

THE METHYLATION OF AMINOPTERIDINES

and

AMINOPYRIMIDINES

a Thesis

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in the

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by

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The work described in this thesis was carried out by the candidate at the Australian National University during the period January 1959 to August 1961. Where the work of others was employed, appropriate references have been included.

N. W. Jacobsen

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Finally, the author wishes to thank his wife for the considerable assistance she rendered by typing the initial draft of this thesis.

PREFACE.

This thesis describes the methylation of a number of aminopteridines and aminopyrimidines. In actual fact however, it consists of much more than an investigation of one type of reaction, since emphasis has been placed upon the chemistry of the methylation products and the manner in which they can be degraded to recognisable compounds that show the position at which substitution occurred.

Factors which may influence the site of methylation in these heterocyclic compounds are also discussed, but because of the diversity that exists in the conditions of methylation (both in this work and in the literature) this cannot be comprehensive.

The main text of the thesis is preceeded by an historical account of the earlier methylations which are relevant to the work undertaken, and is followed by a discussion of the results of the physico-chemical measurements made during the investigations.

COMPOUNDS METHYLATED

Pteridines

4-amino-
4-amino-6,7-dimethyl-
2-amino-4-hydroxy-
4-amino-2-hydroxy-
2-amino-4-hydroxy-6,7-dimethyl-
4-amino-2-hydroxy-6,7-dimethyl-
2,4-diamino-
2,4-diamino-6,7-dimethyl-
4-dimethylamino-
4-dimethylamino-6,7-dimethyl-
6,7-dimethyl-4-methylamino-
4-methylamino-

Pyrimidines

4-amino-2-methylamino-
2,4-diamino-
4,5-diamino-
4,6-diamino-
2,4-diamino-6-hydroxy-
2,4-diamino-6-methylthio-
4,5-diamino-2-methylthio-
4,6-diamino-5-nitro-
2,4,6-triamino-

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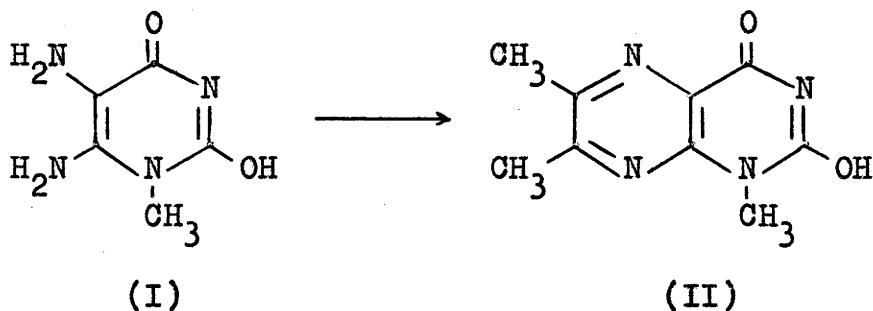
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SECTION 1.

HISTORICAL

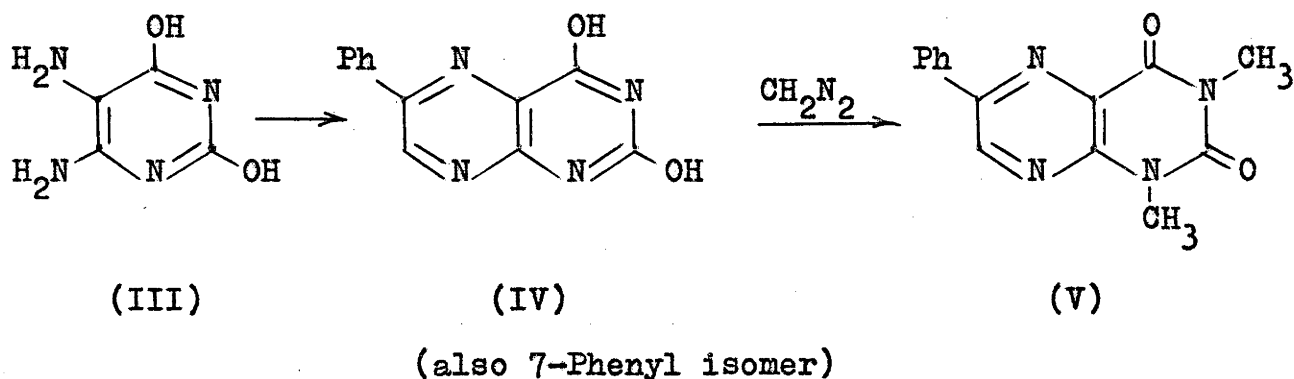
A. Earlier Methylations of Pteridines

Nuclear N-methylated pteridines have been known since Sachs and Meyerheim (1908) first condensed 4,5-diamino-3,6(2,3)-dihydro-2(4)-hydroxy-3-methyl-4(2)-oxypyrimidine (I) with biacetyl to give 1,6,7-trimethyl-lumazine (II).



The introduction of methyl substituents into the pteridine nucleus by direct methylation however, is a process of more recent years. The earliest recorded attempt was made by Wieland, Metzger, Schöpf and Bülow (1933) who tried to structuralise leucopterin (2-amino-4,6,7-trihydroxy-pteridine) by methylation with diazomethane. This first unsuccessful experiment was later partly rectified by Wieland and Decker (1941) who did obtain two isomeric trimethyl derivatives but were unable to determine their constitution.

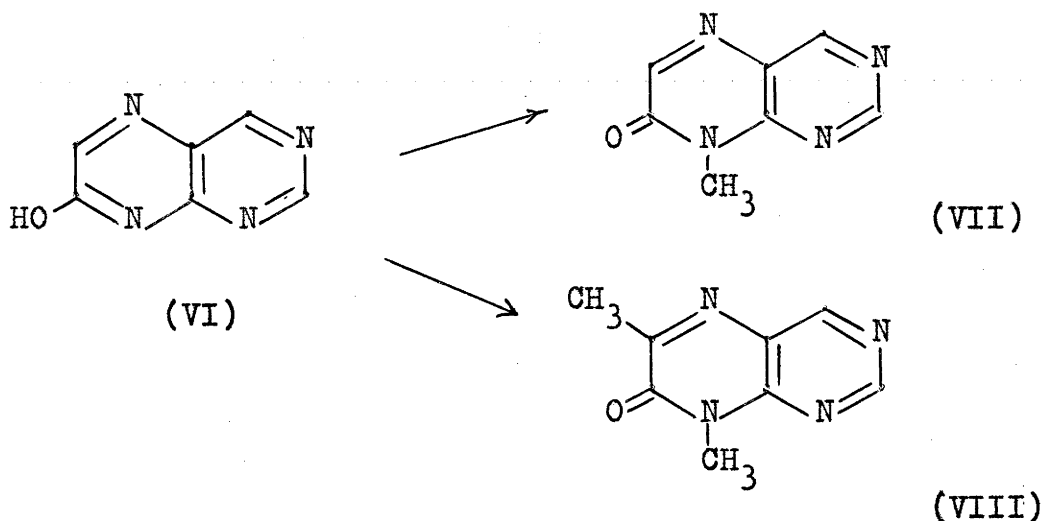
During this interval, Ganapathi (1937) succeeded in methylating a mixture of 2,4-dihydroxy-6-phenylpteridine (IV) and 2,4-dihydroxy-7-phenylpteridine obtained from the condensation of 4,5-diaminouracil (III) and phenylglyoxal. Although unstructuralised at the time, the dimethyl derivative has recently been identified as a complex of 1,2,3,4-tetrahydro-1,3-dimethyl-2,4-dioxo-6-phenylpteridine (V) and the 7-phenyl isomer (Dick, Wood and Logan, 1956).



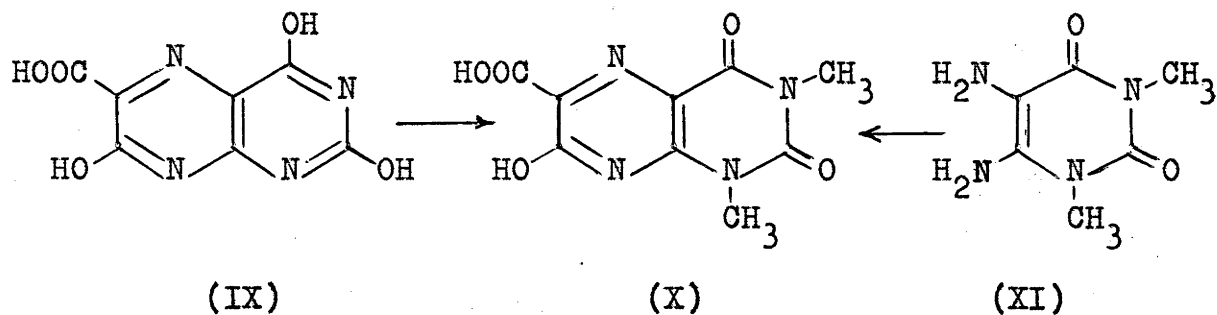
After an interval of many years Tschesche and Korte (1951) claimed that the action of dimethyl sulphate upon 2,4,7-trihydroxypteridine furnished 2,7-dihydroxy-3-methyl-4-pteridone. Unfortunately no evidence is given in the paper to support this structure, which must therefore be assumed to be unproven.

7-Hydroxypteridine (VI) was the first mono-substituted pteridine to be methylated (Albert, Brown and Cheeseman, 1952) but it was not until some years later that Albert, Brown and Wood (1956), after repeating the

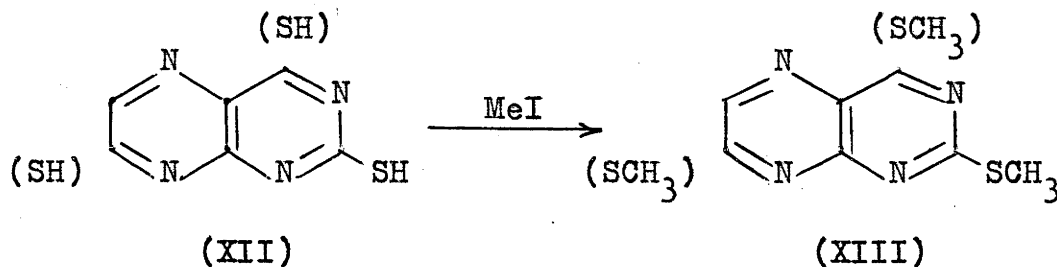
methylation with dimethyl sulphate, identified the product as 8-methyl-7-pteridone (VII). From the second methylation, they also isolated 6,8-dimethyl-7-pteridone (VIII) and this is the first recorded instance of a direct C-methylation in the pteridine series.



Notwithstanding the earlier experiments mentioned above, Pfeleiderer and Geissler (1954) appear the first to fully characterise their methylation product. 2,4,7-trihydroxypteridine-6-carboxylic acid (IX) was methylated with dimethyl sulphate and the product identified as the 1,3-dimethyl derivative (X) by comparison with the hydrolysed condensation product of 4,5-diamino-1,2,3,6-tetrahydro-1,3-dimethyl-2,6-dioxypyrimidine (XI) and diethyl mesoxalate.



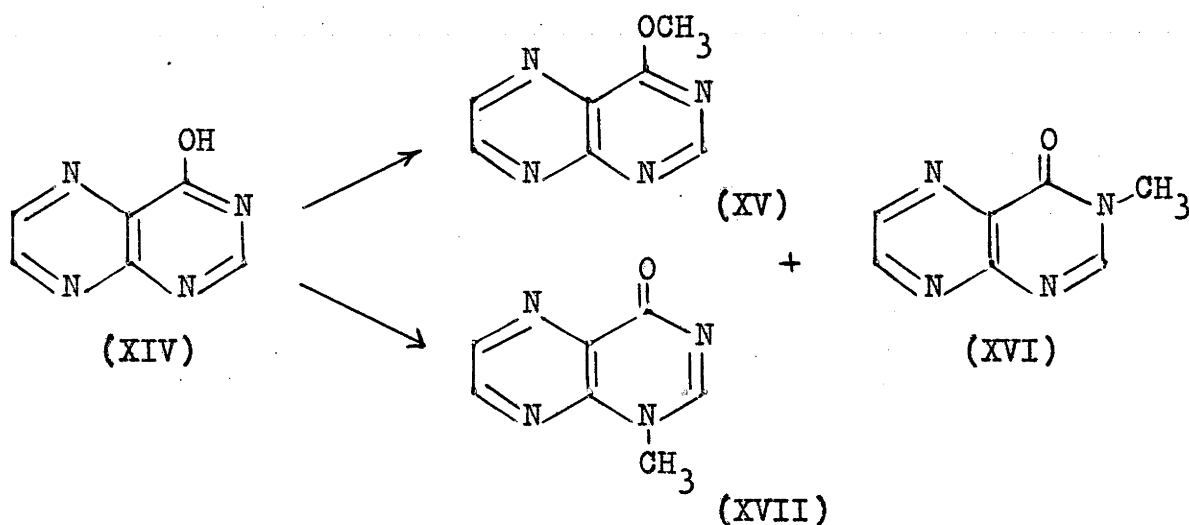
However, almost simultaneously with the publication of Pfleiderer and Geissler's results, appeared those of Albert, Brown and Wood (1954) recording the methylation of 2,4, and 7-mercaptopteridine (XII) with methyl iodide in the presence of alkali, to yield respectively 2,4, and 7-methylthiopteridine (XIII).



These methylations were the first to be recorded in which methyl iodide had been used to methylate a pteridine and the first in which an extranuclear methylation had occurred. The position of methylation in these mercaptopteridines was established by acid hydrolysis leading to the evolution of methanethiol.

In 1956, further work of Albert, Brown and Wood (1956) showed that the position of methylation in the

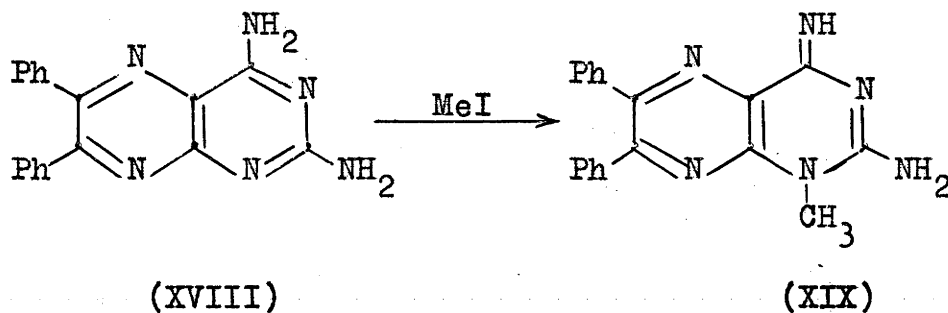
pteridine nucleus could vary with different methylating agents. Thus methylation of 4-hydroxypteridine (XIV) with diazomethane yielded both 4-methoxypteridine (XV) and 3-methyl-4-pteridone (XVI), while with dimethyl sulphate, 1-methyl-4-pteridone (XVII) as well as the 3-methyl isomer were obtained.



An interesting attempt by these workers to methylate unsubstituted pteridine was unsuccessful.

Until the publication of the results to be described in this thesis, there was but one isolated example of the methylation of an aminopteridine. Boon and Bratt (1957) described the methylation of 2,4-diamino-6,7-diphenylpteridine (XVIII) with methyl iodide as giving 2-amino-1,4-dihydro-4-imino-1-methylpteridine (XIX) or its tautomer. This result is supported in part by the work

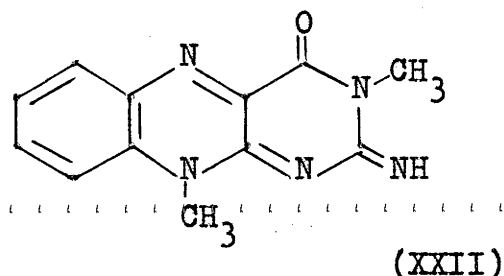
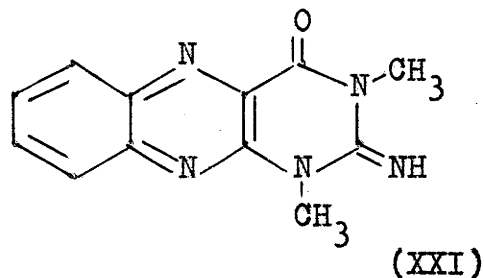
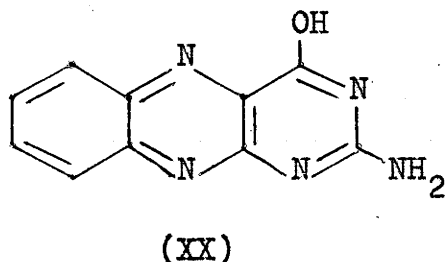
described in this thesis on the methylation of simple 2,4-diaminopteridines.



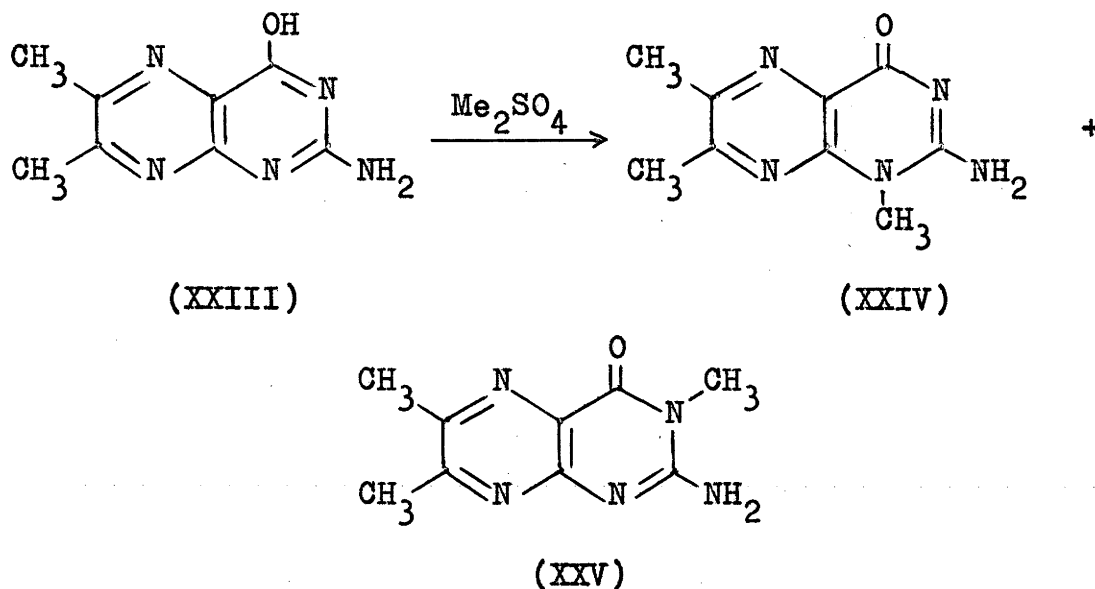
Apart from the early methylations of leucopterin, amino-hydroxy-pteridines have received little attention in such investigations. This is surprising in view of the fact that the 2-amino-4-hydroxy configuration is present in most naturally occurring pteridines. However of recent years two papers describing such methylations have appeared in addition to those describing the work contained in this thesis.

While investigating the chemistry of compounds related to riboflavin, Creswell, Hill and Wood (1959) carried out the methylation of 2-amino-4-hydroxybenzo[g] pteridine (XX) with methyl iodide. The dimethyl derivative obtained was however, not investigated further, despite the fact that the number of possible structures permitted by valency is small: if extranuclear methylation on O or N is excluded as improbable with methyl iodide (as the evidence to be presented in this thesis would

suggest), the dimethyl derivative could only have one of the structures (XXI) or (XXII).



From the work of Angier and Curran (1961), who methylated 2-amino-4-hydroxy-6,7-dimethylpteridine (XXIII) with dimethyl sulphate and obtained 2-amino-1,4-dihydro-1,6,7-trimethyl-4-oxopteridine (XXIV) and its 3,6,7-trimethyl isomer (XXV), the first structure (XXI) would be favoured. On the other hand, evidence to be presented in this thesis on the action of methyl iodide on simple 2-amino-4-hydroxypteridines, suggests that the transannular methylation product (XXII) is equally likely.

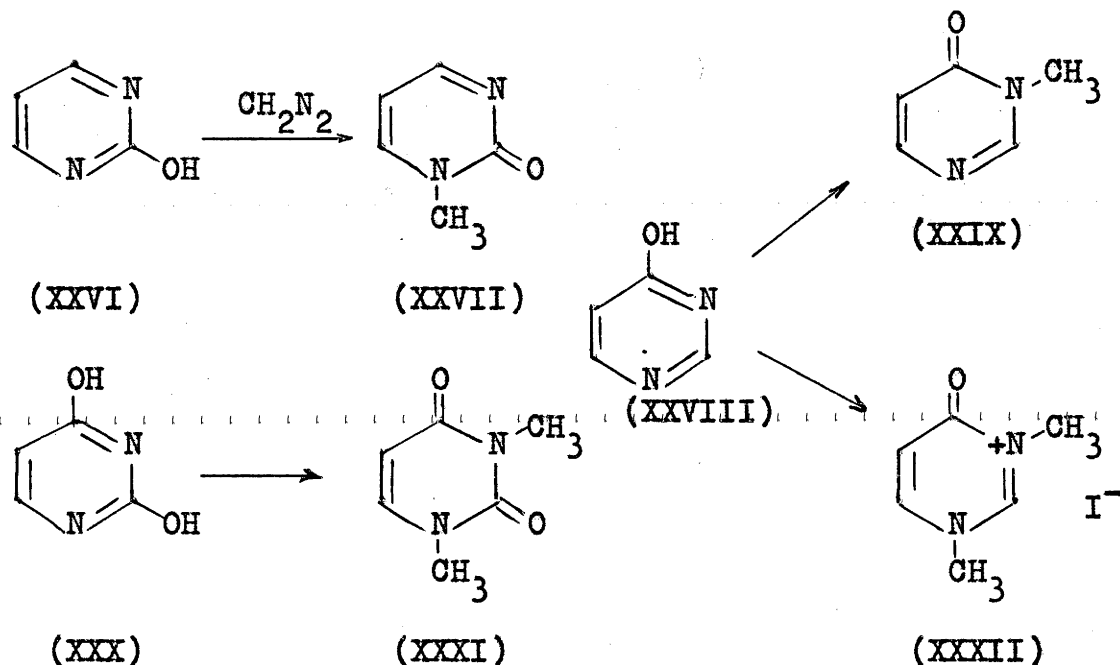


B. Earlier Methylations of Pyrimidines

In contrast to the pteridine series, the literature contains many examples of the direct methylation of pyrimidine derivatives. However, as this section is intended as an introduction to the investigations described later, it is confined to a brief review of relevant simple methylations.

The hydroxypyrimidines feature prominently among the derivatives of the series that have been alkylated. The simplest of these, 2- and 4-hydroxypyrimidine (XXVI and XXVIII) on reaction with ethereal solutions of diazomethane gave 1,2-dihydro-1-methyl-2-oxypyrimidine (XXVII) and 3,4-dihydro-3-methyl-4-oxypyrimidine (XXIX). In these reactions, Brown, Hoerger and Mason (1955a) also isolated smaller amounts of the respective methoxy

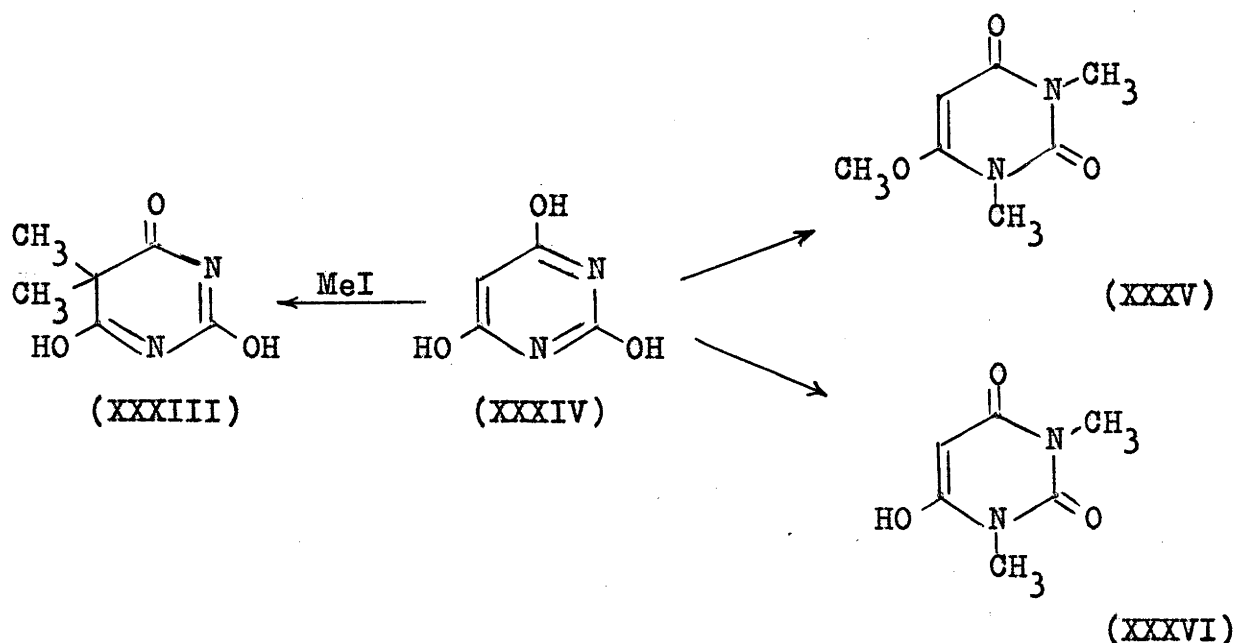
derivatives. They also showed that using methyl iodide, 4-hydroxypyrimidine gave a single quaternary product 1,4(3,4)-dihydro-1,3-dimethyl-4-oxopyrimidinium iodide (XXXII).



Among the dihydroxypyrimidines that have been methylated, it is interesting to observe that the 2,4-dihydroxy derivative (XXX) could not be mono-methylated. Thus the action of diazomethane (Case and Hill, 1930), dimethyl sulphate (Davidson and Baudisch, 1926) and methyl iodide (Johnson and Clapp, 1908) yielded the dimethylated product 1,2,3,4-tetrahydro-1,3-dimethyl-2,4-dioxypyrimidine (XXXI).

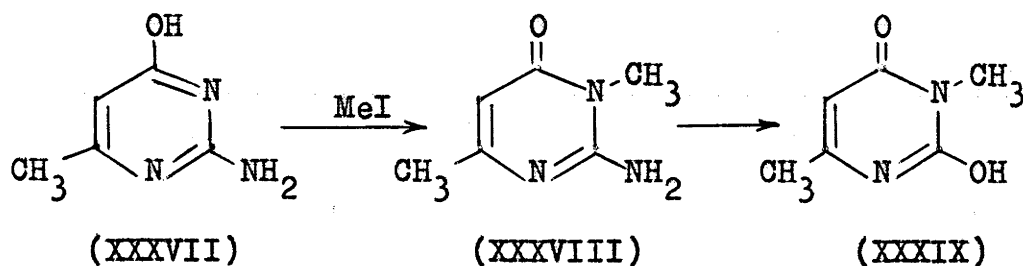
The trihydroxypyrimidines are also particularly prone to poly-methylation. Thus barbituric acid (XXXIV,

2,4,6-trihydroxypyrimidine) and diazomethane (Biltz and Wittek, 1921) gave the trimethyl derivative 1,2,3,4-tetrahydro-6-methoxy-1,3-dimethyl-2,4-dioxypyrimidine (XXXV), while with dimethyl sulphate (Herzig, 1921), 1,2,3,4-tetrahydro-6-hydroxy-1,3-dimethyl-2,4-dioxypyrimidine (XXXVI) was obtained. Barbituric acid was one of the first pyrimidines to be methylated, and it is of interest to compare the reaction of methyl iodide and silver barbiturate to give 5,5-dimethylbarbituric acid (XXXIII; Conrad and Guthzeit, 1881) with the C₍₅₎-methylation of 2,4-diamino-6-hydroxypyrimidine to be described in this thesis.

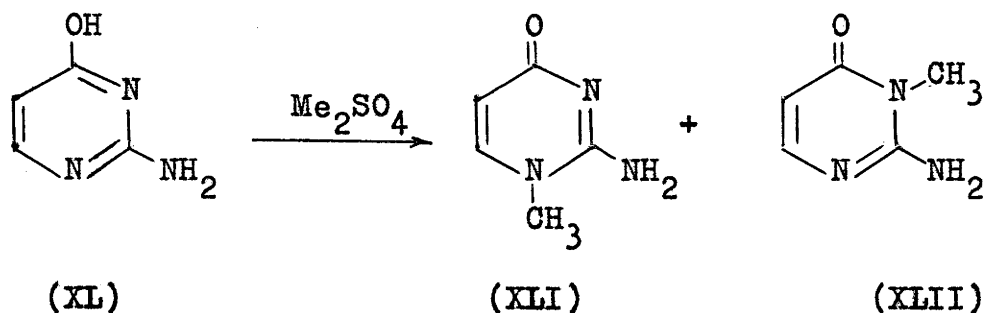


Methylations of aminopyrimidines also feature in the early chemistry of the series. For example, Jaeger (1891) allowed 6-methylisocytosine (2-amino-4-hydroxy-6

-methylpyrimidine, XXXVII) to react with methyl iodide and obtained a product which was later synthesized by Majima (1908) and shown to be 2-amino-1,6-dihydro-1,4-dimethyl-6-oxypyrimidine (XXXVIII) by its hydrolysis to 3,6-dimethyluracil (XXXIX).



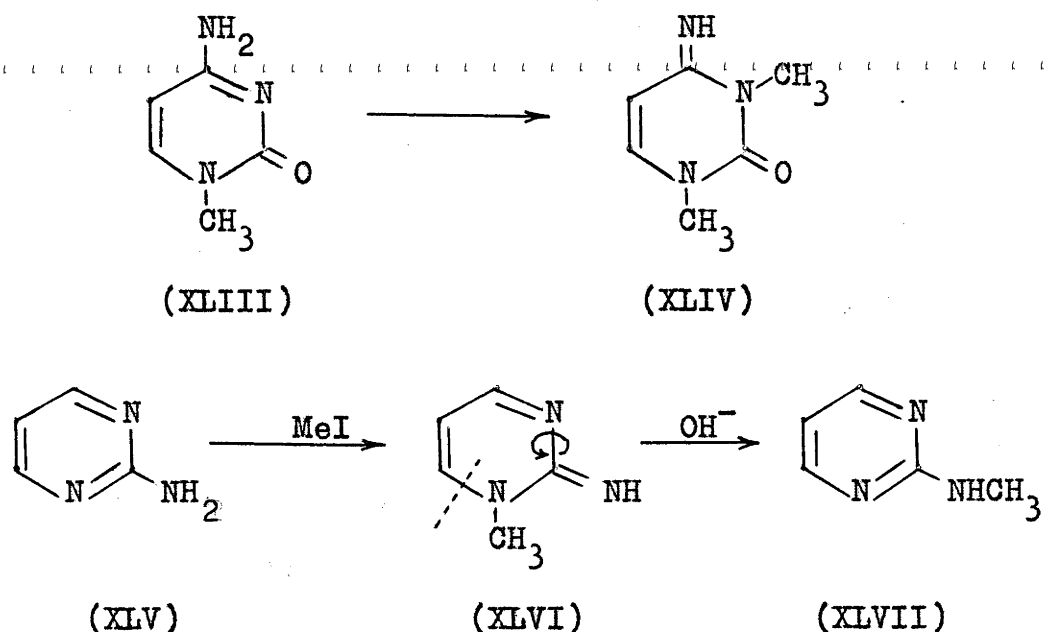
Contrasting in age and content with the above methylation is the recent announcement by Angier and Curran (1961) that dimethyl sulphate and isocytosine (XL) yielded both the 1-methyl (XLI) and 3-methyl (XLII) isomers.



The isomeric cytosine (4-amino-2-hydroxypyrimidine) has rather similar history. First methylated by Johnson and Clapp (1908), it was claimed to yield 1-methylcytosine, but the product did not resemble the

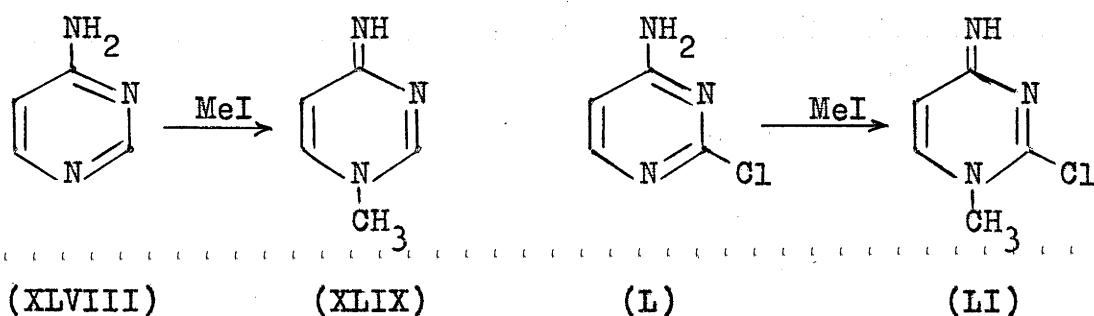
authentic N₍₁₎ derivative made later by Hilbert (1934). Recently Brookes and Lawley (1961) have re-investigated the methylation and have shown that the products are in fact 3-methylcytosine and a dimethyl derivative.

1-Methylcytosine (XLIII) is of interest for another reason. Its methylation by Hilbert (1934) using methyl iodide, yielded 1,2,3,4-tetrahydro-4-imino-1,3-dimethyl-2-oxopyrimidine (XLIV), which was the first iminopyrimidine to be prepared.



Many iminopyrimidines have been prepared of more recent years, and their high basic strengths and their susceptibility towards rearrangement, makes them an interesting class of compound. Thus 2-aminopyrimidine (XLV) on methylation with methyl iodide gave the strong

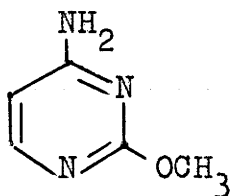
base 1,2-dihydro-2-imino-1-methylpyrimidine (XLVI, Brown, Hoerger and Mason, 1955) which readily rearranged to 2-methylaminopyrimidine (XLVII). Using this transformation, Brown (1961) has established that the mechanism of rearrangement involves hydrolytic ring fission, rotation of the terminal amidine group, and recycloisatation.



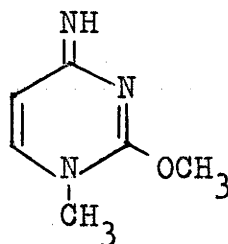
The methyl derivative of 4-aminopyrimidine (XLVIII) was also made by the action of methyl iodide (Brown, Hoerger and Mason, 1955) but of the two possible isomers, only 1,4-dihydro-4-imino-1-methylpyrimidine (XLIX) was obtained. Pyrimidines which contain non-tautomerisable groups (such as $-\text{Cl}$, $-\text{OCH}_3$, $-\text{SCH}_3$) in addition to an amino-substituent can also yield imino-derivatives on methylation. Thus 4-amino-2-chloropyrimidine (L) with methyl iodide in 2-ethoxyethanol, gave 2-chloro-1,4-dihydro-4-imino-1-methylpyrimidine (LI; Curd and Richardson, 1955).

4-Amino-2-methoxypyrimidine (LII) likewise gave the base 1,4-dihydro-4-imino-2-methoxy-1-methylpyrimidine

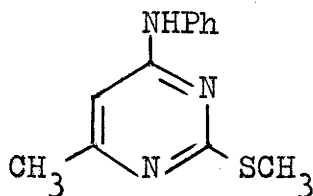
(LIII) when treated with methyl iodide at room temperature (Hilbert, 1934) and 4-anilino-6-methyl-2-methylthio-pyrimidine (LIV) yielded 1,4-dihydro-1,6-dimethyl-2-methylthio-4-phenyliminopyrimidine (LV; Ainley, Curd, Hepworth, Murray and Vasey, 1953).



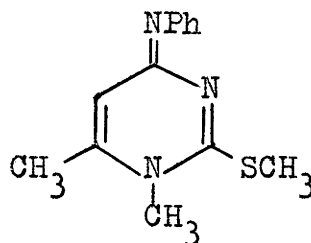
(LII)



(LIII)



(LIV)



(LV)

There are no examples recorded of the synthesis of a di-imine. Thus an attempt to prepare such a derivative of 2,4-diaminopyrimidine by methylation with methyl iodide, gave only a single mono-methyl derivative of indeterminate structure (Brown, Hoerger and Mason, 1955). A further investigation of this reaction is described in this thesis and the structure of the product is now known.

SECTION 2.

DISCUSSION OF THE EXPERIMENTAL RESULTS

A. The Methylating Reagent

Methyl iodide was the reagent used in all the methylations to be described in this thesis. Its exclusive use was not the result of a preconceived plan, but was the outcome of its repeatedly successful use and the following considerations:

(a) Methylations with methyl iodide enabled the unstable imine bases to be isolated and purified as their hydriodide salts, which were not susceptible to the rearrangements and hydrolyses common to the free imine bases.

(b) In the absence of extraneous inorganic salts, the hydriodides were readily isolated and purified.

(c) The hydriodides could be converted almost quantitatively into the hydrochlorides under very mild conditions.

(d) Methylations could be carried out in the anhydrous conditions necessary to avoid hydrolysis.

(e) Reaction temperatures up to 160° could be safely employed.

(f) When alkaline conditions were required, the reagent was successfully used in methanolic sodium methoxide.

While it is true to say that the use of other methylating reagents may have yielded methylation products

differing from those obtained with methyl iodide, such a course would have seriously reduced the range of compounds investigated and may well have resulted in considerable duplication.

B. Methods for Determining the Position of Methylation

Once micro-analysis had established the extent of methylation, one or more of the following procedures were used to establish the position at which methylation had occurred.

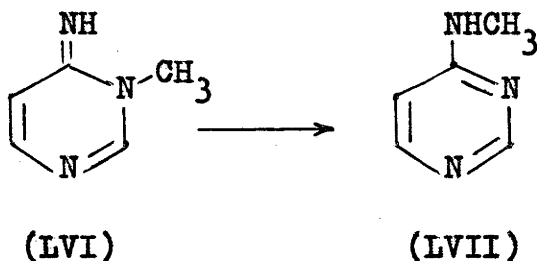
(a) Ionisation Constants. The introduction of a methyl group into an aminopteridine or aminopyrimidine molecule either by C-methylation, extranuclear N-methylation, or by nuclear N-methylation, results in an increase in the strength of the base. With C-methylation and extranuclear N-methylation however, the increase in the basic pK_a constant is invariably less than 1 unit (often less than 0.5 units). Nuclear N-methylation on the other hand has been consistently found to increase the basic strength by the order of 6 pK_a units over that of the unmethylated base (discussed in detail in Section 3). Thus the measurement of the basic pK_a value, while not distinguishing between C- and extranuclear N-methylation, does give a reliable indication when substitution on a ring nitrogen atom has occurred.

With aminohydroxy-pteridines and pyrimidines the situation is somewhat different, since nuclear N-methylation of these compounds does not result in a marked increase in their basic strength. However in such cases, variations in the acidic pK_a constant are often more revealing. For example, the greatest change in the acidic pK_a is observed when all acidic properties are lost due to the oxygen atom being substituted to yield a methoxy derivative. Nuclear N-methylation also greatly weakens the acidic strength and it is not uncommon for the decrease to be 3 or 4 pK_a units. On the other hand, substitution on nuclear carbon atoms or on extranuclear nitrogen atoms only suppresses the pK_a constant by the order of 0.5 units, as it simultaneously increases the basic constant by approximately the same amount.

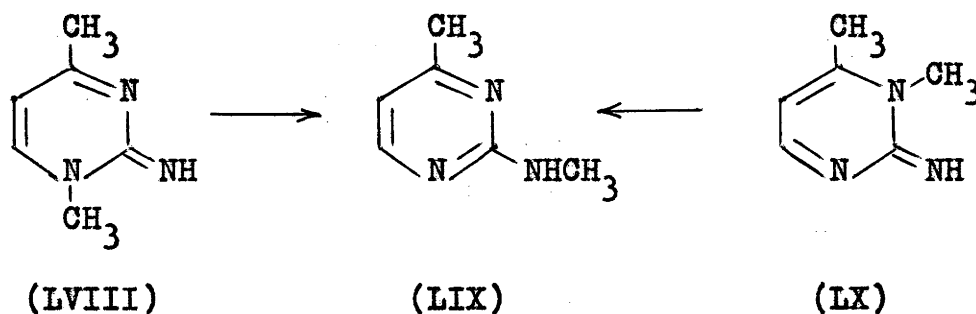
(b) Degradation. Hydrolytic methods were used frequently in the present work for determining the structure of methylation products. The pteridines that were substituted on a nuclear N-atom were found to be particularly susceptible to degradation with aqueous acid and alkali, and by variations in conditions as many as four different breakdown products were obtained. In most cases the structure of any one of the breakdown products sufficed to establish the site of methylation in the pteridine.

Hydrolytic methods for determining structure could not be used extensively in the more stable pyrimidine series.

(c) Rearrangements. The recent proof by Brown (1961) that the rearrangement of nuclear N-methylated aminopyrimidines proceeds by way of hydrolytic ring fission, followed by rotation of the terminal amidine group, and recyclization, has placed this phenomenon on a basis where it can now be used as an aid to structure determination. For example, if methylated 4-amino-pyrimidine yielded 4-methylaminopyrimidine (LVII) on rearrangement, its structure would thereby be established as 3,4-dihydro-4-imino-3-methylpyrimidine (LVI).



However it should be pointed out that the method cannot be used for structuralising the methylation products of 2-amino-derivatives, since, for example, 4-methyl-2-methylaminopyrimidine (LIX) could arise from the rearrangement of either 1,2-dihydro-2-imino-1,4-dimethylpyrimidine (LVIII) or 2,3-dihydro-2-imino-3,4-dimethylpyrimidine (LX)



This type of rearrangement is also found in the pteridine series (Curran and Angier, 1958; Pfleiderer, Liedek, Lohrmann, Rukwied, 1960) but to the present time no studies have been made on the mechanism. However since it is not unreasonable to assume that the same mechanism pertains, the method can be used, with the same limitation, in this series.

(d) Primary Synthesis. The complete synthesis of a methylation product by way of unambiguously prepared intermediates was a most satisfactory method of establishing its structure, and this method was used wherever possible.

(e) Preparation of Derivatives. In some instances where a methylation product could not be prepared by a primary synthesis, its structure was proven by a further transformation into a derivative of known structure. However, inherent in this method is the danger that an unnoticed rearrangement may occur during the synthesis.

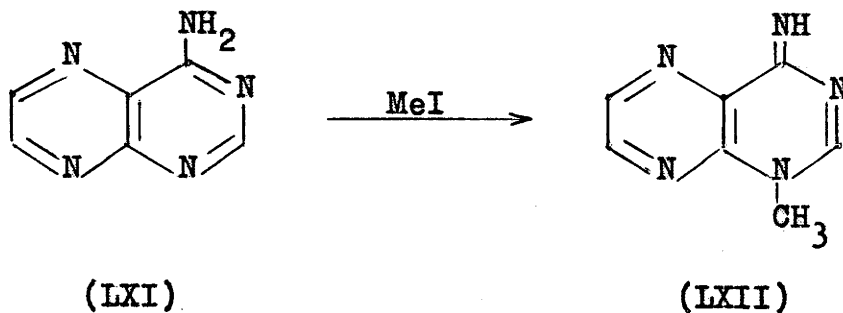
(f) Process of Elimination. This method consists of structuralising a methylation product by direct comparison with, and the elimination of all other, possible isomeric structures. The method was always used in conjunction with further evidence from one or more of the other methods.

C. Methylation of the Aminopteridines

The methylation of the simple aminopteridines are the first to be discussed, followed by more complex examples involving aminopteridines with a second functional group.

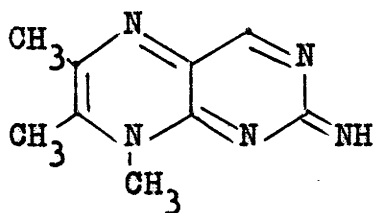
(a) 4-Aminopteridines. The first attempts to methylate 4-aminopteridine under mild conditions similar to those used by Brown, Hoerger and Mason (1955) in the aminopyrimidine series, were unsuccessful. Due perhaps in part to the lower solubility of the pteridine in methanol and methyl iodide, it was found that more vigorous conditions were necessary.

4-Aminopteridine (LXI) was found to react slowly with methyl iodide if the temperature was raised to 140° , and under these conditions gave a single mono-methyl derivative of high basic strength (pK_a 9.5). The methylation product was shown to be 1,4-dihydro-4-imino-1-methylpteridine hydriodide (LXII) by the evidence to be presented below.

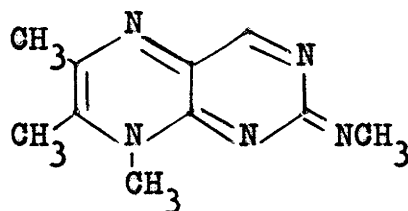


The possibility of the extranuclear N-methylation of the amino group was first precluded by the synthesis of 4-methylaminopteridine and a direct comparison of its basic pK_a constant (3.7) and ultraviolet spectra with those of the methylation product. Nuclear C-methylation could also be precluded for the same reasons, as such bases would have pK_a values of the same order as 4-amino- and 4-methylaminopteridine.

Moreover, the high basic pK_a of the product also enabled the possibility of substitution on N(8) to be discounted, for while these bases are stronger than 4-amino- and 4-methylaminopteridine, they fall far short of the base strength observed for the methylation product. Evidence in support of this last conclusion is found in the work of Fidler and Wood (1957), who record the basic pK_a values of a number of 8-methylated aminopteridines. Thus, 2,8-dihydro-2-imino-6,7,8-trimethylpteridine (LXIII) has pK_a 5.6 and 2,8-dihydro-6,7,8-trimethyl-2-methyliminopteridine (LXIV) has pK_a 6.1.

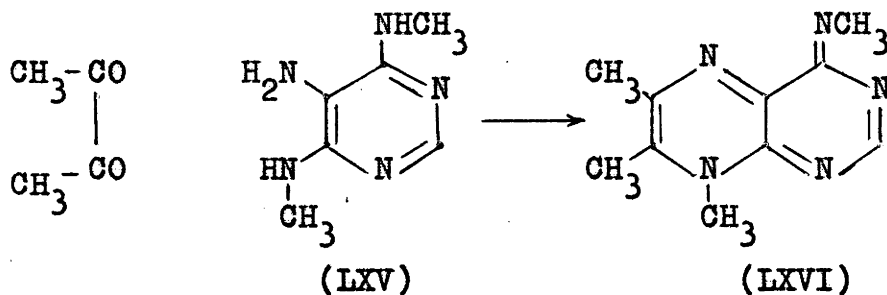


(LXIII)



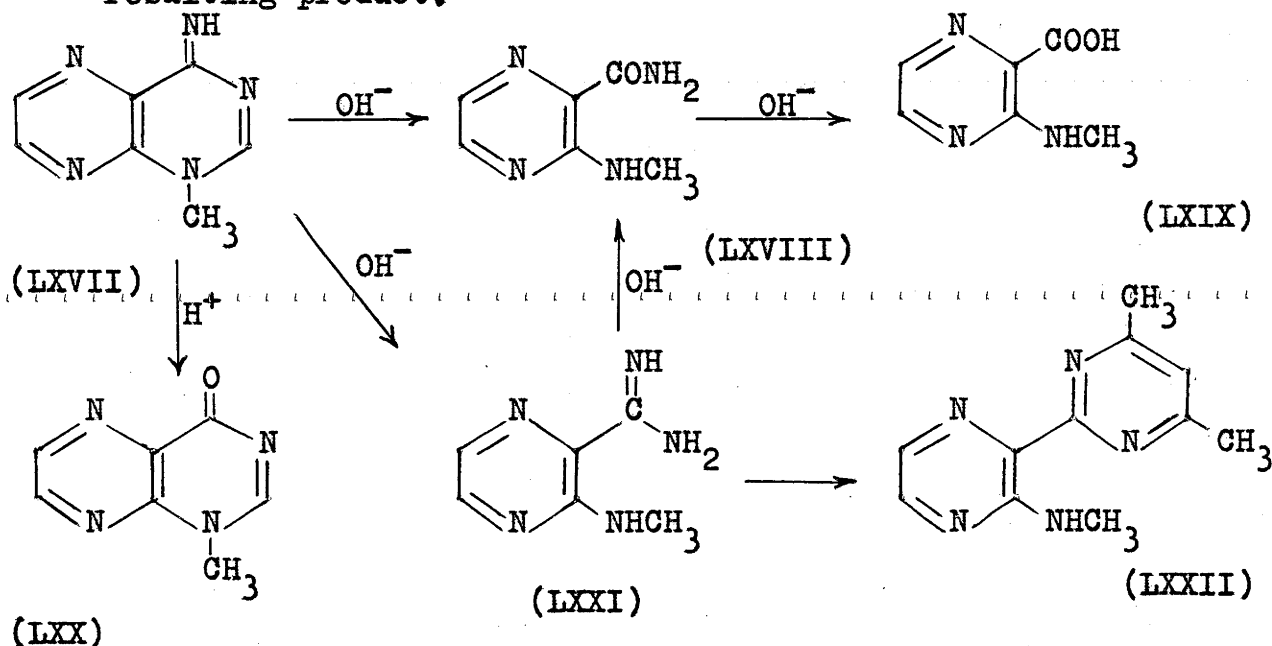
(LXIV)

In order to have a trans-methylated compound that was representative of the present 4-amino- series, 4,8-dihydro-6,7,8-trimethyl-4-methyliminopteridine (LXVI) was prepared by condensation of 5-amino-4,6-bismethylamino-pyrimidine (LXV) with biacetyl. Its basic pK_a of 6.6 was in keeping with the constants of the other $N_{(8)}$ -methylated aminopteridines quoted above.



Because substitution of 4-aminopteridine at position 5 is precluded by valency, there remained but two positions, $N_{(1)}$ and $N_{(3)}$ at which methylation could have occurred. Alkaline hydrolysis of the hydriodide of (LXVII) at 100° readily gave a product identified with 2-carbamoyl-3-methylaminopyrazine (LXVIII) described in the work of Albert, Brown and Wood (1956). By prolonged

heating at 100° in alkali, this amide was further degraded into 2-carboxy-3-methylaminopyrazine (LXIX). The presence of a methylamino substituent in the pyrazine nucleus, showed N₍₁₎ to be the site at which 4-aminopteridine had been methylated and confirmed the structure of the resulting product.



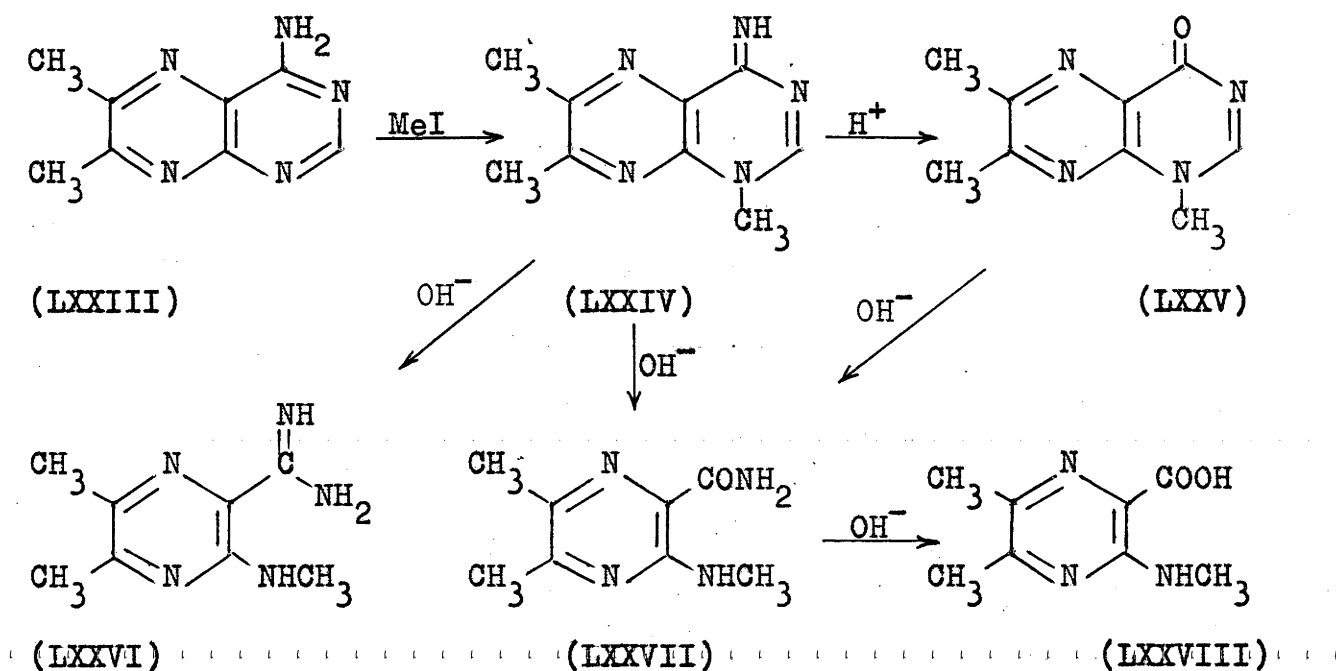
In the hydrolysis of the iminopteridine to give the amide (LXVIII), it is not unreasonable to assume that the reaction proceeds via the intermediate 1-methyl-4-pteridone (LXX). However, that this pteridone was not isolated is not surprising since it is known from the work of Albert, Brown and Wood (1956) to be exceedingly alkali-labile. For this reason, the hydrolysis was repeated, this time with alkali at 0° . Instead of the expected pteridone (LXX), a product of high basic strength (pK_a 9.0)

was obtained. From the analytical results and the evidence that the new base could be further hydrolysed into the amide (LXVIII), it was assigned the structure 2-amidino-3-methylaminopyrazine (LXXI)*. This was confirmed by its condensation with acetylacetone to give 2-(4,6-dimethylpyrimid-2-yl)-3-methylaminopyrazine (LXXII).

Ultimately, the intermediate 1-methyl-4-pteridone (LXX) was obtained from the iminopteridine by acid hydrolysis at 100°, and its identity confirmed by comparison with an authentic specimen prepared by Albert, Brown and Wood (1956).

The methylation of 4-amino-6,7-dimethylpteridine (LXXIII) and the chemistry arising from it, was parallel to that of its homologue described above. Vigorous conditions were again necessary to achieve methylation with methyl iodide, and as before a single mono-methylation product was obtained.

*Some idea of the facility with which the iminopteridine (LXVII) is decomposed in this way, was seen in the attempted measurement of its ultraviolet spectrum. Despite rapid working, the trace of the neutral molecule at pH 11.5 was consistently identical with that of the amidinopyrazine (LXXI)



The strongly basic pK_a (10.5) of the derivative once more precluded extranuclear N-methylation, since that product, 4-methylamino-6,7-dimethylpteridine (LXXIII) is a weak base of pK_a 4.2. Transannular N₍₈₎-methylation was likewise discounted, since as discussed above, the strength of that base would be close to pK_a 6. To distinguish between the remaining possibilities hydrolytic methods were again used.

With hot alkali a facile hydrolysis occurred, and the resulting amide was shown to be 2-carbamoyl-5,6-dimethyl-3-methylaminopyrazine (LXXVII) by further degradation to 2-carboxy-5,6-dimethyl-3-methylaminopyrazine (LXXVIII). The presence of three methyl groups in this pyrazine acid served to confirm the structure of the amide and also that of the original methylation product (LXXIV).

By hydrolysing the iminopteridine (LXXIV) under much milder conditions, 2-amidino-5,6-dimethyl-3-methylaminopyrazine (LXXVI) was obtained and its pK_a of 9.5 and ultraviolet spectra agreed well with those of the homologous amidine.

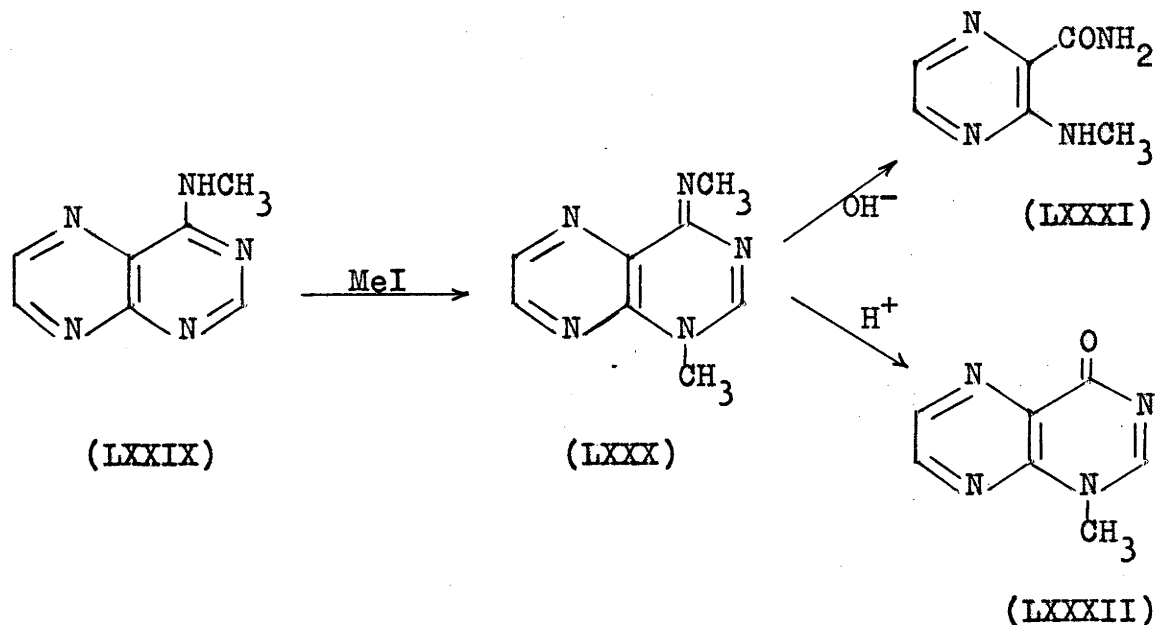
To complete the analogy with the previous series, the acid hydrolysis of the methylation product was studied. The resulting substance was assigned the structure of 1,6,7-trimethyl-4-pteridone (LXXV) on the basis of its further alkaline degradation to the amide (LXXVII).

(b) 4-Methylaminopteridines. This series was studied in order to discover if the small variation in the substituent group would affect the position of methylation or the chemistry of degradation.

4-Methylaminopteridine (LXXIX) was synthesised from its 4-methylthio- precursor, and methylated with methyl iodide under the same vigorous conditions used in the primary- amino series. As before, the single product proved to be a mono-methyl derivative having a base strength (pK_a 10.3) that was significantly comparable with those of the previous methylation products. Therefore, for the reasons already discussed in detail, methylation could not have occurred on the extranuclear nitrogen atom (4-dimethylaminopteridine has pK_a 4.3), on any of the nuclear carbon

atoms, or on the nitrogen atom in position 8. Of the remaining possibilities, N₍₁₎ was naturally favoured if only by analogy with the previous series.

Because the alkaline degradations had proven so effective in structuralising the previous products, the method was again used. Thus with alkali at 100°, the methylated pteridine was degraded into the now familiar 2-carbamoyl-3-methylaminopyrazine (LXXXI) thereby revealing its structure as 1,4-dihydro-1-methyl-4-methyliminopteridine (LXXX).

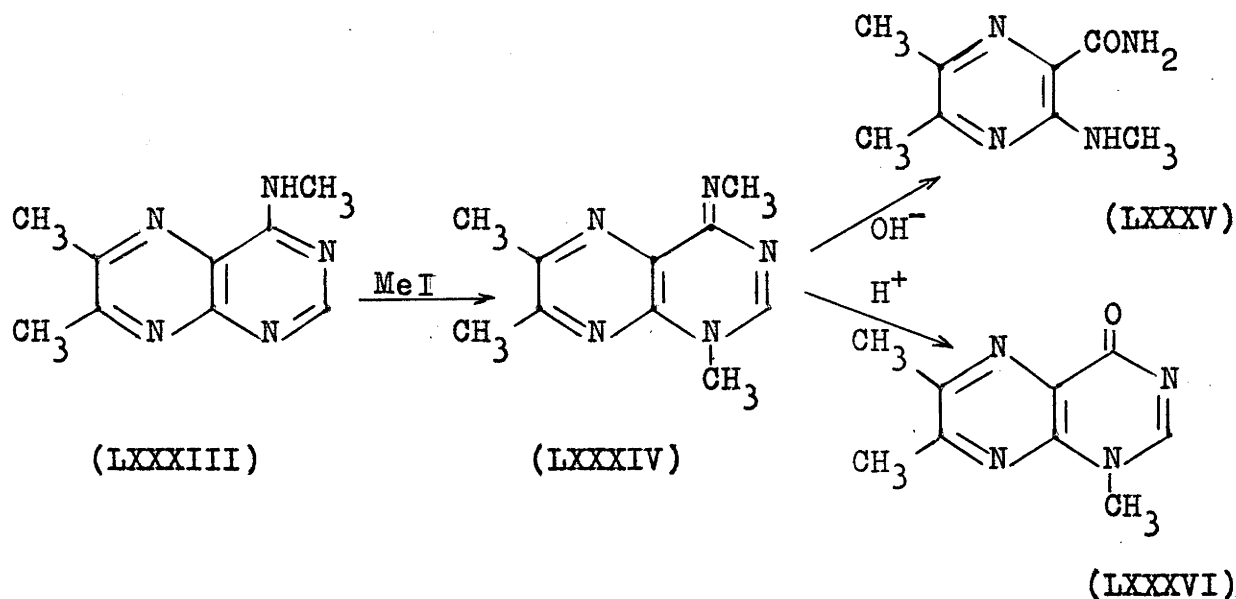


In contrast to the previous series, mild alkaline hydrolysis did not yield an amidinopyrazine. In view of the fact that the more severe alkaline hydrolysis proceeded with the same facility as before, this observation supports

a theory that two routes of alkaline degradation operate. One, in which the imino-group is hydrolysed before ring cleavage, proceeds only slowly at 0° , but is so rapid at 100° that the small stabilising effects introduced by each additional methyl substituent are not apparent. The other, in which ring cleavage to give the amidine occurs first, but which is much slower, is naturally more easily suppressed by the presence of electron-supplying stabilising groups. Acid hydrolysis of the iminopteridine (LXXX) proceeded at a slower rate than in the previous series but yielded the same 1-methyl-4-pteridone (LXXXII).

6,7-Dimethyl-4-methylaminopteridine (LXXXIII) was prepared by methylation of the 4-methylthio precursor in the same manner as its lower homologue. Its methylation was carried out under slightly less vigorous conditions than had hitherto been used, but in other respects the reaction conditions were the same. Once more the product proved to be a mono-methylation derivative with a base constant (pK_a 11.4) considerably in excess of the previous iminopteridines. This clearly indicated again that the base was substituted on either an $N_{(1)}$ or $N_{(3)}$. Degradation with hot alkali, and the isolation of 2-carbamoyl-5,6-dimethyl-3-methyl aminopyrazine (LXXXV), enabled the structure of the imino-base to be identified as 1,4-dihydro-1,6,7-trimethyl-4-

methyliminopteridine (LXXXIV). Further confirmation of the methylation site was obtained by acid hydrolysis to 1,6,7-trimethyl-4-pteridone (LXXXVI). However it is significant, that in order to achieve this degradation with acid, even more vigorous conditions than previously used, were necessary.



From the aforementioned examples, it is of interest to notice the stabilising effect that the electron-donating methyl substituents have on the π electron-depleted system. In this connection, Table 1 summarises the optimum conditions (determined by paper chromatography) for both the acid and alkaline hydrolysis.

Table 1

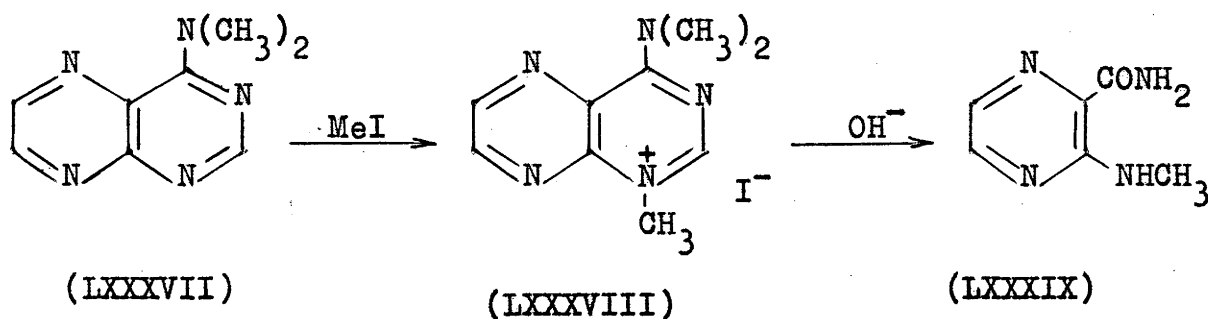
1,4-Dihydro-1-methyl deriv.	Reagent, time (min.) and temp.			Product
4-Imino-	2.5N-HCl	30	100°	1-methyl-4-pteridone
	2.5N-NaOH	20	0°	2-amidino-3-methylaminopyrazine
	N-NaOH	10	100°	2-carbamoyl-3-methylaminopyrazine
4-Imino-6,7-dimethyl	2.5N-HCl	120	100°	1,6,7-trimethyl-4-pteridone
	2.5N-NaOH	10	35°	2-amidino-5,6-dimethyl-3-methylaminopyrazine
	N-NaOH	40	100°	2-carbamoyl-5,6-dimethyl-3-methylaminopyrazine
4-Methylimino-	6.3N-HCl	240	100°	1-methyl-4-pteridone
	N-NaOH	10	100°	2-carbamoyl-3-methylaminopyrazine
6,7-Dimethyl-4-methylamino-	6.3N-HCl	60	130°	1,6,7-trimethyl-4-pteridone
	N-NaOH	40	100°	2-carbamoyl-5,6-dimethyl-3-methylaminopyrazine

While conditions for the alkaline degradations reveal that the methylimino- are not significantly different from the imino-derivatives, it can be seen that the corresponding 6,7-dimethyl analogues are considerably more stable. Acid hydrolysis however, shows even more clearly the steady increase in stability that accompanies the introduction of methyl groups into the pteridine molecule.

(c) 4-Dimethylaminopteridines. The extension of the present series to include the dimethylamino-pteridines, involves more than just the additional effect of another methyl group on the course of a methylation. Clearly, the reactions of these tertiary bases with methyl iodide must be true quaternisations, and the products true pteridinium iodides. This field of pteridine chemistry was completely unexplored, and only in the work of Albert, Brown and Woods (1956) is mention made of one such compound of undetermined constitution.

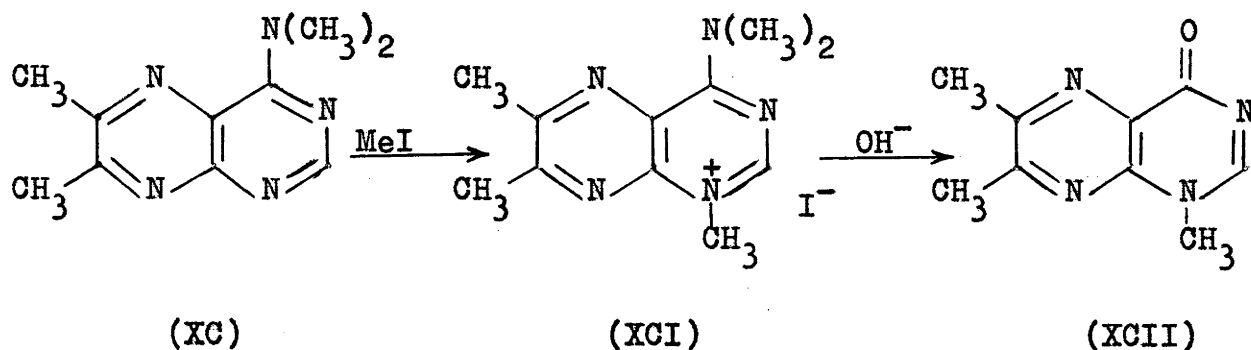
The quaternisation of 4-dimethylaminopteridine (LXXXVII) with methyl iodide occurred under conditions much milder than those used for the primary and secondary amines of the series. The mono-methiodide obtained, proved to be particularly unstable in alkaline solution (preventing the measurement of its pK_a constant) and was

rapidly degraded in hot water at pH 10, to 2-carbamoyl-3-methylaminopyrazine (LXXXIX). Consequently, its structure can be represented by 4-dimethylamino-1-methylpteridinium iodide of which (LXXXVIII) is one of the unperturbed resonance states.



4-Dimethylamino-6,7-dimethylpteridine (XC)

proved to be quaternised with methyl iodide even more readily than its homologue. In fact the reaction proceeded most successfully at room temperature. Again, the product was found to be a mono-methiodide, and by an analogous hydrolysis in hot water its structure was shown to be 4-dimethylamino-1,6,7-trimethylpteridinium iodide (XCI). However, it is significant to the theory of stabilisation mentioned above, that the product isolated from the hydrolysis was the intermediate 1,4-dihydro-1,6,7-trimethyl-4-pteridone (XCII).



Methylation of 2-Amino-4-hydroxypteridines

2-Amino-4-hydroxypteridine was included in the series of compounds methylated because of its importance as the basic nucleus of most naturally occurring pteridines. Moreover, since attempts to methylate 2-aminopteridine had been unsuccessful, this derivative of it offered the opportunity to study the effect of a 2-amino substituent on the course of methylation.

Initial experiments on the methylation of the 2-amino-4-hydroxypteridines were unsuccessful, chiefly for the reason that the compounds were so insoluble in methyl iodide. This difficulty was overcome by incorporating methanol with the methylating agent and by rocking the sealed tubes containing the reaction mixture during the period of heating. For this latter purpose an apparatus based on that described by Gabriel (1905) was used. Also, the temperature at which the methylations were carried out had to be reduced to 100-110° to offset the rapid formation

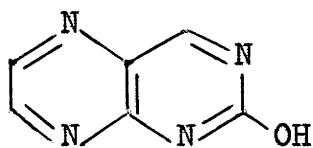
of dimethyl ether. Under these modified conditions, 2-amino-4-hydroxypteridine (CII) gave a single mono-methyl derivative which was isolated as the hydriodide and converted into the hydrochloride.

In contrast to the previous series, the relatively weak basic strength of the methylation product (pK_a 5.4) was of little help in eliminating some of the possible structures, as it represented only a small increase (3 pK_a units) over the base strength of 2-amino-4-hydroxypteridine (pK_a 2.3). More revealing was its anionic pK_a (ca. 11.5). This showed a marked weakening from the acidic strength of 2-amino-4-hydroxypteridine (pK_a 7.9), caused, no doubt, by the hydroxy group being forced into the oxo- form by the process of substitution.

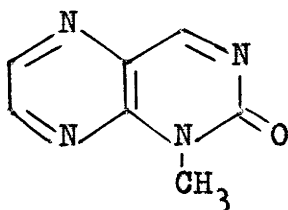
Such an interpretation would clearly preclude all possible nuclear C-methylation products as well as the extranuclear N-methylation product, 4-hydroxy-2-methylaminopteridine, since these would all retain a similar acidic function to that of the original hydroxypteridine.

While this argument proved sound in the light of subsequent work, it was decided to proceed with caution, especially in view of some striking anomalies that exist in the literature. For example, Brown and Mason (1956) record that 2-hydroxypteridine (XCIII) and its nuclear

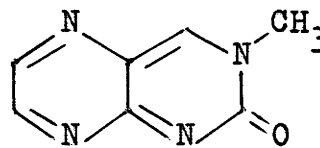
N-methylated products 1-methyl- and 3-methyl-2-pteridone (XCIV; XCV) all have similar acidic constants (pK_a values of 11.13; 11.43; 11.0 respectively).



(XCI)



(XCIV)

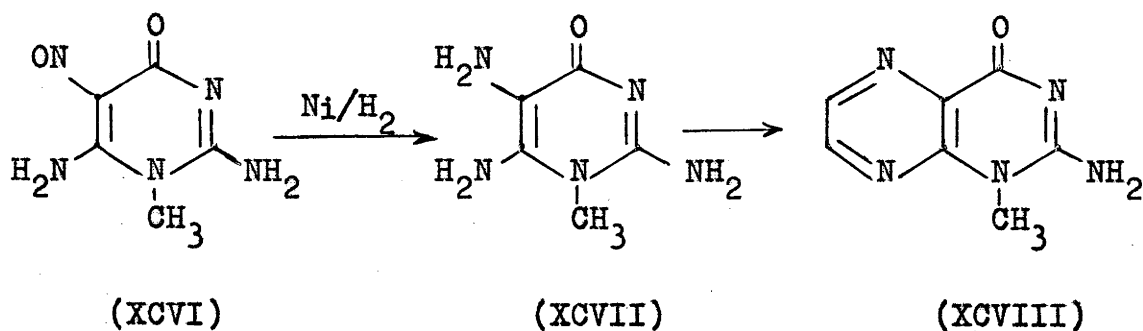


(XCV)

For this reason, all the possible methyl derivatives (with the exception of the C-methylated isomers) were synthesised by unambiguous methods and the properties of each compared directly with those of the actual methylation product.

The first of these prepared was 2-amino-1,4-dihydro-1-methyl-4-oxopteridine*(XCVIII), since, by analogy with the first series, substitution on N₍₁₎ was anticipated. It was synthesised from 2,4-diamino-3,6-dihydro-3-methyl-5-nitroso-6-oxopyrimidine (XCVI) (Roth, Smith and Hultquist, 1951, with corrections by Boon and Bratt, 1957) by reduction to the triamine (XCVII) followed by the usual type of condensation with glyoxal.

* The preparation of this compound by a similar method was later described by Pfeleiderer, Liedek, Lohrmann and Rukwied, (1960).



However the 1-methylpteridone proved to be a much weaker base (pK_a 2.8) than the methylation product (pK_a 5.4) and to differ markedly from it in chromatography and ultraviolet spectra (see figure 1).

4-Hydroxy-2-methylaminopteridine (XCIX)* was next prepared by nitration of 4-amino-6-hydroxy-2-methylaminopyrimidine, catalytic reduction of the 5-nitropyrimidine to give the 4,5-diamino- derivative, and then condensation with glyoxal.

The hydroxypteridine however, was markedly different from the methylation product in chromatography, ultraviolet spectrum (see figure 1) and ionisation constants. It was also of interest to discover that its pK_a values (acidic, 8.16; basic, 1.98) did not vary greatly from those of 2-amino-4-hydroxypteridine (7.92 and 2.31 respectively). However, while the weakening of the acidic function was in

* See foot-note page 35.

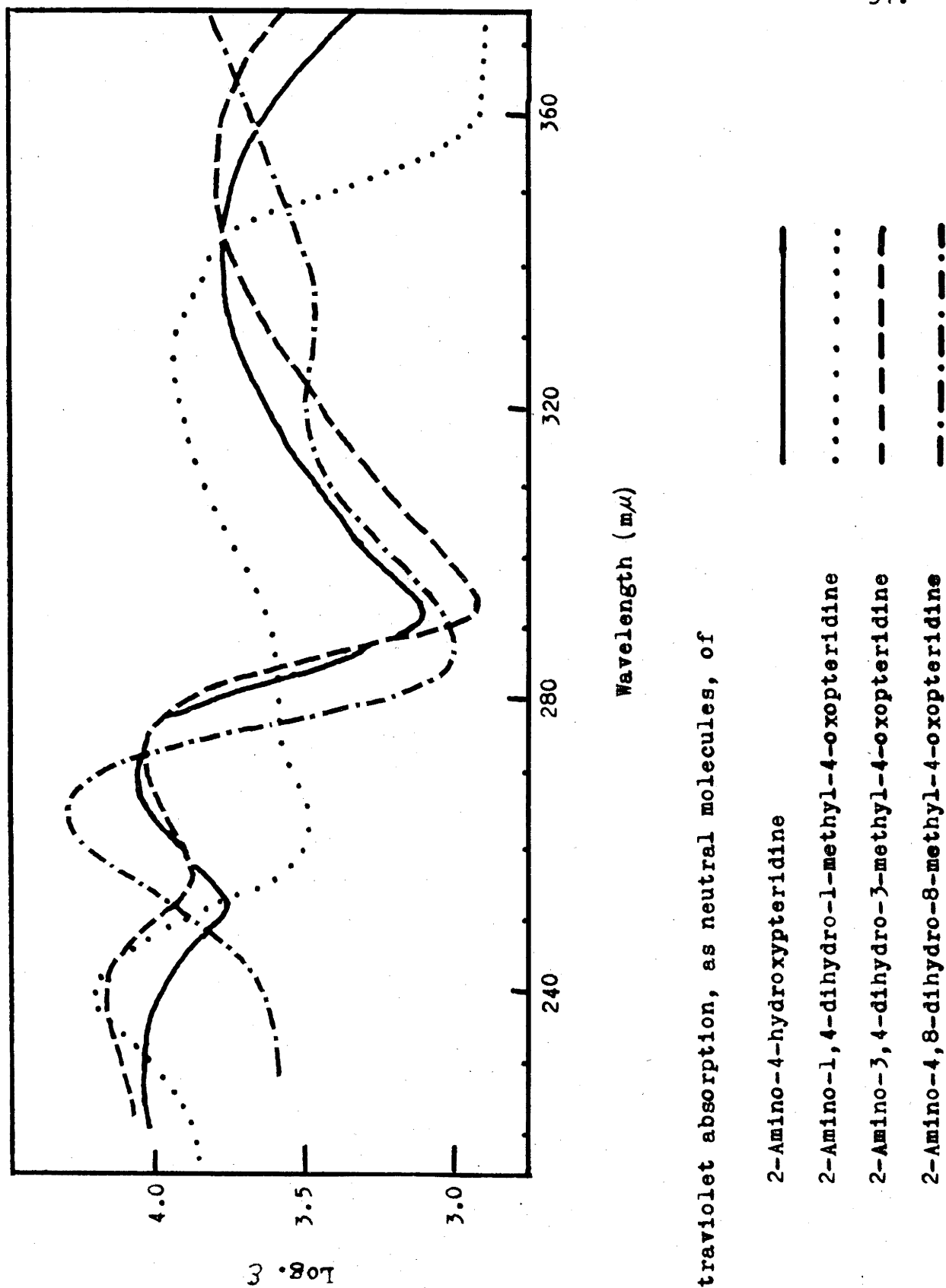


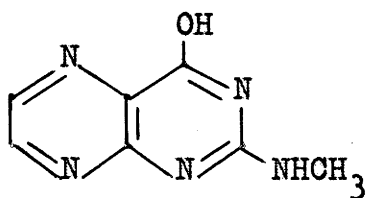
Fig. 1.

accord with expectations, the similar decrease in the base strength was difficult to explain. Nevertheless, there can be little doubt of the validity of the base constant, since a similar value is also quoted by Pfeleiderer, Liedek, Lohrmann and Rukwied (1960).

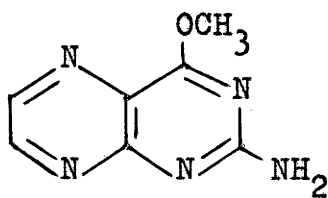
An unambiguous synthesis of 2-amino-4-methoxypteridine (C)*, resulted from the condensation of 2,4,5-triamino-6-methoxypyrimidine with glyoxal. This pteridine did not possess an acidic function, but its basic pK_a (3.46) and ultraviolet spectrum (see figure 1) differed significantly from those of the methylation product.

Of the remaining isomers, 2-amino-3,4-dihydro-3-methyl-4-oxopteridine (CI)* was obtained by the condensation of 2,4,5-triamino-1,6-dihydro-1-methyl-6-oxypyrimidine and glyoxal. Apart from the fact that its basic pK_a (2.25) and spectrum (figure 1) differed from those of the methylation product, it readily rearranged in hot alkali to 4-hydroxy-2-methylaminopteridine (XCIX).

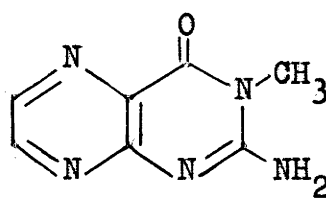
* See foot-note page 35.



(XCIX)

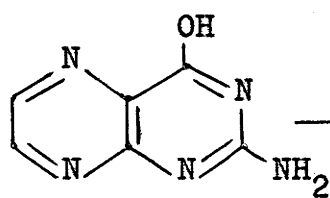


(C)

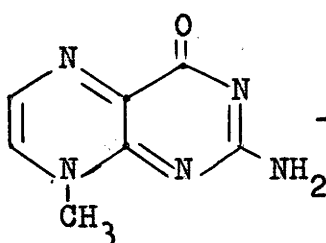


(CI)

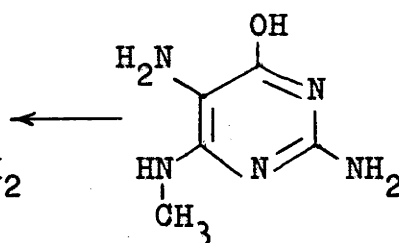
With the elimination of the above compounds from the list of possible methylation products, there remained only 2-amino-4,8-dihydro-8-methyl-4-oxopteridine (CIII) to be considered. This transannularly-methylated pteridine was synthesized unambiguously from 2-amino-4-hydroxy-6-methylamino-5-nitrosopyrimidine (Fidler and Wood, 1957) by catalytic reduction to 2,5-diamino-4-hydroxy-6-methylaminopyrimidine (CIV) followed by condensation with glyoxal.



(CII)



(CIII)



(CIV)

Isolated as the hydrochloride, the pteridine was compared with the methylation product of 2-amino-4-hydroxypteridine and in respect of melting points, ionisation constants, chromatography and ultraviolet spectra,

the compounds were found to be the same. As further proof of their identity, infrared spectral traces of each were recorded and these are reproduced in figure 2.

The direct transannular methylation of 2-amino-4-hydroxypteridine, thus confirmed, is unique and has no parallel in the literature. In all the examples recorded, many of which have been reviewed in Section 1, amino- and hydroxy-pteridines have invariably methylated in the ring bearing the tautomeric substituent. Moreover, this is as expected, and can be justified in terms of resonance stability, as such orientation permits of at least one sextet of delocalised electrons in a Kekulé structure. However in transmethylated pteridines, no such structure is possible and in the $N_{(8)}$ derivative (CIII) only an ortho-ortho arrangement of double bonds can exist.

In an effort to extend this unusual type of methylation to other compounds, the reaction was repeated under the same conditions on 2-amino-4-hydroxy-6,7-dimethylpteridine (CIX). Once again the product obtained was shown to be a mono-methyl derivative with ionisation constants (acidic pK_a ca.12.0; basic pK_a 6.10) and ultraviolet spectrum akin to the former transannularly methylated pteridine.

Comparison of possible isomeric methylation

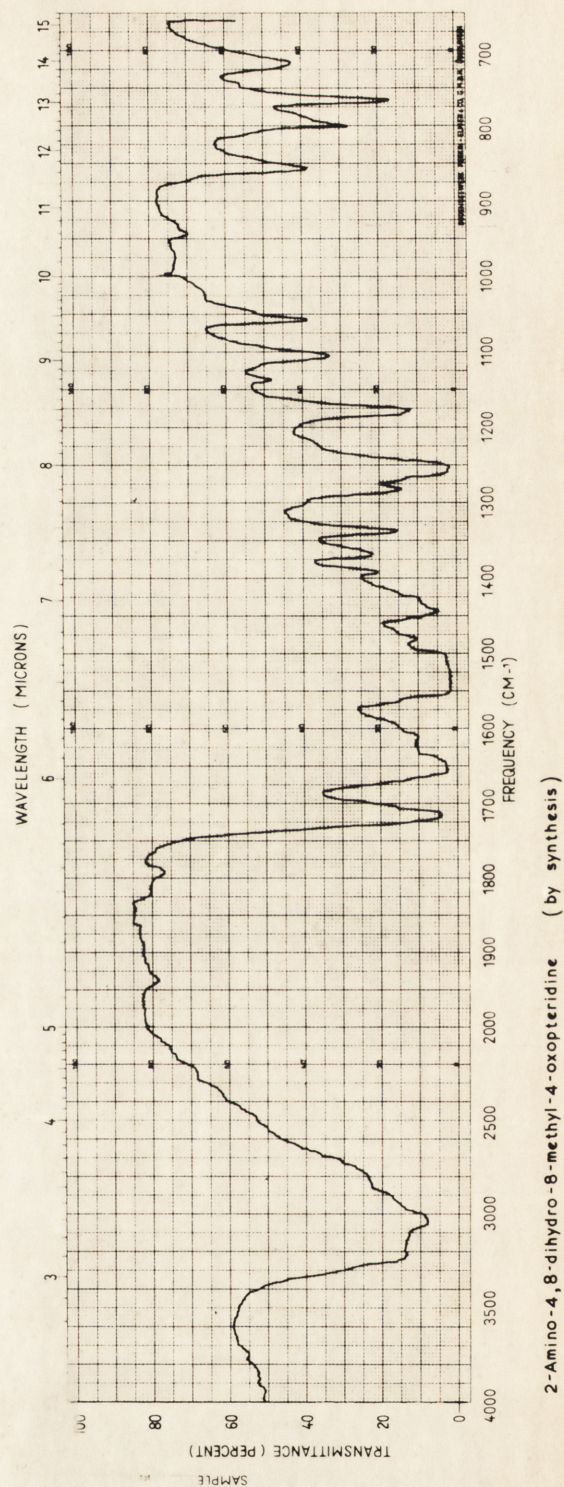
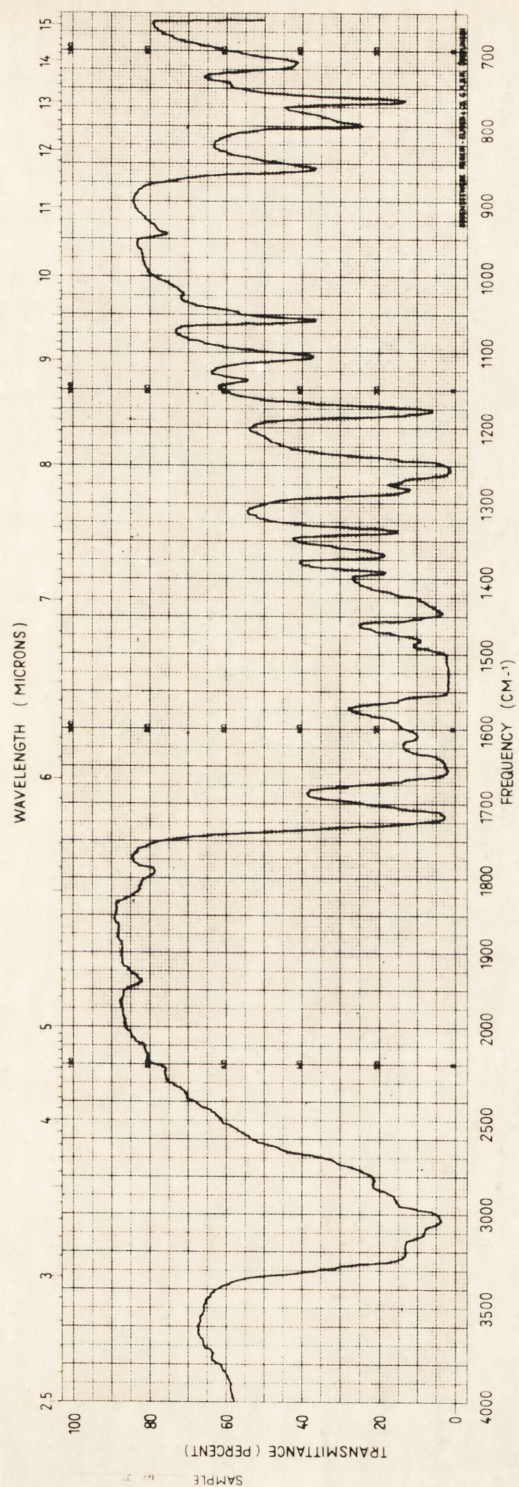
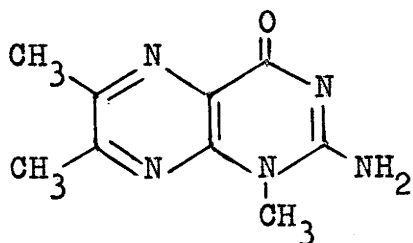
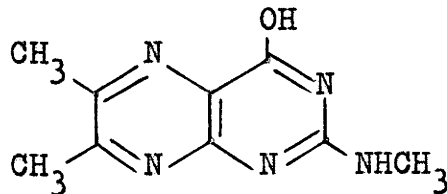


Fig. 2.

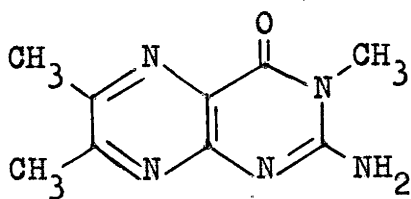
products was rendered much easier in the second case since these had already been described. Thus the properties of 2-amino-1,4-dihydro-1,6,7-trimethyl-4-oxo- (CV), 4-hydroxy-2-methylamino-6,7-dimethyl- (CVI) and 2-amino-3,4-dihydro-4-oxo-3,6,7-trimethyl-pteridine (CVII) prepared by Curran and Angier (1958) and 2-amino-4-methoxy-6,7-dimethylpteridine (CVIII) characterised by Roth, Smith and Hultquist (1951), were similar to those of the lower homologues and differed from the methylation product in the same respects as before.



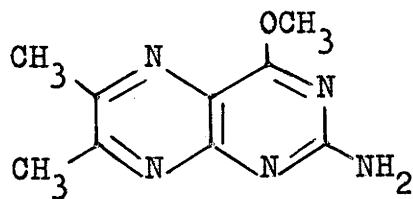
(CV)



(CVI)



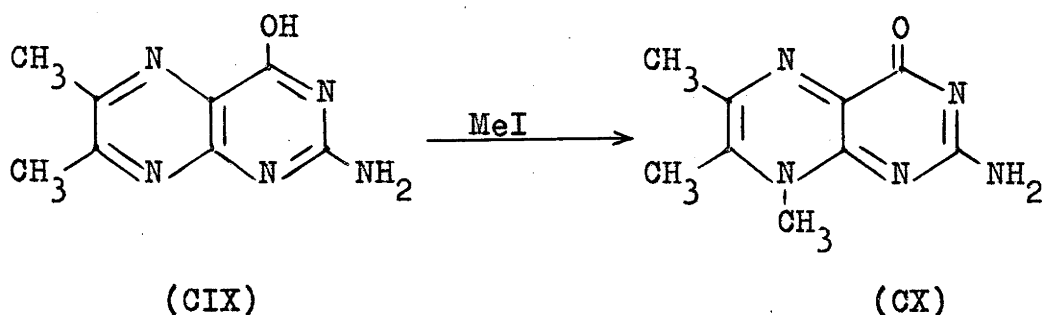
(CVII)



(CVIII)

The transannularly methylated isomer 2-amino-4,8-dihydro-6,7,8-trimethyl-4-oxopteridine (CX) had also been described

as the free base by Fidler and Wood (1957). As the hydrochloride, it was compared directly with the methylation product and identity established by the same physical methods as before.

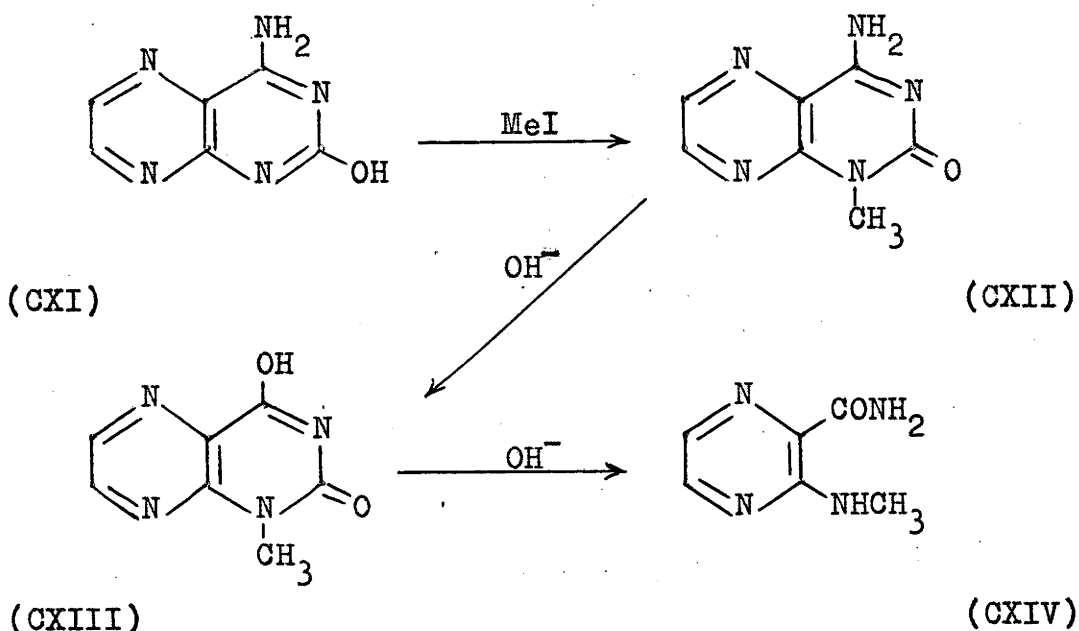


Of considerable consequence in any theory put forward to explain transannular methylation is a recent note by Angier and Curran (1961). These workers report that methylation of 2-amino-4-hydroxy-6,7-dimethylpteridine using dimethyl sulphate in aqueous alkali, gave the 1-methyl (CV), and 3-methyl (CVII) derivatives as the only products. The significance of this discovery is discussed later.

Methylation of 4-Amino-2-hydroxypteridines

To find if the above transannular methylation was a characteristic of the 2-amino-4-hydroxy configuration, it was natural to investigate the isomeric 4-amino-2-hydroxy series. It is disappointing therefore to report that the methylation of 4-amino-2-hydroxypteridine (CXI) under the same conditions was quite unsuccessful. When

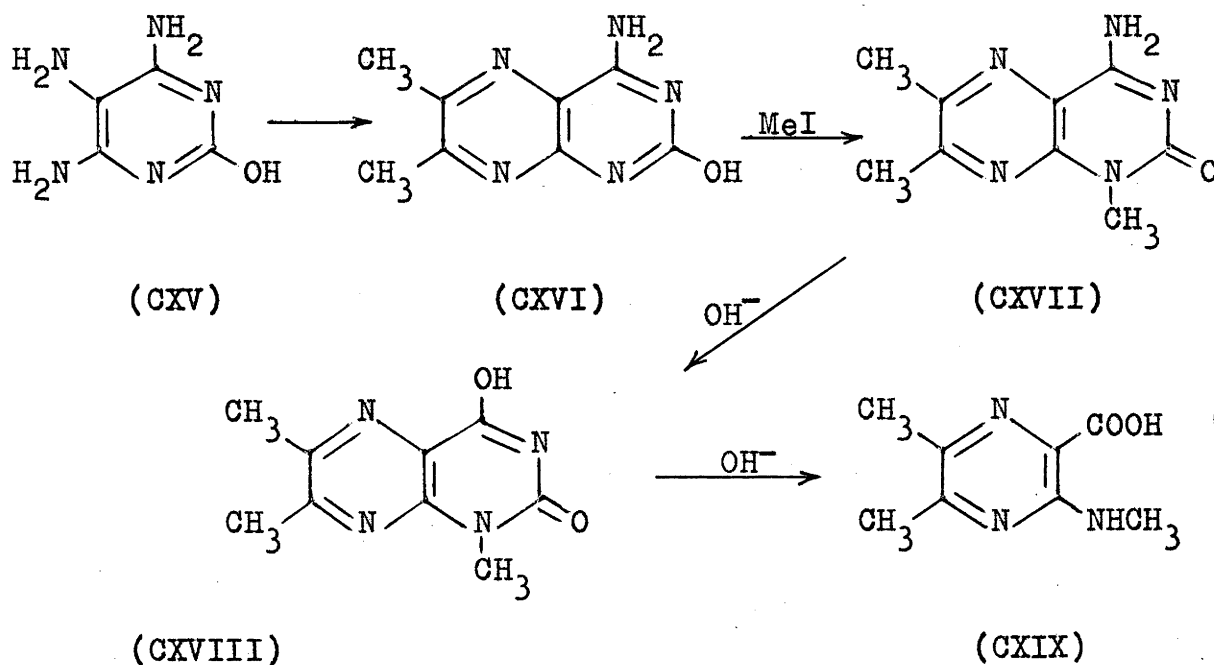
the methylation was carried out under milder conditions and in the presence of an equivalent of sodium methoxide, however, a mono-methyl derivative was obtained. This was shown to be 4-amino-1,2-dihydro-1-methyl-2-oxopteridine (CXII) by hydrolysis with alkali to 1-methyl-lumazine (CXIII) and thence to 2-carbamoyl-3-methylaminopyrazine (CXIV).



The higher homologue, 4-amino-2-hydroxy-6,7-dimethylpteridine (CXVI), which had not previously been described, was prepared from 4,5,6-triamino-2-hydroxypyrimidine (CXV; Bendich, Tinker and Brown, 1948) and glyoxal. Another attempt was made to methylate this analogue using the "N₍₈₎-methylating" conditions, but once more without success. It is worth recording

however, that chromatography showed the presence of 4 or 5 substances in the reaction mixture, none of which could be characterised.

Reverting to the use of milder conditions and sodium methoxide, a single methylation product was produced. Degradation of it with alkali yielded successively, 1,6,7-trimethyl-lumazine (CXVIII; first described by Sachs and Meyerheim, 1908) and 2-carboxy-5,6-dimethyl-3-methylaminopyrazine (CXIX), thus indicating its structure to be 4-amino-1,2-dihydro-1,6,7-trimethyl-2-oxopteridine (CXVII).

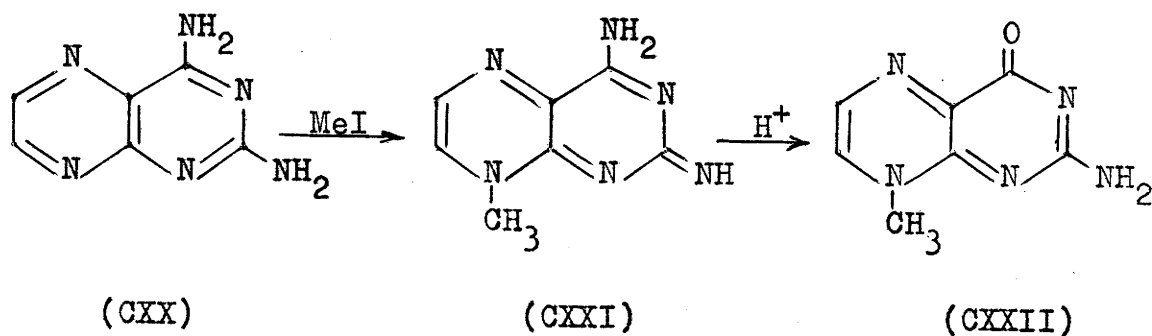


Methylation of 2,4-Diaminopteridines

In the search for further examples of transannular methylation, the series was extended to the diamino-

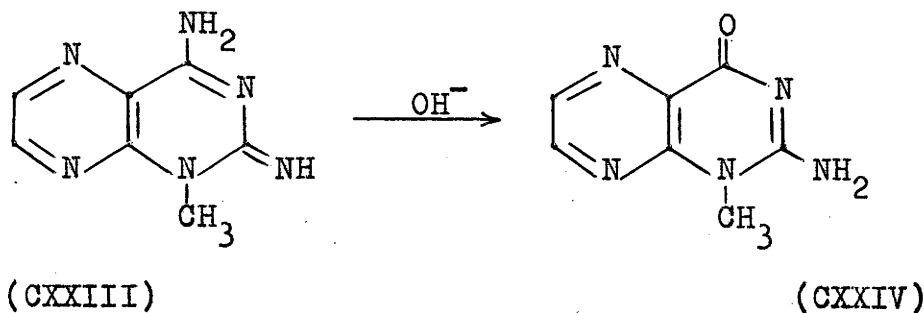
pteridines. 2,4-Diaminopteridine (CXX) was successfully methylated under the conditions previously used to give N₍₈₎-methyl derivatives, but chromatography revealed that two products were present. One of these proved to be the first example of a dihydriodide to be isolated in the series, although analysis confirmed that only mono-methylation had occurred. The presence of the additional hydriodic acid however, is easily explained, since as mentioned earlier, these reactions were always accompanied by the formation of dimethyl ether. Ultraviolet spectroscopy showed that the dihydriodide derivative possessed the strong absorption band in the visible region ($\lambda_{\text{max.}} = 412 \text{ m}\mu$.) that was such a feature of the other N₍₈₎-methyl derivatives described in this work and elsewhere (Fidler and Wood, 1957).

Acid hydrolysis of the dihydriodide produced a pteridine which was shown by its melting point, chromatography and ultraviolet and infrared spectra, to be the hydrochloride of 2-amino-4,8-dihydro-8-methyl-4-oxopteridine (CXXII) thus establishing its diamino precursor as the N₍₈₎-methylated pteridine (CXXI).

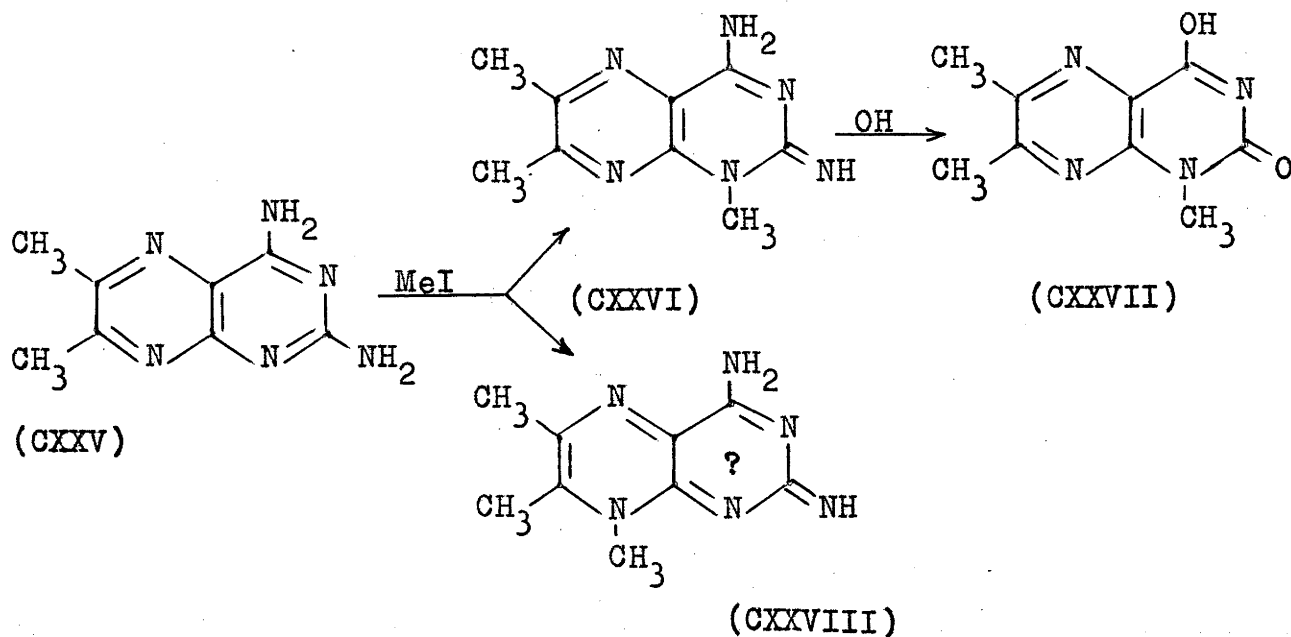


While there is only incomplete evidence to indicate the preferred tautomeric form of the methylation product (CXXI), it has been pictured as the ortho-paraquinonoid form since this is rated to be of lower activation energy (and hence more stable) than the ortho-orthoquinonoid structure of the 2-amino-4-imino configuration (Gore and Phillips, 1949). This concept is expanded further in Section 3.

Another product formed in the methylation of 2,4-diaminopteridine could not be separated from a small quantity of the N₍₈₎-methyl compound (CXXI). Nevertheless, analysis of the mixture showed the two compounds to be isomeric. That this second isomer was in fact the N₍₁₎-methyl derivative, 4-amino-1,2-dihydro-1-methyl-2-iminopteridine (CXXIII), was shown by the isolation of 2-amino-1,4-dihydro-1-methyl-4-oxopteridine (CXXIV) after hydrolysis of the mixture.



In the methylation of the higher homologue, 2,4-diamino-6,7-dimethylpteridine (CXXV), two products were again formed. This time however, the product isolated in the pure state was the $N_{(1)}$ -methyl derivative 4-amino-1,2-dihydro-1,6,7-trimethyl-2-iminopteridine (CXXVI) while the other isomer was obtained only in admixture with the first. The structure of the $N_{(1)}$ -methyl derivative was confirmed by hydrolysis to 1,6,7-trimethyl-lumazine (CXXVII), while by analogy with the previous methylation, the second product is assumed to be the $N_{(8)}$ -methyl pteridine (CXXVIII).



SUMMARY

The methylation of six different 4-aminopteridines with methyl iodide resulted in substitution exclusively on the nuclear nitrogen atom $N_{(1)}$. The two pteridinium compounds prepared were the first of the series to be described.

2-Amino-4-hydroxypteridines reacted in a unique manner with methyl iodide to give the first known examples of pteridines undergoing transannular $N_{(8)}$ -methylation, and the first recorded instances where methylation had occurred in a ring other than the one containing the tautomeric group. The isomeric 4-amino-2-hydroxypteridines could not be methylated in the same manner, but under different conditions yielded the $N_{(1)}$ -methyl derivatives.

In the case of the 2,4-diaminopteridines, both $N_{(8)}$ - and $N_{(1)}$ -methyl derivatives were obtained.

Factors Influencing the Position of Methylation

The unique character of the transannular methylations described above, and the contrast they present to other examples, calls for some attempt to correlate the factors which may influence the position of methylation.

Even though alkylations are regarded classically as nucleophilic substitutions (S_N), it is, as Ingold (1953) points out " a pure convention as to which of two inter-

acting substances is regarded as the reagent and which the substrate or substance on which the reagent acts". In the methylations described in this thesis therefore, the alkyl halide, which is the smaller of the two reactants and the one generally in excess, is regarded as the attacking reagent, while the heterocyclic bases are considered as the substrates. Keeping this convention in mind, the methylations can then be classified as electrophilic substitutions. It follows from the general theory therefore, that substitution may be expected at those sites in the hetero-cyclic nucleus where the electron density is highest.

The insertion of 4 nitrogen atoms into the skeletal structure of naphthalene (as in the molecule of pteridine), results in a strong polarisation of the π electrons and a deactivation towards electrophilic attack at all carbon atoms. Thus the nitrogen atoms, by virtue of their electronegativity, become the sites most susceptible to this type of substitution. For this reason it is suggested that the factors influencing the position of methylation most, will be those which further influence the distribution of electrons throughout the nucleus.

(a) Substituent Groups. In both electrophilic and nucleophilic substitution, the orientation effects from substituent groups in the substrate molecule, are

well established (Ingold, 1953). Thus with electron repelling groups such as -NH_2 and -OH at positions 2 and 4 in the pteridine nucleus, the concentration of π electrons around each of the hetero-atoms will be increased, even though it is not unreasonable to expect the effect to be greatest at $\text{N}_{(1)}$ and $\text{N}_{(3)}$.

Using a method based on the Linear Combination of Atomic Orbitals (L.C.A.O.), Pullman (1960)* has calculated the π electron distribution in the molecules of 4-amino- and 2-amino-4-hydroxy-pteridine and gives the order for the hetero-atoms as

4-Aminopteridine $\text{N}_{(1)} = \text{N}_{(3)} > \text{N}_{(8)} > \text{N}_{(5)}$

2-Amino-4-hydroxypteridine $\text{N}_{(1)} > \text{N}_{(3)} > \text{N}_{(8)} > \text{N}_{(5)}$

While it must be remembered that the distribution of electrons in the unperturbed state of the molecule may not be the same during the approach of an attacking reagent, it is interesting to observe that some correlation with experimental fact does exist. Thus from the results described in this thesis, 4-aminopteridine has been shown

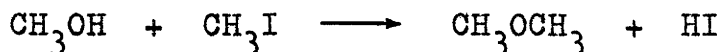
* Recently Brown, R. D. (1961) has calculated the distribution of electric charges in the unsubstituted pteridine and pyrimidine nuclei by a more precise modification of the L.C.A.O. method, and his results differ in some respects from those of Pullman.

to methylate on $N_{(1)}$, while Curran and Angier (1961) report the methylation of 2-amino-4-hydroxypteridine on $N_{(1)}$ and $N_{(3)}$. That $N_{(1)}$ is also the most basic centre in 2-amino-4-hydroxypteridine is seen from spectral evidence presented by Pfeleiderer, Liedek, Lohrmann and Rukwied (1960) who showed that protonation occurs at this site.

The evidence from the calculations of Pullman however, does not explain the absence of a $N_{(3)}$ -methylation product in the first example. Again, as far as the transannular methylation of 2-amino-4-hydroxypteridine is concerned, the order of electron densities gives no indication that exclusive substitution on $N_{(8)}$ is at all probable.

It is possible, though rather improbable, that the arrangement of the substituent groups at positions 2 and 4 may cause substitution to occur on $N_{(8)}$. Thus only those pteridines with 2-amino- groups were methylated on $N_{(8)}$, whereas those with 4-amino- groups gave $N_{(1)}$ -methyl derivatives. Moreover, when 2- and 4-amino- groups were present in the same nucleus, then both $N_{(8)}$ - and $N_{(1)}$ -methyl derivatives were obtained. Despite these considerations, the differences may be more apparent than real, and to result from the relative small number of methylations done under varying conditions.

(b) Ionic Species. In so far as the introduction of a positive or negative charge may alter the electron distribution in the neutral molecule, so may the prevailing ionic species influence the course of methylation. Thus it will be remembered that the $N_{(1)}^-$ and $N_{(3)}^-$ -methylations of 2-amino-4-hydroxypteridine by Curran and Angier (1961), and the $N_{(1)}$ -methylation of 4-amino-2-hydroxypteridine described in this work, were carried out under alkaline conditions that would ensure a predominance of the anionic species. Using the conditions that give rise to $N_{(8)}^-$ substitution however, mention has already been made of the dimethyl ether formed by the interaction of methyl alcohol and methyl iodide.



Since the ether has no basic properties, the hydriodic acid produced renders the methylation medium strongly acidic*, with the result that the pteridines are partly (if not completely) in their protonated form.

It follows therefore, that the presence of a strongly positive centre at $N_{(1)}$ would not only preclude methylation at this site but would reduce the electron density at the adjacent $N_{(3)}$ position. With such a redistribution of π electrons $N_{(8)}$ may well become the position most susceptible to attack.

*As evidence of this fact it will be remembered that one of the $N_{(8)}$ -methyl derivatives was actually isolated as its dihydriodide.

D. Methylation of Aminopyrimidines

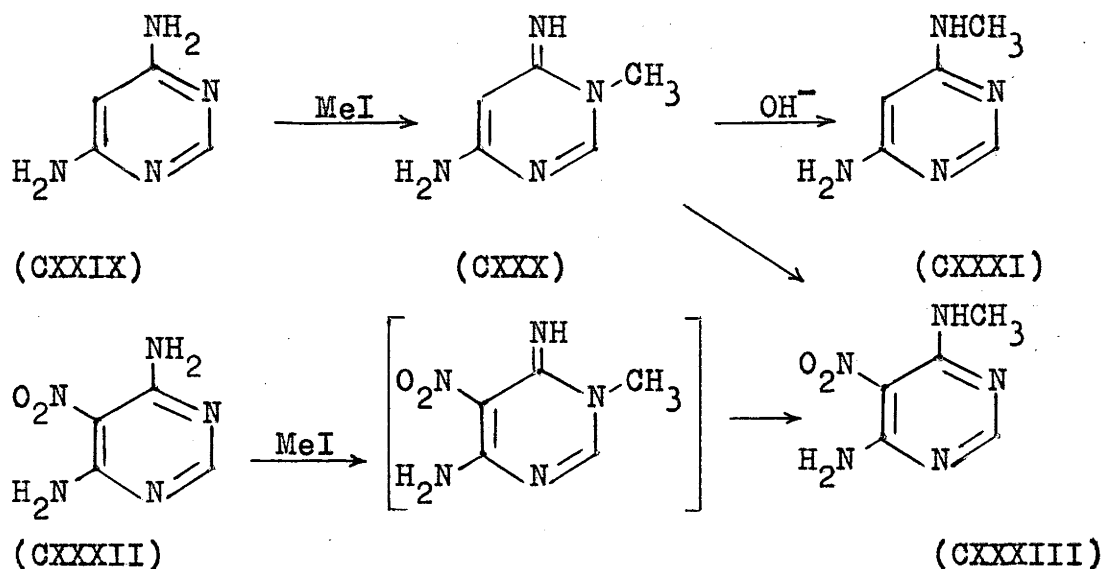
The methylations described in this sub-section were part of a project to extend this aspect of pyrimidine chemistry, but they are in no way intended to be divorced from the preceeding similar study of the pteridines. Thus, in many instances the methylation of a particular pyrimidine also represents an attempt to prepare an intermediate in the synthesis of a methylated pteridine that was unobtainable by direct substitution.

Methylation of 4,6-Diaminopyrimidine

The methylation of 4,6-diaminopyrimidine was investigated in an attempt to provide the necessary pyrimidine precursors for the synthesis of nuclear N-methylated 4-aminopteridines.

4,6-Diaminopyrimidine (CXXIX) was methylated with methyl iodide in methanol under mild conditions similar to those employed in the 4-aminopyrimidine series by Brown, Hoerger and Mason (1955). The mono-methylated product which was isolated both as the hydriodide and hydrochloride salts, had a basic pK_a value (not less than 12.0) far stronger than either 4,6-diaminopyrimidine (pK_a 6.0) or 4-amino-6-methylaminopyrimidine (pK_a 6.3), and comparable to the nuclear N-methylated aminopyrimidines described by Brown and his co-workers.

As 4-amino-6-methylaminopyrimidine was in fact one of the two possible methylation products, there seemed little doubt that the strong base was 4-amino-1,6-dihydro-6-imino-1-methyl-pyrimidine (CXXX). Further confirmation of this structure was obtained from the rearrangement reaction which occurred with facility even at room temperature to yield 4-amino-6-methylaminopyrimidine (CXXXI).



In an attempt to convert the methylated pyrimidine (CXXX) into the precursor of a N-methyl-pteridine, the former was nitrated under the mildest conditions possible. Notwithstanding this precaution, rearrangement accompanied the nitration and the product isolated was 4-amino-6-methylamino-5-nitropyrimidine (CXXXIII).

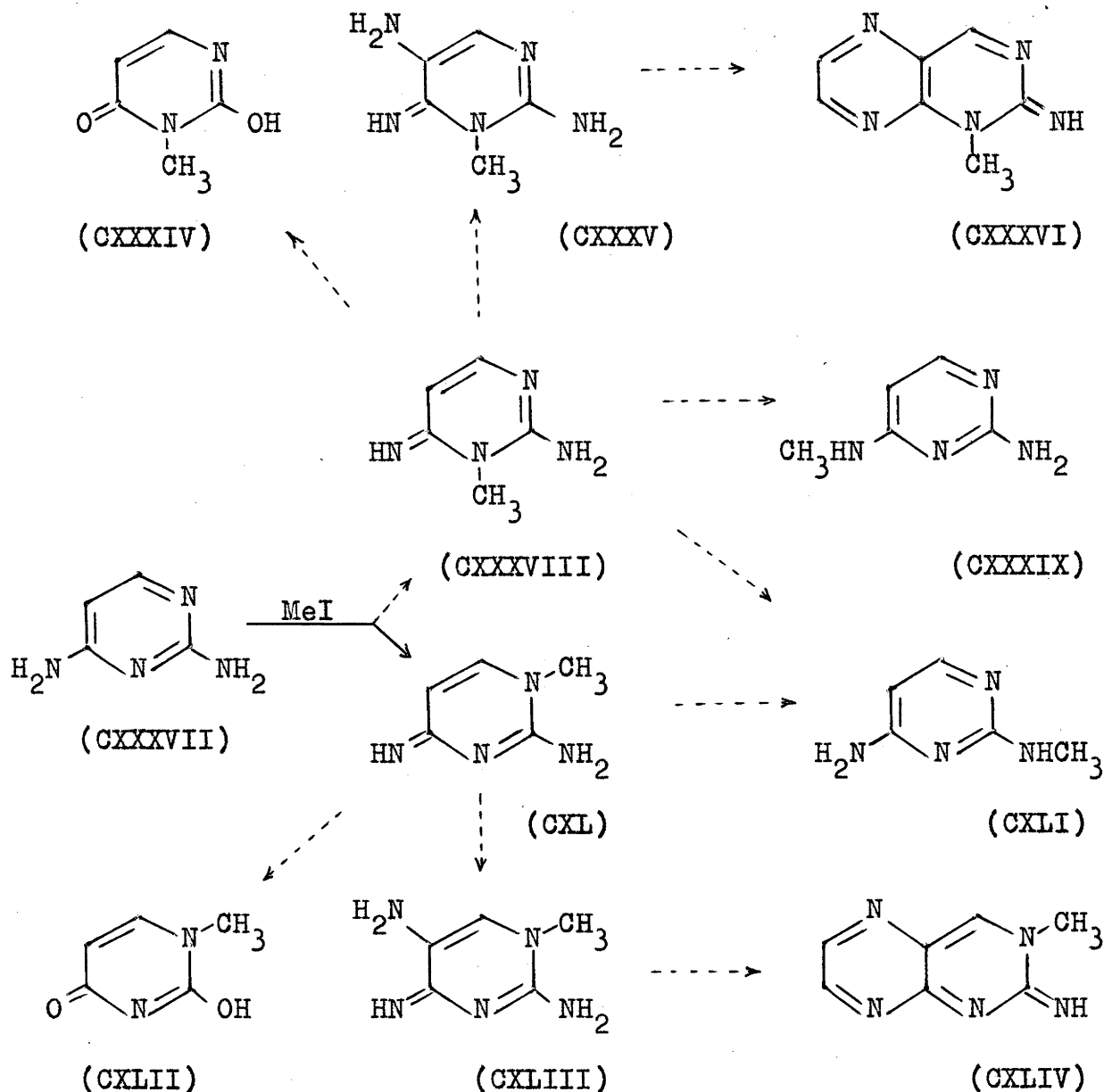
In an attempt to obviate this rearrangement, the nitration stage was carried out prior to methylation.

However, the methylation of 4,6-diamino-5-nitropyrimidine (CXXXII) required more vigorous conditions than did the un-nitrated analogue and the product isolated was again the unwanted rearranged material (CXXXIII). Although in this case there is no proof that methylation did not occur directly on the extranuclear nitrogen atom, it seems more likely that nuclear N-methylation was followed by rearrangement as before.

Methylation of 2,4-Diamino- and 2,4-Diamino-6-mercapto-pyrimidines

Because of the unusual results obtained in the methylation of the 2,4-diaminopteridines, it was of special interest to extend the investigations to the similarly substituted pyrimidines.

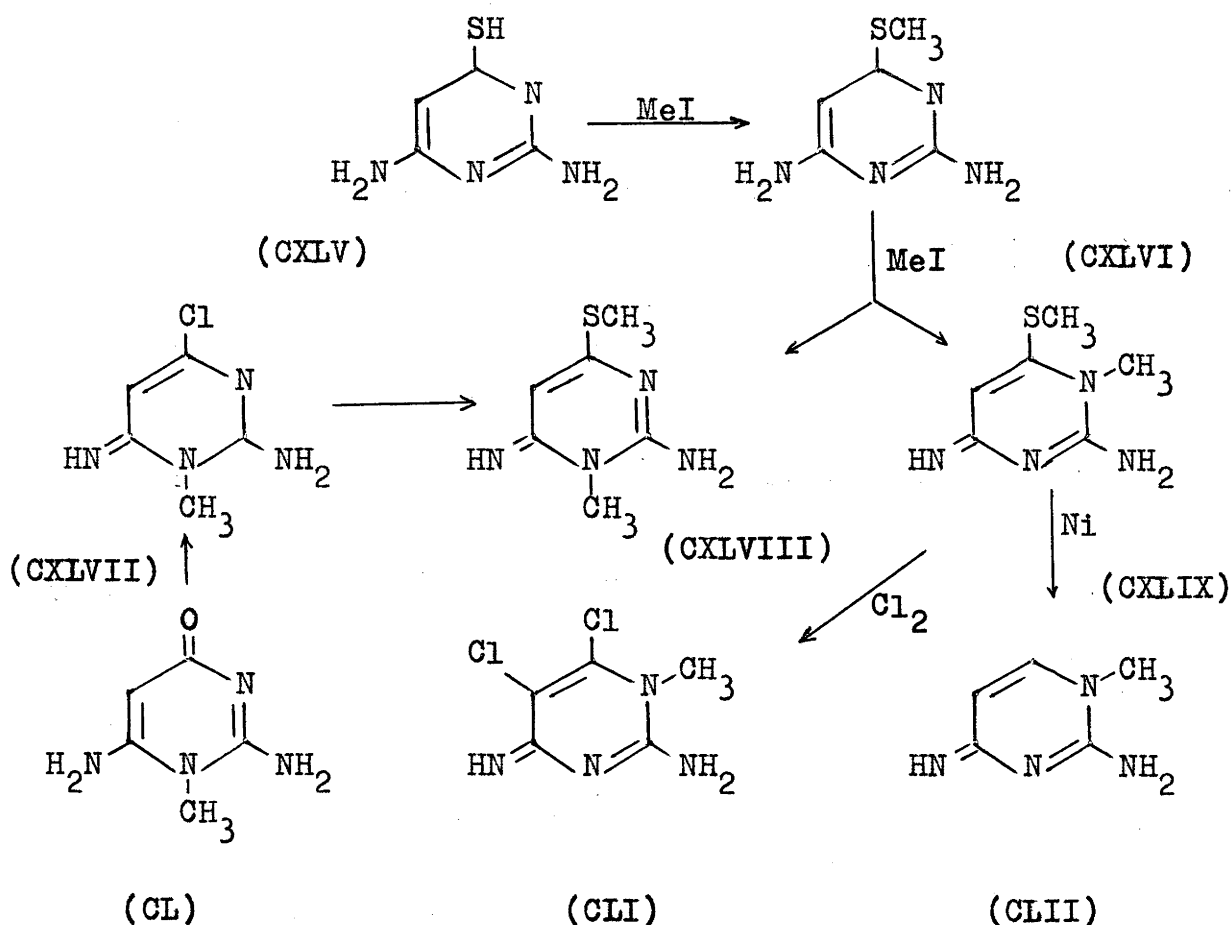
The methylation of 2,4-diaminopyrimidine (CXXXVII) did not require the temperatures above 100° found necessary in the pteridine series, but methanol had again to be used in order to increase the solubility of the starting material. Chromatography revealed that only one product was obtained, and this on isolation, proved to be another strong base (pK_a not less than 12.5). This fact and analogy with previous examples, showed that the methylation product was one of the two nuclear N-methyl derivatives (CXXXVIII) and (CXL). Contrary to expectations however, the product would rearrange neither to 2-amino-4-methylaminopyrimidine (CXXXIX) nor to 4-amino-2-methylaminopyrimidine (CXLI).



An attempt to structuralise the methylation product by hydrolysis to one of the known N-methyluracils (CXXXIV and CXLII, Brown, Hoerger and Mason, 1955) did not succeed.

Another attempt was made, this time to convert the

product, via the 4,5-diaminopyrimidine precursor (CXXXV) or (CXLIII) into an N-methyl derivative of 2-aminopteridine (CXXXVI) or (CXLIV) which could with reasonable certainty be degraded into a recognisable amino- or methylamino- pyrazine. However the synthesis proved unsuccessful in that the methylation product could be neither nitrated nor nitrosated. The methylation product was finally shown to be 2(4)-amino-1,4(1,2)-dihydro-4(2)-imino-1-methylpyrimidine (CXL), by its unambiguous synthesis in the following manner.



2,4-Diamino-6-mercaptopyrimidine (CXLV) had previously been prepared by Elion, Lange and Hitchings (1956), but the conditions used were too severe and made the isolation of the product unsatisfactory. Using optimum conditions determined by chromatography, the mercaptopyrimidine was obtained without difficulty and converted then into the methylthio derivative (CXLVI) by extranuclear S-methylation with methyl iodide.

Methylation of 2,4-diamino-6-methylthiopyrimidine (CXLVI) was carried out using the same conditions as for the 2,4-diamino-pyrimidine, but in this instance, two products were obtained. Analysis revealed that these were both mono-methyl derivatives. Once more the basic strengths of the products were diagnostic of the site of methylation, for whereas 2,4-diamino-6-methylthiopyrimidine had a basic pK_a value 5.6, the constants for the isomeric products were 12.0 and 11.16 respectively. On this evidence, the latter were regarded as the 1-methyl-(CXLVIII) and the 3-methyl-(CXLIX) derivatives. To distinguish between these isomers, one of them, 2(4)-amino-3,4(2,3)-dihydro-4(2)-imino-3-methyl-6-methylthiopyrimidine (CXLVIII) was synthesised by the unambiguous route illustrated. 2,4-Diamino-3,6-dihydro-3-methyl-6-oxopyrimidine (CL; Roth, Smith and Hultquist, 1951: corrections by Boon and Bratt, 1957) was chlorinated with phosphoryl chloride to yield 2(6)-amino-

4-chloro-1,6(1,2)-dihydro-6(2)-imino-1-methylpyrimidine (CXLVII). Reaction of this compound with sodium methyl mercaptide furnished the methylthio- derivative (CXLVIII). The remaining isomer (CXLIX) was therefore, 2(4)-amino-1,4(1,2)-dihydro-4(2)-imino-1-methyl-6-methylthiopyrimidine. Several attempts were made to confirm the structure of this isomer independently by hydrolysis, but without success. In one of these, an attempt to prepare the methylsulphonyl derivative by chlorine oxidation of the methylthio-group (Noell and Robins, 1959; Robins, 1961) gave the unstable 2(4)-amino-5,6-dichloro-1,4(1,2)-dihydro-4(2)-imino-1-methylpyrimidine (CLI). The possibility that the chlorination product was one of the isomeric chloramines or dichloramines, was precluded because it did not possess the oxidizing properties towards hydriodic acid that are so characteristic of such compounds (Taylor and Baker, 1945).

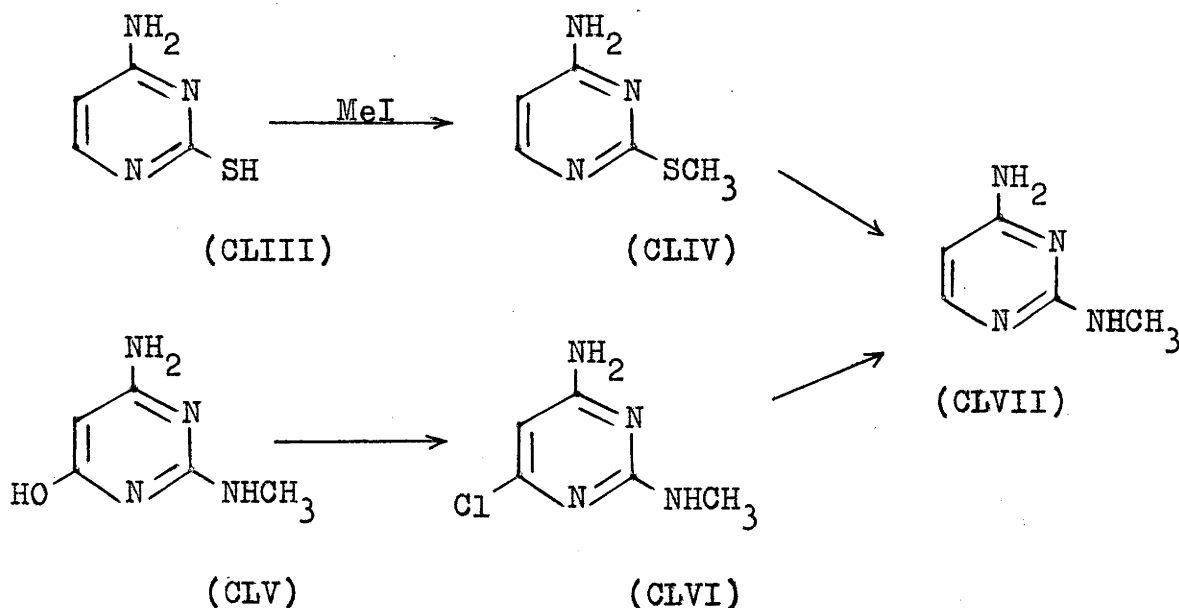
To establish the structure of the methylation product of 2,4-diaminopyrimidine, it remained only to desulphurise one of the methylated methylthiopyrimidines (CXLVIII) or (CXLIX). This was achieved with Raney nickel on the second isomer which gave 2(4)-amino-1,4(1,2)-dihydro-4(2)-imino-1-methylpyrimidine (CLII). Compared as its hydrochloride, the desulphurised product was

identical with the same salt of the methylation product and N₍₁₎ was thus established as the site of substitution.

Methylation of 4-Amino-2-methylaminopyrimidine

This was an extension of the previous methylation study and was designed to discover if the site of methylation would be influenced by a small variation in the adjacent substituent group.

4-Amino-2-methylaminopyrimidine (CLVII) which had not been previously described, was prepared by two different routes. The first of these started with 4-amino-2-mercaptopyrimidine (CLIII) and proceeded via the methylthio derivative (CLIV) which on methylation gave the desired material (CLVII).



The other approach was made from 4-amino-6-hydroxy-2-methylaminopyrimidine (CLV) by chlorination and subsequent catalytic dehalogenation of the derivative (CLVI) using the method of Fidler and Wood (1957).

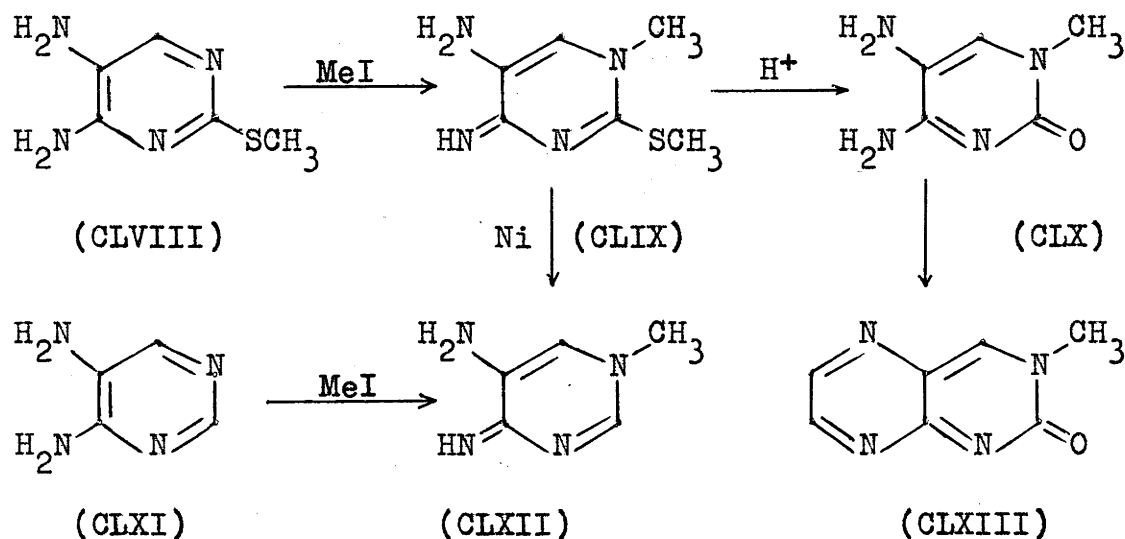
Using methylating conditions similar to those for previous compounds in the series, 4-amino-2-methylaminopyrimidine (CLVII) also afforded a single mono-methyl derivative of high basic strength (pK_a not less than 13). When allowance is made for the small bathochromic displacements induced by an additional methyl group, its ultra-violet spectra are very similar to those of the 1-methyl derivative of 2,4-diaminopyrimidine. It therefore seems probable that substitution on $N_{(1)}$ had again taken place, although this assumption has not yet been independently confirmed.

Methylation of 4,5-Diaminopyrimidine

The mono-methyl derivative isolated from the methylation of 4,5-diaminopyrimidine (CLXI) again possessed the exceptionally high basic strength (pK_a 12.5) that had become so indicative of nuclear N-methylation. By analogy with the methylation of 4-aminopyrimidine (Brown, Hoerger and Mason, 1955), substitution was expected on $N_{(1)}$, and this was negatively supported by unsuccessful attempts to induce a rearrangement. In seeking more positive evidence of structure, degradative methods were of little use, since

neither were the possible breakdown products known, nor could they be readily synthesized. An attempt to condense the methylation product with glyoxal to yield one of the hitherto (Albert, Brown and Wood, 1956) inaccessible methyl-pteridinium salts also failed.

An acceptable solution to the problem was however found by synthesis of the 1-methyl derivative starting from 4,5-diamino-2-methylthiopyrimidine. The following reaction scheme was used.



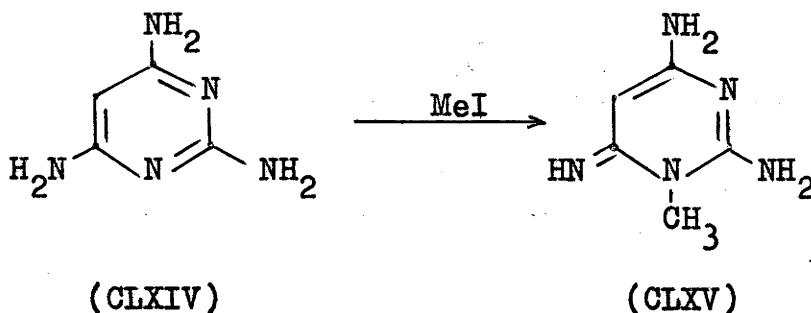
4,5-Diamino-2-methylthiopyrimidine (CLVIII) was methylated without varying the conditions previously used. Analysis of the product confirmed it as a mono-methyl derivative, while its high basic pK_a value (not less than 12.6) left no doubt that substitution had occurred on one

of the nuclear nitrogen atoms. That this site was N₍₁₎ and the product 5-amino-1,4-dihydro-4-imino-1-methyl-2-methylthiopyrimidine (CLIX), was established by its hydrolysis to the known 4,5-diamino-1-methyl-2-pyrimidone (CLX; Brown, 1955). For confirmation, the pyrimidone was converted into 3-methyl-2-pteridone (CLXIII; Albert, Brown and Wood, 1956).

Reductive desulphurisation of the methylthiopyrimidine (CLIX) furnished 5-amino-1,4-dihydro-4-imino-1-methylpyrimidine (CLXII) and this was shown to be identical with the methylation product of 4,5-diamino-pyrimidine.

Methylation of 2,4,6-Triaminopyrimidine

This methylation represented an attempt to prepare an intermediate in the synthesis of the N₍₁₎- and N₍₃₎-methyl derivatives of 2,4-diaminopteridine. The product obtained after refluxing 2,4,6-triaminopyrimidine (CLXIV) in methyl iodide and methanol was a mono-methyl derivative with a basic strength greater than pK_a 12. From a consideration of this evidence and the symmetry of the molecule, the methylation product could be none other than 2,4-diamino-1,6-dihydro-6-imino-1-methylpyrimidine (CLXV) (or a tautomer).



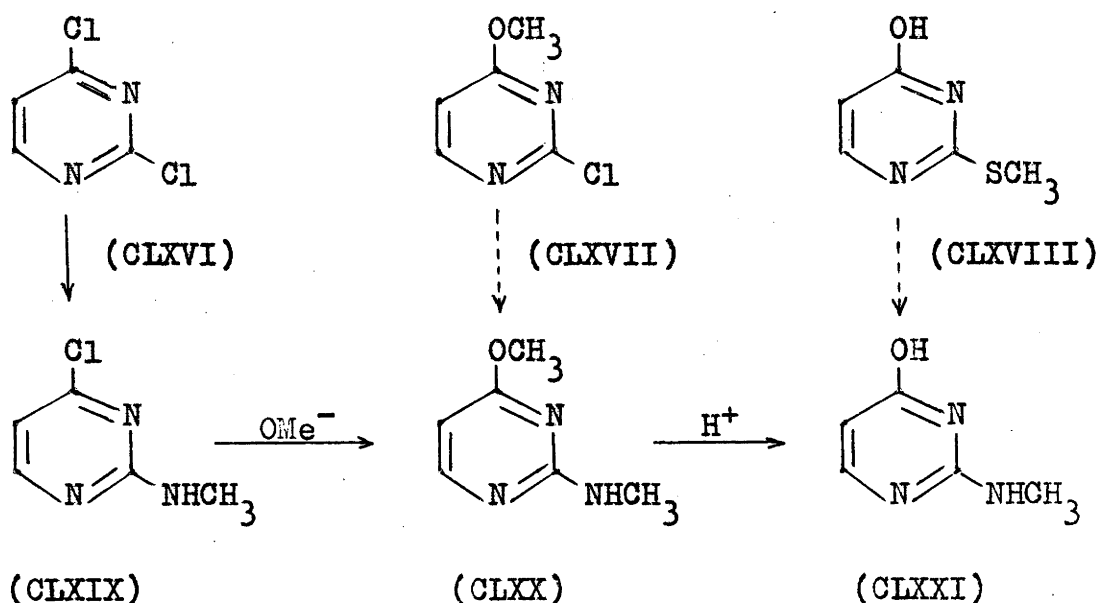
Despite the success of this methylation, the pyrimidine (CLXV) could not be used as a pteridine intermediate because attempts to substitute it (by nitration or nitrosation) in position 5 did not meet with success.

Methylation of 2-Amino-4-hydroxypyrimidine

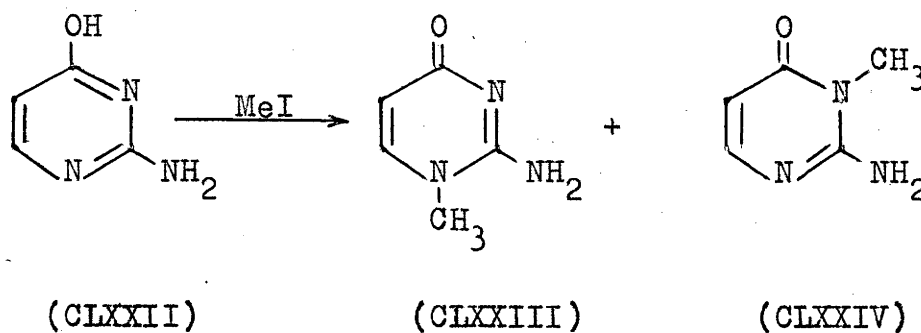
2-Amino-4-hydroxypteridine (isocytosine, CLXXII), on treatment with methyl iodide in anhydrous methanolic sodium methoxide, gave two isomeric products which were separated only with considerable difficulty.

Of the four possible mono-methyl derivatives, only 2-amino-4-methoxypyrimidine had been described previously (Hilbert and Johnson, 1930), and this did not resemble either of the isomers. The synthesis of the remaining extranuclear N-methylation product, 4-hydroxy-2-methylaminopyrimidine (CLXXI), proved to be much more difficult than was anticipated. Repeated attempts to methylamine 4-hydroxy-2-methylthiopyrimidine (CLXVIII) failed to yield the required product, although desulphurisation did occur.

Likewise the use of 2-chloro-4-methoxypyrimidine (CLXVII) in the same type of reaction was unsuccessful. Finally, 4-chloro-2-methylaminopyrimidine (CLXIX) was prepared as the intermediate, by mono-methylation of 2,4-dichloropyrimidine (CLXVI). After separation from the 4-methylamino isomer, it was converted into the 4-methoxy derivative (CLXX) and thence, by acid hydrolysis, to the required 4-hydroxy-2-methylaminopyrimidine (CLXXI).



Examination of this derivative (CLXXI) showed that it did not correspond to either of the isomeric methylation products of isocytosine. It was therefore clear that these were the $\text{N}_{(1)}$ - and $\text{N}_{(3)}$ -methyl derivatives (CLXXIII) and (CLXXIV).

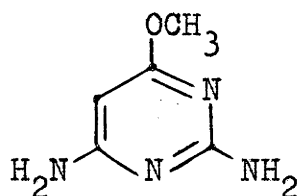


Before the structure of the products could be unequivocally confirmed however, Angier and Curran (1961) described the methylation of isocytosine with dimethyl sulphate and established the two products as the 1-methyl and 3-methyl derivatives. Their products were identical in melting point, chromatography and ultraviolet spectra with the isomers obtained by methylation with methyl iodide.

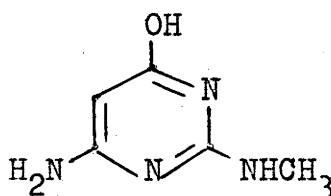
Methylation of 2,4-Diamino-6-hydroxypyrimidine

2,4-Diamino-6-hydroxypyrimidine was methylated in an endeavour to provide an intermediate in the synthesis of either the N₍₁₎- or N₍₃₎-methyl derivatives of the 2-amino-4-hydroxypteridines. Methyl iodide in methanolic sodium methoxide was used and gave a mono-methyl derivative. Characterisation of the product was rendered easier in this instance because three of the possible products, 2,4-diamino-6-methoxy-(CLXXV), 4-amino-6-hydroxy-2-methylamino-(CLXXVI), and 2,4-diamino-3,6-dihydro-3-methyl-6-oxo-pyrimidine

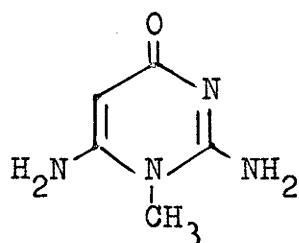
(CLXXVII) had been prepared by Roth, Smith and Hultquist (1951), and none of these resembled the methylation product. The compound was therefore tentatively assigned the structure of the remaining N-methylated isomer, 2,4-diamino-1,6-dihydro-1-methyl-6-oxypyrimidine (CLXXVIII).



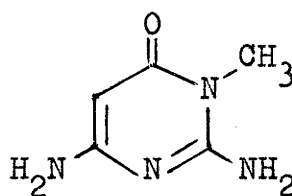
(CLXXV)



(CLXXVI)



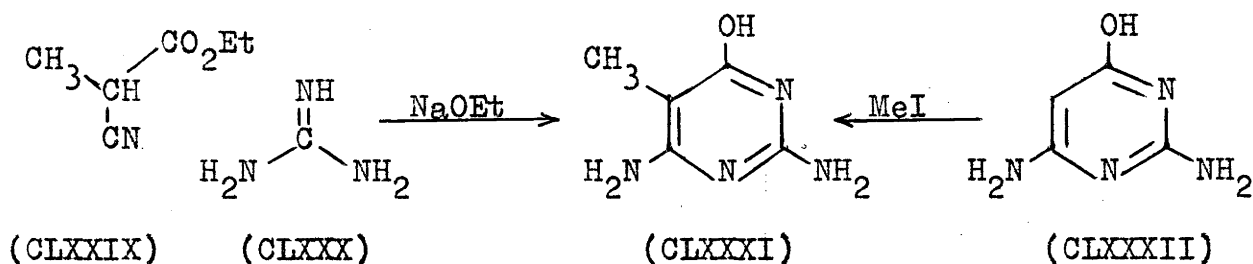
(CLXXVII)



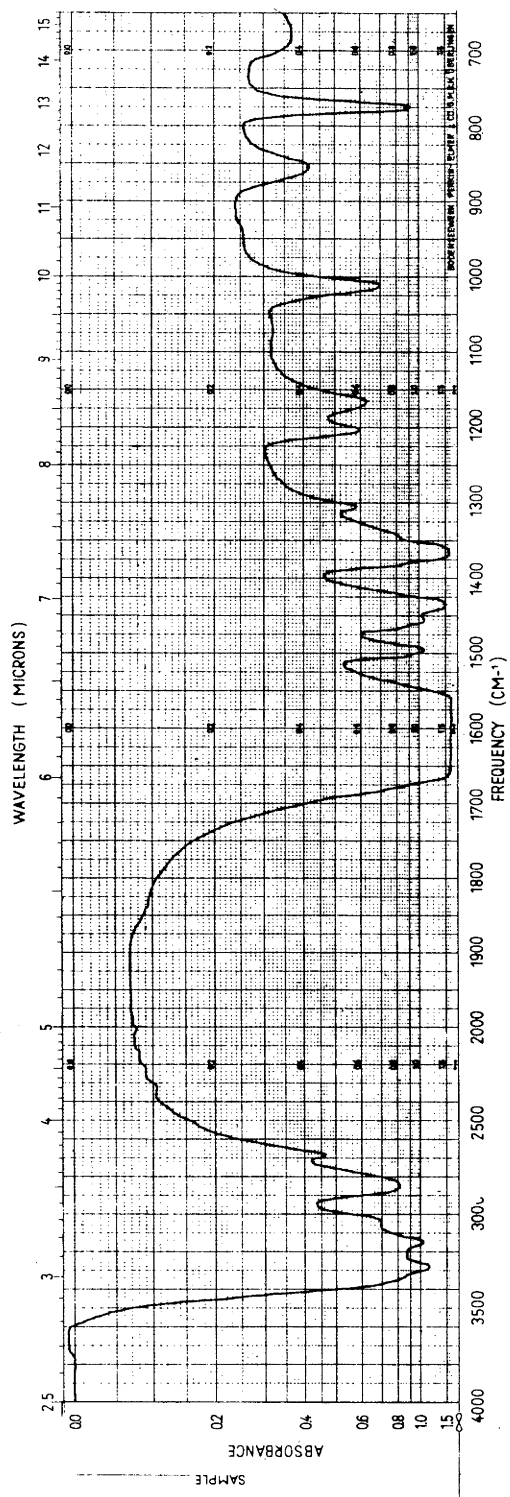
(CLXXVIII)

However, this structure was not consistent with the subsequently observed failure of the pyrimidine to nitrosate or nitrate, especially as its isomer (CLXXVII) was readily substituted in position 5. Moreover, examination of the methylation product showed it to have ionisation constants (acidic pK_a 11.07; basic pK_a 3.61) differing very little from those of 2,4-diamino-6-hydroxypyrimidine (CLXXXII;

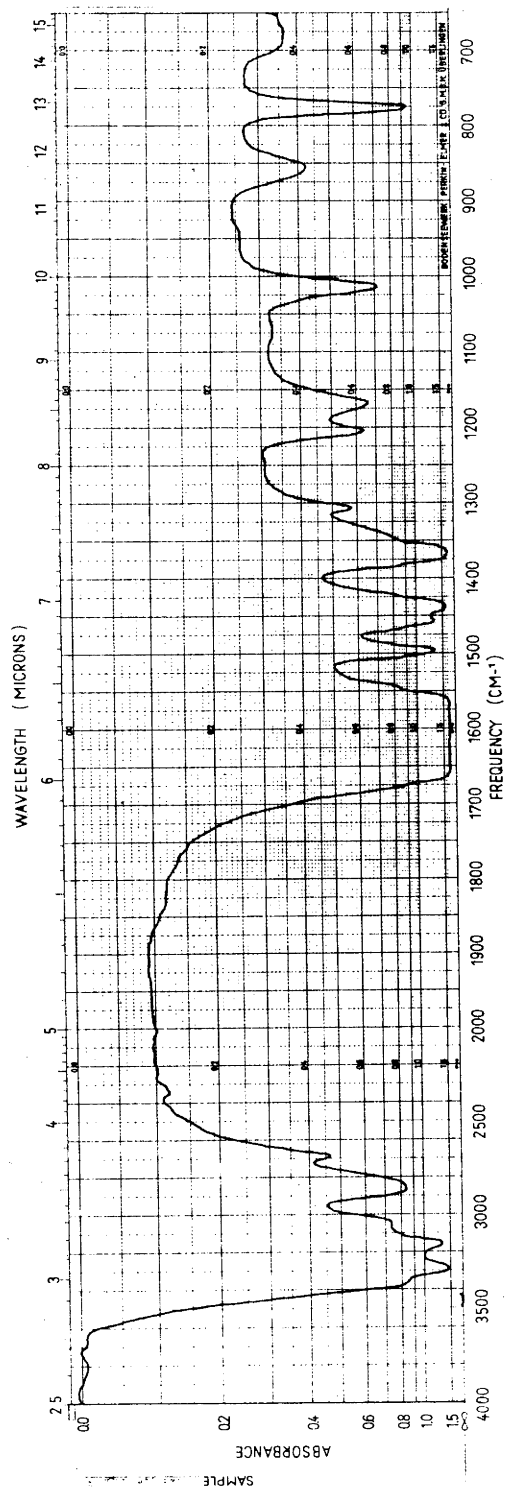
acidic pK_a 10.78; basic pK_a 3.33). This all suggested the possibility that substitution may have occurred on C₍₅₎ and the methylation product was therefore compared with an authentic specimen of 2,4-diamino-6-hydroxy-5-methylpyrimidine (CLXXXI) made by condensation of ethyl 2-cyanopropionate (CLXXIX) with guanidine (CLXXX). They were found to be identical by the usual criteria, and as C-methylation is so infrequent in the pyrimidine series, the identity of the two compounds was reconfirmed by comparison of their infrared spectral traces (see page 70).



In view of these facts, 2,4-diamino-1,6-dihydro-1-methyl-6-oxopyrimidine (CLXXVIII) remains an unknown isomer, despite the mention of it twice in the literature (Roth, Smith and Hultquist, 1951; Yamada, Chibata and Kiguchi, 1958). The compound described in both of these references being, in fact, the 3-methyl isomer (CLXXVII). It is of considerable interest to note that the 1-methyl derivative (CLXXVIII) could theoretically be obtained from



2,4-Diamino-6-hydroxy-5-methylpyrimidine (by methylation)



2,4-Diamino-6-hydroxy-5-methylpyrimidine (by synthesis)

2(4)-amino-1,4(1,2)-dihydro-4(2)-imino-1-methyl-6-methylthiopyrimidine (CXLIX) described earlier in this thesis, but preliminary attempts at hydrolysis did not meet with success.

SUMMARY

2,4-Diamino-, 4,5-diamino-, 4,6-diamino, 4,5-diamino-2-methylthio- and 2,4,6-triamino-pyrimidine were all methylated by methyl iodide on N₍₁₎.

2-Amino-4-hydroxypyrimidine (isocytosine) and 2,4-diamino-6-methylthiopyrimidine gave both N₍₁₎- and N₍₃₎-methyl derivatives.

The methylation product of 2,4-diamino-6-hydroxypyrimidine has been confirmed as 2,4-diamino-6-hydroxy-5-methylpyrimidine, and is an almost unique example of C-methylation in the series.

Factors Influencing the Position of Methylation

In pyrimidine and its symmetrical derivatives, the nuclear nitrogen atoms are equivalent to each other, and for this reason mono-methylation can only lead to one nuclear N-methyl derivative. In the case of unsymmetrical pyrimidines, the relative susceptibilities of the nuclear nitrogen atoms towards methylation may be influenced by the same factors discussed in the pteridine series.

SECTION 3

ANALYSIS OF PHYSICO-CHEMICAL MEASUREMENTS

A. Table of Ionisation Constants and Spectra

Compound	pK_a^a and Concn.	λ max (m μ .) ^b	pH	log ϵ
<u>Pteridine derivatives</u>				
4-Amino ^c		335; 244		3.82; 4.20
cation	3.56	324; 229		3.99; 4.10
4-Amino-6,7-dimethyl		330; 245	6.2	3.88; 4.25
cation	3.80 $\pm$ 0.02 (M/400)	337; 326; 233	1.7	3.96; 4.05; 4.18
1,4-Dihydro-4-imino-1-methyl ^e			12.5 ^h	
cation	9.51 $\pm$ 0.05 (M/200)	<u>350</u> ; 333; 233	7.4	<u>3.87</u> ; 4.00; 4.14
1,4-Dihydro-4-imino-1,6,7-tri- methyl ^e			12.5 ^h	
cation	10.47 $\pm$ 0.06 (M/200)	<u>344</u> ; 330; 237	8.5	<u>3.98</u> ; 4.06; 4.18
1,4-Dihydro-1-methyl-4-methyl- imino ^e			12.5 ^h	
cation	10.34 $\pm$ 0.04 (M/100)	<u>354</u> ; 344; 233	8.3	<u>4.03</u> ; 4.07; 4.16
1,4-Dihydro-1,6,7-trimethyl- 4-oxo		<u>340</u> ; 325; <u>243</u> ; 234	5.0	<u>3.92</u> ; 4.00; <u>4.06</u> ; 4.17
cation	1.73 $\pm$ 0.04 ⁱ	<u>322</u> ; 308	-1.0	<u>3.87</u> ; 4.04
1,4-Dihydro-1,6,7-trimethyl-4- methylimino ^e			13.1 ^h	
cation	11.43 $\pm$ 0.05 (M/100)	354; 341; <u>255</u> ; 237	8.5	4.06; 4.10; <u>3.87</u> ; 4.15
4,8-Dihydro-6,7,8-trimethyl-4- methylimino		383; 284; 241; 215	8.5	3.68; 4.23; 4.06; 4.27
cation	6.64 $\pm$ 0.05 (M/600)	410; <u>295</u> ; 268; 233	4.0	4.03; <u>4.00</u> ; 4.06; 4.09
4-Dimethylamino ^j		362; 241		3.93; 4.15
cation	4.33	344 + 347 + 356; 239		4.09 + 4.10 + 4.04; 4.19
4-Dimethylamino-6,7-dimethyl		355; 244	6.8	3.99; 4.20
cation	4.84 $\pm$ 0.03 (M/400)	<u>353</u> ; 342; 244	2.8	<u>4.12</u> ; 4.16; 4.19
4-Dimethylamino-1-methyl- pteridinium iodide ^g		352; 243		4.11; 4.17
4-Dimethylamino-1,6,7-trimethyl- pteridinium iodide ^g		347; 246		4.19; 4.23
6,7-Dimethyl-4-methylamino		346; 249	6.2	3.97; 4.15
cation	4.17 $\pm$ 0.03 (M/400)	<u>348</u> ; 337; 234	1.7	<u>4.10</u> ; 4.14; 4.19
4-Methylamino		352; 248; 226	5.7	3.88; 4.06; 4.05
cation	3.70 $\pm$ 0.03 (M/200)	<u>352</u> ; 339; <u>251</u> ; 232	1.7	<u>3.99</u> ; 4.06; <u>3.68</u> ; 4.12
4-Amino-2,8-dihydro-2-imino- 8-methyl ^f		<u>365</u> ; 313; 288; 241	11.0	<u>3.23</u> ; 3.92; 3.93; 4.11
cation	8.88 $\pm$ 0.04 ⁱ	412; 330; 270; <u>244</u>	6.0	3.98; 3.52; 4.24; <u>3.73</u>

Pteridine derivatives continued

4-Amino-1,2-dihydro-2-imino-					
1,6,7-trimethyl		368; 262	14.16	3.97; 4.15	
cation	11.90 ± 0.05^i	<u>347</u> ; 335; 282; <u>246</u>	7.5	<u>4.01</u> ; 4.07; 3.64; <u>4.25</u>	
2,4-Diamino		364; 255; 224	8.0	3.86; 4.32; 4.07	
cation	5.32^j	<u>345</u> ; 332; <u>318</u> ; 284; 240	3.0	<u>3.90</u> ; 3.98; <u>3.91</u> ; 3.73; 4.10	
2-Amino-1,4-dihydro-1-methyl-					
4-oxo ⁿ		<u>342</u> ; 327; <u>280</u> ; 239	5.0	<u>3.81</u> ; 3.93; <u>3.62</u> ; 4.21	
cation	2.83 ± 0.03 (M/200)	314; <u>335</u> ; <u>250</u> ; 227	0.13	3.90; <u>3.66</u> ; <u>3.95</u> ; 4.12	
anion	11.5^d	360; 258	14.0	3.90; 4.13	
2-Amino-3,4-dihydro-3-methyl-					
4-oxo ⁿ		355; 274; 240	5.0	3.80; 4.05; 4.18	
cation	2.25 ± 0.03^i	318; 230	0.13	3.88; 4.17	
2-Amino-4,8-dihydro-8-methyl-					
4-oxo		400; 320; 265	8.5	3.98; 3.47; 4.28	
cation	5.42 ± 0.02 (M/200)	389; <u>283</u> ; 261	3.0	4.01; <u>3.99</u> ; 4.14	
anion	11.5^d	314; 284; 239	14.0	3.91; 3.89; 4.26	
2-Amino-4,8-dihydro-6,7,8-tri-					
methyl-4-oxo		407; 315; 267	8.5	4.11; 3.43; 4.35	
cation	6.10 ± 0.02 (M/200)	397; 285; 254	3.0	4.12; 4.13; 4.09	
anion	11.97 ± 0.02^i	370; 308; <u>301</u> ; 228 ^m	14.0	3.88; 4.20; <u>4.18</u> ; 4.33	
4-Amino-1,2-dihydro-1-methyl-2-oxo		345; 288; 245	5.0	3.88; 3.56; 4.13	
cation	2.96 ± 0.02 (M/400)	<u>360</u> ; 341; 238	0.13	<u>3.68</u> ; 3.87; 4.08	
anion	12^d	350; 245	14.0	3.79; 4.04	
4-Amino-1,2-dihydro-1,6,7-tri-					
methyl-2-oxo		<u>360</u> ; 345; 282; 248; 216	7.1	<u>3.93</u> ; 4.04; 3.53; 4.24; 4.18	
cation	3.66 ± 0.03^i	360; 343; <u>264</u> ; 218	0.3	3.91; 4.02; <u>3.82</u> ; 4.29	
2-Amino-4-hydroxy ^j		340; 270; 230	5.25	3.76; 4.05; 4.04	
cation	2.31	315; 220		3.88; 4.1	
anion	7.92	358; 251		3.83; 4.31	
4-Amino-2-hydroxy		<u>350</u> ; 337; <u>286</u> ; 240	7.0	<u>3.87</u> ; 3.94; <u>3.63</u> ; 4.08	
cation	2.99 ± 0.02^i	335; 237	0.83	3.90; 4.08	
anion	9.97^j	375; 255	12.0	3.85; 4.31	
4-Amino-2-hydroxy-6,7-dimethyl		<u>350</u> ; 340; 276; 245	6.5	<u>3.96</u> ; 4.01; 3.48; 4.07	
cation	3.49 ± 0.03^i	<u>350</u> ; 338; <u>256</u> ; 215	1.3	<u>3.97</u> ; 4.03; <u>3.85</u> ; 4.32	
anion	10.69 ± 0.04	370; <u>274</u> ; 254	13.8	3.88; <u>3.78</u> ; 4.29	
2-Amino-4-methoxy ⁿ		274; 239	5.5	3.81; 3.84	
cation	3.46 ± 0.02 (M/200)	285; 237	1.0	3.99; 3.80	

Pteridine derivatives continued

4-Hydroxy-2-methylamino ⁿ		348; 275	5.5	3.75; 4.16
cation	1.98 ± 0.03 (M/200)	317; 236	-0.07	3.87; 4.16
anion	8.16 ± 0.02 (M/400)	365; 260	10.5	3.84; 4.32
Lumazine, dianion ^c		365; 252	13	3.78; 4.23
1-Methyl-lumazine, anion ^k	8.57 ± 0.02 (M/200)	339; 281; 242	10.5	3.89; 3.54; 4.19
3-Methyl-lumazine, anion ^k	8.0	362; 268; 244; 215	10.0	3.81; 3.98; 4.25; 4.02
1,6,7-Trimethyl-lumazine		334; 252; <u>225</u>	7.0	3.98; 3.96; <u>4.07</u>
anion	9.06 ± 0.04 (M/300)	341; <u>280</u> ; 244	11.5	4.03; <u>3.38</u> ; 4.25
6,7,8-Trimethyl-lumazine ^l		404; 276; 256	7.0	4.08; 4.04; 4.16
anion	9.83 ± 0.04 (M/400)	363; 313; <u>304</u> ; 243	12.0	3.78; 4.31; <u>4.28</u> ; 4.19

Pyrazine derivatives

2-Amidino-3-methylamino		361; 256	11.0	3.76; 4.13
cation	8.98 ± 0.06 (M/200)	359; 264	7.0	3.69; 3.93
2-Amidino-5,6-dimethyl-3-methylamino		370; 261	11.5	3.85; 4.11
cation	9.45 ± 0.03 (M/200)	370; 272	7.4	3.77; 3.92
2-(4,6-Dimethylpyrimidin-2-yl)-3-methylamino		375; 263; 223	5.7	3.85; 4.00; 4.01
cation	2.93 ± 0.03 (M/100)	417; 259; 223	0.9	3.85; 4.03; 4.07
2-Carbamoyl-3-methylamino		371; 256	5.0	3.74; 4.15
cation	2.11 ± 0.04 (M/200)	361; 247	0	3.77; 4.15
2-Carbamoyl-5,6-dimethyl-3-methylamino		377; 263	5.0	3.88; 4.17
cation	2.70 ± 0.02 (M/400)	380; 255	0	3.96; 4.20
2-Amino-3-carboxy-5,6-dimethyl				
proton lost	4.46 ± 0.04 (M/200)	343; 248	7.0	3.87; 4.00
proton gained	$> 1^1$	372; 250	-1.0	4.01; 4.08
2-Carboxy-5,6-dimethyl-3-methylamino				
proton lost	4.82 ± 0.04 (M/200)	365; 259	7.0	3.83; 4.13
proton gained		386; 257	-1.0	3.90; 4.13

Pyrimidine derivatives

2-Amino-4-chloro-1,6-dihydro-6-imino-1-methyl cation	9.90 ± 0.04 (M/100)			
4-Amino-6-chloro-2-methylamino cation	3.81 ± 0.01 (M/200)			
2-Amino-1,4-dihydro-4-imino-1-methyl cation	12.5 ^d (M/20)	270; <u>235</u>	9.0	3.83; <u>4.02</u>
2-Amino-1,4-dihydro-4-imino-1-methyl-6-methylthio cation	12.0 ^d (M/10)	288; 226	9.0	4.18; 4.33
2-Amino-3,4-dihydro-4-imino-3-methyl-6-methylthio cation	11.16 (M/50)	302; <u>254</u> ; 242	9.0	4.25; <u>4.18</u> ; 4.19
4-Amino-1,6-dihydro-6-imino-1-methyl cation	12.0 ^d (M/100)	265; 221	10.0	4.03; 4.52
5-Amino-1,4-dihydro-4-imino-1-methyl cation	12.5 ^d (M/20)	292	9.0	3.98
5-Amino-1,4-dihydro-4-imino-1-methyl-2-methylthio cation	12.6 ^d (M/20)	300; 246	9.0	4.00; 4.39
2-Amino-1,4-dihydro-1-methyl-4-oxo ^o cation		260; 216	1.0	3.90; 3.96
anion		260	13.0	3.73
2-Amino-3,4-dihydro-3-methyl-4-oxo ^o cation		256	1.0	3.82
anion		284; 225	13.0	3.99; 3.86
4-Amino-6-hydroxy-2-methylamino cation	3.22 ± 0.04 (M/200)			
anion	11.03 ± 0.06 (M/100)			
4-Amino-2-methylamino cation	7.55 ± 0.01 (M/200)			
4-Amino-6-methylamino cation	6.32 ± 0.02 (M/200)	260; 221 268; 225	8.2 4.2	3.72; 4.58 4.13; 4.38
4-Amino-6-methylamino-5-nitro cation	2.75 ± 0.01 ^b	348; <u>227</u> 341; 238	5.0 -1.0	3.98; <u>4.17</u> 3.83; 4.36

Pyrimidine derivatives continued

2-Chloro-4-methylamino				
cation	2.83 ± 0.03 (M/100)			
4-Chloro-2-methylamino				
cation	2.63 ± 0.01 (M/50)			
2,4-Diamino-6-chloro				
cation	3.57 ± 0.01 (M/200)			
2,4-Diamino-1,6-dihydro-6-imino-1-methyl				
cation	12.7^d (M/40)			
2,4-Diamino-6-hydroxy				
cation	3.33 ± 0.02 (M/100)			
anion	10.78 ± 0.06 (M/100)			
2,4-Diamino-6-hydroxy-5-methyl				
cation	3.61 ± 0.02 (M/100)			
anion	11.07 ± 0.06 (M/100)			
4,5-Diamino-6-hydroxy-2-methylamino				
cation	5.44 ± 0.02 (M/200)			
anion	10.63 ± 0.05 (M/100)			
4,5-Diamino-6-methylamino		279; 216	8.0	4.02; 4.47
cation	5.93 ± 0.01 (M/100)	286; 222	4.0	4.04; 4.22
2,4-Diamino-6-methylthio				
cation	5.46 ± 0.01 (M/200)			
4,5-Diamino-2-methylthio				
cation	5.05 ± 0.01 (M/200)			
1,4-Dihydro-4-imino-1-methyl-2-methylamino				
cation	13.0^d (M/10)	276; <u>237</u>	9.0	3.81; <u>4.18</u>
4-Hydroxy-2-methylamino				
cation	3.93 ± 0.03 (M/100)			
anion	9.82 ± 0.02 (M/200)			

Notes to Ionisation-Spectra Table

- ^a By potentiometric titration in water at 20° (see Albert and Phillips, 1956)
- ^b Inflexions are underlined
- ^c Albert, Brown and Cheeseman (1951)
- ^d Value is approximate
- ^e Spectra measured on buffered solutions of hydrochloride
- ^f Spectra measured on buffered solutions of hydriodide with a balanced I⁻ concentration in reference cell
- ^g Spectrum in unbuffered water and corrected for I⁻
- ^h Species too unstable for measurement of spectrum
- ⁱ Spectrometrically determined at 0.25×10^{-4} M
- ^j Albert, Brown and Cheeseman (1952)
- ^k Pfleiderer (1957)
- ^l Birch and Moye (1958); values determined on supplied specimens
- ^m The peak at 268 mμ recorded by Fidler and Wood (1957) at pH 13 arises from that of the neutral molecule, present to the extent of 10% in their solution
- ⁿ cf. Physical measurements by Pfleiderer, Liedek, Lohrmann and Rukwied (1960)
- ^o cf. Physical measurements by Angier and Curran (1961).

B. Ionisation Constants

It is no exaggeration to say that the information gained from ionisation constants is indispensable to the success of investigations such as are described in this thesis. While the principal use of the constants was as an aid in establishing structure, much other useful information is also provided. Thus accurately determined pK_a values are useful criteria of identity, especially for those compounds that do not have satisfactory melting points. In addition, a small degree of deviation of the pK_a values derived from the various points on the titration curve, is good evidence that the compound is pure. Further, if the constants are redetermined by back-titration, an indication of the stability of a compound in one or more species can be obtained. Finally, for the correlation of ultraviolet spectra and structure, the acidic and basic constants indicate quantitatively the ionic species present in solution at a particular pH, thereby enabling the spectra of pure species to be measured.

The Effect of Methylation on Ionisation Constants

Any variation in the ionisation constants, brought about by the introduction of a methyl group into a molecule, is naturally of particular interest in a study of methylation. Because methyl groups are electron repelling, the

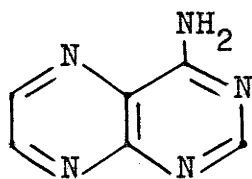
general effect to be expected will be that of base-strengthening and acid-weakening. Reference to the table of ionisation constants shows that this is true. Thus 4-aminopteridine (pK_a 3.6) is made progressively more basic by the introduction of methyl groups either as C- or as extranuclear N-substituents. While the effect from each group is only small (generally less than 0.5 pK_a units) it is accumulative, so that 4-dimethylamino-6,7-dimethylpteridine reaches a basic strength of pK_a 4.8.

In contrast, nuclear N-methylation of an aminopteridine produces an immense increase in the basic strength of from 3 to 6 pK_a units, depending on which of the centres is substituted. Thus the basic pK_a of 1,4-dihydro-4-imino-1-methylpteridine (CLXXVI) is 6 units above that of 4-aminopteridine (CLXXIII). If C- or extranuclear N-methyl groups are added to this iminopteridine, then the usual small increments in basic strength are still observed. In this way 1,4-dihydro-1,6,7-trimethyl-4-methyliminopteridine becomes one of the strongest pteridine bases yet reported (pK_a 11.4).

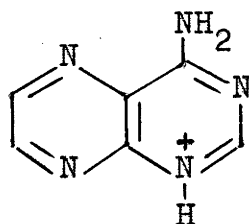
The outstanding basic strengths of the iminopteridines formed by nuclear alkylation calls for some explanation. It is well established that the strength of a base is directly associated with ionic resonance (Albert

and Goldacre, 1944; Albert, Goldacre, and Phillips, 1948; Wheland, 1955). For this reason, if a base attains a greater degree of resonance (and thereby stability) by accepting a proton, there will be a strong tendency for it to do so, and it will be stronger than a base which gains little additional resonance as a cation. It also follows that the larger the extra ionic resonance energy, the greater will be the basic strength of the imine.

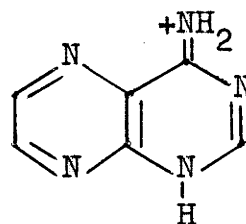
Consider as example, 4-aminopteridine and its imino-analogue 1,4-dihydro-4-imino-1-methylpteridine. The resonance energy of the former is doubtless high even in the neutral molecule (CLXXIII) since it contains two sextets of delocalised π electrons as Kekulé structures. Because of this, the resonance should not be greatly increased in the protonated forms (CLXXIV and CLXXV) and as a result, 4-aminopteridine is only a weak base (pK_a 3.56).



(CLXXIII)



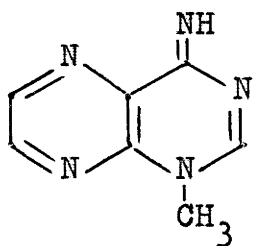
(CLXXIV)



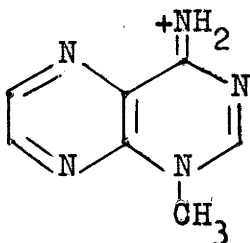
(CLXXV)

The iminopteridine (CLXXVI) however, presents a different set of circumstances. Because its π electrons

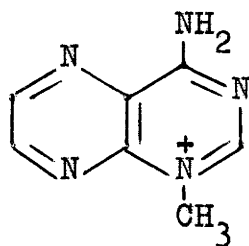
have been localised by nuclear N-methylation, the neutral molecule can have only the structure shown, and its resonance is therefore confined to a separation of positive and negative charges. Protonation however, greatly increases stability in the ion by establishing a resonance hybrid of the para-quinonoid (CLXXVII) and Kekulé (CLXXVIII) structures. The resulting exaltation in resonance is manifested in the observed pK_a of 9.5.



(CLXXVI)



(CLXXVII)

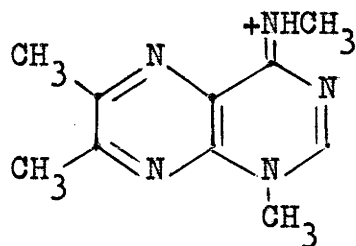


(CLXXVIII)

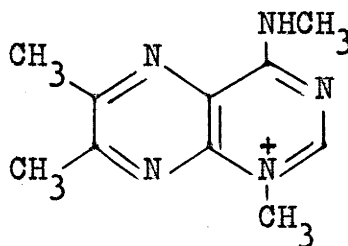
Consideration must now be given to the effect that $N_{(8)}$ -methylation has on the basic strength of aminopteridines. From the table of ionisation constants, it can be seen that while 4,8-dihydro-6,7,8-trimethyl-4-methyliminopteridine (CLXXXI and CLXXXII) is a stronger base than 6,7-dimethyl-4-methylaminopteridine (pK_a 4.2), it is some 3 pK_a units weaker than its $N_{(1)}$ -methyl isomer (CLXXIX and CLXXX)*.

*Exactly the same difference in basic strength is apparent in the 2,4-diaminopteridines series, where the 1,6,7-trimethyl derivative has pK_a 11.9 and the homologous 8-methyl compound has pK_a 8.9.

In seeking to explain the differences in the strength of the $N_{(1)}$ - and $N_{(8)}$ -methyl bases, it is necessary to examine the canonical forms of each resonance hybrid for differences in structure and stability. In the case of $N_{(1)}$ -methyl derivative of 6,7-dimethyl-4-methylaminopteridine, the resonance of the cation is between the highly stable Kekulé (CLXXX) and para-quinonoid (CLXXIX) states.

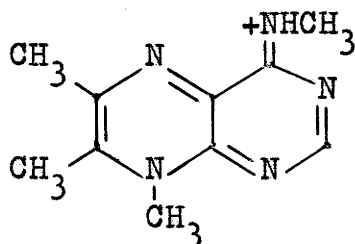


(CLXXIX)

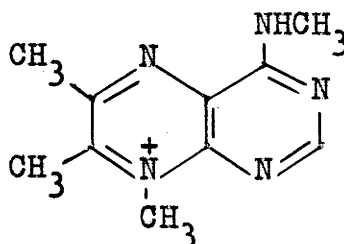


(CLXXX)

For the $N_{(8)}$ -methyl isomer the situation is rather different. The cation may still exist as a stable resonance hybrid of (CLXXXI) and (CLXXXII) thus accounting for its considerable basic strength, but the overall exaltation of resonance resulting from protonation will be less because of the orth-ortho configuration that prevails in one of the canonical forms (CLXXXI) (Gore and Phillips, 1949). Consequently the basicity of the $N_{(8)}$ -methyl derivative is less than that of its $N_{(1)}$ -methyl isomer.



(CLXXXI)

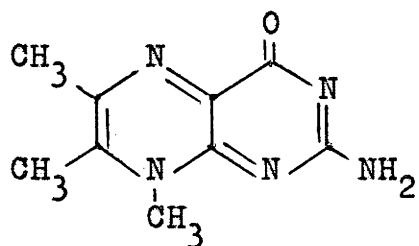


(CLXXXII)

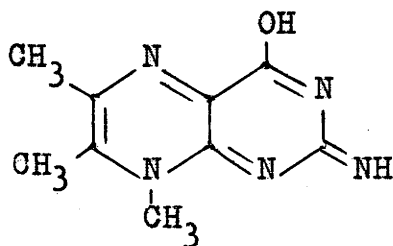
The basic strengths of the methylated amino-hydroxypteridines are quite different. Thus 4-amino-1,2-dihydro-1-methyl-2-oxopteridine has a basic pK_a that, within the limit of experimental error, is identical with that of 4-amino-2-hydroxypteridine (pK_a 3.0). Similarly in the isomeric series, the basic pK_a (2.3) of 2-amino-4-hydroxypteridine is not very different to the basicity of its following methylation products: O-methyl (3.46); NH-methyl (1.98); $N_{(1)}$ -methyl (2.83); and $N_{(3)}$ -methyl (2.25). These constants indicate that the preferred tautomeric form in these cases involves an amino-form (as in CLXXXIII) rather than an imino-form (as in CLXXXIV).

The exception however, comes with the $N_{(8)}$ -methyl derivative which this time has a much higher basic strength of 5.42. That this is an authentic constant is shown by its relationship to the pK_a value of 5.8 reported by Fidler and Wood (1957) for its 6,7-dimethyl homologue (CLXXXIII). No satisfactory explanation can be offered for this

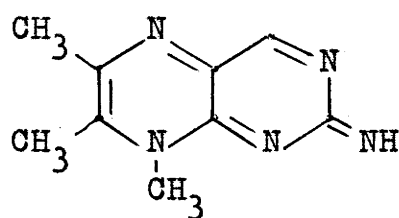
anomalous constant. Because the relatively high basic pK_a approximates to that of 2,8-dihydro-2-imino-6,7,8-trimethylpteridine (pK_a 5.6; CLXXXV), Fidler and Wood have postulated the hydroxy-imino structure (CLXXXIV) for the compound, and in support of this they record an acidic pK_a of 8.9.



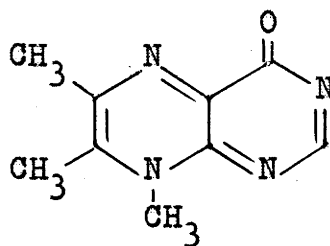
(CLXXXIII)



(CLXXXIV)



(CLXXXV)



(CLXXXVI)

It is believed however, that their conclusion is incorrect because (i) On repetition, no acidic pK_a could be found in the region indicated. The lower limit of the anionic constant was found to be 11.5, which is more in keeping with the acidic function of the $N_{(1)}$ - and $N_{(3)}$ -methyl derivatives. (ii) The basic strength of the 6,7,8-trimethyl compound (CLXXXIII) is in line with that of 4,8-dihydro-6,7,8-trimethyl-4-oxopteridine (CLXXXVI; Brown and

Mason, 1956) which can involve no imine but yet has a basic strength of pK_a 4.7.

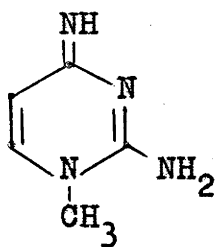
Reference to the table of ionisation constants also reveals the influence that the site of methylation has on the acidic strength of the amino-hydroxypteridines. It will be noticed that the acid-weakening effect of methylation is smallest when substitution occurs on a nuclear carbon atom or extranuclear nitrogen atom. In these cases, the increase in the acidic constant (equivalent to a decrease in acidity) is less than 0.5 pK_a units per methyl group.

Nuclear N-methylation on the other hand causes a marked weakening in acidity since, for the reason discussed above, the hydroxy group is thereby forced into the oxo-form (as in CLXXXIII). When the oxygen atom itself is substituted, either by direct methylation or by synthesis, then the acidic properties are completely destroyed, as exemplified in 2-amino-4-methoxypteridine (CXCVIII).

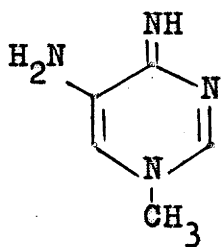
In the pyrimidine series, methylation was found to have the same effect upon the acidic and basic strengths as it had in the pteridine series. Extranuclear N- and C-methylation again caused small increases in basic strength, with the result that 2,4-diamino-6-hydroxy-5-methylpyrimidine, pK_a 3.6 (3.3), 4-amino-2-methylamino-

pyrimidine pK_a 7.55 (7.26, Albert, Goldacre and Phillips, 1948) and 4-amino-6-methylaminopyrimidine pK_a 6.3 (6.01, Brown, 1961) are stronger bases than their unmethylated counterparts (pK_a values for these compounds are shown in parenthesis).

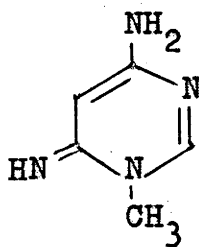
Nuclear N-methylation of aminopyrimidines had already been studied by Brown, Hoerger and Mason (1955) and found to yield imino-derivatives of high basic strength. Their results have been confirmed and extended by the methylation of 2,4-diamino- (7.26, Albert, Goldacre and Phillips, 1948) 4,5-diamino- (6.03, Albert, Brown and Cheeseman, 1952), 4,6-diamino- (6.01, Brown, 1961) and 2,4,6-triamino- (6.84, Albert, Goldacre and Phillips, 1948) pyrimidine to give the strong imine bases (CLXXXVII; pK_a 12.5), (CLXXXVIII; pK_a 12.5), (CLXXXIX; pK_a 12.0), and (CXC; pK_a 12.7) respectively.



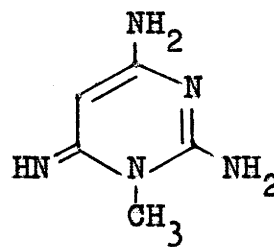
(CLXXXVII)



(CLXXXVIII)



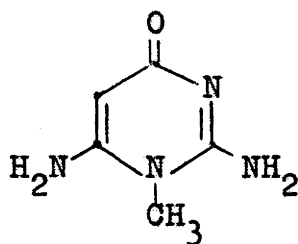
(CLXXXIX)



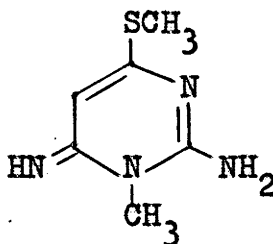
(CXC)

The introduction of a nuclear N-methyl substituent into an amino-hydroxypyrimidine does not produce a strong

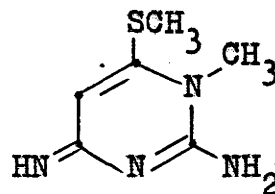
base. As in the pteridine series, it is the oxygen substituent which increases its covalency, and the amino-oxypyrimidines so formed differ little in basic properties from those of the unmethylated pyrimidine. As illustration, 2,4-diamino-3,6-dihydro-3-methyl-4-oxypyrimidine (CXCI) with a basic pK_a 4.22 (Brown, 1961) is less than one pK_a unit above that of 2,4-diamino-6-hydroxypyrimidine (pK_a 3.33). On the other hand, if the second substituent is a group such as $-SCH_3$, $-Cl$, or $-OCH_3$ which cannot increase its covalency in the same way as $-OH$, then methylation on a ring nitrogen again results in a strong base. As illustration of this fact, 2,4-diamino-6-methylthiopyrimidine (pK_a 5.46) gave on methylation the $N_{(1)}$ - and $N_{(3)}$ -methyl derivatives (CXCII and CXCI) which were found to have basic pK_a values of 12.0 and 11.16 respectively.



(CXCI)



(CXCII)



(CXCI)

That the $N_{(3)}$ -methyl isomer (CXCII) is a weaker base than (CXCI) is understandable since the resonance of its cation depends on a contribution from the less stable ortho-quinonoid structures (Albert and Goldacre, 1944).

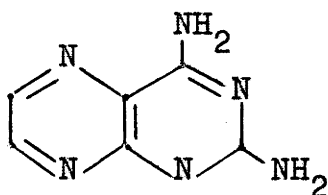
C. Ultraviolet Spectra

The utility of ultraviolet spectra as a criteria of identity has already been indicated throughout Section 2. In this final section, other applications of these physical measurements to the present work will be shown.

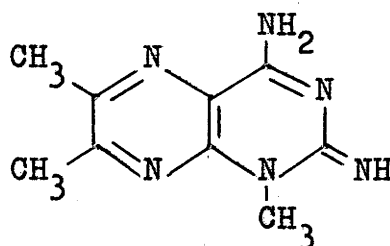
The methyl group is well known as a bathochromic substituent, and when introduced into a molecule it causes a small displacement of the principal features of the ultraviolet spectrum to longer wavelengths (Jones, 1945). This was found to be generally true in the pteridines and pyrimidines studied. By considering the spectra of 4-aminopteridine and its closely similar 6,7-dimethyl derivative, it can be seen that extranuclear N-mono- and dimethylation results in a progressive bathochromic displacement of the long-wavelength band. Spectral features in the lower wavelength region were sometimes less affected. Thus by appreciating the differences likely to be caused by a methyl substituent, it is possible to use the spectrum of the C-methyl derivative when the spectrum of the lower homologue is for some reason unknown.

Nuclear N-methylation of the 4-amino- and 2,4-diamino-pteridines, caused major changes in the ultraviolet spectra, because it produced important structural changes in the heterocyclic molecule. This fact can be observed, for example, by comparing the spectra of 2,4-diaminopteridine

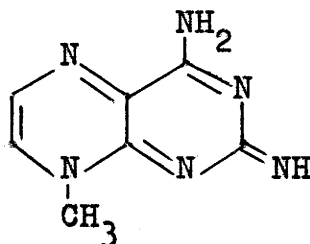
(CXCIV) with the N₍₁₎-methyl (CXCV) and N₍₈₎-methyl (CXCVI) derivatives.



(CXCIV)

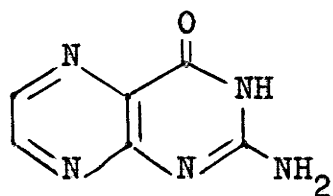


(CXCV)

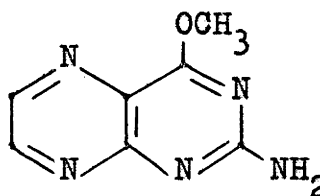


(CXCVI)

In the case of amino-hydroxypteridines, nuclear N-methylation did not cause such pronounced changes in spectra, because these compounds were already in the amide form (as in CXCVII). However O-methylation, in so far as it changed this configuration, did produce major alterations in the spectrum. Comparison of the spectra of 2-amino-4-hydroxy- (CXCVII) and 2-amino-4-methoxy- (CXCVIII) validates this claim.



(CXC VII)

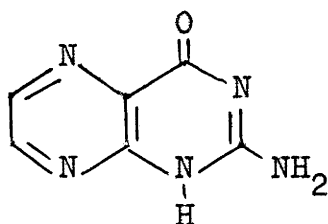


(CXC VIII)

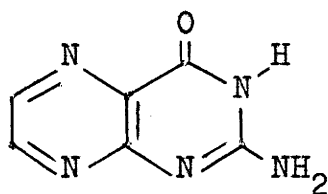
Because it is not unreasonable to expect protonation to occur at the same site as methylation (provided that steric factors do not influence the latter), it is of interest to observe the evidence provided by ultraviolet spectra. If only the major features of the spectra are considered, it can be seen that the cationic spectra of 4-amino-, 4-methylamino-, and 4-dimethylamino-pteridine are similar to those of the corresponding $N_{(1)}$ -methyl derivatives, suggesting that the former group are indeed protonated on $N_{(1)}$. Pfleiderer, Liedek, Lohrmann, and Rukwied (1960) by a similar comparison of cationic spectra, have shown that 2-amino-4-hydroxypteridine (CXC VII) also protonates at this centre.

In view of the importance of 2-amino-4-hydroxypteridines in nature, and their unique $N_{(8)}$ -methylation with methyl iodide, it is of interest to discover their preferred tautomeric form in aqueous solution. Valency permits the pteridine to exist in 9 different forms. Five of these involve an imino configuration and hence can

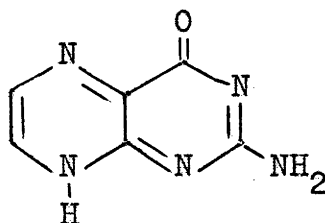
be precluded because of the compound's low basic pK_a (2.3). This has also been confirmed by Pfeleiderer, Liedek, Lohrmann and Rukwied (1960) who have shown the marked similarity in spectra of 2-amino-4-hydroxy- and 2-dimethylamino-4-hydroxy-pteridine, the second of which cannot assume an imino-form. Reference to the table of spectra, reveals that the one amino-hydroxy form can also be eliminated because of the dissimilarity in the spectra of 2-amino-4-hydroxy- and 2-amino-4-methoxy-pteridine. With these eliminations, there remain the three amino-oxo forms (CXCVII, CXCVIII and CXCIX).



(CXCVII)



(CXCVIII)

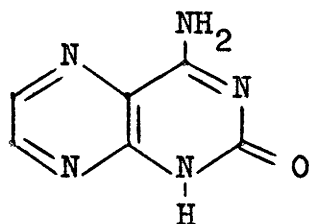


(CXCIX)

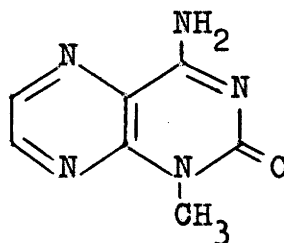
Comparison of the spectrum of 2-amino-4-hydroxy-pteridine as neutral molecule with those of its $N_{(1)}^-$, $N_{(3)}^-$, and $N_{(8)}^-$ -methyl derivatives (see figure page 37)

leaves little doubt that the hydrogen atom occupies position 3 and that 2-amino-3,4-dihydro-4-oxopteridine (CXCVIII) is the predominant tautomer, at least in aqueous solution*.

Similar treatment cannot be accorded to the isomeric 4-amino-2-hydroxypteridine, because the range of methylated reference compounds is incomplete. Nevertheless, all the imino-tautomers can be excluded as before, since the pteridine is also a weak base (pK_a 3.0). The spectrum of its neutral molecule however is not unlike that of 4-amino-1,2-dihydro-1-methyl-2-oxopteridine (CCI) so that 4-amino-1,2-dihydro-2-oxopteridine (CC) may well be the principal tautomer.



(CC)



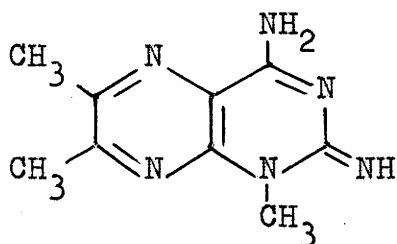
(CCI)

When a direct comparison is not possible, the preferred tautomeric form of an amine can often be deduced by comparing the spectrum of its neutral molecule with the anion spectrum of the corresponding hydroxy derivative

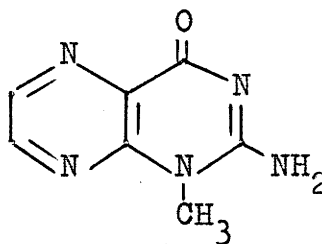
* Pfleiderer, Liedek, Lohrmann and Rukwied (1960) also reported the same conclusion, but on incomplete evidence.

(Jones, 1945). That this relationship is valid in this series, can be seen from the similarity of the neutral molecule spectrum of 2,4-diaminopteridine to the anion spectra of 4-amino-2-hydroxy- and 2-amino-4-hydroxy-pteridine, and even to the di-anion of 2,4-dihydroxypteridine (lumazine).

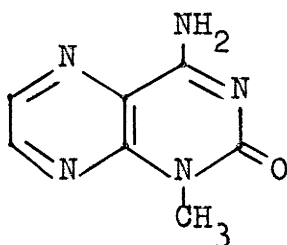
Applying this type of relationship, the preferred tautomeric form of the methylated diamino-pteridines can be found. Thus if allowance is made for the small bathochromic shift resulting from C-methyl groups, the neutral molecule spectrum of 4-amino-1,2-dihydro-2-imino-1,6,7-trimethylpteridine (CCII) approximates more closely to the anion spectrum of 2-amino-1,4-dihydro-1-methyl-4-oxopteridine (CCIII) than to that of 4-amino-1,2-dihydro-1-methyl-2-oxopteridine (CCIV). For this reason the preferred tautomer of the diamine is most likely to be that illustrated by (CCII).



(CCII)

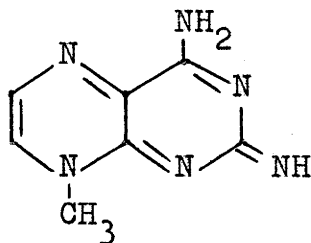


(CCIII)

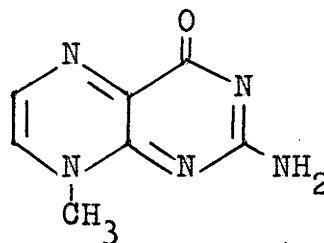


(CCIV)

Similarly in the case of the $N_{(8)}$ -methylated pteridine, the predominant tautomer is probably 4-amino-2,8-dihydro-2-imino-8-methylpteridine (CCV), because its neutral molecule spectrum is very similar to the anion spectrum of 2-amino-4,8-dihydro-8-methyl-4-oxopteridine (CCVI).



(CCV)



(CCVI)

In the series of pyrimidines investigated, it was not possible to interpret the ultraviolet spectra in the manner described for the pteridines, because (i) the range of hydroxy derivatives enabling the application of Jones' rule was limited. (ii) The inherent tendency of the iminopyrimidines to rearrange in alkaline solution, made the measurement of their neutral molecule spectra unreliable. In general however, methylation again caused the small

bathochromic displacements that were observed in the pteridine series, and these are apparent from the spectra included in the table.

SECTION 4.

EXPERIMENTAL

Introduction

Micro-analyses were carried out by Dr. J. E. Fildes, and assistant analysts Mrs. I. Komorowsky and Mr. D. A. Maguire, in the Micro-analytical Section of the Department of Medical Chemistry, Australian National University, Canberra.

Melting points were determined in glass capillaries inserted in an electrically heated copper block (Townson and Mercer Ltd., Type V Melting Point Apparatus) and are uncorrected.

Paper chromatography by the "ascending front" method was used extensively to check the purity of compounds prepared, and where practical, the course of all new reactions. Whatman No.1 and No.4 papers were used with the following solvents: (a) 3% aqueous ammonium chloride, (b) n-butanol-5N-acetic acid (7:3), (c) n-butanol saturated with 24% aqueous ammonia, (d) Bergmann's solvent: iso-propanol 65%; dimethylformamide 22.5%; 90% formic acid 2.5%; water 10%. The paper chromatograms were examined under ultraviolet light of two principal wavelengths, (a) 365 mμ from a mercury vapour lamp and a Wood's glass filter, (b) 254 mμ from a mercury resonance lamp and a Chance Brothers' OX7/19874 filter.

Following the practice of The Chemical Society, the names of new compounds are underlined (in lieu of italics) at their first mention in the body of the experimental text. Names which are headings, however, are also underlined, although this does not indicate that the compound is new.

All new compounds are indexed on the following pages.

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- ^a Other tautomeric forms possible.
- ^b Previously not characterised fully.
- ^c Structure not fully confirmed.
- ^d Compound recently claimed by other workers.
- ^e Compound not isolated.

B. Methylation of Aminopteridines

Methylation of 4-Aminopteridine:

4-Aminopteridine (0.75 g.; Albert, Brown and Cheeseman, 1951) and methyl iodide (7.5 ml.) were heated in a sealed tube at 140° for 4 hours. After evaporating off the excess of methyl iodide, the solid was extracted with boiling ethanol (100 ml.), the extract treated with charcoal and evaporated to a small volume (20 ml.).

Addition of light petroleum (b.p. $60-80^{\circ}$; 20 ml.) and recrystallisation from a similar mixture, produced the yellow 1,4-dihydro-4-imino-1-methylpteridine hydriodide (78%), m.p. 255° (Found: C, 28.9; H, 3.0; I, 43.8.

$C_7H_8IN_5$ requires C, 29.1; H, 2.8; I, 43.9%).

Conversion of Hydriodides into Hydrochlorides:

General Method: The hydriodide, dissolved in cold water (or suspended in sufficient water to ensure that the resulting hydrochloride was fully soluble), was shaken with washed silver chloride (a 5-fold excess) in the absence of light for 1 hour. After filtering off the spent silver halide mixture, the filtrate was evaporated to dryness in vacuo to yield the hydrochloride. Quantitative yields were usually obtained, and in no instance was the product contaminated with iodide ion.

The above imine hydriodide in water (30 parts),

treated in this manner, gave 1,4-dihydro-4-imino-1-methylpteridine hydrochloride (79%). Recrystallised from ethanol (80 parts), it did not melt below 300° (Found: C, 42.5; H, 4.1; N, 35.0. $C_7H_8ClN_5$ requires C, 42.5; H, 4.1; N, 35.4%).

Degradation of 1,4-Dihydro-4-imino-1-methylpteridine

(a) Hydrolysis with 2.5N-hydrochloric acid. The imine hydrochloride (0.2 g.) in 2.5N-hydrochloric acid (20 ml.) was heated on a steam bath for 30 minutes. After adjusting to pH 5, the solution was evaporated to dryness and the residue sublimed ($170^{\circ}/0.01$ mm.). The sublimate (30%), recrystallised from isobutyl methyl ketone (440 parts), had m.p. 222° , and it was identified by mixed m.p., as 1,4-dihydro-1-methyl-4-oxopteridine (Albert, Brown and Wood, 1956). Quantitative paper chromatography indicated a much higher yield (85%) of this pteridone than was actually isolated.

(b) Hydrolysis with cold 2.5N-sodium hydroxide. The imine hydriodide (1 g.) was triturated with ice-cold 2.5N-sodium hydroxide (10 ml.) for 5 minutes. The solid (93%) was filtered off, washed with ice-water (3 X 2 ml.), and recrystallised from light petroleum (b.p. $60-80^{\circ}$; 300 parts) to give 2-amidino-3-methylaminopyrazine, m.p. $108-110^{\circ}$ (Found: C, 47.65; H, 5.9; N, 46.1. $C_6H_9N_5$ requires

C, 47.7; H, 6.0; N, 46.3%). Its hydrochloride, recrystallised from 1:3 ethanol-light petroleum (300 parts), had m.p. 204° (Found: C, 38.45; H, 5.4; Cl, 18.8; N, 37.0. $C_6H_{10}ClN_5$ requires C, 38.4; H, 5.4; Cl, 18.9; N, 37.3%).

The amidine hydrochloride (1.25 g.) acetylacetone (2.65 g.), and potassium carbonate (1.8 g.) in water (15 ml.), were shaken at 45° for 8 hours. After evaporation in vacuo, the residue was boiled with ethanol (2 x 5 ml.), and the extract taken to dryness. Recrystallisation from light petroleum (b.p. $60-80^{\circ}$; 50 parts) gave 2-(4,6-dimethylpyrimidin-2-yl)-3-methylaminopyrazine (40%), m.p. 89° (Found: C, 61.2; H, 6.15; N, 32.35. $C_{11}H_{13}N_5$ requires C, 61.4; H, 6.1; N, 32.5%).

(c) Hydrolysis with hot N-sodium hydroxide. The hydriodide (0.3 g.) was heated in N-sodium hydroxide (3 ml.) at 100° for 10 minutes. The product (64%), which crystallised from the hot solution, was purified by sublimation ($140^{\circ}/0.01$ mm.) and had m.p. 198° , in which form it gave no mixed m.p. depression with an authentic specimen of 2-carbamoyl-3-methylaminopyrazine (Albert, Brown and Wood, 1956).

2-Carbamoyl-3-methylaminopyrazine (0.3 g.) was hydrolysed further by heating, with stirring, on a steam bath in N-sodium hydroxide (10 ml.) for 2.5 hours. After

acidifying to pH 2 and evaporating to dryness, the residue was extracted with hot benzene (2 X 20 ml.). These extracts, on evaporation, yielded 2-carboxy-3-methylamino-pyrazine (72%), which, recrystallised from water (50 parts), had m.p. 181-182° (Found: C, 47.0; H, 4.5; N, 27.4.

$C_6H_7N_3O_2$ requires C, 47.05; H, 4.6; N, 27.45%).

4-Amino-6,7-dimethylpteridine.

4,5,6-Triaminopyrimidine (7.9 g.; Albert, Brown and Cheeseman, 1951) and biacetyl (4.5 ml.) were refluxed in methanol (200 ml.) for 2 hours. The solid was recrystallised from water (160 parts) to give 90% of 4-amino-6,7-dimethylpteridine, m.p. 295° (Daly and Christensen, 1956, describe an alternative synthesis). The pteridine (0.75 g.), suspended in water (35 ml.) and acidified to pH 1 with 6% hydriodic acid, gave on evaporation 4-amino-6,7-dimethylpteridine hydriodide (1.3 g.). Recrystallised from 1:1 ethanol-ethyl acetate (25 parts), it had m.p. 205-208° (decomp.) (Found: C, 32.0; H, 3.5; I, 41.1; N, 22.9. $C_8H_{10}IN_5$ requires C, 31.7; H, 3.35; I, 41.85; N, 23.1%).

Methylation of 4-Amino-6,7-dimethylpteridine.

4-Amino-6,7-dimethylpteridine (2.6 g.) and methyl iodide (20 ml.) were heated in a sealed tube at 140° for 4 hours. Evaporation of the excess of methyl iodide

gave a solid which was dissolved in hot ethanol (320 ml.) and recrystallised by rapid evaporation to a smaller volume (190 ml.), followed by the addition of hot light petroleum (b.p. 80-100°; 20 ml.). The yellow 1,4-dihydro-4-imino-1,6,7-trimethylpteridine hydriodide (89%) was recrystallised from water (15 parts) and from methanol (20 parts) when it showed m.p. 264° (decomp.) (Found: C, 34.0; H, 3.9; I, 40.0; N, 21.9. $C_9H_{12}IN_5$ requires C, 34.1; H, 3.8; I, 40.0; N, 22.1%). The hydriodide (1.59 g.) in water (30 parts) was shaken with silver chloride to yield the imine hydrochloride (80%). It was recrystallised from ethanol (70 parts) and had m.p. 290° (decomp.) (Found: C, 48.0; H, 5.4; N, 31.1. $C_9H_{12}ClN_5$ requires C, 47.9; H, 5.4; N, 31.0%).

Degradation of 1,4-Dihydro-4-imino-1,6,7-trimethylpteridine.

(a) Hydrolysis with 2.5N-hydrochloric acid. The imine hydrochloride (0.7 g.) in 2.5N-hydrochloric acid (4.25 ml.) was heated on a steam bath for 2 hours. On cooling and neutralising to pH 7, the product, 1,4-dihydro-1,6,7-trimethyl-4-oxopteridine (79%), was obtained. Recrystallised from water (20 parts), it had m.p. 217° (Found: C, 56.5; H, 5.4; N, 29.0. $C_9H_{10}N_4O$ requires C, 56.8; H, 5.3; N, 29.45%).

(b) Hydrolysis with cold 2.5N-sodium hydroxide. The imine

hydriodide (0.5 g.) was added to 2.5N-sodium hydroxide (5 ml.) at 0° , precipitating the free base. The temperature was raised to 35° during 5 minutes of stirring. The base dissolved and an oil separated, which, on trituration, solidified and was recrystallised from light petroleum (b.p. $60-80^{\circ}$; 85 parts) to give 2-amidino-5,6-dimethyl-3-methylaminopyrazine (75%), m.p. $131-132^{\circ}$ (Found: C, 53.5; H, 7.2; N, 39.1. $C_8H_{13}N_5$ requires C, 53.6; H, 7.3; N, 39.1%).

(c) Hydrolysis with hot N-sodium hydroxide. The imine hydriodide (1.4 g.) and N-sodium hydroxide (10 ml.) were heated on a steam bath for 40 minutes and gave 2-carbamoyl-5,6-dimethyl-3-methylaminopyrazine (70%). It was recrystallised from water (300 parts) and had m.p. 164° (Found: C, 53.4; H, 6.75; N, 31.1. $C_8H_{12}N_4O$ requires C, 53.3; H, 6.7; N, 31.1%). This amide (0.2 g.) was stirred for 2 hours at 100° with N-sodium hydroxide (20 ml.) The solution was acidified to pH 1 and evaporated to dryness. The residue was extracted with, and then recrystallised from, light petroleum (b.p. $60-80^{\circ}$; 165 parts) giving 45% of 2-carboxy-5,6-dimethyl-3-methylaminopyrazine, m.p. 146° (Found: C, 52.7; H, 5.9; N, 23.15. $C_8H_{11}N_3O_2$ requires C, 53.0; H, 6.1; N, 23.2%).

2-Amino-3-carboxy-5,6-dimethylpyrazine

4-Hydroxy-6,7-dimethylpteridine (1.5 g.; Albert, Brown and Cheeseman, 1951) was refluxed in 10N-sodium hydroxide (20 ml.) for 4 hours. The solution, acidified to pH 2, was evaporated to dryness, and the residue continuously extracted with light petroleum (b.p. 80-100°). Evaporation of the extract gave the pyrazine acid (21%) which, recrystallised from water (110 parts), had m.p. 210-211° (209-210° was recorded by Vogel and Taylor, 1959) (Found: C, 49.95; H, 5.3; N, 24.95. Calc. for $C_7H_9N_3O_2$: C, 50.3; H, 5.4; N, 25.1%)

4-Methylaminopteridine

4-Methylthiopteridine (0.7 g.; Albert, Brown and Wood, 1954) and 3% alcoholic methylamine (35 ml.) were refluxed for 2 hours. After refrigeration, the solid was recrystallised from water (30 parts) to give 4-methylaminopteridine (95%), m.p. 251-252° (Found: C, 52.2; H, 4.35; N, 42.9. $C_7H_7N_5$ requires C, 52.2; H, 4.4; N, 43.45%). The pteridine (0.43 g.), dissolved in water (20 ml.) and acidified to pH 1 with 6% hydriodic acid, gave 4-methylaminopteridine hydriodide (92%) on evaporation. Recrystallised from ethanol (40 parts) it had m.p. 234-235° (decomp.) (Found: C, 29.25; H, 2.8; I, 43.95; N, 24.2. $C_7H_8IN_5$ requires C, 29.1; H, 2.8; I, 43.9; N, 24.2%).

Methylation of 4-Methylaminopteridine

4-Methylaminopteridine (0.5 g.) and methyl iodide (4.5 ml.) were heated in a sealed tube at 140° for 4 hours. After evaporation of the excess of methyl iodide, the residue was recrystallised from ethanol (100 parts) to give the yellow 1,4-dihydro-1-methyl-4-methyliminopteridine hydriodide (75%), m.p. $273-274^{\circ}$ (Found: C, 31.6; H, 3.4; I, 41.75; N, 22.8. $C_8H_{10}IN_5$ requires C, 31.7; H, 3.3; I, 41.9; N, 23.1%). The methyliminopteridine hydrochloride (91%), made from the hydriodide (0.68 g.) by dissolving in water (30 ml.) and shaking with silver chloride, was recrystallised from 23 parts of a mixture of ethyl acetate (70%), ethanol (20%), and water (10%), or alternatively from 20 parts of a mixture of ethanol (75%) and light petroleum (b.p. $60-80^{\circ}$; 25%). It decomposed above 250° without melting (Found: C, 45.4; H, 4.9; Cl, 16.7. $C_8H_{10}ClN_5$ requires C, 45.4; H, 4.8; Cl, 16.75%).

Degradation of 1,4-Dihydro-1-methyl-4-methyliminopteridine

(a) Hydrolysis with 6.3N-hydrochloric acid. The hydrochloride (0.4 g.), in 6.3N-hydrochloric acid (4 ml.) was heated on a steam bath for 4 hours, after which the solution was evaporated to dryness. The solid extracted by boiling isobutyl methyl ketone (5 X 30 ml.) was

purified by sublimation ($170^{\circ}/0.01\text{mm.}$) and by recrystallisation from the aforementioned solvent (440 parts). The hydrolysis product (55%), with m.p. $221-222^{\circ}$, was identified as 1,4-dihydro-1-methyl-4-oxopteridine by comparison with an authentic specimen (Albert, Brown and Wood, 1956).

(b) Hydrolysis with hot N-sodium hydroxide. The hydriodide (0.45 g.) was added to N-sodium hydroxide (4.5 ml.) and the mixture heated on the steam bath for 10 minutes. The product (60%), recrystallised from water (250 parts), had m.p. 196° , and showed no m.p. depression when mixed with 2-carbamoyl-3-methylaminopyrazine (Albert, Brown and Wood, 1956).

6,7-Dimethyl-4-methylthiopteridine

4,5-Diamino-6-methylthiopyrimidine (5 g.; Albert, Brown and Wood, 1954) and biacetyl (2.75 g.) in methanol (38 ml.) were refluxed for 10 minutes. The solid (62%) was recrystallised from water (35 parts) to give 6,7-dimethyl-4-methylthiopteridine, m.p. $120-121^{\circ}$ (Found: C, 52.2; H, 4.9; N, 27.1; S, 15.75. $\text{C}_9\text{H}_{10}\text{N}_4\text{S}$ requires C, 52.4; H, 4.9; N, 27.2; S, 15.55%).

6,7-Dimethyl-4-methylaminopteridine

6,7-Dimethyl-4-methylthiopteridine (2 g.) was refluxed in 5% ethanolic methylamine (60 ml.) for 2

hours. After refrigeration, the product was collected and recrystallised from ethanol (20 parts) to give 6,7-dimethyl-4-methylaminopteridine (95%), m.p. 222-223° (Found: C, 57.15; H, 5.8; N, 36.6. $C_9H_{11}N_5$ requires C, 57.1; H, 5.9; N, 37.0%). The hydriodide, obtained by acidifying a solution of the free base to pH 1.5 with 6% hydriodic acid and evaporating to dryness, was recrystallised from 1:1 ethyl acetate-ethanol (15 parts). It had m.p. 218-220° (Found: C, 33.8; H, 3.7; I, 40.1; N, 22.0. $C_9H_{12}IN_5$ requires C, 34.1; H, 3.8; I, 40.0; N, 22.1%).

Methylation of 6,7-Dimethyl-4-methylaminopteridine

The pteridine (1.25 g.) and methyl iodide (10 ml.) were heated in a sealed tube at 130° for 4 hours. The solid obtained on evaporation was recrystallised from methanol (10 parts), or alternatively from 30 parts of mixture of ethyl acetate (70%), ethanol (20%), and water (10%), to give 1,4-dihydro-1,6,7-trimethyl-4-methylimino-pteridine hydriodide (86%), m.p. 247° (Found: C, 36.6; H, 4.2; I, 38.4; N, 20.9. $C_{10}H_{14}IN_5$ requires C, 36.3; H, 4.3; I, 38.3; N, 21.15%). The methyliminopteridine hydrochloride, obtained from the hydriodide by shaking with silver chloride, was recrystallised from ethanol (12 parts) and from the above three component solvent

(30 parts). It melted ca. 250° (decomp.) (Found: C, 50.25; H, 5.7; N, 29.1. $C_{10}H_{14}ClN_5$ requires C, 50.1; H, 5.9; N, 29.2%).

Degradation of 1,6,7-Trimethyl-4-methyliminopteridine

(a) Hydrolysis with 6.3N-hydrochloric acid. The hydrochloride (0.6 g.) and 6.3N-hydrochloric acid (6.0 ml.) were heated in a sealed tube at 130° for $1\frac{1}{2}$ hours. After neutralizing the darkly coloured solution to pH 7, it was treated with charcoal and evaporated. The residue was dissolved in a minimum of hot water (4 ml.) and the solution refrigerated. The product (50%) was further recrystallised from water (15 parts) and its m.p., $216-217^{\circ}$, was unaltered by admixture with the authentic 1,4-dihydro-1,6,7-trimethyl-4-oxopteridine described above.

(b) Hydrolysis with hot N-sodium hydroxide. The hydriodide (0.45 g.) was heated on a steam bath for 40 minutes together with N-sodium hydroxide (4.5 ml.). 2-Carbamoyl-5,6-dimethyl-3-methylaminopyrazine (70%) which crystallised from the hot solution, was recrystallised from water (300 parts). With m.p. $162-164^{\circ}$ it was characterised against the authentic compound (see above).

5-Amino-4,6-bismethylaminopyrimidine

4,6-Bismethylamino-5-nitropyrimidine (12g.;

Brown, 1954) was hydrogenated in methanol (500 ml.) over Raney nickel. The filtered solution was evaporated in vacuo and the residue recrystallised by dissolution in boiled-out water (30 parts) at 25° and cooling to 0°. When the purification process after hydrogenation was carried out entirely within a nitrogen box, the otherwise deep red 5-amino-4,6-bismethylaminopyrimidine (60%) was obtained as a white solid, m.p. 178-180° (Found: C, 47.0; H, 7.25; N, 45.3. $C_6H_{11}N_5$ requires C, 47.0; H, 7.25; N, 45.7%).

4,8-Dihydro-6,7,8-trimethyl-4-methyliminopteridine

5-Amino-4,6-bismethylaminopyrimidine (1 g.) was refluxed for 15 minutes with biacetyl (1.1g.) in methanol (5 ml.). The solid formed on chilling, recrystallised from light petroleum (b.p. 60-80°; 20 parts), to give the yellow 4,8-dihydro-6,7,8-trimethyl-4-methyliminopteridine, m. p. 113-115° (Found: C, 58.7; H, 6.5; N, 34.3.

$C_{10}H_{13}N_5$ requires C, 59.1; H, 6.45; N, 34.5%).

4-Dimethylaminopteridine

4-Methylthiopteridine (8 g.; Albert, Brown and Wood, 1954) was refluxed for 2 hours with 6% ethanolic dimethylamine (280 ml.). After evaporation, the residue was recrystallised from light petroleum (b.p. 60-80°; 650 parts) and gave, on refrigeration, 4-dimethyl-

aminopteridine (94%), m.p. 165-166°, identified with an authentic specimen prepared by an alternate method (Albert, Brown and Cheeseman, 1952). The hydriodide of the base recrystallised from 1:3 light-petroleum (b. p. 60-80°)-ethanol (85 parts), and had m.p. 244-245° (Found: C, 31.8; H, 3.4; I, 41.8; N, 23.0. $C_8H_{10}IN_5$ requires C, 31.7; H, 3.3; I, 41.9; N, 23.1%).

Methylation of 4-Dimethylaminopteridine

The base (0.35 g.) and methyl iodide (0.62 ml.) in methanol (6.2 ml.) were refluxed for 2 hours. The solvent was evaporated, and the residue recrystallised from 3:2 light petroleum (b.p. 80-100°)-ethanol (225 parts). The yellow 4-dimethylamino-1-methylpteridinium iodide (52%) had m.p. 227-229° (Found: C, 33.7; H, 3.85; I, 40.15; N, 21.8. $C_9H_{12}IN_5$ requires C, 34.1; H, 3.8; I, 40.0; N, 22.1%).

Hydrolysis of 4-Dimethylamino-1-methylpteridinium iodide

The quaternary iodide (0.4 g.) was dissolved in water (10 ml.), the solution adjusted to pH 10 with N-sodium hydroxide, and boiled for 4 minutes. The product (31%) that crystallised from the hot solution was further purified by sublimation (140°/0.01 mm.) and its m.p., 197-198°, was undepressed on admixture with 2-carbamoyl-3-methylaminopyrazine (Albert, Brown and Wood, 1956).

4-Dimethylamino-6,7-dimethylpteridine

6,7-Dimethyl-4-methylthiopteridine (8.3 g.) was aminated by refluxing for 2 hours with 6% alcoholic dimethylamine (245 ml.). Evaporation of the solvent and recrystallisation of the residue from light petroleum (b. p. 60-80°; 125 parts), gave 4-dimethylamino-6,7-dimethylpteridine (90%) with m.p. 138-140° (Found: C, 59.3 ; H, 6.4; N, 34.3. $C_{10}H_{13}N_5$ requires C, 59.1; H, 6.45; N, 34.5%). Its hydriodide salt, formed by the addition of an equivalent of 6% hydriodic acid and recrystallised from ethanol (5 parts), had m.p. 206-207° (Found: C, 36.5; H, 4.4; I, 38.8; N, 20.9. $C_{10}H_{14}IN_5$ requires C, 36.3; H, 4.3; I, 38.3; N, 21.15%).

Methylation of 4-Dimethylamino-6,7-dimethylpteridine

4-Dimethylamino-6,7-dimethylpteridine (0.4 g.), ethyl acetate (40 ml.), and methyl iodide (0.62 ml.) were kept at 20° for 12 hours in a darkened container. The crystals (88%) were collected, washed with ethyl acetate, and dried in vacuo. 4-Dimethylamino-1,6,7-trimethylpteridinium iodide, obtained in this way, had m.p. 153-154° (Found: C, 38.3; H, 4.7; I, 37.2; N, 20.2. $C_{11}H_{16}IN_5$ requires C, 38.3; H, 4.7; I, 36.8; N, 20.3%).

Hydrolysis of 4-Dimethylamino-1,6,7-trimethylpteridinium iodide

The quaternary iodide (0.4 g.) was dissolved in

water (2.5ml.). The red solution was made alkaline to pH 10 and boiled for 4 minutes. On cooling, a crystalline solid (46%), m.p. $210-211^{\circ}$, was deposited. Further recrystallisation from water (20 parts) raised the m.p. to $216-217^{\circ}$, which was unaltered by admixture with authentic 1,4-dihydro-1,6,7-trimethyl-4-oxopteridine (see above).

Methylation of 2-Amino-4-hydroxypteridine

2-Amino-4-hydroxypteridine (2 g.; Cain, Mallette and Taylor, 1946), methyl iodide (15 ml.), and methanol (60 ml.) were rocked in a sealed tube at 110° for 12 hours. The tube was opened at -40° (a precaution against the considerable quantity of dimethyl ether formed) and the solution evaporated (to ca. 15 ml.) until ruby-red crystals commenced to be deposited. These were recrystallised from methanol (140 parts, with concentration) to yield 2-amino-4,8-dihydro-8-methyl-4-oxopteridine hydriodide (35%), decomp. 265° (Found: C, 27.85; H, 2.8; I, 41.1; N, 22.55. $C_7H_8IN_5O$ requires C, 27.55; H, 2.65; I, 41.6; N, 22.95%).

Treatment with silver chloride furnished the hydrochloride which recrystallised from methanol (330 parts, with concentration to 100 parts) had m.p. ca. 285° (decomp) (Found: C, 39.35; H, 3.9; N, 32.7. $C_7H_8ClN_5O$

requires C, 39.35; H, 3.8; N, 32.8%). Paper chromatography revealed the following R_f values:

Butanol - acetic acid	0.02
Butanol - ammonia	0.05
Bergmann's solvent	0.20
Ammonium chloride	0.65

The structure of this hydrochloride was established by its unambiguous synthesis.

2,5-Diamino-4-hydroxy-6-methylaminopyrimidine hydrochloride (0.45 g.; see pyrimidine section) was refluxed for 30 minutes with polyglyoxal (0.14 g.) in methanol (30 ml.). Evaporation of the solution to 10 ml. gave the crystalline pteridine hydrochloride (50%) which, recrystallised from methanol, had m.p. 280-285° (decomp.) (Found: C, 39.35; H, 4.0; N, 32.7%). It was identified by mixed m.p., paper chromatography in the solvent systems shown, and by infrared spectroscopy (see spectral traces in text) with the hydrochloride from the methylation product of 2-amino-4-hydroxypteridine described above.

2-Amino-1,4-dihydro-1-methyl-4-oxopteridine

2,4-Diamino-3,6-dihydro-3-methyl-5-nitroso-6-oxopyrimidine (4 g.; Roth, Smith and Hultquist, 1951; corrections by Boon and Bratt, 1957) in methanol (200 ml.) was hydrogenated over Raney nickel. The spent catalyst was filtered off, washed with hot water (3 X 50 ml.), and

the aqueous washings together with glyoxal monohydrate (2 g.) added to the methanolic filtrate. The yellow solution was evaporated to 80 ml. on a steam bath over a period of 1 hour, then chilled to yield the oxopteridine (74%). Purified by triturating with ice-cold 0.5N-sodium hydroxide (20 ml.) and by recrystallisation from water (60 parts), it had m.p. 335-336° (decomp.) (Pfleiderer, Liedek, Lohrmann and Rukwied, 1960, record m.p. 335-337°) (Found: C, 47.55; H, 4.0; N, 39.45. Calc. for $C_7H_7N_5O$: C, 47.45; H, 4.0; N, 39.55%). In chromatography the following R_f values were obtained:

Butanol - acetic acid	0.27
Butanol - ammonia	0.12
Bergmann's solvent	0.48
Ammonium chloride	0.70

2-Amino-3,4-dihydro-3-methyl-4-oxopteridine

2,4,5-Triamino-1,6-dihydro-1-methyl-6-oxopyrimidine hydrochloride (0.5 g.; Curran and Angier, 1958), polyglyoxal (0.15 g.), and methanol (60 ml.) were refluxed for 1 hour. Evaporation to ca. 5 ml. gave the oxopteridine (70%) which, after recrystallisation from glacial acetic acid (120 parts), decomposed at 319-320°. R_f values from chromatography were as follows:

Butanol - acetic acid	0.35
Butanol - ammonia	0.20
Bergmann's solvent	0.55
Ammonium chloride	0.60

(Pfleiderer, Liedek, Lohrmann and Rukwied, 1960, record m. p. 322° with an 18% yield) (Found: C, 47.65; H, 4.0; N, 39.35. Calc. for $C_7H_7N_5O$: C, 47.45; H, 4.0; N, 39.5%).

4-Hydroxy-2-methylaminopteridine

This pteridine was prepared by two routes:

(a) Primary synthesis: 4,5-Diamino-6-hydroxy-2-methylaminopyrimidine hydrochloride (1 g.; see pyrimidine section) and polyglyoxal (0.3 g.) were refluxed in methanol (125 ml.) for 1 hour. Evaporation, and recrystallisation of the recovered solid by dissolution in hot water (400 parts), addition of ethanol (400 parts) and slow concentration, yielded the pteridine (81%) decomp. ca. 378° (Pfleiderer, Liedek, Lohrmann and Rukwied, 1960, record m.p. $>350^{\circ}$) (Found: C, 47.3; H, 4.0; N, 39.3. Calc. for $C_7H_7N_5O$: C, 47.45; H, 4.0; N, 39.5%).

(b) Rearrangement: 2-Amino-3,4-dihydro-3-methyl-4-oxopteridine (0.25 g.) was heated in N-sodium hydroxide (2.5 ml.) at 100° for 5 minutes. The solution was chilled, acidified to pH 5 with 5% acetic acid, and the

rearranged product (80%) filtered off. Recrystallised, it decomposed ca.378°, and had chromatographic R_f values:

Butanol - acetic acid	0.40
Butanol - ammonia	0.08
Bergmann's solvent	0.67
Ammonium chloride	0.60

It was identified with authentic 4-hydroxy-2-methylamino-pteridine by chromatography and by ultraviolet spectroscopy (see text).

2-Amino-4-methoxypteridine

2,4,5-Triamino-6-methoxypyrimidine sulphate (0.5 g.; Roth, Smith and Hultquist, 1951) and polyglyoxal (0.11 g.) were refluxed in methanolic sodium methoxide (60 ml.; from sodium, 0.085 g.) for 1 hour. The oily residue obtained on evaporation was triturated with water (5 ml.), and the resulting solid recrystallised from water (25 parts). The methoxypteridine (43%) had m.p. 204-205° (Pfleiderer, Liedek, Lohrmann and Rukwied, 1960, record m.p. as 207-209°) (Found: C, 47.4; H, 3.85; N, 39.4. Calc. for $C_7H_7N_5O$: C, 47.45; H, 4.0; N, 39.5%).

Methylation of 2-Amino-4-hydroxy-6,7-dimethylpteridine

2-Amino-4-hydroxy-6,7-dimethylpteridine (1 g.; Cain, Mallette, and Taylor, 1946) was rocked for 5 hours at 100° with methyl iodide (7.5 ml.) and methanol (30 ml.).

Evaporation of the solvents, and recrystallisation of the product from a mixture of methyl iodide (35 parts) and methanol (65 parts), gave 2-amino-4,8-dihydro-6,7,8-trimethyl-4-oxopteridine hydriodide (60%); m.p. 265-270° (decomp.) (Found: C, 32.45; H, 3.5; I, 37.75; N, 20.85. $C_9H_{12}IN_5O$ requires C, 32.45; H, 3.6; I, 38.1; N, 21.0%).

Silver chloride converted this hydriodide into the hydrochloride which, recrystallised from 25% aqueous ethanol (26 parts), had m.p. 255-260° (decomp.) (Found: Cl, 14.8; N, 28.75. $C_9H_{12}ClN_5O$ requires Cl, 14.7; N, 29.0%). The following R_f values were obtained in paper chromatography:

Butanol - Ammonia	0.12
Butanol - acetic acid	0.18
Bergmann's solvent	0.35
Ammonium Chloride	0.75

The hydrochloride was also made unambiguously. 2,5-Diamino-4-hydroxy-6-methylaminopyrimidine hydrochloride (0.6 g.) in methanol (35 ml.), was refluxed with biacetyl (0.31 ml.) for 30 minutes. Evaporation to 10 ml. and addition of ether (10 ml.) gave a solid (93%) which, after recrystallisation, was identified with the product (hydrochloride) from methylation of 2-amino-4-hydroxy-6,7-dimethylpteridine by means of mixed m.p., paper chrom-

atography, and infrared spectroscopy (see spectral traces in text).

Methylation of 4-Amino-2-hydroxypteridine

4-Amino-2-hydroxypteridine (1.35 g.; Taylor and Cain, 1949; Albert, Brown and Cheeseman, 1952), methyl iodide (5.2 ml.), and methanolic sodium methoxide (200 ml.; from sodium, 0.21 g.) were refluxed for 1 hour. Recrystallisation of the insoluble product from water (160 parts) gave 4-amino-1,2-dihydro-1-methyl-2-oxopteridine (75%), m.p. 324-325° (decomp.) (Found: C, 47.6; H, 4.0; N, 39.4. $C_7H_7N_5O$ requires C, 47.45; H, 4.0; N, 39.5%).

Hydrolysis of 4-Amino-1,2-dihydro-1-methyl-2-oxopteridine

This pteridone (0.3 g.) was stirred in N-sodium hydroxide (15 ml.) for 5 hours at 100°. After the solution was acidified to pH 1 and evaporated to dryness, the residue was continuously extracted with hot benzene for 4 hours. Taken to dryness, the extract was sublimed (120°/0.02 mm.) and the sublimate (39%) recrystallised from water (50 parts) to give 2-carboxy-3-methylamino-pyrazine (see above), m.p. and mixed m.p. 180°. The unsublimed part (30%) recrystallised from ethanol (260 parts), and when sublimed (220°/0.02 mm.) had m.p. 285°, undepressed on admixture with 1-methyl-lumazine (Pfleiderer, 1957) (Found: C, 46.95; H, 3.40; N, 31.35. Calc. for

$C_7H_6N_4O_2$: C, 47.2; H, 3.40; N, 31.45%).

4-Amino-2-hydroxy-6,7-dimethylpteridine

4,5,6-Triamino-2-hydroxypyrimidine sulphate (4.8 g.; Bendich, Tinker and Brown, 1948) and sodium hydrogen carbonate (3.4 g.) in water (350 ml.), were stirred on the steam-bath with biacetyl (1.8 g.) for 15 minutes. The resulting solid (3.4 g.) was dissolved in hot water (150 parts) by the addition of hydrochloric acid to pH 2.5, and 4-amino-2-hydroxy-6,7-dimethyl-pteridine, m.p. ca. 340° (decomp.), crystallised on the slow addition of sodium acetate to pH 5. (Found: C, 50.3; H, 4.75; N, 36.15. $C_8H_9N_5O$ requires C, 50.25; H, 4.7; N, 36.6%).

Methylation of 4-Amino-2-hydroxy-6,7-dimethylpteridine

The pteridine (1 g.) and methyl iodide (2.5 ml.) were refluxed for 1 hour in methanolic sodium methoxide (25 ml.; from sodium, 0.13 g.). After chilling, the water-washed solid (84%) was recrystallised from ethanol (350 parts), to give 4-amino-1,2-dihydro-1,6,7-trimethyl-2-oxopteridine, m.p. $314-316^\circ$ (decomp.) (Found: C, 52.65; H, 5.4; N, 33.7. $C_9H_{11}N_5O$ requires C, 52.65; H, 5.4; N, 34.1%).

Hydrolysis of 4-Amino-1,2-dihydro-1,6,7-trimethyl-2-oxopteridine

Hydrolysis of this pteridone (0.93 g.) in

boiling N-sodium hydroxide (19 ml.) for 15 minutes gave, after chilling and adjustment to pH 5, 1,6,7-trimethyl-lumazine (83%), m.p. 328-330° (Sachs and Meyerheim, 1908, record m.p. 328-330°) (Found: C, 52.5; H, 4.8; N, 27.15. Calc. for $C_9H_{10}N_4O_2$: C, 52.4; H, 4.9; N, 27.15%).

1,6,7-Trimethyl-lumazine (0.42 g.) was further hydrolysed by heating with 2.5N-sodium hydroxide (16 ml.) in a steel bomb at 200° for 4 hours. After being treated with charcoal and acidified to pH 2, the solution was evaporated to dryness. The residue was extracted with boiling light petroleum (b.p. 60-80°; 25 ml.) to yield 2-carboxy-5,6-dimethyl-3-methylaminopyrazine (35%). Recrystallised from the above solvent (165 parts), it had m.p. 143-145° unaltered by admixture with an authentic specimen of the pyrazine acid (see above).

Methylation of 2,4-Diaminopteridine

2,4-Diaminopteridine (4 g.), prepared according to Mallette, Taylor and Cain (1947) and purified by the method of Albert, Brown and Cheeseman (1952), was rocked together with methyl iodide (40 ml.) and methanol (80 ml.) at 110° for 1 hour. Evaporation gave a crude solid (8.4 g.) which was extracted with boiling methanol (40 ml.). The residue, twice recrystallised from methanol (160 parts, with concentration), gave 4-amino-2,8-dihydro-2-imino-8-methylpteridine dihydriodide (or tautomer) (0.5 g.) as dark red

crystals, m.p. 236-237° (Found: C, 19.5; H, 2.1; I, 58.75. $C_7H_{10}I_2N_6$ requires C, 19.45; H, 2.3; I, 58.75%).

Evaporation of the initial methanol extract and the mother-liquors from the dihydriodide recrystallisations, left a yellow solid consisting of a mixture of the 8-methylpteridine and its 1-methyl isomer. Attempts to separate these compounds by chromatography and by recrystallisation were unsuccessful.

Degradation of the Methylated 2,4-Diaminopteridines

This was carried out to confirm the structure of the 8-methylpteridine dihydriodide and to establish the structure of the second product.

(a) Hydrolysis of the dihydriodide. The dihydriodide (0.28 g.) was heated at 100° with N-hydrochloric acid (36 ml.) for 30 minutes. To remove iodide ion, the solution was shaken with washed silver chloride (ca. 2 g.) for 1 hour, then filtered and the filtrate evaporated to dryness. The residue was dissolved in water (10 ml.) which was then adjusted to pH 3.5 with 5% ammonia solution and chilled. The solid precipitate (80%) recrystallised from water, (80 parts), giving 2-amino-4,8-dihydro-8-methyl-4-oxopteridine hydrochloride, decomp. 278-283°, identified with authentic material (see above) by mixed m.p., paper chromatography in 4 solvents, and infrared and ultraviolet spectroscopy (see text).

(b) Hydrolysis of the mixture. The mixture (6 g.) was dissolved in 0.5N-sodium hydroxide (125 ml.) at 0°. After 15 minutes at this temperature, the crystalline product (1 g.) was collected and recrystallised from water (60 parts) to give 2-amino-1,4-dihydro-1-methyl-4-oxopteridine, m.p. 336° (decomp.) undepressed by admixture with a specimen of the authentic material described above.

This pteridine (0.5 g.) was hydrolysed further by being refluxed in N-sodium hydroxide (7 ml.) for 15 minutes. The solid (88%) obtained on adjustment to pH 5, was sublimed (220°/0.02 mm.) and recrystallised (ethanol, 260 parts) to give 1-methyl-lumazine m.p. 285° (see above).

2,4-Diamino-6,7-dimethylpteridine

2,4,5,6-Tetraminopyrimidine sulphate (8.0 g.; Cavalieri, Bendich, Tinker and Brown, 1948) and biacetyl (3 ml.) were stirred in water (50 ml.) on a steam bath for 1 hour. The yellow solid (98%) obtained on cooling, was triturated with 0.1N-sodium hydroxide, washed with hot water and ethanol, and recrystallised from dimethylformamide (145 parts) or glacial acetic acid (20 parts) to give the diaminopteridine. It did not melt below 360°, and was chromatographically pure. Despite failure by older methods (Mallette, Cain and Taylor, 1947), it gave a

satisfactory analysis for carbon and hydrogen by the "rapid combustion" method (Belcher and Ingram, 1950) in admixture with vanadium pentoxide, and for nitrogen by the Kjeldahl method (sealed tube) (Found: C, 50.75; H, 5.35; N, 43.75. $C_8H_{10}N_6$ requires C, 50.5; H, 5.3; N, 44.2%).

Methylation of 2,4-Diamino-6,7-dimethylpteridine

This pteridine (2 g.), methyl iodide (20 ml.), and methanol (40 ml.) were rocked together, in a sealed tube, at 110° for 5 hours. After cooling to -40° (to liquify dimethyl ether formed), the tube was opened and the solid (27%) recrystallised from ethanol (40 parts) until it was chromatographically homogeneous (R_f 0.65 in butanol-acetic acid). The 4-amino-1,2-dihydro-2-imino-1,6,7-trimethylpteridine hydriodide (or tautomer) had m.p. $280-285^\circ$ (decomp.) (Found: C, 32.5; H, 3.85; I, 38.15; N, 25.2. $C_9H_{13}IN_6$ requires C, 32.55; H, 3.95; I, 38.2; N, 25.3%).

When the above methylation mixture was heated for 1 hour, and the product (1.3 g.) precipitated with ether (60 ml.), paper chromatography revealed that a second substance (R_f 0.55 in butanol-acetic acid) was also present. It could not be separated by recrystallisation. Analysis, after recrystallisation from a mixture of 65%

methanol - 35% methyl iodide (40 parts), indicated the two products as isomeric (Found for the mixture:

C, 32.4; H, 3.95; I, 37.65; N, 25.1%).

Hydrolysis of 4-Amino-1,2-dihydro-2-imino-1,6,7-trimethyl-pteridine

The homogeneous hydriodide (0.23 g.) was refluxed with N-sodium hydroxide (5 ml.) for 15 minutes. The solution was acidified to pH 5, chilled, and the solid recrystallised from water (250 parts) to give 1,6,7-trimethyl-lumazine (92%), m.p. 327-329° without depression when mixed with authentic material (see above).

C. Methylation of Aminopyrimidines

Methylation of 4,6-Diaminopyrimidine

4,6-Diaminopyrimidine (5 g.), prepared according to the directions of Brown (1950), was refluxed in a mixture of methyl iodide (12 ml.) and methanol (25 ml.) for 3 hours. 4-Amino-1,6-dihydro-6-imino-1-methylpyrimidine (89%) which was obtained on cooling, crystallised from ethanol (90 parts) as needles, m.p. 283-284° (Found: C, 23.8; H, 3.6; I, 50.5; N, 21.95. $C_5H_9IN_4$ requires C, 23.8; H, 3.6; I, 50.35; N, 22.2%). It was converted with silver chloride into the imine hydrochloride which, after recrystallisation from ethanol (35 parts), had m.p. 268-269° (Found: C, 37.5; H, 5.65; Cl, 22.15; N, 34.5.

$C_5H_9ClN_4$ requires C, 37.4; H, 5.65; Cl, 22.1; N, 34.9%).

The Rearrangement of 4-Amino-1,6-dihydro-6-imino-1-methylpyrimidine

The imine hydrochloride (1 g.) was dissolved in N-sodium hydroxide (12.5 ml.). After 6 hours at 20°, the crystals (58%) were removed and the filtrate was adjusted to pH 8-9 with sulphuric acid. After vacuum-evaporation, the residue was boiled with ethyl acetate (50 ml.), and the extract on evaporation gave a second crop (29%). Recrystallised from water (15 parts), the product had m.p. 209-211°, undepressed on admixture with 4-amino-6-methylaminopyrimidine prepared to the directions of Whitehead and Traverso (1958) (Found: C, 48.3; H, 6.4; N, 45.2. Calc. for $C_5H_8N_4$: C, 48.4; H, 6.5; N, 45.1%).

The addition of an equivalent of hydrochloric acid to this base furnished 4-amino-6-methylaminopyrimidine hydrochloride. Recrystallised from ethanol (20 parts) it had m.p. 213-214° (Found: C, 37.45; H, 5.6; Cl, 22.2; N, 34.7. $C_5H_9ClN_4$ requires C, 37.4; H, 5.65; Cl, 22.1; N, 34.9%).

Methylation of 4,6-Diamino-5-nitropyrimidine

4,6-Diamino-5-nitropyrimidine (4 g.; Brown, 1950) and methyl iodide (40 ml.) were heated in a sealed tube at 140° for 6 hours. An aqueous solution (50 ml.) of the

residue from evaporation was adjusted to pH 8-9, and the product (48%) recrystallised from water (330 parts).

4-Amino-6-methylamino-5-nitropyrimidine thus obtained had m.p. 248-250°, undepressed by admixture with authentic material made according to Daly and Christensen (1956).

The nitropyrimidine was also prepared from 4-amino-1,6-dihydro-6-imino-1-methylpyrimidine hydrochloride (see above) by nitration and an accompanying rearrangement. The imine hydrochloride (2.4 g.) in concentrated sulphuric acid (11 ml.) was evacuated at 20° to remove hydrogen chloride. Nitric acid (d.1.5; 4.8 ml.) was added with stirring at 0°, and the mixture was then heated for 30 minutes at 40° before being poured onto ice. Addition of ammonia gave the nitro-compound (85%), m.p. 248-249° (Found: C, 35.2; H, 4.2. Calc. for $C_5H_7N_5O_2$: C, 35.5; H, 4.2%).

4,5-Diamino-6-methylaminopyrimidine

4-Amino-6-methylamino-5-nitropyrimidine (1 g.) was hydrogenated in methanol (100 ml.) over Raney nickel. After filtration, the solvent was evaporated to yield the triamine (60%), which was purified by sublimation at 120°/0.01 mm. to m.p. 187-189° (Found: C, 43.2; H, 6.65; N, 50.05. $C_5H_9N_5$ requires C, 43.15; H, 6.5; N, 50.3%).

Methylation of 2,4-Diaminopyrimidine

2,4-Diaminopyrimidine (1.35 g.; prepared by the

method of Brown, 1950) dissolved in methanol (7 ml.) was refluxed with methyl iodide (7 ml.) for 1 hour. Recrystallisation of the insoluble product by dissolving in boiling ethanol (40 parts) and adding hot ethyl acetate (6 parts), gave 76% of 2(4)-amino-1,4(1,2)-dihydro-4(2)-imino-1-methylpyrimidine hydriodide, m.p. 273-274° (Found: C, 23.7; H, 3.55; I, 50.4; N, 22.1. $C_5H_9N_4I$ requires C, 23.8; H, 3.6; I, 50.35; N, 22.25%). Shaking with an aqueous suspension of silver chloride, converted this into the hydrochloride which, recrystallised from methanol (15 parts), had m.p. 275-276° (Found: C, 37.15; H, 5.65; Cl, 22.35; N, 34.6. $C_5H_9ClN_4$ requires C, 37.4; H, 5.65; Cl, 22.1; N, 34.9%).

This hydrochloride was identical with the Raney nickel desulphurisation product of 2(4)-amino-1,4(1,2)-dihydro-4(2)-imino-1-methyl-6-methylthiopyrimidine hydrochloride described below.

2,4-Diamino-6-mercaptopyrimidine

2,4-Diamino-6-chloropyrimidine (12.0 g.; Pfeleiderer and Lohrmann, 1961) and N-potassium hydrogen sulphide (240 ml.) were heated in a sealed tube at 135° for 12 hours (optimum conditions determined chromatographically; cf. conditions used by Elion, Lange and Hitchings, 1956). On cooling, the mercaptopyrimidine (78%) crystallised readily

(cf. claim of Elion et. al). Recrystallised from water (14 parts) it had m.p. 300-301° (recorded m.p. 309-310°) (Found: C, 33.95; H, 4.4; N, 39.25. Calc. for $C_4H_6N_4S$: C, 33.8; H, 4.25; N, 39.4%).

2,4-Diamino-6-methylthiopyrimidine

2,4-Diamino-6-mercaptopyrimidine (7.5 g.), dissolved in 2.5N-sodium hydroxide (21.1 ml.), was shaken with methyl iodide (3.6 ml.) until crystallisation was complete (10 minutes). The methylthio compound (86%), recrystallised from water (17 parts), had m.p. 200-202° (Found: C, 38.3; H, 5.1; N, 35.85. $C_5H_8N_4S$ requires C, 38.45; H, 5.15; N, 35.9%).

Methylation of 2,4-Diamino-6-methylthiopyrimidine

The pyrimidine (9.5 g.), dissolved in methanol, (135 ml.) was refluxed with methyl iodide (75 ml.) for 24 hours. The insoluble 2(4)-amino-1,4(1,2)-dihydro-4(2)-imino-1-methyl-6-methylthiopyrimidine hydriodide (39%) was filtered from the hot solution and recrystallised from water (25 parts). It melted at 281-282° (Found: C, 24.1; H, 3.6; I, 42.4; N, 18.8. $C_6H_{11}IN_4S$ requires C, 24.15; H, 3.7; I, 42.55; N, 18.8%). Treatment of this hydriodide with silver chloride gave the hydrochloride. Recrystallised from methanol (40 parts) or from water (3 parts), it had m.p. 287-288° (Found: C, 34.6; H, 5.4; Cl. 17.15;

N, 27.0. $C_6H_{11}ClN_4S$ requires C, 34.85; H, 5.35; Cl, 17.15; N, 27.1%).

Evaporation of the above filtrate, and recrystallisation of the residue from water (7 parts), gave the isomeric 2(4)-amino-3,4(2,3)-dihydro-4(2)-imino-3-methyl-6-methylthiopyrimidine hydriodide (22%) with m.p. 248-250° (Found: C, 23.9; H, 3.65; I, 42.45; N, 18.5%).

Reaction with silver chloride furnished the hydrochloride which, recrystallised from methanol (30 parts) or water (2 parts), had m.p. 281-282° and gave a 15° m.p. depression when mixed with the isomeric hydrochloride above (Found: C, 34.9; H, 5.45; Cl, 17.3; N, 26.9%).

The Structures of the Isomeric 2,4-Diamino-6-methylthiopyrimidine Methylation Products

The structures of the two isomeric products obtained in the above methylation were established by (a) the measurement of their high basic pK_a values (12.0 and 11.15 resp.) and (b) the unambiguous synthesis of 2(4)-amino-3,4(2,3)-dihydro-4(2)-imino-3-methyl-6-methylthiopyrimidine hydrochloride in the following series.

2(6)-Amino-4-chloro-1,6(1,2)-dihydro-6(2)-imino-1-methylpyrimidine

2,4-Diamino-3,6-dihydro-3-methyl-6-oxopyrimidine (5.0 g.; Roth, Smith and Hultquist, 1951; with corrections

by Boon and Bratt, 1957) and phosphoryl chloride (60 ml.) were refluxed for 3 hours. After removal of excess phosphoryl chloride in vacuo, the oily residue was poured onto ice, and the solution made alkaline (50% sodium hydroxide) to pH 12. Continuous extraction with ether yielded the chloropyrimidine (17%) which, recrystallised from water (16 parts), decomposed at 192-193° (no precise decomposition is observed if sample is heated slowly to this temperature) (Found: C, 37.9; H, 4.45; Cl, 22.25. $C_5H_7ClN_4$ requires C, 37.85; H, 4.45; Cl, 22.35%).

2(4)-Amino-3,4(2,3)-dihydro-4(2)-imino-3-methyl-6-methylthiopyrimidine

2(6)-Amino-4-chloro-1,6(1,2)-dihydro-6(2)-imino-1-methylpyrimidine (0.5 g.) in 0.5N-ethanolic sodium methyl mercaptide (8 ml.), was refluxed for 15 minutes and the filtered solution evaporated to dryness in vacuo. Water (5 ml.) was added to the residue and the methylthio-pyrimidine (83%) collected. Recrystallised from water (10 parts), it had m.p. 191-192° (Found: C, 42.6; H, 5.9; N, 32.85. $C_6H_{10}N_4S$ requires C, 42.35; H, 5.9; N, 32.95%).

This free base was converted into the hydrochloride. Recrystallised from methanol (30 parts) it had m.p. 280-281° and showed no m.p. depression when mixed with the methylation product of 2,4-diamino-6-methylthiopyrimidine (as the hydrochloride).

Desulphurisation of 2(4)-Amino-1,4(1,2)-dihydro-4(2)-imino-1-methyl-6-methylthiopyrimidine

The process was carried out in two ways.

(a) Desulphurisation with Raney nickel. The methylthio-pyrimidine hydrochloride (0.5 g.) and ammonium chloride (0.5 g.) were dissolved in water (15 ml.) at 80°. Raney nickel (ca. 5 g.) was added portionwise and the mixture refluxed and stirred for 5 hours. Evaporation of the filtered solution and extraction of the residue with hot methanol (7 ml.), yielded the desulphurised product (64%). Recrystallised from methanol (15 parts), it had m.p. 274-275°, unchanged on admixture with the methylation product of 2,4-diaminopyrimidine (as the hydrochloride).

(b) Desulphurisation with chlorine. The methylthio-pyrimidine hydrochloride (0.68 g.) was suspended in anhydrous methanol (20 ml.) and dry chlorine gas passed into the stirred mixture for $\frac{1}{2}$ hour at such a rate that the temperature did not rise above 10° with external cooling from crushed ice. After stirring at 10° for a further $\frac{1}{2}$ hour and concentrating (to 10 ml.) in vacuo, the product (66%) was collected and recrystallised from 95% ethanol (60 parts), to give 2(4)-amino-5,6-dichloro-1,4(1,2)-dihydro-4(2)-imino-1-methylpyrimidine hydrochloride, m.p. 269-270° (Found: C, 25.7; H, 3.1; Cl (ionic), 15.15.

$C_5H_6Cl_2N_4.HCl$ requires C, 26.15; H, 3.05; Cl (ionic), 15.45%). This compound showed no oxidising properties when tested with potassium iodide solution.

This hydrochloride (0.2 g.), dissolved in water (4 ml.) and made alkaline to pH 12, yielded the free pyrimidine base m.p. 173° (decomp.) (Found: C, 30.9; H, 3.2; Cl, 36.8; N, 28.6. $C_5H_6Cl_2N_4$ requires C, 31.1; H, 3.15; Cl, 36.75; N, 29.0%).

4-Amino-2-methylthiopyrimidine

4-Amino-2-mercaptopyrimidine (30 g.) was dissolved in 2.5N-sodium hydroxide (95 ml.) and shaken with methyl iodide (18 ml.) for $\frac{1}{2}$ hour. The methylthio-derivative (88%), washed with water and recrystallised from a mixture of 13 parts of light petroleum (b.p. $60-80^{\circ}$) and 3 parts of ethanol, had m.p. $123-125^{\circ}$ (m.p. $125-126^{\circ}$ recorded by Wheeler and Bristol, 1905).

4-Amino-6-chloro-2-methylaminopyrimidine

4-Amino-6-hydroxy-2-methylaminopyrimidine (5 g.) and phosphoryl chloride (30 ml.) were refluxed for 1 hour. After concentrating in vacuo, the residue was poured onto ice, neutralised to pH 7 and extracted with ether (5 x 100 ml.). The dried ($MgSO_4$) extracts, on evaporation gave the chloropyrimidine (35%). Recrystallised from a mixture (1:1) of light petroleum (b.p. $80-100^{\circ}$) and ethanol (50 parts) it had m.p. $198-200^{\circ}$ (Found: C, 37.95; H, 4.55;

Cl, 22.25; N, 35.25. $C_5H_7N_4Cl$ requires C, 37.85; H, 4.45; Cl, 22.35; N, 35.35%).

4-Amino-2-methylaminopyrimidine

(a) 4-Amino-6-chloro-2-methylaminopyrimidine (0.4 g.) was hydrogenated in water (30 ml.) over 5% palladium-charcoal (0.3 g.) in the presence of magnesium oxide (0.3 g.). The mixture was heated to 100° , filtered, and the filter-cake extracted with acetone (2 x 10 ml.). 2.5N-Sodium carbonate (2 ml.) was added to the filtrate and washings, and these then taken to dryness. Extraction with hot isobutyl methyl ketone (75 ml.) gave 4-amino-2-methylaminopyrimidine (80%) m.p. $123-126^\circ$, raised on sublimation ($110^\circ/0.04$ mm.) to m.p. $131^\circ-133^\circ$ (Found: C, 48.55; H, 6.35; N, 44.65. $C_5H_8N_4$ requires C, 48.4; H, 6.5; N, 45.1%).

(b) 4-Amino-2-methylthiopyrimidine (14 g.) and 30% aqueous methylamine (50 ml.) were heated in a sealed tube for 20 hours at 155° . Evaporation and trituration of the residue with isobutyl methyl ketone (4 x 10 ml.) gave a solid (53%) m.p. $120-123^\circ$, which was recrystallised from ethyl acetate (4 parts) and sublimed, as above, to m.p. $131-133^\circ$. It was undepressed on admixture with the product from (a).

Methylation of 4-Amino-2-methylaminopyrimidine

Methyl iodide (3.75 ml.) was added to 4-amino-2-methylaminopyrimidine (1.5 g.) dissolved in methanol (2 ml.)

under reflux. After 3 hours, the solution was chilled and the product (81%) recrystallised from ethanol (10 parts) when it showed m.p. 228-231° (Found: C, 26.8; H, 3.95; I, 47.5; N, 20.8. $C_6H_{11}IN_4$ requires C, 27.1; H, 4.15; I, 47.7; N, 21.05%). The methylation product was converted into the hydrochloride by shaking with silver chloride. Recrystallised from ethanol (18 parts) it had m.p. 288-289° (Found: C, 41.35; H, 6.35; Cl, 20.25; N, 32.25. $C_6H_{11}ClN_4$ requires C, 41.25; H, 6.35; Cl, 20.3; N, 32.1%).

Methylation of 4,5-Diaminopyrimidine

4,5-Diaminopyrimidine (5 g.; Brown, 1952), methanol (80 ml.) and methyl iodide (30 ml.) were refluxed for 3 hours. Concentration to 15 ml. and chilling gave 5-amino-1,4-dihydro-4-imino-1-methylpyrimidine hydriodide (77%), with m.p. 175-177° after recrystallisation from 90% ethanol (3 parts) or absolute ethanol (20 parts) (Found: C, 23.85; H, 3.55; I, 49.9; N, 22.15. $C_5H_9IN_4$ requires C, 23.8; H, 3.6; I, 50.35; N, 22.25%). Shaking a solution of the hydriodide with silver chloride yielded the hydrochloride m.p. 213-214°, recrystallised from boiling ethanol (30 parts) by the addition of ethyl acetate (30 parts) (Found: C, 37.45; H, 5.6; N, 34.85. $C_5H_9ClN_4$ requires C, 37.4; H, 5.65; N, 34.9%). The hydrochloride was also prepared

by the desulphurisation of 5-amino-1,4-dihydro-4-imino-1-methyl-2-methylthiopyrimidine to be described below.

Methylation of 4,5-Diamino-2-methylthiopyrimidine

4,5-Diamino-2-methylthiopyrimidine (5 g.), prepared by the method of Albert and Brown (1954), methanol (30 ml.), and methyl iodide (20 ml.) were refluxed for 1 hour.

5-Amino-1,4-dihydro-4-imino-1-methyl-2-methylthiopyrimidine hydriodide (31%) obtained from the chilled solution, was recrystallised from ethanol (30 parts), after which it had m.p. 234-235° (Found: C, 24.25; H, 3.7; I, 42.7; N, 18.6. $C_6H_{11}IN_4S$ requires C, 24.15; H, 3.7; I, 42.55; N, 18.8%). Silver chloride converted it into the hydrochloride which was recrystallised from methanol (23 parts) or water (3 parts) and had m.p. 276-277° (Found: C, 34.7; H, 5.45; N, 26.95. $C_6H_{11}ClN_4S$ requires C, 34.85; H, 5.35; N, 27.1%).

Desulphurisation of 5-Amino-1,4-dihydro-4-imino-1-methyl-2-methylthiopyrimidine

(a) Hydrolytic: The pyrimidine hydriodide (1 g.) was refluxed in 6N-hydrochloric acid (50 ml.) for 5 hours, and the solution then evaporated to dryness. The residue was redissolved in water (5 ml.), purified with charcoal, and the solution adjusted to pH 9. Recrystallised once more from water (20 parts), the pyrimidone (53%) had

decomp. $250-255^{\circ}$ (Brown, 1955, records 4,5-diamino-1-methyl-2-pyrimidone as decomposing above 220°). The hydrolysis product yielded a picrate. Recrystallised from water (150 parts) it had m.p. 239° (decomp.) unaltered by admixture with the picrate from authentic 4,5-diamino-1-methyl-2-pyrimidone (Found: C, 35.45; H, 3.1; N, 25.45. $C_{11}H_{11}N_7O_8$ requires C, 35.75; H, 3.0; N, 25.55%).

This hydrolysis product (0.25 g.) was further confirmed as the pyrimidone by condensation in water (8 ml.) with glyoxal monohydrate (0.15 g.), to give 3-methyl-2-pteridone monohydrate (0.24 g.). Recrystallised from water (35 parts) it had decomp. from 212° , and m.p. ca. 280° (decomp.) unchanged when mixed with the authentic pteridone prepared by Albert, Brown and Wood (1956). (Found: C, 46.8; H, 4.55; N, 31.0. Calc. for $C_7H_6N_4O \cdot H_2O$: C, 46.65; H, 4.5; N, 31.1%).

(b) Reductive. Raney nickel (ca. 5 g.) was added portionwise to a solution of the pyrimidine hydrochloride (0.5 g.) and ammonium chloride (0.5 g.) in water (15 ml.) at 80° . After a 4 hours refluxing, the mixture was filtered, the filtrate evaporated, and the residue extracted with boiling ethanol (15 ml.). Addition of light petroleum (b.p. $80-100^{\circ}$; 10 ml.) to the extract, precipitated ammonium chloride, while a further addition (8 ml.) furnished the

desulphurised product 5-amino-1,4-dihydro-4-imino-1-methylpyrimidine hydrochloride (28%) in the form of glistening plate-like crystals. Recrystallised from ethanol (30 parts) by the addition of ethyl acetate (30 parts), it had m.p. 213° unchanged on admixture with the methylation product of 4,5-diaminopyrimidine (as the hydrochloride) described earlier.

Methylation of 2,4,6-Triaminopyrimidine

2,4,6-Triaminopyrimidine (5 g.), synthesised by Traube's method (1904), was refluxed with methanol (60 ml.) and methyl iodide (12 ml.) for 6 hours. The solid (47%) was recrystallised from water (7 parts) to give 2,4-diamino-1,6-dihydro-6-imino-1-methylpyrimidine hydriodide (or tautomers), m.p. $309-310^{\circ}$ (Found: C, 22.5; H, 3.8; I, 47.45; N, 26.05. $C_5H_{10}N_5I$ requires: C, 22.5; H, 3.75; I, 47.5; N, 26.2%).

Methylation of 2-Amino-4-hydroxypyrimidine (Isocytosine)

Isocytosine (1.1 g.), prepared according to Caldwell and Kime (1940), was heated under reflux with methanolic sodium methoxide (12 ml.; from sodium, 0.23 g.) and methyl iodide (1.5 ml.) for $1\frac{1}{2}$ hours. After evaporation, the residue was dissolved in water (25 ml.), shaken with silver carbonate (2.7 g.), and filtered. The filtrate was adjusted to pH 7 with hydrochloric acid and again clarified.

Extraction of the residue from evaporation with hot ethanol (2 x 100 ml.) and concentration of the extracts to 20 ml. gave a solid (0.5 g.). Concentration of the mother-liquors to 5 ml. gave a further yield (0.2 g.). Paper chromatography in butanol-acetic acid revealed that both crops contained two substances, R_f 0.60 and R_f 0.35.

Repeated recrystallisation of the larger crop from ethanol (30 parts) gave 2-amino-3,4-dihydro-3-methyl-4-oxopyrimidine (3-methylisocytosine), m.p. 257-260°, R_f 0.60 (Angier and Curran, 1961, record a corrected m.p. 262-266° and R_f 0.62) (Found: C, 47.8; H, 5.45; N, 33.15. Calc. for $C_5H_7N_3O$: C, 48.0; H, 5.65; N, 33.6%).

Recrystallisation of the smaller crop from ethanol (15 parts) gave 2-amino-1,4-dihydro-1-methyl-4-oxopyrimidine (1-methylisocytosine), m.p. 283-285°, R_f 0.35 (Angier and Curran, 1961, gave m.p. 275-280°, R_f 0.38) (Found: C, 48.2; H, 5.55; N, 33.25%). Ultraviolet spectroscopy further established the identity of the above isomers with those prepared by Angier and Curran.

2-Chloro-4-methylamino- and 4-chloro-2-methylamino-pyrimidines

2,4-Dichloropyrimidine (40 g.), prepared by the method of Whittaker (1951), was added to a 7.5% solution of methylamine in ethanol (300 ml.) cooled in ice to 5°.

The pressure bottle was stoppered and contents stirred during the initial reaction which raised the temperature to 35°. After a further 18 hours at room temperature, the mixture was evaporated to dryness, suspended in water (200 ml.) containing ammonia (S.G. 0.9; 15 ml.), and rapidly steam distilled. 4-Chloro-2-methylamino-pyrimidine (10%) obtained from the steam distillate, was recrystallised from ethanol (4 parts) and had m.p. 123-124° (Found: C, 41.9; H, 4.3; Cl, 24.8; N, 28.85. $C_5H_6ClN_3$ requires C, 41.8; N, 4.2; Cl, 24.7; N, 29.25%).

The aqueous residue, on chilling, gave 2-chloro-4-methylaminopyrimidine (30%). Recrystallised from water (10 parts) it had m.p. 128-129° which was depressed 35° on admixture with the 4-chloro isomer (Found: C, 41.65; H, 4.3; Cl, 24.55; N, 29.0%).

4-Methoxy-2-methylaminopyrimidine

4-Chloro-2-methylaminopyrimidine (1 g.) was refluxed in methanolic sodium methoxide (15 ml.; from sodium, 0.2 g.) for 2 hours. The residue obtained on evaporation was sublimed (70°/20 mm.) to give the methoxy-pyrimidine (93%), m.p. 55-57° (Found: C, 51.6; H, 6.4; N, 30.2. $C_6H_9N_3O$ requires C, 51.8; H, 6.5; N, 30.2%).

4-Hydroxy-2-methylaminopyrimidine

4-Methoxy-2-methylaminopyrimidine (0.45 g.) and

concentrated hydrochloric acid (2.3 ml.) were heated on a steam-bath for 1 hour. Adjusted to pH 6 with 10N-sodium hydroxide and refrigerated, the solution deposited the hydroxypyrimidine (62%). Recrystallised from ethanol (30 parts) it had m.p. 214-215° (the isomeric 2-hydroxy-4-methylaminopyrimidine prepared by Brown, 1955, has m.p. 275-278°) (Found: C, 48.0; H, 5.7; N, 33.3. $C_5H_7N_3O$ requires C, 48.0; H, 5.65; N, 33.6%).

Methylation of 2,4-Diamino-6-hydroxypyrimidine

2,4-Diamino-6-hydroxypyrimidine (5.0 g.; VanAllan, 1952), methanolic sodium methoxide (30 ml.; from sodium, 0.92 g.), and methyl iodide (5 ml.) were refluxed for 1 hour. The residue on evaporation was triturated with cold water (20 ml.) to yield 2,4-diamino-6-hydroxy-5-methylpyrimidine (22%). Recrystallised from water (6 parts) or 50% ethanol (18 parts) or absolute ethanol (120 parts), it had m.p. 308-310° (decomp.), undepressed by admixture with authentic material prepared below.

Ethyl 2-Cyanopropionate

Ethyl cyanoacetate (22.6 g.) was dissolved in methanolic sodium methoxide (65 ml.; from sodium, 4.6 g.). Methyl iodide (12.5 ml.) was added slowly (10 minutes), with cooling and stirring, and the mixture refluxed for 30 minutes. Water (180 ml.) was then added, and the

product extracted with ether (3 x 80 ml.). After drying (calcium chloride), the ethereal extracts were evaporated and the oil fractionated. The ethyl 2-cyanopropionate (57%), b.p. $68-74^{\circ}/12$ mm., was shown by gas chromatography to be uncontaminated with ethyl cyanoacetate present to some extent in higher fractions. A sample redistilled for analysis had b.p. $68^{\circ}/12$ mm. (Gagnon and Boivin, 1949, record b.p. $87-88^{\circ}/15$ mm.) (Found: C, 56.3; H, 7.0; N, 11.0. Calc. for $C_6H_9NO_2$: C, 56.7; H, 7.15; N, 11.0%).

2,4-Diamino-6-hydroxy-5-methylpyrimidine

Ethyl 2-cyanopropionate (9.5 g.) was added with stirring to a mixture of guanidine hydrochloride (7.2 g.) and ethanolic sodium ethoxide (45 ml.; from sodium 3.45 g.). After refluxing for 2 hours, the mixture was evaporated to dryness. The residue, dissolved in water (30 ml.) and neutralised to pH 7 with acetic acid, gave 2,4-diamino-6-hydroxy-5-methylpyrimidine (56%). Recrystallised from water (6 parts) or from ethanol (120 parts) it had m.p. $308-310^{\circ}$ (decomp.) (Found: C, 42.7; H, 5.6; N, 39.9. $C_5H_8N_4O$ requires C, 42.85; H, 5.75; N, 40.0%).

2-Amino-4-hydroxy-6-methylaminopyrimidine

2-Amino-4-chloro-6-hydroxypyrimidine (8 g.; Boon and Leigh, 1951) and 10% ethanolic methylamine (50 ml.)

were rocked in a sealed tube at 100° for 6 hours. After refrigeration, the product (63%) was collected and recrystallised from methanol (85 parts) to m.p. $255-257^{\circ}$ (Fidler and Wood, 1957, record m.p. $255-257^{\circ}$) (Found: C, 43.0; H, 6.1; N, 39.65. Calc. for $C_5H_8N_4O$: C, 42.85; H, 5.75; N, 40.0%).

2-Amino-4-hydroxy-6-methylamino-5-nitrosopyrimidine

2-Amino-4-hydroxy-6-methylaminopyrimidine (1.0 g.) was suspended in hot water (15 ml.) at $70-80^{\circ}$. Acetic acid (0.75 ml.) was added, followed by slow addition of sodium nitrite (0.5 g.) in water (1.5 ml.). After stirring 20 minutes on the steam bath, the nitroso-pyrimidine (83%), decomp. $304-308^{\circ}$, (Fidler and Wood, 1957, give m.p. as above 300°) was filtered off from the hot mixture, and washed with water and hot methanol (Found: C, 35.5; H, 4.02. Calc. for $C_5H_7N_5O_2$: C, 35.5; H, 4.15%).

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

2-Amino-4-hydroxy-6-methylamino-5-nitrosopyrimidine (1.0 g.) suspended in water (20 ml.) was reduced by

(a) Hydrogen over Raney Nickel at $45-50^{\circ}$ and atmospheric pressure. When the hydrogen uptake was complete (1 hour), the mixture was filtered into dilute hydrochloric acid (0.006 moles) and the solution

evaporated to dryness in vacuo to yield the same hydrochloride (68%) as described in part (b).

(b) Dithionite (2.25 g.) added portionwise to the suspension at 80°. After chilling, the crystalline triamine (0.9 g.) was filtered off and dissolved in methanol (10 ml.) containing an equivalent amount of hydrochloric acid. Addition of ether (10 ml.) precipitated the hydrochloride (85%) which, recrystallised from methanol (160 parts by concentration to 20 parts), had m.p. 237-238° (decomp.) (Found: C, 31.4; H, 5.5; Cl, 18.25. $C_5H_{10}ClN_5O$ requires C, 31.35; H, 5.25; Cl, 18.5%).

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

4-Amino-6-hydroxy-2-methylaminopyrimidine (5 g.; Roth, Smith and Hultquist, 1951) was added during 30 minutes to stirred nitric acid (d. 1.5; 20 ml.) at 5-10°. After stirring for a further 30 minutes at this temperature, the mixture was poured onto ice. Washing the solid in boiling water (350 ml.) and recrystallisation from water (1500 parts), gave the nitropyrimidine (73%), m.p. 348-350° (decomp.) (Found: C, 32.7; H, 3.8. $C_5H_7N_5O_3$ requires C, 32.45; H, 3.8%).

4,5-Diamino-6-hydroxy-2-methylaminopyrimidine hydrochloride

4-Amino-6-hydroxy-2-methylamino-5-nitropyrimidine (2.5 g.) in methanol (250 ml.) was hydrogenated over Raney nickel. After filtration, the catalyst was washed with hot water (50 ml.), and the filtrate and washings were added to N-hydrochloric acid (18 ml.) and evaporated to dryness. Addition of ethanol (15 parts) to the residue (1.9 g.) dissolved in hot water (5 parts), gave the diamine hydrochloride (74%), m.p. 275-277° (decomp.) (Found: C, 31.45; H, 5.5; N, 36.3. $C_5H_{10}ClN_5O$ requires, C, 31.35; H, 5.25; N, 36.55%).

D. Physico-chemical Measurements

(a) Ionisation constants. These were determined by both the potentiometric and spectrometric methods described by Albert and Phillips (1956). The larger proportion of constants quoted in the text were determined by the potentiometric method. However, for those compounds where (a) their solubility in water at 20° was less than $10^{-3}M$, or (b) their constant was an extreme one, such that the logarithm of the dilution factor could not be made less than the pK, the more laborious spectrometric method was used.

In a number of instances, pK_a values determined potentiometrically at high pH and high concentration, have been calculated as thermodynamic constants by the use of activity terms to replace those of concentration in equation (1)

$$pK_a = pH + \log \frac{[BH] + [OH]}{[B] - [OH]} \quad (1)$$

Where pK_a is the basic constant, $[BH]$ represents the concentration of the protonated base and $[B]$ the concentration of the free base. The activity terms were calculated from equation (2)

$$a_{BH} = f \times [BH] \quad (2)$$

and the activity coefficient f from equation (3)

$$- \log f = 0.5 \left\{ \frac{\sqrt{BH}}{1 + \sqrt{BH}} - 0.2 [BH] \right\} \quad (3)$$

An important limiting factor in potentiometrically determined high basic pK_a or low acidic pK_a values, is the accuracy with which pH values above 12 can be measured using a glass electrode. For this reason, a pK_a quoted above 12 may be subject to a correction of up to + 0.2 or + 0.3 pK_a units, and is therefore only significant as a minimum lower limit of the constant.

(b) Ultraviolet absorption spectra. These traces were obtained using either the Perkin-Elmer "Spectracord" 4000A, or the Shimadzu Recording Spectrophotometers. In every instance, the extinction coefficients at wavelengths of maximum absorption and inflection were rechecked on the manually operated Hilger "Uvispek" H700/305 Quartz Spectrophotometer. Each compound for investigation was dissolved in an aqueous solution of buffer of low ultraviolet absorption and having a pH at least 2 units away from the compound's closest pK_a value. By this method, the spectral traces were of a pure species with never more than 1% contamination from another species of the molecule.

(c) Infrared absorption spectra. The spectral traces for comparison purposes, were obtained using the Perkin-Elmer 21 Spectrophotometer fitted with a sodium chloride prism and using potassium bromide discs containing 0.5% of the specimen compound.

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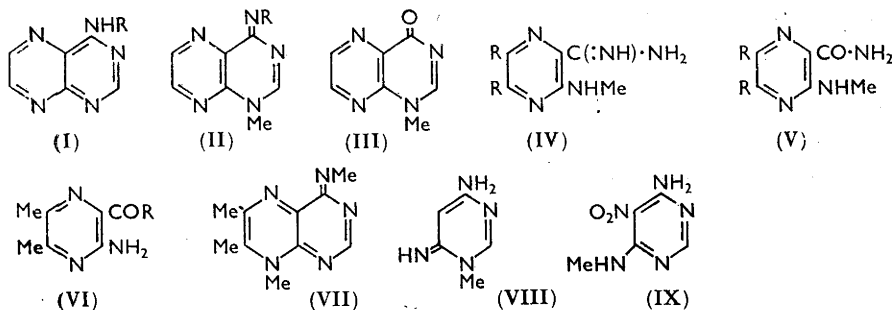
402. Pteridine Studies. Part XII.¹ The Methylation of 4-Aminopteridine.

By D. J. BROWN and N. W. JACOBSEN.

Methylation of 4-aminopteridine is found to occur on N₍₁₎. The resulting 1,4-dihydro-4-imino-1-methylpteridine, a strong base, is hydrolysed by acid to 1,4-dihydro-1-methyl-4-oxopteridine, and quickly degraded by hot alkali to 2-carbamoyl-3-methylaminopyrazine, whereby the structure is confirmed. However, with cold alkali, it yields 2-amidino-3-methylaminopyrazine which gives the above carbamoyl derivative only slowly in hot alkali. 4-Methylaminopteridine undergoes similar methylation and degradation. 4-Dimethylaminopteridine yields its 1-methiodide, the first quaternised pteridine to be described. The p*K*_a values and ultraviolet spectra of the imines and their degradation products are discussed.

An intermediate, 4-amino-1,6-dihydro-6-imino-1-methylpyrimidine, is shown to rearrange at 20° in alkali, and during nitration, to 4-amino-6-methylaminopyrimidine and its 5-nitro-derivative respectively.

With methyl iodide 4-aminopyrimidine undergoes methylation on N₍₁₎ only² to give 4,4-dihydro-4-imino-1-methylpyrimidine. Similar treatment of 4-hydroxypteridine gives a mixture of three methyl derivatives.³ However, only a single methylation product was formed from 4-aminopteridine, namely, 1,4-dihydro-4-imino-1-methylpteridine (II; R = H), as the following evidence shows.



Methylation of the amino-group was first excluded by preparation of 4-methylaminopteridine (I; R = Me) from 4-methylthiopteridine. The p*K*_a (3.7) and ultraviolet spectrum of this base differed markedly from those of the methylation product (p*K*_a 9.5), and the hydriodides gave a depression of the melting points on admixture.

When treated at 100° for a few minutes with dilute alkali, the methylation product gave the known³ 2-carbamoyl-3-methylaminopyrazine (V; R = H), thus indicating N₍₁₎ as the position of methylation in the pteridine. In an attempt to isolate the presumed intermediate pteridone (III), alkali at 0° was used. The resulting compound, which was not the expected pteridone (III), was further degraded to the pyrazine (V; R = H) only on prolonged treatment with boiling alkali. This behaviour, analysis, and a highly basic *K*_a of 9 suggested that it was 2-amidino-3-methylaminopyrazine (IV; R = H) and this was confirmed by condensation with acetylacetone to give 2-(4,6-dimethylpyrimidin-2-yl)-methylaminopyrazine. Thus two routes of alkaline degradation operate: one, rapid at 100° but negligible at 0°, in which the amino-group is hydrolysed before ring cleavage; and another, which proceeds steadily even at 0° and involves ring cleavage to amidine and

subsequent very slow hydrolysis to amide. That the presumed pteridone intermediate (III) of the first (hot) degradation could not be isolated is not surprising as it is known³ to be exceedingly alkali-labile. On the other hand, acid-degradation of the original methylation product readily gave this pteridone (III).

When 4-amino-6,7-dimethylpteridine was treated with methyl iodide, again a single product resulted. Brief alkaline treatment at 0° in this case gave the free unstable imine, and more prolonged treatment gave the amidine (IV; R = Me). Hot alkali gave an amide having structure (V; R = Me) or (VI; R = NHMe); the second possibility was excluded by further hydrolysis to a pyrazinecarboxylic acid which was not 3-amino-5,6-dimethylpyrazine-2-carboxylic acid (VI; R = OH) (prepared unambiguously by alkaline degradation of 4-hydroxy-6,7-dimethylpteridine). The acid, which still contained three methyl groups, was therefore 5,6-dimethyl-3-methylaminopyrazine-2-carboxylic acid, and so the original product was 1,4-dihydro-4-imino-1,6,7-trimethylpteridine. Acid-hydrolysis gave 1,4-dihydro-1,6,7-trimethyl-4-oxopteridine, degraded by alkali to the above pyrazine-amide.

4-Methylaminopteridine (I; R = Me) and its 6,7-dimethyl derivative (prepared *via* the methylthio-analogue) behaved similarly with methyl iodide, giving the 1-methyl-4-methylimino-derivative (II; R = Me) and its 1,6,7-trimethyl analogue, respectively, and these were degraded by acid and alkali to the pyrazines formed from the corresponding imines. Amidines, which would retain the extra methyl group, could not be isolated.

A representative 8-methylated imine was synthesised by reducing 4,6-bismethylamino-5-nitropyrimidine⁴ to the 5-amino-derivative and condensing this with biacetyl, to give 4,8-dihydro-6,7,8-trimethyl-4-methyliminopteridine (VII). Attempts to prepare a 3-methylated imine were unsuccessful. A possible intermediate, 4,5-diamino-1,6-dihydro-6-imino-1-methylpyrimidine, was first approached by methylation of 4,6-diamino-5-nitropyrimidine, but this gave 4-amino-6-methylamino-5-nitropyrimidine (IX). Methylation of 4,6-diaminopyrimidine was more promising, giving 4-amino-1,6-dihydro-6-imino-1-methylpyrimidine (VIII) (and/or its tautomer) the structure of which was confirmed by a pK_a of 12 and by non-identity with 4-amino-6-methylaminopyrimidine prepared by successive aminations of 4,6-dichloropyrimidine.⁵ Nitration of the imine under a variety of conditions, however, gave only 4-amino-6-methylamino-5-nitropyrimidine (IX), which had undergone the rearrangement familiar under alkaline conditions in the pyrimidine series^{6,2} but also known under nitrating conditions in the pyridine series.⁷ The imine (VIII) also rearranged rapidly in cold N-alkali to give 4-amino-6-methylaminopyrimidine in good yield.

4-Dimethylaminopteridine and its 6,7-dimethyl derivative, with methyl iodide, gave products which can be only quaternary methiodides. Methylation had taken place at N₁₀ to give the salt (X) because, when boiled at pH 10, the first product was converted through 1,4-dihydro-1-methyl-4-oxopteridine (III) into 2-carbamoyl-3-methylaminopyrazine (V; R = H), and the second methiodide gave 1,4-dihydro-1,6,7-trimethyl-4-oxopteridine.

The stability of 1,4-dihydro-4-imino-1-methylpteridine is progressively increased towards acid-hydrolysis by the introduction of methyl groups (see Table 1). In alkaline degradation, the methylimino- are not significantly different from the imino-derivatives but the 6,7-dimethyl grouping increases stability in each case. This is understandable on the basis of electron-contribution from methyl groups to a system which is depleted of π -electrons.

Concerning the ionisation constants, Table 2 shows that 4-aminopteridine (pK_a 3.6) is made progressively more basic by C-methylation and by extranuclear N-methylation. By these means, 4-dimethylamino-6,7-dimethylpteridine reaches pK_a 4.8. Nuclear N-methylation, however, produces an effect of another order. The iminopteridines so formed are the most strongly basic members of the series yet reported. They are some 6 pK_a units stronger than the corresponding amino-derivatives and thus resemble the analogous 1,4-dihydro-4-imino-1-methylpyrimidine and (VIII), which bear similar relation

respectively to 4-aminopyrimidine² and to 4-amino-6-methylaminopyrimidine (Table 2). The addition of methyl groups to compound (II; R = H) increases its basic strength by the usual small increments and 1,4-dihydro-1,6,7-trimethyl-4-methyliminopteridine

TABLE 1. *Optimum conditions for hydrolysis of iminopteridines.*

1,4-Dihydro-1-methyl deriv.	Reagent, time (min.), temp.	Product	Yield ^a (%)
4-Imino	2.5N-HCl; 30; 100° ^b	4-Oxo-analogue	30; 85 ^c
	2.5N-NaOH; 20; 0°	(IV; R = H)	93
	N-NaOH; 10; 100°	(V; R = H)	64
4-Imino-6,7-dimethyl	2.5N-HCl; 120; 100°	4-Oxo-analogue	79 ^b
	2.5N-NaOH; 10; 35°	(IV; R = Me)	75
	N-NaOH; 40; 100°	(V; R = Me)	70
4-Methylimino	6.3N-HCl; 240; 100° ^b	4-Oxo-analogue	55; 82 ^c
	N-NaOH; 10; 100°	(V; R = H)	60
6,7-Dimethyl-4-methylimino	6.3N-HCl; 60; 130° ^d	4-Oxo-analogue	50
	N-NaOH; 40; 100°	(V; R = Me)	70

^a Isolated. ^b Optimum conditions determined initially by paper chromatography. ^c Yield estimated by paper chromatography, elution, and spectroscopy. ^d Sealed tube.

reaches pK_a 11.4. The basic strength of its transannular-methylated isomer (VII) is less, but still marked. That compounds (II) and (VII) are strong bases, is understandable because the cations must be resonance hybrids involving a quaternised amine of the *N*-methylpyridinium type. Resonance in the neutral molecule involves separation of

TABLE 2.

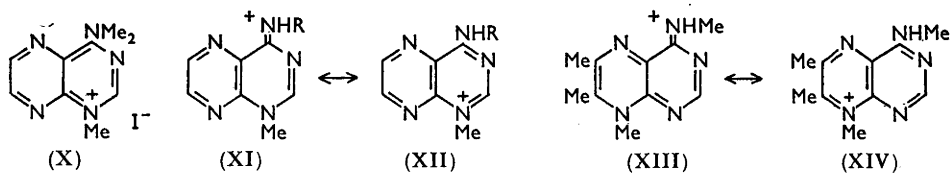
Compound	pK_a ^a and concn. (M)	λ_{max} . (m μ) ^b	pH	log ϵ
<i>Pteridine derivatives.</i>				
4-Amino ^c cation	3.56	335; 244		3.82; 4.20
4-Methylamino cation	3.70 $\pm$ 0.03 (200)	324; 229 352; 248; 226 352; 339; 251; 232	5.7 1.7	3.99; 4.10 3.88; 4.06; 4.05 3.99; 4.06; 3.68; 4.12
4-Dimethylamino ^d cation	4.33	362; 241 344 + 347 + 356; 239		3.93; 4.15 4.09 + 4.10 + 4.04; 4.19
4-Amino-6,7-dimethyl cation	3.80 $\pm$ 0.02 (400)	330; 245 337; 326; 233	6.2 1.7	3.88; 4.25 3.96; 4.05; 4.18
6,7-Dimethyl-4-methylamino cation	4.17 $\pm$ 0.03 (400)	346; 249 348; 337; 234	6.2 1.7	3.97; 4.15 4.10; 4.14; 4.19
4-Dimethylamino-6,7-dimethyl cation	4.84 $\pm$ 0.03 (400)	355; 244 353; 342; 244	6.8 2.8	3.99; 4.20 4.12; 4.16; 4.19
1,4-Dihydro-4-imino-1-methyl ^e cation	9.51 $\pm$ 0.05 (200)	350; 333; 233	12.5 ^f 7.4	3.87; 4.00; 4.14
1,4-Dihydro-1-methyl-4-methyl-imino ^e cation	10.34 $\pm$ 0.04 (100)	354; 344; 233	12.5 ^f 8.3	4.03; 4.07; 4.16
1,4-Dihydro-4-imino-1,6,7-trimethyl ^e cation	10.47 $\pm$ 0.06 (200)	344; 330; 237	8.5 13.1 ^f	3.98; 4.06; 4.18
1,4-Dihydro-1,6,7-trimethyl-4-methylimino ^e cation	11.43 $\pm$ 0.05 (100)	354; 341; 255; 237	8.5	4.06; 4.10; 3.87; 4.15
4-Dimethylamino-1-methyl-pteridinium iodide ^g		352; 243		4.11; 4.17
4-Dimethylamino-1,6,7-trimethylpteridinium iodide ^g		347; 246		4.19; 4.23
4,8-Dihydro-6,7,8-trimethyl-4-methylimino cation	6.64 ^h $\pm$ 0.05 (600)	383; 284; 241; 215 410; 295; 268; 233	8.5 4.0	3.68; 4.23; 4.06; 4.27 4.03; 4.00; 4.06; 4.09
1,4-Dihydro-1,6,7-trimethyl-4-oxo ^j cation	1.73 $\pm$ 0.04 ^k	340; 325; 243; 234 322; 308	5.0 -1.0	3.92; 4.00; 4.06; 4.17 3.87; 4.04

TABLE 2. (Continued.)

Compound	pK_a^a and concn. (M)	$\lambda_{max.}$ (m μ) ^b	pH	log ϵ
<i>Pyrazine derivatives.</i>				
2-Amidino-3-methylamino cation	8.98 $\pm$ 0.06 (200)	361; 256 359; 264	11.0 7.0	3.76; 4.13 3.69; 3.93
2-Amidino-5,6-dimethyl-3-methyl- amino cation	9.45 $\pm$ 0.03 (200)	370; 261 370; 272	11.5 7.4	3.85; 4.11 3.77; 3.92
2-(4,6-Dimethylpyrimidin-2-yl)-3- methylamino cation	2.93 $\pm$ 0.03 (100)	375; 263; 223 417; 259; 223	5.7 0.9	3.85; 4.00; 4.01 3.85; 4.03; 4.07
2-Carbamoyl-3-methylamino cation	2.11 $\pm$ 0.04 (200)	371; 256 361; 247	5.0 0	3.74; 4.15 3.77; 4.15
2-Carbamoyl-5,6-dimethyl-3- methylamino cation	2.70 $\pm$ 0.02 (400)	377; 263 380; 255	5.0 0	3.88; 4.17 3.96; 4.20
2-Amino-3-carboxy-5,6-dimethyl proton lost proton gained	4.46 $\pm$ 0.04 (200) >1 ^k	343; 248 372; 250	7.0 -1.0	3.87; 4.00 4.01; 4.08
2-Carboxy-5,6-dimethyl-3-methyl- amino proton lost proton gained	4.82 $\pm$ 0.04 (200)	365; 259 386; 257	7.0 -1.0	3.83; 4.13 3.90; 4.13
<i>Pyrimidine derivatives.</i>				
4-Amino-6-methylamino cation	6.32 $\pm$ 0.02 (200)	260; 221 268; 225	8.2 4.2	3.72; 4.58 4.13; 4.38
4-Amino-6-methylamino-5-nitro cation	2.75 $\pm$ 0.01 ^k	348; 227 341; 238	5.0 -1.0	3.98; 4.17 3.83; 4.36
4,5-Diamino-6-methylamino cation	5.93 $\pm$ 0.01 (100)	279; 216 286; 222	8.0 4.0	4.02; 4.47 4.04; 4.22
4-Amino-1,6-dihydro-6-imino-1- methyl ^e cation	11.98 $\pm$ 0.04 (100)	265; 221	14.0 ^f 10.0	4.03; 4.52

^a Potentiometric titration (see Albert and Phillips, *J.*, 1956, 1294) in water at 20°. ^b Inflections in italics. ^c Albert, Brown, and Cheeseman, *J.*, 1951, 474. ^d *Idem*, *J.*, 1952, 4219. ^e Spectra measured on buffered solutions of hydrochloride. ^f Solution too unstable for measurement of spectrum. ^g Spectrum in unbuffered water and corrected for iodide ion. ^h Cf. 5-6 for 2,8-dihydro-2-imino-6,7,8-trimethylpteridine (Fidler and Wood, *J.*, 1957, 4157). ⁱ Cf. 1,4-dihydro-1-methyl-4-oxopteridine (ref. 11). ^k Spectrometrically determined at $0.25 \times 10^{-4}M$ (cf. footnote a).

charges and is therefore small enough to be neglected. Hence, to explain the high pK_a values, it will suffice to discuss factors which increase resonance in the cations. The molecule (II) gives a resonant cation having the extreme forms (XI) and (XII). Both are



highly stable, (XI) because it contains only Kekulé and paraquinonoid structures,⁸ and (XII) because the positive charge is on the most electron-rich nitrogen atom.⁹ The resonance hybrid of the cation of (VII) should be rather less stable because (XIII) has only orthoquinonoid forms (which are not as low in energy as are paraquinonoid forms),⁸ and (XIV) has the positive charge on a nitrogen atom far less rich in electrons.⁹ Thus although compounds (II) and (VII) are both highly basic, the former is the stronger.

2-Aminopyrazine is weakly basic¹⁰ (pK_a 3.14), and 2-methylaminopyrazine might reasonably be expected to be a little more basic. The addition of an amide grouping in 2-carbamoyl-3-methylaminopyrazine lowers the pK_a to 2.1, and the apparent figure falls to 1.5 when a carboxy-group replaces the amide. However, it is possible that the amino-acid

s zwitterionic, and therefore the figure for proton gain is a measure of the enhanced acidic strength of the carboxy-group, and the figure above 4 (proton loss) is a measure of basic strength. The corresponding amidinopyrazines (IV) are quite strong bases (pK_a 9), but on cyclization to a pyrimidinylpyrazine, their pK_a falls to 2.9.

In their ultraviolet spectra, 4-aminopteridine and its closely similar 6,7-dimethyl derivative, both as neutral molecule and cation, show the usual progressive bathochromic shift of the long-wavelength band with extranuclear *N*-mono- and di-methylation. This shift is also evident in changing from the cations of 1,4-dihydro-4-imino-1-methylpteridine and its 6,7-dimethyl derivative to the methylimines. It is reasonable to expect protonation of 4-aminopteridine to occur at the same site as methylation does, provided that steric factors do not influence the latter. The implication of N_1 as basic centre is independently upheld by the close similarity of the cationic spectra of the amino-, methylamino-, and dimethylamino-pteridines to those of the imine cations and the two quaternary iodides in water. Exceptional is the transannular imine (VII) which, with its bathochromic shift of the long-wavelength band, bears much the same relation to the intra-annular imines as does 4,8-dihydro-6,7,8-trimethyl-4-oxopteridine¹¹ to 1,4-dihydro-1,6,7-trimethyl-4-oxopteridine. Unlike the other pyrazines in Table 2, the pyrimidinyl-methylamino-derivative shows a strong bathochromic shift of its long-wavelength band on forming the visibly yellow monocation.

EXPERIMENTAL

1,4-Dihydro-4-imino-1-methylpteridine.—4-Aminopteridine¹² (0.75 g.) and methyl iodide (7.5 ml.) were heated at 140° for 4 hr. The solid was extracted with boiling ethanol (100 ml.), and the extract treated with charcoal and evaporated to 20 ml. Addition of hot light petroleum (b. p. 60–80°; 20 ml.) and recrystallisation from a similar mixture produced the yellow *imine hydriodide* (78%), m. p. 255° (Found: C, 28.9; H, 3.0; I, 43.8. $C_7H_8IN_5$ requires C, 29.1; H, 2.8; I, 43.9%). This salt was shaken for 3 hr. with silver chloride (1 part) in water (30 parts). The filtrate was evaporated and the residue recrystallised from ethanol (80 parts) to give 79% of the *hydrochloride*, m. p. >300° (Found: C, 42.5; H, 4.1; N, 35.0. $C_7H_8ClN_5$ requires C, 42.5; H, 4.1; N, 35.4%).

1,4-Dihydro-4-imino-1,6,7-trimethylpteridine.—4,5,6-Triaminopyrimidine¹³ (7.9 g.) and biacetyl (4.5 ml.) were refluxed in methanol (200 ml.) for 2 hr. The solid was recrystallised from water (160 parts), to give 90% of 4-amino-6,7-dimethylpteridine, m. p. 295° (made by another route,¹⁴ also m. p. 295°). It was methylated as above (89%). The *imine hydriodide*, recrystallised from methanol (20 parts), had m. p. 264° (Found: C, 34.0; H, 3.9; I, 40.0; N, 21.9. $C_9H_{12}IN_5$ requires C, 34.1; H, 3.8; I, 40.0; N, 22.1%). The *hydrochloride* had m. p. 290° (decomp.) (Found: C, 48.0; H, 5.4; N, 31.1. $C_9H_{12}ClN_5$ requires C, 47.9; H, 5.4; N, 31.0%).

4-Methylaminopteridine.—4-Methylthiopteridine¹⁵ (0.7 g.) and 3% alcoholic methylamine (35 ml.) were refluxed for 2 hr. After refrigeration, the solid (95%) was recrystallised from water (30 parts) to give 4-methylaminopteridine, m. p. 251–252° (Found: C, 52.2; H, 4.35; N, 42.9. $C_7H_7N_5$ requires C, 52.2; H, 4.4; N, 43.45%). Its *hydriodide* (from ethanol, 40 parts) had m. p. 234–235° (Found: C, 29.25; H, 2.8; I, 43.95; N, 24.2. $C_7H_8IN_5$ requires C, 29.1; H, 2.8; I, 43.9; N, 24.2%).

1,4-Dihydro-1-methyl-4-methyliminopteridine.—4-Methylaminopteridine was treated with methyl iodide as above, to give the orange *methyliminopteridine hydriodide* (75%), m. p. 274° (vac.) (Found: C, 31.6; H, 3.4; I, 41.75; N, 22.8. $C_8H_{10}IN_5$ requires C, 31.7; H, 3.3; I, 41.9; N, 23.1%). The *hydrochloride* made from it (91%) was recrystallised from 23 parts of a mixture of ethyl acetate 70%, ethanol 20%, and water 10% (Found: C, 45.4; H, 4.9; Cl, 16.7. $C_8H_{10}ClN_5$ requires C, 45.4; H, 4.8; Cl, 16.75%).

1,4-Dihydro-1,6,7-trimethyl-4-methyliminopteridine.—4,5-Diamino-6-methylthiopyrimidine¹⁵ (5 g.) and biacetyl (2.75 ml.) in methanol (38 ml.) were refluxed for 10 min. The solid (62%) was recrystallised from water (35 parts), to give 6,7-dimethyl-4-methylthiopteridine, m. p. 120–121° (Found: C, 52.2; H, 4.9; S, 15.75; N, 27.1. $C_9H_{10}SN_4$ requires C, 52.4; H, 4.9; S, 15.55; N, 27.2%). Treatment with boiling ethanolic methylamine as above, and recrystallisation from ethanol (20 parts), gave 6,7-dimethyl-4-methylaminopteridine (95%), m. p. 223° (Found:

C, 57.15; H, 5.8; N, 36.6. $C_9H_{11}N_5$ requires C, 57.1; H, 5.9; N, 37.0%). Its *hydriodide* (from 1:1 ethyl acetate-ethanol; 15 parts) had m. p. 218—220° (Found: C, 33.8; H, 3.7; I, 40.1; N, 22.0. $C_9H_{12}IN_5$ requires C, 34.1; H, 3.8; I, 40.0; N, 22.1%). Methylation as for the analogues above, followed by recrystallisation from methanol (10 parts), gave the *methyliminopterin hydriodide* (86%), m. p. 247° (Found: C, 36.6; H, 4.2; I, 38.4; N, 20.9. $C_{10}H_{14}IN_5$ requires C, 36.3; H, 4.3; I, 38.3; N, 21.15%). The *hydrochloride* had m. p. ca. 250° (decomp.) (Found: C, 50.25; H, 5.7; N, 29.1. $C_{10}H_{14}ClN_5$ requires C, 50.1; H, 5.9; N, 29.2%).

4,8-Dihydro-6,7,8-trimethyl-4-methyliminopterin.—4,6-Bismethylamino-5-nitropyrimidine⁴ (12 g.) was hydrogenated in methanol over Raney nickel. The filtered solution was evaporated *in vacuo* and the residue twice recrystallised by dissolution in boiled-out water (30 parts) at 25°, filtration, and cooling to 0°. When the process after hydrogenation was conducted entirely within a nitrogen box, the otherwise deep red 5-amino-4,6-bismethylaminopyrimidine was a white solid, m. p. 178—180° (Found: C, 47.0; H, 7.25; N, 45.3. $C_6H_{11}N_5$ requires C, 47.0; H, 7.25; N, 45.7%). The triamine (1 g.) was refluxed for 15 min. with biacetyl (1.1 g.) in methanol (5 ml.). The solid formed on chilling recrystallised from light petroleum (20 parts), to give the yellow *pteridine*, m. p. 113—115° (Found: C, 58.7; H, 6.5; N, 34.3. $C_{10}H_{13}N_5$ requires C, 59.1; H, 6.45; N, 34.5%).

4-Dimethylamino-1-methylpteridinium Iodide.—4-Methylthiopteridine¹⁵ (8 g.) was refluxed for 2 hr. with 5% alcoholic dimethylamine (280 ml.). After evaporation, the residue was extracted with boiling light petroleum (b. p. 60—80°; 3 × 2 l.), and refrigeration gave 4-dimethylaminopterin (94%), m. p. 165—166° (lit.¹⁶ 164—165°, by another route). The *hydriodide* [from 1:3 light petroleum (b. p. 60—80°)-ethanol; 85 parts] had m. p. 244—245° (Found: C, 31.8; H, 3.4; I, 41.8; N, 23.0. $C_8H_{10}IN_5$ requires C, 31.7; H, 3.3; I, 41.9; N, 23.1%). The base (0.35 g.) and methyl iodide (0.62 ml.) in methanol (6.2 ml.) were refluxed for 2 hr., the solvent was removed, and the residue recrystallised from 3:2 light petroleum (b. p. 80—100°)-ethanol (225 parts). The yellow quaternary *iodide* (52%) had m. p. 227—229° (Found: C, 33.7; H, 3.85; I, 40.15; N, 21.8. $C_9H_{12}IN_5$ requires C, 34.1; H, 3.8; I, 40.0; N, 22.1%).

4-Dimethylamino-1,6,7-trimethylpteridinium Iodide.—6,7-Dimethyl-4-methylthiopteridine (8.3 g.) was aminated with dimethylamine as above. The crude product was extracted with light petroleum (b. p. 60—80°; 2 × 1 l.) giving 4-dimethylamino-6,7-dimethylpteridine (90%), m. p. 138—140° (Found: C, 59.3; H, 6.4; N, 34.3. $C_{10}H_{13}N_5$ requires C, 59.1; H, 6.45; N, 34.5%). Its *hydriodide* had m. p. 206—207° (Found: C, 36.5; H, 4.4; I, 38.8; N, 20.9. $C_{10}H_{14}IN_5$ requires C, 36.3; H, 4.3; I, 38.3; N, 21.15%). After the base (0.2 g.) and methyl iodide (0.31 ml.) in ethyl acetate (20 ml.) had been kept at 20° for 12 hr., the crystals (8%) were collected, washed with ethyl acetate, and dried *in vacuo*. The quaternary *iodide* had m. p. 153—154° (Found: C, 38.3; H, 4.7; I, 37.2; N, 20.2. $C_{11}H_{16}IN_5$ requires C, 38.3; H, 4.7; I, 36.8; N, 20.3%).

1,4-Dihydro-1-methyl-4-oxopteridine.—Formed as indicated in Table 1, the pteridone was purified by extraction from the dry residue with isobutyl methyl ketone and sublimation at 170°/0.01 mm. It had m. p. 222°, unaltered by admixture with authentic material.³ With alkali it gave 2-carbamoyl-3-methylaminopyrazine.

1,4-Dihydro-1,6,7-trimethyl-4-oxopteridine.—The acid solutions (see Table 1) were treated with charcoal, adjusted to pH 7, and evaporated to dryness, and the residues recrystallised from water (20 parts), to give the *oxopteridine*, m. p. 216—217° (Found: C, 56.5; H, 5.4; N, 29.0. $C_9H_{10}ON_4$ requires C, 56.8; H, 5.3; N, 29.45%). It was also obtained in 46% yield by boiling a solution (adjusted to pH 10) of 4-dimethylamino-1,6,7-trimethylpteridinium iodide (0.4 g.) in water (2.5 ml.) for 4 min.

2-Amidino-3-methylaminopyrazine.—1,4-Dihydro-4-imino-1-methylpteridine *hydriodide* (2.54 g.) was triturated with ice-cold 2.5N-sodium hydroxide (25 ml.) for 5 min. The solid (97%) was filtered off, washed with ice-water (3 × 5 ml.), and recrystallised from light petroleum (b. p. 60—80°; 300 parts), to give the *amidine*, m. p. 108—110° (Found: C, 47.65; H, 5.9; N, 46.1. $C_6H_9N_5$ requires C, 47.7; H, 6.0; N, 46.3%). Its *hydrochloride*, from 1:3 ethanol-light petroleum (300 parts), had m. p. 204° (Found: C, 38.45; H, 5.4; Cl, 18.8; N, 37.0. $C_6H_{10}ClN_5$ requires C, 38.4; H, 5.4; Cl, 18.9; N, 37.3%).

2-Amidino-5,6-dimethyl-3-methylaminopyrazine.—1,4-Dihydro-4-imino-1,6,7-trimethylpteridine *hydriodide* (0.5 g.) was added to 2.5N-sodium hydroxide (5 ml.) at 0°, precipitating the free base. The temperature was raised to 35° during 5 min., the base dissolved, and an o

pared. On trituration it solidified, and recrystallised from light petroleum (b. p. 60–80°; 5 parts) to give the *amidine* (75%), m. p. 131–132° (Found: C, 53.5; H, 7.2; N, 39.1). $\text{C}_8\text{H}_{13}\text{N}_5$ requires C, 53.6; H, 7.3; N, 39.1%).

2-Carbamoyl-3-methylaminopyrazine.—This pyrazine arose from alkaline hydrolysis of 4-dihydro-4-imino-1-methylpteridine or its methylimino-analogue (see Table 1), or (in 30% yield) by boiling a 4% aqueous solution of 4-dimethylamino-1-methylpteridinium iodide at H 10 for 4 min. In each case the solid had m. p. 196° undepressed on admixture with authentic material.³

2-Carbamoyl-5,6-dimethyl-3-methylaminopyrazine.—Formed as in Table 1, from an imine (1.4 g.) and sodium hydroxide solution (10 ml.), and recrystallised from water (300 parts), the *carbamoylpyrazine* had m. p. 164° (Found: C, 53.4; H, 6.75; N, 31.1. $\text{C}_8\text{H}_{12}\text{ON}_4$ requires C, 53.3; H, 6.7; N, 31.1%). This amide (0.2 g.) was stirred at 100° for 2 hr. with *N*-sodium hydroxide (20 ml.). The solution was acidified to pH 1 and evaporated to dryness. The residue was extracted with, and then recrystallised from, light petroleum (b. p. 60–80°; 165 parts), giving 45% of 5,6-dimethyl-2-methylaminopyrazine-3-carboxylic acid, m. p. 146° (Found: C, 52.7; H, 5.9; N, 23.15. $\text{C}_8\text{H}_{11}\text{O}_2\text{N}_3$ requires C, 53.0; H, 6.1; N, 23.2%).

4-Hydroxy-6,7-dimethylpteridine¹² (1.5 g.) was refluxed in 10*N*-sodium hydroxide (20 ml.) for 4 hr. Treatment as above and recrystallisation from water (110 parts) gave 2-amino-5,6-dimethylpyrazine-3-carboxylic acid (20%), m. p. 210–211° (cf. 209–210°, by another route¹⁷) (Found: C, 49.9; H, 5.3; N, 24.95. Calc. for $\text{C}_7\text{H}_9\text{O}_2\text{N}_3$: C, 50.3; H, 5.4; N, 25.1%).

2-(4,6-Dimethylpyrimidin-2-yl)-3-methylaminopyrazine.—2-Amidino-3-methylaminopyrazine hydrochloride (1.25 g.), acetylacetone (2.65 g.), and potassium carbonate (1.8 g.) in water (15 ml.) were shaken at 45° for 8 hr. After evaporation *in vacuo* the residue was boiled with ethanol (2 × 50 ml.), and the extract taken to dryness. Recrystallisation from light petroleum (b. p. 60–80°; 50 parts) gave the *pyrimidinylpyrazine* (40%), m. p. 89° (Found: C, 61.2; H, 6.15; N, 32.35. $\text{C}_{11}\text{H}_{13}\text{N}_5$ requires C, 61.4; H, 6.1; N, 32.5%).

4-Amino-1,6-dihydro-6-imino-1-methylpyrimidine.—4,6-Diaminopyrimidine¹⁸ (5 g.) and methyl iodide (12 ml.) in methanol (25 ml.) were refluxed for 3 hr. The *iminopyrimidine hydriodide* (89%) crystallised from ethanol (90 parts) as needles, m. p. 284° (Found: C, 23.8; H, 3.6; I, 50.5. $\text{C}_5\text{H}_9\text{IN}_4$ requires C, 23.8; H, 3.6; I, 50.35%). It was converted with silver chloride into the *hydrochloride* which after recrystallisation from ethanol (35 parts) had m. p. 268–269° (Found: C, 37.5; H, 5.65; Cl, 22.15; N, 34.5. $\text{C}_5\text{H}_9\text{ClN}_4$ requires C, 37.4; H, 5.65; Cl, 22.1; N, 34.9%). This differed from the isomeric 4-amino-6-methylaminopyrimidine *hydrochloride*, m. p. 213–214°, prepared from the base⁵ and recrystallised from ethanol (20 parts) (Found: C, 37.45; H, 5.6; Cl, 22.2; N, 34.7%).

The above imine hydriodide (1 g.) was dissolved in *N*-sodium hydroxide (12.5 ml.). After 6 hr. at 20°, the crystals (58%) were removed and the filtrate was adjusted to pH 8–9 with sulphuric acid. After vacuum-evaporation the residue was boiled with ethyl acetate (50 ml.), and the extract on evaporation gave a second crop (29%). Recrystallised from water (15 parts), the product had m. p. 209–211°, undepressed on admixture with 4-amino-6-methylaminopyrimidine⁵ (Found: C, 48.3; H, 6.4; N, 45.2. $\text{C}_5\text{H}_8\text{N}_4$ requires C, 48.4; H, 6.5; N, 45.1%). The hydrochloride (m. p. 213°) prepared from it was identical with the authentic salt above.

4-Amino-6-methylamino-5-nitropyrimidine.—(a) 4,6-Diamino-5-nitropyrimidine¹⁸ (4 g.) and methyl iodide (40 ml.) were heated at 140° for 6 hr. An aqueous solution of the residue from evaporation was adjusted to pH 8–9 and after recrystallisation from water (330 parts) the nitro-compound had m. p. 248–250° undepressed by admixture with authentic material.¹⁹ (b) 4-Amino-1,6-dihydro-6-imino-1-methylpyrimidine hydrochloride (2.4 g.) in concentrated sulphuric acid (11 ml.) was evacuated at 20° to remove hydrogen chloride. Nitric acid (*d* 1.5; 4.8 ml.) was added with stirring at 0°, and the mixture was heated for 30 min. at 40°, then poured on ice. Addition of ammonia gave the nitro-compound (85%). Hydrogenation in methanol over Raney nickel followed by sublimation at 120°/0.01 mm. gave pale yellow 4,5-diamino-6-methylaminopyrimidine (60%), m. p. 187–189° (Found: C, 43.2; H, 6.65; N, 50.0. $\text{C}_5\text{H}_9\text{N}_5$ requires C, 43.15; H, 6.5; N, 50.3%).

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THE UNIQUE TRANSANNULAR METHYLATION OF
2-AMINO-4-HYDROXYPTERIDINE

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AMINO- and hydroxy-pteridines have invariably methylated on a nuclear-nitrogen atom of the ring bearing the substituents. Thus 4-amino-¹, 4-methylamino-¹, 2,4-diamino-², 4-amino-2-hydroxy-², 2-hydroxy-³, and 4-hydroxy-³, pteridines all methylate entirely on N₍₁₎ with the exception of the last example which also gives an O- and N₍₃₎-methyl derivative. Moreover, 6-hydroxy-, 7-hydroxy-, and 6,7-dihydroxy-, pteridines give respectively 5-, 8-, and 5,8-di-, methylated derivatives³.

On the other hand, 2-amino-4-hydroxypteridine (I), which is the fundamental nucleus of most known natural

¹ D.J. Brown and N.W. Jacobsen, J. chem. Soc. 1978 (1960).

² D.J. Brown and N.W. Jacobsen, unpublished work.

³ A. Albert, D.J. Brown, and H.C.S. Wood, J. chem. Soc. 2066 (1956).

pteridines, is now shown to undergo transannular methylation yielding 2-amino-4,8-dihydro-8-methyl-4-oxopteridine (VI). This phenomenon may be of particular interest if suggestions of N₍₈₎-substitution in natural pteridines (as in riboflavine) prove well founded⁴.

2-Amino-4-hydroxypteridine (I) and methanolic methyl iodide at 100° for 12 hours yield a single ruby-coloured hydroiodide, C₇H₈IN₅O. Since methylation on N₍₅₎ is precluded by valency, the base must have one of the five structures (II-VI). These have now all been unambiguously prepared.

4-Hydroxy-2-methylaminopteridine (II), dec. 374-378°, resulted from condensing glyoxal with 4,5-diamino-6-hydroxy-2-methylaminopyrimidine, made from 4-amino-6-hydroxy-2-methylaminopyrimidine⁵ via its 5-nitro derivative. Compound (II) was also made from (V) in alkali by a now familiar type of rearrangement^{6,7}. 2-Amino-4-methoxypteridine (III),

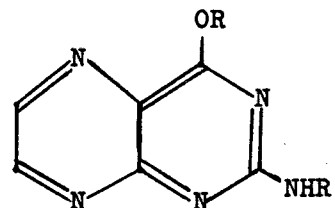
⁴ Inter alia., H.S. Forrest and H.K. Mitchel, J. Amer. chem. Soc. **76**, 5658 (1954); T. Masuda, T. Kishi, M. Asai, and S. Kuwada, Chem. pharm. Bull. **7**, 366 (1959); R. Tschesche, F. Korte, and L. Reichle, Z. Naturforsch. **10B**, 346 (1955); E.C. Taylor and H.M. Loux, J. Amer. chem. Soc. **81**, 2474 (1959).

⁵ B. Roth, J.M. Smith, and M.E. Hultquist, J. Amer. chem. Soc. **73**, 2864 and 2869 (1951).

⁶ D.J. Brown, E. Hoerger, and S.F. Mason, J. chem. Soc. 4035 (1955); D.J. Brown, Nature, in press.

⁷ W.V. Curran and R.B. Angler, J. Amer. chem. Soc. **80**, 6095 (1958).

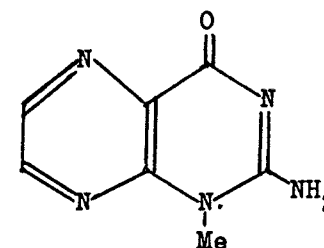
m.p. 204-205° (dec.), 2-amino-1,4-dihydro-1-methyl-4-oxopteridine (IV), m.p. 336° (dec.), and its 3,4-dihydro-3-methyl-isomer (V), dec. 319-320°, were made by similar condensations from respectively 2,4,5-triamino-6-methoxypyrimidine⁵, 2,4,5-triamino-3,6-dihydro-3-methyl-6-oxopyrimidine^{5,7}, and its 1,6-dihydro-1-methyl-isomer⁷.



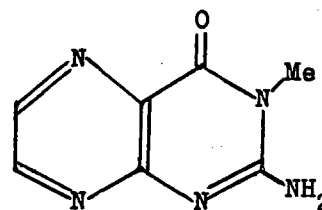
(I) R = R' = H

(II) R=H; R' = Me

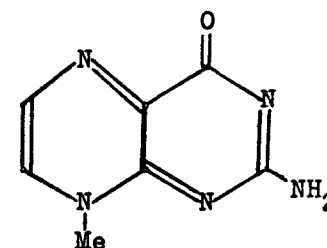
(III) R=Me; R' = H



(IV)



(V)



(VI)

In addition, mild alkaline hydrolysis of 2(4)-amino-1,4(1,2)-dihydro-4(2)-imino-1-methylpteridine² yielded (IV), and the position of methylation was confirmed by further degradation to 2-carboxy-3-methylaminopyrazine². The remaining isomer (VI) was made from 2,5-diamino-4-hydroxy-6-methylaminopyrimidine⁸ and glyoxal. The hydrochloride of

⁸ W.E. Fidler and H.C.S. Wood, J. chem. Soc. 4157 (1957).

(VI) was identified with that of methylated-(I) by its decomposition point (280-285°; unchanged by admixture), by paper chromatography in six solvent systems, and by infrared spectrum.

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1/123. *Pteridine Studies. Part XIV.¹ Methylation of 2-Amino-4-hydroxypteridine and Related Compounds.*

By D. J. BROWN and N. W. JACOBSEN.

2-Amino-4-hydroxypteridine is shown to undergo an unprecedented transannular methylation on N₆, confirmed by an unambiguous synthesis of the product. 4-Amino-2-hydroxypteridine, however, forms a 1-methyl derivative, whose structure is proved by alkaline degradation to 3-methylaminopyrazine-2-carboxylic acid. 2,4-Diaminopteridine gives a mixture of 1- and 8-methyl derivatives.

pK_a values and ultraviolet spectra of the products and related compounds indicate that the predominant tautomer of "2-amino-4-hydroxypteridine" (the fundamental unit of naturally occurring pteridines) is 2-amino-3,4-dihydro-4-oxopteridine, and that the isomeric "4-amino-2-hydroxypteridine" is probably in the form of 4-amino-1,2-dihydro-2-oxopteridine. Application of Jones's rule suggests that the 1-methyl derivative of 2,4-diamino-6,7-dimethylpteridine exists largely as the tautomer, 4-amino-1,2-dihydro-2-imino-1,6,7-trimethylpteridine.

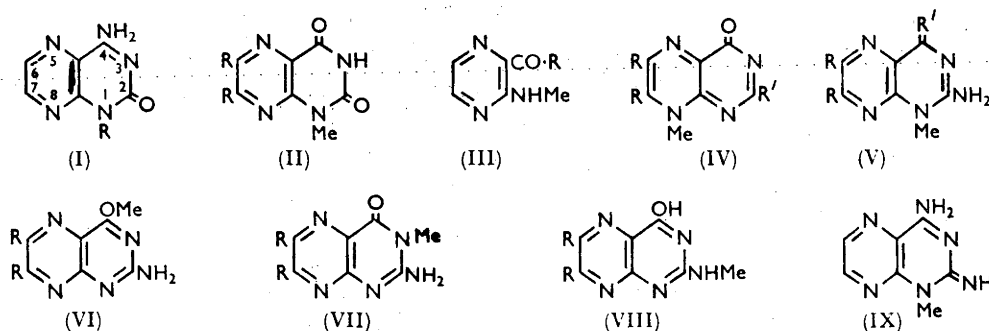
4-AMINOPTERIDINE is readily converted by methyl iodide into 1,4-dihydro-4-imino-1-methylpteridine² but attempts to isolate a product on methylation of 2-aminopteridine have so far failed. However, 4-amino-2-hydroxy-, 2-amino-4-hydroxy-, and 2,4-diaminopteridines proved more amenable to such treatment.

4-Amino-2-hydroxypteridine gave a single monomethyl derivative which was shown to be 4-amino-1,2-dihydro-1-methyl-2-oxopteridine (I; R = Me) by alkaline degradation to 1-methyl-lumazine³ (II; R = H) and to 3-methylaminopyrazine-2-carboxylic acid (III; R = OH). The structure of the pyrazine was proved by preparation from its known⁴ amide (III; R = NH₂). 4-Amino-2-hydroxy-6,7-dimethylpteridine (prepared from 4,5,6-triamino-2-hydroxypyrimidine and biacetyl), when methylated similarly, gave the 6,7-dimethyl derivative of (I), the structure of this being confirmed by alkaline hydrolysis to 1,6,7-trimethyl-lumazine⁵ (II; R = Me) and then to the known² 5,6-dimethyl-3-methylaminopyrazine-2-carboxylic acid.

As in the above cases, other amino- and hydroxy-pteridines have always been methylated only in the ring bearing the substituents. Thus 4-methylamino-,² and 2-⁴ and 4-hydroxypteridine give 1-methyl derivatives though the last gives also an *O*- and a 3-methyl derivative; 6- and 7-hydroxy- and 6,7-dihydroxy-pteridine⁴ give, respectively, 5- and 8-methyl and 5,8-dimethyl derivatives. It was, therefore, unexpected that 2-amino-4-hydroxypteridine should undergo transannular methylation, yielding exclusively 2-amino-4,8-dihydro-8-methyl-4-oxopteridine (IV; R = H; R' = NH₂). The 8-alkylated pteridines found in Nature⁶ may arise in this way. The structure of the product was shown as follows.

Because methylation at position 5 is precluded by valency, there are five possible structures (V; R = H, R' = O), (VI-VIII; R = H), and (IV; R = H, R' = NH₂). The first of these compounds was synthesised in another connexion (see below); 2-amino-4-methoxypteridine and 2-amino-3,4-dihydro-3-methyl-4-oxopteridine were prepared from pyrimidine precursors^{7,8} by processes more simple than those recently described;⁹ and 4-hydroxy-2-methylaminopteridine was made by a new route from 4-amino-6-hydroxy-2-methylaminopyrimidine by nitration, reduction to the 4,5-diamino-analogue, and condensation with glyoxal. None of these substances resembled the methylation product of 2-amino-4-hydroxypteridine, but the remaining isomer, 2-amino-4,8-dihydro-8-methyl-4-oxopteridine (made from 2,5-diamino-4-hydroxy-6-methylaminopyrimidine¹⁰ and glyoxal) proved identical with it (compared as hydrochlorides).

It seemed wise to confirm this with the 6,7-dimethyl homologues. 2-Amino-4-hydroxy-6,7-dimethylpteridine gave a single methyl derivative similar in spectra and pK_a values to the previous product (IV; $R = H$; $R' = NH_2$). Identity with four of the five possible isomers, (V; $R = Me$, $R' = O$) and (VI–VIII; $R = Me$), can be eliminated by their published constants.^{7,8} The fifth isomer¹⁰ (IV; $R = Me$, $R' = NH_2$) proved, on comparison of hydrochlorides, to be identical with the methylation product, confirming that 8-alkylation had again taken place.

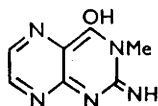


2,4-Diaminopteridine gave two methyl derivatives. As already reported,¹¹ and although it has not been obtained pure, one of these has structure (IX) or (V; $R = H$, $R' = NH$), as shown by the following degradation of impure material. With ice-cold sodium hydroxide solution it rapidly gave 2-amino-1,4-dihydro-1-methyl-4-oxopteridine (V; $R = H$, $R' = O$) which on brief hydrolysis in warm alkali gave 1-methyl-lumazine (II; $R = H$). This in turn was degraded by prolonged hydrolysis to the acid (III; $R = OH$), which had been synthesised unambiguously as mentioned above. The possibility that the initial hydrolysis product has the 4-amino-structure (I; $R = Me$) rather than (V; $R = H$, $R' = O$) is excluded by synthesis and confirmation of the structure of the former (see above), as well as by a recent unambiguous synthesis⁹ of the latter. The formation of the 1-methyl derivative (IX) parallels that of its 6,7-diphenyl derivative reported by Boon and Bratt.¹²

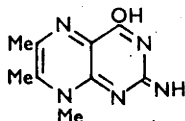
The second methylated product was obtained pure. It was not degraded by alkali and its spectrum differed from that of 4-amino-1,2-dihydro-2-imino-1,6,7-trimethylpteridine and 2,4-diaminopteridine (see Table). This precludes its being the 1-methyl derivative (IX) or 2-amino-4-methylamino- or 4-amino-2-methylamino-pteridine (the last two would resemble 2,4-diaminopteridine spectrographically). Of the remaining possibilities the 8-methylated derivative is strongly suggested by the extraordinarily long-wave-length absorption, typical of transannularly methylated imino- and oxo-pteridines shown in the Table and elsewhere.^{2,10,13–15} This is confirmed by the similarity of the spectrum of its neutral molecule to that of the anion of 2-amino-4,8-dihydro-8-methyl-4-oxopteridine, as would be expected of the 8-methyl isomer according to the R.N. Jones rule.¹⁶ Moreover, its basic strength (pK_a 8.9) is intermediate between those of the parent pteridine (pK_a 5.3) and its 1,6,7-trimethyl derivative (pK_a 11.9), in line with analogous cases of transannularly methylated iminopteridines.^{2,10} Transannular methylation was finally proved by acid hydrolysis in good yield to 2-amino-4,8-dihydro-8-methyl-4-oxopteridine (IV; $R = H$, $R' = NH_2$).

Like its simpler homologue, 2,4-diamino-6,7-dimethylpteridine gave two products on methylation. The major one was degraded by alkali to 1,6,7-trimethyl-lumazine⁵ (II; $R = Me$) and is therefore the 1-methyl derivative. The minor product could not be obtained entirely free from the major one but analysis of the mixture indicated that the constituents are isomeric, and by analogy it is assumed to be the 8-methyl derivative.

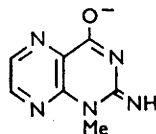
The Table shows that the amino-hydroxypteridines methylated on O, N₍₁₎, N₍₃₎, or the extranuclear N-atom, are quite weak bases, of $pK_a < 3.5$, indistinguishable in this respect from their unmethylated precursors. This indicates that the preferred tautomeric form in these cases involves an amino-form (as in VII) rather than an imino-form (as in X), which must be more strongly basic.^{2,17} On the other hand, 2,4-diamino-1,6,7-trimethylpteridine, which must involve an imine as (IX) or (V; $R' = NH$, $R = Me$), is an exceptionally strong base of pK_a ca. 12.



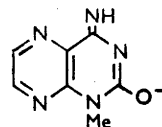
(X)



(XI)



(XII)



(XIII)

The basic strengths of 8-methylated 2-amino-4-hydroxypteridines are markedly greater than those of the 1- and 3-methyl isomers. Thus the simple derivative (IV; $R = H$, $R' = NH_2$) has pK_a 5.4, and its 6,7-dimethyl derivative has pK_a 6.1. That the basic strength of the latter approximates to that of 2,8-dihydro-2-imino-6,7,8-trimethylpteridine (5.6) led Fidler and Wood¹⁰ to postulate the hydroxy-imino-structure (XI) for the compound, and a pK_a of 8.9 was assigned to the hydroxy-group. We have been unable to confirm the latter constant, but find an anionic pK_a 12, which is more in line with those of the 1- and 3-methyl analogues that are forced into an hydroxy-imino-form such as (XII) only at high pH values. The recorded value of 8.9 may have arisen from the formation of 6,7,8-trimethyl-lumazine (pK_a 9.8) by partial hydrolysis of the amino-group during measurement. Moreover, the enhanced basic strength of compound (IV; $R = Me$, $R' = NH_2$) is in line with that of 4,8-dihydro-6,7,8-trimethyl-4-oxopteridine¹³ (IV; $R = Me$, $R' = H$), which can involve no imine but has a comparably enhanced basic strength (pK_a 4.7) when compared with the isomeric 1,4-dihydro-1,6,7-trimethyl-4-oxopteridine² (pK_a 1.7). It is, therefore unwarranted, in the face of accepted general principles,¹⁷ to assume the hydroxy-imino-form (XI) at the expense of the usual amino-oxo-forms (IV; $R = H$ or Me , $R' = NH_2$).

It is of special interest to discover the preferred tautomeric form of 2-amino-4-hydroxypteridine in aqueous solution because this structure is common to almost all of the natural pteridines. Valency permits this substance to exist in 9 forms. The five imino-forms (three hydroxy-imino-tautomers with the mobile hydrogen atom severally at positions 1, 3, and 8, as well as two imino-oxo-tautomers each with two hydrogen atoms at positions 1,3 and 1,8) are precluded by the weakly basic nature of 2-amino-4-hydroxypteridine (pK_a 2.3) because all known pteridine and related imines are much stronger bases.^{2,10} This has been confirmed by the marked similarity in spectra⁹ of 2-amino-4-hydroxy- and 4-hydroxy-2-dimethylamino-pteridine, the second of which cannot assume an imino-form. A similar conclusion can be drawn from a comparison of their 6,7-dimethyl derivatives.⁷ The one possible amino-hydroxy-form can also be eliminated by dissimilarity in spectra⁹ between 2-amino-4-hydroxy- and 2-amino-4-methoxy-pteridine. There remain the three amino-oxo-forms with hydrogen located severally at positions 1, 3, and 8. Comparison of the spectrum of 2-amino-4-hydroxypteridine as neutral molecule with those of its 1-, 3-, and 8-methyl derivatives (see Figure) leaves little doubt that the hydrogen occupies position 3 and that 2-amino-3,4-dihydro-4-oxopteridine is the predominant tautomer, at least in aqueous solution, as believed also by Pfeleiderer *et al.*⁹ This stands in contrast to its methylation at position 8 and its reported protonation⁹ at position 1, but is in line with the recorded preference^{13,18} for an α -cyclic amide at the expense of a γ -vinylogous cyclic amide where the choice exists in 1,3-diazines.

Similar treatment cannot be accorded to 4-amino-2-hydroxypteridine because the

range of methylated reference compounds is incomplete. However, its weak basic strength and the close similarity of its spectrum (neutral molecule) to that of 4-amino-1,2-dihydro-1-

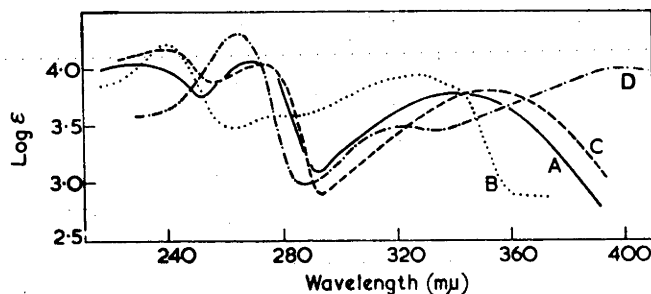
Compound	pK _a ^a and concn.	λ _{max.} (mμ) ^b	pH	log ε
<i>Pteridine derivatives</i>				
4-Amino-1,2-dihydro-1-methyl-2-oxo cation	2.96 ± 0.02 (M/400)	345; 288; 245 360; 341; 238	5.0 0.13	3.88; 3.56; 4.13 3.68; 3.87; 4.08
anion	ca. 12	350; 245 360; 345; 282; 248; 216	14.0 7.1	3.79; 4.04 3.93; 4.04; 3.53; 4.24; 4.18
4-Amino-1,2-dihydro-1,6,7-trimethyl-2-oxo cation	3.66 ± 0.03 ^c	360; 343; 264; 218 350; 337; 286; 240	0.3 7.0	3.91; 4.02; 3.82; 4.29 3.87; 3.94; 3.63; 4.08
4-Amino-2-hydroxy cation	2.99 ± 0.02 ^{c, d}	335; 237	0.83	3.90; 4.08
anion	9.97 ^e	375; 255	12.0	3.85; 4.31
2-Amino-1,4-dihydro-1-methyl-4-oxo cation	2.83 ± 0.03 (M/200)	See Figure — ^f	5.0 —	— —
anion	ca. 11.5	360; 258 See Figure	14.0 5.0	3.90; 4.13 —
2-Amino-3,4-dihydro-3-methyl-4-oxo cation	2.25 ± 0.03 ^e	— ^f	—	—
2-Amino-4,8-dihydro-8-methyl-4-oxo cation	5.42 ± 0.02 (M/200)	400; 320; 265 389; 283; 261	8.5 3.0	3.98; 3.47; 4.28 4.01; 3.99; 4.14
anion	ca. 11.5	314; 284; 239 407; 315; 267	14.0 8.5	3.91; 3.89; 4.26 4.11; 3.43; 4.35
2-Amino-4,8-dihydro-6,7,8-trimethyl-4-oxo cation	6.10 ± 0.02 (M/200) ^g	397; 285; 254	3.0	4.12; 4.13; 4.09
anion	11.97 ± 0.02 ^c	370; 308; 301; 228 ^h See Figure	14.0 5.25	3.88; 4.20; 4.18; 4.33 —
2-Amino-4-hydroxy ⁱ cation	2.31	315; <220	—	3.88; >4.1
anion	7.92	358; 251	—	3.83; 4.31
4-Amino-2-hydroxy-6,7-di-methyl cation	3.49 ± 0.03 ^e	350; 338; 256; 215	1.3	3.97; 4.03; 3.85; 4.32
anion	10.69 ± 0.04	370; 274; 254 365; 313; 288; 241	13.8 11.0	3.88; 3.78; 4.29 3.23; 3.92; 3.93; 4.11
4-Amino-2,8-dihydro-2-imino-8-methyl ^j cation	8.88 ± 0.04 ^e	412; 330; 270; 244 368; 262	6.0 14.16	3.98; 3.52; 4.24; 3.73 3.97; 4.15
4-Amino-1,2-dihydro-2-imino-1,6,7-trimethyl cation	11.90 ± 0.05 ^e	347; 335; 282; 246 364; 255; 224	7.5 8.0	4.01; 4.07; 3.64; 4.25 3.86; 4.32; 4.07
2,4-Diamino cation	5.32 ^e	345; 332; 318; 284; 240	3.0	3.90; 3.98; 3.91; 3.73; 4.10
1-Methyl-lumazine, anion ^k	8.57 ± 0.02 (M/200)	339; 281; 242	10.5	3.89; 3.54; 4.19
3-Methyl-lumazine, anion ^k	8.0	362; 268; 244; 215 334; 252; 225	10.0 7.0	3.81; 3.98; 4.25; 4.02 3.98; 3.96; 4.07
1,6,7-Trimethyl-lumazine anion	9.06 ± 0.04 (M/300)	341; 280; 244	11.5	4.03; 3.38; 4.25
6,7,8-Trimethyl-lumazine ^l anion	9.83 ± 0.04 (M/400)	404; 276; 256 363; 313; 304; 243	7.0 12.0	4.08; 4.04; 4.16 3.78; 4.31; 4.28; 4.19
Lumazine, dianion ^m	—	365; 252	—	3.78; 4.23

^a By potentiometric titration in water at 20° (cf. Albert and Phillips, *J.*, 1956, 1294). For values for other compounds see Experimental section. ^b Inflexions in italics. ^c Spectrometrically determined. ^d Cf. approx. value of 3.2 by potentiometric titration, ref. 19. ^e From ref. 19. ^f For spectrum see ref. 9. ^g Cf. 5.85 given in ref. 10. ^h The peak at 268 mμ recorded by Fidler and Wood (ref. 10) at pH 13 arises from that of the neutral molecule, present to the extent of 10% in their solution. ⁱ Constants measured on dihydriodide with balanced I⁻ concentration in reference cell. ^j Spectrum from ref. 3. ^k Prep.: ref. 14 and Birch and Moye, *J.*, 1958, 2622; values determined on supplied specimens; cf. pK_a 9.86 in ref. 15. ^l Values from Albert, Brown, and Cheeseman, *J.*, 1951, 474.

methyl-2-oxopteridine (I; R = Me) suggests that (unlike its isomer) it carries a hydrogen atom at position 1, the principal tautomer being 4-amino-1,2-dihydro-2-oxopteridine (I; R = H).

The spectrum of the neutral molecule of 4-amino-1,2-dihydro-2-imino-1,6,7-trimethylpteridine reveals the compound's tautomeric form. If allowance is made for the usual small bathochromic shift resulting from the C-methyl groups, the spectrum approximates more closely to that of the anion of 2-amino-1,4-dihydro-1-methyl-4-oxopteridine (XII) than to that of the anion of 4-amino-1,2-dihydro-1-methyl-2-oxopteridine (XIII). Application of Jones's rule¹⁶ (that the spectrum of an amino-derivative is similar to that of the anion of the corresponding hydroxy-derivative) suggests that the methylated diamine

Absorption, as neutral molecules, of: A, 2-amino-4-hydroxypteridine; B, 2-amino-1,4-dihydro-1-methyl-4-oxopteridine; C, 2-amino-3,4-dihydro-3-methyl-4-oxopteridine; D, 2-amino-4,8-dihydro-8-methyl-4-oxopteridine.



is probably best represented as 4-amino-1,2-dihydro-2-imino-1,6,7-trimethylpteridine. Simple examples supporting the validity of the rule in this series are the similarity of the spectra of 2,4-diaminopteridine (neutral molecule) to those of the anions of 4-amino-2-hydroxy- and 2-amino-4-hydroxy-pteridine and the dianion of lumazine (see Table).

EXPERIMENTAL

Analyses were done by Dr. J. E. Fildes and staff.

3-Methylaminopyrazine-2-carboxylic Acid.—1,4-Dihydro-1-methyl-4-methyliminopteridine hydrochloride² (0.45 g.) was stirred in *N*-sodium hydroxide (10 ml.) at 100° for 2.5 hr. The solution was adjusted to pH 1 with hydrochloric acid and evaporated to dryness. The powdered residue was extracted with boiling benzene (2 × 20 ml.), and the solid obtained on evaporation was recrystallized from water (50 parts) to give the *pyrazine* (72%), m. p. 182° (decomp.) (Found: C, 47.0; H, 4.5; N, 27.4. C₆H₇N₃O₂ requires C, 47.05; H, 4.6; N, 27.4%). The same product was obtained similarly from 1,4-dihydro-4-imino-1-methylpteridine² and from 3-methylaminopyrazine-2-carboxamide.⁴

4-Amino-1,2-dihydro-1-methyl-2-oxopteridine.—4-Amino-2-hydroxypteridine^{19,20} (1.35 g.), methyl iodide (5.2 ml.), and methanolic sodium methoxide (200 ml.; from sodium, 0.21 g.) were refluxed for 1 hr. Recrystallization of the solid from water (160 parts) gave the *methyl-pteridine* (75%), m. p. 324–325° (decomp.) (Found: C, 47.6; H, 4.0; N, 39.4. C₇H₇N₃O requires C, 47.45; H, 4.0; N, 39.5%).

This pteridine (0.3 g.) was stirred in *N*-sodium hydroxide (15 ml.) for 5 hr. at 100°. The solution was adjusted to pH 1 and evaporated to dryness, and the residue continuously extracted with benzene. The extract was taken to dryness and sublimed at 120°/0.02 mm. to give 3-methylaminopyrazine-2-carboxylic acid (39%), m. p. and mixed m. p. 180°. The un-sublimed part (30%), when recrystallized from ethanol and sublimed (220°/0.02 mm.), had m. p. 285°, undepressed on admixture with 1-methyl-lumazine.

4-Amino-1,2-dihydro-1,6,7-trimethyl-2-oxopteridine.—4,5,6-Triamino-2-hydroxypyrimidine sulphate²¹ (4.8 g.) and sodium hydrogen carbonate (3.4 g.) in water (350 ml.) were stirred on

the steam-bath with biacetyl (1.8 g.) for 15 min. The resulting solid (3.4 g.) dissolved in hot water (150 parts) on addition of hydrochloric acid to pH 2.5, and the 4-amino-2-hydroxy-6,7-dimethylpteridine, m. p. ca. 340° (decomp.), crystallized on slow addition of sodium acetate to pH 5 (Found: C, 50.3; H, 4.75; N, 36.15. $C_8H_9N_5O$ requires C, 50.25; H, 4.7; N, 36.6%).

This pteridine (1 g.) and methyl iodide (2.5 ml.) were refluxed for 1 hr. in methanolic sodium methoxide (25 ml.; from sodium, 0.13 g.). After chilling, the water-washed solid (84%) recrystallized from ethanol (350 parts), to give the trimethyloxopteridine, m. p. 314—316° (decomp.) (Found: C, 52.65; H, 5.4; N, 33.7. $C_9H_{11}N_5O$ requires C, 52.65; H, 5.4; N, 34.1%). Hydrolysis in boiling N-sodium hydroxide (25 parts) for 15 min. gave, after adjustment to pH 5, 1,6,7-trimethyl-lumazine (83%), m. p. 328—330° (lit.,⁵ 328—330°) (Found: C, 52.5; H, 4.8; N, 27.15. Calc. for $C_9H_{10}N_4O_2$: C, 52.4; H, 4.9; N, 27.15%). This substance (0.42 g.) was further heated with 2.5N-sodium hydroxide (16 ml.) at 200° for 4 hr. After treatment with charcoal and evaporation, the residue recrystallized from light petroleum (b. p. 60—80°; 165 parts) to give 5,6-dimethyl-3-methylaminopyrazine-2-carboxylic acid (35%), m. p. and mixed m. p. (ref. 2) 143—145°.

2-Amino-4,8-dihydro-8-methyl-4-oxopteridine.—2-Amino-4-hydroxypteridine (2 g.), methyl iodide (15 ml.), and methanol (60 ml.) were rocked at 110° for 12 hr. The tube was opened at -40° (dimethyl ether!), and the solution on evaporation to 15 ml. deposited red crystals (1.3 g.). Recrystallization from methanol (140 parts) with concentration gave the oxopteridine hydriodide, (decomp.) 265° (Found: C, 27.85; H, 2.8; I, 41.1; N, 22.55. $C_7H_8IN_5O$ requires C, 27.55; H, 2.65; I, 41.6; N, 22.95%). Treatment with silver chloride furnished the hydrochloride (from methanol, 330 parts, with concentration), m. p. ca. 285° (decomp.) (Found: C, 39.35; H, 3.9; N, 32.7. $C_7H_8ClN_5O$ requires C, 39.35; H, 3.8; N, 32.8%).

This pteridine was also made unambiguously. 2-Amino-4-hydroxy-6-methylamino-5-nitrosopyrimidine was reduced with sodium dithionite¹⁰ or hydrogenated over Raney nickel. An equivalent amount of aqueous hydrochloric acid was added to the crude base (0.9 g.), suspended in methanol (10 ml.). Addition of ether (10 ml.) precipitated 2,5-diamino-4-hydroxy-6-methylaminopyrimidine hydrochloride (85%), which, recrystallized from methanol, had m. p. 237—238° (decomp.) (Found: C, 31.4; H, 5.5; Cl, 18.25. $C_5H_{10}ClN_5O$ requires C, 31.35; H, 5.25; Cl, 18.5%). This hydrochloride (0.45 g.) was refluxed for 30 min. with polyglyoxal (0.14 g.) in methanol (30 ml.); evaporation to 10 ml. then gave a solid (50%) which after recrystallization was identified with the pteridine hydrochloride by mixed m. p., chromatography in six systems, and infrared spectroscopy.

2-Amino-4,8-dihydro-6,7,8-trimethyl-4-oxopteridine.—2-Amino-4-hydroxy-6,7-dimethylpteridine^{2a} (1.0 g.) was rocked for 5 hr. at 100° with methyl iodide (7.5 ml.) and methanol (30 ml.). Evaporation and recrystallization from methyl iodide-methanol (35:65) gave the oxopteridine hydriodide (60%), m. p. 265—270° (decomp.) (Found: C, 32.45; H, 3.5; I, 37.75; N, 20.85. $C_9H_{11}IN_5O$ requires C, 32.45; H, 3.6; I, 38.1; N, 21.0%). Silver chloride converted it into the hydrochloride which, recrystallized from 25% aqueous ethanol (26 parts), had m. p. 255—260° (decomp.) (Found: Cl, 14.8; N, 28.75. $C_9H_{12}ClN_5O$ requires Cl, 14.7%; N, 29.0%). It was also prepared in 93% yield from 2,5-diamino-4-hydroxy-6-methylaminopyrimidine hydrochloride and biacetyl (see homologue above), and the products from both routes were identified by mixed m. p., chromatography, and spectroscopy.

2-Amino-2-methoxypteridine.—2,4,5-Triamino-6-methoxypyrimidine sulphate⁷ (0.5 g.) and polyglyoxal (0.11 g.) were refluxed for 1 hr. in methanolic sodium methoxide (from sodium, 0.085 g.). The oily residue obtained on evaporation was triturated with water (5 ml.), and the resulting solid recrystallized from water (25 parts). The aminomethoxypteridine (0.14 g.) had m. p. 204—205° (lit.,⁹ 207—209°) and pK_a 3.46 $\pm$ 0.02 (m/200; 20°) (Found: C, 47.4; H, 3.85; N, 39.4. Calc. for $C_7H_7N_5O$: C, 47.45; H, 4.0; N, 39.5%).

2-Amino-3,4-dihydro-3-methyl-4-oxopteridine.—2,4,5-Triamino-1,6-dihydro-1-methyl-6-oxopyrimidine hydrochloride⁹ (0.5 g.), polyglyoxal (0.15 g.), and methanol (60 ml.) were refluxed for 1 hr. Evaporation to ca. 5 ml. gave the oxopteridine (70%) which after recrystallization from glacial acetic acid decomposed at ca. 320° (lit.,⁹ 322°) (18%) (Found: C, 47.65; H, 4.0; N, 39.35. Calc. for $C_7H_7N_5O$: C, 47.45; H, 4.0; N, 39.5%).

4-Hydroxy-2-methylaminopteridine.—4-Amino-6-hydroxy-2-methylaminopyrimidine⁷ (5 g.) was added during 30 min. to stirred nitric acid (d 1.5; 20 ml.) at 5—10°. After a further 30 min., the mixture was poured on ice. Washing the solid in boiling water (350 ml.) and recrystallization from water (1500 parts) gave 4-amino-6-hydroxy-2-methylamino-5-nitropyrimidine

(73%), m. p. 348—350° (decomp.) (Found: C, 32.7; H, 3.8. $C_5H_7N_5O_3$ requires C, 32.45; H, 3.8%). This nitro-compound (2.5 g.) was hydrogenated over Raney nickel in methanol (250 ml.), and the catalyst filtered off and washed with hot water (50 ml.). The filtrate and washings were added to *N*-hydrochloric acid (18 ml.) and evaporated to dryness. Addition of ethanol (30 ml.) to the residue (1.9 g.) in hot water (10 ml.) gave 4,5-diamino-6-hydroxy-2-methylaminopyrimidine hydrochloride, m. p. 275—277° (decomp.) (Found: C, 31.45; H, 5.5; N, 36.3. $C_5H_{10}ClN_5O$ requires C, 31.35; H, 5.25; N, 36.55%).

The diamine hydrochloride (1 g.) and polyglyoxal (0.3 g.) were refluxed in methanol (125 ml.) for 1 hr. The solid recovered by evaporation was recrystallized by dissolution in hot water (400 parts), addition of hot alcohol (400 parts), and concentration. 4-Hydroxy-2-methylaminopteridine (0.69 g.) decomposed at ca. 378° (lit.,⁹ >350°) and had pK_a 1.98 ± 0.03 (m/200), and 8.16 ± 0.02 (m/400) (Found: C, 47.3; H, 4.0; N, 39.3. Calc. for $C_7H_7N_5O$: C, 47.45; H, 4.0; N, 39.5%).

4-Amino-2,8-dihydro-2-imino-8-methylpteridine (or Tautomer).—2,4-Diaminopteridine²³ (4 g.), methyl iodide (40 ml.), and methanol (80 ml.) were rocked together at 110° for 1 hr. Evaporation gave a crude solid (8.4 g.) which was extracted with boiling methanol (40 ml.). The residue, twice recrystallized from methanol (160 parts) with concentration, gave the 8-methylpteridine dihydriodide (0.5 g.) as dark red crystals, m. p. 236—237° (Found: C, 19.5; H, 2.1; I, 58.75. $C_7H_{10}I_2N_6$ requires C, 19.45; H, 2.3; I, 58.75%). The dihydriodide (0.28 g.) was heated at 100° with *N*-hydrochloric acid (36 ml.) for 30 min. The solution was shaken with silver chloride (ca. 2 g.) for 1 hr., then filtered and the filtrate was evaporated *in vacuo* to dryness. The residue was dissolved in water (10 ml.) which was then adjusted to pH 3.5 with 5% ammonia solution and chilled. The solid precipitate (80%) recrystallized from water (80 parts), giving 2-amino-4,8-dihydro-8-methyl-4-oxopteridine hydrochloride, identified with authentic material by mixed m. p., paper chromatography in 4 solvents, and infrared and ultraviolet spectroscopy.

The initial methanol extract and the mother-liquors from the dihydriodide recrystallization gave, on evaporation, a yellow solid consisting of the 8-methylpteridine and its 1-methyl isomer. The mixture (6 g.) was dissolved in 0.5*N*-sodium hydroxide (125 ml.) and kept at 0° for 15 min. Recrystallization of the precipitate (1 g.) from water gave 2-amino-1,4-dihydro-1-methyl-4-oxopteridine, m. p. 336° (decomp.) (lit.,⁹ 335—337°) (Found: C, 47.55; H, 4.0; N, 39.5. Calc. for $C_7H_7N_5O$: C, 47.45; H, 4.0; N, 39.5%). This pteridine (0.5 g.) was refluxed in *N*-sodium hydroxide (7 ml.) for 15 min. Adjustment to pH 5, followed by sublimation and recrystallization of the solid (0.44 g.) from ethanol (260 parts), gave 1-methyl-lumazine, m. p. 285° (lit.,³ 290—291°) (Found: C, 46.95; H, 3.4; N, 31.35. Calc. for $C_7H_8N_4O_2$: C, 47.2; H, 3.4; N, 31.45%). The methyl-lumazine was refluxed in *N*-sodium hydroxide for 3 hr. and the solution then adjusted to pH 1—2. The residue obtained on evaporation was extracted with boiling benzene to give 3-methylaminopyrazine-2-carboxylic acid identical with authentic material.

4-Amino-1,2-dihydro-2-imino-1,6,7-trimethylpteridine (or Tautomer).—2,4-Diamino-6,7-dimethylpteridine was prepared according to directions by Mallett *et al.*²³ It was triturated with dilute aqueous sodium hydroxide, washed with hot water, and recrystallized from dimethylformamide (145 parts) or glacial acetic acid (20 parts). It was then chromatographically homogeneous, and despite failure by older methods²³ it gave a satisfactory analysis for carbon and hydrogen by the "rapid combustion" method in admixture with vanadium pentoxide, and for nitrogen by the Kjeldahl method (sealed tube) (Found: C, 50.75; H, 5.35; N, 43.75. $C_8H_{10}N_6$ requires C, 50.5; H, 5.3; N, 44.2%).

This pteridine (2 g.), methyl iodide (20 ml.), and methanol (40 ml.) were rocked together at 110° for 5 hr. The tube was opened at -40° (dimethyl ether!) and the solid (27%) recrystallized from ethanol (40 parts). The iminopteridine dihydriodide had m. p. 280—285° (decomp.) (Found: C, 32.5; H, 3.85; I, 38.15; N, 25.2. $C_9H_{13}IN_6$ requires C, 32.55; H, 3.95; I, 38.2; N, 25.3%).

The hydriodide (0.23 g.) was refluxed with *N*-sodium hydroxide (5 ml.) for 15 min. Adjustment to pH 5 and recrystallization from water (250 parts) then gave 1,6,7-trimethyl-lumazine (92%), m. p. and mixed m. p. 327—329°.

When the methylation mixture was heated for 1 hr. and then treated with ether (60 ml.), a solid (1.3 g.) was precipitated. Paper chromatography revealed that repeated recrystallization was ineffective in separating all the above imine from a yellow isomeric hydriodide (Found, for the mixture: C, 32.4; H, 3.95; I, 37.65; N, 25.1%).

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