THE SYNTHESIS AND PROPERTIES OF SOME PYRROLO[2,3-d]

PYRIMIDINES OF POTENTIAL BIOLOGICAL IMPORTANCE

By

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~ SUMMARY.

Methods available for the synthesis of pyrrolo[2,3-d]pyrimidines are reviewed in detail and the biological significance of these compounds is discussed.

Three possible synthetic routes to pyrrolo[2,3-d]pyrimidines are investigated.

1. A Fischer-Indole type of synthesis from pyrimidylhydrazones.

Several pyrimidyl-6-hydrazones are readily prepared from the corresponding 6-hydrazinopyrimidines. These hydrazones cyclise on heating, to give the corresponding pyrrolo[2,3-d]pyrimidines, when either a small group or no substituent is present at position 4 in the pyrimidine ring. Hydrazones with bulky groups in this position could not be induced to cyclise. 1,3-Dimethyl-2,4-dioxopyrimidin-6-hydrazones are converted to pyrrolopyrimidines by acid-catalysed cyclisation. The mechanisms involved in these cyclisations are discussed.

2. Direct Methods from 2-amino-3-substituted pyrroles.

The preparation of derivatives of 2-amino-3-cyano-4-phenylpyrrole, dialkyl 2-aminopyrrole-3,4-dicarboxylates and 3-amido-2-aminopyrrole are described but attempts to convert them to pyrrolo[2,3-d]pyrimidines, by methods previously used in the synthesis of fused pyrimidines, were unsuccessful.

Dimethyl 2-aminopyrrole-3,4-dicarboxylate reacts with phenyl isocyanate in pyridine to give dimethyl 3-phenylpyrrolo[1,2-a]-[1,3,5]-triazin-2,4-dione-6,7-dicarboxylate, an example of a previously unreported ring system. This assigned structure is confirmed by spectroscopic studies.

The reluctance of 2,3-disubstituted pyrroles to undergo cyclisation to pyrrolo[2,3-d]pyrimidines is attributed to deactivation of groups in

position 3 of the pyrrole ring.

3. Indirect methods from 2-amino-3-cyanopyrroles.

The reaction of 2-amino-3-cyano-4-phenylpyrrole with carbon disulphide
in pyridine yields 4-imino-5-phenylpyrrolo[2,3-d]-[3,1]thiazin-2(1H)-thione.
This compound isomerises to 5-phenylpyrrolo[2,3-d]pyrimidin-2,4(1H,3H)-
dithione and its reactions with methylating agents and aqueous alkali are
investigated. A possible mechanistic explanation of these reactions is
discussed.

4-Imino-5-phenylpyrrolo[2,3-d]-[3,1]thiazin-2(1H)-thione reacts with
primary amines to give 3-substituted-4-iminopyrrolo[2,3-d]pyrimidines and
the mechanism of this reaction is outlined. 3-Cyano-2-dimethylthiomethylene-
amino-4-phenylpyrrole condenses with primary amines providing a new route
for the synthesis of fused pyrimidine ring systems. The methylation of
5-phenylpyrrolo[2,3-d]pyrimidin-2,4(1H,3H)-dithione is described.

The 3-substituted-4-iminopyrrolo[2,3-d]pyrimidines prepared during
the work did not undergo a Dimroth rearrangement when treated with alkali
but were converted to the corresponding 3-alkylpyrrolo[2,3-d]pyrimidin-
4-ones.

The mass spectra of the pyrrolo[2,3-d]pyrimidines and of some pyrrole
and pyrimidine intermediates prepared in the course of this work are
recorded and possible fragmentation pathways are suggested for many of these
compounds.

CONTENTS

INTRODUCTION

EXPERIMENTAL

PYRROLO[2,3-d]PYRIMIDINES

NOMENCLATURE   Pyrrolo[2,3-d]pyrimidine (I) may be regarded as an analogue of purine (2) in which the nitrogen at position 7 has been replaced by a -CH= group. Indeed, many examples of the pyrrolo[2,3-d] pyrimidine ring system have been synthesised as possible antimetabolites to naturally occurring purine derivatives, and the pyrrolopyrimidine (I) has often been referred to as 7-deazapurine. In 1913, Johnson and Kohmann[1] referred to pyrrolo[2,3-d]pyrimidine as 1,3,9-pyrimazole and, as late as 1959, Schueler and Suranyi,[19] named some of its derivatives as 1,5,7-triazaindans. However, such systems of nomenclature are rarely used.
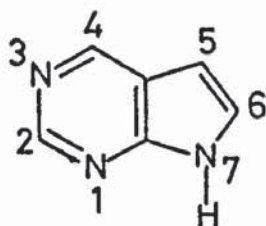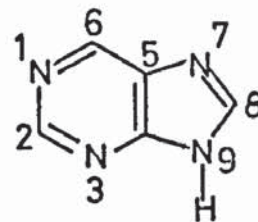
(1)   (2)

SYNTHESIS   The procedures which have been applied to the synthesis of the pyrrolo[2,3-d]pyrimidine ring system may be divided into two, those using pyrimidines as starting materials and those using 2-amino-3-cyanopyrroles. Synthetic routes using pyrimidines have proved very versatile and numerous pyrrolopyrimidines have been prepared in this way. .However pyrrole intermediates have provided direct access to certain biologically important compounds which were otherwise unobtainable.

I SYNTHESES OF PYRROLO|2,3-d|PYRIMIDINES FROM PYRIMIDINES.

The methods which have been used to convert pyrimidines to the fused pyrrolopyrimidine system may be subdivided according to the nature of the pyrrole ring precursor employed (Fig. 1). Four types of synthesis are shown and of these type I has been the most versatile.
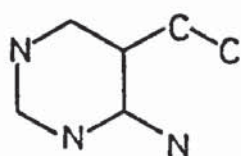
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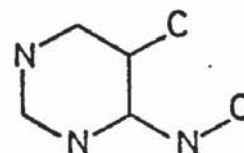
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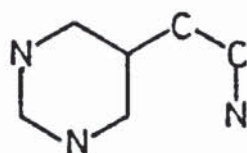
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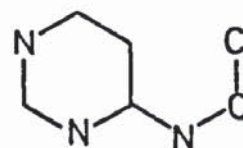
TYPE 4

Figure 1.

A. SYNTHESES OF TYPE I.

These invariably consist of a pyrimidine system where cyclisation is effected by attack of a basic amino group on an electron defficient carbon atom, (Fig. 2). Syntheses of this type may be further subdivided according to the nature of the pyrimidine starting material used.
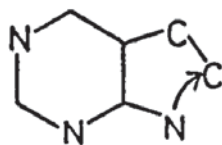
Figure 2.

A.I.   Syntheses from 4-aminopyrimidyl-5-acetic acid derivatives.

The first pyrrolo|2,3-d|pyrimidine (5) was synthesised by Johnson[2] in 1911, the cyclisation being effected by heating ethyl 4-chloro-2-ethylthiopyrimidyl-5-acetate (3) with ammonia.

(3)   (4)   (5)
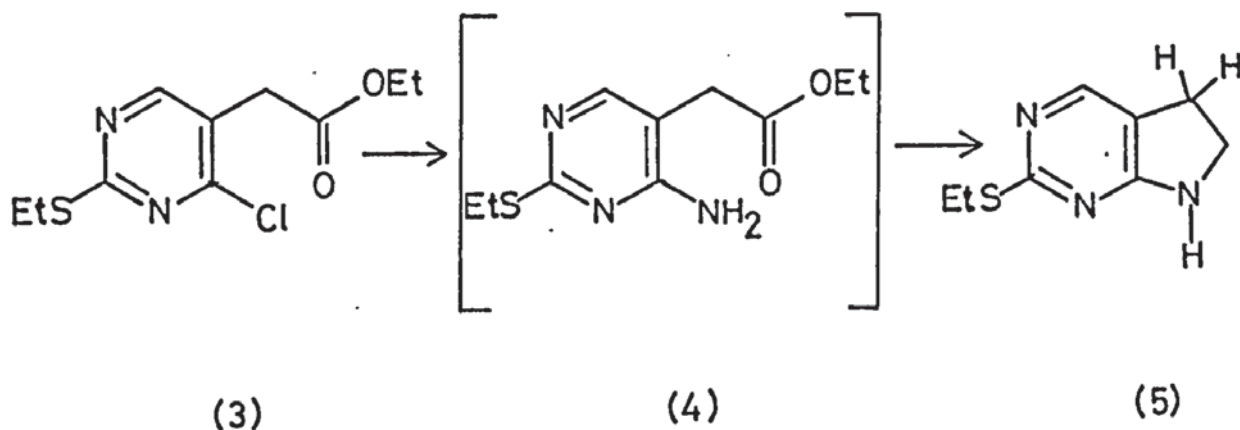
The reaction presumably proceeds *via* the 4-aminopyrimidine (4), (although this was not isolated), which undergoes intramolecular nucleophilic cyclisation to the pyrrolopyrimidine (5).

A later attempt, by Johnson and Kohmann,[1] to convert a 4-amino-pyrimidylacetic acid derivative (6) to the corresponding pyrrolo|2,3-d| pyrimidine (10), was hindered by their failure to prepare the starting material in a sufficiently pure form, and the cyclised product was

isolated only in small yield.

In a similar manner the 2-methylpyrrolo[2,3-$\underline{d}$] pyrimidine dione (11) has been prepared[3] from the corresponding 4-aminopyrimidylacetic ester (7)
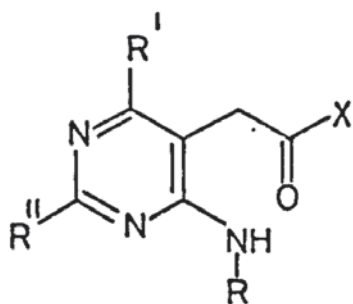
(6) R=H, R'=OH, R"=OH, x=~~OE2T~~ OC₂H₅

(7) R=H, R'=OH, R"=CH₃, x=OC₂H₅

(8) R=CH₃, R'=H, R"=CH₃, x=OH

(9) R=CH₃, R'=H, R"=CH₃, x=OCH₃
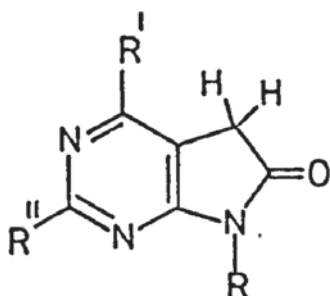
(10) R=H, R'=OH, R"=OH

(11) R=H, R'=OH, R"=CH₃

(12) R=CH₃, R'=H, R"=CH₃

In an attempt[4] to esterify 2-methyl-4-methylaminopyrimidyl-5-acetic acid (8), by treatment with hydrogen chloride in refluxing ethanol, 2,7-dimethylpyrrolo[2,3-$\underline{d}$]pyrimidin-6(5H)-one (12) was produced. The monomethylester (9), (prepared by a different method), also cyclised readily to the same pyrrolopyrimidine (12) when treated with ammonia.

This route has been used to convert other pyrimidyl acetic acid derivatives to the corresponding pyrrolopyrimidines. Treatment of the

hydrazide (13), for example, with amyl nitrite also furnished 2,7-dimethylpyrrolo[2,3-d]pyrimidin-6(5H)-one (12).[4] This result was surprising since the very similar 4-aminopyrimidyl-5-acetic hydrazide (14), when treated in the same way, produced the urethane (16) by a normal Curtius rearrangement.

It was suggested[4] that the azide (15), (a necessary intermediate in the formation of the urethane), is a good acylating agent. The 4-methylamino group is acylated giving the pyrrolopyrimidine (12) while the less basic free amino group does not react and the compound undergoes a Curtius rearrangement to the urethane (16).

(13) R=CH₃
(14) R=H

(15)

R=CH₃

R=H

(12)

(16)
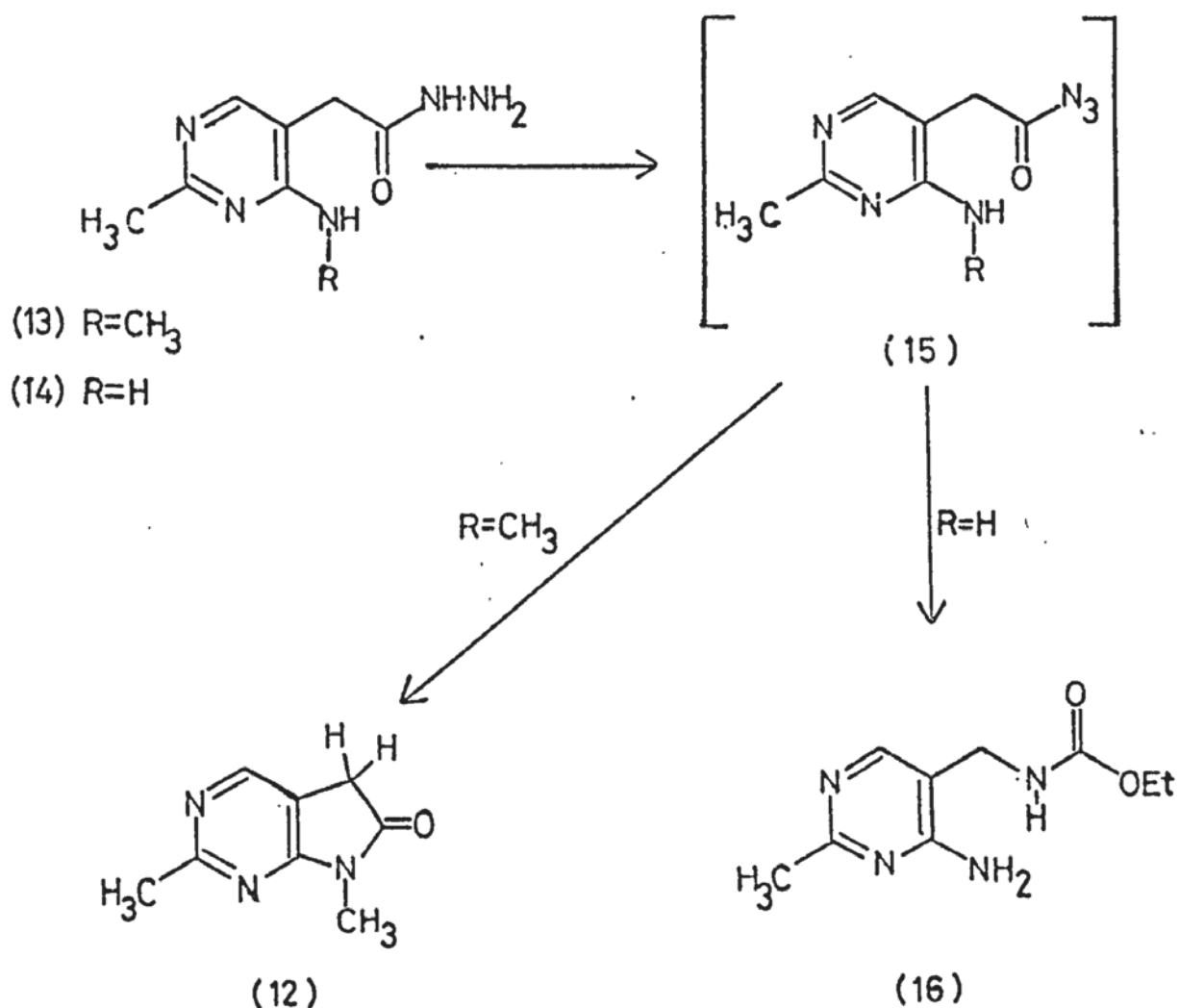
When pyrimidylacetic acids with substituents on the carbon α to the acid function are used as starting materials, 5-substituted pyrrolo-

pyrimidines are obtained. Thus, Pfleiderer et al[5] described the preparation

of some bipyrimidyl acetate derivatives (20) from 4-amino-1,3-dimethyl-

pyrimidin-2,4(1H,3H)-dione (17), (and similar compounds (18) and (19)),

and was able to convert them to 5-substituted pyrrolo[2,3-d]pyrimidines

(21) either by treatment with alkali or by thermal cyclisation.

(17) R=CH₃, R'=H
(18) R=H, R'=H
(19) R=CH₃, R'=CH₃

R	R'
CH₃	C₂H₅
CH₃	H
H	C₂H₅
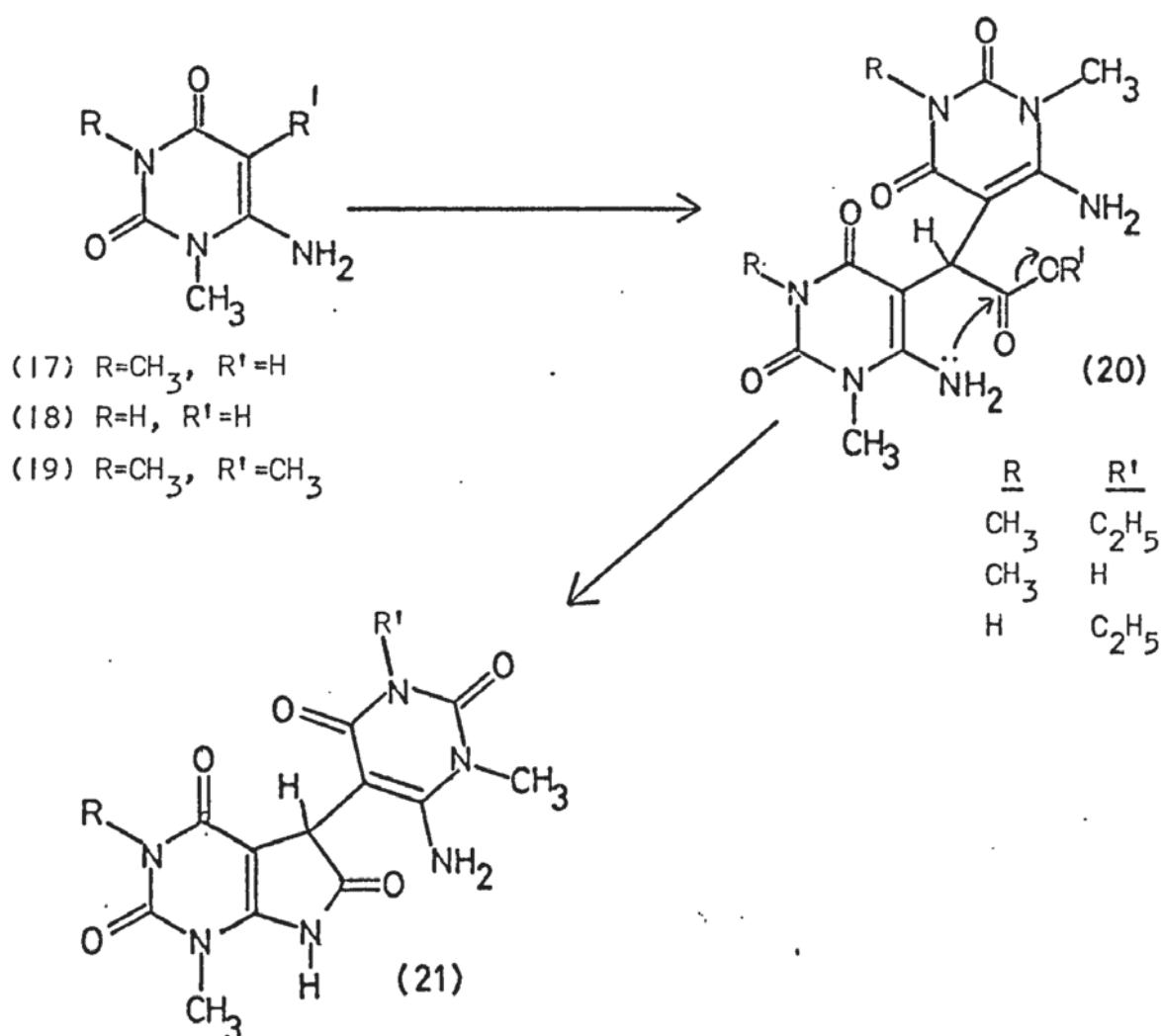
A.2 Syntheses from 4-aminopyrimidyl-5-acetaldehyde and its derivatives.

In 1960, West, Ledig and Hitchings described[6] the preparation of

the acetals (22) and their conversion to pyrrolo[2,3-d]pyrimidines (24).

At the same time Davoll,[7] working independently, reported the synthesis

of the same compounds (22) by a different procedure, and their cyclisation

to pyrrolopyrimidines by methods essentially similar to those described

by Hitchings and his coworkers.
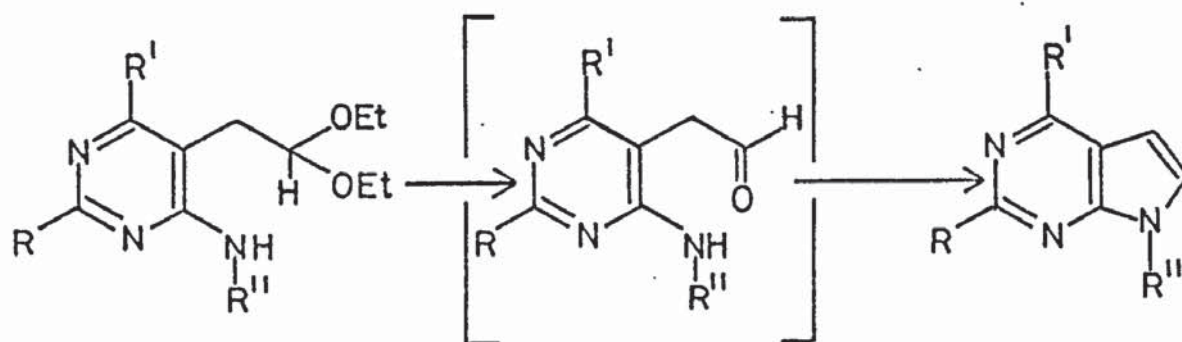
(22) R''=H   (23) R''=H   (24) R''=H

West et al[8]   West and Beauchamp[10]

R, R'=OH, SH or NH_2 $R=CH_3$, C_2H_5, $\underline{n}-C_3H_7$, C_6H_5

R''=H   R'=OH, R''=H or CH_3
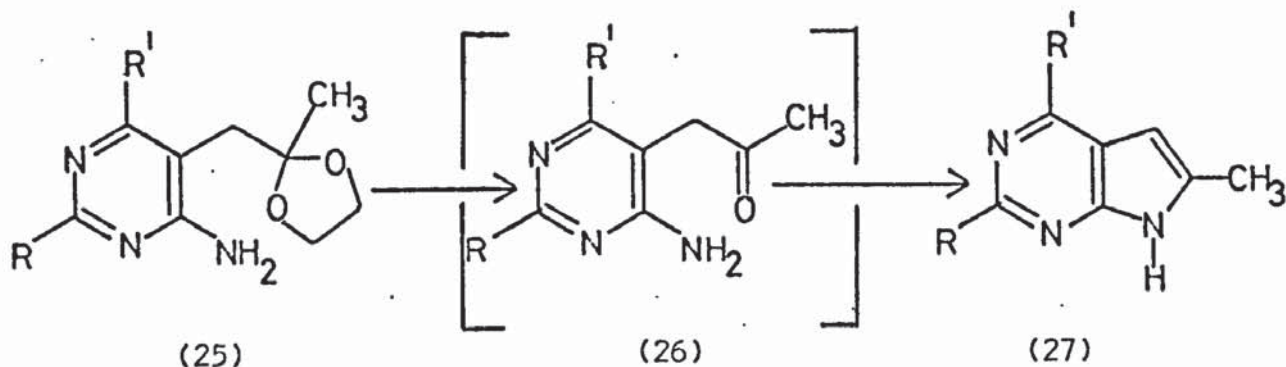
(25)   (26)   (27)

Davoll[9]

When R'=OH, R=H, NH_2, OH or SH, R''=H.

When R'=NH_2, R=H, NH_2, OCH_3 or SH, R''=H.

Reactions of this type proceed *via* the intermediate 4-aminopyrimidin-5-acetaldehydes (23) which are liberated by acid hydrolysis of the acetals. These intermediates (23), were not isolated however, since they were immediately converted to the corresponding pyrrolopyrimidines (24). A similar method has been used[7] to prepare 6-methylpyrrolo[2,3-$\underline{d}$]pyrimidines (27) from 4-aminopyrimidines (25) containing a potential 5-acetonyl group,

the intermediate 5-acetonyl-4-aminopyrimidines (26) being converted immediately to the pyrrolopyrimidines (27).

A slight variation of these syntheses has involved the preparation of some 4-aminopyrimidines[7](28) with a 2,3-dihydroxypropyl group at the 5-position. These compounds could be oxidised to 4-aminopyrimidyl-5-acetaldehydes (29), which were converted to the expected pyrrolo[2,3-d] pyrimidines (30), (31) and (32). It is of interest to note that the parent ring system, pyrrolo[2,3-d]pyrimidine, was readily prepared from 4-mercaptopyrrolo[2,3-d]pyrimidine (by desulphurisation) and from pyrrolo[2,3-d]pyrimidin-4(3H)-one (via the 4-chloro compound), both of which were synthesised by the methods described above in this section.

(28) (29) (30) R=H, R'=OH
(31) R=SCH$_3$, R'=OH
(32) R=SCH$_3$, R'=NH$_2$

In continuation of the work described above[6] West and Beauchamp[8] reported their preparation of further examples of the pyrimidine acetals (22) and the conversion of these compounds into the pyrrolopyrimidines (24). Later in the same year (1960) Kopecky and Michl[9] used an identical procedure to prepare some pyrrolo[2,3-d]pyrimidines (33).

$X = S, O, NH$

The 4-amino-5-diethoxyethylpyrimidines (22) described above were prepared by ring synthesis, using either 2-substituted malononitriles or ethyl cyanoacetates, and hence they all contained a free amino group at the 4- position of the pyrimidine ring. Obviously such compounds are of little use in the preparation of 7-alkylpyrrolo[2,3-d]pyrimidines, but Montgomery and Hewson[10] overcame the problem by devising a new preparative route to 4-alkylamino-5-(2,2-diethoxyethyl)-pyrimidines (34), (Fig. 3), which were readily converted to the corresponding pyrrolo-pyrimidines (35).

Bisagni et al[11] have reported a variation of this synthetic method. In 1969, they described their synthesis of some 4-chloro-5-acetonyl-pyrimidines (36). These compounds reacted readily with primary amines to give the 6-methylpyrrolo[2,3-d]pyrimidines (38). The reaction presumably proceeds via the 4-amino-5-acetonylpyrimidine (37) although this was not isolated.

Figure 3

(36)

(37)

(38)

R=SCH$_3$ or CH$_3$

R'=H, C$_6$H$_5$, C$_6$H$_4$-o(OH)

    CH$_2$-furyl, CH$_2$-tetrahydro furyl.
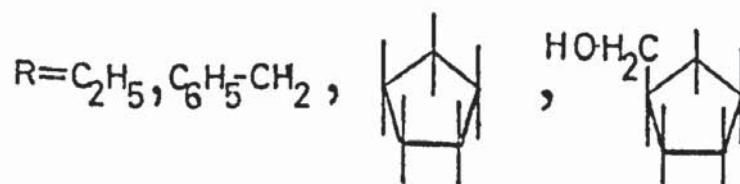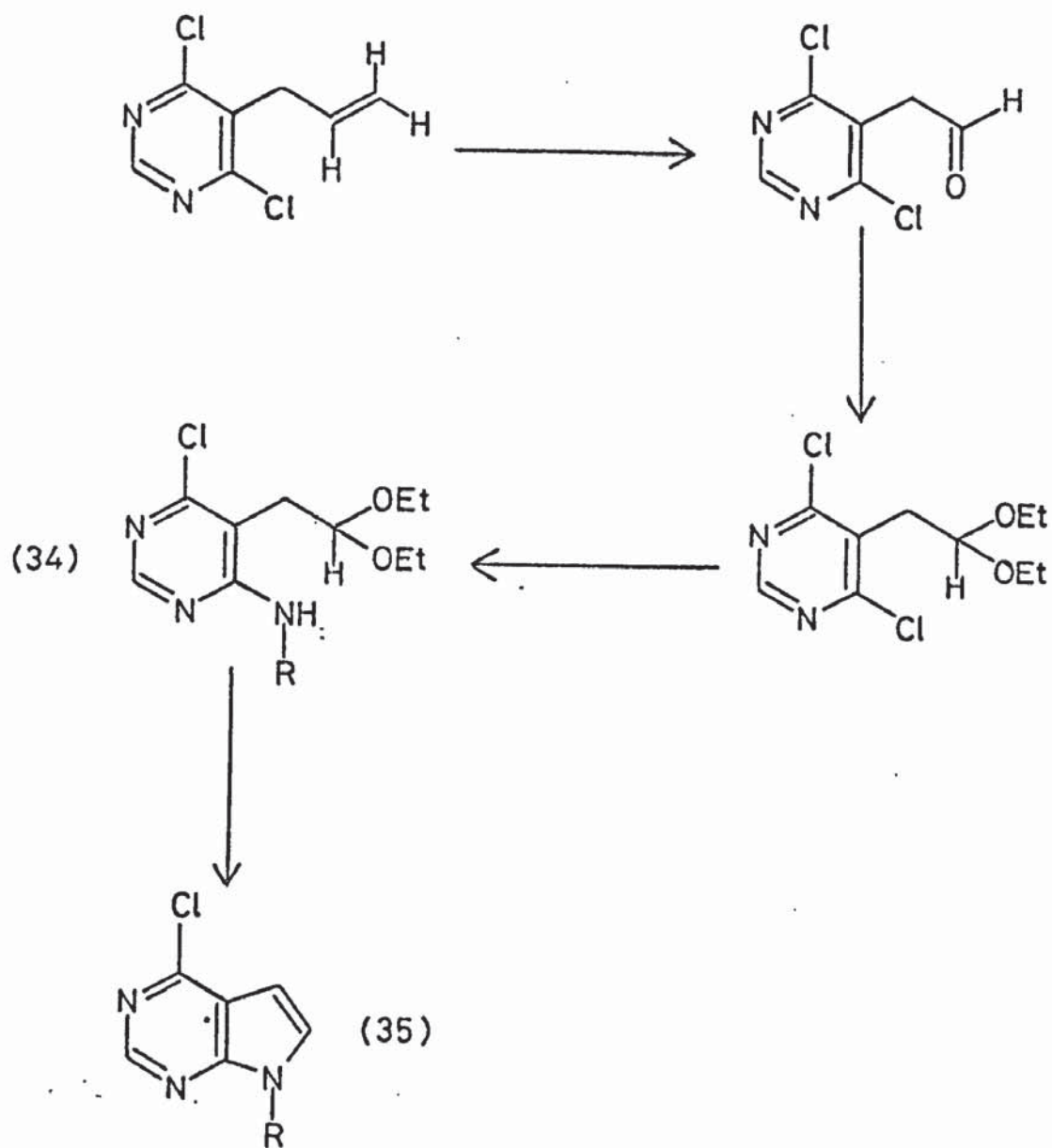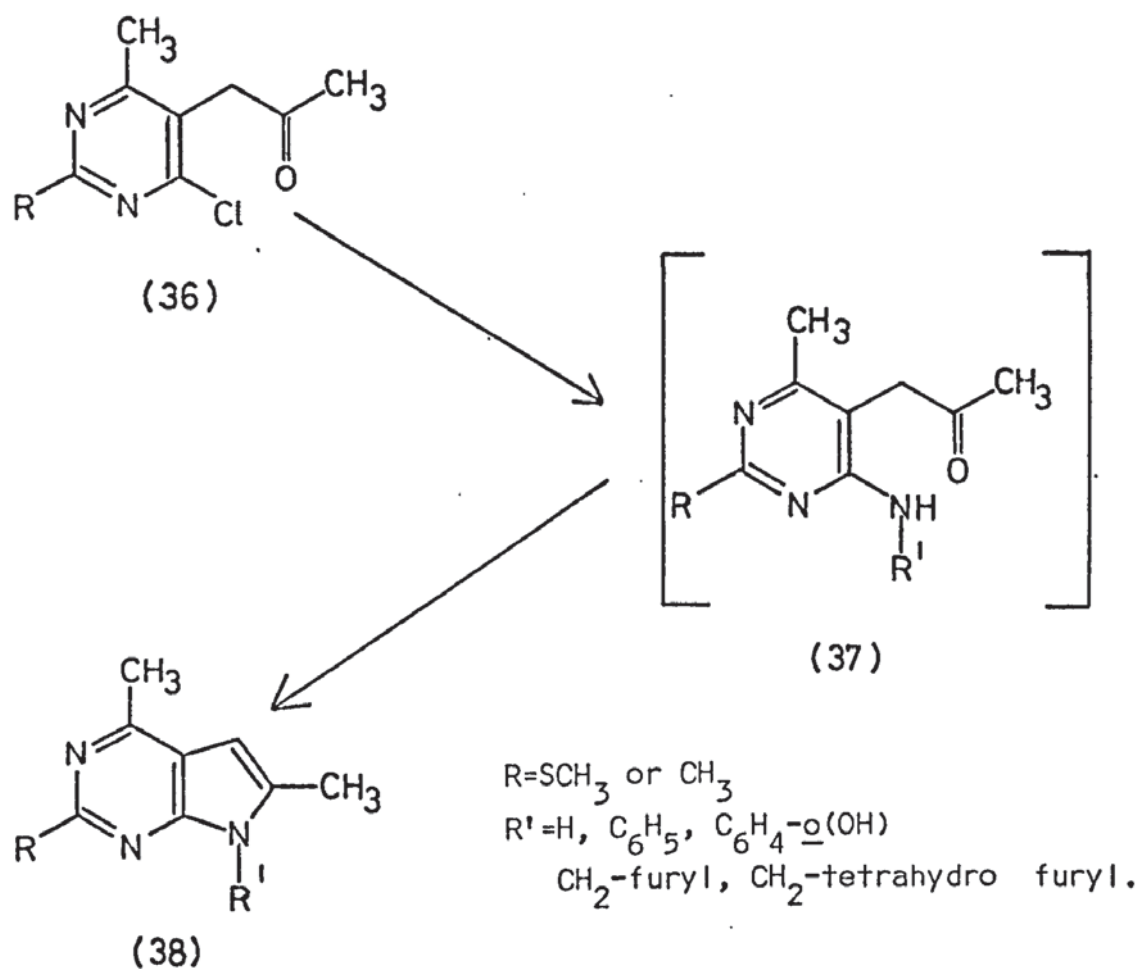
In later publications[12,13] this route has been explored more fully and many 2,4,7-substituted derivatives of the pyrrolo[2,3-d]pyrimidine ring system have been synthesised (Fig. 4) from the corresponding 4-chloro-5-acetonylpyrimidines.

R=H, CH$_3$

R'=OH, Cl, NH$_2$, SH, substituted amino

R''=H, CH$_3$, CH$_2$CH$_2$OH, furfuryl

    CH$_2$ C$_6$H$_5$, tetrahydrofuryl,

    ribofuranosyl, NH$_2$, NHCO-NC$_5$H$_5$
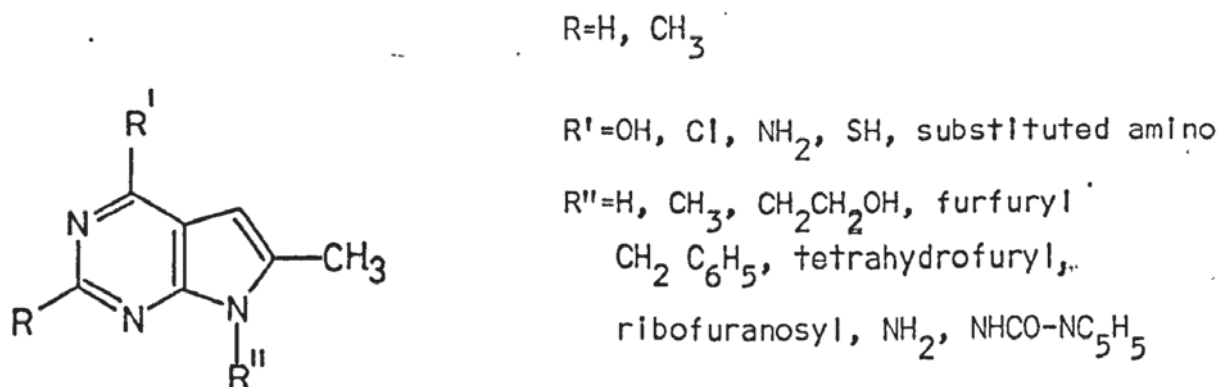
Figure 4

A.3. Synteses from 4-aminopyrimidines.

This type of synthesis proceeds by the reaction of a 4-amino-
pyrimidine derivative with a compound containing two electron-defficient
carbon atoms (Fig. 5). The first stage in the reaction is presumably
a substitution at position 5 of the pyrimidine ring which is then
followed by attack of the amino function, at the second electrophilic
centre, to furnish the cyclised product.
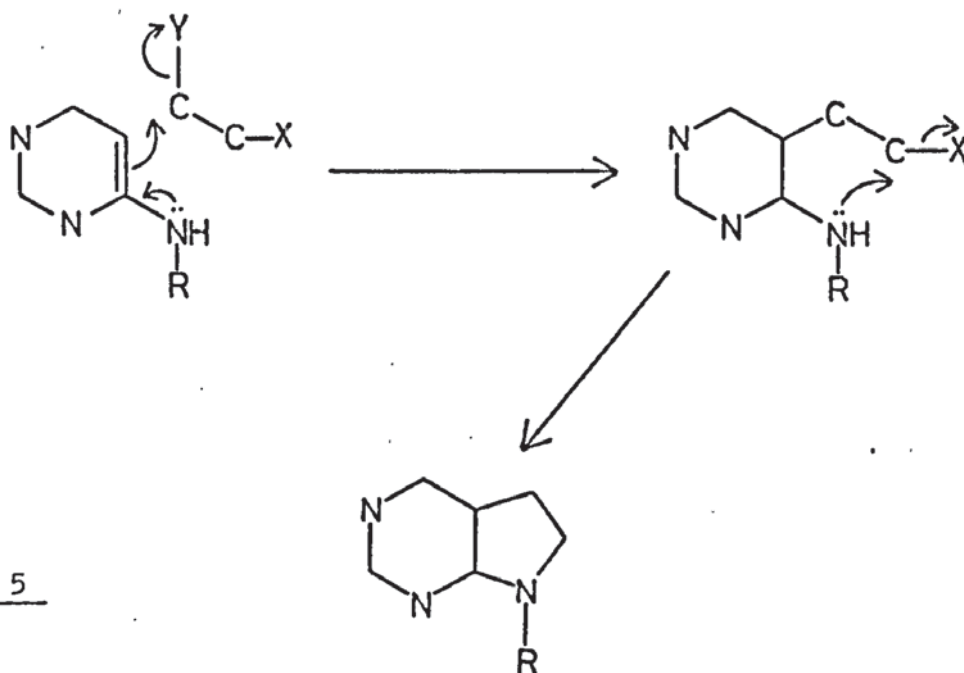
Figure 5

Most of these reactions consist of condensations of the amino-
pyrimidine with aldehydes or ketones which have halogenated α-carbon
atoms. The first example was described by West, Ledig and Hitchings[6]
in the same publication where many syntheses of the type described
in A.2. above were reported. These authors obtained the pyrrolopyrimidine
(45) directly by condensation of 6-aminouracil (43) with chloroacetalde-
hyde. When the reaction was carried out with a similar aminopyrimidine

(39) the 2-methylmercaptopyrrolo[2,3-_d_]pyrimidinone (40) was produced

again without the isolation of any intermediate.[14] In a similar manner

this route has been used[14] to prepare 7-alkylpyrrolo[2,3-_d_]pyrimidines

(41) and (42) and also the N ⎯ methylpyrrolopyrimidine dione (46),

from 6-amino-1,3-dimethyluracil (44).
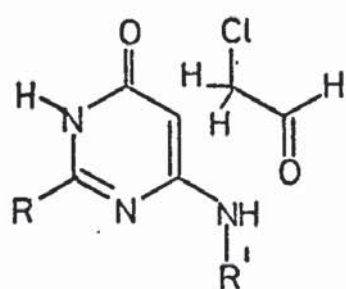
(39) R=CH₃S, R'=H
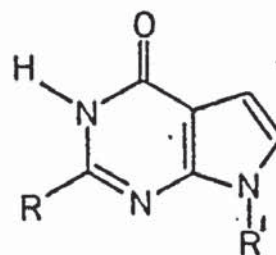
(40) R=CH₃S, R'=H
(41) R=NH₂, R'=_n_-C₃H₇
(42) R=NH₂, R'=CH₂-C₆H₅
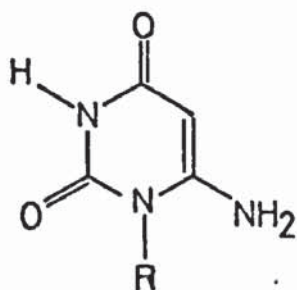
(43) R=H
(44) R=CH₃
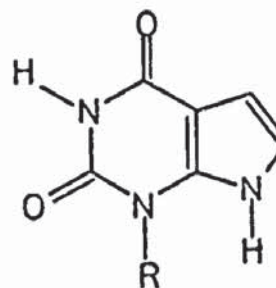
(45) R=H
(46) R=CH₃
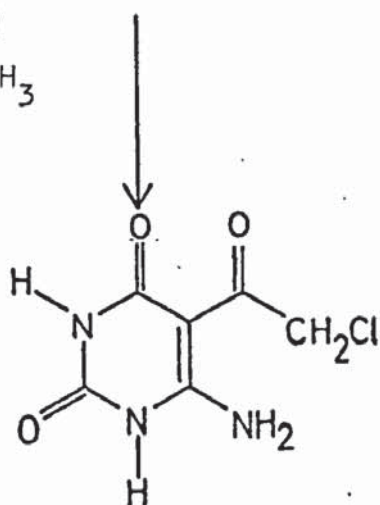
(47)
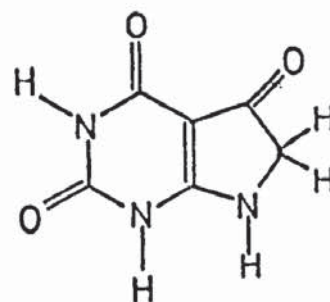
(48)

A slight modification of the procedure has been reported[14] which involved the use of an acid chloride and, in this case, an intermediate (47) was isolated. Condensation of 6-aminouracil with chloroacetyl chloride gave the 5-chloroacetylpyrimidine (47), which when treated with boiling aqueous ammonia cyclised to 2,4,5-pyrrolo[2,3-d]pyrimidine trione (48).

Other α-halocarbonyl compounds may condense with 4-aminopyrimidines and two other extensions of this synthetic method have been reported. Recently Yoneda et al[15] have studied the reaction of 6-amino-1,3-dimethyl-uracil (49) with phenacyl bromides. In neutral conditions the reaction product was the expected 6-phenylpyrrolopyrimidine (52). A similar condensation with p-chloro and p-bromophenacyl bromides gave the analogous 6-substituted pyrrolo[2,3-d]pyrimidines (53) and (54) respectively. When the reaction was carried out in the presence of acetic acid, however, the product was the 5-phenylpyrrolo[2,3-d]pyrimidine (59). Ogura et al[16] have described the reaction of substituted aminouracils (50) and (51) with ethyl α-bromoacetate. The resulting 7-alkylpyrrolo[2,3-d]pyrimidinones (55) and (56) were obtained in 26% and 10% yields respectively without isolation of any intermediates. When 6-amino-1,3-dimethyluracil (49) was induced to react with ethyl α-bromoacetate an intermediate 4-aminopyrimidyl-5-acetic ester (58) was produced which cyclised readily to the corresponding pyrrolopyrimidine (57) when treated with polyphosphoric acid.

(49) R=H
(50) R=CH₃
(51) R=C₆H₅

(52) R =H
(53) R=Cl
(54) R=Br

(59)

(55) R=CH₃
(56) R=C₆H₅
(57) R=H

(58)
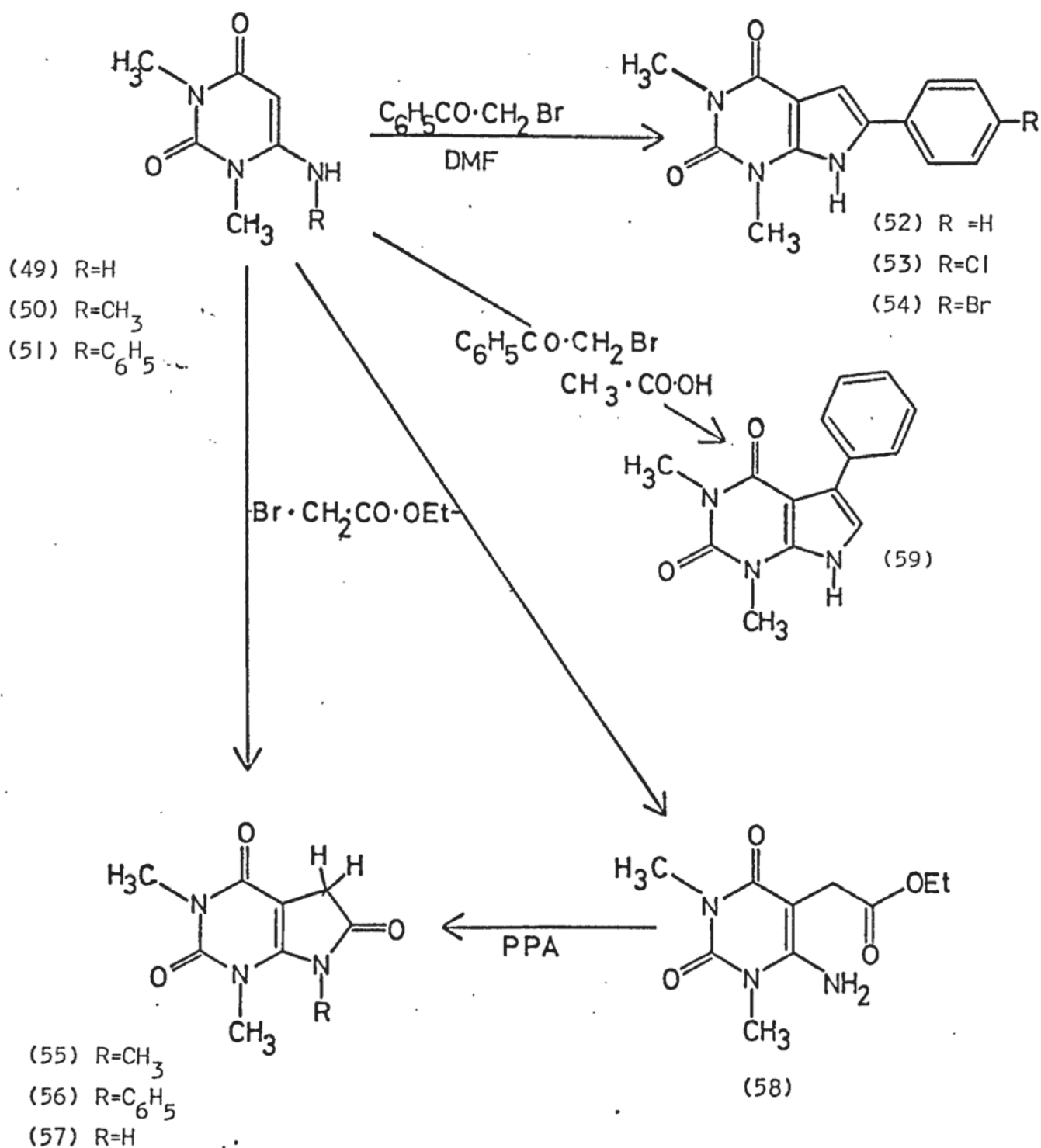
The two-carbon fragments which react with aminopyrimidines in these synthetic methods need not have halogen atoms incorporated into their structures. The formal positive charge on the pyridinium cation in phenacylpyridinium bromide increases the reactivity of the methylene carbon atom. Taylor and Garcia[17] were able to induce this compound to

react with 1,3-dimethyl-6-anilinouracil (60) to furnish 1,3-dimethyl-6,7-diphenylpyrrolo[2,3-d]pyrimidin-2,4(1H,3H)-dione (61) in 15% yield.

(60) (61)
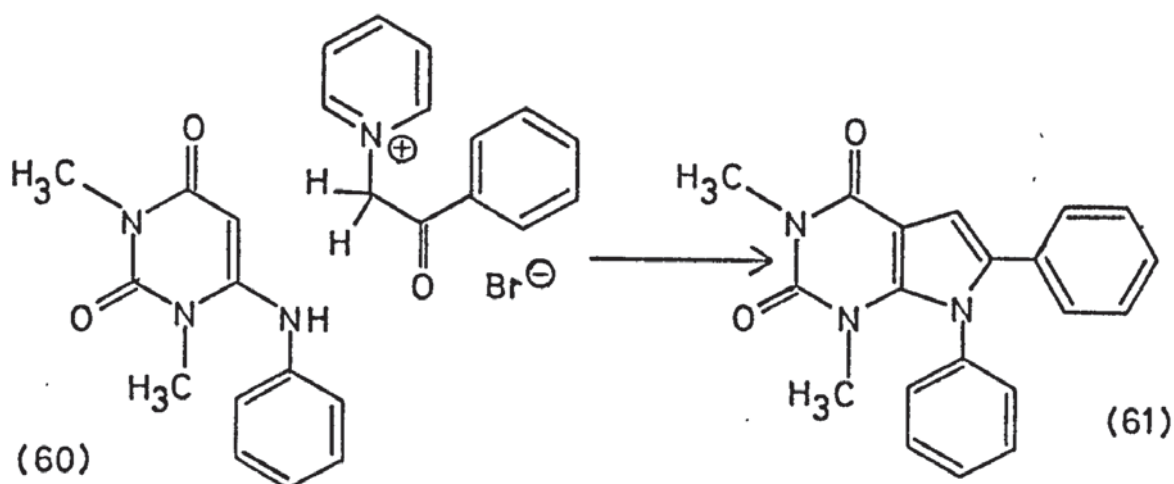
B. SYNTHESES OF TYPE 2.

The only reported examples of this type of synthesis are those due to Kim and Santilli.[18] Substituted 4-aminopyrimidines (62) were prepared, which had a methylene group incorporated into their structures, which was activated by a second functional group such as a carbethoxy or cyano group. The treatment of such compounds with sodium ethoxide apparently gives the carbanions (63) which spontaneously undergo an intra-molecular Dieckmann-type cyclisation to the pyrrolopyrimidines (65) which tautomerise to the corresponding 5-hydroxy-2-phenylpyrrolo[2,3-d] pyrimidines, in excellent yields, as their sodium salts. Acidification furnishes the free pyrrolopyrimidines (64).

(62)

(63)

(64)

(65)

R	X
H	COOEt
CH$_3$	CN
C$_2$H$_5$	CN
n-C$_4$H$_9$	CN
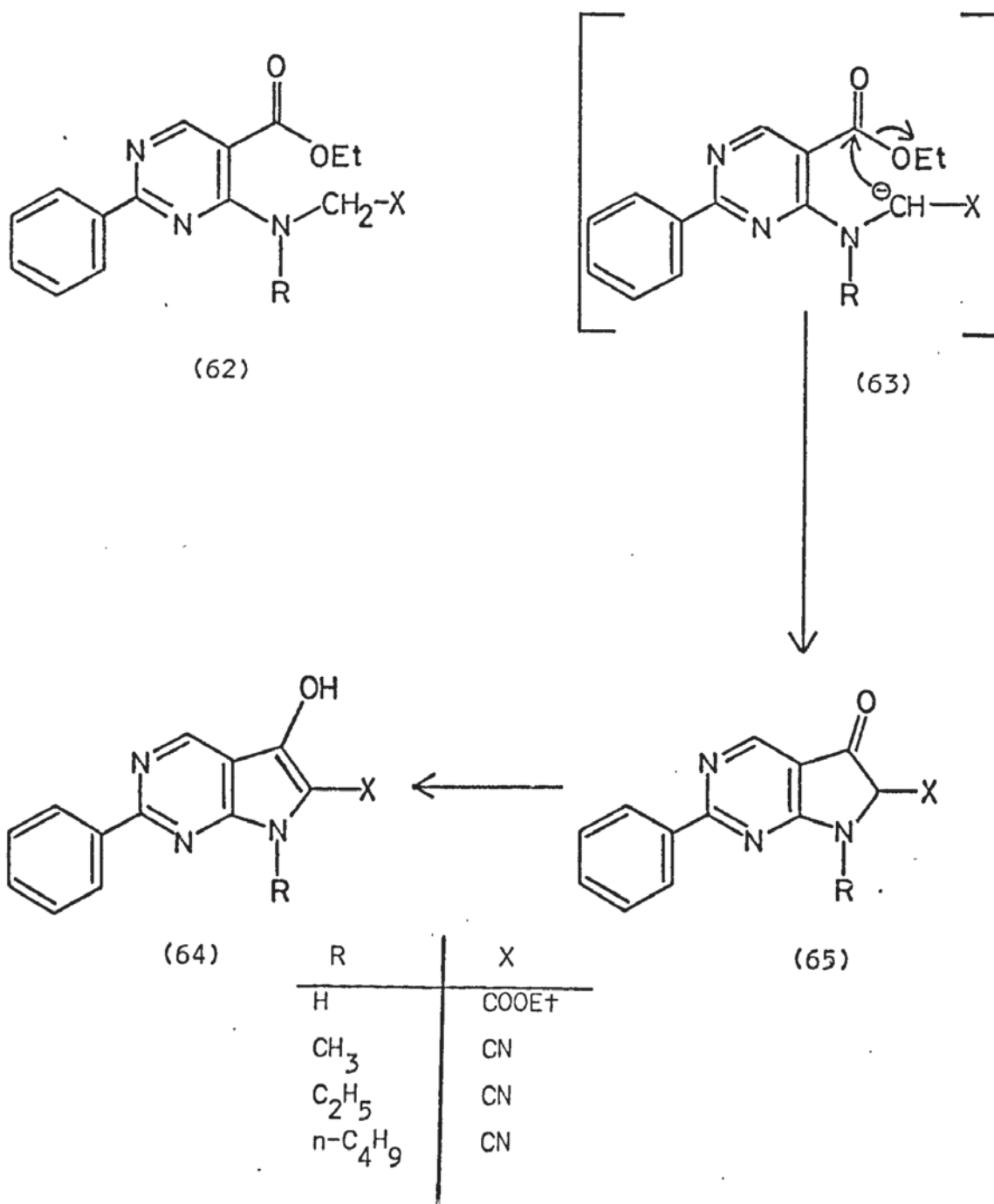
C. SYNTHESES OF TYPE 3.

Syntheses of this type involve preparation of a pyrimidyl-5-ethylamine derivative which undergoes intramolecular nucleophilic cyclisation to the 6-position when a suitable leaving group is present at that position, (Fig. 6).

Figure 6

Only two such cyclisations have been effected,[19,20] both of which involved the treatment of 4-hydroxypyrimidines (66) and (69) with phosphorylchloride or similar reagents. The reactions presumably proceed *via* the 4-chloro compounds (67) and (70) although these were not isolated. In this manner the dihydro-pyrrolopyrimidines (68) were synthesised[19] from the 4-hydroxy-5-ethylaminopyrimidines (66). Similarly Partyka[20] reported the synthesis of the pyrimidylacetamides (69) and their conversion to the 6-chloropyrrolo[2,3-d]pyrimidines (71).

(66)   (67)   (68)
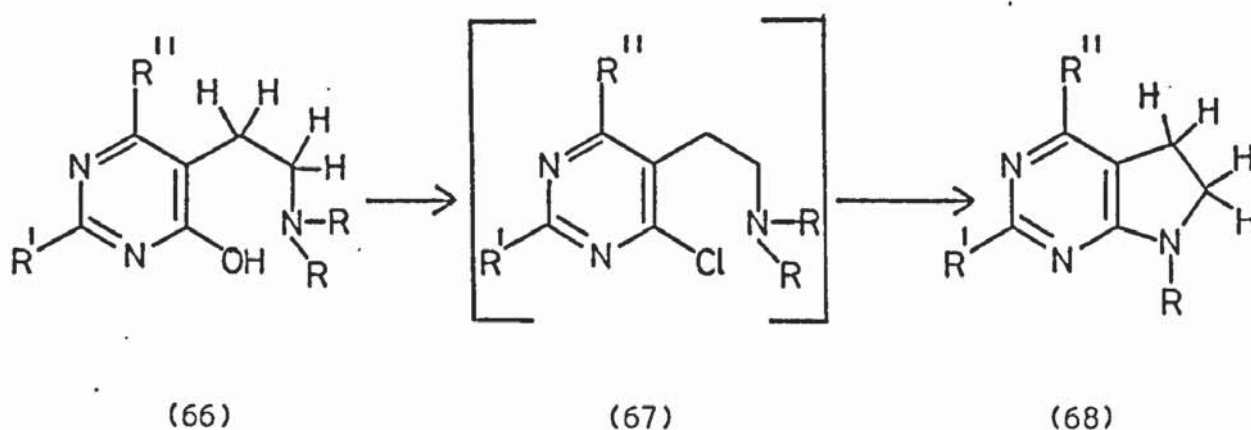
Schueler and Suranyi[19]

    R   = alkyl

    R' = alkyl or aryl

    R'' = amino (usually).

(69) → (70) → (71)
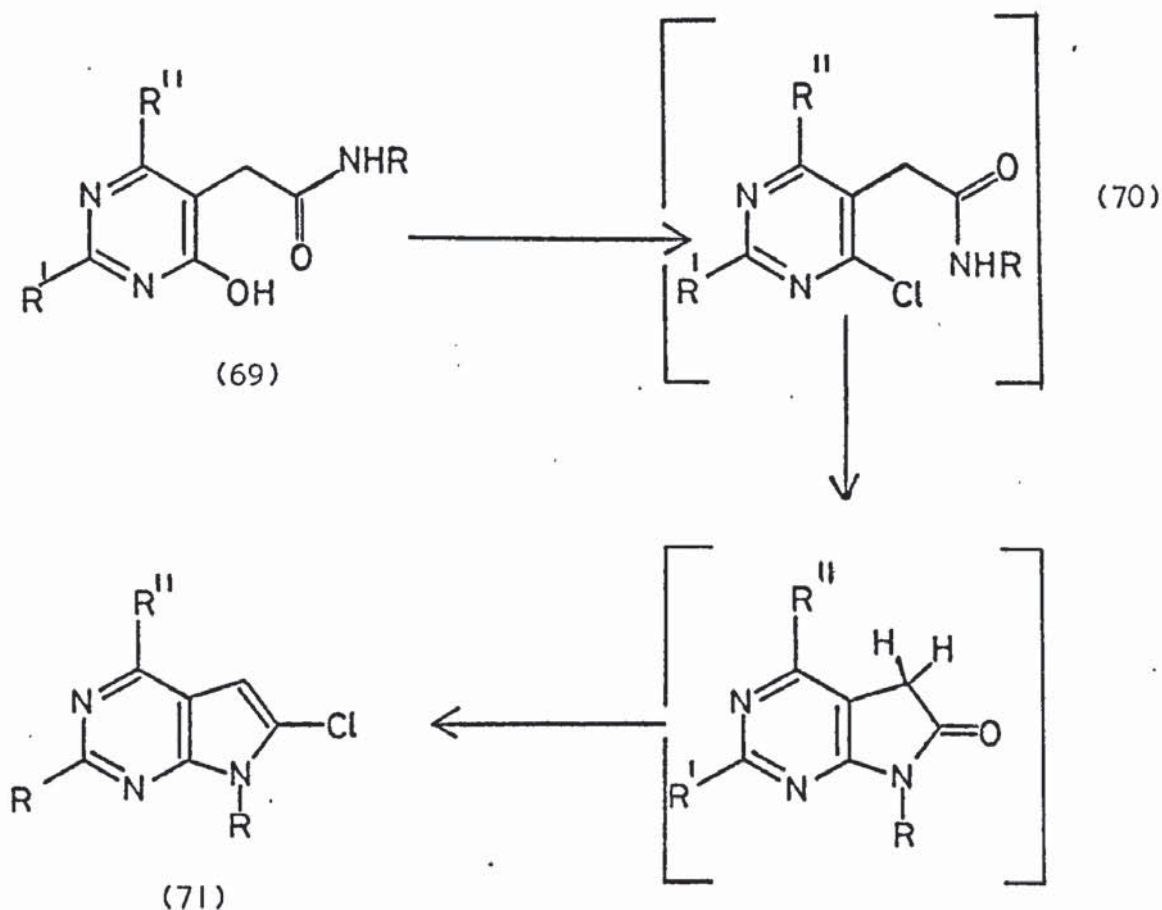
Partyka[20]

R=alkyl, aryl, aralkyl, $CH\equiv C-CH_2$, $(C_2H_5)-N-(CH_2)_2$.

R'=CH_3, CH_3S, C_2H_5S, C_6H_5S, SH.

R"=CH_3 or H.

D. SYNTHESES OF TYPE 4.

Two quite different synthetic methods have been included in this section, photocyclisations of 4-anilinopyrimidines (which produce benzoderivatives of the pyrrolo[2,3-d]pyrimidine ring system), and thermal cyclisations of pyrimidyl hydrazones. The latter reaction, a thermally-induced Fischer indole cyclisation, is included for convenience although a study of the reaction mechanism might warrant its inclusion as a synthesis of type I.

D.I. Photocyclisations of 4-Anilinopyrimidines

4-Anilinopyrimidines, when irradiated, cyclise to give pyrimido[4,5-b] indoles[21] (72).
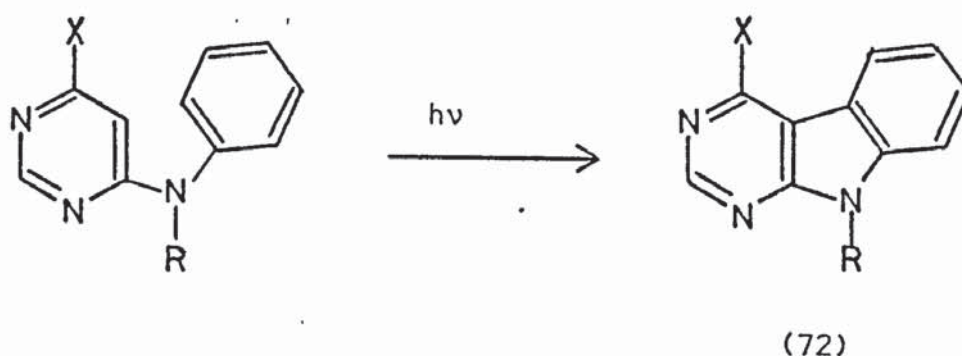
x=Cl or H

D.2. Thermal Cyclisation of pyrimidyl hydrazones

Crooks and Robinson[22] have described a thermally induced conversion of pyrimidyl-4-hydrazones to pyrrolo[2,3-d]pyrimidines, a similar reaction to Fischer's Indole synthesis. The hydrazones (73) and (74) in solution in digol, were heated for several hours to give the pyrimido[4,5-b]indole (76) and the pyrrolo[2,3-d]pyrimidine (77) respectively. Similar treatment of isobutyraldehyde 4-pyrimidyl-hydrazone (75) gave none of the expected 5,5-dimethylpyrrolo[2,3-d] pyrimidine the only reaction product being 5,6-dimethylpyrrolo[2,3-d] pyrimidine (77) produced by rearrangement of the 5,5-dimethyl analogue.

When the experimental work described in this thesis had been completed, Senda and Hirota[23] published a short communication describing a synthesis of 5,6-disubstituted pyrrolo[2,3-d]pyrimidines which is similar to that described in this work. However, no analytical data or spectroscopic evidence was supplied and, to this date, no corroborating details have appeared in the literature.
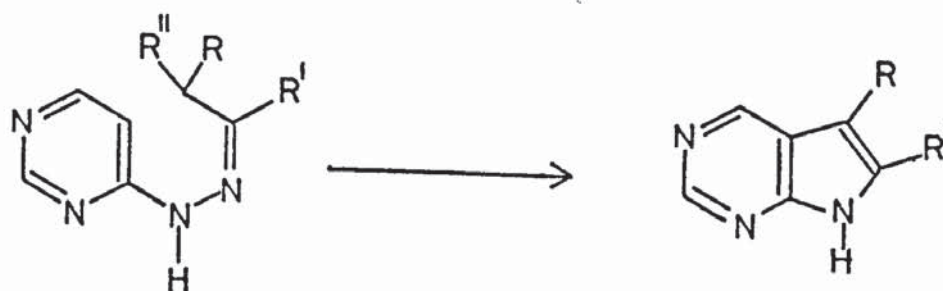
(73) R+R'=(CH$_2$)$_4$, R''=H

(74) R=CH$_3$, R'=CH$_3$, R''=H

(75) R=CH$_3$, R'=H, R''=CH$_3$

(76) R+R'=(CH$_2$)$_4$

(77) R=CH$_3$, R'=CH$_3$

This synthetic method consists of the condensation of 1,3-dimethyl-6-hydrazinopyrimidin-2,4(1H,3H)-diones (78) with aldehydes or ketones. The resulting hydrazones (79) were converted *in situ* to the corresponding pyrrolopyrimidines (80) by heating them under reflux in high boiling solvents. In a few cases the hydrazinopyrimidine was heated under reflux with the carboxyl compound in tetralin or ethylene glycol and the pyrrolopyrimidine (80) was obtained directly, without isolation of the intermediate hydrazone. Attempts to induce a pyrrole ring closure by treatment of the hydrazones (79) with acidic catalysts were unsuccessful. This latter result is not in agreement with the experimental findings reported later in this thesis.
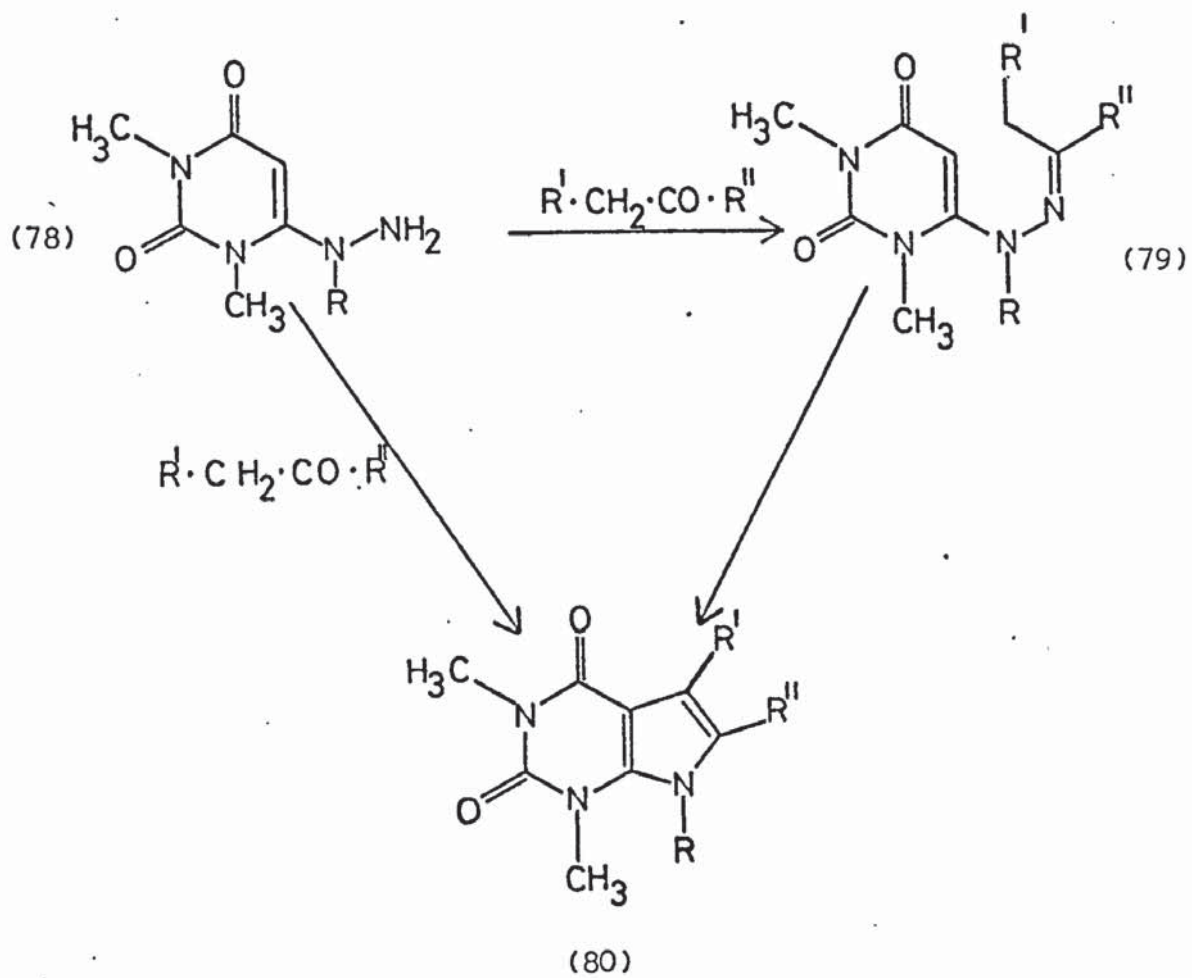
R	R'	R''
H	CH_3	CH_3
H	CH_3	H
H	C_6H_5	H
H	H	CH_3
CH_3	CH_3	CH_3
H	CH_3	C_6H_5
H	$(CH_2)_4$	

II SYNTHESES OF PYRROLO[2,3-d]PYRIMIDINES FROM 2-AMINO-3-CYANOPYRROLES

All synthetic procedures to the pyrrolo[2,3-d]ring system *via* pyrroles have involved the use of 2-amino-3-cyanopyrroles. On the basis of the method used to construct the pyrimidine ring from these precursors, syntheses of this type may be classified as either cyclisations *via* an intermediate alkoxymethylenamino derivative or ring closures effected by condensation with formamidine acetate.

A. SYNTHESES INVOLVING CYCLISATION OF AN INTERMEDIATE ALKOXYMETHYLENEAMINO DERIVATIVE

Condensation of the 2-amino-3,4-dicyanopyrrole (81) with methyl orthoformate followed by treatment with alcoholic ammonia gave a 4-amino-5-cyanopyrrolo[2,3-d]pyrimidine[24,25] (96). The reaction proceeds *via* the methoxymethyleneamino compound (84) which may be isolated if required, or treated directly with ammonia to produce an intermediate amidine (87) which cyclises immediately to the pyrrolopyrimidine (96). The ethoxymethylene derivatives (85) and (86) afforded the pyrrolo-pyrimidines (97) and (98) respectively when similarly treated.

Alkoxymethyleneaminoderivatives may also be prepared from the 4-substituted pyrrole amino-nitriles (82)and (83). However, in this case, either the 4-iminopyrrolo[2,3-d]pyrimidines (90) and (91) or the amidines (88) and (89) respectively, are isolated depending on the nature of the amine used in the reaction. The 4-iminopyrrolopyrimidines (90) and (91)rearrange smoothly to the 4-alkylamino compounds (92) and (93) respectively.[24,25]

This route has also been explored by other workers[26] to prepare the pyrrolopyrimidines (94) and (95).
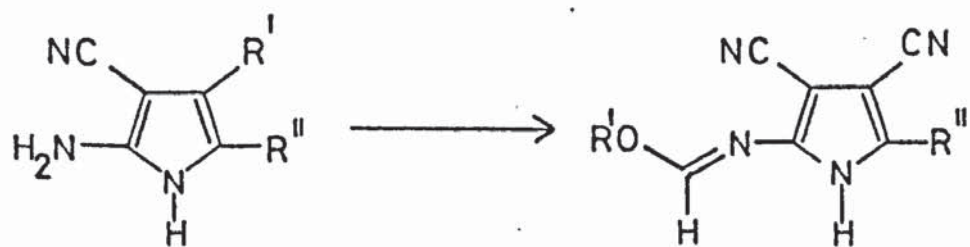
(81) R'=CN, R''=SH
(82) R'=CH$_3$, R''=H
(83) R'=C$_6$H$_5$, R''=H

(84) R''=SH, R'''=CH$_3$
(85) R''=SCH$_3$, R'''=CH$_3$
(86) R''=SC$_2$H$_5$, R'''=C$_2$H$_5$
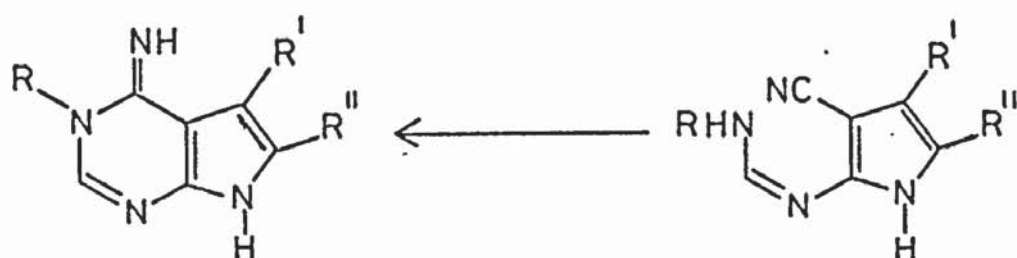
(90) R=alkyl, R'=CH$_3$,
     R''=H
(91) R=alkyl, R'=C$_6$H$_5$
     R''=H

(87) R=H, R'=CN, R''=SH
(88) R=alkyl, R'=CH$_3$, R''=H.
(89) R=alkyl, R'=C$_6$H$_5$, R''=H.
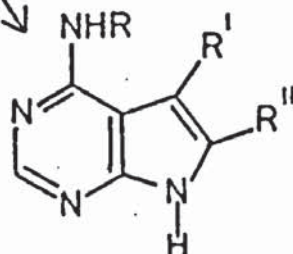
(92) R^O=H,   R=alkyl, R'=CH$_3$,   R''=H,
(93) R^O=H,   R=alkyl, R'=C$_6$H$_5$, R''=H,
(94) R^O=H,   R=CH$_3$, R'=CN,   R''=SCH$_3$
(95) R^O=CH$_3$, R=CH$_9$, R'=CN,   R''=SCH$_3$
(96) R^O=H,   R=H,   R'=CN,   R''=SH
(97) R^O=H,   R=H,   R'=CN,   R''=SCH$_3$
(98) R^O=H,   R=H,   R'=CN,   R''=SC$_2$H$_5$

B.   SYNTHESIS BY CONDENSATION WITH FORMAMIDINE ACETATE.

Taylor and Hendess[24,25] demonstrated that the amino nitrile (99)
could be induced to react with formamidine acetate to yield 4-amino-5-
cyano-6-mercaptopyrrolo[2,3-d]pyrimidine (101) directly, although the
reaction was accompanied by considerable decomposition of the pyrrole.
A similar reaction reported by Robins and coworkers,[26,27] using 2-amino-
5-bromo-3,4-dicyanopyrrole (100) furnished the corresponding 4-amino-
pyrrolo[2,3-d]pyrimidine (102) in 65% yield.
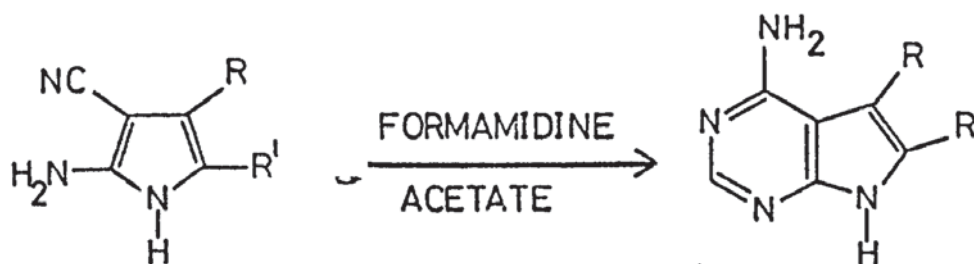
(99)   R=CN, R'=SH

(100)   R=CN, R'=Br.

(101)   R=CN, R'=SH

(102)   R=Cn, R'=Br.

THE BIOLOGICAL IMPORTANCE OF PYRROLO|2,3-d|PYRIMIDINES

Recent interest in the synthesis of derivatives of the pyrrolo[2,3-d] pyrimidine ring system has arisen from the discovery of three naturally occurring nucleoside antibiotics, whose chemical structures incorporated a pyrrolopyrimidine moiety. These nucleoside antibiotics are all derivatives of 4-amino-7-β-ribofuranosyl [2,3-d]pyrimidine and vary only in their possession of different substituents at position 5.

I DISCOVERY AND ELUCIDATION OF THE STRUCTURES OF THE PYRROLO[2,3-d]

PYRIMIDINE NUCLEOSIDE ANTIBIOTICS

The first of these antibiotics to be discovered was toyocamycin. It was isolated from various species of *streptomyces* by several independent workers[28-38] who provided the compound with a variety of names. The absolute proof that antibiotic 1037, unamycin B, vengicide, antibiotic E-212 and toyocamycin were identical was provided by the total synthesis of toyocamycin by Robins and his coworkers[26] and the structure was shown to be 4-amino-5-cyano-7-β-D-ribofuranosylpyrrolo|2,3-d| pyrimidine (103).

Tubercidin (104) was the second pyrrolopyrimidine nucleoside antibiotic isolated, and is an analogue of adenosine in which the nitrogen at position 7, (of the purine ring system), has been replaced by a carbon atom. Like toyocamycin this compound has also been isolated[39-41] from several species of *streptomyces*.

In 1963, Rao and Renn[42], obtained the third pyrrolo|2,3-d|pyrimidine nucleoside antibiotic from the culture filtrates of a strain of *streptomyces rimosus*. This compound was named Sangivamycin (105) and differs from toyocamycin only in its possession of an amido, (instead of a cyano), group at position 5.
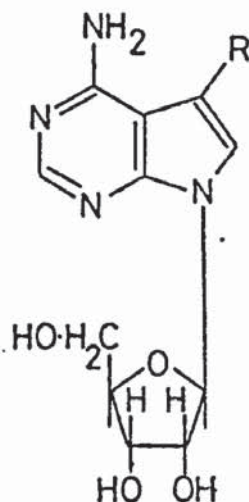
Toyocamycin   (103)   R=CN

Tubercidin   (104)   R=H

Sangivamycin   (105)   R=CONH$_2$

II BIOLOGICAL ACTIVITY IN PYRROLO|2,3-d|PYRIMIDINE DERIVATIVES.

In addition to their antibiotic nature, these pyrrolopyrimidines are renowned for antitumour properties and cytotoxic effects on mammalian cells. Few other actions on living systems have been reported, the only important one being a central nervous system depressant action.[18] Pyrrolo[2,3-d]pyrimidine nucleosides have proved to be the most biologically useful derivatives of the ring system and the value of free pyrrolopyrimidine bases will probably depend on the ease with which they are converted to the corresponding nucleosides in vivo.

IIA. Antimicrobial Activity.

Toyocamycin strongly inhibits *Candida albicans, Cryptococcus neoformans* and *Mycobacterium tuberculosis* but its effect on a large range of gram-positive and gram-negative, fungi and yeasts was much less marked.[35] Tubercidin also inhibited *Mycobacterium tuberculosis* and *Candida albicans* but, like toyocamycin, had little activity against gram positive bacteria, yeast or fungi.[39,43,44] It has also been reported that tubercidin inhibited the growth of both RNA and DNA viruses. Sangivamycin showed little or no antibacterial or antifungal activity.[45]

IIB. Cytotoxic Effects.

Despite their relatively undistinguished activity against micro-organisms, the pyrrolo[2,3-d]pyrimidine antibiotics evoked further interest when their highly cytotoxic effect on mammalian cell strains was discovered. Toyocamycin was shown to be a significant antitumour compound[46] and was particularly active against NF sarcoma cells. Tubercidin is also very toxic to NF mouse sarcoma cells and several reports have described the toxic effects of this compound both on mammalian cells grown in vitro and on experimental tumours.[46,47,49-56] On this basis tubercidin was selected for clinical trial and, when toxicity problems had been overcome by changing the route of administration, significant regression of malignancy was reported.[58] Sangivamycin, which had shown virtually no antibiotic effect, displayed very significant antitumour properties. The compound showed marked activity against leukaemia L1210 in mice and iscytotoxic towards He La cells.[42] The inhibition of sarcoma 180 and carcinoma 755 tumours by sangivamycin was less obvious. Sangivamycin has undergone clinical trial in the treatment of leukaemia and, furthermore, it showed no toxicity in

maximally tolerated doses, in humans.[48] As a result of these encouraging observations many derivatives, involving changes in the amino group, the amide group or the pentose residue, of sangivamycin, have been examined for antileukaemic properties. [45]

IIC. Central Nervous System Depressant Activity

Kim and Santilli[18] have reported the preparation of a series of 2,5-disubstituted-6-cyanopyrrolo[2,3-d]pyrimidine derivatives which had a depressant effect on the central nervous system of mice when administered either orally or intraperitoneally.

III   MODE OF ACTION OF PYRROLO[2,3-d]PYRIMIDINE NUCLEOSIDES IN

BIOLOGICAL SYSTEMS.

Of the three nucleoside antibiotics discussed so far the mechanism of action of tubercidin has been studied in most detail. The modes of action of toyocamycin and sangivamycin are less well documented but, since they are structurally very similar to tubercidin, one may speculate that their inhibitory effects are related to those of tubercidin. However one must consider that the functional and steric presence of a substituent at position 5 in the pyrrolo[2,3-d]pyrimidine ring system may alter the participation of these compounds in enzymic reactions.

Examinations of the methods by which these pyrrolopyrimidine anti-biotics exert their toxic effects on microorganisms and mammalian cells have resulted in the explanation of these effects in terms of three properties, the incorporation of the compounds into nucleic acids, their interference with protein synthesis and some undefined effect on cellular utilisation of glucose.

IIIA. The Effects on Nucleic Acids.

The glycosidic bond in tubercidin, unlike that of adenosine is resistant to acid cleavage. This property is consistent with the resistance of the antibiotic to cleavage by adenosine phosphorylase.[43] Tubercidin is a substrate for adenosine kinase which induces its conversion to tubercidin 5'-monophosphate (TuMP).[59] Since little enzymic cleavage with adenosine phosphorylase (or adenosine deaminase) is likely, TuMP is readily produced in many cells. Further phosphorylations of TuMP to the di- and triphosphates, (TuDP and TuTP respectively), are carried out by appropriate kinases, and in a similar manner toyocamycin and sangivamycin form the corresponding triphosphates, ToTP and SaTP respectively.

TuTP interferes with nucleic acid function in two ways. It is known to be incorporated into RNA as a nucleotide[56] and, since TuDP and TuTP are substrates of ribonucleotide reductase systems, it is also converted to the deoxyribonucleotide and then incorporated into DNA.[56] Toyocamycin has been shown to be incorporated into RNA and DNA in a similar manner,[60,61] and SaTP is also converted to the 2'-deoxyribonucleotide, although SaDP is not.[60,61]

The possible consequences of the incorporation of these compounds into nucleic acids are three-fold. Their location in DNA may disrupt both replication and transcription processes and their involvement in RNA structures may be expected to have adverse effects on translation processes with consequent disruption of protein synthesis. (See Fig. 7).

It seems likely that all three of these sites of interference with cellular processes are involved and tubercidin is known to inhibit both RNA and DNA viruses,[44] and RNA, DNA and protein synthesis in mouse

fibroblasts,[56] KB cells[58] and L cells.[56]
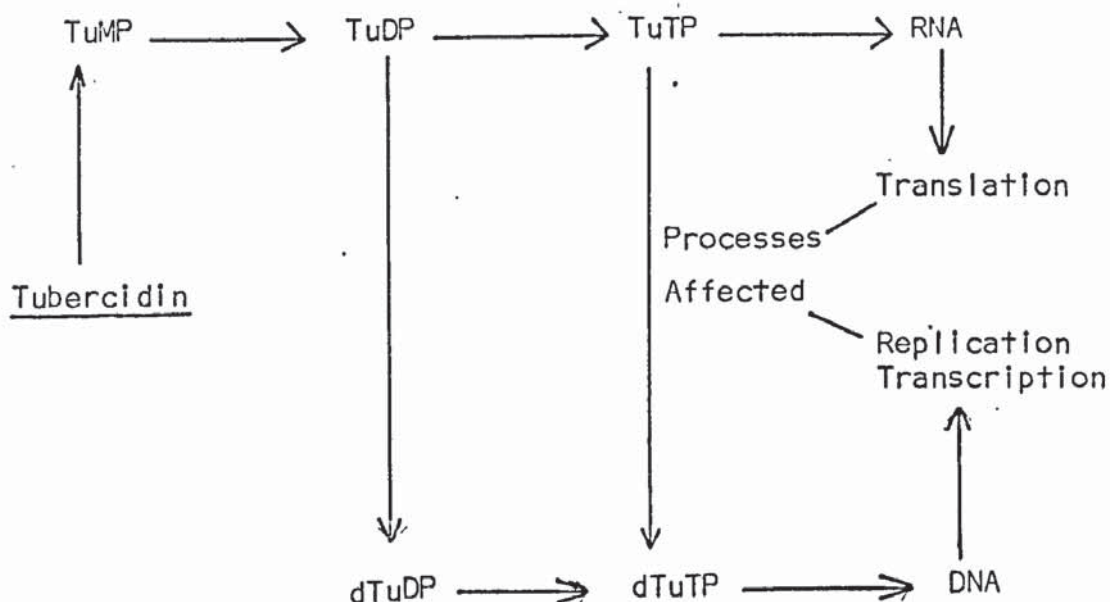
Figure 7

IIIB. The Effects on Protein Synthesis.

In addition to their incorporation into the synthesis of RNA (IIIA above), there is evidence that these pyrrolopyrimidine antibiotics specifically inhibit the synthesis of certain ribosomal RNA structures. Since the ribosomes are the only known sites of protein synthesis in living cells, this phenomenon may explain, to some extent, the profound inhibition of protein synthesis which has been observed in the presence of these compounds.

Studies in mouse fibroblasts[62] showed that, according to examination of the sucrose gradient centrifugation profile, low concentrations of toyocamycin completely inhibited the synthesis of 28-S and 18-S RNA, although the 45 and 45-S subunits were not affected. Tavitian et al[62]

suggested that when toyocamycin becomes incorporated into the precursor ribosomal RNA, cleavage into the 28S and 18S subunits is not possible. This result may partially explain the inhibition of protein synthesis produced by these three pyrrolopyrimidine antibiotics.

Perhaps an equally important factor is , the incorporation of the pyrrolopyrimidines into the 3' termini of transfer RNA (tRNA) molecules.[63] In a normal cell amino-acyl synthetase enzymes catalyse the transfer of amino acids to their specific tRNA molecules. The resulting amino-acyl-tRNA compounds diffuse to the ribosomes where peptide bond formation occurs and proteins are formed. In order to participate in this reaction aminoacids must first be activated by adenosine triphosphate (ATP) but, despite their close structural similarity to ATP, TaTP, ToTP and SaTP cannot replace it in this activation process although ToTP and SaTP can competitively inhibit ATP, since their substituents at the 5 position favour their formation of complexes with amino-acyl synthesase. Therefore it appears that interference with ATP is not the solution to the protein synthesis inhibition problem. However all three pyrrolo-pyrimidine antibiotics are incorporated into the end of the tRNA molecule which joins. with the activated amino acid. This end of the tRNA molecule usually terminates in a cytosine-cytosine-adenosine (CCA) coding and all three compounds can replace adenosine in this 3' terminal sequence.[63] tRNA with tubercidin located at the 3'terminus was able to esterify amino acids but the acceptor activity of tRNA containing sangivamycin or toyocamycin was reduced. It was shown[63] that aminoacyl tRNA which contained tubercidin actually performed normally in peptide bond formation at the ribosomes, although at a reduced rate.

In conclusion the inhibition of protein synthesis produced by the

pyrrolo|2,3-d|pyrimidine antibiotics is probably due, at least in part, to their interference with ribosomal RNA production and to their incorporation into tRNA molecules.

IIIC. Effects on Glucose Utilisation.

Uretsky et al[63] stated that these effects shown by the pyrrolo-pyrimidine nucleosides *in vivo* could not explain their profound inhibitory effect on protein synthesis in intact cell preparations. They suggested that the activity in these cases might be an indirect one where tubercidin, for example, interfered with some critical reaction which is vital for cellular function. It was suggested[43] that impairment of protein synthesis is due to the interference of tubercidin with utilisation of glucose, thereby presumably depriving the cell of the necessary metabolic energy it requires for protein synthesis. This theory was supported experiment-ally by the reversal of tubercidin-induced growth inhibition, in one bacterial species, by administration of pyruvate.[43] (See IIID. below).

IIID. Effects on Cellular Cofactor Functions.

Incorporation of tubercidin into nicotinamide deazaadenine dinucleotide, an analogous process to the formation of Nicotinamide adenine dinucleotide (NAD) has been reported.[43] Since NAD is an important hydrogen receptor in oxidative reactions, the replacement of adenosine by tubercidin may be expected to produce important changes in the cellular metabolic processes. One may speculate that it is for this reason that cells display reduced ability to utilise glucose in the presence of tubercidin, (see IIIC. above), but there is no experimental evidence for such a mechanism at present.

IV THE SYNTHESIS OF PYRROLO[2,3-d]PYRIMIDINE NUCLEOSIDE ANTIBIOTICS.

In order to verify the structural assigments of tubercidin, toyocamycin and sangivamycin, Tolman, Robins and Townsend[27] described a total synthesis of these three pyrrolopyrimidine antibiotics. This synthesis was accomplished by a condensation of a trisubstituted pyrrolo[2,3-d]pyrimidine (107) with the tetra-o-acetyl sugar (108), and constitutes a versatile synthetic method for these and other pyrrolo-pyrimidine nucleosides.

4-amino-6-bromo-5-cyanopyrrolo[2,3-d]pyrimidine (106) was prepared in the manner previously described in this thesis (P. 26). Since electron-withdrawing groups in a purine moiety had previously been shown to facilitate the fusion reaction with an acetylated sugar, Robins and his coworkers prepared the 4-acylaminopyrrolo[2,3-d]pyrimidine (107) from the free 4-amino compound (105). Condensation of 1,2,3,5-tetra-o-acetyl-β-D-ribofuranose (108) with this 4-acylaminopyrrolopyrimidine.(107), in the presence of a small amount of catalyst, gave the tetra-acylated nucleoside (109) as a syrup, which was not purified, but was deactylated by reaction with methanolic ammonia to 4-amino-6-bromo-5-cyano-7-β-D-ribofuranosylpyrrolo[2,3-d]pyrimidine (110). This latter compound could easily be converted to tubercidin (111), (by debromination, hydrolysis of the nitrile and decarboxylation), to toyocamycin (112), (by debromination) and to sangivamycin (113), (by hydrolysis of the nitrile and debromination).

(106) → (107) + (108)

(109) → (110)
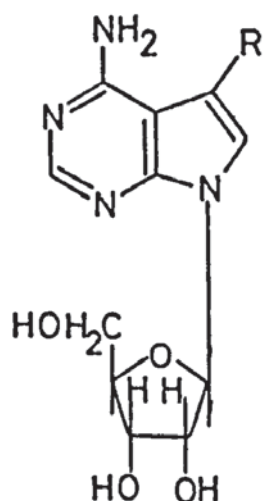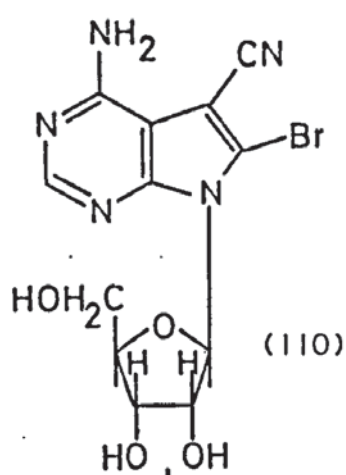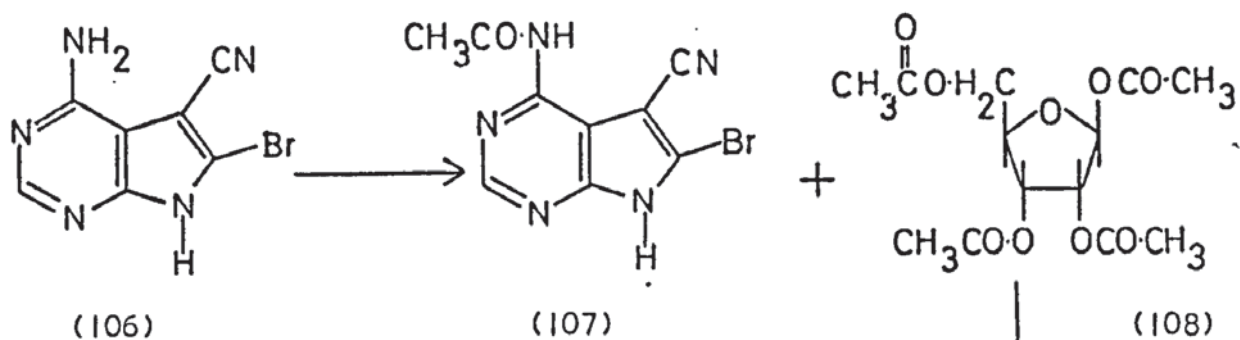
(111) R=H, Tubercidin

(112) R=CN, Toyocamycin

(113) R=CONH$_2$, Sangivamycin
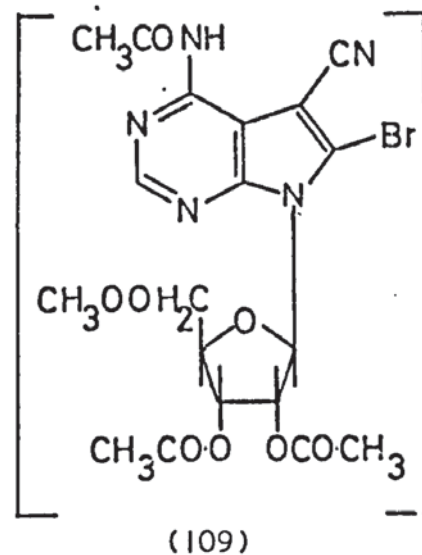
DISCUSSION

In the introduction to this thesis all the known syntheses of pyrrolo[2,3-d]pyrimidines have been reviewed and the biological properties of these compounds have been summarised. The aim of this work was to investigate new synthetic routes to these pyrrolopyrimidines and to briefly examine their chemical and biological properties. Three synthetic methods have been studied; the first concerned cyclisation of 6-substituted pyrimidines and the second and third involved the use of pyrrole precursors.

I   SYNTHESES OF PYRROLO[2,3-d]PYRIMIDINES FROM PYRIMIDYL HYDRAZONES

A thermally induced conversion of pyrimidyl-4-hydrazones to pyrrolo [2,3-d]pyrimidines has been reported by Crooks and Robinson[22] and the scope of this synthetic method has been described earlier in this thesis (P.20). The only compounds prepared by this route prior to the commencement of this thesis were the 5,6-dimethylpyrrolo[2,3-d]pyrimidine (77) and the pyrimido[4,5-b]indole (76). This chapter concerns an investigation of the cyclisation of 2-. and 2,4-disubstituted pyrimidyl-6-hydrazones, with and without acid catalysts.

A.   THE SYNTHESIS OF 2-METHYL-,2,4-DIMETHYL- AND
     2,4-DIPHENYLPYRROLO[2,3-d]PYRIMIDINES

A.I.   Preparation of pyrimidyl-6-hydrazones.

2,4-Dimethyl-6-hydrazinopyrimidine (114) was prepared by the treatment of 4-chloro-2,6-dimethylpyrimidine[64] with hydrazine hydrate, in the manner described by Miller and Rose.[65] It was discovered that when this hydrazinopyrimidine (114) was heated on a steam bath with various ketones, (either alone or in ethanolic solution), the corresponding hydrazones

were produced. Thus treatment with cyclohexanone, benzylmethyl ketone,

butanone and acetophenone gave the corresponding hydrazones (117), (118),

(119), (120) in 87%, 36%, 43% and 54% yields respectively.

The infrared spectra of these products all contained a strong

absorption between 3225 and 3300cm^{-1} which was assigned to the NH

stretching vibration. Strong absorptions observed between 1585 and

1610cm^{-1} indicated the presence of the exocyclic -C=N- bond. The NMR

spectra of these four hydrazones were also consistent with the proposed

structures (117) - (120). All the spectra had two singlets between 7.5

and 7.7τ corresponding to the 2- and 4- methyl groups and in all cases

a singlet between 3.06 and 3.28τ, assigned to the proton at the pyrimidine

5 position, was observed. In three spectra a broad, exchangeable singlet,

variable in position, was visible and this absorption was attributed to

the NH proton.

(114) R=CH$_3$, R'=CH$_3$

(115) R=H, R'=CH$_3$

(116) R=C$_6$H$_5$, R'=C$_6$H$_5$

(117) R=CH$_3$, R'=CH$_3$, R''+R'''=(CH$_2$)$_4$

(118) R=CH$_3$, R'=CH$_3$, R''=CH$_3$, R'''=C$_6$H$_5$

(119) R=CH$_3$, R'=CH$_3$, R''=CH$_3$, R'''=CH$_3$

(120) R=CH$_3$, R'=CH$_3$, R''=C$_6$H$_5$, R'''=H

(121) R=H, R'=CH$_3$, R''+R'''=(CH$_2$)$_4$

(122) R=C$_6$H$_5$, R'=C$_6$H$_5$, R''+R'''=(CH$_2$)$_4$

(123) R=C$_6$H$_5$, R'=C$_6$H$_5$, R''=C$_6$H$_5$, R'''=H.
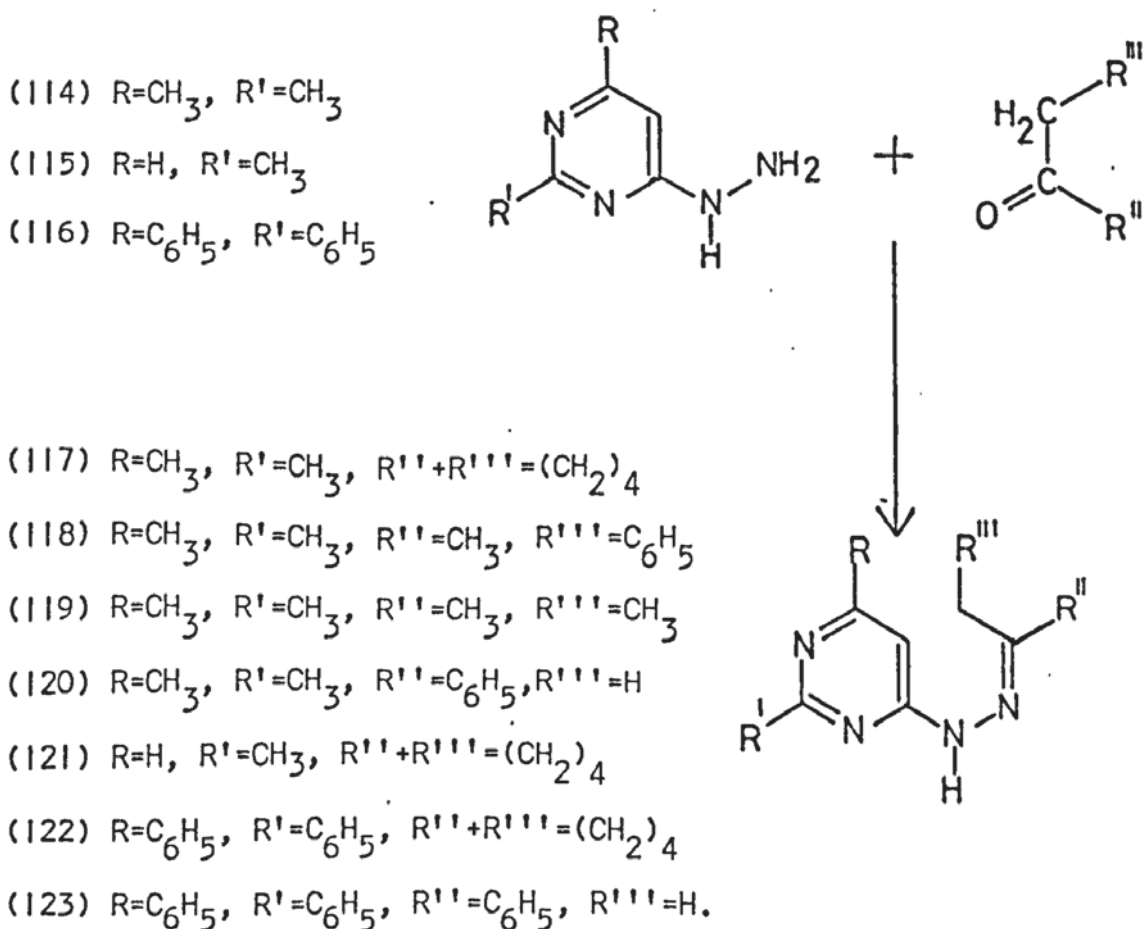
2-Methyl-4-hydrazinopyrimidine (115) was prepared from the corresponding 4-hydroxy compound[66] *via* the 4-chloropyrimidine, by the method described by Miller and Rose.[65] This pyrimidyl hydrazine, like the 2,4-dimethylhydrazino compound (114), readily reacted with cyclohexanone to furnish the corresponding hydrazone (121) in 54% yield. The infrared spectrum of the hydrazone (121) contained the typical NH stretching absorption at $3200cm^{-1}$ and a strong peak at $1580cm^{-1}$, corresponding to -C=N- stretching, was also observed. An examination of the NMR spectrum provided confirmation of the structure (121). A singlet absorption at 7.53τ, corresponding to the 2-methyl group, was observed and, an AB quartet, (assigned to the protons at positions 5 and 6 of the pyrimidine ring) was visible as doublets at 3.08 and 1.85τ respectively. Multiplets between 7.6 and 7.9τ and between 8.1 and 8.6τ correspond to the cyclohexyl protons.

2,4-Diphenyl-6-hydrazinopyrimidine (116) was readily prepared[65] according to the method used for the methyl- and dimethylpyrimidyl hydrazines (115) and (114) respectively. This compound condensed very readily with cyclohexanone and acetophenone, (in ethanol), to give the hydrazones (122) and (123) in yields greater than 70%. The infrared spectra of these two compounds contained NH absorptions at higher wavelengths, (3375 and $3350cm^{-1}$ respectively), than those observed in the spectra of the alkylpyrimidyl hydrazones prepared above. The NMR spectra were consistent with the expected structures which were additionally confirmed by mass spectral and analytical evidence.

A.2. Cyclisation of pyrimidyl-6-hydrazones under acidic conditions.

No attempts to prepare pyrrolo[2,3-<u>d</u>]pyrimidines by this method were made since protonation of basic nitrogen atoms, in acidic media,

deactivates the pyrimidine nucleus towards electrophilic attack and thus would be expected to prevent cyclisation.[67] This is discussed in more detail later in this chapter.

A.3. Cyclisation of pyrimidyl-6-hydrazones by a non-catalytic thermal technique.

Robinson[22] (P.20) prepared the pyrrolo|2,3-d|pyrimidines (76) and (77), by heating solutions of the corresponding hydrazones in high-boiling solvents for several hours, and attempts to cyclise the pyrimidyl-6-hydrazones (117) - (123) were made by essentially the same method. Each hydrazone was heated under reflux in digol or trigol, (in a nitrogen atmosphere), until no further evolution of ammonia occurred or, if no basic gas was detected, until the reaction mixture began to decompose.

(124) R=CH$_3$, R'=CH$_3$, R''+R'''=(CH
(125) R=H, R'=CH$_3$, R''+R'''=(CH$_2$)
(125a) R=Ph, R'=Ph, R''+R'''=(CH$_2$)
(125b) R=Ph, R'=Ph, R''=C$_6$H$_5$, R'''

(128)

(126) R'=C$_6$H$_5$, R''=CH$_3$
(126a) R'=CH$_3$, R''=CH$_3$
(126b) R'=H, R''=C$_6$H$_5$

(127)
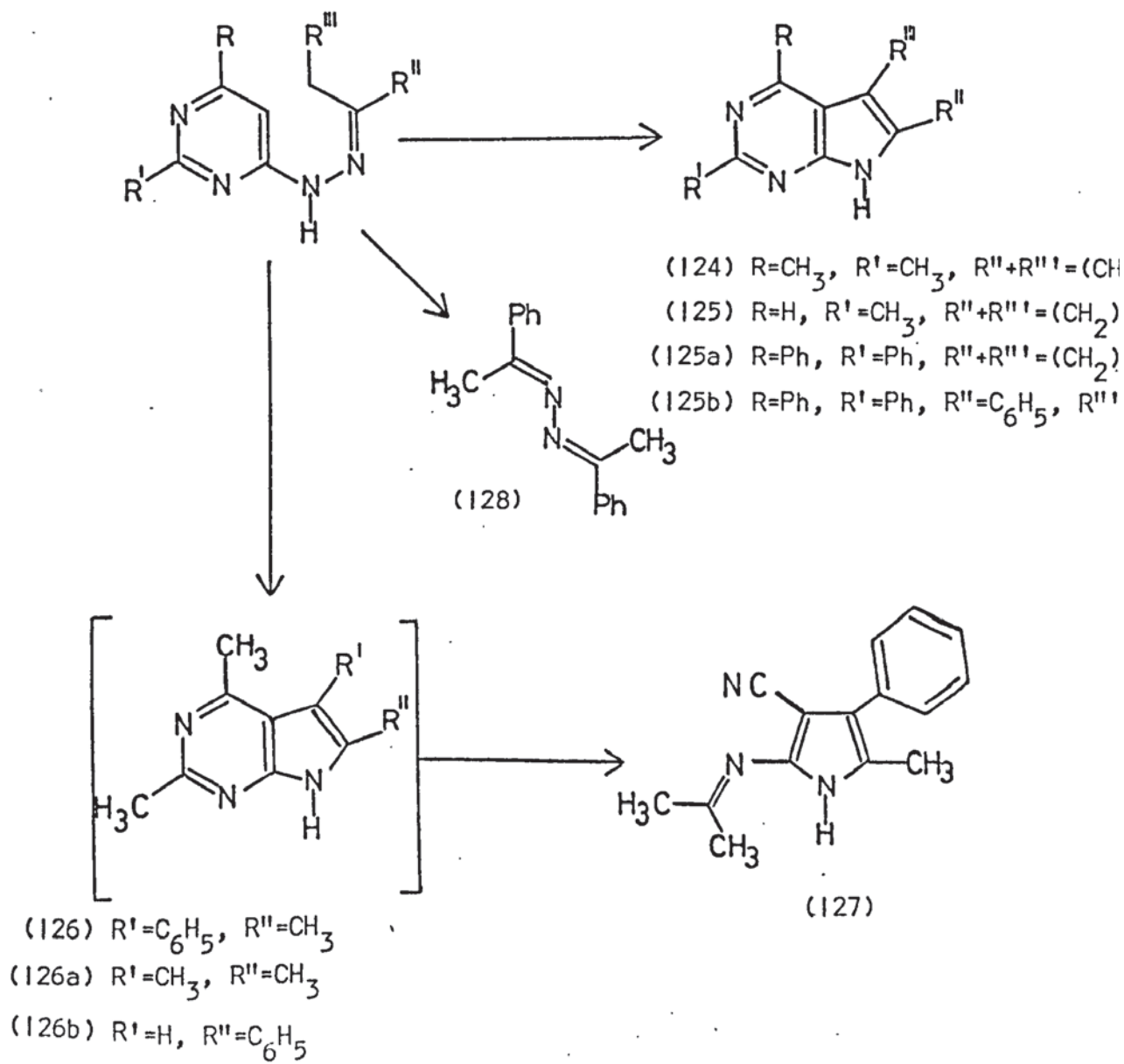
The cyclohexanone 2,4-dimethylpyrimidinyl-6-hydrazone (117), when subjected to these conditions cyclised to 2,4-dimethyl-5,6,7,8-tetrahydropyrimido[4,5-b]indole (124) in 45% yield. The infrared spectrum contained a strong NH absorption at 3150cm^{-1} (lower than in the spectrum of the corresponding hydrazone (117). Examination of the NMR spectrum of this pyrrolo[2,3-d]pyrimidine indicated the absence of a proton at position 5 of the pyrimidine ring and provided confirmation of the proposed structure.

Similar treatment of the butanone hydrazone (119) was less successful. Although ammonia was evolved during the reaction, (indicating that cyclisation was taking place), only a dark gum, which could not be purified, was isolated from the reaction mixture.

An attempted cyclisation of the acetophenone 2,4-dimethylpyrimidyl-6-hydrazone (120) produced none of the expected pyrrolopyrimidine. Ammonia was not evolved during the reaction and the NMR and infrared spectra of the only product isolated were consistent with those expected from a compound containing two equivalent methyl groups, two equivalent phenyl rings and no free NH group. It is tentatively suggested that this product is the dihydrazone (128).

The hydrazone (118) also reacted abnormally when subjected to non-catalytic thermal cyclisation conditions. Ammonia was evolved during the reaction and examination of the elemental analysis data and the mass spectrum of the only product isolated, did not contradict the proposed pyrrolopyrimidine structure (126). However the infrared spectrum contained a typical C≡N stretching absorption at 2225cm^{-1} and the only NH absorption occurred at a wavenumber too high (3275cm^{-1}) to be consistent with a pyrrolo[2,3-d]pyrimidine structure. (None of the infrared

spectra of the pyrrolopyrimidines prepared during the course of this work contained absorptions, attributable to pyrrole NH stretching which occurred above $3200 cm^{-1}$). Further work must be done to elucidate the structure of this anomalous product but the tetrasubstituted pyrrole (127) is consistent with the available spectroscopic and analytical evidence. It is suggested that such a product must have been formed by the thermally induced decomposition of the pyrrolopyrimidine (126) under the severe reaction conditions.

Cyclohexanone 2-methylpyrimidyl-4-hydrazone (121) was readily converted to the 2-methylpyrimido[4,5-b]indole (125) in almost quantitative yield. The infrared spectrum of the product contained a typical NH absorption at $3125 cm^{-1}$, and the presence of a singlet at 1.80τ, (and the absence of any absorption at $3.0 - 3.3\tau$), in the NMR spectrum, indicated that cyclisation to the pyrrolopyrimidine had occurred. It is very significant that a pyrimidyl hydrazone with no substituent at position 4 should cyclise more readily than its 2,4-disubstituted derivative. Such an observation suggests that some steric factor is involved in the reaction mechanism and this postulate, (which is discussed more fully later in this chapter), is supported by evidence obtained from attempted cyclisations of pyrimidyl-6-hydrazones with a bulky group at position 4. Thus the two diphenylpyrimidyl hydrazones (122) and (123), when subjected to vigorous thermal conditions for many hours, produced only unchanged starting material.

B. THE SYNTHESIS OF 1,3-DIMETHYLPYRROLO[2,3-d]PYRIMIDIN-2,4-DIONES.

Since the cyclisations of pyridyl and pyrimidyl hydrazones are inhibited by acid catalysts, thermally induced Fischer indole syntheses have provided a valuable route to pyrrolopyridines and pyrrolopyrimidines.[6]

However, the long reaction times and severe conditions involved in non-catalytic techniques, cause decomposition of reaction mixtures and consequently impure products are often isolated. Therefore, it was decided to prepare pyrimidyl hydrazones, with non-basic nitrogen atoms in the pyrimidine ring, which were expected to undergo both acid-catalysed and thermally induced conversions to the corresponding pyrrolo[2,3-d]pyrimidines.

B.I. Preparation of 1,3-dimethyl-2,4-dioxopyrimidin-6-hydrazones.

Pfleiderer and Schündehütte[68] described the preparation of 1,3-dimethyl-4-hydrazinopyrimidin-2,6(1H,3H)-dione (132) and during the course of this work, the preparative method has been modified to increase the yield of this compound. The 6-hydroxypyrimidin-2,4-dione (130) was heated under reflux with phosphoryl chloride to give the 6-chloropyrimidine (131). Treatment of this compound with hydrazine hydrate in ethanol gave the hydrazinopyrimidine (132) in 90% yield, (compared with 82% reported by the above authors[68]).
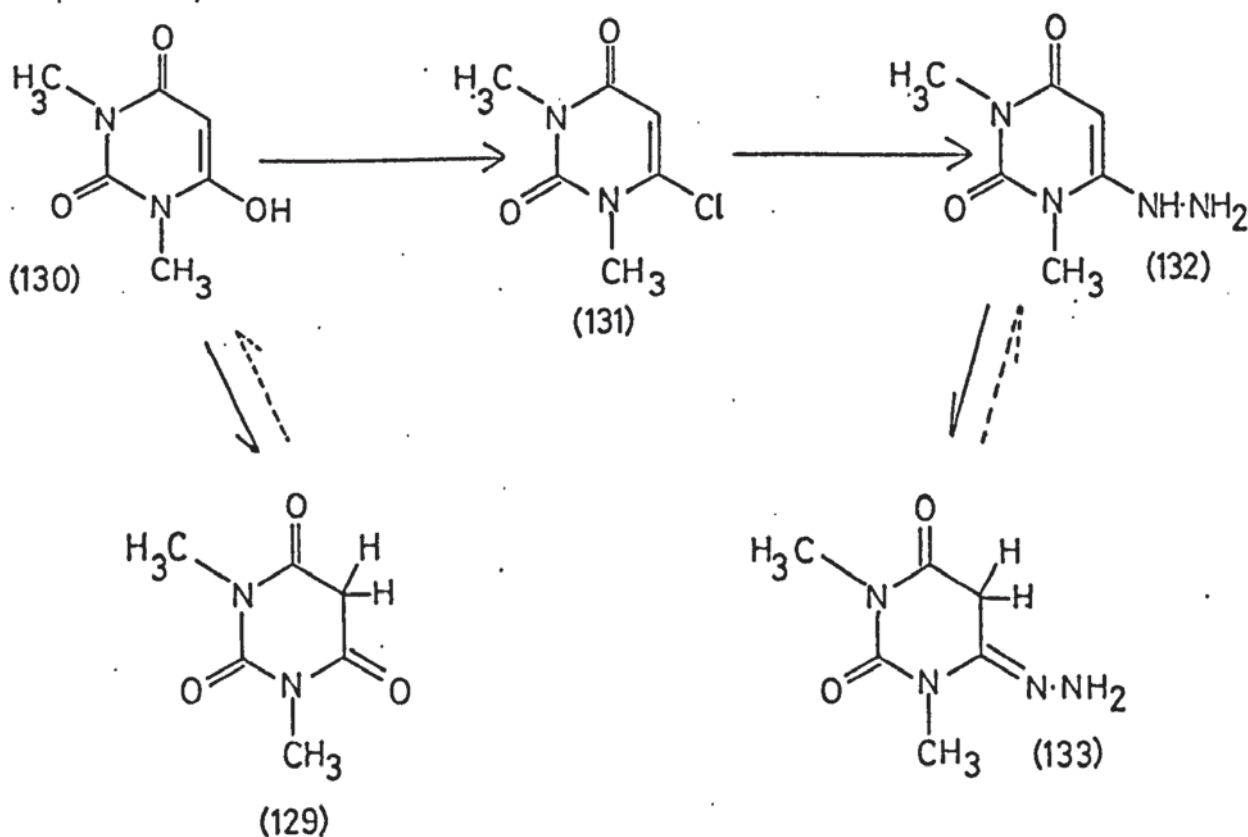
Examination of the NMR spectrum of the compound, described[68] as the 4-hydroxypyrimidine dione (130), revealed the presence of singlets at 6.73 (6H) and 6.37τ (2H) respectively. This indicates that the compound exists as the symmetrical pyrimidine trione tautomer (129). The NMR spectrum of the chloropyrimidine (131) contains a singlet at 4.19τ, and singlets, attributed to the 2- and 4- CH_3 groups, at 6.48 and 6.76τ respectively. This finding is consistent with the chloropyrimidine structure (131) which is not conducive to tautomerism. The NMR spectrum of the hydrazinopyrimidine was not determined but the compound, (on the basis of observations, described below, of similar structures), is assumed to adopt the aminoimine tautomeric form (133).

This hydrazinopyrimidine dione, when heated with a solution of cyclo-hexanone in ethanol gave a product, to which the hydrazone structure (134) was assigned, on the basis of its NMR spectrum. The spectrum contained, (in addition to the cyclohexyl proton multiplets and N-CH_3 singlets), a singlet at 6.08τ corresponding to 2 protons, which dis-appeared when the sample was deuterated. This indicates that the compound adopts the imino tautomeric form and the amino form is present to a negligible extent under the conditions in which the NMR spectrum was determined.

The methylene group at position 5 in the imino tautomer (134) contains two acidic protons which readily exchange with deuterium in solution. Hence the singlet attributed to these protons in the NMR spectrum, was readily removed on the addition of deuterium oxide.

(133)

(134)-(138)

(139)

(134) $R+R' = (CH_2)_4$

(135) $R=C_6H_5, R'=CH_3$

(136) $R=CH_3, R'=CH_3$

(137) $R=CH_3, R'=H$

(138) $R=C_6H_5, R'=H$
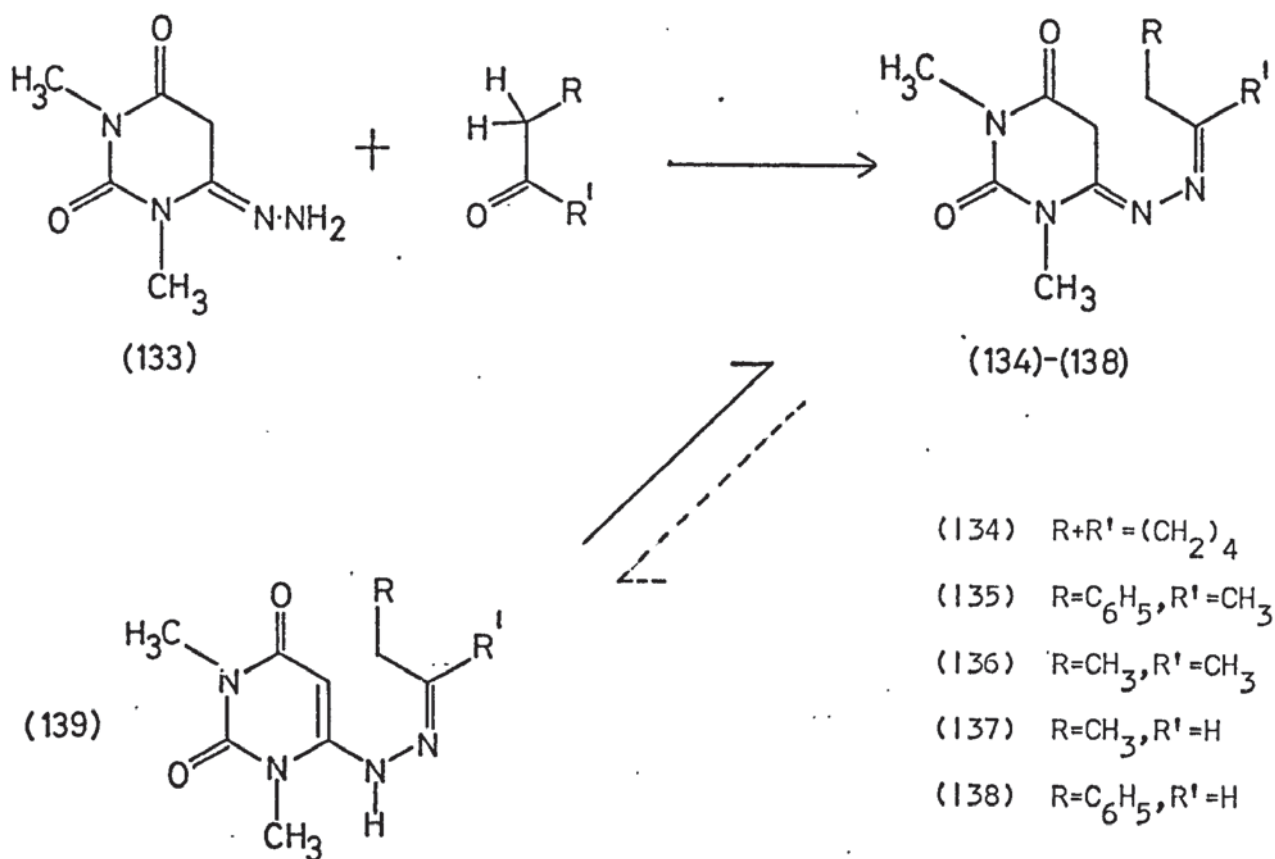
The hydrazinopyrimidine (133) condensed in a similar manner with benzyl methyl ketone and butanone to produce compounds to which the structures (135) and (136) respectively have been assigned. The NMR spectra of these hydrazones all contain the expected singlet (6.05-6.10τ) corresponding to the protons of the active methylene group at position 5 in the imino tautomer.

Propionaldehyde and phenylacetaldehyde also condensed readily with the 4-hydrazino, pyrimidine-2,6-dione (133) to furnish the corresponding hydrazones. NMR spectra of these products were not determined but they have been assigned the structures (137) and (138) respectively on the

basis of the evidence for imino-enamine tautomerism described above. It is of interest to note that the infrared spectra of the hydrazones (134)–(138) all contained a strong absorption between 3150 and 3225cm^{-1} which is assignable to NH stretching. The spectra were obtained from nujol mulls or potassium bromide discs and this would indicate that the hydrazones exist as the amino form (139) in the solid state. In most cases where imino-enamine tautomerism occurs, the imino form predominates[69] but it is suggested that in the solid state, where intermolecular hydrogen bonding effects are more important than in solutions with non-polar solvents, some change to the amino form occurs.

The infrared spectra of the hydrazones (134) – (138) all contain either one or two very broad peaks between 1680 and 1695cm^{-1} which correspond to the absorptions due to the carbonyl groups at positions 2 and 4 in the pyrimidine nucleus. All spectra also contain a strong peak at 1620cm^{-1} which may be attributed to –C=N– stretching.

TABLE I. Hydrazones prepared from 1,3-dimethyl-4-hydrazino-pyrimidin-2,6(1H,3H)-dione
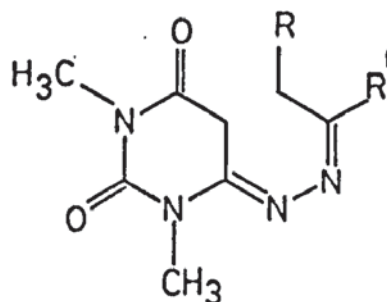
KETONE	REACTION PRODUCT	YIELD
Cyclohexanone	(134)R+R'=(CH$_2$)$_4$	78%
benzyl methyl ketone	(135)R=C$_6$H$_5$,R'=CH$_3$	83%
butanone	(136)R=CH$_3$,R'=CH$_3$	61%
phenylacetaldehyde	(138)R=C$_6$H$_5$,R'=H	69%

The only product isolated was assigned the bipyrimidylhydrazine structure (145), on the basis of infrared and mass spectral evidence.

(140) R+R' = (CH$_2$)$_4$

(141) R=C$_6$H$_5$, R'=CH$_3$

(142) R=CH$_3$, R'=H

(143) R=C$_6$H$_5$, R'=H

(144) R=CH$_3$, R'=CH$_3$

(145)
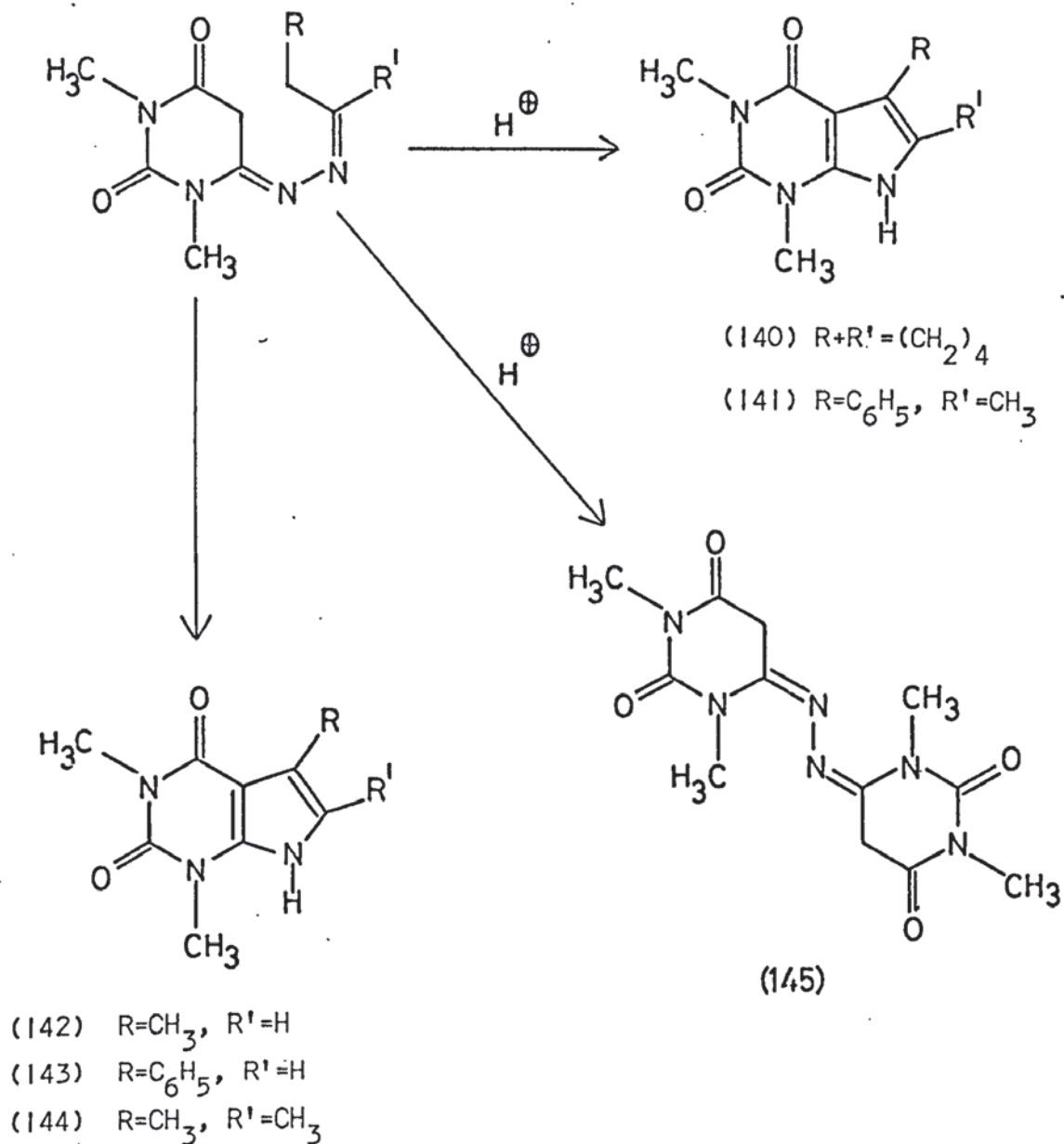
Attempted preparations of the 5-methyl and 5-phenylpyrrolopyrimidines (142) and (143) by this method were unsuccessful. Heating the corresponding hydrazones (137) and (138) respectively, with acetic acid for several hours provided only unchanged starting material.

The failure of some of these dimethylpyrimidine ·diones to cyclise

under acidic conditions provides information about the reaction mechanism.
Evidently the pyrimidine ring nitrogen atoms, although they are not basic,
contribute to deactivation of position 5 in the pyrimidine nucleus.

B.3. Cyclisation of 1,3-dimethyl-2,4-dioxopyrimidin-6 hydrazones by a
non-catalytic thermal technique.

The method used for thermal cyclisation of the pyrimidyl hydrazones,
described in section A, was applied to the dioxopyrimidine diones. Cyclo-
hexanone 1,3-dimethylpyrimidyl-4-hydrazone-2,6(1H,3H)-dione (134) was
heated under reflux in digol for 24 hours. Ammonia was evolved during the
reaction and the pyrimido[4,5-b]indole (140) was isolated (in 80% yield).
Similar treatment of the dimethylpyrimidyl hydrazone (136) prepared from
butanone gave the expected tetramethylpyrrolo[2,3-d]pyrimidine (144) which
could not be prepared by treatment of the same hydrazone with acid (B.2.
above).
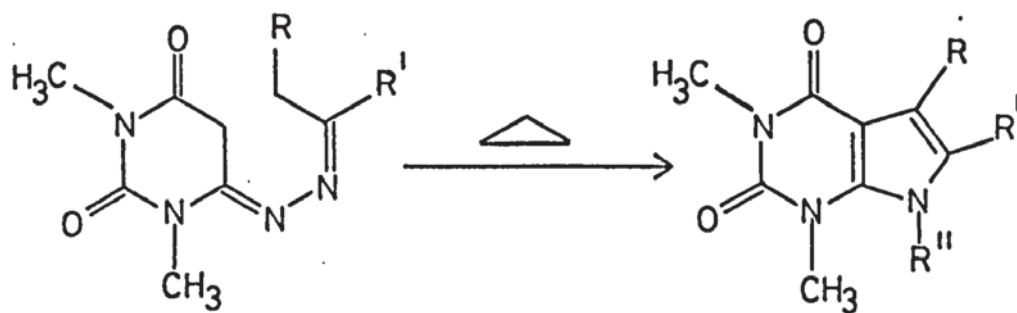
(134) R+R'=(CH$_2$)$_4$

(136) R=CH$_3$,R'=CH$_3$

(140) R+R'=(CH$_2$)$_4$,R''=H

(144) R=CH$_3$,R'=CH$_3$,R''=H

(142) R=CH$_3$,R'=H,R''=H

(143) R=C$_6$H$_5$,R'=H,R''=H

(146) R=H,R'=CH$_3$,R''=H

(147) R=H,R'=C$_6$H$_5$,R''=H

(148) R=CH$_3$,R'=CH$_3$,R''=CH$_3$

A short communication[23] reporting the thermally-induced conversion of 2,4-dioxopyrimidin-6-hydrazones to the corresponding pyrrolo|2,3-d| pyrimidines has been discussed in the introduction (P. 20). This publication appeared after the completion of the experimental work described in this thesis and claims the preparation of the pyrrolo-pyrimidines (140), (142), (143) and (144) (described above), by a non-catalytic thermal technique. These authors[23] also reported the synthesis of the 6-methyl, the 5-methyl-6-phenyl- and the pentamethyl-pyrrolo-pyrimidines (146), (147) and (148) respectively. The cyclised compounds described in this publication[23] were prepared by heating the corresponding hydrazone precursors which were identified by these authors as the enamine tautomers (139). It is also of interest to note that these workers were unable to effect a pyrrole ring-closure by heating these hydrazones with acidic catalysts such as zinc chloride or concentrated sulphuric acid; a surprising observation in the light of the results discussed above (B.2).

C.   THE MECHANISMS INVOLVED IN FISCHER INDOLE CYCLISATIONS

Several observations have been made about the chemical processes involved in the Fischer indole reaction but the mechanism is still not fully understood.[67] In this work both acidic and neutral conditions have been employed to effect cyclisation and two different reaction mechanisms are proposed.

C.1.   Cyclisation in acidic conditions.

The first stage in acid-catalysed cyclisation of aryl hydrazones is formation of an enehydrazine tautomer[67] (150) from the hydrazone (149) and this is aided by the presence of acid catalysts. The enehydrazine intermediate (150) undergoes intramolecular electrophilic cyclisation into

the aromatic nucleus, with concurrent fission of the N-N bond, producing

the diimine (151), which has been isolated in several cases, but usually

cyclises immediately to the indole (152).
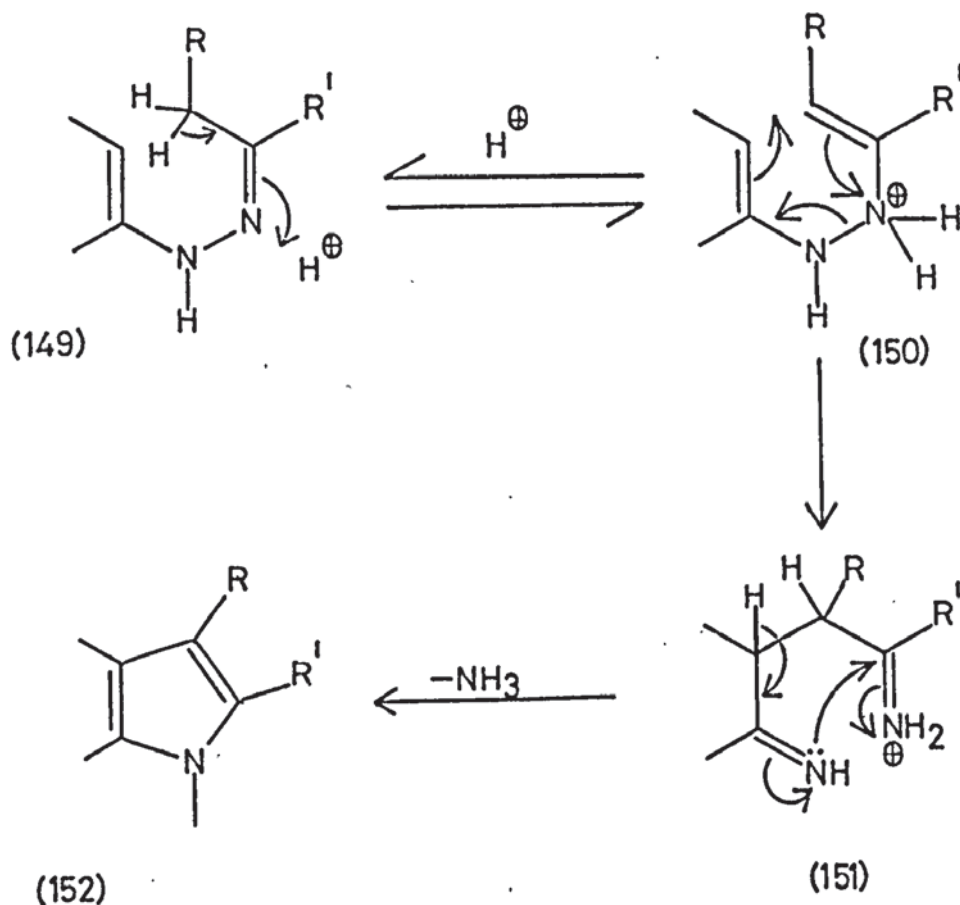
In acidic conditions one pyrimidine heteroatom protonates relatively

easily and the resulting positive charge accommodated by the ring causes

position 5 to become electron defficient and therefore deactivated towards

electrophilic attack. It has been postulated[67] that pyrimidyl hydrazones

do not cyclise to pyrrolopyrimidines, in acidic conditions, for this

reason.

The basic strength of a ring nitrogen atom is considerably reduced

when it becomes part of a cyclic amide structure. N,N'-dimethyluracil

derivatives are fixed in such a cyclic amide form and it is suggested that the 1,3-dimethyl-2,4-dioxopyrimidyl-6-hydrazones (section B.1) undergo cyclisation to the corresponding pyrrolopyrimidine diones because the alkylated heteroatoms do not protonate in the weakly acidic conditions and hence, position 5 is not deactivated towards intramolecular electrophilic cyclisation.

The only obvious alternative to the above mechanism is a nucleophilic attack at the aromatic nucleus in the enehydrazine structure (150). Such a postulate must infer that nitrophenylhydrazones and pyrimidylhydrazones cyclise more readily than phenylhydrazones and this proposal is unsubstantiated.

C.2. Cyclisation by thermal techniques.

Senda and Hirota[23] have recently suggested that thermally induced indolisations of the 2,4-dioxopyrimidyl-6-hydrazones (153) proceed *via* an intramolecular nucleophilic cyclisation (Fig. 8). No evidence for this claim has yet been published and a more feasible alternative may be provided. All thermally-induced Fischer indole reactions may be explained in terms of a concerted mechanism.[67]

The conversion of the enehydrazine (154) to the diimine (155), which involves formation of a C-C σ bond and breaking of the hydrazine N-N σ bond, is analogous to the Cope rearrangement and may be explained in terms of the conservation of orbital symmetry. The enehydrazine structure (154) may be regarded as consisting of two separate similar vinylamine radicals (Fig. 10).

(153)
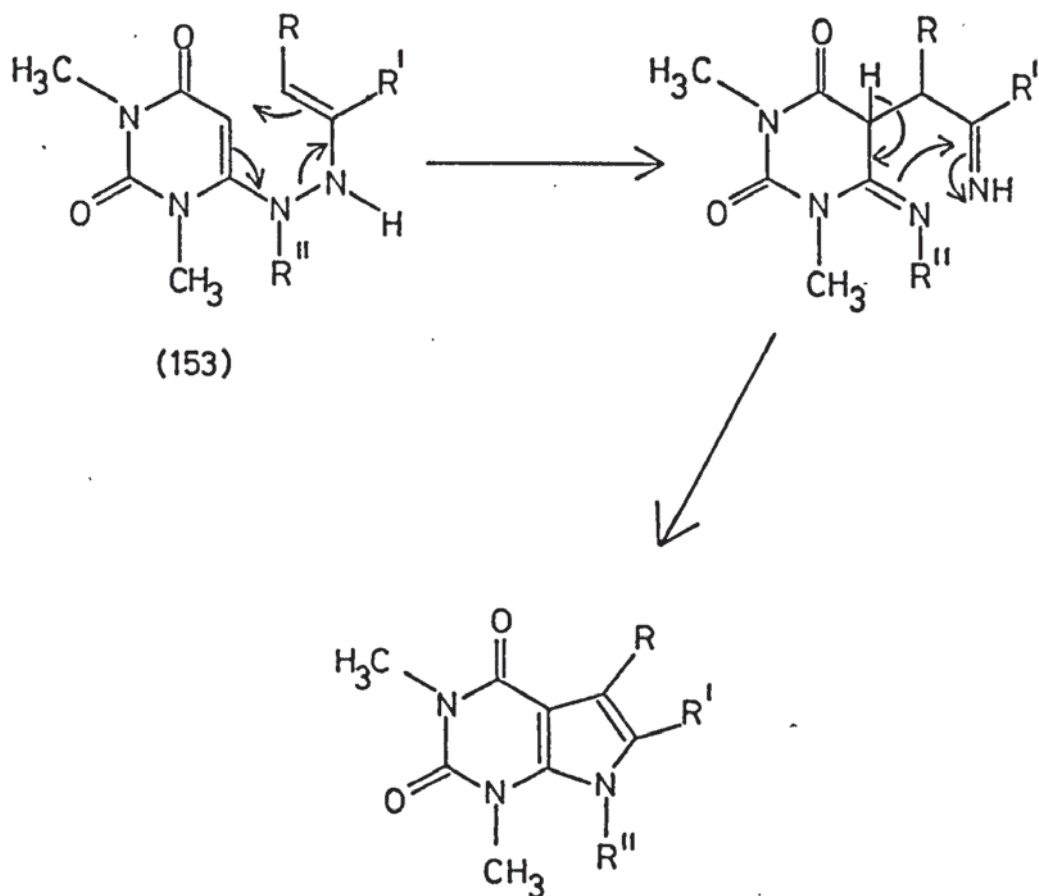
Figure 8

Each radical is drawn so that only its highest occupied orbital is shown. However three different orbitals exist: two are occupied orbitals and one is an antibonding orbital which is unoccupied (Fig. 9). Three electrons are accommodated in these orbitals: two in the lowest occupied orbital and one in the highest occupied orbital.

Lowest occupied orbital (contains 2 electrons)

Highest occupied orbital (contains 1 electron)

Unoccupied orbital

Figure 9.

(154)

(155)

(155)

(155)

$-NH_3$

(156)
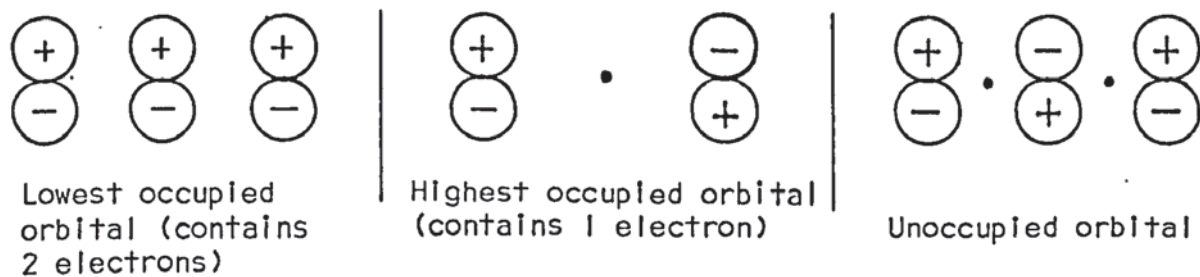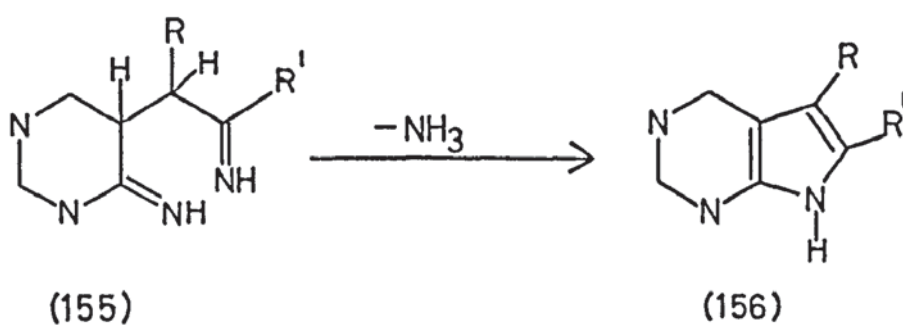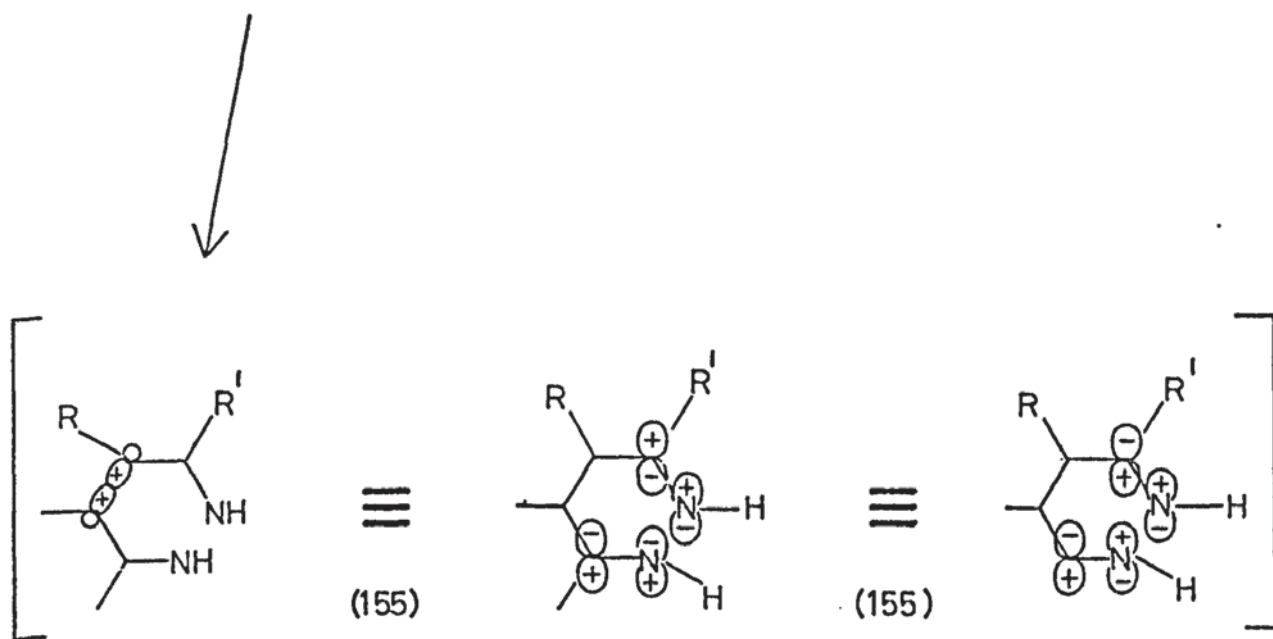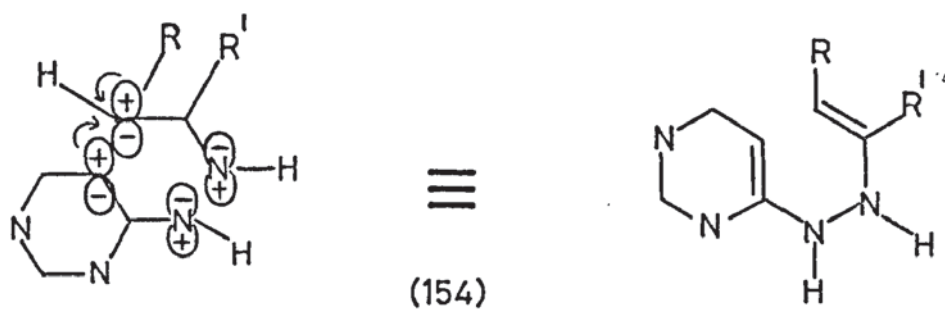
Figure 10

After the conrotatory twist (Fig. 10) has occurred one electron from the highest occupied orbital of each radical participates in formation of the new σ bond. Two electrons now remain in the lowest occupied orbital of each radical and may adopt two forms, the typical C=N bonding and anti-bonding π orbitals (Fig. 10). The resulting structures now represent the diimine intermediate (155) which readily undergoes conversion to the pyrrolo pyrimidine (156).

This mechanism, which may be applied to all thermally induced non - catalytic Fischer indole cyclisations, is confirmed by evidence that steric factors affect the ease with which cyclisations occur. The 2-methylpyrimidyl-6-hydrazone (121), unsubstituted at position 4, cyclises in almost quantitative yield, while 2,4-diphenylpyrimidyl-6-hydrazones (122) and (123) could not be induced to react. It is postulated that steric crowding in the presence of phenyl rings hinders formation of the 6-centred biradical structure (154) and as a result the above |3,3| sigmatropic shift is prevented. Thermal cyclisations of 2,4-dioxopyrimidyl hydrazones proceeded smoothly and it is suggested that the carbonyl group at the pyrimidine 4-position causes insufficient steric crowding to affect the spacial arrangement of the atoms in the 6-centre enehydrazine structure (154). Failure to cyclise the acetophenone 2,4-dimethylpyrimidyl-6-hydrazone (120) may be attributed to difficulty in forming the enehydrazine tautomer; aryl hydrazones of acetone and acetaldehyde have proved difficult to convert to indolised products,[66] presumably for this reason.

II SYNTHESIS OF PYRROLO[2,3-d]PYRIMIDINE FROM 2,3-DISUBSTITUTED PYRROLES.

In the introduction to this thesis two methods for preparing pyrrolo [2,3-d]pyrimidines from 2-amino-3-cyanopyrroles have been described (P.23- 25) and these are the only reported syntheses of the ring system from pyrrole precursors. Several methods for the synthesis of other fused pyrimidine ring systems have been reported in the literature and the aim of the work described in this chapter was to investigate the application of some of these methods to the preparation of pyrrolo[2,3-d]pyrimidines from pyrrole precursors.

A. SYNTHESIS FROM DERIVATIVES OF 2-AMINO-3-CYANO-4-PHENYLPYRROLES.

A.I. Preparation of 2-amino-3-cyano-4-phenylpyrrole and its derivatives.

2-Amino-3-cyano-4-phenylpyrrole (157) was prepared by condensation of malononitrile and ω-aminoacetophenone under basic conditions, according to the method described by Gewald.[72] The compound was unstable when exposed to the atmosphere for several hours but treatment with acetic anhydride furnished the stable 2-acetamidopyrrole (158) in 73% yield.

(158) R=CH₃

(159) R=-C₆H₄-p(NO₂)

(160) R=-NH-C₆H₅
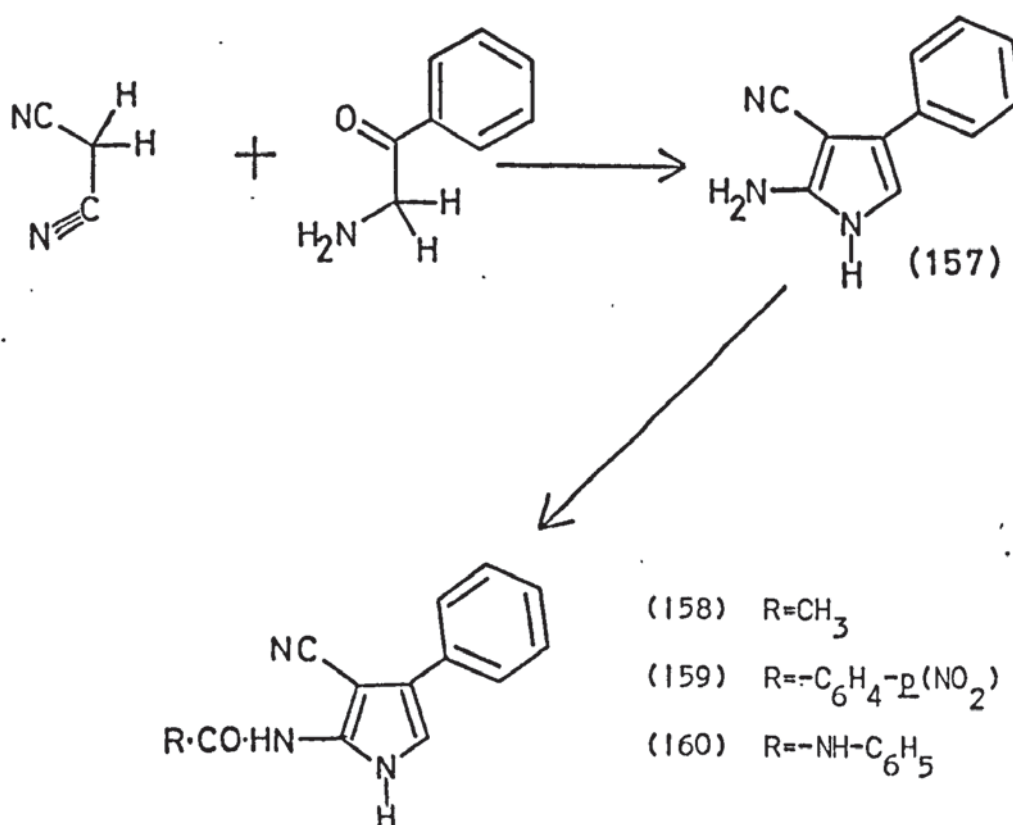
Treatment of this aminonitrile (157) with $\underline{p}$-nitrobenzoyl chloride

gave the benzamidopyrrole (159) in 12% yield and a similar condensation,

of the same pyrrole (157) with phenyl isocyanate in dry benzene, yielded

the pyrolylurea (160).

A.2. <u>Attempted cyclisations of 2-amino-3-cyano-4-phenylpyrrole and its</u>
<u>derivatives to pyrrolo[2,3-d]pyrimidines.</u>

The conversion of $\underline{o}$-aminonitriles to fused 4-aminopyrimidines by

heating the former compounds with formamide has been reviewed[71] and the

reactions of 2-amino-3,4-dicyanopyrroles with formamidine acetate have

been described above, (P.25). Treatment of the 2-amino-3-cyanopyrrole

(157) with formamide produced a very impure solid which could neither be

induced to crystallise nor separated from a large amount of polymeric

material. However, the mass spectrum of the crude solid was not in-

consistent with that expected from the pyrrolopyrimidine dione (167) but

no evidence for the presence of the desired aminopyrrolopyrimidine (163)

was obtained.

A general procedure for the conversion of $\underline{o}$-aminonitriles to condensed

2,4-diaminopyrimidines is treatment of the former compounds with guanidine,

(liberated in situ from the hydrochloride salt), and these reactions have

been reported in detail.[71] Treatment of the aminonitrile (157) in this

manner produced only a dark tarry solid, (which could not be purified

sufficiently to obtain spectroscopic data), and none of the expected

diaminopyrrolo[2,3-d]pyrimidine (164).

(157) R=H
(158) R=COCH$_3$
(160) R=CO.NH.C$_6$H$_5$

(161)

(162)

(163) R=H
(164) R=NH$_2$
(165) R=CH$_3$

(166)

(167) R=H, R'=H, x=O
(168) R=CH$_3$, R'=H, x=O
(169) R=OH, R'=C$_6$H$_5$, x=NH
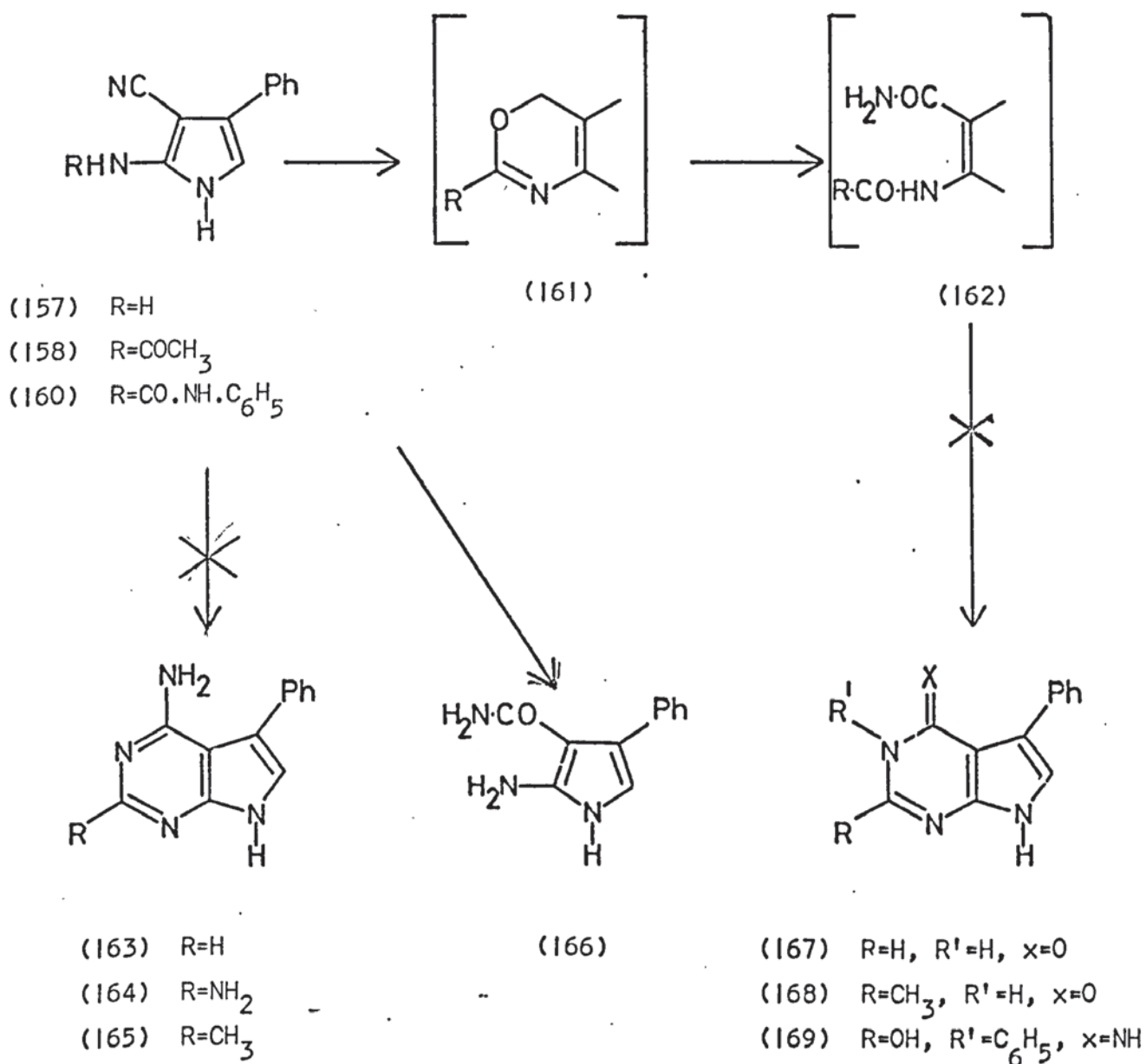
o-Acylaminonitriles have also been used as precursors of fused pyrimidinones and the method of choice for this conversion is their treatment with dry hydrogen chloride gas in absolute ethanol[71]. The reaction is reputed to proceed _via_ the cyclisation of the diamides (162), which are produced _in situ_ from fused oxazine structures (161). Treatment of 2-acetamido-3-cyano-4-phenylpyrrole (158) in this way afforded the 2-amino-3-amidopyrrole (166), despite the use of "superdry" ethanol,

acid-dried hydrogen chloride and the normal laboratory methods for excluding moisture from such preparations. The NMR spectrum of the product contained no absorption assignable to a methyl group and was thus incompatible with the expected pyrrolopyrimidinone structure (168). The presence of a phenyl group was indicated by a broad multiplet at $\tau 2.38$ while a singlet at $\tau 5.03$, (corresponding to two protons), was attributed to protonation of the pyrrole ring, at the unsubstituted 2-position, by the solvent, (trifluoroacetic acid).

Treatment of the same acylamino compound (158) with dry ethanolic ammonia, in a sealed vessel at 100°, gave the unchanged starting material and none of the expected 2-methyl-4-aminopyrrolo[2,3-d]pyrimidine (165).

The ureas formed from the reaction of o-aminonitriles, with iso-cyanates and isothiocyanates have been studied[72,73] and are useful intermediates in the syntheses of fused pyrimidines. However, treatment of the pyrrolylurea (160) with sodium methoxide or pyridine yielded only the starting material and similarly no cyclised product was obtained when the same compound (160) was heated under reflux with glacial acetic acid. An attempt to prepare the 4-iminopyrrolopyrimidine (169), by heating the urea with phosphoryl chloride, also failed and resulted only in decomposition of the reaction mixture.

The difficulty experienced in preparing pyrrolo[2,3-d]pyrimidines from 2-amino-3-cyanopyrrole derivatives may be due to the increased basicity of the amino group and the decreased activity of the cyano group, in these compounds, compared with the same groups in o-aminobenzonitrile. In ba conditions, it is suggested that the lack of reactivity of the nitrile group could be due to the delocalisation of charge in the pyrolyl anion (170). Attempted cyclisations of the above pyrroles (157), (158) and

(160), failed in basic conditions.

(170)
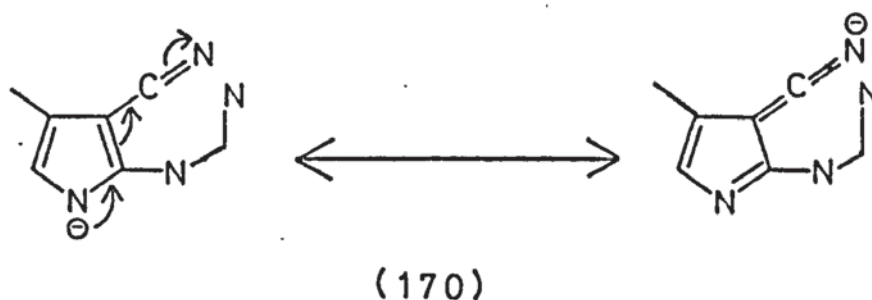
Attempts to synthesise pyrrolopyrimidines in acidic conditions caused decomposition of the reaction mixtures and the general instability of pyrrole aminonitrile derivatives indicated the advisability of synthesising more stable pyrrole precursors.

B.   SYNTHESES FROM PYRROLE o-AMINOESTER DERIVATIVES

B.I.   Preparation of 2-aminopyrrole-3,4-dicarboxylates

Treatment of dimethylpyrrole-3,4-dicarboxylate[75] (171) with a mixture of sulphuric and nitric acids for 24 hours gave the dinitropyrrole (173); and the mononitropyrrole (174) was obtained when the reaction time was shortened to half an. hour. The NMR and infrared spectra of this latter compound were consistent with the structure (174): singlets at τ6.15 and 6.00 indicated the presence of two methoxycarbonyl groups and the singlet at τ2.75 was attributed to the proton at position 5 of the pyrrole ring. The infrared spectrum showed a typical NH absorption at 3225cm^{-1}, two strong C=O absorptions at 1735 and 1705cm^{-1}, and peaks at 1520 and 1360cm^{-1} which were consistent with the presence of a nitro group in the molecule. Addition of this nitropyrrole (174) and methyl iodide to an ethanolic solution of sodium methoxide gave the N-methylpyrrole (175).

Diethyl pyrrole-3,4-dicarboxylate (172) was prepared from diethyl 2-formylsuccinate[76] by a slight modification of the method described by Cornfield and Jones.[77] The NMR and infrared spectra of this pyrrole were consistent with the reported structure,[77] and treatment of this diester (172) with a mixture of sulphuric and nitric acids gave the mono-nitropyrrole (176) with infrared and NMR spectra consistent with the proposed structure.

Treatment of the three 2-nitropyrrole-3,4-diesters (174) (175) and (176) with hydrogen, in the presence of a palladium charcoal catalyst, gave the corresponding 2-aminopyrroles (178) (179) and (180) respectively and the spectroscopic properties of these products were consistent with the proposed structures.

(171) R=CH$_3$

(172) R=C$_2$H$_5$

(173) R=CH$_3$, R'=H, x=NO$_2$

(174) R=CH$_3$, R'=H, x=H

(175) R=CH$_3$, R'=CH$_3$, x=H

(176) R=C$_2$H$_5$, R'=H, x=H

(177) R=H, R'=H, x=H

(178) R=CH$_3$, R'=H, R''=H.

(179) R=CH$_3$, R'=CH$_3$, R''=H.

(180) R=C$_2$H$_5$, R'=H, R''=H.

(181) R=C$_2$H$_5$, R'=H, R''=COCH$_3$

(182) R=H, R'=H, R''=H.
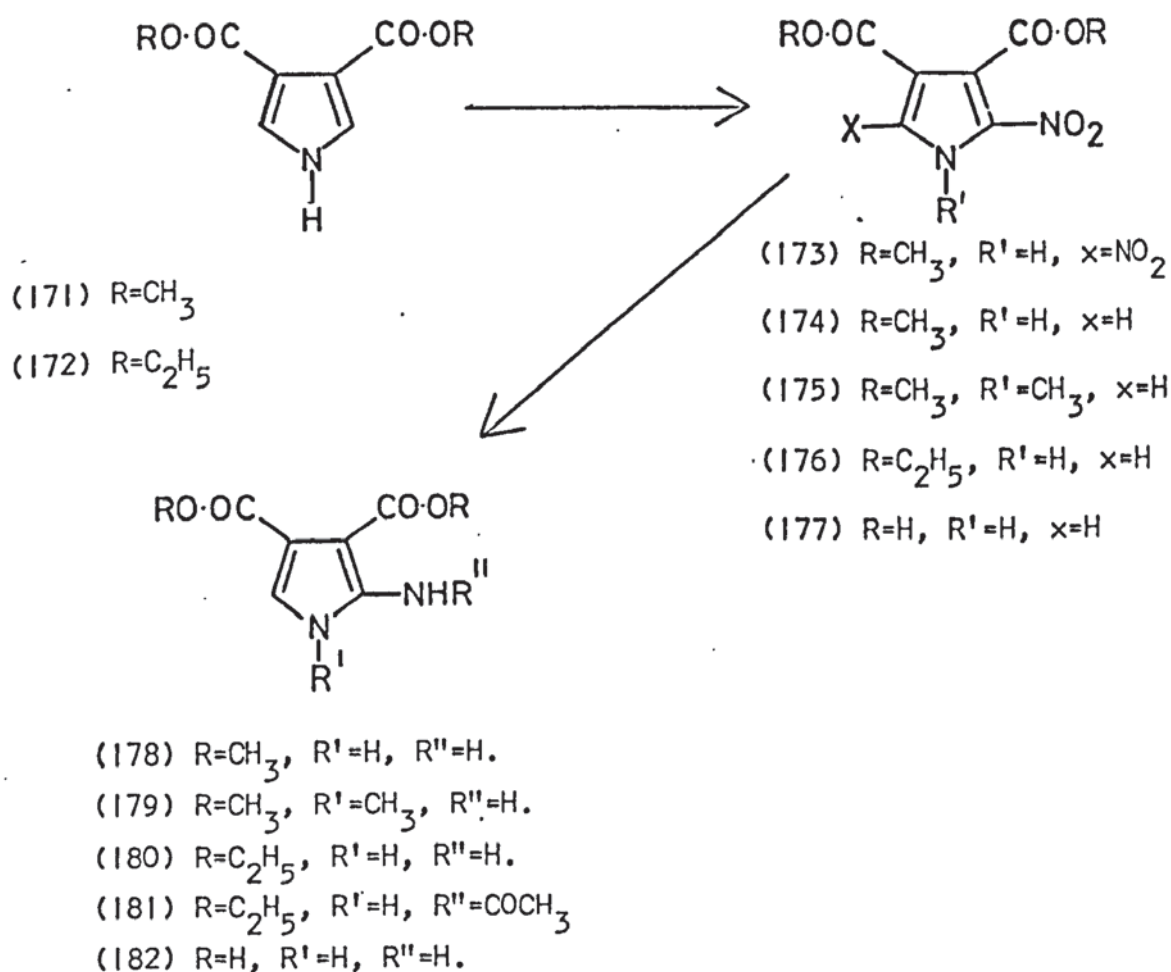
The diethylpyrrole aminoester (180) was readily acetylated when treated with acetyl chloride in the presence of base and the 2-acetyl-aminopyrrole-3,4-dicarboxylate (181) was produced in 70% yield. The infrared spectrum of this compound contained C=O absorptions at 1730, 1680 and 1670cm^{-1} and the NMR spectrum contained a singlet at τ7.75 which was assigned to the acetamido methyl group.

It was intended to prepare the 2-aminopyrrole-3,4-di~~ester~~ *acid* (182) and to convert it to pyrrolo[2,3-d]pyrimidines *via* an oxazine intermediate, (see next section C). However the low yield of 2-nitropyrrole-3,4-di~~carboxylate~~ *-ic acid* (177), obtained by alkaline hydrolysis of the dimethylpyrrole diester (174), was not considered conducive to the success of such a multistage synthesis and no attempt was made to reduce this nitro compound to the 2-aminopyrrole (182).

B.2. Cyclisations of 2-aminopyrrole-3-esters.

Several purine derivatives have been prepared[79-82] from the ureides formed by the reaction of imidazole amino esters with isocyanates and isothiocyanates. The cyclisations of the ureides and thioureides were accomplished by treatment with alkali (Fig. 11).

$R = CH_3, C_6H_5$

$X = O, S$
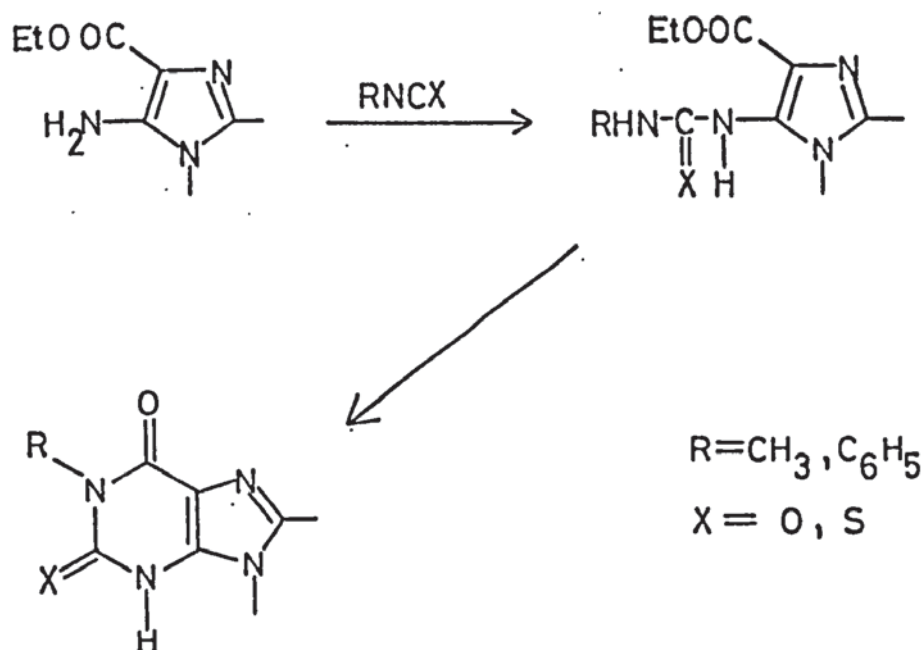
Figure 11

More recently[83] pteridines were synthesised by the reaction of methyl 2-aminopyrazine-3-carboxylates with isocyanates and isothiocyanates. Pyridine was used as a solvent and basic catalyst and the pteridine (183), for example, was obtained in 96% yield without the isolation of any ureide intermediate.

(183)
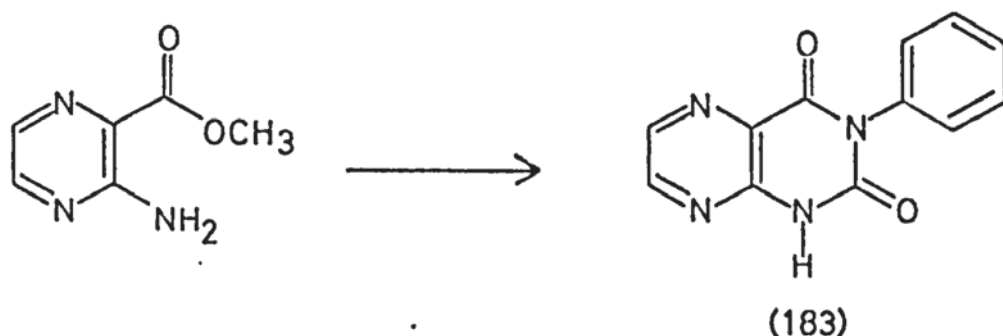
It was intended to utilise reactions of this type in a pyrrolo[2,3-d] pyrimidine synthesis and accordingly, dimethyl 2-aminopyrrole-3,4-dicarboxy-late (178) was dissolved in anhydrous pyridine and heated under reflux with freshly distilled, dry phenyl isocyanate. Removal of solvents and a small amount of diphenylurea gave a product with spectroscopic and analytical data which were inconsistent with the expected pyrrolopyrimidine structure (184). The infrared spectrum contained absorptions at 1750, 1720, 1715 and 1680 cm^{-1} which were attributed to C=O groups, and there was a strong NH absorption at 3225 cm^{-1}. The NMR spectrum contained a singlet at τ6.20 (6H) which was attributed to the ester methyl groups. The singlet at τ2.43 indicated the presence of an unsubstituted position in the pyrrole ring and the singlet at τ2.50 was assigned to a phenyl group. Analytical data and accurate mass measurements of the molecular ion peak, in the mass spectrum, indicated that the molecular formula was $C_{14}H_{11}N_3O_4$ and, on the basis of this and the above evidence the compound was deduced to be the pyrrolo[1,2-a]-s-triazine (187).

(178) R=H
(188) R=CH₃

(184) R=H
(189) R=CH₃

(185a)
(185b)

— Ph NCO / Pyridine —

(186a)
(186b)

(187)
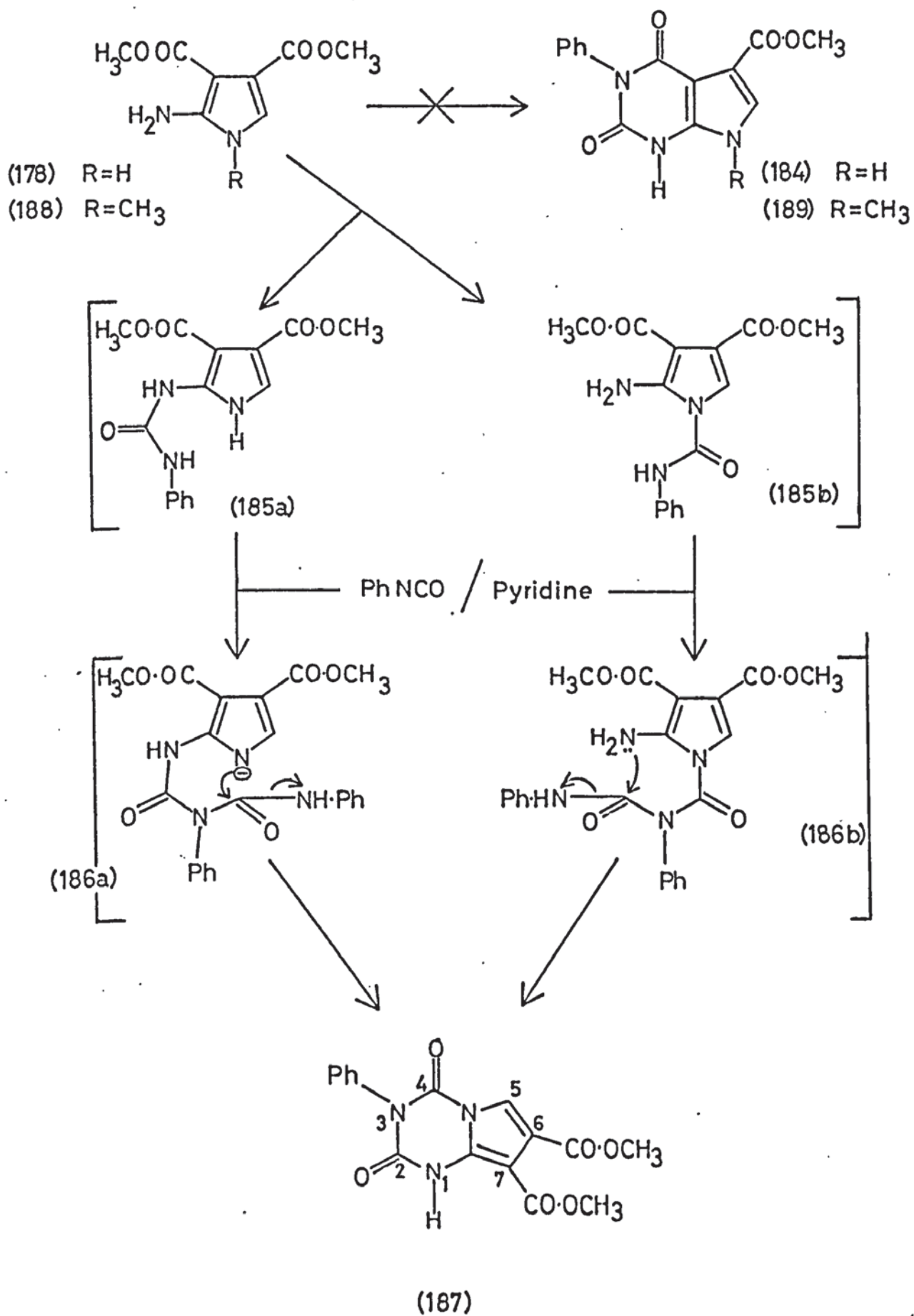
·This is the first synthesis of a pyrrolo[1,2-a]-[1,3,5]triazine which has been reported but further work has been done[84] to investigate the versatility of this preparative method. It is postulated that the reaction proceeds *via* either the ureide (185a) or the isomeric pyrrol-1-yl urea (185b). These intermediates may then be converted by reaction with a molecule·of phenylisocyanate,to the isomeric disubstituted biurets (186a) and (186b) respectively, either of which might be expected to undergo ring closure to the pyrrolotriazine (187), with elimination of aniline. This reaction would appear to be enhanced by the formation of pyrrolyl anions which not only aid the formation of the biurets (186a) and (186b), but also deactivate the 3-ester carbonyl function towards nucleophilic attack, thus preventing formation of the pyrrolo[2,3-d]pyrimidine (184). Therefore it was decided to attempt to cyclise a pyrrole aminoester which had no labile proton on the ring nitrogen. However, treatment of dimethyl 2-amino-1-methylpyrrole-3,4-dicarboxylate (188) with phenyl isocyanate in pyridine gave only an intractable tar and none of the expected 7-methyl-pyrrolo[2,3-d]pyrimidine (189). The failure of this reaction may be attributed to the instability of the starting material, which decomposed even at room temperature.

Several o-acylaminobenzaldehydes and phenyl ketones (190) react readily with ammonia to give high yields of the corresponding quinazolines (192)[85], and the synthesis has been extended to the preparation of 4-quinazolones (193)[85] from N-acylanthranilic acid (191) and ammonia. It was proposed to employ a similar reaction in a synthesis of a pyrrolo[2,3-d] pyrimidin-4-one (194) from diethyl 2-acetamidopyrrole-3,4-dicarboxylate (181).
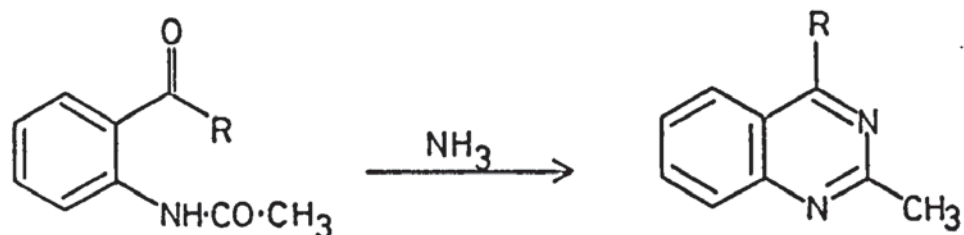
(190) R = alkyl, aryl, H

(191) R = OH

(192) R = alkyl, aryl

(193) R = OH

(181)

(194)

conc.
aqueous
NH₃

(195)

(180)
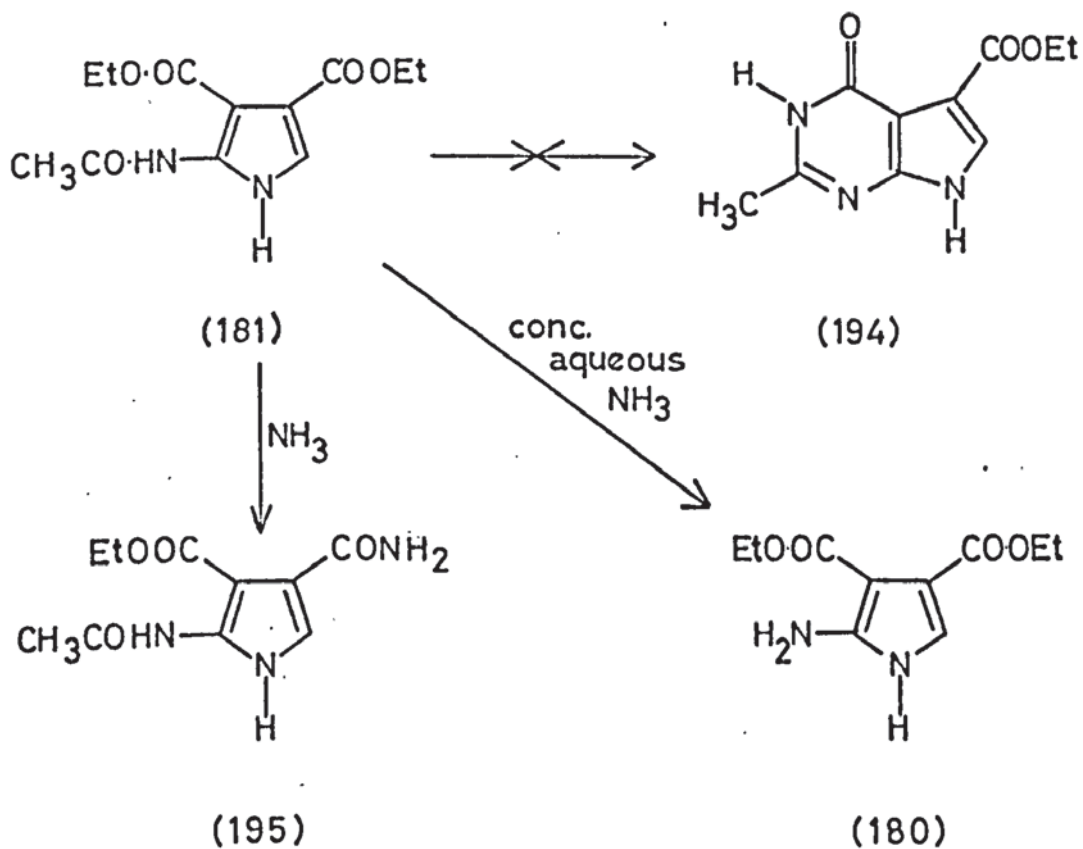
The acylaminoester (181), when treated with concentrated aqueous

ammonia, was hydrolysed to the free aminopyrrole (180). A solution of

the same pyrrole (181) in ethanolic ammonia, stirred at room temperature

for 30 days, furnished a compound to which the diamide structure (195)

was assigned. The NMR spectrum indicated that the compound possessed
only one ethyl group; the singlet at $\tau 7.50$ was attributed to the acetamido
methyl group. The infrared spectrum of the product contained strong
absorptions at 1680, 1660 and 1650cm^{-1}, (which were attributed to C=O
stretching), and absorptions at 3400 and 3200cm^{-1} which were typical of
NH stretching. In the 2-acetamidopyrrole diester starting material (181)
the marked difference in the positions, (1680 and 1730cm^{-1}), at which the
carbonyl functions at positions 3 and 4 respectively absorb, may be
attributed to a delocalisation of the lone pair electrons of the acetamido
nitrogen atom, which exerts a mesomeric effect on the 3-carbonyl function
but leaves the group at position 4 unaffected. The absence of this
higher absorption in the infrared spectrum of the product (195) indicates
that ammonolysis has occurred at position 4 and not at the deactivated
ester carbonyl group at position 3 to give the 3-amidopyrrole (195a).

(181)   (195a)
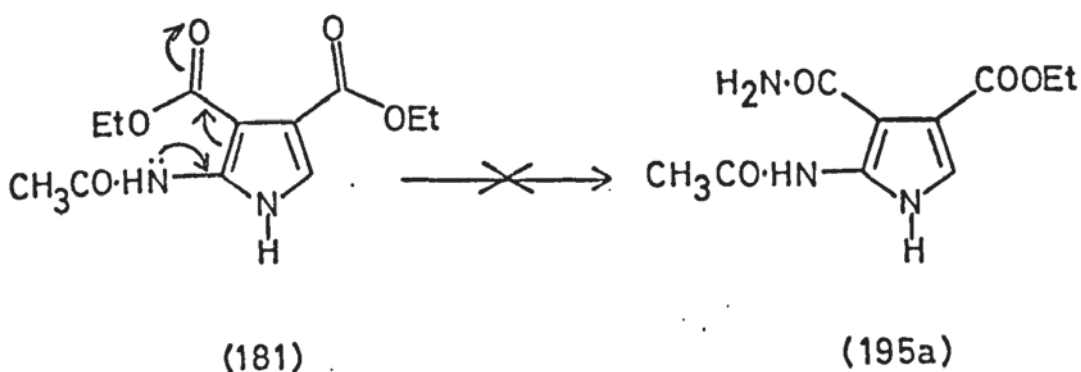
Treatment of the acetamidopyrrole diester (181) with ethanolic
ammonia at 100° resulted in hydrolysis of the acetamido group and the
aminoester (180) was produced.

The failure to obtain the pyrrolo[2,3-d]pyrimidines (194) from this
ammonolysis reaction is undoubtedly due to the lack of reactivity of the
carbonyl function at position 3 of the pyrrole ring. It is suggested

that ammonia, a strong base, removes the acidic ring proton and the resulting negative charge accommodated by the pyrrole nucleus, deactivates the 3 carbethoxy group towards nucleophilic attack.

Ammonolysis reactions of this type proceed to the pyrrolopyrimidines *via* the conversion of an o-acylaminoester to an o-acylaminoamide and, since the attempts described had failed, it was decided to prepare pyrrole o-aminoamides directly and endeavour to cyclise them to the desired pyrrolo[2,3-d]pyrimidines.

C. SYNTHESES FROM PYRROLE o-AMINOAMIDES

Many fused pyrimidine ring systems have been synthesised from o-aminoamides and their derivatives. Some of these precursors have contained 5-membered rings with an sp^2-hybridised -NH- group and, for example, the o-aminoamides and o-aminothioamides of imidazole (196) and pyrazole (197) have been used in the preparation of purines (198)[86] and pyrazolopyrimidines (199)[87] , respectively.

(196) Y=N, Z=CH
(197) Y=CH, Z=N

(198) Y=N, Z=CH
(199) Y=CH, Z=N
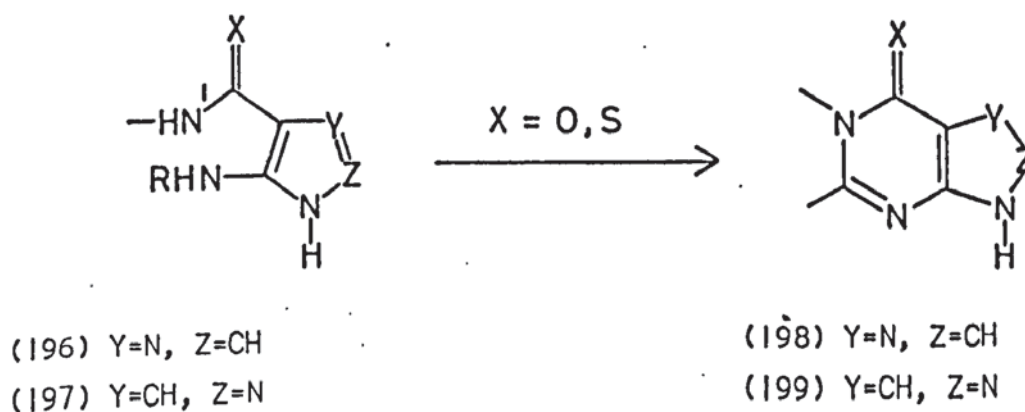
C.I. Preparation of 3-amido-2-aminopyrroles.

Attempts were made to convert the readily available 2-amino-3-cyanopyrrole (157) and its acyl derivatives (158) and (159) to the

corresponding 3-amidopyrroles (166), (200) and (201) respectively.

(157) R=H

(158) R=COOCH$_3$

(159) R=CO.C$_6$H$_4$-p-NO$_2$
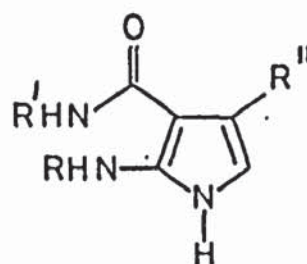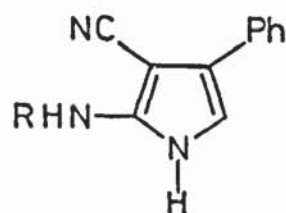
(166) R=H, R'=H, R''=C$_6$H$_5$

(200) R=COCH$_3$, R'=H, R''=C$_6$H$_5$

(201) R=CO-C$_6$H$_4$pNO$_2$, R'=H, R''=C$_6$H$_5$

(202) R=H, R'=H, R''=-COOCH$_3$

(203) R=H, R'=C$_6$H$_5$, R''=-COOCH$_3$

(204) R=H, R'=CH$_2$C$_6$H$_5$, R''=-COOCH$_3$

Treatment of the acetamidonitrile (158) with concentrated sulphuric

acid, at room temperature, gave the desired 2-acetamido-3-amino-4-phenyl-

pyrrole in 80% yield, with an infrared spectrum consistent with this

structure. Similar treatment of the aminonitrile (157) with concentrated

sulphuric acid gave only intractable tars and, when the compound reacted

with a mixture of sodium hydroxide and hydrogen peroxide a solid product

was obtained which could not be sufficiently purified for identification

purposes. The required product, 2-amino-4-phenylpyrrole-3-carboxamide

was (166) eventually obtained by simultaneous deacetylation and hydration

of the acylaminonitrile (158), as described earlier in this thesis,

(section A.2).

An attempt to prepare the amide (201) by treatment of the p-nitro-benzoylaminopyrrole (159) with alkaline hydrogen peroxide gave only a dark intractable tarry solid and p-nitrobenzoic acid.

Since attempts to react the 2-aminopyrrole-3,4-diesters, described in section IIB, with ammonia had afforded neither the required pyrrolo-pyrimidines nor a useful intermediate o-aminoamide, it was proposed to treat dimethyl 2-nitropyrrole-3,4-dicarboxylate (174) with ammonia and some amines to synthesise the o-nitroamides (205) (206) (207), which might be capable of reduction to the desired o-aminoamides (202) (203) and (204) respectively. However, only unchanged starting material was obtained when ammonia or aniline was heated with the diester (174) and treatment with benzylamine at 100° gave a dark intractable tar.

(174)

(205) R=H
(206) R=C_6H_5
(207) R=$CH_2 \cdot C_6H_5$
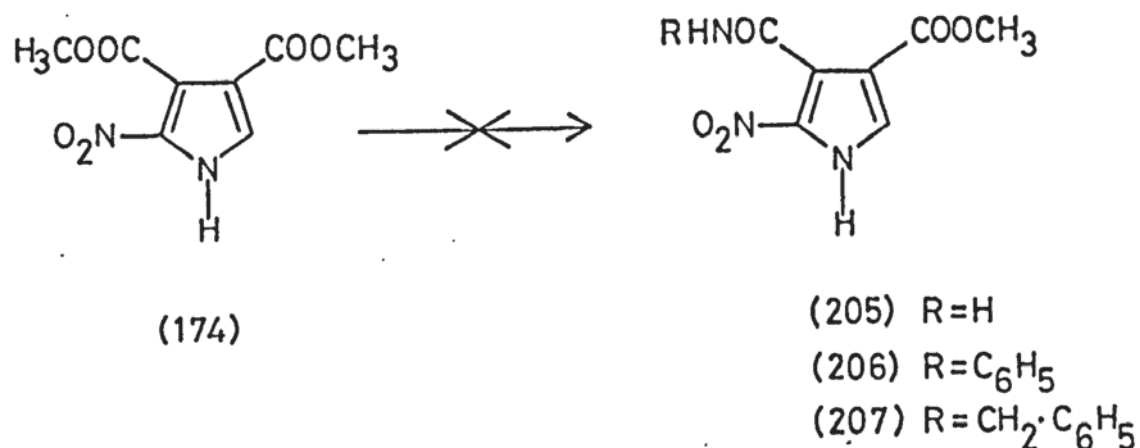
Treatment of the 2-aminopyrrole-3-carboxamide (200) with phosphoryl chloride produced only starting material and the reaction mixture appeared to be decomposing. A solution of the same pyrrole (200) in digol gave the 3-cyanopyrrole (158) when heated under reflux in a nitrogen atmosphere. An attempt to convert the diamide (200) to the pyrrolo-pyrimidine (208), by treatment with dilute hydrochloric acid, effected

deacetylation of the former compound to the 2-aminopyrrole amide (166).
When the same pyrrole precursor (200) was heated with the monoethyl
ester of polyphosphoric acid (PPET) only unchanged starting material was
obtained.
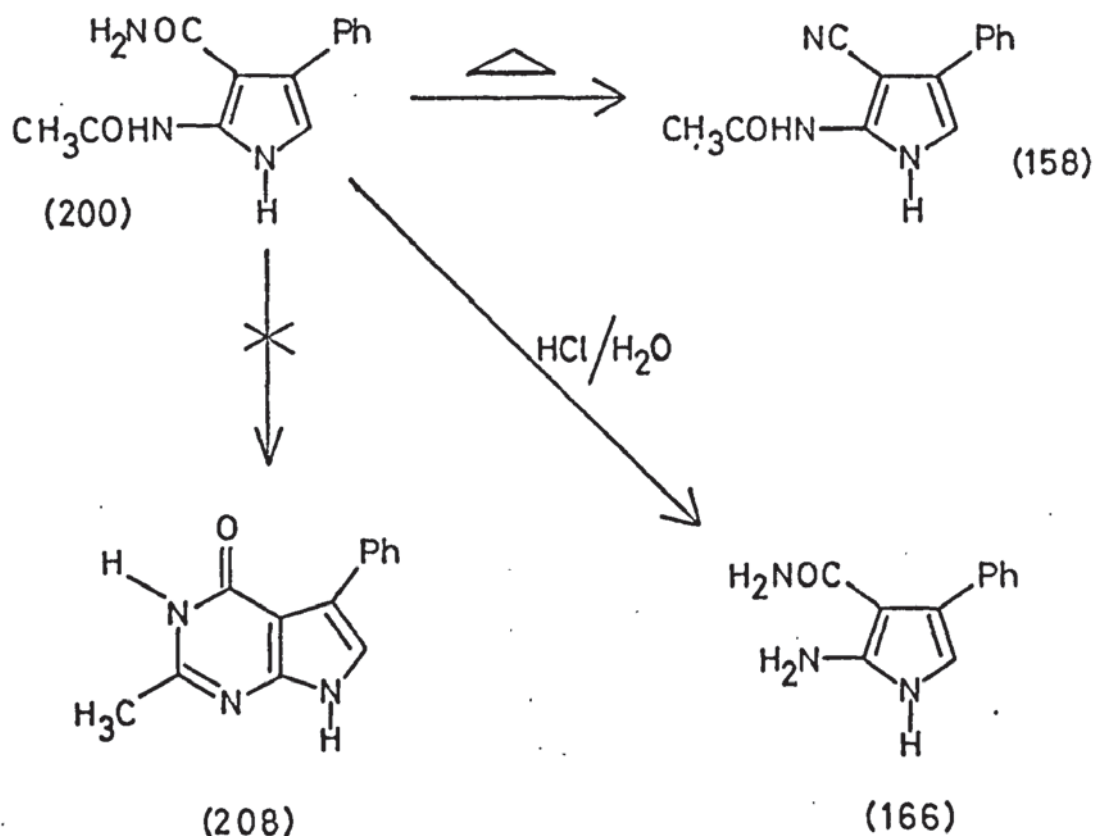
From the lack of success associated with the conversion of the
diamide (200) to a pyrrolopyrimidine it was deduced that routes of this
type were unlikely to prove valuable. Therefore no attempt was made to
cyclise the pyrrole o-aminoamide (166); instead it was decided to
investigate the preparation of intermediates in the synthesis of the
pyrrolo[2,3-d]pyrimidine ring system which could be produced from pyrrole
derivatives but which were likely to be more stable and versatile
precursors.

III   PYRROLO[2,3-d]PYRIMIDINES FROM 4-IMINO-5-PHENYLPYRROLO[2,3-d]-[3,1] THIAZIN-2(1H)-THIONE

In his review of the quinazolines, Williamson[85] described the conversion of anthranilic acids to benzooxazinones which reacted exothermally with ammonia and most amines to give high yields of the corresponding quinazolones (209). The reaction appears to provide a general synthetic route to fused pyrimidines and was extended by later workers to the preparation of various pyrido pyrimidine ring systems (210) (211) and (212).[88] In all the cases the reaction proceeds *via* cyclisation of the amide (213).[88]

(209) X=Y=Z=CH

(210) X=N, Y=CH, Z=CH

(211). X=CH, Y=N, Z=CH

(212) X=CH, Y=CH, Z=N
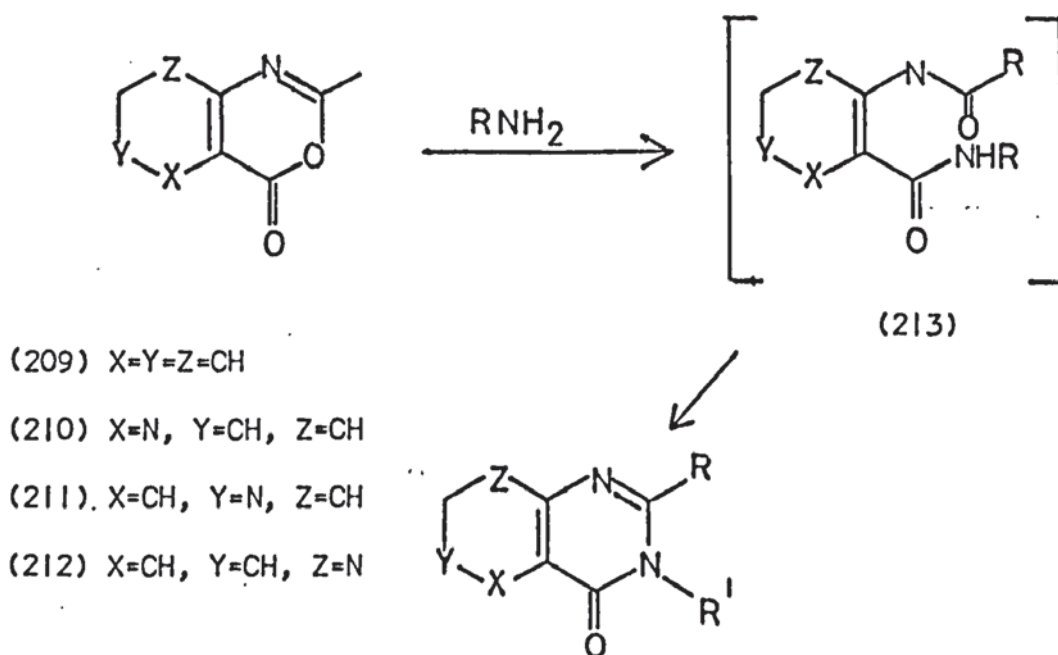
The rearrangement of m-thiazines (214) to pyrimidines is also a known reaction[70,89] and several fused pyrimidine triones (215) have been prepared[90,91,92] from the corresponding fused m-thiazines (214). It was proposed to prepare a pyrrolothiazine from a readily available pyrrole precursor, and convert it, by conventional methods, to the corresponding

pyrrolopyrimidines.
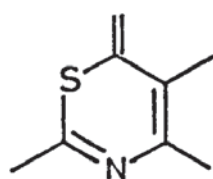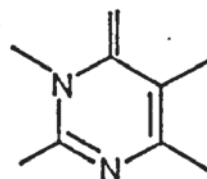
(214)   (215)

A. THE SYNTHESIS OF A PYRROLO[2,3-d]-m-THIAZINE AND ITS CONVERSION TO A PYRROLOPYRIMIDINE DITHIONE.

When 2-amino-3-cyano-4-phenylpyrrole (157) was treated with carbon disulphide in pyridine a yellow crystalline product was obtained which was deduced to be 4-imino-5-phenylpyrrolo[2,3-d]-[3,1]thiazin-2(1H)-thione (219), on the basis of spectroscopic and analytical data. The latter compound is the first example of the previously unreported pyrrolo[2,3-d]-m-thiazine ring system and its structure was confirmed by the presence of a strong absorption at 3400cm^{-1} in the infrared spectrum. Examination of the mass spectrum indicated that loss of CS_2 from the molecular ion is a facile fragmentation pathway and this also tends to corroborrate the thiazine thione structure (219).
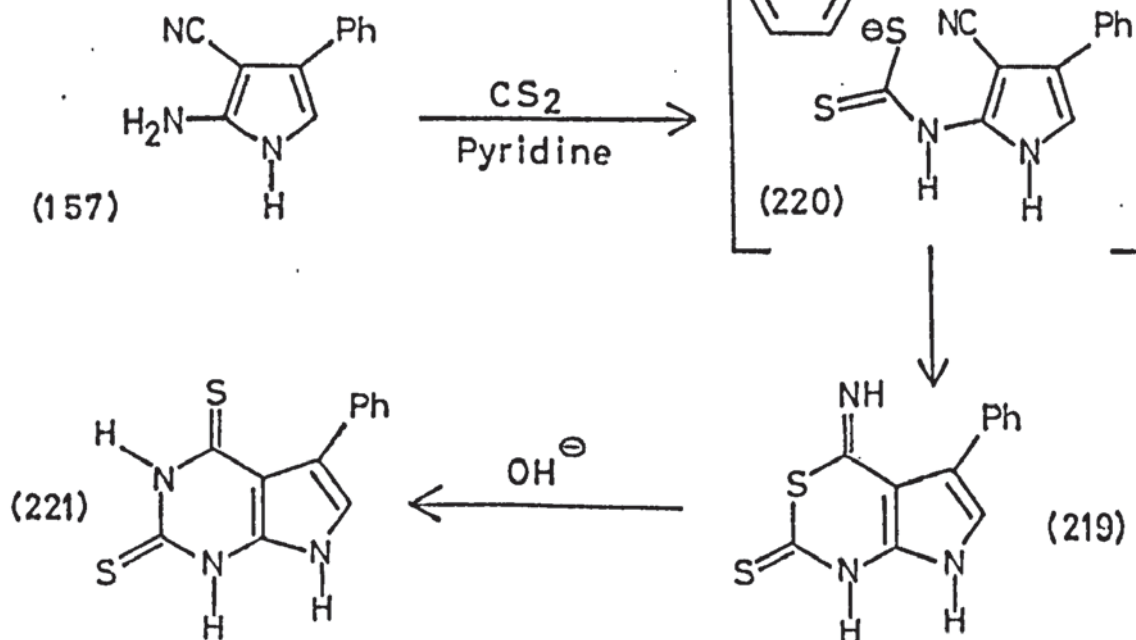
In a previously reported conversion of o-aminonitriles to similar fused pyrimidine thiones, Taylor et al[92] suggested that reactions of this type proceed via an intermediate dithiocarbamate salt and, although no such intermediate was isolated in the reaction described above, it seems probable that the pyrrolyl dithiocarbamic acid derivative (220) is an intermediate, in the formation of the pyrrolothiazine (219).

Treatment of this fused thiazine (219) with dilute alkali on a steam bath, gave a compound with the same elemental analysis data as the thiazine but with radically different infrared and mass spectra. The infrared spectrum contained no absorption compatible with the imino NH function and the mass spectrum provided no evidence of CS_2 loss from the molecular ion; the major fragmentation pathways involved either loss of SH or loss of CNS from the parent molecular ion. The same product could be obtained by increasing the reaction time of the 2-amino-3-cyanopyrrole (157), with carbon disulphide and pyridine, to 20 hours. On the basis of this evidence, and in the light of Taylor's work[92] on similar conversions, the product was assigned the pyrrolopyrimidine dithione structure (221), which is isomeric with the pyrrolo[2,3-d]thiazine (219). The mechanism of this isomerisation is discussed in section B below.

B. SOME PROPERTIES OF 4-IMINO-5-PHENYLPYRROLO[2,3-d]-
[3,1]THIAZIN-2(1H)-THIONE.

The conversion of the pyrrolo[2,3-d]-m-thiazine (219) to the pyrrolo-pyrimidine dithione (221), by treatment with aqueous alkali, has been described in section A. However, when methyliodide was added to a solution of the same fused thiazine (219) in aqueous alkali at room temperature, a product was isolated, in 91% yield, which had a typical C=N stretching absorption in its infrared spectrum. The NMR spectrum indicated the

presence of two equivalent methyl groups and the structure of the compound was deduced to be 3-cyano-2-dimethylthiomethyleneamino-4-phenyl-pyrrole (224).

Treatment of this product with refluxing aqueous alkali produced the pyrrole aminonitrile (157) in 70% yield, after several hours, thus providing further confirmation for the dimethylthiomethyleneamino structure (224).

The behaviour of the pyrrolothiazine (219) in aqueous alkali is illustrated in figure 12. It is suggested that the thiazine (219) is rapidly and irreversibly converted to the pyrrolopyrimidine dithione (221), by attack of hydroxyl ion at position 2, when the solution is heated. The thiazine is in equilibrium at room temperature with the pyrrolyl dithiocarbamic acid (222), (which is formed by abstraction of the proton from the imino function by hydroxyl ion), and the introduction of an alkyl halide may be considered to disrupt this equilibrium by promoting formation of the dithiocarbamic ester (223) which is subsequently converted irreversibly to the dimethylthiomethyleneamino compound (224). Such a mechanism is supported by the production of a small amount of the pyrrole aminonitrile (157), (presumably formed by hydrolysis of the dithiocarbamate (222), in the conversion of the pyrrolothiazine (219) to the fused pyrimidine dithione(221).

A water-soluble compound, to which the hydroiodide structure (225) was assigned, is produced when the pyrrolothiazine (219) is treated with methyl iodide in methanol. The NMR spectrum of this product contains a peak at $\tau 7.13$ (3H) which was assigned to the S-CH$_3$ group at position 2. The structure was also confirmed by the mass spectrum which displayed a molecular ion corresponding to a loss of HI from the proposed molecular

(219)

(157)

(221)

(222)

(223)

(224)
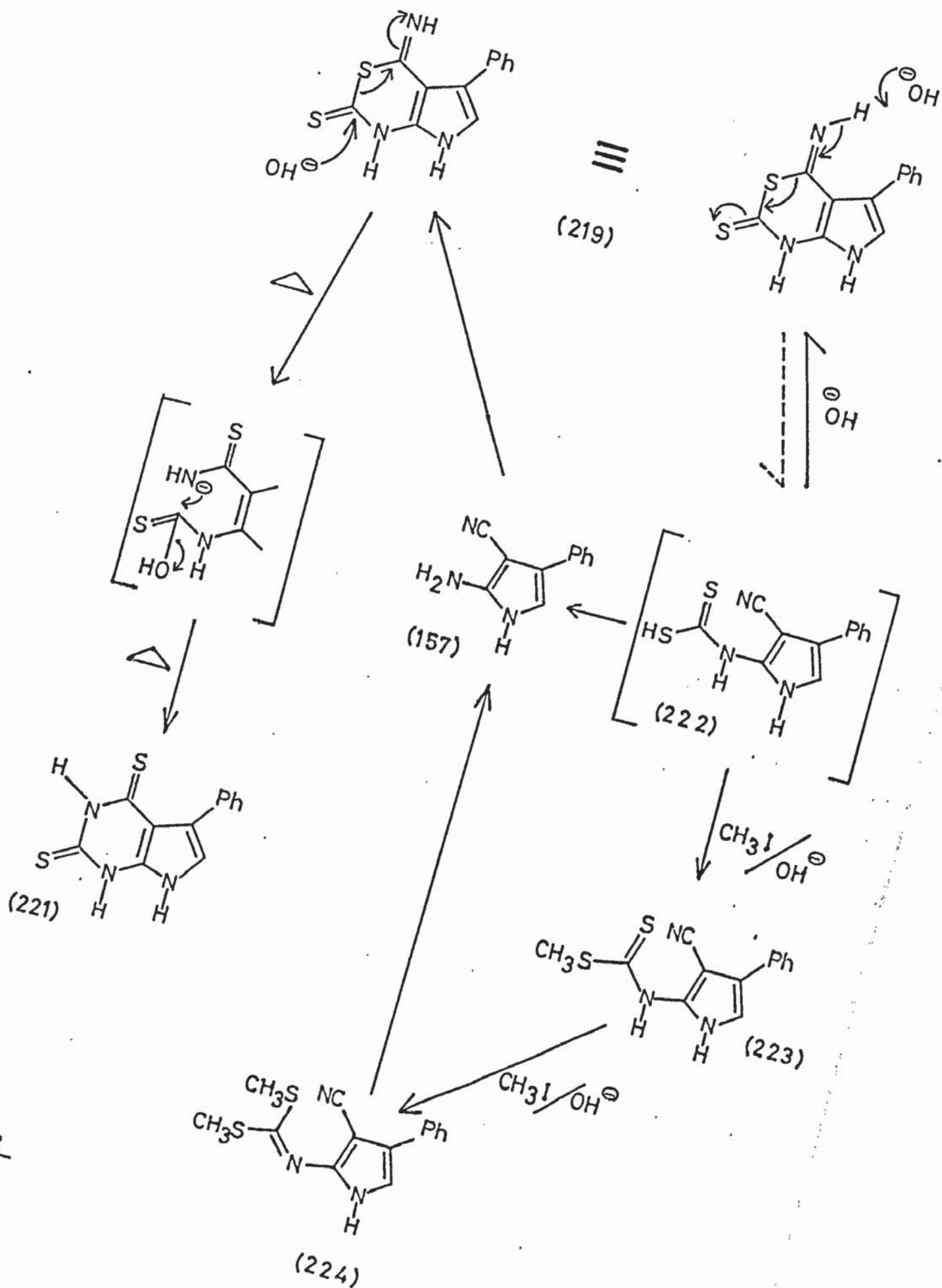
formula.
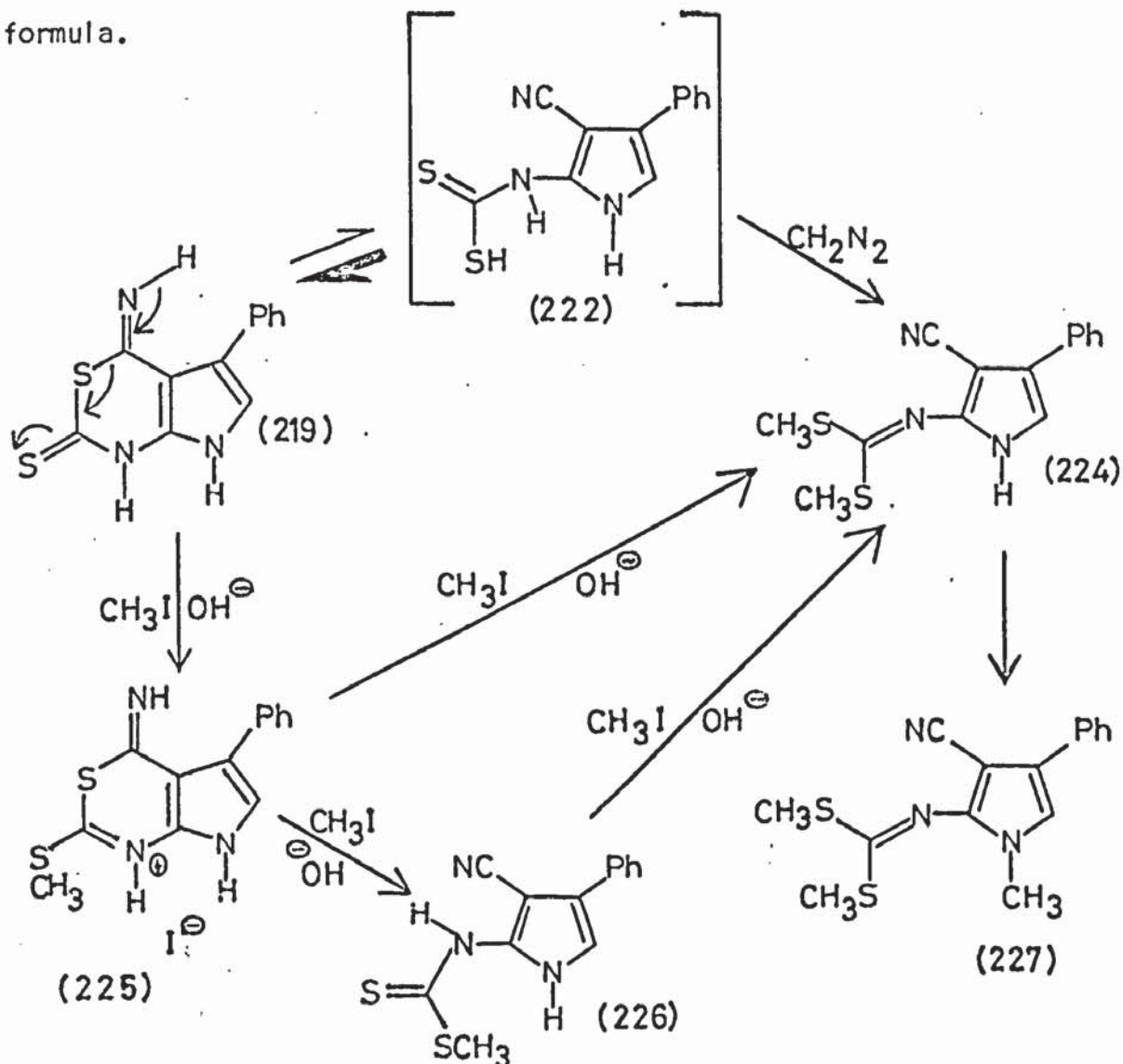
Treatment of this fused thiazine hydroiodide with aqueous alkali
gave an unstable compound which, on the basis of infrared and mass spectral
evidence, was tentatively assigned the thioamide structure (226). The
reaction of this compound with methyl iodide in aqueous alkali gave the
dimethylthiomethyleneaminopyrrole (224). This provides some confirmation
for the suggested structure (226) but further work must be done to
corroborate this assignment.

A mixture of the dimethylthiopyrroles (224) and (227) was produced
when the pyrrolothiazine (219) was treated with the neutral methylating
agent diazomethane for several hours at room temperature. Prolongation

of the reaction resulted In an Increased yield of the N-methylpyrrole

(227) and a decrease In the amount of the I-unsubstituted product (224).

Such a reaction must Involve Initial deprotonation of the Imino function

In the pyrrolothiazine (219) followed by methylation of the resulting

dithiocarbamic acid derivative (222) by diazomethane, (see also Fig.12, P.75)

C. THE SYNTHESIS OF 3-SUBSTITUTED-4-IMINOPYRROLO[2,3-d]PYRIMIDIN-2(1H)-
   THIONES.

It was discovered that 3-substituted-4-iminopyrrolo[2,3-d]pyrimidines (2:

- (239) could be synthesised by the reaction of 4-imino-5-phenylpyrrolo

[2,3-d]-[3,1]thiazin-2(1H)-thione (219) with primary amines. Aqueous

solutions of the appropriate amines furnished varying amounts of the

pyrrolopyrimidine dione (221) In addition to the 3-substituted pyrrolo-

pyrimidines but the use of anhydrous amines In sealed reaction vessels

afforded only the desired 3-substituted compounds (232) - (239), usually

In high yield, (Table 2). This reaction provides a new and versatile

synthetic route to several previously unreported pyrrolopyrimidines.

The Infrared spectra of these products (232) - (239) were consistent

with the proposed structures and all contained a sharp absorption at ca.

3400cm^{-1} which was attributed to the Imino NH group at position 4. The

mass spectra of the compounds all Indicated that loss of NCS from the

molecular Ion Is a major fragmentation pathway and were all consistent

with the proposed 3-substituted 4-Imino structures (232) - (239).

Further evidence for these Imino structures was obtained and Is described

later In this thesis (P.88).

(219)   RNH₂ →   (232)-(239)   +   (221)

RNH₂/H₂O
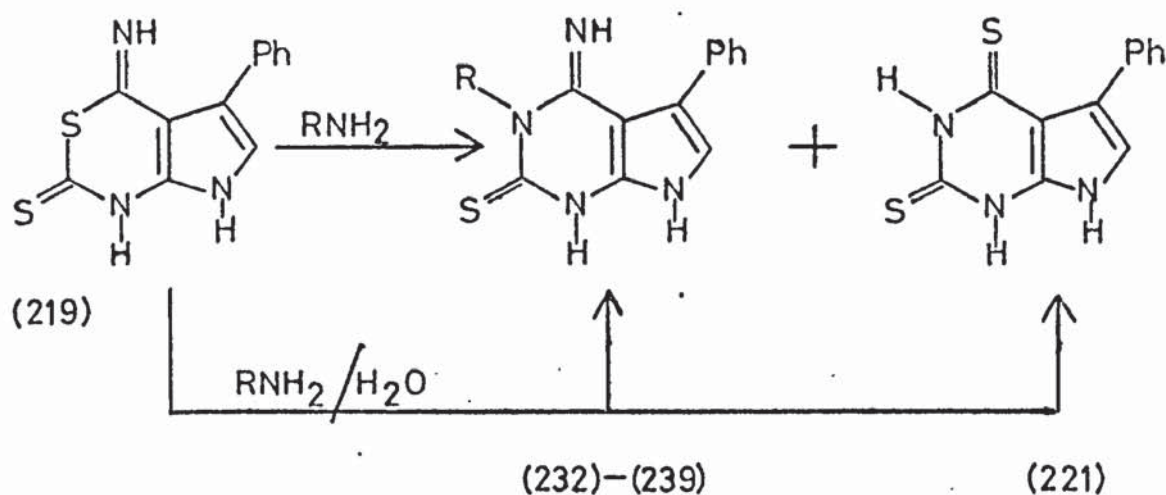
R	Reaction Conditions	% Yield of 3-substituted pyrrolo[2,3-d]pyrimidine	% yield of (221)
(232) n-C₄H₉	A 25 mins. B 20 mins.	87 91	2 .-
(233) C₂H₅	A 20 mins. B 20 mins.	53 86	30 -
(234) CH₃	A 15 mins.	54	40
(235) H	B 2 hours	43	10
(236) NH₂	A 5 mins.	91	2
(237) -CH₂-C₆H₅	B 4,5 hours	94	-
(238) C₆H₅	C 3 hours	33	-
(239) p-CH₃.C₆H₄	B 5 hours	45	-

A. (219) and an aqueous solution of the amine heated under reflux

B. (219) and the anhydrous amine heated in a closed vessel at 100°.

C. (219) and the amine heated under reflux in a nitrogen atmosphere.

Table 2.

Treatment of the fused thiazine (219) with isopropylamine (anhydrous or aqueous) gave none of the expected pyrrolopyrimidine (240). The only product isolated (apart from a small amount of the dithione (221) in aqueous conditions), was a compound to which the thiourea structure (241) was assigned. The analytical data was consistent with the expected 3-isopropylpyrrolo[2,3-d]pyrimidine structure (240) but the infrared spectrum contained a large absorption at 2225 cm^{-1}, typical of C≡N stretching and the mass spectrum contained a small molecular ion which appeared to decompose by loss of C_3H_7NH.

(219)   (240)

(241)
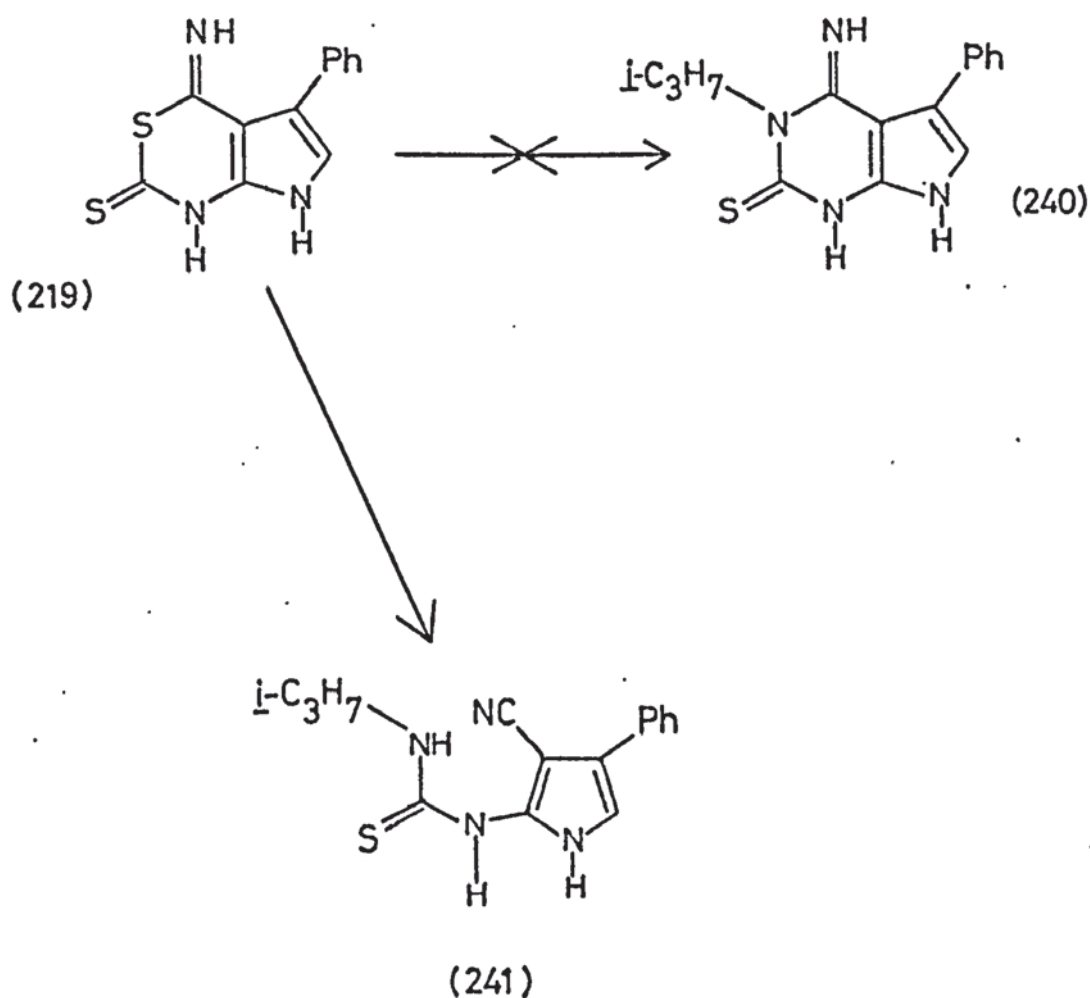
It is probable that the pyrrolyl thiourea (241) is formed from some intermediate in the synthesis of the pyrrolopyrimidine (240) which is unable to cyclise to the latter compound because of the steric crowding caused by the bulky isopropyl group. If this is so the isolation of this compound provides information about the mechanism by which primary amines react with the pyrrolo[2,3-d]-m-thiazine (219).

The conversion of the 4-iminopyrrolothiazine (219) to the pyrrolo-pyrimidine (221) with aqueous alkali may be explained only by attack of the hydroxyl anion at position 2, (section B above). There seems to be no reason why the two nucelophiles RNH_2 and OH^θ should attack the thiazine ring at two different positions; and therefore, it may be postulated that the reaction of the thiazine with amines proceeds by attack of the nucelophile at the electron-defficient thione carbon at postion 2 of the pyrrolothiazine ring system (219), to give the intermediate o-thioamido thioureide (242). Such a compound may cyclise to the pyrrolopyrimidines (232)-(239) or, in the presence of the sterically hindering isopropyl group, may be converted to the 3-cyanopyrrolylthiourea (241). It is possible that cyanopyrrole derivatives of this type (241), (if they are normally formed when less bulky substituents are present), also cyclise to the pyrrolopyrimidines (232)-(239), but this is unlikely because the o-cyanoureide (160) failed to cyclise to the pyrrolopyrimidine (169) (see section II, p.58). The author's suggestion that nucelophilic attack on the thiazine (219) occurs at position 2 is apparently contradicted however by Wagner's and Rothe's suggestion[90] that the fused thiazine dithiones (228) are converted to the quinazoline dithiones (230) *via* the amides (229), since such a postulate necessarily infers that initial amine attack occurs at position 4 in the fused thiazine (228). This is supported by

the isolation of some o-aminothioamides (231) from their reactions, presumably formed by decomposition of the substituted dithiocarbamic acids (229).

$$R = H, CH_3 \qquad R' = H, CH_3, CH(CH_3)_2, C_6H_5, p\text{-}Cl\text{-}C_6H_4.$$
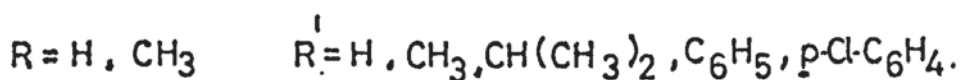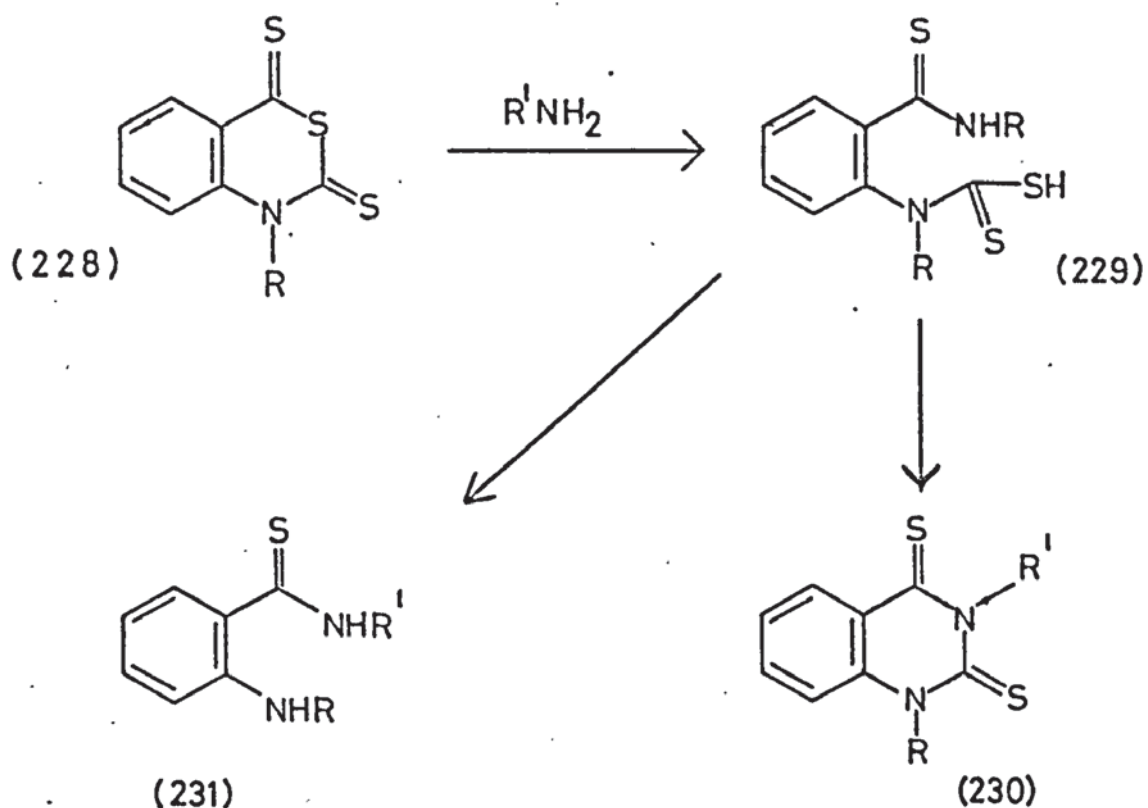
Nevertheless it may not be possible to apply this hypothesis to the pyrrolothiazine system (219) under discussion, which has an imino group at position 4 as opposed to the thione function in the benzothiazine (228). Extrapolation to the pyrrole system also involves the postulated formation of the intermediate amidine (243), which must rearrange to the 3-cyano compound (241) *via* the dithiocarbamic acid (244) but no decomposition product from (244), e.g. the aminonitrile (157), was isolated. Further- more formation of the thioamide (231) does not prove that the quinazoline thiones (230) are formed *via* the phenyldithiocarbamic acid (229): attack may

occur at both position 2, with formation of the pyrrolopyrimidine, and at
position 4, to form the o-aminothioamide (231).

(232)-(239) X=S

(169) X=O, R=Ph

(219)

(219)

(242)

(157)

(243)

(241) R=i·C$_3$H$_7$, X=S

(160) R=Ph, X=O

RNH$_2$

(244)

RNH$_2$

(219)
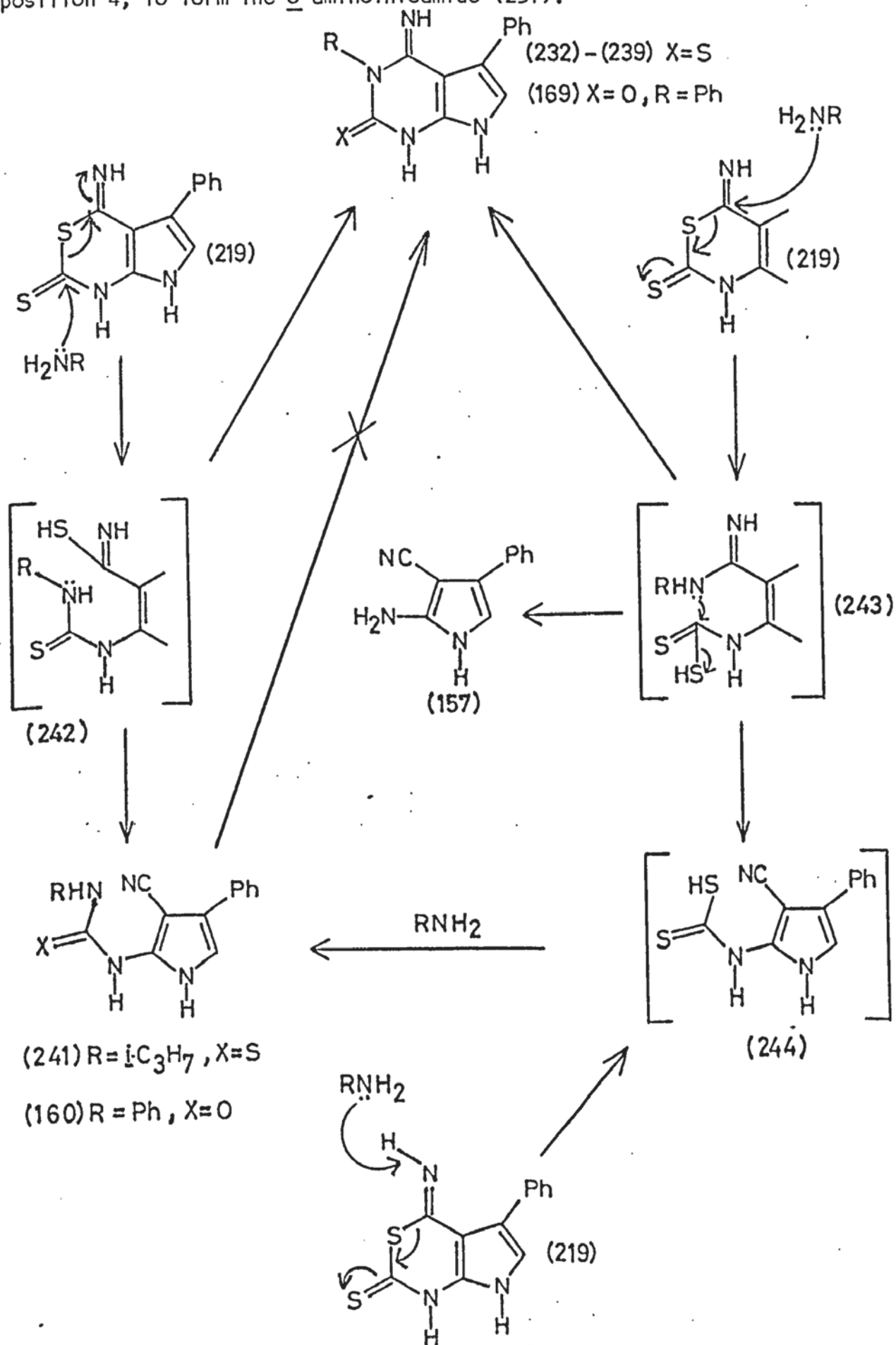
The 3-cyanopyrrolylthiourea (241) may also be theoretically prepared by abstraction of the proton from the imino function of the thiazine (219). However, because neither the resulting dithiocarbamic acid (244) nor pyrroles like (241) and (160) seem capable of conversion to the pyrrolopyrimidines (232)-(239) such a mechanism seems unlikely.

The reaction of a 4-iminopyrrolo[2,3-d]-m-thiazine with primary amines seems to provide a general route to 3-substituted pyrrolo[2,3-d] pyrimidines. The synthesis of other pyrrole amino nitriles has been described in the literature[71,72,97-102] and the use of these and both aliphatic and aromatic amines in the reaction should prove the versatility of this new synthetic route.

D. THE SYNTHESIS OF 3-SUBSTITUTED-4-IMINO-2-METHYLTHIOPYRROLO[2,3-d] PYRIMIDINES.

Attempted cyclisation of 3-cyanopyrroles possessing a variety of amino functions at position 2 have been discussed earlier in this thesis (p. 56-58), but no conversion of such a compound to a pyrrolopyrimidine has been effected. The presence of methylthio groups, (which are known to have good leaving group properties), in the 3-cyanopyrrole (224) led to an attempt to convert this compound to amidine-like intermediates, (246) and (247) which, it was felt, might cyclise to the required pyrrolo [2,3-d]pyrimidines (248)-(251).

Accordingly, 3-cyano-2-dimethylthiomethyleneamino-4-phenylpyrrole (224) was heated with various aliphatic amines and ammonia, either in sealed reaction vessels or under reflux in aqueous solution. Products were obtained which were assigned the pyrrolopyrimidine structures (248)-(251), (Table 3), on the basis of analytical data and spectroscopic evidence, and this reaction provides a new synthesis of fused pyrimidine ring systems.
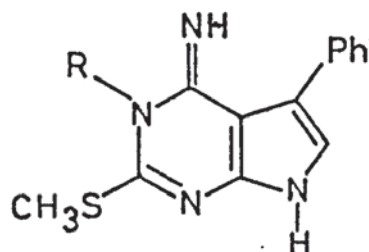
	R	REACTION CONDITIONS	% YIELD OF PYRROLOPYRIMIDINE
(248)	H	A, $100°$ 1 hour	93
(249)	CH_3	B, 16 hours	88
(250)	C_2H_5	A, $100°$ 3 hours	99
(251)	$n\text{-}C_4H_9$	A, $110°$ 12 hours	92
A. (224) and the amine heated in a closed vessel			
B. (224) and an aqueous solution of the amine heated under reflux.			

Table 3.

The compounds (249) (250) and (251) all had a strong absorption at 3350 cm^{-1} in their infrared spectra which was assigned to the imino NH at position 4. Strong absorptions at 3500, 3350 and 3200 cm^{-1} in the infrared spectrum of the only 3-unsubstituted compound (248), which are presumably due to NH_2 stretching modes indicate that the compound exists as the 4-amino tautomeric form. The spectra of all four pyrrolopyrimidines contained an absorption at 3100 cm^{-1} which was assigned to the NH function in the pyrrole moiety. The NMR spectrum of the n-butyl compound (251) contained a singlet (3H) at τ 7.47 which was assigned to the 2-methylthio substituent and also, multiplets at $\tau 8.0 - 9.2$ (7H) and $\tau 5.8$ (2H)

corresponding to the n-butyl group. The singlet at τ3.38 was attributed

to the proton at the unsubstituted position 6 and the symmetrical

multiplet at τ2.60 was typical of the absorption expected from a 5-phenyl

substituent. Further evidence in support of the 4-imino-2-methylthio-

pyrrolopyrimidine structures (248)-(251) was provided by an alternative

synthesis of some of these compounds (Section E below).
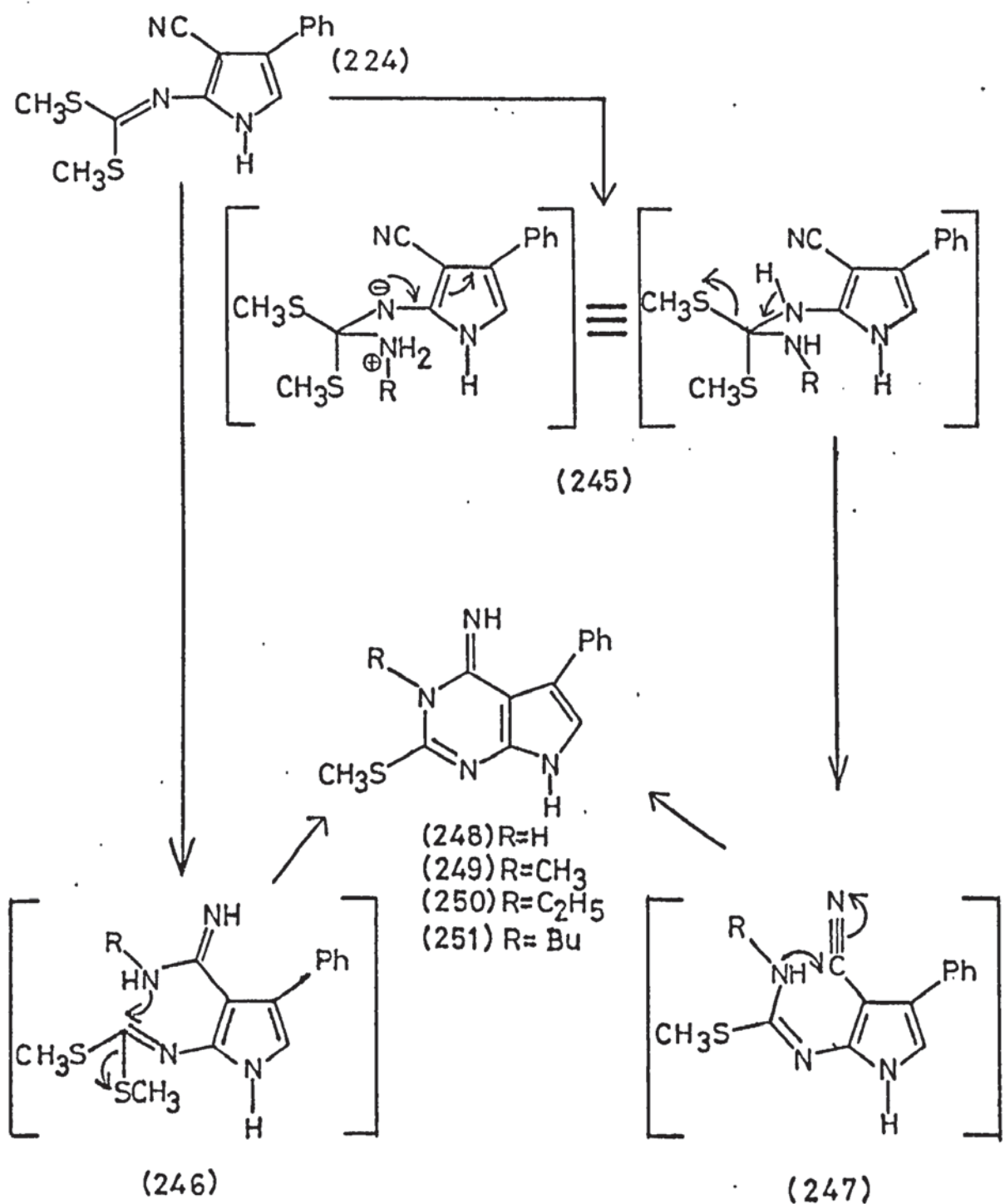
This reaction of the dimethylthiomethyleneaminopyrrole (224) with primary amines may proceed by either of two mechanisms. Attack of the amine may occur either at the unsaturated carbon atom in the methylene-amino substituent at position 2, giving the S-methylthiourea intermediate (247), or at the nitrile group directly, producing the amidine (246). Both intermediates (246) and (247) might be expected to cyclise to the N-alkylpyrrolo[2,3-d]pyrimidines (248)-(251). Nucleophilic substitution at a trigonal carbon atom usually proceeds by a second order mechanism, (known as the tetrahedral mechanism), when the carbon atom is doubly bonded to oxygen, nitrogen or sulphur.[93,94,95,96] In the reaction under discussion, this tetrahedral mechanism involves attack of the amine at the trigonal carbon atom in the side chain of the pyrrole (224), to give an intermediate (245), containing both the amine function and the two methylthio groups, which then decomposes to the S-methyl thiourea derivative (247). The intermediate (245) is stabilised however, since the negative charge on the exocyclic imino nitrogen atom may be delocalised over the pyrrole nucleus and indeed, over the nitrile group also. It is suggested therefore that such a mechanism is more likely than one involving direct attack of the amine at the cyano group to give the intermediate (246).

The reaction appears to provide a general synthetic route to pyrrolo [2,3-d]pyrimidines from readily available pyrrole precursors. It seems probable that the dimethylthiomethyleneaminopyrrole (224) and many similar compounds might be easily prepared from the aminonitrile (157) thus making possible the preparation of new 2-substituted derivatives · of the ring system. A number of other 2-amino-3-cyanopyrroles[71,72,97,98,99,100,101,102] have been reported in the literature and the use of

these in the above reaction would be expected to increase its versatility. Similarly, an extension of the synthesis to o-aminonitriles of other cyclic systems should provide a general route to fused pyrimidines.

E. SOME PROPERTIES OF THE PYRROLOPYRIMIDINES PREPARED FROM 4-IMINO-5-PHENYLPYRROLO[2,3-d]-[3,1]THIAZIN-2(IH)-THIONE.

Methylation of some of the pyrrolopyrimidines prepared in the preceeding sections (A,C,E) was investigated, in basic conditions. Treatment of the N-methylpyrrolopyrimidine thione (234) with methyl iodide and aqueous alkali furnished the 3-methyl-2-methylthiopyrrolo[2,3-d] pyrimidine (249) which had also been prepared from the dimethylthiomethyl-eneaminopyrrole (224) by the method described in Section D above. Similarly the 3-butyl-2-methylthiopyrrolopyrimidine (251) was prepared by methylation of the thione (232) and this synthesis provides further confirmation of the pyrrolopyrimidine structures (248)-(251).

(234) R=CH$_3$, X=NH
(232) R=n-C$_4$H$_9$, X=NH
(221) R=H, X=S.

(224)

(252)

(249) R=CH$_3$
(251) R=n-C$_4$H$_9$
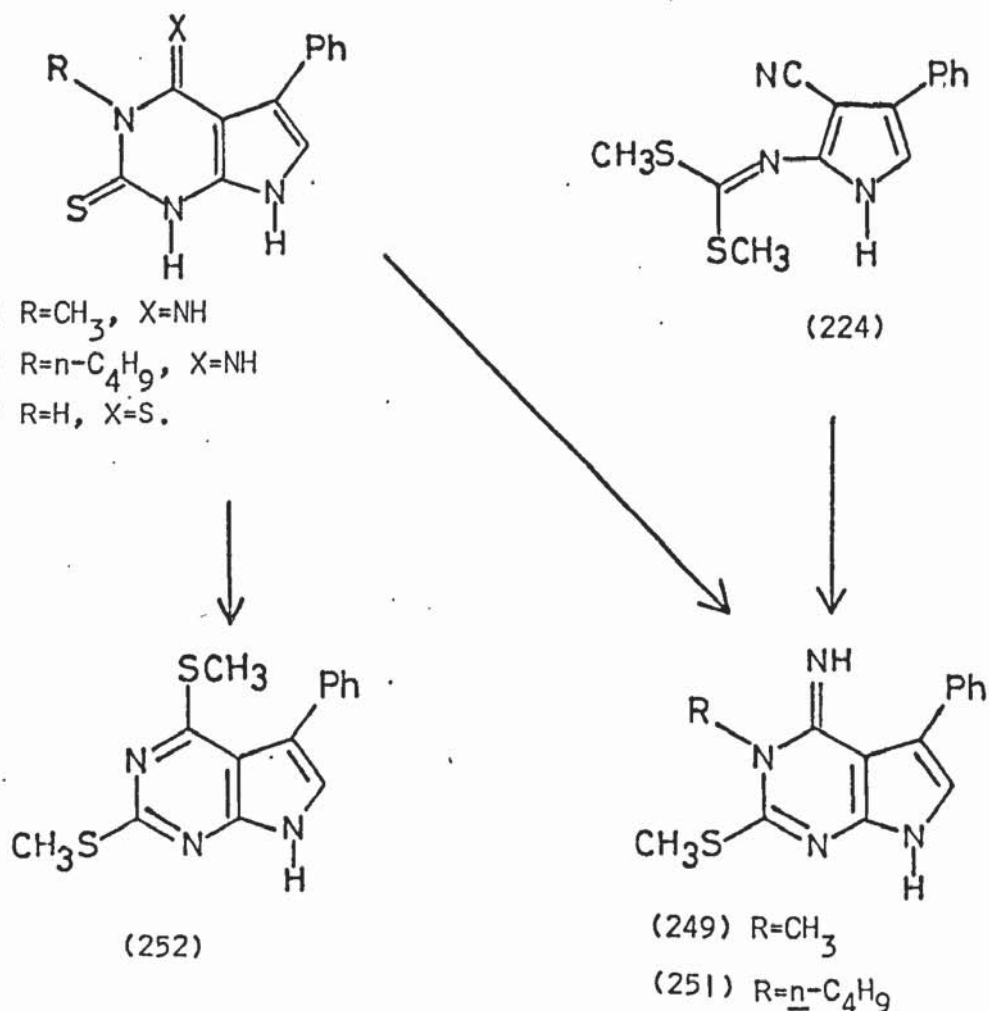
Treatment of the pyrrolopyrimidine dithione (221) with methyl

iodide and aqueous alkali gave a compound to which the 2,4-dimethylthio-

pyrrolo[2,3-d]pyrimidine structure (252) was attributed. The NMR

spectrum of the product was consistent with this proposed structure: the

two singlets at τ7.41 and τ7.31 were assigned to the methyl groups; the

singlet at τ2.91 was attributed to the proton at the unsubstituted position

6 and the multiplet at τ25-26 was typical of that due to a 5-phenyl

substituent.

Taylor and McKillop[71] have described the isomerisation of fused

3-substituted-4-iminopyrimidines to 4-substituted-aminopyrimidines and

this reaction, which is known as the Dimroth rearrangement, has also

been reported in examples of the pyrrolo[2,3-d]pyrimidine ring system,[25]

with no substituent at position 2. Accordingly, the 3-methyl-4-imino-

pyrrolo[2,3-d]pyrimidine (234) was treated with hot aqueous alkali for

several hours until dissolution was attained. No evidence for the

occurrence of a Dimroth rearrangement was obtained, however, and the

analytical data and spectroscopic properties of the only isolated product

were consistent with the fused pyrimidin-4-one structure (253). Similarly,

3-ethyl-4-imino-5-phenylpyrrolo[2,3-d]pyrimidin-2(1H)-thione (233) gave

the pyrrolo[2,3-d]pyrimidin-4-one (254) when treated in the same manner,

but again no product expected from a Dimroth rearrangement was isolated.

The isolation of the two pyrrolopyrimidinones (253) and (254), however,

provides further proof that the pyrrolopyrimidines described in sections

C and D, above, exist as the imino forms and not as the substituted 4-

amino compounds (255).

(234) R=CH₃
(233) R=C₂H₅

(253) R=CH₅
(254) R=C₂H₅

(255)
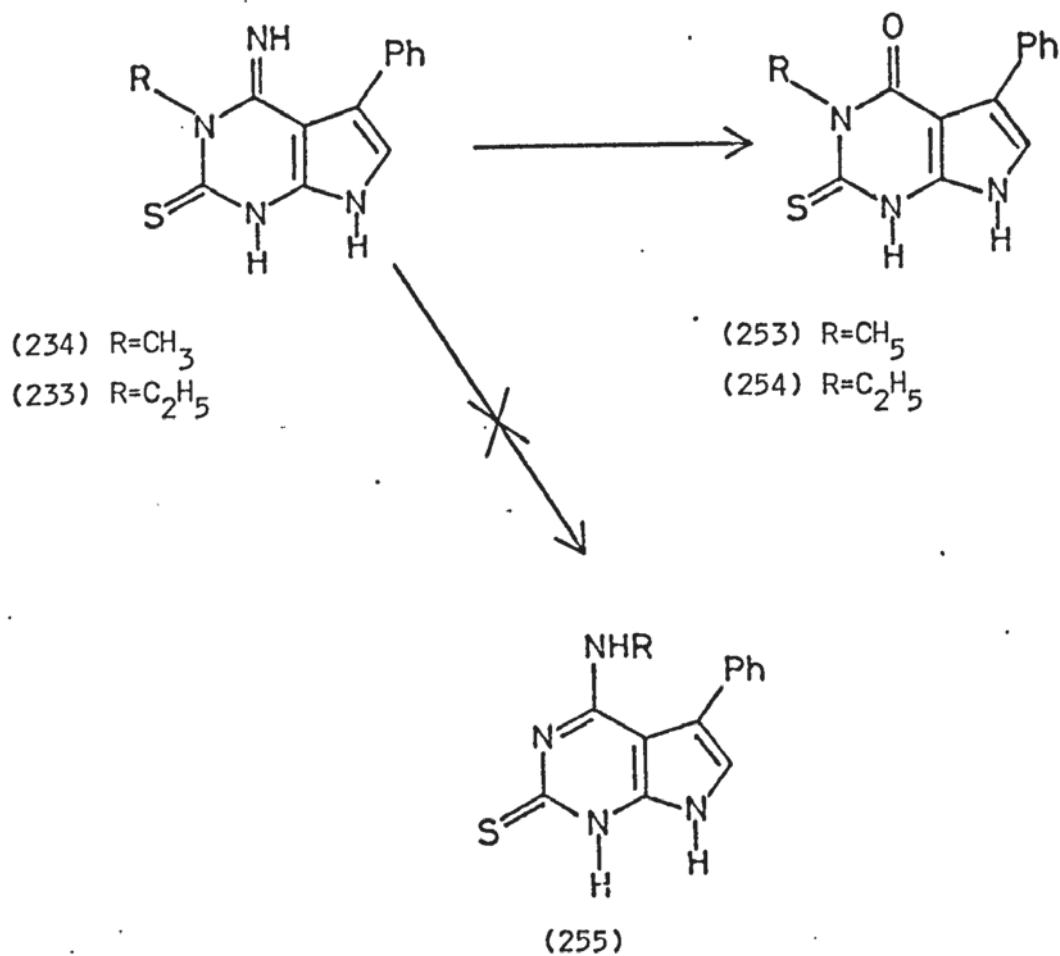
Further work must be done to investigate the chemical properties of the sulphur-containing pyrrolo[2,3-d]pyrimidine analogues described above in this thesis, it is suggested, however, that these compounds would behave similarly to the corresponding purines[86] with respect to desulphurisation and nucleophilic displacement of thiol and alkylthio substituents in the pyrimidine moiety.

EXPERIMENTAL

Infrared spectra were determined, either as nujol mulls (Nujol) or potassium bromide discs (KBr), with a Unicam S.P. 200 spectrophotometer. The most intense peaks in the spectra, and those which were easily assignable, are shown.

Nuclear magnetic resonance spectra were determined, using tetramethylsilane as internal standard, at 60 MHz, with a Varian A-60A spectrometer. All the peaks are assigned in terms of τ values and abbreviations used in the interpretation of NMR spectra are: s=singlet; d=doublet; t=triplet; q=quartet; m=multiplet; J=coupling constant; a=removed on deuteration.

Mass spectra were determined with an A.E.I. MS9 spectrometer operating at 100 µamps and 70eV. M, signifies the molecular ion peak.

Melting points are uncorrected and reaction temperatures are those of an external oil bath.

I THE SYNTHESIS OF PYRROLO[2,3-d]PYRIMIDINES VIA PYRIMIDYL-6-HYDRAZONES

<u>Cyclohexanone</u> 2,4-dimethylpyrimidyl-6-<u>hydrazone</u> (117).

2,4-Dimethyl-6-hydrazinopyrimidine (2.0g, twice recrystallised from ethanol) and cyclohexanone (1.6g) were heated on a steam bath for 15 minutes. The resultant yellow viscous liquid was triturated with light petroleum (b.p. 60-80°) at 0° to give the <u>pyrimidyl hydrazone</u> (3.2g, 87%), colourless needles, m.p. 129-130°, (from cyclohexane).

Found: C, 66.1; H, 8.3; N, 25.8%; $\underline{M}$, 218.

$C_{12}H_{18}N_4$ requires: C, 66.1; H, 8.3; N, 25.7%; $\underline{M}$, 218.

$\nu_{max.}$ (Nujol) 3225(NH), 1600, 1570, 1450, 1430, 1120, 950cm^{-1}.

$\tau(CCl_4)$ 8.33(6H, m, cyclohexyl [3',4' and 5'] protons),

7.70(3H, s, C$\underline{H}_3$),

7.67(3H, s, C$\underline{H}_3$,

7.65(4H, m, cyclohexyl [2' and 6'] protons),

3.28(1H, s, 5-$\underline{H}$),

2.40(1H, broad s, N$\underline{H}$)[a].

<u>Benzyl methyl ketone</u> 2,4-dimethylpyrimidyl-6-<u>hydrazone</u> (118).

2,4-Dimethyl-6-hydrazinopyrimidine (5.0g), benzyl methyl ketone (5.0g) and ethanol (5cm^3) were heated together on a steam bath for 20 minutes. The solvent was removed *in vacuo* and the residual yellow gum mixed intimately with neutral aluminium oxide. The mixture was extracted continuously for 18 hours with light petroleum (b.p. 60-80°), in a suitable apparatus. Concentration of the extract in vacuo gave the <u>pyrimidyl hydrazone</u> (3.9g, 37%), yellow rosettes, m.p. 66-68° (from light petroleum, b.p. 60-80°)

Found: C, 70.0; H, 7.0; N, 21.0%; $\underline{M}$, 254.

$C_{15}H_{18}N_4$ requires: C, 70.8; H, 7.1; N, 21.6%; $\underline{M}$, 254.

$\nu_{max.}$ (Nujol)   3225(NH), 1590, 1420, 1175, 1115, 985, 710cm^{-1}.

τ(CHCl$_3$)   8.22(3H, s, -N=C'-C$\underline{H}_3$),

7.60(3H, s, 4-C$\underline{H}_3$),

7.50(3H, s, 2-C$\underline{H}_3$)

6.37(2H, s, -C$\underline{H}_2$-Ph),

3.06(1H, s, 5-$\underline{H}$),

2.72(5H, broad s, phenyl protons),

2.0-2.2(1H, broad s, N$\underline{H}$)[a].

Butanone 2,4-dimethylpyrimidyl-6-hydrazone (119)

2,4-Dimethyl-6-hydrazinopyrimidine (2.0g), butanone (1.1g) and ethanol (10cm^3) were heated on a steam bath for 5 minutes and the solvent then removed in vacuo. The resulting gum was triturated with light petroleum (b.p. 60-80°) to give the pyrimidyl hydrazone (1.4g, 43%), m.p. 190-193°.

Found: $\underline{M}$, 192.

C$_{10}$H$_{16}$N$_4$ requires: $\underline{M}$, 192.

$\nu_{max.}$ (KBr)   3300(NH), 2900(aliphatic CH), 1650, 1610, 1560, 1390 and 1040cm^{-1}.

τ(CCl$_4$)   8.92(3H, t, J=7Hz, CH$_2$-C$\underline{H}_3$),

8.18(3H, s, N=C-C$\underline{H}_3$)

7.78(2H, q, J=7Hz, C$\underline{H}_2$-CH$_3$),

7.72(3H, s, 4-C$\underline{H}_3$),

7.67(3H, s, 2-C$\underline{H}_3$),

5.33(1H, broad s, N$\underline{H}$)[a],

3.25(1H, s, 5-$\underline{H}$).

Acetophenone 2,4-dimethylpyrimidyl-6-hydrazone (120).

2,4-Dimethyl-6-hydrazinopyrimidine (1.0g) and acetophenone (0.9g) were mixed and heated together on a steam bath for 15 minutes. The reaction mixture was cooled and the resulting gummy solid triturated with ethanol to give a pale-yellow solid, (1.1g, 54%), m.p. 130-133°, with NMR and infrared spectra which were consistent with the structure of the expected hydrazone.

$\nu_{max.}$ (Nujol)   3225(NH), 1585, 1450, 1165, 760, 690cm^{-1}.

τ(CDCl$_3$)   7.89(3H, s, -N=C-C$\underline{H}_3$),

7.69(3H, s, 4-C$\underline{H}_3$),

7.62(3H, s, 2-C$\underline{H}_3$),

3.12(1H, s, 5-$\underline{H}$),

2.0-2.4(2H, m, phenyl $\underline{o}$-protons),

2.5-2.8(3H, m, phenyl m- and $\underline{p}$-protons).

Cyclohexanone 2-methylpyrimidyl-4-hydrazone (121).

A solution of 4-hydrazino-2-methylpyrimidine (1.3g) in ethanol (*ca.* 20cm^3) was heated on a steam bath with cyclohexanone (1.0g) for 10 minutes. Removal of the solvent gave a yellow oil which was extracted several times with light petroleum. Concentration of the combined extracts *in vacuo* caused a yellow solid to crystallise, which was collected and sublimed *in vacuo* at 100° to give the pyrimidyl hydrazone (0.6g, 24%), m.p. 115-116°, colourless needles (from light petroleum, b.p. 60-80°).

Found: C, 58.8; H, 7.8; N, 25.2%; $\underline{M}$, 204.

C$_{11}$H$_{16}$N$_4$·H$_2$O requires: C, 59.4; H, 8.1; N, 25.2%; $\underline{M}$, 204.

$\nu_{max.}$ (KBr)   3200(NH), 1580, 1560, 1440, 1220, 1080, 980cm^{-1}.

$\tau(CDCl_3)$ 8.1-8.6(6H, m, cyclohexyl [3',4' and 5'] protons,

7.6-7.9(4H, m, cyclohexyl [2' and 6'] protons),

7.53(3H, s, 2-C$\underline{H}_3$),

3.08(1H, d, J=6Hz, 5-$\underline{H}$),

1.85(1H, d, J=6Hz, 6-$\underline{H}$).

Cyclohexanone 2,4-diphenylpyrimidyl-6-hydrazone (122).

2,4-Diphenyl-6-hydrazinopyrimidine (2.5g), cyclohexanone (1.0g) and ethanol were heated on a steam bath for 20 minutes and then cooled. Filtration gave the pyrimidylhydrazone (2.3g, 70%), pale yellow needles, m.p. 158-159°, (from ethanol: petroleum spirit, 1:4).

Found: C, 77.0; H, 6.5; N, 16.1%; $\underline{M}$, 342.

$C_{22}H_{22}N_4$ requires: C, 77.2: H, 6.4; N, 16.4%; $\underline{M}$, 342.

$\nu_{max.}$ (Nujol) 3375(NH), 1640(C=N), 1590, 1570, 1550, 1400, 690cm^{-1}

$\tau(CDCl_3)$ 8.28(6H, m, cyclohexyl [3',4' and 5'] protons),

7.59(4H, m, cyclohexyl [2' and 6'] protons),

6.25(1H, s, N$\underline{H}$)[a],

2.71(1H, s, 5-$\underline{H}$).

2.30-2.65(6H, $\underline{m}$, 2- and 4-phenyl [m- and p-] protons)

1.30-1.80(4H, m, 2- and 4-phenyl [o-] protons).

Acetophenone 2,4-diphenylpyrimidyl-6-hydrazone (123).

2,4-diphenyl-6-hydrazinopyrimidine (2.5g), acetophenone (1.2g) and ethanol (5cm^3) were heated on a steam bath for 30 minutes. The reaction mixture was cooled and filtered to give the pyrimidylhydrazone (2.6g, 75%), colourless felted needles, m.p. 181-182°, (from ethanol: light petroleum, 3:1).

Found: C, 79.0; H, 5.5; N, 15.3%; $\underline{M}$, 364.

$C_{24}H_{20}N_4$ requires: C, 79.1; H, 6.6; N, 15.4%; $\underline{M}$, 364.

$v_{max.}$ (Nujol) 3350(NH), 1595, 1575, 1555, 1410, 1390, 750, 690cm^{-1}.

τ(TFA) 7.77(3H, m, C$\underline{H}_3$),

2.0-3.3(15H, m, phenylprotons),

2.94(1H, s, 5-$\underline{H}$)

2,4-Dimethyl-5,6,7,8-tetrahydropyrimido[4,5-b]indole (124).

A solution of cyclohexanone 2,4-dimethylpyrimidyl-6-hydrazone (2.3g) in digol (30cm^3) was heated under reflux in a nitrogen atmosphere for 24 hours. The reaction mixture was cooled, poured into water (250 cm^3) and the resulting pale yellow solution was extracted with ether (4X30cm^3). The extracts were dried (calcium chloride) and the solvent then removed in vacuo to give the pyrrolopyrimidine (0.95g, 45%), colourless needles, m.p. 243-245° (from ethanol: light petroleum |b.p. 60-80°|, 3:1).

Found: C, 71.4; H, 7.4; N, 20.8%; $\underline{M}$, 201.

$C_{12}H_{15}N_3$ requires: C, 71.6; H, 7.4; N, 20.9%; $\underline{M}$, 201.

$v_{max.}$ (KBr) 3150(NH), 1620, 1580, 1420, 1260, 1140, 760cm^{-1}.

τ(CDCl$_3$) 8.12(4H, m, cyclohexyl|6' and 7'|protons),

7.29(3H, s, 4-C$\underline{H}_3$),

7.26(3H, s, 2-C$\underline{H}_3$),

7.15(4H, m, cyclohexyl|5' and 8'|protons).

Attempted preparation of 2,4,5,6-tetramethylpyrrolo[2,3-d]pyrimidine (126a)

Butanone 2,4-dimethylpyrimidyl-6-hydrazone (2.0g) in digol (30cm^3) was heated under reflux for 24 hours, in a nitrogen atmosphere. The reaction mixture was cooled, poured into ice-water and the resulting solution was extracted several times with ether. Removal of the solvent from the combined extracts produced a dark gum which could not be purified.

Attempted preparation of 2,4-dimethyl-6-phenylpyrrolo[2,3-d]pyrimidine (126b

Acetophenone 2,4-dimethylpyrimidyl-6-hydrazone (1.0g) in digol (20cm^3)

was heated under reflux for 24 hours. The reaction mixture was cooled, poured onto ice and extracted with ether several times. Removal of the solvent from the combined extract gave an impure product, the NMR and infrared spectra of which were not consistent with the expected pyrrolo-pyrimidine structure.

$\nu_{max.}$ (Nujol) 1600, 1440, 1360, 755 and 685cm^{-1}.

τ(CDCl$_3$) 7.66(3H, s).

2.45-2.75(3H, m)

1.90-2.30(2H, m).

Attempted preparation of 2,4,6-trimethyl-5-phenylpyrrolo[2,3-d]pyrimidine (126).

Benzyl methyl ketone 2,6-dimethylpyrimidyl-6-hydrazone (1.50g) was heated under reflux with digol (30cm^3) in a nitrogen atmosphere for 24 hours. The reaction mixture was cooled, poured into water (300cm^3) and extracted with ether (6 X 50cm^3). The ether extracts were washed with a little water, dried (magnesium sulphate) and then concentrated to dryness *in vacuo*. The residual black tar was extracted several times with light petroleum (b.p. 60-80°). Concentration of the extracts to small volume caused a product to crystallise. Filtration gave 3-cyano-2-dimethyl-methyleneamino-5-methyl-4-phenylpyrrole (0.45g, 32%), pale yellow needles, m.p. 218-219° (from light petroleum, b.p. 60-80°).

Found: C, 75.8; H, 6.3; N, 17.5%; M, 237.

C$_{15}$H$_{15}$N$_3$ requires: C, 75.9; H, 6.3; N, 17.7%; M, 237.

$\nu_{max.}$ (KBr) 3275(NH), 2225(C≡N), 1600, 1420, 1250, 775 and 705cm^{-1}.

2-Methyl-5,6,7,8-tetrahydropyrimido[4,5-b]indole (125).

A solution of cyclohexanone 2-methylpyrimidyl-4-hydrazone (0.40g) in digol (30cm^3) was heated under reflux, in a nitrogen atmosphere, for 24 hours. The reaction mixture was poured into water at 0° and the

resulting yellow solution extracted with ether (10 X 20cm^3). The solvent was removed in vacuo from the combined extracts to give the pyrrolo-pyrimidine (0.36g, 98%), colourless needles, m.p. 270-272°, (from ethanol: light petroleum|b.p. 60-80°|, 3:1).

Found: C, 66.7; H, 7.0; N, 20.3%; M, 187.110585

C$_{11}$H$_{13}$N$_3$.½H$_2$O requires: C, 67.3; H, 7.1; N, 20.5%; M, 187.110942.

$\nu_{max.}$ (Nujol) 3125(NH), 1620, 1580, 1560, 1440, 1380, 1130, 695cm^{-1}.

τ(CDCl$_3$) 8.4-8.9(4H, m, 6-$\underline{H}_2$ and 7-$\underline{H}_2$),

7.6-8.1(4H, m, 5-$\underline{H}_2$ and 8-$\underline{H}_2$),

7.72(3H, s, 2-C$\underline{H}_3$),

1.80(1H, s, 4-$\underline{H}$).

Attempted preparation of 2,4-diphenyl-5,6,7,8-tetrahydropyrimido[4,5-b]-indole (125a).

A solution of cyclohexanone 2,4-diphenylpyrimidyl-6-hydrazone (1.00g) in trigol (12cm^3) was heated under reflux, for 4 hours, in a nitrogen atmosphere. The reaction mixture was cooled, poured into water (150 cm^3) and the resulting yellow solution extracted with chloroform (3 X 50cm^3). The extract was washed with water (3 X 20cm^3), and dried (calcium chloride). Removal of the solvent in vacuo gave an oil which, when triturated with ether, produced an off-white solid. This product decomposed to an intractable black tar on exposure to the atmosphere.

Replacement of trigol by digol in the above procedure gave ,(after 24 hours), a product with an infrared spectrum and m.p. identical to those of the starting material.

Attempted preparation of 2,4,6-triphenylpyrrolo[2,3-d]pyrimidine (125b).

A solution of acetophenone 2,4-diphenylpyrimidyl-6-hydrazone (1.00g) in digol (10cm^3) was heated under reflux for 12 hours . The reaction mixture was allowed to cool, poured onto ice-water and left to stand for 48 hours, whereupon a solid separated. Filtration gave a yellow solid with infrared spectrum and m.p. identical to those of the starting material.

1,3-Dimethylpyrimidin-2,4,6(1H,3H,5H)-trione (129).

This compound was prepared (according to the method described by Pfleiderer and Shündehütte[68], from N,N'-dimethylurea and malonic acid.

τ(CDCl$_3$)   6.73(6H, s, 2- and 4-CH$_3$),

6.37(2H, s, 5-H$_2$).

4-Chloro-1,3-dimethylpyrimidin-2,6(1H,3H)-dione (131).

1,3-Dimethylpyrimidyl-2,4,6(1H,3H,5H)-trione   (10.0g) and phosphoryl chloride (30cm^3) were heated under reflux for 15 minutes. The excess phosphoryl chloride was removed *in vacuo* and the residual gum cooled and poured into ice-water. The precipitated chloropyrimidine was collected, washed with water and dried (4.0g). The filtrates and washings were extracted with chloroform (5 X 30cm^3) and the combined extracts dried (magnesium sulphate) and concentrated *in vacuo* to give a further 5.5g (overall yield 84%) of the chloropyrimidine, m.p. 110-111°. (The method described by Pfleiderer *et al*[68] gave the chloropyrimidine, m.p. 109-110°, in 79% yield).

τ(CCl$_4$)   6.76(3H, s, CH$_3$),

6.48(3H, s, CH$_3$),

4.19(1H, s, 5H).

1,3-Dimethyl-4-hydrazinopyrimidin-2,6(1H,3H-dione (133).

A solution of 4-chloro-1,3-dimethylpyrimidin-2,6(1H,3H-dione (5.0g) in ethanol (20cm^3) was heated under reflux and vigorously stirred. Hydrazine hydrate (15cm^3) was rapidly added and the reaction mixture heated until no further crystals separated. The reaction mixture was cooled and filtered to give the hydrazinopyrimidine (4.4g, 90%), m.p. 238-40°, (The method described by Pfleiderer et al[68] gave the hydrazinopyrimidine in 82% yield).

$\nu_{max.}$ (Nujol) 3360(NH), 3320(NH), 1700(C=O), 1660(C=O), 1630, 1600, 1530, 1460, 765 and 755cm^{-1}.

Cyclohexanone 1,3-dimethylpyrimidin-4-hydrazone-2,6(1H,3H)dione (134)

1,3-Dimethyl-4-hydrazinopyrimidin-2,6(1H,3H)dione (2.00g), cyclohexanone (1.20g) and ethanol (15cm^3) were heated on a steam bath for 5 minutes. The resulting yellow solution was filtered and concentrated in vacuo until the product began to crystallise. Filtration gave the pyrimidylhydrazone (1.93g, concentration of the filtrates and washings gave a further 0.35g, overall yield 78%), colourless plates m.p. 134-135°, (from ethanol).

Found: C, 57.4; H, 7.2; N, 22.3%; $\underline{M}$, 250.

$C_{12}H_{18}N_4O_2$ requires: C, 57.6; H, 7.2; N, 22.4%; $\underline{M}$, 250.

$\nu_{max.}$ (Nujol) 3150, 1680(C=O), 1675(C=O), 1620(C=N), 1590, 1440, 1220, 785, 755cm^{-1}.

τ(CDCl$_3$) 8.37(6H, m, cyclohexyl [3',4' and 5']protons),

7.2-7.9(4H, m, cyclohexyl [2' and 6']protons,

6.72(3H, s, C$\underline{H}_3$),

6.58(3H, s, C$\underline{H}_3$),

6.08(2H, s, -CO-C$\underline{H}_2$-)[a].

Benzyl methyl ketone 1,3-dimethylpyrimidyl-4-hydrazone-2,6(1H,3H)-dione
(135).

1,3-dimethyl-4-hydrazinopyrimidin-2,4(1H, 3H)-dione (2.00g), benzyl
methyl ketone (1.70g) and ethanol (10cm^3) were heated under reflux for
15 minutes. The yellow solution was filtered and concentrated, in
vacuo, until a solid separated. Filtration gave the pyrimidyl hydrazone
(2.80g, 83%) colourless needles, m.p. 133.5-134°, (from 1,2-dimethoxy-
ethane).

Found: C, 60.2; H, 6.3; N, 18.4%; $\underline{M}$, 286.

$C_{15}H_{18}N_4O_2 \cdot {}^2/_3H_2O$ requires: C, 60.4; H, 6.4; N, 18.8%, $\underline{M}$, 286.

$\nu_{max.}$ (KBr) 3200, 1680, 1630, 1620(C=N), 1190, 750,
700cm^{-1}.

τ(CDCl$_3$) 8.10(3H, s, -N=C-CH$_3$),

6.72(3H, s, -N-CH$_3$),

6.59(3H, s, -N-CH$_3$),

6.47(2H, s, -CH$_2$-Ph),

6.05(2H, s, -CO-CH$_2$-)^a.

Butanone 1,3-dimethylpyrimidyl-4-hydrazone-2,6(1H,3H)-dione (136)

1,3-Dimethyl-4-hydrazinopyrimidin-2,6(1H,3H)-dione (5.50g) and
butanone (8.00g) were heated on a steam bath with ethanol (50cm^3) for
15 minutes. The yellow solution was filtered, concentrated *in vacuo*
and allowed to stand overnight. Filtration gave the pyrimidylhydrazone
(6.10g, 84%) colourless prisms, m.p. 130-132°, (from ethanol).

Found: C, 53.5; H, 7.1; N, 25.0%; $\underline{M}$, 224.128232.

$C_{10}H_{16}N_4O_2$ requires: C, 53.5; H, 7.1; N, 25.0%; $\underline{M}$, 224.127318.

$\nu_{max.}$ (Nujol) 3225, 1690(C=O), 1620(C=N), 1600, 1540, 1440,
1190, 800, 755cm^{-1}.

$\tau(CDCl_3)$ 8.88(3H, t, J=7.5Hz, $CH_2-C\underline{H}_3$),

8.06(3H, s, $N=C-C\underline{H}_3$),

7.67(2H, d, J=7.5Hz, $C\underline{H}_2-CH_3$),

6.72(3H, s, $N-C\underline{H}_3$),

6.58(3H, s, $N-C\underline{H}_3$),

6.10(2H, s, $-CO-C\underline{H}_2$).

Propionaldehyde 1,3-dimethylpyrimidyl-4-hydrazone-2,6(1H,3H)-dione (137).

1,3-Dimethyl-4-hydrazinopyrimidin-2,4(1H, 3H)-dione (2.40g), propionaldehyde (3.00g) and ethanol (30cm^3) were heated under reflux for 20 minutes. The pale yellow solution was filtered and concentrated in vacuo until a solid separated. Filtration gave the pyrimidyl-hydrazone (1.8g, 61%), colourless needles, m.p. 196-191° (from ethanol).

Found: C, 51.2; H, 6.7; N, 26.5%; $\underline{M}$, 210.112454

$C_9H_{14}N_4O_2$ requires: C, 51.4; H, 6.7; N, 26.6%; $\underline{M}$, 210.111669.

$\nu_{max.}$ (Nujol)   3200, 1695(C=O), 1620(C=N), 1595, 1570, 1470, 785, 760cm^{-1}.

An NMR spectrum was not obtained since no suitable solvent was available.

Phenylacetaldehyde 1,3-dimethylpyrimidyl-4-hydrazone-2,6(1H,3H)-dione (138).

1,3-dimethyl-4-hydrazinopyrimidin-2,4(1H, 3H)-dione (1.0g) and phenylacetaldehyde were dissolved in ethanol and the resulting solution heated on a steam bath for 15 minutes. This pale yellow solution was filtered, concentrated *in vacuo* and cooled to 0°. Filtration gave the hydrazone (1.1g, 69%), colourless felted needles, m.p. 157° decomp., (from dimethylformamide: water, 4:1).

Found: C, 61.7; H, 6.0; N, 20.8%; $\underline{M}$, 272.

$C_{14}H_{16}N_4O_2$ requires   C, 61.7; H, 5.9; N, 20.6%; $\underline{M}$, 272.

$\nu_{max.}$ (Nujol) 3225, 3100(aromatic C-H), 1690(C=O), 1620(C=N),

1565, 1460, 1200, 1080, 790, 755 and 740cm^{-1}.

An NMR spectrum was not obtained since no suitable solvent was available.

1,3-Dimethyl-5,6,7,8-tetrahydropyrimido|4,5-b|indol-2,4(1H,3H)-dione (140).

Method A.   Cyclohexanone 1,3-dimethylpyrimidyl-4-hydrazone-2,6(1H,3H-dione (0.40g) and glacial acetic acid (15cm^3) were heated together on a steam bath for several hours. A white solid separated as the solution was cooled and filtration gave the dimethylpyrrolopyrimidine dione (0.10g, concentration of the filtrates and washings gave a further 0.10g, overall yield 54%), colourless needles, m.p. 325° decomp. (from ethanol).

Found: C, 60.6; H, 6.5; N, 17.3%; M, 233.

$C_{12}H_{15}N_3O_2 \cdot 1/3H_2O$ requires: C, 60.3; H, 6.5; N, 17.6%; M, 233.

$\nu_{max.}$ (KBr)   3200(NH), 2800(aliphatic CH), 1680(C=O),

1670(C=O), 1640, 1620, 1540, 1450, 1345

and 740cm^{-1}.

$\tau|CD_3)_2SO|$ 7.30-7.60(4H, m, 6-$\underline{H}_2$ and 7-$\underline{H}_2$),

6.53(6H, s, 1-C$\underline{H}_3$ and 3-C$\underline{H}_3$),

6.40-6.70(4H, m, 5-$\underline{H}_2$ and 8-$\underline{H}_2$).

Method B.   A solution of cyclohexanone 1,3-dimethylpyrimidyl-4-hydrazone-2,6(1H,3H)-dione (0.40g) in digol (20cm^3) was heated under reflux in a nitrogen atmosphere for 24 hours. The reaction mixture was cooled and poured into ice-water, whereupon a pale yellow solid separated. Filtration gave the pyrrolopyrimidine dione (0.30g, 80%), with infrared spectrum and m.p. identical to those of a sample prepared by method A.

4-Phenyl-1,3,6-trimethylpyrrolo|2,3-d|pyrimidin-2,4(1H,3H)-dione (141).

Benzyl methyl ketone 1,3-dimethylpyrimidyl-4-hydrazone-2,6(1H,3H)-

dione (0.50g) and glacial acetic acid ($8cm^3$) were heated under reflux for 6 hours. The solvent was removed *in vacuo* and the residual gum triturated with ether to give the trimethylpyrrolopyrimidine (0.20g, 42%), colourless needles, m.p. 300° decomp., (from dimethyl formamide: water, 3:1).

Found: C, 64.9; H, 5.6; N, 15.6%; M, 269.

$C_{15}H_{15}N_3O_2.1/2H_2O$ requires: C, 64.7; H, 5.7; N, 15.1%; M, 269.

$\nu_{max.}$ (KBr) 3150(NH), 1680(C=O), 1670(C=O), 1620, 1540, 1290, 980, 740 and $695cm^{-1}$.

An NMR spectrum was not obtained since no suitable solvent was available.

1,3,5,6-Tetramethylpyrrolo[2,3-d]pyrimidin-2,4(1H,3H)-dione (144).

Method A. A solution of butanone 1,3-dimethylpyrimidyl-4-hydrazone-2,6(1H, 3H)-dione (2.00g) in digol ($24cm^3$) was heated under reflux in a nitrogen atmosphere for 7 hours. The reaction mixture was cooled and poured into water at 0° and the resulting yellow solution extracted with chloroform. The extracts were washed with water, dried (magnesium sulphate) and concentrated to dryness *in vacuo*. The residual dark gum was triturated with ethyl acetate to give the pyrrolopyrimidine (1.10g, 60%), colourless needles, m.p. 300° decomp., (from ethanol).

Found: C, 57.5; H, 6.2; N, 20.1%; M, 207.100471.

$C_{10}H_{13}N_3O_2$ requires: C, 57.9; H, 6.3; N, 20.3%; M, 207.100770.

$\nu_{max.}$ (Nujol) 3200(NH), 1690(C=O), 1640, 1620, 1470, 1300, $745cm^{-1}$.

An NMR spectrum was not obtained since no suitable solvent was available.

Method B. Butanone 1,3-dimethylpyrimidyl-4-hydrazone-2,6-(1H,3H)-dione (1.0g) and glacial acetic acid ($10cm^3$) were heated on a steam bath for 14 hours. The solvent was removed *in vacuo* and the

residual yellow gum was triturated with ether to give 1,2-di-(1,3-dimethylpyrimidin-4-yl-2,4(1H,3H)-dione)-hydrazine (0.3g, 22%), colourless needles, m.p. 7320° (from dimethylformamide: water, 3:1).

Found: $\underline{M}$, 308.123695

$C_{12}H_{16}N_6O_4$ requires: $\underline{M}$, 308.123294

$\nu_{max.}$ (Nujol) 1710(C=O), 1660(C=O), 1600, 1460, 1360, 1300, 1280 and 755cm^{-1}.

Attempted preparation of 1,3,5-trimethylpyrrolo[2,3-d]pyrimidin-2,4 (1H,3H)-dione (142).

Propionaldehyde 1,3-dimethylpyrimidyl-4-hydrazone-2,6(1H,3H)-dione (1.0g) was heated under reflux with glacial acetic acid for 7 hours. The solution was filtered and the solvent removed in vacuo. Trituration of the residual oil with ether gave a product with an infrared spectrum and m.p. which were identical to those of the starting material.

Attempted preparation of 1,3-dimethyl-5-phenylpyrrolo[2,3-d]pyrimidin-2,4(1H,3H)-dione (143).

A solution of phenylacetaldehyde 1,3-dimethylpyrimidyl-4-hydrazone-2,6(1H,3H)-dione (0.5g)in glacial acetic acid 5cm^3 was heated at 110° for 16 hours. Removal of the solvent in vacuo afforded a yellow gum which when triturated with ether gave a product with an infrared spectrum and m.p. identical to those of the starting material.

II SYNTHESES AND REACTIONS OF 2,3-DISUBSTITUTED PYRROLES.

A. DERIVATIVES OF 2-AMINO-3-CYANO-4-PHENYLPYRROLE

2-Amino-3-cyano-4-phenylpyrrole (157).

Malononitrile (5.94g) and sodium hydroxide (6.60g) were dissolved in 80% aqueous ethanol (100cm^3) and the resulting solution was heated very vigorously under reflux and stirred violently. A solution of ω-aminoacetophenone hydrochloride (12.87g) in 80% aqueous ethanol (150cm^3) was added dropwise over 15 minutes. When the addition was complete the ·orange solution was heated for a further 5 minutes, cooled to room temperature as quickly as possible and poured slowly, with stirring, onto ice. The precipitated yellow solid was washed thoroughly with ice-water (10 X 100cm^3), dried *in vacuo* at 70° and then continuously extracted with petroleum ether (b.p. 40-60°). The extracts were discarded and the residual pale yellow solid dried *in vacuo* to give the 2-amino-3-cyano-pyrrole (7.10g, 52%), m.p. 175° decomp. (Gewald[70] quoted an m.p. of 172-174°).

Found: <u>M</u>, 183.079602.

C$_{11}$H$_9$N$_3$ requires: <u>M</u>, 183.079643.

ν_{max}. 3400(NH), 3250(NH), 2225(C≡N), 1600, 1500, 1495, 1470, 1380, 740cm^{-1}.

2-acetamido-3-cyano-4-phenylpyrrole (158).

2-amino-3-cyano-4-phenylpyrrole (0.60g) and acetic anhydride (6cm^3) were heated together on a steam bath for 10 minutes. The reaction mixture was cooled to 0° and the solid filtered off to give the acetamidopyrrole (0.55g, 75%) colourless plates, m.p. 290° decomp., (from methanol).

Found: C, 68.5; H, 5.1; N, 17.9%; $\underline{M}$, 225.090350.

$C_{13}H_{11}N_3O$ requires: C, 69.3; H, 4.9; N, 18.6%; $\underline{M}$, 225.090207.

$\nu_{max.}$ (Nujol) 3300(NH), 3225(NH), 2250(C≡N), 1665(C=O),

1620, 1470, 1320, 1280, 745cm^{-1}.

τ(DMSO) 2.94(IH, s, 5-$\underline{H}$),

2.2-2.7(5H, m, phenylprotons).

3-Cyano-4-phenyl-2-p-nitrobenzoylaminopyrrole (159).

2-Amino-3-cyano-4-phenylpyrrole (1.80g) and p-nitrobenzoyl chloride (1.80g) were heated under reflux with pyridine (10cm^3) for 30 minutes. The solution was cooled and the solid which separated was collected by filtration, washed thoroughly with hot methanol and dried to give the acylaminopyrrole (0.40g, 12%), m.p. 234-236°.

Found: $\underline{M}$, 332.0908813.

$C_{18}H_{12}N_4O_3$ requires: $\underline{M}$, 332.090933

$\nu_{max.}$ (Nujol) 3400(NH), 3250(NH), 2250(C≡N), 1650(C=O).,

1600, 1530(NO$_2$), 1350(NO$_2$), 1290cm^{-1}.

N-(3-cyano-4-phenylpyrrol-2-yl)-N'-phenylurea. (160)

2-Amino-3-cyano-4-phenylpyrrole (0.90g) and freshly distilled phenyl isocyanate (0.70g) were heated under reflux in dry benzene (50cm^3) for 15 hours. The solution was cooled and filtered to give the pyrrolylurea (0.80g, 54%), colourless needles, m.p. 199-200°, (from benzene).

Found: C, 70.7; H, 4.7; N, 18.1%; $\underline{M}$, 302.116184.

$C_{18}H_{14}N_4O$ requires: C, 71.5; H, 4.6; N, 18.5%; $\underline{M}$, 302.116754.

$\nu_{max.}$ (nujol) 3380(NH), 3375(NH), 3300(NH), 2250(C≡N),

1695(C=O), 1640, 1600, 1560, 750cm^{-1}.

Attempted preparation of 4-Amino-5-phenylpyrrolo[2,3-d]pyrimidine (163).

2-Amino-3-cyano-4-phenylpyrrole (0.40g) was heated under reflux in a

nitrogen atmosphere with formamide (20cm^3). The red reaction mixture was cooled and poured onto ice and a purple solid separated which was collected, washed with water and dried to give a product (0.30g), which could not be purified further.

Found $\underline{M}$, 211.074923.

Attempted preparation of 2,4-diamino-5-phenylpyrrole (164).

Guanidine hydrochloride (0.90g) was added to a solution of sodium (1.00g)in absolute ethanol (100cm^3) and the resulting mixture thoroughly shaken. 2-Amino-3-cyano-4-phenylpyrrole (0.70g) was added, the mixture heated under reflux for 2.5 hours and then allowed to stand overnight. The solution was filtered and the solvent then removed *in vacuo* to leave a black solid which was extracted several times with hot water and dried to give a product (0.60g) which could not be further purified.

The reaction of 2-acetamido-3-cyano-4-phenylpyrrole with dry ethanolic hydrogen chloride.

See the preparation of 3-amido-2-amino-4-phenylpyrrole in section IIC.

Attempted preparation of 4-amino-2-methyl-5-phenylpyrrolo[2,3-d]pyrimidine (165).

A solution of 2-acetamido-3-cyano-4-phenylpyrrole (0.50g) in absolute ethanol (30cm^3) was saturated with ammonia at 0° and then heated in a stainless steel bomb at 100° for 20 hours. The reaction vessel was cooled and opened, and the solvent removed *in vacuo* to give a solid (0.45g, 90%) with an infrared spectrum and melting point identical to those of the starting material.

Attempted preparation of 4-imino-3,5-diphenylpyrrolo[2,3-d]pyrimidin-2(1H,3H)-one (169).

Method A.   A solution of N-(3-cyano-4-phenylpyrrolo-2-yl)-N' phenylurea (0.05g) in dimethylformamide (1cm^3) was heated under reflux with glacial acetic acid (0.10g), in a nitrogen atmosphere, for 18 hours. The reaction mixture was allowed to cool and then poured onto ice. The pH of the resulting solution was adjusted to 5.1 whereupon a purple solid precipitated which was washed well with water and dried to give a product (0.23g), m.p. 170-180° decomp., which could not be purified further.

ν_{max} (Nujol) 2250cm^{-1} (C≡N).

Method B.   N-(3-cyano-4-phenylpyrrol-2-yl)-N'-phenylurea. (0.10g) was heated under reflux with phosphoryl chloride (2cm^3) until dissolution was complete.   The excess phosphoryl chloride was removed *in vacuo* and the residual gum triturated with ethyl acetate to give a crystalline solid which on exposure to the atmosphere rapidly decomposed to a black intractable tar.

Method C.   N-(3-cyano-4-phenylpyrrol-2-yl)-N'-phenylurea (0.20g) was heated under reflux with pyridine (5cm^3) for 4 hours.   Removal of the solvent *in vacuo* gave a solid whose infrared spectrum was identical to that of the starting material.

Method D.   N-(3-cyano-4-phenylpyrrol-2-yl)-N'-phenylurea (0.20g) was heated under reflux with sodium methoxide (0.02g) in methanol (20cm^3) for 16 hours.   The solvent was removed *in vacuo* and the residual solid washed with hot water and dried to give a compound (0.15g) whose infra-red spectrum was identical to that of the starting material.   Acidification of the washings and filtrates precipitated a further 0.01g (overall yield 80%).

, ### B. DERIVATIVES OF PYRROLE o-AMINOESTERS

Dimethyl-2-nitropyrrole-3,4-dicarboxylate (174).

Dimethylpyrrole-3,4-dicarboyxlate (6.0g) was dissolved in sulphuric acid (120g). Fuming nitric acid (2.3g) was added and the solution stirred at room temperature for 0.5 hour. The yellow reaction mixture was poured onto ice and the precipitated solid collected by filtration, washed with water and dried to give the nitropyrrole (5.0g, 67%), colourless prisms, m.p. 170-171° (from benzene).

Found: C, 41.4; H, 3.6; N, 12.0%; $\underline{M}$, 228.

$C_8H_8N_2O_6$ requires: C, 42.1; H, 3.5; N, 12.2%; $\underline{M}$, 228.

$\nu_{max.}$ (KBr) 3225(NH), 1735(C=O), 1705(C=O), 1520(NO$_2$)
1470, 1360(NO$_2$), 1330, 1080cm^{-1}.

τ(CDCl$_3$)   6.15(3H, s, 4-COOC$\underline{H}_3$),
6.00(3H), s, 3-COOCH$_2$CH$_3$
2.75(1H), s, 5-$\underline{H}$).

Dimethyl 2,5-dinitropyrrole-3,4-dicarboxylate (173).

A solution of diethylpyrrole-3,4-dicarboxylate (0.43g) and fuming nitric acid (0.22cm^3) in sulphuric acid (7cm^3) was stirred at room temperature for 40 hours. The yellow reaction mixture was poured onto ice and the precipitated white solid collected, washed with water and dried to give the dinitropyrrole (0.38g, 59%), m.p. 145-7°.

Found: $\underline{M}$, 273.

$C_8H_7N_3O_8$ requires: $\underline{M}$, 273.

$\nu_{max.}$ (KBr) 3150(NH), 1730(broad, C=O), 1560, 1550, 1530(NO$_2$),
1350(NO$_2$), 1240, 1100cm^{-1}.

τ(CDCl$_3$) 6.03(6H, s, 3 and 4-COOC$\underline{H}_3$).

Dimethyl 1-methyl-2-nitropyrrole-3,4-dicarboxylate (175).

Dimethyl 2-nitropyrrole-3,4-dicarboxylate (1.90g) and methyl iodide (4.20g) were added to a solution of sodium methoxide (0.53g) in absolute ethanol (15cm^3) at 0°. The temperature throughout the addition was maintained below 20° and the reaction mixture was then left to stand at room temperature for 20 hours. Filtration gave the N-methylpyrrole (1.60g, 80%), colourless needles, m.p. 174-175°, (from ethanol).

Found: C, 43.8; H, 4.1; N, 11.2%; $\underline{M}$, 242.054317.

$C_9H_{10}N_2O_6$ requires: C, 44.6; H, 4.1; N, 11.5%; $\underline{M}$, 242.053879.

$\nu_{max.}$ (Nujol) 1730(C=O), 1710(C=O), 1510(NO$_2$), 1460, 1330(NO$_2$), 1300, 1090, 780cm^{-1}.

An NMR spectrum was not obtained since no suitable solvent was available.

Diethylpyrrole-3,4-dicarboxylate[77] (172).

Diethyl-2-diethoxymethylsuccinate was prepared from diethyl-2-formylsuccinate as described by Cornfield and Jones.[77]

To a mixture of sodium hydride (*ca.* 20g) and absolute ether (500cm^3), absolute ethanol (10cm^3) was added. The mixture was stirred and diethyl-2-diethoxymethylsuccinate (200g) and triethyl orthoformate (107g) were added dropwise. The mixture was stirred for 24 hours and then poured into ice-water. (The ether layer was evaporated to give unchanged starting material). The residual aqueous solution was acidified at 0° with dilute sulphuric acid and extracted with ether (4 x 150cm^3). The extracts were evaporated to give an oil which distilled at 135-140° (1.1mm) to give diethyl-1-formyl-2-diethoxymethylsuccinate (170g, 76%).

Diethyl-1-formyl-2-diethoxymethylsuccinate was readily converted to diethylpyrrole-3,4-dicarboxylate by the method described by the above

authors.[77]

$\nu_{max.}$ (KBr) 3250(NH), 2950(aliphatic CH), 1700(C=O),

1430, 1280, 1130, 1050, 760cm^{-1}.

τ(CDCl$_3$) 8.40(6H, t, J=7Hz, 3 and 4-COOCH$_2$CH$_3$),

5.44(4H, q, J=7Hz, 3 and 4-COOCH$_2$CH$_3$),

2.32(2H, d, J=3Hz, 2-and 5-H),

—1.10(1H, broad s, NH).

Diethyl-2-nitropyrrole-3,4-dicarboxylate (176).

To a solution of diethylpyrrole-3,4-dicarboxylate (1.9g) in

concentrated sulphuric acid (13cm^3), fuming nitric acid (0.5g) was added

and the mixture stirred for 24 hours at room temperature and then

poured onto ice. The pH was adjusted to 10 (dilute aqueous ammonia) and

then to 6(dilute sulphuric acid). The mixture was thoroughly extracted

with ether and the combined extracts were washed (water), dried

(magnesium sulphate) and evaporated to give the 2-nitropyrrole (2.0g,

87%), colourless needles, m.p. 127-128°, (from ethyl acetate:light

petroleum, b.p. 60-80°, 1:1.

Found: C, 47.1; H, 4.7; N, 11.1%; M, 256.

$C_{10}H_{12}N_2O_6$ requires: C, 46.9; H, 4.7; N, 10.9%; M, 256.

$\nu_{max.}$ (KBr) 3200(NH), 2950(aliphatic CH), 1735(C=O),

1700(C=O), 1530(NO$_2$), 1500, 1350(NO$_2$), 1310,

1220, 1180, 1010cm^{-1}.

τ(CDCl$_3$) 8.68(3H, t, J=7Hz, 4-CH$_2$-CH$_3$),

8.60(3H, t, J=7Hz, 3-CH$_2$-CH$_3$),

5.66(2H, q, J=7Hz, 4-CH$_2$-CH$_3$),

5.60(2H, q, J=7Hz, 3-CH$_2$-CH$_3$);

2.46(1H, s, 5-H),

-1.30(1H, broad s, -NH).

Dimethyl-2-aminopyrrole-3,4-dicarboxylate (178).

Dimethyl-2-nitropyrrole-3,4-dicarboxylate (4.0g) was dissolved in ethanol ($160cm^3$) containing 10% palladium on charcoal (0.4g). The resulting mixture was shaken with hydrogen at 4 atmospheres for 2.5 hours and then filtered through celite. The filtrates and washings were decolourised by boiling with charcoal. Removal of the solvent *in vacuo* gave the crude aminopyrrole (3.7g, 94%), m.p. 162-164°, (an analytical sample was not prepared since no suitable solvent for recrystallisation could be found).

<div style="text-align:center">

Found: $\underline{M}$, 198.062942.

</div>

$C_8H_{10}N_2O_4$ requires: $\underline{M}$, 198.064051..

$\nu_{max.}$ (Nujol) 3450(NH), 3275(NH), 3200(NH), 1720(C=0), 1700(C=0), 1520, 1380, 1330, 1300, $1080cm^{-1}$.

τ(acetone) 6.18(3H, s, 3-COOC$\underline{H}_3$),

6.06(3H, s, 4-COOC$\underline{H}_3$),

2.31(1H, s, 5-$\underline{H}$).

Dimethyl 2-amino-1-methylpyrrole-3,4-dicarboxylate (179).

A solution of dimethyl 1-methyl-2-nitropyrrole-3,4-dicarboxylate (1.20g) in absolute ethanol ($200cm^3$) was poured onto 10% palladium on charcoal (0.12g) and shaken with hydrogen (6 atmospheres) for 11 hours. The mixture was filtered through celite and the filtrate concentrated *in vacuo* until a grey solid separated. Filtration gave the aminopyrrole (0.40g). Concentration of filtrates and washings produced a further 0.30g (overall yield 55%). An analytical sample was not obtained since the aminopyrrole, although stable at 0°, decomposed at room temperature.

<div style="text-align:center">

Found: $\underline{M}$, 212.080294.

</div>

$C_9H_{12}N_2O_4$ requires: $\underline{M}$, 212.079700.

$\nu_{max.}$ (Nujol) 3475(NH), 3350(NH), 1710(C=O), 1670(C=O),

1600, 1300 and 1050cm^{-1}.

Diethyl 2-aminopyrrole-3,4-dicarboxylate (180).

A solution of diethyl 2-nitropyrrole-3,4-dicarboxylate (1.00g) in

ethanol (150cm^3) was shaken with hydrogen (4 atmospheres) and 10%

palladium on charcoal for 3 hours. The mixture was filtered through

celite and the solvent removed from the filtrates and ethanolic washings

to give the pyrrole aminoester (0.95g, 93%) colourless prisms, m.p.

203-205°, (from 1,2-dimethoxyethane).

Found: C, 53.1; H, 6.2; N, 12.3%; $\underline{M}$, 226.

$C_{10}H_{14}N_2O_4$ requires: C, 53.1; H, 6.2; N, 12.4%; $\underline{M}$, 226.

$\nu_{max.}$ (Nujol) 3450(NH), 3300 (NH), 3200(NH), 1700(C=O),

1665(C=O), 1610, 1290, 1180 and 1050cm^{-1}.

τ(DMSO) 8.40(6H, t, J=7.5Hz, 3 and 4-COOCH$_2$C$\underline{H}_3$),

5.52(4H, t, J=7.5Hz, 3 and 4-COOC$\underline{H}_2$CH$_3$),

2.90(1H, s, 5-$\underline{H}$),

-0.80(1H, s, N$\underline{H}$)^a.

Diethyl 2-acetamidopyrrole-3,4-dicarboxylate (181).

Diethyl 2-aminopyrrole-3,4-dicarboxylate (2.26g), 1,2-dimethoxy-

ethane (120cm^3), potassium carbonate (5.0g) and acetyl chloride (1.0cm^3)

were stirred together at 45° for 20 hours. The mixture was cooled and

filtered and the washings and filtrates evaporated to dryness *in vacuo*

to give the acetamidopyrrole (1.90g, 70%).

$\nu_{max.}$ (KBr) 3400(NH), 1730(C=O), 1680(C=O), 1670(C=O), 1620,

1340, 1320, 1185, 1075 and 770cm^{-1}.

τ(CDCl$_3$) 8.67(3H, t, J=7Hz, COOCH$_2$C$\underline{H}_3$),

8.62(3H, t, J=7Hz, COOCH$_2$C$\underline{H}_3$),

7.75(3H, s, COCH$_3$),

2.95(1H, d, J=2Hz, 5-H),

-0.15(1H, s, NH.CO),

-1.15(1H, broad s, pyrrole NH).

2-Nitropyrrole-3,4-dicarboxylate (177).

Dimethyl 2-nitropyrrole-3,4-dicarboxylate (0.50g) was dissolved in

20% aqueous sodium hydroxide and the resulting yellow solution heated on a

steam bath for 30 minutes and then allowed to cool. The solution was

acidified with dilute hydrochloric acid and then continuously extracted

with ether for 18 hours in a suitable apparatus. Removal of the solvent

(*in vacuo*) from the extract gave the pyrrole dicarboxylic acid (0.25g,

57%) m.p. 220° decomp.

Found: M, 200.007509.

C$_6$H$_4$N$_2$O$_6$ requires: M, 200.006931.

ν$_{max.}$ (Nujol) 3450(OH), 3200(NH), 1710(C=O), 1685(C=O),

1530(NO$_2$), 1360(NO$_2$), 1280, 1195, 835cm^{-1}.

τ(acetone) 3.22(2H, broad s, 3 and 4-COOH)^a,

2.31(1H, d, 5-H).

Attempted preparation of methyl 3-phenylpyrrolo[2,3-d]pyrimidin-2,4(1H,3H)-

dione-5-carboxylate (184).

Method A.   Dimethyl 2-aminopyrrole-3,4-dicarboxylate (0.50g) and

freshly distilled phenyl isocyanate (0.30g) were heated under reflux in

sodium-dried benzene (20cm^3) for 1 hour. The solvent was removed *in*

vacuo to give a tarry solid which when triturated with ethanol gave a

solid with a melting point and infrared spectrum identical to those of

the starting material.

Method B.   Dimethyl 2-nitropyrrole-3,4-dicarboxylate (0.40g) and freshly distilled phenyl isocyanate (1.00g) were dissolved in anhydrous pyridine (10cm^3). The solution was heated under reflux for 3 hours, in a carefully dried apparatus. The solvent was removed *in vacuo* and the resultant dark gum triturated with chloroform to give diphenylurea (0.15g). The chlorofrom washings and filtrates were concentrated *in vacuo* and the resultant tarry solid triturated with acetone to give Dimethyl-3-phenylpyrrolo[1,2-a]-[1,3,5]-triazin-2,4-dione-6,7-dicarboxylate (0.35g, 51%), colourless needles, m.p. 263-264°, (from methanol).

Found: C, 55.8; H, 4.0; N, 12.3%; M, 343.080109.

C$_{14}$H$_{11}$N$_3$O$_4$ requires: C, 55.9; H, 3.8; N, 12.3%; M, 343.080427.

$\nu_{max.}$ (Nujol)   3225(NH), 1750(C=O), 1720(C=O), 1715(C=O), 1680(C=O), 1600, 1460, 1380, 1220, 1160, 740, 720cm^{-1}.

$\tau|(CD_3)_2SO|$ 6.20(6H, s, 6- and 7-COOC$\underline{H}_3$), 2.50(5H, s, phenyl protons), 4.43(1H, s, 5-$\underline{H}$).

Attempted preparation of methyl 7-methyl-3-phenylpyrrolo|2,3-d|pyrimidin-2,4(1H,3H)-dione-5-carboxylate (189).

A solution of dimethyl 2-amino-1-methylpyrrole-3,4-dicarboxylate (0.25g) in anhydrous pyridine (1.60g) was heated under reflux with freshly distilled phenyl isocyanate (0.60g) in a nitrogen atmosphere for 1 hour. The reaction mixture was allowed to cool to room rempterature and was extracted with ether, to remove pyridine. The residual black tar was extracted several times with ethyl acetate and removal of the solvent from the combined extracts gave a gum, which when triturated with light

petroleum (b.p. 40-60°) gave diphenylurea (0.60g); with an infrared

spectrum and m.p. identical to those of an authentic sample.

Attempted preparation of ethyl-2-methylpyrrolo[2,3-d]pyrimidin-4(3H)-

one-5-carboxylate.

Method A.   A solution of diethyl 2-acetamidopyrrole-3,4-dicarboxyl-

ate (0.20g) in absolute ethanol (20cm³) was heated with ammonia solution

(30cm³, d. 0.88), in a steel bomb at 100°, for 18 hours. The reaction

vessel was cooled and opened and the solvents were removed *in vacuo* to

give a purple solid which was washed with ether and dried to give diethyl

2-aminopyrrole-3,4-dicarboxylate (0.10g, 41%) with m.p. and both infra-

red and mass spectra identical to those of a sample prepared by the

method described above.

Method B.   A solution of diethyl-2-acetamidopyrrole-3,5-dicarboxyl-

ate (0.5g) in ethanol (100cm³) was saturated with ammonia at 0° and

stirred at room temperature for 30 days. Removal of the solvent *in vacuo*

left a tarry solid which, when triturated with ether, gave ethyl 2-

acetamido-4-amidopyrrole-5-carboxylate (0.2g, 45%), pink-grey needles,

m.p. 285° decomp. (from aqueous ethanol).

Found: C, 49.2; H, 5.4; N, 17.6%; $\underline{M}$, 239.091680.

$C_{10}H_{13}N_3O_4$ requires: C, 49.3; H, 5.5; N, 17.2%; $\underline{M}$, 289.090598.

$\nu_{max.}$ (Nujol) 3400(NH), 3200(NH), 1680(C=O), 1660(C=O),

1650(C=O), 1410, 1390, 1120 and 750cm⁻¹.

τ(TFA) 8.53(3H, t, J=7Hz, 3-COOCH$_2$C$\underline{H}_3$),

7.50(3H, s, 2-NH.CO.C$\underline{H}_3$),

5.52(2H, q, J=7Hz, 3-COOC$\underline{H}_2$CH$_3$),

2.30(1H, d, 5-$\underline{H}$).

Treatment of a similar solution of diethyl-2-acetamidopyrrole-3,4-dicarboxylate at 100° in a steel bomb for 18 hours, gave only diethyl-2-aminopyrrole-3,4-dicarboxylate, when the solvents were removed. The infrared spectrum and m.p. of the product were identical with those of a sample prepared by the method described above (P. 113).

C. DERIVATIVES OF PYRROLE o-AMINOAMIDES.

2-Acetamido-3-amido-4-phenylpyrrole (200).

2-acetamido-3-cyano-4-phenylpyrrole (0.54g) was dissolved in a solution of sulphuric acid (3.80g) in water (0.40g). After standing at room temperature for 1.5 hours the reaction mixture was poured slowly, with stirring, onto ice. The orange precipitate was collected, washed well with water and dried at 80° *in vacuo* to give the pyrrole diamide (0.47g, 80%), colourless needles, m.p. 191-193°, (from aqueous ethanol).

Found: C, 64.8; H, 5.7; N, 16.5%; $\underline{M}$, 243.098385.

$C_{13}H_{13}N_3O_2$ requires: C, 64.2; H, 5.4; N, 17.3%; $\underline{M}$, 243.100770.

$\nu_{max.}$ (Nujol) 3350(NH), 3175(NH), 1660(C=O), 1665(C=O), 1595, 1390, 1360cm^{-1}.

An NMR spectrum was not obtained because no suitable solvent was available.

3-Amido-2-amino-4-phenylpyrrole (166).

Method A. Dry hydrogen chloride was bubbled through a mixture of 2-acetamido-3-cyano-4-phenylpyrrole (1.25g) and ethanol (30cm^3) for 10 hours at room temperature, (the temperature initially rose to 55°). The reaction mixture was then heated under reflux for 10 minutes, cooled and filtered to give 2-amino-3-amido-4-phenylpyrrole (1.05g, 94%), colourless plates, m.p. 250° decomp. (from acetic acid).

Found: C, 56.0; H, 5.3; N, 17.5%; $\underline{M}$, 201.091073.

$C_{11}H_{11}N_3O.2H_2O$ requires: C, 55.7; H, 6.3; N, 17.7%; $\underline{M}$, 201.090207.

$\nu_{max.}$ (NuJol) 3250(NH), 3100(NH), 3050(NH), 1690(C=O),

1660, 1630(NH_2), 1470, 1320, 770cm^{-1}.

τ(TFA) 5.03(2H, s, 2-$\underline{CH}_2$[due to protonation by the

solvent]),

2.38(5H, m, phenyl protons).

__Method B.__   2-acetamido-3-amido-4-phenylpyrrole (0.41g), hydro-
chloric acid (15cm^3) and water (5cm^3) were heated under reflux for 45
minutes. Hydrochloric acid (2cm^3) was added, the mixture heated for a
further 15 minutes and then cooled to room temperature. The red solution
was filtered and concentrated *in vacuo* until a pink solid crystallised
out. Filtration gave 2-amino-3-amido-4-phenylpyrrole (0.08g, 24%) with
an infrared spectrum and m.p. identical to those of a sample prepared
by the method described above.

__Method C.__   2-Amino-3-cyano-4-phenylpyrrole (0.20g) was dissolved
in sulphuric acid (1.00g) and the solution allowed to stand at room
temperature for 18 hours. The reaction mixture was poured into water
and the yellow solid which separated was collected, but it rapidly
decomposed to an intractable tar. The filtrates were basified gradually
(20% aqueous sodium hydroxide) until no further solid precipitated.
Filtration gave a product with a m.p. and infrared spectrum identical to
those of the starting material.

__Method D.__   2-Amino-3-cyano-4-phenylpyrrole (1.00g) was dissolved
in sulphuric acid (6cm^3) and the solution warmed on a steam bath for
5 minutes. The brown viscous reaction mixture was poured into water
(40cm^3) whereupon a small amount of a brown solid separated but rapidly
decomposed to an intractable black tar on exposure to the atmosphere.

The filtrate was basified gradually to pH 10 (20% aqueous sodium hydroxide) and a solid precipitated which also decomposed to a brown intractable tar.

Method C.   2-Amino-3-cyano-4-phenylpyrrole (0.50g), hydrogen peroxide ($3cm^3$, '100 vol'), 25% aqueous sodium hydroxide ($2cm^3$) and ethanol ($5cm^3$) were heated together on a steam bath for 1 hour. The yellow solution was filtered and concentrated *in vacuo* whereupon a yellow solid separated. Filtration gave a product which could not be purified sufficiently to obtain any satisfactory spectroscopic data.

Attempted preparation of 3-amido-2-p-nitrobenzoylamino-4-phenylpyrrole (201).

3-Cyano-4-phenyl-2-p-nitrobenzoylaminopyrrole (0.15g), 20% aqueous sodium hydroxide ($3cm^3$) and 3% aqueous hydrogen peroxide ($5cm^3$) were heated together on a steam bath for 30 minutes. 3% aqueous hydrogen peroxide ($2cm^3$) was added and the mixture refluxed for 1 hour, and then allowed to cool. The solution was extracted with ether ($3 \times 10cm^3$) to remove some coloured impurity, and then acidified (dilute hydrochloric acid). The precipitated solid had a m.p. and infrared spectrum identical to those of p-nitrobenzoic acid. Removal of the solvent from the aqueous filtrates and washings gave only inorganic material and a dark intractable tarry solid.

Attempted preparation of methyl 3-amido-2-nitropyrrole-4-carboxylate (205).

A solution of dimethyl 2-nitropyrrole-3,4-dicarboxylate (1.00g) in ethanol ($50cm^3$) was saturated at 0° with ammonia. The solution was heated in a steel bomb at 150° for 24 hours and then cooled to room temperature. The reaction vessel was opened and the solvent removed *in vacuo* to give a dark viscous liquid which was triturated with ether to give a yellow solid whose m.p. and infrared spectrum were identical to those of the

starting material.

Attempted preparation of methyl 2-nitro-3-N-phenylcarbamoylpyrrole-4-carboxylate. (206).

Dimethyl 2-nitropyrrole-3,4-dicarboxylate (1.00g) and freshly distilled aniline (0.50g) were heated under reflux in petroleum spirit (30cm^3, b.p. 60-80°). The reaction mixture was allowed to cool and then filtered to give a yellow solid whose infrared spectrum and melting point were identical to those of the starting material.

Attempted preparation of methyl 2-nitro-3-Nphenylcarbamoylpyrrole-4-carboxylate (207).

Dimethyl 2-nitropyrrole-3,4-dicarboxylate (1.00g) and benzylamine (0.50g) were heated together for I hour at 100°. The resultant dark viscous liquid was triturated with several solvents but could not be induced to solidify.

Attempted preparation of 2-methyl-5-phenylpyrrolo[2,3-d]pyrimidin-4(3H)-one (168).

Method A. 2-Acetamido-3-amido-4-phenylpyrrole (0.20g) was heated on a steam bath with phosphoryl chloride (0.50g) for I hour. The darkly coloured reaction mixture was cooled, poured onto ice and then extracted with ether. The extracts were dried (magnesium sulphate) and the solvent removed *in vacuo* to give a green solid (0.15g, 75%) whose infrared spectrum and m.p. were identical to those of the starting material.

Method B. A solution of 2-acetamido-3-amido-4-phenylpyrrole (0.50g) in digol (20cm^3) was heated under reflux for 3 hours in a nitrogen atmosphere. The reaction mixture was cooled and poured onto ice and the

dark solid which separated was recrystallised from methanol (charcoal) to give 2-acetamido-3-cyano-4-phenylpyrrole (0.35 g, 76%) with an infra-red spectrum and m.p. identical to those of a sample prepared by the method described above in this section.

Method C.　　2-acetamido-3-amido-4-phenylpyrrole (0.05g) and ethylpolyphosphate (0.50g) were heated on a steam bath for I hour. The reaction mixture was poured into water and extracted with chloroform. Removal of the solvent *in vacuo* gave a solid with a m.p. and infrared spectrum identical to those of the starting material.

Method D.　　See the preparation of 3-amido-2-amino-4-phenylpyrrole (166), methods A and B, above.

III   <u>SYNTHESES FROM 4-IMINO-5-PHENYLPYRROLO[2,3-d]-[3,1]THIAZIN-2(1H)THIONE.</u>

<u>4-Imino-5-phenylpyrrolo[2,3-d]-[3,1]thiazin-2(1H)-thione. (219)</u>

2-Amino-3-cyano-4-phenylpyrrole (5.0g), carbon disulphide (50cm^3) and pyridine (40cm^3) were stirred together at 30°. The temperature was slowly raised to 60-65°, the solution stirred and refluxed for 2 hours, and then allowed to cool overnight. Addition of diethyl ether (100cm^3) gave the pyrrolothiazine (5.4g, 76%), yellow plates, m.p. >360° (from a large volume of methanol).

Found: C, 55.6; H, 3.4; N, 16.2; S, 24.7%; <u>M</u>, 259.024450.

$C_{12}H_9N_3S_2$ requires: C, 55.3; H, 3.3; N, 15.9; S, 24.4%; <u>M</u>, 259.023789.

$\nu_{max.}$ 3400(=NH), 3250(thiazine NH), 3100(pyrrole NH), 1630, 1590, 1550, 1520, 1425, 1390, 960cm^{-1}.

<u>5-Phenylpyrrolo[2,3-d]pyrimidin-2,4 (1H,3H)-dithione(221).</u>

<u>Method A.</u>   4-Imino-5-phenylpyrrolo[2,3-d] [3,1]thiazin-2(1H)-thione (0.2g) was heated with 5% aqueous potassium hydroxide solution (5cm^3), on a steam bath for 30 minutes. The solution was cooled and the precipitated 2-amino-3-cyano-4-phenylpyrrole (0.03g, 21%) was collected and washed several times with water. The filtrate and washings were acidified with acetic acid to precipitate the <u>pyrrolopyrimidine dithione</u> (0.10g, 50%). m.p. >360°. The product was purified by precipitation with acetic acid from solution in 5% aqueous potassium hydroxide.

Found: C, 55.1; H, 3.4; N, 15.9; S, 23.6%; <u>M</u>, 259.023179.

$C_{12}H_9N_3S_2$ requires: C, 55.6; H, 3.5; N, 16.2; S, 24.7%; <u>M</u>, 259.023789.

$\nu_{max.}$ 3250(pyrimidine NH), 3100(pyrrole NH), 1625, 1570, 1.170, 1040cm^{-1}.

Method B.   2-Amino-3-cyano-4-phenylpyrrole (0.5g), carbon disulphide (5cm^3) and pyridine (4cm^3) were heated under reflux for 20 hours. The solution was cooled and treated with ether to give the dithione (0.3g, 42%) with infrared spectrum, mass spectrum and m.p. identical to those of the product obtained by method A.

4-Imino-2-methylthio-5-phenylpyrrolo[2,3-d]-[3,1]thiazine hydroiodide (225).

4-Imino-5-phenylpyrrolo[2,3-d]-[3,1]thiazin-2(1H)-thione (0.5g) and methyl iodide (1.5g) were boiled under reflux in methanol (25cm^3) for I hour. The solution was filtered hot, concentrated to small volume *in vacuo* and allowed to cool. Addition of ether (25cm^3) produced a pale yellow precipitate of the pyrrolo[2,3-d]-[3,1]thiazinium iodide (0.72g, 93%), yellow needles, m.p. 197-8°, (from a mixture of methanol and ethyl acetate).

　　　　Found: C, 39.0; H, 3.0; N, 10.2; S, 15.3%; $\underline{M}$, ($\underline{M}^+$-HI), 273.038161.

C$_{13}$H$_{12}$N$_3$S$_2$I requires: C, 38.9; H, 3.0; N, 10.4; S, 15.9%; $\underline{M}$, ($\underline{M}^+$-HI), 273.039429.

　　　　$\nu_{max.}$ (NUJOL) 3400(imino NH), 3200(pyrrole NH), 2800 (=NH-) 1620, 1580, 1460, 940cm^{-1}.

　　　　τ(TFA) 7.13(3H, s, I-C$\underline{H}_3$),

　　　　　　　　2.62(IH, d, J=2.5Hz, 6-$\underline{H}$),

　　　　　　　　2.38(5H, s, 5-phenyl protons)

3-Cyano-2-dimethylthiomethyleneamino-4-phenylpyrrole (224).

Method A.   A solution of 4-imino-5-phenylpyrrolo[2,3-d]-[3,1] thiazin-2(1H)-thione (1.0g) and methyl iodide (2.0g) in 5% aqueous potassium hydroxide (25cm^3) was stirred for I hour at room temperature. Filtration gave the dimethylthiomethyleneamino pyrrole (1.01g, 91%), colourless

prisms, m.p. 139-139.5° (from ethylacetate).

Found: C, 58.8; H, 4.4; N, 14.6; S, 22.1%; $\underline{M}$, 287.053633.

$C_{14}H_{13}N_3S_2$ requires: C, 58.5; H, 4.5; N, 14.6; S, 22.3%; $\underline{M}$, 287.055088.

$\nu_{max.}$ (KBr)   3250(NH), 2240(C≡N), 1580, 1550, 1460, 1440, 1010, 940cm^{-1}.

τ(CDCl$_3$)   7.45(6H, s, C$\underline{H}_3$S-C-S.C$\underline{H}_3$),

3.25(1H, d, 5-$\underline{H}$)

2.2-2.7(6H, m, N$\underline{H}$ and phenylprotons).

<u>Method B.</u>   Methyl iodide (2.0g) was added to a stirred solution of 4-imino-2-methylthio-5-phenylpyrrolo[2,3-$\underline{d}$]-[3,1]thiazine hydroiodide (0.80g) in 5% aqueous potassium hydroxide (25cm^3). After 30 minutes at room temperature the reaction mixture was filtered to give 3-cyano-2-dimethylthiomethyleneamino-4-phenylpyrrole (0.57g, 99%) with an infrared spectrum and m.p. identical to those of a sample prepared by Method A.

<u>Method C.</u>   To a solution of methyl 3-cyano-4-phenylpyrrole-2-dithiocarbamate (0.05g) in 5% aqueous potassium hydroxide (5cm^3), methyl iodide (0.10g) was added. The reaction mixture was filtered to give 3-cyano-2-dimethylthiomethyleneamino-4-phenylpyrrole (0.03g, 59%), with an infrared spectrum and m.p. identical to those of a sample prepared by Method A.

<u>The reaction of 3-cyano-2-dimethylthiomethyleneamino-4-phenylpyrrole with aqueous alkali.</u>

3-cyano-2-dimethylthiomethyleneamino-4-phenylpyrrole (0.10g) was heated under reflux with 5% aqueous potassium hydroxide (8cm^3) for 3 hours, until solution was effected. The reaction mixture was cooled to 0° and filtered to give 2-amino-3-cyano-4-phenylpyrrole (0.05g, 70%) with an infrared spectrum and m.p. identical to those of a sample prepared

by the method described above (P.105).

The reaction of 4-imino-2-methylthio-5-phenylpyrrolo[2,3-d]-[3,1]-thiazine hydroiodide with aqueous alkali.

A solution of 4-imino-2-methylthio-5-phenylpyrrolo[2,3-d]-[3,1]-thiazine hydroiodide (0.90g) in 5% aqueous potassium hydroxide (15cm^3) was stirred at room temperature for I hour. The green solution was acidified with hydrochloric acid and the precipitated solid collected, washed with water and dried to give methyl 3-cyano-4-phenylpyrrole-2-dithiocarbamate (226) (0.90g, 100%). Attempts to recrystallise the product resulted in its decomposition.

Found: $\underline{M}$, 273.037897.

$C_{13}H_{11}N_3S_2$ requires: $\underline{M}$, 273.039439.

3-cyano-2-dimethylthiomethyleneamino-I-methyl-4-phenylpyrrole (227).

4-Imino-5-phenylpyrrole[2,3-d]-[3,1]thiazin -2(IH)-thione (1.0g) was stirred with diazomethane (ca 3.0g) in diethyl ether for 24 hours. The solvent was allowed to evaporate and the resulting yellow gum was chromatographed on a neutral alumina column using light petroleum (b.p. 40-60°), diethyl ether as eluent. Removal of the solvent from the first fraction collected gave the N-methylpyrrole (0.3g, 26%), colourless prisms, m.p. 154-5° (from ethylacetate).

Found: C, 59.6; H, 5.2; N, 13.8; S, 21.5%; $\underline{M}$, 301.070388.

$C_{15}H_{15}N_3S_2$ requires: C, 59.8; H, 5.0; N, 13.9; S, 21.3%; $\underline{M}$, 301.070737.

$\nu_{max.}$ 2250(C≡N), 1540, 1340, 1110, 970cm^{-1}.

$\tau(CDCl_3)$ 7.42(3H, s, N-$\underline{CH}_3$),

6.56(3H, s, s-$\underline{CH}_3$),

6.31(3H, s, s-$\underline{CH}_3$),

3.20(IH, s, 5-$\underline{H}$),

2.2-2.7(5H, m, phenyl protons).

A second fraction was eluted from the column. Removal of the solvent *in vacuo* gave 3-cyano-2-dimethylthiomethyleneamino-4-phenylpyrrole (224) (0.4g, 36%), with a m.p. and infrared spectrum identical to those of samples prepared by the methods described above.

The yield of (227) was increased, (35%), and that of (224) decreased, (9%), when the reaction time was increased to 36 hours.

3-n-Butyl-4-imino-5-phenylpyrrolo[2,3-d]pyrimidin-2(1H,3H)-thione (232).

Method A.   4-imino-5-phenylpyrrolo[2,3-d]-[3,1]-thiazin-2(1H)-thione (0.50g) was heated under reflux for 25 minutes with a solution of n-butylamine (0.50g) in water (10cm^3). The reaction mixture was cooled to 0° and the yellow solid filtered off thoroughly washed with water to give the N-alkylated pyrrolo[2,3-d]pyrimidine (0.50g, 87%),yellow plates, m.p. 230-231° (from a large volume of 95% ethanol).

Found: C, 64.5; H, 6.1; N, 18.7; S, 10.9%; $\underline{M}$, 298.125620.
$C_{16}H_{18}N_4S$ requires: C, 64.4; H, 6.0; N, 18.8; S, 10.7%; $\underline{M}$, 298.125212.

$\nu_{max.}$ (Nujol) 3420(imino NH), 3200(thioamide NH), 3100(pyrrole NH), 1640, 1560, 1540, 1075, 755cm^{-1}.

An NMR spectrum was not obtained since no suitable solvent was available.

Acidification of the aqueous filtrates and washings, from the above product gave 5-phenylpyrrolo[2,3-d]pyrimidin-2,4(1H,3H)-dithione (0.01g 2%) with a m.p. and infrared spectrum identical to those of a sample prepared by the methods described above.

Method B.   4-imino-5-phenylpyrrolo[2,3-d]-[3,1]thiazin-2(1H)-thione (0.2g) and n-butylamine (1.0g) were mixed in a thick-walled glass tube and cooled in liquid nitrogen. The tube was evacuated, sealed by fusing the glass and, after standing at room temperature for 3 hours

heated at 100° for 20 minutes. The reaction vessel was again cooled in liquid nitrogen, opened and allowed to stand at room temperature for several hours. The yellow solid was collected and triturated with ether to give the pyrrolopyrimidine (0.21g, 91%) with a m.p. and infrared spectrum identical to those of a sample prepared by method A.

3-Ethyl-4-imino-5-phenylpyrrolo[2,3-d]pyrimidin-2(1H,3H)-thione (233).

Method A. 4-Imino-5-phenylpyrrolo[2,3-d]-[3,1]thiazin-2(1H)-thione (1.0g) was heated under reflux with 50% ^{v}/v aqueous ethylamine (15cm^3) for 20 minutes. Yellow plates separated and the reaction mixture was cooled and filtered. The product was washed thoroughly with water and dried at 110° to give the pyrrolopyrimidine (0.55g, 86%), yellow plates, m.p. 245-246°, (the solid was purified by washing with a mixture of ethanol and acetone).

Found: C, 62.0; H, 5.1; N, 20.6%; $\underline{M}$, 270.093385.

$C_{14}H_{14}N_4S$ requires: C, 62.2; H, 5.2; N, 20.7%; $\underline{M}$, 270.093913.

$\nu_{max.}$ (Nujol) 3410(imino NH), 3200(thioamide NH), 3100(pyrrole NH), 1635, 1560, 1530, 1050, 750cm^{-1}.

τ(TFA) 8.46(3H, t, J=7Hz, N-CH$_2$-$\underline{CH}_3$),

5.30(2H, q, J=7Hz, N-$\underline{CH}_2$-CH$_3$),

3.15(2H, s, 1-N$\underline{H}$ and 6-$\underline{H}$),

2.75(5H, s, phenylprotons).

The aqueous filtrates and washings were acidified (glacial acetic acid) to give 5-phenylpyrrolo[2,3-d]pyrimidin-2,4-(1H,3H)-dithione (0.30g, 30%) with a m.p. and infrared spectrum identical to those of an authentic sample.

Method B.   4-Imino-5-phenylpyrrolo$[2,3-\underline{d}]$-$[3,1]$thiazin-2(1H)-thione (0.50g) was added to anhydrous ethylamine (ca. $5cm^3$) in a thick-walled glass tube cooled in liquid nitrogen. The tube was evacuated, sealed by fusing the glass and heated at 100° for 20 minutes. The reaction mixture was treated as described above, under the preparation of the 3-butyl derivative (P.126).to give the pyrrolopyrimidine (0.45g, 86%) with an infrared spectrum and m.p. identical to those of a sample prepared by Method A.

4-Imino-3-methyl-5-phenylpyrrolo$[2,3-\underline{d}]$pyrimidin-2(1H,3H)-thione (234).

4-Imino-5-phenylpyrrolo$[2,3-\underline{d}]$-$[3,1]$thiazin-2(1H)-thione (1.0g) was heated under reflux with 25% aqueous methylamine solution ($20cm^3$) for 15 minutes. The bright yellow plates which separated were filtered off, thoroughly washed with water and dried at 100° to give the N-methyl-pyrrolopyrimidine (0.53g, 54%). m.p. 240 decomp. The product was purified for analysis by washing with hot methanol.

Found: C, 60.7; H, 4.7; N, 21.7; S, 12.2%; $\underline{M}$, 256.079120.

$C_{13}H_{12}N_4S$ requires: C, 60.9; H, 4.7; N, 21.9; S, 12.5%; $\underline{M}$, 256.078264.

$\nu_{max.}$ (Nujol)   3420(imino NH), 3200(thioamide NH), 3100(pyrrole NH),

1640, 1570, 1540, 1410, $1055cm^{-1}$.

τ(TFA)   6.01(3H, s, 3-C$\underline{H}_3$),

3.15(2H, broad s, 1-N$\underline{H}$ and 6-$\underline{H}$),

2.72(5H, s, phenylprotons).

The aqueous filtrates and washings were acidified (glacial acetic acid) to give a fine yellow precipitate of 5-phenylpyrrolo$[2,3-\underline{d}]$ pyrimidin-2,4(1H,3H)-dithione (0.40g, 40%) with an infrared spectrum and m.p. identical to those of samples prepared by the methods described above.

4-<u>Amino</u>-5-<u>phenylpyrrolo</u>[2,3-<u>d</u>]<u>pyrimidin</u>-2(1H)-<u>thione</u> (235).

4-Amino-5-phenylpyrrolo[2,3-<u>d</u>]-[3,1]thiazin-2(1H)-thione (2.0g) was
heated in a steel bomb with liquid ammonia (20cm^3) at 100° for 2 hours.
The reaction vessel was cooled, opened and left to stand until all gases
had dispersed. The residual yellow gum was triturated with ethyl acetate
to give a crude solid which was washed several times with hot aqueous
acetone to give the 4-<u>aminopyrrolo</u>[2,3-<u>d</u>]<u>pyrimidine</u> (0.80g, 43%), pale
yellow prisms, m.p. >360°. (The product was purified for analysis by
washing with hot acetone.

Found: C, 56.2; H, 4.0; N, 21.8; S, 12.8%; M, 242.063599
$C_{12}H_{10}N_4S2/3H_2O$ requires: C, 56.7; H, 4.4; N, 22.0; S, 12.6%; M, 242.062615.

$\nu_{max.}$ (Nujol) 3375(NH), 3300(NH), 3100(NH), 1645, 1590, 1580,
1530, 1490, 1170, 1150, 755, 700cm^{-1}.

An NMR spectrum was not obtained since no suitable solvent was
available.

Concentration of the acetone washings gave 5-phenylpyrrolo[2,3-<u>d</u>]
pyrimidin-2,4(1H,3H)-dithione (0.20g, 10%) with an infrared spectrum and
m.p. identical to those of samples prepared by the methods described
above.

3-<u>Amino</u>-4-<u>imino</u>-5-<u>phenylpyrrolo</u>[2,3-<u>d</u>]<u>pyrimidin</u>-2(1H,3H)-<u>thione</u> (236).

4-Amino-5-phenylpyrrolo[2,3-<u>d</u>]-[3,1]thiazin-2(1H)-thione (1.0g) and
a solution of hydrazine hydrate (1.0g) in water (5cm^3) were heated on a
steam bath for 5 minutes. The yellow solid which precipitated was
collected, washed with water and dried to give the 3-<u>aminopyrrolopyrimidine</u>
(0.9g, 91%), colourless plates, m.p. 290° decomp., (from aqueous dimethyl
formamide).

Found: C, 54.9; H, 4.8; N, 26.2%; $\underline{M}$, 257.073619.

$C_{12}H_{11}N_5S.\frac{1}{2}H_2O$ requires: C, 54.2; H, 4.5; N, 26.3%; $\underline{M}$, 257.073513.

$\nu_{max.}$ (Nujol) 3400(NH), 3275(NH), 3225(NH), 3150(NH), 1630, 1530, 1470, 1130, 1020 and 750cm^{-1}.

An NMR spectrum was not obtained since no suitable solvent was available.

The aqueous filtrates and washings were acidified (glacial acetic acid) to give a yellow precipitate of 5-phenylpyrrolo[2,3-$\underline{d}$]pyrimidin-2,4(1H,3H)-dithione (0.02g, 2%) with an infrared spectrum and m.p. identical to those of samples prepared by the methods described above.

3-Benzyl-4-imino-5-phenylpyrrolo|2,3-d|pyrimidin-2(1H,3H)-thione (237).

Method A.   A thick-walled glass tube containing 4-imino-5-phenyl-pyrrolo[2,3-$\underline{d}$] [3,1]thiazin-2(1H)-thione (0.5g) and benzylamine (1.5g) was cooled in liquid nitrogen, evacuated and sealed by fusing the glass. When the tube attained room temperature it was heated to 100° and maintained at this temperature for 4½ hours. The vessel was opened in liquid nitrogen and then allowed to attain room temperature. The residual gummy solid was triturated with ether to give the 3-benzylpyrrolo[2,3-$\underline{d}$] pyrimidine (0.60g, 94%), m.p. 234-6° (the product was purified by washing with hot methanol and sublimation at 220°, 1mm).

Found: C, 68.4; H, 5.0; N, 16.8; S, 9.8%; $\underline{M}$, 332.110441.

$C_{19}H_{16}N_4S$ requires: C, 68.7; H, 4.8; N, 16.9; S, 9.6%; $\underline{M}$, 332.109563.

$\nu_{max.}$ (Nujol) 3430(imino NH), 3150(thioamide and pyrrole NH), 1640, 1600, 1570, 1535, 1470cm^{-1}.

An N.M.R. spectrum was not obtained since no suitable solvent was available.

4-<u>Imino</u>-3,5-<u>diphenylpyrrolo</u>[2,3-<u>d</u>]<u>pyrimidin</u>-2(1H,3H)-<u>thione</u> (238).

A mixture of 4-imino-5-phenylpyrrolo[2,3-<u>d</u>]-[3,1]thiazin-2(1H)-thione (1.0g) and aniline (1.0g) was heated under reflux for 3 hours in a nitrogen atmosphere and then allowed to cool to room temperature. Ether (30cm^3) was added and the precipitated brown solid collected. Further washing with ether gave the <u>diphenylpyrrolopyrimidine</u> (0.4g, 33%) yellow prisms, m.p. >300° (from dilute aqueous ethanol).

Found: C, 65.6; H, 4.5; N, 16.8; S, 9.7%; <u>M</u>, 318.093321.

$C_{18}H_{14}N_4S.\frac{1}{2}H_2O$ requires: C, 66.0; H, 4.5; N, 17.1; S, 9.8%; <u>M</u>, 318.093913.

$\nu_{max.}$ 3400(imino NH), 1620, 1600, 1570, 1460, 750cm^{-1}.

4-<u>Imino</u>-3-(4-<u>methylphenyl</u>)-5-<u>phenylpyrrolo</u>[2,3-<u>d</u>]<u>pyrimidin</u>-2(1H,3H)-<u>thione</u> (239).

A mixture of 4-imino-5-phenylpyrrolo[2,3-<u>d</u>]-[3,1]thiazin-2(1H)-thione (0.50g) and 4-methylaniline (1.50g) was heated in a sealed glass tube at 100° for 5 hours. The tube was cooled and opened and ether was added to the reaction mixture to give the <u>pyrrolopyrimidine</u> (0.29g, 45%), m.p. 207-8°. An analytical sample was not obtained.

Found: <u>M</u>, 332.107507.

$C_{19}H_{16}N_4S$ requires: <u>M</u>, 332.109563.

$\nu_{max.}$ (Nujol) 3400(NH), 3300(NH), 3150(NH), 1620, 1590, 1470, 1385, 1245, 800, 755, 710cm^{-1}.

N-(3-<u>cyano</u>-4-<u>phenylpyrrol</u>-2-<u>yl</u>)-N'-<u>isopropylthiourea</u> (241).

<u>Method A.</u> A mixture of 4-imino-5-phenylpyrrolo[2,3-<u>d</u>]-[3,1]thiazin-2(1H)-thione (0.25g), isopropylamine (0.50g) and water (3cm^3) was heated on a steam bath for 30 minutes. The reaction mixture was cooled, filtered washed with water and dried to give the <u>thiourea</u> (0.23g, 84%), colourless needles, m.p. 240° decomp., (from water: acetone, 1:3).

Found: C, 63.3; H, 5.8; N, 19.9%; $\underline{M}$, 284.108572.

$C_{15}H_{16}N_4S$ requires: C, 63.4; H, 5.6; N, 19.7%; $\underline{M}$, 284.109563.

$\nu_{max.}$ (Nujol) 3325(NH), 3175(NH), 3125(NH), 2225(C≡N), 1630, 1540, 1490, 1250, 750, 690cm^{-1}.

An NMR spectrum was not obtained because no suitable solvent was available.

Acidification of the aqueous filtrates and washings gave 5-phenyl-pyrrolo[2,3-$\underline{d}$]pyrimidin-2,4(1H,3H)-dithione (0.05g, 2%) with an infrared spectrum and m.p. identical to those of samples prepared by the methods described above.

Method B. 4-Imino-5-phenylpyrrolo[2,3-$\underline{d}$]-[3,1]thiazin-2(1H)-thione (0.50g) and isopropylamine (1.50g) were mixed in a thick-walled glass tube. The tube was evacuated, sealed by fusing the glass and heated at 110° for 4.25 hours. When the tube was opened an all gases allowed to disperse, addition of ether gave the thiourea (0.47g, 85%) with an infrared spectrum and m.p. identical to those of a sample prepared by method A.

4-Amino-2-methylthio-5-phenylpyrrolo[2,3-$\underline{d}$]pyrimidine (248).

3-cyano-2-dimethylthiomethyleneamino-4-phenylpyrrole (0.73g) and liquid ammonia (ca. 5cm^3) were placed in a thick-walled glass vessel immersed in liquid nitrogen. The vessel was evacuated, sealed by fusing the glass and allowed to attain room temperature. The temperature was raised to 100° and maintained for 1 hour until no solid remained in the reaction mixture. The reaction vessel was opened in liquid nitrogen and the ammonia allowed to disperse at room temperature over several hours leaving the 4-aminodeazapurine, (0.61g, 93%), colourless prisms, m.p. 290-2°, (from ethanol).

Found: C, 59.8; H, 4.7; N, 21.5%; $\underline{M}$, 256.079369.

$C_{13}H_{12}N_4S$ requires: C, 59.9; H, 4.8; N, 21.5%; $\underline{M}$, 256.078264.

$\nu_{max.}$ (Nujol) 3500(NH), 3350(NH), 3200(NH), 3100(NH), 1645,

1610(NH$_2$), 1560, 1470, 1270, 960, 810, 785,

760, 710cm^{-1}.

4-Imino-3-methyl-2-methylthio-5-phenylpyrrolo[2,3-d]pyrimidine (249).

Method A.   3-Cyano-2-dimethylthiomethyleneamino-4-phenylpyrrole

(0.3g) and 25% aqueous methylamine (10cm^3) were heated under reflux for

16 hours. The reaction mixture was cooled and filtered in an ice bath

to give the 3-methylpyrrolopyrimidine (0.25g, 88%), colourless needles,

m.p. 260° decomp., (from 95% ethanol).

Found: C, 62.4; H, 5.3; N, 20.6; S, 11.5%; $\underline{M}$, 270.094978.

$C_{14}H_{14}N_4S$ requires: C, 62.2; H, 5.2; N, 20.7; S, 11.8%; $\underline{M}$, 270.094978.

$\nu_{max.}$ (Nujol) 3350(imino NH), 3100(pyrrole NH), 1620, 1575,

1475, 1140, 1110, 760cm^{-1}.

Method B.   4-Imino-3-methyl-5-phenylpyrrolo[2,3-d]pyrimidine-2(1H,

3H)-thione (0.30g), 5% aqueous sodium hydroxide solution (10cm^3) and

methyl iodide (1.0g) were heated under reflux on a steam bath for 1.5

hours. The solution was cooled and filtered to give the pyrrolopyrimidine

(0.30g, 95%) with an infrared spectrum and m.p. identical to those of a

sample prepared by method A.

3-Ethyl-4-imino-2-methylthio-5-phenylpyrrolo[2,3-d]pyrimidine (250).

3-Cyano-2-dimethylthiomethyleneamino-4-phenylpyrrole (0.50g) and

anhydrous ethylamine (*ca.* 10cm^3) were heated at 100° for 3 hours in a

thick-walled glass tube according to a procedure similar to that

described for the 4-aminopyrrolopyrimidine (248), above. The reaction

vessel was opened in liquid nitrogen, allowed to attain room temperature

and the resulting yellow solid was collected to give the 3-ethylpyrrolo-
pyrimidine (0.50g, 99%), colourless needles, m.p. 230° decomp., (from
absolute ethanol).

Found: C, 63.0; H, 5.7; N, 19.5; S, 11.5%; $\underline{M}$, 284.10789.

$C_{15}H_{16}N_4S$ requires: C, 63.4; H, 5.6; N, 19.7; S, 11.3%; $\underline{M}$, 284.109563.

$\nu_{max.}$ (Nujol) 3350(imino NH), 3100(pyrrole NH), 1620, 1580, 1520,
1460, 1110, 770cm^{-1}.

3-Butyl-4-imino-2-methylthio-5-phenylpyrrolo[2,3-d]pyrimidine (251).

Method A. 3-Cyano-2-dimethylthiomethyleneamino-4-phenylpyrrole
(1.00g) and n-butylamine (5cm^3) were mixed intimately in a thick-walled
glass tube and heated for 12 hours at 110° according to the procedure
described for the 4-aminopyrrolopyrimidine (248) above. The tube was
opened and the residual gummy solid triturated with ether to give the
pyrrolopyrimidine (1.00g, 92%), colourless prisms, m.p. 187-188°, (from
ethanol).

Found: C, 64.9; H, 6.4; N, 17.9; S, 10.5%; $\underline{M}$, 312.140398.

$C_{17}H_{20}N_4S$ requires: C, 65.4; H, 6.4; N, 17.9; S, 10.2%; $\underline{M}$, 312.140861.

$\nu_{max.}$ (Nujol) 3350(imino NH), 3100(pyrrole NH), 1620, 1580, 1530,
1470, 1110, 775cm^{-1}.

τ(CDCl$_3$) 8.0-9.2(7H, m, C$\underline{H}_3$-C$\underline{H}_2$.C$\underline{H}_2$-CH$_2$-N)

7.47(3H, s, SC$\underline{H}_3$)

5.80(2H, m, CH$_3$-CH$_2$-CH$_2$-C$\underline{H}_2$-N)

3.38(1H, s, 6-$\underline{H}$)

2.60(5H, m, phenylprotons).

Method B. 3-$\underline{n}$-Butyl-4-imino-5-phenylpyrrolo[2,3-d]pyrimidin-2(1H,
3H)-thione (0.1g), 5% aqueous sodium hydroxide solution (3cm^3) and methyl
iodide (0.5g) were heated underreflux for 1 hour. The reaction mixture

was cooled and filtered to give the 2-methylthiopyrrolopyrimidine
(0.1g, 95%) with an infrared spectrum and m.p. identical to those of a
sample prepared by method A.

2,4-Dimethylthio-5-phenylpyrrolo[2,3-d]pyrimidine (252).

5-Phenylpyrrolo[2,3-d]pyrimidin-2,4(1H,3H)-dithione (0.5g) was
dissolved in 5% aqueous potassium hydroxide (30cm^3). Methyl iodide
(2.0g) was added and the mixture heated under reflux for 10 minutes. The
reaction was cooled to 0° and filtered to give the dimethylthiopyrrolo-
pyrimidine (0.5g, 90%), colourless prisms, m.p. 247-8° (from ethylacetate).

Found: C, 58.1; H, 4.8; N, 14.4; S, 22.6%; M, 287.052526.

$C_{14}H_{13}N_3S_2$ requires: C, 58.5; H, 4.5; N, 14.6; S, 22.3%; M, 287.055088.

$\nu_{max.}$ (KBr) 3150(NH), 1600, 1550, 1530, 1260, 1240, 890cm^{-1}.

τ(CDCl$_3$) 7.41(3H, s, 4-SCH$_3$),

7.31(3H, s, 2-SCH$_3$),

2.91(1H, s, 6-H),

2.5-2.6(5H, m, phenyl protons).

3-Methyl-5-phenylpyrrolo[2,3-d]pyrimidin-2(1H)-thione-4(3H)-one (253).

4-Imino-3-methyl-5-phenylpyrrolo[2,3-d]pyrimidin-2(1H)-thione (0.2g)
in 5% aqueous potassium hydroxide (10cm^3) was heated on a steam bath for
24 hours. The solution was cooled and a small amount of insoluble matter
was removed by filtration. Acidification of the filtrate gave the
pyrrolopyrimidine (0.18g, 87%), colourless prisms, m.p. 310° decomp.
(from ethanol).

Found: C, 60.4; H, 4.3; N, 16.3; S, 12.6%; M, 257.

$C_{13}H_{11}N_3OS$ requires: C, 60.7; H, 4.3; N, 16.3; S, 12.5%; M, 257.

$\nu_{max.}$ 3300(pyrimidine NH), 3150(pyrrole NH), 1650

(C=O), 1615, 1600, 1470, 1380, 1290, 1250, 770cm^{-1}.

3-Ethyl-5-phenylpyrrolo[2,3-d]pyrimidin-2(1H)-thione-4(3H)-one (254).

3-Ethyl-4-imino-5-phenylpyrrolo[2,3-d]pyrimidin-2(1H)-thione (0.15g) was heated under reflux with 5% aqueous potassium hydroxide (20cm^3) for 20 hours. Acidification of the red solution gave the pyrrolopyrimidinone (0.13g, 86%), colourless needles, m.p. 300° decomp., (from aqueous ethanol).

Found: C, 61.3; H, 4.9; N, 15.2; S, 11.5%; M, 271.077529.

C$_{14}$H$_{13}$N$_3$OS requires: C, 62.0; H, 4.8; N, 15.5; S, 11.8%; M, 271.077929.

$\nu_{max.}$ 3350(pyrimidine NH), 3150(pyrrole NH), 1635 (C=O), 1610, 1450, 1200, 760cm^{-1}.

IV   MASS SPECTRA

MASS SPECTRA

The mass spectra of the pyrrolo|2,3-d|pyrimidines prepared during the course of this work are recorded. In addition the spectra of several intermediate substances are included.

In the following diagrams the vertical and horizontal axes represent "Relative Abundance" and "m/e" respectively. In some cases possible framentation pathways are shown and these are supported, in many cases by the presence of peaks due to metastable ions (+) and by accurate mass measurements of specific peaks (e.g. 259). Fragment losses supported by ion source determinations are indicated by ←

(117)

(119)

(118)

HCN — CH₃CN — CH₃CN — C₇H₇

C₂H₂ — C₂H₂

(121)

HCN — CH₃CN — C₃H₃· — HCN — C₂H₅·

CH₃-Pyr-NH· — HCN — CH₃·

HCN — HCN — CH₃CN·HCN — C₄H₇·

CH₂C·NH — C₆H₉N

HCN

(125)

HCN ── CH₃CN ── C₂H₄ / HCN

CH₃CN

159

187

28 51 77 91 118

(134)

C₄H₈

CH₃NCO ── C₃H₇·

150

C₂H₅·

55 70 41 28 96 141 150 168 194 207 221 250

(135)

(137)

(140)

HCN — CO — CH₃NCO — C₂H₄ — 233

Fragmentation scheme for compound (140):

- HCN (93) — CO (148) — CH₃NCO — C₂H₄ — 233
- C₂H₄ (148) — CO (176) — CH₃NCO —
- CH₃NCO (120) — C₄H₈ (177)
- HCN (92) — CO (119) — CO (147) — (CH₃)₂N₂ (175)

(141)

HCN — CH₃ — CO — CH₃NCO — 269

Fragmentation scheme for compound (141):

- HCN — CH₃
- CH₃ — HCN — CO — CH₃NCO
- CO — CH₃ (212)

(144)

(158)

(159)

(187)

(195)

(219)

(221)

(224)

(227)

(232)

(235)

(236)

(237)

(238)

(239)

C₇H₈N
106

76

91

149

183

225

274

299

337

2-NH₂-3-CN-4-Ph-PYRROLE

C₇H₇·

NCS·

C₇H₉N

(241)

28

17

44

31

101

129

168

183

191

201

226

259

284

(246)

(249)

(250)

(251)

(252)

(253)

C_2H_5

O

Ph

N

S

N
H

N
H

(254)

HNCS/C_2H_5N·

HCNO — CN· — C_2H_5· — ·S

271

C_2H_5NH·

184

HCN — ·S

211   238

18

28

44

64

77

102

128

140

155

169

210 211

227

238 239

10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270

V BIOLOGICAL TESTING RESULTS

Female mice (strain CBA/LAC) were innoculated, by subcutaneous (inguinal) transplant, with either R_1 lymphoma or TLX/5 Ascites tumours. Compounds were injected intraperitoneally, as solutions in arachis oil, and the percentage increase in survival time of injected animals over a control group was measured.

Compound (140)

Cell innoculum:- 1.5×10^6

Tumour: R_1 lymphoma

Dosage schedule:- Daily x 5.

DOSE mg/Kg	NUMBER OF ANIMALS	DAYS OF TREATMENT	DAYS OF DEATH	RANGE OF DAYS OF DEATH	AVERAGE	% INCREASE IN SURVIVAL TIME OVER CONTROL
CONTROL	10	-	8,8,8,9,9, 9,9,9,9,9,	8-9	8.7	-
6	5	3-7	8,8,8,9,9.	8-9	8.4	-3.4
30	5	3-7	8,8,8,9,9.	8-9	8.4	-3.4
150	5	3-7	8,8,9,9,9.	8-9	8.6	-1.1

Compound (234)

Cell innoculum:- approximately 10^5

Tumour:- TLX/5 ascites

Dosage schedule: TLX/5 ascites

DOSE mg/Kg	NUMBER OF ANIMALS	DAYS OF TREATMENT	DAYS OF DEATH	RANGE OF DAYS OF DEATH	AVERAGE	% INCREASE IN SURVIVAL TIME OVER CONTROL
80	5	3,4,5,6,7.	10,10,10,10,11.	10-11	10.2	0.9%
200	5	3,4,5,6,7.	10,10,10,10,11	10-11	10.2	0.9%
control	10	untreat-ed	10,10,10,10,10,10,10,10,10,11	10-11	10.1	

Compound (219)

Cell innoculum:- approximately 10^5

Tumour:- TLX/5 ascites

Dosage Schedule:- daily x 5

DOSE mg/Kg	NUMBER OF ANIMALS	DAYS OF TREATMENT	DAYS OF DEATH	RANGE OF DAYS OF DEATH	AVERAGE	% INCREASE IN SURVIVAL TIME OVER CONTROL
160	5	3,4,5,6,7.	9,9,9,9,10	9-10	9.2	-6.2%
400	5	3,4,5,6,7.	10,9,10,10,10	9-10	9.8	0%
control	10	untreat-ed	9,9,10,10,10,10,10,10,10,10,	9-10	9.8	

Compound (221)

Cell innoculum:- approximately 10^5

Tumour:- TLX/5 ascites

Dosage schedule:- daily x 5.

DOSE mg/Kg	NUMBER OF ANIMALS	DAYS OF TREATMENT	DAYS OF DEATH	RANGE OF DAYS OF DEATH	AVERAGE	% INCREASE IN SURVIVAL TIME OVER CONTROL
160	5	3,4,5,6,7.	10,10,10,10,10	10	10	2.0%
400	5	3,4,5,6,7.	9,10,10,10,10	9-10	9.8	0%
control	10	untreated	9,9,10,10,10,10,10,10,10,10	9-10	9.8	

Compound (252)

Cell innoculum:- approximately 10^5

Tumour:- TLX/5 ascites

Dosage schedule:- daily x 5.

DOSE mg/Kg	NUMBER OF ANIMALS	DAYS OF TREATMENT	DAYS OF DEATH	RANGE OF DAYS OF DEATH	AVERAGE	% INCREASE IN SURVIVAL TIME OVER CONTROL
160	5	3,4,5,6,7	9,10,10,11,11	9-11	10.2	0.9%
400	5	3,4,5,6,7	9,10,10,11,11	9-11	10.2	0.9%
control	10	untreated	10,10,10,10,10,10,10,10,10,11	10-11	10.1	

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