hand, if the lumazine acts as both acceptor and donor of the four carbon atoms, then two molecules of the pteridine (59; R = D - ribityl) are required to be enzymically converted into one molecule of riboflavin (58; R = D - ribityl) and the isotope experiments of Plaut[78] indicate that the latter is the case.



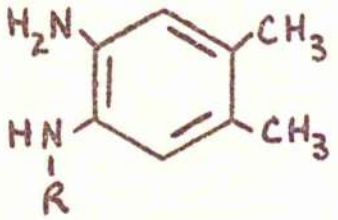
R.S.R. 1 : 2 : 0·5

Thus, 6,7 - dimethyl - ^{14}C - 8 - D - ribityllumazine was enzymically converted into riboflavin and chemical degradation of the latter by chromic acid oxidation to acetic acid resulted in a ratio of relative molar specific radioactivities for " G - compound ", riboflavin and acetic acid of 1:2.2:0.33 which is in good agreement with the expected values shown above.

Further proof of this mechanism would come with the isolation and identification of a product resulting from the

removal of a 4 - carbon unit from the lumazine derivative (59; R = D - ribityl) but such a compound has not yet been detected in the reaction mixtures.

The accumulated evidence indicates that riboflavin biosynthesis starts from the pyrimidine, rather than the benzenoid, end of the molecule. The only reasonable alternative route to riboflavin, that from 2 - amino - 4,5 - dimethyl - 1 - D - ribitylamino - benzene (61; R = D - ribityl), is ruled out owing to the non-stimulatory effect of this compound[59] and a precursor-product relationship could not be shown using other compounds related to the aromatic ring portion of riboflavin in the medium of ascomycetes.[51,59,96] In addition, it has been demonstrated that label from glycine - ^{15}N is recovered in the pyrazine and not the pyrimidine ring of the flavin[97] with cultures of A. gossypii, ^{15}N from uniformly labelled purine is transferred as a unit to the vitamin by cells of E. ashbyii, and " G - compound " is probably an intermediate.



(61)

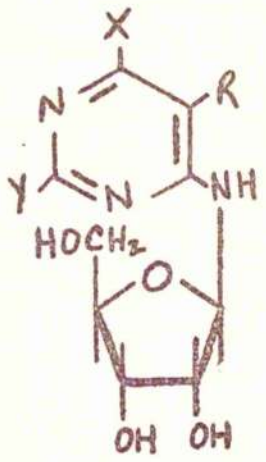
(d) Mechanism of Formation of the Ribityl Side Chain.

Thus far, nothing has been said of the origin of the ribityl group at position 9 of the riboflavin molecule.

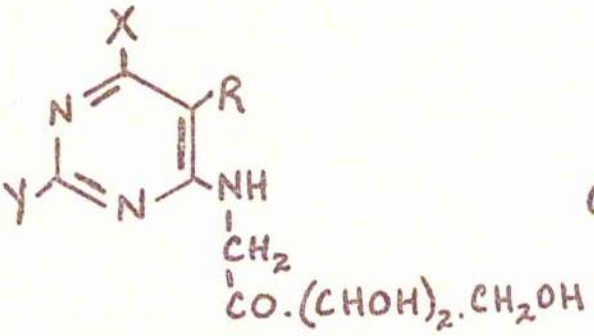
Plaut and Broberg[98] showed that carbon atoms 1,2 and 6 of glucose are all incorporated into the ribityl side chain but the labelling pattern is such as to preclude any simple explanation of mechanism.

McNutt and Forrest[90] using uniformly labelled guanosine observed that purine ribosides are not superior to the free bases, the diaminopyrimidine moiety, but not the ribosyl group, being incorporated into riboflavin in the mould E. ashbyii. However, Weygand's hypothesis[99] for the transformation of purines into pteridines suggests that the pyrimidine glycoside (62; R = NH_2), derived from a purine nucleoside, could undergo an Amadori rearrangement to the ketose (63; R = NH_2). Stereospecific reduction of the carbonyl group of the latter could give a D - ribityl side chain as found in riboflavin.

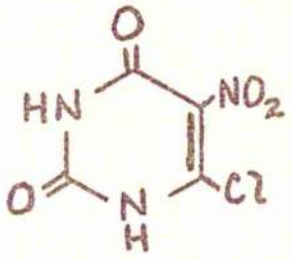
Neilson and Wood[100] in fact synthesised 1 - (2',6' - dihydroxy - 5' - nitro - 4' - pyrimidinylamino) - 1 - deoxy - D - erythropentulose (63; R = NO_2; X = Y = OH) by condensing 4 - chloro - 5 - nitrouracil (64) with 1 - amino - 1 - deoxy - D - erythro-pentulose (65).



(62)



(63)



(64)

$NH_2.CH_2.CO.(CHOH)_2.CH_2OH$

(65)

Stereospecific reduction of the carbonyl group of the pyrimidine (63; R = NO_2; X = Y = OH) followed by reduction of the nitro group and condensation with the dimer of 3,4 - dimethyl - o - benzoquinone gave riboflavin. This mechanism for the formation of the ribityl group seems a feasible one especially since Weygand's route[99] to pteridines from purines <u>via</u> an Amadori rearrangement is generally accepted.

The fact that "G - compound" contains a ribityl side chain on the N_8 atom shows that the introduction of the ribityl group into the riboflavin molecule occurs before the synthesis of the o - xylene ring and possibly prior to the synthesis of the pyrazine ring. Thus, "G - compound" could be formed from the pyrimidine (63; R = NH_2; X = Y = OH) by stereospecific reduction of the carbonyl group, followed by condensation with diacetyl.

SYNTHESIS OF 8 - SUBSTITUTED PTERIDINES.

The methods which have been employed for the syntheses of 8 - substituted pteridines belong to one of the following categories: (i) synthesis from diaminopyrimidines, (ii) synthesis from pyrazine derivatives, or (iii) direct alkylation of pteridines.

1. From Diaminopyrimidines.

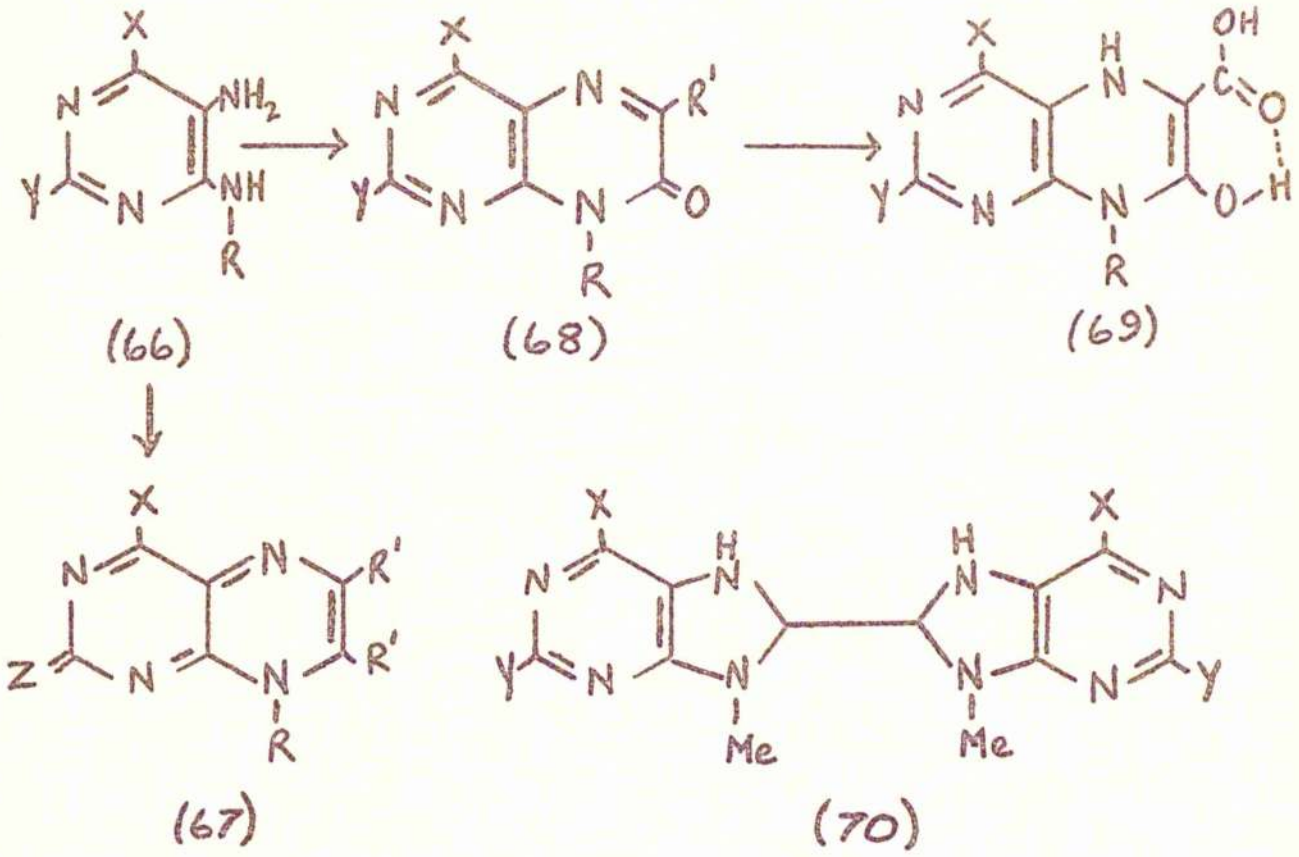
By far the most common of the three procedures is the reaction of diaminopyrimidines with dicarbonyl compounds, condensation taking place readily in the majority of cases.

A few typical reactions have been chosen to illustrate their use in the synthesis of 8 - substituted pteridines and the list given here is by no means exhaustive.

Some simple 8 - alkyl pteridines have been prepared by methods involving the condensation of a suitable 4 - substituted - amino - 5 - aminopyrimidine with a 1,2 - diketone. For example, Fidler and Wood[101] synthesised the pteridine (67; R = R' = Me; X = OH; Z = NH) by reaction of the pyrimidine (66; R = Me; X = OH; Y = NH_2) with diacetyl. Mention has been made, in a previous section, of the synthesis of 8 - D - ribitylpteridines by a similar procedure from the pyrimidine (66; X = Y = OH; R = D - ribityl).

An interesting side reaction was reported by Wood *et al.*[49,102] whereby attempted syntheses of some 8 - methylpteridines of the

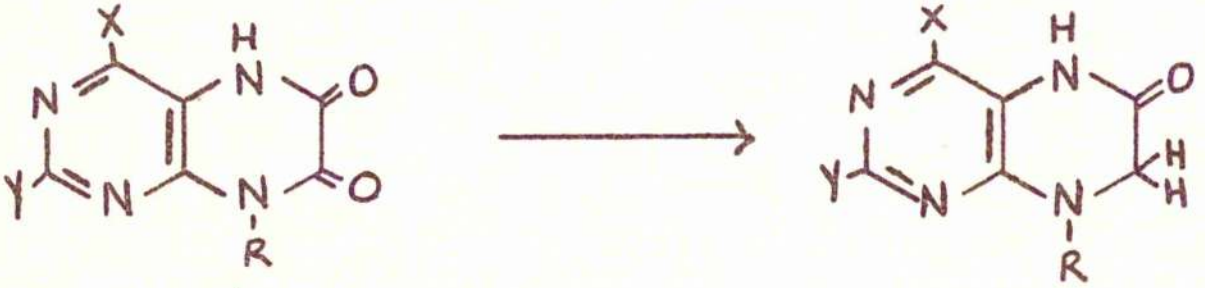
type (67; R = Me; R' = H), involving the reaction of the appropriate pyrimidines with glyoxal, led to the formation of bisdihydropurinyls (70), which were produced presumably by reaction of 1 molecule of glyoxal with 2 molecules of the pyrimidine.



7 - oxo - 8 - substituted pteridines have been prepared by condensation of the diaminopyrimidine derivative (66) with oxalic acid[1], ethyl oxalate,[1] ethyl pyruvate,[103] ethyl glyoxylate hemiacetal,[101,104] or ethyl oxomalonate.[42,103] The last mentioned reagent gives, of course, 8 - substituted pteridine - 6 - carboxylic acids (68; R' = COOH) which can be decarboxylated by heating at 200 - 250°,[42,105] or by treatment with sodium amalgam.[2] However, when these pteridine - 6 -

carboxylic acids are treated with sodium borohydride, a 5,8 - dihydropteridine (69) results,[105] hydrogen bonding taking place between the 7 - hydroxyl group and the carbonyl oxygen atom of the 6 - carboxyl group.

It is notable that the 6,7 - dioxopteridines (71), obtained by reacting pyrimidines of the type (66) with ethyl oxalate and sodium ethoxide, can be converted into the corresponding 7,8 - dihydropteridines (72) by reduction with sodium amalgam[2] and hence is equivalent to the reduction of an amide group. This type of reaction must depend on the depletion of electrons from the carbonyl group undergoing the reaction (i.e. at position 7), due to the proximity of the 6 - carbonyl group, since oxygen is not removed by sodium amalgam from 7 - hydroxypteridine[106] nor from 7 - oxo- pteridine - 6 - carboxylic acids.[2]

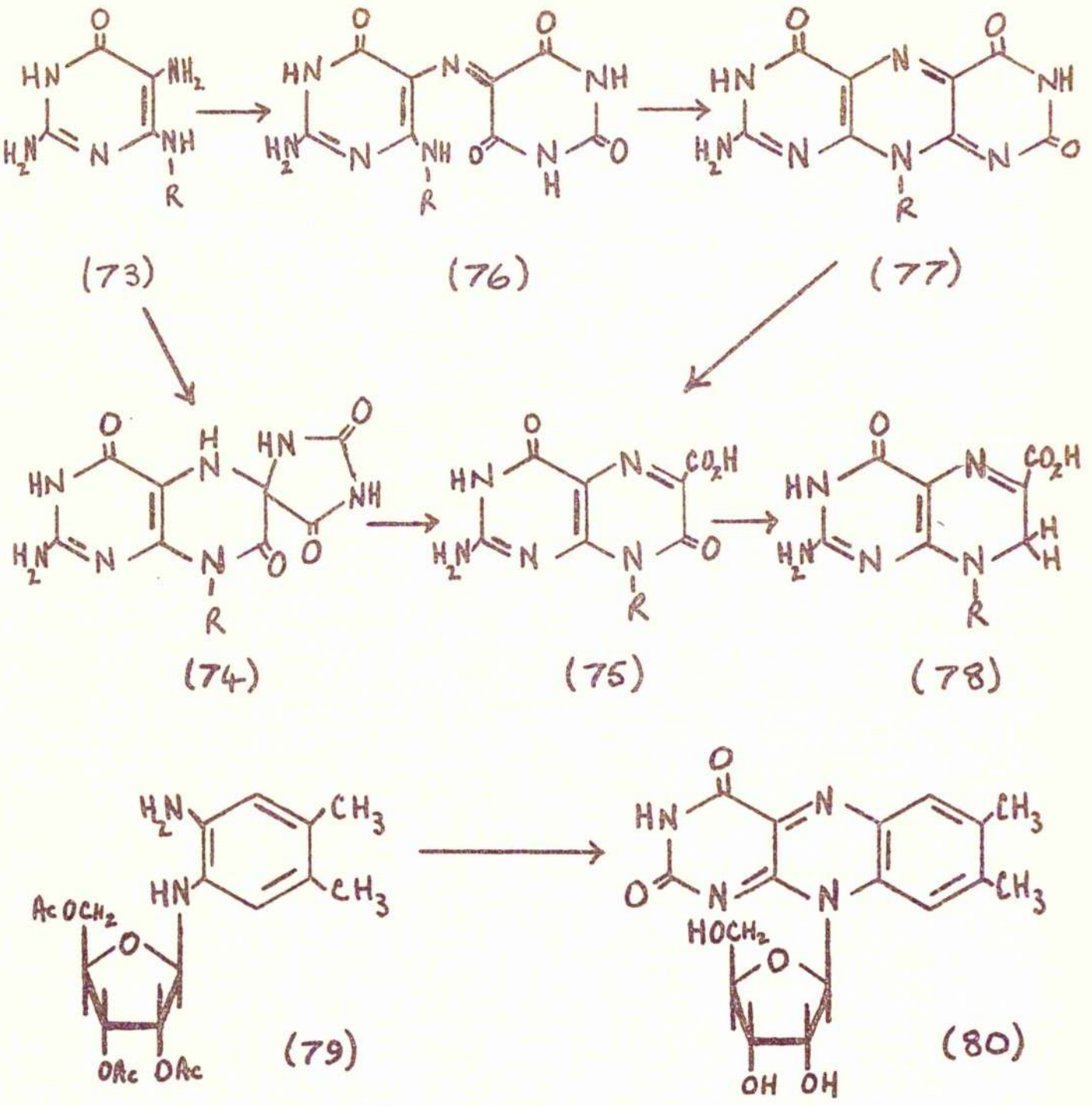


(71) (72)

An alternative method of obtaining 7 - oxo - pteridine - 6 - carboxylic acids (68; R' = COOH) has been discovered by Taylor and Loux[44] who condensed the diaminopyrimidine (73; R = alkyl) with alloxan in alkaline solution. An interesting feature of this reaction is that it appears to follow different routes

depending on the order of the addition of the alloxan and alkali, but the final product (75; R = alkyl) is always the same. Thus, if the alkali is added in sufficient quantity to make the solution of the diaminopyrimidine approximately 0.5 N with respect to sodium hydroxide, and then treated with alloxan, the product (75; R = alkyl) is formed via a spiro intermediate (74; R = alkyl). However, if the alloxan is added directly to the reduction mixture containing the diaminopyrimidine, a deep violet colour due to the anil (76; R = alkyl) appears which then fades on addition of alkali. Therefore, in the latter case the product (75; R = alkyl) is obtained by alkaline degradation of a 9 - substituted pyrimidopteridine (77; R = alkyl). Methods for the reduction of the lactam grouping in the 7 - oxo - pteridine - 6 - carboxylic acids (75; R = alkyl) were investigated by Taylor and Loux[44] and this was finally achieved under Clemmensen conditions, reduction by lithium aluminium hydride being inapplicable due to solubility difficulties.

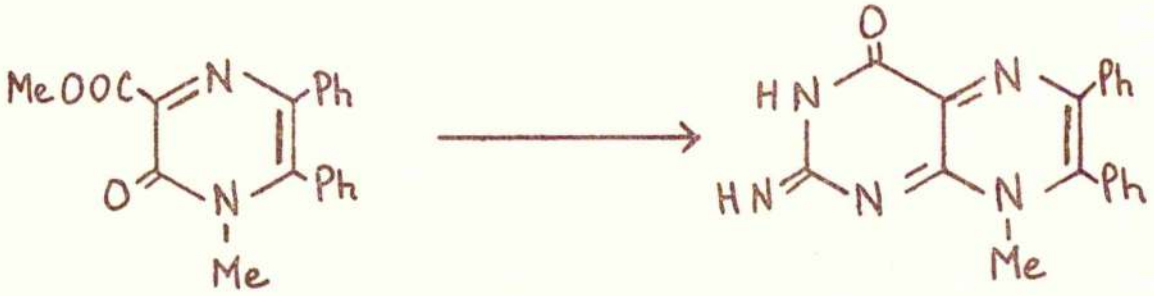
It is perhaps pertinent at this point to mention that Kuhn and Strobele[107] obtained isoalloxazine - 9 - glycosides by treating the o - xylidine glycoside acetate (79) with alloxan followed by deacetylation to give the flavin glycoside (80) which was remarkably labile, being hydrolysed even by cold dilute acetic acid or 0.1 N sodium hydroxide and it was very much more sensitive to light than riboflavin.



5 - Amino - 4 - substituted aminopyrimidines have also been condensed with α - hydroxyketones, such as benzoin,[1,101,108] to form 8 - substituted pteridines and with halo compounds, for example chloroacetyl chloride,[1] yielding 6 - oxo - 8 - substituted pteridines.

2. From Pyrazine Derivatives.

Another approach to the synthesis of 8 - substituted pteridines was provided by Dick, Fidler and Wood[49] whose starting material was a pyrazine derivative rather than a diaminopyrimidine. The synthesis was achieved by fusion of 1,2 - dihydro - 3 - methoxy - carbonyl - 1 - methyl - 2 - oxo - 5,6 - diphenylpyrazine (81) with guanidine carbonate to give the 8 - methylpteridine (82) in 93% yield.

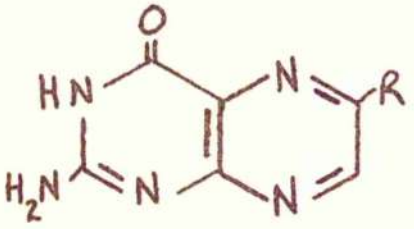


(81) (82)

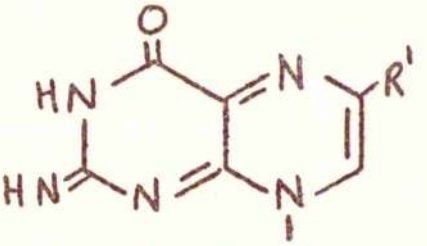
3. Direct Alkylation of Pteridines.

Simple amino - and hydroxy - pteridines are usually alkylated on a nuclear nitrogen atom of the ring which bears the substituents. Thus, 4 - amino -,[109] 4 - methylamino -,[109] 4 - amino - 2 - hydroxy -,[110] 2 - hydroxy -,[111] and 4 - hydroxy[111] - pteridines are all methylated only on N_1 with exception of the last example which also gives an O - and N_3 - methyl derivative. Furthermore, 6 - hydroxy -, 7 - hydroxy - , 2,4 - dihydroxy -, and 6,7 - dihydroxy - pteridines give respectively 5 - methyl -, 8 - methyl - , 1,3 - dimethyl - , and 5,8 - dimethyl derivatives.[111] On the other hand, this rule is somewhat disrupted in the case of

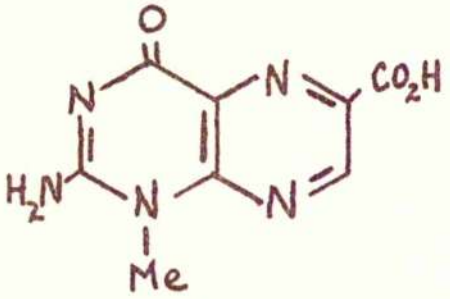
2 - amino - 4 - hydroxypteridine (83; R = H) which is the fundamental nucleus of most known natural pteridines. In contrast to the examples quoted above, this pteridine undergoes transannular methylation yielding exclusively 2 - amino - 4,8 - dihydro - 8 - methyl - 4 - oxopteridine (84; R = Me; R' = H).[110] The structure of the latter was elucidated by (a) preparing unambiguously all of the five possible monomethylated pteridines, methylation on N_5 being precluded by valency considerations, and (b) comparing their physical properties with the product obtained from the methylation reaction.



(83)



(84)



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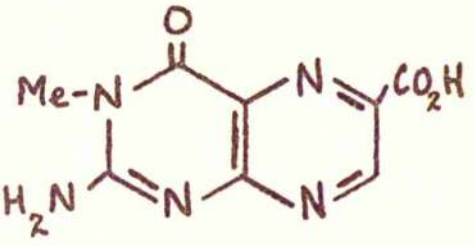

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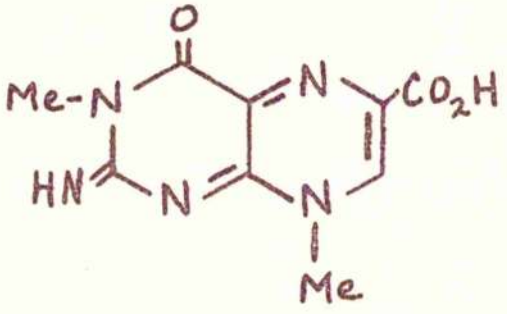


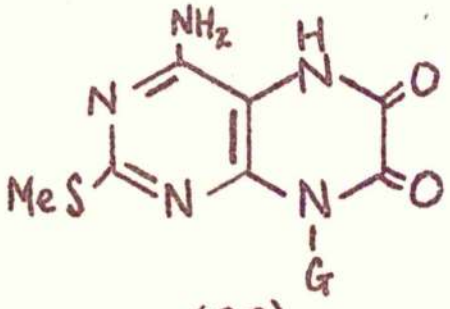
(87)

Recently, Angier and Curran[112] examined the direct methylation of 2 - amino - 4 - hydroxypteridine - 6 - carboxylic acid (83; R = COOH) which gave a variety of products depending on the conditions employed. Methylation of this pteridine in acetic acid - dimethylformamide solution yielded the 8 - methylpteridine - 6 - carboxylic acid (84; R = Me; R' = COOH) supporting Brown & Jacobsen's transannular alkylation. However, when (83; R = COOH) was methylated in alkaline solution, a mixture of the 1 - methyl -, 3 - methyl -, and 3,8 - dimethyl - derivatives (85 to 87) was obtained. Angier and Curran[112] concluded that the neutral molecule (83; R = COOH) undergoes preferential N - methylation in the pyrazine ring at position 8, while in a basic solution the resulting negative charge is located primarily in the pyrimidine ring so that methylation occurs preferentially at the 1 - and 3 - N atoms. Treatment of the 3 - methyl derivative with methyl sulphate in dimethylformamide containing a small amount of sulphuric acid resulted in a slow and incomplete reaction to form the 3,8 - dimethyl compound (87), indicating that methylation is inhibited by acid and that it is actually the neutral molecule which is readily methylated in the 8 - position.

Pteridine - 8 - glycosides.

All of the methods outlined in (1) to (3) above could theoretically be used as model reactions for the synthesis of pteridine - 8 - glycosides; indeed, many of them were carried out with such an aim in view. The 6,7 - dioxo - pteridine -

glucoside (88; G = D - glucosyl) of Todd et al.[1] and the 7 - oxo - pteridine - glucosides of the type (89; X = O; or X = NH; G = D - glucosyl) of Forrest and Lohrmann[113] were synthesised from the appropriate diaminopyrimidines by the Isay reaction. However, no pteridine - 8 - glycoside strictly analogous to the purine nucleosides has yet been synthesised; none with a structure similar to (84; R = glycosyl; R' = H), for example, which is the pteridine analogue of guanosine.



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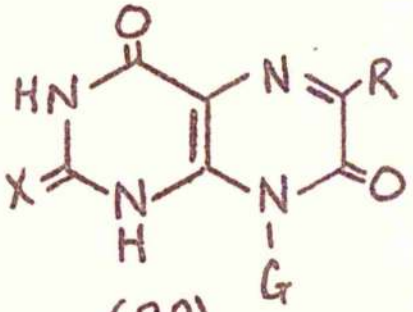


(89)

R = H; Me; OH; COOH.

Pfleiderer and Lohrmann[114] reported the direct alkylation of 7 - oxopteridines with halosugars in presence of silver carbonate, but instead of the expected N - glycosides they obtained pteridine - 7 - O - glycosides.

A new method of synthesising N - glycosides of uric acid has recently been achieved by Birkofer et al.[115] utilising triethylsilyl derivatives of the purine, which on treatment with a halosugar in presence of silver perchlorate yielded the uric acid 3 - glycosides. This technique may prove to be applicable to pteridines but is not discussed further in this thesis.

PART I

CHEMICAL ASPECTS OF THE BIOSYNTHESIS OF RIBOFLAVIN.

THEORETICAL

The chemical studies of Cresswell and Wood[33] prompted a closer examination of the chemical reactions involved in the biosynthetic route to riboflavin (96; R = D - ribityl) from 5 - amino - 4 - D - ribitylaminouracil (92; R = D - ribityl) which is thought to be a precursor of the vitamin. As mentioned earlier, these authors postulated the formation of riboflavin *via* a pteridine intermediate (94; R = D - ribityl) which they synthesised without isolation. Subsequent treatment of the intermediate at pH 1 - 6 or at pH 13 gave riboflavin.

In order to study further the feasibility of this suggested biosynthetic route to riboflavin, attempts were made to isolate the above intermediate so that its effect on flavinogenesis could be examined. The initial problem of the isolation of the very labile pteridine intermediate was only partially solved. This pteridine was synthesised according to the method of Cresswell and Wood[33] by reaction of the dimer of diacetyl with the diaminopyrimidine (91; R = D - ribityl). This reaction gives in addition small amounts of another pteridine to which the aforementioned authors assigned structure (95; R = D - ribityl). The desired pteridine (94; R = D - ribityl) was separated by large scale paper chromatography its isolation being possible only on paper chromatogram strips.

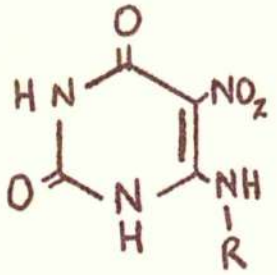
A strain of the microorganism Nematospora gossypii (or Ashbya gossypii,) which was kindly supplied by Prof. E.O. Morris (R.C.S.T., Glasgow) proved to be too weakly flavinogenic for our purposes. The paper chromatogram strips of the pteridine intermediate were therefore sent to Dr. G.W.E. Plaut (University of Utah) for microbiological investigation but due to its extreme instability the pteridine did not survive the journey. Recently, however, Asai and Kuwada[95] confirmed the formation of riboflavin from the pteridine (94; R = D - ribityl) by the action of acid but reported that this pteridine did not give riboflavin in vitro by the action of the enzyme from the mycelium of E. ashbyii, while 6,7 - dimethyl - 8 - D - ribityl - lumazine (92; R = D - ribityl) when treated with the enzyme under the same conditions did, in fact, produce riboflavin.

Attention was next focussed on the two 8 - D - ribityl - pteridine derivatives isolated by Masuda[6,70] from cultures of E. ashbyii, namely, 6,7 - dimethyl - 8 - D - ribityllumazine ("G - compound") (92; R = D - ribityl) and 7,8 - dihydro - 2,4 - dihydroxy - 6 - methyl - 7 - oxo - 8 - D - ribityl- pteridine ("V - compound") (93; R = D - ribityl), with a view to examining their role in the biosynthetic pathway to riboflavin from a chemical standpoint.

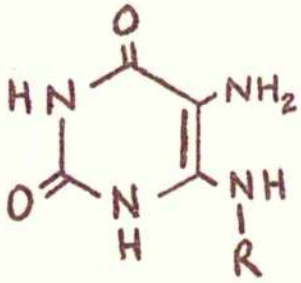
The biological conversion of "G - compound" into "V - compound" has been previously mentioned in the Historical Section. A chemical study of this conversion which was thought to be

desirable is now described.

"G - compound" and "V - compound" were synthesised from 5 - amino - 4 - D - ribitylaminouracil (91; R = D - ribityl) by condensation with diacetyl and pyruvic acid respectively.



(90)



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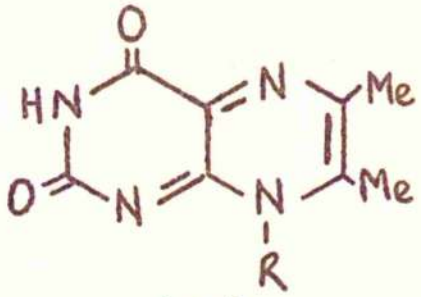
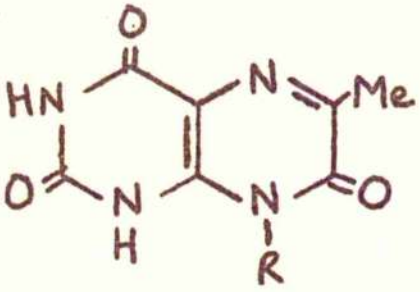
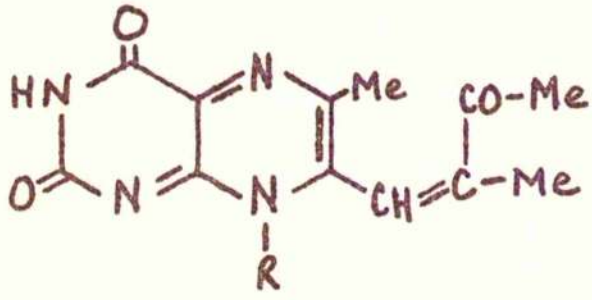


(92)



(93)

(94)



(95)

(96)

It was thought that if the "quinonoid" pteridine (92; R = D - ribityl) could be reduced to the corresponding 5,6,7,8 - tetrahydropteridine or the 7,8 - dihydro derivative these might then undergo a displacement reaction with pyruvic acid to form the 6 - methyl - 7 - oxopteridine (93; R = D - ribityl). It was decided that a model reaction using the less expensive 8 - (2' - hydroxyethyl) - pteridine (92; R = CH_2CH_2OH) would prove valuable. The 5 - aminopyrimidine (91; R = CH_2CH_2OH), obtained by hydrogenation of the 5 - nitro compound (90; R = CH_2CH_2OH), was isolated as the hydrochloride. (The isolation of diaminopyrimidines of this type as hydro - chlorides gave pteridine products of higher yield and purity). Subsequent condensation of the amine hydrochloride with diacetyl in methanol gave the lumazine derivative (92; R = CH_2CH_2OH). The 6 - methyl - 7 - oxopteridine (93; R = CH_2CH_2OH) was furnished by a similar condensation of the amine hydrochloride with pyruvic acid.

Two mols. of hydrogen were consumed by the quinonoid pteridine (92; R = CH_2CH_2OH) when hydrogenated over pre-reduced platinum oxide, indicating the formation of a tetrahydropteridine. Removal of the catalyst and concentration of the solution <u>in vacuo</u>, however, yielded a product, the ultraviolet spectra of which suggested that it was a 7,8 - dihydropteridine which had presumably been formed by oxidation of the tetrahydro - pteridine during the isolation procedure.

Structure (97; R = CH_2CH_2OH; R' = H) was assigned to the product on the basis of (a) its ultraviolet spectra in acid and alkali which were similar to those of a known 7,8 - dihydropteridine (see Fig. 1); (b) hydrogenation when 1 mol. of hydrogen was consumed; (c) rapid aerial oxidation on heating in acid or alkaline solution to give the original lumazine derivative (92; R = CH_2CH_2OH); and (d) elementary analysis.

(97)

(98)

Treatment of the 7,8 - dihydropteridine (97; R = CH_2CH_2OH; R' = H) with pyruvic acid in water or in acid solution yielded the lumazine (92; R = CH_2CH_2OH) in addition to a mixture of decomposition products. None of the 6 - methyl - 7 - oxopteridine (93; R = CH_2CH_2OH) could be detected. When the reaction was carried out in alkaline solution there was again a complex mixture of products but one of these was identified by Rf (6 solvent systems) and ultra violet spectra as the 6 - methyl - 7 - oxopteridine (93; R = CH_2CH_2OH). It will subsequently be shown that this is probably formed <u>via</u> the quinonoid pteridine (92; R = CH_2CH_2OH) which arises by oxidation of the 7,8 -

dihydro compound (see above). The alternative routes from this quinonoid lumazine to the 6 - methyl - 7 - oxo - pteridine are (a) oxidation of the 7 - methyl group, which at first sight seemed unlikely, and/or (b) displacement of the carbon atoms at positions 6 and 7 and their methyl groups by a pyruvate residue.

Hydration of 8 - Substituted Pteridines.

It is convenient here to digress slightly to consider the hydration of 8 - substituted pteridines as this led to a better understanding of the steps involved in the above problem.

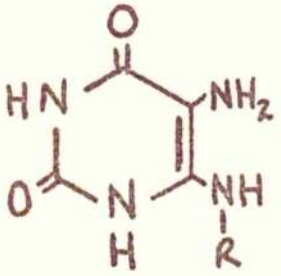
At this time Hemmerich[116] was investigating the ultra-violet spectra of 8 - substituted pteridines of the type (92) and suggested that the anomalous strong hypsochromic shift from λ_{max} 408 mμ at pH 5.8 to λ_{max} 316 mμ at pH 13 was due to the formation of the anion of the "hydrated" form (97; R' = OH) in the strongly alkaline medium. He also showed that the hydration was promoted by metal ions giving colourless chelates such as (98).

Albert,[117] on the other hand, was of the opinion that the hydrated form was represented by the peak at ca. 410 mμ and the anhydrous form by the peak at ca. 310 mμ. His conclusions are based on the following observations: (a) 4 - hydroxypteridine on reduction to its 7,8 - dihydro derivative exhibits a bathochromic and not a hypsochromic shift, which phenomenon is also found in the conversion of quinoline to dihydroquinoline, and (b) 8 - methyl -

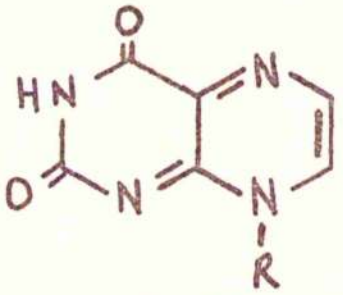
lumazine (99; R = Me), unlike the 8 - D - ribityl derivative, retains the 410 mμ peak in addition to the new peak at 310 mμ on increasing the pH from 1 to 13. Albert suggests that the cation and neutral species of 8 - methyllumazine are therefore fully hydrated but the anion is a hydrated - anhydrous equilibrium mixture. Thus, he argues, a methyl group at position 8 cannot prevent a certain amount of hydration (represented by the 410 mμ band) but bulkier groups make it sterically impossible for the water molecule to remain there.

It was decided to examine more closely this question of hydration of 8 - substituted pteridine derivatives. 8 - (2' - Hydroxyethyl) - lumazine (99; R = CH_2CH_2OH) was synthesised by condensation of glyoxal with 5 - amino - 4 - (2' - hydroxy - ethylamino) - uracil hydrochloride (91; R = CH_2 CH_2OH) in methanol and its ultraviolet spectra recorded. On changing the pH from 1 to 13, the 410 mμ peak completely disappeared and a new peak at 310 mμ was observed. It was found moreover that the oxidation of 8 - (2' - hydroxyethyl) - lumazine (99; R = CH_2CH_2OH) to the 7 - oxo derivative (100; R = CH_2CH_2OH) takes place much more readily under alkaline than under neutral or acid conditions. This confirms that nucleophilic attack by the hydroxyl ion has taken place at position 7 and argues in favour of the anion being the hydrated species (i.e. represented by the 310 mμ band) in agreement with Hammerich's theory. Furthermore, when simple alkyl groups are present in the 7 or 8 positions then there is a certain steric hindrance to the hydration process,

exemplified by the presence of the 410 mμ peak in 8 - methyllumazine.



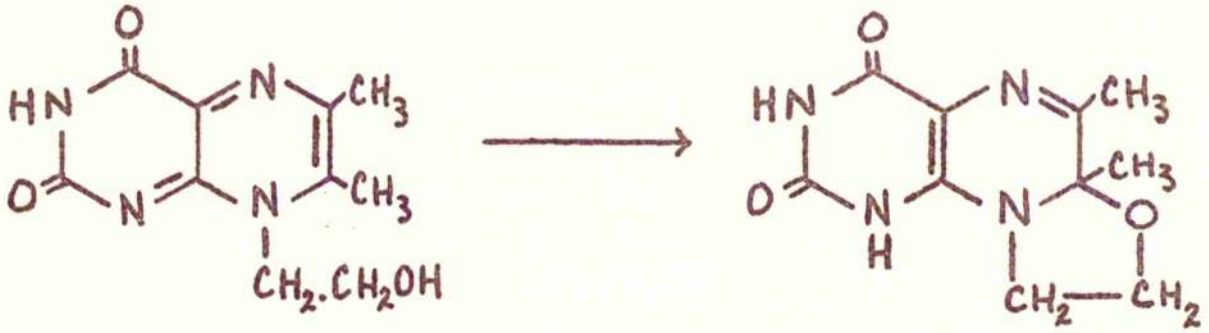
(91)



(99)

(100)

The greater ease of hydration when a hydroxyethyl - or similar group, such as D - ribityl, is present in position 8 can be explained by the increased possibility of hydration due to intramolecular addition as in the following scheme:



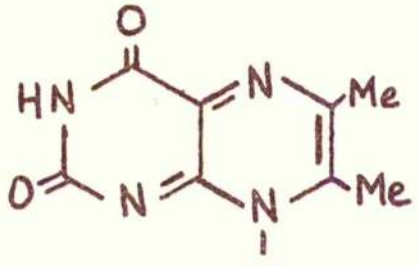
From the ultraviolet spectrum at pH 13 of 8 - (2' - hydroxy - ethyl) - lumazine (99; R = CH_2CH_2OH), $\lambda_{max.}$ 231, 282, 311 mμ and that of the corresponding 6,7 - dimethyl derivative, $\lambda_{max.}$ 231, 283, 316 mμ , the methyl group in position 7 is apparently exerting a little influence on the hydration in alkaline solution, which is thought to be an intramolecular reaction.

Perhaps the best evidence for the anion of 8 - substituted

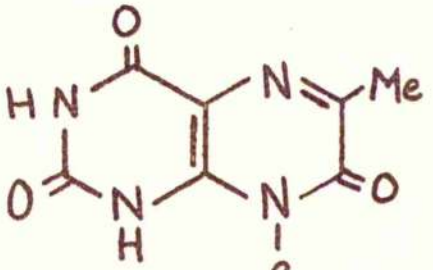
pteridines being in the hydrated form is to be found in the comparison of the ultraviolet spectra at pH 13 of the lumazine (92; R = CH_2CH_2OH), $\lambda_{max.}$ 231, 283, 316 mμ with that of the corresponding 7,8 - dihydropteridine (97; R = CH_2CH_2OH; R' = H) $\lambda_{max.}$ 231, 283, 317 mμ , which are virtually identical (see Fig. 2). Reacidification of the alkaline solution of the 7,8 - dihydro - pteridine gave the absorption maxima of the neutral molecule of the 7,8 - dihydropteridine showing that the latter had not merely been oxidised to the 6,7 - dimethyllumazine (92: R = CH_2CH_2OH).

Displacement Reactions.

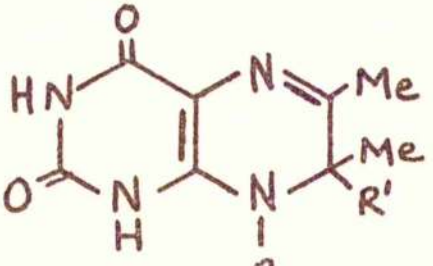
Having reasonably established that 8 - substituted pteridines of the type (92) exist in the hydrated form (97; R' = OH) in alkaline solution, it seemed possible that the compound (97; R = D - ribityl; R' = OH), which is a carbinolamine, is an intermediate in the biological conversion of Masuda's "G - compound" (92; R = D - ribityl) into "V - compound" (93: R = D - ribityl).



(92)



(93)



(97)

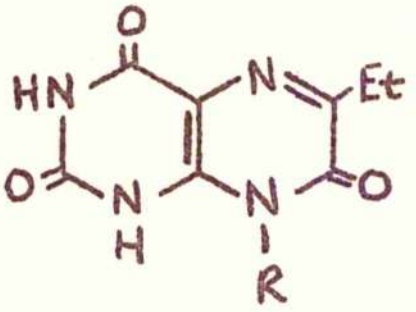
It has now been found [118] that this transformation can be brought about *in vitro* by refluxing the lumazine (92; R = CH_2CH_2OH; or R = D - ribityl) with pyruvate for 8 hr. at pH 13 to give the 6 - methyl - 7 - oxopteridine (93: R = CH_2CH_2OH; or R = D - ribityl) identified by ultraviolet and infrared spectral comparison and by paper chromatography with authentic samples. This, however, did not rule out the possibility of oxidative removal of the 7 - methyl group as an alternative route to the 7 - oxopteridine.

An aqueous solution of 6,7 - dimethyl - 8 - D - ribityl - lumazine (92; R = D - ribityl) when left exposed to the air suffered no change after 7 days but on standing for several weeks a little of the 6 - methyl - 7 - oxopteridine (93; R = D - ribityl) was detected by paper chromatography. Similarly, an alkaline solution of the lumazine derivative through which oxygen passed was slowly converted to the 6 - methyl - 7 - oxopteridine, refluxing for 15 hr. being required for complete conversion. Thus, oxidation is also chemically feasible for the conversion of (92) into (93).

When the reaction of the lumazine (92) with pyruvate at pH 13 was carried out under oxygen - free nitrogen, the 6 - methyl - 7 - oxopteridine (93) was obtained as before. The conversion of (92) into (93) was also observed when the former was kept in the presence of pyruvate at 37° and pH 13 for 9 days but the product was contaminated by other decomposition products. The pH was

varied and the conversion was found to take place at pH values down to 7.3 but the lower the pH employed, the longer the reaction time required.

One final factor in these displacement reactions still remained doubtful. The conversion took place in the absence of oxygen but was it possible for the pyruvic acid to be acting as an oxidising agent and the conversion thereby still following an oxidative mechanism? This hypothesis was readily tested and proved not to be the case. The lumazine (92; R = CH_2CH_2OH) was refluxed under nitrogen in alkaline solution as before but α - oxobutyric acid was substituted for pyruvic acid. The product isolated was the 6 - ethyl - 7 - oxopteridine (101; R = CH_2CH_2OH), no trace of the 6 - methyl - 7 - oxopteridine being detected.

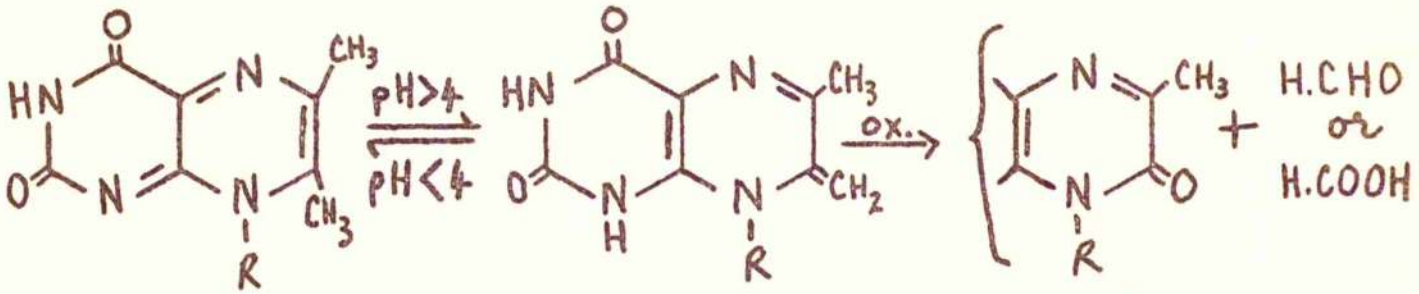


(101)

Thus, in the biological conversion of the 6,7 - dimethyl - lumazine (92) into the 6 - methyl - 7 - oxopteridine (93) both (a) oxidative removal of the 7 - methyl group, and (b) nucleophilic attack at position 7 of the lumazine followed by reaction with pyruvate, seem to be chemically feasible pathways.

However, recent biochemical work indicates that route (a)

may have preference over route (b). Thus, Asai et al.[119] treated 6,7 - dimethyl - 8 - D - ribityllumazine ("G - compound") (92; R = D - ribityl) with the enzyme from E. ashbyii and detected the presence of "V - compound" (93; R = D - ribityl) in the reaction mixture in addition to formaldehyde, formic acid and riboflavin, which they state is explained by the following reactions: -



(92) (102) (93)

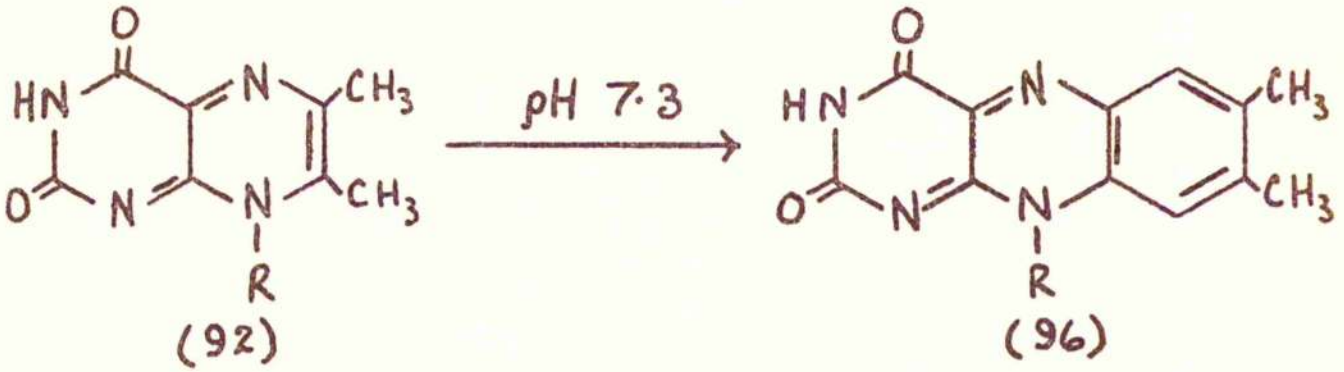
When pyruvic acid and 5 - amino - 4 - D - ribitylaminouracil were treated with the same enzyme under the same conditions, these authors did not detect "V - compound". This seems surprising.

The postulated intermediate (102) is also unlikely to be the primary intermediate as it has already been shown that the lumazine (92) in alkaline solution is hydrated at position 7 and its ultra-violet spectrum is that of a 7,8 - dihydropteridine whereas a structure such as (102) which has an increased chromophoric system would be similar to that of (93). This is not the case. Plaut's recent isotope experiments[120] are more significant and confirm that the biological conversion of "G - compound" into "V - compound" follows an oxidative mechanism. Thus "V - compound" produced enzymically from "G - compound" labelled specifically in the 6,7 -

dimethyl groups, contained half the molar specific radioactivity of the radioactive lumazine, indicating removal of only one of the methyl groups during the conversion.

Synthesis of Riboflavin and Related Compounds.

Whilst investigating the above pyruvate displacement reactions, it was observed that when a solution of 8 - (2' - hydroxyethyl) - 6,7 - dimethyllumazine (92; R = CH_2CH_2OH) in a phosphate buffer of pH 7.3 was refluxed under nitrogen in the dark for 15 hours, the main product was the isoalloxazine derivative (96; R = CH_2CH_2OH). This transformation occurred in the absence of enzymes or any other source of carbon. A similar reaction with 6,7 - dimethyl - 8 - D - ribityllumazine (92; R = D - ribityl) furnished riboflavin (96; R = D - ribityl) in good yield.[121]



(92) (96)

This confirmed Plaut's observation[78] that all the carbon atoms of the o - xylene moiety of riboflavin are derived from the same lumazine (92; R = D - ribityl). In other words, 2 molecules of this lumazine ("G - compound") react under certain conditions to

give 1 molecule of riboflavin. It was decided to ascertain the limits of these conditions and attempt to define a mechanism for the transformation. For this purpose, the lumazine derivative (92; R = CH_2CH_2OH) was used as a model compound, being more readily available than the ribityl analogue.

In order to ensure that the phosphate ions had no role to play in the transformation, the reaction was repeated using formate and borate buffers of the same pH value. This yielded similar results. The pH value was varied but it was found that the original pH of 7.3 (biological pH) was the optimum for iso - alloxazine formation, the yields being almost halved by raising or lowering the pH of the reaction mixture by 1 unit.

The conversion of the lumazine (92) into the isoalloxazine (96) also occurred in strongly acid medium but with reduced yield and purity. The isoalloxazine was detected by paper chromatography after refluxing the lumazine in 4N - sulphuric acid but much decomposition also occurred. 2N - Hydrochloric acid produced somewhat better results than the stronger acid particularly when the lumazine solution was maintained at room temperature in the dark for 6 weeks in a tightly stoppered flask.

Effect of Additives.

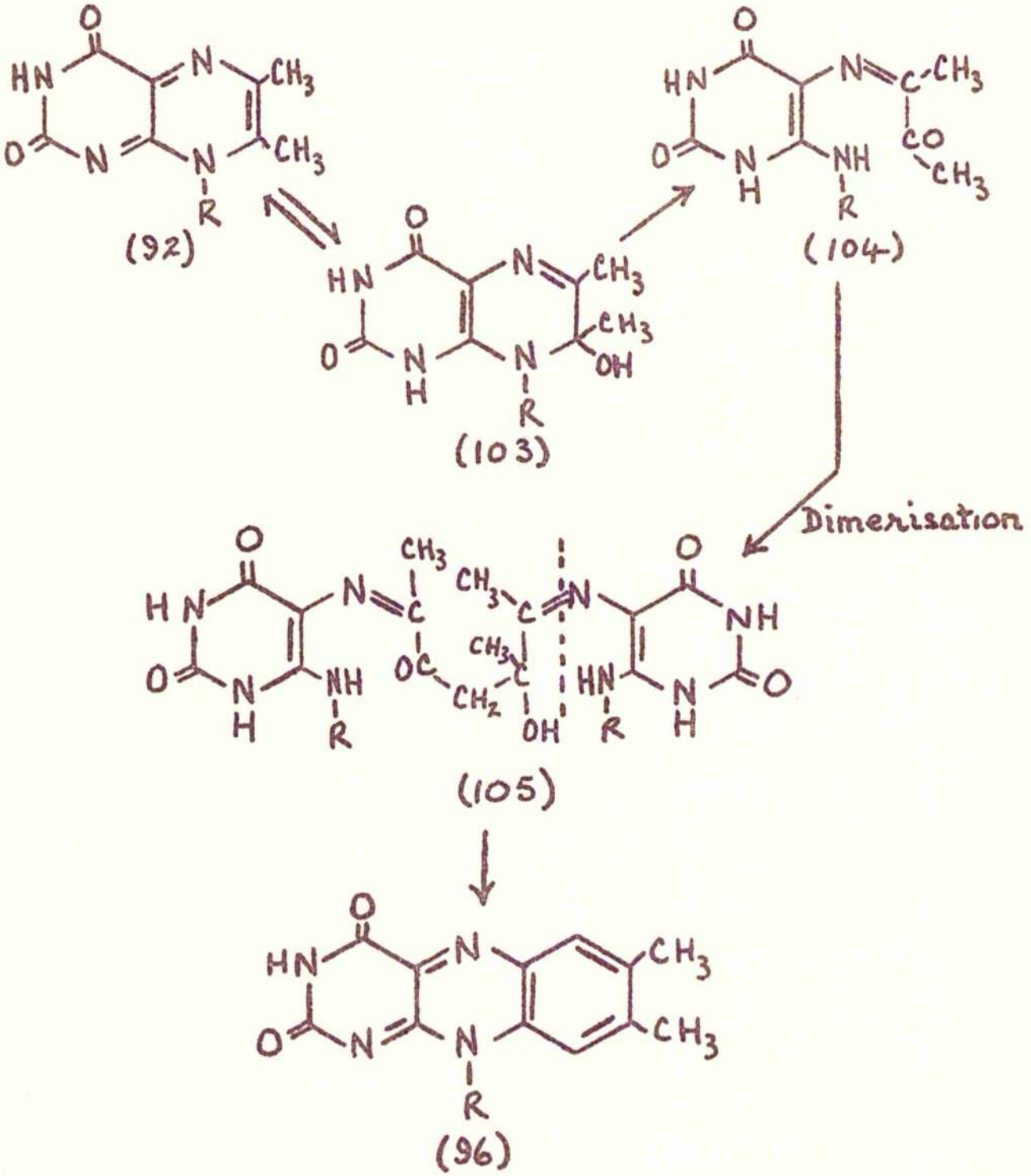
The effect of various additives on the reaction was next examined. Plaut[120] reported that reducing agents increased the rate of riboflavin production in his enzymic conversions of

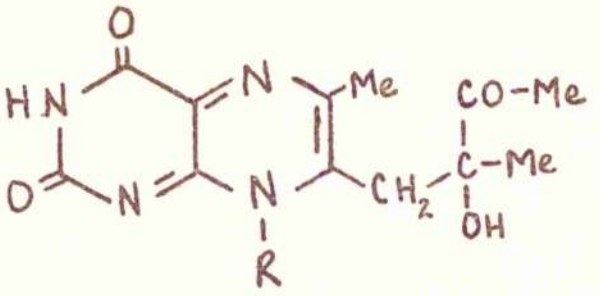
"G - compound". However, in the *in vitro* experiments described here, the addition of 2 - mercapto - ethanol had no effect on the "rate" or yield of the reaction. (The term "rate" refers to the approximate time taken for disappearance of the lumazine starting material as detected by paper chromatographic examination at frequent intervals). The addition of 1 equivalent of diacetyl to the reaction mixture had likewise no apparent effect on either the yield or "rate" of the reaction. On the other hand, the addition of nickel ions was found to accelerate the conversion and to furnish a slight increase in yield.

Possible Mechanism for Conversion of (92) into (96).

It is suggested that the mechanism of the transformation of the lumazine (92) into the isoalloxazine (96), and presumably of the corresponding enzyme reaction, involves the following steps. (a) Ring-opening of the pyrazine ring in the quinonoid pteridine (92) takes place, being initiated by nucleophilic attack at position 7 (92 → 103 → 104). (The displacement experiments previously described clearly indicate that the pyrazine ring - closure in the quinonoid pteridines such as (92) is reversible). (b) Aldol condensation involving two molecules of (104) occurs to give the large molecule (105) which is in effect a derivative of dimeric diacetyl, as suggested by Birch.[122] An alternative to the mechanism *via* the rather cumbersome molecule (105) is that a diacetyl moiety is cleaved from one molecule of (104) and reacts

with the second molecule of (104) to form the "intermediate" (94) of Cresswell and Wood[33] which then cyclises to the iso - alloxazine (96). However, if this were so, the addition of one equivalent of diacetyl to the reaction mixture should have doubled the yield of isoalloxazine which was not the case.



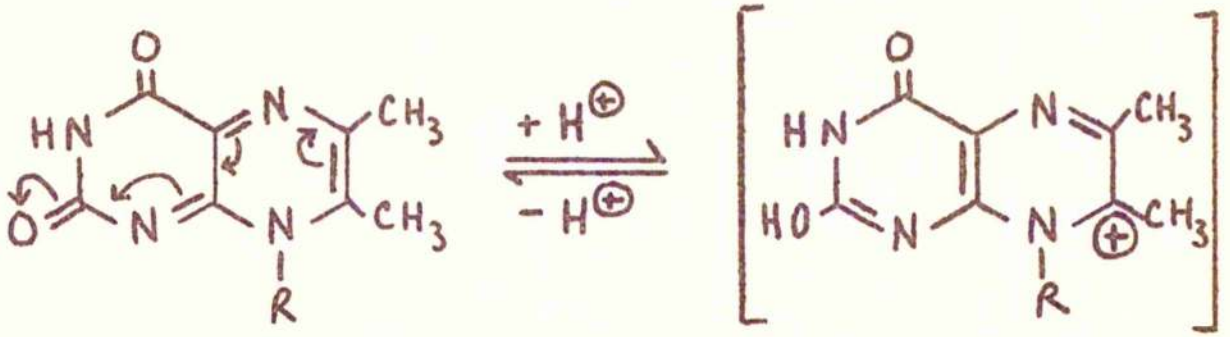


(94)

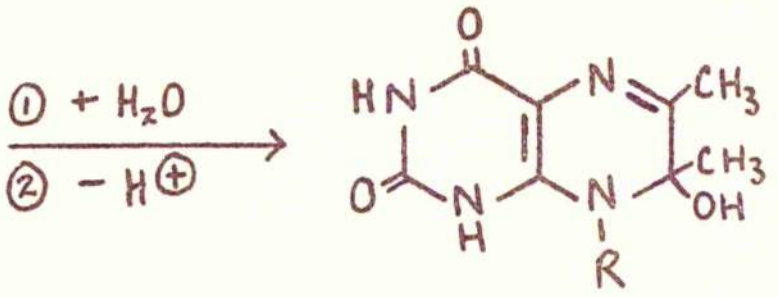
(c) Cyclisation of the intermediate (105) with loss of the diaminopyrimidine portion, on the right hand side of the dotted line, gives the isoalloxazine (96). This is similar to the cyclisation of (94) reported by Cresswell and Wood.[33]

It was surprising to find that the conversion of the lumazine (92; R = D - ribityl) into riboflavin occurred also in a strongly acid medium albeit in much reduced yield. The fact that it occurs at all in acid solution seems to deny the first step of the mechanism, that of hydration which appears to occur only in alkaline solution.

A tentative mechanism for riboflavin production in acid solution is suggested in the scheme below.



(92) (106)



(103)

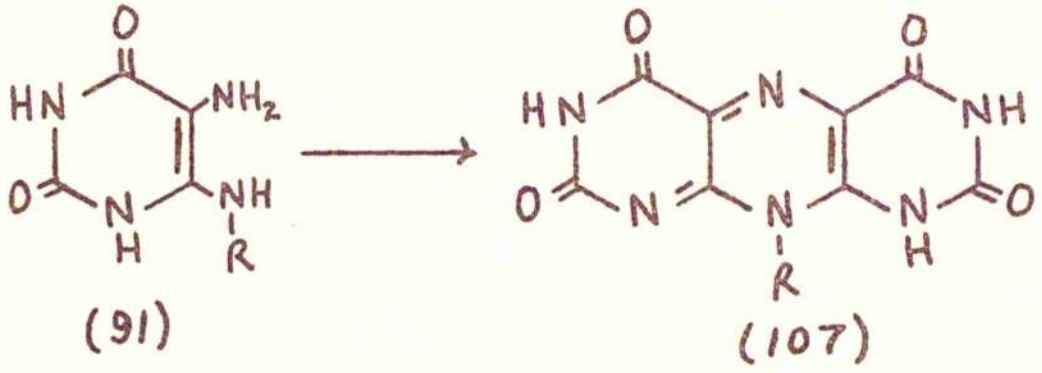
The carbonium ion (106) undergoes addition of water followed by elimination of a proton to give the hydrated lumazine (103), the remaining stages to the isoalloxazine taking place as before. Although aldol condensations are normally base - catalysed, it is known that they can also take place in an acid medium (e.g. the formation of phorone from acetone[123]). No evidence, however, has yet been found for the presence of the hydrated species (103) in strongly acid medium.

Isolation of Intermediates formed in the Conversion of (92) into (96).

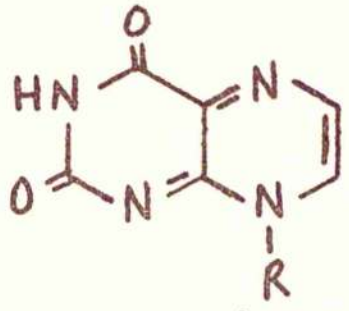
Attempts were made in vain to isolate intermediates formed in the production of the isoalloxazine (96) from the lumazine (92). The addition of hydroxylamine or 2,4 - dinitrophenylhydrazine to the reaction mixture produced a mixture of no less than eight components as detected by paper chromatography and attempts at isolation ended in failure.

According to the proposed mechanism, a 4 - substituted - amino - 5 - aminopyrimidine moiety is cleaved from the intermediate (105), as shown on the right hand side of the dotted line.

Evidence for the presence of the diaminopyrimidine (91) has been found. In the model reactions using the lumazine (92; R = CH_2CH_2OH), a small amount of the pyrimidopteridine (107; R = CH_2CH_2OH) was isolated. (Cresswell, Neilson and Wood[124] have shown that such pyrimidopteridines can be readily formed from diaminopyrimidines such as (91)). Furthermore, the addition of glyoxal to the reaction mixture from the lumazine (92; R = CH_2CH_2OH) at pH 7.3, yielded a little of the pteridine (99; R = CH_2CH_2OH).



(91) (107)



(99)

This is, however, not the complete answer as Plaut's isotope studies[120] point to the presence of a pyrimidine ("X"), in biological systems in which riboflavin has been produced enzymically from "C - compound". This pyrimidine ("X") is <u>not</u>

the diaminopyrimidine (91; R = D - ribityl). His general mechanism as represented by the equation below agrees, however, on the whole with the mechanism proposed above.

2 x "G - compound" ——→ Riboflavin + "X".

The composition of "X" has not yet been elucidated by Plaut but he has shown that radioactivity from 6,7 - dimethyl - 8 - D - ribityllumazine (2 - ^{14}C) is incorporated in "X" and the latter on alkaline degradation yields radioactive urea. Therefore C_2 of the lumazine remains associated with the two nitrogen atoms in "X". On the other hand, when the label was placed in the 6 and 7 methyl groups of the lumazine, the isolated compound "X" was inactive and the latter yielded inactive urea on degradation. Radioactivity from the lumazine (4,8a - ^{14}C) was recovered in "X". Thus, the carbon atoms 2,4 and 8a of the original lumazine were retained in "X". Thus, "X" would appear to be a pyrimidine derivative but its precise structure is, as yet, unknown.

2 - Amino - 4 - hydroxy - 8 - substituted Pteridines.

It was decided to apply the displacement studies and the formation of isoalloxazine derivatives to the 2 - amino - 4 - hydroxypteridine series, the latter being the most common naturally occurring pteridine nucleus.

Synthesis of the 8 - Substituted Pteridines.

2 - Amino - 4 - chloro - 6 - hydroxypyrimidine[1] was

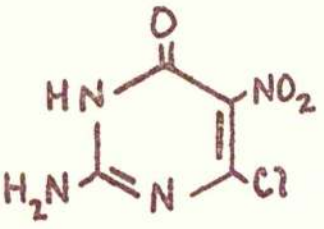
nitrated under carefully controlled conditions yielding the 5 - nitro compound (108). This nitro - chloropyrimidine was condensed with a suitable primary amine to give the corresponding 4 - substituted - aminopyrimidine (109) which on hydrogenation afforded the key diaminopyrimidine (110), isolated as the hydrochloride. Subsequent treatment of (110) with diacetyl or pyruvate yielded the pteridines (111) and (112) respectively.

Conversion of the 6,7 - Dimethylpteridine (111) to the 6 - Methyl - 7 - oxopteridine (112).

The same pattern as found in the 2 - oxopteridine series was followed. In the absence of oxygen, displacement by pyruvate occurred to give the 6 - methyl - 7 - oxopteridine (112) at pH 13. Oxidation of the pteridine (111) to (112) also occurred even more readily than with the corresponding 2 - oxo compound but a longer refluxing time was still required for the complete conversion *via* oxidation than *via* displacement by pyruvate in the absence of oxygen. Again, the optimum conditions for the conversion, whether by oxidation or displacement was at pH 13 although the reaction also took place much more slowly at pH 7.3. The hydrated pteridine (113) would thus appear to be an intermediate in both reactions.

Synthesis of Isoalloxazine 2 - Imines. (115).

2,10 - Dihydro - 4 - hydroxy - 10 - (2' - hydroxyethyl) - 2 - imino - 7,8 - dimethylbenzo[g]pteridine (115; R = CH_2CH_2OH)



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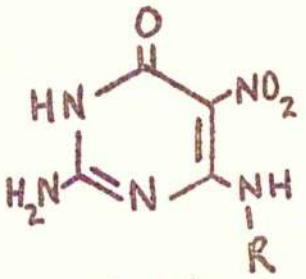

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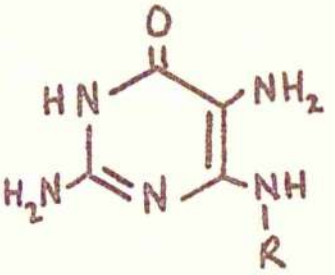

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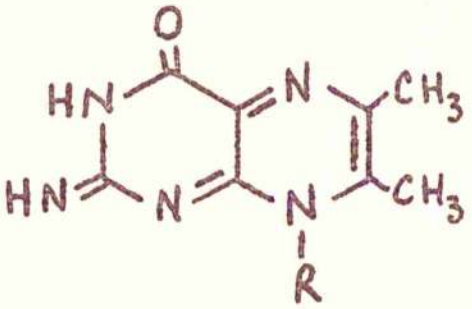
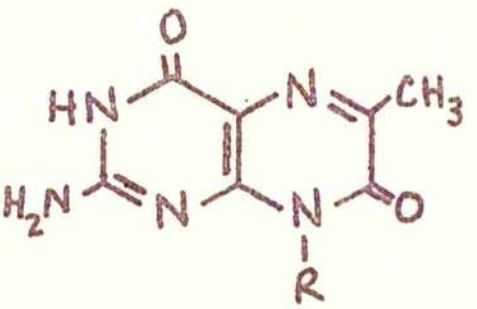


(111)



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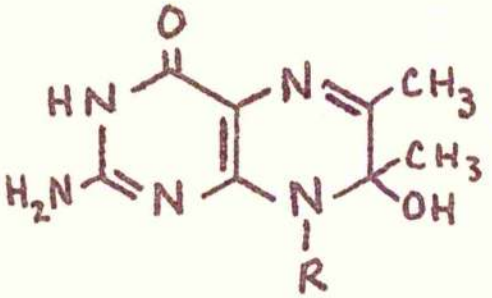

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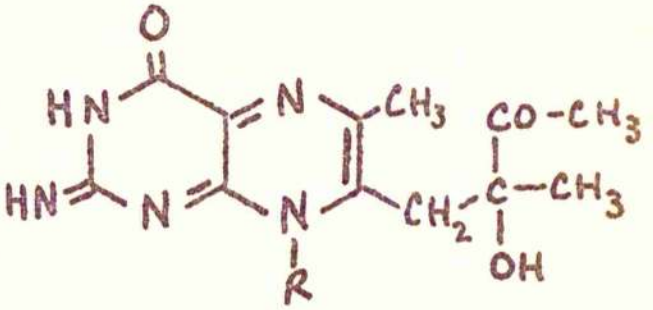

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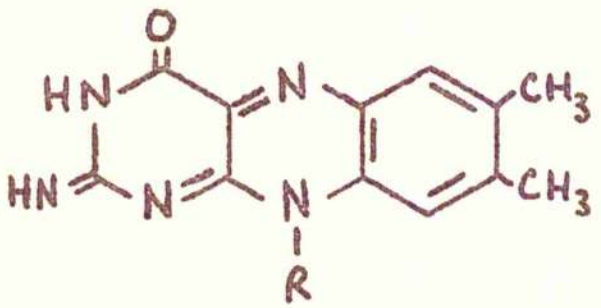


(115)

(116)

was synthesised in three different ways.

(i) A synthesis similar to that of Cresswell and Wood[33] for riboflavin was carried out by reacting the dimer of diacetyl with the diaminopyrimidine (110; R = CH_2CH_2OH) to give the iso - alloxazine - 2 - imine (115; R = CH_2CH_2OH) presumably *via* the intermediate (114; R = CH_2CH_2OH) by analogy with Cresswell and Wood's reaction.

(ii) The ribityl side-chain of 2 - imino - riboflavin (115; R = D - ribityl) (a sample of which was kindly supplied by Dr. P. Hemmerich, University of Basle) was cleaved with periodate to give the aldehyde (116) which on reduction with sodium boro - hydride furnished the isoalloxazine - 2 - imine (115; R = CH_2CH_2OH).

(iii) The 6,7 - dimethylpteridine (111; R = CH_2CH_2OH) was refluxed under nitrogen in a phosphate buffer of pH 7.3 for 24 hours. During this time any solid product which formed was removed by filtration at intervals of 6 hours to keep decomposition to a minimum. The product had identical Rf values in 6 solvent systems and ultraviolet spectra with those obtained in (i) and (ii) but in this case the product could not be obtained completely free from impurities.

EXPERIMENTAL.

Infrared spectra were determined for nujol mulls and potassium chloride discs. Ultraviolet spectra were determined on Unicam SP600 and Perkin - Elmer 137U.V. spectrophotometers.

Paper chromatograms were developed by the ascending technique, the solvent systems being, (A) n - butanol - 5 N. acetic acid (7:3); (B) 3% aqueous ammonium chloride; (C) n - propanol - ammonium hydroxide (S.G. 0.880) - water (40: 1: 20); (D) 3% aqueous ammonium formate; (E) n - butanol - ethanol - water (50 :15: 35); (F) n - butanol - benzene - water - methanol (1: 1: 1: 2), and they were viewed in ultraviolet light of wavelengths 254 and 365 mμ .

Yields of substances which have no definite m.p. refer to the stage when they appeared homogeneous on paper chromatograms.

Barbituric Acid.[125]

Ethyl malonate (320g.) and dry urea (120g.) were dissolved in absolute ethanol (1000cc.) and added to a sodium ethoxide solution (46g. sodium in 1000cc. absolute ethanol). The mixture was refluxed for 7 hr. in an oil bath at 100 - 110° and hot water (2000cc. at 50°) was added, followed by concentrated hydrochloric acid (188cc.). After stirring, the solution was filtered and left to stand overnight at 0°. The crystalline product was collected,

washed with cold water (200cc.) and oven-dried at 100 - 110° for 2 hr. to give white crystals (192g.; 75%), m.p. 238 - 240° (dec.) (lit.,[125] m.p. 240°).

2,4,6 - Trichloropyrimidine.[126]

Barbituric acid (63g.) was added portionwise over 10 min. to a mixture of diethylaniline (121cc.) and phosphoryl chloride (198cc.) and the whole was heated under reflux for 15 - 20 min., cooled and poured on to ice (1000g.). The mixture was extracted with ether (4 x 250cc.) and the combined ether extracts were dried over anhydrous sodium sulphate. The ether was removed under vacuum and the oily residue was fractionally distilled at reduced pressure, the fraction of b.p. 103°/ 35 mm. Hg. being collected to give the trichloropyrimidine (70g.; 76%) as a clear viscous oil. (Care was exercised in handling this product due to its highly vesicant properties).

4 - Chlorouracil.[33]

2,4,6 - Trichloropyrimidine (74g.) was heated under reflux for 16 hr. in a solution of sodium hydroxide (64.5g.) in water (650cc.). On cooling, the sodium salt of 4 - chlorouracil separated and this was converted to the free pyrimidine by the addition of concentrated hydrochloric acid (80cc.). The product was collected, recrystallised from the minimum amount of water and dried over silica gel to give 4 - chlorouracil as fine white needles (53g.; 90%), m.p. 301° (dec.).

4 - Chloro - 5 - nitrouracil.[33]

Dry - 4 - chlorouracil (5g.) was partially dissolved in 36 N - sulphuric acid (6cc.) at $< 40^{\circ}$ and nitric acid (d,1.5; 5.3cc.) was added slowly at 0°, stirring until the solid had completely dissolved. The solution was kept at room temperature for 30 min. by which time the product had separated, and was then poured on to ice (20g.). The white product was rapidly collected, washed with cold water (2 x 20cc.) and ether (50cc.) and dried in vacuo over phosphorus pentoxide. Yield 2.5g., m.p. 220 - 221.° (lit.,[33] 220 - 222°).

D - Ribose Oxime.[21]

Sodium ethoxide solution (3.5g. sodium in 75cc. absolute ethanol) was added to a solution of dry hydroxylamine hydrochloride (10.8g.) in absolute ethanol (225cc.) and the mixture was allowed to stand overnight. After filtration of the sodium chloride, the filtrate was heated to 70° and the hot solution was treated portion-wise with D - ribose (15g.). The mixture was kept at room temperature overnight, then the product was collected and washed with ethanol and ether to give D - ribose oxime (14.21g.; 86%), m.p. 139 - 140° (lit.,[21] 140°).

2,6 - Dihydroxy - 5 - nitro - 4 - D - ribitylaminopyrimidine.[33]

D - Ribose oxime (6.6g.) was dissolved in water (200cc.) and hydrogenated over platinum oxide catalyst (2g.) which had previously been pre-reduced. When the uptake of hydrogen was complete the

catalyst was removed and the resulting D - ribitylamine solution was treated with a solution of 4 - chloro - 5 - nitro - uracil (3.9g.) in ethanol (200cc.) at room temperature. After 24 hr. the solid (1g.) which had formed was collected and shown to be identical in its physical properties to an authentic sample of 4 - amino - 2,6 - dihydroxy - 5 - nitropyrimidine. The filtrate was shown, by paper chromatography, to contain two other pyrimidines which were separated by ion - exchange chromatography. The solution was treated with ammonia and formic acid until buffered at pH 10.7, added to a column of the resin (Amberlite CG400; formate form), and the column thoroughly washed with ammonium formate buffer (pH 10.7). No product was eluted at this stage. The pH of the buffer solution was changed to 7.4 and a bright yellow pyrimidine of unknown constitution was eluted. ($\lambda_{max.}$ 322 mμ at pH 1; $\lambda_{max.}$ 334 mμ at pH 13.). On changing to a buffer of pH4, the second pyrimidine was eluted and the solution was concentrated to ca. 10cc. in vacuo at $< 40^{\circ}$. Ethanol (500cc.) was added and the resulting white precipitate was collected, freed from ammonium formate by boiling in ethanol (150cc.) and filtered. (2.6g.), m.p. 202 - 204° (lit.,[33] 203 - 204°).

Diacetyl Dimer.[31]

N - Potassium hydroxide solution (350cc.) was added dropwise over a period of 1 hr. to a stirred solution of diacetyl (100g.) in water (300cc.) while the reaction mixture was maintained at 0°. The pale yellow solution, after acidification with 10% sulphuric

acid, was extracted with ether (4 x 250cc.). The ethereal extract was dried over anhydrous sodium sulphate and the solvent was evaporated *in vacuo* giving a yellow viscous liquid (51g.) which was fractionally distilled at 0.3 mm., the fraction, b.p. 85° being collected. Redistillation at 0.15 mm. (b.p. 79°) yielded a pale yellow gum which crystallised on standing at room temperature for several days. The yellow solid was recrystallised from ether - light petroleum (60 - 80°) and so gave white crystals, m.p. 55 - 57° (lit.,[31] 51 - 54°). The I.R. spectrum of the product agreed with that of Birch and Moye[31].

Reaction of Diacetyl Dimer with 5 - Amino - 2,6 - dihydroxy - 4 - D - ribitylaminopyrimidine.[33]

2,6 - Dihydroxy - 5 - nitro - 4 - D - ribitylaminopyrimidine (0.25g.) was dissolved in water (20cc.) and hydrogenated using pre-reduced Adam's catalyst (0.1g.). When the hydrogen uptake was complete the flask was removed from the apparatus under nitrogen and the remainder of the procedure carried out in a cabinet in an atmosphere of nitrogen. The catalyst was filtered and the filtrate was treated with a solution of diacetyl dimer (0.15g.) in water (10cc.). The light orange mixture was heated at 90° on a water bath for 15 min. during which time the solution darkened in colour. Paper chromatography showed the presence of two compounds, one exhibiting orange fluorescence and the other, yellow fluorescence when viewed in ultraviolet light. The solution was concentrated to *ca.* 5cc. and chromatographed on several Whatman No. 17 filter

papers using solvent system (A). The yellow fluorescing band was eluted with water and the eluate was freeze - dried to give a gum which could not be crystallised. All attempts to isolate this compound were hindered by its extreme instability, being converted to its dehydrated form (the orange - fluorescing component), to riboflavin and to degradative products. The yellow - fluorescing component had, however, a 'lifetime' of a few days when stored as a dry paper chromatogram band in a sealed polythene bag.

6,7 - Dimethyl - 8 - D - ribityllumazine. (Masuda's "G - compound").

2,6 - Dihydroxy - 5 - nitro - 4 - D - ribitylamino - pyrimidine (2g.) was dissolved in water (200cc.) and hydrogenated over palladised charcoal (10% Pd.; 1g.). Attempts to isolate the resulting diaminopyrimidine as the hydrochloride using the procedure described subsequently for the hydroxyethylamino compound, were unsuccessful and the following steps were carried out in an atmosphere of nitrogen. The catalyst was removed, the filtrate was adjusted to pH 2 by dropwise addition of 2 N - hydrochloric acid and diacetyl (5cc.) was added. The reaction mixture was heated in a water bath at 80° in the dark for 30 min. Treatment with a little animal charcoal followed and after filtration the solution was concentrated in vacuo to ca. 10cc. Hot ethanol was added until

the product began to separate, refrigeration completing the crystallisation to give yellow needles (1.21g.), m.p. 268° (dec.). Recrystallisation from a little water by the technique of Pfleiderer and Nübel[74] yielded yellow needles, m.p. 271° (dec.) [lit.,[74] 270 - 271° (dec.)]. The ultraviolet spectra and Rf values of the product were identical to those described in the literature.[33]

7,8 - Dihydro - 2,6 - dihydroxy - 6 - methyl - 7 - oxo - 8 - D - ribitylpteridine. (Masuda's "V - compound").

2,6 - Dihydroxy - 5 - nitro - 4 - D - ribitylamino - pyrimidine (500 mg.) was hydrogenated over palladised charcoal (10% Pd.; 200 mg.). The resulting 5 - aminopyrimidine was condensed with pyruvic acid (100% ; 0.4 cc.) by heating at pH 1 in a water bath at 90° for 1 hr. in the dark in an atmosphere of nitrogen. The solution was concentrated to ca. 5cc., treated with a little charcoal and the charcoal was extracted with 5% aqueous pyridine after washing with water. The charcoal treatment and elution procedure were repeated with the filtrate, and the combined aqueous pyridine extracts concentrated *in vacuo* to ca. 1cc. Ethanol (50cc.) was added and the mixture was refrigerated to complete the precipitation of a pale yellow solid. The product was dissolved in water (5cc.) and finally purified by large scale paper chromatography using Whatman No.17 papers. The violet bands (mean Rf 0.30 in solvent system [C]) were cut out, eluted with water and the combined aqueous extracts were

evaporated to dryness under vacuum to give a white solid (103 mg.) which was crystallised from 80% aqueous ethanol, m.p. 260° (dec.) [lit.,[91] 263° (dec.)]. The Rf values and ultraviolet spectra were identical to those reported in the literature.[74]

2,6 - Dihydroxy - 4 - (2' - hydroxyethylamino) - 5 - nitro - pyrimidine.[33]

4 - Chloro - 5 - nitrouracil (16.7g.) in ethanol (200 cc.) was treated with ethanolamine (12cc.) and heated, with stirring, on a steam bath under reflux for 15 min. On cooling, the hydroxyethylamino pyrimidine separated as fine pale yellow needles. Recrystallisation from aqueous ethanol yielded white needles (16.5g.), m.p. 218 - 219° (lit.,[33] 217 - 219°).

5 - Amino - 2,6 - dihydroxy - 4 - (2' - hydroxyethylamino) - pyrimidine Hydrochloride.

4 - (2' - Hydroxyethylamino) - 5 - nitrouracil (6.6g.) was suspended in water (250cc.) and 2N - sodium hydroxide solution was added dropwise until the solid just dissolved. The nitro group was reduced by hydrogenation over Raney nickel and the catalyst filtered off, the filtrate being collected in a solution of concentrated hydrochloric acid (30cc.) in water (30cc.). This acidic solution was concentrated to ca. 20cc. in vacuo without delay. (Otherwise formation of 2,10 - dihydro - 9 - (2' - hydroxyethyl) - 4,6,8 - trihydroxy - 2 -

oxopyrimido [5,4 - g] pteridine occurs due to oxidative self - condensation of the diaminopyrimidine). Refrigeration of the solution yielded a white crystalline solid, which on rapid recrystallisation from 98% aqueous methanol gave the hydro - chloride as white feathery crystals (4.1g.), m.p. 252 - 254° (dec.). (Found : C, 32.7; H, 4.6; N, 25.8; Cl, 15.6. $C_6H_{11}N_4O_3Cl$ requires C, 32.35; H, 4.9; N, 25.2; Cl, 15.95%). The product may be stored under vacuum over phosphorus pentoxide for a few days but storage for longer periods results in decomposition.

8 - (2' - Hydroxyethyl) - lumazine.

(a) 4 - (2' - Hydroxyethylamino) - 5 - nitrouracil (500 mg.) was dissolved in 0.1N - sodium hydroxide (10cc.) and sodium dithionite was added until the yellow colour disappeared. The pH was adjusted to 1 - 2 with 2N - hydrochloric acid and a solution of glyoxal monohydrate (0.2g.) in water (10cc.) was added. The solution was heated on a steam bath for 20 min., filtered hot after treating with charcoal, and refrigerated to give yellow crystals (0.28g.). Repeated recrystallisation from aqueous methanol/ether yielded yellow crystals (0.15g.), m.p. 280 - 285° (dec.).

(b) A much superior method of synthesising this lumazine derivative was as follows: 5 - amino - 4 - (2' - hydroxyethyl-amino) - uracil hydrochloride (0.9g.) was suspended in methanol

(20cc.) and a solution of polyglyoxal (0.4g.) in methanol (30cc.) was added. The mixture was refluxed with stirring for 30 min., the colour changing through deep red to brown. After filtering hot the filtrate was allowed to cool in the refrigerator yielding brown prisms. Recrystallisation from aqueous iso - propanol, with charcoaling, gave the lumazine as orange crystals (650 mg.), m.p. 286 - 288° (dec.). (Found : C, 46.3; H, 3.5; N, 26.7. $C_8H_8N_4O_3$ requires C, 46.15; H, 3.85; N, 26.9%).

8 - (2' - Hydroxyethyl) - 6,7 - dimethyllumazine.

5 - Amino - 4 - (2' - hydroxyethylamino) - uracil hydro - chloride (3.4g.) was suspended in methanol (200cc.) and diacetyl (3.5cc.) in methanol (50cc.) immediately added. The mixture was heated with stirring on a steam bath under reflux for 30 min., filtered hot and the filtrate was cooled in the refrigerator. The lumazine derivative separated as bright yellow needles (1.81g.), m.p. 270 - 273° (dec.) [lit.,[33] 270° (dec.)] . The ultraviolet absorption spectra and Rf values of the product were in good agreement with those reported in the literature.[33]

7,8 - Dihydro - 2,4 - dihydroxy - 8 - (2' - hydroxyethyl) - 6 - methyl - 7 - oxopteridine.

5 - Amino - 4 - (2' - hydroxyethylamino) - uracil hydro - chloride (1g.) was suspended in methanol (75cc.) and pyruvic acid (100%; 0.8g.) was added at once. The mixture was refluxed

with stirring for 1 hr., filtered hot and the filtrate was cooled in the refrigerator whereupon the product deposited. Recrystallisation from water gave the 7 - oxopteridine as long white needles (0.92g.), m.p. $> 350^{\circ}$. (Found : C,45.2; H,4.5; N, 23.8. $C_9H_{10}N_4O_4$ requires C,45.4; H, 4.2; N, 23.5%).

7,8 - Dihydro - 2,4 - dihydroxy - 8 - (2' - hydroxyethyl) - 6,7 dimethylpteridine.

8 - (2' - Hydroxyethyl) - 6,7 - dimethyllumazine (0.6g.) was dissolved in water (100cc.) and hydrogenated at atmospheric pressure and temperature, using pre-reduced platinum oxide (0.2g.) as catalyst, until the uptake of hydrogen was complete (ca. 1 hr.) 2 Mols. of hydrogen were consumed. The catalyst was removed by filtration, the volume of the filtrate was reduced to ca. 20cc. in vacuo at $< 40^{\circ}$ and the solution was refrigerated. Pale yellow crystals (0.25g.), m.p. 260° (dec.), of the 7,8 - dihydropteridine separated, being further supplemented by 0.2g. from the mother liquor on concentration. A portion of the product was rapidly recrystallised from water for analysis. (Found : C, 50.7; H,6.0; N,23.3. $C_{10}H_{14}N_4O_3$ requires C,50.4; H, 5.9; N, 23.5 %).

Oxidation and Reduction of 7,8 - Dihydro - 2,4 - dihydroxy - 8 - (2' - hydroxyethyl) - 6,7 - dimethylpteridine.

(a) A solution of the 7,8 - dihydropteridine (20 mg.) in 0.1N - sodium hydroxide solution (10cc.) was heated on a steam bath for 30 min. Paper chromatography and ultraviolet

spectroscopy showed that it had been re-converted into 8 - (2' - hydroxyethyl) - 6,7 - dimethyllumazine.

(b) The 7,8 - dihydropteridine (200 mg.) in water (50cc.) was hydrogenated over pre-reduced platinum oxide (50 mg.). The volume of hydrogen consumed was 19cc. at N.T.P., i.e. 1 mol. of hydrogen per mol. of dihydropteridine.

Reaction of 7,8 - Dihydro - 2,4 - dihydroxy - 8 - (2' - hydroxy - ethyl) - 6,7 - dimethylpteridine with Pyruvic Acid.

(a) The dihydropteridine (50 mg.) was dissolved in water (5cc.) and pyruvic acid (99%; 0.2cc.) was added. The solution was heated on a steam bath for 30 min. yielding a mixture of products, one of which was identified as 8 - (2' - hydroxyethyl) - 6,7 - dimethyllumazine, which was formed by oxidation of the starting material.

(b) The dihydropteridine (50 mg.) was dissolved in 0.1N - hydrochloric acid (5cc.) and pyruvic acid (99%; 0.2cc.) was added. On heating the solution as in (a) above, the same mixture of products was obtained.

(c) The dihydropteridine (100 mg.) was dissolved in 0.1N - sodium hydroxide (10cc.) and pyruvic acid (99%; 0.4cc.) was added. The solution was heated on the steam bath for 5 hr. The reaction mixture was again a complex mixture of products but in addition to the oxidation product 8 - (2' - hydroxyethyl) - 6,7 - dimethyllumazine, a compound, which was identical in Rf values

in solvent systems (A) to (E) and in ultraviolet absorption spectrum with 7,8 - dihydro - 2,4 - dihydroxy - 8 - (2' - hydroxyethyl) - 6 - methyl - 7 - oxopteridine, was detected.

Reaction of 8 - (2' - Hydroxyethyl) - 6,7 - dimethyllumazine with Pyruvic Acid.

(a) 8 - (2' - Hydroxyethyl) - 6,7 - dimethyllumazine (100 mg.) was dissolved in 0.1N - sodium hydroxide solution (20cc.) and pyruvic acid (100%; 0.08cc.) was added. The pH of the solution was adjusted to 13 by the dropwise addition of N - sodium hydroxide and the reaction mixture was refluxed in the dark for 8 hr. Adjustment to pH 4 and refrigeration of the solution overnight yielded pale yellow crystals. Recrystallisation from water (10cc.) produced off-white needles, which on further recrystallisation (charcoal) gave long fine white needles (40 mg.), m.p. $> 300^{\circ}$. The product was identical in Rf values (5 different solvent systems), in ultraviolet and in infrared spectra with an authentic sample of 7,8 - dihydro - 2,4 - dihydroxy - 8 - (2' - hydroxyethyl) - 6 - methyl - 7 - oxopteridine.

(b) The above procedure was repeated except that the reaction was carried out under oxygen - free nitrogen when 10 hr. refluxing was required to complete the reaction but the yield of product was similar and its purity slightly higher.

(c) The reaction (a) was repeated in the absence of pyruvic acid and exposed to the atmosphere. This resulted in the formation of the same product but with reduced yield. The refluxing time had to be increased from 8 hr. to 15 hr. for the reaction to go to completion.

(d) The dimethyllumazine derivative (10 mg.) was dissolved in 0.1N - sodium hydroxide solution and pyruvic acid (100%; 1 drop) was added. The pH was adjusted to 13 and the reaction mixture was heated at 37° in the dark. Examination at various intervals of time by paper chromatography showed that the starting material had completely reacted after 9 days forming the 6 - methyl - 7 - oxopteridine (major product) in addition to other decomposition products.

(e) The procedure described in (a) was repeated at pH values 11.5, 10.5 and 9.5 with similar results but the refluxing time had to be increased from 8 hr. to 15 - 19 hr. for complete reaction. (Light from a 60 - watt tungsten lamp placed 6 in. from the reaction mixture had no apparent effect on any of the above reactions).

Reaction of 6,7 - Dimethyl - 8 - D - ribityllumazine with Pyruvic Acid.

(a) The ribityllumazine derivative (300 mg.) was dissolved in 0.1N - sodium hydroxide solution (15cc.) and pyruvic acid (100%; 0.16cc) was added. N - Sodium hydroxide solution was

added dropwise to readjust the pH to 13 and the solution was refluxed in the dark under oxygen-free nitrogen for 10 hr. The reaction mixture was concentrated to ca. 5cc., the pH adjusted to 1 and hot ethanol (50cc.) was added. On refrigeration, a brown precipitate resulted which was dissolved in the minimum amount of water and chromatographed in bulk on several Whatman No.17 papers [solvent system (F)] . The band, mean Rf 0.50, fluorescing blue in ultraviolet light, was cut out and eluted with water. After concentration to ca. 2cc. the eluate was chromatographed on Whatman No.17 papers [solvent system (C)] . The blue-fluorescing band, mean Rf 0.30, was eluted with water and on concentration a white solid (92 mg.) separated. This product was identical with an authentic specimen of Masuda's "V - compound" in Rf values [solvents (A) to (F)] and in ultraviolet and infrared spectra.

(b) The ribityllumazine derivative (10 mg.) was dissolved in a phosphate buffer solution (20cc.), pH 7.3 and pyruvic acid (1 drop) was added. The pH was readjusted to 7.3 and the solution was refluxed under nitrogen in the dark. Paper chromatography showed the reaction to be complete after 18 hr. refluxing. The product was identical in Rf [solvent systems (A) to (F)] and in ultraviolet spectra with Masuda's "V - compound".

6 - Ethyl - 7,8 - dihydro - 2,4 - dihydroxy - 8 - (2' - hydroxy - ethyl) - 7 - oxopteridine.

A suspension of 2,6 - dihydroxy - 4 - (2' - hydroxyethyl -

amino) - 5 - nitropyrimidine (1g.) in water (20cc.) was made strongly alkaline by addition of N - sodium hydroxide solution (10cc.) whereby a yellow solution resulted. The solution was heated on a steam bath and sodium dithionite was added until the yellow colour disappeared. The pH was adjusted to 5 by dropwise addition of concentrated hydrochloric acid and without delay a solution of α - oxo - butyric acid (1g.) in water (10cc.) was added. After heating for 1 hr. at 90° the solution was allowed to cool to room temperature and then refrigerated. White crystals separated which on recrystallisation from water yielded the 6 - ethyl - 7 - oxopteridine as white needles (820 mg.; 70%), m.p. 329 - 331° (dec.). (Found : C,47.7 ; H, 4.5 ; N, 22.0. $C_{10}H_{12}N_4O_4$ requires C, 47.6 ; H, 4.8 ; N, 22.2 %).

Reaction of 8 - (2' - Hydroxyethyl) - 6,7 - dimethyllumazine with α - Oxo - butyric Acid.

The 6,7 - dimethyllumazine derivative (500 mg.) was dissolved in 0.1N - sodium hydroxide solution (40cc.) and α - oxo - butyric acid (500 mg.) in 0.1N - sodium hydroxide (5cc.) was added. The pH was adjusted to 12 and the solution was refluxed in the dark in an atmosphere of oxygen - free nitrogen for 15 hr. Glacial acetic acid was added to give pH 4 and the solution was concentrated to ca. 10cc. On refrigeration a pale yellow solid deposited. Recrystallisation from water (charcoal) produced white crystals (210 mg.), m.p. 327 - 330° (dec.). The product was identical in

Rf (6 solvent systems) and in ultraviolet and infrared spectra with an authentic sample of 6 - ethyl - 7,8 - dihydro - 2,4 - dihydroxy - 8 - (2' - hydroxyethyl) - 7 - oxopteridine.

Oxidation of 6,7 - Dimethyl - 8 - D - ribityllumazine.

(a) 6,7 - Dimethyl - 8 - D - ribityllumazine (1.5 mg.) was dissolved in water (1cc.) and left exposed to the air. The solution was examined at various time intervals by paper chromatography [solvent systems (B) and (C)]. After 7 days no change was observed but on standing several weeks a blue fluorescent spot was detected on the chromatogram when viewed in ultraviolet light. This compound has the same Rf (6 solvent systems) and ultraviolet spectra as Masuda's "V - compound".

(b) 6,7 - Dimethyl - 8 - D - ribityllumazine (5 mg.) was dissolved in 0.1N - sodium hydroxide solution (5cc.) and oxygen was passed through the solution in the dark. There was still an appreciable amount of starting material present after 14 days at room temperature although a small amount of Masuda's "V - compound" was also detected by paper chromatography and ultraviolet spectra of the eluted blue - fluorescent spots. When the reaction mixture was refluxed, the starting material was completely converted into the 6 - methyl - 7 - oxopteridine derivative in 15 hr.

9 - (2' - Hydroxyethyl) - 6,7 - dimethyl - isoalloxazine from Riboflavin.

Riboflavin (150 mg.) was treated with a solution of sodium

metaperiodate (260 mg.) in water (100cc.) and left in the dark at room temperature overnight with stirring. The resulting yellow solid was washed with water, a little methanol and then with ether. The solid (60 mg.) was added to a solution of sodium borohydride (20 mg.) in water (100cc.) and stirred for 10 min. On standing overnight at room temperature a yellow precipitate separated (40 mg.), m.p. 299 - 301° (lit.,[127] 300 - 301°). The product was identical in Rf (6 solvent systems) and in ultraviolet spectra with an authentic sample of 9 - (2' - hydroxyethyl) - 6,7 - dimethyl - isoalloxazine.[33]

Conversion of 8 - (2' - Hydroxyethyl) - 6,7 - dimethyllumazine into 9 - (2' - Hydroxyethyl) - 6,7 - dimethyl - isoalloxazine.

(i) In Phosphate Buffer, pH 7.3.

8 - (2' - Hydroxyethyl) - 6,7 - dimethyllumazine (1g.) was dissolved in phosphate buffer solution (50cc.), pH 7.3, and the solution was refluxed in an atmosphere of oxygen - free nitrogen in the dark for 15 hr. Pale brown crystals separated and were collected, (250 mg. ; 41%). These were recrystallised from water (ca. 300cc.) to give orange prisms (185 mg.; 30%), m.p. 299 - 300° (lit.,[127] 300 - 301°). The product was identical in Rf (6 solvent systems), in ultraviolet and infrared spectra with an authentic sample of the isoalloxazine derivative.[33] The mother liquor showed the presence of a few other compounds in relatively small amounts. Half of the mother liquor was subjected to large scale paper chromatography after concentration to small

bulk, Whatman No.17 papers being used. The band which fluoresced pale blue in ultraviolet light was cut out and extracted with water. Concentration in vacuo yielded 2,10 - dihydro - 4,6,8 - trihydroxy - 10 - (2' - hydroxyethyl) - 2 - oxo - pyrimido [5,4 - g] pteridine as a yellow powder (20 mg.) which was identified by Rf and ultraviolet spectra. The remainder of the mother liquor on treatment with glyoxal (0.5g.) and heating on a steam bath for 30 min. gave an additional yellow - green fluorescing spot on the paper chromatogram. This compound had the same Rf values (4 solvents) and ultraviolet spectra as 8 - (2' - hydroxyethyl) - lumazine.

(ii) In Formate Buffer, pH 7.3.

The dimethyllumazine derivative (100 mg.) was refluxed in the dark under oxygen - free nitrogen for 20 hr. in a formate buffer (15cc.), pH 7.3. The product was identical with that obtained in (i), the yield of pure isoalloxazine being 17 mg. (28%).

(iii) In Borate Buffer, pH 7.3.

The dimethyllumazine derivative (1g.) was dissolved in a borate buffer solution (50cc.), pH 7.3 and refluxed in the dark for 15 hr. under oxygen - free nitrogen yielding brown crystals (260 mg.) which on recrystallisation from water gave orange crystals (190 mg.; 31%), m.p. 298 - 300°.

(iv) In Phosphate Buffer, pH 6.3.

The reaction (i) was carried out as before but using a

phosphate buffer, pH 6.3. Refluxing for 27 hr. was required for complete disappearance of starting material from the paper chromatogram. Yield of the isoalloxazine was only 18%.

(v) In Borate Buffer, pH 8.6.

The reaction (i) was again repeated but with a borate buffer, pH 8.6 and refluxing for 30 hr. Paper chromatography indicated that some starting material still remained. The isoalloxazine derivative was isolated in 15% yield.

(vi) In Strongly Acid Medium.

(a) The dimethyllumazine derivative (100 mg.) was dissolved in 4N - sulphuric acid (15cc.) and refluxed in the dark under nitrogen for 17 hr. The reaction mixture was carefully neutralised with a concentrated solution of sodium hydroxide. Paper chromatography showed the presence of the hydroxyethyl - dimethyl - isoalloxazine in addition to several decomposition products. The isoalloxazine, however, defied any attempts at isolation.

(b) 8 - (2' - Hydroxyethyl) - 6,7 - dimethyllumazine (100mg.) was dissolved in 2N - hydrochloric acid (5cc.) and kept at room temperature in the dark for 6 weeks in a tightly stoppered flask. During this time brown crystals separated. Recrystallisation from water produced orange crystals (11 mg.; 18%) which were identical to the products obtained in (i) to (v).

(c) A solution of the lumazine derivative (500 mg.) in 2N - hydrochloric acid (20cc.) was refluxed under nitrogen in the dark

for 18 hr. by which time no starting material remained. The resulting black solid was recrystallised twice from water (charcoal) to give orange crystals (42 mg.; 14%), identical with the products obtained above in (i) to (v).

(vii) Effect of Addition of Diacetyl.

Reaction (i) was repeated but with addition of diacetyl (0.4 cc.; ca. 1 equiv.) before refluxing. The reaction still required 15 hr. to go to completion and the yield of pure iso - alloxazine derivative (165 mg.; 26%) was similar to that obtained in (i).

(viii) Effect of Addition of 2 - Mercapto - ethanol.

The addition of 2 - mercapto - ethanol (1cc.; ca. 3 equiv.) neither altered the reaction time nor the yield of isoalloxazine derivative (29%) obtained when carried out as described in (i).

(ix) Effect of Addition of a Nickel Salt.

A solution of 8 - (2' - hydroxyethyl) - 6,7 - dimethyl - lumazine (1g.; 4.2 m. mol.) in phosphate buffer solution (30 cc.), pH 7.3 was treated with a solution of nickel sulphate (325 mg.; 2.1 m. mol.) in phosphate buffer solution (30cc.), pH 7.3. The mixture was refluxed under nitrogen in the dark for 12 hr. by which time the starting material had completely reacted. On cooling, a brown solid (617 mg.) separated. Recrystallisation (twice) from water yielded the orange crystals (210 mg.; 35%) of 9 - (2' - hydroxyethyl) - 6,7 - dimethyl - isoalloxazine, m.p. 299 - 301°.

(x) Attempts to Isolate Intermediate/s formed in the Reaction.

(a) The dimethyllumazine derivative (500 mg.) was dissolved in phosphate buffer solution (25cc.), pH 7.3, containing hydroxylamine hydrochloride (300 mg.). The pH having been readjusted to 7.3, the solution was refluxed under nitrogen in the dark for 9 hr. On refrigeration the crude iso - alloxazine derivative separated (120 mg.). The mother liquor was concentrated to ca. 10cc. and chromatographed in bulk on several Whatman No.17 papers [solvent system (F)]. No fewer than 8 components were detected under ultraviolet light. The absorption band which was located almost at the solvent front was cut out and eluted with methanol but insufficient material was isolated for characterisation. The pale blue band was eluted with water and on concentration yielded a yellow solid (8 mg.) whose ultra - violet and infrared spectra and Rf (6 solvents) were identical with those of 2,10 - dihydro - 4,6,8 - trihydroxy - 10 - (2' - hydroxyethyl) - 2 - oxo - pyrimido [5,4 - g] pteridine. No further components could be isolated.

(b) The above reaction was repeated but with the addition of 2,4 - dinitro - phenylhydrazine (425 mg.) instead of the hydroxylamine hydrochloride. Again the isoalloxazine deposited in similar yield but the mother liquors contained a complex mixture of at least 12 components, one of which fluoresced pale blue in ultraviolet light and had the same Rf values as the pyrimidopteridine isolated in (a).

Conversion of 6,7 - Dimethyl - 8 - D - ribityllumazine into Riboflavin.

6,7 - Dimethyl - 8 - D - ribityllumazine (1g.) was dissolved in a phosphate buffer solution (50cc.), pH 7.3. Refluxing for 15 hr. in the dark in an atmosphere of oxygen - free nitrogen produced the crude product as brown crystals (318 mg.; 55%) which were collected and recrystallised from water (twice) to give orange crystals (220 mg.; 38%), m.p. 290° (lit.,[21] 292°). The product was identical with an authentic sample of riboflavin (commercial) in infrared and ultraviolet spectra and in Rf (6 solvent systems).

2 - Amino - 4,6 - dihydroxypyrimidine.[128]

Sodium (100 g.) was dissolved in ethanol (1500 cc.), dry powdered guanidine hydrochloride (200 g.) was added, followed by diethyl malonate (320 g.) and the mixture was refluxed for 2 hr. at 110°. Ethanol (ca. 600 cc.) was removed by distillation in vacuo, water (2000 cc.) was added, and the solution was acidified with glacial acetic acid. The precipitate was filtered and dried at 110° to give the pyrimidine (145g.; 54.5%), m.p. > 360°.

2 - Amino - 4,6 - dichloropyrimidine.[129]

2 - Amino - 4,6 - dihydroxypyrimidine (50g.) was refluxed at 100° for 3 hr. with redistilled phosphoryl chloride (200 cc.) and redistilled N,N - diethylaniline (50 cc.). Excess phosphoryl chloride and N,N - diethylaniline were removed by distillation

in vacuo and the residual dark viscous mass was poured on to crushed ice (600 g.). The mixture was stirred until all oily material had dissolved. The brown solid was then collected, washed with 2N - sodium hydroxide solution to remove unchanged phenolic material, and dried. Recrystallisation from ethanol (charcoal) yielded the dichloropyrimidine as colourless needles (21 g.; 65%), m.p. 220 - 221° (lit.,[129] 221°).

2 - Amino - 4 - chloro - 6 - hydroxypyrimidine.[1]

2 - Amino - 4,6 - dichloropyrimidine (15g.) was heated under reflux for 6 hr. in N - sodium hydroxide solution (150 cc.). The solution was filtered hot, cooled and acidified with glacial acetic acid. The precipitate was collected and dried in air at 110°. Crystallisation from aqueous ethanol yielded the pyrimidine as white needles (10.3 g.; 89%), m.p. 260 - 262° (lit.,[1] 261°).

2 - Amino - 4 - chloro - 6 - hydroxy - 5 - nitropyrimidine.[130]

2-Amino - 4 - chloro - 6 - hydroxypyrimidine (5g.) was dissolved in concentrated sulphuric acid (6cc.) at $< 45°$ and fuming nitric acid (d, 1.5; 5.3 cc.) was cautiously added with stirring and cooling in an ice bath. After 30 min. the mixture was poured on to ice (ca. 20g.) with rapid manual stirring. The precipitated product was collected, washed quickly with ice-cold water (2 x 20cc.), with ethanol (20cc.) and finally with ether. The resulting yellow powder, 2 - amino - 4 - chloro - 6 -

hydroxy - 5 - nitropyrimidine (4.5g.), m.p. $> 360^{\circ}$, was stored under vacuum over phosphorus pentoxide. (Found : C, 23.25; H, 2.3; N, 26.5. Calc. for $C_4H_3N_4O_3Cl$. H_2O C, 23.0; H, 2.4; N, 26.85%).

2 - Amino - 6 - hydroxy - 4 - (2' - hydroxyethylamino) - 5 - nitropyrimidine.

2 - Amino - 4 - chloro - 6 - hydroxy - 5 - nitropyrimidine (9.25g.) was suspended in ethanol (400 cc.) and redistilled ethanolamine (6cc.) in ethanol (100cc.) was added. The mixture was refluxed with stirring for 20 min. On cooling, the crude product was filtered and washed with ethanol and ether. Crystallisation from water (2000 cc.) (with charcoal) yielded white lustrous plates of 2 - amino - 6 - hydroxy - 4 - (2' - hydroxyethylamino) - 5 - nitropyrimidine (8.4 g.; 80%), m.p. 296 - 297°. (Found : C, 33.2; H, 4.0; N, 32.3. $C_6H_9N_5O_4$ requires C, 33.5; H, 4.2; N, 32.6%).

2,5 - Diamino - 6 - hydroxy - 4 - (2' - hydroxyethylamino) - pyrimidine Hydrochloride.

2 - Amino - 6 - hydroxy - 4 - (2' - hydroxyethylamino) - 5 - nitropyrimidine (7.67g.) was suspended in water (100 cc.) and 2N - sodium hydroxide was added dropwise until the solid dissolved. The solution was hydrogenated over Raney nickel until the uptake of hydrogen was complete and the mixture was then filtered into a solution of concentrated hydrochloric acid

(30 cc.) in water (30 cc.). The filtrate was immediately concentrated under vacuum to ca. 20cc. when white crystals separated. Recrystallisation by rapid dissolution in methanol and refrigeration yielded white crystals of the diaminopyrimidine hydrochloride (7g.; 89%), m.p. 217 - 220°. This product should be used soon after its preparation as it turns pink (probably due to oxidative self-condensation) on standing. It may be stored over phosphorus pentoxide in vacuo for a few days. (Found : C, 32.65; H, 5.7; N, 31.2; Cl, 16.5. $C_6H_{11}N_5O_2$.HCl requires C, 32.5; H, 5.4; N, 31.6; Cl, 16.0%).

2,8 - Dihydro - 4 - hydroxy - 8 - (2' - hydroxyethyl) - 2 - imino - 6,7 - dimethylpteridine Hydrochloride.

2,5 - Diamino - 6 - hydroxy - 4 - (2' - hydroxyethylamino) - pyrimidine hydrochloride (2.6g.) was suspended in methanol (100cc.) and a solution of diacetyl (2.6 cc.) in methanol (20 cc.) was added. The mixture was refluxed for 30 min. and after cooling was filtered to give yellow crystals of the pteridine hydrochloride which were recrystallised from 2N - hydrochloric acid. (Yield, 2.4g.; 75%), m.p. 285 - 288° (dec.). (Found : C, 44.7; H, 5.4; N, 26.2; Cl, 13.0. $C_{10}H_{13}N_5O_2$.HCl requires C, 44.2; H, 5.2; N, 25.8; Cl, 13.0%).

2 - Amino - 7,8 - dihydro - 4 - hydroxy - 8 - (2' - hydroxyethyl) - 6 - methyl - 7 - oxopteridine.

(a) 2,5 - Diamino - 6 - hydroxy - 4 - (2' - hydroxyethylamino)- pyrimidine hydrochloride (2.2g.) was dissolved in water (40cc.) and

pyruvic acid (100%; 1cc.) was added. The solution was refluxed for 30 min., cooled and filtered giving lustrous yellow crystals. Recrystallisation from water (ca. 400 cc./g.) with a little charcoal yielded the pteridine as pale yellow needles (1.5g.), m.p. 335 - 340° (dec.). (Found : C, 45.9; H, 4.9; N, 30.0. $C_9H_{11}N_5O_3$ requires C, 45.6; H, 4.65; N, 29.5%).

(b) 2,5 - Diamino - 6 - hydroxy - 4 - (2' - hydroxyethylamino)-pyrimidine hydrochloride (4.4g.) was dissolved in water (30cc.) and ethyl pyruvate (3cc.) in methanol (10cc.) was added. The solution was heated with stirring under reflux for 2 hr. Refrigeration yielded lustrous yellow crystals (3.7g.) which were identical with those obtained from (a).

2 - Amino - 6 - hydroxy - 4 - methylamino - 5 - nitropyrimidine.

2 - Amino - 4 - chloro - 6 - hydroxy - 5 - nitropyrimidine (3.81g.) was suspended in ethanol (150cc.) and treated with an alcoholic solution of methylamine (33% w/v; 5cc.). The mixture was refluxed for 15 min., cooled and filtered, the residue being then washed with ethanol and ether to give a yellow powder. This crude product was purified by suspension in hot water, dropwise addition of 4N - sodium hydroxide until complete solution was obtained and acidification of the hot solution with glacial acetic acid. On cooling, the nitropyrimidine separated as white needles (3.2g.) which were dried with ethanol and ether. M.p. $> 360°$. (Found : C, 32.7; H, 4.1; N, 37.95. $C_5H_7N_5O_3$ requires C, 32.4; H, 3.8; N, 37.8%).

2,5 - Diamino - 6 - hydroxy - 4 - methylaminopyrimidine Hydrochloride.

2 - Amino - 6 - hydroxy - 4 - methylamino - 5 - nitropyrimidine (4.38g.) was suspended in water (250cc.) and 2N - sodium hydroxide was added dropwise until the solid just dissolved. The solution was hydrogenated over Raney nickel and, when the uptake of hydrogen was complete, it was filtered into a solution of concentrated hydrochloric acid (25cc.) in water (25cc.). This acidic solution was immediately concentrated in vacuo until the product began to separate, complete precipitation being accomplished by addition of methanol (20cc.) and ether (10cc.). The crude product was recrystallised from aqueous methanol (98%) to give the hydrochloride as white crystals (4g.), m.p. 236° (dec.), [lit.,[110] 237 - 238°(dec.)]. (Found : C, 31.55; H, 5.05; N, 36.2; Cl, 18.8. Calc. for $C_5H_9N_5O.HCl$ C, 31.3; H, 5.2; N, 36.6; Cl, 18.5%).

2,8 - Dihydro - 4 - hydroxy - 2 - imino - 6,7,8 - trimethylpteridine Hydrochloride.[110]

2,5 - Diamino - 6 - hydroxy - 4 - methylaminopyrimidine hydro - chloride (4.3g.) was suspended in methanol (100cc.) and a solution of diacetyl (2.5cc.) in methanol (25cc.) was added. The mixture was heated under reflux on a steam bath with stirring for 40 min., filtered hot and the filtrate was allowed to cool. On refrigeration of the solution yellow needles separated. Recrystallisation from 2N -

hydrochloric acid yielded yellow needles (3.5g.) which decomposed without melting at 260 - 263° [lit.,[110] 255 - 260° (dec.)]. The product was identical in ultraviolet spectrum with that of Brown and Jacobsen.[110]

Reaction of 2,8 - Dihydro - 4 - hydroxy - 8 - (2' - hydroxyethyl) - 2 - imino - 6,7 - dimethylpteridine with Pyruvate.

(a) The hydrochloride of the 2 - imino - 6,7 - dimethyl - pteridine (100mg.) was treated with 0.1N - sodium hydroxide solution (20cc.) and sodium pyruvate (100mg.). The pH was adjusted to 12 and the mixture was refluxed in the dark under nitrogen for 7 hr. Brown crystals deposited on refrigeration of the reaction mixture. Recrystallisation from water (with a little charcoal) gave pale yellow needles (32mg.) of 2 - amino - 7,8 - dihydro - 4 - hydroxy - 8 - (2' - hydroxyethyl) - 6 - methyl - 7 - oxopteridine. The product was identical in its physical properties with an authentic sample which was prepared as described previously.

(b) The above reaction also took place in the absence of pyruvate but in this case the presence of oxygen and a longer refluxing period were necessary.

(c) The reaction described in (a) was repeated but at pH 7.3 using a phosphate buffer in place of the sodium hydroxide solution. A refluxing period of 18 hr. was required and the yield (22mg.) of the 6 - methyl - 7 - oxopteridine derivative was somewhat reduced. Paper chromatography of the mother liquor indicated the formation

of some 2,10 - dihydro - 4 - hydroxy - 10 - (2' - hydroxyethyl) - 2 - imino - 7,8 - dimethylbenzo [g] pteridine identified by its Rf values (6 solvent systems).

2,10 - Dihydro - 4 - hydroxy - 10 - (2' - hydroxyethyl) - 2 - imino - 7,8 - dimethylbenzo[g]pteridine.

(a) 2 - Amino - 6 - hydroxy - 4 - (2' - hydroxyethylamino) - 5 - nitropyrimidine (500mg.) was dissolved in a mixture of water (10cc.) and 2N - sodium hydroxide solution (3cc.). The nitro - pyrimidine was reduced by sodium dithionite in the usual way, taken to pH 4 by addition of 2N - hydrochloric acid and treated with diacetyl dimer (500mg.) in water (5cc.). Heating at 90° for 15 min. gave an orange solution, the pH of which was adjusted to 1 with concentrated hydrochloric acid and the solution was refluxed in the dark for 30 min. Solid sodium bicarbonate was carefully added to give pH 7 and the mixture was cooled in the refrigerator. A yellow-brown solid (145 mg.) resulted which was shown by paper chromatography to be a mixture containing 2 components. These were separated by large scale paper chromatography [solvent system (A)] on Whatman No.17 papers. The yellow fluorescing band was eluted with 0.1N - hydrochloric acid and neutralised with sodium bicarbonate giving the 2 - imino - 7,8 - dimethylbenzo[g]pteridine derivative (25mg.), m.p. $> 320°$, identified by its ultraviolet spectrum. (Found : C, 58.6; H, 5.4; N, 24.9. $C_{14}H_{15}N_5O_2$ requires C, 58.95; H, 5.3; N, 24.6%).

(b) 2 - Imino - riboflavin (100mg.) was treated with a solution of sodium metaperiodate (180mg.) in water (30cc.) and stirred in the dark overnight. The resulting reddish brown solid was collected and washed with water, with a little methanol and with ether. The solid (70mg.) was treated with a solution of sodium borohydride (30mg.) in water (30cc.) and stirred for 30 min. The mixture, on standing overnight at room temperature, deposited a reddish brown solid which was dissolved in 0.1N - hydrochloric acid (10cc.), treated with charcoal and filtered hot. The filtrate was carefully adjusted to pH 7 by addition of solid sodium bicarbonate and refrigerated to give yellow-brown crystals (56 mg.; 83%). The product was identical with that obtained in (a) in all respects.

(c) A solution of 2,8 - dihydro - 4 - hydroxy - 8 - (2' - hydroxyethyl) - 2 - imino - 6,7 - dimethylpteridine hydrochloride (1g.) in phosphate buffer (25cc.), pH 7.3, was refluxed in the dark in an atmosphere of oxygen - free nitrogen for 24 hr. At intervals of 6 hr. any solid product which had formed was removed by filtration in a hood under nitrogen. The resulting brown crystals were combined and recrystallised by dissolving in 0.5N - hydrochloric acid (15cc.), treating with charcoal, and adding sodium bicarbonate to the hot filtrate until the pH was increased to 7. The product (260mg.; 49%) could not be obtained in a completely pure state, samples always containing some

decomposition products. The purification was further hampered by the instability of the product which was identical in Rf (6 solvent systems) with the samples obtained in (a) and (b) above.

PART II.

PTERIDINE - 8 - GLYCOSIDES.

THEORETICAL.

Most of the naturally occurring pteridines so far isolated have the 2 - amino - 4 - hydroxypteridine nucleus and with this in mind, methods were sought whereby 2 - amino - 4 - hydroxypteridine - 8 - glycosides might be synthesised. These pteridine - 8 - glycosides are analogous to the nucleosides in the purine series and might therefore play an important role in biological mechanisms.

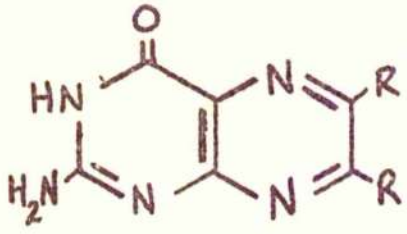
The synthetic difficulties encountered in this part of the work have proved to be formidable but nevertheless interesting. The two main avenues of approach which were considered to be worthy of investigation were (i) direct alkylation of the pteridine nucleus, and (ii) synthesis from 4,5 - diaminopyrimidine precursors.

1. Direct Alkylation.

The methods which are available for the synthesis of 8 - alkyl - pteridines by direct alkylation of the pteridine have been reviewed in the Historical Section. The successful transannular methylation of 2 - amino - 4 - hydroxypteridine by Brown and Jacobsen[110] indicated that pteridine - 8 - glycosides could perhaps be formed by condensation of a pteridine of the type (117) with a halosugar such as (118 ; Bz = COC_6H_5). This reaction, however, was found to be hindered by the insolubility of the pteridine (117 ; R = Me or R = H) in organic solvents.

A suspension of the pteridine (117 ; R = Me or R = H) in N,N - dimethylformamide or in toluene was treated with the halosugar (118 ;

Bz = COC_6H_5) at various temperatures under neutral conditions and also in the presence of sodium bicarbonate or acetic acid. In all of these cases the pteridine derivative was recovered unchanged. Strong bases in which the pteridine is soluble could not be used as solvent as halosugars are converted into 1,2 - glycoseens [131] under these conditions. Furthermore, N_1 - and N_3 - substitution are more likely to take place in alkaline solution. [112]



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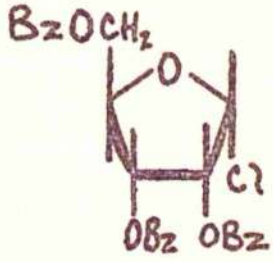

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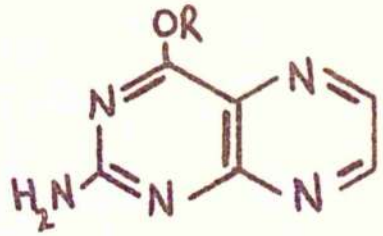


(119)

2 - Amino - 4 - hydroxypteridine (117 ; R = H) was treated with D - ribose in the presence of polyphosphoric ester after the manner of Schramm et al.,[132] who isolated adenosine from reaction of adenine with D - ribose and polyphosphoric ester, but once more insolubility of the starting material was a hindrance. In order to overcome this solubility problem, 2 - amino - 4 - hydroxypteridine (117 ; R = H) was acetylated, the 2 - acetylaminopteridine was dissolved in N,N - dimethylformamide and the halosugar (118 ; Bz = COC_6H_5) was added. The reaction was carried out at 37° and also at higher temperatures but the pteridine derivative was recovered unchanged and prolonged refluxing led only to the decomposition of the ribose derivative.

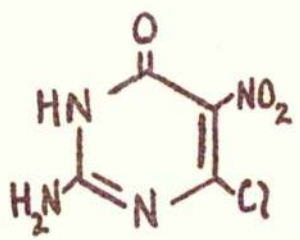
The benzyloxypteridine (119 ; R = $CH_2C_6H_5$) of Pfleiderer and

Lohrmann,[133] which is soluble in hot xylene was synthesised and condensation with the halosugar (118 ; Bz = COC_6H_5) was attempted, but this also ended in failure.

2. Synthetic Approach.

The obvious method of synthesising 8 - substituted pteridines is to condense a 5 - amino - 4 - substituted - aminopyrimidine (e.g. 110) with a 1,2 - dicarbonyl compound such as diacetyl or glyoxal and some studies using model compounds were therefore carried out.

The compounds (110 ; R = Me or R = CH_2CH_2OH) were isolated as their hydrochlorides, as described in Part I, and reacted with glyoxal and with diacetyl to give the appropriate pteridines (120 ; R = Me or R = CH_2CH_2OH ; R' = H or R' = Me). These reactions took place readily in methanolic solution at reflux temperature.



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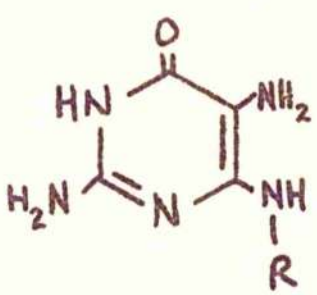

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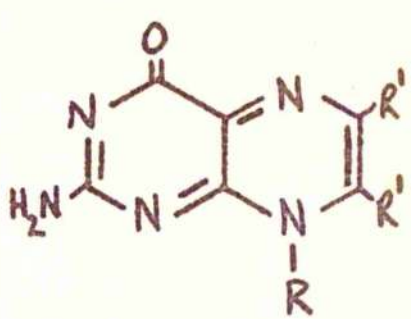

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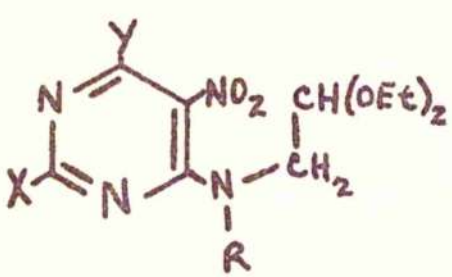

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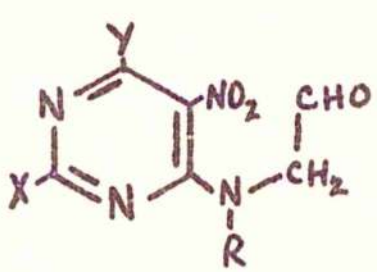

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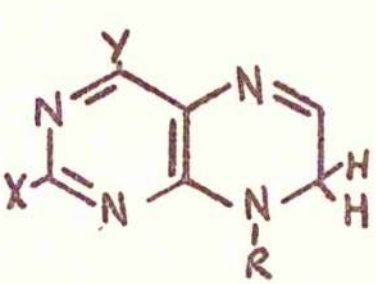
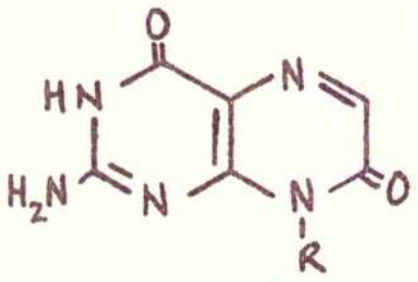


(123)

Albert and Matsuura[134] synthesised 2 - hydroxy - 7,8 - dihydro - pteridine(123; X = OH ; Y = R = H) by hydrolysing the acetal (121 ; X = OH ; Y = R = H) to the aldehyde (122 ; X = OH ; Y = R = H). On catalytic hydrogenation cyclisation to the 7,8 - dihydropteridine readily took place. This offered an alternative route to the model pteridines of type (120). The acetal (121 ; X = NH_2 ; Y = OH ; R = Me) was easily synthesised by reacting the diethyl acetal of methylamino - acetaldehyde with the 4 - chloro - 5 - nitropyrimidine (108) but acid hydrolysis to the corresponding aldehyde (122 ; X = NH_2 ; Y = OH ; R = Me) proved troublesome. A mixture of two unknown pyrimidines, neither of which analysed for the aldehyde, was obtained. This difficulty was overcome by first reducing the nitro group with an aqueous solution of sodium borohydride containing a trace of palladised - charcoal according to the method of Neilson, Wood and Wylie.[135] Heating at 80° for 2 hr. in dilute mineral acid then hydrolysed the acetal and yielded the pteridine (120 ; R = Me ; R' = H) which was formed due to the facile oxidation of the 7,8 - dihydro - pteridine (123 ; X = NH_2 ; Y = OH ; R = Me).

Pteridines of the type (120 ; R = alkyl ; R' = H) underwent rapid oxidation in alkaline solution to the corresponding 7 - oxopteridine (124). This oxidation took place much more readily than that of the 6,7 - dimethylpteridines (120 ; R = alkyl ; R' = Me) which is described in Part I. Having no methyl group in position 7, the pteridines (120 ; R = alkyl ; R' = H) are more readily hydrated to give the intermediate

(125 ; R = alkyl) which is then easily dehydrogenated to the 7 - oxo - pteridine (124 ; R = alkyl).



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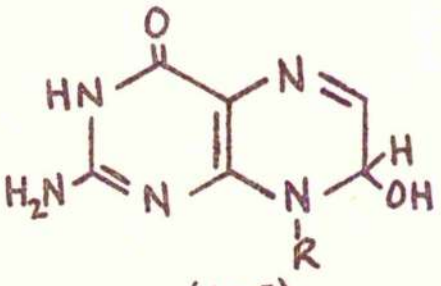
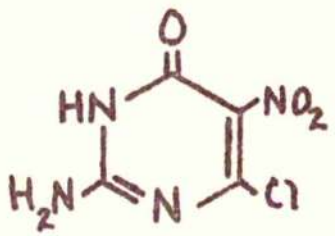


(125)

Having investigated the synthesis and reactions of these model 8 - substituted pteridines, the way appeared clear for the synthesis of pteridine - 8 - glycosides by similar procedures. The initial problem lay in the synthesis of a suitable diaminopyrimidine glycoside (e.g. 110 ; R = glycosyl).

When 2,3,4,6 - tetraacetylglucosylamine (126), which is a weaker base than the model alkylamines used above, was refluxed in dry ethanol with the nitro - chloropyrimidine (108), the expected condensation did not take place and the main sugar - containing product isolated was N,N -α - di - (D - glucosyl) - amine octaacetate. This was presumably formed by condensation of two molecules of the glucosylamine derivative with elimination of ammonia.



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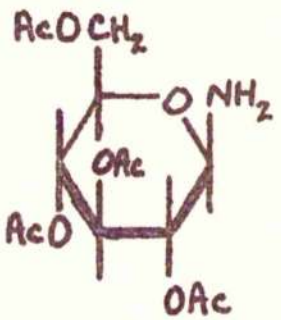

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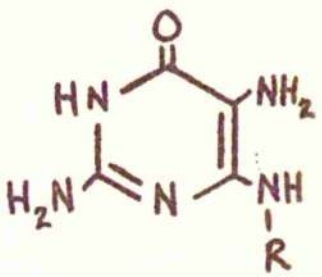

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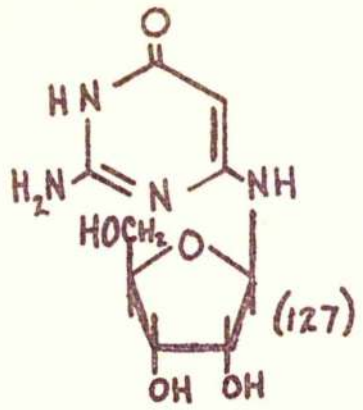
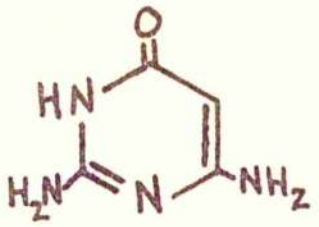
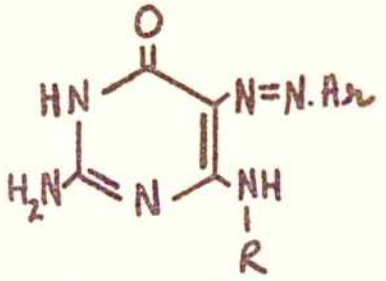


(127)



(128)



(129)

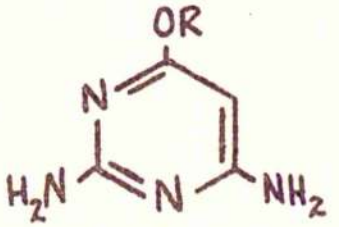
Rembold[136] reported the synthesis of the riboside (127) which he obtained from the direct condensation of 2,4 - diamino - 6 - hydroxy - pyrimidine (128) with D - ribose in presence of ammonium chloride. His attempts to nitrosate this riboside (127) were unsuccessful due to hydrolysis of the glycosidic link. It seemed likely that Rembold's riboside (127) could be converted to the diaminopyrimidine riboside (110 ; R = D - ribosyl) by coupling with a suitable diazonium salt under neutral conditions, thereby protecting the glycosidic link, followed by reduction of the resulting azo compound (129).

The riboside (127) was synthesised and its properties were investigated. Mild acid hydrolysis by 0.1N - hydrochloric acid yielded 2,4 - diamino - 6 - hydroxypyrimidine (128) and D - ribose which were detected by paper chromatography. The riboside gave a positive Dische and Schwarz test for pentoses and in addition, paper chromatography showed, in ultraviolet light, a single absorption spot which gave a positive reaction for sugar using the periodate - Schiff's reagent technique of Baddiley et al.[137] The elementary analysis was in agreement with structure (127) assigned by Rembold.[136] However, on coupling with p - nitrobenzene diazonium chloride or with 2,4 - dichloro -

benzene diazonium chloride, the riboside link was cleaved and the 2,4 - diaminopyrimidine (129 ; R = H) was obtained in each case. These reactions were carried out under both neutral and alkaline conditions, the diazonium solution being neutralised just before addition of the riboside. Coupling with p - carboxybenzene diazonium chloride in glacial acetic acid, as suggested by Hemmerich,[116] suffered a similar fate. The reason for this facile hydrolysis which is more typical of an O - glycoside than an N - glycoside, is inexplicable. The only alternative possibilities are that the sugar is attached to a ring nitrogen atom or to the carbon atom at position 5. Such glycosides, however, would only be hydrolysed with difficulty.[138]

The ultraviolet absorption spectra of Rembold's riboside were compared (Fig. 3) with those of the 4 - (2' - hydroxyethylamino) - pyrimidine (130 ; R = CH_2CH_2OH) and the 6 - alkoxypyrimidine (131 ; R = Me or R = $CH_2C_6H_5$).

(130)



(131)

The spectra of the riboside ($\lambda_{max.}$ 266, 276 and 268 mμ at pH 1, 7.4 and 13 respectively) are similar to those of the hydroxyethylamino - pyrimidine (130 ; R = CH_2CH_2OH), ($\lambda_{max.}$ 267, 268 and 265 mμ at pH 1, 7.4 and 13 respectively) except for the discrepancy of 8 mμ at pH 7.4. On the other hand, the ultraviolet spectra of the alkoxypyrimidine (131 ;

R = Me or R = $CH_2C_6H_5$) ($\lambda_{max.}$ 276 and 266 mμ at pH 1 and pH 7.4 respectively) are also similar to those of the riboside at pH 7.4 and pH 13 respectively. The riboside is easily hydrolysed by 0.1N - hydrochloric acid even at room temperature and it is possible that it is the spectrum of the cation of the diaminopyrimidine (130; R = H) which is being recorded at pH 1 (represented by the 266 mμ peak), and not that of the riboside. The other two peaks quoted above for the riboside (i.e. at 276 and 268 mμ) could be assigned to the cation and neutral molecule respectively, thereby agreeing with structure (131). Determination of pK_a values was complicated by the facile hydrolysis of the riboside.

The "basic" pK_a (4.71) of the riboside (see Appendix, Table I) was found to lie between those of the 6 - hydroxypyrimidines (130 ; R = H) (pK_a, 3.33) and (130 ; R = CH_2CH_2OH) (pK_a, 2.96) on the one hand and those of the alkoxypyrimidines (131; R = Me) (pK_a, 5.25) and (131 ; R = $CH_2C_6H_5$) (pK_a, 5.21) on the other.

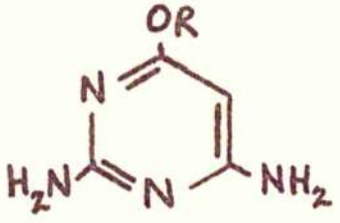
The "acidic" pK_a (10.73) of the riboside was similar to those of the 6 - hydroxypyrimidines (130 ; R = H or R = CH_2CH_2OH) while the alkoxypyrimidines (131 ; R = Me or R = $CH_2C_6H_5$) have, of course, no "acidic" pK_a. The riboside is not hydrolysed at room temperature by 0.1 N - sodium hydroxide indicating that the "acidic" pK_a value of 10.73 is in fact that of the riboside and not that of the diaminopyrimidine (130 ; R = H).

The infrared evidence is ambiguous. Thus, in the 3000 $cm.^{-1}$ region the overlapping of the frequency ranges of the OH and NH

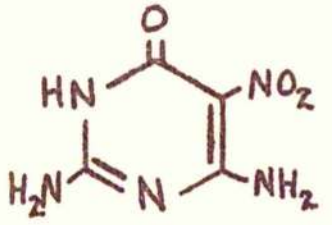
absorptions introduces difficulties, whilst in the 1600 cm.$^{-1}$ region absorption can arise from NH deformations, carbonyl vibrations and vibrations of ring double bonds.

The above evidence is inconclusive but seems to favour an N - glycoside, which is very easily hydrolysed in acid but not so readily in alkali. The failure to isolate the coupled riboside in neutral or alkaline conditions may be due to the facile hydrolysis of the coupled riboside under these conditions.

Attempts to condense D - ribose with other pyrimidines (131 ; R = $CH_2C_6H_5$) and (132) were unsuccessful ; in the latter case this was presumably due to the deactivation of the ring by the 5 - nitro group.

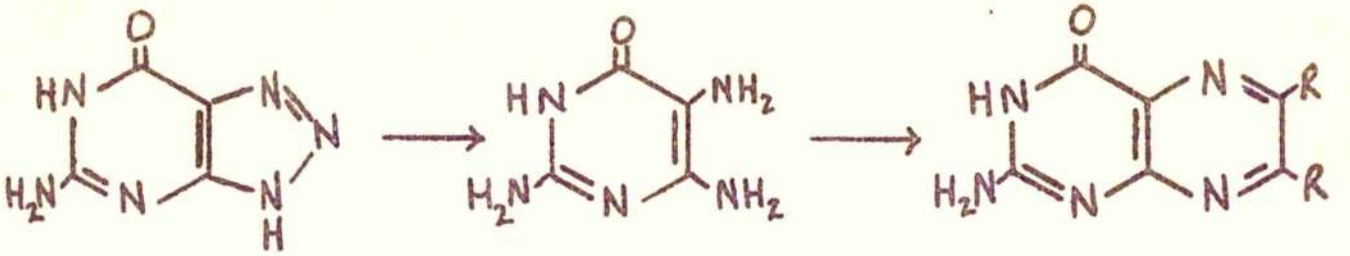


(131)

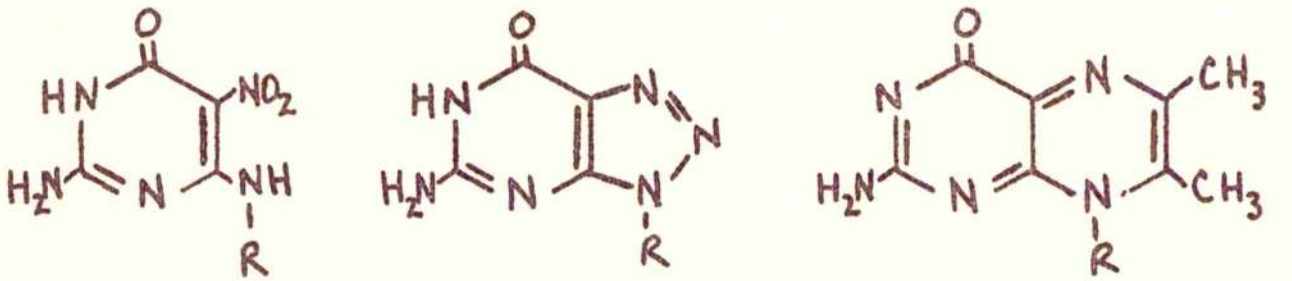


(132)

A fresh approach to the problem was then made. It was observed that several workers [139 - 144] had reported the cleavage of N - N bonds by heating with an excess of Raney nickel. It was decided to investigate the possibility of N - N bond cleavage of azapurine glycosides by this method to provide the required 4,5 - diaminopyrimidine glycoside.



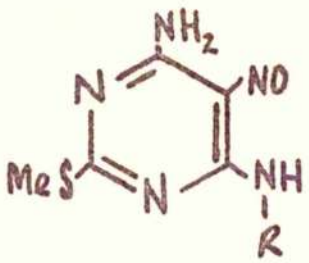
(133) (134) (117)



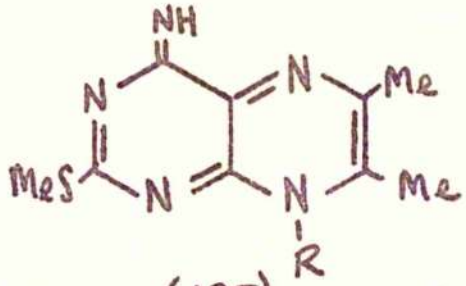
(109) (135) (111)

Initially, as a model reaction, 8 - azaguanine (133) was refluxed for 9 hr. in aqueous ammonia in presence of an excess of Raney nickel and, after removal of the nickel, treatment with diacetyl yielded 2 - amino - 4 - hydroxy - 6,7 - dimethylpteridine (117 ; R = Me) in almost 20% yield. This seemed encouraging. 8 - Aza - 9 - (2' - hydroxyethyl) - guanine (135 ; R = CH_2CH_2OH) was synthesised by catalytic reduction of the 5 - nitropyrimidine (109 ; R = CH_2CH_2OH) and subsequent treatment with nitrous acid. However, this 9 - substituted azapurine (135 ; R = CH_2CH_2OH) and also the corresponding 9 - D - ribityl compound (135 ; R = D - ribityl), which was kindly supplied by Dr. Davoll, Parke Davis and Company, yielded negligible quantities of the expected pteridines (111 ; R = CH_2CH_2OH or R = D - ribityl) when treated as above. The substituent in the 9 - position must hinder cleavage of the N - N bond in some way

as the 9 - substituted azapurines were recovered unchanged.



(136)

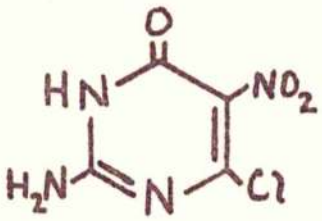


(137)

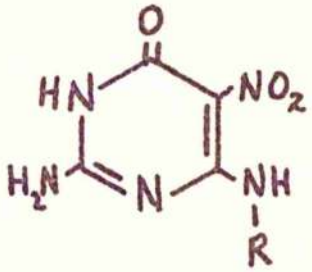
(138)

Yet another attempt at pteridine - 8 - glycoside synthesis was made starting from the 5 - nitrosopyrimidine (136 ; R = D - mannosyl)[145] which was hydrogenated over palladised-charcoal and treated with diacetyl. Instead of yielding the expected 8 - mannosylpteridine (137 ; R = D - mannosyl), the alternative route for cyclisation took place to give the pteridine (138 ; R = D - mannosyl). This cyclisation route is not the one which is usually preferred.[1]

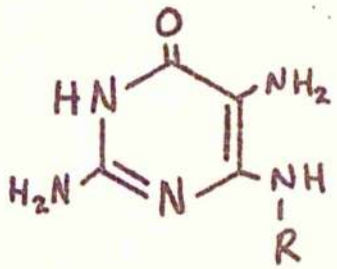
Recently, Forrest and Lohrmann[113] prepared the nitropyrimidine glycoside (109 ; R = tetraacetylglucosyl) by a method similar to the unsuccessful attempt described previously but using more drastic conditions. They treated the nitrochloropyrimidine (108) in a mixture of dry dioxan and ethanol at 100° in a stoppered flask with 2', 3', 4', 6' - tetraacetylglucosylamine.



(108)



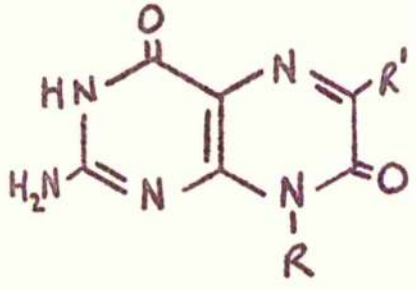
(109)



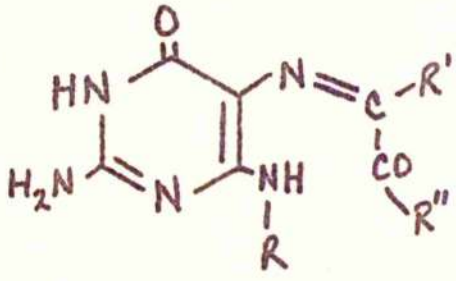
(110)

This reaction was repeated and the desired pyrimidine glycoside (109 ; R = tetraacetylglucosyl) was obtained, a by - product again being H,N - α - di - (D - glucosyl) - amine octaacetate.[146]

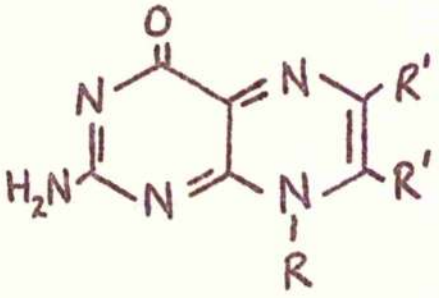
Forrest and Lohrmann[113] prepared a series of 7 - oxopteridine - 8 - glucosides of the type (139 ; R = D - glucosyl) by reduction of the nitropyrimidine (109 ; R = tetraacetylglucosyl) and condensation of the resulting diaminopyrimidine with ethyl pyruvate, ethyl glyoxylate hemiacetal, or diethyl mesoxalate, followed by deacetylation of the glycoside tetraacetate.



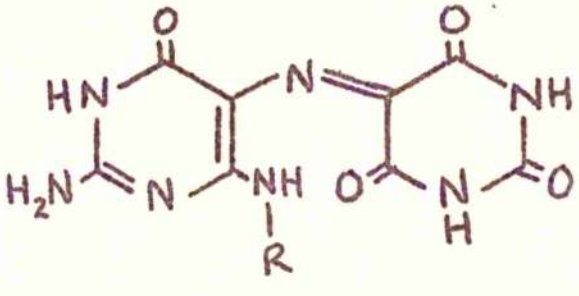
(139)



(140)



(120)

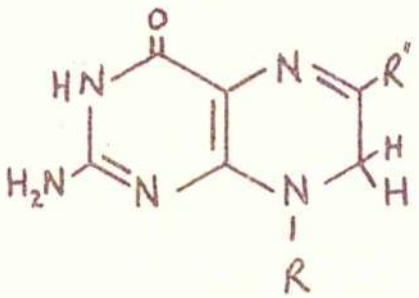


(141)

It was decided to apply this reaction to the synthesis of 8 - glycosylpteridines of the type (120) by reaction of the 4,5 - diamino - pyrimidine glucoside (110 ; R - tetraacetylglucosyl) with diacetyl or glyoxal. Surprisingly, these reactions did not proceed beyond the stage of the Schiff's bases (140 ; R = tetraacetylglucosyl ; R' = R'' = Me or R' = R'' = H) which were identified by paper chromatography, which showed an absorption spot in ultraviolet light in each case, and by comparison of their ultraviolet spectra (Fig. 4) with the spectrum of the Schiff's base (140 ; R = CH_2CH_2OH ; R' = H ; R'' = OEt).

Deacetylation of the nitropyrimidine glucoside tetraacetate (109 ; R = tetraacetylglucosyl) with sodium methoxide furnished the water - soluble pyrimidine glucoside (109 ; R = D - glucosyl). Reduction of this glucoside and condensation with diacetyl or glyoxal again stopped at the Schiff's base stage.

Condensation of the diaminopyrimidine (109 ; R = tetraacetyl - glucosyl) with alloxan gave a bright yellow product which analysed for the Schiff's base (141 ; R = tetraacetylglucosyl).



(142)

As a final effort, it was decided to attempt to reduce the 7 - oxopteridine (139 ; R = D - glucosyl ; R' = H) to the reduced pteridine (142 ; R = D - glucosyl ; R' = H) which would then possibly be readily dehydrogenated to the desired pteridine (120 ; R = D - glucosyl ; R' = H). Lithium aluminium hydride reduction and also the technique of Brown and Subba Rao[147] for the conversion of disubstituted amides to amines using a mixture of sodium boro - hydride and aluminium chloride, were ineffective due to the insolubility of the 7 - oxopteridine glucoside in solvents suitable for these reactions. Attempts to isolate the 7 - oxopteridine glycoside tetraacetate before deacetylation were unsuccessful as the alkaline conditions required for the cyclisation of the Schiff's base also deacetylated the sugar. The free 7 - oxopteridine glucoside could not be reacetylated due to solubility difficulties.

Time did not permit further investigation into this intriguing synthetic problem, the solution to which has, as yet, been so evasive.

EXPERIMENTAL.

2 - Amino - 4 - hydroxypteridine.[148]

2,4,5 - Triamino - 6 - hydroxypyrimidine sulphate monohydrate[149] (10g.) in water (200cc.) was treated with a solution of polyglyoxal (3g.) in water (15cc.) and the mixture was heated on a steam bath for 15 min. with stirring. The red precipitate which formed was dissolved by addition of 4N - sodium hydroxide solution, the solution was filtered hot and the filtrate was treated with 5N - sulphuric acid until the pH was decreased to 3. After cooling, the crude product was collected and washed with water, ethanol and ether. The solid was dissolved in the minimum amount of 2N - sodium hydroxide solution, treated with charcoal and filtered hot. To the filtrate was added an equal volume of 10N - sodium hydroxide giving a pale brown precipitate which was collected and washed with 4N - sodium hydroxide, ethanol and ether. This sodium salt was dissolved in water and adjusted to pH 3 with 5N - sulphuric acid. On cooling, the pale yellow 2 - amino - 4 - hydroxypteridine (5.2g. ; 82%) separated. M.p. $> 360^{\circ}$.

2 - Amino - 4 - hydroxy - 6,7 - dimethylpteridine.[150]

2,4,5 - Triamino - 6 - hydroxypyrimidine sulphate monohydrate (10g.) was suspended in water (700cc.) at 80°. A solution of diacetyl (5cc.) in water (100cc.) was added to the hot mixture and the heating was continued for 2 hr. with stirring. On cooling, a yellow

precipitate formed. The solid was collected, dried and dissolved in 9N - ammonium hydroxide solution (1000cc.). Concentration in vacuo until solid just appeared and acidification to pH 4 yielded the purified pteridine as a pale yellow powder (3.9g. ; 53%), m.p. > 300°.

1 - O - Acetyl - 2,3,5 - tri - O - benzoyl - β - D - ribofuranoside.[151]

D - Ribose (25g.) was dissolved in dry methanol (535cc.) with stirring and a solution of dry hydrogen chloride (2.2g.) in dry methanol (24cc.) was slowly added until Fehling's test for the reducing group became negative. The hydrogen chloride was neutralised by addition of dry pyridine (50cc.) and the solvent was distilled off under reduced pressure. Pyridine (50cc.) was added and the distillation was continued until a viscous yellow oil remained which was dissolved in a mixture of chloroform (135cc.) and dry pyridine (295cc.). The solution was cooled in an ice-salt bath and slowly, with stirring, treated with benzoyl chloride (97cc.). The hetero - geneous mixture was kept in the refrigerator overnight and poured into iced water (1000cc.) with stirring. The organic phase was separated and the aqueous layer extracted with chloroform (3 x 70cc.). The extracts were combined with the original organic phase, washed with water (2 x 100cc.) and dried over anhydrous sodium sulphate. After removal of the solvent at reduced pressure, there remained a red viscous oil which was dissolved in a mixture of glacial acetic acid (40cc.) and acetic anhydride (93cc.). The solution was cooled in an ice bath and concentrated sulphuric acid (13cc.) was slowly added with stirring. The reaction mixture was left overnight in the refrigerator and then

with stirring, poured into iced water (1000cc.) giving a tacky solid. The aqueous phase was thrice decanted and replaced with fresh water. Crystallisation from 10 parts isopropanol yielded white feather-like crystals which were dried over phosphorus pentoxide. Yield 35g., m.p. 130 - 131°. (lit.,[151] 131 - 132°).

Attempted Condensation of α- Chloro - 2,3,5 - tri - O - benzoyl - D - ribofuranoside with 2 - Amino - 4 - hydroxy - 6,7 - dimethylpteridine.

(a) To anhydrous ether (100cc.) saturated with hydrogen chloride at 0° was added acetyl chloride (3.6cc.) and 1 - O - acetyl - 2,3,5 - tri - O - benzoyl - β - D - ribofuranose (3.6g.). Hydrogen chloride was passed through the solution at 0° until the solid completely dissolved. After 4 days at 3° in a stoppered flask, the solution was evaporated to dryness in vacuo. The evaporation was repeated twice with portions (20cc.) of dry benzene giving a clear colourless gum which was dissolved in redistilled N,N - dimethylformamide (10cc.). A suspension of finely powdered 2 - amino - 4 - hydroxy - 6,7 - dimethyl - pteridine (1.7g.) in N,N - dimethylformamide (250cc.) at 37° was treated with the solution of the chlorosugar and the mixture was kept at 37° for 12 days. At the end of this time, unchanged 2 - amino - 4 - hydroxy - 6,7 - dimethylpteridine (1.5g.) was recovered and no other pteridine was detected by paper chromatography.

(b) The procedure described in (a) was repeated but with the addition of sodium bicarbonate (10g.) to the dimethylformamide solution containing the pteridine and chlorosugar. Again no condensation occurred.

(c) The α- chlorosugar was prepared as in (a) from the 1 - O -

acetyl derivative and dissolved in dry toluene (10cc.). This solution was added to a suspension of 2 - amino - 4 - hydroxy - 6,7 - dimethyl - pteridine (1.3g.) in dry toluene (250cc.) containing sodium bicarbonate (15g.). The mixture was thoroughly stirred and heated under reflux for 32 hr. Again the pteridine starting material was recovered unchanged while the sugar had decomposed.

(d) The experiments (b) and (c) were repeated replacing the sodium bicarbonate with acetic acid and also variations in temperature were tried without effect.

Attempted Condensation of D - Ribose with 2 - Amino - 4 - hydroxy - pteridine in presence of Polyphosphoric Ester.

2 - Amino - 4 - hydroxypteridine (1.2g.), suspended in dry re-distilled N,N - dimethylformamide (100cc.), was treated with conc. hydrochloric acid (0.5cc.) followed by the addition of polyphosphoric ester[132] (3 - 4g.), the reaction mixture being thoroughly stirred. A solution of D - ribose (200 mg.) in N,N - dimethylformamide (50cc.) was dropped in slowly to the stirred reaction mixture. After stirring for 22 hr. in a glycerine bath at 50° the mixture was cooled and filtered. Crude unchanged 2 - amino - 4 - hydroxypteridine (0.8g.) only was recovered and no other pteridine was observed on paper chromatography of the reaction mixture.

2 - Acetylamino - 4 - hydroxypteridine.[152]

2 - Amino - 4 - hydroxypteridine (2g.), suspended in acetic anhydride (140cc.), was boiled under reflux for 7 hr. and the hot

solution was filtered. On cooling, cream needles separated which were collected and dried by suction. Recrystallisation from hot water (130cc.) (charcoal) yielded fine white needles (1.1g.), decomp. 283° (lit.,[152] 285°).

Reaction of 2 - Acetylamino - 4 - hydroxypteridine and α - Chloro - 2,3,5 - tri - O - benzoyl - D - ribofuranoside.

The α - chlorosugar was prepared as before from 1 - O - acetyl - 2,3,5 - tri - O - benzoyl - β - D - ribofuranose (7.2g.). The clear colourless gum was dissolved in dry N,N - dimethylformamide (7cc.) and a solution of the pteridine (0.9g.) in dry N,N - dimethylformamide (40cc.) was added. Heating under reflux for 10 hr. yielded mainly unchanged 2 - acetylamino - 4 - hydroxypteridine. Paper chromatography showed several weak blue spots in ultraviolet light but the concentrations of these compounds were too small for identification purposes and were not increased by further refluxing of the reaction mixture which only led to decomposition of the sugar component.

2 - Amino - 4 - benzyloxypteridine.[133]

A solution of 2,4,5 - triamino - 6 - benzyloxypyrimidine[133] (0.7g.) in methanol (20cc.) was added to a warm solution of polyglyoxal (0.2g.) in methanol (20cc.) and boiled under reflux for 2 hr. The methanol was distilled off under vacuum and the brown residue was extracted with several portions (20cc.) of not too hot xylene. On cooling, a yellow - brown precipitate separated which was further purified by recrystallisation from water (charcoal) giving the pteridine as yellow needles (100mg.),

m.p. 220 - 223° (lit.,[133] 220 - 223°).

Attempted Condensation of 2 - Amino - 4 - benzyloxypteridine with α - Chloro - 2,3,5 - tri - O - benzoyl - D - ribofuranoside.

(a) The chlorosugar was prepared as described previously from 1 - O - acetyl - 2,3,5 - tri - O - benzoyl - β - D - ribofuranose (200mg.). The clear colourless gum was dissolved in dry xylene (5cc.) and added to a solution of 2 - amino - 4 - benzyloxypteridine (100mg.) in dry xylene (60cc.). Refluxing for 40 hr. had no effect on the benzyloxypteridine which was recovered unchanged and the sugar eventually decomposed.

(b) The above experiment was repeated in the presence of acetic acid (0.5cc.) and then again in presence of sodium bicarbonate but the end result was similar.

2 - Amino - 4,8 - dihydro - 8 - (2' - hydroxyethyl) - 4 - oxopteridine Hydrochloride.

2,5 - Diamino - 6 - hydroxy - 4 - (2' - hydroxyethylamino) - pyrimidine hydrochloride (8.3g. ; 0.038 mol.) was suspended in methanol (200 cc.) and a solution of polyglyoxal (2.3g.) in methanol (100cc.) was added. The mixture was boiled under reflux for 30 min., the colour of the solution changing to deep red and finally brown. The product was filtered and augmented by concentration of the filtrate in vacuo. The resulting brown crystals were dissolved in 2N - hydrochloric acid (300cc.), heated on a steam bath and treated with charcoal. After a few minutes the mixture was filtered hot, washing the charcoal with hot

2N - hydrochloric acid. The filtrate was concentrated under vacuum until solid separated, complete precipitation being achieved by the addition of a little methanol and refrigeration giving the pteridine hydrochloride as yellow crystals (3.5g. ; 39%), m.p. $> 300^{\circ}$. A portion was recrystallised from 2N - hydrochloric acid for analysis. (Found: C, 39.8 ; H, 4.3 ; N, 28.6 ; Cl, 14.3. $C_8H_{10}N_5O_2Cl$ requires C, 39.4 ; H, 4.1 ; N, 28.75 ; Cl, 14.6%).

2 - Amino - 7,8 - dihydro - 4 - hydroxy - 8 - (2' - hydroxyethyl) - 7 - oxopteridine.

(a) Ethyl glyoxylate hemiacetal[153] (4.5g.) was added to a solution of 2,5 - diamino - 6 - hydroxy - 4 - (2' - hydroxyethylamino) - pyrimidine hydrochloride (4.43g.) in water (100cc.). After standing at room temperature for 30 min. the precipitate was filtered giving the intermediate Schiff's base which was dissolved in N - sodium bicarbonate solution (70cc.) and boiled under reflux for 15 min. Glacial acetic acid (7cc.) and water (1400cc.) were added and the mixture was boiled with a little charcoal and filtered. On cooling pale brown crystals separated which were purified by dissolving in 2N - ammonium hydroxide solution, treating with charcoal, and filtering hot. To the hot filtrate was added glacial acetic acid dropwise until a slight turbidity formed and pale yellow crystals separated on cooling. The 7 - oxopteridine (3.2g.) was washed with water, ethanol and ether, m.p. $> 360^{\circ}$. (Found : C,43.25 ; H, 4.2 ; N, 31.4. Calc. for $C_8H_9N_5O_3$ C, 43.05 ; H, 4.0 ; N, 31.4%).

(b) 2 - Amino - 4,8 - dihydro - 8 - (2' - hydroxyethyl) - 4 - oxopteridine hydrochloride (200mg.) was dissolved in water (10cc.) and N - sodium hydroxide was added dropwise until pH 10. The solution was heated on a steam bath for 4 hr. and filtered. Charcoal was added to the filtrate which was further heated for 20 mins. and filtered hot. The charcoal treatment was repeated and the hot filtrate was acidified with glacial acetic acid. On refrigeration brown crystals separated. Purification was achieved as in (a) by dissolution in dilute ammonium hydroxide solution, treatment with charcoal and acidification to give pale yellow crystals (65mg.) which were identical in Rf (6 solvent systems), in ultraviolet and infrared spectra with the product from (a).

2 - Amino - 6 - hydroxy - 4 - (N,N - methyl - 2', 2' - diethoxyethyl - amino) - 5 - nitropyrimidine.

2 - Amino - 4 - chloro - 6 - hydroxy - 5 - nitropyrimidine (7.65g.; 0.04 mol.) was suspended in ethanol (60cc.) and methylaminoacetaldehyde diethyl acetal (14.0cc. ; 0.1 mol.) was added. The solution was refluxed for 15 min., cooled and filtered. The product was recrystal - lised from isopropanol giving the pyrimidine as yellow hexagonal plates (6.5g. ; 60%), m.p. 183 - 184°. (Found: C, 43.8 ; H, 5.95 ; N, 23.5. $C_{11}H_{19}N_5O_5$ requires C, 43.85 ; H, 6.3 ; N, 23.3%).

2 - Amino - 4,8 - dihydro - 8 - methyl - 4 - oxopteridine Hydrochloride.

(a) 2,5 - Diamino - 6 - hydroxy - 4 - methylaminopyrimidine hydrochloride (2.8g.) was refluxed with stirring for 1 hr. with poly - glyoxal (0.9g.) in methanol (100cc.). The solution was concentrated to ca. 20cc. when a brown precipitate formed. This precipitate was

crystallised from 2 N - hydrochloric acid (charcoal) yielding the hydrochloride as yellow prisms (2.2g.), decomp. 280 - 285° [lit.,[110] ca. 285° (dec.)].

(b) Palladised charcoal (10% Pd. ; 90 mg.) was suspended in water (10cc.) and a solution of sodium borohydride (1.67g. ; 44m. mol.) in water (50cc.) was added with stirring. This was immediately followed by the dropwise addition of a solution of 2 - amino - 6 - hydroxy - 4 - (N,N - methyl - 2',2' - diethoxyethylamino) - 5 - nitro - pyrimidine (3.9g. ; 14.5 m. mol.) in 0.5N - sodium hydroxide solution (50cc.), the stirring being continued until the disappearance of the yellow colour and then for a further 10 min. The catalyst was removed by filtering through celite into 2.5 N - hydrochloric acid solution (50cc.) and the filtrate was immediately concentrated to ca. 20cc. Methanol (30cc.) was added and the precipitated inorganic salts were removed by filtration. The filtrate was heated on a steam bath for 2 hr. and refrigerated to give a pale brown precipitate. Repeated recrystallisation from 2 N - hydrochloric acid (charcoal) yielded the pteridine hydrochloride as prisms (1.1g. ; 39%), decomp. 280 - 283°. The product was identical with that from (a).

2 - Amino - 7,8 - dihydro - 4 - hydroxy - 8 - methyl - 7 - oxopteridine.

(a) To a solution of 2,5 - diamino - 6 - hydroxy - 4 - methyl - aminopyrimidine hydrochloride (8g.) in water (100cc.) was added glyoxylic acid hydrate (4g.) in water (70cc.) and the mixture was thoroughly stirred. After 30 min. at room temperature the cream

crystals of the intermediate Schiff's base (6.7g.) were collected and washed with water, ethanol and ether. This Schiff's base was dissolved in N - sodium bicarbonate solution (140cc.) and boiled under reflux for 15 min. Glacial acetic acid (30cc.) was added to the hot solution followed by water (300cc.) and the mixture was boiled for 5 min. On cooling, a dark brown precipitate separated. The crude product was collected, dissolved in hot 2 N - ammonium hydroxide solution, treated with charcoal and filtered. The hot filtrate was acidified with glacial acetic acid, the precipitation of the product being completed by refrigeration. Pale cream crystals of the 7 - oxopteridine were obtained in the form of needles (3.5g.), m.p. 350°. A portion was recrystallised from a large quantity of water for analysis. (Found : C, 43.8 ; H, 3.3 ; N, 36.5. Calc. for $C_7H_7N_5O_2$ C, 43.5 ; H, 3.6 ; N, 36.3%).

(b) 2 - Amino - 4,8 - dihydro - 8 - methyl - 4 - oxopteridine hydrochloride (600mg.) was dissolved in water (30cc.) and N - sodium hydroxide solution was added dropwise to give pH 10. After heating for 3½ hr. at 80° the mixture was filtered hot, the filtrate was treated with charcoal and was further heated for 10 min. The charcoal was removed by filtration and the hot filtrate was acidified with glacial acetic acid yielding brown crystals on refrigeration. The crude product was purified as in (a) giving a product (210mg.) which was identical in all respects with that obtained by the procedure described in (a).

Condensation of 2,4 - Diamino - 6 - hydroxypyrimidine with D - Ribose.[136]

D - Ribose (7,8g. ; 0.05 mol.) and ammonium chloride (0.5g.) were dissolved in a solution of ethanol (150cc.) and water (50cc.). 2,4 - Diamino - 6 - hydroxypyrimidine[154] (10.3g. ; 0.08 mol.) was added and the misture was heated on a steam bath with stirring for 3½ hr. The solution was filtered and the filtrate was treated with acetone (300cc.). The acetone was carefully decanted from the resulting gum which was dissolved in water (50cc.). To this aqueous solution methanol (250cc,) was added giving a pale yellow precipitate which was rapidly filtered and ground with methanol and then with ether, the precipitate being pressed dry. The product was hygroscopic and was dried *in vacuo* in a desiccator over phosphorus pentoxide. The crude product was dissolved in 1 : 1 dry N,N - dimethylformamide - anhydrous methanol (50cc./g.), filtered hot and the filtrate was cooled in ice to give the glycoside as a pale yellow solid (7.1g.) which was stored *in vacuo* over phosphorus pentoxide. The product ("Rembold's riboside") gave a positive Dische and Schwarz test for pentoses. Paper chromatography in solvent systems (B) and (F) showed a single absorption spot, when viewed under ultraviolet light, which gave a positive reaction for sugar using the technique of Baddiley *et al.*[137] (Found : C, 41.5 ; H, 5.7 ; N, 22.2. Calc. for $C_9H_{14}N_4O_5$ C, 41.9 ; H, 5.4 ; N,21.7%).

Acid Hydrolysis of "Rembold's Riboside".

The riboside (0.2g.) was kept overnight at room temperature in 0.1 N - hydrochloric acid (20cc.) yielding 2,4 - diamino - 6 - hydroxy - pyrimidine and ribose in addition to some unchanged riboside, which

were detected in the reaction mixture by paper chromatography (4 solvent systems). Complete hydrolysis was achieved by refluxing in 0.1N - hydrochloric acid for 2 hr.

Alkaline Hydrolysis of "Rembold's Riboside".

The riboside (0.2g.) was not affected on standing overnight in 0.1N - sodium hydroxide solution (20cc.). Only slight hydrolysis of the riboside occurred after refluxing the riboside (0.2g.) in 2N - sodium hydroxide solution (20cc.) for 4 hr.

Coupling Reactions of 2,4 - Diamino - 6 - hydroxypyrimidine Riboside.

(a) p - Nitroaniline (0.26g.) was dissolved in a mixture of water (10cc.) and concentrated hydrochloric acid (1.5cc.) and was diazotised at 0° by the portionwise addition of sodium nitrite (0.14g.) in water (10cc.) with stirring. The diazonium solution was added rapidly to a cold freshly prepared solution of the riboside (0.52g.) in water (20cc.). The resulting solution was immediately poured into a solution of sodium bicarbonate (1.6g.) in water (100cc.) at 0° and the mixture was left in ice for 2 hr. The red precipitate was filtered and shown to be identical with 2,4 - diamino - 6 - hydroxy - 5 - p - nitro - benzeneazopyrimidine by comparison with an authentic sample prepared by coupling 2,4 - diamino - 6 - hydroxypyrimidine with the same reagent. Hydrolysis was also indicated by the paper chromatogram technique of Baddiley et al.[137]

Variations in the above technique were carried out in an attempt to prevent hydrolysis occurring.

(i) The diazonium solution was neutralised with sodium bicarbonate solution just before addition of the riboside solution.

(ii) A strongly alkaline (sodium hydroxide) solution of the riboside was added to the neutralised (sodium bicarbonate) solution of the diazonium salt.

However, the product obtained in all cases was the same.

(b) 2,4 - Dichloroaniline (0.27g.) was dissolved in water (15cc.) containing conc. hydrochloric acid (0.65cc.) and diazotised by adding a solution of sodium nitrite (0.12g.) in water (10cc.) portionwise at 0°. The solution was neutralised by pouring into an aqueous solution of sodium bicarbonate (1.3g. in 20cc.). A solution of the riboside (0.5g.) in water (10cc.) was immediately added to the neutralised diazonium solution producing a bright orange precipitate. After 2 hr. the precipitate was collected, washed well with water and dried in a vacuum desiccator over calcium chloride. This product was identical with that obtained by treating diazotised 2,4 - dichloroaniline with 2,4 - diamino - 6 - hydroxypyrimidine at 0°. Furthermore, coupling in strongly alkaline solution gave the same product.

(c) A solution of p - amino - benzoic acid (2g.) in a mixture of water (10cc.) and conc. hydrochloric acid was cooled to 0°. A solution of sodium nitrite (1.2g.) in water (5cc.) was added and the mixture was cooled to -5° in an ice-salt bath. White fluffy needles of p - carboxy - benzene diazonium chloride[116] separated which were washed with tetra - hydrofuran and sucked dry. The pyrimidine riboside (0.5g.) was dissolved in glacial acetic acid (20cc.) and cooled to room temperature.

The above diazonium salt was added to the acetic acid solution of the riboside whereby a brown precipitate was obtained. Filtration of the mixture after 2 hr. at room temperature yielded a pale brown solid (0.31g.), m.p. $> 320^{\circ}$, which was identical with that obtained from the reaction of the diazonium salt with 2,4 - diamino - 6 - hydroxypyrimidine.

2 - Amino - 6 - hydroxy - 4 - (2' - hydroxyethylamino) - pyrimidine Hydrochloride.[42]

2 - Amino - 4 - chloro - 6 - hydroxypyrimidine (2g.) and ethanol - amine (2cc.) were heated in an oil bath at 140° for 30 min. On cooling, the mixture was diluted with absolute ethanol (80cc.) and 18% ethanolic hydrogen chloride (4cc.). After standing in ice for 1 hr. the mixture was filtered and the product was washed with ethanol (40cc.) and ether (20cc.). The pyrimidine hydrochloride (1.72g.) was dried at room temperature, m.p. 200 - 203° (lit.,[42] 203°).

2,4 - Diamino - 6 - methoxypyrimidine.[155]

2,4 - Diamino - 6 - chloropyrimidine (2g.) was heated under reflux for 1 hr. with a solution of sodium methoxide (1g. sodium in 50cc. methanol). The precipitated sodium chloride was removed by filtration and the filtrate was evaporated to dryness under vacuum. The white solid was recrystallised from ethanol to give white crystals (1.7g.), m.p. 160 - 162° (lit.,[155] 140 - 145°; lit.,[133] 162 - 163°).

2,4 - Diamino - 6 - chloropyrimidine.

A suspension of 2 - amino - 4,6 - dichloropyrimidine (50g.) in 10% (w/v) aqueous ammonia (600cc.) was heated at 120° for 2 hr. in a

litre bomb fitted with a mechanical stirrer. After cooling to room temperature the mixture was filtered and the product was recrystallised from water (10cc./g.) (charcoal) to give the pyrimidine as white crystals (35g.; 79%), m.p. 199 - 200° (lit.,[156] 197 - 200°). (Found : C, 33.3 ; H, 3.3 ; N, 38.95 ; Cl, 25.0. Calc. for $C_4H_5N_4Cl$ C, 33.2 ; H, 3.5 ; N, 38.8 ; Cl, 24.6%).

2,4 - Diamino - 6 - benzyloxypyrimidine.[133]

Sodium (6g.) was dissolved in benzyl alcohol (160cc.) and dry 2,4 - diamino - 6 - chloropyrimidine (35g.) was added. The mixture was heated with stirring at 150 - 160° for 3 hr. in an oil bath, excluding moisture, and then the excess benzyl alcohol was completely removed by distillation under vacuum. The residual gum was dissolved in benzene (500cc.), charcoal was added and the mixture was heated on a steam bath for 15 min. The charcoal was removed by filtration and the filtrate on cooling yielded white prisms, m.p. 105 - 107°. Recrystallisation from benzene gave the pyrimidine as lustrous white crystals (35g.; 67%), m.p. 107 - 109° (lit.,[133] 108 - 109°).

Attempted Condensation of 2,4 - Diamino - 6 - benzyloxypyrimidine with D - Ribose.

(a) D - Ribose (1.5g.; 0.01 mol.), ammonium chloride (0.2g.) and 2,4 - diamino - 6 - benzyloxypyrimidine (4.32g.; 0.02 mol.) were added to anhydrous ethanol (60cc.) and refluxed for 8 hr. On concentration of the reaction mixture the diamino - benzyloxypyrimidine was recovered unchanged.

(b) D - Ribose (0.95g.) was heated under reflux with the diamino - benzyloxypyrimidine (2.16g.) and ammonium chloride (0.1g.) in benzene (150cc.). The flask was attached to a Dean and Stark apparatus for removal of any water which may have formed during the reaction. After refluxing for 6 hr. the reaction mixture was concentrated yielding mainly the diamino - benzyloxypyrimidine mixed with ammonium chloride. The ribose had largely decomposed.

2,4 - Diamino - 6 - hydroxy - 5 - nitropyrimidine.

2 - Amino - 4 - chloro - 6 - hydroxy - 5 - nitropyrimidine (1g.) was dissolved in ethanol (100cc.) and ammonium hydroxide solution (S.G. 0.880) was added until the solution was alkaline to litmus. The solution was refluxed for 30 min., concentrated *in vacuo* until a precipitate began to separate and adjusted to pH 5 by addition of 5 N - sulphuric acid. The product (0.6g.) was collected, washed with water and dried, m.p. $> 300^{\circ}$. The product was identical with an authentic sample[157] of 2,4 - diamino - 6 - hydroxy - 5 - nitropyrimidine.

Attempted Condensation of 2,4 - Diamino - 6 - hydroxy - 5 - nitro - pyrimidine with D - Ribose.

D - Ribose (0.5g.) and ammonium chloride (0.05g.) were dissolved in a mixture of ethanol (20cc.) and water (5cc.). 2,4 - Diamino - 6 - hydroxy - 5 - nitropyrimidine (0.6g.) was added and the mixture was heated with stirring on a steam bath for 20 hr. On concentration the pyrimidine starting material (0.58g.) was recovered unchanged.

2 - Amino - 4 - hydroxy - 6,7 - dimethylpteridine from 8 - Azaguanine.

8 - Azaguanine (0.5g.) was dissolved in 5 N - ammonium hydroxide (40cc.), Raney nickel (ca. 6g.) was added and the mixture was refluxed for 9 hr. The nickel was filtered from the hot solution, washed with hot 5 N - ammonium hydroxide solution and the combined filtrate and washings were treated with diacetyl (0.5cc.). The solution was boiled gently for 2 hr. by which time much of the excess ammonia had been removed and the product began to separate. The pH of the solution was adjusted to 4 by dropwise addition of 5 N - sulphuric acid and the mixture was cooled to room temperature. The yellow solid (120 mg. ; 19%) which formed was identical in its Rf (6 solvent systems) and ultraviolet spectra with an authentic sample of 2 - amino - 4 - hydroxy - 6,7 - dimethylpteridine, prepared as described previously. A shorter or longer refluxing time with Raney nickel led to decreased yields of the pteridine.

8 - Aza - 9 - (2' - hydroxyethyl) - guanine.

2 - Amino - 6 - hydroxy - 4 - (2' - hydroxyethylamino) - 5 - nitropyrimidine (1.29g. ; 6 m. mol.) was suspended in water (100cc.) and 4 N - sodium hydroxide solution was added dropwise until the solid just dissolved. The solution was hydrogenated over Raney nickel at room temperature, the catalyst was filtered and washed with a small amount of hot water. The filtrate was quickly cooled to 5° in an ice bath and sodium nitrite (0.69g. ; 10 m. mol.) was added. The pH of the solution was adjusted to 4 by dropwise addition of glacial acetic acid

and the temperature was kept below 5° for 30 min. The pale brown solid was collected and washed with cold water, ethanol and ether. The solid (1.02g.) was suspended in water (300cc.) and 9 N - ammonium hydroxide solution was added dropwise until complete dissolution had occurred. The solution was treated with charcoal and boiled. After filtration of the charcoal the filtrate was allowed to cool yielding white needles which were recrystallised from water to give the pure azapurine (785 mg. ; 70%). (Found : C, 36.55 ; H, 4.3 ; N, 42.8. $C_6H_8N_6O_2$ requires C, 36.7 ; H, 4.1 ; N, 42.85%).

Action of Excess Raney Nickel on 8 - Aza - 9 - (2' - hydroxyethyl) - guanine.

8 - Aza - 9 - (2' - hydroxyethyl) - guanine (0.69g.) was dissolved in 9 N - ammonium hydroxide solution (30cc.) and Raney nickel (7g.) was added. The mixture was refluxed for 9 hr., the nickel was removed by filtration and the filtrate was treated with diacetyl (0.5cc.). The pH of the solution was adjusted to 4 by dropwise addition of glacial acetic acid and after 2 hr. heating on the steam bath the solution was refrigerated. The brown solid (360mg.) obtained was shown to be unchanged starting material by comparison of Rf values (6 solvent systems) and ultraviolet spectra.

Action of Excess Raney Nickel on 8 - Aza - 9 - D - ribitylguanine.

8 - Aza - 9 - D - ribitylguanine (0.5g.) was dissolved in water (40cc.) with the aid of heat. Raney nickel (7g.) was added and the mixture was refluxed for 8 hr. Filtration of the catalyst was

followed by the addition to the filtrate of a solution of diacetyl (0.5cc.) in water (70cc.). Dropwise addition of glacial acetic acid reduced the pH of the solution to 4 and the solution was heated on a steam bath for 2 hr. The original 9 - substituted - 8 - azapurine (0.32g.) separated on refrigeration.

6 - Amino - 4 - D - mannosylamino - 2 - methylmercapto - 5 - nitroso - pyrimidine.[145]

A suspension of 6 - amino - 4 - D - mannosylamino - 2 - methyl - mercaptopyrimidine (4.5g.) in water (250cc.) was treated at 0° with a solution of sodium nitrite (3g.) in water (50cc.) and glacial acetic acid (9cc.) was added with stirring. The solution was kept at 0° for 1 hr. and for an additional 1 hr. at room temperature. The blue precipitate was collected, washed with ice-cold water and recrystallised from water (ca. 2000cc.) to give the nitrosopyrimidine as fine purple needles (3g.), m.p. 231 - 233° (dec.) [lit.,[145] 230 - 231° (dec.)].

4 - D - Mannosylamino - 2 - methylmercapto - 6,7 - dimethylpteridine.

A suspension of 6 - amino - 4 - D - mannosylamino - 2 - methyl - mercapto - 5 - nitrosopyrimidine (0.5g.) in water (100cc.) was hydrogenated over palladised-charcoal (10% Pd; 200mg.). The catalyst was removed by filtration and the filtrate was treated with diacetyl (0.3cc.). After heating the solution at 80° for 20 min. the volume was reduced in vacuo to ca. 20cc. and refrigerated. The resulting crystals were recrystallised from water (charcoal) yielding the pteridine as "off-white" needles (0.1g.), m.p. 250 - 252° (dec.). The product gave

a positive Molisch's test and the paper chromatogram indicated the presence of a sugar by the technique of Baddiley et al.[137] The ultraviolet spectrum was similar to that of 4 - amino - 6,7 - dimethyl - 2 - methylmercaptopteridine. In addition, on a paper chromatogram it showed one blue fluorescent spot under ultraviolet light. (Found: C, 44.9 ; H, 5.5 ; N, 17.6. $C_{15}H_{21}N_5O_5S.H_2O$ requires C, 44.9 ; H, 5.4; N, 17.45%).

Acetobromoglucose.[158]

A mixture of D - glucose monohydrate (99g.) and acetic anhydride (420cc.) was treated cautiously with conc. sulphuric acid (4 drops), cooling momentarily in cold water to prevent the reaction becoming too vigorous. Within about 10 min. the glucose had almost completely dissolved and the temperature of the reaction mixture was approximately 100°. The flask was loosely stoppered and heated on a steam bath for 2 hr. Distillation under reduced pressure removed a mixture of acetic acid and acetic anhydride (ca. 300cc.). Acetic anhydride (90cc.) was added to the resulting warm viscous syrup, the mixture warmed slightly on the steam bath until the solution became homogeneous and a solution of hydrogen bromide (45% in acetic acid; 540cc.) was added. The flask was then sealed with a rubber stopper and allowed to stand at 5° over - night. The excess hydrogen bromide, acetic acid and acetic anhydride were removed by distillation at not more than 60° under reduced pressure. A crystalline solid separated, was treated with dry isopropyl ether (450cc.) and dissolved by warming carefully. The solution was cooled

rapidly to 45°, allowed to cool slowly to room temperature and was finally refrigerated at 5° for 2 hr. The acetobromoglucose was collected and washed with dry isopropyl ether (75cc.) giving white crystals (180g.), m.p. 87 - 88° (lit.,[158] 87 - 88°). The product was stored over sodium hydroxide in vacuo.

2,3,4,6 - Tetra - O - acetylglucosylazide.[159]

Freshly prepared acetobromoglucose (34g.) was stirred in aceto - nitrile (50cc.) with finely powdered sodium azide (17g.) and heated under reflux on a steam bath for 4 hr., moisture being excluded. The sodium bromide and excess sodium azide were filtered from the hot solution. The filter cake was well washed with chloroform and the combined filtrate and washings were concentrated under vacuum to give a viscous syrup which was dissolved in a little ether. The ethereal solution was kept at - 10° for 30 min. when the fine crystalline material was collected. Recrystallisation from methanol (150cc.) afforded white crystals (17.1g.; 55%), m.p. 126 - 129° (lit.,[159] 129°).

2,3,4,6 - Tetra - O - acetylglucosylamine.[160]

The above glucosylazide tetraacetate (20g.) was hydrogenated in ethyl acetate (250cc.) using platinum oxide (1g.) as catalyst, the apparatus being frequently evacuated and fresh hydrogen introduced. (The completion of the reaction was indicated by the disappearance of the azide peak in the infrared spectrum). The reaction mixture was filtered and the filtrate was evaporated to dryness in vacuo giving a white solid which was recrystallised from ethanol. The product (16g.), m.p. 126° (lit.,[160] 126°) was stored over phosphorus pentoxide in vacuo.

4 - (2',3',4',6' - Tetraacetylglucosylamino) - 2 - amino - 6 - hydroxy - 5 - nitropyrimidine.[113]

2,3,4,6 - Tetra - O - acetylglucosylamine (31g.) was dissolved in a mixture of dry dioxan (80cc.) and dry ethanol (55cc.). 2 - Amino - 4 - chloro - 6 - hydroxy - 5 - nitropyrimidine (8g.) was added portionwise to the glucosylamine solution and the mixture was heated under reflux with stirring for 3 hr. After cooling, the mixture was filtered and the filtrate was poured into water (400cc.). Extraction with chloroform (8 x 100cc.) containing 5% v/v N,N - dimethylformamide followed and the combined extracts were dried over anhydrous sodium sulphate. The sodium sulphate was removed by filtration and the filtrate was evaporated to dryness under vacuum to give a pale yellow gum. Fractional crystallisation from methanol yielded initially N,N -α- di - (D - glucosyl) - amine octaacetate (12.5g.), m.p. 216 - 218°, $[\alpha]_D$ -89° (chloroform) [lit.,[146] m.p. 213 - 216°, $[\alpha]_D$ -87° (chloroform)], followed by the separation of the desired pyrimidine glucoside. Recrystallisation of the glucoside from N,N - dimethylformamide/water gave white needles (11.2g.; 53%), m.p. 296 - 300° (dec.) [lit.,[113] 295 - 300° (dec.)].

Deacetylation of 4 - (2',3',4',6' - Tetraacetylglucosylamino) - 2 - amino - 6 - hydroxy - 5 - nitropyrimidine.

The glucoside tetraacetate (1.5g.) was suspended in warm absolute ethanol (75cc.) and sodium ethoxide solution (3cc.) (5g. sodium in 100cc. dry ethanol) was added dropwise over a period of 30 min. The mixture was refluxed on a steam bath for an additional 1 hr., with stirring, care

being taken to have the solution kept alkaline by addition of more ethoxide when necessary. The mixture was cooled, filtered and the residue was washed quickly with dry ethanol and acetone. Recrystallisation from the minimum amount of aqueous ethanol gave the glucoside as a pale yellow solid (0.98g.: 98%), which was hygroscopic and decomposed without melting at ca. 200 - 210°. (Founds C, 34.1; H, 4.7; N, 19.6. $C_{10}H_{15}N_5O_8.H_2O$ requires C, 34.2; H, 4.8; N, 19.95%).

2 - Amino - 8 - D - glucosyl - 7,8 - dihydro - 4 - hydroxy - 6 - methyl - 7 - oxopteridine.[113]

(a) 4 - (2',3',4',6' - Tetraacetylglucosylamino) - 2 - amino - 6 - hydroxy - 5 - nitropyrimidine (4g.) was suspended in methanol (70cc.) and hydrogenated over Raney nickel. Ethyl pyruvate (4cc.) was added to the hydrogenation mixture which was then refluxed for 2 hr. under nitrogen. The mixture was filtered hot and the red filtrate was evaporated carefully to dryness in vacuo. The syrupy residue was repeatedly dissolved in chloroform (25cc.) and the solvent was removed each time under vacuum. After addition of anhydrous ether a yellow solid (3.5g.) formed. This solid was recrystallised from ethanol to give yellow needles which were dissolved in warm ethanol (80cc.) and a conc. solution of sodium ethoxide was added dropwise until a dense precipitate appeared. Heating on the steam bath for 30 min., keeping the solution alkaline, yielded a yellow precipitate (2.2g.) which was recrystallised several times from water/acetone (charcoal). Decomp. $>$ 250°.

(b) 2 - Amino - 4 - D - glucosylamino - 6 - hydroxy - 5 - nitro - pyrimidine (500mg.) was dissolved in water (20cc.) and reduced with

sodium dithionite. Ethyl pyruvate (0.5cc.) was added and heated on a steam bath for 2 hr. On cooling, yellow crystals (60mg.) separated which were identical with those obtained in (a).

2 - Amino - 8 - D - glucosyl - 7,8 - dihydro - 4 - hydroxy - 7 - oxo - pteridine.[113]

4 - (2',3',4',6' - Tetraacetylglucosylamino) - 2 - amino - 6 - hydroxy - 5 - nitropyrimidine (1.1g.) was reduced in methanol (25cc.) as in the above procedure and the mixture was filtered into a solution of ethyl glyoxylate hemiacetal (1cc.) in methanol (5cc.). After refluxing under nitrogen for 50 min., the solution was concentrated carefully under vacuum to ca. 10cc. Water (20cc.) was added giving yellow crystals of the Schiff's base ($\lambda_{max.}$ 253, 288, 356 mμ at pH 13). These crystals were dissolved in methanol (30cc.) and dilute sodium methoxide solution was added dropwise to give pH 7 - 8. The solution was refluxed for 3 hr. and allowed to cool whereupon a yellow solid (0.9g.) deposited. The 7 - oxopteridine was dried over phosphorus pentoxide in vacuo.

Reaction of 4 - (2',3',4',6' - Tetraacetylglucosylamino) - 2 - amino - 6 - hydroxy - 5 - nitropyrimidine with 1,2 - Dicarbonyl Compounds.

(a) The pyrimidine glucoside tetraacetate (1.5g.) was suspended in methanol (25cc.) and hydrogenated over Raney nickel. The catalyst was removed by filtration and the filtrate was collected into a solution of diacetyl (0.6cc.) in methanol (10cc.). This solution was immediately refluxed under nitrogen for 10 hr. The yellow solid was filtered. Its ultraviolet absorption spectrum and its appearance on paper chromatography

suggested that it was the intermediate Schiff's base which had not cyclised to the expected 8 - glucosylpteridine. The Schiff's base could not be induced to form the pteridine by heating under various conditions of pH and temperature.

(b) The above reaction was repeated under anhydrous conditions in presence of dehydrating agents, e.g. zinc chloride in dry methanol or phosphorus pentoxide in dry benzene, but cyclisation to the 8 - glucosyl - pteridine was not effected.

(c) Reaction (a) was repeated with glyoxal in place of diacetyl but again the reaction stopped at the Schiff's base stage.

(d) The pyrimidine glucoside tetraacetate (1g.) was suspended in ethyl acetate (40cc.) and triethylamine (2cc.) was added. The mixture was hydrogenated over pre-reduced platinum oxide (100mg.) and filtered. The filtrate was collected into a solution of alloxan (0.35g.) and boric acid (0.4g.) in acetic acid (50cc.). The solution was heated at 37° under anhydrous conditions for 16 hr. The resulting brown crystals (250mg.) were recrystallised from water to give yellow crystals (200mg.) of the Schiff's base, m.p. > 300°. (Found: C, 42.15; H, 4.8; N, 16.05. $C_{22}H_{25}N_7O_{13}$. $1\frac{1}{2}H_2O$ requires C, 42.4; H, 4.5; N, 15.8%).

(e) The tetraacetylglucoside was deacetylated as described previously and reactions (a) and (c) were carried out with the free glucoside in aqueous medium. In each case, the reactions did not proceed beyond the formation of the Schiff's base, identified by ultraviolet spectroscopy and comparison with the deacetylated products of reactions (a) and (c).

APPENDIX.

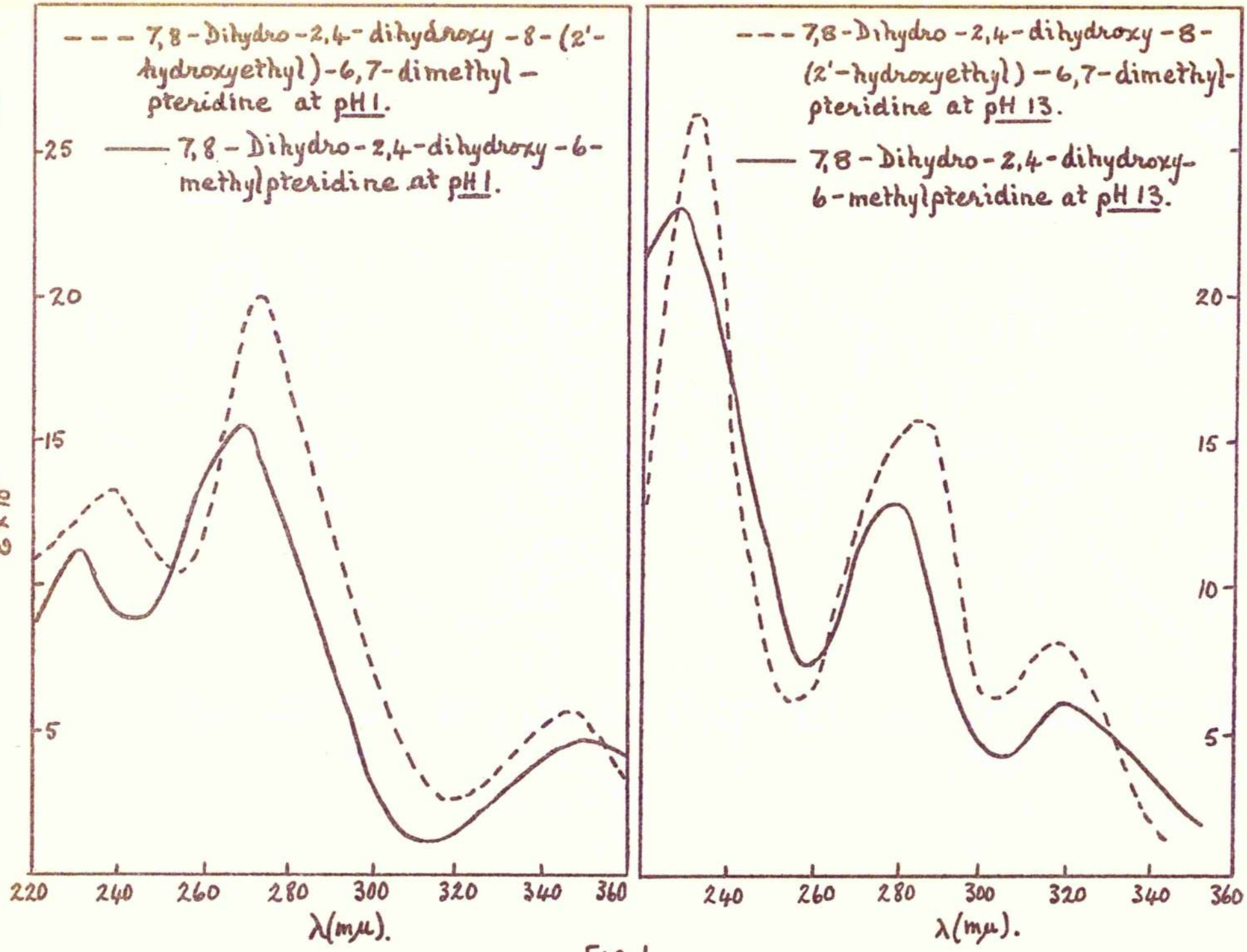

- - - 7,8-Dihydro-2,4-dihydroxy-8-(2'-hydroxyethyl)-6,7-dimethyl-pteridine at pH 1.
—— 7,8-Dihydro-2,4-dihydroxy-6-methylpteridine at pH 1.
ε × 10⁻³
25
20
15
5
220
240
260
280
300
320
340
360
λ(mμ).
- - - 7,8-Dihydro-2,4-dihydroxy-8-(2'-hydroxyethyl)-6,7-dimethyl-pteridine at pH 13.
—— 7,8-Dihydro-2,4-dihydroxy-6-methylpteridine at pH 13.
20
15
10
5
240
260
280
300
320
340
360
λ(mμ).

FIG. 1

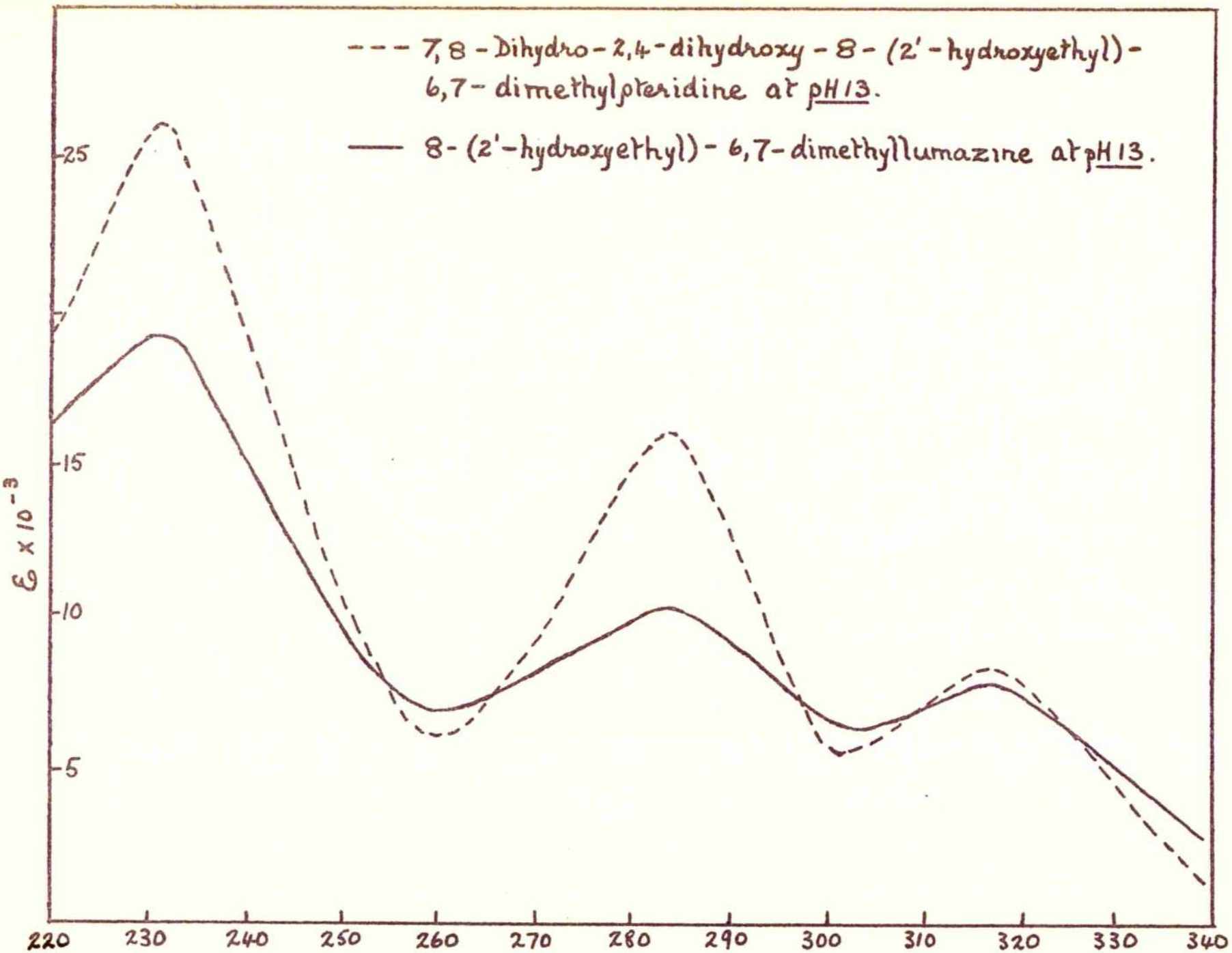

--- 7,8 - Dihydro - 2,4 - dihydroxy - 8 - (2' - hydroxyethyl) - 6,7 - dimethylpteridine at pH 13.
—— 8 - (2' - hydroxyethyl) - 6,7 - dimethyllumazine at pH 13.
ε x 10⁻³
25
15
10
5
220
230
240
250
260
270
280
290
300
310
320
330
340
λ (mμ).

FIG. 2.

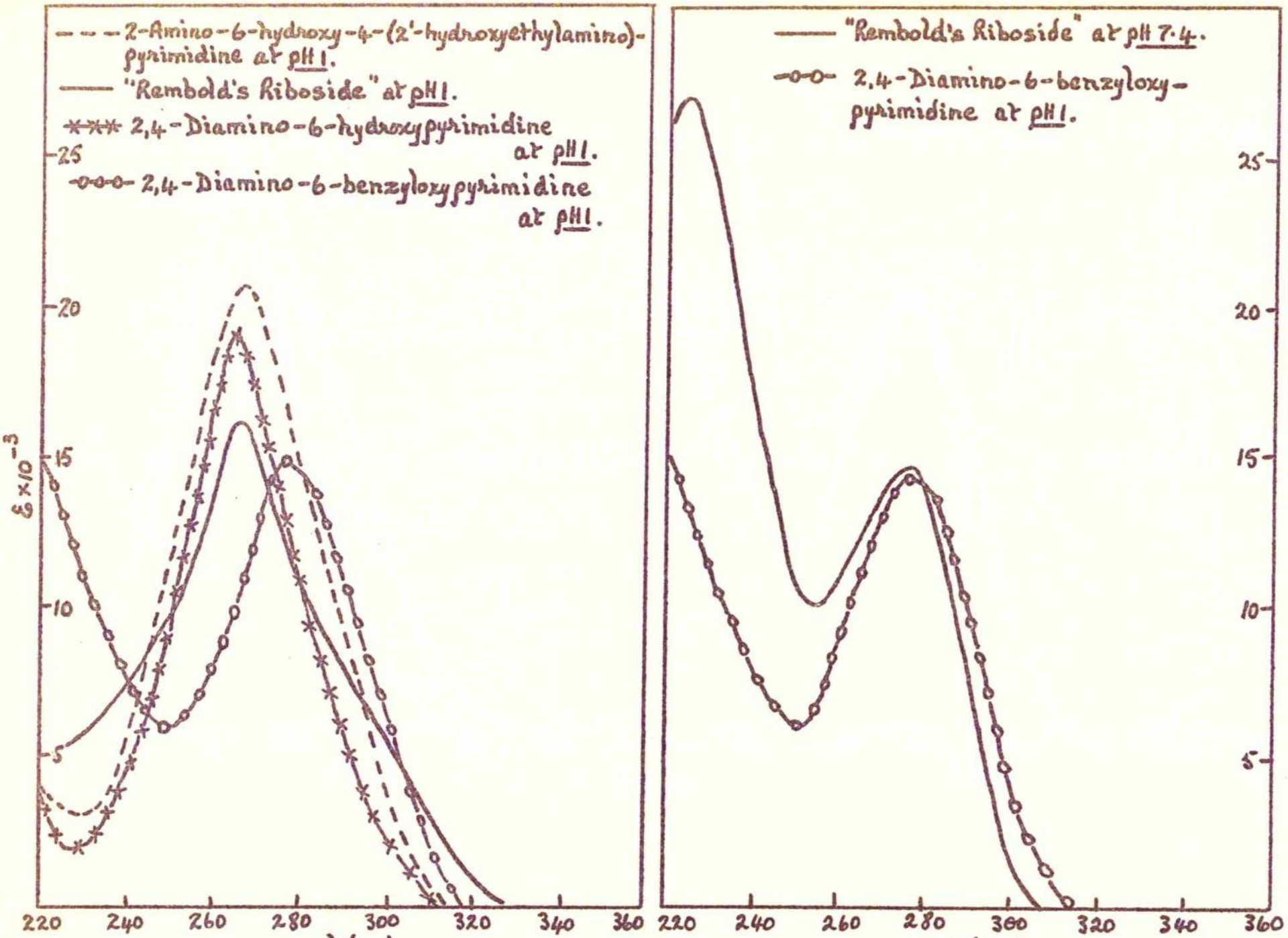

- - - - 2-Amino-6-hydroxy-4-(2'-hydroxyethylamino)-pyrimidine at pH 1.
—— "Rembold's Riboside" at pH 1.
*** 2,4-Diamino-6-hydroxypyrimidine at pH 1.
-o-o-o- 2,4-Diamino-6-benzyloxypyrimidine at pH 1.
—— "Rembold's Riboside" at pH 7·4.
-o-o- 2,4-Diamino-6-benzyloxy-pyrimidine at pH 1.
ε × 10⁻³
25
20
15
10
5
220
240
260
280
300
320
340
360
λ(mμ).

FIG. 3.

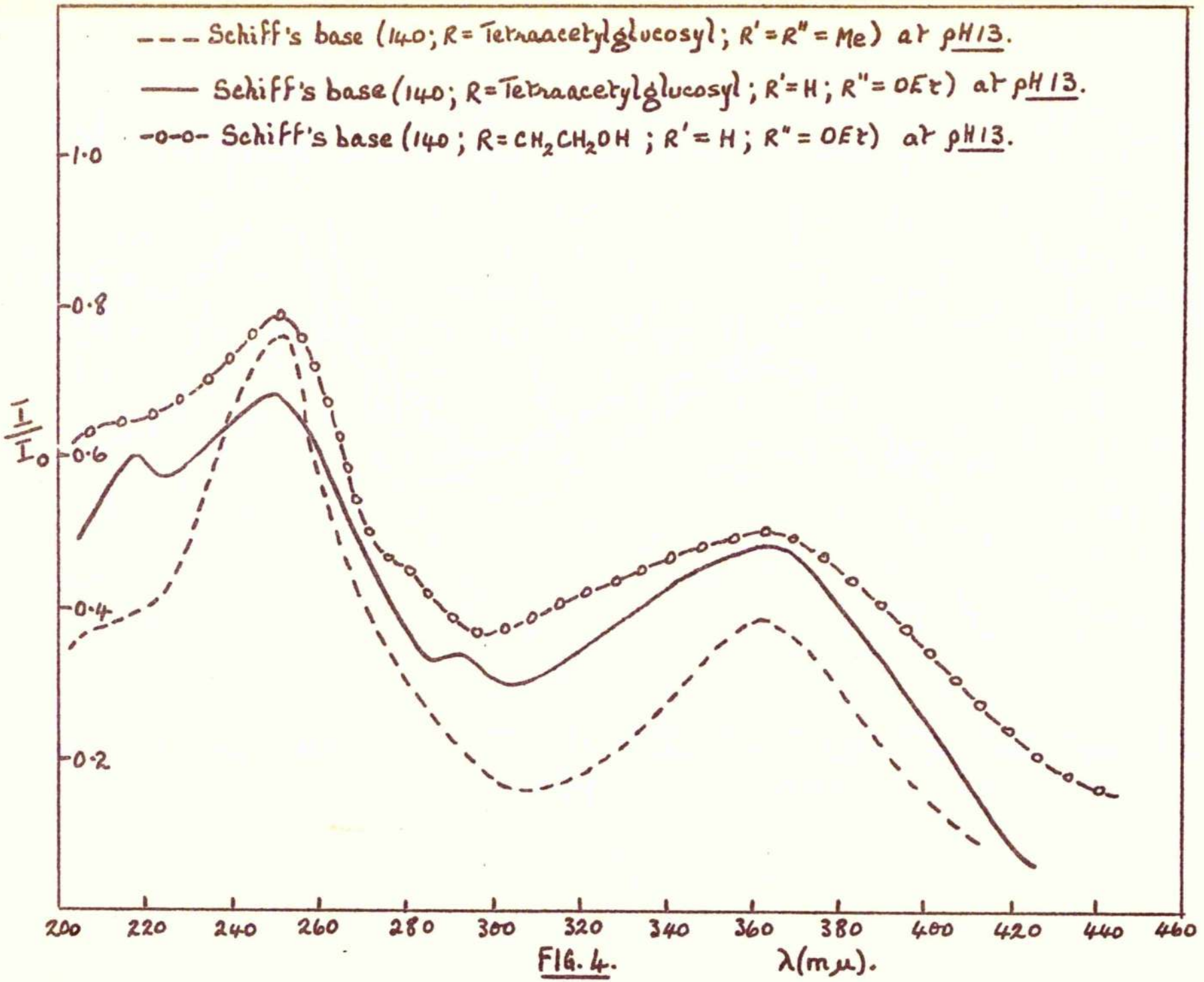

--- Schiff's base (140; R = Tetraacetylglucosyl; R' = R'' = Me) at pH 13.
—— Schiff's base (140; R = Tetraacetylglucosyl; R' = H; R'' = OEt) at pH 13.
-o-o- Schiff's base (140; R = CH2CH2OH; R' = H; R'' = OEt) at pH 13.
I/I0
1·0
0·8
0·6
0·4
0·2
200
220
240
260
280
300
320
340
360
380
400
420
440
460
λ(mμ).

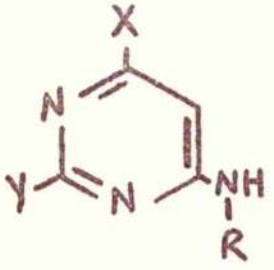
FIG. 4.

TABLE I. ULTRAVIOLET ABSORPTION SPECTRA OF PYRIMIDINES.

Compound.

(+) = cation, (o) = neutral mol., (-) = anion.

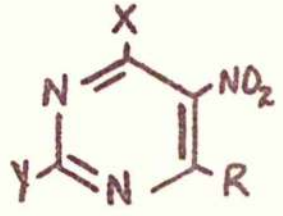
s = shoulder.



		pKa	$\lambda_{max.}$(mμ). ε values in parenthesis.	
X = OH; Y = NH_2; R = H.	(+)	3.33[a]	264(19,000)	pH 1
	(o)	10.78[a]	266(15,900)	pH 7.4
	(-)	—	261(10,680)	pH 13
R = CH_2CH_2OH	(+)	2.96 ± 0.07	267(20,850)	pH 1
	(o)	10.29 ± 0.1	268(16,200)	pH 7.4
	(-)	—	265(15,900)	pH 13
Rembold's Riboside	(+)	4.71 ± 0.05	266(16,150)	pH 1
	(o)	10.73 ± 0.1	276(14,600)	pH 7.4
	(-)	—	268(11,850)	pH 13
X = OMe; Y = NH_2; R = H	(+)	5.25 ± 0.04	276(13,350)	pH 1
	(o)	—	266(10,900)	pH 7.4
X = $OCH_2C_6H_5$; Y = NH_2; R = H	(+)	5.21 ± 0.06	277(14,550)	pH 1
	(o)	—	267(11,680)	pH 7.4
X = Cl; Y = NH_2; R = H			300(9,000)	pH 1
			284(7,000)	pH 13

[a]Brown, "The Pyrimidines", 1962, p.473, Interscience.

5 - Nitropyrimidines.

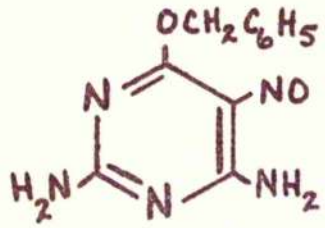


	$\lambda_{max.}$(mμ). ε values in parenthesis.	
X = Y = OH; R = Cl	280(8,200), 310[a](7,800)	pH 1

TABLE I (Contd.)

	$\lambda_{max.}$ (mμ). ε values in parenthesis.	
X = Y = OH; R = NHMe	224(18,750), 321(11,200)	pH 1
	218(15,200), 334(13,900)	pH 13
R = $NHCH_2CH_2OH$	228(23,150), 324(13,510)	pH 1
	217(17,800), 334(11,420)	pH 13
R = $NHCH_2(CHOH)_3CH_2OH$	228(23,700), 323(13,600)	pH 1
	220(17,000), 339(17,300)	pH 13
X = OH; Y = NH_2; R = Cl	300(8,600)	pH 1
R = NH_2	231(15,100), 332(13,200)	pH 1
	233(9,200), 345(11,800)	pH 13
R = NHMe	217(22,100), 335(13,820)	pH 1
	222(10,000), 348(10,200)	pH 13
R = $NHCH_2CH_2OH$	219(15,500), 336(11,500)	pH 1
	217(12,400), 348(12,600)	pH 13
R = NH-D-glucosyl	238(12,300), 334(11,100)	pH 1
	233(8,570), 346(12,600)	pH 13
R = NH-Tetraacetyl-D-glucosyl.	238(12,100), 335(11,000)	pH 1
	233(8,420), 344(12,350)	pH 13
R = $N(Me)CH_2CH(OEt)_2$	220(23,200), 364(18,400)	pH 13.

5 - Nitrosopyrimidines.

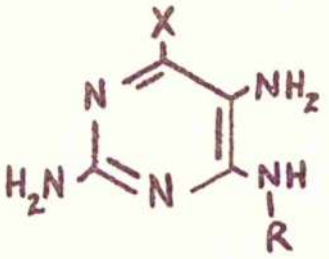


	271(10,200), 314(5,000)	pH 1
	270^{s}(2,900), 324(9,100)	pH 13

TABLE I (Contd.)

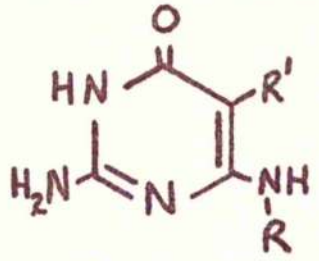
$\lambda_{max.}$ (mμ). & values in parenthesis.

4,5 - Diaminopyrimidines.

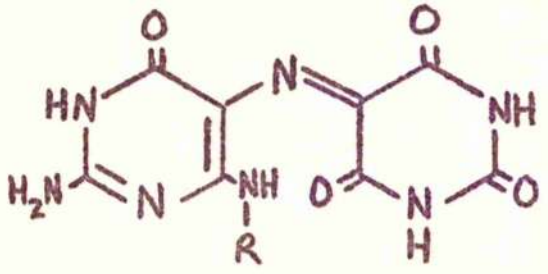


X = OH; R = H	263(15,800)	pH 1
	279(7,950)	pH 13
R = Me	265(15,750)	pH 1
	273(12,500)	pH 13
R = CH_2CH_2OH	272(17,800)	pH 1
	281(13,200)	pH 13
X = $OCH_2C_6H_5$; R = H	280(13,200)	pH 1
	288(11,500)	pH 13

Miscellaneous Pyrimidines.



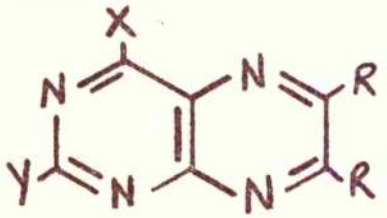
	$\lambda_{max.}$ (mμ).	
R = D - glucosyl; R' = - N=CHCHO	250, 359	pH 13
R = D - glucosyl; R' = - N=C(Me)COMe	252, 360	pH 13
R = Tetraacetyl - glucosyl; R' = -N=C(Me)COMe	252, 361	pH 13
R = Tetraacetyl - glucosyl; R' = -N=CHCOOEt	253, 288, 356	pH 13
R = CH_2CH_2OH; R' = -N=CHCOOEt.	251, 276[s], 362	pH 13



R = D - glucosyl	234, 446	pH 1
	232, 280, 456	pH 13

TABLE II ULTRAVIOLET ABSORPTION SPECTRA OF PTERIDINES.

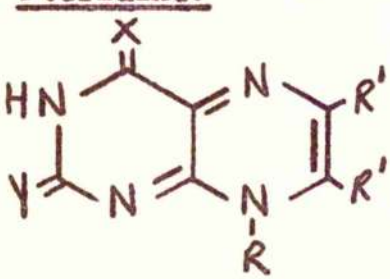
Pteridine.



$\lambda_{max.}$(m). ε values in parenthesis.

X = OH; Y = NH_2; R = H	312(6,980)	pH 1
	250(19,800), 358(7,500)	pH 13
R = Me	254(4,700), 324(7,100)	pH 1
	254(19,510), 359(7,700)	pH 13
X = $OCH_2C_6H_5$; Y = NH_2; R = H	214(23,100), 333(10,000)	pH 1
	231(20,350), 260[s](8,920), 360(7,500)	pH 13
X-NH-D-mannosyl; Y = SMe; R=Me	238(15,650), 267(17,000), 294(10,700) } 346(16,900), 357(15,500) }	pH 1
	249(18,650), 269(20,850), 355(10,950)	pH 13
X = NH_2; Y = SMe; R = Me	233(16,100), 265(18,800), 292(9,400) } 340(14,650), 354(13,500) }	pH 1
	268(18,600), 352(9,800)	pH 13

Pteridine.



X = Y = O; R = CH_2CH_2OH; R' = H	259(19,000), 276[s](11,000), 407(10,300)	pH 1
	231(23,700), 282 (15,200), 311(9,820)	pH 13
R = CH_2CH_2OH; R' = Me	256(14,500), 276[s](9,900), 406(11,150)	pH 1
	231(18,500), 283 (10,900), 316(8,150)	pH 13
R = D - ribityl; R' = Me	255(12,800), 276[s](8,750), 405(10,000)	pH 1
	229(13,700), 280 (11,000), 314(7,900)	pH 13
X = O; Y = NH; R = Me; R' = H	262(13,500), 280[s](9,710), 388(10,210)	pH 1
	238(18,050), 282 (7,620), 316(8,100)	pH 13

TABLE II (Contd.)

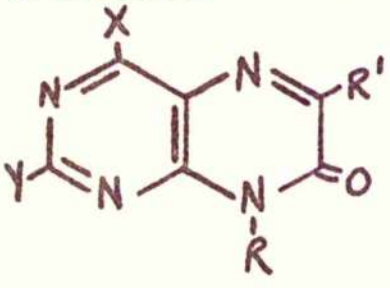
λ_{max}(mμ). ε values in parenthesis.

X = O; Y = NH; R = CH_2CH_2OH; R' = H	263(13,900), 279^s(10,800), 395(9,900)	pH 1
	242(17,950), 284 (6,900), 316(7,600)	pH 13
R = R' = Me	255(12,300), 285 (13,500), 396(13,600)	pH 1
	230(22,900), 303^s(17,300), 307(17,500), 371(8,850)	pH 13
R = CH_2CH_2OH; R' = Me	256(15,000), 287 (13,600), 400(13,600)	pH 1
	230(22,300), 286^s(9,750), 310(13,750), 372(3,500)	pH 13

Pteridine.

Y = O; R = D - ribityl	258,	276^s,		412	pH 1
Y = NH; R = CH_2CH_2OH	250,	288,	304^s,	426	pH 1

Pteridine.



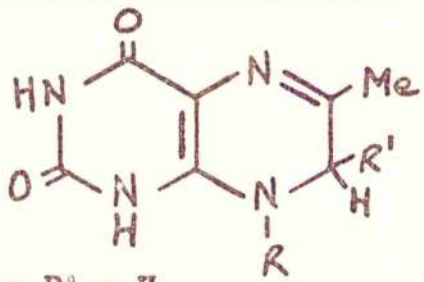
X = Y = OH; R = CH_2CH_2OH; R' = Me	281(10,500), 326(10,800)	pH 1
	222(12,200), 258(7,300), 290(6,000), 350(11,200)	pH 13
R = D - ribityl; R' = Me	280(11,900), 326(11,500)	pH 1
	222(13,000), 290(11,000), 348(12,800)	pH 8
	220(13,100), 258(7,000), 286(7,200), 350(12,820)	pH 13

TABLE II (Contd.)

λ_{max} (mμ). ε values in parenthesis.

X = Y = OH; R=CH_2CH_2OH; R'= Et	283(10,200), 329(10,300)	pH 1
	220(18,900), 262(7,350), 292(8,400), 354(12,600)	pH 13
X = OH; Y = NH_2; R = Me; R'= H	264(9,000), 294(11,200), 345(13,800)	pH 1
	265(15,950), 280^s(5,900), 360(16,700)	pH 13
R=CH_2CH_2OH; R' =H	262(9,100), 292(11,700), 346(14,100)	pH 1
	262(16,200), 286(6,400), 362(19,600)	pH 13
R=D - glucosyl; R'=H	261(5,100), 292(9,500), 349(12,000)	pH 1
	262(10,150), 280^s(3,900), 366(12,600)	pH 13
R=CH_2CH_2OH; R'=Me	296(10,350), 344(12,400)	pH 1
	260(13,000), 284(4,800), 357(14,400)	pH 13
R=D-glucosyl; R'=Me	295(11,100), 342(9,950)	pH 1
	259(9,100), 279^s(4,300), 360(10,810)	pH 13

7,8 - Dihydropteridines.



R = R' = H	228(11,200), 267(15,400), 350(4,500)	pH 1
	226(23,000), 276(13,000), 318(6,500)	pH 13
R = CH_2CH_2OH; R' = Me	239(13,300), 272(20,000), 346(5,500)	pH 1
	231(26,300), 283(15,300), 317(8,300)	pH 13

TABLE III

ULTRAVIOLET ABSORPTION SPECTRA OF ISOALLOXAZINE DERIVATIVES.

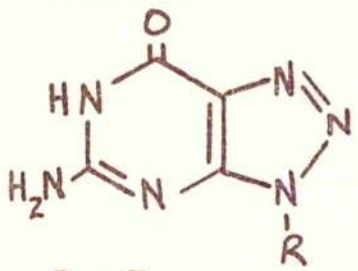
Compound.

λmax. (mμ). ε values in parenthesis.

X = Y = O;	R = CH_2CH_2OH	268(28,300), 377(10,200), 443(11,100)	pH 1
		272(33,600), 356(12,000), 450(12,300)	pH 13
	R = D - ribityl	268(35,440), 376(10,810), 445(11,600)	pH 1
		271(31,800), 356(10,650), 447(10,600)	pH 13
X = O; Y = NH;	R = CH_2CH_2OH	267(24,050), 385(11,200), 442(14,000)	pH 1

TABLE IV. ULTRAVIOLET ABSORPTION SPECTRA OF AZAPURINES.

Compound.

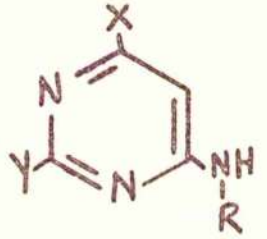


λmax.(mμ). ε values in parenthesis.

R = H	251(5,100), 264^{s}(4,850)	pH 1
	282(10,910)	pH 13
R = CH_2CH_2OH	252(9,150), 270^{s}(6,600)	pH 1
	278 (9,850)	pH 13
R = D - ribityl	254(11,600), 272^{s}(8,300)	pH 1
	280(10,250)	pH 13

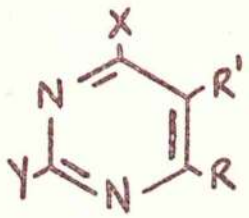
TABLE V. Rf VALUES OF PYRIMIDINES.

Compound.



	Solvent Systems.					
	(A)	(B)	(C)	(D)	(E)	(F)
X = OH; Y = NH_2; R = H	0.21	0.32	0.37	0.29	—	0.28
Rembold's Riboside	0.09	0.57	0.16	0.54	—	0.12

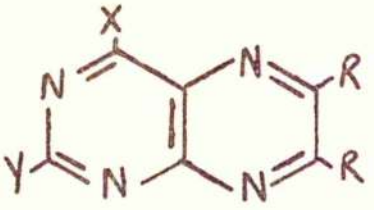
Compound.



X = Y = OH; R' = NO_2; R = NHMe	0.26	0.60	—	0.56	—	—
R=$NHCH_2CH_2OH$	0.25	0.65	—	0.62	—	—
R=NH-D-ribityl	0.10	0.61	—	0.59	—	—
X = OH; Y = NH_2; R' = NO_2; R = NH_2	—	—	0.18	—	—	0.24
R = NHMe	0.32	0.49	0.36	0.45	—	0.55
R=$NHCH_2CH_2OH$	0.24	0.53	0.32	0.50	—	0.50
R=NH-D-glucosyl	0.05	0.70	0.18	0.66	—	0.24
R=NH-Tetraacetyl-D-glucosyl	0.70	0.42	0.68	0.38	—	0.87
R=N(Me)$CH_2CH(OEt)_2$	—	0.70	0.79	0.66	—	0.78
X = $OCH_2C_6F_5$; Y = NH_2; R=NH_2; R'=NO	0.73	0.24	0.84	0.21	—	0.75
R'=NH_2	0.60	0.32	0.73	0.28	—	0.65
X = NH_2; Y = SMe; R=NH-D-mannosyl; R'=NO	0.25	0.47	0.52	0.44	—	0.56

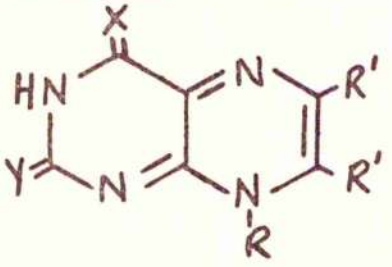
TABLE VI. Rf VALUES OF PTERIDINES.

Compound.



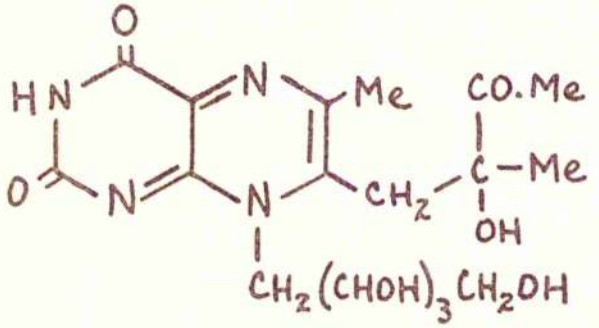
	Solvent Systems.					
	(A)	(B)	(C)	(D)	(E)	(F)
X = OH; Y = NH_2; R = H	0.31	0.48	0.30	0.44	—	0.39
R = Me	0.43	—	0.34	—	0.51	0.44
X = $OCH_2C_6H_5$; Y = NH_2; R = H	0.83	0.38	0.70	0.35	—	0.89
X = NH-D-mannosyl; Y = SMe; R=Me	0.50	0.65	0.70	0.62	—	0.78

Compound.



X = Y = O; R = CH_2CH_2OH; R' = H	0.10	0.76	0.36	0.73	—	0.32
R = CH_2CH_2OH; R' = Me	0.18	0.71	0.72	0.65	—	—
R = D - ribityl; R' = Me	0.10	0.74	0.31	0.71	0.27	0.34
X = O; Y = NH; R = Me; R' = H	—	0.78	—	0.75	—	0.34
R = CH_2CH_2OH; R' = H	—	0.78	0.35	0.74	—	0.38
R = R' = Me	—	0.75	—	0.72	—	0.43
R = CH_2CH_2OH; R'=Me	0.10	0.79	—	0.76	—	0.52

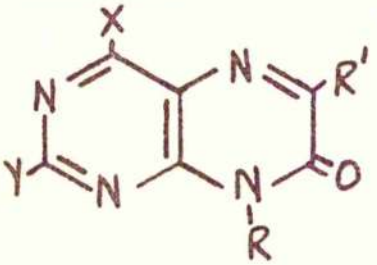
Compound.



	0.18	0.65	0.14	0.62	—	—

TABLE VI. (Contd.)

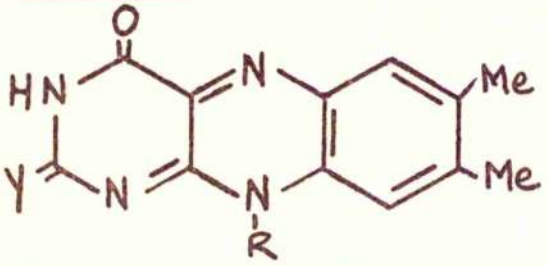
Compound.



		Solvent Systems.					
		(A)	(B)	(C)	(D)	(E)	(F)
X = Y = OH;	R = CH_2CH_2OH; R' = Me	0.17	0.60	0.44	0.56	—	0.51
	R = CH_2CH_2OH; R' = Et	0.26	0.67	0.53	0.63	—	0.65
	R = D-ribityl; R' = Me	0.05	0.65	0.30	0.61	0.26	0.50
X = OH; Y = NH_2;	R = Me; R' = H	0.24	0.49	0.36	0.45	—	0.37
	R = CH_2CH_2OH; R' = H	0.24	0.58	0.39	0.54	—	0.48
	R = D-glucosyl; R' = H	0.04	0.74	0.23	0.72	—	0.20
	R = R' = Me	—	0.43	—	0.41	—	—
	R = CH_2CH_2OH; R' = Me	0.25	0.59	—	0.55	—	0.50
	R = D-glucosyl; R' = Me	—	0.76	—	0.72	—	—

TABLE VII. Rf VALUES OF ISOALLOXAZINE DERIVATIVES.

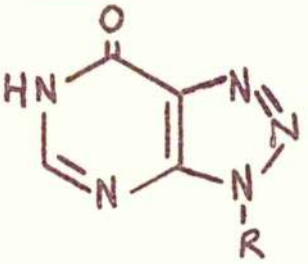
Compound.



		Solvent Systems.					
		(A)	(B)	(C)	(D)	(E)	(F)
Y = O;	R = CH_2CH_2OH	0.40	0.32	0.45	0.28	—	0.52
	R = D - ribityl	0.22	0.37	0.55	0.39	0.39	0.34
	R = CH_2CHO	—	0.45	—	0.43	—	—
Y = NH;	R = CH_2CH_2OH	0.22	0.21	0.40	0.22	—	0.45
	R = D - ribityl	0.15	0.42	0.28	0.40	—	0.28
	R = CH_2CHO	—	0.40	—	0.38	—	—

TABLE VIII. Rf VALUES OF AZAPURINES.

Compound.



Solvent Systems.

	(A)	(B)	(C)	(D)	(E)	(F)
R = H	0.38	0.40	0.27	0.37	—	0.27
R = CH_2CH_2OH	0.26	0.63	0.37	0.60	—	0.51
R = D - ribityl	0.06	0.70	0.23	0.68	—	0.34

Solvent Systems.

(A) n - Butanol/5N - acetic acid (aqueous) (7:3).

(B) 3% Aqueous ammonium chloride.

(C) n - Propanol/ammonium hydroxide (S.G. 0.880)/water (40:1:20).

(D) 3% Aqueous ammonium formate.

(E) n - Butanol/ethanol/water (50:15:35).

(F) n - Butanol/benzene/water/methanol (1:1:1:2).

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