T H E S I S

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by

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

C O N T E N T S.

Section I.

A New Synthesis of 2-Amino-4-Hydroxypteridines,

and

A Synthesis of 2:8-Dihydro-4-hydroxy-2-imino-8-methyl-pteridines.

Section II.

Methylation of 2:4-Dihydroxy-6- and -7-phenylpteridine and Related Topics.

S E C T I O N   I

A New Synthesis of 2-Amino-4-hydroxypteridines,

and

A Synthesis of 2:8-Dihydro-4-hydroxy-2-imino-8-
-methylpteridines.

SUMMARY

1. A new method is reported for the synthesis of 2-amino-4-
   -hydroxypteridines, namely, the condensation of 2-chloro-3-
   -methoxycarbonylpyrazines with guanidine salts. This method
   will enable the 2-position in this important type of pteridine
   molecule to be labelled isotopically at the final stage in
   the synthesis.

   A route to 2-chloro-3-methoxycarbonylpyrazines starting
   from readily available aliphatic compounds has been
   developed.

   The use of the aldehyde-binding reagent, sodium hydrogen
   sulphite in the condensation of α:β-dicarbonyl compounds with
   aminomalonamide has been shown to facilitate the reaction.
   Contrary to a previous report, the condensation of methyl
   glyoxal with aminomalonamide, with or without an aldehyde-
   -binding reagent present, gave 2-hydroxy-6-methylpyrazine-
   -3-carboxyamide. This result has since been supported by
   more recent publications by two independent research teams.

2. A method has been developed for the synthesis of 2:8-dihydro-
   -4-hydroxy-2-imino-8-methylpteridines from pyrazine derivatives
   This preliminary investigation will probably provide a
   suitable synthetic route to pteridine Mg glycosides, which
   have been postulated to occur in nature.

   The methylation of 2-hydroxy-5:6-diphenyl-3-carboxylic
   acid and its methyl ester have been studied.

INTRODUCTION.

Pteridine Syntheses.

Synthesis from more complex substances.

The earliest successful synthesis of the 1:3:5:8-
-tetra-azanaphthalene ring system (I) was carried out in
1895 by Kuhling[1]. "Tolualloxazine" (II) was oxidised to
2:4-dihydroxypteridine-6:7-dicarboxylic acid, which was
decarboxylated in two stages to 2:4-dihydroxypteridine.   No
other example of this approach is known, but it is of special
interest in that it was achieved about the same time as
Hopkins[2] carried out his investigations on the pigments of
butterfly wings, and antedated any other synthesis by twelve
years.
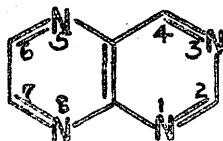
I   II
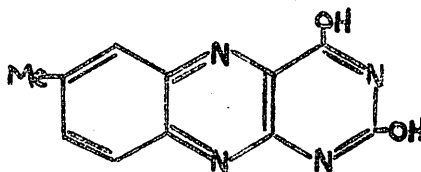
### Syntheses from pyrimidines.

The conventional methods for the synthesis of the
pteridine ring system employ a condensation of a 4:5-
-diaminopyrimidine (IV) with an α,β-dicarbonyl compound (III),
an α-halocarbonyl compound, an α-keto-alcohol or derivatives
of these compounds.   The first example of such a synthesis
was Isay's preparation[3] of 6:7-diphenylpteridine (V; R' = R² =

Ph, R^3 = R^4 = H) from 4:5-diaminopyrimidine (IV; R^3 = R^4 = H)

and benzil (III; R' = R^2 = Ph).

The reaction was more intensively studied by Sachs

and Meyerheim[4] in 1908.

III   IV   V
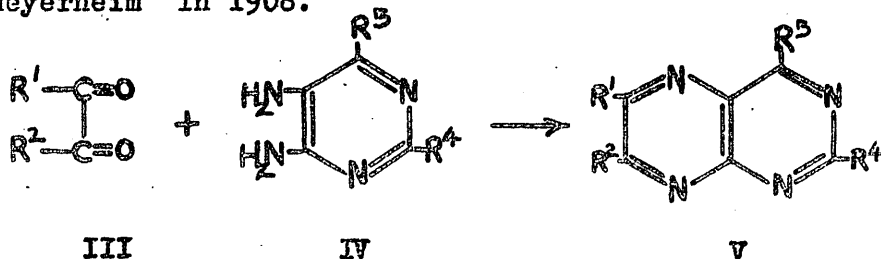
All pyrimidines containing primary amino-groups in

both the 4- and 5- positions appear to be suitable and the

reaction has been widely exploited because of the availability

of the pyrimidine intermediates. 2:5:6-Triamino-4-hydroxy-

pyrimidine (IV; R^4 = NH_2, R^3 = OH) has been used extensively

for the synthesis of 2-amino-4-hydroxypteridines, and has

provided the only route to the increasing number of naturally

occurring pteridines[5]. It is an essential intermediate in

all reported syntheses of the biologically important compound,

pteroylglutamic acid (P.G.A.)[6] (see below).

The use of N-substituted pyrimidine intermediates

such as 5:6-diamino-1:2:3:4-tetrahydro-1:3-dimethyl-2:4-

-dioxopyrimidine (VI) has been much explored[4,7,8,9] for the

syntheses of 1- and 3-alkyl-2- and -4-pteridones (e.g. VII).
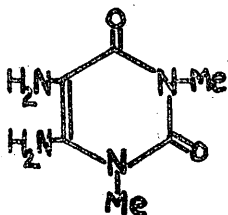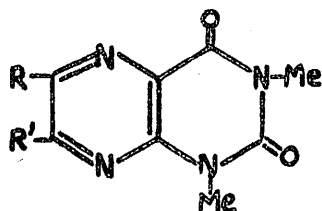
VI   VII

    The Isay reaction can be carried out under neutral, acid, or alkaline conditions; neutral conditions usually give the best yields.

    The scope of this reaction has been increased by the replacement of the dicarbonyl compounds by α-substituted carbonyl compounds. The α-halocarbonyls 1:3 dichloroacetone, α,β-dichloropropaldehyde, and α-bromotetronic acid have been condensed with 2:5:6-triamino-4-hydroxypyrimidine[10]. The first synthesis[11] of pteroylglutamic acid (XI) depended on the simultaneous condensation at pH4 of 1:2-dibromopropion-aldehyde (IX) with triamino-4-hydroxypyrimidine (X) and p-aminobenzoylglutamic acid (VIII).
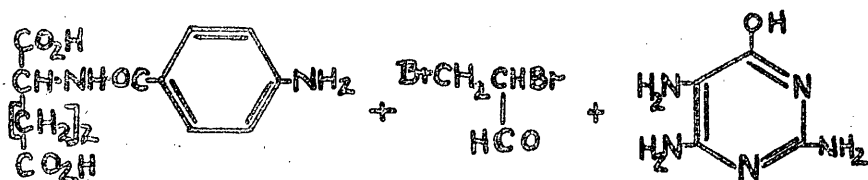
VIII   IX   X
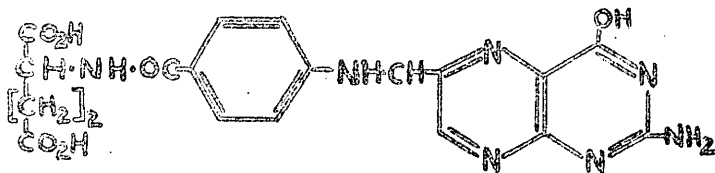
XI

The above synthesis requires the loss of two hydrogen atoms, apparently effected by disproportionation, and the yield is poor (15%), but this was improved by the addition of an oxidising agent[12,13,14]. 1:2-Dibromo-propionaldehyde (IX) has been replaced in the above Waller synthesis with 1:1-dichloro-3-bromoacetone[15] or 1:1:3--tribromoacetone[14,16]. With these reagents, loss of the elements of hydrogen halide makes it unnecessary to use an oxidising agent. If the three reactants are not present simultaneously, however, 7-substituted pteridines are sometimes obtained[11]. In a later improvement of the synthesis[17], (IX) was first condensed with pyridine to give 1-(2-bromo-2-formylethyl)-pyridinium bromide (XII), which reacted with triamino-4-hydroxypyrimidine (X) to give a quaternary salt (XIII). The latter reacted with p-amino-benzoyl-L-glutamic acid (VIII) to give pteroylglutamic acid (XI).
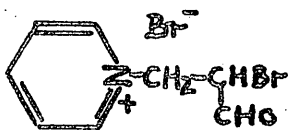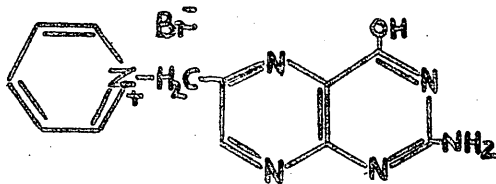
XII   XIII

Aldehydo- and keto-alcohols react with triamino-
-4-hydroxypyrimidine, but not so far as is known, with
monosubstituted derivatives of 4:5-diaminopyrimidine. The
most common reaction products are 7-alkyl-2-amino-5:6-dihydro-
-4-hydroxypteridines (XIV), which are spontaneously oxidised,
in air, to the corresponding pteridines (XV). Thus with
hydroxy-acetone and dihydroxy acetone, 2-amino-4-hydroxy-7-
-methylpteridine (XV; R = Me) was obtained[18]. In the latter
case loss of the elements of water takes the place of
dehydrogenation.

XIV   XV
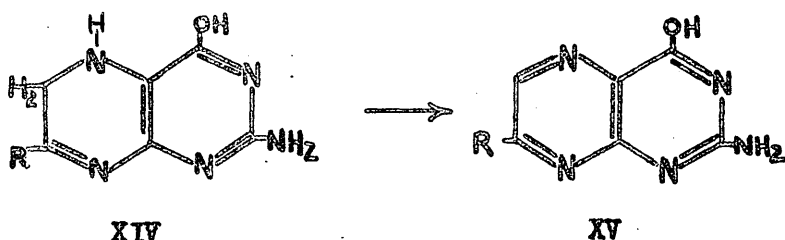
Glucose and fructose react simultaneously by both
mechanisms each giving a mixture of the same 2-amino-4-hydroxy-
-7-tri-(and -tetra-)-hydroxybutylpteridines[18,19]. In the
presence of hydrazine, glucose and fructose give, the 6-tetra-
hydroxybutyl isomer and this has been attributed to the
formation of an ozazone, which enables dehydrogenation to
precede formation of the pteridine ring[19].

Aromatic keto-alcohols (e.g. benzoin) give pairs of
stable isomeric dihydropteridines; 7:8-dihydropteridines are
formed in the presence of acetic acid and different, apparently,

5:6-dihydropteridines in its absence[20,21].

The above synthesis (i.e. using 4:5-diamino-
-pyrimidines) suffers from the major draw-back that when
the carbonyl compound employed is not symmetrical two isomers
can arise. This has led to difficulties in separation and
identification. When, however, the dicarbonyl compound is
an acid (or ester) highly acid conditions tend to favour
the formation of the 6-hydroxypteridines, whereas mildly acid
or neutral conditions tend to favour that of 7-hydroxy-
pteridines[22,23,24]. Variation of the pH of the reaction
medium may, however, only alter the relative proportions of the
different isomers formed, still leaving the problem of their
separation.

When the unsymmetrical dicarbonyl compound is neither
an acid nor an ester, the most successful attempts to
influence orientation have involved the use of aldehyde- and
ketone- binding reagents (such as hydrazine or sodium hydrogen
sulphite); the use of which tends to force an alkyl group
into the 6-position[14,18,19,25]. Thus triamino-4-hydroxy-
pyrimidine and methyl glyoxal give 2-amino-4-hydroxy-6-methyl-
pteridine if the methylglyoxal is first allowed to react with
hydrazine[18], but the 7-methyl isomer is obtained if hydrazine
is omitted[18,26].

Unambiguous syntheses (from pyrimidines).

The synthesis, utilizing 4-chloro-5-nitropyrimidines, independently developed by Polonovski and coworkers[27] and by an I.C.I. team[28], is of particular interest because it gives an unambiguously orientated 6-substituted pteridine. Thus 2:4-dichloro-5-nitropyrimidine was condensed with ethyl glycinate to give ethyl-2-chloro-5-nitro-4-pyrimidylamino-acetate (XVI). The 5-nitro group was catalytically reduced and the product was cyclised, by treatment with boiling water, to yield 2-chloro-7:8-dihydro-6-hydroxypteridine (XVII). Various dichloro- and monochloropyrimidines have been used[27,28] and the 5-nitro group has been replaced by phenylazo[29]. Dehydrogenation of the 7:8-dihydroproducts (e.g. XVII) to pteridines is readily effected by cold alkaline potassium permanganate[22].

XVI   XVII
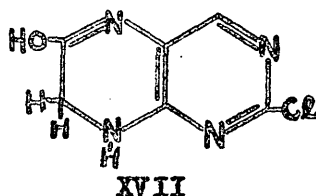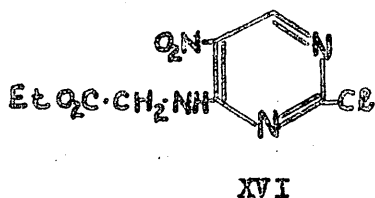
The other unambiguous synthesis involves the condensa-tion of 4-amino-5-nitrosopyrimidines (XIX; R' = R² = NH_2, or R' = R² = OH) with ketones containing an active methylene adjacent to the carbonyl group [e.g. ethyl phenyl ketone (XVIII)] giving pteridines such as (XX)[30].

XVIII   XIX   XX
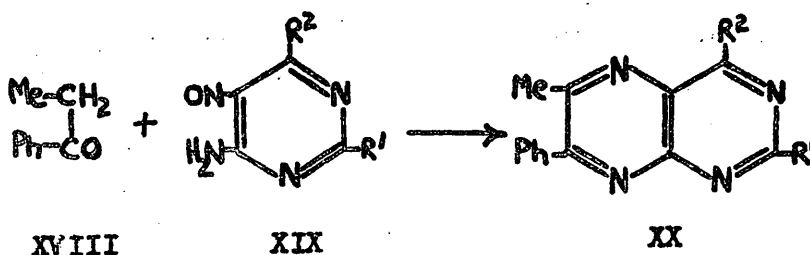
This method has been extended to the syntheses

of 7-amino and 7-hydroxypteridines (XXI; R^3 = NH_2 or OH) by

replacement of the ketone (XVIII) with arylacetonitriles and

aryacetylchlorides respectively[31]. Cyanacetic acid has also

been used to obtain 7-amino-6-carboxypteridines (e.g. XXII)[32].

At present, the scope of this reaction is limited by the lack

of monosubstituted and unsubstituted 4-amino-5-nitrosopyrimi-

dines.

XXI   XXII
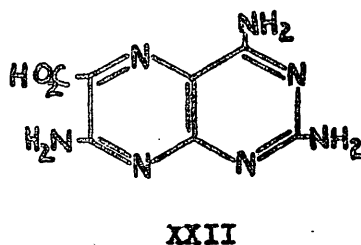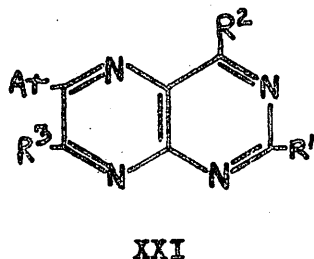
Syntheses from pyrazines.

Before commencement of this work only two methods

had been reported in the literature for the syntheses of

pteridine derivatives from pyrazine intermediates. This was

due to the limited availability of suitable pyrazines and the

relatively undeveloped nature of this field[33].

Gabriel and Sonn[54] in 1907 treated pyrazine-2:3-
-dicarboxyamide (XXIII), prepared from pyrazine-2:3-dicarboxylic
acid obtained by the oxidation of quinoxaline[35], with 2 moles
of potassium hypobromite and obtained 2:4-dihydroxypteridine
(XXIV) in poor yield.

XXIII   XXIV
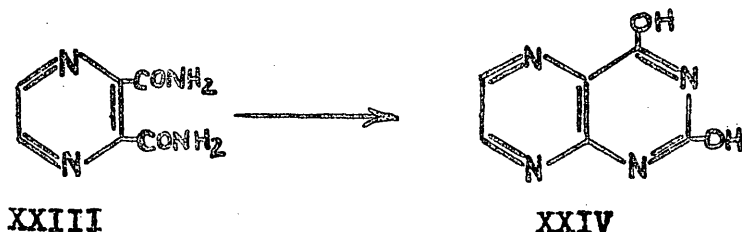
In more recent studies, Albert and coworkers[36] have
synthesised 4-hydroxypteridine (XXVI; R = OH) from 2-amino-
pyrazine-3-carboxyamide (XXV) by heating the pyrazine with
ethylorthoformate and acetic anhydride under reflux.   Likewise
2-aminopyrazine-3-carboxythioamide yielded 4-mercaptopteridine
(XXVI; R = SH)[36].

XXV   XXVI

This reaction has since been extended to the syntheses
of pteridines, N-substituted in the pyrimidine ring.   Thus
cyclisation of 2-aminopyrazine-3-carboxymethylamide (XXVII)
with a mixture of acetic anhydride and formic acid gave

3:4-dihydro-3-methyl-4-oxopteridine (XXVIII) in 47% yield[37].
The pyrazine-carboxymethylamide (XXVII) was prepared from
2:4-dihydroxypteridine (XXIV) by a series of reactions
involving initial degradation with alkali[25] to obtain 2-amino-
pyrazine-3-carboxylic acid, the ester of which was converted
to (XXVII) by treatment with aqueous methylamine[37].

XXVII   XXVIII
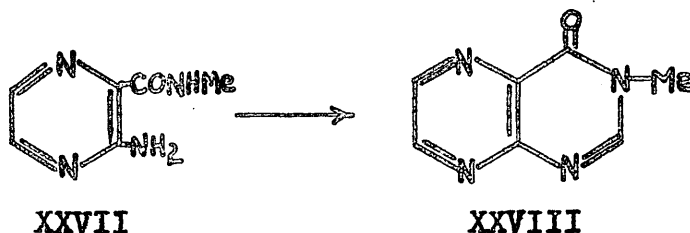
Further exploitation of this reaction has been made
possible by Taylor's studies[38,39] on the aminolytic cleavage
of 2:4-dihydroxypteridines with organic amines (primary and
secondary).   This work has made available a greater variety
of 2-aminopyrazine-3-carboxyalkyl-(or aryl-) amides.   Thus
2:4-dihydroxy-6:7-diphenylpteridine reacted with refluxing
benzylamine to give 2-amino-5:6-diphenylpyrazine-3-carboxy -
benzylamide (XXIX), which was recyclised to 3-benzyl-3:4-
-dihydro-4-oxo-6:7-diphenylpteridine (XXX) with a mixture of
formic acid and acetic anhydride (or better ethyl orthoformate
and acetic anhydride)[38,39].   Cyclisation with formamadine
yielded 4-hydroxy-6:7-diphenylpteridine, the N-benzyl group
being lost during ring closure.

XXIX   XXX
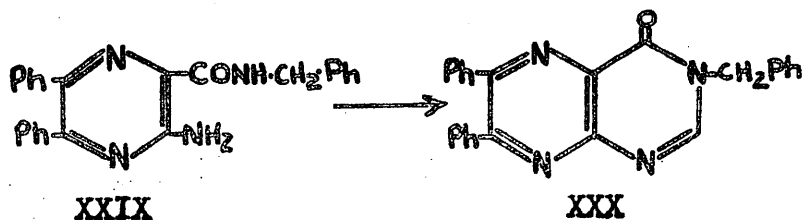
In an alternative method of cyclisation developed by Taylor[39], (XXIX) was heated with ethyl chloroformate to give 2-(ethoxycarbonyl)amino-5:6-diphenylpyrazine-3-carboxy-benzylamide (XXXI), which was cyclised to 3-benzyl-3:4-dihydro-2-hydroxy-4-oxo-6:7-diphenylpteridine (XXXII) with sodium ethoxide.

XXXI   XXXII
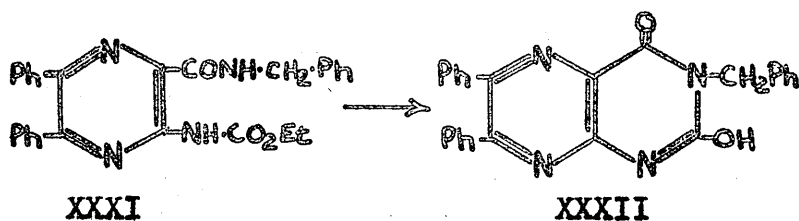
More recently Taylor has described further methods of cyclising (XXIX) and the corresponding thioamide without publishing full experimental details[40].

A few syntheses of pteridines from 2-amino-3-cyano--5:6-diphenylpyrazine (XXXIV) have also been summarily reported by Taylor[40]. This important intermediate arose from the accidental discovery of the novel cleavage of 4-mercapto-5:6-diphenylpteridine with chloroacetic acid in the presence of potassium carbonate. The mechanism of this reaction has not yet been elucidated[41].

Heating the cyanopyrazine (XXXIV) with methyliso-
thiourea hydroiodide gave 2-amino-4-hydroxy-6:7-diphenyl-
pteridine (XXXV) and not the expected 2:4-diamino derivative[40].

XXXIV   XXXV
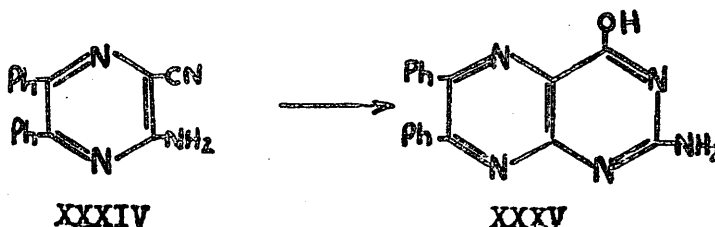
8-Substituted Pteridine Syntheses.

Because of the structural relationships between the
natural pteridines, purines and isoalloxazines it was
suggested by Todd and coworkers[42] in 1951 that pteridines
analogous to the purine nucleosides (e.g. guanosine (XXXVI)
and riboflavine (XXXVII) might be expected to occur in nature.
The 8-position of the pteridine ring corresponds to the
9-position in purines and isoalloxazines, and the most
probable substituent in 8-substituted pteridines would be
expected to be a sugar such as ribose.
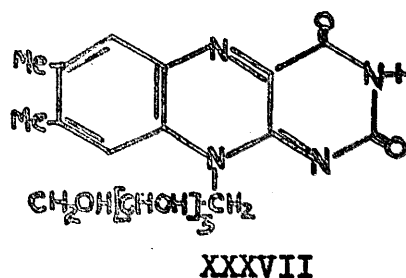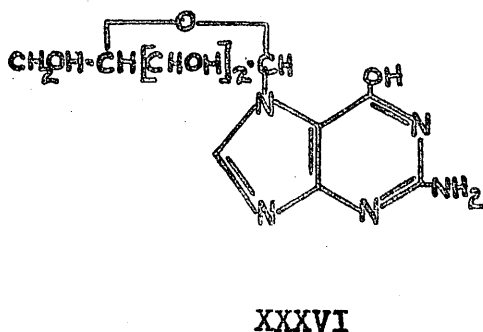
XXXVI   XXXVII

Strehler[43] has reported the isolation of the pigment,
luciferesceine, from the head of the firefly, Photinus pyralis,
and indicated that it is probably an iminoribityl pteridine.
The structure (XXXVIII) has been tentatively suggested[44].
More recently Forrest and Mitchell[45] have reported the
isolation of a yellow eye pigment from the sepia mutant of
Drosophila melanogaster and its probable identification as
the 8-lactyl derivative of 2-amino-7:8-dihydro-4-hydroxy-
pteridine-6-carboxylic acid. The modified structure (XXXIX)
has been suggested by Wood[46] to account for the absorption
at long wavelength observed in the ultra-violet (pH 11,
maxima at 268, and 440 mu; pH 1, maxima at 279, and 409 mu).

The widespread occurrence of folic acid and the
citrovorum factor[50] in nature, and the variety of combined
forms in which they appear to occur in animal tissues are
also suggestive of forms which may contain sugar moieties.
The most suitable place for the linkage of a sugar to the
citrovorum factor would be at $N_{(8)}$ since $N_{(5)}$ is formylated.
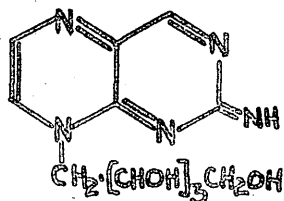
XXXVIII
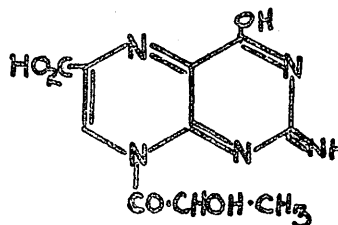
XXXIX

Several syntheses of 8-substituted pteridines from 5-amino-4-substituted aminopyrimidine intermediates have been reported. Todd and coworkers[42] cyclised 5-amino-6-anilino--2:4-dimethyl pyrimidine (XL) by conversion with chloroacetyl-chloride to the 5-chloroacetamido derivative (XLI), which on treating with silver carbonate gave 7:8-dihydro-6-hydroxy--1:4-dimethyl-8-phenylpteridine (XLII). 2:5-Diamino-6-ethyl-amino-4-hydroxypteridine (XLIII) failed to cyclise on similar treatment, but condensed with oxalic acid to give 2-amino-8--ethyl-7:8-dihydro-4:6-dihydroxy-7-oxopteridine (XLIV), and with benzoin to give 2-amino-8-ethyl-7:8-dihydro-4-hydroxy--6:7-diphenylpteridine (XLV).
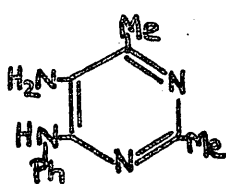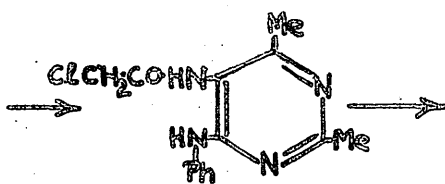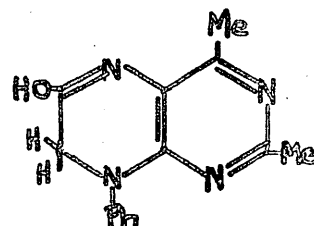
XL   XLI   XLII
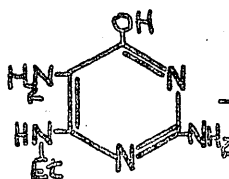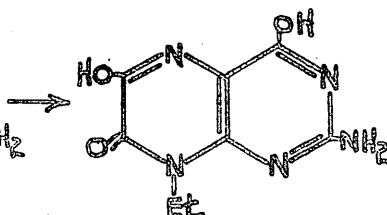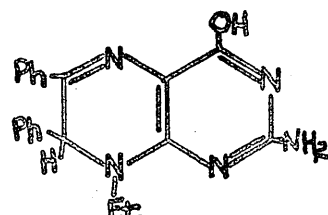
XLIII   XLIV   XLV

The last compound (XLV) was employed in the structural investigations on the citrovorum factor to show that the formyl group was attached to the $N_{(5)}$ rather than the $N_{(8)}$ postion[47].

Todd's team[42] attempted to extend their synthesis, to 2-amino-4-hydroxypteridine $N_{(8)}$ glycosides, but they were unable to prepare the appropriately substituted pyrimidine intermediates. However, they succeeded in preparing 4-amino-8-D-glucosyl-7:8-dihydro-6-hydroxy-7-oxo-2-methylthio-pteridine (XLVII) by reacting 4:5-diamino-2-methylthio-6-D-tetra-acetyl-glucosylaminopyrimidine (XLVI) with ethyl oxalate in the presence of sodium ethoxide.

XLVI   XLVII
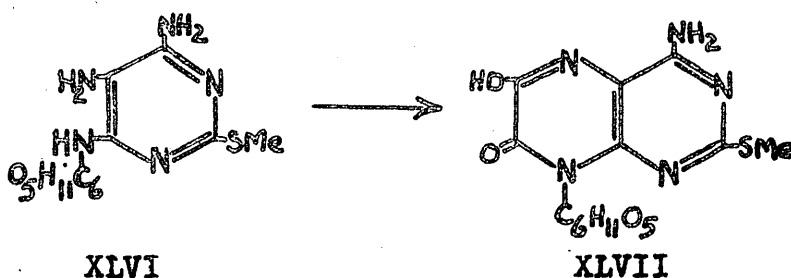
Condensation of ethyl oxomalonate with 2:5-diamino-6β-hydroxyethylamino-4-hydroxypyrimidine (XLVIII) has been shown to give the 8-β-hydroxyethyl derivative of ethyl isoxanthopterin-6-carboxylate (XLIX)[48]

XLVIII   XLIX
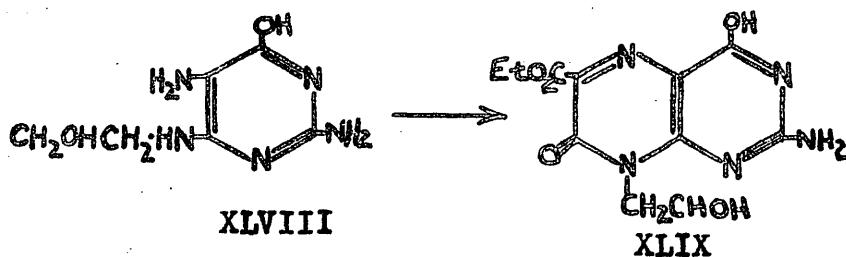
More recently Taylor and Loux[49] have previously

reported the ring closure of 5-amino-8-ethylaminopyrimidines

(e.g. L) with alloxan in dilute alkaline solution, to obtain

8-ethyl-7:8-dihydro-7-oxopteridine-6-carboxylic acids

(e.g. LI).

L   LI
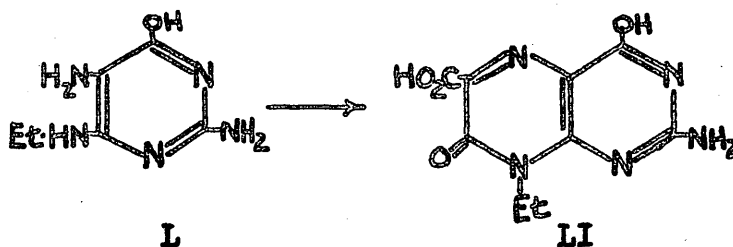
THEORETICAL.

A NEW SYNTHESIS OF 2-AMINO-4-HYDROXYPTERIDINES.

In the few reported cases where pteridines have been synthesised from pyrazine intermediates, the pyrazine-carboxyamides used, were obtained by degradation of bicyclic hetero-ring systems, which contained a preformed pyrazine ring, e.g. quinoxaline[34], or 2:4--dihydroxypteridines[36,37,38,39]. The latter were synthesised from pyrimidine intermediates in the first instance.

It therefore seemed desirable to investigate a synthesis utilizing pyrazine intermediates which could be prepared from simple and readily available aliphatic compounds.

Furthermore, while the above methods would enable the 2-position in the pteridine nucleus to be labelled isotopically in the final stage of a synthesis, they do not increase the availability of 2-amino-4-hydroxypteridines. All the naturally occurring pteridines, so far as is yet known, contain this grouping. A synthesis of this type of compound which permits introduction of a labelled atom at the final stage is likely to be of considerable importance to biochemists.

Syntheses of pteroylglutamic acid labelled with ^{14}C at the 2- and 9-positions were described by Weygand and coworkers[51,52] in 1952. The compound labelled at the 2-position (with ^{14}C) was prepared by converting radioactive barium carbonate (via a guanidine salt) to triamino-4-hydroxy-

pyrimidine labelled at postion 2.   The pyrimidine was then
used in the usual Waller synthesis[11].   It would obviously
be much better if the labelling was carried out in the last
stage of a synthesis.

It was also considered that a synthesis utilizing
pyrazine intermediates would help to overcome the problem of
the formation of a mixture of 6- and 7- isomers experienced in
the conventional Isay synthesis when unsymmetrical $\alpha:\beta$-
-dicarbonyl compounds are condensed with 4:5-diaminopyrimidines.

Early syntheses of pyrazines from simple aliphatic
compounds although numerous, are mainly of the autocondensation
type giving pyrazines, unsuitable as potential pteridine
intermediates[55].   However in 1949 Jones[54] described the
synthesis of 2-hydroxypyrazine-3-carboxyamides (LIV),
consisting of the condensation of $\alpha:\beta$-dicarbonyl compounds
(LII) with aminomalonamide (LIII).

LII   LIII   LIV
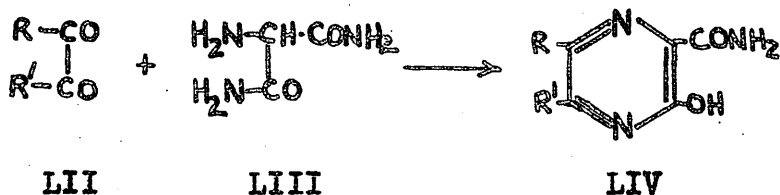
These hydroxypyrazine-carboxyamides would make
ideal starting materials for more versatile derivatives,
which could be condensed to pteridines, and also converted

to pyrazines already shown to be capable of cyclisation.

It has been shown that hydroxypyrazine (LV) and 3,5, and 6-alkyl substituted analogues can be readily converted to 2-chlorpyrazines (LVI) with phosphorus oxychloride either alone or mixed with phosphorus penta-chloride[55,56]. The effect of a carboxyamide or alkoxy-carbonyl group at the adjacent 3-position was not investigated.

LV   LVI   LVII
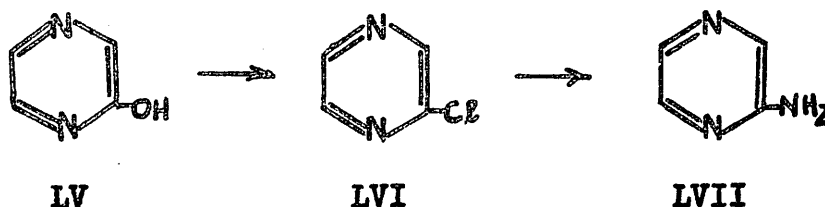
Since in pyrazine (a syn 1:4-diazine) the halogen is always ortho to a negative nitrogen atom, the monohalo-pyrazines resemble the α-pyridyl halides in the reactivity of the halogen. Although chloropyrazine gives a negative test for "active" halogen with alcoholic silver nitrate, the bromo analogue gives a positive test, and the halogen in chloropyrazines is sufficiently labile to allow these compounds to be used in general syntheses of phenols, thiols, ethers and primary amines. Treatment of chloropyrazine with 28% aqueous ammonia at 200° gives aminopyrazine (LVII) in 80% yield[55]. A similar replacement in the case of α-pyridyl chloride requires a reaction temperature of 250°

and the presence of a catalyst[57].

Esters of pyrazine-carboxylic acids (LVIII) readily react with ammonia and amines (primary and secondary) to give amides (LIX) and there are numerous references to such reactions in the literature[25,34,37,55,58]. The majority of these amides were synthesised by Dalmer and Walter[59] because of their purported physiological behaviour as analeptics.

LVIII   LIX
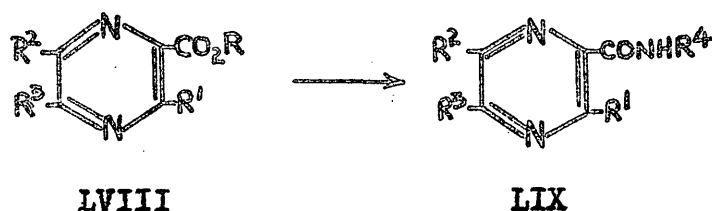
Because of the reactivity of both halo- and alkoxycarbonyl-pyrazines to amino compounds generally it was considered that 2-chloro-3-methoxycarbonylpyrazines (LX) would constitute the most favourable intermediates in a new synthesis of the pteridine ring system, when reacted with 1:1-diamino compounds (e.g. guanidine, urea etc.) under suitable conditions. It was also envisaged that these compounds could be readily converted to 2-aminopyrazine-3- -carboxyamides or N-substituted derivatives (LXI). Methods for the cyclisation of which have been outlined[36,37,39,40].

LX   LXI
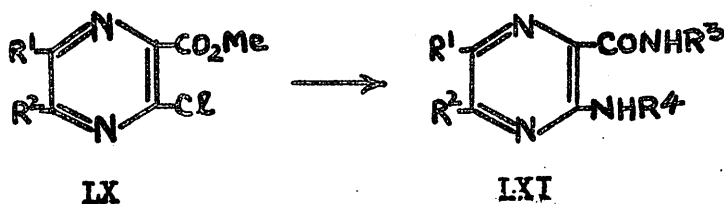
Aminomalonamide (LXVI), the key reactant in Jones'
synthesis[54] of 3-hydroxypyrazinecarboxyamides was obtained in
high yields by allowing a solution of aminomalonic ester
(LXV) in several volumes of alcohol saturated with ammonia
to stand at room temperature for several days.   The unstable
amino-ester (LXV) was best prepared from malonic ester
(LXIII) by nitrosation - according to the general method of
Cherchez[60] - to ethyl (hydroximino) malonate (LXIV), which
was catalytically reduced under pressure to the amino-ester
(LXV)[61].   This method is superior to that used by Jones,
which depended on an aluminium amalgam reduction of the
hydroximino compound and isolation of (LXV) as its
hygroscopic hydrochloride[62].   It was found that the crude
amino-ester obtained by catalytic reduction could be used
directly without purification by distillation, as the
malondiamide, which separated along with the aminomalonamide
in the last stage, was readily removed by extraction of the
crude product with warm alcohol, the aminomalonamide being

relatively insoluble.

$$H_2C:(CO_2Et)_2 \longrightarrow HON = C:(CO_2Et)_2$$

(LXIII)   (LXIV)

$$H_2N.CH:(CONH_2)_2 \longleftarrow H_2N.CH:(CO_2Et)_2$$

(LXVI)   (LXV)
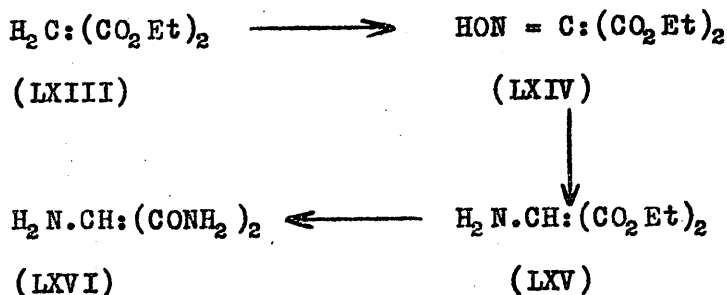
Condensation of aminomalonamide with glyoxal under the conditions described by Jones[54] gave 2-hydroxypyrazine--3-carboxyamide (LXVIII) in yields less than half of that quoted (i.e. 90%). Variation of the reaction conditions or use of polyglyoxal led to no improvement, and tarry reaction mixtures were occasionally observed. Investigation of the reaction showed that replacement of glyoxal, by its sodium hydrogen sulphite derivative (LXVII) and the use of approximately 3 moles of sodium hydroxide (12.5 $\underline{N}$) resulted in greatly improved yields (75%), when the reaction was carried out at room temperature.

Muehlmann and Day in a recent publication[63], have also reported that they could not reproduce Jones' result in this preparation. Further examination of the reaction conditions led them to use glyoxal sodium hydrogen sulphite also. They, however, avoided the use of strong alkali, and carried out the reaction at 80° in aqueous solution, to obtain an 85%

yield of the free pyrazine carboxyamide.

The pyrazine-carboxyamide (LXVIII) was hydrolysed to the carboxylic acid (LXIX) by warming on a water bath with 4N-sodium hydroxide.

LXVII   LXVI   LXVIII   LXIX
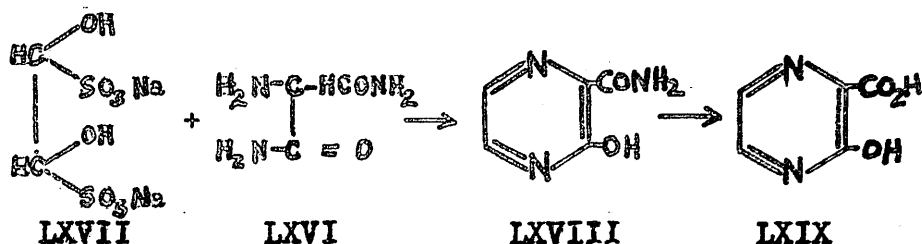
This acid (LXIX) was obtained previously from 2:4--dihydroxypteridine (LXX), by cleavage with strong alkali to 2-aminopyrazine-3-carboxylic acid (LXXI), and treatment of the latter with nitrous acid[64].

LXX   LXXI   LXIX
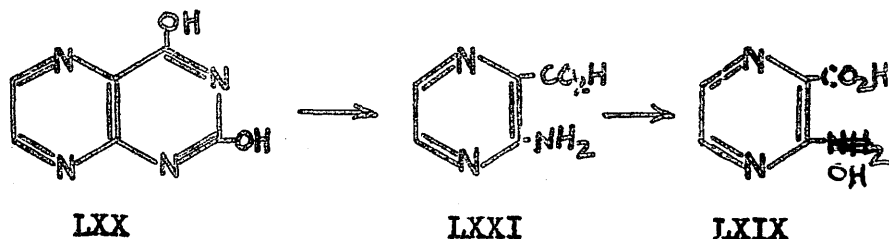
The pyrazine carboxylic acid (LXIX) was converted to the methyl ester (LXXII) by solution in boiling methanol and treatment with dry hydrogen chloride. The hydroxy-ester gave the 2-chloro derivative (LXXIII) in high yield (81%) on reaction with refluxing phosphorus oxychloride[56,65].

LXXII   LXXIII   LXXIV
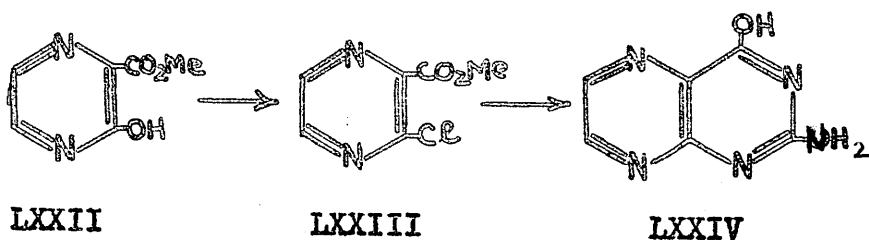
Condensation of 2-chloro-3-methoxycarbonylpyrazine (LXXIII) with free guanidine was catalysed with sodium methoxide in absolute methanol, and 2-amino-4-hydroxypteridine (LXXIV) was isolated in 20% yield, after refluxing for 30 hours. Reduction of the reaction period lowered the yield (e.g. 10 hours, 7%) and attempts to increase the yield by carrying out the reaction in a sealed tube at higher temperatures resulted in considerable decomposition and equally poor yields (e.g. 10 hours at 109°, 5%). The use of potassium tert-butoxide in boiling tert-butyl alcohol did not increase the yield. However, when an intimate mixture of the pyrazine (LXXIII) and guanidine carbonate was heated at 170° for 30 minutes, an 89% yield of the pteridine (LXXIV) was obtained.

The identity of this material with a specimen of 2-amino-4-hydroxypteridine prepared from triamino-4-hydroxy-pyrimidine (LXXV) and glyoxal[66] was established by paper chromatography and confirmed by comparison of the infra-red and ultra-violet spectra.

LXXV   LXXIV
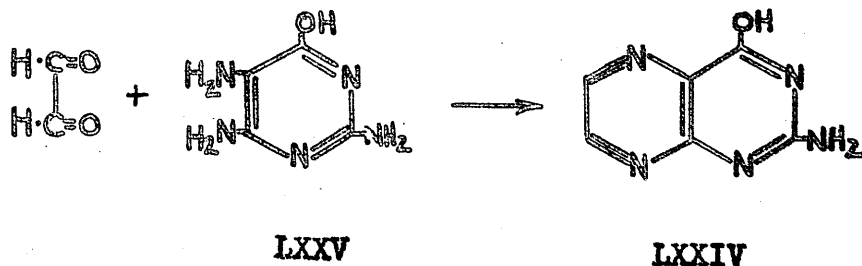
A similar fusion of urea with 2-chloro-3-methoxy-carbonylpyrazine did not give 2:4-dihydroxypteridine nor did reaction with S-methylthiourea in methanol or pyridine yield 4-hydroxy-2-methylmercaptopteridine.

The versatility of this intermediate (LXXIII) is enhanced by the fact that it is readily converted to 2-amino or 2-methylaminopyrazine-carboxyamide or -N-substituted carboxyamides by reaction with ammonia and/or alkylamines, and methods for the cyclisation of such compounds to pteridine derivatives have been reported recently[36,37,38,39]. Thus Albert and coworkers[65] have shown that ammonia at 20° selectively converted the ester to chloropyrazine-3-carboxy-amide (LXXVI) which gave 2-methylaminopyrazine-3-carboxyamide (LXXVII) on treatment with alcoholic methylamine at 120°. Cyclisation of this pyrazine to 1:4-dihydro-1-methyl-4-oxo-pteridine (LXXVIII) was effected with a refluxing mixture of formic acid and acetic anhydride[65]

LXXIII   LXXVI   LXXVII

LXXVIII
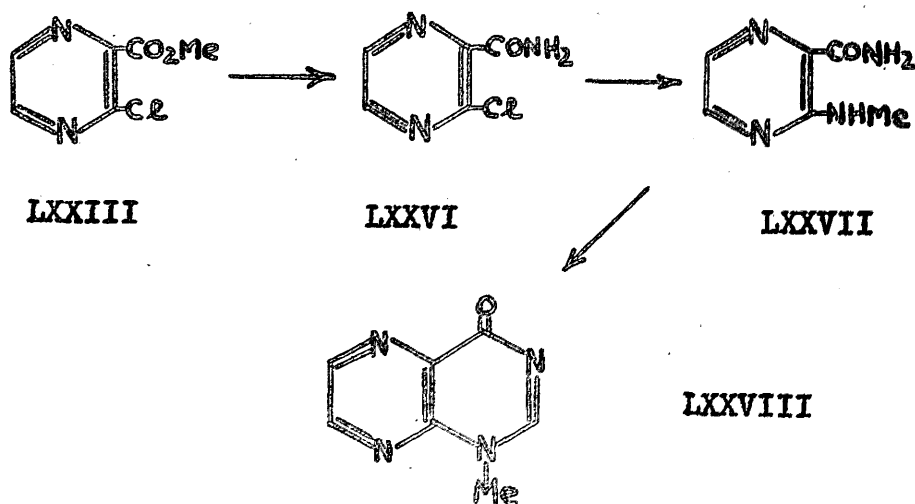
The synthesis of 2-amino-4-hydroxy-5:6-diphenylpteridine (LXXXIII) from 2-hydroxy-5:6-diphenylpyrazine-3-carboxyamide (LXXIX)[54] was carried out in a similar manner.

The hydrolysis of the pyrazine amide (LXXIX) was best effected by heating with strong ethanolic alkali in a steel bomb at 180° for 6.5 hours, because of the extreme insolubility of its monosodium salt in refluxing aqueous alkali.

Chlorination of the hydroxy-ester (LXXXI) to obtain 2-chloro-3-methoxycarbonyl-5:6-diphenylpyrazine (LXXXII) was carried out in 81% yield by heating with phosphorus oxychloride in a sealed glass tube at 160°. The use of refluxing phosphorus oxychloride, or a mixture of phosphorus oxychloride and phosphorus pentachloride at 80° proved ineffective. The observation of Karmas and Spoerri[56], that large alkyl or aryl groups in the remote 5- and 6-positions effectively hinder the replacement of the 2-hydroxyl by chlorine, when an alkyl group is at the 3-position, was supported in this case. Thus 2-hydroxy-3-methoxycarbonylpyrazine was chlorinated (see above) under conditions similar to those employed by Karmas and Spoerri for hydroxypyrazines substituted only at the 3-position with simple alkyl groups, and the 3-methoxycarbonyl group did not hinder substitution any more than a 3-ethyl group; however more vigorous conditions were required for the 5:6-diphenyl analogue.
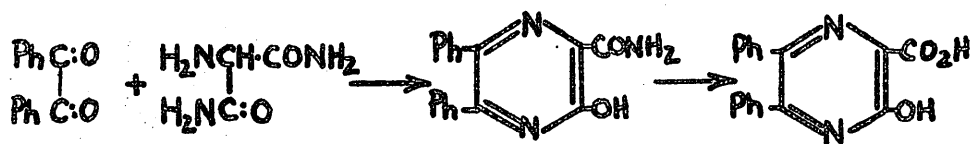
LXVI   LXXIX   LXXX
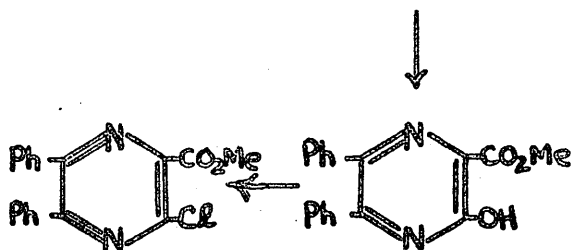
LXXXII   LXXXI
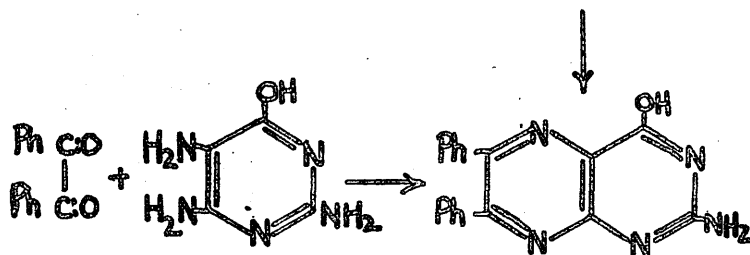
LXXV   LXXXIII
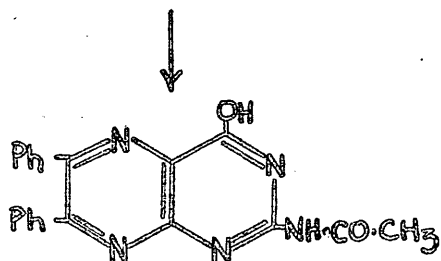
LXXXIV

The chloro-ester (LXXXII) was condensed with
guanidine carbonate by heating an intimate mixture at 170°
with the formation of 2-amino-4-hydroxy-6:7-diphenylpteridine
(LXXXIII) (70%). The identity of the pteridine was confirmed
by comparison of the infra-red and ultra-violet spectra with
that of an authentic specimen prepared[68] by condensation of
benzil with triamino-4-hydroxypyrimidine (LXXV). Increased
yields by this route were obtained by using the dihydro-
chloride salt of the pyrimidine instead of the bisulphite salt.

Acetylation of the pteridine with acetic anhydride
containing a trace of concentrated sulphuric acid gave the
2-acetamido derivative (LXXXIV), which unlike the parent
compound melted below 360°. A mixed melting point of the
acetyl derivatives from both routes showed no depression.

Attempts to condense 2-chloro-3-methoxycarbonyl-5:6-
-diphenylpyrazine (LXXXII) with guanidine in the presence
of sodium methoxide, under the conditions used for the parent
compound, gave none of the expected pteridine, but a low
melting product was formed. The acidic properties, and
analytical results indicated that this was either 2-hydroxy-
-3-methoxycarbonyl-5:6-diphenylpyrazine or 2-methoxy-5:6-
-diphenylpyrazine-3-carboxylic acid (LXXXV). It was not
identical with the hydroxy ester (LXXXI). Re-esterification
with methanol and dry hydrogen chloride to give a methyl ester

(LXXXVI) confirmed that the compound was the methoxy acid
(LXXXV).

LXXXII   LXXXV   LXXXVI

LXXXVII   LXXXVIII   LXXXIX
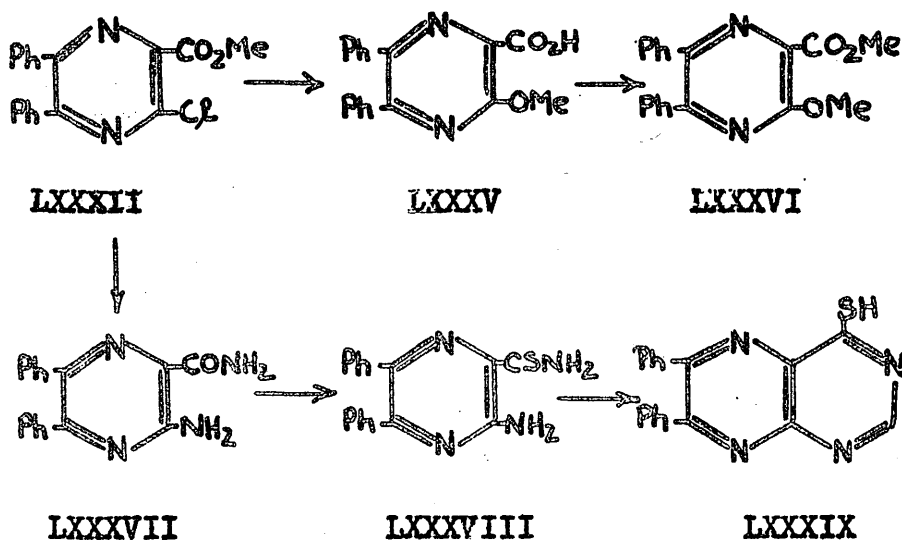
Reaction of the chloro-ester (LXXXII) with concentrated
ammonium hydroxide at 180° gave 2-amino-5:6-diphenylpyrazine-
3-carboxyamide (LXXXVII).   This compound was previously
prepared by Taylor[38] by degradation of 2:4-dihydroxy-6:7-
-diphenylpyrazine with ammonia and could be cyclised by either
of the methods previously outlined[38,39].   Taylor actually
converted (LXXXVII) to the thioamide (LXXXVIII) with
phosphorus pentasulphide before cyclisation, with a mixture of
ethylorthoformate and acetic anhydride, to obtain 4-mercapto-
-5:6-diphenylpteridine (LXXXIX).

It would appear that this new method, i.e. condensing
a chloropyrazine-ester with guanidine, could be applied to the
synthesis of 2-amino-4-hydroxy-6-methylpteridine, which is an

intermediate in one of the most flexible methods for the
synthesis of pteroylglutamic acid and its analogues[69,70].
The starting material for this synthesis would be 2-hydroxy-
-5-methylpyrazine-3-carboxyamide (XC), and this compound has
been obtained by Jones[54] from the condensation of methyl
glyoxal with aminomalonamide.

LXVI   XC   XCVI
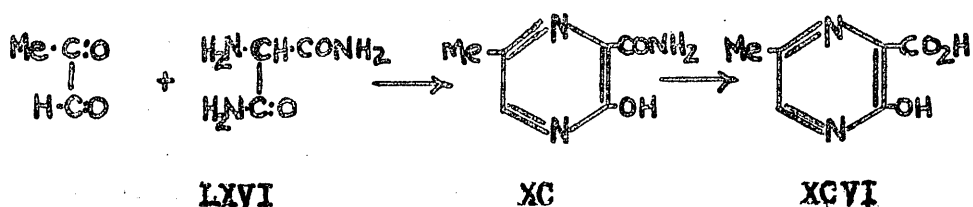
That this type of reaction gives only 3:5-disubstituted-
-2-hydroxypyrazines and none of the isomeric 3:6-disubstituted
compounds was confirmed by Spring and his coworkers[71] who
investigated the condensation of methyl glyoxal and phenyl
glyoxal with α-amino-hydroxamic acids. Thus methyl glyoxal
and DL-alanine hydroxamic acid (XCI) gave the cyclic hydroxamic
acid (XCII) which was identified by reduction to 2-hydroxy-3:5-
-dimethylpyrazine (XCIII).

XCI   XCII   XCIII
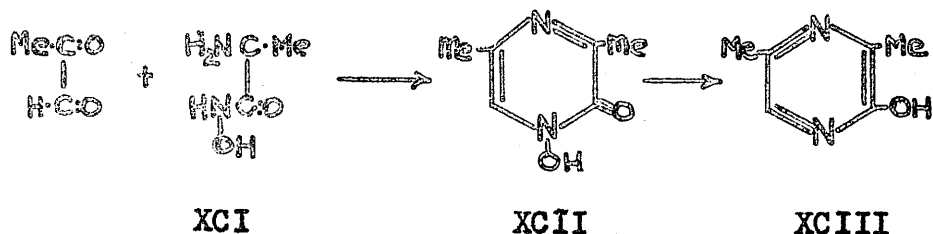
Repetition of Jones' condensation[54] of freshly prepared methyl glyoxal[72] with aminomalonamide (LXVI) did not give the product m.p. 243-244° (decomp.) and shown to be 2-hydroxy-5-methylpyrazine-3-carboxyamide (XC)[54]. There resulted instead a compound melting sharply at 219-220° (decomp.) but analysing for the pyrazine carboxyamide (XC) or its 6-methyl isomer (XCV). Hydrolysis of this new product with $5\underline{N}$-sodium hydroxide gave a carboxylic acid, m.p. 188-189° (decomp.) which agrees with the m.p. 183-184° (decomp.) recorded by Jones for 2-hydroxy-6-methylpyrazine-3-carboxylic acid (XCV), prepared from 2-amino-6-methylpyrazine-3-carboxylic acid (XCIV)[25] by treatment with nitrous acid. The 5-methyl acid (XCVI) described by Jones melts at 155-157°.

XCIV   XCV   XCVI
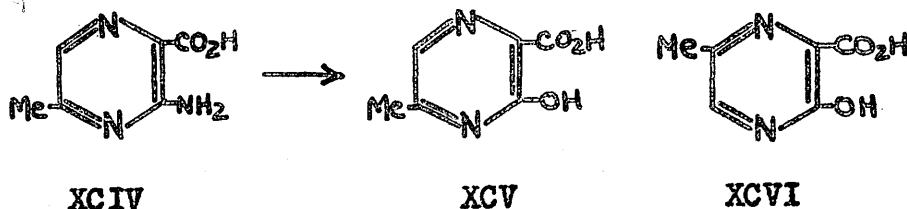
The product (m.p. 219-200°) obtained in the condensation must therefore be 2-hydroxy-6-methylpyrazine-3-carboxyamide (XCVII), i.e. the other isomer, which was not obtained by Jones[54]. Rigorous chromatographic examination of the reaction mixture failed to disclose the presence of any of the expected 5-methyl isomer.

H·C:O   H₂N·CH·CONH₂   →   (XCVII structure)   →   (XCV structure)

Me·C:O   H₂N·C:O

LXVI   **XCVII**   **XCV**
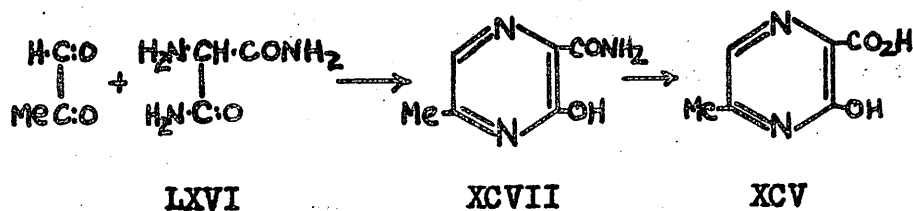
The use of aldehyde- and ketone- binding reagents (such as sodium hydrogen sulphite and hydrazine) to influence the orientation in reactions employing unsymmetrical α:β-dicarbonyl compounds has proved successful in the pteridine series. The use of these reagents tends to force an alkyl group into the 6-position of the pteridine nucleus[14,18]. Neither reagent affected the orientation of this reaction. Sodium hydrogen sulphite facilitated the condensation, however, and 2-hydroxy-6-methylpyrazine-3-carboxyamide (XCVII) was obtained in the absence of the usual basic catalyst.

The hydroxy-acid (XCV) was converted to 2-chloro-3-methoxycarbonyl-6-methylpyrazine(XCIX) via the hydroxy-ester (XCVIII) in the usual way. Fusion of the chloro-ester (XCIX) with guanidine carbonate (C) gave 2-amino-4-hydroxy-7-methyl-pteridine (CI) in 80% yield. The inra-red spectrum of this compound was identical with that of an authentic specimen[19] prepared from triamino-4-hydroxy-pyrimidine (LXXV) and methyl glyoxal, and different from that of 2-amino-4-hydroxy-6-methyl-pteridine (CIII) prepared from the same reactants, but employing

XCVII   XCV   XCVIII

C   XCIX
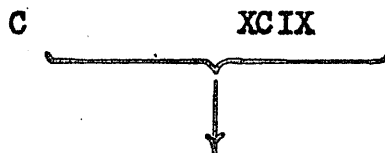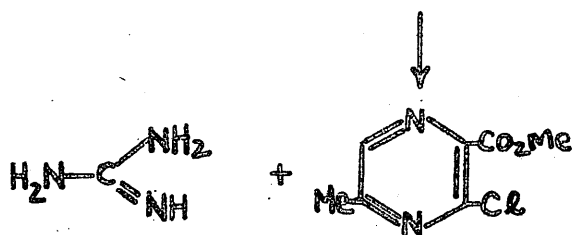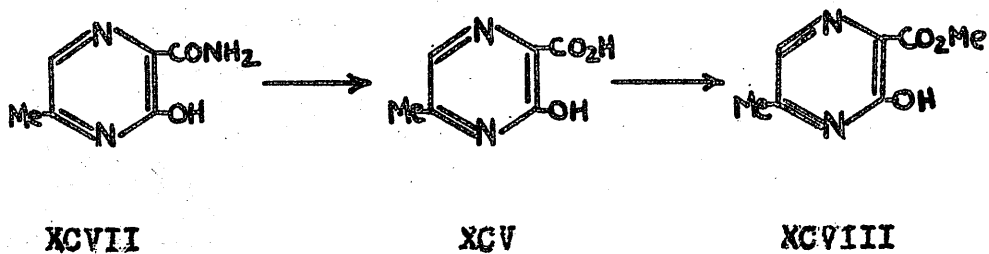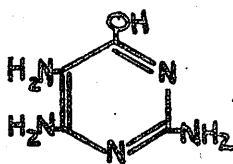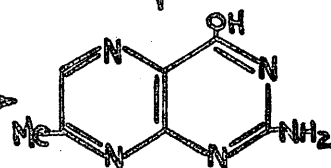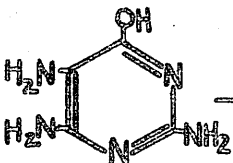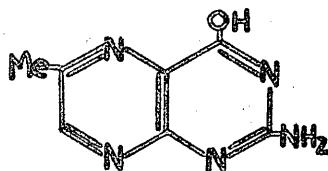
LXXV   CI

CII   LXXV   CIII

the aldhyde binder, hydrazine (CII), to force the methyl group into the 6-position[18]. This evidence confirmed the earlier conclusion, that the methyl group in the original pyrazinecarboxyamide (XCVII) was in the 6-position.

It has been reported by Karmas and Spoerri[56] that repetition of the condensation of methyl glyoxal with alanine (CIV) at first yielded the 2-hydroxy-3:6-dimethylpyrazine (CV). However reinvestigation of the reaction by these workers using freshly prepared methyl glyoxal always gave 2-hydroxy-3:5- -dimethylpyrazine (CVI) the product originally obtained by Jones[54].
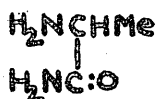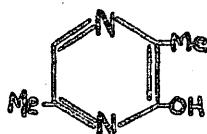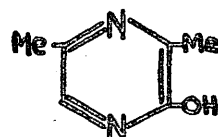
| CIV | CV | CVI |

The same workers[56] also reported that the condensation of methyl glyoxal with glycine gave a mixture of 2-hydroxy-6- -methylpyrazine (CVII) 8%, and the 5-methyl isomer (CVIII), 27%.
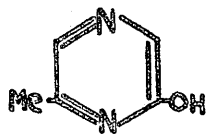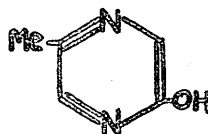
| CVII | CVIII |

Since the publication of the above results[73], the conclusions concerning the condensation of methyl glyoxal with aminomalonamide have been confirmed by two independent research groups.

Muehlmann and Day[63] could not reproduce Jones's result. They obtained a methyl-2-hydroxypyrazine-3-carboxyamide m.p. 227° (decomp.) whereas that reported by Jones had m.p. 243-244° (decomp.). The corresponding carboxylic acids had m.p. 205° (decomp.) and m.p. 155-157° (decomp.). These workers considered that their acid (m.p. 205°) was identical with 2-hydroxy-6-methylpyrazine-3-carboxylic acid [m.p. 183-184° (decomp.)], prepared by Jones[54] from the corresponding 2-amino compound, and reported by him to depress the m.p. (155-157°) of his acid. Muehlmann and Day concluded that they were dealing with the 6-methylpyrazine series while Jones had obtained the 5-substituted series. These results are in agreement with those reported above, viz. amide, m.p. 219-220° (decomp.), and acid m.p. 188-189° (decomp.)

These workers also found that enhanced yields were obtained if the methyl glyoxal was allowed to react with excess sodium hydrogen sulphite before the aminomalonamide was added. Whilst the reaction was carried out at 80°, sodium hydroxide was still added during the reaction period. The yield of 45% is still 25% less than that reported above.

The Russian workers Gortinskaya and Shchukina[67]
condensed methyl glyoxal with aminomalonamide as described
by Jones[54] and hydrolysed the product hoping to obtain
2-hydroxy-5-methylpyrazine-3-carboxylic acid, which they
intended to decarboxylate to obtain 2-hydroxy-5-methylpyrazine
(CVIII).   However, the carboxylic acid obtained had m.p.
180-182°, (decomp.) [cf. m.p. 155-157° (decomp.) given by
Jones].   Decarboxylation of this acid gave 2-hydroxy-6-
-methylpyrazine (CVII), m.p. 249-250° previously reported by
Karmas and Spoerri[56] (m.p. 250-251°).   The 5-methyl isomer
(CVIII) has m.p. 126-128°.

XCV   CVII   CVIII
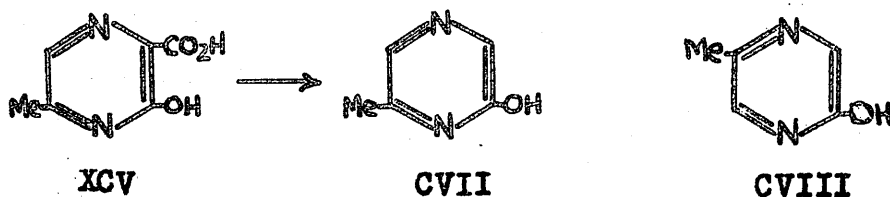
The Russian workers considered that the acid (m.p.
155-157°) obtained by Jones was actually impure 2-hydroxy-
-6-methylpyrazine-3-carboxylic acid (XCV) since their acid
initially melted at 155-157°, but became 180-182° after
recrystallisation.   They did not comment on the depressed
melting point observed by Jones on mixing his product with
authentic 2-hydroxy-6-methylpyrazine-3-carboxylic acid, nor
did they compare the m.p.(not given) of their amide with that
recorded by Jones (m.p. 243-244°) which is much higher than
that of the 6-methyl isomer (m.p. 219-220°).   It is therefore
probable that the products obtained by Jones belonged to the
5-methyl series.

2-Chloro-3-cyanopyrazines.

Whilst the initial investigation of a new synthetic route to pteridine derivatives was directed at the synthesis of 2-chloro-3-methoxycarbonylpyrazines and their cyclisation, it was realised that other pyrazines, particularly other 2-halo derivatives, could be synthesised by utilizing essentially the same intermediates. It was envisaged that chlorination of the hydroxypyrazine-3-carboxyamides or -3-carboxylic acids would also yield suitably reactive inter-mediates.

Treatment of 2-hydroxy-5:6-diphenylpyrazine-3--carboxyamide (LXXIX) with a mixture of phosphorus oxychloride and phosphorus pentachloride at 80°, resulted in dehydration of the amide group, as well as, replacement of the 2-hydroxy group with chlorine to give 2-chloro-3-cyano-5:6-diphenyl-pyrazine (CIX) in almost 100% yield.

LXXXIX   CIX   CX
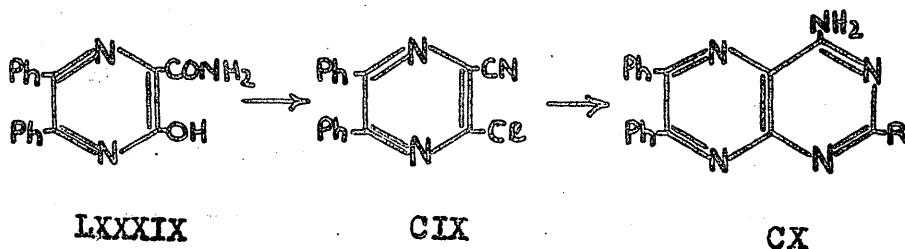
During the investigation of this compound as a suitable pteridine precursor, American workers[74] reported its synthesis by the same method, and further work was discontinued.

These workers briefly outlined the cyclisation of
this pyrazine, to 4-aminopteridines, with various reagents.
Fusion of 2-chloro-3-cyano-5:6-diphenylpyrazine (CIX) with
guanidine carbonate gave 2:4-diamino-6:7-diphenylpteridine
(CX); R = NH_2) in 65% yield.   Likewise fusion with thiourea and
urea gave the 2-mercapto (CX; R = SH) and the 2-hydroxy
(CX; R = OH) analogues in 51% and 59% yields, respectively.

Methods for the conversion of the cyanopyrazine (CIX)
into 2-amino-5:6-diphenylpyrazine-3-carboxyamide (CXI) and
2-amino-3-cyano-5:6-diphenylpyrazine (CXII) were also outlined.
The cyclisation of these compounds, which were obtained
previously by the degradation of pteridines was discussed in
the introduction.   The preparation of the amide (CXI) from
2-chloro-3-methoxycarbonyl-5:6-diphenylpyrazine was described
above (page 29).

CXI   CIX   CXII
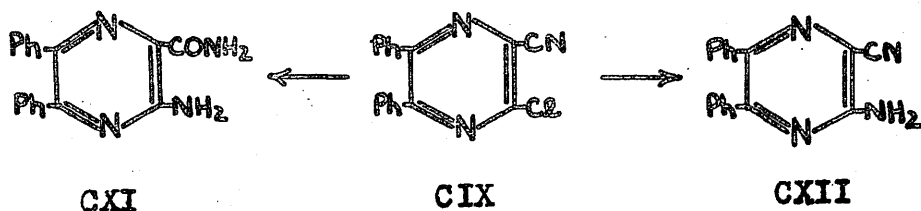
These reactions further demonstrate the utility and
potential flexibility of this new approach to the synthesis of
pteridine derivatives which uses pyrazine intermediates that
are readily available from aliphatic compounds.   These

pyrazine compounds are easier to work with than the more
conventional and frequently unstable diaminopyrimidines,
which have to be handled as their salts.   As the latter
generally possess no sharp melting point, and are insoluble
in organic solvents, they are therefore difficult to purify.

SYNTHESIS OF 2:8-DIHYDRO-4-HYDROXY-2-IMINO-8-METHYLPTERIDINES.

All the reported syntheses of 8-substituted pteridines
have involved the use of unstable 5-amino-4-substituted amino-
pyrimidines and have resulted in the formation of 8-substituted-
-7-pteridones or 8-substituted-7:8-dihydropteridines[42,47,48,49,]
(see introduction page 12).   Difficulties in preparing and
cyclising the appropriately substituted pyrimidines, which are
required to give 2-amino-4-hydroxypteridine $N_{(8)}$ glyosides have
been reported[42].   Further, the cyclisation of certain
pyrimidines has given products other than N_8 substituted
pteridines.   For example ethyl oxomalonate reacted with
triamino-6-methylaminopyrimidine (CXIII) to give a mixture of 2-amino-
-7-hydroxy-4-methylaminopteridine-6-carboxylic acid (CXIV) and
2:4-diamino-7:8-dihydro-8-methyl-7-oxopteridine-6-ethoxycarbonyl
(CXV)[48].

CXIII   CXIV   CXV
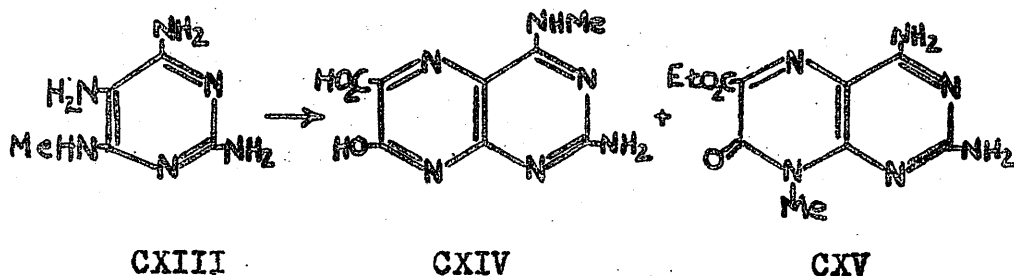
A route utilizing suitable pyrazine derivatives could
overcome many of these difficulties.   It was considered that
the β-oxo-ester function present in 1-alkyl-1:2-dihydro-3-methoxy-
carbonyl-2-oxopyrazines (CXVIII) might have reactivity similar
to that of the β-keto-esters normally used for pyrimidine
syntheses[86], and would condense with guanidine to give a
pteridine (CXIX), which, unlike those previously synthesised,
has a transannular bond system similar to that postulated for
luciferesceine[44] and the _Drosophila melanogaster_ eye pigment[46]
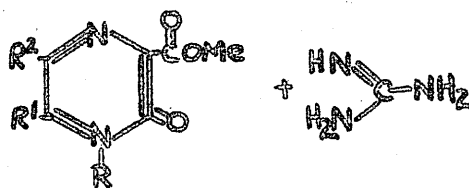
CXVIII
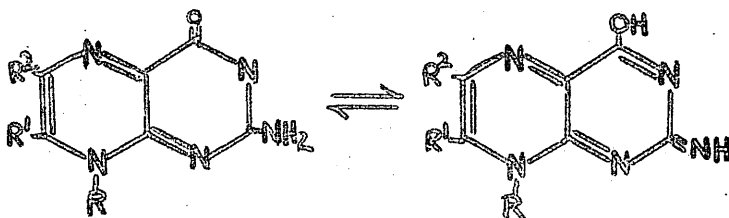
CXIXa   CXIXb

In order to test the feasibility of this route preliminary investigations have been limited to the preparation of 8-methylpteridines. This required the synthesis of 1:2-dihydro-3-methoxycarbonyl-1-methyl-2-oxo-pyrazines, which could best be prepared by N-methylation of the 2-hydroxy-3-methoxycarbonylpyrazines, already investigated in this section.

Only one case of the methylation of a hydroxypyrazine has been reported. Dutcher[78] treated hydroxypyrazine (CXX) with diazomethane and obtained a crystalline monomethyl derivative which he considered to be 1:2-dihydro-1-methyl-2--oxopyrazine (CXXI). 2-Methoxypyrazine (CXXII), a liquid, has since been prepared[76] and may also have been present in the mother liquors from the reaction. Diazomethylation of hydroxyl groups α to a ring nitrogen generally gives rise to a mixture of O- and N-methyl derivatives[65,77,78].
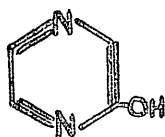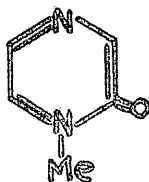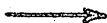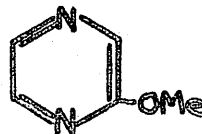
CXX   CXXI   CXXII

Treatment of 2-hydroxy-3-methoxycarbonyl-5:6-diphenyl-pyrazine (CXXIII) with diazomethane gave a mixture of the O- and N- methyl derivatives (CXXIV, and CXXV). Likewise

reaction with methyl iodide and potassium carbonate in refluxing dry acetone gave a similar mixture in the ratio 2:1.
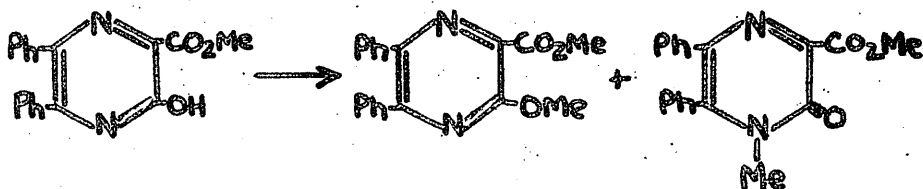
CXXIII CXXIV CXXV

Exclusive N-methylation of hydroxyheterocyclic compounds capable of lactam-lactim tautomerism is best effected with methyl sulphate[65,77,78]. Methylation of the hydroxycarboxylic acid (CXXVI) with methyl sulphate at 35° in aqueous solution at pH8, gave 1:2-dihydro-1-methyl--2-oxo-5:6-diphenylpyrazine -3-carboxylic acid (CXXVII) which yielded the ester (CXXV) on treatment with diazomethane.
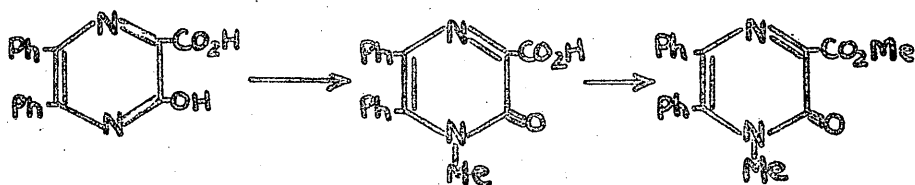
CXXVI CXXVII CXXV

α-Alkoxyquinolines and 2:4-dimethoxypyrimidine (CXXVIII) give N-methyl derivatives (e.g. CXXX) on reaction with methyl iodide[60,61,82]. The intermediate pentavalent nitrogen compounds (e.g. CXXIX) are unstable and dissociate with the production of

the N-methyl derivatives.   Treatment of the methoxy ester
(CXXIV) with methyl iodide at 180° in a sealed tube yielded
only a trace of the N-methyl isomer (CXXV).

CXXVIII   CXXIX   CXXX
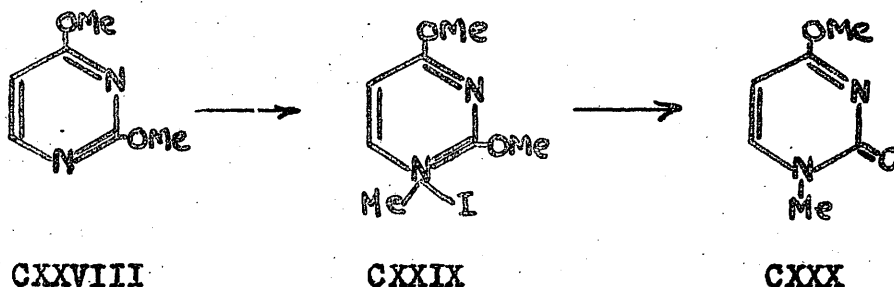
Reaction of the N-methylester (CXXV) with free guanidine
in refluxing methanol or in the presence of sodium methoxide
was unsuccessful.   However fusion of the pyrazine (CXXV) with
guanidine carbonate at 180-200° gave 2:8-dihydro-4-hydroxy-2-
-imino-8-methyl-6:7-diphenylpteridine (CXXXI).   This compound
was also synthesised using an alternative route, by Mr. W. E.
Fidler, of this Department, who condensed benzil with 2:5-
-diamino-4-hydroxy-6-methylaminopyrimidine (CXXXII).   The
infra-red and ultra-violet spectra of both products were
identical.

CXXV   CXXXI   CXXXII
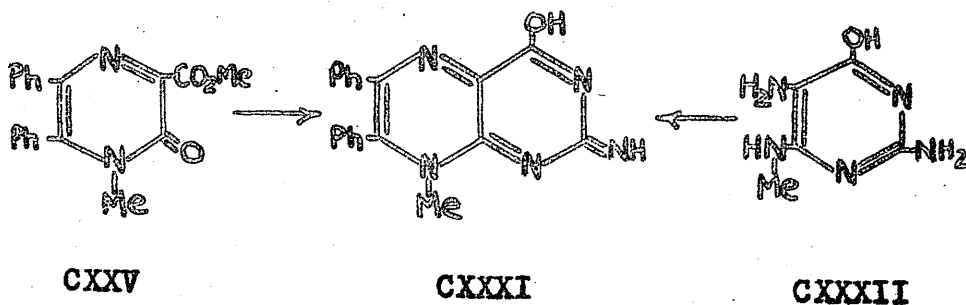
The use of 1:2-dihydro-3-methoxycarbonyl-1-methyl-2-
-oxopyrazine (CXXXIII) in the above synthesis will enable
the preparation of the unsubstituted pteridine (CXXXIV), which
cannot be obtained by the alternative route from the 5-amino-
-6-methylaminopyrimidine (CXXXII).   The condensation of
glyoxal with 5-amino-6-methylaminopyrimidines has been shown
to give bisdihydropurinyl compounds (e.g. CXXXV) and not
8-methylpteridines[83] (e.g. CXXXIV).

CXXXIII   CXXXIV

CXXXV
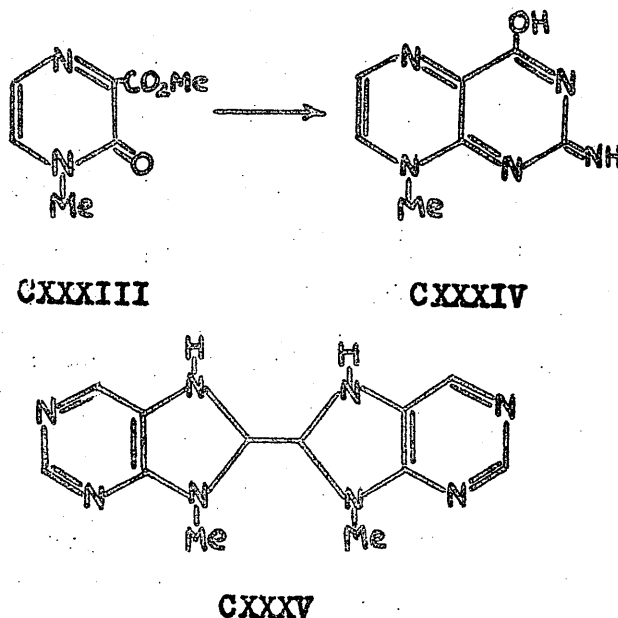
It appears that this method would offer a suitable route
to pteridine N_8 glycosides.   This would require the synthesis
of suitable pyrazine glycosides which have not yet been
studied.   However various methods have been developed in the
purine and pyrimidine nucleoside field for the introduction
of a sugar moiety at a ring nitrogen atom[84,85].

EXPERIMENTAL

Melting points were determined using a standard N.P.L. thermometer.

Paper chromatography was performed on No.1 Whatman paper by the ascending method using (separately) butanol--5N-acetic acid (7:3) and aqueous ammonium chloride 3%, and the chromatograms were viewed in ultra-violet light of wave-lengths 254 and 365 mu. Yields of substances that have no definite melting point refer to the stage when they appeared chromatographically homogeneous.

Substances were dried in air at 110° unless otherwise stated.

Ultra-violet absorption spectra were determined with a Unicam SP.500 spectrophotometer and E denotes intensity of absorption.

Ethyl-aminomalonate[60,61]. - Malonic ester (480 g., 3 mol.) was dissolved in glacial acetic acid (540 g., 9 mol.), and a saturated aqueous solution of sodium nitrite (621 g., 9 mol.) was added dropwise to the stirred solution over a period of 2 hours, the temperature being kept below 20°. The mixture was allowed to stand overnight, water (1.5 l.), and ether (3 l.) were added and the layers allowed to separate. The aqueous layer was extracted with more ether (2 x 1.5 l.). The combined extracts were washed with 5% sodium bicarbonate

(1.5 1.), 20% aqueous urea (2 x 500 c.c.) water (2 x 1 l.),
and dried (magnesium sulphate) and the ether removed by
distillation in vacuo to give 450 g., 80%, of ethyl
(hydroximino) malonate.

The isonitroso ester (100 g.) was dissolved in absolute
alcohol (50 c.c.), and hydrogenated rapidly at room temperature
at 4-5 atmospheres pressure, using 10% palladised charcoal[87]
(4 g.) as catalyst. The catalyst was filtered off and the
alcohol removed under reduced pressure. The ethyl-aminomalonate
was distilled at pressures not exceeding 1 mm.; yield 78 g.,
84%, b.p. 58-60° at 0.07 mm., N_D^{20} 1.4320.

Aminomalonamide[84]. - Ethyl-aminomalonate (67 g.) was
dissolved in saturated ethanolic ammonia (500 c.c.) and the
solution allowed to stand at room temperature for 7 days.
The product which separated as a yellow solid, was collected,
washed with ethanol, and dried; 44 g., 98%, m.p. 187-188°
(decomp.). The aminomalonamide was used without further
purification.

2-Hydroxypyrazine-3-carboxyamide. - Glyoxal sodium
hydrogen sulphite hemihydrate[88] (35 g.) was dissolved in
water (150 c.c.) and a solution of aminomalonamide (15.5 g.)
in water (100 c.c.) added. 12.5 N-Sodium hydroxide (36 c.c.)
was added dropwise to the stirred solution. The mixture was

chilled overnight and the crystalline sodium salt of 2-hydroxypyrazine-3-carboxyamide collected, washed with ice cold water, and suspended in warm water (60 c.c.). Acetic acid (20 c.c.) was added and the suspension stirred for 2 hours before being chilled and the crystalline product collected, 14 g., 75%, m.p. 268° (decomp.). A sample was recrystallized from water, as pale yellow needles, m.p. 270° (decomp.).

Found: C,43.1; H,3.5; N,30.0.

Calc. for $C_9H_6O_2N_3$: C,43.2; H,3.6; N,30.2%

Condensation of aminomalonamide with aqueous glyoxal under the conditions described by Jones[54] gave tarry reaction mixtures and poor yields ($<$ 40%) of the pyrazine. Muehlmann and Day have also reported the use of the sodium hydrogen sulphite derivative of glyoxal for this reaction, in a recent publication[63].

2-Hydroxypyrazine-3-carboxylic acid. - 2-Hydroxypyrazine-3-carboxyamide (14 g.) and 4N-sodium hydroxide (100 c.c.) were heated on a water bath for 12 hours. The resulting solution was acidified with concentrated hydrochloric acid to pH4, chilled, and the precipitate collected. The product was purified by dissolving in aqueous sodium bicarbonate, and the solution treated with charcoal and filtered. The filtrate was acidified with hydrochloric acid, and allowed to cool.

The crystalline solid was collected, washed with ice-cold water, and dried at 100° (10.8 g., 77%); the m.p. was 218-219° (decomp.), undepressed when mixed with a specimen prepared from 2-amino-pyrazine-3-carboxylic acid[64].

Found: C,42.8; H,3.0; N,20.2.

Calc. for $C_5H_4O_3N_2$: C,42.9; H,2.9; N,20.0%

2-Hydroxy-3-methoxycarbonylpyrazine[65]. - A suspension of 2-hydroxypyrazine-3-carboxylic acid (6.5 g.) in dry boiling methanol (250 c.c.) was treated with dry hydrogen chloride until it had dissolved (20 min.), and the solution was refluxed for a further 2 hours, before being concentrated in vacuo to 50 c.c. Ice-water (220 c.c.) was added cautiously to the chilled solution, and the pH adjusted to 3 with sodium hydrogen carbonate. Sodium chloride (55 g.) was added and the solution extracted with ethyl acetate (600 c.c.) for 24 hours. The extract was dried, boiled with charcoal (3 g.) for 10 min., filtered, and evaporated in vacuo. The yellow residue (6 g.) was sublimed at 130° (bath)/0.05 mm., giving 2-hydroxy-3-methoxycarbonylpyrazine, 4.7 g., 65%, m.p. 151-152°

2-Chloro-3-methoxycarbonylpyrazine[65]. - 2-Hydroxy-3--methoxycarbonylpyrazine (3.4 g.) and freshly distilled phosphorus oxychloride (20 c.c.), containing one drop of 10N-hydrochloric acid, were refluxed for 3 hours, then taken to dryness at 80° in vacuo. The residue was poured onto

crushed ice (140 g.), and the mixture stirred for 20 min.
The pH was adjusted to 6 with ammonium hydroxide (S.G., 0.88)
sodium chloride (68 g.) added, and the mixture extracted with
ethyl acetate (8 x 35 c.c.).   The combined extracts were
dried (sodium sulphate), and evaporated in vacuo.   The
resulting pale brown oil on distillation (b.p. 50-52°/0.04 mm.)
gave 2-chloro-3-methoxycarbonylpyrazine, as white plates,
3.15 g., 82%, m.p. 31-32°.

2-Amino-4-hydroxypteridine. - (a) 2-Chloro-3-
methoxycarbonylpyrazine (1.0 g.) and guanidine carbonate
(2.0 g.) were finely powdered and the mixture heated at 170°
(bath) for 30 min., with occasional stirring, when effervescence
took place, and the mixture turned brown.   On cooling, the
residual solid was dissolved in boiling water (50 c.c.) and
the solution was filtered.   After treatment with charcoal,
and filtration, the boiling solution was brought to pH5 with
3N-hydrochloric acid.   The pale yellow solid which separated,
was collected at 90-100°, washed with boiling water (25 c.c.)
and ethanol (15 c.c.), and dried at 100° to give 2-amino-4-
-hydroxypteridine (0.84 g., 89%), m.p. > 360°.

A sample (0.37 g.) was dissolved in hot 2N-sodium
hydroxide (1.8 c.c.), the solution filtered hot, and 10N-sodium
hydroxide (1.8 c.c.) added to the filtrate.   The yellow sodium

salt which separated on cooling, was collected, washed with
2.5-$\underline{N}$ sodium hydroxide, and air dried to give fine yellow
needles.   These were dissolved in boiling water (9 c.c.)
and the hot solution was poured slowly into 3$\underline{N}$-acetic acid
(30 c.c.).   The pale yellow solid which separated was
collected, washed as before, and dried at 135°.

Found: C,43.9; H,3.0;   N,42.6.

Calc. for $C_6H_5ON_5$:   C,44.2; H,3.1;   N,42.9%

Light absorption in 0.1N-sodium hydroxide: Max. at 255
(E = 16,000), and 358 mu (E = 6,600).

(b)   Guanidine hydrochloride (1.11 g.) was added to a
solution of sodium (1.06 g.) in dry methanol (40 c.c.).
The solution was filtered from sodium chloride, and 2-chloro-
-3-methoxycarbonylpyrazine (2.0 g.) was added.   A clear,
bright scarlet solution resulted on shaking, and was refluxed
for 30 hours, during which further precipitation of sodium
chloride took place.   The filtered solution was diluted with
water (60 c.c.), and the pH of the boiling solution was
adjusted to 4.5 with warm glacial acetic acid.   The yellow
bulky precipitate was collected and purified as above, 0.375 g.,
20%, m.p. > 360°.

Found: C,44.5; H,3.1; N,43.0%

The yield fell appreciably when the period of refluxing
was reduced (e.g. 10 hours, yield 7%), or when the reaction

was carried out at higher temperatures in a sealed tube (e.g. heating at 109° for 10 hours, yield 5%).

(C) [66] A solution of 2:5:6-triamino-4-hydroxypyrimidine dihydrochloride (2.14 g.) in water (20 c.c.) was heated to 70° and treated with 26.5% aqueous glyoxal (2.2 c.c.). After 24 hours the precipitate (1.3 g.) was collected and purified as in section (a), m.p.> 360°.

The ultra-violet and infra-red spectra of the above products were identical.

2-Hydroxy-5:6- diphenylpyrazine-3-carboxyamide[54]. - A mixture of benzil (21 g., 0.1 mol.) and powdered aminomalonamide (11.7 g., 0.1 mol.) in 50% aqueous ethanol (350 c.c.) was heated to 70° and 12.5 N-sodium hydroxide (10 c.c., 0.125 mol.) was added with stirring. A clear brown solution resulted and then suddenly it became almost solid with a crystalline precipitate. The cooled mixture was filtered and the solid was washed with acetone (500 c.c.) and water (250 c.c.). The sparingly soluble sodium salt was suspended in acetone (200 c.c.) and treated with concentrated hydrochloric acid (20 c.c.). The resulting clear solution was diluted with water (500 c.c.) and the 2-hydroxy-5:6-diphenylpyrazine-3- carboxyamide which separated, was collected after chilling the mixture overnight. The product was recrystallised from acetone/water as yellow needles, 25 g., 83%, m.p. 175° (decomp.)

Found: C,70.1; H,4.3; N,14.3.

Calc. for $C_{17}H_{13}O_2N_3$:   C,70.1; H,4.5; N,14.4%.

2-Hydroxy-5:6-diphenylpyrazine-3-carboxylic acid. -
2-Hydroxy-5:6-diphenylpyrazine-3-carboxyamide (8.0 g.) and
sodium hydroxide (8.0 g.) in ethanol (110 c.c.) were heated in
a steel bomb at 180° for 6.5 hours.   After cooling, warm water
(150 c.c.) was added, and the ethanol evaporated.   The hot
solution was filtered, concentrated hydrochloric acid was
added to pH4, and the yellow precipitate was collected after
chilling.   Recrystallisation from aqueous acetone gave
2-hydroxy-5:6-diphenylpyrazine-3-carboxylic acid as golden
needles, 7.28 g., 91% m.p. 216-217° (decomp.).

Found: C,69.7; H,3.9; N,9.4.

$C_{17}H_{12}O_3N_2$ requires   C,69.8; H,4.1; N,9.6%.

2-Hydroxy-3-methoxycarbonyl-5:6-diphenylpyrazine. -
2-Hydroxy-5:6-diphenylpyrazine-3-carboxylic acid (7.5 g.) in
dry boiling methanol (300 c.c.) was treated with dry hydrogen
chloride until it had completely dissolved (about 20 min.) and
the solution was refluxed for a further 2 hours.   On cooling
the reaction mixture, 2-hydroxy-3-methoxycarbonyl-5:6-diphenyl-
pyrazine was deposited as yellow needles.   These were collected,
and the mother liquors concentrated to 50 c.c. in vacuo when
a second crop was obtained.   Recrystallisation from methanol
gave the hydroxy-ester as fine yellow needles, 6.65 g., 89%,

m.p. 204-205°.

Found: C,70.7; H,4.7; N,8.9.

$C_{18}H_{14}O_3N_2$ requires   C,70.6; H,4.6; N,9.1%.

2-Chloro-3-methoxycarbonyl-5:6-diphenylpyrazine. -
2-Hydroxy-3-methoxycarbonyl-5:6-diphenylpyrazine (3.5 g.) and
redistilled phosphorus oxychloride (23 c.c.) containing one
drop of concentrated sulphuric acid, were mixed in a Carius
tube.   The mixture was heated at 110° (bath) for 10 min., when
evolution of hydrogen chloride had ceased.   The tube was then
sealed and heated at 160° for 5.5 hours.   The dark yellow
solution was poured on cracked ice (200 g.), and stirred for
30 min., during which a buff solid separated.   This was
collected, washed with water, and recrystallised from methanol,
to give small plates of 2-chloro-3-methoxycarbonyl-5:6-
-diphenylpyrazine, 3.0 g., 81%, m.p. 113-114°.   Recrystallisa-
tion from methanol-light petroleum (b.p. 60-80°) followed by
sublimation gave a sample of m.p. 116-116.5°.

Found: C,66.6; H,4.0; N,8.9; Cl,11.5.

$C_{18}H_{13}O_2N_2Cl$ requires   C,66.6; H,4.0; N,8.6; Cl,11.9%.

The yield at 150° was only 50%, and at 190° 14%.   The
use of a mixture of phosphorus oxychloride and diethylaniline
(cf.79), or of phosphorus oxychloride and phosphorus
pentachloride[56] was unsuccessful.

<u>2-Amino-4-hydroxy-6:7-diphenylpteridine.</u> - (a) A
finely powdered mixture of 2-chloro-3-methoxycarbonyl-5:6-
-diphenylpyrazine (0.2 g.) and guanidine carbonate (0.4 g.),
was heated at 170° (bath) for 30 min., with occasional
stirring; effervescence took place. The mixture was
extracted with boiling water to leave an orange solid (0.230 g.),
which was purified by dissolution in hot 2N-sodium hydroxide
(20 c.c.), filtration, and the warm filtrate was poured into
boiling glacial acetic acid (10 c.c.).   The yellow solid
which separated was collected at 90-100°, washed with warm
water, ethanol, and ether, and dried to give 2-amino-4-hydroxy-
-6:7-diphenylpteridine, 0.13 g., 70%, m.p. > 360°.   Recrystall-
isation from dimethylformamide gave a yellow microcrystalline
solid which was dried at 135°.

Found: $C,68.3$; $H,4.0$; $N,22.4$

Calc. for $C_{18}H_{13}ON_5$: $C,68.6$; $H,4.2$; $N,22.2\%$.

Light absorption in 0.1N-sodium hydroxide: Max. at 270
($E = 20,900$), and 380 mu ($E = 13,000$).

(b) (cf.68). - A solution of 2:5:6-triamino-4-hydroxy-
pyrimidine dihydrochloride (2.4 g.) in warm water (25 c.c.)
was added to a solution of benzil (2.5 g.) in boiling ethanol
(75 c.c.).   The resulting clear yellow solution on refluxing,
rapidly deposited a yellow crystalline solid.   After 4 hours

the mixture was filtered while still warm, and the residue washed with water, and acetone. The product was purified as above, 3 g., 85%, m.p. > 360°.

Found: C,68.3; H,4.0; N,21.8%.

Cain, Mallette and Taylor,[68] using the bisulphite derivative of the pyrimidine obtained the pteridine in 54% yield.

The infra-red and ultra-violet spectra of the above products were identical, and a mixture of their acetyl derivatives (see below) showed no depression of m.p.

2-Acetamido-4-hydroxy-6:7-diphenylpteridine. - A mixture of 2-amino-4-hydroxy-6:7-diphenylpteridine (50mg.) and redistilled acetic anhydride (2 c.c.) containing 3 drops of concentrated sulphuric acid, was heated on a steam bath for 1 hour. The dark solution was cooled, poured into water (15 c.c.) with stirring, and the mixture chilled for 2 hours. The yellow precipitate was collected washed with 2N-sodium bicarbonate, water, and acetone. Recrystallisation from aqueous ethanol gave 2-acetamido-4-hydroxy-6:7-diphenylpteridine, as pale yellow blades, 40 mg., 70%, m.p. 236-238°.

Found: C,67.1; H,4.1.

$C_{20}H_{16}O_2N_5$ requires C,67.3; H,4.2%.

2-Methoxy-5:6-diphenylpyrazine-3-carboxylic acid. -

To a solution of sodium (0.06 g.) in dry methanol (7 c.c.) were added guanidine hydrochloride (0.06 g.) and 2-chloro--3-methoxycarbonyl-5:6-diphenylpyrazine (0.2 g.). The mixture was refluxed for 12 hours on a water bath during which a white bulky precipitate separated. The solid was collected, dissolved in boiling water, and the filtrate taken to pH4 with glacial acetic acid. Crystallisation occurred on cooling to give fluffy white needles, (0.096 g.), m.p. 179° (decomp.). The filtrate from the reaction mixture was concentrated _in vacuo_, warm water was added to dissolve any solid material, and the solution was filtered from traces of slime. The clear filtrate was adjusted to pH4 with glacial acetic acid, and on cooling, a further deposit of crystals was formed (0.04 g.) m.p. 175° (decomp.). Total yield 73%. Recrystallisation from aqueous methanol gave 2-methoxy-5:6-diphenylpyrazine-3-carboxylic acid as small white needles, m.p. 180-181° (decomp.).

Found: C,70.7; H,4.3; N,8.9.

$C_{18}H_{14}O_3N_2$ requires C,70.6; H,4.6; N,9.2%.

Care must be taken during the preparation of this material that it does not become mixed with its sodium salt which

crystallises readily from water as white plates, m.p.
254-256°(decomp.).   The same methoxy-acid, m.p. and mixed
m.p. 178-180° (decomp.), was obtained from 2-chloro-3-
-methoxycarbonyl-5:6-diphenylpyrazine and sodium methoxide
in the absence of guanidine.

2-Methoxy-3-methoxycarbonyl-5:6-diphenylpyrazine. -
2-Methoxy-5:6-diphenylpyrazine-3-carboxylic acid (0.2 g.) was
esterified with methanol and dry hydrogen chloride, using the
method employed for the esterification of the corresponding
hydroxy-acid (see page 52).   2-Methoxy-3-methoxycarbonyl-5:6-
-diphenylpyrazine (0.2 g., 95%) was recrystallised from
methanol to give fluffy white needles, m.p. 118.5-119°.

Found: C,71.2; H,5.0; N,8.6.

$C_{19}H_{16}O_3N_2$ requires   C,71.2; H,5.0; N,8.8%.

2-Amino-5:6-diphenylpyrazine-3-carboxyamide. - A
mixture of 2-chloro-3-methoxycarbonyl-5:6-diphenylpyrazine (0.2
g.) and concentrated ammonium hydroxide (S.G., 0.88; 4 c.c.)
in a sealed Carius tube was heated at 180° for 16 hours.
After cooling, the reaction mixture was evaporated to dryness
in vacuo and the yellow residue extracted with cold water
(5 c.c.).   Recrystallisation of the final residue from
aqueous ethanol gave 2-amino-5:6-diphenylpyrazine-3-carboxy-
amide, as pale yellow needles, 0.17 g., 95%, m.p. 203-204°.

Found: C,70.2; H,5.0; N,19.1

Calc. for $C_{17}H_{14}ON_4$: C,70.3; H,4.9; N,19.3%.

Taylor[38] gives m.p. 203.5-205°.

Methyl glyoxal[72]. - Selenium dioxide (245 g.) and

acetone (1.2 l.) were heated under reflux for 3-4 hours,

carefully during the initial stages when the reaction was

exothermic. The yellow liquid product was decanted, the

black residue washed with acetone (200 c.c.), and the whole

liquid fractionated up to 80° to remove the excess of acetone,

as a pale yellow azeotropic mixture (b.p. 56.5°) containing

about 1% methyl glyoxal (retained as the initial material for

further preparations). The residual liquid was fractionated

under reduced pressure. The yield of methyl glyoxal

(containing a little water) b.p. 54-70°/ 50 m.m. was 40 g.

2-Hydroxy-6-methylpyrazine-3-carboxyamide. - (a)

Freshly prepared methyl glyoxal (9 g.) in water (12 c.c.)

was cooled to -20° and finely powdered aminomalonamide (15 g.)

was added. With stirring, and keeping the temperature below

0° , 12.5 N-sodium hydroxide (10 c.c.) was added dropwise

over a period of 30 mins. After stirring for 2 hours, the

mixture was allowed to stand for 2 days at 0°. The yellow

sodium salt which separated was collected, washed with a

small volume of water, and suspended in moist acetone (250 c.c.).

The mixture was adjusted to pH4 with concentrated hydrochloric acid, and the buff solid (13.0 g., 70%) which separated was collected, washed with ice-cold water and acetone, and dried. Rigorous chromatographic examination of the product, and the mother-liquors, did not reveal any fluorescent material other than the solid isolated. Recrystallisation from methanol (charcoal) gave 2-hydroxy-6-methylpyrazine-3-carboxyamide as pale yellow needles, m.p. 219-220° (decomp.).

Found: C,46.9; H,4.7; N,27.4.

$C_6H_7O_2N_3$ requires C,47.1; H,4.6; N,27.5%.

(b) Freshly prepared methylglyoxal (12 g.) in water (30 c.c.) was treated with sodium hydrogen sulphite (10 g.) and the mixture was set aside at room temperature for 30 min. Finely powdered aminomalonamide (20 g.) was added, and the mixture was heated on a water-bath until the solid had dissolved. On overnight refrigeration a semi-solid buff mass separated. 12.5 N-Sodium hydroxide (20 c.c.) was added to the stirred mixture and the sodium salt of the product, which separated as a bright yellow precipitate, was collected, after chilling, and washed with a small volume of water, acetone and dried, 19.2 g., 70%. A sample (1 g.) was converted to the free hydroxypyrazine, m.p. 219-220° (decomp.) as before (section a)

was concentrated in vacuo, on a water bath, to 10 c.c., and chilled. The yellow crystalline product was collected and washed with a little dry methanol. Recrystallisation from methanol gave 2-hydroxy-3-methoxycarbonyl-6-methylpyrazine as clear needles, 1.1 g., 100%, m.p. 174-175° (decomp.)

Found: C,49.8; H,4.7; N,16.6.

$C_7H_8O_3N_2$ requires   C,50.0; H,4.8; N,16.7%.

2-Chloro-3-methoxycarbonyl-6-methylpyrazine. -
2-Hydroxy-3-methoxycarbonyl-6-methylpyrazine (0.3 g.) was refluxed with redistilled phosphorus oxychloride (6 c.c.), containing one drop of concentrated sulphuric acid, for 5 hours. The reaction mixture was poured onto cracked ice (100 g.) and stirred for 30 min. Ethyl acetate (30 c.c.) was added to the ice cold solution, and the pH was adjusted to 8 with concentrated ammonia. The aqueous layer was saturated with sodium chloride, and the solution was extracted with ethyl acetate for 24 hours. The yellow extract was dried and evaporated to dryness in vacuo to give a brown gum which crystallised on the addition of methanol. Crystallisation from methanol, using charcoal, gave 2-chloro-3-methoxycarbonyl--6-methylpyrazine, 0.2 g., 61%, as small yellow needles, m.p. 82-83°. Recrystallisation from light petroleum (b.p. 60-80°) gave clear plates, m.p. 84-85°.

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Found: C,45.2; H,3.6.

C₇H₇O₂N₂Cl requires   C,45.1; H,3.8%.

2-Amino-4-hydroxy-7-methylpteridine. - (a) A finely powdered mixture of 2-chloro-3-methoxycarbonyl-6-methylpyrazine (135 mg.) and guanidine carbonate (0.4 g.) was heated at 165-175° (bath) for 30 min. with occasional stirring. The mixture turned light orange at 125° and slowly deepened to a dull caramel colour as the temperature was raised. After cooling the solid was dissolved in boiling water (7 c.c.), charcoaled, and filtered. The warm filtrate was poured into warm glacial acetic acid (4 c.c.). The lemon coloured solid which separated was collected at 90-100° on a sintered glass funnel, washed with water, ethanol and ether, 105 mg., 80%, m.p. > 300°. The product was purified by dissolving in 2N-sodium hydroxide (2.5 c.c.), the solution filtered and 12N-sodium hydroxide (2.5 c.c.) added. The mixture was warmed to dissolve precipitated solid, and allowed to cool slowly, when the sodium salt separated as lemon needles, which were collected and washed with 5N-sodium hydroxide (5 c.c.). The sodium salt was dissolved in warm water (4 c.c.) and the clear solution added to boiling acetic acid (3 c.c.). The 2-amino--4-hydroxy-7-methylpteridine which separated was collected and washed with warm water, ethanol, and ether, and dried at 135° in vacuo.

Found: C,44.9; H,4.2.

Calc. for $C_7H_7ON_5 \cdot \frac{1}{2}H_2O$: C,45.2; H,4.3%.

(b)[19]   Methyl glyoxal (1 c.c.) was added to a suspension of 2:5:6-triamino-4-hydroxypyrimidine sulphate (3.66 g.) in water (20 c.c.) containing hydrated sodium acetate (3.96 g.). The mixture, which became orange-red, was heated on a water bath for 1 hour, when the colour turned buff.   The suspended solid was filtered off, washed with a little water, and added to warm 5$\underline{N}$-sodium hydroxide (50 c.c.), when the sodium salt separated, almost immediately.   After chilling, the solid was collected washed with 5$\underline{N}$-sodium hydroxide (30 c.c.), ice-cold water (10 c.c.), and ethanol.   The product was purified as above, m.p. > 300°

Found: C,45.0; H,4.3%.

The infra-red spectra of the above products were identical and different from that of the 6-methyl isomer (see below).

2-Amino-4-hydroxy-6-methylpteridine[18].   A solution of methyl glyoxal (0.6 g.) in water (10 c.c.) was treated with 90% hydrazine hydrate (1 c.c.) and after 30 min. at room temperature the solution was added to a suspension of 2:5:6--triamino-4-hydroxypyrimidine sulphate (2.3 g.) in water (25 c.c.) containing anhydrous sodium acetate (1.38 g.) and boric acid (1.0 g.). The mixture was stirred on the water bath

in a gentle stream of nitrogen for 4 hours. After cooling
the solid was collected and the crude product was crystallised
as its sodium salt by addition to 5N-sodium hydroxide. The
yellow needle shaped crystals were collected and dissolved in
warm water (charcoal) and sufficient warm 12N-sodium hydroxide
added to the filtrate to make 5N. The sodium salt which
crystallised out was collected and washed with 5N-sodium
hydroxide and a little ice-cold water. The residue was
dissolved in warm water and the solution poured into sufficient
warm acetic acid to give pH4-5. The 2-amino-4-hydroxy-6-
-methylpteridine which separated was collected at 90-100°,
washed with water, ethanol, and ether; 1.0 g., m.p.> 300°.

2-Chloro-3-cyano-5:6-diphenylpyrazine. - A stirred
mixture of 2-hydroxy-5:6-diphenyl-3-carboxyamide (0.5 g.),
phosphorus pentachloride (608 mg.) and redistilled phosphorus
oxychloride (7 c.c.) were heated at 85°(bath) for 3 hours,
under anhydrous conditions. The clear red solution was
cooled, poured onto cracked ice (75 g.) and the mixture
stirred for 30 min. The yellow solid which separated was
filtered off, washed with cold water and dried, m.p. 204-206°
(decomp.). Recrystallisation from aqueous acetone, charcoal,
gave 2-chloro-3-cyano-5:6-diphenylpyrazine as fine yellow
needles, 0.5 g., 100%, m.p. 208-210°. A sample for analysis
was sublimed to give a white solid, m.p. 210°.

Found: C,69.7; H,3.6; N,14.4; Cl,12.3

$C_{17}H_{10}N_3Cl$ requires   C,70.0; H,3.5; N,14.4; Cl,12.2%.

A similar preparation has since been briefly reported by Taylor and Paudler[74] but no m.p. or analysis were given.

Methylation of 2-hydroxy-3-methoxycarbonyl-5:6-
-diphenylpyrazine. - (a) With methyl iodide and potassium carbonate. (cf.89). A mixture of 2-hydroxy-3-methoxycarbonyl-
-5:6-diphenylpyrazine (0.5 g.), dry potassium carbonate (0.5 g.) and methyl iodide (0.45 c.c.) in dry acetone (20 c.c.), was heated under reflux for 20 hours. The suspended inorganic salts were filtered off and washed with dry acetone (10 c.c.), and the yellow filtrate evaporated to dryness in vacuo. The resulting residue was extracted with warm chloroform (3 x 10 c.c.) and the extracts evaporated to dryness in vacuo to give a yellow solid which was crystallised from methanol, 0.48 g., m.p. 110-175°. Fractional recrystallisation of this material from benzene, containing a trace of petrol(b.p. 60-80°), gave 1:2-dihydro-3-methoxycarbonyl-1-methyl-2-oxo-5:6-diphenyl-
pyrazine, as yellow prisms, 0.3 g., m.p. and mixed m.p. with authentic material (see below) 185-186°.

The clear mother liquors were evaporated to dryness, and the white solid recrystallised from aqueous methanol to give 2-methoxy-3-methoxycarbonyl-5:6-diphenylpyrazine, as fluffy white needles, 0.15 g., m.p. and mixed m.p. with an authentic

specimen (see page 57) 118-119°. Light absorption in
ethanol: Max. at 206 (E = 27,000), 222 (E = 23,200)
271 (E = 13,200) and 338 mu (E = 12,400).

(b)   With diazomethane. - The hydroxy ester (1 g.) was
treated with 7 equivs. of diazomethane (from 7.2 g. of
toluene-p-sulphonylmethylnitrosamide[90]) in ether-methanol.
After standing overnight, the solution was evaporated to
dryness in vacuo, and the resulting mixture of O- and N-
-methyl isomers (0.95 g.) separated by fractional recrystallisa-
tion, as in section a.

1:2-Dihydro-1-methyl-2-oxo-5:6-diphenylpyrazine-3-
-carboxylic acid. - 2-Hydroxy-5:6-diphenylpyrazine-3-carboxylic
acid (2.5 g.) was treated with 2N-sodium hydroxide (8.7 c.c.,
2 equivs.), and boiling water (40 c.c.) added to dissolve the
precipitated disodium salt.   To the clear solution (adjusted
to pH8-9) at 35°, methyl sulphate (5 c.c.) was added dropwise
during 30 min., and the whole stirred for 2 hours longer.
The pH was kept at 8 throughout by means of 2N-sodium hydroxide.
After standing overnight at room temperature the mixture was
filtered, and the filtrate acidified with concentrated
hydrochloric acid to pH3.   The yellow precipitate was collected,
after chilling, washed with water, and dried.   Recrystallisation
from chloroform-petrol (b.p. 60-80°) and finally from benzene
gave 1:2-dihydro-1-methyl-2-oxo-5:6-diphenylpyrazine-3-
carboxylic acid, as yellow prisms, 1.66 g., 66%, m.p.203-204°

(decomp.).

Found: C,70.7; H,4.3; N,9.4.

C$_{18}$H$_{14}$N$_2$O$_3$ requires   C,70.6; H,4.6; N,9.2%.

Light absorption in ethanol: Max. at 204 (E = 20,600),
243 (E = 10,400), 274 (E = 11,600), and 348 mu(E=8,850).

1:2-Dihydro-3-methoxycarbonyl-1-methyl-2-oxo-5:6-
-diphenylpyrazine. - A solution of 1:2-dihydro-1-methyl-2-oxo-
-5:6-diphenylpyrazine-3-carboxylic acid (0.96 g.) in methanol
(50 c.c.) was treated with 4 equivs. of diazomethane (from
4.3g. of toluene-p-sulphonylmethylnitrosamide[90]) in ether-
methanol.   After standing overnight the solution was evaporated
in vacuo to small bulk and allowed to crystallise.   The product
was collected, washed with water, methanol, and dried, 0.86 g.,
86%, m.p. 184-186°.   Recrystallisation from aqueous methanol
gave 1:2-dihydro-3-methoxycarbonyl-1-methyl-2-oxo-5:6-diphenyl-
pyrazine as yellow prisms, m.p. 186-187°.

Found: C,71.5; H,4.9; N,8.5.

C$_{19}$H$_{16}$N$_2$O$_3$ requires   C,71.2; H,5.0; N,8.8%.

Light absorption in ethanol: Max. at 206 (E = 22,000),
267, (E = 17,000), and 370 mu (E = 8,800).

Treatment of 2-methoxy-3-methoxycarbonyl-5:6-
-diphenylpyrazine with methyl iodide. - The methoxy ester
(45mg.) and methyl iodide (2 c.c.) were heated in a sealed

tube at 180° for 60 hours. After cooling, chloroform (15 c.c.) was added, and the dark solution washed with 0.1N-sodium thiosulphate (2 x 10 c.c.), water (2 x 10 c.c.), dried (magnesium sulphate) and evaporated to dryness in vacuo. Recrystallisation of the faintly yellow solid (40 mg.) from methanol gave starting material as white needles, 35 mg., m.p. and mixed m.p. 118-119°. Paper chromatography of the mother liquors indicated the presence of a trace of 1:2-dihydro-3--methoxycarbonyl-1-methyl-2-oxo-5:6-diphenylpyrazine as well as more starting material.

2:8-Dihydro-4-hydroxy-2-imino-8-methyl-6:7-diphenyl-pteridine. - A finely powdered mixture of 1:2-dihydro-3--methoxycarbonyl-1-methyl-2-oxo-5:6-diphenylpyrazine (0.61 g.) and guanidine carbonate (2.0 g.) was heated slowly to 175° (bath) with stirring. The mixture became deep yellow, and after 30 min. the temperature was raised to 200° and the heating continued for another 30 min. On cooling, the residual orange solid was stirred with warm water (20 c.c.) for 10 min. The mixture was cooled and the pH adjusted to 7 with acetic acid. The yellow solid was collected and washed liberally with water, ethanol, and warm chloroform, and dried, 0.55 g. 89%, m.p. ≫ 300°. Recrystallisation from a large volume of dimethyl-forinamide gave 2:8-dihydro-4-hydroxy-2-imino-8-methyl-6:7--diphenylpteridine as a microcrystalline solid m.p. ≫ 300°

Found: N, 20.9.

$C_{19}H_{16}N_5O$ requires   N, 21.3%.

Light absorption in 0.1N-sodium hydroxide: Max at 218 ($E = 39,000$), 267 ($E = 24,000$) and 380 mu ($E = 13,700$).

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S E C T I O N II

Methylation of 2:4-Dihydroxy-6- and -7-phenyl-
pteridine, and Related Topics.

SUMMARY

The condensation of 5:6-diamino-2:4-dihydroxypyrimidine
and phenylglyoxal has been investigated, and the nature of
the products obtained by previous investigators, has been
established.   The methylation of these substances has been
studied.

The condensation of phenylglyoxal with 5:6-diamino-2:4-
dihydroxypyrimidine and 5:6-diamino-1:2:3:4-tetrahydro-1:3-
-dimethyl-2:4-dioxopyrimidine in the presence of sodium
hydrogen sulphite gave 7-phenylpteridines.   Hitherto it
has been generally accepted that when aldehyde binding reagents
are used, in this type of reaction, the alkyl or aryl group
will be in the 6-position.

Unequivocal synthesis of 1:2:3:4-tetrahydro-1:3-dimethyl-
-2:4-dioxo-6-phenylpteridine and the 7-phenyl isomer have been
carried out. Mixtures of these compounds (in the ratio 2:1 and
1:2) have been shown to form crystalline complexes (with definite
melting points) which failed to separate on recrystallisation.
Alkaline degradation of these N-alkylpteridones led to pyrazine
derivatives.   The effect of organic bases on the 7-phenyl
compound has been studied and some interesting results are
reported and discussed.

The condensation of aminomalonamide and phenylglyoxal
has been reinvestigated, and the product, m.p. 252-253°, has
been identified as 2-hydroxy-6-phenylpyrazine-3-carboxyamide.

Unambiguous structures have been established for the pteridines obtained by condensation of triamino-4-hydroxy-pyrimidine with $\omega\omega$-dichloroacetophenone, with phenylglyoxal, and with ω-nitroacetophenone.

INTRODUCTION.

ALKYLATION OF PTERIDINES.

Until recently the alkylation of hydroxy-pteridines has seldom been attempted.   Early work was carried out by Ganapati[1], who reported that treatment of "6(or 7)-phenyl-lumazine" (2:4-dihydroxy-6(or 7)-phenylpteridine) with diazomethane gave a dimethyl derivative of unknown constitution.   Wieland and Decker[2] treated leucopterin (I) with diazomethane and obtained a mixture of two trimethyl derivatives of unknown structure which were more soluble in alcohol and water than the starting material.   Eight years earlier Wieland and coworkers[3] had stated that leucopterin did not react with diazomethane;   this earlier failure was ascribed to the absence of water in the reaction mixture.   One of these compounds β-trimethyl-leucopterin was sufficiently soluble in phenol to enable the molecular-weight to be determined.   This fact, together with improved nitrogen analysis, led to the adoption of the correct molecular formulae for leucopterin and Xanthopterin[2,4].   Tetrahydroxypteridine on treatment with diazomethane gave a tetramethyl derivative also of unknown constitution[2].   Methylation of 2:4:7-trihydroxypteridine (II) with methyl sulphate (at 35° and pH8) gave a monomethyl derivative.   A structure has been put forward for this compound[5], but the orientation has not been completely proved.
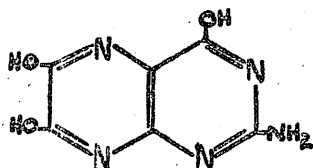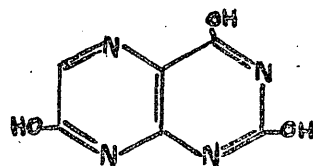
I   II

More recently Albert and coworkers[6] have carried out a
systematic investigation of the methylation of the four
mono-hydroxypteridines.   A variety of methylating agents
were used, and the products included N-, O-, and C-methyl
derivatives.   In general however N-methyl-pteridones are
formed.   Treatment of 4-hydroxypteridine (III) with methyl
sulphate in alkaline solution (pH8) gave a mixture of 3:4-
-dihydro-3-methyl-4-oxopteridine (V) and 1:4-dihydro-1-methyl-
-4-oxopteridine (IV) in the ratio 2:1.   Ethereal diazomethane
on the other hand gave a mixture of 4-methoxypteridine (VI)
and the 3-methylpteridone (V) in the ratio 1:3.

The same research school[7] had previously reported
that methylation of 7-hydroxypteridine (VII) with molecular
quantities of diazomethane gave 7:8-dihydro-8-methyl-7-
oxopteridine (IX) and only a trace of 7-methoxypteridine (VIII).
A large excess of diazomethane resulted in dimethylation
giving 7:8-dihydro-6:8-dimethyl-7-oxopteridine (X).   The
latter was also obtained by treating (IX) with an excess of

III

IV

+

VI

+

V

VII

VIII

+

X
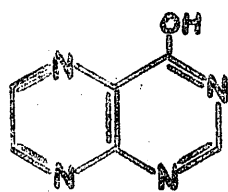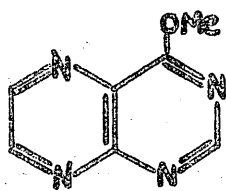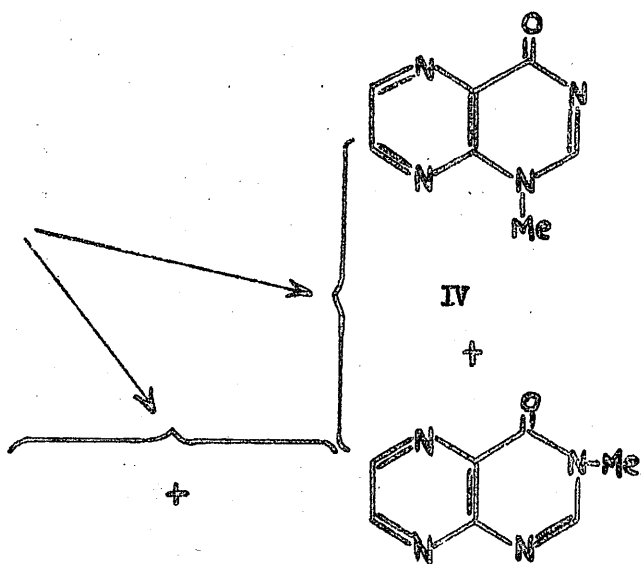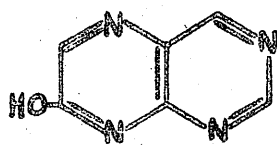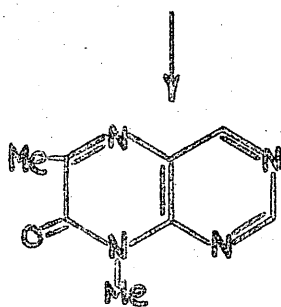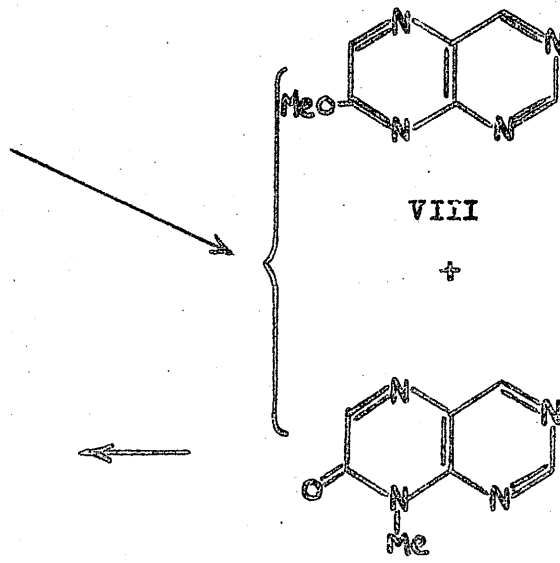
IX

diazomethane.

The 8-methyl-7-pteridone (IX) was also obtained in better yield by the use of methyl sulphate in water at pH8, when an excess of the reagent was used, about 5% of the dimethylated compound (X) was also formed. The introduction of the second methyl group at position 6 is a rare example of C-methylation in an electron deficient series. Methylation on a carbon atom bearing a highly negative charge is well known, e.g. in the pyrolle and indole series, but electron diagrams show that in pteridine all the carbon atoms are positive[8]. The above example was explained along the lines of C-methylation of ethyl β-diethylaminocrotonate[9] and the 1:2-dihydroisoquinolines[10] viz., that resonance between the three atom systems (XI) and (XII) produces a hybrid with a negatively charged carbon atom which is attacked by a methyl cation and, finally a proton is eliminated[11]. The equivalent of (XII) with 8-methyl-7-pteridone is (XIII), which contains a five atom system vinylogous with (XII). This accounts for C-methylation by methyl sulphate but that by diazomethane probably involves addition across the 5:6 double bond to give at first a 1:2:3-triazoline (XIV) just as in the reaction of benzylideneaniline with diazomethane[12].

$$>N - \underset{|}{C} = \underset{|}{C}H \qquad \longleftrightarrow \qquad >\overset{\oplus}{N} = \underset{|}{C} - \overset{\ominus}{\underset{|}{C}}H$$

(XI)   (XII)
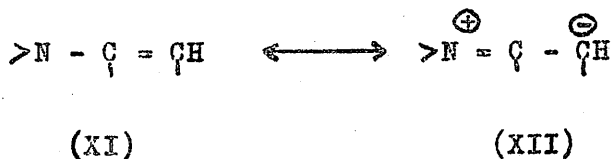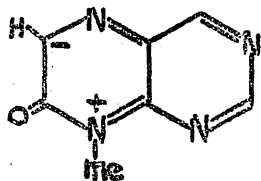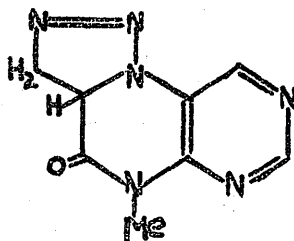
XIII   XIV

The early failure of 2-hydroxypteridine (XV) and
6-hydroxypteridine (XVII) to react with diazomethane and
aqueous methylsulphate was thought to be associated with the
formation of very stable monohydrates by these pteridines[6a].
However both have been shown to be capable of methylation[6b]
with methyl iodide or methyl sulphate in methanolic sodium
methoxide, giving 1:2-dihydro-1-methyl-2-oxopteridine (XVI)
and 5:6-dihydro-5-methyl-6-oxopteridine (XVIII) as mono-
hydrates.   The latter is produced in low yield (14%) and
forms crystalline complexes with 6-hydroxypteridine (in the
ratios, 2:1 and 5:2).
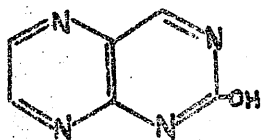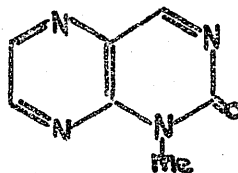
XV   XVI
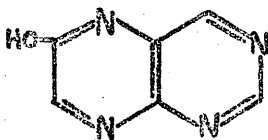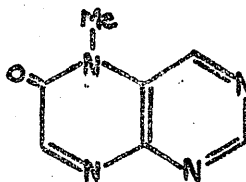
XVII   XVIII

Methyl sulphate methylation of 2:4-dihydroxypteridine (XIX) (in aqueous suspension at pH8) and 6:7-dihydroxypteridine (XXI) (in sodium methoxide) gave the NN'-dimethyl derivatives (XX) and (XXII) respectively[6b].
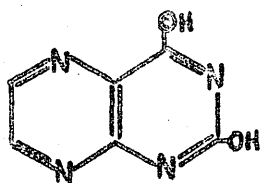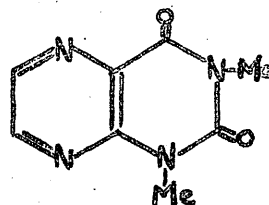
XIX　　　　　XX
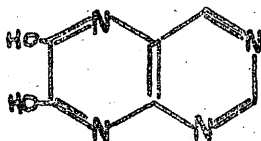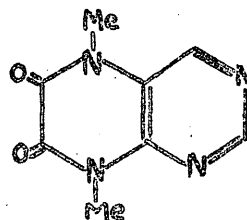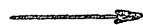
XXI　　　　　XXII

One example of alkylation other than methylation has been reported. Taylor[13] found that treatment of 4-hydroxy-6:7-diphenylpteridine (XXIII) with benzyl chloride under mild alkaline conditions gave 3:4-dihydro-3-benzyl-4-oxopteridine (XXIV). Further examination of this reaction has revealed that a small quantity ($\leqslant$ 1%) of the isomeric 1-benzylpteridone (XXV) is also formed[14].

82
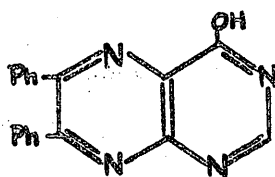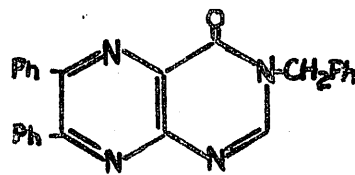
XXIII   XXIV
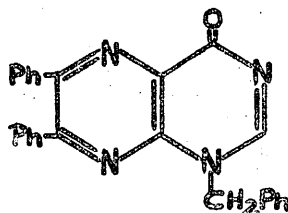
XXV

The alkylation of some mercaptopteridines has been reported. Treatment of 4-hydroxy-2-mercaptopteridine (XXVI) with ethyl bromide and sodium ethoxide gives the S-ethyl derivative (XXVII)[15]. Methyl iodide and sodium hydroxide give the S-methyl derivatives (e.g. XXIX) with 2,4, and 7--monomercaptopteridines (e.g. XXVIII)[16].
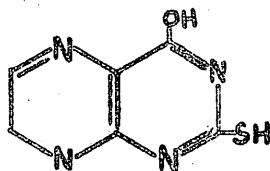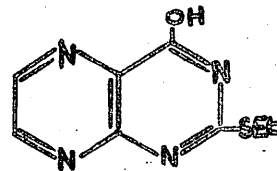
XXVI   XXVII
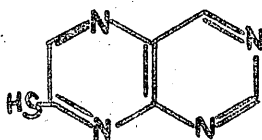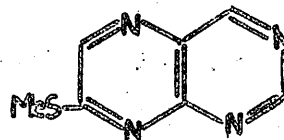
XXVIII   XXIX

SYNTHESES OF N-METHYLPTERIDONES.

Syntheses of N-methylpteridones from N-methyl and NN'-dimethylpyrimidones were reported prior to the recent systematic study of the methylation of hydroxypteridines[17,18]. For example benzil (XXX) reacted with 5:6-diamino-1:2:3:4--tetrahydro-2:4-dioxopyrimidine (XXXI) to give the NN'-dimethyl-pteridone (XXXII).

| XXX | XXXI | XXXII |
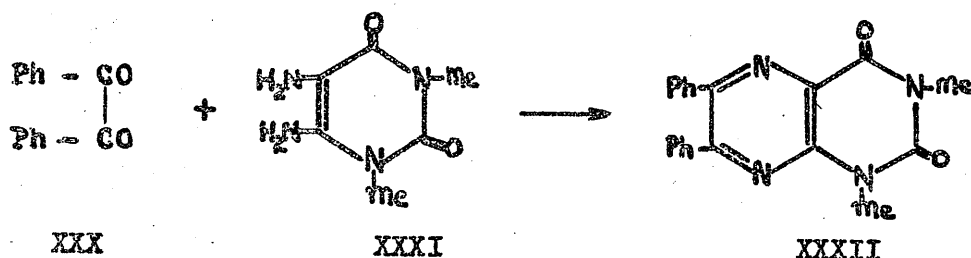
More recent syntheses connected with the alkylation studies have generally involved ring cyclisation of a substituted pyrazine amide[6,13]. Thus 1:4-dihydro-1-methyl--4-oxo-pteridine (IV) was prepared by cyclisation of 2-methylaminopyrazine-3-carboxyamide (XXXIII) with a mixture of acetic anhydride and formic acid.[6,13]

| XXXIII | IV |
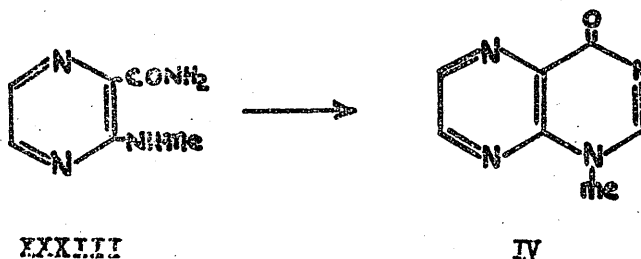
CLEAVAGE BY ALKALI.

1. Pteridones.

The outstanding property of the N-methylpteridones is the ease with which they are degraded by mild alkali. The parent hydroxypteridines are relatively stable to mild alkali.

Pteridine (XXXIV) itself is readily degraded by both dilute acid and alkali. Treatment with refluxing N-sulphuric acid for five minutes gives 2-amino-3-formylpyrazine (XXXV), whilst 2N-sodium carbonate, when hydroxylamine is present, rapidly produces 3-formyl-2-pyrazinylformamidine oxime (XXXVI)[6b].
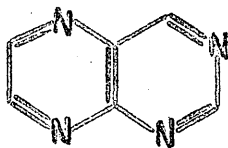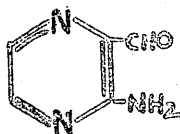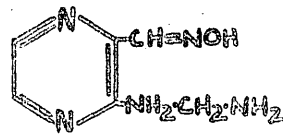
| XXXIV | XXXV | XXXVI |

This instability has been attributed to the electron attracting character of the four-ring nitrogens which leads to partial localisation, on the nitrogen atoms of the 10π electrons originating from the six carbons and four nitrogens. Thus the aromatic stabilisation normally conferred by the presence of these π electrons is much diminished. The tendency of the pteridine nucleus to lose the pyrimidine (rather than the pyrazine) ring on acid and alkaline hydrolyses is consonant with the electron-density diagrams[8] which show

the pyrimidine ring to be more polar and hence more readily attacked. Substitution of the pteridine nucleus by electron-releasing groups restores this deficit of electrons and thus permits normal aromatic stabilisation. However at least two such groups are generally necessary and their efficacy varies somewhat with the positions at which they are inserted[19]. Tri- and tetrasubstitution at the 2:4:6 and 7- positions confers remarkable stability, as is indicated by the inertness of the naturally occurring leucopterin and xanthopterin[3,8,20,21].

In the N-alkylpteridones the stabilising effect of the electron releasing hydroxyl groups of the parent hydroxy-pteridine has been removed. Further the ability of the hydroxypteridines to form an anion, where the negative charge is distributed over the pteridine ring system, tends to inhibit attack by similarly charged hydroxyl ions. Such a stabilisation is impossible with an N-alkylpteridone as it cannot form a simple anion, and hydrolytic attack followed by ring cleavage occurs with ease[6,13]. For example 2:4--dihydroxypteridine requires to be heated with 10N-sodium hydroxide for 2 hours at 180° before ring opening occurs[22].

1:2:3:4-Tetrahydro-1:3-dimethyl-2:4-dioxopteridine (XXXVII) is hydrolysed to 2-methylaminopyrazine-3-carboxymethylamide

(XXXVIII) by refluxing with N-sodium hydroxide for 1 minute.

XXXVII   XXXVIII
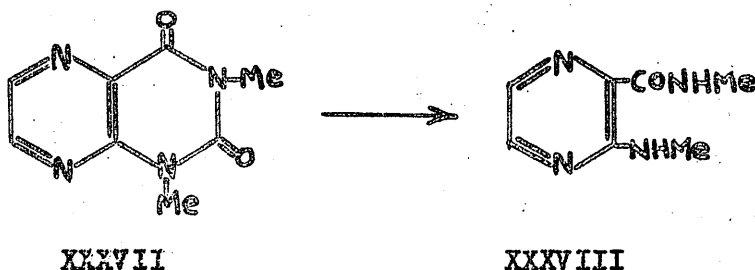
This lability of N-alkylpteridones has been used to determine the structure of the alkylation products of hydroxypteridines by degrading them to pyrazine or pyrimidine compounds[6,13]. Thus the isomeric 3-methyl-4-pteridone (V) and 1-methyl-4-pteridone (IV) readily yielded 2-aminopyrazine-3-carboxymethylamide (XXXIX) and 2-methylaminopyrazine-3-carboxyamide (XL) respectively with mild alkali.

V   XXXIX

IV   XL
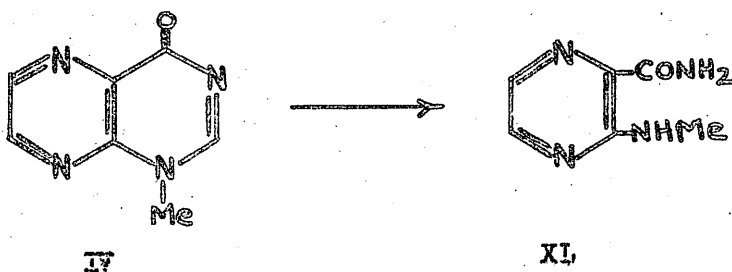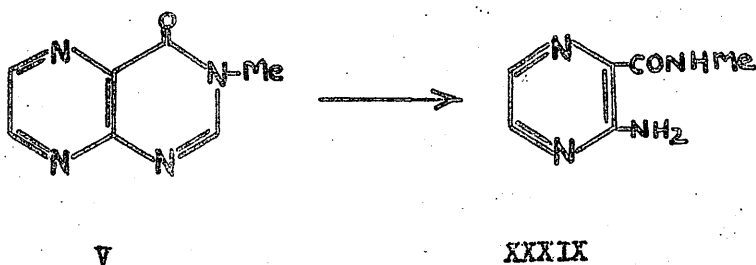
The 1-methyl-4-pteridone (IV) is quantitatively decomposed after 5 minutes at pH12, while the parent 4-hydroxypteridine requires refluxing for 1 hour with 10N-sodium hydroxide to effect cleavage[19]. Methoxy pteridines (e.g. VI) are not cleaved by mild alkali, but are rapidly hydrolysed to the corresponding hydroxy compounds[19,25].

VI   III
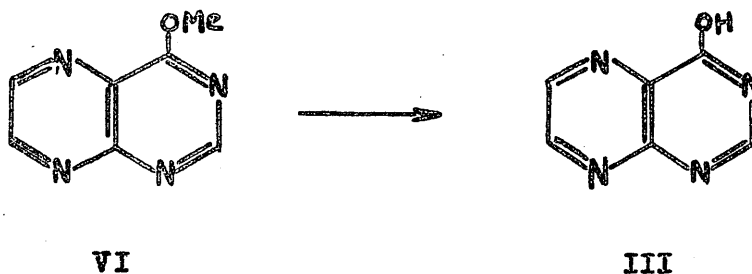
2. Hydroxy- and Aminopteridines.

Although the introduction of electron releasing groups into pteridine stabilises the nucleus, probably every known pteridine may be cleaved by basic hydrolysis provided sufficiently vigorous conditions are employed. The cleavage of lumazines to pyrazines with strong alkali was developed by Weijlard, Tishler and Erickson[22] as a route to substituted 2-aminopyrazine-3-carboxylic acids. Thus 2:4-dihydroxypteridine (XLII) yields 2-aminopyrazine-3-carboxylic acid (XLIII) (94%) on autoclaving at 170° for 2 hours with 10N-sodium hydroxide[22].

XLII   XLIII
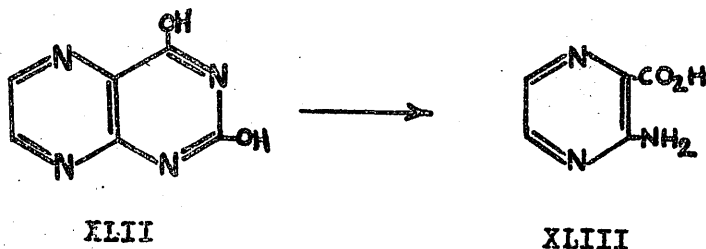
2-Amino-4-hydroxy- and 2:4-diaminopteridines are also
cleaved to pyrazines under similar conditions, and the
reaction has been employed as a means of establishing the
structure of various pteridine derivatives monosubstituted
in the pyrazine ring.   The decarboxylated product [2-amino-
-4-hydroxy-6-methylpteridine (XLIV)] formed by condensation
of triamino-6-hydroxypyrimidine with methyl $\gamma\gamma$-dimethoxy-
acetoacetate was cleaved by alkali to 2-amino-5-methyl-
pyrazine-3-carboxylic acid (XLV), thus establishing the
structure of the initial condensation product as 2-amino-4-
-hydroxypteridine-6-acetic acid[23].   This result played an
important part in the fundamental structural work on folic
acid.

XLIV   XLV
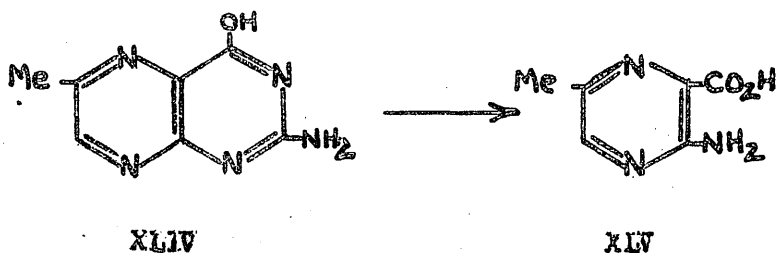
Similarly, the reaction product of triamino-6-hydroxy-
pyrimidine and methyl glyoxal acetal was shown to be 2-amino-
-4-hydroxy-7-methylpteridine (XLVI) by cleavage to 2-amino-6-

-methylpyrazine-3-carboxylic acid (XLVII)[23] . The latter
pyrazine was also obtained by cleavage of the reaction
product of tetra-aminopyrimidine and methyl glyoxal, thus
establishing the structure of the product as 2:4-diamino-7-
-methylpteridine[24] .

XLVI   XLVII
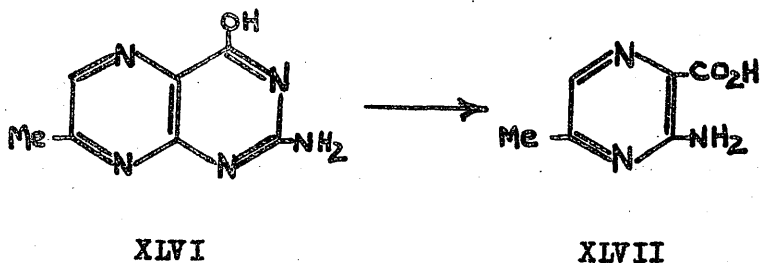
THEORETICAL.

The first alkylation of a pteridine was described in 1937 when Ganapati[1] reported that treatment of "6 or 7-phenyllumazine" (2:4-dihydroxy-6(or 7)-phenylpteridine) with diazomethane gave a dimethyl derivative of unknown constitution. It has been shown recently that diazomethylation of simple hydroxypteridines can give either N-, O-, or C- methyl derivatives[6]. The 6 (or 7)-phenyllumazine (LIII) was prepared in the first instance by condensing the unsymmetrical dicarbonyl compound, phenyl glyoxal (LI) with 5:6-diamino-2:4-dihydroxypyrimidine (LII); a reaction which could result in the formation of either the 6- or 7-phenyl isomer or a mixture of both (LIII).
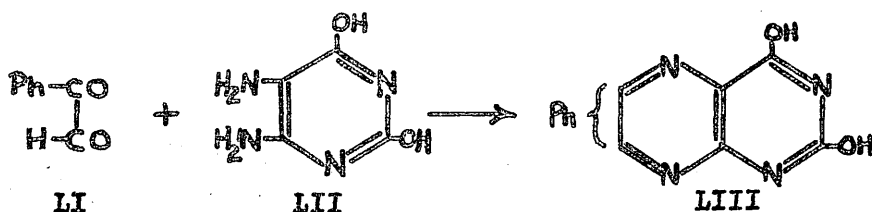
In view of the complete absence of knowledge concerning the constitution of these products, it was decided to reinvestigate the reactions, and establish the nature of the dimethyl compound obtained by Ganapati (m.p. 278°).

Condensation of phenylglyoxal hydrate with 5:6-diamino-2:4-dihydroxypyrimidine sulphate in dilute acetic acid gave an apparently homogeneous product, m.p. > 300°. Treatment

of this substance with diazomethane, followed by crystallisa-
tion from 80% formic acid, gave an apparently homogeneous
dimethyl derivative, m.p. 237-238° (Ganapati gave m.p. 278°).
Alkaline degradation of the dimethyl compound however, gave
results which could not readily be explained[26], and the
presence of a mixture of isomers was suspected. No methoxyl
groups were detected by the Zeisel method of estimation.
Condensation of phenyl glyoxal (LI) with 5:6-diamino-1:2:3:4-
-tetrahydro-1:3-dimethyl-2:4-dioxopyrimidine (LV) in 10%
hydrochloric acid gave the same dimethyl compound, m.p.
237-238°. Synthesis of the dimethylpyrimidine (LV) from
NN'-dimethylurea (LIV), by the following series of reactions[18]
located the methyl groups on the ring nitrogens.

LIV

LV
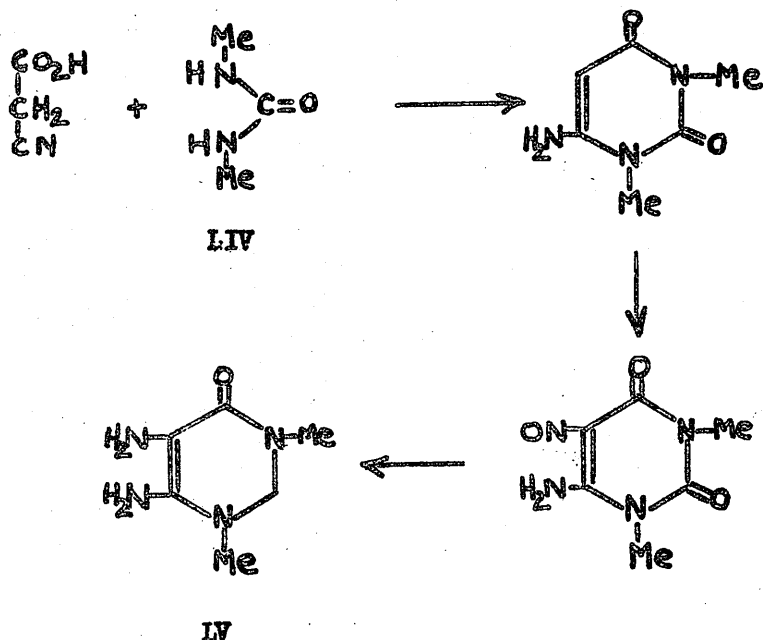
This establishes that NN'-methylation of the pteridine has taken place, and the mixture, if present, must therefore consist of the 6- and 7- phenyl isomers.

Only recently Pfleider[27] has reported that reaction of the pyrimidine (LV) with methyl pyruvate (LVI) gives rise to a mixture of the two possible products (LVIIa, b)

LVI   LV   LVIIa,b
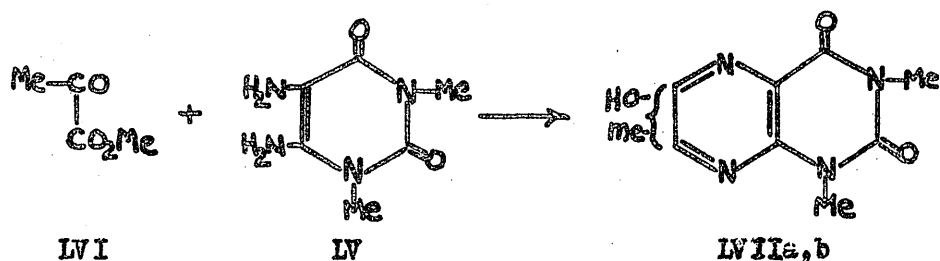
Where an unsymmetrical dicarbonyl compound has been used in the synthesis of pteridines, the use of aldehyde- and ketone-binding reagents tends to force an alkyl or aryl group into the 6-position[28,29]. Condensation of phenyl-glyoxal with 5:6-diamino-2:4-dihydroxypyrimidine (LII) in the presence of sodium hydrogen sulphite, followed by methylation of the product with diazomethane, gave a dimethyl compound, m.p. 299-300°, identical with that obtained by reaction of phenylglyoxal and 5:6-diamino-1:2:3:4-tetrahydro--1:3-dimethyl-2:4-dioxopyrimidine (LV) in the presence of sodium hydrogen sulphite. A mixed melting point of this material and that of m.p. 237-238° showed no depression, and melted over a considerable range. Unequivocal syntheses of

1:2:3:4-tetrahydro-1:3-dimethyl-2:4-dioxo-6-phenylpteridine

(LIX), and the 7-phenyl isomer (LX) were therefore essential.
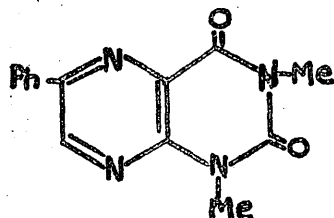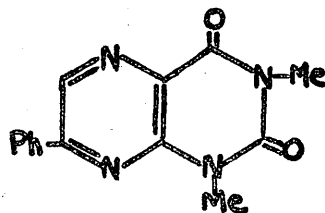
LIX   LX

Condensation of 2:4:6-triamino-5-nitrosopyrimidine

(LXII) with carbonyl compounds possessing a suitably reactive

α-methylene group [such as ethyl phenyl ketone (LXI)] has

been shown to give pteridines of unambiguous structure

(e.g. LXIII)[30].

LXI   LXII   LXIII
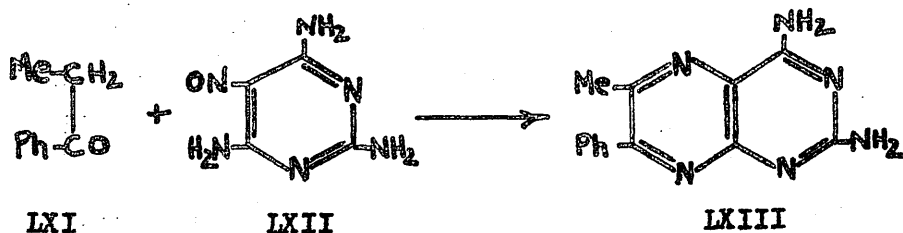
Attempts to condense phenylacetaldehyde (LXIV) with

6-amino-1:2:3:4-tetrahydro-1:3-dimethyl-5-nitroso-2:4-dioxo-

pyrimidine (LXV) in refluxing glacial acetic acid or at higher

temperatures (up to 200°) using the same solvent in a sealed

glass tube[30] were unsuccessful. However, addition of the

nitrosopyrimidine (LXV) to an excess of refluxing phenyl

acetaldehyde (LXIV) gave 1:2:3:4-tetrahydro-1:3-dimethyl-2:4-

-dioxo-6-phenylpteridine (LIX) in 41% yield, m.p. 251-253°.

LXIV   LXV   LIX
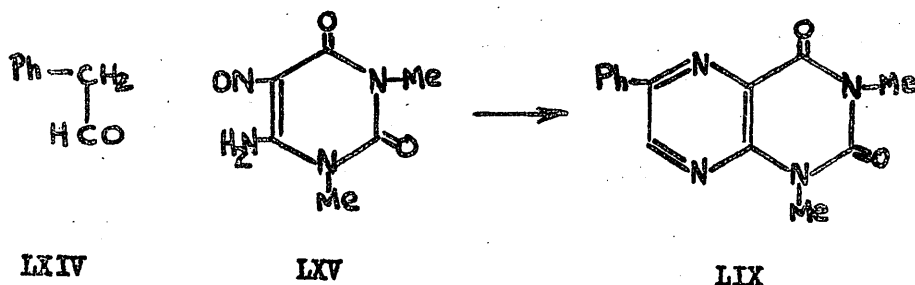
A similar condensation of acetophenone (LXVI) with (LXV) gave 1:2:3:4-tetrahydro-1:3-dimethyl-2:4-dioxo-7- -phenylpteridine (LX), m.p. 298-300°.

LXVI   LXV   LX
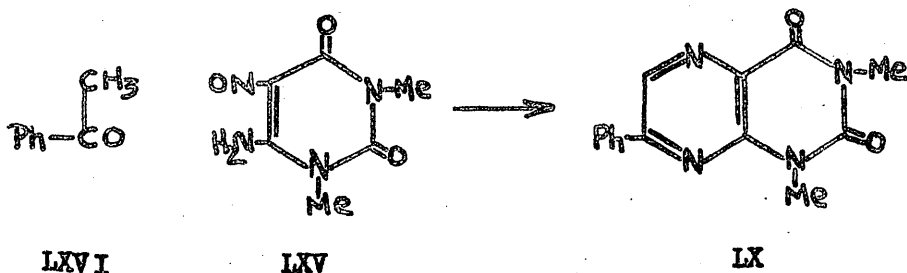
The latter material was identical with that obtained above from the condensation of phenylglyoxal and 5:6-diamino- -1:2:3:4-tetrahydro-1:3-dimethyl-2:4-dioxopyrimidine (LV) in the presence of sodium hydrogen sulphite. Hitherto, it has been generally accepted that when these aldehyde-binding reagents are used, the products will have the alkyl or aryl group in the 6-position[31]. One exception to this rule has already been reported by Albert and coworkers[16], who found that reaction of methyl glyoxal (LXVII) and 4:5-diamino- pyrimidine (LXVIII) always gave 7-methylpteridine (LXIX)

whether an aldehyde-binder was present in the reaction mixture or not.

LXVII   LXVIII   LXIX

It would therefore appear necessary to establish the configuration of the product from such an Isay reaction in each instance.

A mixture of 1:2:3:4-tetrahydro-1:3-dimethyl-2:4-dioxo--6-phenylpteridine (2 parts) and the 7-phenyl isomer (1 part) formed a complex, m.p. 236-238°. Another complex, m.p. 278°, was obtained when the ratio was reversed. It is therefore considered that the material originally obtained by Ganapati[1] from the condensation of phenylglyoxal with 5:6-diamino-2:4-dihydroxypyrimidine in acetic acid solution is a mixture (LIII) of 2:4-dihydroxy-6-phenylpteridine and the 7-phenyl isomer. Methylation of this material with diazomethane gives a mixture of the corresponding NN'-dimethyl compounds (LIX and LX).

LIII   LIX   and   LX

The apparent discrepancy between the melting points given by Ganapati for the methylation product (278°) and that subsequently observed on repeating the reactions (237-238°) would appear to result from a reversal of the relative proportions of the 6- and 7-phenyl isomers formed in the initial condensation.

The constant solubility, ultra-violet adsorption spectrum, and m.p. of the mixed dimethyl derivatives, through successive recrystallisations, together with the failure of routine paper chromatography to separate these isomers is worthy of note in a series where much reliance is placed upon these criteria[25].

The condensation of phenylglyoxal with 5:6-diamino-2:4--dihydroxypyrimidine in strong ammoniacal solution has been reported[22]. No attempt was made to determine the homogenity of the product apart from degradation with 80% sulphuric acid at 220° to an unknown phenyl-aminopyrazine (m.p. 130-131°) in poor yield (14.5%). Repetition of this condensation gave a 2:4-dihydroxy-phenylpteridine, which on methylation with diazo-methane, gave a dimethyl derivative m.p. 299-300°, identical with the material obtained above. The initial condensation product was therefore 2:4-dihydroxy-7-phenylpteridine (LIIIb) and the pyrazine degradation product (m.p. 130-131°) was 6-phenyl-2-aminopyrazine (LXX).

LIIIb   LX   LXX
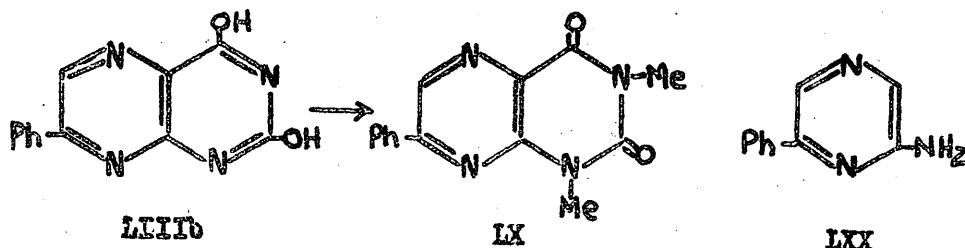
The preparation of 2:4-dihydroxy-7-phenylpteridine
carried out in the presence of sodium hydrogen sulphite
(see previously, yield 87%) is preferred to that employing a
strong aqueous ammonium hydroxide medium[22].   In repeating the
latter method dark gummy reaction mixtures and poor yields
(20%) were encountered.   A synthesis of the 6-phenyl isomer
was not achieved.   Attempts to condense phenylacetaldehyde
with 6-amino-2:4-dihydroxy-5-nitrosopyrimidine (LXXI) or
6-amino-4-hydroxy-2-methylthio-5-nitrosopyrimidine (LXXII)
under a variety of conditions proved unsuccessful (cf.[32])
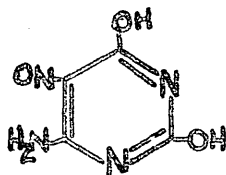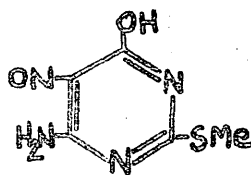
LXXI   LXXII

The ease with which N-alkylpteridones can be degraded
with dilute alkali has been illustrated and discussed   in the
introduction to this section[6,15].   Fischer[35] has also pointed
out that purines become more subject to hydrolysis by alkali

upon N-methylation.   1:2:3:4-Tetrahydro-1:3-dimethyl-2:4-
-dioxo-6-phenylpteridine (LIX) and the 7-phenyl isomer (LX)
showed surprising stability to hydrolysis by aqueous alkali.
Refluxing with 2N-sodium hydroxide for 1 hour left 40% of the
former and 47% of the latter, unchanged. Increasing the
concentration of the alkali to 5N and the reaction time to
3 hours did not effect complete degradation; 27% and 35%
respectively remained unchanged.   This apparent stability
is due to the lack of solubility of these pteridones in aqueous
alkali.   Thus 1:2:3:4-tetrahydro-1:3-dimethyl-2:4-dioxo-6-
-phenylpteridine (LIX) gave a quantitative yield of 2-methyl-
amino-5-phenylpyrazine-3-carboxymethylamide (LXXIII) on
refluxing with 0.1 N-potassium hydroxide in ethanol for
15 minutes.   Similar treatment of the 7-phenyl isomer (LX)
gave 2-methylamino-6-phenylpyrazine-3-carboxymethylamide (LXXV).
More drastic hydrolysis (16 hours at 150-200°) of either the
pteridones (LIX, LX) or the methylamides (LXXIII, LXXV) with
ethanolic sodium hydroxide gave the corresponding 2-methylamino-
-5(and 6)-phenylpyrazine-3-carboxylic acids (LXXIV, LXXVI).

These acids (LXXIV, and LXXVI) were also isolated in
small quantities, together with starting material and the
corresponding methylamides (LXXIII and LXXV), from the aqueous
alkali degradation of the N-methylpteridones (LIX, and LX).
They are formed in this reaction by the secondary hydrolysis of

LIX

LXXIII

LXXIV

LX

LXXV

LXXVI
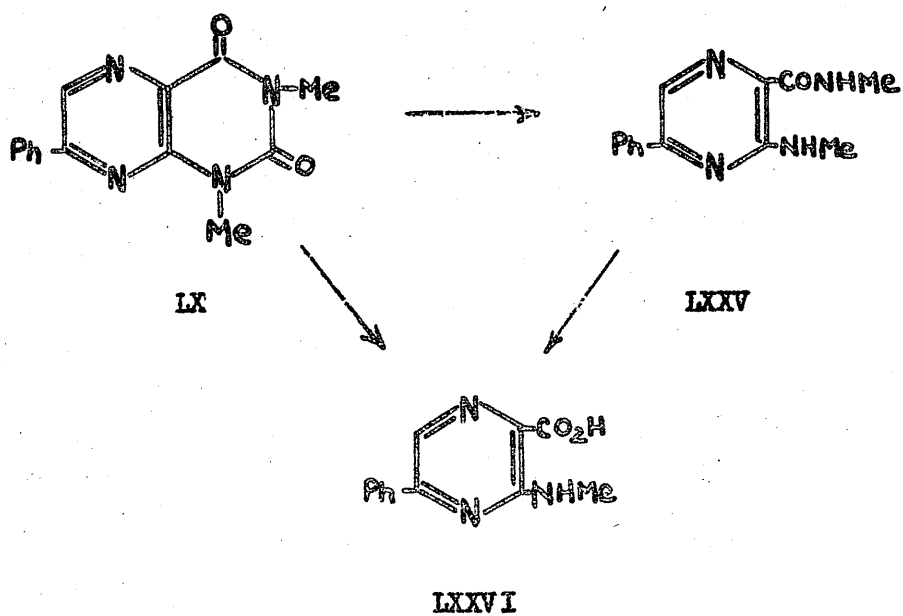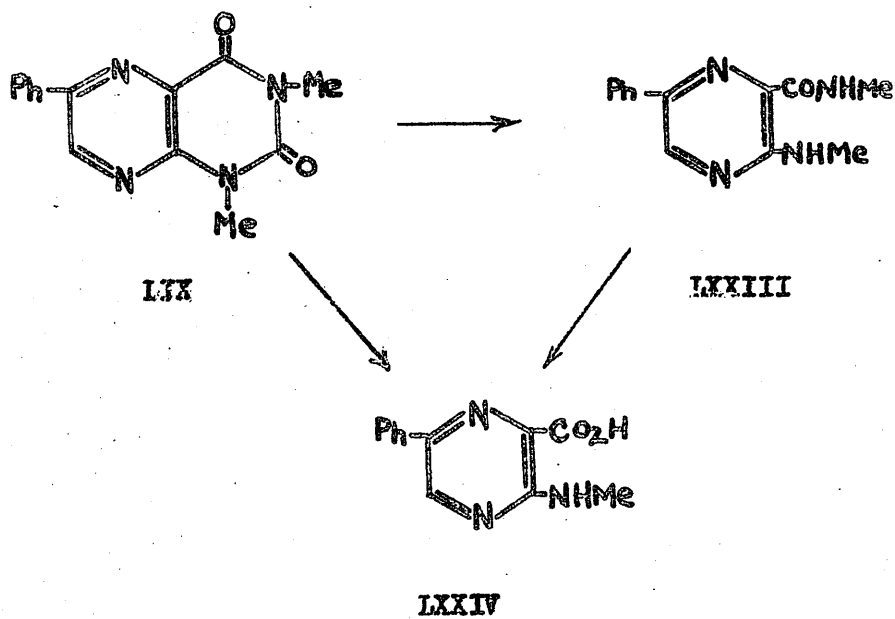
the methylamides (LXXIII and LXXV), the main degradation

products, which would appear to result from initial

nucleophilic attack of the hydroxyl ion at C_2, followed by

ring opening. They probably do not result from a dual cleavage

of the pteridine ring system since hydrolysis of the pure

methylamides, under the conditions used for the degradation

of the parent pteridones, gave a greater yield of the

carboxylic acids in each case, and in the briefer ethanolic

alkali degradations only the respective methylamide was formed.

These degradations are to be compared with the reported

hydrolysis of 3:4-dihydro-3-methyl-4-oxopteridine (LXXVII)

with N-sodium hydroxide when 2-aminopyrazine-3-carboxymethyl-

amide (35%) (LXXVIII) and the corresponding acid (50%) (LXXIX)

were formed simultaneously, by fission of the pteridine ring

system in two different ways[6a].

LXXVII   LXXVIII   LXXIX
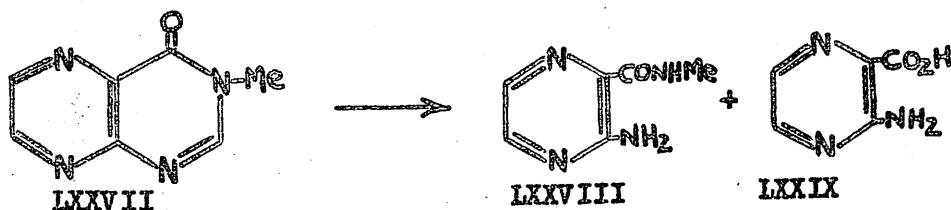
Like the N-methylpurines, the N-methylpteridones are

less sensitive to acid than to alkali.   1:2:3:4-Tetrahydro-

-1:3-dimethyl-2:4-dioxo-7-phenylpteridine (LX) was unchanged

by refluxing 5N-hydrochloric acid.

Pteridines which undergo cleavage with alkali would
be expected to be attacked by other nucleophilic reagents,
and the structural features influencing the degree of
lability towards alkali should govern the latter cases as
well.   Thus 2:4-dihydroxy-6:7-diphenylpteridine (LXXX),
which was degraded under forcing alkaline conditions (17%
aqueous sodium hydroxide under reflux for 35 hours) to give
3-amino-5:6-diphenylpyrazine carboxylic acid[22], was shown by
Taylor[34] to be more readily cleaved by benzylamine.   Refluxing
with the amine for 5 minutes gave 2-(3-benzylureido)-5:6-
-diphenylpyrazine-3-carboxybenzylamide (LXXXII) in 42% yield,
while longer heating led to further cleavage of the ureido
substituent to give 2-amino-5:6-diphenyl-3-carboxybenzylamide
(LXXXIII) in 60% yield.   Similar types of pyrazine products
resulted when the secondary amines, piperidine and morpholine,
were used[34].

LXXX   LXXXI

LXXXII   LXXXIII
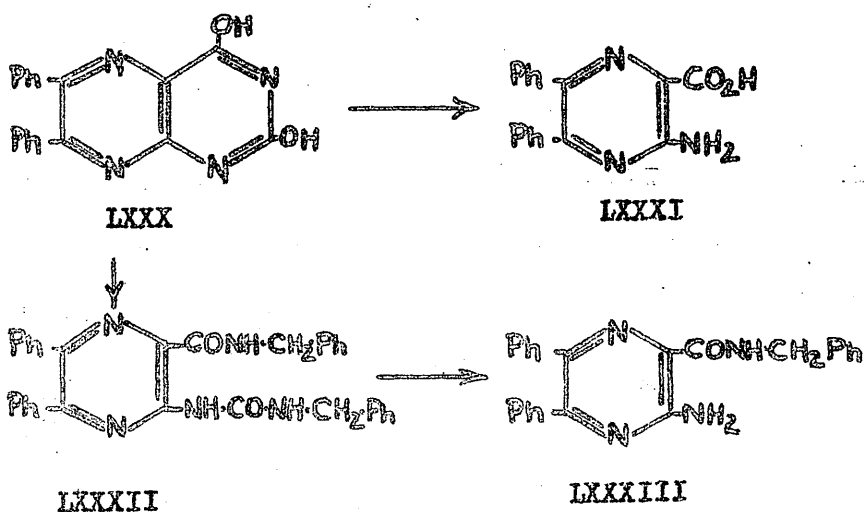
The mechanism of these cleavages involves initial nucleophilic attack at C_4 by the amine, followed by ring cleavage at the $N_3 - C_4$ linkage. This gives rise to a 2-ureidopyrazine-3-carboxyamide (LXXXIV) which undergoes immediate ammonolysis to give the first isolated product a 2-(substitutedureido) pyrazine-3-carboxyamide (LXXXV). Further ammonolysis of the latter leads to the final product a substituted-2-aminopyrazine-3-carboxyamide (LXXXVI) and a 1:3-disubstituted urea (LXXXVII).

Whilst the unsubstitued 2-ureidopyrazine (LXXXIV) was never isolated, evidence for its participation was found in the cleavage of (LXXX) with hydrazine which gave a mixture of the expected 3-amino-5:6-diphenylpyrazinoic acid hydrazide (LXXXVIII) and a small amount of 3-amino-2-hydroxy-3:4-dihydro-5:6-diphenylpteridine (LXXXIX). The latter could only result from recyclisation of the 2-ureidopyrazine intermediate (XC).

Although the alkaline cleavage of N-methylpteridones has recently been reported[6,15], the effect of organic amines has not been studied. Treatment of 1:2:3:4-dihydro-1:3-dimethyl--2:4-dioxo-7-phenylpteridine (LX) with refluxing benzylamine failed to effect cleavage, and still more drastic conditions (16 hours in a sealed tube at 210°) left the pteridone unchanged. This was extremely surprising in view of the

LXXX

LXXXIV

LXXXV

LXXXVI

LXXXVII

LXXX

XC

LXXXIX

LXXXVIII
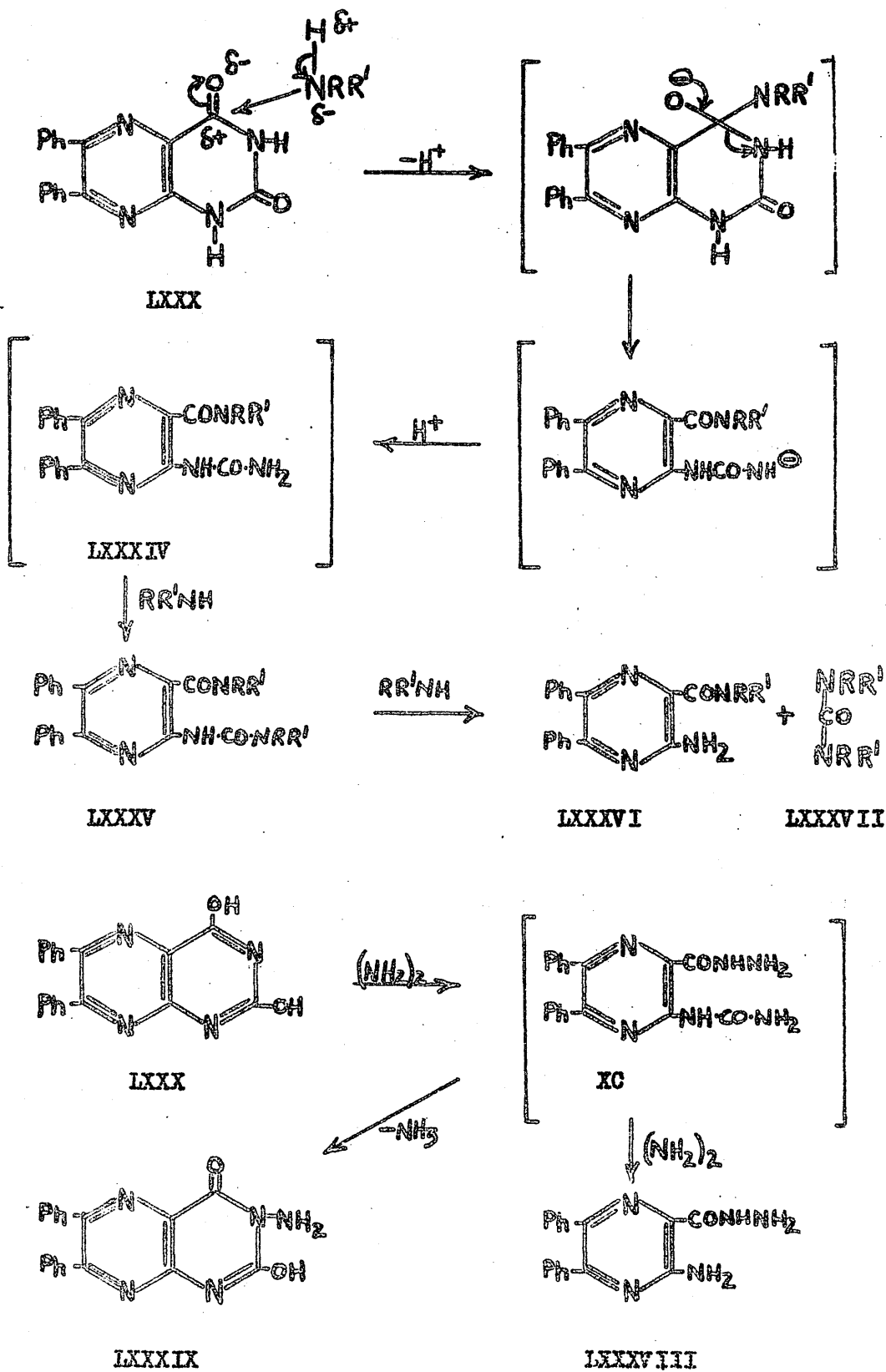
favourable electronic conditions existing in the pteridone for nucleophilic attack, and evidenced by the lability to hydroxyl ion, when in solution.

However suspension in refluxing hydrazine hydrate led to a rapid attack and the formation of a single product, 3-methyl-amino-5-phenylpyrazinoic acid hydrazide (XCI), which was identified by synthesis from 3-methylamino-5-phenylpyrazinoic acid (LXXVI), via the methyl ester (XCII), by treatment of the latter with hydrazine.

LX   XCI

LXXVI   XCII
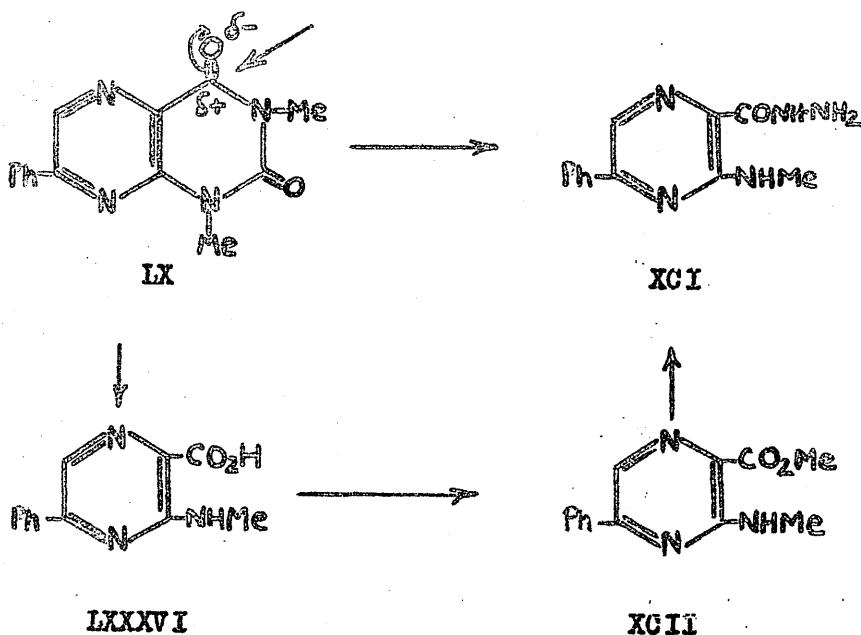
Reaction of (LX) with ethanolic ammonia under anhydrous conditions also led to ready attack, with the formation of 2-methylamino-6-phenylpyrazine-3-carboxyamide (XCIII).
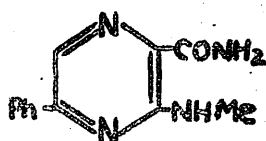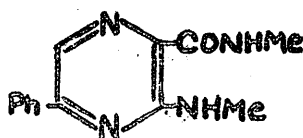
XCIII   LXXV

Treatment of the corresponding pyrazine-3-carboxymethyl-amide (LXXV) under similar conditions did not give (XCIII). This would indicate that initial attack occurred at C_4 and not at C_2, and that cleavage of the pteridone by ammonia and hydrazine probably follows a similar route to that suggested for the aminolytic cleavage of 2:4-dihydroxypteridines[24].

A study of a Stuart model of the pteridone (LX) indicated that the resistance to attack by benzylamine was probably due to steric factors. The presence of the methyl groups on the ring nitrogens exhibit a blocking effect to the large benzylamine molecule, while the smaller nucleophilic agents, ammonia, hydrazine or hydroxyl ion are not inhibited from attacking the positive carbon centres.

The condensation of aminomalonamide and phenylglyoxal, and the unambiguous synthesis of 2-amino-4-hydroxy-7-phenylpteridine.

Condensation of aminomalonamide with phenylglyoxal, using 12.5N-sodiumhydroxide as catalyst, has been shown[36] to give 2-hydroxy-5(or 6)-phenylpyrazine-3-carboxyamide, m.p. 213-216° (decomp.) but the position of the phenyl group was

not determined. Repetition of this reaction, which was not
described in detail, gave a compound, m.p. 252-253° (decomp.)
in poor yield, analysing for 2-hydroxy-5(or 6)-phenylpyrazine-
-3-carboxyamide. A similar condensation between aminomalon-
amide and methyl glyoxal[37], (see Section I page 31) gave 2-
-hydroxy-6-methylpyrazine-3-carboxyamide whereas earlier work
by Jones[36] had given the 5-methyl compound. The use of
aldehyde binding reagents in this type of condensation has
already been investigated (Section I, page 32), and, as
expected, sodium hydrogen sulphite facilitated the reaction.
The same product, m.p. 252-253°, was obtained in the absence
of the usual basic catalyst, in greatly increased yields.
Use of these aldehyde-binding reagents enables the condensation
to take place at room temperature or above, without the
formation of black intractable tars.

The above product was hydrolysed to a carboxylic acid,
m.p. 217° (decomp.), and the methyl ester of which heated
at 155° with phosphorus oxychloride in a sealed tube gave
2-chloro-3-methoxycarbonyl-5(or 6)-phenylpyrazine, m.p.81-83°.

Reaction of the chloro-ester with alcoholic methylamine
at 140° gave 2-methylamino-6-phenylpyrazine-3-carboxymethyl-
amide (LXXV) identical with the product already obtained from
the alkaline degradation of 1:2:3:4-tetrahydro-1:3-dimethyl-
-2:4-dioxo-7-phenylpteridine (LX). This establishes that the

phenyl group in the above pyrazine derivatives is in the
6-position and that the original product was therefore
2-hydroxy-6-phenylpyrazine-3-carboxyamide (XCIV). The
material (m.p.213-216°) obtained by Jones[36] is presumably
the 5-phenyl isomer.

XCIV   XCV

LXXV   XCVII   XCVI

LX
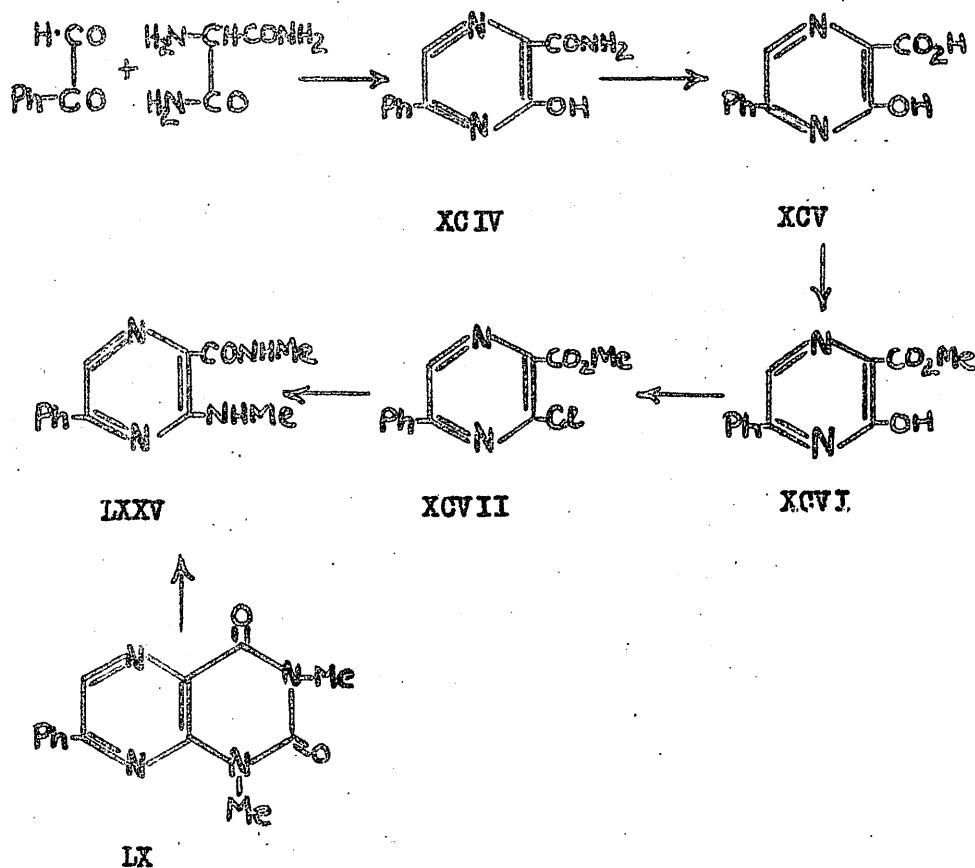
King and Spensley[38] reported the synthesis of 2-amino-
-4-hydroxy-6-phenylpteridine (CIV) from triamino-6-hydroxy-
pyrimidine (C) and $\omega\omega$-dichloroacetophenone (CIII), and of
the 7-phenyl isomer (CII) from the same pyrimidine and either

phenylglyoxal or ω-nitroacetophenone (XCIX). Because of
the absence of melting points, these pteridines were difficult
to distinguish.   However degradation with 4N-sodium
hydroxide at 170° for 20 hours gave rise to two distinct
series of pyrazine derivatives but none of the products were
known or synthesised unambiguously[38].   The condensation of
triamino-6-hydroxypyrimidine (C) and ω-nitroacetophenone
(XCIX) in the absence of bisulphite yielded a Schiff's base
intermediate (CI) which gave a colour with Folin-Denis
reagent[39].   From this it was deduced by King and Spensley
that the 5-amino group was still present in the Schiff's base
(CI) and that cyclisation of the latter, with dithionite gave
the 7-phenylpteridine (CII).

XCIX   C   CI

XCV   CII
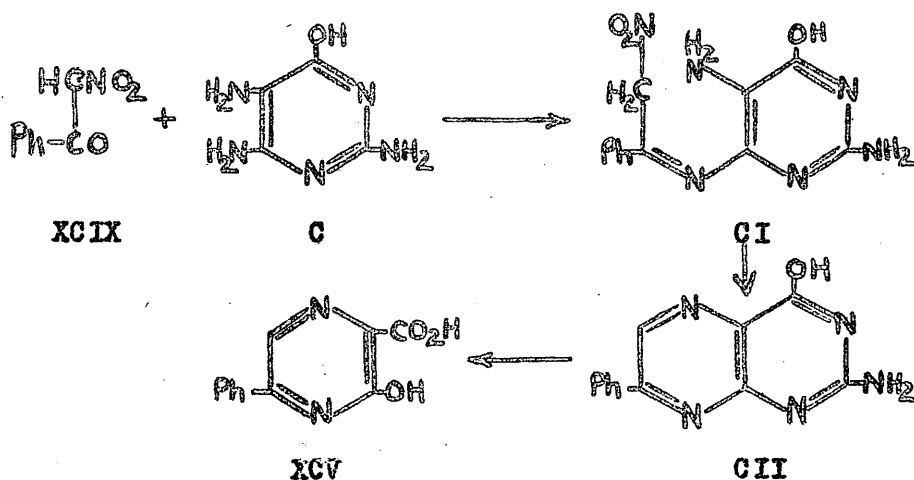
As the product obtained when ωω-dichloroacetophenone
(CIII) was condensed with the pyrimidine (C) gave rise to a
different series of pyrazine derivatives, it was concluded[38]

that it must be the isomeric 2-amino-4-hydroxy-6-phenyl-pteridine (CIV).

CIII   C   CIV
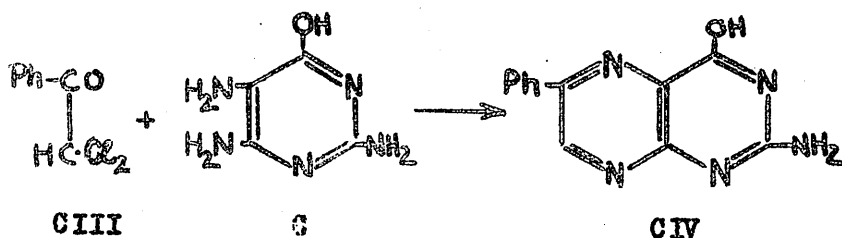
Alkaline degradation of the product (CII) from ω-nitroacetophenone gave 2-hydroxy-6-phenylpyrazine-3--carboxylic acid (XCV) m.p. 208-209° (ethyl ester, m.p. 112-114°).[38] This compound appears to be identical with the carboxylic acid, m.p. 217° (ethyl ester, m.p. 112-114°) obtained from 2-hydroxy-6-phenylpyrazine-3-carboxyamide (XCIV) (see above).

However repetition of the condensation of triamino-6--hydroxypyrimidine (C) with $\omega\omega$-dichloroacetophenone, with phenylglyoxal, and with ω-nitroacetophenone gave in each case 2-amino-4-hydroxy-6-phenylpteridine (CIV). The infra-red spectra of these products were all identical and different from the 7-phenyl isomer (CII), which was unambigu-ously prepared by condensing 2-chloro-3-methoxycarbonyl-6--phenylpyrazine (XCVII) with guanidine carbonate (cf.[37]).

XCVII   CII

The infra-red spectrum of a specimen of "2-amino-4-
-hydroxy-7-phenylpteridine" kindly supplied by Dr. P. C.
Spensley was identical with that of the 6-phenyl isomer
(CIV) and different from that of the authentic 7-phenyl
compound (CII).

The Schiff's base formed on repetition of the condensa-
tion of the triamino-6-hydroxypyrimidine (C) and ω-nitro-
acetophenone did not give a colour with Folin-Denis reagent[39].
Cyclisation of the anil with sodium dithionite, followed by
alkaline degradation of the pteridine gave 2-hydroxy-5-phenyl-
pyrazine-3-carboxylic acid (CV), m.p. 200° (ethyl ester,
m.p. 158-159°).

CIV   CV

It is difficult to explain the difference between these
results and those of King and Spensley. The orientation of
the products obtained when unsymmetrical dicarbonyl compounds

and their derivatives are condensed with 4:5-diaminopyrimidines appears to be unpredictable, and different products can be obtained under apparently identical conditions.   It is therefore essential that these structures should be verified in each instance.

EXPERIMENTAL

Melting points were determined using a standard N.P.L. thermometer.

Paper chromatography was performed on No. 1 Whatman paper by the ascending method using (separately) butanol-5N--acetic acid (7:3) and aqueous ammonium chloride 3%, and the chromatograms were viewed in ultra-violet light of wave-lengths 254 and 365 mu. Yields of substances that have no definite melting point refer to the stage when they appeared chroma- tographically homogeneous.

Substances were dried in air at 110° unless otherwise stated.

Ultra-violet absorption spectra were determined with a Unicam S.P. 500 spectrophotometer and E denotes intensity of absorption.

5:6-Diamino-2:4-dihydroxypyrimidine sulphate[40]. - Powdered urea (11.3 g.) and ethyl cyanoacetate (10.6 c.c.) were added to a solution of sodium (4.6 g.) in absolute ethanol (75 c.c.). The mixture was heated under reflux for 2 hours and then filtered hot. The sodium salt of 6-amino-2:4--dihydroxypyrimidine thus obtained was washed with alcohol and dissolved in water (100 c.c.). Ice (50 g.) and sodium nitrite (8 g.) were added and the mixture added dropwise to a mixture of acetic acid (24 g.), water (25 c.c.) and ice (75 g.).

Ammonium hydroxide (S.G., 0.88) was then added until the
solution became alkaline, when the rose-coloured salt of
6-amino-2:4-dihydroxy-6-nitrosopyrimidine was filtered off.
The salt was suspended in hot water (250 c.c.), sodium
hydrosulphite (40 g.) added, and the stirred mixture heated
until boiling.   The mixture was chilled and the buff-coloured
precipitate of 5:6-diamino-2:4-dihydroxypyrimidine sulphate
collected by filtration.   The product was purified by
dissolving in 6% aqueous sodium hydroxide containing a little
sulphite, charcoaled, and the clarified solution poured into
boiling 10% sulphuric acid (110 c.c.).   After chilling, the
pyrimidine sulphate was filtered off, washed with water, and
dried, 13 g., 62%.

6-Amino-1:2:3:4-tetrahydro-1:3-dimethyl-5-nitroso-2:4-
-dioxopyrimidine monohydrate[18]. -   N N'-Dimethylurea (88 g.),
cyanacetic acid (85 g.) and acetic anhydride (202 c.c.) were
heated, with the exclusion of moisture, at 60° for 3 hours.
The excess anhydride and the acetic acid formed during the
reaction were removed under reduced pressure.   A 5% sodium
hydroxide solution (500 c.c.) was added slowly to the cooled,
stirred residue, whereupon the 6-amino-1:2:3:4-tetrahydro-1:3-
-dimethyl-2:4-dioxopyrimidine precipitated.   A solution of
sodium nitrite (83 g.) in water (500 c.c.) was added to the

cooled, stirred mixture and it was acidified by the dropwise addition of acetic acid (120 c.c.) over a period of 1 hour. The stirring was continued for an additional 2 hours at room temperature. The mixture was thoroughly cooled, the red-violet precipitate was filtered and washed with water, 95% ethanol and finally with ether; yield 162 g. (80%), m.p. 233° (decomp.).

5:6-Diamino-1:2:3:4-tetrahydro-1:3-dimethyl-2:4-dioxo-pyrimidine.[18] - The product from the previous reaction (80.8 g.) was stirred and concentrated ammonium hydroxide (400 c.c.) was added. The yellow-orange ammonium salt was warmed on a steam bath, stirred, and a solution of sodium hydrosulphite (220 g.) in water (1 l.) was added during a 20 minute period. The salt dissolved and the solution underwent a series of colour changes. The solution was stirred and heated for 15 minutes, filtered while hot and the filtrate cooled. The precipitate was used without further purification; m.p. 209° (decomp.), yield 90%.

Mixed dimethyl compounds, m.p. 237-238° - (a)[2] Phenyl-glyoxal hydrate[46] (3 g.), 5:6-diamino-2:4-dihydroxypyrimidine sulphate (5 g.), glacial acetic acid (125 c.c.) and water (875 c.c.) were gently refluxed for 1 hour. The mixture was cooled, and filtered yielding 3.73 g. of crude product, which was crystallised from aqueous ethanol or (better) NN-dimethyl-formamide to give orange-yellow needles, m.p.> 300°, 2.65 g.,

84%. The solubility in these solvents, and the ultra-violet absorption spectrum [in 0.1 N-sodium hydroxide; Max. at 208 (E = 20,600), 273 (E = 14,200), and 352 mu (E = 13,600)] were constant. Only one spot was obtained on paper chromatography in the above solvent systems.

This material was treated with diazomethane (8 equivalents) in ether[1], methanol being added to initiate the reaction. The product was recrystallised from 80% formic acid giving pale yellow needles, m.p. 237-238° (Ganapati[1] gives m.p. 278°).

Found: C,62.4; H,4.5; N,20.7%.

Light absorption in ethanol: Max. at 203 (E = 19,500), 230 (E = 12,400), 280 (E = 18,500), and 357 mu (E = 12,400).

(b) 5:6-Diamino-1:2:3:4-tetrahydro-1:3-dimethyl-2:4-dioxo-pyrimidine (1 g.) was dissolved in a mixture of 10% hydrochloric acid (25 c.c.) and ethanol (15 c.c.); and phenylglyoxal hydrate (1 g.) was added. The solution was refluxed for 1 hour, cooled, and the product collected. Recrystallisation from 80% formic acid (15 c.c.) gave pale yellow needles (1.3 g., 83%), m.p. 237-238°.

2:4-Dihydroxy-7-phenylpteridine. - Phenylglyoxal hydrate[46] (2 g.) was dissolved in water (100 c.c.), a solution of sodium hydrogen sulphite (d.1.34; 15 c.c.) added and the mixture set aside for 1 hour. A solution of 5:6-diamino-2:4-dihydroxy-pyrimidine sulphate (2.3 g.) and sodium sulphite hydrate (8 g.)

in water (200 c.c.) was added, and the mixture heated under reflux for 1 hour.   After cooling, acetic acid was added with stirring to give pH 4-5.   The product which separated was collected by filtration, washed with water, ethanol, ether and dried.   Recrystallisation from NN-dimethylformamide gave 2:4- -dihydroxy-7-phenylpteridine as pale yellow needles, (2.5 g.; 87%), m.p. > 300°.

$$\text{Found:} \qquad C, 60.0; \ H, 3.2; \ N, 23.1.$$

$C_{12}H_8O_2N_4$ requires $C, 60.0; \ H, 3.4; \ N, 23.3\%$.

Light absorption in 0.1N-sodium hydroxide: Max. at 208 ($E = 21,000$) and 352 ($E = 16,100$); inflexion at 260-265 mu ($E = 8,000$).

1:2:3:4-Tetrahydro-1:3-dimethyl-2:4-dioxo-7-phenylpteridine. - (a) Acetophenone (10 g.) and 6-amino-1:2:3:4-tetrahydro- -1:3-dimethyl-5-nitroso-2:4-dioxopyrimidine (1 g.) were refluxed gently for 30 min.   During this period an acetophenone-water mixture was allowed to distill off and the volume was restored with fresh acetophenone (ca. 3 c.c.).   The dark reaction mixture was cooled and ether (50 c.c.) added.   After standing overnight, the solid was collected, washed with ether, and dried to give a tan powder (0.5 g.; 35%).   Recrystallisation from 80% formic acid (charcoal) gave 1:2:3:4-tetrahydro-1:3- -dimethyl-2:4-dioxo-7-phenylpteridine as fine yellow needles, m.p. 298-300°.

Found: C,62.6; H,4.3; N,20.5.

$C_{14}H_{12}O_2N_4$ requires   C,62.7; H,4.5; N,20.9%.

Light absorption in ethanol: Max. at 204 (E = 23,500),
225 (E = 22,700), 260 (E = 9,400), 282 (E = 9,000), and
354 mu (E = 19,300).

(b)   2:4-Dihydroxy-7-phenylpteridine (2 g.) was stirred for
1 hour with 8 equivalents of diazomethane (from 21g. of
p-tolylsulphonylmethylnitrosamide[41]) in ether-methanol, when
vigorous evolution of nitrogen took place.   After standing
overnight, the product was collected and recrystallised from
80% formic acid to give the pteridone (2 g.; 90%), m.p. 299-300°,
undepressed when mixed with above material.

Found: C,62.8; H,4.6; N,20.8%.

(c)   To phenylglyoxal hydrate (2 g.) in water (25 c.c.) was
added a solution of sodium hydrogen sulphite (d.1.34; 15 c.c.),
and the mixture was allowed to stand for 1 hour. 5:6-Diamino-
-1:2:3:4-tetrahydro-1:3-dimethyl-2:4-dioxopyrimidine (2 g.) and
sodium sulphite, hydrated, (8 g.) in water (100 c.c.) were added
and the solution heated under reflux for 40 min.   On cooling,
the dimethylpteridone crystallised rapidly.   Recrystallisation
from 80% formic acid gave pale yellow needles, m.p. and mixed
m.p. 299-300°.

1:2:3:4-Tetrahydro-1:3-dimethyl-2:4-dioxo-6-phenylpteri-
dine. - Phenylacetaldehyde (5 g.) was refluxed gently and
6-amino-1:2:3:4-tetrahydro-1:3-dimethyl-5-nitroso-2:4-dioxo-
pyrimidine (0.5 g.) was added slowly.   After refluxing for
25 min., the dark red mixture, was cooled and ether was added.
The orange-yellow solid (0.32 g., 41%) which separated, was
collected after standing overnight and washed with ether.
Recrystallisation from 80% formic acid gave 1:2:3:4-tetrahydro-
-1:3-dimethyl-2:4-dioxo-6-phenylpteridine as clear lemon
needles, m.p. 251-253°.

Found: C,62.9; H,4.3; N,20.8.

$C_{14}H_{12}O_2N_4$ requires   C,62.7; H,4.5; N,20.9%.
Light absorption in ethanol: Max. at 203 (E = 20,000), 280
(E = 22,400) and 360 mu(E=9,600).

Mixtures of this material and the 7-phenylpteridone in
the ratio 2 to 1 gave m.p. 236-238° and in the ratio 1 to 2
gave m.p. 278°.

2:4-Dihydroxy-7-phenylpteridine[22].   - To a warm solution
of 5:6-diamino-2:4-dihydroxypyrimidine sulphate (5.7 g.) in
water (75 c.c.) and concentrated ammonium hydroxide (15 c.c.)
was added phenylglyoxal hydrate (5.7 g.).   The mixture was
refluxed for 1 hour, cooled, and acidified with acetic acid.
The product was filtered off, washed with water and alcohol
m.p.> 300;   0.2 g.;   20%.

1:2:3:4-Tetrahydro-1:3-dimethyl-2:4-dioxo-7-phenylpteridine. - The above product (0.2 g.) was suspended in an ethereal solution of diazomethane [from p-tolylsulphonyl-methylnitrosamide[41] (2.06 g.)], methanol (3 c.c.) added, and the mixture stirred for 2 hours. After standing overnight the yellow residue was filtered off, washed with ether and dried. The product was recrystallised from 80% formic acid as pale yellow needles (1.5 g.) m.p. and mixed m.p. with authentic 7-phenylpteridone 298-300°.

Unsuccessful syntheses of 2:4-dihydroxy-6-phenylpteridine. - (a) A mixture of 4-amino-2:6-dihydroxy-5-nitrosopyrimidine (0.5g.) and phenylacetaldehyde (5 g.) was heated under reflux for 30 min. The orange brown mixture was cooled, shaken with ether (100 c.c.) and the buff coloured solid filtered off, and washed by suspension in ether (30 c.c.), filtered and dried, 0.33 g. Paper chromatography indicated the formation of a material with Rf's similar to 2:4-dihydroxy-7-phenylpteridine. However attempts to purify this material by crystallisation or solution in 2N-sodium hydroxide, followed by precipitation with acid, gave tarry residues.

(b) Timmis[32] has shown that in condensations similar to that attempted above replacement of the 2-hydroxypyrimidine by the more soluble 2-methylthiopyrimidine gives more satisfactory results. However reaction of phenylacetaldehyde with

4-amino-6-hydroxy-2-methylthio-5-nitrosopyrimidine[42] under a
variety of conditions (reflux, refluxing ethanol/sodium ethoxide,
refluxing dry ethylene glycol alone, or sodium added) failed to
yield the desired product.

2-Methylamino-6-phenylpyrazine-3-carboxymethylamide. -
1:2:3:4-Tetrahydro-1:3-dimethyl-2:4-dioxo-7-phenylpteridine
(0.2 g.) was dissolved in refluxing ethanol (200 c.c.) and warm
0.3 N-ethanolic potassium hydroxide (100 c.c.) was added with
shaking.   After refluxing for 15 min., the solution was cooled
and neutralised by the addition of glacial acetic acid (1.7 c.c.).
The ethanol was removed in vacuo on a water bath, and the yellow
residue suspended in water (50 c.c.) and extracted with chloro-
form (3 x 60 c.c.).   The combined extracts were dried
(magnesium sulphate) and evaporated to give a yellow solid.
Crystallisation from light petroleum (b.p. 60-80°) gave
2-methylamino-6-phenylpyrazine-3-carboxymethylamide as long
yellow needles (99%), m.p. 112-114°.

Found: C,64.7; H,5.7; N,23.1

$C_{13}H_{14}ON_4$ requires   C,64.4; H,5.8; N,23.1%.

2-Methylamino-6-phenylpyrazine-3-carboxylic acid. -
(a)   1:2:3:4-Tetrahydro-1:3-dimethyl-2:4-dioxo-7-phenylpteridine
(1.05 g.) and sodium hydroxide (1 g.) in ethanol (35 c.c.) were
heated in a steel bomb at 200° for 16 hours.   After cooling,
water (50 c.c.) was added, and the ethanol evaporated.   The hot

solution was filtered, concentrated hydrochloric acid was
added to pH4, and the yellow precipitate was collected after
chilling.   2-Methylamino-6-phenylpyrazine-3-carboxylic acid
(0.76 g., 80%) was recrystallised from ethanol, fluffy needles,
m.p. 178-179° (decomp.)

$$\text{Found:} \quad C, 62.7; \quad H, 4.5; \quad N, 18.2$$

$C_{12}H_{11}O_2N_3$ requires   C,62.9; H,4.8; N,18.3%.

(b) This acid was also obtained in 85% yield on similar
hydrolysis of 3-methylamino-6-phenylpyrazine-3-carboxymethyl-
amide.

3-Methoxycarbonyl-2-methylamino-6-phenylpyrazine. - A
suspension of 2-methylamino-6-phenylpyrazine-3-carboxylic acid
(85 mg.) in refluxing dry methanol (200 c.c.) was treated with
dry hydrogen chloride for 10 min.   The yellow solution
deepened in colour and was refluxed for a further 2 hours,
before concentrating in vacuo to 10 c.c.   Water (10 c.c) was
added and the solution chilled.   The product separated as
fine, dark yellow needles, which were filtered off, washed
with methanol, water, and dried (80 mg., 93%).   The 3-
-methoxycarbonyl-2-methylamino-6-phenylpyrazine was recrystall-
ised from aqueous methanol, as fine yellow needles.   A sample
was sublimed for analysis m.p. 134-135°.

$$\text{Found:} \quad C, 64.3; \quad H, 5.2; \quad N, 17.3.$$

$C_{13}H_{13}O_2N_3$ requires   C,64.2; H,5.4; N,17.3%

<u>Treatment of 1:2:3:4-tetrahydro-1:3-dimethyl-2:4-dioxo-</u>
<u>-7-phenylpteridine with aqueous alkali.</u> - The pteridone (3 g.)
was refluxed with 2N- sodium hydroxide (150 c.c.) for 1 hour.
The mixture was chilled overnight and the yellow precipitate
(2.66 g.) was collected, washed with water and dried. The
solid was extracted with boiling light petroleum (b.p. 60-80°;
4 x 50 c.c.) leaving a residue (1.4 g., 47%) of 1:2:3:4-tetra-
hydro-1:3-dimethyl-2:4-dioxo-7-phenylpteridine, m.p. 299-300°.
The bright yellow petroleum extract was concentrated, and on
cooling, 2-methylamino-6-phenylpyrazine-3-carboxymethylamide
(1.2 g. 45%) crystallised as long yellow needles, m.p. 112-114°.
The alkaline filtrate was acidified with concentrated hydro-
chloric acid to pH4 and extracted with chloroform (5 x 50 c.c.).
The combined extracts were dried (sodium sulphate) and evaporated
to dryness to give a yellow solid. Crystallisation from
ethanol gave 2-methylamino-6-phenylpyrazine-3-carboxylic acid
(0.08 g., 3%) m.p. 178-179° (decomp.).

An identical hydrolysis of 2-methylamino-6-phenylpyrazine
-3-carboxymethylamide (0.5 g.) with 2N-sodium hydroxide (25 c.c.)
gave the carboxylic acid, m.p. 178-179° (decomp.) (0.032 g. 7%)

When the pteridone was refluxed with 5N alkali for 3 hours,
56% of the amide and 9% of the acid were produced.

<u>2-Methylamino-5-phenylpyrazine-3-carboxymethylamide.</u> -
1:2:3:4-tetrahydro-1:3-dimethyl-2:4-dioxo-6-phenylpteridine

(0.2 g.) was dissolved in refluxing ethanol (200 c.c.) and warm 0.3$\underline{N}$-ethanolic potassium hydroxide (100 c.c.) was added and the mixture refluxed for 15 min.   The reaction mixture was worked up as for the 6-phenyl isomer (see above) to give 2-methylamino-5-phenylpyrazine -3-carboxymethylamide.   The product was recrystallised from light petroleum (b.p. 60-80°); fine yellow needles, 98%, m.p. 92-94°.

<div style="text-align:center">Found: C,64.5; H,5.8; N,23.3.</div>

<div style="text-align:center">$C_{13}H_{14}ON_4$ requires   C,64.4; H,5.8; N,23.1%.</div>

2-Methylamino-6-phenylpyrazine-3-carboxylic acid. -

(a) 1:2:3:4-Tetrahydro-1:3-dimethyl-2:4-dioxo-6-phenylpteridine (1.05 g.) and sodium hydroxide (1 g.) in ethanol (30 c.c.) were heated in a steel bomb at 140° for 17 hours.   After cooling, water (50 c.c.) was added and the ethanol evaporated. The warm aqueous solution was filtered, the pH adjusted to 4 with concentrated hydrochloric acid, and the mixture chilled. The canary yellow crystalline solid was collected, washed with water, and dried, (0.82 g., 91%).   The 2-methylamino-5- -phenylpyrazine-3-carboxylic acid was recrystallised from aqueous methanol, soft yellow needles, m.p. 173-174°.

<div style="text-align:center">Found: C,63.2; H,4.6; N,18.5.</div>

<div style="text-align:center">$C_{12}H_{11}O_2N_3$ requires   C,62.9; H,4.8; N,18.3%.</div>

(b)   This acid was also obtained in 100% yield on similar hydrolysis of 3-methylamino-5-phenylpyrazine-3-carboxymethyl-amide.

3-Methoxycarbonyl-2-methylamino-5-phenylpyrazine. -
2-Methylamino-5-phenylpyrazine-3-carboxylic acid (0.5 g.) in
refluxing dry methanol (25 c.c.) was treated with dry hydrogen
chloride for 20 min. and the mixture refluxed for a further
2 hours, before concentrating in vacuo to 10 c.c. The yellow
solution was poured into ice cold water (50 c.c.), the resulting
solution neutralised with sodium carbonate, and extracted with
chloroform (5 x 50 c.c.). The combined extracts were dried
(magnesium sulphate) and evaporated in vacuo to give a yellow
gum, which crystallised.   3-Methoxy-carbonyl-2-methylamino-5-phenyl-
pyrazine was recrystallised from chloroform methanol, thick
yellow needles, 0.45 g., 85%, m.p. 140-141°.

Found: C,64.5; H,5.2; N,17.1.

$C_{13}H_{13}O_2N_3$ requires   C,64.2; H,5.4; N,17.3%.

Treatment of 1:2:3:4-tetrahydro-1:3-dimethyl-2:4-dioxo-6-
phenylpteridine with aqueous alkali. -   The dimethylpteridone
(0.6 g.) was refluxed with 2N-sodium hydroxide (30 c.c.) and
the reaction mixture worked up as described for the 7-phenyl
isomer (see above) to give 40% of starting material, (m.p.
252-253°), 52% of methylamide (m.p. 92-94°), and 8% of
carboxylic acid (m.p. 172-173°).

An identical hydrolysis of the pure methylamide gave
33% of the carboxylic acid (m.p. 172-173°).

When the pteridone was heated with 5N-alkali for
3 hours 60% of the amide, 12% of the acid, and 27% of starting
material were obtained.

Acid treatment of 1:2:3:4-tetrahydro-1:3-dimethyl-2:4-
-dioxo-7-phenylpteridine. - The dimethylpteridone (0.5 g.)
was heated with 5N-hydrochloric acid (40 c.c.) under reflux
for 6 hours.   Paper chromatography indicated that no reaction
had taken place and the starting material (0.47 g.) was
recovered, m.p. 298-300°.

Treatment of 1:2:3:4-tetrahydro-1:3-dimethyl-2:4-dioxo-
-7-phenylpteridine with benzylamine. - The pteridone (0.5 g.)
and benzylamine (15 c.c.) were heated in a sealed glass tube
at 260° for 24 hours.   The clear yellow solution on cooling
deposited crystals, which were collected, washed with ethanol
and dried, 0.46 g., m.p. and mixed m.p. with starting material
298-300°.   Paper chromatography of the filtrate and washings
indicated the presence of starting material only.

3-Methylamino-5-phenylpyrazinoic acid.hydrazide. -
(a) 1:2:3:4-Tetrahydro-1:3-dimethyl-2:4-dioxo-7-phenylpteridine
(0.5 g.) was suspended in 85% hydrazine hydrate (15 c.c.) and
the mixture heated under reflux for 6 hours.   The orange-red
mixture was chilled and the suspended deep yellow needles
collected, washed with water, methanol, and dried.   The

3-methylamino-5-phenylpyrazinoic acid hydrazide was recrystallised from chloroform/methanol, fluffy yellow needles, 0.37 g., 80%, m.p. 215-216.5°.

Found: C,59.3; H,5.2; N,28.9.

$C_{12}H_{13}O N_5$ requires   C,59.3; H,5.4; N,28.8%.

(b)   A mixture of 3-methoxycarbonyl-2-methylamino-6-phenyl-pyrazine (0.1 g.) and 85% hydrazine hydrate (2 c.c.) was heated under reflux for 30 min., chilled and the crystalline product collected, washed with water and dried.   The acid hydrazide was recrystallised from chloroform-methanol, soft yellow needles, 0.08 g., 80%, m.p. 216-217°.   A mixed melting point with a sample from the previous experiment showed no depression.

2-Methylamino-6-phenylpyrazine-3-carboxyamide. -
(a) 1:2:3:4-Tetrahydro-1:3-dimethyl-2:4-dioxo-7-phenylpteridine (250 mg.), absolute alcohol (20 c.c.) and liquid ammonia (5 c.c.) were heated together in a steel bomb at 210° for 20 hours.   The resulting yellow solution was filtered and the clear filtrate evaporated in vacuo to give an orange yellow solid.   The residue was extracted with boiling light petroleum (4 x 30 c.c.) and the combined extracts crystallised to give 2-methylamino-6-phenylpyrazine-3-carboxyamide, fine yellow needles, 0.168 g., 79%.   The product was recrystallised

from chloroform/petrol (b.p. 60-80°) lemon coloured needles, m.p. 188-189°.

Found: C,63.1; H,4.9; N,24.7.

$C_{12}H_{12}ON_4$ requires C,63.1; H,5.3; N,24.6%

(b)   A mixture of 3-methoxycarbonyl-2-methylamino-6-phenyl-pyrazine (0.13 g.) and saturated ethanolic ammonia (5 c.c.) were heated in a sealed tube at 125° for 5 hours.   The cooled reaction mixture was filtered and the filtrate evaporated to dryness in vacuo.   The 2-methylamino-6-phenylpyrazine-3--carboxyamide was recrystallised from chloroform/petrol (b.p. 60-80°), clusters of yellow needles, 0.10 g. 80%, m.p. 187-188°.

A mixed melting point with a sample from the previous experiment showed no depression.

Treatment of 2-methylamino-6-phenylpyrazine-3-carboxy-methylamide (0.25 g.) with dry ethanol and liquid ammonia under the same conditions used for the pteridone (section (a) above) gave the starting material m.p. 112-114°, 0.24 g.

2-Hydroxy-6-phenylpyrazine-3-carboxyamide. - (a) Phenyl-glyoxal hydrate (5.4 g.) in water (25 c.c.) was treated with aqueous sodium hydrogen sulphite (d.,1.34; 50 c.c.), and the mixture was allowed to stand at room temperature for 45 min. Aminomalonamide (see experimental Section I) (3.9 g.) in water

(30 c.c.) was added, and the cloudy solution was heated on the steam bath for 2.5 hours, during which a yellow crystalline precipitate separated.   This was collected, washed with water and ethanol, and dried.   Recrystallisation from ethanol gave 2-hydroxy-6-phenylpyrazine-3-carboxyamide as yellow needles, m.p. 252-253° (decomp.), 4.81 g., 62%.

Found: C,61.7; H,4.3; N,19.3.

$C_{11}H_9O_2N_3$ requires   C,61.4; H,4.2; N,19.5%

(b)   The same product, in much smaller yield 16%, was obtained when the condensation was carried out as described by Jones[36].

The product obtained Jones had m.p. 213-216° (decomp.).

(c)   A mixture of $\omega\omega$-dichloroacetophenone (1.62 g.) (see below), aminomalonamide (1 g.) and sodium acetate (3.5 g.) in water (50 c.c.) was refluxed for 3 hours, concentrated to 20 c.c. and chilled.   The yellow precipitate was collected and washed with water.   Recrystallisation from ethanol gave 2-hydroxy-6-phenylpyrazine-3-carboxyamide, as yellow needles, 0.25 g., 14%, m.p. and mixed m.p. 251-252° (decomp.).

2-Hydroxy-6-phenylpyrazine-3-carboxylic acid. -
2-Hydroxy-6-phenylpyrazine-3-carboxyamide (1.3 g.), sodium hydroxide (1.1 g.) and ethanol (30 c.c.) were heated in a steel bomb at 150° for 16 hours.   The yellow gelatinous product was dissolved in water (50 c.c.), and the ethanol

evaporated.   The warm solution was filtered, the filtrate acidified to pH4 with concentrated hydrochloric acid, and the heavy yellow precipitate collected, washed with water and dried. The 2-hydroxy-6-phenylpyrazine-3-carboxylic acid was recrystallised from ethanol, yellow needles, 1.25 g., 96%, m.p. 217° (decomp.).

Found: C,60.9; H,3.5; N,13.0.

Calc. for $C_{11}H_8O_3N_2$:   C,61.1; H,3.7; N,13.0%.

This acid gave a mid-blue fluorescence in ultra-violet light.   King and Spensley[38] report a similar fluorescence and m.p. 208-209° (decomp.).

2-Hydroxy-3-ethoxycarbonyl-6-phenylpyrazine. - A solution of the above acid (0.3 g.) in refluxing dry ethanol (4 c.c.) containing 2 drops of concentrated sulphuric acid was heated for 2 hours.   On cooling the product crystallised as pale yellow prisms, 0.27 g. Recrystallisation from ethanol gave the ester as clear prisms, m.p. 112-114°.

Found: C,63.5;   H,4.7;   N,11.7

Calc. for $C_{13}H_{12}O_3N_2$:   C,63.9;   H,5.0;   N,11.5%

King and Spensley[38] give m.p. 112°.

2-Hydroxy-3-methoxycarbonyl-6-phenylpyrazine. - 2-Hydroxy-6-phenylpyrazine-3-carboxylic acid (2.75 g.) in dry boiling methanol (150 c.c.) was treated with dry hydrogen chloride until it had completely dissolved (about 20 min.).

The clear orange solution was refluxed for a further 2 hours.
When the reaction mixture was cooled, 2-hydroxy-3-methoxy-
carbonyl-6-phenylpyrazine was deposited as white prismatic
needles. The acidic mother liquors were concentrated in vacuo
to ca. 75 c.c. and after the addition of water (75 c.c.), the
solution was neutralised with ammonium hydroxide (S.G.0.88),
the temperature being kept below 10°. The solution was
extracted with chloroform (4 x 100 c.c.) and the combined
extracts after drying (sodium sulphate) were evaporated
in vacuo to give a yellow solid. Crystallisation from methanol
(charcoal) gave a second crop of the methyl ester (total yield
2.7 g., 92%). A sample recrystallised from chloroform-methanol
had m.p. 172-173°.

Found: C,62.7; H,4.3; N,12.4.

$C_{22}H_{10}O_3N_2$ requires   C,62.6; H,4.4; N,12.2%

2-Chloro-3-methoxycarbonyl-6-phenylpyrazine. -
2-Hydroxy-3-methoxycarbonyl-6-phenylpyrazine (1 g.) and
redistilled phosphorus oxychloride (14 c.c.) containing one
drop of concentrated sulphuric acid were mixed in a Carius tube.
The mixture was heated at 115° (bath) for 30 min.; evolution
of hydrogen chloride had then ceased. The tube was sealed and
heated at 155° for 16 hours. The cooled reaction mixture was
poured on cracked ice (150 g.), and the mixture stirred for

for 30 min. The product, which separated, was collected, washed with water, and dried (0.94 g., 87%). Recrystallisation from chloroform/methanol, and finally from methanol gave the chloro-ester as clear prisms, m.p. 81-83°.

Found: C,57.5; H,3.5; N,11.4; Cl,13.9.

$C_{12}H_9O_2N_2Cl$ requires   C,57.9; H,3.6; N,11.3; Cl,14.3%.

2-Methylamino-6-phenylpyrazine-3-carboxymethylamide. - 2-Chloro-3-methoxycarbonyl-6-phenylpyrazine (0.1 g.) and 33% ethanolic methylamine (2 c.c.) were heated in a Carius tube at 140° for 6 hours. The yellow solution was evaporated to dryness in vacuo, absolute methanol (10 c.c.) was added, and the solution again taken to dryness to leave a yellow residue which was extracted with chloroform (3 x 10 c.c.). The combined extracts were concentrated, and on the addition of light petroleum (b.p. 60-80°), 2-methylamino-6-phenylpyrazine-3-carboxymethyl-amide (65 mg., 67%) crystallised as long yellow needles, m.p. 112-113°. A mixed m.p. with the methylamide obtained by degradation of 1:2:3:4-tetrahydro-1:3-dimethyl-2:4-dioxo-7--phenylpteridine showed no depression.

2-Amino-4-hydroxy-7-phenylpteridine. - An intimate mixture of 2-chloro-3-methoxycarbonyl-6-phenylpyrazine (0.2 g.) and guanidine carbonate (0.5 g.) was heated at 170-180° (bath) for 30 min. with occasional stirring. The yellow reaction

mixture was cooled, and warm water (40 c.c.) was added. The resulting suspension was heated to 95°, and glacial acetic acid was added to pH4-5 when further precipitation took place. The pale yellow solid was collected at 90-100°, washed with water, ethanol and ether, and dried to give 2-amino-4-hydroxy-7-phenylpteridine (170 mg., 88%) m.p. > 300°. The product was purified by dissolving in hot 2N-sodium hydroxide, and reprecipitating with acetic acid at 95-100°, and was dried at 135°.

Found: C,59.9; H,3.8; N,29.4

Calc. for $C_{12}H_9ON_5$: C,60.2; H,3.8; N,29.3%.

ωω-Dichloroacetophenone[43]. - Dry chlorine was passed into a solution of acetophenone (24 g.) in glacial acetic acid (100 c.c.). Initially the mixture turned yellow in colour prior to a distinct rise in temperature, and at 25° the colour rapidly faded. The reaction temperature was not allowed to exceed 60°, by controlling the flow of chlorine, which was continued until the solution became yellow and an increase in weight of 20 g. had occurred. The reaction mixture was poured over crushed ice (600 g.), stirred several times, and allowed to stand until the ice melted. The product which separated as a heavy oil was collected, treated with benzene (20 c.c.) and the water and benzene distilled off in vacuo to leave

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$\omega\omega$-dichloroacetophenone as a clear oil (33 g.).

ω-Nitroacetophenone[44,45] . - A mixture of redistilled benzaldehyde (16 g.) and nitromethane (9.6 g.) was cooled to - 10°, anhydrous potassium carbonate (4 g.) was added with stirring, during 45 min., the temperature being kept below 10°. After 1 hour acetic acid (10 c.c.) and then water (100 c.c.) were added. The aqueous layer was decanted and the heavy oily phenylnitromethylcarbinol obtained was washed with water[44].

The aqueous layer was extracted with ether. The extracts were combined with the oily layer along with more ether (100 c.c.) and washed with 10% sodium bisulphite (2 x 75 c.c.), 5% sodium bicarbonate (2 x 75 c.c.) and finally with water. The ether solution was dried (magnesium sulphate) and concentrated in vacuo at 40°, to give 18.7 g. of the carbinol. The latter (15.7 g.) was oxidised with potassium dichromate (15.7 g.) in acetic acid (75 c.c.), by the method used by Long and Troutman[46], to give ω-nitroacetophenone (11.7 g.), m.p. 105-106°.

2-Amino-4-hydroxy-6-phenylpteridine.[38] - (a) Triamino-4--hydroxy pyrimidine dihydrochloride (4.5 g.) in 50% aqueous ethanol (80 c.c.) was treated with sodium acetate (13.5 g.) followed by $\omega\omega$-dichloroacetophenone (3.8 g.) and the mixture heated under reflux for 1.5 hours. 2-Amino-4-hydroxy-6-

phenylpteridine separated (3.1 g., 60%), and was purified

by dissolving in hot 2N-sodium hydroxide, and collecting

the sodium salt, which crystallised on cooling.   The pteridine

(m.p. > 360°) was obtained by dissolving the salt in water

and acidifying the solution to pH2.

(b)   Triamino-4-hydroxypyrimidine was condensed with

phenyl glyoxal as described by King and Spensley[38] for the

preparation of the 7-phenyl isomer.   The infra-red spectrum

of the purified product was identical with that of the material

prepared from ωω-dichloroacetophenone, and different from the

spectrum of the authentic 2-amino-4-hydroxy-7-phenylpteridine

prepared above.

(c)(i) 2:4-Diamino-6-hydroxy-5-(2-nitro-1-phenylethylideneamino)-

-pyrimidine. - To a clear solution of triamino-4-hydroxy-

pyrimidine dihydrochloride (5 g.) in water (50 c.c.), powdered

sodium acetate (10 g.) and then ω-nitroacetophenone (4 g.) in

warm 50% aqueous ethanol (200 c.c.) were added.   A crystalline

precipitate gradually separated from the reddish-orange mixture

in the cold.   After standing for 24 hours the 2:4-diamino-6-

-hydroxy-5-(2-nitro-1-phenylethylideneamino)-pyrimidine was

filtered off and recrystallised from 50% ethanol, orange needles,

4.52 g., 63%, m.p. > 300°.   The Schiff's base gave no

colouration in ammoniacal solution with the Folin-Denis reagent.[39]

Found: C,47.2; H,4.4; N,27.8.

$C_{12}H_{12}O_3N_6.H_2O$ requires C,47.1; H,4.6; N,27.5%

(ii) A suspension of the above Schiff's base (3.3 g.) in refluxing 25% ethanol (75 c.c.) was treated with sodium dithionite (15 g.), added in portions during 1 hour. The suspended solid became canary-yellow, and after an additional hour the mixture was allowed to cool. The product, which contained sulphur, was collected, dissolved in 2N-sodium hydroxide, filtered and the clear filtrate acidified with concentrated hydrochloric acid to pH2. The 2-amino-4-hydroxy--6-phenylpteridine was collected washed with water, ethanol and dried, 2.7 g., m.p. $\geqslant$ 360°. Identical with material prepared in sections (a) and (b).

2-Hydroxy-5-phenylpyrazine-3-carboxylic acid. - The pteridine (0.63 g.) from (c) was heated with 4N-sodium hydroxide (10 c.c.) at 180° for 20 hours, and the product isolated by diluting the solution with water (10 c.c.), and acidifying the filtered solution to pH2. The 2-hydroxy-5--phenylpyrazine-3-carboxylic acid was crystallised from chloroform/ethanol, yellow prisms, 180 mg., m.p. 200° (decomp.) (ethyl ester, m.p. 158-159°[38]). In dilute acid solution this acid gave a brilliant pale green ultra-violet fluorescence, quite distinct from that of the isomeric 6-phenylpyrazine; the mixed m.p. with the latter was 187-190°

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