SYNTHESIS AND PROPERTIES OF SOME NOVEL IMIDAZOPYRIDINES

BY

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University of Aston in Birmingham

To My Parents

and

Teresa

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SUMMARY

The synthesis and biological properties of known imidazo [4,5-b]pyridines and imidazo[4,5-c]pyridines is reviewed and the rationale behind the work is discussed.

The chemical work concerned the preparation and properties of imidazo -[4,5-b]- and -[4,5-c]- pyridines. Several novel imidazopyridines were prepared by methods involving ring-cyclisation of substituted diaminopyridines and these methods are discussed. Attempts to prepare 2-amino-3H-imidazo[4,5-b]pyridine and 2-amino-1H-imidazo[4,5-c]pyridine were unsuccessful.

Attempts to prepare novel pyridine precursors yielded 2-amino-6-hydroxy-3-phenylazopyridine hydrochloride, 2,3-diamino-6-hydroxy-pyridine dihydrochloride, 2,3-diamino-6-methylpyridine dihydrochloride and 2-amino-4-nitropyridine 1-oxide.

Partial catalytic hydrogenation of 7-nitro-3H-imidazo[4,5-b] pyridine 4-N-oxide yielded 7-amino-3H-imidazo[4,5-b]pyridine 4-N-oxide, which on treatment with phosphoryl chloride yielded 5,7-dichloro-3H-imidazo[4,5-b]pyridine. This dichloro derivative was similarly prepared by treatment of 7-nitro-3H-imidazo[4,5-b] pyridine 4-N-oxide with phosphoryl chloride.

Fusion of 2,3-diamino-6-methylpyridine dihydrochloride with urea gave 5-methyl-3H-imidazo[4,5-b]pyridine-2(1H)-one which underwent nitration and N-oxidation reactions to give respectively, 5-methyl-6-nitro-3H-imidazo[4,5-b]pyridine-2(1H)-one and 1(3)-N-acetyl-5-methyl-imidazo[4,5-b]pyridine-2(3H)-one 4-N oxide.

Reaction of 2-chloro-3,4-diaminopyridine and 3,4,5-

triaminopyridine trihydrochloride with urea gave respectively, 4-chloro-1H-imidazo[4,5-c] pyridine-2(3H)-one and 7-amino-1H-imidazo [4,5-c] pyridine-2(3H)-one. 4-Chloro-1H-imidazo[4,5-c] pyridine-2(3H)-one on N-oxidation gave 4-chloro-1H-imidazo [4,5-c] pyridine-2(3H)-one 5-N-oxide. Similarly, 1H-imidazo [4,5-c] pyridine-2(3H)-one gave the 5-N-oxide.

Seventy two of the compounds prepared in the present study were tested for mutagenicity in the Ames test without liver activation. Ten of these compounds were also tested with liver activation but no significant changes in reversion rates were observed. Fourteen compounds were mutagenic without liver activation and the dose-response and mutagenic potency of these compounds is included in the biological results section.

7-Nitro-3H-imidazo[4,5-b] pyridine 4-N-oxide, 6-hydroxylaminopurine and 2-amino-4-nitropyridine 1-oxide were shown to be base-pair substitution mutagens. 2-Nitrophenyl-imidazo -[4,5-b]- and -[4,5-c]- pyridine derivatives showed potent frameshift mutagenic activity and it is postulated that these compounds are structurally similar to the potent mutagen, 2-nitrosofluorene.

Activation of nitro compounds by bacterial nitro-reductases and the toxicity of some of the compounds to Salmonella are discussed in detail.

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INTRODUCTION

IMIDAZO[4,5-b] PYRIDINES

Nomenclature

Several systems of nomenclature have been used to describe this ring system but under I.U.P.A.C. rules, it is known as imidazo[4,5-b]pyridine and this name is listed in Chemical Abstracts as an approved name.

3H-Imidazo[4,5-b]pyridine (1) may be regarded as an analogue of purine (2) in which the -N= at position 1 has been replaced by a -CH= group (see Scheme 1). Many examples of this ring system have been synthesised as possible antimetabolites to naturally occurring purines and these compounds have often been referred to as 1-deazapurines. For example, the 7-amino derivative has been called 1-deaza-adenine (3)[1].

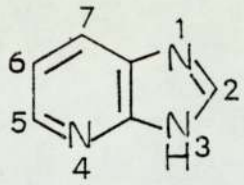
In 1948, Petrow and Saper[2] referred to some imidazo[4,5-b] pyridine derivatives as 4-azabenziminazoles and as late as 1963, Kurihara et al.[3] were using this method of nomenclature. Adler and Albert[4], in 1963, refer to 3H-imidazo[4,5-b]pyridine as 3,4-diazaindole. However, neither of these systems of nomenclature are in general use today.

Synthesis

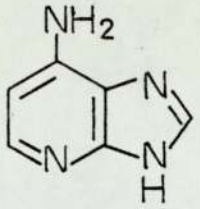
All the imidazo[4,5-b]pyridine derivatives which have been made have been prepared from pyridine precursors. These precursors have been of type (4a) (see Scheme 1).

Cyclisation of the pyridine precursor appears to be a two step process which is initiated by attack on an electron-deficient carbon atom in the cyclising reagent molecule by the electron-rich nitrogen in the 3-position (see 4b in Scheme 1) leading to the formation of an uncyclised intermediate (4c). This intermediate, which can be isolated, undergoes intramolecular cyclisation with

SCHEME 1



(1)



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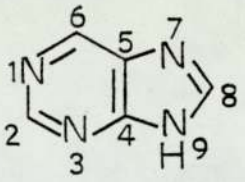
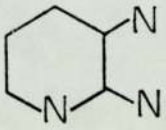
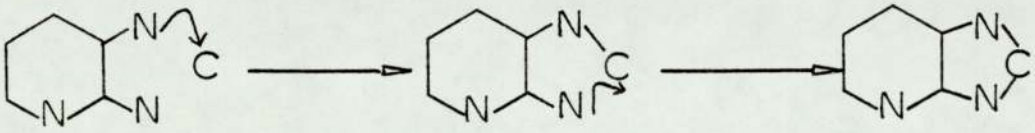


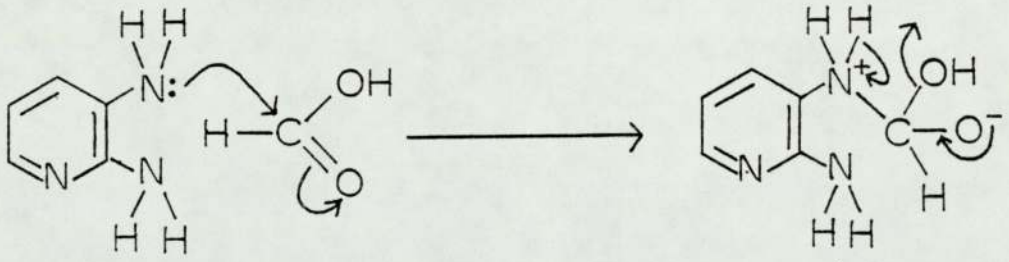
(2)



(4a)

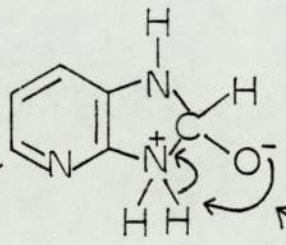


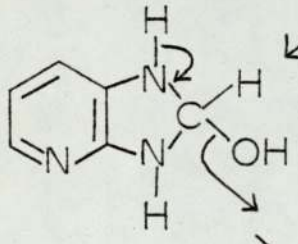
(4b) (4c) (4d)

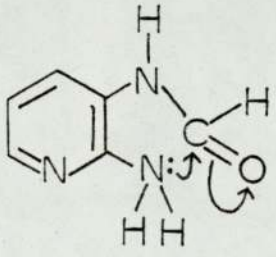


(51)

$-H_2O$

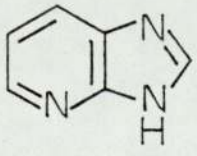






(5)

$-H_2O$



(1)

the loss of a suitable leaving group to give the imidazo[4,5-b] pyridine (4d).

The synthesis of imidazo[4,5-b]pyridines may be subdivided according to the nature of the cyclising agent and the subsequent substituents found at position 2 of the formed imidazo[4,5-b] pyridine.

(i) Preparation with $\geqslant$C - H substituent at position 2.

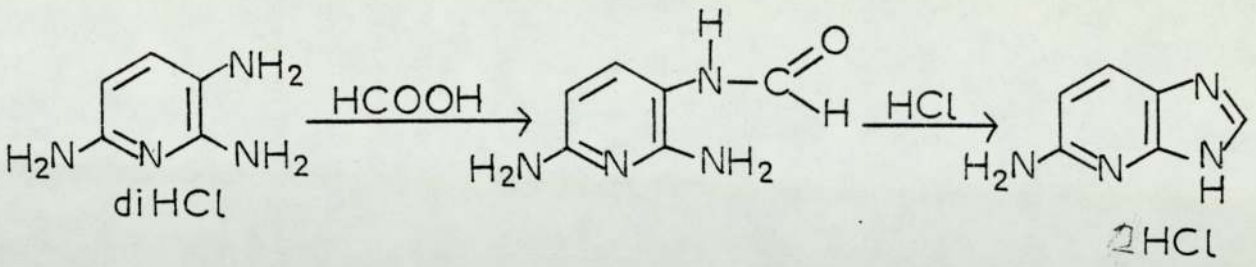
Formic acid

In 1948, Petrow and Saper[2] described the synthesis of some pyrido[3,2-d]pyrimidine and imidazo[4,5-b]pyridine derivatives including 3H-imidazo[4,5-b]pyridine (1). This compound was prepared by the cyclisation of 2,3-diamino pyridine (51) with formic acid. It can be seen from the mechanism proposed in Scheme 1 that the uncyclised intermediate, 2-amino-3-formylamino pyridine (5), is formed. However, this compound was not isolated by Petrow and Saper.[2]

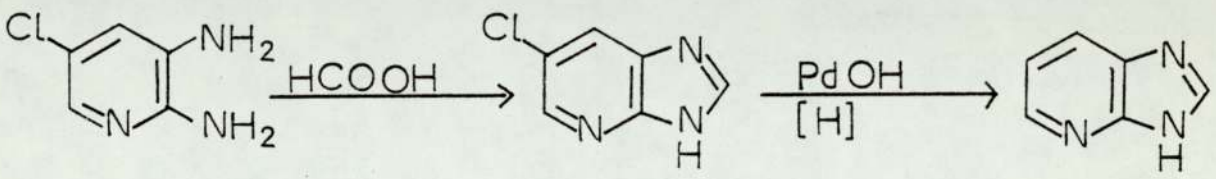
Kögl, van der Want and Salemink[5] also reported the synthesis of (1) from 2,3-diaminopyridine (51) and formic acid in the presence of copper acetate but the yield was poor.

In 1949, Vaughan, Krapcho and English[6] isolated the formyl intermediate, 2,6-diamino-3-formylaminopyridine (9), by treatment of 2,3,6-triaminopyridine dihydrochloride (8) with formic acid and sodium formate (see Scheme 2). Cyclisation of (9) to 5-amino-3H-imidazo[4,5-b]pyridine hydrochloride (7) was effected by heating on a steam bath with concentrated hydrochloric acid. Vaughan and his co-workers also made 6-chloro-3H-imidazo[4,5-b]pyridine (10) by evaporation of a solution of 5-chloro-2,3-diaminopyridine (11) in formic acid to dryness. (10) was hydrogenated with palladium hydroxide catalyst to yield 3H-imidazo[4,5-b]pyridine (1) but the

SCHEME 2



(8) (9) (7)



(11) (10) (1)

(15) R_1=Cl, R_2=H.

(16) R_1=H, R_2=Cl.

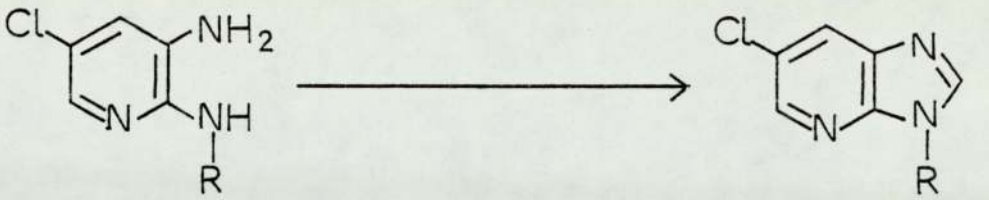
(8) R_1=H, R_2=NH_2. diHCl

(12) R_1=Cl, R_2=H.

(13) R_1=H, R_2=Cl.

(14) R_1=H, R_2=NH_2.

SCHEME 3



(18) R = CH_3.

(19) R = C_2H_5.

(22) R = $CH_2C_6H_5$.

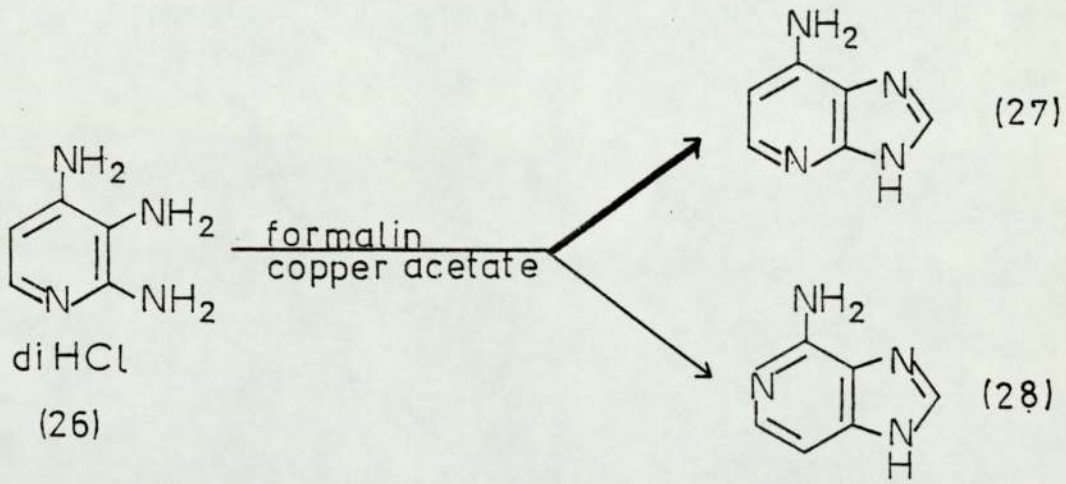
(23) R = $CH_2 \cdot CH_2 \cdot N(CH_3)_2$.

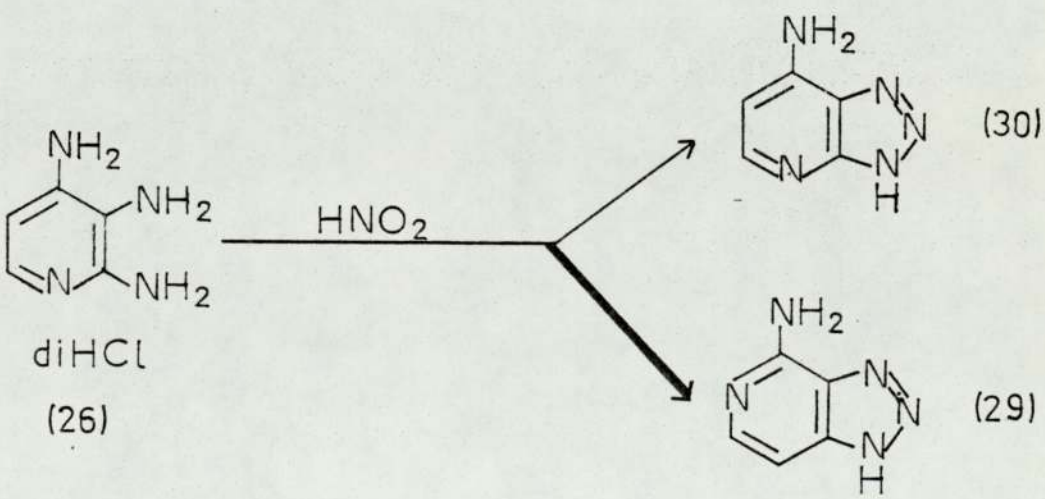
(20) R = CH_3.

(21) R = C_2H_5.

(24) R = $CH_2C_6H_5$.

(25) R = $CH_2 \cdot CH_2 \cdot N(CH_3)_2$.





yield was poor (see Scheme 2).

Korte[7] repeated Petrow and Saper's work in 1952 when he prepared 3H-imidazo[4,5-b]pyridine (1), but he isolated the formyl intermediate (5) which was sublimed in vacuo to form the imidazopyridine (1). Korte also prepared 7-chloro-3H-imidazo[4,5-b] pyridine (12), 5-chloro-3H-imidazo[4,5-b]pyridine (13) and 5-amino-3H-imidazo[4,5-b]pyridine (14) by treatment of 4-chloro-2,3-diaminopyridine (15), 6-chloro-2,3-diaminopyridine (16) and 2,3,6-triaminopyridine dihydrochloride (8) respectively with formic acid to produce the formyl intermediate, which was sublimed to afford the product (see Scheme 2).

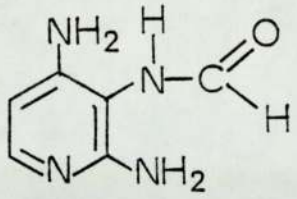
In 1959, Takahashi, Yoneda and Oishi[8] used formic acid to cyclise 3-amino-5-chloro-2-methylaminopyridine (18) and 3-amino-5-chloro-2-ethylaminopyridine (19) to 6-chloro-3-methylimidazo [4,5-b]pyridine (20) and 6-chloro-3-ethyl-imidazo[4,5-b]pyridine (21) respectively (see Scheme 3). Similar reactions were carried out with 3-amino-2-benzylamino-5-chloropyridine (22) and 3-amino-5-chloro-2-(2-dimethylaminoethylamino) pyridine (23) to prepare the corresponding imidazo[4,5-b]pyridines (24) and (25) respectively (see Scheme 3).

In 1960, similar work was reported by Takahashi, Kanematsu, Ohishi and Mizutani[9]. They described the synthesis of 1-substituted-6-chloro-imidazo[4,5-b]pyridines from the corresponding precursors by treatment with formic acid.

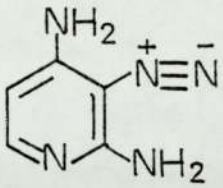
Formaldehyde

In 1949, Roche Products submitted a patent[10] describing the preparation of an imidazopyridine made from the reaction of 2,3,4-triamino pyridine dihydrochloride (26) with a 40% solution of formaldehyde in the presence of copper acetate. The authors were

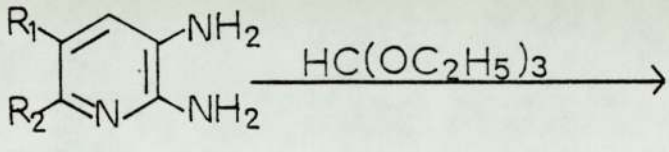
SCHEME 4



(31)

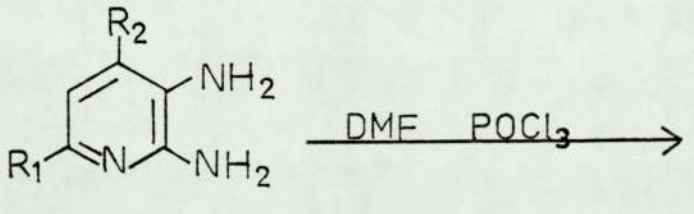


(32)



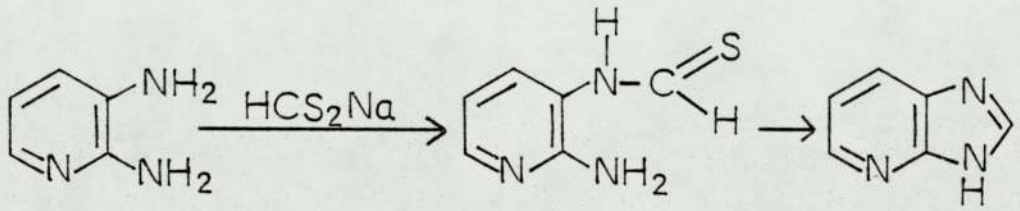
(17) $R_1 = H$ $R_2 = NH_2$.
(33) $R_1 = SO_2NH_2$ $R_2 = H$.

(14) $R_1 = H$ $R_2 = NH_2$.
(34) $R_1 = SO_2NH_2$ $R_2 = H$.

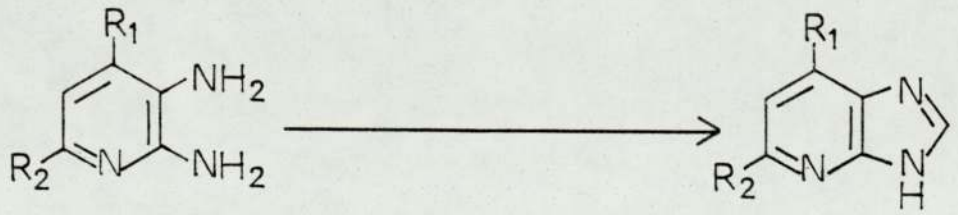


(15) $R_1 = H$ $R_2 = Cl$.
(35) $R_1 = R_2 = Cl$.

(12) $R_1 = H$ $R_2 = Cl$.
(36) $R_1 = R_2 = Cl$.



(51) (37) (1)



(40) $R_1 = H$ $R_2 = F$.
(42) $R_1 = F$ $R_2 = H$.

(41) $R_1 = H$ $R_2 = F$.
(43) $R_1 = F$ $R_2 = H$.

uncertain whether 7-amino-3H-imidazo[4,5-b]pyridine (27) (1-deaza-adenine) or 4-amino-1H imidazo[4,5-c]pyridine (28) (3-deaza-adenine) had been prepared (see Scheme 3).

Kögl, van der Want and Salemink[5] performed the same reaction and published their results concurrently. They claimed that the only product produced was (27). However, in 1969, De Roos and Salemink[12] re-examined the cyclisation of 2,3,4-triaminopyridine dihydrochloride, using several ring closure procedures and showed that although the major product was (27), (28) was always obtained as well.

Triethylorthoformate

In 1948, Kögl, van der Want, and Salemink[5] effected the cyclisation of 2,3,4-triaminopyridine dihydrochloride with triethyl-orthoformate to obtain mainly 1-deaza-adenine as product.

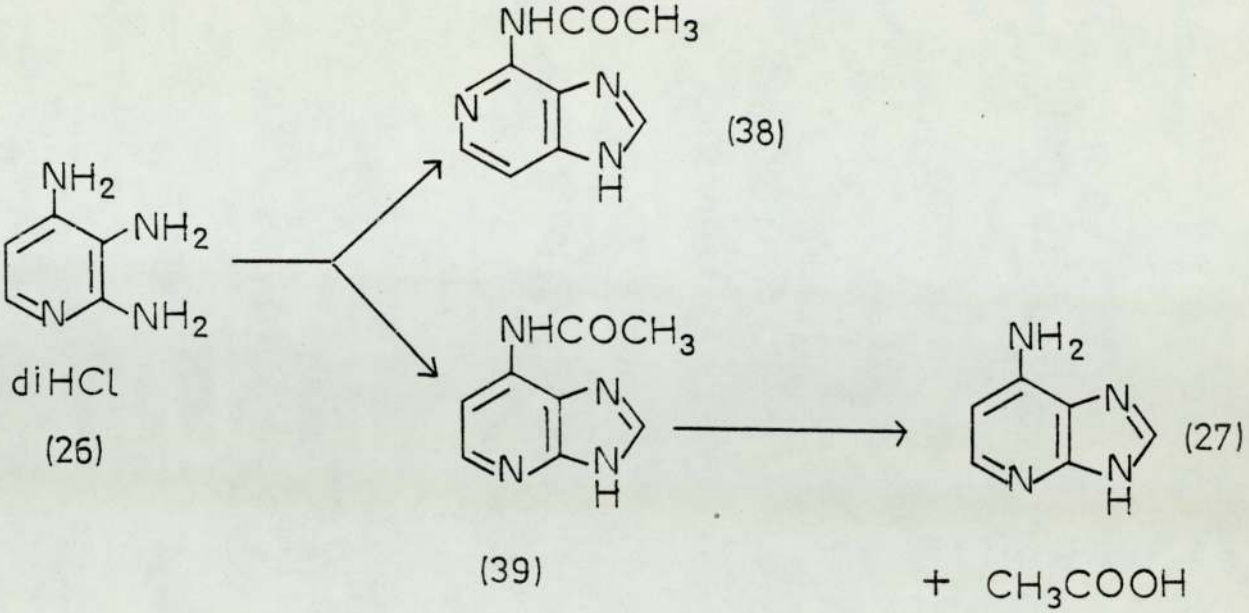
Graboyes and Day[11] used triethylorthoformate in 1957 to cyclise 2,3,6-triaminopyridine dihydrochloride (17) and 2,3-diamino-pyridine-5-sulphonamide hydrochloride (33) to 5-amino-3H-imidazo [4,5-b]pyridine (14) and 3H-imidazo[4,5-b]pyridine-6-sulphonamide (34) respectively (see Scheme 4).

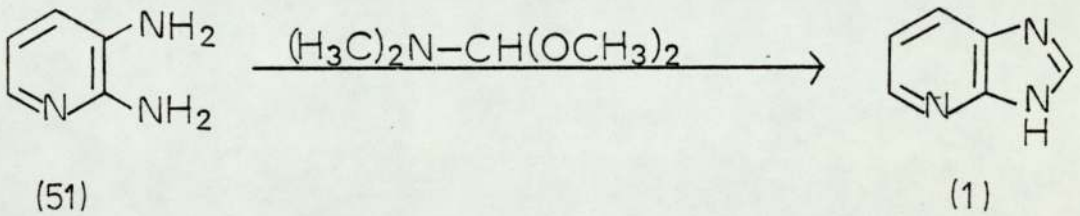
In 1960, Chatterjee, Dhar, Anand and Dhar[13] reported that they obtained better yields of 3H-imidazo[4,5-b]pyridine when triethylorthoformate was used to cyclise 2,3-diaminopyridine instead of formic acid.

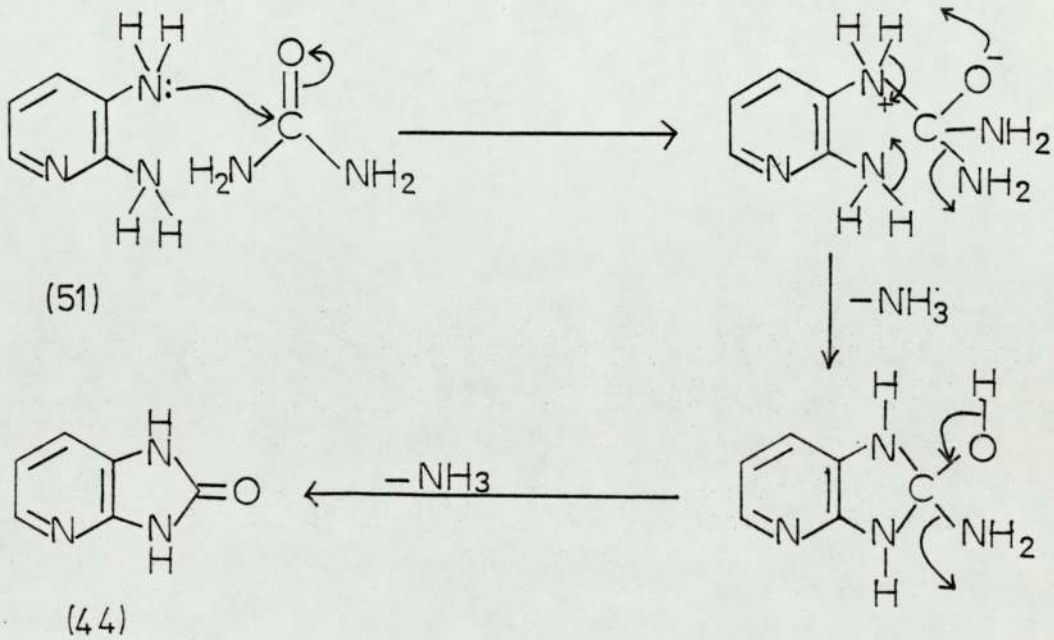
Phosphoryl Chloride and Dimethylformamide

In 1961, Clark and Lister[14] described the use of a mixture of phosphoryl chloride and dimethylformamide for conversion of 4,5-diaminopyrimidines into purines. A feature of the method was the ease with which very weakly basic diamines were converted into purines under mild conditions. Consequently, in 1969, De Roos and Salemink[12] used the method to effect the ring cyclisation of 4-chloro-2,3-diaminopyridine (15) and 4,6-dichloro-2,3-diaminopyridine

SCHEME 5


NHCOCH3
(38)
NH2
NH2
NH2
diHCl
(26)
NHCOCH3
(39)
NH2
(27)
+ CH3COOH


NH2
NH2
(H3C)2N–CH(OCH3)2
(51)
(1)


(51)
–NH3
–NH3
(44)

(35) to 7-chloro-3H-imidazo[4,5-b]pyridine (12) and 5,7-dichloro-3H-imidazo[4,5-b]pyridine (36) respectively, in excellent yields (see Scheme 4). However, in 1972, Schelling and Salemink[15] reported that a better yield of (36) was obtained when (35) was ring-cyclised with formic acid.

Sodium thioformate

In 1948, Kögl, van der Want and Salemink[5] obtained a poor yield of 3H-imidazo[4,5-b]pyridine by treatment of 2,3-diamino pyridine with formic acid in the presence of copper acetate. However, when 2,3-diaminopyridine was treated with aqueous sodium thioformate, the thioformyl pyridine intermediate (37) was obtained which could be cyclised in high yield in boiling pyridine to 3H-imidazo[4,5-b]pyridine (1) (see Scheme 4).

Diethoxymethyl acetate

Montgomery and Hewson[16], in 1965, used diethoxymethyl acetate to effect the cyclisation of 2,3,4-triaminopyridine dihydrochloride to yield a mixture of 4-acetamido-1H-imidazo[4,5-c]pyridine (38) and 7-acetamido-3H-imidazo[4,5-b]pyridine (39) (see Scheme 5). (39) was the major product and could be deacetylated to 1-deaza-adenine (27).

Kroon, van den Brink, Vlietstra and Salemink[17] used diethoxy-methyl acetate in 1976 for the cyclisation of some fluoro-substituted 2,3-diaminopyridines (40) and (42) to yield the corresponding mono-substituted imidazo[4,5-b]pyridines (41) and (43) (see Scheme 4).

N,N-Dimethylformamide dimethyl acetal

Stanovnik and Tisler[18] used this reagent in 1974 to convert 2,3-diaminopyridine (51) to 3H-imidazo[4,5-b]pyridine (1) (see Scheme 5).

(ii) Preparation with $\rangle C=O$ substituent at position 2

Urea

Petrow and Saper[2] reported in 1948 that 3H-imidazo[4,5-b] pyridine-2(1H)-one (44) was produced when a finely ground equimolar mixture of 2,3-diaminopyridine (51) and urea were heated together. The reaction mechanism is similar to that described for formic acid and 2,3-diaminopyridine but in this case two molecules of ammonia are produced instead of water (see Scheme 5).

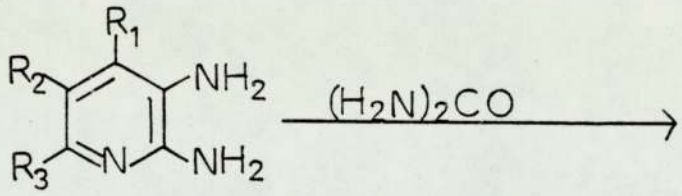
Petrow and Saper also made 6-bromo-3H-imidazo[4,5-b]pyridine-2(1H)-one (45) by the same method from 5-bromo-2,3-diaminopyridine (46).

In 1959, Israel and Day[19] made 6-bromo-7-methyl-3H-imidazo [4,5-b]pyridine-2(1H)-one (47) by heat treatment of a finely ground mixture of urea and 5-bromo-2,3-diamino-4-methylpyridine (48). 6-Bromo-5-methyl-3H-imidazo[4,5-b]pyridine-2(1H)-one (49) was similarly prepared from 5-bromo-2,3-diamino-6-methylpyridine (50) (see Scheme 6).

Carbonyl chloride (phosgene)

Vaughan, Krapcho and English[6] are the only workers who have reported the use of phosgene to cyclise substituted 2,3-diamino-pyridines. This may well be because the extreme toxicity of the reagent outweighs its effectiveness as a cyclising reagent. Treatment of 5-chloro-2,3-diaminopyridine (52) in hydrochloric acid with phosgene yielded 6-chloro-3H-imidazo[4,5-b]pyridine-2(1H)-one (53) (see Scheme 6). The mechanism of the cyclisation is similar to urea and formic acid, but hydrochloric acid is produced instead of ammonia and water respectively.

SCHEME 6



(51) $R_1 = R_2 = R_3 = H$

(46) $R_1 = R_3 = H$ $R_2 = Br$

(48) $R_1 = CH_3$ $R_2 = Br$ $R_3 = H$

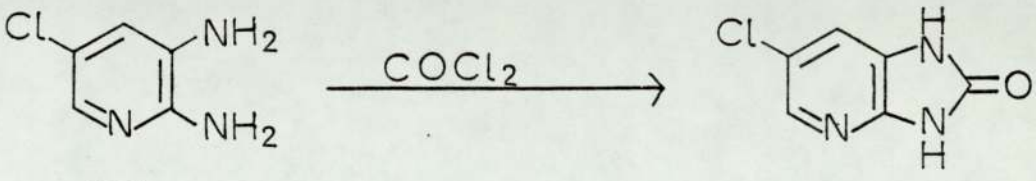
(50) $R_1 = H$ $R_2 = Br$ $R_3 = CH_3$

(44) $R_1 = R_2 = R_3 = H$

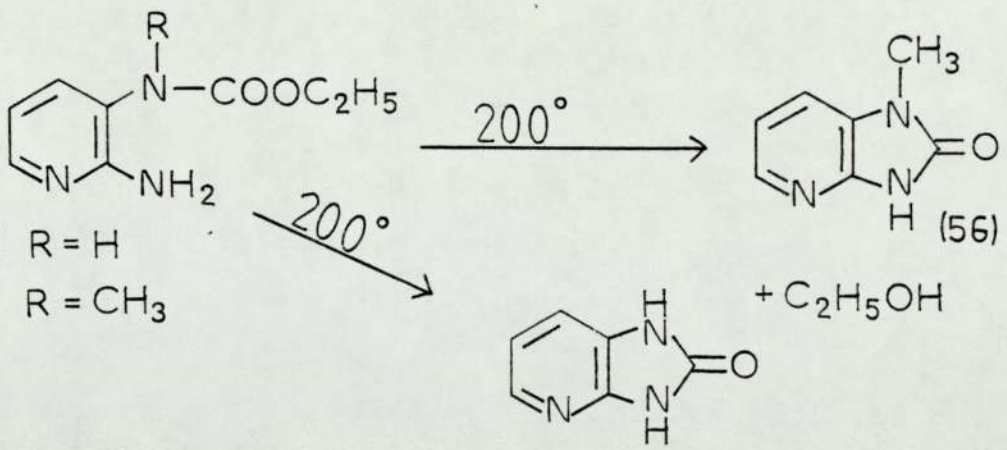
(45) $R_1 = R_3 = H$ $R_2 = Br$

(47) $R_1 = CH_3$ $R_2 = Br$ $R_3 = H$

(49) $R_1 = H$ $R_2 = Br$ $R_3 = CH_3$



(52) (53)

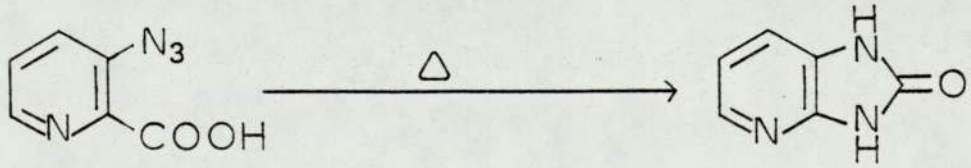


(55) R = H

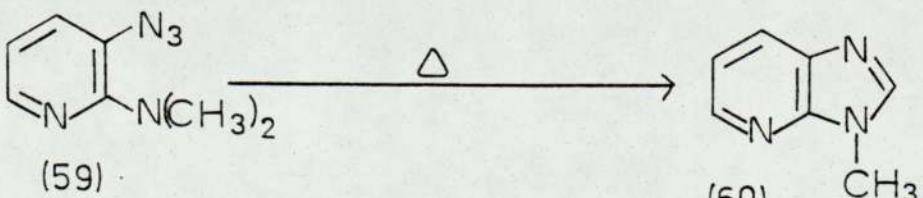
(54) R = CH_3

(56)

(44)



(58) (44)



(59) (60)

Substituted pyridine carbamates

In 1957, Clark-Lewis and Thompson[20] reported that pyrolysis of ethyl N-methyl-2-amino-3-pyridine carbamate (54) and ethyl-2-amino-3-pyridine carbamate (55) respectively gave 1-methyl-2-hydroxy-imidazo[4,5-b]pyridine (56) and 3H-imidazo[4,5-b] pyridine-2(1H)-one (44) (see Scheme 6).

Pyrolytic Cyclisation

Harrison and Smith[140] reported in 1959 that when 3-azido-nicotinic acid (58) was heated in hot toluene or xylene, pyrolytic cyclisation occurred to yield 3H-imidazo[4,5-b]pyridine-2(1H)-one (44) (see Scheme 6) and in 1966, Smalley[141] reported the preparation of 3-methyl-imidazo[4,5-b]pyridine (60) by pyrolytic cyclisation of 3-azido-2-dimethylaminopyridine (59).

(iii) Preparation with $\diagdown C=S$ substituent at position 2

Thiophosgene

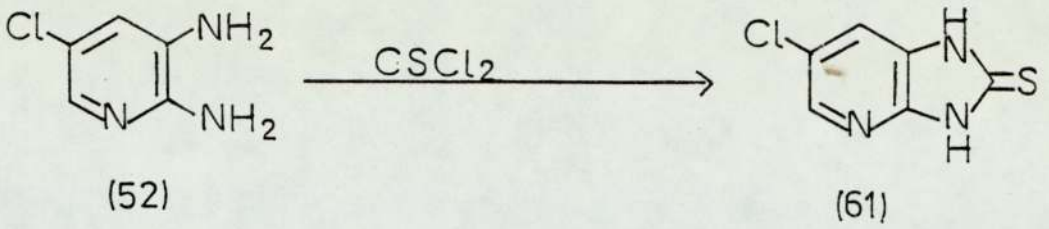
In 1949, Vaughan, Krapcho and English[6] reported that treatment of a substituted 2,3-diaminopyridine in hydrochloric acid with thiophosgene produced the corresponding substituted imidazopyridine-thione. They described the preparation of 6-chloro-3H-imidazo [4,5-b]pyridine-2(1H)-thione (61) from 5-chloro-2,3-diaminopyridine (52) but the yield was very poor (< 5%) (see Scheme 7).

No further use appears to have been made of thiophosgene as a cyclising reagent in the synthesis of imidazopyridines, but this is probably due to its toxicity and the low yields obtained.

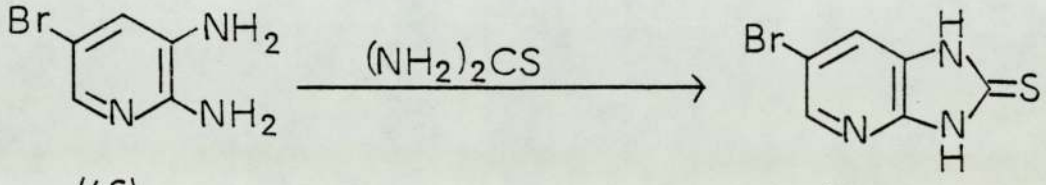
Thiourea

Petrow and Saper[2] fused together 5-bromo-2,3-diaminopyridine (46) and thiourea to yield 6-bromo-3H-imidazo[4,5-b]pyridine-2 (1H)-thione (62) (see Scheme 7). The product was identical to that obtained by treatment of 5-bromo-2,3-diaminopyridine with carbon disulphide and potassium hydroxide.

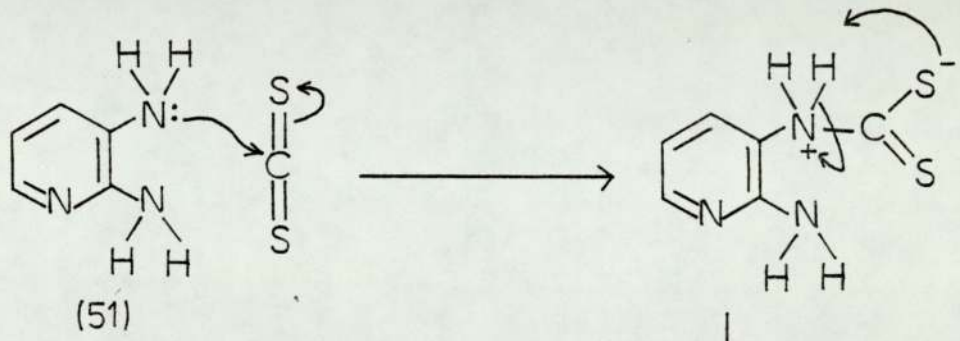
SCHEME 7



(52) (61)



(46) (62)

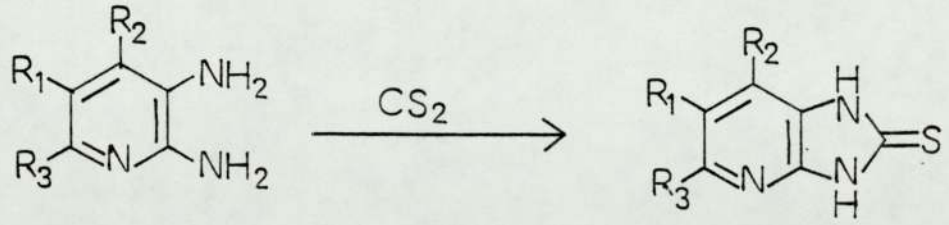


(51) (63)

Carbon disulphide

Petrow and Saper[2], in 1948, prepared 3H-imidazo[4,5-b] pyridine-2(1H)-thione (63) by treatment of 2,3-diaminopyridine (51) with carbon disulphide and ethanol. The proposed mechanism of the reaction is set out in Scheme 7. These two workers similarly prepared 6-bromo-3H-imidazo[4,5-b]pyridine-2(1H)-thione (64) from 5-bromo-2,3-diaminopyridine (46) but potassium hydroxide was used in place of ethanol.

In 1959, Israel and Day[19] made 6-bromo-7-methyl-3H-imidazo [4,5-b] pyridine-2(1H)-thione (65) and 6-bromo-5-methyl-3H-imidazo [4,5-b]pyridine-2(1H)-thione (66) from 5-bromo-2,3-diamino-4-methylpyridine (48) and 5-bromo-2,3-diamino-6-methylpyridine (50) respectively with carbon disulphide in potassium hydroxide solution (see below).



(51) $R_1 = R_2 = R_3 = H$.

(46) $R_1 = Br$ $R_2 = R_3 = H$.

(48) $R_1 = Br$ $R_2 = CH_3$ $R_3 = H$.

(50) $R_1 = Br$ $R_2 = H$ $R_3 = CH_3$.

(63) $R_1 = R_2 = R_3 = H$.

(64) $R_1 = Br$ $R_2 = R_3 = H$.

(65) $R_1 = Br$ $R_2 = CH_3$ $R_3 = H$.

(66) $R_1 = Br$ $R_2 = H$ $R_3 = CH_3$.

(iv) Preparations with $\rangle C=$ - R alkyl or aryl substituents in position 2

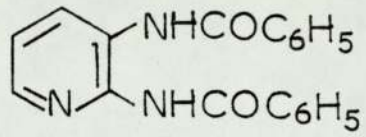
Anhydrides

Takahashi and Yajima, in 1946[21], reported the synthesis of 2-phenyl- and 2-cinnamyl- 3H-imidazo[4,5-b]pyridine by treatment of 2,3-diaminopyridine (under unstated conditions) with benzoic anhydride and cinnamic anhydride respectively. However, in 1964, Garmaise and Komlossy[22] found that when they heated benzoic anhydride with 2,3-diaminopyridine at 180° for 2 h, the only identifiable product was 2,3-dibenzamidopyridine (67) (see Scheme 8). This result threw doubt on the validity of other results published from the same laboratory at this time. Takahashi, Yoshikawa and Ichikawa[23], for example, claimed that when 2,3-diaminopyridine was heated with acetic anhydride, 2-methyl-3H-imidazo[4,5-b]pyridine was obtained. Indeed, this agreed with Chichibabin and Kirsanov's[24] findings in 1927, but when Garmaise and Komlossy[22] refluxed 2,3-diaminopyridine and acetic anhydride in the expectation of obtaining the 2-methyl derivative, only 2,3-diacetamidopyridine could be isolated.

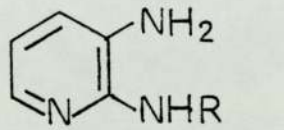
In 1959, Takahashi, Yoneda and Oishi[8] heated 3-amino-5-chloro-2-methylaminopyridine (68) and 3-amino-5-chloro-2-ethylaminopyridine (69) with acetic anhydride to produce 2,3-dimethylimidazo[4,5-b] pyridine (70) and 3-ethyl-2-methyl-imidazo[4,5-b]pyridine (71) respectively (see Scheme 8).

In 1963, Kurihara and Chiba[3] heated 5-bromo-2,3-diaminopyridine with acetic anhydride at 110° and obtained 5-bromo-2,3-diacetamido-pyridine (72) which was heated at 315° to obtain 6-bromo-2-methyl-3H-imidazo [4,5-b]pyridine (73) with loss of acetic acid (see Scheme 8).

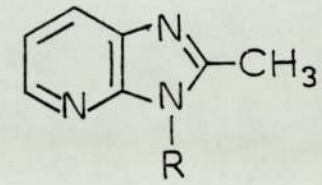
SCHEME 8



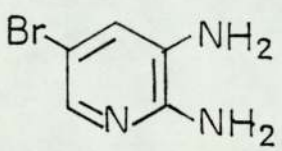
(67)



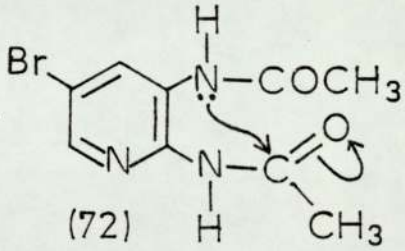
(68) R = CH_3
(69) R = C_2H_5



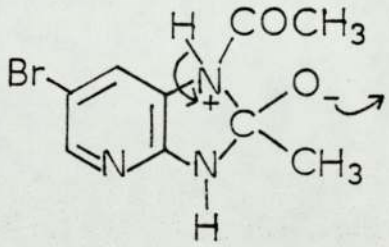
(70) R = CH_3
(71) R = C_2H_5

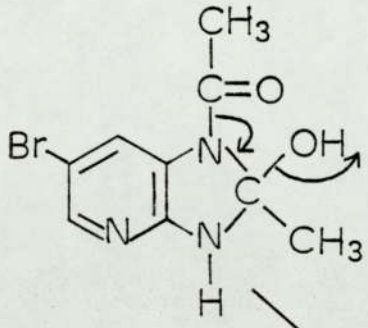


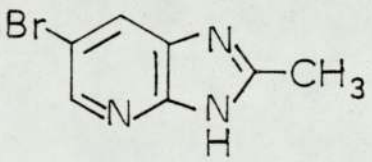
(46)



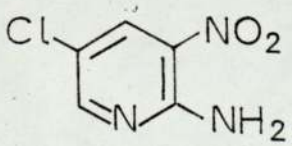
(72)



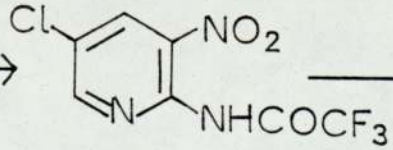




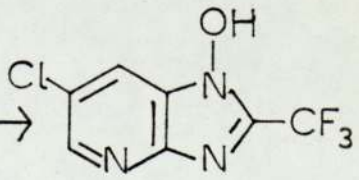
(73)



(74)



(75)



(76)

In 1975, concurrently with the present work, Doherty and Fuhr[25] used trifluoroacetic anhydride to prepare substituted imidazo[4,5-b]pyridines. (The trifluoroacetic anhydride is not strictly the cyclising agent but it is convenient to mention the method of synthesis here.) 2-Amino-5-chloro-3-nitropyridine (74) was treated with trifluoroacetic anhydride and the resulting 5-chloro-3-nitro-2-trifluoroacetamidopyridine (75) was cyclised by hydrogenation to give 6-chloro-1-hydroxy-2-trifluoromethyl-imidazo [4,5-b]pyridine (76) (see Scheme 8). Several imidazo[4,5-b] pyridines were made in this way and a second patent[26] was submitted to include a number of difluoroalkanoic anhydrides, thus greatly increasing the number of substituted imidazopyridines.

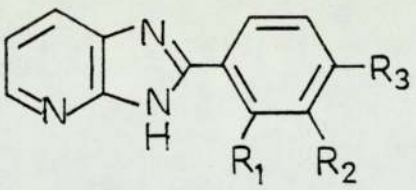
Carboxylic acids

Takahashi and Yajima[21], in 1946, claimed to have made 2-(o-aminophenyl)-3H-imidazo[4,5-b]pyridine (77) by heating 2,3-diamino-pyridine with anthranilic acid (under unstated conditions), but when Garmaise and Komlossy[22] repeated this work in 1964, they only managed to isolate a salt of anthranilic acid and 2,3-diaminopyridine. Garmaise and Komlossy readily obtained the cyclised product (77) by condensing 2,3-diaminopyridine with anthranilic acid in polyphosphoric acid. Indeed, polyphosphoric acid was found to be a useful and convenient condensing agent for the preparation of several aryl derivatives e.g. (77) (78) (79) (80) (see Scheme 9).

Baldwin, Lumma, Novello, Ponticello and Sprague[27] reported in 1977 on the use of polyphosphoric acid in the preparation of 2-(3-pyridyl)- and 2-(4-pyridyl)-3H-imidazo[4,5-b]pyridine, (81) and (82) respectively (see Scheme 9).

Chatterjee, Jain and Anand[28] reported in 1965 that condensation of 2,3-diaminopyridine with mandelic acid (83b) in boiling xylene

SCHEME 9

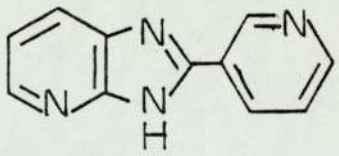


(78) $R_1 = R_2 = H$ $R_3 = CH_3$

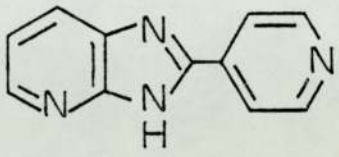
(77) $R_1 = NH_2$ $R_2 = R_3 = H$

(79) $R_1 = R_2 = H$ $R_3 = Cl$

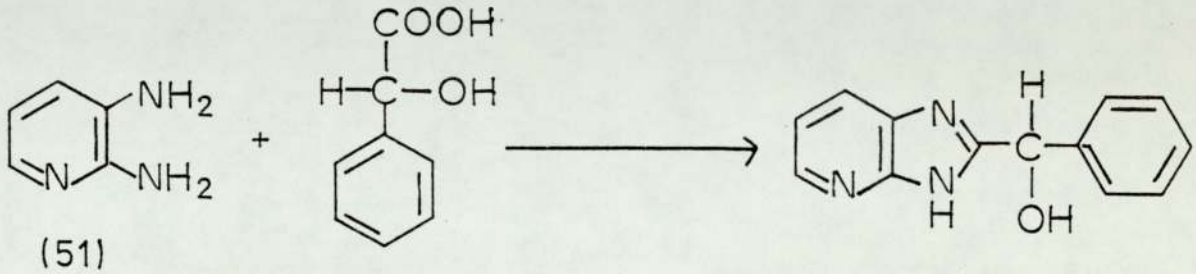
(80) $R_1 = R_2 = H$ $R_3 = F$



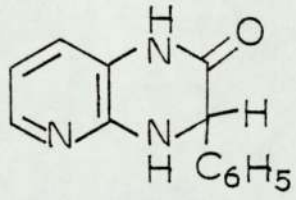
(81)



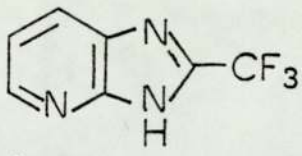
(82)



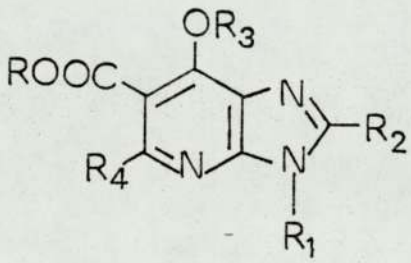
(51) (83b) (83a)



(84)



(85)



(86)

R = H or lower alkyl.

R_1 = H, lower alkyl, phenyl.

R_2 = H, lower alkyl, phenyl.

R_3 = lower alkyl, lower alkenyl, phenyl, substituted phenyl.

R_4 = H, lower alkyl, phenyl.

yielded 2-(α-hydroxybenzyl)-3H-imidazo[4,5-b]pyridine (83a). They also reported that (83a) could be prepared by fusion of 2,3-diaminopyridine with mandelic acid at 150°. However, Berner, Reinshagen and Koch[29] have questioned the validity of these results in a more recent report, claiming that Chatterjee et al.[28] had prepared the isomeric pyridopyrazinone (84) instead of the imidazopyridine claimed (see Scheme 9).

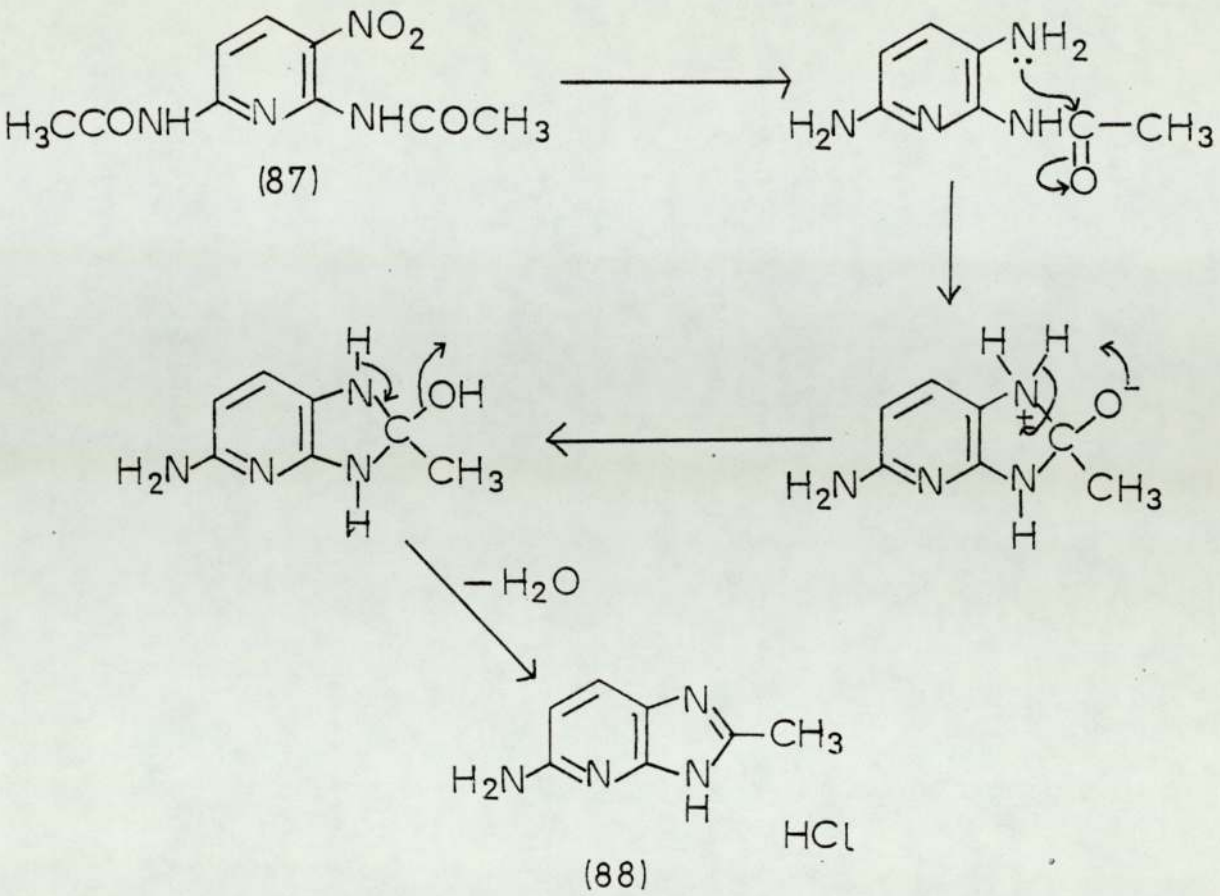
Fisons Ltd.[30] submitted a patent in 1966 which described the preparation of some 2-substituted imidazo[4,5-b]pyridines which were prepared in a search for new compounds with analgesic properties. 2,3-Diaminopyridine was treated with a trihaloacetic acid or functional derivative in an inorganic acid halide medium to obtain the product. e.g. 2,3-diaminopyridine was treated with trifluoroacetic acid to obtain 2-trifluoromethyl-3H-imidazo[4,5-b] pyridine (85) (see Scheme 9).

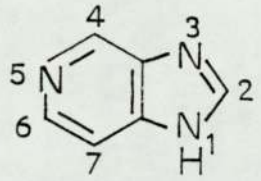
Regensburg and Tegernheim[31] submitted a patent in 1977 describing the preparation of some polysubstituted imidazo[4,5-b] pyridines of the general formula (86). The compounds described were prepared by ring cyclisation of the appropriate substituted pyridine with an organic acid of formula R_2 - COOH (see Scheme 9).

Stannous chloride

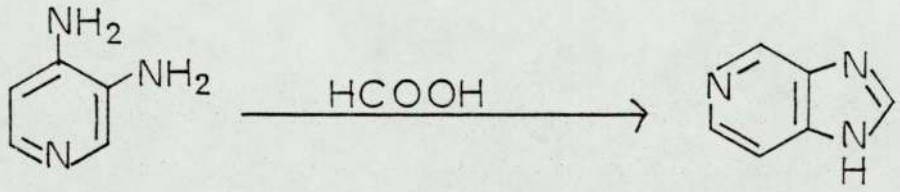
In 1947, Bernstein, Stearns, Shaw and Lott[32] reported the preparation of the first imidazo[4,5-b]pyridine derivative by treatment of 2,6-diacetamido-3-nitropyridine (87) with stannous chloride and hydrochloric acid to form 5-amino-2-methyl-3H-imidazo [4,5-b]pyridine hydrochloride (88). Presumably, the -nitro group is reduced first followed by ring cyclisation to the imidazopyridine. At some stage in the reaction, deacetylation occurs, but at what point is uncertain (see Scheme 10).

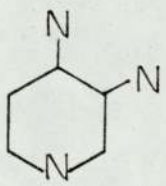
SCHEME 10





(89)





(90)

IMIDAZO[4,5-c]PYRIDINES

Nomenclature

The nomenclature of the imidazo[4,5-c]pyridine ring system is similar to that of the -[4,5-b]- system and although different systems of nomenclature have been used to describe this ring system, under generally accepted rules it is known as imidazo[4,5-c] pyridine (89). The imidazo[4,5-c]pyridine can be regarded as an analogue of purine (2) in which the -N= at position 3 has been replaced by a -CH= group. Derivatives of the -[4,5-c]- system have been referred to in the literature as 3-deazapurines[1,5,12]. In 1963, Adler and Albert[4] referred to 1H-imidazo[4,5-c]pyridine as 3,5-diazaindole and as recently as 1966, Barlin[33] named some derivatives as 1,3,5-triazaindenes.

Synthesis

Many of the methods used to prepare imidazo[4,5-b]pyridine derivatives are applicable and have been used as such to prepare -[4,5-c]- derivatives as well. The majority of -[4,5-c]- derivatives have been prepared from pyridine precursors of type (90) but three cases exist where the bicyclic ring system has been formed from imidazole precursors.

From Pyridine Precursors

(i) Preparation with $\rangle C-H$ substituent at position 2

Formic acid

In 1938, Weidenhagen and Weeden[34] treated 3,4-diaminopyridine (92) with formic acid but did not obtain the expected 1H-imidazo [4,5-c]pyridine (91). Instead, a 3-N-formyl derivative was obtained which, on treatment with hydrochloric acid yielded formic acid and 3,4-diaminopyridine. (91) was obtained by heating 3,4-diaminopyridine and formic acid in a sealed tube at 140° in the

presence of copper salts. However, in 1956, Albert and Pederson[35] treated 3,4-diaminopyridine with formic acid and obtained 1H-imidazo [4,5-c]pyridine after removal of the excess formic acid in vacuo.

In 1949, Salemink and van der Want[36] prepared 4-hydroxy-1H-imidazo[4,5-c]pyridine (93) and 4-hydroxy-6-methyl-1H-imidazo [4,5-c]pyridine (94) by treatment of 3,4-diamino-2-hydroxypyridine dihydrochloride (95) and 3,4-diamino-2-hydroxy-6-methylpyridine (96) respectively with formic acid (see Scheme 11).

Formaldehyde

In 1948, Kögl, van der Want and Salemink[5] prepared a mixture of 1- and 3- deaza-adenine by the reaction of 2,3,4-triaminopyridine dihydrochloride with formaldehyde solution (40%) in the presence of copper acetate. However, 4-amino-1H-imidazo[4,5-c]pyridine (28) was the minor product.

Triethylorthoformate

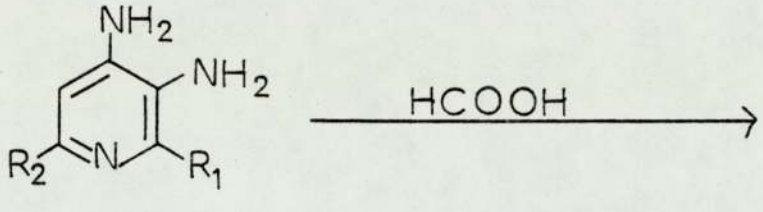
In 1966, Rousseau and Robins[37] used a 1 : 1 mixture of acetic anhydride and triethylothoformate to ring cyclise some substituted 3,4-diaminopyridines (97) (98) (99) (103) to the corresponding imidazopyridines (100) (101) (102) (104) (see Scheme 11).

Barlin[33] used the same method and conditions in 1966 to effect the ring cyclisation of 2-chloro-3,4-diaminopyridine (103) and 2-chloro-4,5-diaminopyridine (97) to 4-chloro-1H-imidazo[4,5-c] pyridine (104) and 6-chloro-1H-imidazo[4,5-c]pyridine (100) respectively.

Sodium thioformate

In 1948, Kögl, van der Want and Salemink[5] obtained an excellent yield of 1H-imidazo[4,5-c]pyridine (105) by heating the thioformyl derivative (106), prepared by treatment of 3,4-diaminopyridine with sodium thioformate in pyridine (see Scheme 11).

SCHEME 11

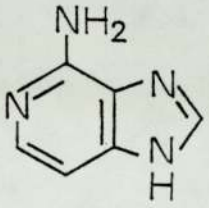


(95) R_1 = OH R_2 = H

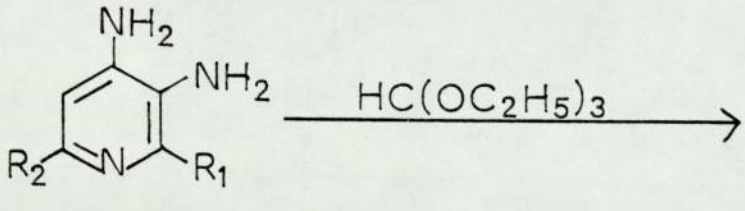
(96) R_1 = OH R_2 = CH_3

(93) R_1 = OH R_2 = H

(94) R_1 = OH R_2 = CH_3



(28)



(97) R_1 = H R_2 = Cl

(98) R_1 = Cl R_2 = Cl

(99) R_1 = H R_2 = NH_2

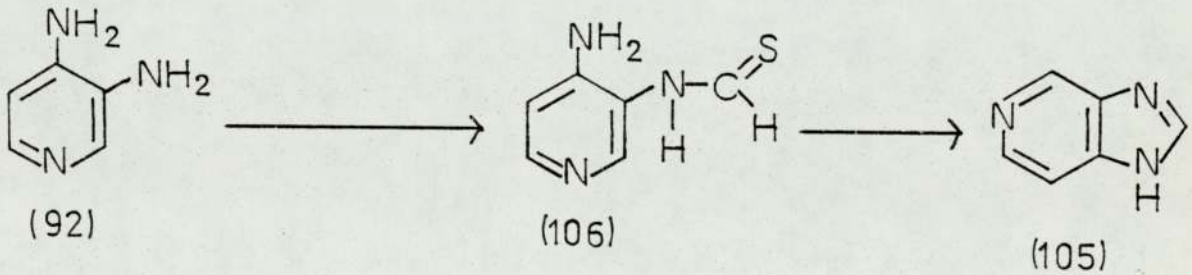
(103) R_1 = Cl R_2 = H

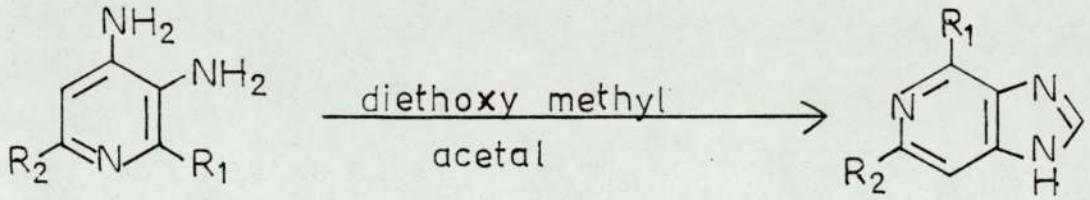
(100) R_1 = H R_2 = Cl

(101) R_1 = Cl R_2 = Cl

(102) R_1 = H R_2 = NH_2

(104) R_1 = Cl R_2 = H





(110) R_1 = F R_2 = H

(111) R_1 = H R_2 = F

(112) R_1 = F R_2 = F

(107) R_1 = F R_2 = H

(108) R_1 = H R_2 = F

(109) R_1 = F R_2 = F

Diethoxymethyl acetate

Montgomery and Hewson[16] used diethoxymethyl acetate to ring cyclise 2,3,4-triaminopyridine dihydrochloride to 1- and 3-deaza-adenines. 3-Deaza-adenine was the minor product and could be purified by several recrystallisations from water.

Diethoxymethyl acetate was used as recently as 1976, when Kroon, van den Brink, Vlietstra and Salemink[17] prepared some mono- and di- fluoro substituted imidazo[4,5-c]pyridines (107) (108) (109) from the corresponding substituted diaminopyridines (110) (111) (112) (see Scheme 11).

N,N-Dimethylformamide dimethyl acetal

Stanovnik and Tisler[18] used this reagent in 1974 to ring cyclise 3,4-diaminopyridine (92) to 1H-imidazo[4,5-c]pyridine (91) and it was claimed that the reagent was preferable to other reagents used previously because of the low temperatures required for cyclisation to occur (see Scheme 12).

(ii) Preparation with $\rangle C=O$ substituent at position 2

Urea

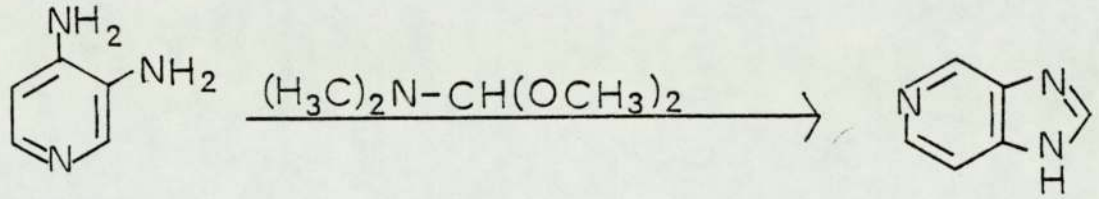
In 1966, Barlin[33] prepared 1H-imidazo[4,5-c]pyridine-2(3H)-one (113) by heating 3,4-diaminopyridine (92) and urea at 160° for one hour. 6-Chloro-1H-imidazo[4,5-c]pyridine-2(3H)-one (114) was similarly prepared from 2-chloro-4,5-diaminopyridine (98). Yutilov and Svertilova[38], as recently as 1974, have described the preparation of 4-chloro-1H-imidazo[4,5-c]pyridine-2(3H)-one (115) from 2-chloro-3,4-diaminopyridine (103) (see Scheme 12).

(iii) Preparation with $\rangle C=S$ substituent at position 2

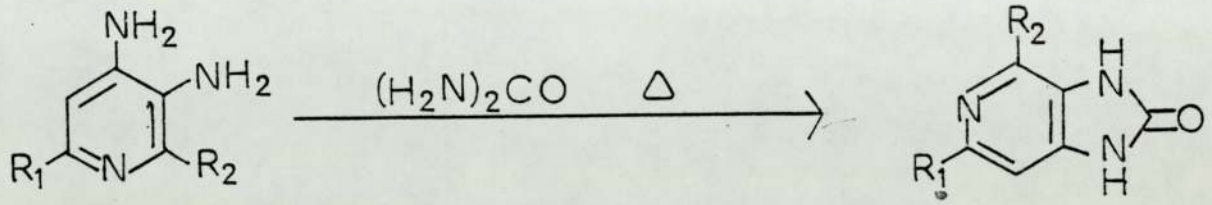
Carbon Disulphide

Clark-Lewis and Singh,[39] in 1962, treated 3,4-diaminopyridine (92) with carbon disulphide in ethanol to produce 1H-imidazo[4,5-c]

SCHEME 12

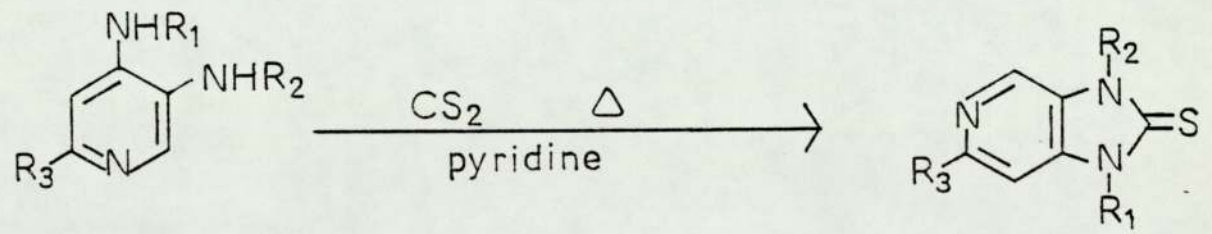


(92) (91)



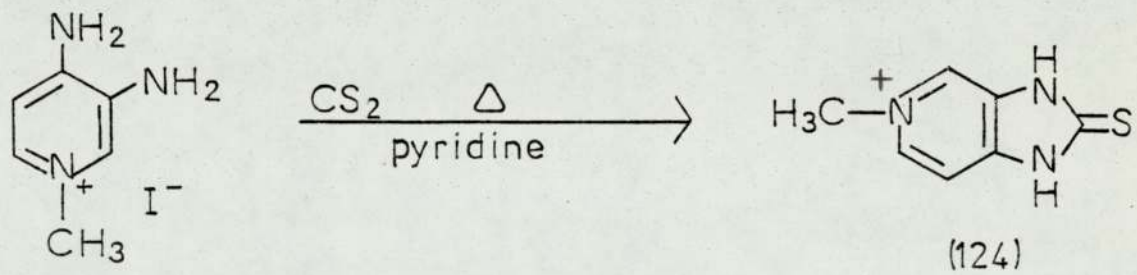
(92) $R_1 = R_2 = H$
(98) $R_1 = Cl\ R_2 = H$
(103) $R_1 = H\ R_2 = Cl$

(113) $R_1 = R_2 = H$
(114) $R_1 = Cl\ R_2 = H$
(115) $R_1 = H\ R_2 = Cl$



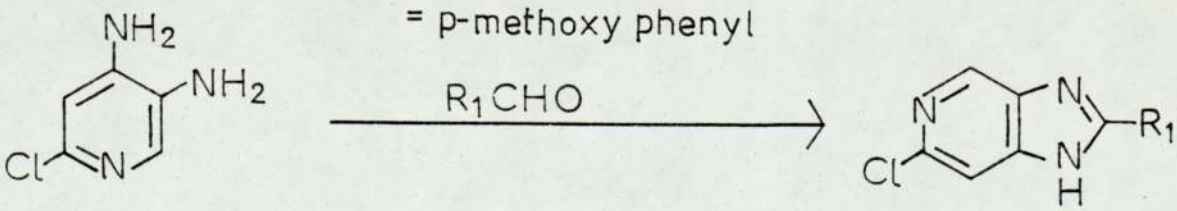
(92) $R_1 = R_2 = R_3 = H$
(120) $R_1 = CH_3\ R_2 = R_3 = H$
(121) $R_1 = R_3 = H\ R_2 = CH_3$
(122) $R_1 = R_2 = H\ R_3 = Cl$

(116) $R_1 = R_2 = R_3 = H$
(117) $R_1 = CH_3\ R_2 = R_3 = H$
(118) $R_1 = R_3 = H\ R_2 = CH_3$
(119) $R_1 = R_2 = H\ R_3 = Cl$



(123) (124)

$R_1 = C_6H_5$
= p-methoxy phenyl



pyridine-2(3H)-thione (116) and in 1966, Barlin[33] used a similar method to prepare several N-methylated imidazopyridinethiones (117) (118) (119) (124) but ethanol was used instead of pyridine for the reactions (see Scheme 12).

(iv) Preparation with $\diagdown C - R$ aryl or alkyl substituents at position 2

Aldehydes

The first reported attempt to prepare imidazo[4,5-c]pyridine derivatives was in 1938 when Weidenhagen and Weeden[34] reacted 3,4-diaminopyridine with different aliphatic and aromatic aldehydes in the presence of copper acetate in an attempt to obtain copper complexes of the 2-substituted imidazo[4,5-c]pyridines. The copper complexes of the imidazopyridine derivatives were only obtained after long boiling and the 2-substituted imidazo[4,5-c] pyridines only after heating in sealed tubes at 130° - 150° in low yields (see Scheme 13).

As recently as 1977, Yutilov and Kovaleva[40] have prepared some 2,6-disubstituted imidazo[4,5-c]pyridines by reaction of 6-substituted-3,4-diaminopyridines with aromatic aldehydes in the presence of an oxidising agent (see Scheme 12).

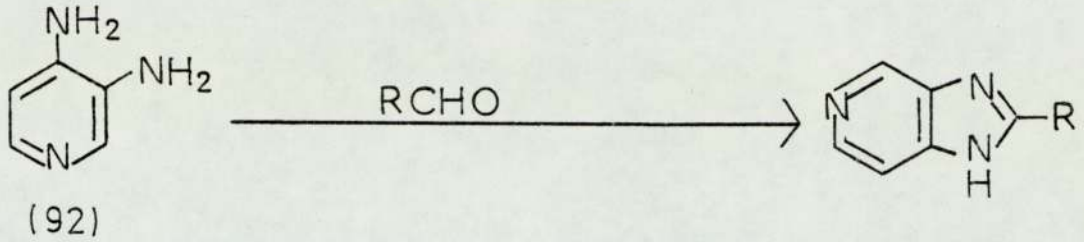
Acetic anhydride

In 1962, Knobloch and Kühne[41] reacted 3,4-diaminopyridine (92) with acetic anhydride to obtain the product 2-methyl-1H-imidazo[4,5-c]pyridine (125) (see Scheme 13).

2-Ethylhexanol

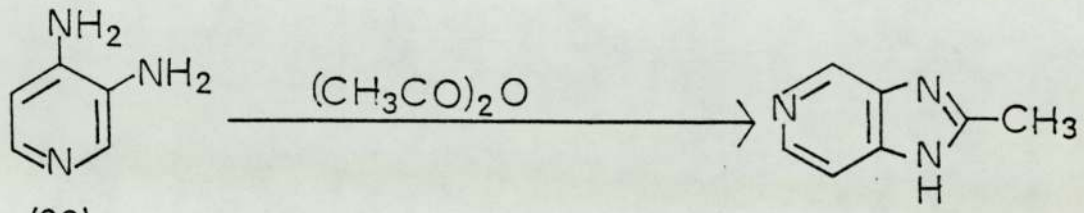
In 1963, Edwards[42] prepared some 2-alkylimidazo[4,5-c] pyridines by heating the appropriate disubstituted pyridine in 2-ethylhexanol (see Scheme 13).

SCHEME 13

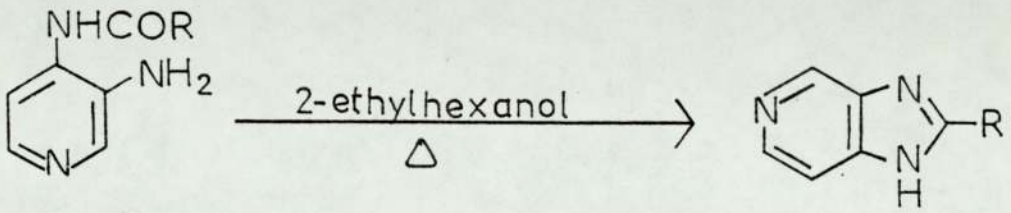


(92)

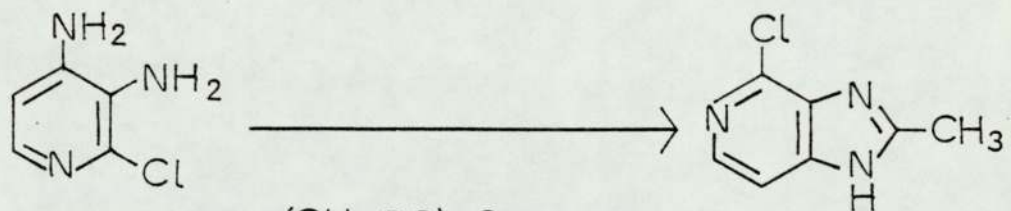
R = H, CH_3, C_2H_5, p-methoxyphenyl



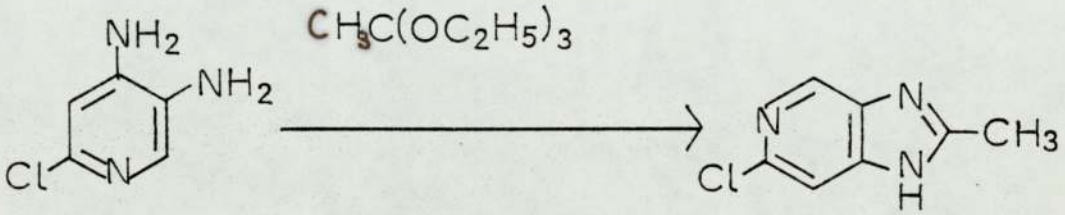
(92) (125)



R = CH_3, C_2H_5, C_3H_7, C_4H_9.



(103) (126a)



(98) (126b)

Triethylorthoacetate

In 1966, Barlin[33] reacted 2-chloro-4,5-diaminopyridine (98) with a 1 : 1 mixture of acetic anhydride and triethylorthoacetate to obtain 6-chloro-2-methyl-1H-imidazo[4,5-c]pyridine (126b). A similar reaction was performed in 1973 by Stetsenko and Miroschnichenko[43] when they treated 2-chloro-3,4-diaminopyridine (103) with a 1 : 1 mixture as above to obtain 4-chloro-2-methyl-1H imidazo[4,5-c]pyridine (126a) (see Scheme 13).

From Imidazole Precursors

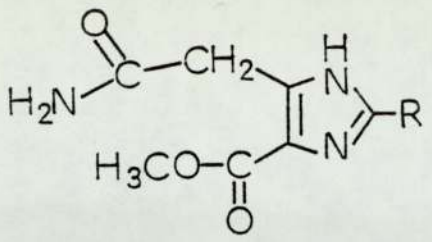
In 1963, Robins et al.,[44] prepared four imidazo[4,5-c] pyridine derivatives (127) (128) (129) (130) from imidazole precursors. Cyclisation was effected by boiling with sodium ethoxide in ethanol (131) (132) (133) and in the case of the 2,4,6-trihydroxy derivative (134), boiling aqueous sodium carbonate.

The proposed mechanism of the reaction is outlined in Scheme 14 using (127) as an example.

In 1973, Rousseau, May, Robins and Townsend[45] prepared 3-deaza-6-thioguanine (137) from an imidazole precursor, namely 4(5)-cyano-5(4)-methylcyano-1H(3H)-imidazole (135) which was treated in ether with anhydrous ~~potassium~~ hydrogen bromide to produce 6-amino-4-bromo-1H-imidazo[4,5-c]pyridine (136) (see Scheme 14). Attempts to achieve ring closure with other similar acids were unsuccessful. It is interesting that cyclisation occurred in one specific direction, with no formation of the isomeric 4-amino-6-bromo-1H-imidazo[4,5-c] pyridine. Reaction of an aqueous suspension of (136) with thiourea in the presence of a catalytic amount of formic acid gave 6-amino-1H-imidazo[4,5-c]pyridine-2(3H)-thione (137) (see Scheme 14).

The work of Robins et al.,[44] was important in laying the foundations for the preparation of the elusive 3-deazaguanine (138).

SCHEME 14

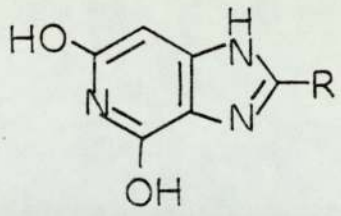


(131) R = H

(132) R = Cl

(134) R = O

(133) R = S

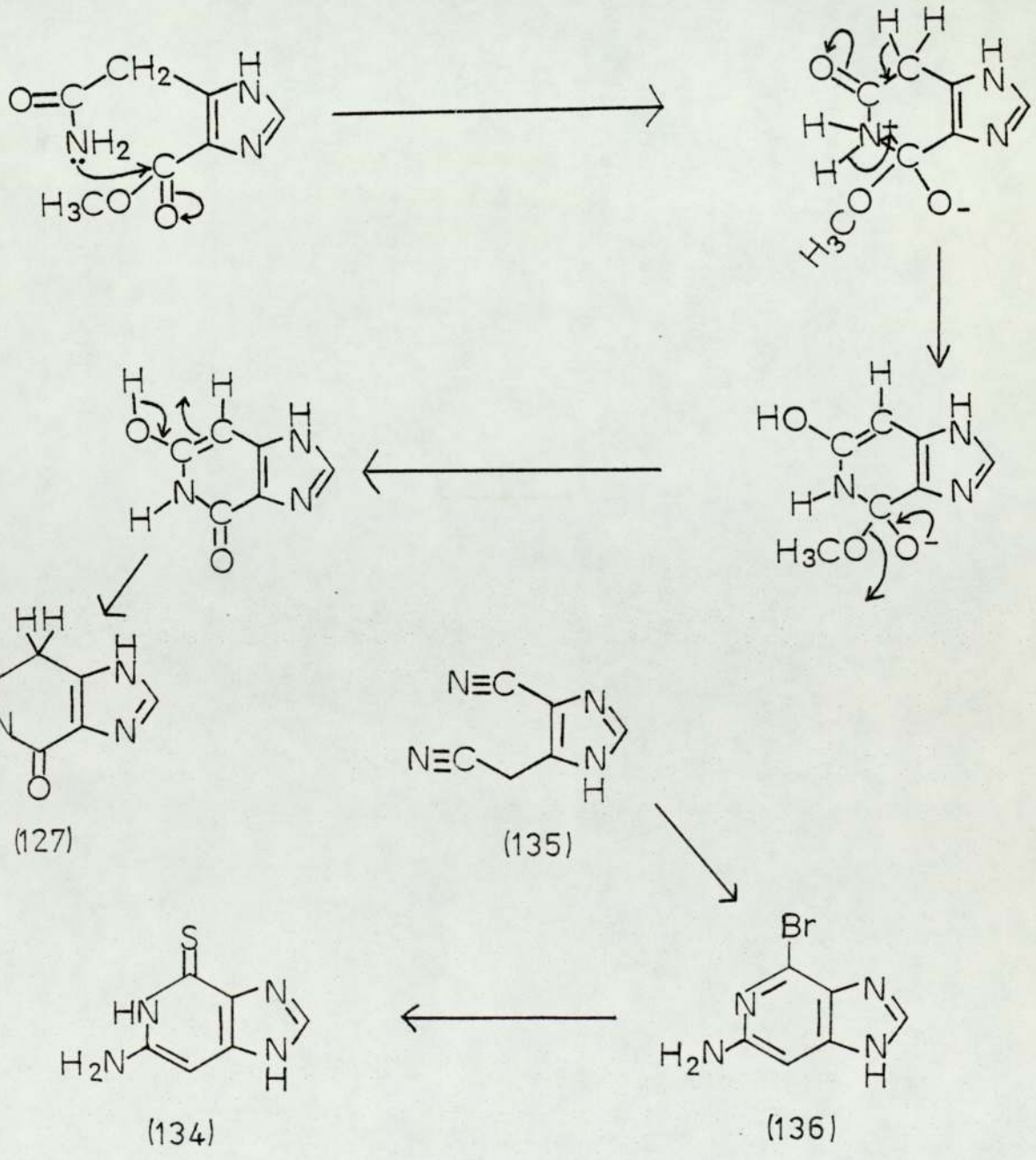


(127) R = H

(128) R = Cl

(129) R = O

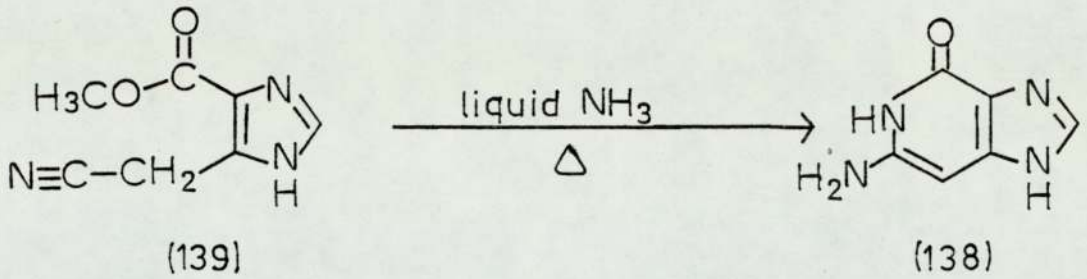
(130) R = S



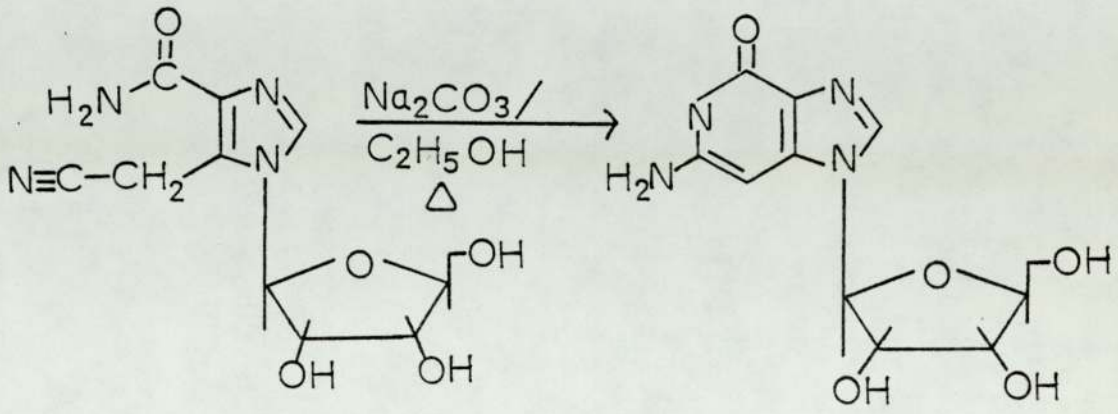
In 1976, Cook et al.[46] reported the preparation of (138) from an imidazole precursor. Two years earlier, in 1974, DeBode and Salemink[47] had reported an unsuccessful attempt to prepare the compound from a pyridine precursor. The key intermediate in the preparation of 3-deazaguanine was methyl-5(4)-cyanomethylimidazole-4(5)-carboxylate (139) which yielded the product (138) on treatment with liquid ammonia (see Scheme 15).

3-Deazaguanosine (141) and 3-deazaguanylic acid (140) were also prepared by cyclisation of the appropriate imidazole precursor (see Scheme 15).

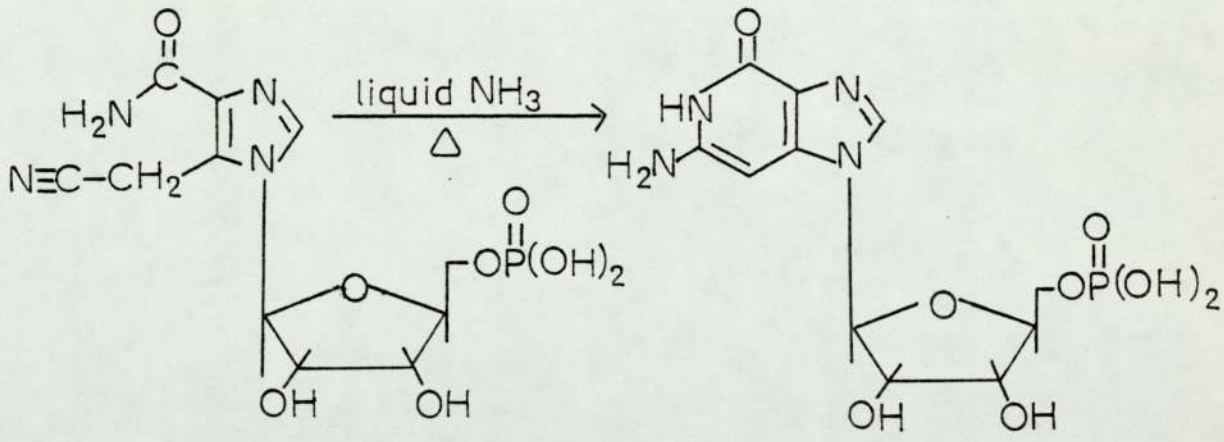
SCHEME 15



(139) (138)



(140)



(141)

B. BIOLOGICAL ACTIVITY

(i) Anti-viral activity

In 1958, Hollinshead and Smith[48] reported that 2-(α-hydroxy benzyl)-benzimidazole (HBB 142) had anti-viral activity against enteroviruses and in 1961, Eggers and Tamm[55,49] showed that this activity was due to prevention of the production of infective viral RNA of susceptible viruses. This was confirmed in 1962, when Eggers and Tamm[50] showed that HBB (142) inhibited virus induced ribonucleotide synthesis.

Following Hollinshead and Smith's discovery, many new benzimidazole derivatives and structural analogues were prepared and tested,[56,57,58] several of which were active. In 1965, Chatterjee, Jain and Anand[28] prepared and tested the imidazo -[4,5-b]- and -[4,5-c]- pyridine structural analogues of HBB but neither of these compounds showed any activity against Ranikhet Disease, polio, herpes or measles viruses (see Scheme 16).

This ~~negative~~ lack of activity was surprising, especially as CNDO calculations for (143) showed that electron distribution did not differ fundamentally from that of HBB. This fact prompted Berner, Reinshagen and Koch[29] to repeat the synthesis of (143) as described

by Chatterjee et al.,[28] and they demonstrated that, instead of the imidazopyridine, Chatterjee and his co-workers had prepared the isomeric pyridopyrazinone (145). See Page 11. Berner, Reinshagen and Koch prepared (143) by unambiguous synthesis and it was shown to be an inhibitor of acid-sensitive viruses in vitro.

It seemed reasonable to expect that imidazopyridine analogues of essential purines would also possess anti-viral activity. This was borne out by the fact that Brants, Graafland and Kerling[51] showed that 1-deaza-adenine was active against tobacco mosaic virus in excised tomato roots cultivated in vitro.

In 1962, Babbar and Chowdhury[52] showed that 3-β-D-ribofuranosyl-imidazo[4,5-b]pyridine (146) (see Scheme 16) was similarly effective against Ranikhet disease virus, and as recently as 1975, Allen et al.[53] have shown that 3-deazaguanine (138) (see Scheme 15) possesses a broad spectrum of anti-viral activity. In vitro studies showed that 3-deazaguanine had marked activity against parainfluenza, herpes, vaccinia, vesicular stomatitis and rhino-viruses. Moderate activity was shown against human and murine cytomegalo-virus, pseudorabies virus and myxoma virus. In vivo studies showed that intracerebral injection of mice with (138) after herpes virus increased the survivor numbers by 30%. However, only slight activity was demonstrated against parainfluenza virus (Sendai) but 3-deazaguanosine (140) significantly increased the survivor numbers (50 - 80%). It is possible that the inactivity of (138) is due to an inability to ribosylate to the active (140) (see Scheme 15).

(ii) Cytotoxic activity

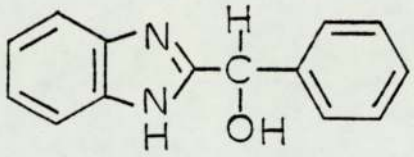
In 1957, Kidder and Dewey[54] tested 1-deazaguanine (147) for anti-tumour activity on adenocarcinoma 755 in C57 black, line 6

mice but the results were negative. The tumours grew at the same rate as the controls and the average time of death was not prolonged. It was suggested that this surprising result was due to the insolubility of (147) and the consequent use of ineffectual concentrations. This suggestion was reinforced when Gorton, Ravel and Shive[59] showed that (147) had some anti-tumour activity against mouse mammary carcinoma C 3H strain grown in eggs. It was, however, suggested that this inhibitory action was not specific for the tumour. 1- (27) and 3- (28) Deaza-adenine both appeared to lack cytotoxic activity and when Bennett and Smithers,[60] in 1964, evaluated both (27) and (28) as potential feedback inhibitors of purine synthesis, they found that they were inactive at the highest concentrations studied.

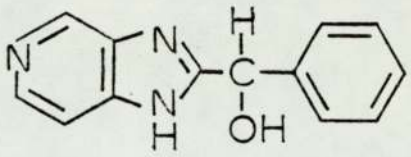
Montgomery and Hewson[16,61,62] were currently involved in the preparation and evaluation of imidazopyridine analogues of the anti-tumour agent, 6-mercaptopurine (148) (see Scheme 16). The two analogues, (149) and (150), were found to be cytotoxic against cell cultures but the 1-deaza analogue (150), which was the more potent of the two, was still 300 times less potent than 6-mercaptopurine. These workers then prepared and tested 3-deaza-6-methylmercaptopurine ribonucleoside (151) and compared its activity with the parent compound (152). The parent compound was 1000 times more inhibitory than the deaza-analogue and Montgomery and Hewson[61] proposed that the 3-N was necessary for enzymic binding of the molecule to either adenosine kinase or to the allosteric site of PRPP-glutamine amidotransferase.

In a more recent paper, Montgomery and Hewson[62] reported the preparation of the 1-deaza- analogue (153) and also the ribosylated deazapurine itself (146). Both (153) and (146) were

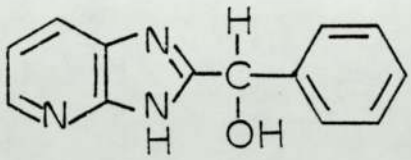
SCHEME 16



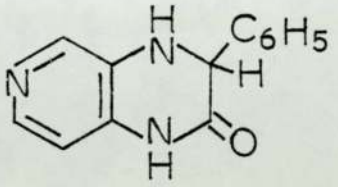
HBB (142)



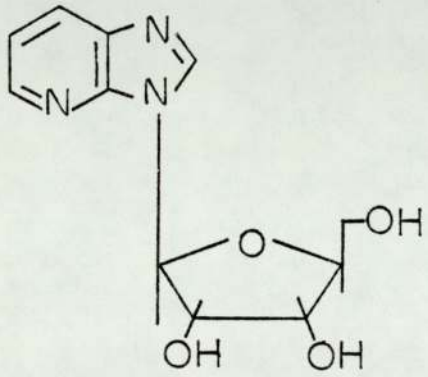
(143)



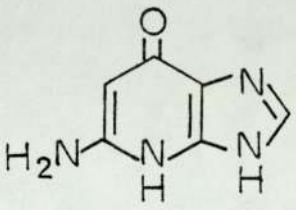
(84)



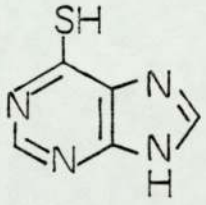
(145)



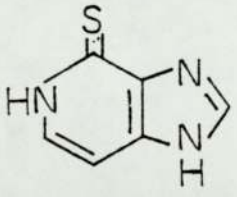
(146)



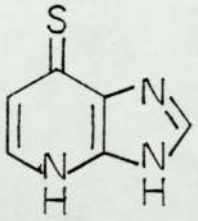
(147)



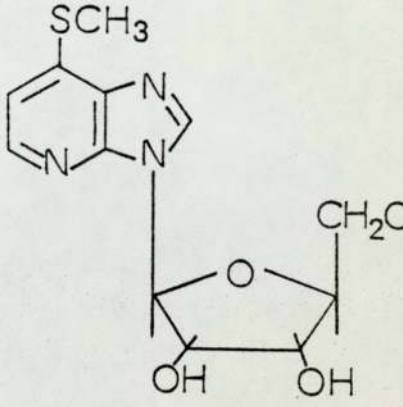
(148)



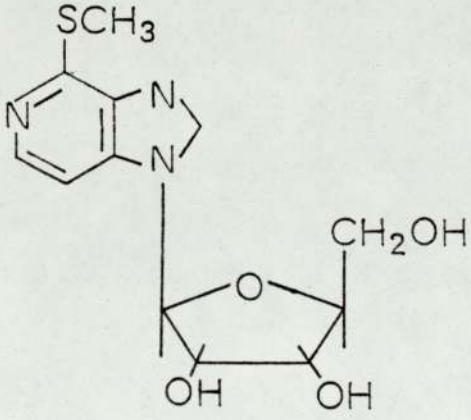
(149)



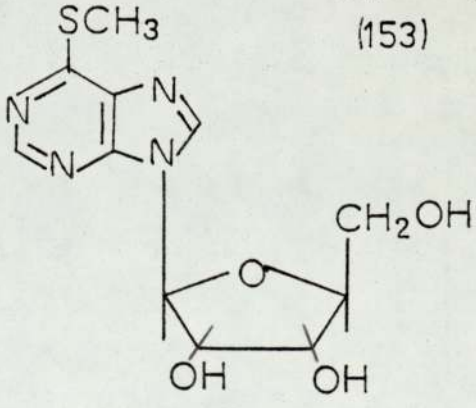
(150)



(153)



(151)



(152)

found to be cytotoxic, although they were only 0.25% as active as the corresponding purine nucleosides. On the basis of these results, it was proposed that although the N-3 of the purine ring appears to be essential for biological activity of the purine molecule, N-1 may not be essential even though it is contributory.

In 1975, concurrently with the present work, Brantsevich *et al.*[63] described some sugar derivatives of various imidazo [4,5-*c*] pyridines which inhibited growth of Sarcoma 37 in mice by up to 70%. One example cited was 1-(tetra-o-acetyl-β-D-galactopyranosyl)-2-methyl-4-chloro-imidazo[4,5-*c*]pyridine.

In the same year, Khwaja *et al.*[64] described *in vivo* and *in vitro* anti-tumour activity of the recently prepared 3-deaza-guanine (138), demonstrating the potential use of this compound as a new and powerful anti-tumour agent.

(iii) Herbicidal activity

In 1966, two patents[65, 30] were submitted claiming that some newly prepared substituted imidazo[4,5-*b*]pyridines had a herbicidal action against certain types of plants. Since then, several patents[67,68,69,25] have been submitted for imidazopyridines of similar basic structure exhibiting herbicidal activity (154) (see Scheme 17). The $-CF_2-$ moiety in the 2-position is essential for activity but the actual mode of action of this herbicidal activity on a molecular level is unknown. Bond and Corbett[70] carried out work on the mode of action and metabolism of some of these compounds but their results were inconclusive.

(iv) Rodenticidal activity

Two patents[71,72] have been submitted since the present work commenced in 1975, claiming that some novel substituted imidazo [4,5-*b*]pyridines have a rodenticidal action. The series of

compounds had the general structural formula (155) (see Scheme 17).

Rats which were fed 1-allyloxy-5,6-dichloro-2-(1,1,2,2-tetrafluoroethyl)-1H-imidazo[4,5-b]pyridine died within 48 hours and the autopsy showed that death was due to internal haemmorhage. The second patent[72] was a refinement of the first and described a successful field experiment using 5,6-dichloro-2-heptafluoropropyl-1-methoxy-imidazo[4,5-b]pyridine (156).

(v) Anti-microbial activity

Vaughan, Krapcho and English[6] reported in 1949 that certain imidazo[4,5-b]pyridines showed no anti-microbial activity against strains of Mycobacterium, Erssipelothrix, Pneumococcus, Streptococcus and Pasteurella multocida. Markees and Kidder[66] suggested that this was not surprising because the positions of substituents were different from those of natural products.

In 1952, Dimmling and Hein[73] tested 4-amino-1H-imidazo[4,5-c] pyridine (28), previously prepared by Roche Products[10] and Kögl, van der Want and Salemink[5], showed that it had anti-microbial activity against eleven different types of bacteria tested. This was not surprising, since the imidazo[4,5-c]pyridine nucleus occurs in the streptothricin family of antibiotics.[94]

Kidder and Dewey[54] and Gorton, Ravel and Shive[59] in 1957 and 1958 respectively, both demonstrated anti-microbial growth inhibitory effects of 5-amino-3H-imidazo[4,5-b]pyridine-7(4H)-one(1-deaza-guanine) (147). Kidder and Dewey also showed that 1-deaza-adenine (27) possessed anti-microbial growth inhibitory activity against Tetrahymena pyriformis strain W and that this inhibitory activity was antagonised by adenine. 1-Deazaguanine was also found to possess activity, but the low solubility prevented a half maximal inhibitory concentration from being obtained.

Similarly, Gorton, Ravel and Shive found that 1-deazaguanine

was a very effective inhibitor of L. casei and moderately effective against other organisms. However, S. faecalis and E. coli were not inhibited in their growth even at high concentrations. It was also found that guanine reversed the toxicity of (147) over a broad range of concentrations and prompted them to suggest that (147) exerted its primary effect as a competitive guanine antagonist.

In 1971, De Roos and Salemink[1] reported on the anti-microbial inhibitory activity of 3-deaza-adenine and 3-deazahypoxanthine. Both compounds were tested on S. faecalis ATCC 8043 and were shown to inhibit the strain by more than 50%.

There have been no reports on the potential anti-microbial activity of 3-deazaguanine but the compound has only recently been synthesised.

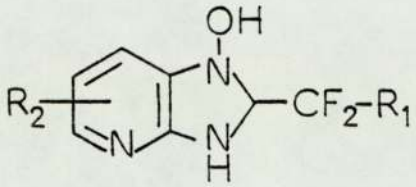
(vi) Pharmacological Activity

Adler and Albert[4], and Vohra et al.[74] have tested imidazo -[4,5-b]- and -[4,5-c]- pyridine, (1) and (91) respectively, in vivo by injecting mice intra-peritoneally. Both compounds induced convulsions, paralysis and shock. More specific pharmacological activity is listed below.

Analgesic effects. A few references appear in the literature describing the analgesic activity of imidazopyridine derivatives. In 1961, Hoffmann et al.[75] submitted a patent describing the preparation of some imidazo[4,5-c]pyridine derivatives with analgesic activity and in 1963, Kurihara and Chiba[3] prepared some -[4,5-b]- derivatives, e.g. 6-bromo-2-methyl-3H-imidazo[4,5-b] pyridine (73) (see Scheme 17).

Cardiovascular effects. In 1964, Talik and Brekiesz[76] described the preparation of several new imidazo[4,5-c]pyridine derivatives in a search for new compounds to lower blood pressure

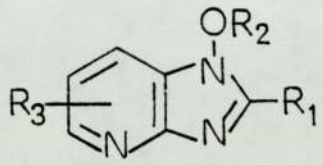
SCHEME 17



(154)

R_1 = H, Cl, F, CF_3.

R_2 = NH_2, Cl, F, Br, CN, NO_2, lower alkyl.

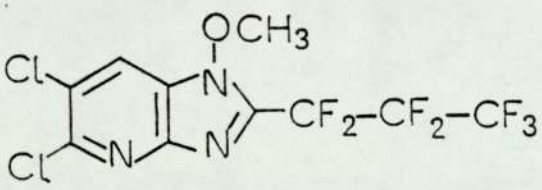


(155)

R_1 = fluoroalkyl

R_2 = H alkyl, alkenyl, acyl.

R_3 = Cl, F, Br.



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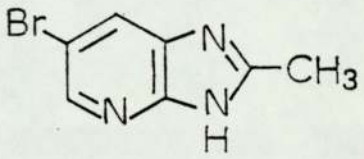
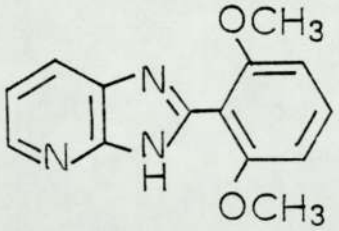
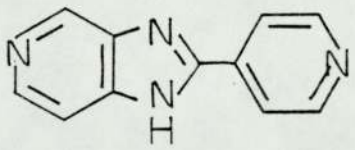


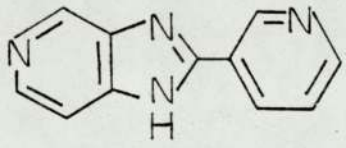
(73)



(157)



(159)



(158)

and in 1975, concurrently with the present work, Kutter, Austel and Diederen[77] submitted a patent describing the synthesis of 67 compounds which lowered blood pressure and had a positive inotropic effect on the heart, e.g. 2-(2,6-dimethoxyphenyl)-3H-imidazo[4,5-b]pyridine (157) (see Scheme 17).

Belz, Nuebling and Zimmer[78] reported in 1976 that (157) had been tested in 8 healthy volunteers and the results showed that (157) was a short acting drug with a high positive inotropic action which was easily controlled.

Psychotropic effects. A United States Patent[31] was submitted in 1977 which described the preparation of some polysubstituted imidazo[4,5-b]pyridine derivatives which were useful for the relief of anxiety and tension states. They also exhibited anti-inflammatory activity and reduced carageenin induced inflammation in experimental animals.

(vii) Xanthine Oxidase Inhibition

Baldwin et al.[27] prepared and tested (81) (82) (158) and (159) in 1977 as potential inhibitors of the enzyme xanthine oxidase but only (158) and (159) showed activity (see Scheme 17).

C. PURINE ANTIMETABOLITES AND CANCER CHEMOTHERAPY

(i) Introduction

The action of cytotoxic drugs, such as the antimetabolites, is non selective with respect to normal and neoplastic cells. When these drugs are used in doses sufficient to inhibit the rate of growth of tumour cells, they will also have an effect on normal cells possessing a high turnover rate. Most anticancer drugs possess a therapeutic index that is virtually unity - that is, at an effective therapeutic level, toxic effects are also observed.

By definition, antimetabolites rely for their action upon successful competition with normal metabolites for particular enzymes, the metabolite being, in fact, the substrate which is loosely bound to the corresponding enzyme and in the normal way enables it to function in the required utilisation of substrate. The antimetabolite having displaced the substrate, the enzyme cannot function normally. The structure of the antimetabolite must be such that it can combine at those points on the enzyme which normally accommodate the substrate. Thus both antimetabolite and metabolite (substrate) must have similar structural features necessary for binding.

Structures and Lethal Synthesis

Three purine antimetabolites are commercially available, 6-mercaptopurine (160), 6-thioguanine (161) and azathioprine (162). Others have been shown to possess powerful anti-tumour activity

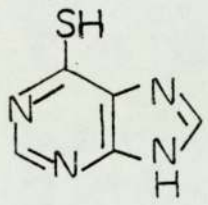
but their toxicity prevents their use in practise, e.g. 6-chloropurine (163)[138] and 6-hydroxylaminopurine (164)[127] (see Scheme 18).

The purine antimetabolites all appear to have the same mechanism of action. They are converted in the cell to nucleotides in which form they are active as inhibitors of purine synthesis. The conversion of the inactive antimetabolite to the active nucleotide is called "lethal synthesis". The mechanism by which "lethal synthesis" occurs involves the direct conversion of the purine antimetabolite to the nucleotide by a nucleotide pyrophosphorylase. This is called the salvage pathway (see Scheme 18).

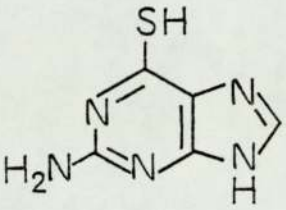
Pyrimidine antimetabolites, e.g. 5-fluorouracil cannot utilise this pathway, and they are converted to their nucleotides by a two step mechanism in which the pentose is added first and then the nucleoside is phosphorylated. There is no purine nucleoside phosphorylase in mammalian cells, but there are purine nucleoside kinases, so some purine nucleosides, like methylmercaptopurine riboside, can be phosphorylated by this reaction.

If antimetabolites must be converted to an active form in the cell then one might predict that resistance to that antimetabolite could arise by a mutation which decreases or abolishes the activity of the converting enzyme system, provided that such a change was compatible with continued cell viability. This has certainly proved to be the case with some cell lines and tumours that are resistant to 6-mercaptopurine. The loss of this enzyme function is not lethal since the principal route of purine biosynthesis <u>de novo</u> involves a different pathway and the salvage pathway can be eliminated without injuring the cell. The compound 6-methylmercaptopurine ribonucleoside (6-MMPR) is taken into mammalian cells and phosphorylated by a kinase, probably adenosine kinase.

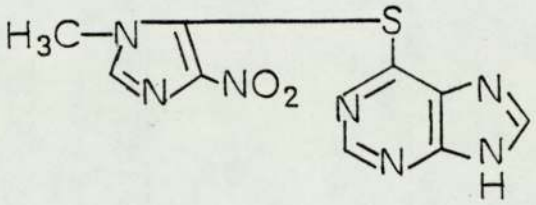
SCHEME 18



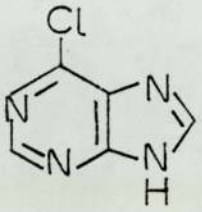
(160)



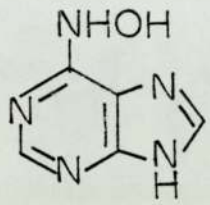
(161)



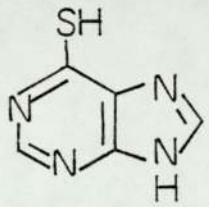
(162)



(163)

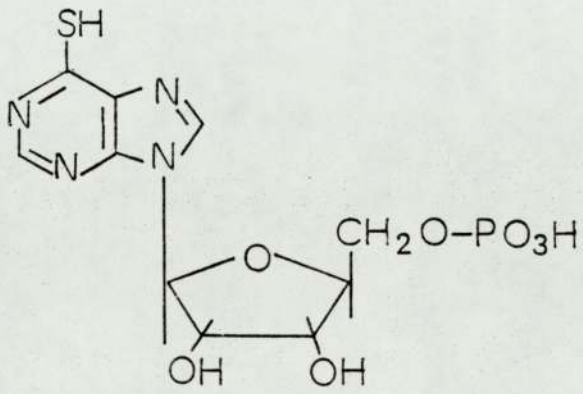


(164)



+ phosphoribosylpyrophosphate

nucleotide
pyrophosphorylase



6-mercaptopurine–ribose–phosphate

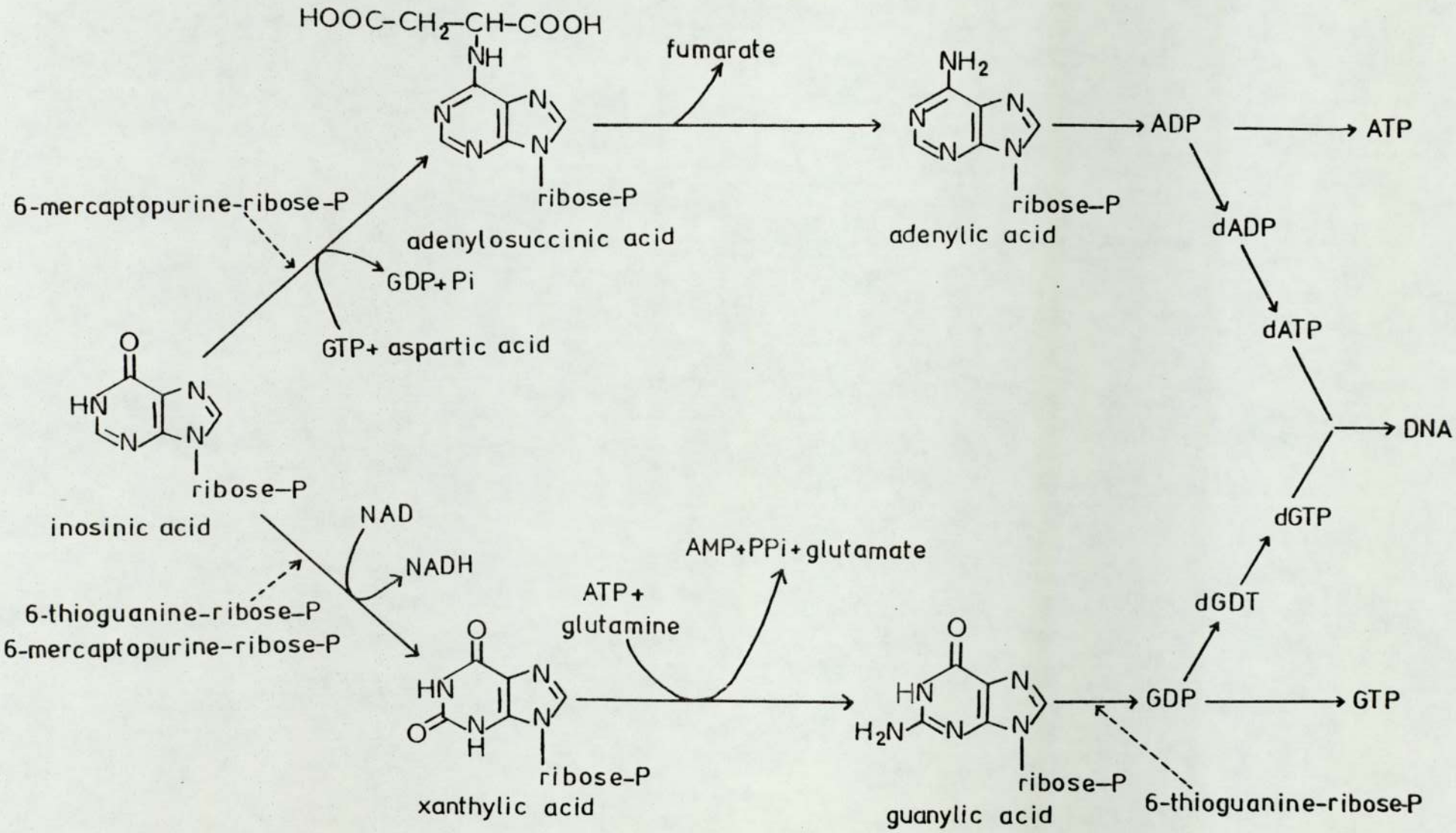
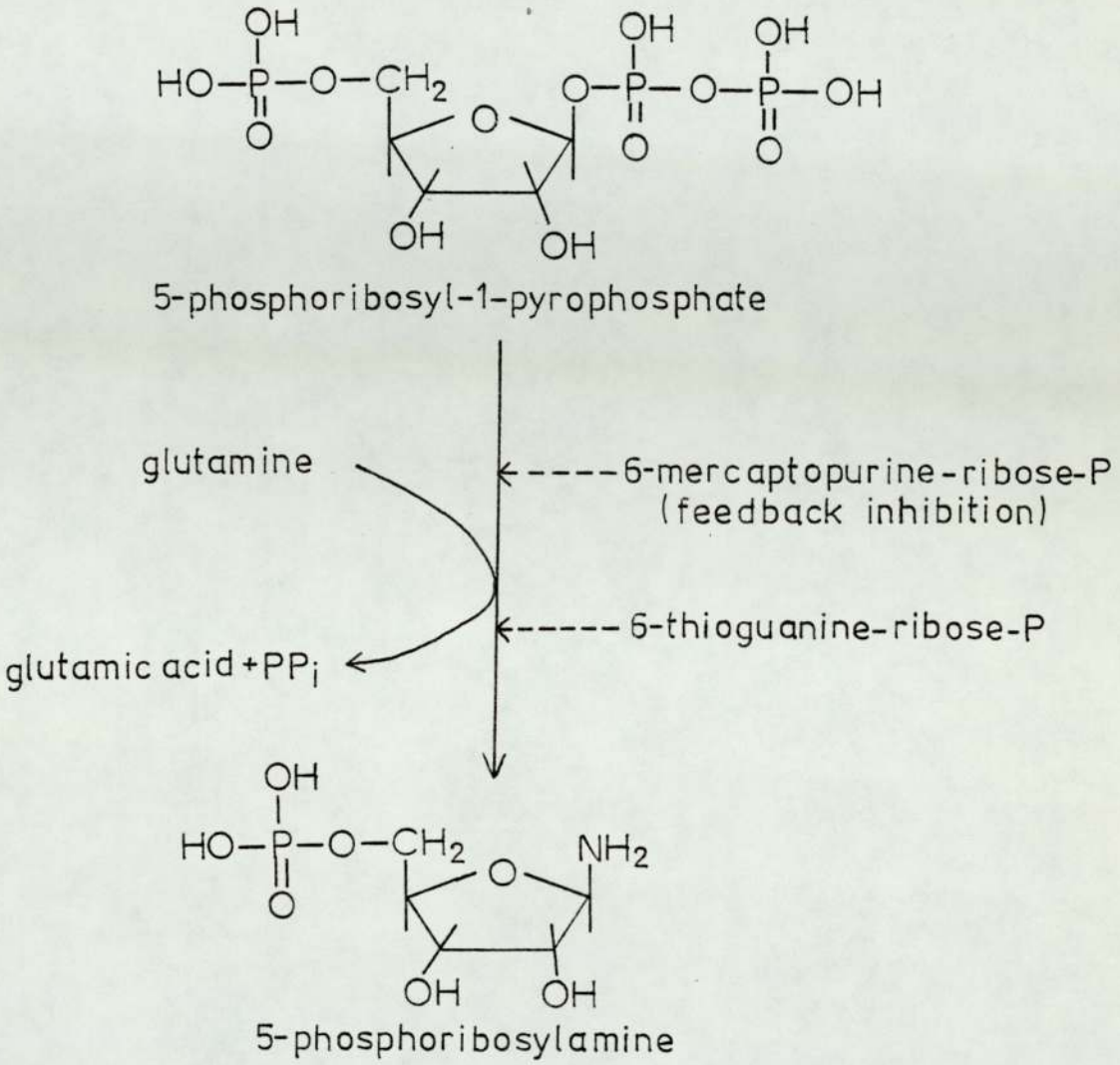

HOOC-CH2-CH-COOH
NH
fumarate
NH2
ADP
ATP
6-mercaptopurine-ribose-P
ribose-P
adenylosuccinic acid
adenylic acid
dADP
GDP+Pi
GTP+ aspartic acid
dATP
DNA
ribose-P
inosinic acid
NAD
NADH
AMP+PPi+glutamate
dGTP
6-thioguanine-ribose-P
6-mercaptopurine-ribose-P
ATP+
glutamine
dGDT
GDP
GTP
ribose-P
xanthylic acid
ribose-P
guanylic acid
6-thioguanine-ribose-P

FIGURE 1

The resulting nucleotide is a potent inhibitor of purine synthesis. Resistance to 6-mercaptopurine resulting from a deficiency of nucleotide pyrophosphorylase can thus be circumvented with 6-MMPR, an antimetabolite which utilises a different type of lethal synthesis.

(ii) Mechanism of Action

6-Mercaptopurine-riboside-phosphate (6MPRP, thioinosine monophosphate) is a structural analogue of inosine monophosphate, the common precursor of both adenylic acid and guanylic acid. It interacts with a number of enzymes active in purine metabolism, it inhibits NAD synthesis, and it interferes with coenzyme A function. Inhibition of the three reactions is responsible for reducing the production of purines in the cell and the consequent inhibition of nucleic acid synthesis. 6MPRP inhibits the conversions of inosinic to adenylosuccinic acid and xanthylic acid, thus preventing the synthesis of adenylic and guanylic acid (Figure 1). A third site of inhibition of purine synthesis occurs at the first step of the de novo synthesis of purines (Figure 2). The amidotransferase that catalyses the transfer of an amino group to the 5-phosphoribosyl-1-pyrophosphate is a regulatory enzyme that is inhibited by the products of the purine synthetic pathway. Thus, when the concentration of the adenosine and guanosine phosphates rise in the cell they interact with a regulatory site and function as a "pseudo feedback inhibitor" of purine biosynthesis. The most potent pseudo feedback inhibitor of the amidotransferase is 6-methylmercaptopurine nucleotide, the analogue of 6-mercaptopurine that follows a different pathway of lethal synthesis and is cytotoxic for certain 6-mercaptopurine resistant tumours.



De Novo Purine Synthesis Step1

FIGURE 2

The ribonucleotide derivative of 6-thioguanine also inhibits the conversion of inosinic acid to adenylosuccinic acid and xanthylic acid, and acts as a pseudo-feedback inhibitor of the amidotransferase. In addition, 6-thio-GMP competitively inhibits guanylate kinase, the enzyme that phosphorylates GMP to GDP (see Figure 1). Although small amounts of 6-thioguanine become

incorporated into DNA, this does not appear to contribute significantly to the cytotoxic effect. 6-mercaptopurine is apparently not incorporated into DNA either.

(iii) Specific uses in cancer chemotherapy

The three purine antimetabolites mentioned earlier are used to maintain remissions in both acute lymphoblastic and acute myeloblastic leukemia and in combination with other drugs to induce remissions. In addition, they are useful in the treatment of chronic myelocyticleukemia that is refractory to busulphan.

D. THE USE OF THE AMES TEST AS A BIOLOGICAL SCREEN TO DETECT COMPOUNDS OF POTENTIAL INTEREST IN CANCER CHEMOTHERAPY

The Ames test is a simple bacterial test for detecting chemical mutagens. The compounds are tested on petri plates with several specially constructed mutants of Salmonella typhimurium.[79,80,81] These mutant bacterial strains are all histidine auxotrophs and mutations are scored by counting the number of revertant colonies. The test has been modified by adding homogenates of rat liver directly to the petri plates thus incorporating an important aspect of mammalian metabolism into the in vitro test. In this way, a number of mutagens which are not mutagenic per se but require metabolic activation can be detected.[79,80,82]

(i) The bacterial tester strains

There are several standard bacterial tester strains containing different types of histidine mutations. One strain (TA 1535) can be used to detect mutagens causing base-pair substitutions and two (TA 1538 and TA 1537) to detect various kinds of frameshift mutagens. The molecular basis of the frameshift mutations in these strains has been investigated. Frameshift mutations occur by shifted pairing in repetitive sequences of DNA and frameshift mutagens can be very specific for the particular sequences they mutate. TA 1538 has a repetitive sequence -C-G-C-G-C- near the site of the histidine mutation[83], and is reverted well by mutagens such as 2-nitrosofluorene. The other frameshift tester strain, TA 1537, appears to have a run of C's at the site of the mutation[79], and is reverted well by mutagens such as 9-aminoacridine. In

addition to the histidine mutation, each tester strain contains two additional mutations that greatly increase the sensitivity to mutagens. One of these causes loss of the excision repair system and the other loss of the lipopolysaccharide barrier which coats the surface of the bacteria.[79]

In 1975, Ames and his co-workers developed two new strains (TA100 and TA98) by incorporating a resistance transfer plasmid (R factor) in the standard strains TA1535 and TA1538 respectively. These new strains were found to be extremely sensitive in detecting mutagens such as aflatoxin B_1, 4-nitroquinoline N-oxide and many polycyclichydrocarbons which were detected only weakly in the earlier strains used. The strains used in mutagenesis testing are shown in Table 1 below[84]

TABLE 1

Histidine Mutation			Additional Mutations		
his G46	his C3076	his D3052	LPS	Repair	R factor
TA1535	TA1537	TA1538	rfa	ΔuvrB	-
TA100		TA98	rfa	ΔuvrB	+R
(TA1975)	(TA1977)	TA1978***	rfa	+	-
TA92		TA2420	+	+	+R
TA1950	TA1952	TA1534	+	ΔuvrB	-
TA2410			+	ΔuvrB	+R
TA1530	TA1532	TA1964	Δgal	ΔuvrB	-
TA2631		TA2641	Δgal	ΔuvrB	+R
	TA2637		rfa	ΔuvrB	+R

N.B. All strains were originally derived from S. typhimurium LT_2. Wild type genes are indicated by a +. The deletion (Δ) through uvrB also includes the nitrate reductase (chl) and biotin (bio) genes. The Δgal strains (and the rfa uvrB strains) have a single deletion through gal chl bio uvrB. The rfa repair$^+$ strains have a mutation in gal E. R = pkM_{101}.

*** TA1538/TA1978 is recommended.

R : R factor strains.

For general mutagen testing, Ames et al.[84] recommend that three standard tester strains are used, TA1535, TA1537 and TA1538 in combination with the two R-factor strains TA100 and TA98. For screening purposes, TA1538 may be deleted, as TA98 appears to be more sensitive than TA1538 for the detection of all mutagens so far tested on the two strains.[84] The other frameshift tester strain, TA1537, is still considered useful though and McCann et al.[81] recommend that TA1535 be used in addition to TA100 because it has a much lower spontaneous mutation rate and is therefore more convenient and sensitive for the detection of mutagens that do not preferentially revert TA100.

Strain TA1978 can be used in a repair test in combination with TA1538.[79,85] This is not a mutagenicity test, but indicates whether an agent is killing bacteria by damage to DNA that can be repaired by the uvrB excision repair system.

(ii) The Ames test as a potential screen

The testing of novel compounds for biological activity in animals, deleterious or potentially useful, is extremely expensive, time consuming and sometimes unreliable. Attempts are therefore being made to develop or adapt cheaper, quicker and simpler short term in vitro tests. The Ames test is one of these tests and it gives specific information about the mutagenicity of a chemical

or metabolite, albeit in a bacterial system. At the present time, the test is very much under scrutiny, largely due to the so far unproved supposition that chemical mutagenicity is related to chemical carcinogenesis. Because of the political, economic and scientific implications if this relationship is proved, much work is in progress in this area.

Several cancer chemotherapeutic agents have been tested for their *in vivo* carcinogenicity,[86,87] and some are suspected of being carcinogenic in man. This is not surprising when their mode of action is considered. Several reports have also appeared recently describing the mutagenic activity of several of these agents.[88,89,90,91,92]

In 1977, Benedict *et al.*[93] tested seventeen cancer chemotherapeutic agents for possible mutagenic activity in the Ames test. Fourteen of those tested were positive but actinomycin D, bleomycin and methotrexate showed no activity. Benedict *et al.*[93] suggested that the possible reason for actinomycin D and bleomycin giving a negative result was due to inability of the molecule to pass through the bacterial cell envelope. Work is in progress at the moment to examine the mutagenicity of these two compounds in other *Salmonella* tester strains.

The non mutagenicity of methotrexate is not surprising when the mode of action is considered. Indeed, several dihydrofolate reductase inhibitors have been recently tested in the Ames test and they also failed to show mutagenic activity.[139]

The work of Benedict and his co-workers showed that, except in three specific and explicable cases, the cancer chemotherapeutic agents tested in the Ames test gave a positive result. In searching for new cancer chemotherapeutic agents, with the exception

of potential dihydrofolate reductase inhibitors, the Ames test may well be useful as a primary screen to isolate those compounds which are mutagenic in the test and therefore of potential use in the field of cancer chemotherapy.

E. THE RAISON D'ETRE

Derivatives of both the imidazo -[4,5-b] - and -[4,5-c] - pyridine ring system are known and have been synthesised before but because of the many different types of biological activity exhibited by these compounds, it was of interest to prepare further imidazopyridine derivatives and investigate their potential biological activity. Many of the biological properties exhibited by the imidazopyridines indicate, on the molecular level, possible interaction with DNA in the cell. This particularly applies to cytotoxic properties but also includes anti-viral, cell inhibitory, rodenticidal and herbicidal properties. The Ames test therefore seemed an excellent choice for a biological test to use in order to assess these compounds. This view was reinforced when, concurrently with the present work, Benedict et al.[93] reported the testing of cytotoxic drugs used in cancer chemotherapy in the Ames test.

DISCUSSION

A. CHEMICAL

B. BIOLOGICAL

PAGE

A. CHEMICAL DISCUSSION

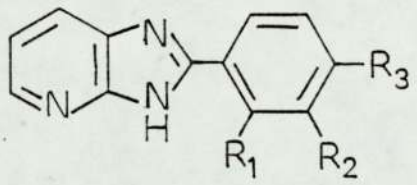
The chemical discussion will be arranged in sections according to the starting materials used and the reactions attempted using these compounds.

(i) 2,3-DIAMINOPYRIDINE (51)

Although 2,3-diaminopyridine (51) was available commercially, it was also prepared in the laboratory from 2-aminopyridine by known methods.[2, 108]

The present study confirmed that polyphosphoric acid is an excellent reagent for ring-cyclisation of the diamine (51) with substituted organic acids to prepare 2-substituted imidazo[4,5-b] pyridines.[22]

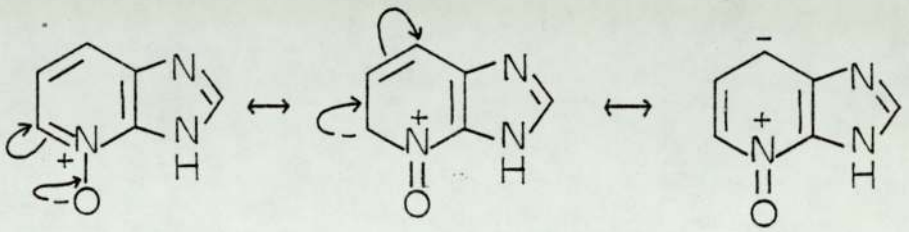
Several new compounds, (166) (167) (168) (169) (170) and (171) were prepared by this method in excellent yield (see below). The mechanism of the reaction is similar to the cyclisation of formic acid with the diamine (51) which has been mentioned earlier (Page 2 , Scheme 1).



	R_1	R_2	R_3		R_1	R_2	R_3
(166)	H	NO_2	H	(169)	H	Br	H
(167)	Cl	H	H	(171)	H	H	NO_2
(168)	H	Cl	H	(170)	H	H	Br

3H-Imidazo[4,5-b]pyridine (1), previously prepared by refluxing the diamine (51) with formic acid,[(2)] was prepared in a higher yield by reaction of (51) and formic acid in polyphosphoric acid (PPA). An examination of the n.m.r. spectrum confirmed the structure. A singlet absorption at 1.4 τ was assigned to the 2-H proton, and the protons at positions 5, 6 and 7 exhibited typical AMX splitting.

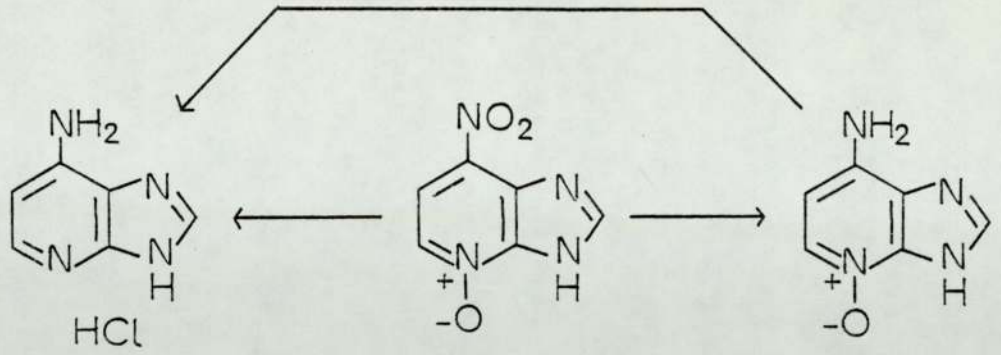
3H-Imidazo[4,5-b]pyridine 4-N-oxide (172) was first prepared by reaction of the imidazopyridine (1) with hydrogen peroxide and acetic acid at 80°.[102] The reaction was repeated at a temperature of 100° and the yield was increased considerably. N.m.r. studies indicated that the N-oxide function was exerting the expected "back donation effect" (see below).



(172)

Typical AMX splitting was again observed for the protons at positions 5, 6 and 7.

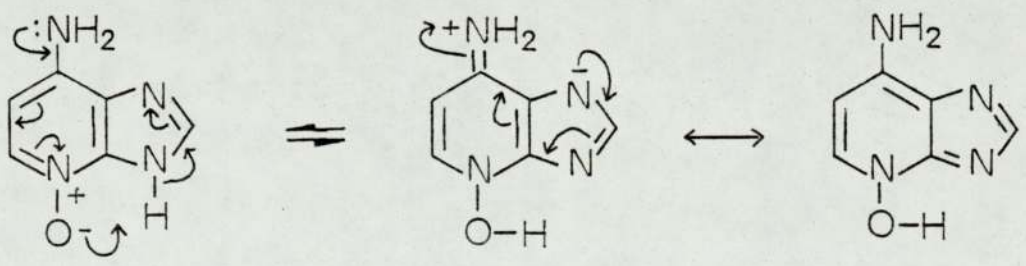
7-Nitro-3H-imidazo[4,5-b]pyridine 4-N-oxide (173) was prepared in 1966 from the N-oxide (172), but only in low yield.[102] The reaction was repeated but the isolation procedure was modified to increase the yield. The same authors[102] report the hydrogenation of the nitro N-oxide (173) in the presence of Raney nickel to yield 7-amino-3H-imidazo[4,5-b]pyridine hydrochloride (3). This work was repeated but a different compound was isolated which appeared to be 7-amino-3H-imidazo[4,5-b]pyridine 4-N oxide (174). An identical product was also obtained when palladium on charcoal and platinum oxide catalysts were used.



(3) (173) (174)

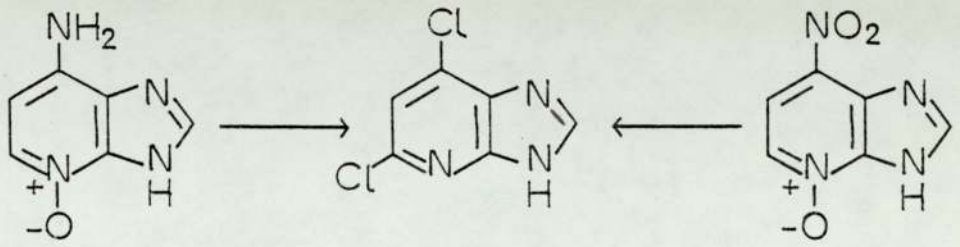
The 7-amino compound (3) was finally obtained when the nitro N-oxide (173) was hydrogenated with palladium on charcoal in a mixture of glacial acetic acid and acetic anhydride. The imidazo pyridine (3) was also obtained by hydrogenation of the amino N-oxide (174) as above.

The amino N-oxide (174) which was characterised by analysis, mass spectra, accurate mass, infrared and n.m.r., occurred as orange needles. The highly coloured nature of the compound was unusual and it could suggest that tautomeric quinonoid structures such as (174b) are present (see below).



(174) (174a) (174b)

The N-oxide (172) was treated with phosphorous oxychloride to yield 5-chloro-3H-imidazo[4,5-b]pyridine (175). Under similar conditions, the nitro N-oxide (173) and amino N-oxide (174) reacted to form 5,7-dichloro-3H-imidazo[4,5-b]pyridine (176) (see below) which has previously been prepared by ring



(174) (176) (173)

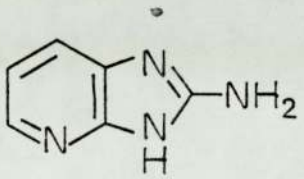
closure of 4,6-dichloro-2,3-diaminopyridine.[12] The preparation of the dichloro derivative (176) from the nitro N-oxide (173) was expected. However, nucleophilic substitution in the amino N-oxide (174) was surprising.

The dichloro derivative (176) was treated with ethanolic ammonia solution in an attempt to prepare either 5-amino-7-chloro -3H-imidazo[4,5-b]pyridine (240) or 5,7-diamino-3H-imidazo [4,5-b]pyridine (241). However, only starting material was recovered.

3H-Imidazo[4,5-b]pyridine-2(1H)-thione (63), previously prepared by treatment of the diamine (51) with carbon disulphide,[2] was prepared using thiophosgene.

Robins prepared some 8-aminopurines by fusion of substituted 4,5-diaminopyrimidines with guanidine,[98] but attempts to repeat this reaction using the diamine (51) to prepare

2-amino-3H-imidazo[4,5-b]pyridine (243) were unsuccessful. Cyanogen bromide, which has also been used to prepare 8-amino-purines by reaction with diaminopyrimidines,[97] was equally unsuccessful when reacted with the diamine (51) in preparing the 2-amino derivative (243).

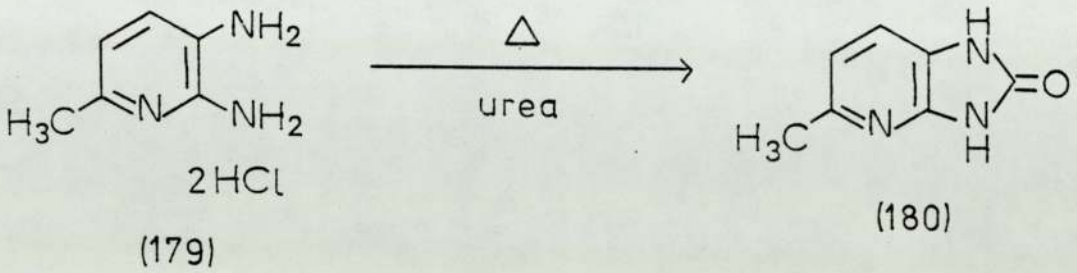


(243)

(ii) 2,3-DIAMINO-6-METHYLPYRIDINE DIHYDROCHLORIDE (179)

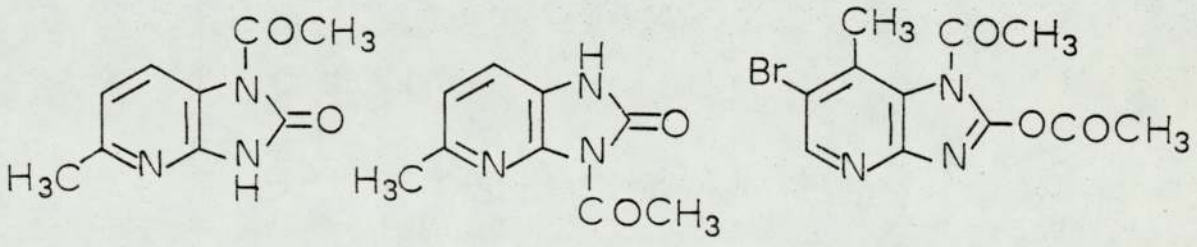
Nitration of 2-amino-6-methylpyridine yields[108] the 2-amino -3-nitro- (178) and 2-amino-5-nitro- (244) derivatives which can be separated by steam distillation.[130] 2-Amino-6-methyl-3-nitropyridine (178) was catalytically hydrogenated to yield the diamine (179) as the dihydrochloride salt.

Fusion of the diamine (179) with urea yielded 5-methyl-3H-imidazo [4,5-b]pyridine-2(1H)-one (180). N.m.r. and infrared spectra were consistent with the proposed structure: a singlet at 7.15τ was attributed to the methyl group at position 5 and the two doublets at 1.72τ and 2.4τ were attributed to the protons at positions 7 and 6 respectively. The infrared spectrum showed a strong C=O absorption at 1720 cm^{-1}. C, H and N analysis confirmed the structure.



(179) (180)

Israel and Day[19] prepared 6-bromo-7-methyl-3H-imidazo [4,5-b]pyridine-2(1H)-one (47) and they claimed that when this imidazopyridone (47) was refluxed with acetic anhydride, 1-N-acetyl-2-acetoxy-6-bromo-7-methyl-imidazo[4,5-b]pyridine (245) was obtained. The imidazopyridone (180) was treated with acetic anhydride under identical conditions in the expectation of obtaining 1-N-acetyl-2-acetoxy-5-methyl-imidazo[4,5-b]pyridine (246). However, n.m.r. and infrared spectroscopic evidence suggests that the compound isolated was either the 1-N-acetyl- (182) or 3-N-acetyl- (182a) derivative.

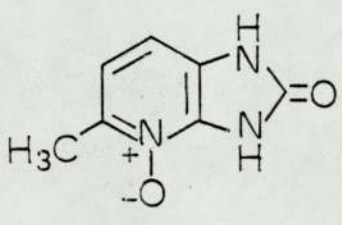


(182) (182a) (245)

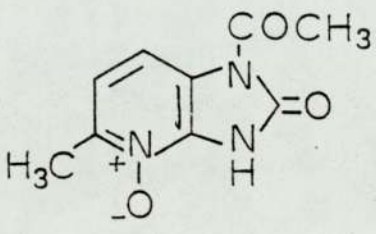
An acetoxy C = O absorption in the infrared spectrum would not appear below 1750 cm^{-1} but the C = O absorption in the product

occurred at 1720 cm^{-1}. Singlets in the n.m.r. at 7.8τ and 7.6τ have been attributed to the methyl group of the N-acetyl substituent and the 5 position on the ring respectively.

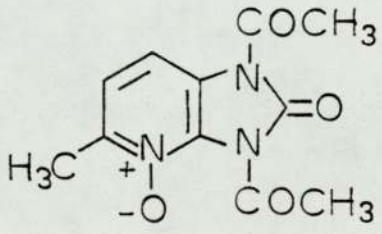
When the imidazopyridone (180) was heated with glacial acetic acid and hydrogen peroxide in the expectation of obtaining the 4-N-oxide (185), the 1-N-acetyl 4-N-oxide (183) was isolated and after this compound (183) was refluxed with acetic anhydride, the di-N-acetyl 4-N-oxide was obtained (184). The 4-N-oxide was finally prepared by hydrolysis of the 1-N-acetyl 4-N-oxide (183) derivative. It was postulated that the mono-N-acetyl 4-N-oxide derivative (183) was acetylated in the 1 position because the 3 position was less favourable due to possible steric hindrance by the N-oxide group. There was no evidence of acetoxy formation from C=O absorption values in the infrared spectra and the n.m.r. showed a singlet at 7.8τ which was attributed to the N-acetyl methyl group for (183) and (184) with an integration of 3 and 6 respectively.



(185)



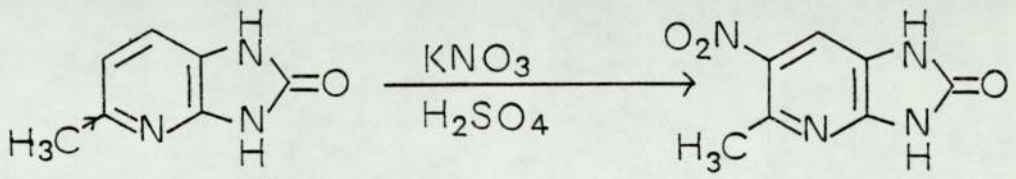
(183)



(184)

The imidazopyridone (180) was treated with a mixture of potassium nitrate and sulphuric acid to yield 5-methyl-6-nitro-

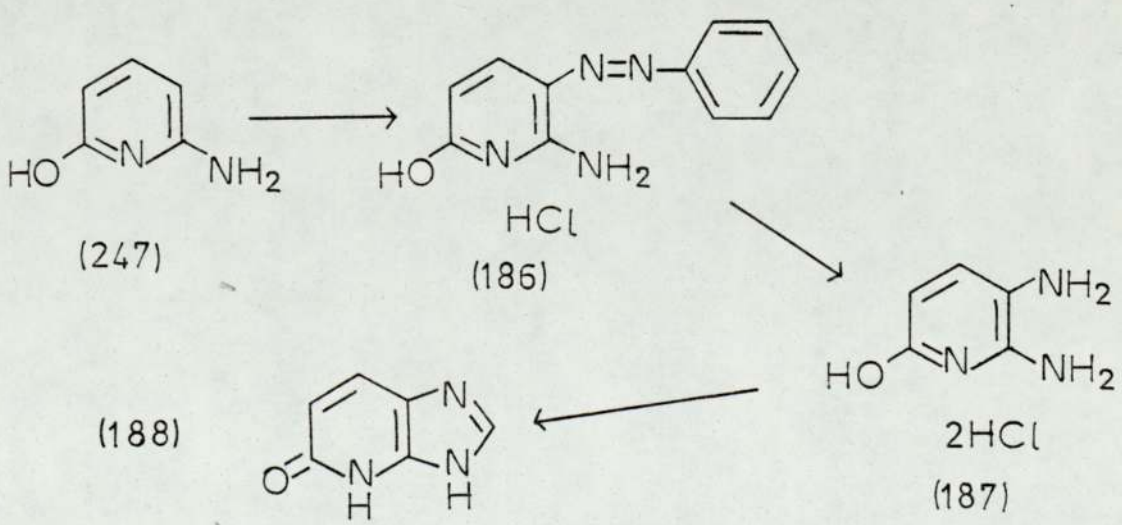
3H-imidazo [4,5-b] pyridine-2(1H)-one (181). The infrared spectrum showed absorptions at 1540 cm^{-1} and 1340 cm^{-1} which were attributed to the nitro group and the accurate mass confirmed the empirical formula. When the n.m.r. spectrum of the imidazopyridone (180) and the nitro-imidazopyridone (181) were compared, it was noted that the methyl singlet and ring proton were both deshielded and it is therefore proposed that nitration had occurred at the 6 position.



(180) (181)

(iii) 2,3-DIAMINO-6-HYDROXYPYRIDINE DIHYDROCHLORIDE (187)

The diamine (187) was prepared by catalytic hydrogenation of 2-amino-6-hydroxy-3-phenylazopyridine hydrochloride (186) which itself was obtained by coupling benzene diazonium chloride with 2-amino-6-hydroxypyridine (247). Treatment of the diamine (187) with a mixture of triethylorthoformate and acetic anhydride yielded 3H-imidazo [4,5-b]pyridine-5(4H)-one (188).

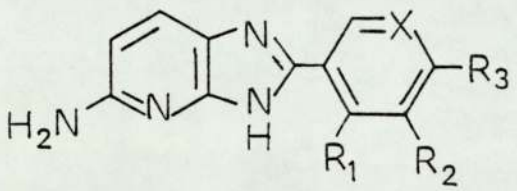


(247) (186) (187) (188)

(iv) 2,3,6-TRIAMINOPYRIDINE DIHYDROCHLORIDE (190)

The triamine (190) was prepared by catalytic hydrogenation of 2,6-diamino-3-phenylazopyridine hydrochloride (189)[6] which itself was obtained by coupling benzene diazonium chloride with 2,6-diaminopyridine (248).[131]

5-Amino-2-substituted imidazo[4,5-b] pyridine derivatives were obtained under similar conditions to those described for 2,3-diaminopyridine (51) (Page 37) by reaction of the triamine (190) with a substituted carboxylic acid in polyphosphoric acid (PPA) (see below).

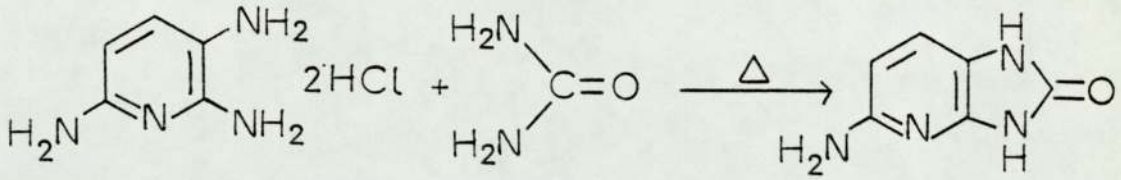


	R_1	R_2	R_3	X		R_1	R_2	R_3	X
(196)	H	NO_2	H	C	(191)	H	H	Br	C
(197)	H	Br	H	C	(192)	H	Cl	H	C
(198)	H	H	H	C	(193)	H	H	NO_2	C
(199)	H	H	H	N	(194)	CH_3	H	H	C
(200)	H	H	CH_3	C	(195)	H	H	Cl	C

The triamine (190) has been ring-cyclised with sodium formate to prepare 5-formylamino-3H-imidazo[4,5-b]pyridine (9) which was treated with concentrated hydrochloric acid to prepare the hydrochloride salt of 5-amino-3H-imidazo[4,5-b]pyridine (7) (see Scheme 2).[6] The free base of the imidazopyridine (7)

has also been prepared by treatment of the triamine (190) with formic acid.[11] In the present work, the triamine (190) was treated with triethylorthoformate to prepare the hydrochloride salt of the imidazopyridine (7).

2,3,6-Triaminopyridine dihydrochloride (190) was fused with urea to yield 5-amino-3H-imidazo[4,5-b] pyridine-2(1H)-one (201) (see below). The product was characterised by infrared and mass spectral data. N.m.r. data was not available because of the insolubility of the compound. The infrared spectrum contained absorptions at 3400 cm^{-1} and 1700 cm^{-1} which were attributed to the NH_2 and C=O substituents respectively. Accurate mass determination confirmed the empirical formula.

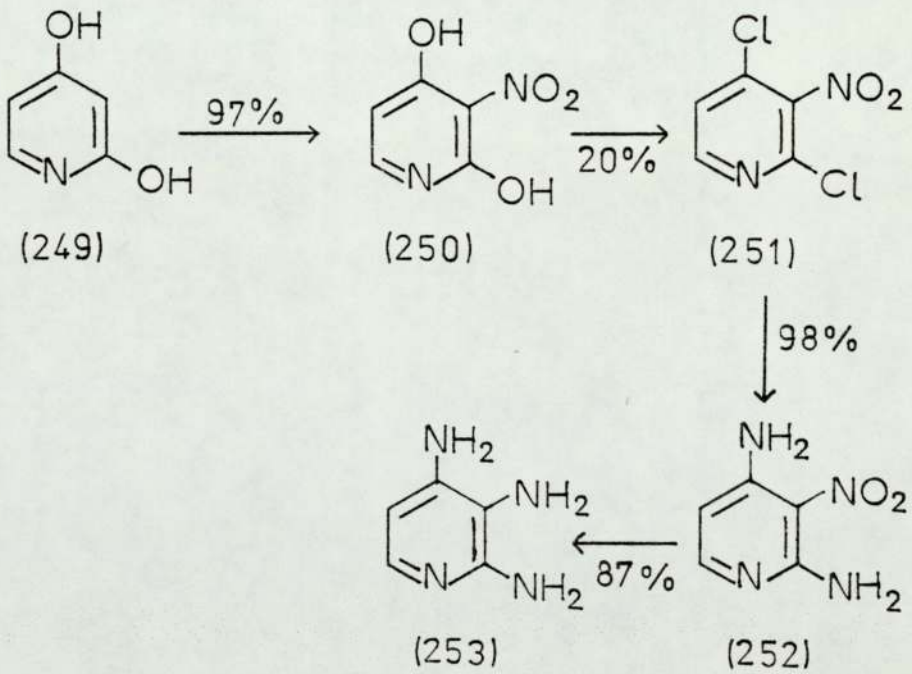


(190) (201)

Reaction of either thiourea or thiophosgene with the triamine (190) yielded 5-amino-3H-imidazo[4,5-b]pyridine 2(1H)-thione (202). The infrared and n.m.r. spectra were consistent with the proposed structure: two doublets in the n.m.r. at 2.73τ and 3.75τ were assigned to protons at 7 and 6 positions respectively. Accurate mass determination confirmed the empirical formula.

(v) 2,3,4-TRIAMINOPYRIDINE SERIES

The preparation of 2,3,4-triaminopyridine is the subject of a patent[132] and this patented method was followed in the present work. The reaction steps and yields are outlined below. Although the patent claimed a yield of 72% for the step from the dihydroxy nitro compound (250) to the dichloro compound (251), this was never achieved in the present work even after several modifications and therefore, because of the low yield for this step, this method for preparation of the triamine (253) was abandoned.

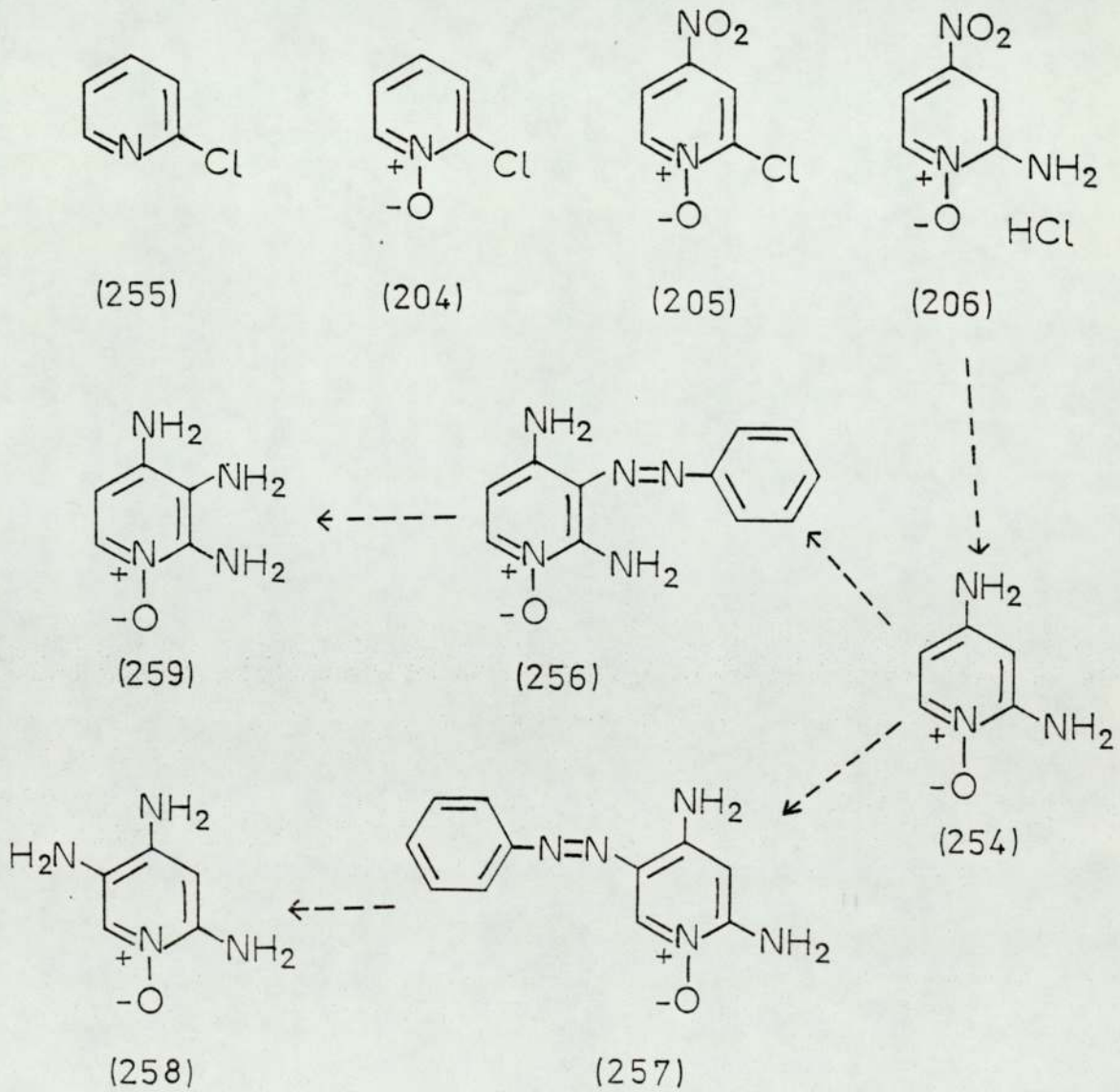


(249) (250) (251) (252) (253)

(vi) 2-CHLOROPYRIDINE SERIES

The failure to prepare the triamine (253) on a quantitative scale led to a search for possible new routes to useful precursors. It was postulated that if 2,4-diaminopyridine 1-oxide (254) could be prepared, coupling of this diamine (254) with benzene diazonium chloride could yield azo derivatives, (256) and (257), which on

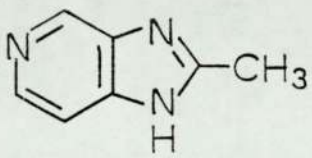
reduction would yield the triamines (259) and (258) respectively (see below). Work on the series was abandoned because of the time involved, but not before the useful 2-amino-4-nitropyridine 1-oxide (206) had been prepared from 2-chloro-4-nitropyridine 1-oxide (205). The n.m.r. and infrared spectra were consistent with the proposed structure: the n.m.r. showed typical AMX splitting and the infrared spectrum had absorptions at 1550 cm^{-1} and 1360 cm^{-1} which were attributed to the NO_2 group. C, H and N analysis and accurate mass determination confirmed the structure.



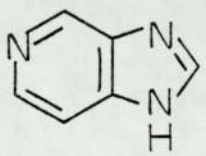
(vii) 3,4-DIAMINOPYRIDINE (92)

The diamine (92) was prepared by catalytic hydrogenation of 4-amino-3-nitropyridine (209).[42] It was also obtained commercially.

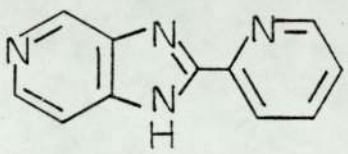
The diamine (92), a substituted carboxylic acid and polyphosphoric acid (PPA) were heated together to yield 2-substituted imidazo[4,5-c] pyridine derivatives (see below). The conditions were similar to those described for 2,3-diaminopyridine (see Page 37). 1H-Imidazo[4,5-c] pyridine (91) was prepared by this method in high yield.



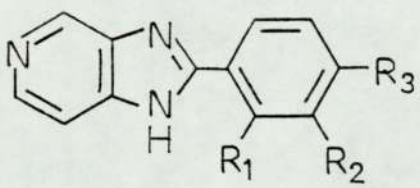
(210)



(91)



(216)



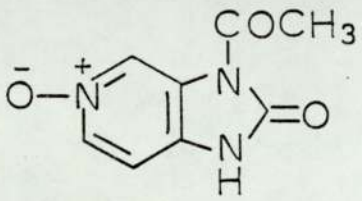
	R_1	R_2	R_3		R_1	R_2	R_3
(213)	H	H	H	(215)	H	H	NO_2
(211)	H	H	Cl	(217)	NO_2	H	H
(212)	H	Cl	H	(218)	H	H	NH_2
(214)	OH	H	H				

1H-Imidazo[4,5-c]pyridine-2(3H)-one (113) was prepared in the present study by fusion of the diamine (92) with urea.[33] The imidazopyridone (113) was also prepared by treatment with phosgene.

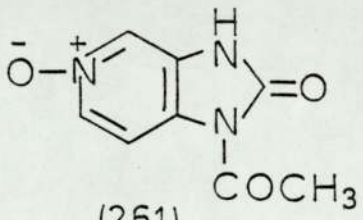
Yutilov and Svertilova[133] claimed, in 1973, that the imidazopyridone (113) could be nitrated directly in the 4 position using

potassium nitrate and sulphuric acid to prepare 4-nitro-1H-imidazo [4,5-c]pyridine-2(3H)-one (260). However, attempts to repeat this reaction proved unsuccessful and only starting material was recovered.

When the imidazopyridone (113), glacial acetic acid and hydrogen peroxide were heated together, either 3-N-acetylimidazo [4,5-c]pyridine-2(1H)-one 5-N-oxide (220) or the 1-N-acetyl derivative (261) were prepared (see below). The infrared and



(220)

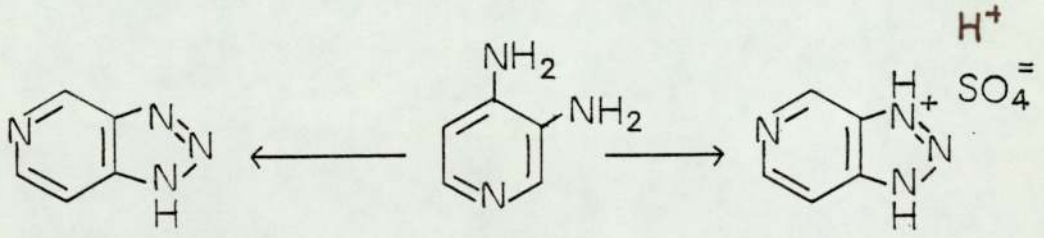


(261)

n.m.r. spectra were consistent with the structure proposed: a singlet at 7.8τ was attributed to the N-acetyl methyl group, two doublets at 2.25τ and 1.4τ were attributed to protons at positions 7 and 6 respectively and the proton at position 4 occurred as a singlet at 1.2τ. The infrared spectrum had a C=O absorption at 1700 cm^{-1}. Accurate mass determination confirmed that N-oxidation had occurred.

Thiourea was fused with the diamine (92) to prepare 1H-imidazo[4,5-c]pyridine-2(3H)-thione (116). C, H and N analysis confirmed the preparation. The imidazopyridinethione (116) has previously been prepared by treatment of the diamine (92) with carbon disulphide.[33]

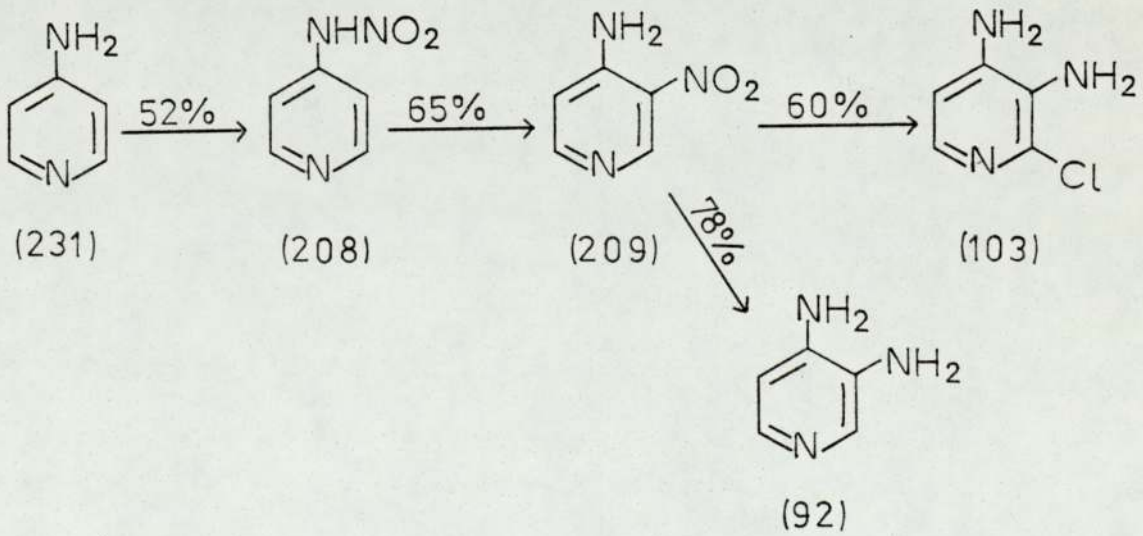
Reaction of 3,4-diaminopyridine (92) with nitrous acid yielded 1H-triazolo[4,5-c]pyridine as the sulphate salt (202). The free base was also prepared (201). The n.m.r. spectra of the free base showed two doublets at 1.36τ and 1.99τ which were assigned to the 6 and 7 ring protons respectively. A singlet at 0.38τ was assigned to the proton at position 4. Accurate mass determination confirmed the empirical formula. The structure of the triazolopyridine sulphate (202) was confirmed by n.m.r., infrared and accurate mass determination. The n.m.r. spectrum showed two doublets at 1.0τ and 1.38τ and a singlet at -0.69τ which were assigned to the 6, 7 and 4 protons respectively.



(201) (92) (202)

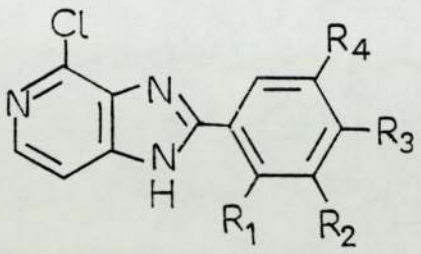
(viii) 2-CHLORO-3,4-DIAMINOPYRIDINE (103)

The diamine (103) was prepared by a modification to a known method[99] and the yield was increased from 30% to 60% (see below).



(231) (208) (209) (103) (92)

Reaction of the diamine (103) with the appropriate substituted carboxylic acid in polyphosphoric acid (PPA) yielded 4-chloro-2-substituted imidazo[4,5-c]pyridine derivatives (see below).

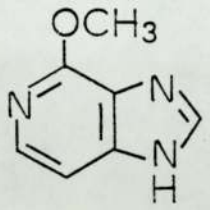


	R_1	R_2	R_3	R_4		R_1	R_2	R_3	R_4
(207)	Cl	H	H	H	(203)	H	NO_2	H	H
(208)	H	Cl	H	H	(204)	H	NO_2	H	NO_2
(209)	H	H	Cl	H	(205)	H	H	CH_3	H
(210)	H	H	NO_2	H	(206)	CH_3	H	H	H
(212)	H	H	Br	H	(213)	H	H	H	H

1H-Imidazo[4,5-c]pyridine-4(5H)-thione (116), previously prepared from 4-chloro-1H-imidazo[4,5-c]pyridine (104)[99] was treated with dimethyl sulphate to yield 4-methylmercapto-1H-imidazo-[4,5-c]pyridine (215). The methylmercapto derivative (215) has previously been made from the thione derivative (116) by treatment with methyliodide.[1]

6-Chloropurine (228) is susceptible to nucleophilic attack at the 6 position and many compounds have been made in this way e.g. 6-methoxypurine (262),[134] 6-phenoxypurine (263),[134] 6-hydroxyl-aminopurine (164).[127] The importance of both ring nitrogens is demonstrated by the fact that the deaza-analog, 4-chloro-1H-imidazo-[4,5-c]pyridine (104), does not undergo nucleophilic substitution so readily. Indeed, reactions to prepare 4-methoxy- (264),

4-hydroxylamino- (265), 4-hydrazino- (266) and 4-amino- (28) were unsuccessful



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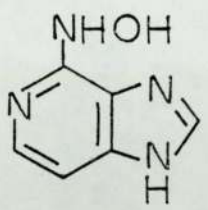

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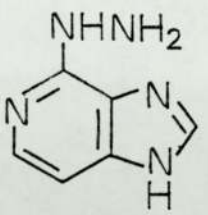


(266)

When 2-chloro-3,4-diaminopyridine (103) and urea were fused, 4-chloro-1H-imidazo[4,5-c]pyridine-2(3H)-one (115) was formed. Concurrently with the present work, Yutilov and Svertilova[38] prepared this compound by the same method. N.m.r. and infrared spectra were consistent with the proposed structure: doublets at 2.15τ and 1.55τ were assigned to the 7 and 6 ring protons respectively. The infrared spectrum showed an absorption at 1720 cm^{-1} which was attributed to the C=O group. Accurate mass determination confirmed the empirical formula.

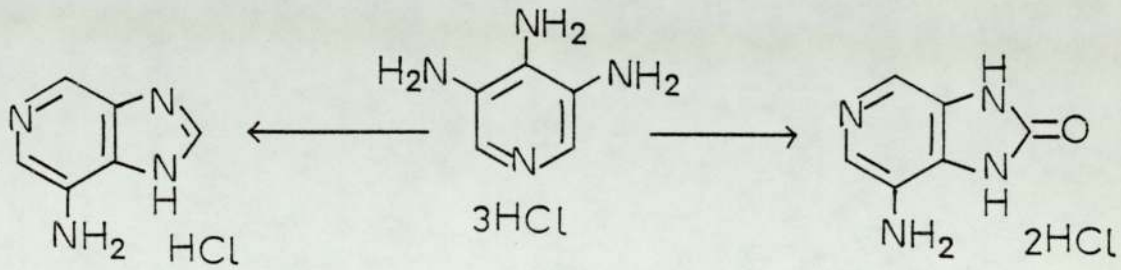
(ix) 3,4,5-TRIAMINOPYRIDINE TRIHYDROCHLORIDE (216)

The triamine (216) was prepared by catalytic hydrogenation of 4-amino-3,5-dinitropyridine (267).[11]

7-Amino-1H-imidazo[4,5-c]pyridine hydrochloride (217) was prepared by treatment of the triamine (216) with triethylorthoformate. The disulphate of the imidazopyridine (217) has previously been prepared by treatment of the triamine (216) with formic acid[11] but a pure sample of the hydrochloride (217) was not obtained. The n.m.r. and infrared spectra were consistent with the proposed

structure and C, H and N analysis confirmed the structure.

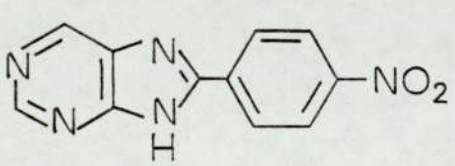
Treatment of the triamine (216) with urea yielded 7-amino-1H-imidazo[4,5-c]pyridine-2(3H)-one dihydrochloride (218). C, H and N analysis and spectroscopic data was in accord with the proposed structure.



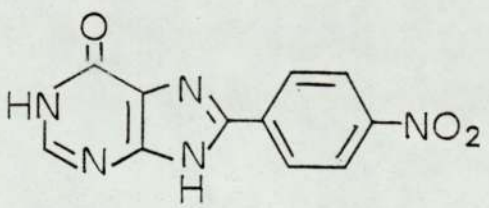
(217) (216) (218)

(x) 4,5-DIAMINOPYRIMIDINE (268)

The diamine (268), which was available commercially, was reacted with p-nitrobenzoic acid in polyphosphoric acid (PPA) to yield 8-(p-nitrophenyl)-9H-purine dihydrochloride (220). Similarly, 4,5-diamino-6-hydroxypyrimidine hemisulphate (269) was reacted with p-nitrobenzoic acid to prepare 8-(p-nitrophenyl)-9H-purine-6(1H)-one isolated as the dihydrochloride (219). The structures proposed were in accord with the n.m.r. and infrared spectra and the empirical formula was confirmed by accurate mass determination.



(220)



(219)

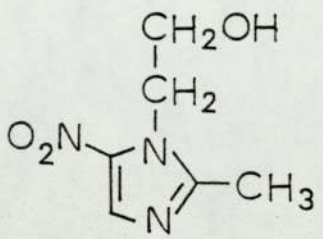
PAGE

B. BIOLOGICAL DISCUSSION

(i) Mutagenicity of nitro compounds

McCann, Choi, Yamasaki and Ames[112] reported, in 1975, that nitro compounds were frequently positive in the Ames test. They suggested that this was because nitro-reductase enzymes in Salmonella metabolise the nitro compounds to an active mutagen. McCann and Ames[113] reported that the mutagenic potency would be high because the active form would have a low probability of reacting with non DNA components before reaching the DNA target.

A number of nitro compounds tested here (see Results Table 5) have been positive without liver activation. Furthermore, 2-(p-nitrophenyl)-1H-imidazo[4,5-c]pyridine dihydrochloride (215) showed no change in the number of revertants with liver activation (see Graph 2). The dilemma arises as to whether a comparison can be drawn between mammalian cells and Salmonella. Rosenkranz and Speck[114], in 1975, found that metronidazole (229), which is mutagenic

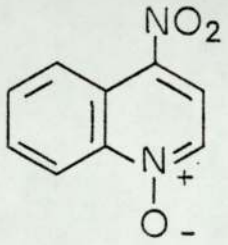


(229)

in TA100 without liver activation, was non mutagenic in a nitro-reductase deficient derivative unless S-9 mix was added. It would therefore seem reasonable to accept that a mutagenic response in the Ames test by nitro compounds can be projected to indicate

mutagenicity in mammalian systems.

Rosenkranz and Speck[114] suggest that the active metronidazole metabolite is either the hydroxylamino or amino derivative. However, other evidence contradicts this conclusion and McCann *et al.*[112] found that 4-nitroquinoline N-oxide (230) (4NQNO) had a mutagenic potency of 2906 revertants/nmole in strain TA100 but 4-hydroxylaminoquinoline N-oxide (231) had a mutagenic potency of only 76 revertants/nmole. This suggests that the active metabolite



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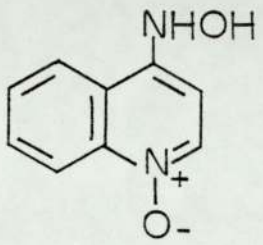


(231)

is a reduction product which is intermediate between the nitro and hydroxylamino species. This is in accord with the fact that in the present study, 2-(*p*-nitrophenyl)-1*H*-imidazo[4,5-*c*]pyridine dihydrochloride (215) was found to be a potent frameshift mutagen but the corresponding amino compound, 2-(*p*-aminophenyl)-3*H*-imidazo [4,5-*c*]pyridine (218), failed to show any mutagenic activity.

Studies by Ames, Gurney, Miller and Bartsch[115], in 1972, on the mutagenesis of 2-acetylaminofluorene (232) (see Figure 3) and its metabolites showed that the order of potency as frameshift mutagens was 2-nitroso (233) > 2-hydroxylamino >> N-hydroxy-2-acetylamino (234) > 2-amino (235) (see Figure 3). These results suggested that in this case the active metabolite could be the nitroso (233) derivative.

In 1977, Garner and Nutman[116] reported some structure-activity relationships of some mutagenic azo dyes and their reduction products. They suggested that the active compounds formed by the bacterial nitro-reductases were N-hydroxyl derivatives. These are relatively unreactive themselves towards nucleic acids and two further possible mechanisms were proposed:-

(a) The N-hydroxy is esterified by a bacterial enzyme.

(b) The N-hydroxy compounds react with each other to form dyes of multiringed character.

Preliminary experiments carried out by ourselves with substituted aminopyridines supports the conclusions of Garner and Nutman but further liver activation studies would be required to confirm their findings. 4-Aminopyridine (231), 4-nitramino-pyridine (208), 4-amino-3-nitropyridine (209), 2,3,6-triaminopyridine dihydrochloride (190) and 2,6-diamino-3-phenylazopyridine hydrochloride (189) were negative without liver activation but 2-amino-4-nitropyridine 1-oxide (206) was positive as a base-pair and frameshift mutagen (see Graph 16). The findings of Garner and Nutman[116] that amino groups require metabolism by liver mixed function oxidases to N-hydroxyl compounds, suggest that liver activation studies of the amino substituted imidazopyridines (see Figure 7) would be of interest.

Although the Ames test is extremely sensitive for the detection of nitro-mutagens, not all nitro compounds tested here were mutagens. 5-Methyl-6-nitro-3H-imidazo[4,5-b]pyridine-2 (1H)-one (181), for example, showed no mutagenic activity (see Figure 6).

(ii) The relationship between electrophilicity and mutagenicity

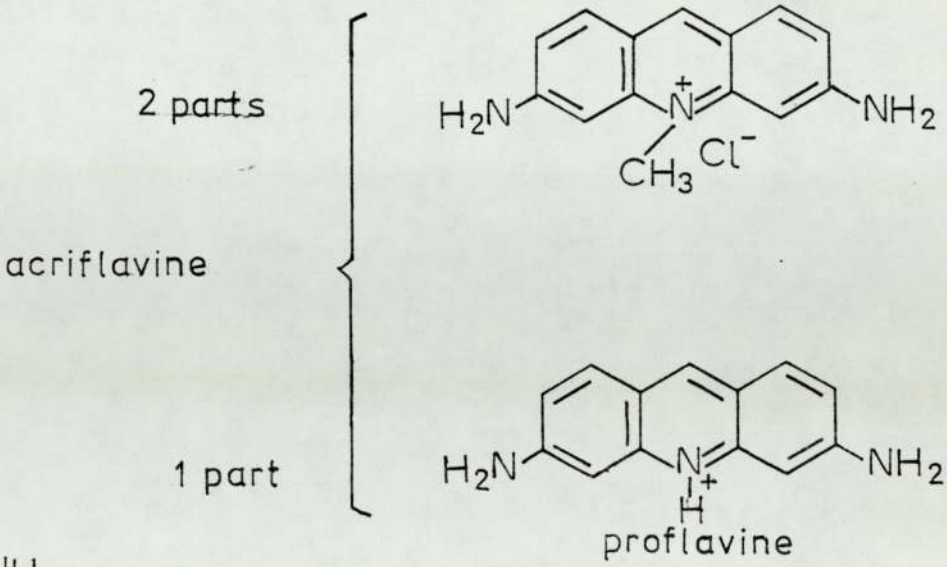
Polycyclic planar aromatic compounds, such as the acridines (see Figure 3), which intercalate in the DNA base-pair stack are mutagens that cause additions and deletions of bases in a gene. It is thought that the intercalation causes mispairing in the DNA during replication, repair or recombination. These mutagens are called frameshift mutagens because the reading of the messenger RNA is shifted by the addition or deletion of a base, and this effect distinguishes them from other mutagens that cause base-pair substitutions[117,118].

In 1972, Ames, Sims and Grover[119] suggested that when an intercalating agent also had an electrophilic sidechain attached to it which could react with DNA, a more potent frameshift mutagen was obtained. Carcinogenic polycyclic hydrocarbons, such as benzo (a)pyrene (236) (see Figure 3), were cited as examples. These compounds, which are known to intercalate with DNA, are converted into electrophilic epoxides by microsomal enzyme systems[119]. Ames et al.[115] studied the effect of reactive groups attached to simple intercalating mutagens and found that the nitroso and hydroxylamino groups were the most active.

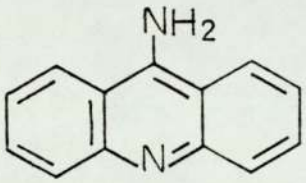
Ames et al.[115] also reported that intercalating mutagens which form covalent bonds with DNA, such as 2-nitrosofluorene (233) (see Figure 3), are much more active in reverting frameshift strains without the excision repair system than in strains able to repair damaged DNA. The absence of this repair system had no effect on the potency of simple intercalating mutagens such as 9-aminoacridine (237) (see Figure 3). In this way, Ames et al.[115] obtained indirect evidence that the more potent frameshift mutagens

Figure 3

Some well known frame-shift mutagens.



(238)



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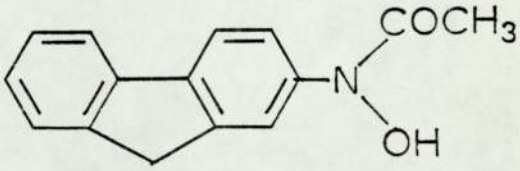

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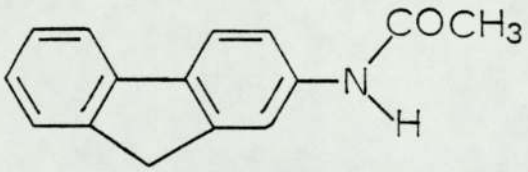

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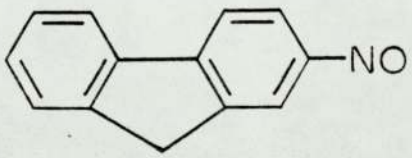

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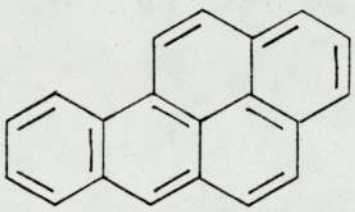

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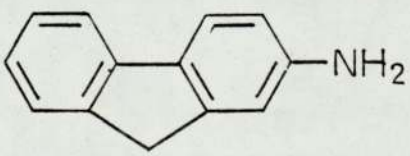
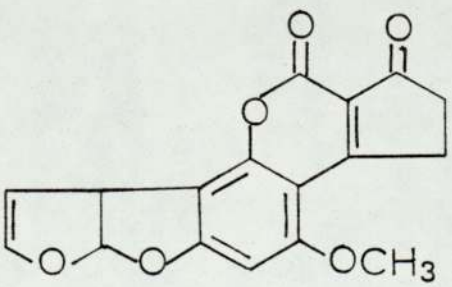


(235)



(239)

were reacting covalently with DNA, as well as intercalating, by comparing their mutagenic effect on strains with ($uvrB^+$) and without ($uvrB^-$) excision repair. These workers found that this difference in mutation frequency between $uvrB^+$ and $uvrB^-$ strains was about 100-fold with 2-nitrosofluorene (233). Slater, Anderson and Rosenkranz[120] also demonstrated the importance of excision repair when they showed that 2-nitrosofluorene (233) and N-hydroxyl-2-acetylaminofluorene (234) were more inhibitory against a strain of Escherichia coli lacking DNA polymerase 1, a component of excision repair.

The concepts proposed to explain frameshift mutagenesis have now been generally accepted. Garner and Nutman[116], in 1977, summarised these concepts and reiterated that reversion of the frameshift mutants can only take place when the mutagen itself induces a frameshift of the DNA reading frame, and in most cases intercalation of a compound into the DNA helix is insufficient to induce this effect. That is to say, to be a mutagen, it must be fixed into position by reacting chemically with the DNA. They point out, however, that there are exceptions to this, both acriflavine (238) and 9-aminoacridine (237) (see Figure 3) are both potent frameshift mutagens in replicating bacteria without undergoing covalent reaction with DNA.

The present study is in accord with the electrophilic theory of mutagenesis. Except in the case of 4-chloro-2-methyl-1H-imidazo [4,5-c]pyridine (211) (see Graph 6), all the compounds which showed frameshift mutagenic activity contained a nitrophenyl substituent in the 2-position which, after activation by bacterial nitro-reductases, was likely to react with DNA to hold the molecule

in position, e.g. (220), (166) and (203) (see Figure 4).

Although the compounds with a nitro group in the phenyl ring were strong mutagens, e.g. (220), (215) and (204) (see Table 5), compounds which differed only very slightly were inactive, e.g. (209), (191), (208) and (212) (see Figures 7 and 8). It is probable that these molecules which show negative activity are capable of intercalation in the same way as the mutagenic derivatives because the planar structure of the molecules remains unaltered. This confirms Garner and Nutman's[116] proposals that in most cases both intercalation and a chemical fixation of the molecule in DNA is necessary in order to induce a mutation.

The ease of reversion of TA1538 decreased progressively in the 2-nitrophenyl-imidazopyridine derivatives with the para-nitro derivatives having a higher mutagenic potency than the corresponding meta-nitro derivative, which was more potent than the ortho derivative (see Table 5).

The mutagenic properties of the nitrophenyl substituted imidazopyridine derivatives may appear surprising. However, bond lengths and angles of known molecules have been used to calculate the molecular size of these compounds and there is a similarity in size and structure to the highly mutagenic 2-nitrosofluorene (233) and related compounds. Appendix 1 demonstrates the similarity in size of compound (215) and 2-nitrobiphenyl, an anolog of 2-nitrosofluorene. This similarity does offer an explanation for the mutagenicity of this group of chemicals.

(iii) Toxicity

Ames et al.[115] suggested that the toxicity of chemicals to Salmonella tester strains is due, in most cases, to excessive

damage to the DNA. Two distinct groups with toxic activity were noted in the present study:-

(a) Compounds which were toxic at high doses (>500 µg/plate) and showed no mutagenic activity at lower doses, e.g. (211), (212), (214) and (197) (see Figures 5 - 8).

(b) Compounds which were toxic at high doses and mutagenic at lower doses (see Figure 4).

Compounds in group (a) do not appear to be toxic by a mutational mechanism because no mutagenic activity is present at any dose below the toxic level.

In our work, structure-activity observations show that imidazo[4,5-c]pyridine derivatives require either a halogeno-phenyl substituent in the 2-position or a chlorine atom in the 4-position when a methylphenyl substituent is in the 2-position. The imidazo[4,5-b]pyridine derivatives require a 5-amino group with a halogeno-phenyl substituent in the 2-position (see Figures 7 and 8). The planarity and intercalating ability of these molecules suggest that the toxic mechanism may involve DNA but as an inhibitor of synthesis rather than by an active mutational mechanism.

The toxic mechanism of the group (b) compounds is more easily accountable. The suggestion, by Ames *et al.*[115] that bacterial toxicity is usually due to excessive DNA damage suggests that this is the likely cause of toxicity. The mutagenic activity occurs at a much lower dosage than the toxic activity, and it is probable that as the mutagen concentration is increased, the number of lethal mutations in the bacteria increase leading to massive cell death.

Although 2-amino-4-nitropyridine 1-oxide (206) was a potent base-pair mutagen and toxic to Salmonella at higher doses, 2-chloro-4-nitropyridine 1-oxide (205) was toxic at high doses but non mutagenic. This is interesting because it parallels the mutagenically inactive but toxic halogen containing analogs of the frameshift mutagens (see above), and is an example of a toxic but mutagenically inactive chlorine containing base-pair analog.

(iv) Strain sensitivity and R-plasmids

Ames et al.[115] recommend the use of 5 strains for standard mutagenesis testing (see Table 2).

Although Ames et al.[115] suggest that TA1538 can be omitted because TA98 is more sensitive for the detection of all mutagens so far tested, observations in the present study indicate that any general screen used to test imidazopyridines or related compounds should include TA1538 (see Table 3).

The sensitivity of the strains used to test the compounds resembling 2-nitrosofluorene (233) is in good agreement with the results of McCann et al.[81] for this compound. In order to compare the effect of the R-plasmid on the sensitivity of TA1538 to different compounds, the mutagenic potency in TA1538 was divided by that for TA98 (see Table 3).

Table 2

Some chemical mutagens and their strain sensitivity

Type	Strain	R plasmid	Chemical Mutagen
base-pair mutagenesis	TA100	+	4NQNO (ii) diethylsulphate (iv) ethyl methane sulphonate (v)
	TA1535	o	4NQNO (ii) diethylsulphate (iv) ethyl methane sulphonate (v)
frameshift mutagenesis	TA1538	o	2-nitrosofluorene (i) 4NQNO (ii) benzo(a)pyrene (iii)
	TA98	+	2-nitrosofluorene (i) 4NQNO (ii) benzo(a)pyrene (iii)
	TA1537	o	9-amino acridine proflavine

(i) equal reversion in TA98 and TA1538[81]

(ii) increased reversion in TA100 compared to TA1535[81]

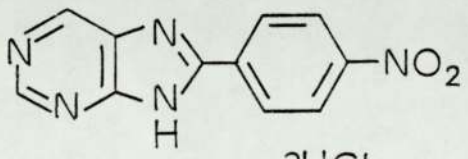
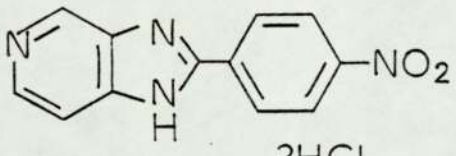
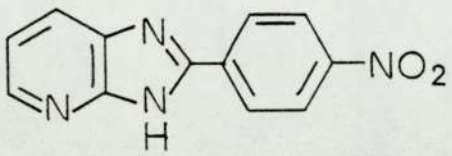
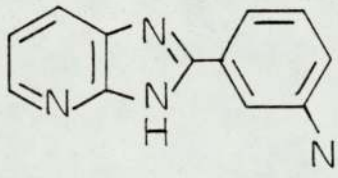
(iii) increased reversion in TA98 compared to TA1538[81]

(iv) decreased reversion in TA100 compared to TA1535[81]

(v) equal reversion in TA100 and TA1535[81]

Table 3

The effect of R-plasmid on the sensitivity of TA1538 to 2-nitrophenyl imidazo pyridines and related compounds

COMPOUND	mut. pot. TA1538* / mut. pot. Ta98
(220)  2HCl	$\frac{231}{115} = 2.1$
(215)  2HCl	$\frac{324}{152} = 2.1$
(171) 	$\frac{6.9}{3.9} = 1.76$
(166) 	$\frac{0.75}{0.24} = 3.1$

*mutagenic potency in revertants/n mole

obtained from Table 5

The reasons for the lowered sensitivity of TA98 are unknown. The R-plasmid usually enhances mutagenesis, e.g. 4NQNO (230) and aflatoxin B_1 (239) but the mechanism is not fully understood at present although recombinational repair does appear to be involved. McCann et al.[81] put forward four points which support this view.

(a) Certain mutagens cause damage to the DNA but are not mutagenic directly and it is postulated that the mutations are caused by errors introduced when the damage is repaired by error-prone recombinational repair mechanisms. Kondo et al.[121] showed that 4NQNO, furylfuramide, niridazole and trimethylphosphate were not mutagenic in strains with a recA mutation.

(b) With these mutagens the R-plasmid causes a marked increase in mutagenesis.

(c) The effect of the R-plasmid on reversion of the hisG46 mutation by these chemical mutagens, by uv, or spontaneously cannot be detected in a strain with a recA mutation but can when there is a uvrB or pol mutation.

(d) Other mutagens, such as ethyl methane sulphonate and 2-nitrosofluorene appear to be relatively independent of the rec system in causing mutations, and these mutagens are not stimulated by the presence of R-plasmids.

Several workers[81, 122, 123, 124, 125] have implicated recombinational repair in the postulated mechanism for R-plasmid stimulated mutagenesis but to date, the definitive nature is unknown.

Although the simple alkylating agents do not cause frameshift mutations, even in the strains containing R-plasmids, many of the reactive frameshift mutagens, such as 2-nitrosofluorene (233) revert

the base-pair substitution R-plasmid strain TA100. It has been postulated by McCann et al.[81] that this is due to error-prone recombinational repair after DNA damage. It is still not clear however, why some of the reactive frameshift mutagens are stimulated by the R-plasmid strain TA100 but not the R-plasmid strain TA98. The fact that compounds (215) (220) (203) (171) (166) (Table 5) revert TA100 reinforces the suggestion that they act in a similar way to the frameshift mutagen 2-nitrosofluorene (233).

The structural analogue of 4NQNO, 7-nitro-3H-imidazo[4,5-b] pyridine 4-N-oxide (173), although much weaker, appears to act in a similar way as it is only effective in reverting TA100 (see Graph 11). However, 6-hydroxylaminopurine (164) showed less activity in TA100 than in TA1535 (see Graph 14) but these results are difficult to interpret because of the toxicity of the chemicals to Salmonella.

(v) Base-pair substitution mutagenesis

The mechanism of base-pair substitution mutagenesis involves the incorporation of false bases into the DNA structure in place of the normal bases, leading to changes in the reading frame which can result in mutations.[126]

2-Amino-4-nitropyridine 1-oxide (206) was shown to be a potent base-pair substitution mutagen without liver activation (see Graph 16). Weak frameshift activity was also present and the order of mutagenic potency in the sensitive strains was TA100 >> TA98 > TA1538. It is possible that after in situ nitro-reductase activation, (206) is acting as a false base and is incorporated directly into the DNA. The nitro N-oxide (206) should be classified separately from the 2-nitrophenyl substituted imidazo-

pyridines because TA100 is more sensitive to it than TA98 or TA1538.

6-Hydroxylaminopurine (6HAP) (164) is a potent base-pair substitution mutagen (Graph 14). The structural similarity between 6HAP and the DNA base purines, especially adenine, coupled with the high reactivity of the hydroxylamino moiety, gives the molecule high activity as a potential false base in DNA. The exact molecular mechanism by which 6HAP exerts its mutagenic action is unknown but it would be of interest to test 1-deaza-adenine (3) and 1-deaza-adenine-3-N-oxide (174) with liver activation.

6-chloropurine (163), which has been shown to possess anti-tumour cytotoxic activity[138] was inactive in the test without liver activation. When tested with liver activation, the results were inconclusive (see Figure 9).

(vi) Implications for possible use in cancer chemotherapy screening procedures

Further liver activation work is necessary on some of the amino containing compounds but the results so far show that some of the compounds prepared are potent frameshift mutagens in the Ames test without liver activation. These compounds may be of value in cancer chemotherapy despite the fact that they resemble the well known mutagen and carcinogen, 2-nitrosofluorene. Evidence to date indicates that cancer chemotherapeutic agents in present day use are both mutagenic and carcinogenic.[93] Further investigation using animal in vivo tests are necessary before assessment is possible. It is interesting to note that 6HAP, which was found to be a potent mutagen in this study, has previously been tested in rats and found to be extremely cytotoxic against a number of tumours.[127]

In conclusion, the Ames test does offer valuable information about compounds of potential use in cancer chemotherapy, and because of the low cost, reliability and speed involved compared to animal *in vivo* studies, it is of use as a primary screen for compounds with cytotoxic activity.

EXPERIMENTAL

A. CHEMICAL

B. BIOLOGICAL

(1) MATERIALS and METHODS

(2) RESULTS

PAGE

A. CHEMICAL

Infrared spectra were determined as potassium bromide (KBr) discs, unless otherwise stated, 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 (NMR) spectra were determined, unless otherwise stated, with tetramethylsilane as internal standard, on a Varian A60-A spectrometer. All the peaks are assigned in terms of τ values. Abbreviations used in the interpretation of NMR spectra are:

s = singlet; d = doublet; t = triplet; q = quartet;

J = coupling constant; a = removed by deuteration.

Mass spectra and accurate mass measurements were determined on an A.E.I. MS9 (relative intensities in parentheses). M, signifies the molecular ion peak.

Melting points are uncorrected and reaction temperatures above 100° are those of an external oil bath.

(i) Reactions with 2,3-diaminopyridine

2-Amino-3-nitropyridine (165).

Prepared by the method of Pino and Zehrung,[108] m.p. 163°, (lit.[108] 163°-164°).

2,3-Diaminopyridine (51).

Prepared by the general method of Petrow and Saper,[2] m.p. 117°, (lit.[2] 118°-119°).

2-(m-Nitrophenyl)-3H-imidazo[4,5-b]pyridine (166).

2,3-Diaminopyridine (1.1 g), m-nitrobenzoic acid (1.71 g) and polyphosphoric acid (36 g) were stirred at 160° for 2 h, cooled, diluted with water (100 ml) and neutralised with potassium carbonate. The dark brown precipitate was collected and boiled with water (25 ml) to yield the dark-brown, water insoluble imidazopyridine which was recrystallised from dimethylformamide to produce light yellow needles (1.95 g, 89%), m.p. > 300° (sublimed).

ν max (KBr) 1600, 1520 (NO_2), 1360 (NO_2) 1240, 1120, 830, 780, 700 cm^{-1}.

Found, C, 59.6; H, 3.7; N, 23.6%.

Required for $C_{12}H_8N_4O_2$, C, 60.0; H, 3.3; N, 23.3%

Found, M, 240.06421.

Required for $C_{12}H_8N_4O_2$, M, 240.064721.

m/e 241 (18), 240 (92), 195 (5), 194 (100), 193 (24), 168 (7), 167 (24), 166 (9), 140 (29), 103 (16), 73 (26).

No suitable solvent was available for the determination of an n.m.r. spectrum.

2-(o-Chlorophenyl)-3H-imidazo[4,5-b]pyridine (167)

2,3-Diaminopyridine (1 g), o-chlorobenzoic acid (1.44 g) and polyphosphoric acid (34 g) were stirred at 180° for 3 h, cooled, diluted with water (100 ml) and neutralised with potassium carbonate. The red precipitate was collected, recrystallised several times from absolute ethanol and treated with activated charcoal to yield the imidazopyridine (1.3 g, 62%), m.p. 189°, white feathery solid.

Found, M, 229.04121.

Required for $C_{12}H_8Cl^{35}N_3$, M, 229.04067.

Found, M, 231.03745.

Required for $C_{12}H_8Cl^{37}N_3$, M, 231.03772.

ν max. (KBr) 3000, 1450, 1410, 1320, 1120, 1050, 780, 740 cm^{-1}.

Found, C, 62.4; H, 3.4; N, 18.5%.

Required for $C_{12}H_8ClN_3$, C, 62.7; H, 3.5; N, 18.3%.

τ TFA. 1.15[1H, d, J = 6Hz, 5-H]
2.01[1H, t, J = 5Hz, 6-H]
2.33[5H, m, 3'4'5'6'7-H]

2-(m-Chlorophenyl)-3H-imidazo[4,5-b]pyridine (168)

2,3-Diaminopyridine (1 g), m-chlorobenzoic acid (1.44 g) and polyphosphoric acid (30 g) were stirred at 180° for 4 h, cooled, diluted with water (100 ml) and neutralised with potassium carbonate. The red precipitate was collected and washed with hot water (100 ml) to yield the crude imidazopyridine which was treated with activated charcoal and recrystallised from methanol to give the pure imidazopyridine as colourless crystals (1.8 g,

85%), m.p. 287°-288°.

Found, M, 229.04097.

Required for $C_{12}H_8N_3Cl^{35}$, M, 229.04067.

Found, M, 231.03818.

Required for $C_{12}H_8N_3Cl^{37}$, M, 231.03772.

ν max. (KBr) 3050, 1600, 1460, 1365, 1260, 890, 800, 760, 740, 710 cm^{-1}.

Found, C, 62.6; H, 3.4; N, 18.2%.

Required for $C_{12}H_8ClN_3$, C, 62.9; H, 3.5; N, 18.3%

2-(m-Bromophenyl)-3H-imidazo[4,5-b]pyridine (169)

2,3-Diaminopyridine (3 g), m-bromobenzoic acid (5.52 g) and polyphosphoric acid (45 g) were stirred at 180° for 3.5 h, cooled, diluted with water (300 ml) and neutralised with potassium carbonate. The red precipitate was collected and washed with boiling water (25 ml) to yield the imidazopyridine (6.5 g, 87%). Purification was effected by dissolving in concentrated hydrochloric acid and precipitating with concentrated ammonium hydroxide solution. Further purification was effected by sublimation, m.p. >300°, off-white feathery solid.

Found, C, 52.5; H, 2.9; N, 15.6%.

Required for $C_{12}H_8BrN_3$, C, 52.7; H, 2.9; N, 15.4%.

ν max. (KBr) 3100, 1600, 1410, 1260, 800, 760, 730 cm^{-1}.

No suitable solvent was available for the determination of an n.m.r. spectrum.

Found, M, 272.99032.

Required for $C_{12}H_8N_3Br_1^{79}$, M, 272.99021.

m/e 275 (100), 274 (51), 273 (100), 195 (11), 194 (56), 193 (7), 184 (8), 183 (3), 182 (8), 181 (3), 168 (2), 167 (6), 166 (3), 157 (4), 155 (4), 140 (8), 103 (8), 102 (8), 97 (8), 96 (7), 90 (16), 78 (3), 77 (8), 76 (8).

2-(p-Bromophenyl)-3H-imidazo[4,5-b]pyridine (170)

2,3-Diaminopyridine (3 g), p-bromobenzoic acid (5.52 g) and polyphosphoric acid (50 g) were stirred at 180° for 3 h, cooled, diluted with water (250 ml) and neutralised with potassium carbonate. The red-brown precipitate was collected and washed with hot water (25 ml). The pure imidazopyridine was obtained by sublimation of the red-brown solid (7.1 g, 95%) m.p. >300°, white feathery solid.

Found, C, 52.6; H, 2.8; N, 15.2%.

Required for $C_{12}H_8BrN_3$, C, 52.7; H, 2.9; N, 15.4%.

ν max. (KBr) 3100, 1600, 1440, 1410, 1280, 1070, 1020, 950, 840, 780, 730 cm^{-1}.

τ (TFA) 1.05[1H, d, J = 6Hz, 5-H]
1.20[1H, d, J = 6Hz, 7-H]
1.14[1H, t, 6-H]
2.05[4H, m, 2'3'5'6'-H]

Found, M, 272.99021.

Required for $C_{12}H_8N_3Br$, M, 272.99003.

m/e 276 (15), 275 (100), 274 (47), 273 (100), 272 (28), 249 (13), 248 (6), 247 (13), 233 (14), 232 (9), 231 (14), 197 (80), 196 (100), 195 (60), 194 (12), 185 (10), 184 (5), 183 (10), 182 (4), 169 (13), 168 (40), 167 (27), 166 (12), 158 (40), 156 (37), 140 (69), 137 (37), 136 (41), 103 (72), 102 (100), 97 (81).

2-(p-Nitrophenyl)-3H-imidazo[4,5-b]pyridine (171)

2,3-Diaminopyridine (1.1 g), p-nitrobenzoic acid (1.71 g) and polyphosphoric acid (37 g) were stirred at 170° for 3 h. The reaction mixture was allowed to cool and 50 ml of water was added. The solution was neutralised with potassium carbonate and the brown solid which was produced was collected by filtration, washed with water and dried in an oven overnight at 100°. The brown solid was sublimed to produce the yellow imidazopyridine (1.8 g, 74%), bright yellow feathery solid, m.p. >300° (resublimed).

ν max. (KBr) 1600, 1520 (NO_2), 1350(NO_2), 1260, 860, 710 cm^{-1}.

No suitable solvent was available for the determination of an n.m.r. spectrum.

Found, M, 240.06437.

Required for $C_{12}H_8N_4O_2$, M, 240.064721.

m/e 241 (14), 240 (100), 194 (44), 167 (17), 149 (18), 140 (12), 85 (17), 84 (10), 83 (14), 81 (17), 76 (12), 71 (31), 70 (14), 69 (30).

Found, C, 60.0; H, 3.2; N, 23.2%

Required for $C_{12}H_8N_4O_2$, C, 60.0; H, 3.3; N, 23.3%.

3H-Imidazo[4,5-b]pyridine (1)

2,3-Diaminopyridine (14.9 g), formic acid (98/100%, 20 ml) and polyphosphoric acid (83 g) were stirred at 190° for 4 h, cooled, diluted with water (300 ml) and neutralised with potassium carbonate. The solution was extracted with chloroform continuously for 12 h and the chloroform removed in vacuo to leave the crude imidazopyridine (14.9 g, 91%). The crude product was treated with activated charcoal and recrystallised from methanol several times, m.p. 154° (lit.[2] 154°), colourless needles.

Found, C, 60.0; H, 4.2; N, 35.0%.

Calc. for $C_6H_5N_3$, C, 60.5; H, 4.2; N, 35.3%.

ν max. (nujol) 2900 (nujol), 1620, 1580, 1460 (nujol), 1380 (nujol), 1315, 1280, 1230, 1110, 960, 780 cm^{-1}.

τ $(CD_3)_2SO$ 1.4[1H, s, 2-H]

1.5[1H, q, J_{56} = 4.5Hz, J_{57} = 1.5Hz, 5-H]

1.85[1H, q, J_{75} = 1.5Hz, J_{76} = 7.5Hz, 7-H]

2.65[1H, q, J_{65} = 4.5Hz, J_{67} = 7.5Hz, 6-H]

3H-Imidazo[4,5-b]pyridine 4-N-oxide (172)

3H-Imidazo| 4,5-b |pyridine (9.7 g), glacial acetic acid (70 ml) and hydrogen peroxide (100 vol, 14.1 ml) were heated on a steam bath at 100° for 3 h. A further aliquot of hydrogen peroxide (14.1 ml) was added and heating was continued for another 4.5 h. The solvent was removed in vacuo to leave the yellow N-oxide (10.0 g, 91%), m.p. 250° (lit.[102] 252°), (from methanol).

Found, M, 135.0433.

Calc. for $C_6H_5N_3O$, M, 135.043259.

ν max. (KBr) 2900, 1700, 1600, 1450, 1310, 1240, 940, 870, 800, 750, 710 cm^{-1}.

τ $(CD_3)_2SO$ 1.4[1H, s, 2-H]

1.67[1H, q, J_{56} = 6Hz, J_{57} = 1Hz, 5-H]

2.25[1H, q, J_{75} = 1Hz, J_{76} = 8Hz, 7-H]

2.65[1H, q, J_{76} = 8Hz, J_{65} = 6Hz, 6-H]

7-Nitro-3H-imidazo[4,5-b]pyridine 4-N-oxide (173)

3H-Imidazo[4,5-b]pyridine 4-N-oxide (8.4 g), fuming nitric acid (d 1.5, 50 ml) and glacial acetic acid (75 ml) were heated at 100° for 5 h. The reaction mixture was cooled and neutralised with 40% sodium hydroxide solution to yield the nitro-N-oxide (9.2 g, 82%), m.p. 288° (lit.[102] 289°), (from water), light brown needles.

ν max. (nujol) 3300, 3100, 2900 (nujol), 1600, 1520 (NO_2), 1460 (nujol), 1380 (nujol), 1360 (NO_2), 1260, 1230, 1160, 1070, 740 cm^{-1}.

τ (TFA) 1.28[1H, s, 2-H]

1.43[1H, d, J = 6Hz, 6-H]

1.90[1H, d, J = 6Hz, 5-H]

7-Amino-3H-imidazo[4,5-b]pyridine 4-N-oxide (174)

7-Nitro-3H-imidazo[4,5-b]pyridine 4-N-oxide (2.6 g) was catalytically hydrogenated with palladium on charcoal 5% in absolute ethanol (150 ml) at a pressure of 3 atm. for 5 h at

room temperature. The reaction mixture was filtered and removal of solvent *in vacuo* yielded the *amine N-oxide* (1.1 g, 51%), m.p. 247-248°, orange needles (from ethanol).

An identical product was obtained under the same conditions when Raney Nickel catalyst was used. Platinum oxide in water under similar conditions also gave the amine N-oxide .

ν max. (KBr) 3200, 1660, 1620, 1530, 1440, 1410, 1360, 1300, 1320, 1220, 820, 660 cm^{-1}.

Found, *M*, 150.053620.

Required for $C_6H_6N_4O$, *M*, 150.054157.

m/e 150 (5), 149 (4), 135 (11), 134 (100), 108 (5), 107 (36), 106 (11), 80 (18), 79 (15), 67 (9), 66 (5), 65 (5), 64 (5), 55 (5), 54 (7), 53 (27).

Found, C, 47.5; H, 4.1; N, 37.0%.

Required for $C_6H_6N_4O$, C, 48.0; H, 4.0; N, 37.3%.

τ $(CD_3)_2SO$ 3.1[1H, d, J = 8Hz, 6 - H]
1.6[1H, d, J = 8Hz, 5 - H]
1.5[1H, s, 2 - H]
2.5[1H, s(a), 7 - NH_2]

7-*Amino*-3H-*imidazo*[4,5-b]*pyridine hydrochloride* (3)

(a) 7-Nitro-*3H*-imidazo[4,5-*b*]pyridine 4-N-oxide (0.6 g), palladium charcoal 5% (0.6 g), glacial acetic acid (130 ml) and acetic anhydride (10 ml) were hydrogenated at 3 atm. for 6 h. The solvent was removed *in vacuo* and after the addition of a few drops of concentrated hydrochloric acid, the 1-deaza-adenine hydrochloride crystallised out (0.42 g, 74%), white plates,

m.p. >300° (lit.[102] 328°).

(b) Under similar conditions, 7-amino-3H-imidazo[4,5-b] pyridine 4-N-oxide (0.3 g) was reduced to yield an identical product (2.8 g, 82%).

Found, C, 42.2; H, 4.1; N, 32.7%.

Calc. for $C_6H_7ClN_4$, C, 42.4; H, 4.1; N, 32.9%.

ν max. (KBr) 3200, 2700, 1660, 1620, 1560, 1510, 1430, 1330, 1320, 1220, 940, 800 cm^{-1}.

Found, M, 134.059140.

Calc. for $C_6H_6N_4$, M, 134.059243.

m/e 136 (2), 135 (10), 134 (100), 119 (2), 118 (1), 117 (1), 108 (5), 107 (52), 106 (17), 94 (8), 93 (3), 81 (6), 80 (48), 79 (42), 78 (5), 77 (5), 69 (13), 68 (7), 67 (25), 66 (8), 60 (10).

τ $(CD_3)_2SO$ 3.05[1H, d, J = 7Hz, 6 - H]
1.79[1H, d, J = 7Hz, 5 - H]
1.28[1H, s, 2 - H]

5-Chloro-3H-imidazo[4,5-b]pyridine (175)

3H-Imidazo[4,5-b]pyridine 4-N-oxide (0.3 g) and phosphoryl chloride were heated at 100° for 6 h and the phosphoryl chloride removed in vacuo to leave a solid residue which was treated with crushed ice. The solution was neutralised with concentrated ammonium hydroxide solution and extracted continuously with chloroform for 12 h. The chloroform was reduced in volume in vacuo and the imidazopyridine crystallised out (0.3 g, 77%), m.p. 258° (ethanol).

Found, M, 153.00959.

Required for $C_6H_4N_3Cl^{35}$, M, 153.00937.

Found, M, 155.00625.

Required for $C_6H_4N_3Cl^{37}$, M, 155.00642.

m/e 156 (3), 155 (28), 154 (11), 153 (100), 127 (7), 126 (3), 125 (13), 124 (3), 118 (5), 117 (25), 98 (7), 91 (4), 90 (11), 63 (13), 62 (7), 61 (3).

ν max. (KBr) 1600, 1580, 1380, 1340, 1280, 1220, 1100, 940, 820 cm^{-1}.

Found, C, 47.2; H, 2.5; N, 27.3%.

Required for $C_6H_4ClN_3$, C, 47.0; H, 2.6; N, 27.5%.

5,7-Dichloro-3H-imidazo[4,5-b]pyridine (176)

(a) 7-Nitro-3H-imidazo[4,5-b] pyridine 4-N-oxide (2 g) and phosphoryl chloride (100 ml) were heated on a steam bath for 2.5 h. The solvent was removed in vacuo and the residue suspended in ice. The dichloro-product was obtained as a yellow precipitate by neutralisation of the suspension with concentrated ammonium hydroxide and was recrystallised from boiling water to give light yellow needles (1.8 g, 86%), m.p. 273-274° (lit.[12] 275°).

(b) 7-Amino-3H-imidazo[4,5-b]pyridine 4-N-oxide (0.2 g) was heated with phosphoryl chloride at 100° for 3 h and the dichloro compound isolated as above (0.2 g 80%), m.p. 274°.

(a) Found, M, 186.97018.

Calc. for $C_6H_3Cl_2{}^{35}N_3$, M, 186.9704.

Found, M, 190.96441.

Calc. for $C_6H_3Cl_2{}^{37}N_3$, M, 190.9645.

(b) Found, M, 186.97101.

Calc. for $C_6H_3Cl_2{}^{35}N_3$, M, 186.9704.

Found, M, 190.96492.

Calc. for $C_6H_3Cl_2{}^{37}N_3$, M, 190.9645.

ν max. (KBr) 3010, 1550, 1440, 1350, 1330, 1250, 1140, 950, 910, 810 cm^{-1}.

τ $(CD_3)_2SO$ 1.07[1H, s, 2-H]
1.86[1H, s, 6-H]

m/e 191 (3), 190 (2), 189 (16), 188 (2), 187 (23), 155 (3), 154 (30), 153 (10), 152 (100), 151 (2), 140 (4), 127 (7), 126 (2), 125 (20), 124 (3), 100 (23), 99 (6), 98 (65), 97 (2), 91 (4), 90 (12), 89 (15), 88 (3), 77 (4), 76 (3), 75 (4), 74 (4), 73 (7), 72 (4).

Treatment of 5,7-dichloro-3H-imidazo[4,5-b]pyridine (176) with alcoholic ammonia solution

5,7-Dichloro-3H-imidazo[4,5-b]pyridine (0.3 g) and absolute ethanol saturated at 0° with ammonia gas (20 ml) were heated in a steel bomb at 100° for 12 h. The ethanol was removed in vacuo to leave a product which was identified spectroscopically as starting material (0.3 g).

3H-Imidazo[4,5-b]pyridine-2(1H)-one (44)

Prepared by the method of Petrow and Saper[2], m.p. 274° (lit.[2] 274°).

3H-Imidazo[4,5-b]pyridine-2(1H)-thione (63)

Thiophosgene (1 ml) was added dropwise to a stirred solution of 2,3-diaminopyridine (0.6 g) in concentrated hydrochloric acid (10 ml) and water (10 ml). The reaction mixture was stirred at room temperature for 24 h and basified with concentrated ammonium hydroxide solution to yield the imidazopyridine (0.65 g, 78%), m.p. >300° (lit.[2] >300°).

ν max. (KBr) 3500, 3000, 1650, 1500, 1420, 1380, 1320, 1170, 1040, 980, 740 cm^{-1}.

Found, M, 151.02036.

Calc. for $C_6H_5N_3S$, M, 151.02042.

m/e 151 (67), 135 (6), 124 (7), 123 (6), 119 (6), 94 (7), 93 (33), 92 (8), 78 (9), 77 (7), 76 (100), 66 (13), 65 (9), 64 (10).

3H-Triazolo[4,5-b]pyridine (177)

A solution of sodium nitrite (3.4 g) in water (9.72 ml) at 5° was added dropwise to a stirred solution of 2,3-diaminopyridine (3.27 g) in concentrated hydrochloric acid (14.7 ml) and water (29.4 ml) at 5°. The reaction mixture was stirred at 5° for 1 h, neutralised with concentrated ammonium hydroxide solution and extracted continuously with chloroform for 12 h. The chloroform was removed in vacuo to leave the crude product which was recrystallised from water (2.8 g, 78%), m.p. 195° (lit.[106] 196°).

ν max. (KBr) 1580, 1520, 1400, 1340, 1260, 1180, 980, 930, 805, 790 cm^{-1}.

Found, M, 120.04423.

Calc. for $C_5H_4N_4$, M, 120.04359.

m/e 121 (7), 120 (65), 97 (3), 96 (2), 95 (3), 94 (3), 93 (15), 92 (100), 91 (6), 87 (3), 85 (17), 83 (23), 81 (4), 79 (3), 78 (2), 77 (4), 71 (7), 70 (3), 69 (8), 67 (6), 66 (29), 65 (75), 64 (38), 63 (12), 57 (13), 56 (5), 55 (11), 54 (3), 53 (13), 52 (36).

Found, C, 46.5; H, 3.1; N, 46.8%.

Calc. for C_5H_4N, C, 50.0; H, 3.3; N, 46.6%.

τ $(CD_3)_2SO$ 1.12[1H, q, J_{56} = 4Hz, J_{57} = 1Hz, 5-H]

1.4 [1H, q, J_{57} = 1Hz, J_{67} = 8.5Hz, 7-H]

2.35[1H, q, J_{56} = 4Hz, J_{67} = 8.5Hz, 6-H]

Reaction of 2,3-diaminopyridine with cyanogen bromide

2,3-Diaminopyridine (1.9 g), cyanogen bromide (1.9 g) and methanol (110 ml) were refluxed for 2 h and then cooled. Addition of ether produced a green precipitate which was collected and immediately dissolved in concentrated ammonium hydroxide solution. The solution was extracted with chloroform continuously for 12 h, the chloroform removed in vacuo and the residue dissolved in ethanol (50 ml) and treated with hydrogen chloride gas. A product precipitated on addition of ether but on exposure to air, rapid decomposition occurred and spectroscopic analysis was not possible.

Attempted fusion of guanidine with 2,3-diaminopyridine

Guanidine hydrochloride (1.12 g) was added to a solution of sodium (0.27 g) in absolute ethanol (10.8 ml) and the sodium chloride filtered off. The filtrate was reduced in volume in vacuo to leave the syrupy guanidine base. 2,3-Diaminopyridine (1 g) was

added to the guanidine base and heated at 250° for 0.5 h. The residue was cooled and boiled up with potassium hydroxide solution (2N, 75 ml). The alkaline solution was extracted with ethyl acetate continuously for 6 h, dried with sodium sulphate and the solvent removed in vacuo to leave a solid residue. The solid residue was dissolved in absolute ethanol and hydrogen chloride gas bubbled through to obtain the hydrochloride which was precipitated by addition of ether. However, on exposure to air, the product decomposed to a charred mass almost instantaneously and spectroscopic analysis was not possible.

(ii) Reactions with 2,3-diamino-6-methylpyridine dihydrochloride

2-Amino-6-methyl-3-nitropyridine (178)

Prepared by the general method of Pino and Zehrung, m.p. 140° (lit.[108] 141°).

2,3-Diamino-6-methylpyridine dihydrochloride (179)

2-Amino-6-methyl-3-nitropyridine, palladium on charcoal (5%, 0.2 g) and ethanol (200 ml) were hydrogenated overnight at normal temperature and pressure. The reaction suspension was filtered through sintered glass into concentrated hydrochloric acid and the diaminopyridine dihydrochloride separated out as white needles (1.7 g, 85%) m.p. >300°.

Found, M, 123.

Required for $C_6H_7N_3O_2$, M, 123.

ν max. (KBr) 1660, 1630, 1550, 1520, 1470, 1420, 1370, 1300, 1140, 1100, 1040, 840 cm^{-1}

5-Methyl-3H-imidazo[4,5-b]pyridine-2(1H)-one (180)

2,3-Diamino-6-methylpyridine dihydrochloride (1 g) and urea (1 g) were finely mixed by grinding in a mortar and then fused at 240° in a metal bath for 0.5 h. The resultant white solid was washed with cold water (20 ml) and the imidazopyridine recrystallised from absolute ethanol (0.88 g, 79%), m.p. 250°, fluffy white solid.

Found, C, 56; H, 4.6; N, 28.4%.

Required for $C_7H_7N_3O$, C, 56.4; H, 4.7; N, 28.2%.

ν max. (KBr) 3500, 3100, 1720 (C=O), 1620, 1460, 1260, 1120, 810, 770, 710 cm^{-1}.

τ (TFA) 1.72[1H, d, J = 8Hz, 7-H]
2.4 [1H, d, J = 8Hz, 5-H]
7.15[3H, s, 5-CH_3]

Found, M, 149.058070.

Required for $C_7H_7N_3O$, M, 149.058908.

m/e 149 (23), 134 (11), 133 (8), 121 (9), 120 (11), 119 (14), 111 (17), 110 (8), 109 (11), 107 (8), 106 (8), 105 (18), 97 (26), 96 (11), 95 (21), 94 (10), 91 (11), 85 (20), 84 (11), 83 (25), 82 (12), 81 (22), 71 (32), 70 (16), 69 (34), 68 (11), 67 (20), 55 (55), 44 (86), 43 (61), 42 (18), 41 (50), 40 (100).

5-Methyl-6-nitro-3H-imidazo[4,5-b]pyridine-2(1H)-one (181)

A mixture of potassium nitrate (730 mg) and concentrated sulphuric acid (4 ml) at 0° was added dropwise with stirring to a mixture of 5-methyl-3H-imidazo[4,5-b]pyridine-2(1H)-one (1 g)

and concentrated sulphuric acid (4 ml) cooled to 0° in an ice-salt bath. The reaction mixture was heated on a steam bath for 2 h, cooled, poured onto crushed ice and neutralised with concentrated ammonium hydroxide to give a yellow precipitate. This was collected, dried and extracted with ethyl acetate in a soxhlet apparatus to give the pure nitro-compound (0.8 g, 62%), m.p. $>300^{\circ}$, yellow needles (from ethyl acetate).

ν max. (KBr) 1740 (C=O), 1680, 1540 (NO_2), 1500, 1340 (NO_2), 1000, 840, 710 cm^{-1}.

τ (TFA) 1.13[1H, s, 6-H]

6.9 [3H, s, 5-CH_3]

Found, M, 194.04348.

Required for $C_7H_6N_4O_3$, M, 194.043986.

m/e 194 (9), 193 (51), 178 (4), 177 (100), 162 (30), 150 (9), 149 (38), 148 (33), 147 (13), 135 (23), 134 (16), 123 (8), 122 (58), 121 (19), 120 (15), 106 (19), 105 (10), 95 (14), 94 (15), 93 (25), 92 (13), 83 (21), 80 (15), 79 (22), 78 (47), 77 (16), 66 (17).

Treatment of 5-methyl-6-nitro-3H-imidazo[4,5-b]pyridine-2(1H)-one (181) with hydrochloric acid

5-Methyl-7-nitro-3H-imidazo[4,5-b]pyridine-2(1H)-one (1 g) and concentrated hydrochloric acid (20 ml) were refluxed for 18 h. The hydrochloric acid was removed in vacuo and the residue treated with crushed ice and concentrated ammonium hydroxide solution. The tan precipitate was collected and

extracted with chloroform continuously for 12 h. The chloroform was removed *in vacuo* to leave starting material (0.95 g).

1-Acetyl-5-methyl-3H-imidazo[4,5-b]pyridine-2(1H)-one (182)

5-Methyl-3H-imidazo[4,5-b]pyridine-2(1H)-one (0.3 g) was refluxed with acetic anhydride (5 ml) and three drops of concentrated sulphuric acid for 0.5 h. The 1-acetyl compound crystallised out on cooling (0.29 g, 74%), m.p. 274°.

τ $(CD_3)_2SO$ 7.8[3H, s, 1-Ac]
7.6[3H, s, 5-Me]
3.25[1H, d, 8Hz, 6-H]
2.15[1H, d, 8Hz, 7-H]

ν max. (KBr) 3400, 3050, 1720 (C=O), 1620, 1520, 1450, 1250, 800, 710 cm^{-1}.

Found, M, 149.05880.

Required for $C_7H_7N_3O$, M, 149.058908.

m/e 192 (60), 193 (7), 150 (43), 149 (100), 148 (37), 121 (25), 120 (14), 94 (4), 80 (11), 79 (7), 78 (11).

1-Acetyl-5-methyl-3H-imidazo[4,5-b]pyridine-2(1H)-one 4-N-oxide (183)

5-Methyl-3H-imidazo[4,5-b]pyridine-2(1H)-one (0.5 g), glacial acetic acid (3.0 g) and hydrogen peroxide (100 vol, 0.5 ml) were heated at 100° for 3 h. A further aliquot of hydrogen peroxide (0.5 ml) was added and heating continued for a further 3 h. The yellow solution was reduced in volume *in vacuo* to yield the light yellow N-oxide (0.53 g, 76%), m.p. 277° (from glacial acetic acid).

Found, M, 165.05425.

Required for $C_7H_7N_3O_2$, M, 165.05382.

ν max. (KBr) 3100, 1710 (C=O), 1620, 1520, 1380, 1280, 1210, 1010, 840, 820, 760 cm^{-1}.

τ (TFA) 7.8[3H, s, 1-Ac]
7.27[3H, s, 5-Me]
2.58[1H, d J=8Hz, 6-H]
2.09[1H, d J=8Hz, 7-H]

1,3-Diacetyl-5-methyl-3H-imidazo[4,5-b]pyridine-2(1H)-one 4-N-oxide (184)

1-Acetyl-5-methyl-3H-imidazo[4,5-b]pyridine-2(1H)-one 4-N-oxide (0.5 g) was refluxed for 0.25 h with acetic anhydride (5 ml). On standing, the diacetyl compound (184) crystallised out as light yellow needles (0.51 g, 85%), m.p. 283-284°.

Found, M, 165.05370.

Required for $C_7H_7N_3O_2$, M, 165.05382.

ν max. (KBr) 1720 (C=O), 1620, 1520, 1410, 1210, 820, 780 cm^{-1}.

τ (TFA) 7.8[6H, s, 1 and 3 Ac.]
7.25[3H, s, 5-Me]
2.6[1H, d, J=8Hz, 6-H]
2.07[1H, d, J=8Hz, 7-H]

5-Methyl-3H-imidazo[4,5-b]pyridine-2(1H)-one 4-N-oxide (185)

The 1-Acetyl compound (183) (0.5 g) was boiled with water for 1 h. The volume was reduced in vacuo to yield the N-oxide

in quantitative yield m.p. 268°-269°.

Found, M, 165.05352.

Required for $C_7H_7N_3O_2$, M, 165.05382.

ν max. (KBr) 3500 (N - H), 3100, 1700 (C = O), 1620, 1410, 1220, 1000, 820, 780, 760 cm^{-1}.

τ (TFA) 7.25 [3H, s, 5 - Me]

2.57 [1H, d, J = 8Hz, 6 - H]

2.1 [1H, d, J = 8Hz, 7 - H]

(iii) Reactions with 2,3-diamino-6-hydroxypyridine dihydrochloride

2-Amino-6-hydroxy-3-phenylazopyridine hydrochloride (186)

A solution of sodium nitrite (2.43 g) in water (34.7 ml) at 5° was added to a cooled (0°-5°) solution of aniline (3.1 ml) in concentrated hydrochloric acid (17.4 ml) and water (17.4 ml). The resultant solution, cooled to 5°, was added to a suspension of 2-amino-6-hydroxypyridine (5 g) in concentrated hydrochloric acid (34.7 ml) and water (34.7 ml) previously cooled to 0°. The solution was stirred at 5° for 6 h and then allowed to stand overnight at room temperature. The brick red product was filtered off, (8.5 g, 87%), m.p. 233°.

Found, M, 214.

Required for $C_{11}H_{10}N_4O$, M, 214.

m/e 214 (20), 213 (100), 211 (20), 137 (85), 112 (27), 110 (40), 109 (82), 105 (18), 94 (71), 93 (46), 92 (15), 91 (15), 82 (29), 81 (55), 79 (16), 78 (16), 77 (93), 66 (47), 65 (42), 64 (15).

2,3-Diamino-6-hydroxypyridine dihydrochloride (187)

2-Amino-6-hydroxy-3-phenylazopyridine hydrochloride (1 g), palladium on charcoal (5%, 0.1 g) and ethanol (150 ml) were hydrogenated overnight at room temperature and normal pressure. The reaction suspension was filtered through sintered glass into concentrated hydrochloric aicd (2 ml). The diaminopyridine dihydrochloride separated out as colourless needles (0.5 g, 86%) m.p. >300°.

ν max. (KBr) 3400, 2900, 1650, 1600, 1580, 1360, 1270, 1200, 1130, 1060, 840, 760, 660 cm^{-1}.

Found, M, 125.

Required for $C_5H_7N_3O$, M, 125.

3H-Imidazo[4,5-b]pyridine-5(4H)-one (188)

Prepared by the method of Graboyes and Day,[11] off white needles, m.p. >300°, (lit.[11] 311°).

An identical product was obtained by refluxing 2,3-diamino-6-hydroxypyridine dihydrochloride (0.5 g) with triethylorthoformate (5 ml) for 2.5 h. The solvent was removed in vacuo and after addition of water (2 ml), the solution was made neutral with sodium hydroxide solution (10%) and cooled overnight in a refridgerator to yield the product (0.38 g, 90%).

ν max. (KBr) 3400, 3000, 1640 (C=O), 1560, 1500, 1340, 1260, 1120, 980, 820 cm^{-1}

τ $(CD_3)_2SO$ 2.1[1H, s, 2-H]

2.24[1H, d, J_{67} = 9Hz, 7-H]

3.69[1H, d, J_{67} = 9Hz, 6-H]

Found, M, 135.04275.

Calc. for $C_6H_5N_3O$, M, 135.043259.

m/e 136 (15), 135 (100), 119 (1), 118 (1), 109 (2), 108 (26), 107 (27), 106 (2), 82 (1), 81 (15), 80 (16), 79 (2), 78 (1).

(iv) Reactions with 2,3,6-triaminopyridine dihydrochloride

2,6-Diamino-3-phenylazopyridine hydrochloride (189)

Prepared by the method of Ostromislensky,[109] m.p. 203° (lit.[109] 204°).

2,3,6-Triaminopyridine dihydrochloride (190)

Prepared by the method of Vaughan, Krapcho and English,[6] m.p. 228° (lit.[6] 230°).

5-Amino-2-(p-bromophenyl)-3H-imidazo[4,5-b]pyridine dihydrochloride (191)

2,3,6-Triaminopyridine dihydrochloride (5.9 g), p-bromobenzoic acid (5.96 g) and polyphosphoric acid (68 g) were stirred at 170° for 3 h and then cooled. After the addition of water (500 ml), the solution was neutralised with potassium carbonate and a green solid was collected and washed with hot water (50 ml). The imidazopyridine was purified as the dihydrochloride by boiling with ethanolic hydrogen chloride (activated charcoal) and precipitating with ether to give an off-white feathery product (8.1 g, 83%), m.p. >300° (sublimed).

ν max. (KBr) 3400, 3150, 1660, 1440, 1400, 1310, 1140, 1020, 820 cm^{-1}.

τ (TFA) 0.83[1H, d, J = 9Hz, 7 - H]

1.36[4H, m, 2'3'5'6' - H]

1.98[1H, d, J = 9Hz, 6 - H]

Found, M, 288.000580.

Required for $C_{12}H_9BrN_4$, M, 288.001110.

m/e 288 (100), 290 (90), 289 (27), 209 (12), 183 (7), 182 (24), 181 (9), 180 (24), 119 (11), 114 (6), 113 (10), 112 (16), 108 (24), 107 (16), 105 (24), 104 (16), 103 (14), 97 (27), 81 (38), 80 (19).

5-Amino-2-(m-chlorophenyl)-3H-imidazo[4,5-b]pyridine (192)

2,3,6-Triaminopyridine dihydrochloride (1 g), m-chlorobenzoic acid (0.8 g) and polyphosphoric acid (28 g) were stirred at 180° for 3 h, cooled, diluted with water (200 ml) and neutralised with potassium carbonate. The brown precipitate was collected and boiled with water (25 ml) to yield a brown, water insoluble product. The dihydrochloride was prepared by boiling with ethanolic hydrogen chloride solution, treating with activated charcoal and addition of ether and trituration (1.2 g, 84%), m.p. >300°, white feathery solid.

ν max. (KBr) 3300, 3100, 1660, 1460, 1320, 1140, 970, 810, 720 cm^{-1}.

Found, C, 51.0; H, 3.9; N, 25.3%.

Required for $C_{12}H_{11}Cl_2N_4$, C, 51.2; H, 3.9; N, 19.9%.

No suitable solvent was available for the determination of an n.m.r. spectrum.

Found, M, 244.05167.

Required for $C_{12}H_9N_4Cl^{35}$, M, 244.05157.

Found, M, 246.04821.

Required for $C_{12}H_9N_4Cl^{37}$, M, 246.04862.

m/e 247 (8), 246 (37), 245 (23), 244 (100), 243 (15), 141 (6), 140 (13), 139 (14), 138 (30), 137 (5), 123 (4), 122 (6), 111 (11), 107 (23), 106 (11), 104 (9), 102 (6), 80 (4), 79 (16).

5-Amino-2-(p-nitrophenyl)-3H-imidazo[4,5-b]pyridine (193)

2,3,6-Triaminopyridine dihydrochloride (0.627 g), p-nitrobenzoic acid (0.5 g) and polyphosphoric acid (30 g) were stirred at 180° for 2 h, cooled, diluted with water (100 ml) and neutralised with potassium carbonate. The solution was extracted with chloroform continuously for 12 h and the chloroform removed in vacuo to yield the crude imidazopyridine (0.51 g, 78%) which was recrystallised from dimethylformamide, light brown needles, m.p. 220°-221°.

ν max. (KBr) 3400, 3300, 3200, 1640, 1600, 1510 (NO_2), 1440, 1340 (NO_2), 1100, 860, 700 cm^{-1}.

Found, M, 255.07599.

Required for $C_{12}H_9N_5O_2$, M, 255.075619.

m/e 255 (68), 226 (21), 225 (100), 209 (59), 210 (10), 208 (10), 196 (9), 191 (15), 180 (9), 153 (11), 120 (12), 119 (59), 118 (12), 107 (12), 106 (9), 104 (17), 103 (14), 92 (16), 91 (12), 85 (13), 83 (21), 80 (24), 79 (24).

Found, C, 56.0; H, 3.2; N, 27.1%.

Required for $C_{12}H_9N_5O_2$, C, 56.5; H, 3.5; N, 27.5%.

No suitable solvent was available for the determination of an n.m.r. spectrum.

5-Amino-2-(o-methylphenyl)-3H-imidazo[4,5-b]pyridine (194)

2,3,6-Triaminopyridine dihydrochloride (1.0 g), o-toluic acid (0.7 g) and polyphosphoric acid (27 g) were stirred at 180° for 3 h and then cooled. Water (150 ml) was added and the solution neutralised with potassium carbonate to yield the imidazopyridine (0.6 g, 53%). After washing with hot water (25 ml), the product was recrystallised from boiling water to give a white feathery solid, m.p. 221°.

ν max. (KBr) 3020, 1600, 1470, 1420, 1380, 1310, 1240, 820 cm^{-1}.

Found: M, 224.105990, 224.10622.

Required for $C_{13}H_{12}N_4$, M, 224.106191.

m/e 225 (21), 224 (100), 223 (59), 222 (7), 209 (4), 208 (6), 207 (8), 206 (10), 147 (15), 118 (20), 117 (12), 116 (11), 91 (13), 80 (25).

Found, C, 69.2; H, 5.3; N, 24.9%.

Required for $C_{13}H_{12}N_4$, C, 69.6; H, 5.4; N, 25.0%.

No suitable solvent was available for the determination of an n.m.r. spectrum.

5-Amino-2-(p-chlorophenyl)-3H-imidazo[4,5-b]pyridine (195)

2,3,6-Triaminopyridine dihydrochloride (1 g), p-chlorobenzoic acid (0.796 g) and polyphosphoric acid (47 g) were stirred at

190° for 2.5 h, cooled, diluted with water (100 ml) and neutralised with potassium carbonate. The off-white precipitate was collected and boiled with water (25 ml) to yield the water insoluble imidazopyridine (1.1 g, 89%), m.p. >300°, (ethanol), white feathery solid.

ν max. (KBr) 3400, 1620, 1590, 1440, 1400, 1300, 1220, 1180, 1000, 950, 800, 720 cm^{-1}.

Found, M, 244.05113.

Required for $C_{12}H_9Cl^{35}N_4$, M, 244.05157.

Found, M, 246.04766.

Required for $C_{12}H_9Cl^{37}N_4$, M, 246.04862.

m/e 247 (8), 246 (39), 245 (22), 244 (100), 243 (12), 141 (4), 140 (13), 139 (14), 138 (33), 137 (6), 122 (7), 111 (9), 107 (23), 106 (12), 81 (4), 80 (34), 79 (14).

Found, C, 58.5; H, 3.6; N, 22.6%.

Required for $C_{12}H_9ClN_4$, C, 59.0; H, 3.7; N, 23.0%.

No suitable solvent was available for the determination of an n.m.r. spectrum.

5-Amino-2-(m-nitrophenyl)-3H-imidazo[4,5-b]pyridine (196)

2,3,6-Triaminopyridine dihydrochloride (1 g), m-nitrobenzoic acid (0.85 g) and polyphosphoric acid (30 g) were stirred at 170° for 2 h, cooled, diluted with water (100 ml) and neutralised with potassium carbonate. The brown precipitate was collected and boiled with water (25 ml) to yield the light brown, water insoluble imidazopyridine (1.1 g, 85%), m.p. 181-183° (methanol), light

brown feathery solid.

Found, M, 255.07637.

Required for $C_{12}H_9N_5O_2$, M, 255.075619.

ν max. (KBr) 3400, 1660, 1620, 1530 (NO_2), 1480, 1350 (NO_2), 820, 720 cm^{-1}.

Found, C, 56.1; H, 3.4; N, 27.1%.

Required for $C_{12}H_9N_5O_2$, C, 56.5; H, 3.5; N, 27.5%.

No suitable solvent was available for the determination of an n.m.r. spectrum.

5-Amino-2-(m-bromophenyl)-3H-imidazo[4,5-b] pyridine dihydrochloride (197)

2,3,6-Triaminopyridine dihydrochloride (3 g), m-bromobenzoic acid (3 g) and polyphosphoric acid (55 g) were stirred at 200° for 3 h, cooled, diluted with water (300 ml) and neutralised with potassium carbonate. The green precipitate was collected and boiled with water (25 ml) to yield the crude imidazopyridine which was recrystallised twice with ethanolic hydrogen chloride solution, treated with activated charcoal and precipitated with ether to give the dihydrochloride salt (4.1 g, 82%), m.p. >300°, light yellow feathery solid.

Found, M, 288.000991.

Required for $C_{12}H_9BrN_4$, M, 288.001110.

ν max. (KBr) 3300, 3100, 1650, 1450, 1400, 1310, 1240, 1130, 960, 800, 710 cm^{-1}.

No suitable solvent was available for the determination of an n.m.r. spectrum.

Found, C, 39.5; H, 3.0; N, 15.2; Cl, 19.1%.

Required for $C_{12}H_{11}BrCl_2N_4$, C, 40.0; H, 3.1; Cl, 19.4; N, 15.5%.

5-Amino-2-phenyl-3H-imidazo[4,5-b]pyridine dihydrochloride (198)

2,3,6-Triaminopyridine dihydrochloride (7.66 g), benzoic acid (5.2 g) and polyphosphoric acid (53 g) were stirred at 180° for 4 h. The reaction mixture was cooled and after addition of 350 ml of water, the solution was neutralised with potassium carbonate. The yellow product was collected and washed with 100 ml of hot water. The dihydrochloride was obtained in a pure form by recrystallisation from ethanolic hydrogen chloride (8 g, 83%), yellow feathery solid, m.p. >300°.

ν max. (KBr) 3300, 3100, 1650, 1460, 1310, 1130, 960, 780, 700 cm^{-1}.

τ (TFA) 1.06[1H, d, J = 9Hz, 7 - H]
1.49[2H, m, 2'6' - H]
1.7 [3H, m, 3'4'5' - H]
2.19[1H, d, J = 9Hz, 6 - H]

Found, M, 210.09007.

Required for $C_{12}H_{10}N_4$, M, 210.090542.

m/e 212 (1), 211 (15), 210 (100), 209 (1), 194 (1), 184 (1), 183 (3), 182 (2), 168 (2), 167 (7), 166 (1), 165 (2), 107 (12), 106 (3), 105 (19),

104 (36), 103 (4), 97 (3), 96 (1), 95 (2), 80 (20), 79 (8), 78 (2), 77 (12), 76 (3).

5-Amino-2-(3-pyridyl)-3H-imidazo[4,5-b]pyridine (199)

2,3,6-Triaminopyridine dihydrochloride (0.7 g), iso-nicotinic acid (0.427 g) and polyphosphoric acid (31 g) were stirred at 180° for 5.5 h, cooled, diluted with water (100 ml) and neutralised with potassium carbonate. The brown precipitate was collected and boiled with water (10 ml) to yield the imidazopyridine which was recrystallised from water and treated with activated charcoal to give feathery yellow crystals, (0.61 g, 82%), m.p. 291°.

Found, M, 211.08611.

Required for $C_{11}H_9N_5$, M, 211.085791.

ν max. (KBr) 3300, 3150, 1660, 1600, 1420, 1320, 1120, 1080, 960, 810, 700 cm^{-1}.

Found, C, 62.7; H, 4.2; N, 32.8%.

Required for $C_{11}H_9N_5$, C, 62.6; H, 4.3; N, 33.1%.

No suitable solvent was available for the determination of an n.m.r. spectrum.

5-Amino-2-(p-methylphenyl)-3H-imidazo[4,5-b]pyridine (200)

2,3,6-Triaminopyridine dihydrochloride (2 g), p-toluic acid (1.4 g) and polyphosphoric acid (33 g) were stirred at 180° for 3 h, cooled, diluted with water (300 ml) and neutralised with potassium carbonate. The yellow precipitate was collected and washed with hot water (50 ml) to yield the imidazopyridine (2.01 g, 88%), m.p. >300° (from boiling water), off-white feathery solid.

ν max. (KBr) 3300, 3150, 1620, 1420, 1280, 1120, 820, 720 cm^{-1}.

Found, C, 69.5; H, 5.6; N, 25.3%.

Required for $C_{13}H_{11}N_4$, C, 69.6; H, 5.4; N, 25.0%.

No suitable solvent was available for the determination of an n.m.r. spectrum.

Found, M, 224.10645.

Required for $C_{13}H_{12}N_4$, M, 224.106191.

m/e 225 (22), 224 (100), 223 (20), 117 (13), 116 (38), 115 (5), 114 (5), 110 (6), 107 (17), 91 (12), 80 (20), 79 (6), 65 (7).

5-Amino-3H-imidazo[4,5-b]pyridine-2-(1H)-one (201)

2,3,6-Triaminopyridine dihydrochloride (1.2 g) and urea (1.2 g) were finely ground in a mortar and then heated at 170° for 10 minutes in a metal bath. The solid was broken up, boiled with water (25 ml) and filtered to give a brown, water-insoluble solid which was dissolved in hot dilute ammonium hydroxide, treated with activated charcoal and reprecipitated with glacial acetic acid to produce the yellow imidazopyridine (0.8 g, 87%), m.p. >300°, yellow feathery solid.

ν max. (KBr) 3400 (NH_2), 3150, 1700 (C=O), 1640, 1600, 1440, 1240, 1040, 1020, 900 cm^{-1}.

Found, M, 150.054470.

Required for $C_6H_6N_4O$, M, 150.054157.

m/e 151 (9), 150 (100), 149 (9), 148 (42), 147 (26),

123 (3), 122 (11), 121 (13), 120 (45), 107 (8), 106 (10), 105 (6), 104 (5), 96 (5), 95 (6), 94 (5), 93 (18), 81 (6), 80 (80), 79 (83), 78 (59), 77 (14), 70 (11), 69 (15), 68 (13).

No suitable solvent was available for the determination of an n.m.r. spectrum.

Attempted preparation of 3H-imidazo[4,5-b]pyridine-2,5(1H,4H)-dione

A solution of sodium nitrite (0.4 g) in water (3.4 ml) at 5° was added dropwise to a stirred solution of 5-amino-3H-imidazo[4,5-b]pyridine-2(1H)-one (1 g) in concentrated sulphuric acid (0.7 ml) and water (30 ml) at 10°. The reaction mixture was allowed to attain room temperature and stirred for 2 h. The temperature was increased to 90° for 0.25 h and the solution filtered while still hot to yield a brown product (0.88 g) which was shown to be imidazopyridine starting material spectroscopically.

5-Amino-3H-imidazo[4,5-b]pyridine-2(1H)-thione (202)

(a) Thiophosgene (1 ml) was added dropwise to a stirred solution of 2,3,6-triaminopyridine dihydrochloride (0.6 g), concentrated hydrochloric acid (10 ml) and water (10 ml). The raction mixture was stirred at room temperature for 24 h and the yellow precipitate (0.08 g) collected. The filtrate was basified with concentrated ammonium hydroxide to yield the imidazopyridine which was purified by dissolving in hot dilute ammonium hydroxide solution and reprecipitating with glacial acetic acid, (0.39 g, 83%), m.p. >300°.

(b) 2,3,6-triaminopyridine dihydrochloride (2 g) and thiourea (3 g) were finely divided and heated at 230° for 0.5 h to yield an identical product to above (1.31 g, 85%), m.p. >300°.

(a) Found, M, 166.03199.

Required for $C_6H_6N_4S$, M, 166.03131.

(b) Found, M, 166.03118.

Required for $C_6H_6N_4S$, M, 166.03131.

ν max. (KBr) 3400, 3200, 1640, 1500, 1460, 1350, 1310, 1170, 820, 650 cm^{-1}.

τ $(CD_3)_2SO$ 2.73[1H, d, J = 9Hz, 7 - H]

3.75[1H, d, J = 9Hz, 6 - H]

4.2 [2H, s broad[a], 5 - NH_2]

m/e 168 (6), 167 (12), 166 (100), 141 (8), 109 (19), 108 (28), 81 (10), 80 (23), 79 (23), 76 (23), 73 (6), 64 (8).

5-Amino-3H-imidazo[4,5-b]pyridine dihydrochloride (7)

2,3,6-Triaminopyridine dihydrochloride (10 g) and triethyl-orthoformate (500 ml) were refluxed for 3 h. The solvent was removed in vacuo and the residue dissolved in absolute ethanol and hydrogen chloride gas bubbled through the solution. After cooling, addition of ether precipitated the dihydrochloride as a yellow solid (8.5 g, 81%), m.p. 292° (lit.[6] 292°), light yellow needles (from ethanolic hydrogen chloride).

ν max. (KBr) 3300, 3100, 1660, 1620, 1550, 1480,

1380, 1330, 1260, 810, 750 cm^{-1}.

τ $(CD_3)_2SO$ 1.92 [1H, d, J = 9Hz, 7 - H]

3.16 [1H, d, J = 9Hz, 6 - H]

Found, M, 134.05873.

Calc. for $C_6H_6N_4$, M, 134.059243.

m/e 136 (2), 135 (9), 134 (100), 133 (2), 119 (2), 108 (2), 107 (21), 106 (4), 94 (1), 92 (2), 91 (1), 90 (1), 81 (2), 80 (2), 79 (1), 67 (3), 65 (2), 64 (3), 63 (1).

3H-Triazolo[4,5-b]pyridine-5(4H)-one (203)

This was prepared by the method of Graboyes and Day,[11] m.p. 282° (lit.[11] 280°-282°), yield 54%.

(v) Reactions with 2-chloropyridine

2-Chloropyridine 1-oxide (204)

Prepared by the method of Finger and Starr[95], m.p. 65° (lit.[95] 66°).

2-Chloro-4-nitropyridine 1-oxide (205)

Prepared by the method of Finger and Starr,[95], m.p. 153° (lit.[95] 153°).

2-Amino-4-nitropyridine 1-oxide hydrochloride (206)

2-Chloro-4-nitropyridine 1-oxide (3.2 g) and 20 ml of absolute ethanol saturated with ammonia at 0° were heated in a steel bomb at 100° for 13 h. The resultant brown solution was reduced in volume in vacuo to yield a sticky tan solid.

Concentrated hydrochloric acid (10 ml) and petroleum ether (100°-120°, 20 ml) were added and after trituration the yellow amine hydrochloride separated out (1.6 g, 56%), bright yellow plates, m.p. 171° (ethanol-hydrogen chloride).

ν max. (KBr) 3350, 3100, 2600, 1660, 1550 (NO_2), 1360 (NO_2), 1190, 900, 840 cm^{-1}.

Found, M, 155.03269.

Required for $C_5H_5N_3O_3$, M, 155.033087.

m/e 155 (100), 156 (7), 139 (14), 109 (25), 99 (12), 98 (8), 97 (14), 95 (5), 94 (5), 93 (14), 92 (18), 91 (15), 85 (29), 84 (13), 83 (16), 82 (27), 81 (11), 79 (12), 71 (42), 70 (34), 69 (25), 66 (24), 65 (18), 64 (14).

Found, C, 31.0; H, 3.1; N, 25.3; Cl, 18.0%.

Required for $C_5H_6ClN_3O_3$, C, 31.4; H, 3.1; Cl, 18.3; N, 25.1%.

The base was obtained as a deep yellow solid by trituration of the hydrochloride salt with concentrated ammonium hydroxide.

ν max. (KBr) 3400, 1640, 1580, 1540, 1360, 1220, 960, 800, 750, 660 cm^{-1}.

τ $(CD_3)_2SO$ 2.6 [1H, q, J_{35} = 2.5Hz, J_{56} = 7.5Hz, 5-H]

2.05 [1H, d, J_{35} = 2.5Hz, 3-H]

1.55 [1H, d, J_{56} = 7.5Hz, 6-H]

(vi) Reactions with 3,4-diaminopyridine

4-Nitraminopyridine (208)

Prepared by the method of Edwards,[42] m.p. 243° (lit.[99] 244°).

4-Amino-3-nitropyridine (209)

Prepared by the method of Edwards,[42] m.p. 199°-200° (lit.[99] 200°).

3,4-Diaminopyridine (92)

Prepared by the method of Edwards,[42] m.p. 218°-220° (lit.[39] 218°-219°).

2-Methyl-1H-imidazo[4,5-c]pyridine (210)

3,4-Diaminopyridine (2 g), glacial acetic acid (1.13 ml) and polyphosphoric acid (39 g) were heated at 190° with stirring for 3.5 h, cooled, diluted with water (140 ml) and neutralised with potassium carbonate. The solution was continuously extracted with chloroform for 12 h. The imidazopyridine was obtained by reducing the solvent in vacuo, light yellow needles, m.p. 169° (lit.[41] 170°-171°), (2.0 g, 82%).

ν max. (nujol) 2900, 1630, 1590, 1460, 1380, 1280, 1220, 1030, 800, 700 cm^{-1}

Found, C, 63.0; H, 5.2; N, 31.7%.

Calc. for $C_7H_7N_3$, C, 63.2; H, 5.3; N, 31.6%.

τ $(CD_3)_2SO$ 1.07[1H, s, 4-H]

1.62[1H, d, J = 5Hz, 6-H]

2.43[1H, d, J = 5Hz, 7-H]

7.46[3H, s, 2-CH_3]

Found, M, 133.06327.

Calc. for $C_7H_7N_3$, M, 133.063994.

m/e 134 (7), 133 (100), 132 (42), 119 (2), 118 (2), 107 (3), 106 (9), 105 (12), 104 (8), 94 (3), 93 (12), 92 (8), 91 (4), 85 (7), 83 (12), 79 (17), 78 (4), 77 (4), 67 (4), 66 (20), 65 (20), 64 (14), 63 (4), 57 (6), 55 (3), 54 (3), 53 (3).

2-(p-Chlorophenyl)-1H-imidazo[4,5-c]pyridine (211)

3,4-Diaminopyridine (1 g), p-chlorobenzoic acid (1.44 g) and polyphosphoric acid (40 g) were stirred at 180° for 4 h, cooled, diluted with water (200 ml) and neutralised with potassium carbonate. The white precipitate was collected and boiled with water (25 ml) to give the crude imidazopyridine which was purified by dissolving in concentrated hydrochloric acid and precipitating with 40% sodium hydroxide solution. Final recrystallisation was effected from dimethyl formamide to produce the pure imidazopyridine (1.8 g, 86%), m.p. >300°, white feathery solid.

Found, M, 229.04049.

Required for $C_{12}H_8N_3Cl^{35}$, M, 229.04067.

Found, M, 231.03721.

Required for $C_{12}H_8N_3Cl^{37}$, M, 231.03772.

ν max. (nujol) 2900 (nujol), 1460 (nujol), 1380 (nujol), 1620, 1590, 1290, 1100, 1030, 940, 810, 730 cm^{-1}.

τ (TFA) 1.33[1H, d, J = 7Hz, 6-H]

1.75[1H, d, J = 7Hz, 7-H]

1.97[5H, m, 2'3'4'5'6'-H]

2-(m-Chlorophenyl)-1H-imidazo[4,5-c]pyridine (212)

3,4-Diaminopyridine (1 g), m-chlorobenzoic acid (1.44 g) and polyphosphoric acid (26 g) were stirred at 180° for 4 h, cooled, diluted with water (100 ml) and neutralised with potassium carbonate. The white precipitate was boiled with water (25 ml) to yield the white, water insoluble imidazopyridine (1.8 g, 86%), m.p. >300°.

ν max. (nujol) 2900 (nujol), 1620, 1590, 1460 (nujol), 1380 (nujol), 1290, 1030, 960, 800, 720 cm^{-1}.

τ (TFA) 0.55[1H, s, 4-H]
1.4 [1H, d, J = 7Hz, 6-H]
1.8 [1H, d, J = 7Hz, 7-H]
2.05[3H, m, 4'5'6'-H]
2.5 [1H, m, 2'-H]

Found, M, 229.04073.
Required for $C_{12}H_8N_3Cl^{35}$, M, 229.04067.
Found, M, 231.03794.
Required for $C_{12}H_8N_3Cl^{37}$, M, 231.03772.
m/e 232 (7), 231 (38), 230 (23), 229 (100), 228 (10), 194 (18), 140 (8), 139 (4), 138 (13), 137 (8), 231/2, 229/2, 114 (9), 111 (10), 193/2, 92 (9), 175/2, 162/2, 75 (8), 65 (15), 64 (11).

2-Phenyl-1H-imidazo[4,5-c]pyridine (213)

3,4-Diaminopyridine (0.97 g), benzoic acid (1.2 g) and polyphosphoric acid were stirred at 180° for 3.5 h, cooled, diluted with water (60 ml) and the solution neutralised with

potassium carbonate. The white solid which precipitated out was collected, washed with hot water (20 ml) and recrystallised from boiling water to yield the imidazopyridine (1.41 g, 90%), m.p. 230°-231°, white feathery solid.

ν max. (nujol) 2900, 1620, 1590, 1460, 1380, 1280, 1220, 1030, 810, 700 cm^{-1}.

τ $(CD_3)_2SO$ 0.98[1H, s, 4-H]
1.73[1H, d, J = 7Hz, 6-H]
2.14[1H, d, J = 7Hz, 7-H]
2.43[2H, m, 2'6'-H]
2.8 [3H, m, 3'4'5'-H]

Found, M, 195.07961.

Required for $C_{12}H_9N_3$, M, 195.079643.

m/e 195 (17), 194 (100), 193 (19), 168 (11), 167 (9), 149 (6), 141 (9), 128 (9), 105 (10), 104 (18), 103 (11), 85 (11), 77 (23), 71 (17), 69 (10), 65 (16), 64 (11).

Found, C, 73.4; H, 4.4; N, 21.2%.

Required for $C_{12}H_9N_3$, C, 73.8; H, 4.6; N, 21.5%.

2-(o-Hydroxyphenyl)-1H-imidazo[4,5-c]pyridine (214)

3,4-Diaminopyridine (0.5 g), salicylic acid (0.633 g) and polyphosphoric acid (21 g) were stirred at 180° for 4 h, cooled, diluted with water (80 ml) and neutralised with potassium carbonate. The imidazopyridine appeared as a white precipitate and after washing with hot water (20 ml), it was recrystallised from ethanol

(0.696 g, 72%), m.p. 117°, white feathery solid.

Found, C, 68.1; N, 20.1; H, 4.2; O, 7.7%.

Required for $C_{12}H_9N_3O$, C, 68.3; N, 19.9; H, 4.3; O, 7.6%.

ν max. (nujol) 3550 (OH), 2900, 1640, 1600, 1460, 1380, 1300, 1260, 1040, 760, 710 cm^{-1}.

No suitable solvent was available for the determination of an n.m.r. spectrum.

Found, M, 211.07478.

Required for $C_{12}H_9N_3O$, M, 211.074557.

m/e 211 (15), 210 (100), 184 (7), 182 (56), 181 (29), 157 (10), 156 (4), 155 (5), 149 (6), 120 (5), 105 (9), 102 (5), 92 (5), 91 (7), 85 (9), 77 (7), 71 (10), 69 (6), 66 (11), 65 (10), 64 (8), 63 (6), 57 (19), 56 (5).

2-(p-Nitrophenyl)-1H-imidazo[4,5-c]pyridine dihydrochloride (215)

3,4-Diaminopyridine (1 g), p-nitrobenzoic acid (1.53 g) and polyphosphoric acid (21 g) were stirred at 190° for 4 h, cooled diluted with water (100 ml) and neutralised with potassium carbonate. The yellow precipitate was collected and boiled with water (25 ml) to yield the base product. This was boiled with an ethanolic hydrogen chloride solution to produce the dihydrochloride as fine, light yellow needles, (1.1 g, 87%), m.p. >300°.

diHCl ν max. (nujol) 2900, 1640, 1530 (NO_2), 1470, 1380, 1350 (NO_2) 1320, 960, 860, 830, 720 cm^{-1}.

diHCl Found, C, 46; H, 3.2; Cl, 22.7; N, 17.9; O, 10.2%

Required for $C_{12}H_{10}Cl_2N_4O_2$, C, 46.1; H, 3.2; Cl, 23.0; N, 17.8%.

base ν max. (KBr) 3000, 1630, 1610, 1600, 1520 (NO_2), 1430, 1350 (NO_2), 1310, 1290, 950, 860, 740 cm^{-1}.

No suitable solvent was available for the determination of an n.m.r. spectrum.

Found, M, 240.065420.

Required for $C_{12}H_8N_4O_2$, M, 240.064720.

m/e 240 (100), 241 (19), 239 (2), 209 (14), 195 (13), 194 (62), 193 (16), 192 (3), 182 (17), 181 (4), 167 (11), 166 (6), 141 (5), 140 (22), 139 (7), 104 (5), 103 (6), 102 (5), 77 (6), 76 (11), 75 (5), 66 (4), 65 (12), 64 (10), 63 (4).

2-(2-Pyridyl)-1H-imidazo[4,5-c]pyridine (216)

3,4-Diaminopyridine (0.5 g), nicotinic acid (0.56 g) and polyphosphoric acid (24 g) were stirred at 180° for 3.5 h, cooled, diluted with water (60 ml) and neutralised with potassium carbonate to precipitate a pink solid which was collected and boiled with water (25 ml) to yield the imidazopyridine (0.91 g, 90%), m.p. 220°, light pink feathery solid (50 - 50 ethanol - water).

ν max. (nujol) 2900, 1460, 1380 (nujol), 1600, 1580, 1540, 1300, 960, 920, 820, 740, 710 cm^{-1}.

Found, M, 196.0746.

Required for $C_{11}H_8N_4$, M, 196.074892.

m/e 197 (6), 196 (100), 195 (15), 170 (3), 169 (9), 168 (12), 106 (3), 105 (27), 104 (3), 98 (2), 92 (3), 79 (17), 78 (21), 65 (8), 64 (7), 63 (3).

Found, C, 67.1; H, 4.1; N, 28.7%.

Required for $C_{11}H_8N_4$, C, 67.3; H, 4.1; N, 28.6%.

2-(o-Nitrophenyl)-1H-imidazo[4,5-c]pyridine (217)

3,4-Diaminopyridine (1 g), o-nitrobenzoic acid (1.53 g) and polyphosphoric acid (35 g) were stirred at 180° for 2 h, cooled, diluted with water (75 ml) and neutralised with potassium carbonate. The solution was continuously extracted with chloroform for 12 h and the chloroform removed in vacuo to leave a tacky solid which was triturated with petroleum ether (60° - 80°) to produce the nitro-imidazopyridine, (1.4 g, 63%), m.p. 145°, dark brown needles (ethanol).

ν max. (nujol) 2900, 1620, 1580, 1530 (NO_2), 1460, 1380, 1350 (NO_2), 980, 860, 830, 790, 730 cm^{-1}.

Found, M, 240.064280.

Required for $C_{12}H_8N_4O_2$, M, 240.064721.

m/e 240 (30), 223 (24), 211 (11), 210 (6), 209 (6), 208 (9), 198 (13), 197 (100), 196 (10), 170 (31), 169 (10), 150 (11), 135 (11), 109 (16), 104 (19), 91 (30), 79 (11), 77 (12), 76 (11), 64 (14).

Found, C, 55.5; H, 3.1; N, 22.9%.

Required for $C_{12}H_8N_4O_2$, C, 60.0; H, 3.3; N, 23.3%.

No suitable solvent was available for the determination of an n.m.r. spectrum.

2-(p-Aminophenyl)-1H-imidazo[4,5-c]pyridine (218)

3,4-Diaminopyridine (1 g), p-aminobenzoic acid (1.25 g) and polyphosphoric acid (37 g) were stirred at 180° for 4 h, cooled, diluted with water (100 ml) and neutralised with potassium carbonate. The solution was extracted continuously with chloroform for 12 h and then the chloroform was removed *in vacuo* to yield the crude imidazopyridine, (1.54 g, 80%), m.p. >300°, light yellow feathery solid (ethanol).

Found, M, 210.08286.

Required for $C_{12}H_{10}N_4$, M, 210.082717.

ν max. (KBr) 3400 (NH_2), 3300, 3200, 1600, 1500, 1440, 1280, 1180, 840 cm^{-1}.

m/e 210 (15), 209 (100), 208 (15), 207 (17), 206 (5), 205 (5), 182 (4), 181 (11), 180 (6), 120 (4), 119 (7), 118 (9), 105 (13), 104 (6), 92 (6), 91 (5).

Found, C, 68.3; H, 4.7; N, 26.5%.

Required for $C_{12}H_{10}N_4$, C, 68.6; H, 4.8; N, 26.7%.

No suitable solvent was available for the determination of an n.m.r. spectrum.

1H-Imidazo[4,5-c]pyridine (91)

3,4-Diaminopyridine (2.2 g), formic acid (98/100%, 4.4 ml) and polyphosphoric acid (40 g) were stirred at 185° for 4 h, cooled

diluted with water (100 ml) and neutralised with potassium carbonate. The solution was continuously extracted with chloroform for 12 h and the chloroform removed *in vacuo* to leave the crude imidazopyridine. This was purified by treatment with activated charcoal and several recrystallisations from ethyl acetate, (2.1 g, 88%), m.p. 171° (lit. 168°-169°[35]).

Found, C, 60.4; H, 4.2; N, 35.1%.

Calc. for $C_6H_5N_3$, C, 60.5; H, 4.2; N, 35.3%.

ν max. (KBr) 1620, 1580, 1440, 1330, 1250, 1180, 1120, 1030, 940, 800 cm^{-1}.

τ (D_2O) 1.75[1H, s, 2-H]
2.09[1H, s, 4-H]
2.23[1H, d, J = 6Hz, 6-H]
3.02[1H, d, J = 6Hz, 7-H]

m/e 119 (11), 118 (100), 117 (9), 92 (6), 91 (27), 90 (7), 66 (5), 65 (9), 64 (22), 63 (16), 62 (7), 52 (16), 51 (6), 41 (13).

τ $(CD_3)_2$ SO 0.75[1H, s, 2-H]
1.3 [1H, s, 4-H]
1.43[1H, d, J = 6Hz, 6-H]
2.13[1H, d, J = 6Hz, 7-H]

1H-Imidazo[4,5-c]pyridine 5-N-oxide (219)

1H-Imidazo[4,5-c]pyridine (5 g), glacial acetic acid (50 ml) and hydrogen peroxide (100 vol, 10 ml) were heated at 100° for 3 h. A further aliquot of hydrogen peroxide (10 ml)

was added and heating continued for a further 3 h. The solvent was removed in vacuo to yield the N-oxide (4.4 g, 79%), m.p. 230° (lit.[101] 220°-250°).

ν max (KBr) 3100, 1640, 1620, 1550, 1390, 1330, 1260, 940, 900, 830, 750 cm^{-1}.

τ $(CD_3)_2SO$ 0.37[1H, s, 2-H]
0.8 [1H, s, 4-H]
1.2 [1H, d, J = 6Hz, 6-H]
1.56[1H, d, J = 6Hz, 7-H]

Attempted nitration of 1H-imidazo[4,5-c]pyridine 5-N-oxide (219)

1H-Imidazo[4,5-c]pyridine 5-N-oxide (0.5 g), fuming nitric acid (d 1.5, 3 ml) and glacial acetic acid (4.5 ml) were heated at 100° for 4 h and, after cooling, neutralised with sodium hydroxide solution (40%). The neutralised solution was extracted with chloroform continuously for 10 h but on removal of the chloroform in vacuo, starting material was recovered (0.5 g).

1H-Imidazo[4,5-c]pyridine-2(3H)-one (113)

(a) Phosgene gas was bubbled through a stirred solution of 3,4-diaminopyridine (1 g) in hydrochloric acid (20%, 60 ml) at 0° for 3 h. The solution was neutralised with concentrated ammonium hydroxide solution and the white precipitate was filtered off. The filtrate was extracted with chloroform continuously for 15 h and on cooling, the imidazopyridine crystallised out (0.8 g, 65%), m.p. 302° (lit.[33] 304°-305°), fine white needles (boiling water).

(b) Prepared by the method of Barlin,[33] m.p. 303°.

ν max (KBr) 1720 (C=O), 1630, 1500, 1270, 1200, 1160, 1040, 1000, 820, 720 cm^{-1}.

τ TFA 1.1 [1H, s, 4-H]

1.35 [1H, d, J = 6Hz, 6-H]

2.03 [1H, d, J = 6Hz, 7-H]

Attempted nitration of 1H-imidazo[4,5-c]pyridine-2(3H)-one (113)

A solution of potassium nitrate (0.37 g) in concentrated sulphuric acid (2 ml) at 0° was added dropwise to a stirred solution of 1H-imidazo[4,5-c]pyridine-2(3H)-one (0.5 g) in concentrated sulphuric acid (2 ml) at 0°. The reaction mixture was heated at 120° for 2 h, cooled and poured onto crushed ice. The solution was neutralised with concentrated ammonium hydroxide to yield a light yellow product. Spectroscopic analysis showed it to be starting material.

3-N-Acetyl-imidazo[4,5-c]pyridine-2(1H)-one 5-N-oxide (220)[1]

1H-Imidazo[4,5-c]pyridine-2 (3H)-one (0.5 g), glacial acetic acid (3.0 g) and hydrogen peroxide (100 vol, 0.5 ml) were heated for 3 h at 100°. A further aliquot (0.5 ml) of hydrogen peroxide was added and heating continued for a further 3 h. The solvent was removed in vacuo to leave the crude N-oxide. Treatment with activated charcoal and recrystallisation from dilute acetic acid yielded the product (0.56 g, 97%), m.p. 270°-295°.

Found, M, 151.03791.

Required for $C_6H_5N_3O_2$, M, 151.038173.

ν max. (KBr) 3100, 1700 (C=O), 1500, 1380, 1270, 1150, 1000, 860, 800, 720 cm^{-1}.

τ TFA 2.25[1H, d, J = 7Hz, 7 - H]

1.4 [1H, d, J = 7Hz, 6 - H]

1.2 [1H, s, 4 - H]

7.8 [3H, s, N-acetyl]

1H-Imidazo[4,5-c]pyridine-2(3H)-thione (116)

3,4-Diaminopyridine (2 g) and thiourea (4 g) were finely mixed in a mortar and heated at 250° for 0.5 h. The resultant solid was boiled up with water (200 ml) which had been acidified to pH 5 with glacial acetic acid, and filtered. The filtrate was reduced in volume by one half in vacuo and neutralised with concentrated ammonium hydroxide solution to yield the imidazopyridine on cooling (2 g, 72%). Purification was effected by dissolving in hot 20% acetic acid and precipitation with concentrated ammonium hydroxide solution to yield light yellow needles, m.p. >300° (lit.[33] >300°).

Found, C, 47.8; H, 2.9; N, 27.5%.

Calc. for $C_6H_5N_3S$, C, 47.7; H, 3.3; N, 27.8%.

ν max (KBr) 3000, 1620, 1540, 1400, 1280, 1220, 1200, 1160, 1040, 810 cm^{-1}.

Found, M, 151.02112.

Calc. for $C_6H_5N_3S$, M, 151.02042.

Attempted fusion of 3,4-diaminopyridine (92) with guanidine hydrochloride.

3,4-Diaminopyridine (0.5 g) and guanidine hydrochloride (0.435 g) were finely mixed in a mortar and heated at 260° for

0.25 h to produce a gum on cooling. Trituration with ethyl acetate yielded a product which was identified spectroscopically as 3,4-diaminopyridine (0.4 g).

1H-Triazolo[4,5-c]pyridine sulphate (202)

A solution of sodium nitrite (0.5 g) in water (1.62 ml) at 5° was added dropwise with stirring to a solution of 3,4-diaminopyridine (0.545 g) in concentrated sulphuric acid (2.4 ml) and water (4.9 ml) at 5°. The reaction mixture was stirred for 2 h at 5° and the light yellow crystalline sulphate was collected by filtration (0.525, 53%), m.p. 193°-194°.

ν max. (KBr) 3000, 1640, 1610, 1440, 1380, 1340, 1300, 1020, 840, 800, 720 cm^{-1}.

τ $(CD_3)_2SO$ -0.69[1H, s, 4-H]
0.37[3H, s^a, 1,2,4N-H]
1.0 [1H, d, J = 7Hz, 6-H]
1.38[1H, d, J = 7Hz, 7-H]

Found, M, 120.04395.
Required for $C_5H_4N_4$, M, 120.043594.
m/e 120 (8), 92 (100), 64 (22), 63 (79), 62 (31), 61 (12), 57 (12), 56 (6), 55 (16), 52 (38), 51 (15).

1H-Triazolo[4,5-c]pyridine (201)

The filtrate obtained after collecting the yellow crystalline sulphate (above) was made alkaline with concentrated ammonium hydroxide solution and extracted continuously with chloroform for 9 h. The chloroform was reduced in volume in vacuo and the triazolopyridine appeared as light yellow crystals (0.45 g, 36%),

m.p. 241° (lit.[105] 243°-245°).

ν max. (KBr) 3050, 2950, 2400, 2100, 1620, 1460, 1440, 1330, 1320, 1180, 1150, 1040, 980, 820, 790 cm^{-1}.

τ $(CD_3)_2SO$ 0.38[1H, s, 4-H]
1.36[1H, d, J = 6Hz, 6-H]
1.99[1H, d, J = 6Hz, 7-H]

Found, M, 120.04395.

Required for $C_5H_4N_4$, M, 120.043594.

m/e 120 (17), 121 (3), 111 (7), 109 (8), 107 (11), 106 (100), 97 (12), 96 (4), 95 (13), 94 (4), 93 (6), 92 (21).

(vii) Reactions with 2-chloro-3,4-diaminopyridine

2-Chloro-3,4-diaminopyridine (103)

4-Amino-3-nitropyridine (20.2 g), concentrated hydrochloric acid (320 ml) and anhydrous stannous chloride (160 g) were refluxed gently for 3 h and cooled. The solid which crystallised out was collected, suspended in water (100 ml) and excess 40% sodium hydroxide added to yield the product (12.6 g, 60%), m.p. 216° (lit.[99] 218°).

ν max. (KBr) 3350 (NH_2), 3200, 2900, 1660, 1640, 1580, 1550, 1520, 1300, 1200, 1100, 810 cm^{-1}.

τ $(CD_3)_2SO$ 2.66[1H, d, J = 5Hz, 6-H]
3.55[1H, d, J = 5Hz, 5-H]
4.3 [2H, broad s[a], NH_2]
5.4 [2H, broad s[a], NH_2]

Found, M, 143.

Required for $C_5H_6ClN_3$, M, 143.

m/e 146 (4), 145 (30), 144 (13), 143 (100), 133 (6), 132 (6), 131 (10), 130 (5), 119 (5), 118 (7), 117 (7), 116 (4), 115 (9), 107 (29), 80 (77), 57 (15), 56 (7), 55 (10), 54 (18), 53 (58), 52 (18).

4-Chloro-2-(m-nitrophenyl)-1H-imidazo[4,5-c]pyridine (203)

2-Chloro-3,4-diaminopyridine (0.5 g), m-nitrobenzoic acid (0.583 g) and polyphosphoric acid (44 g) were stirred at 190° for 2 h, cooled, diluted with water (100 ml) and neutralised with potassium carbonate. An off-white solid was collected and boiled with water (25 ml) to yield the insoluble imidazopyridine (0.75 g, 78%), m.p. >300°, white feathery solid (from ethanol).

ν max. (KBr) 1620, 1580, 1530 (NO_2), 1350 (NO_2), 1230, 1200, 960, 820, 720 cm^{-1}.

τ (TFA) 0.83[1H, s, 2'-H]
1.32[3H, m, 4'5'6'-H]
1.69[1H, d, J = 6Hz, 6-H]
2.08[1H, d, J = 6Hz, 7-H]

Found, M, 274.026040.

Required for $C_{12}H_7ClN_4O_2$, M, 274.025750.

m/e 276 (42), 275 (20), 274 (100), 230 (32), 229 (14), 228 (74), 193 (21), 192 (68), 166 (17), 165 (42), 140 (11), 139 (8), 138 (11), 77 (13), 76 (18), 75 (14).

4-Chloro-2-(3,5-dinitrophenyl)-1H-imidazo[4,5-c]pyridine (204)

2-Chloro-3,4-diaminopyridine (0.5 g), 3,5-dinitrobenzoic acid (0.741 g) and polyphosphoric acid (32 g) were stirred at 170° for 2.5 h, cooled, diluted with water (100 ml) and neutralised with potassium carbonate. The brown precipitate was collected and boiled with water (25 ml) to produce the light brown, insoluble imidazopyridine (0.99 g, 89%), m.p. >300°, light yellow feathery solid (ethanol).

ν max. (KBr) 3050, 1640, 1540 (NO_2), 1340 (NO_2), 1220, 1070, 920, 730 cm^{-1}.

No suitable solvent was available for the determination of an n.m.r. spectrum.

Found, M, 319.011520.

Required for $C_{12}H_6ClN_5O_4$, M, 319.010820.

Found, M, 321.008200.

Required for $C_{12}H_6ClN_5O_4$, M, 321.007870.

m/e 322 (11), 321 (43), 320 (23), 319 (100), 303 (7), 289 (10), 275 (20), 274 (11), 273 (53), 229 (20), 228 (20), 227 (50), 226 (30), 192 (11), 191 (15), 190 (23), 180 (7), 179 (8).

Found, C, 44.9; H, 1.7; N, 21.6%.

Required for $C_{12}H_6ClN_5O_4$, C, 45.1; H, 1.8; N, 21.9%.

4-Chloro-2-(p-methylphenyl)-1H-imidazo[4,5-c]pyridine (205)

2-Chloro-3,4-diaminopyridine (0.5 g), p-toluic acid (0.475 g) and polyphosphoric acid (36 g) were stirred at 170° for 2.5 h, cooled, diluted with water (100 ml) and neutralised with potassium

carbonate. The white precipitate was collected and boiled with water (25 ml) to produce the white, insoluble imidazopyridine (0.75 g, 88%), m.p. 243°-244°, white feathery solid (dimethylformamide).

ν max. (KBr) 1620, 1580, 1500, 1440, 1300, 1220, 940, 810, 720 cm^{-1}.

τ (TFA) 0.69[1H, d, J = 6Hz, 6-H]
1.13[1H, d, J = 6Hz, 7-H]
1.25[2H, d, J = 8Hz, 2'6'-H]
1.84[2H, d, J = 8Hz, 3'5'-H]
7.12[3H, s, 4'-CH_3]

Found, M, 243.055390.
Required for $C_{13}H_{10}ClN_3$, M, 243.056320.
Found, M, 245.052600.
Required for $C_{13}H_{10}ClN_3$, M, 245.053370.
m/e 246 (9), 245 (44), 244 (32), 243 (100), 242 (14), 209 (9), 208 (16), 207 (56), 206 (19), 180 (9), 179 (21), 178 (8), 150 (16), 119 (11), 118 (11), 117 (10), 116 (11), 207/2 (15), 91 (32), 77 (10), 71 (10), 65 (16), 64 (18).

4-Chloro-2-(o-methylphenyl)-1H-imidazo[4,5-c]pyridine (206)

2-Chloro-3,4-diaminopyridine (0.5 g), o-toluic acid (0.475 g) and polyphosphoric acid (30 g) were stirred at 170° for 2 h, cooled, diluted with water (100 ml) and neutralised with potassium carbonate. The white precipitate was collected and boiled with water (25 ml) to yield the white, insoluble imidazopyridine (0.6 g,

70%), m.p. 110°, off-white feathery solid (ethanol).

ν max. (KBr) 1600, 1570, 1420, 1300, 1220, 950, 820 730 cm^{-1}.

τ (TFA) 1.17[1H, s, J = 6Hz, 6 - H]
1.6 [1H, s, J = 6Hz, 7 - H]
2.33[4H, m, 3'4'5'6' - H]
7.44[3H, s, 2' - CH_3]

Found, M, 245.053710.

Required for $C_{13}H_{10}ClN_3$, M, 245.053370.

m/e 245 (42), 246 (10), 244 (42), 243 (100), 209 (7), 208 (13), 207 (42), 206 (53), 205 (8), 181 (7), 180 (7), 179 (13), 154 (6), 153 (6), 152 (7), 117 (10), 116 (32), 115 (5), 105 (7), 104 (8), 103 (10), 102 (7), 91 (27), 90 (18), 89 (18), 77 (14), 76 (8), 75 (6), 71 (9), 69 (8), 65 (18), 64 (26).

4-Chloro-2-(o-chlorophenyl)-1H-imidazo[4,5-c]pyridine (207)

2-Chloro-3,4-diaminopyridine (0.5 g), o-chlorobenzoic acid (0.545 g) and polyphosphoric acid were stirred at 200° for 1 h, cooled, diluted with water (200 ml) and neutralised with potassium carbonate. The white precipitate was collected and washed with water (25 ml) to yield the imidazopyridine (0.75 g, 82%), m.p. 104°-106° (from boiling water), white feathery solid.

ν max. (KBr) 1610, 1570, 1440, 1300, 1220, 1050, 990, 950, 760 cm^{-1}.

No suitable solvent was available for the determination of an n.m.r. spectrum.

Found, M, 263.00124.

Required for $C_{12}H_7Cl_2{}^{35}N_3$, M, 263.00169.

Found, M, 266.99669.

Required for $C_{12}H_7Cl_2{}^{37}N_3$, M, 266.99579.

m/e 266 (25), 265 (77), 264 (25), 263 (100), 229 (25), 228 (41), 227 (29), 194 (98), 165 (48), 164 (80).

Found, C, 54.3; H, 2.5; N, 15.6%.

Required for $C_{12}H_7Cl_2N_3$, C, 54.8; H, 2.7; N, 16.0%.

4-Chloro-2-(m-chlorophenyl)-1H-imidazo[4,5-c]pyridine (208)

2-Chloro-3,4-diaminopyridine (0.5 g), m-chlorobenzoic acid (0.545 g) and polyphosphoric acid (43 g) were stirred at 170° for 2 h, cooled, diluted with water (100 ml) and neutralised with potassium carbonate. The white precipitate was collected and washed with hot water (25 ml) to yield the imidazopyridine (0.8 g, 87%), m.p. >300° (from boiling water), white feathery solid.

Found, M, 263.00187.

Required for $C_{12}H_7Cl_2{}^{35}N_3$, M, 263.00169.

Found, M, 264.99834.

Required for $C_{12}H_7Cl_2{}^{35}{}^{37}N_3$, M, 264.99874.

Found, M, 266.99604.

Required for $C_{12}H_7Cl_2{}^{37}N_3$, M, 266.99579.

ν max. (KBr) 3100, 1620, 1570, 1420, 1300, 1220, 950 720 cm^{-1}.

τ (TFA) 1.26[1H, d, J = 7Hz, 6 - H]

1.72[2H, m, 7 - H 2' - H]

2.18[3H, m, 4'5'6' - H]

m/e 266 (15), 265 (67), 264 (21), 263 (100), 267 (15), 231 (3), 230 (14), 229 (20), 228 (33), 227 (27), 190 (14), 163 (11), 136 (10), 135 (13), 134 (10), 123 (7), 112 (8), 111 (8), 109 (17), 89 (22), 73 (16), 62 (31).

4-Chloro-2-(p-chlorophenyl)-1H-imidazo[4,5-c]pyridine (209)

2-Chloro-3,4-diaminopyridine (0.5 g), p-chlorobenzoic acid (0.545 g) and polyphosphoric acid (38 g) were stirred at 200° for 0.5 h, cooled, diluted with water (100 ml) and neutralised with potassium carbonate. The white precipitate was collected and boiled with water (25 ml) to yield the white, water insoluble imidazopyridine (0.59 g, 91%), m.p. >300°.

Found, C, 54.5; H, 2.6; N, 15.6%.

Required for $C_{12}H_7Cl_2N_3$, C, 54.8; H, 2.7; N, 16.0%.

ν max. (KBr) 1610, 1570, 1480, 1420, 1300, 1220, 1090, 940, 840, 810 cm^{-1}.

τ (TFA) 0.9 [1H, d, J = 7Hz, 6 - H]

1.35[4H, m, 2'3'5'6' - H]

1.85[1H, d, J = 7Hz, 7 - H]

m/e 267 (18), 266 (18), 265 (68), 264 (28), 263 (100), 229 (12), 228 (20), 227 (33), 226 (41), 201 (10), 193 (8), 192 (14), 165 (9), 140 (9), 139 (15),

138 (18), 137 (15), 114 (9), 227/2, 229/2, 113 (8), 263/2, 265/2, 111 (24), 102 (11), 91 (25), 75 (23).

4-Chloro-2-(p-nitrophenyl)-1H-imidazo[4,5-c]pyridine (210)

2-Chloro-3,4-diaminopyridine (0.5 g), p-nitrobenzoic acid (0.583 g) and polyphosphoric acid (34 g) were stirred at 180° for 3 h, cooled, diluted with water (100 ml) and neutralised with potassium carbonate. The yellow precipitate was boiled with water (25 ml) to yield the water insoluble imidazopyridine (0.79 g, 82%), m.p. 282°-284° (from dimethylformamide), light yellow feathery solid.

Found, M, 274.

Required for $C_{12}H_7ClN_4O_2$, M, 274.

ν max. (KBr) 1600, 1580, 1520 (NO_2), 1450, 1420, 1350 (NO_2), 1230, 950, 720 cm^{-1}.

τ (TFA) 1.43[1H, d, J = 6Hz, 6-H]
1.5 [4H, m, 2'3'5'6'-H]
1.75[1H, d, J = 6Hz, 7-H]

m/e 277 (8), 276 (40), 275 (20), 274 (100), 246 (11), 245 (7), 244 (33), 230 (16), 229 (9), 228 (44), 218 (6), 216 (16), 208 (16), 207 (11), 193 (22), 192 (58), 191 (8), 181 (6), 180 (11), 167 (7), 161 (16), 165 (33), 141 (6), 140 (8), 139 (8), 138 (10).

4-Chloro-2-methyl-1H-imidazo[4,5-c]pyridine (211)

2-Chloro-3,4-diaminopyridine (0.5 g), glacial acetic acid

(2 g) and polyphosphoric acid (33 g) were stirred at 180° for 3 h, cooled, diluted with water (150 ml) and neutralised with potassium carbonate. The brown solution was extracted continuously with chloroform for 12 h and after treatment with activated charcoal, the chloroform was removed in vacuo to yield the imidazopyridine (0.45 g, 78%), m.p. 153°-155° (from chloroform), fine white needles.

ν max. (KBr) 1640, 1600, 1460, 1300, 1220, 1040, 990 810 cm^{-1}.

τ (TFA) 0.98[1H, d, J = 7Hz, 6-H]
1.45[1H, d, J = 7Hz, 7-H]
6.8 [3H, s, 2-CH_3]

Found, M, 167.02532.
Calc. for $C_7H_6N_3Cl^{35}$, M, 167.02502.
m/e 167 (3), 168 (1), 149 (3), 132 (100), 131 (24), 130 (64), 107 (3), 106 (15), 105 (19), 104 (17), 94 (4), 93 (18), 92 (14), 91 (7), 80 (7), 79 (30), 78 (15), 77 (3), 71 (6), 67 (5), 66 (31), 65 (35), 64 (15).

4-Chloro-2-(p-bromophenyl)-1H-imidazo[4,5-c]pyridine (212)

2-Chloro-3,4-diaminopyridine (0.5 g), p-bromobenzoic acid (0.702 g) and polyphosphoric acid (30 g) were stirred at 200° for 1.5 h, cooled, diluted with water (250 ml) and neutralised with potassium carbonate. The white precipitate was collected and boiled with water (25 ml) to yield the white, water insoluble imidazopyridine (0.95 g, 89%), m.p. >300°.

Found, C, 46.6; H, 2.3; N, 13.6%.

Required for $C_{12}H_7ClBrN_3$, C, 46.9; H, 2.3; N, 13.7%.

ν max. (KBr) 1620, 1580, 1480, 1420, 1230, 940, 840, 810 cm^{-1}.

τ (TFA) 1.33[1H, d, J = 7Hz, 6 - H]

1.73[1H, d, J = 7Hz, 7 - H]

1.95[4H, m, 2'3'5'6' - H]

Found, M, 306.95175.

Required for $C_{12}H_7N_3Br_1^{79}Cl_1^{35}$, M, 306.95123.

Found, M, 308.94871.

Required for $C_{12}H_7N_3Cl_1^{35}Br_1^{81}$, M, 308.94926.

Found, M, 310.94582.

Required for $C_{12}H_7N_3Br_1^{81}Cl_1^{37}$, M, 310.94631.

m/e 309 (34), 308 (23), 307 (100), 306 (20), 305 (75), 275 (4), 274 (12), 273 (23), 272 (10), 271 (18), 230 (7), 228 (20), 193 (16), 192 (27), 184 (5), 183 (6), 182 (5), 181 (5), 165 (11), 90 (20), 102 (16).

4-Chloro-2-phenyl-1H-imidazo[4,5-c]pyridine (213)

2-Chloro-3,4-diaminopyridine (0.6 g), benzoic acid (0.511 g) and polyphosphoric acid (23 g) were stirred at 190° for 4 h, cooled, diluted with water (60 ml) and neutralised with potassium carbonate. The white precipitate was collected and washed with hot water (25 ml) to yield the imidazopyridine (0.41 g, 51%), m.p. 210° (from boiling water), white feathery solid.

ν max. (KBr) 1620, 1590, 1470, 1440, 1290, 1220, 1030, 960,700 cm^{-1}.

Found, M, 229.04073.

Required for $C_{12}H_8Cl^{35}N_3$, M, 229.04067.

Found, M, 231.03745.

Required for $C_{12}H_8N_3Cl^{37}$, M, 231.03772.

m/e 231 (7), 229 (19), 230 (4), 195 (29), 194 (100), 193 (41), 192 (10), 169 (7), 168 (16), 167 (8), 155 (3), 142 (4), 141 (11), 140 (9), 128 (12), 104 (30), 103 (15), 92 (11), 91 (7), 90 (8), 77 (26), 65 (17).

Found, C, 62.5; H, 3.3; N, 18.0%.

Required for $C_{12}H_8ClN_3$, C, 62.9; H, 3.5; N, 18.3%.

No suitable solvent was available for the determination of an n.m.r. spectrum.

4-Chloro-1H-imidazo[4,5-c]pyridine-2(3H)-one (115).

2-Chloro-3,4-diaminopyridine (4.1 g) and urea (1.7 g) were finely mixed and fused at 240° for 0.5 h. Recrystallisation from 50% acetic acid yielded the imidazopyridine (4.0 g, 83%), m.p. >300° (lit.[38] 300°).

ν max. (KBr) 1720 (C=O), 1620, 1500, 1270, 1180, 1060, 1000, 890, 820, 780, 760 cm^{-1}.

τ (TFA) 1.55[1H, d, J = 7Hz, 6-H]

2.15[1H, d, J = 7Hz, 7-H]

Found, M, 169.00450.

Calc. for $C_6H_4N_3OCl_1^{35}$, M, 169.00428.

Found, M, 171.00123.

Calc. for $C_6H_4N_3OCl^{37}$, M, 171.00133.

m/e 172 (3), 171 (30), 170 (9), 169 (100), 145 (4), 143 (8), 137 (3), 136 (3), 135 (3), 106 (13), 105 (15), 80 (9), 79 (15), 78 (11), 64 (10), 53 (19), 52 (21), 51 (8), 44 (18), 43 (8).

1-N-Acetyl-4-chloro-1H-imidazo[4,5-c]pyridine -2(3H)-one 5-N-oxide (214)

4-Chloro-1H-imidazo[4,5-c]pyridine-2(3H)-one (1.0 g), glacial acetic acid (12 g) and hydrogen peroxide (100 vol, 2 ml) were heated on a steam bath at 100° for 3 h. A further aliquot of hydrogen peroxide was added (1 ml) and heating continued for a further 2 h. The yellow reaction mixture was reduced in volume in vacuo to yield the product (0.95 g, 70%), m.p. >300°.

τ (TFA) 7.8 [3H, s, 1-N-Ac]

1.42[1H, d, J = 7Hz, 6-H]

2.21[1H, d, J = 7Hz, 7-H]

ν max. (KBr) 3000, 2700, 1720 (C=O), 1500, 1470, 1180, 1000, 990, 820, 750 cm^{-1}.

Found, M, 184.99942.

Required for $C_6H_4Cl_1^{35}N_3O_2$, M, 184.99920.

m/e 187 (2), 186 (23), 185 (8), 184 (67), 183 (4), 171 (4), 170 (35), 169 (11), 168 (100), 167 (3), 151 (7), 141 (8), 133 (7), 122 (17), 107 (7),

106 (17), 105 (21), 95 (19), 87 (10).

4-Chloro-1H-imidazo[4,5-c]pyridine (104)

Prepared by the method of Rousseau and Robins[37] from 2-chloro-3,4-diaminopyridine and the method of Mizuno et al.[101] from 1H-imidazo[4,5-c]pyridine 5-N-oxide, m.p. 242° (lit.[37] 243°).

1H-Imidazo[4,5-c]pyridine-4(5H)-thione (116)

Prepared by the method of Rousseau and Robins, m.p. >300° (lit.[37] 365°).

4-Methylmercapto-1H-imidazo[4,5-c]pyridine (215)

Dimethyl sulphate (0.25 ml) was added dropwise to a stirred solution of 1H-imidazo[4,5-c]pyridine-4(5H)-thione (0.5 g) in sodium hydroxide (2N, 2 ml) and water (3.1 ml). The reaction mixture was left to stand for 24 h at room temperature and the resultant methylmercapto-imidazopyridine was collected and recrystallised from boiling water (0.4 g, 73%), m.p. 215° (lit.[1] 217°), light tan crystals.

ν max. (KBr) 3000, 2800, 1580, 1450, 1400, 1260, 1200, 950, 850, 800 cm^{-1}.

τ $(CD_3)_2SO$ 1.62[1H, s, 2-H]
1.71[1H, d, J = 5Hz, 6-H]
2.56[1H, d, J = 5Hz, 7-H]
7.4 [3H, s, S-Me]

Found, M, 165.03627.

Calc. for $C_7H_7N_3S$, M, 165.03606.

m/e 165 (100), 166 (19), 164 (89), 151 (10), 133 (15),

132 (29), 120 (35, 119 (81), 118 (30), 105 (17), 93 (12), 92 (45), 91 (14), 64 (35, 52 (17).

(viii) Reactions with 3,4,5-triaminopyridine trihydrochloride

3,4,5-Triaminopyridine trihydrochloride (216)

Prepared by the method of Graboyes and Day,[11] m.p. 274°-275° (lit.[103] 275°-276°).

7-Amino-1H-imidazo[4,5-c]pyridine hydrochloride (217)

3,4,5-Triaminopyridine trihydrochloride (1 g) and triethylorthoformate (10 ml) were refluxed with stirring for 3 h. The triethylorthoformate was removed in vacuo and the residue refluxed for a further hour with concentrated hydrochloric acid and cooled to yield the imidazopyridine hydrochloride (0.8 g, 89%), m.p. >300°.

Found, M, 134.05924.

Required for $C_6H_6N_4$, M, 134.05901.

m/e 135 (17), 134 (100), 133 (6), 125 (10), 124 (50), 108 (4), 107 (26), 106 (11), 97 (8), 96 (15), 81 (5), 80 (25), 79 (11), 78 (4), 77 (7), 70 (11), 69 (19), 68 (4), 67 (13).

ν max. (KBr) 3300, 3000, 1660, 1570, 1460, 1400, 1260, 1130, 1090, 840, 730 cm^{-1}.

ν max. (KBr) base 3300, 1650, 1610, 1570, 1510, 1440, 1340, 1190, 1160, 870 cm^{-1}.

Found, C, 42.0; H, 3.9; N, 32.5%.

Required for $C_6H_7ClN_4$, C, 42.4; H, 4.1; N, 32.9%

τ $(CD_3)_2SO$ 1.8[1H, s, 6-H]

1.6[1H, s, 4-H]

0.9[1H, s, 2-H]

1.4[2H, s(a), 7-NH_2]

7-Amino-1H-imidazo[4,5-c]pyridine-2(3H)-one dihydrochloride (218)

3,4,5-Triaminopyridine trihydrochloride (1 g) and urea (0.304 g) were finely mixed in a mortar and heated at 320° for 0.5 h. The resultant solid was boiled with water to yield the white, water insoluble imidazopyridine base. The white solid was recrystallised from ethanolic hydrogen chloride to yield the dihydrochloride salt (0.85 g, 89%), m.p. >300°.

Found, M, 150.05416.

Required for $C_6H_6N_4O_1$, M, 150.05396.

ν max. (KBr) 3200, 3000, 1680, 1640, 1570, 1490, 1420, 1230, 1000, 820, 740 cm^{-1}.

Found, C, 32.0; H, 3.5; N, 25.0%.

Required for $C_6H_8Cl_2N_4O$, C, 32.4; H, 3.6; N, 25.2%.

τ (TFA) 1.77[1H, s, 6-H]

1.65[1H, s, 4-H]

(ix) Reactions with 4,5-diaminopyrimidines

8-(p-Nitrophenyl)-9H-purine-6(1H)-one dihydrochloride (219)

4,5-Diamino-6-hydroxypyrimidine hemisulphate (2 g), p-nitrobenzoic acid (1.92 g) and polyphosphoric acid (35 g) were stirred at 210° for 3 h, cooled, diluted with water (200 ml) and neutralised

with potassium carbonate. The yellow precipitate was collected and boiled with water (25 ml) to yield the purine base which was recrystallised from ethanolic hydrogen chloride solution to give the dihydrochloride salt (3 g, 89%), m.p. >300°, light yellow needles (ethanolic hydrogen chloride).

HCl ν max. (KBr) 3300, 3200, 1650 (C=O), 1600, 1530 (NO_2), 1470, 1360 (NO_2) cm^{-1}.

base ν max. (KBr) 1680, 1580, 1620, 1530 (NO_2), 1350 (NO_2) 860, 720 cm^{-1}.

τ (TFA) 1.16[1H, s, 2-H]

1.52[4H, m, 2'3'5'6'-H]

Found, M, 257.054330.

Required for, M, $C_{11}H_7N_5O_3$, 257.054884.

m/e 258 (15), 257 (100), 241 (13), 230 (64), 225 (43), 211 (21), 202 (15), 200 (16), 172 (36), 157 (41), 156 (31), 155 (14), 150 (28), 149 (95), 145 (18), 141 (41), 130 (18), 129 (45), 128 (18), 103 (45), 97 (41), 95 (54), 83 (54), 81 (73), 77 (50), 69 (100).

Found, C, 40.0; H, 2.7; N, 21.0; Cl, 20.9%.

Required for $C_{11}H_9Cl_2N_5O_3$, C, 40.1; H, 2.7; Cl, 21.3; N, 21.3%.

8-(p-Nitrophenyl)-9H-purine dihydrochloride (220)

4,5-Diaminopyrimidine (1 g), p-nitrobenzoic acid (1.52 g) and polyphosphoric acid (40 g) were stirred at 180° for 3.5 h, cooled, diluted with water (200 ml) and neutralised with potassium carbonate. The brown precipitate was collected and recrystallised

from ethanolic hydrogen chloride to yield the purine dihydrochloride on addition of ether as light orange needles, m.p. >300°, (1.8 g, 82%).

ν max. (KBr) 3050, 1640 (C=O), 1600, 1580, 1520 (NO_2), 1340 (NO_2), 1270, 940, 860, 710 cm^{-1}.

τ $(CD_3)_2SO$ 0.46[1H, s, 2-H]
0.71[1H, s, 6-H]
1.3 [4H, m, 2'3'5'6'-H]

Found, M, 241.05916.

Required for $C_{11}H_7N_5O_2$, M, 241.059970.

m/e 242 (4), 241 (100), 212 (7), 211 (48), 195 (15), 178 (50), 177 (6), 176 (9), 168 (7), 152 (10), 138 (17), 120 (17), 119 (16), 118 (8), 117 (5), 115 (9), 74 (10), 73 (35), 71 (28), 69 (26).

3H-Triazolo[4,5-d]pyrimidine-7(6H)-one sulphate (221)

A solution of sodium nitrite (2 g) in water (7 ml) at 0° was added dropwise with stirring to a suspension of 4,5-diamino-6-hydroxypyrimidine hemisulphate (2 g) in concentrated sulphuric acid (5 ml) and water (18 ml) at 0°. The yellow suspension changed to a green solution over a period of 0.25 h and this solution was stirred at 5° for 3 h to yield the yellow triazolopyrimidine sulphate which had precipitated out (1.2 g, 45%), m.p. >300°.

ν max. (KBr) 1720 (C=O), 1600, 1570, 1390, 1260, 1100, 920, 800, 720, 690 cm^{-1}.

τ $(CD_3)_2SO$ 1.82[1H, s, 5-H]

Found, M, 137.03311.

Required for $C_4H_3N_5O$, M, 137.033757.

m/e 137 (100), 136 (13), 135 (3), 109 (12), 108 (9), 107 (53), 106 (9), 105 (2), 99 (6), 98 (8), 97 (9), 96 (6), 95 (9), 94 (5), 85 (13), 84 (8), 83 (16), 82 (16), 81 (21), 80 (35), 73 (10), 72 (7), 71 (20), 70 (15), 69 (22), 68 (13), 67 (20), 66 (33), 65 (13), 64 (85).

6-Methylmercaptopurine (222)

Prepared by the method of Hitchings and Elion,[110] m.p. 219° (lit.[100] 220°).

9H-Purine-8(7H)-one (223)

Prepared by the method of Robins,[98] m.p. >300° (lit.[98] >300°).

9H-Purine-8(7H)-thione (224)

Prepared by the method of Albert and Brown,[97] m.p. >300° (lit.[97] >300°).

6-Hydroxylaminopurine (164)

Prepared by the method of Giner-Sorolla and Bendich,[111] m.p. 258° (lit.[96] 260°).

6,6'-Azoxypurine (226)

Prepared by the method of Giner-Sorolla,[96] m.p. >300° (lit.[96] >350°).

B. BIOLOGICAL

(1) MATERIALS AND METHODS

(i) Test Chemicals

The structures and identification are as described in the chemical experimental section. Metronidazole was kindly supplied by May and Baker Ltd., Dagenham, Essex. 9-Aminoacridine, purine, 6-mercaptopurine, 6-methoxypurine, 6-chloropurine, 4-aminopyridine and 2-aminofluorene were obtained from the Aldrich Chemical Company, Dorset.

(ii) Preparation of Solutions of Test Chemicals

Solutions were prepared in sterile screw cap glass bottles and because most of the test chemicals were insoluble in water, dimethylsulfoxide (DMSO) was always used as the vehicle. Solutions were prepared in DMSO at a concentration of 10 mg ml^{-1}. The mutagen solutions were stored at -20^{o} for extended periods and did not lose their activity.

(iii) Other Chemicals and Biological Media

The chemicals used were of Analar grade. Dimethylsulfoxide, $MgSO_4.7H_2O$, citric acid.H_2O, K_2HPO_4 anhydrous, $NaNH_4HPO_4.4H_2O$, D-glucose, NaCl, $MgCl_2.6H_2O$, KCl, $Na_2HPO_4.7H_2O$, NaH_2PO_4 and phenobarbitone sodium were obtained from BDH Chemicals, Poole, Dorset. D-Biotin, L-histidine, glucose-6-phosphate and NADP were obtained from the Sigma Chemical Company, London. Nutrient Broth (Oxoid Code CM1) was obtained from Oxoid Ltd., London. "Lab M" agar was from London Analytical and Bacteriological Media Ltd.

(iv) Equipment

The liquid nitrogen refrigerator was obtained from Union Carbide, U.K. Ltd. Sterile, plastic, screw capped vials were from Gibco Bio-Cult, Ltd., Hounslow. The Potter-Elvehjem apparatus

with teflon pestle (model K41) was from Tri-R Instruments, U.S.A. The centrifuge used was model MSE High Speed 18.

(v) Bacterial Tester Strains

The strains used were TA100, TA1535, TA98, TA1538 and TA1537 and were kindly supplied by Dr. Bruce Ames, Department of Biochemistry, University of California, U.S.A. The strains, upon receipt, were grown in nutrient broth overnight at 37° and stored at -196°. Subsequently the samples were checked for deletions at histidine, uvrB and rfa (deep rough) sites. The presence of the R-plasmid in TA98 and TA100 was also checked by the method recommended by Ames et al.[84]

(vi) Storage of Bacterial Tester Strains

The tester strains were stored in liquid nitrogen (-196°) in 2 ml sterile plastic screw capped vials containing 0.1 ml of dimethylsulfoxide.

Duplicate frozen cultures of each tester strain were prepared, one of which was stored as a master copy and only opened when regeneration of the frozen stocks was required. The other was used routinely to obtain fresh cultures for mutagenesis testing. Inoculation was effected by scraping a wooden applicator stick over the frozen culture surface, dipping into sterile nutrient broth (∿50 ml) and incubating overnight at 37°.

(vii) Minimal Glucose Agar

The following stock solutions were prepared separately in distilled water and autoclaved.

A. agar 2.5% $^{w}/_{v}$

B. glucose 10% $^{w}/_{v}$

C. Vogel-Bonner E

water 670 ml

$MgSO_4.7H_2O$ 10 g

Citric acid.H_2O 100 g

K_2HPO_4.anhydrous 500 g

$NaNH_4HPO_4.4H_2O$ 175 g

D. Solution C diluted x10

Minimal Glucose Agar was made by mixing aseptically

A. 3 l

B. 1 l

D. 1 l

(viii) Top Agar

Agar 0.6% $^{w}/_{v}$

NaCl 0.5% $^{w}/_{v}$

dissolved in distilled water, distributed in 2 ml aliquots and autoclaved. For use, aliquots were melted and kept at 45°. 0.1 ml of a sterile filtered solution of 1.0 mM L-histidine HCl and 1.0 mM biotin was added to each 2 ml aliquot. The trace of histidine in the top agar allows all the bacteria on the plate to undergo several divisions. This growth and division is necessary for mutagenesis to occur in the case of many mutagens. The slight background that grows up also allows any inhibition by the compound to be seen. Further increase in the amount of histidine on the plate enhances mutagenesis, but also causes heavy growth of the background lawn which obscures the revertants.

(ix) Induction of Rat Liver Enzymes for Mutagen Activation

The induction procedure was based on a modification to the method of Ames et al.[84] Rat liver enzymes were induced with phenobarbitone. Male rats (University Animal House) of approximately 200 g each were maintained on Purina laboratory chow. Phenobarbitone sodium solution (0.4 g/l in tap water) was given as drinking water

ad libitum to the rats for five days before sacrifice. The food was removed 12 h before sacrifice on the fifth day of induction. The rats were stunned by a blow to the head and killed by cervical dislocation.

(x) Preparation of Liver Homogenate Fraction

The preparation of liver S-9 fraction was similar to the procedure of Ames et al.[84] All steps were carried out at 0 - 4° using cold, sterile solutions and glassware. After sacrifice, the liver was dissected out using previously sterilised instruments in aseptic conditions and placed in a pre-weighed beaker containing 0.15 M KCl (approx. 1 ml/g wet liver). The beaker and contents were re-weighed and the livers were transferred to a fresh beaker and 0.15 M KCl added (3 ml/g wet liver), minced with sterile scissors and homogenised in a Potter-Elvehjem apparatus with a teflon pestle. The homogenate was centrifuged for 10 min at 9000 g and the supernatant (the S-9 fraction) was decanted off. The fresh S-9 fraction was distributed in 5 ml portions in sterile, plastic tubes and stored in a liquid nitrogen refrigerator. As required, sufficient of the S-9 fraction was warmed to 0° and kept on ice. The unused portion was discarded at the end of the day.

(xi) Preparation of S-9 Mix

S-9 mix contains per ml:

S-9 0.1 ml

$MgCl_2$ 8 µmoles

KCl 33 µmoles

glucose-6-phosphate 5 µmoles

NADP 4 µmoles

sodium phosphate pH 7.4 100 µmoles

Stock solutions of NADP (0.1 M) and glucose-6-phosphate (1 M) were prepared with sterile water in sterile tubes and stored at -20°. The stock salt solution (0.4 M $MgCl_2$, 1.65 M KCl) and phosphate buffer (0.2 M, pH 7.4) were sterilised by autoclaving and stored in the refrigerator. S-9 mix was freshly prepared each day but could be kept on ice for several hours before use. Bacterial contamination of S-9 mix was minimal and the solution was not therefore sterilised by filtration.

(xii) Mutagenesis Assay on Plates

To tubes of top agar at 45° were added (in order)

- 0.1 ml biotin-histidine solution
- test chemical in DMSO ($\leq$ 0.1 ml)
- 0.1 ml of an overnight culture grown in nutrient broth at 37°
- 0.5 ml, 1 ml, 1.5 ml of S-9 mix corresponding to 50 µl, 100 µl and 150 µl respectively of S-9/plate.

For primary screening, the test chemical was added in three concentrations, 10 µg, 100 µg and 1000 µg/plate. Volumes of DMSO varied but were never greater than 0.5 ml. If the primary screen indicated mutagenic action, a dose-response curve using smaller intervals was carried out.

The bacteria could remain at 45° for a few minutes without harm, but the S-9 mix was extremely sensitive to elevated temperatures, and was only left at this temperature for a few seconds.

The contents of the test tube were mixed (by rotating the tube between the palms) and poured onto minimal glucose agar plates. Uniform distribution of the top agar on the surface of the plates was accomplished by gently tilting and rotating the uncovered

plate. Within an hour, the plates were placed in an incubator at 37° and after 48 h, the colonies were counted. The presence of a light background lawn of growth, due to the trace amounts of histidine added, was confirmed.

When test chemicals were screened by spot testing, the test chemical was left out of the top agar and instead was applied to the plate surface after the top agar containing the bacteria, histidine-biotin solution and S-9 mix had been poured.

(xiii) Controls

In each experiment, positive mutagenesis controls were routinely included to confirm the reversion properties of each strain. The characteristic reversion patterns of the strains used to some diagnostic mutagens are shown in Table 4 . Positive controls using chemicals requiring metabolic activation confirmed that the S-9 mix was active. In the present group of experiments, 2-aminofluorene was spot tested but Ames et al.[84] used the polycyclic hydrocarbon benzo(a)pyrene. When activation experiments were carried out, two control plates without the test chemical but with S-9 mix and bacteria were included. In experiments without activation, control plates were included without the test chemical but with DMSO (0.1 ml) and the bacterial strain (0.1 ml) added in order to obtain the spontaneous reversion rate of the bacterial culture being used. Spontaneous revertant colonies on control plates without test chemical were slightly less than those of Ames et al.[84] which are quoted below:-

20(TA1535) 7(TA1537) 25(TA1538)

160(TA100) 40(TA98)

Table 4

Standard Mutagens used as Positive Controls

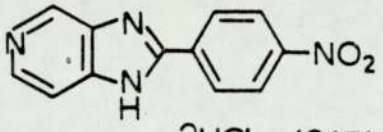
Mutagen	S-9	TA1535	TA100	TA1538	TA98	TA1537
metronidazole	-	-	+	-	-	-
9-aminoacridine	-	-	-	-	-	+
6-hydroxylaminopurine	-	+	+	-	-	-
2-aminofluorene	+	-	+	+	+	-

(2) RESULTS

Introduction

Figure 4 gives the structure of compounds positive in the Ames Test without liver activation. Figures 5 - 8 give the structures of compounds negative in the Ames test without liver activation and figure 9 gives the structures of compounds tested with liver activation. A "T" in figures 4 - 9 indicates a compound which was toxic at high dosage. Graphs 1 - 16 show the dose-response relationship for the mutagenic compounds in the sensitive strains. The calculated least squares line of regression is drawn for each graph and the correlation coefficient (R) has been calculated. Graphs 17 - 21 compare the sensitivity of the mutagenic compounds in different strains and figure 10 gives the key to each compound. Table 5 lists the mutagenic compounds tested and gives the mutagenic potency in revertants/nmole for each sensitive strain. Tables 6 - 9 list the experimental data on compounds tested with and without liver activation for which inconclusive results were obtained. The experimental data on compounds with a negative mutagenic response have not been included because of the volume of data involved.

Structure of compounds positive in the Ames test.
(without activation)



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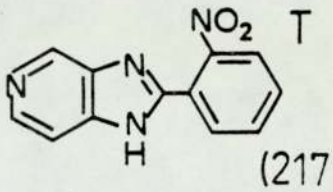

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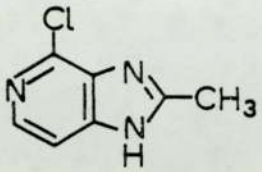

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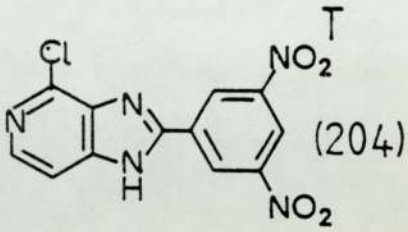

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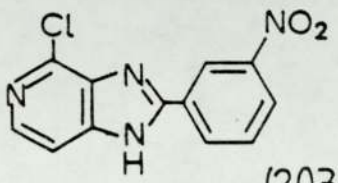

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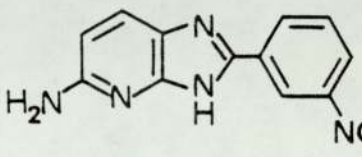

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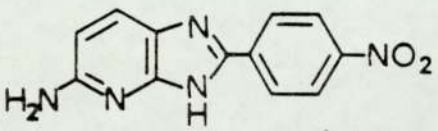

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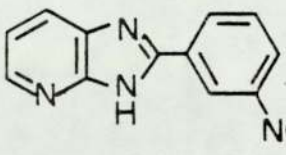

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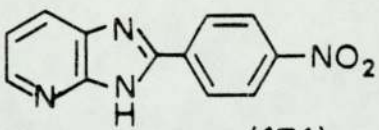

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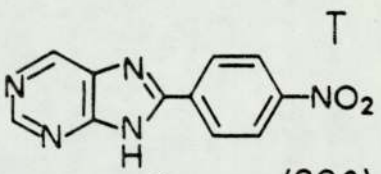

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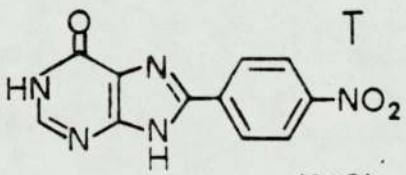

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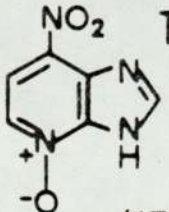

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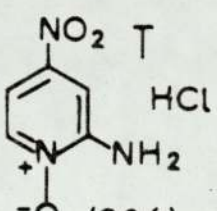

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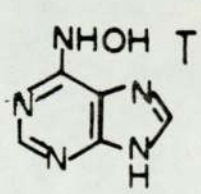
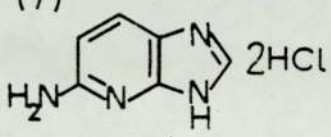
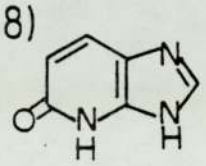


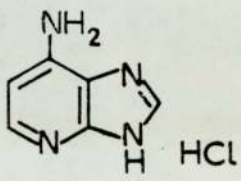
(164)

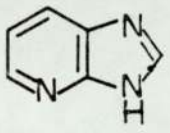
Figure 4

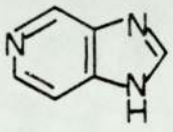
Negative results in Ames test.
(without activation)

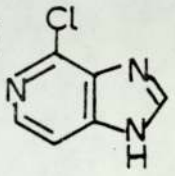
(7) 

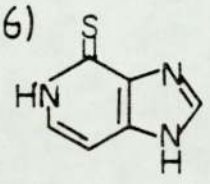
(188) 

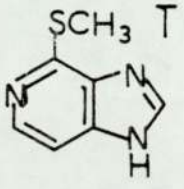
(3) 

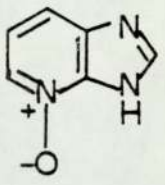
(1) 

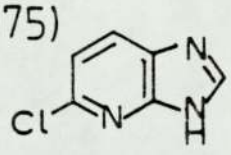
(91) 

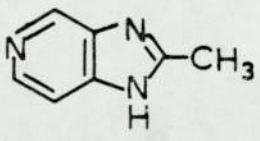
(104) 

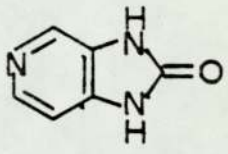
(116) 

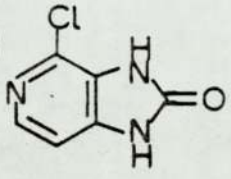
(215) 

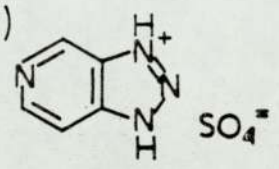
(172) 

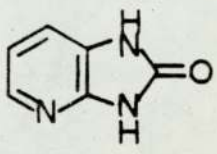
(175) 

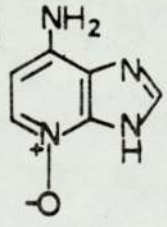
(210) 

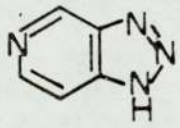
(113) 

(115) 

(202) 

(44) 

(174) 

(201) 

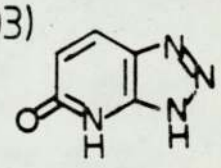
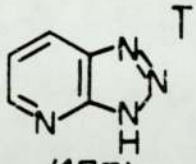
(203) 

Figure 5

Structure of compounds negative in the Ames test
(without activation)



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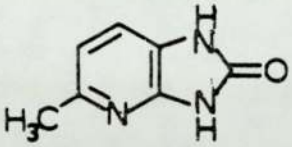
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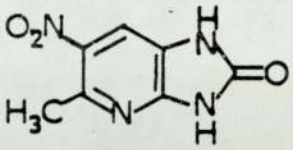
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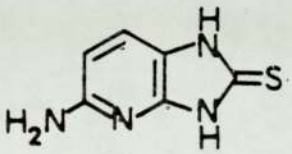
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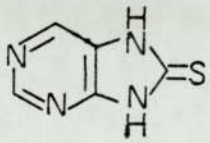
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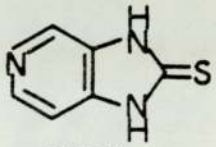
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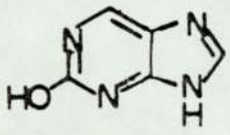
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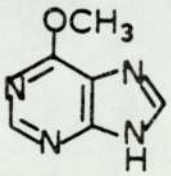
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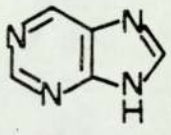
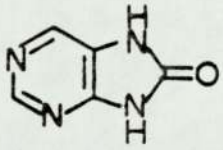
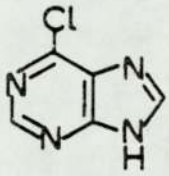
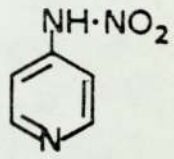
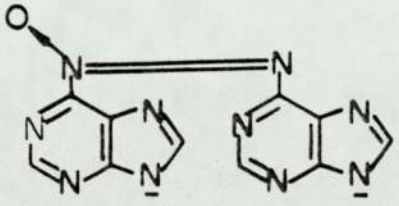

(229)


(223)


(163)


(208)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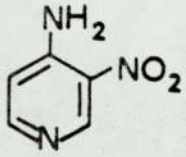
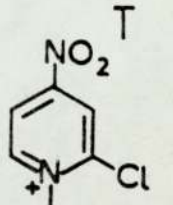
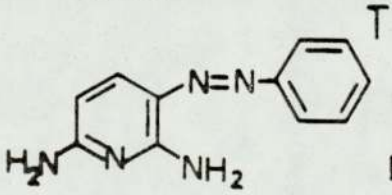
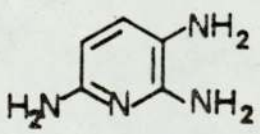

(209)



(205)



HCl
(189)


diHCl
(190)

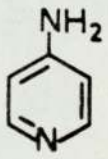
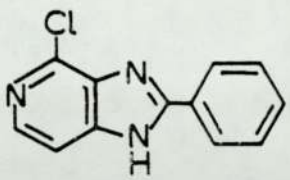
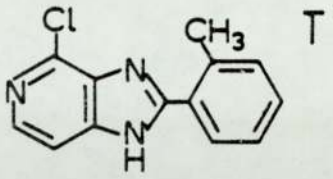

(231)

Figure 6

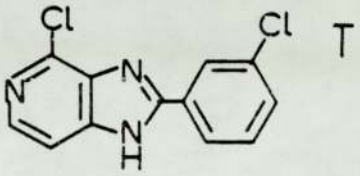
Structure of compounds negative in the Ames test.
(without activation)



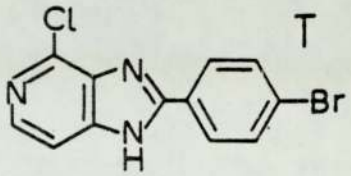
(213)



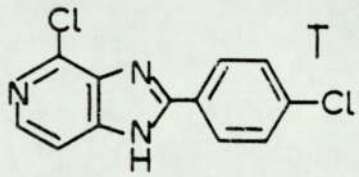
(206)



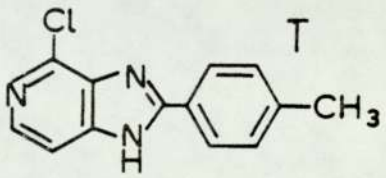
(208)



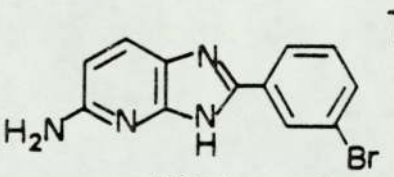
(212)



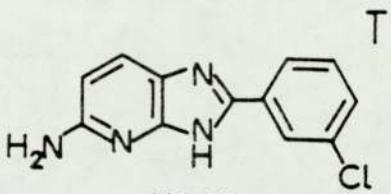
(209)



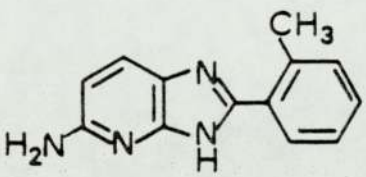
(205)



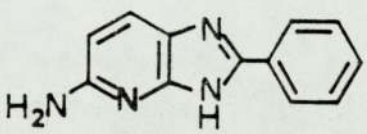
(197) 2HCl



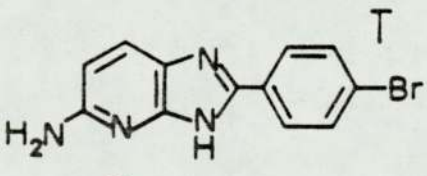
(192)



(194)

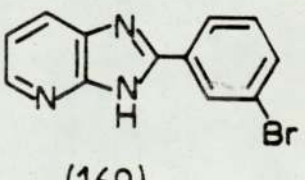


(198)



2HCl

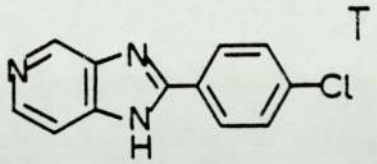
(191)



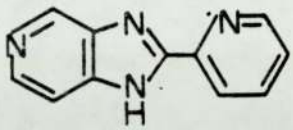
(169)

Figure 7

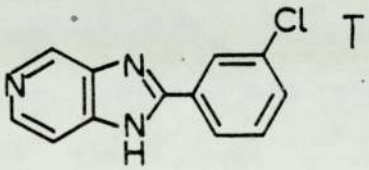
Structure of compounds negative in the Ames test.
(without activation)



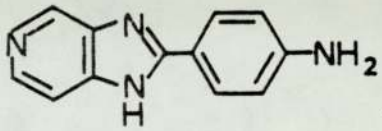
(211)



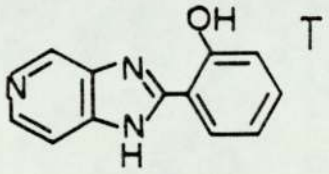
(216)



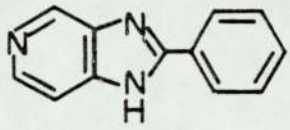
(212)



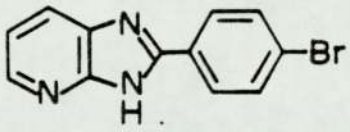
(218)



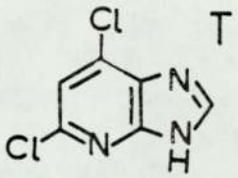
(214)



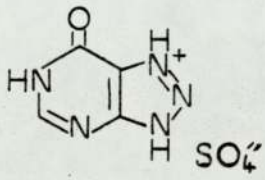
(213)



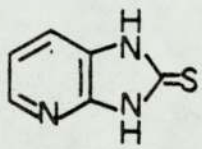
(170)



(176)



(221)

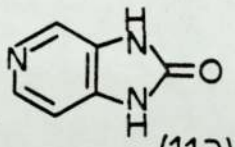


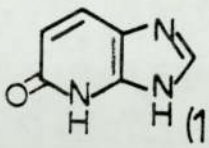
(63)

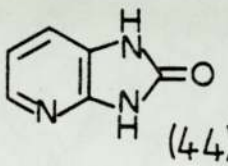
Figure 8

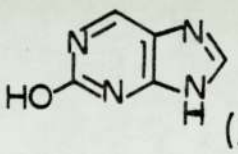
Compounds tested in the Ames Test (with Activation)

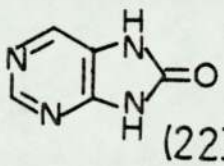
negative


(113)

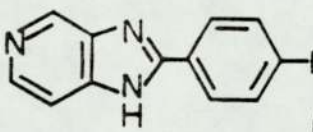

(188)


(44)

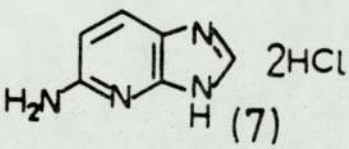

(230)

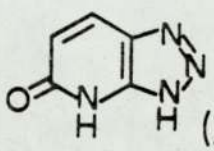

(223)

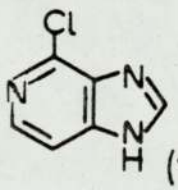
Positive (unchanged)


(215)

Inconclusive


(7)


(203)


(104)

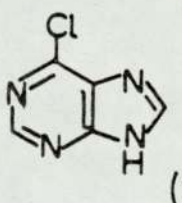
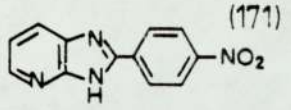
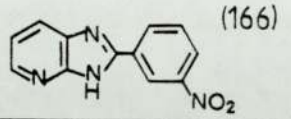
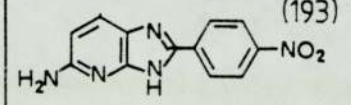
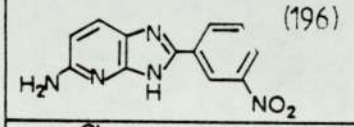
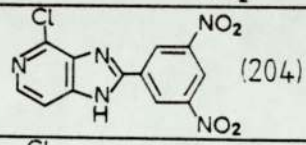
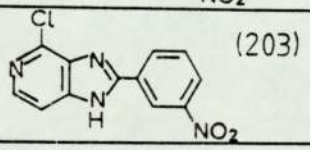
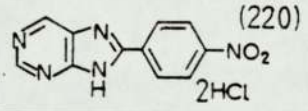
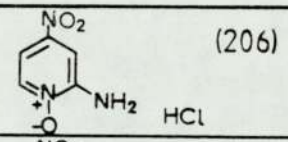
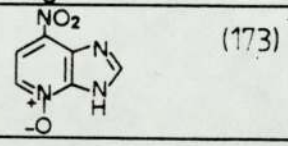
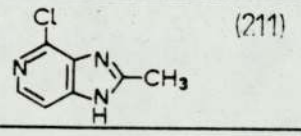
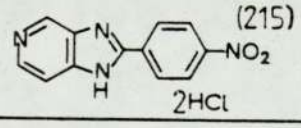
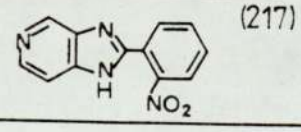

(163)

Figure 9

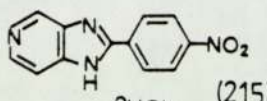
TABLE 5

MUTAGENIC COMPOUNDS

(strain and revertants/nmole)

 (171)	TA 1538 6·9 TA 98 3·9 TA 1537 0·05 TA 100 0·02
 (166)	TA 1538 0·75 TA 98 0·24 TA 1537 0·07 TA 100 0·08
 (193)	TA 98 6·0 TA 100 1·0
 (196)	TA 98 0·16 TA 100 1·2
 (204)	TA 1537 2·7 TA 98 2·9
 (203)	TA 1537 13·7 TA 100 9·5 TA 98 14·3
 (220)	TA 1538 231 TA 98 115 TA 100 18·5
 (206)	TA 100 12·3 TA 98 0·8 TA 1535 0·4
 (173)	TA 100 0·13
 (211)	TA 98 009 TA 1538 0·146 TA 100 0·014
 (215)	TA 1538 324 TA 98 152 TA 100 11
 (217)	TA 98 0·3 TA 1537 0·2 TA 100 0·1

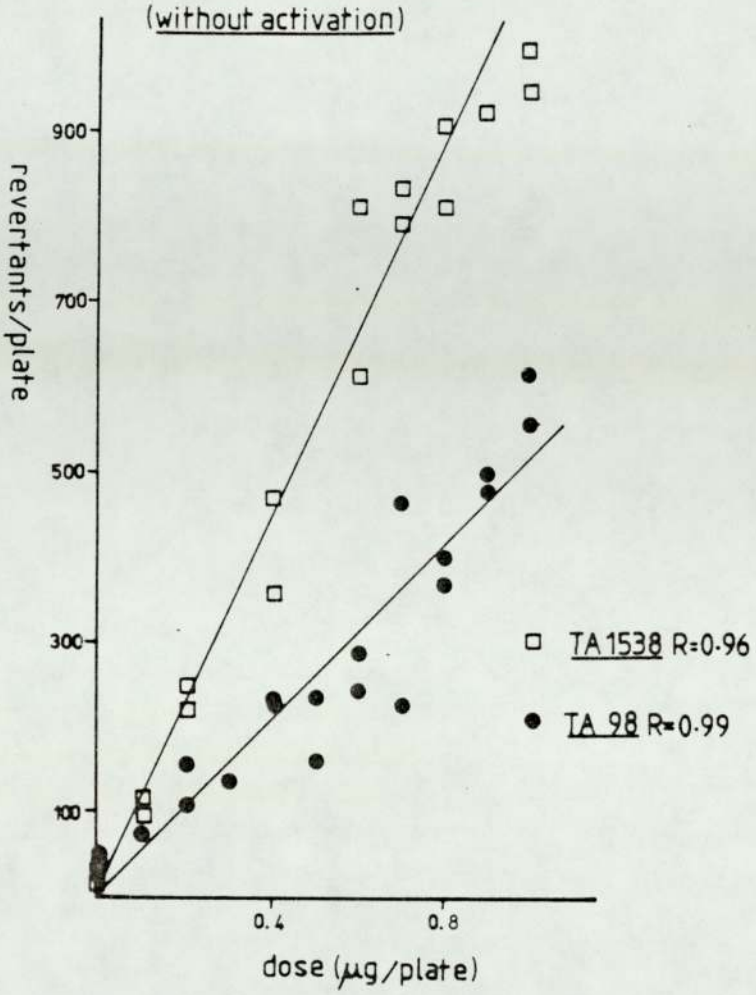
The dose / response of

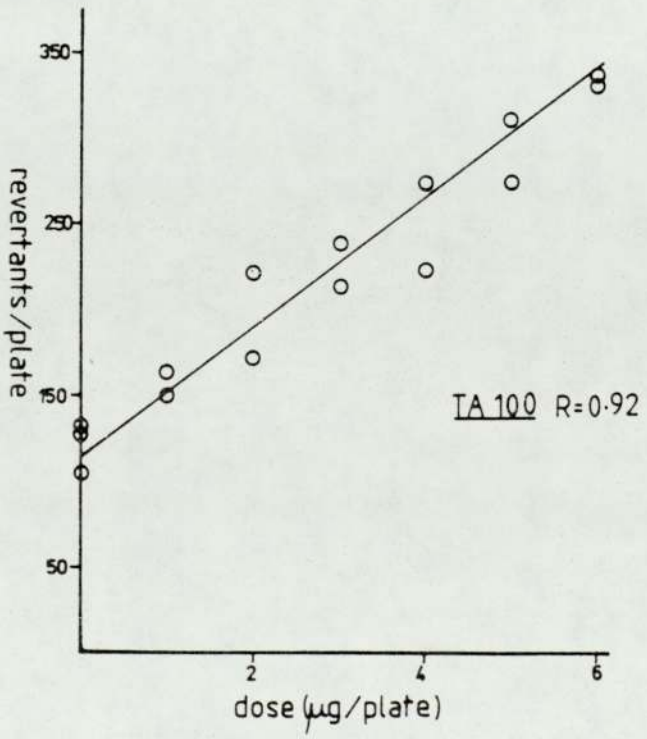


2HCl (215)

in TA 1538, TA 98 and TA 100.

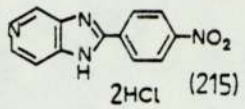
(without activation)





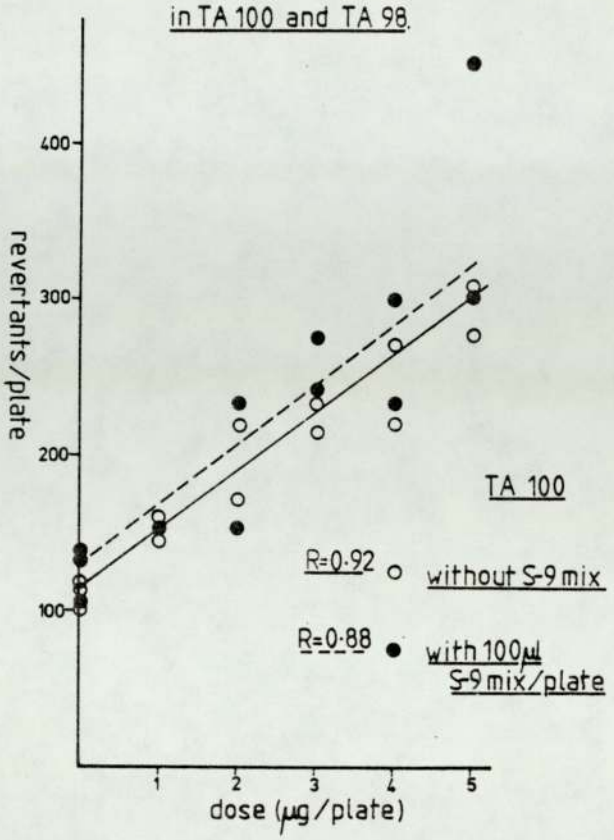
Graph 1

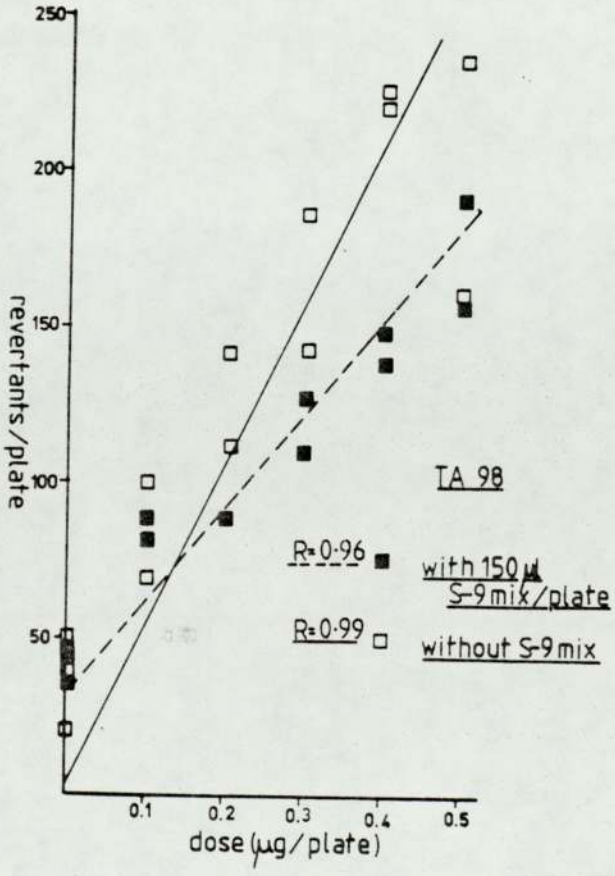
The comparative dose/responses with and without S-9 mix of



2HCl (215)

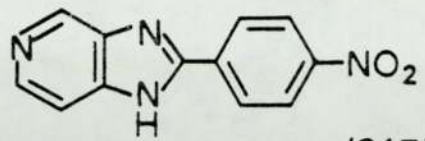
in TA 100 and TA 98.





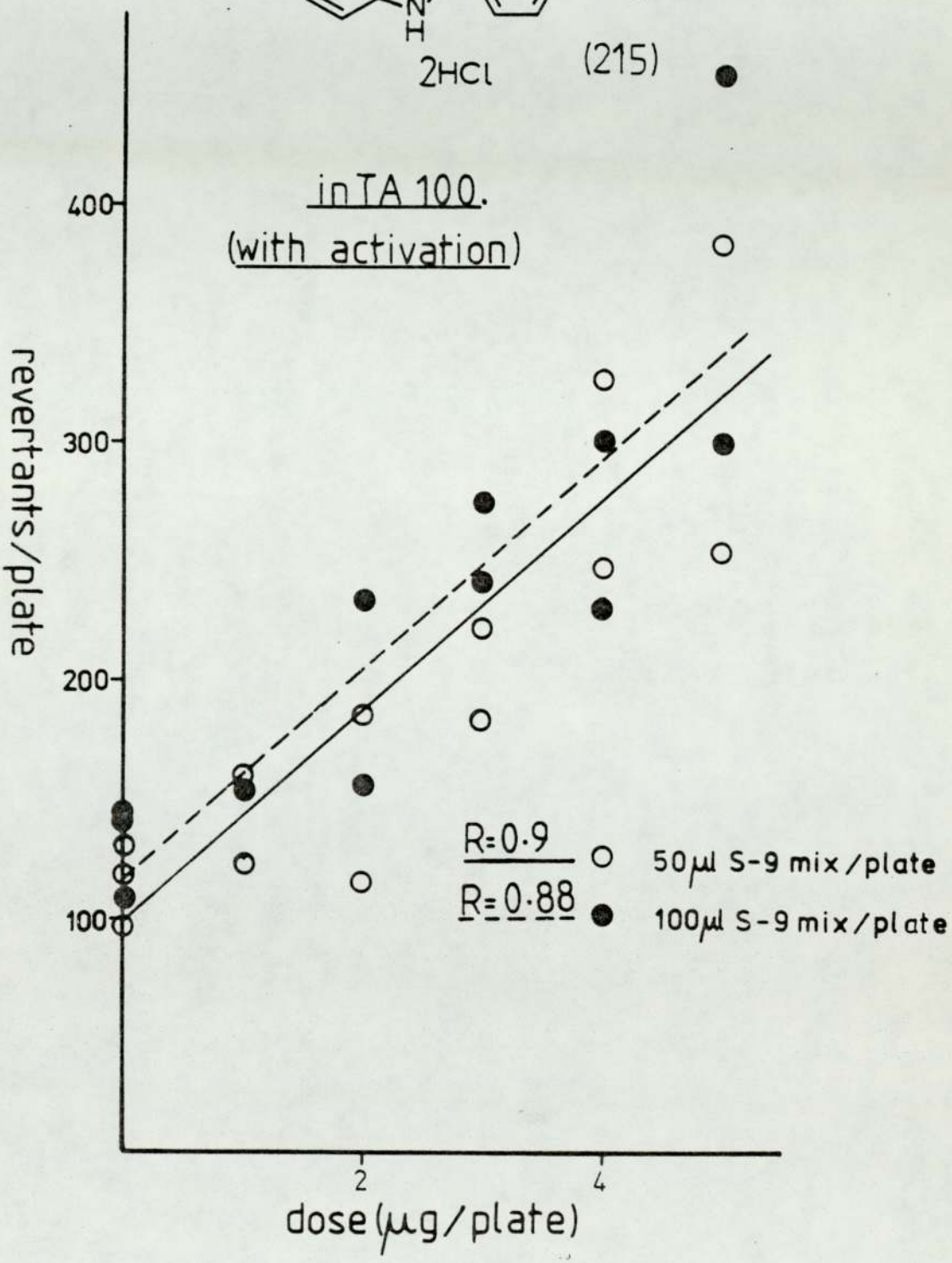
Graph 2

Dose / Response of



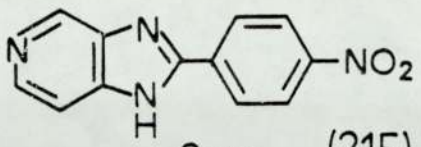
2HCl (215)

in TA 100.
(with activation)



Graph 3

Dose / Response of

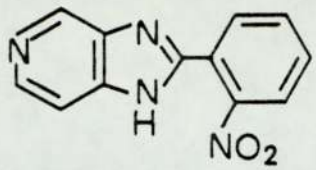


2HCl (215)

in TA 98.
(with activation)

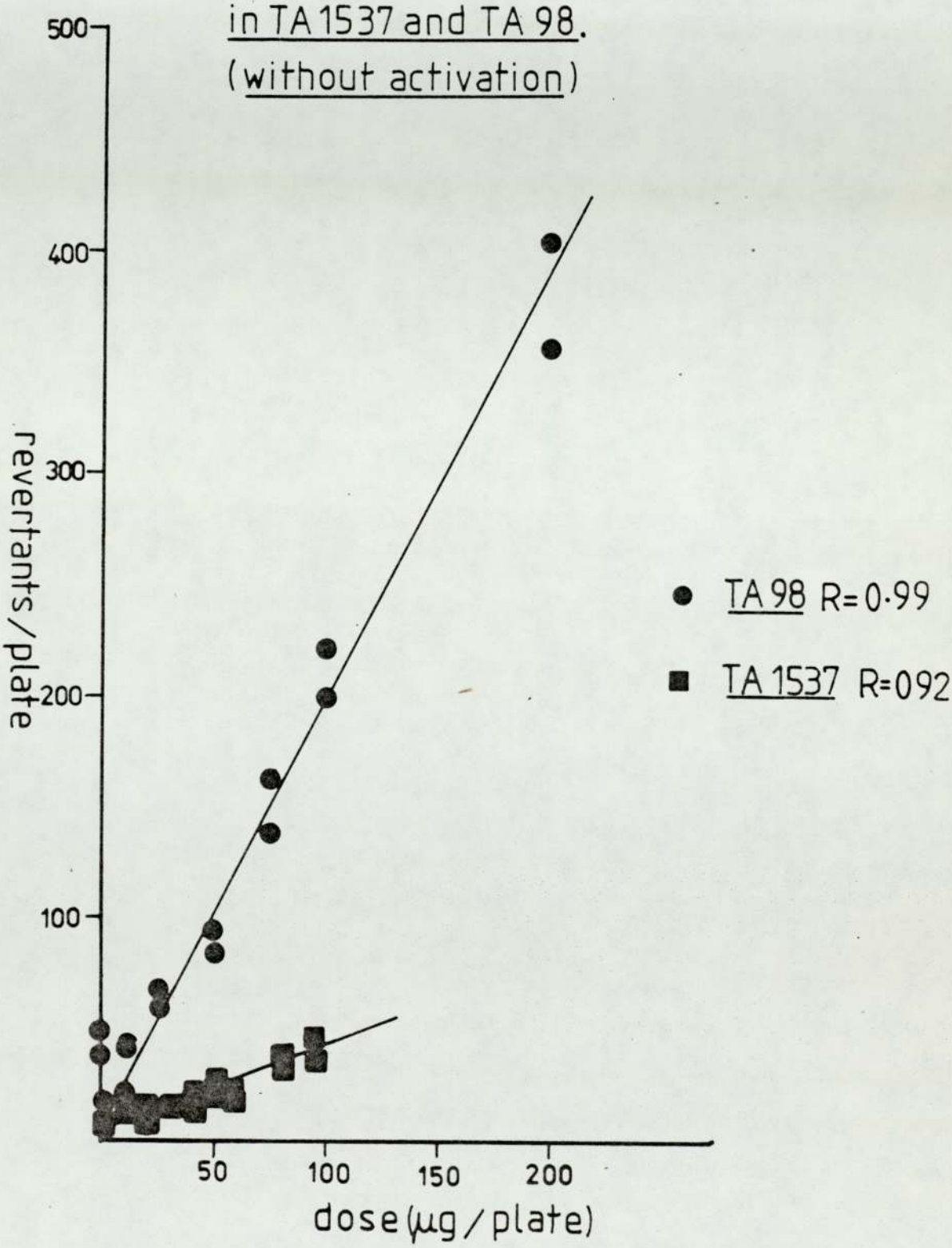
Graph 4

The dose/response of



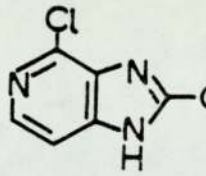
(217)

in TA 1537 and TA 98.
(without activation)



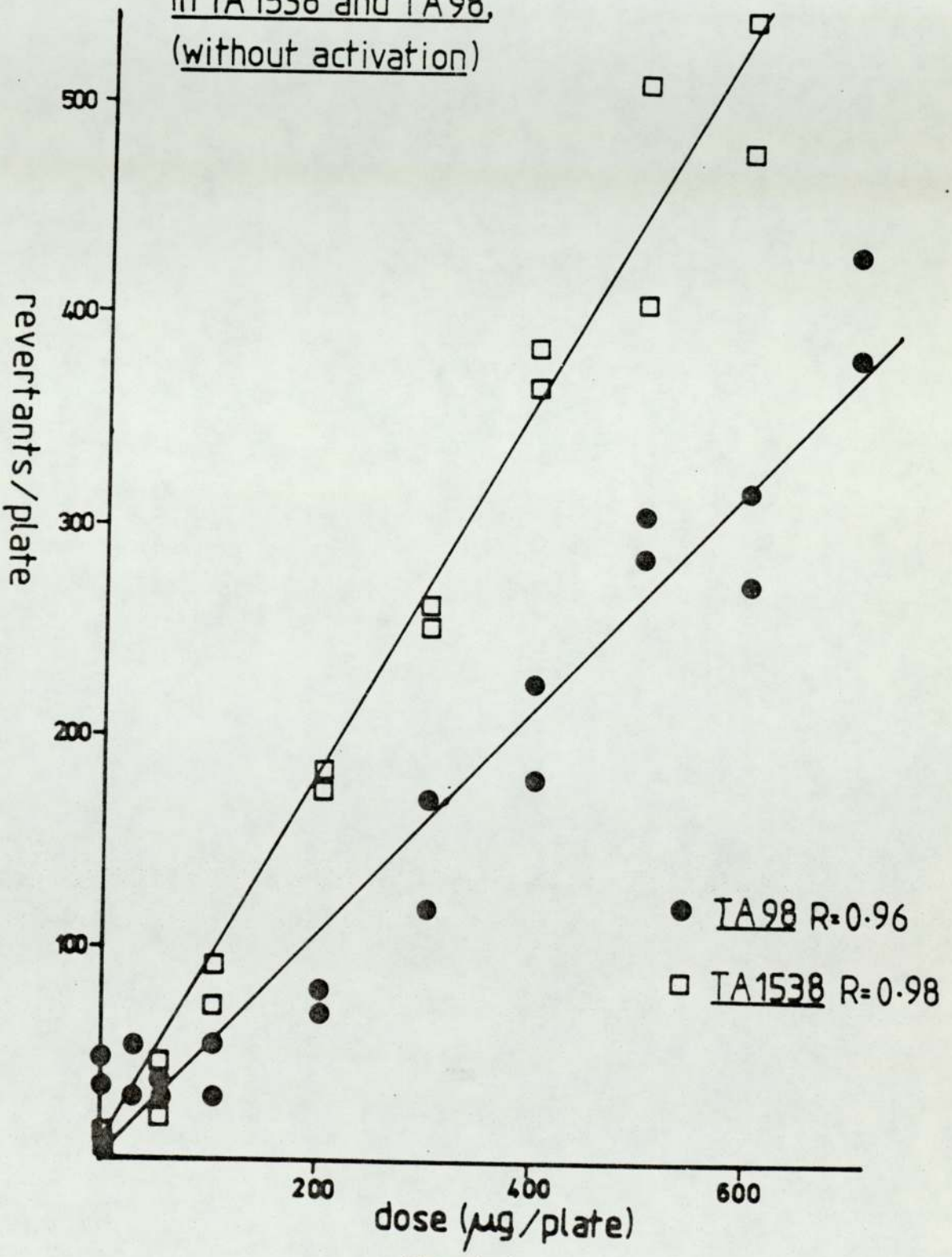
Graph 5

The dose/response of



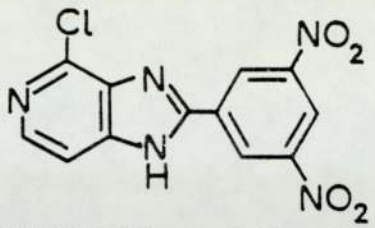
(211)

in TA1538 and TA98,
(without activation)



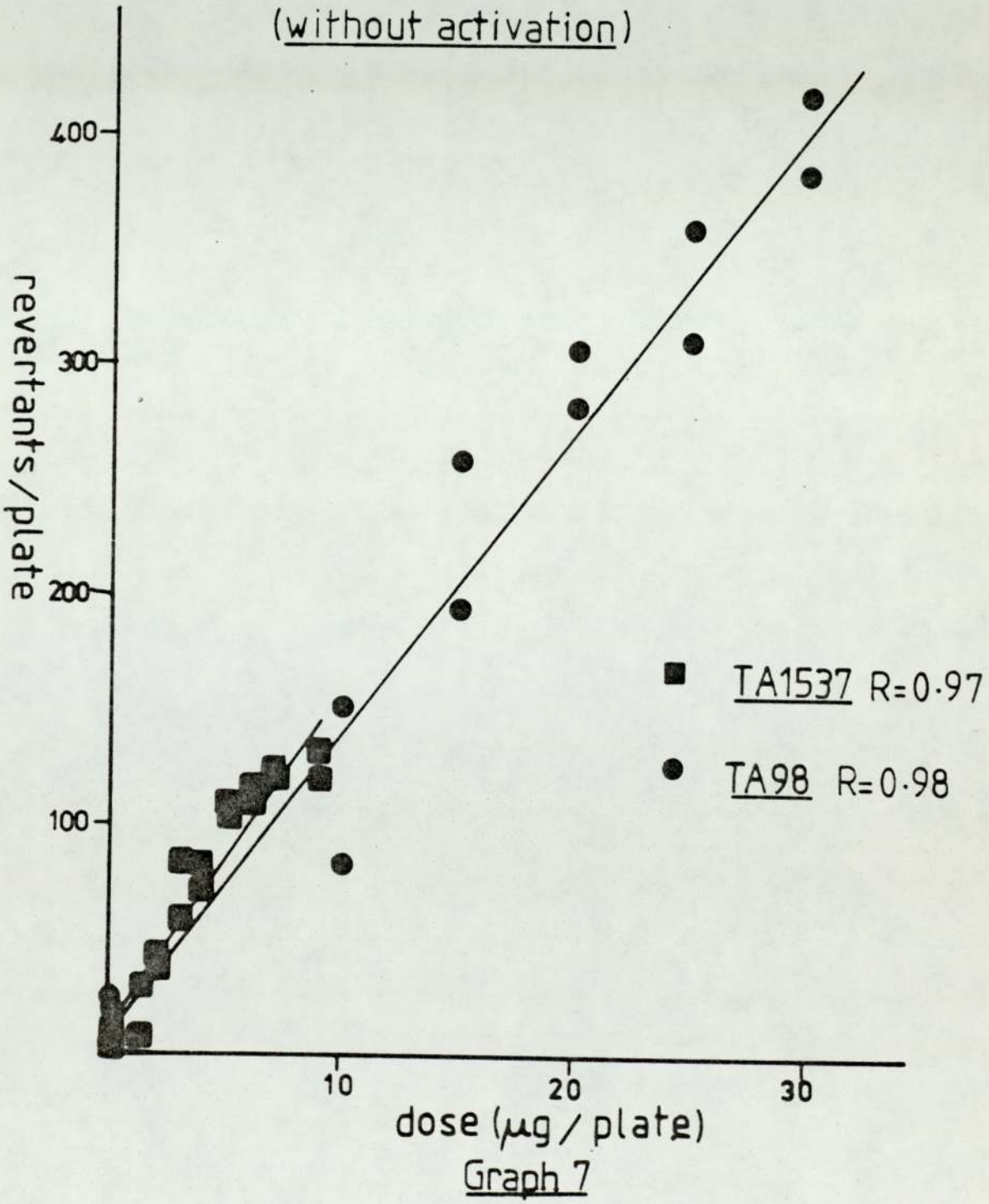
Graph 6

The dose/response of



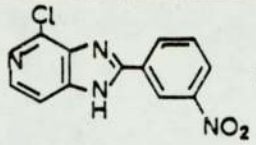
(204)

in TA1537 and TA 98.
(without activation)



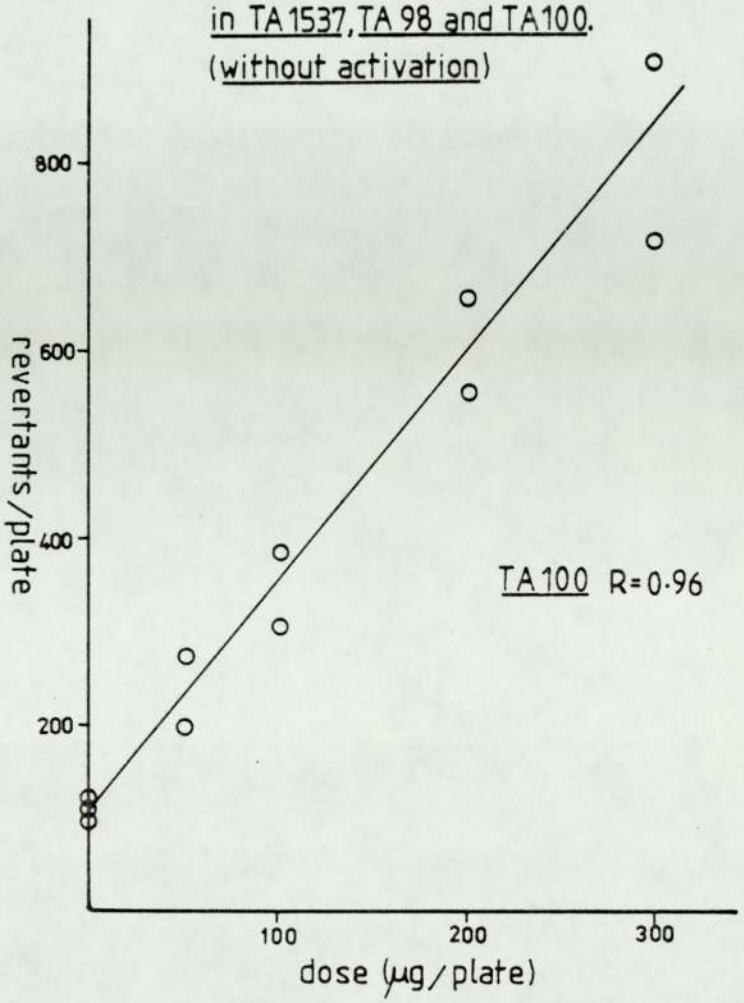
Graph 7

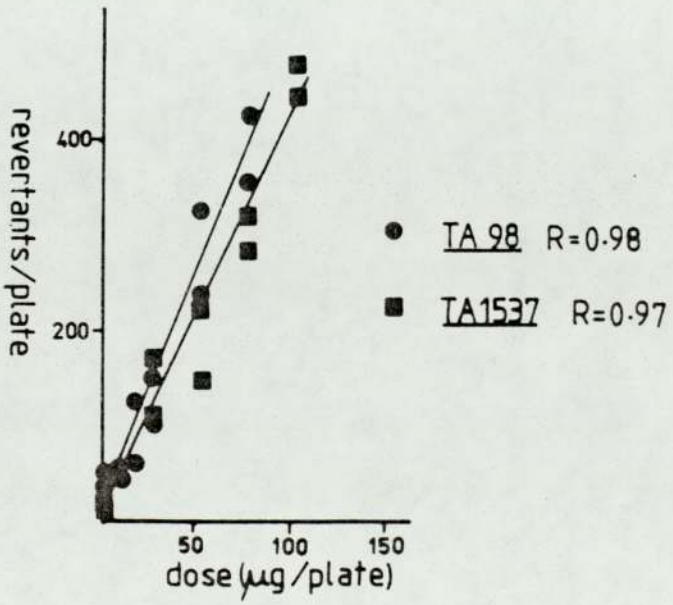
The dose/response of



(203)

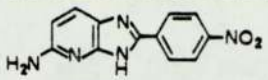
in TA1537, TA 98 and TA100.
(without activation)





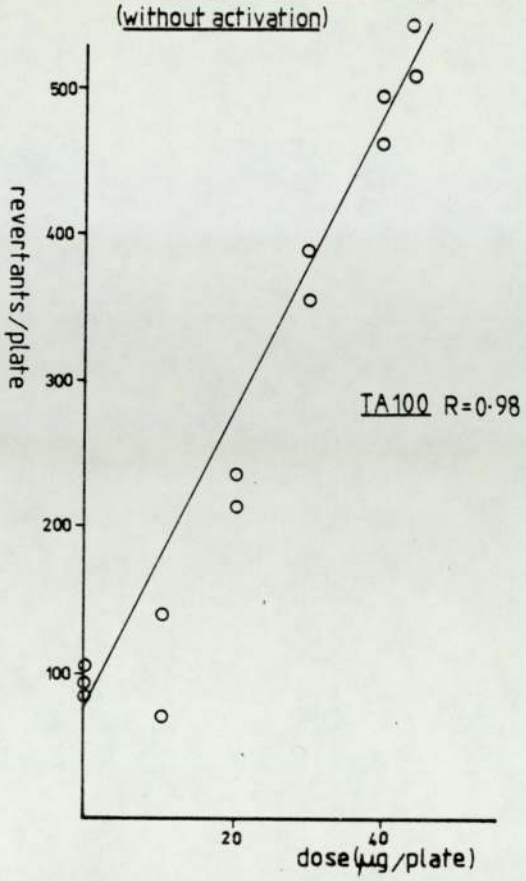
Graph 8

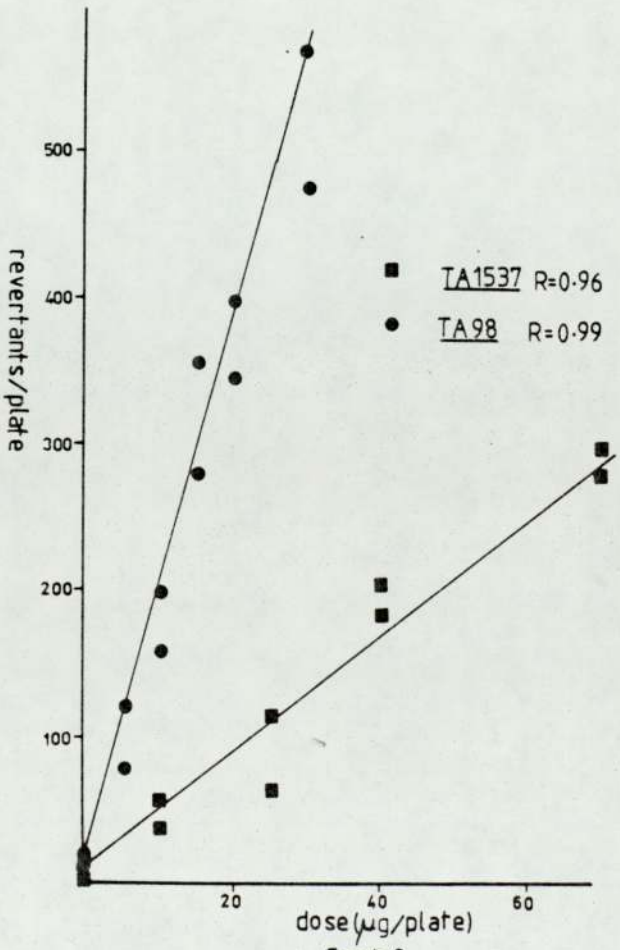
The dose/response of



(193)

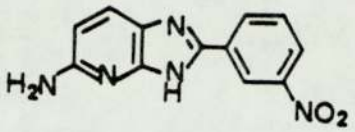
in TA1537, TA 98 and TA 100.
(without activation)





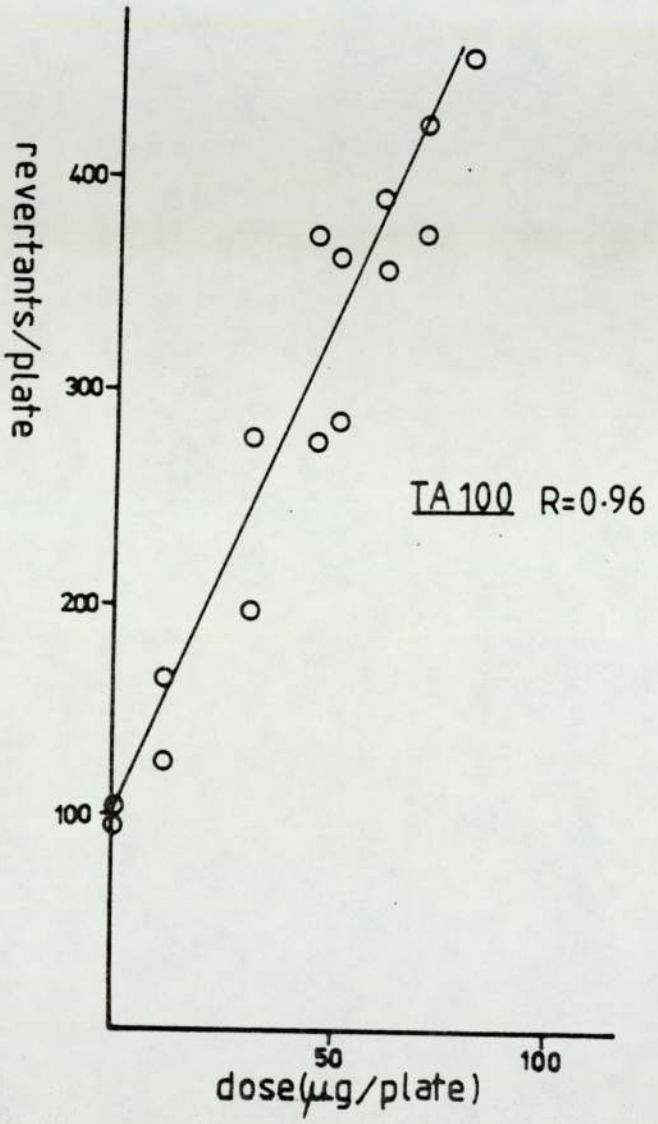
Graph 9

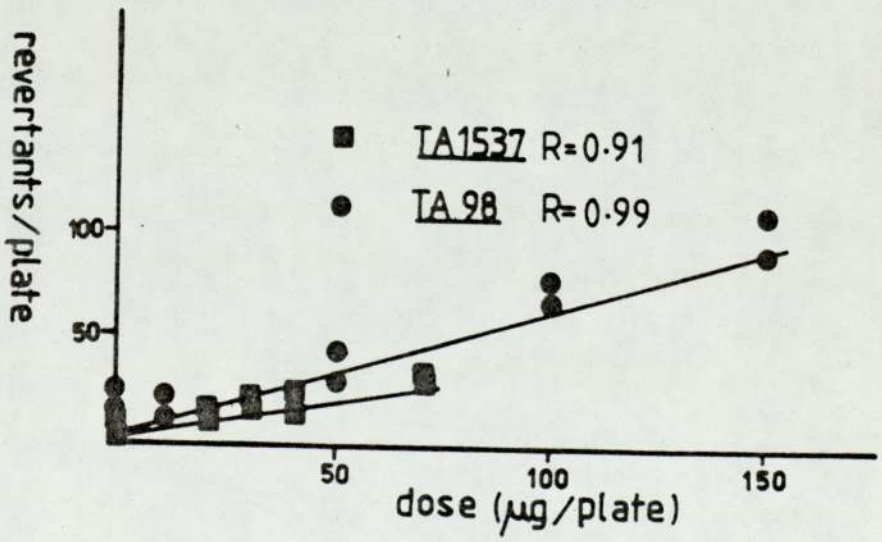
The dose / response of



(196)

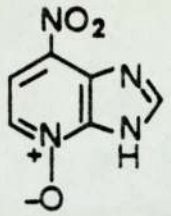
in TA1537, TA 98 and TA 100.
(without activation)





Graph 10

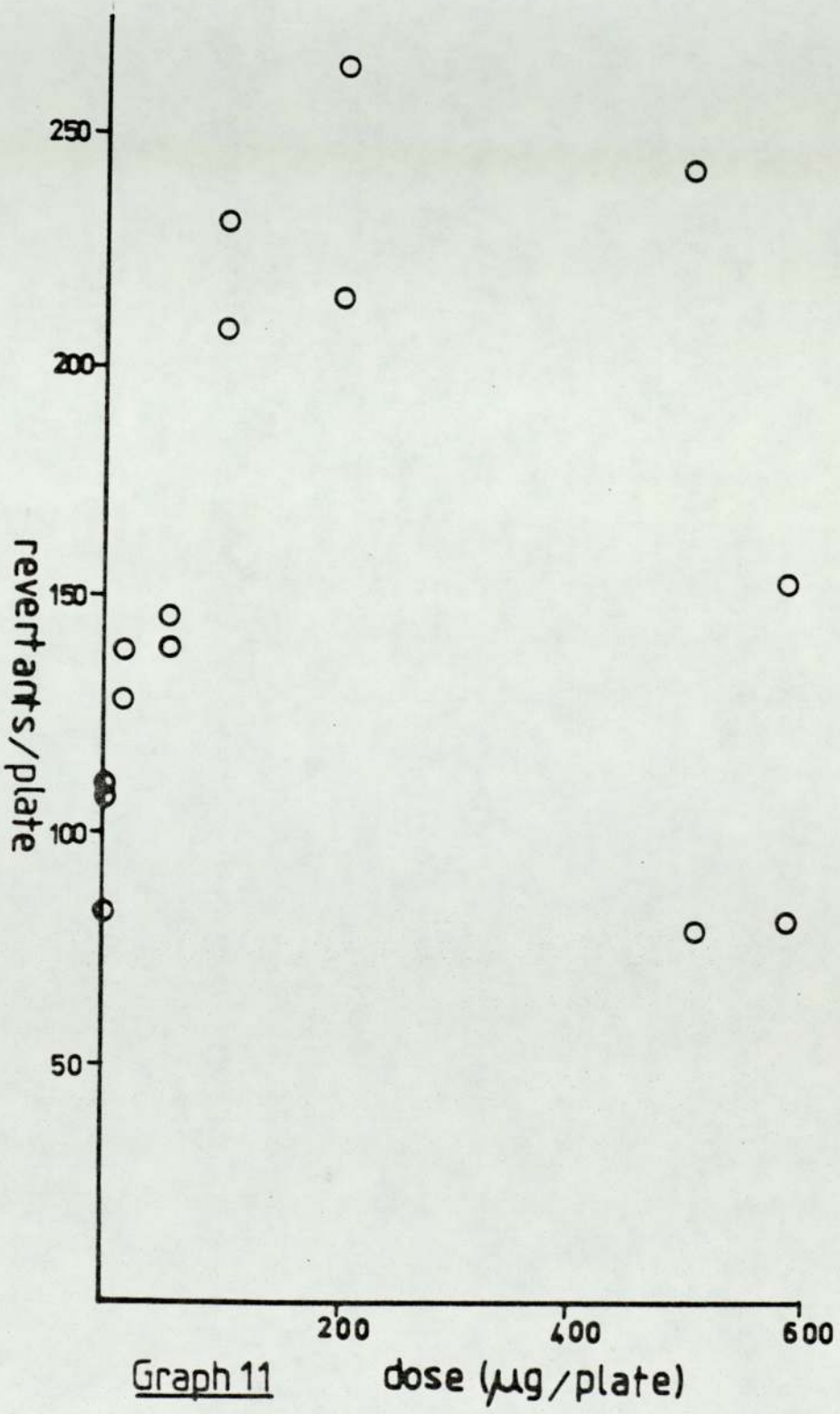
The dose/response of



(173)

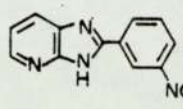
in TA100.

(without activation)



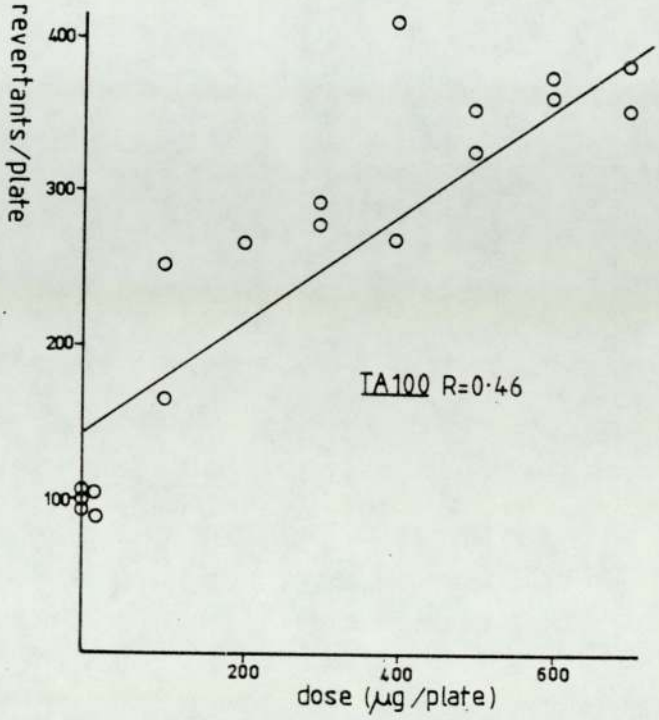
Graph 11

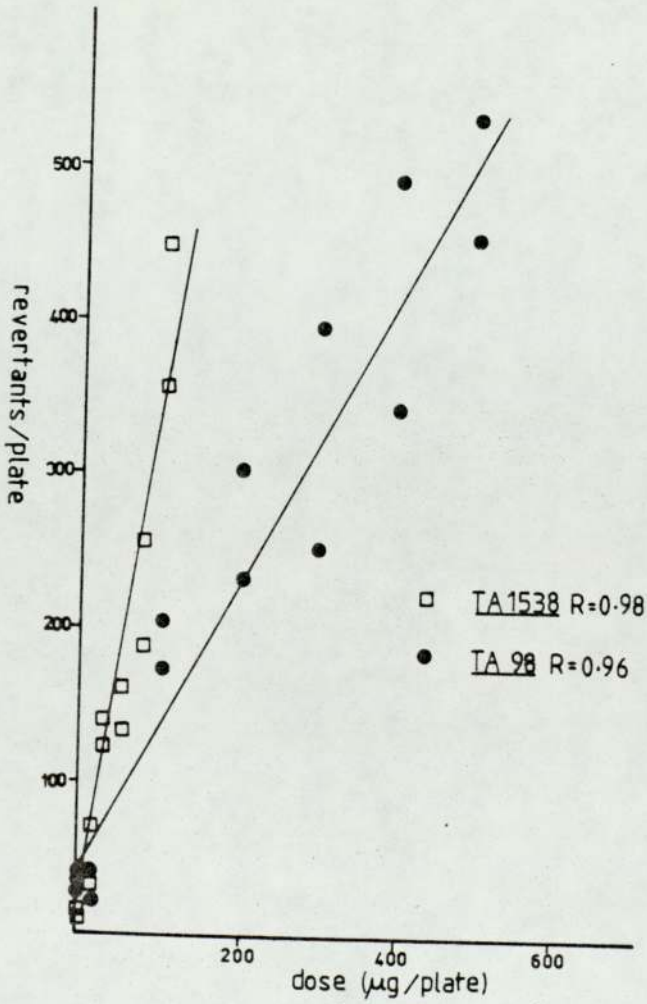
The dose / response of



(166)

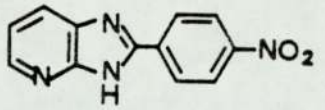
in TA1538, TA 98 and TA100
(without activation)





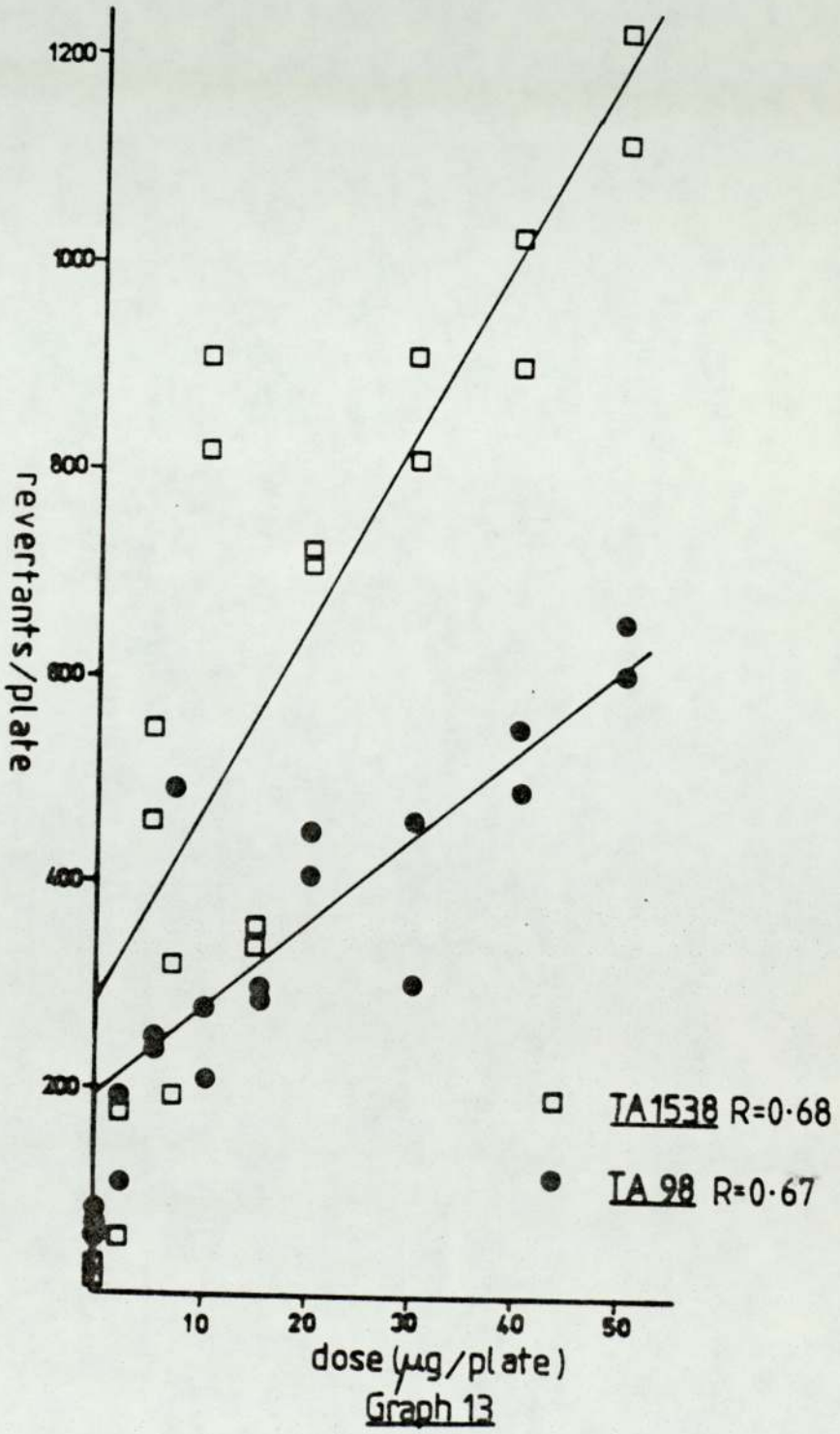
Graph 12

The dose/response of



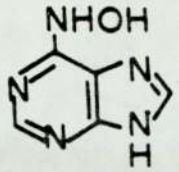
(171)

in TA1538 and TA98.
(without activation)



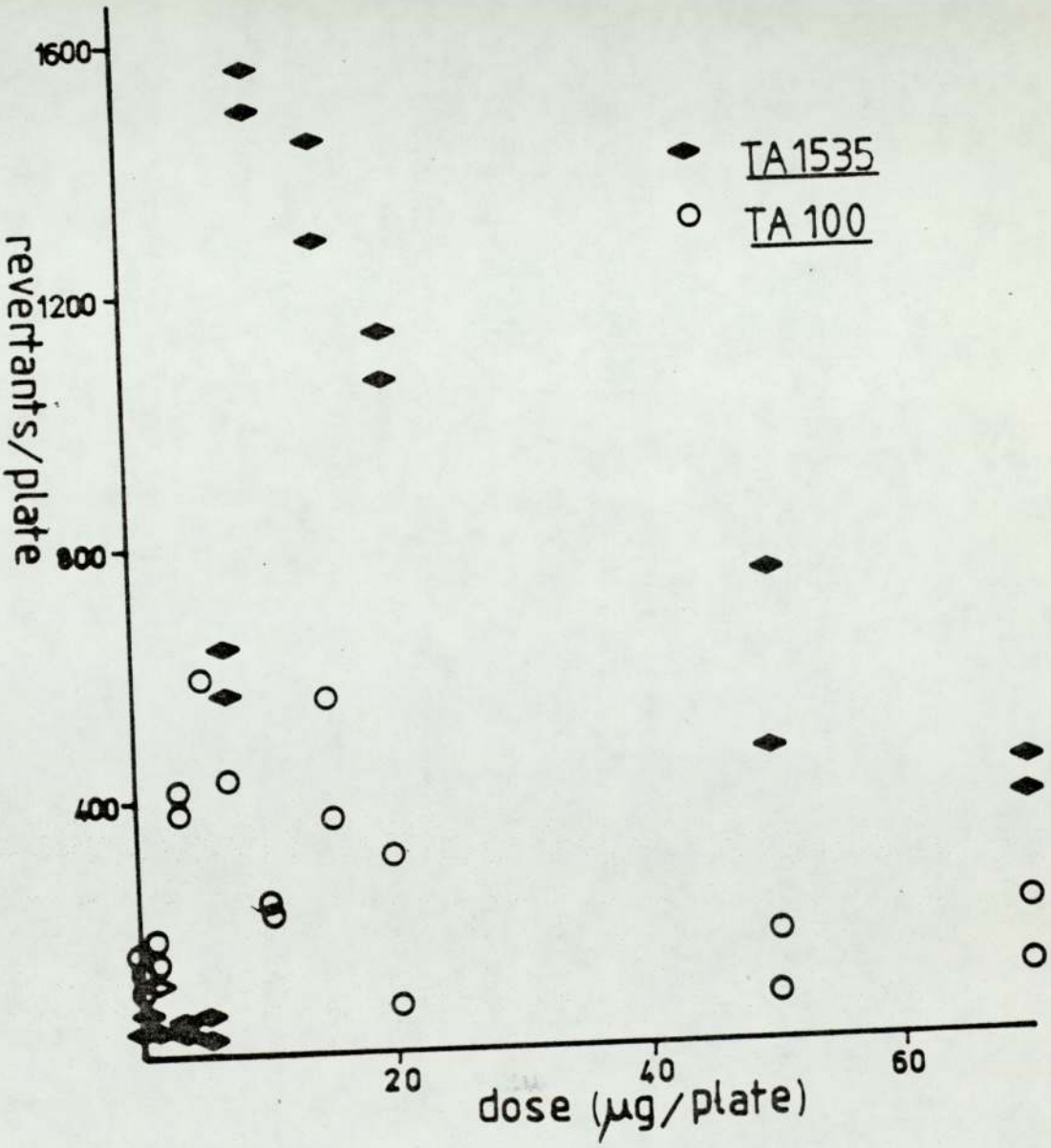
Graph 13

The dose/response of



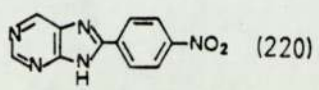
(164)

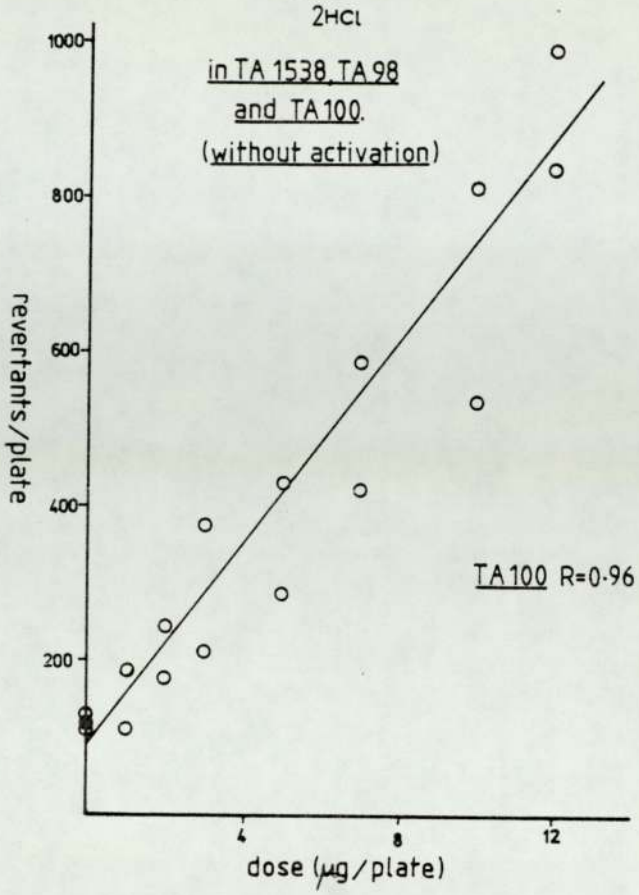
in TA1535 and TA 100.
(without activation)

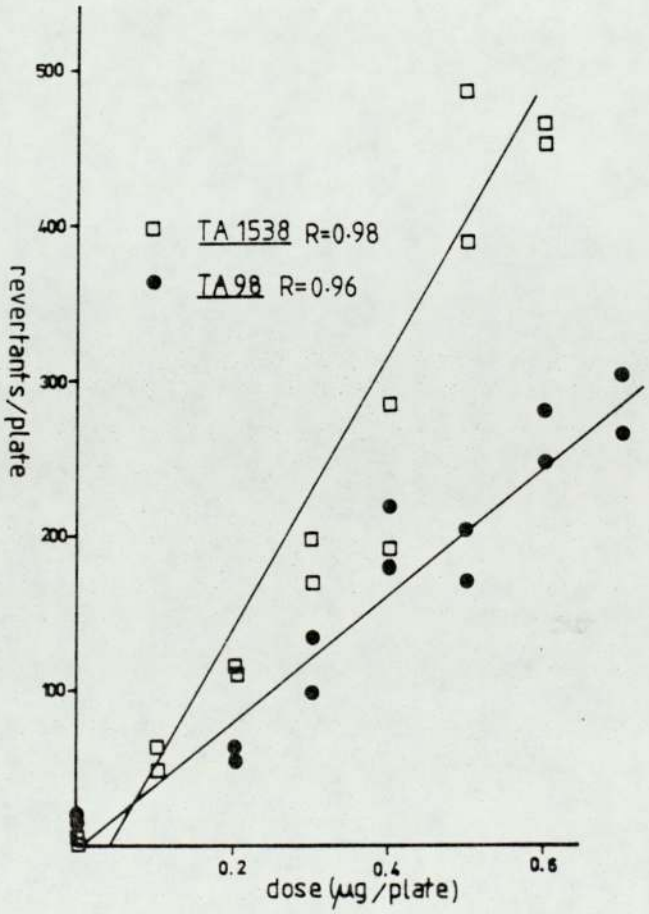


Graph 14

The dose/response of

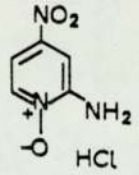






Graph 15

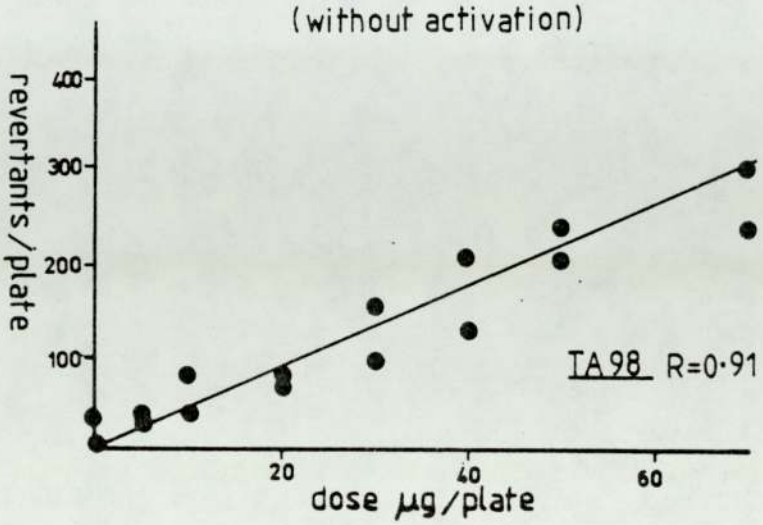
The dose/response of

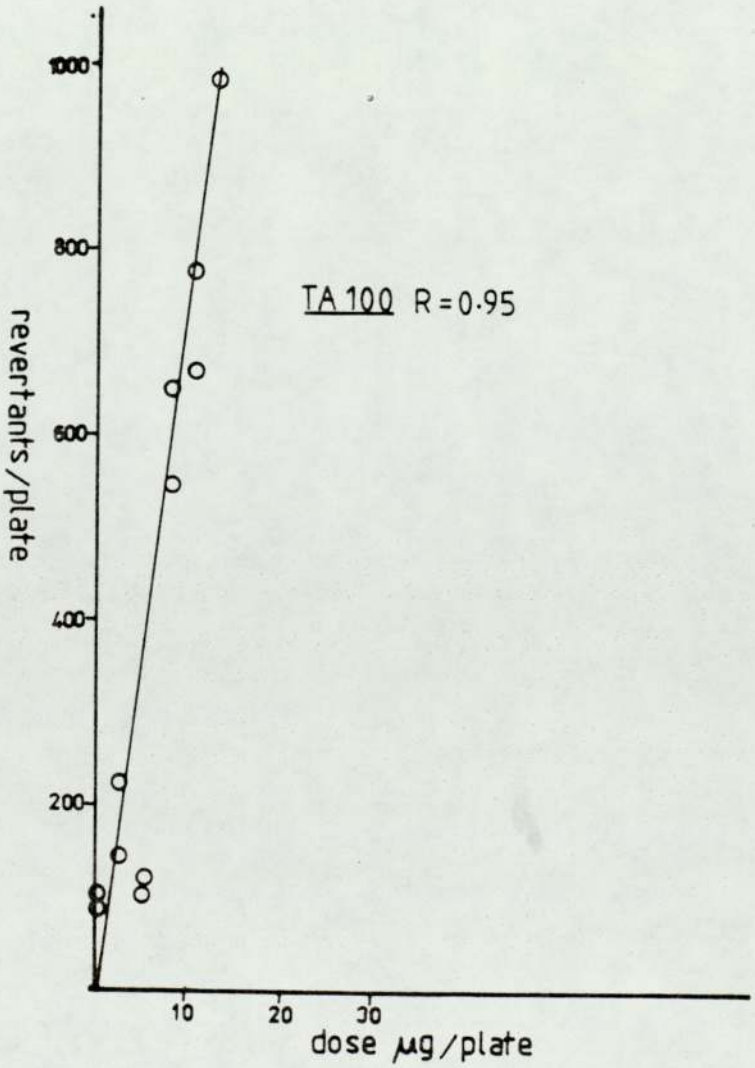


(206)

in TA 98 and TA100.

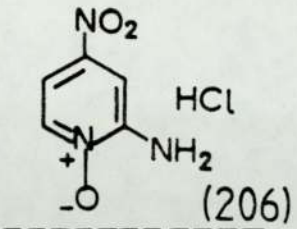
(without activation)



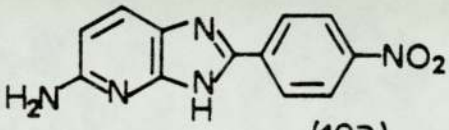


Graph 16

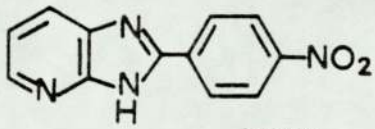
Key to graphs comparing mutagenic activity of several compounds in TA 100, TA1538, TA1537 and TA 98.



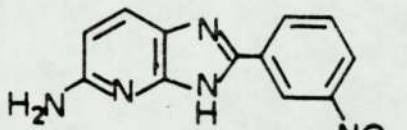
(206)



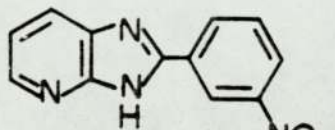
(193)



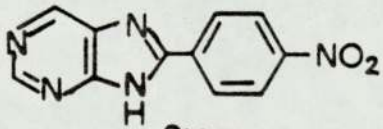
(171)



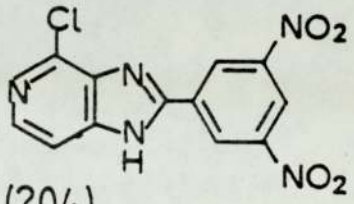
(196)



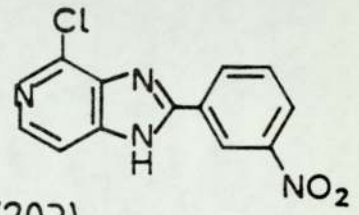
(166)



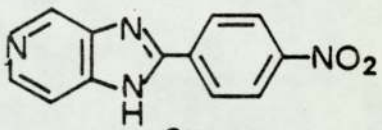
2HCl (220)



(204)

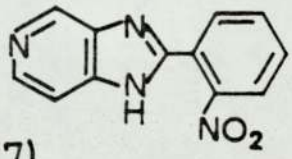


(203)

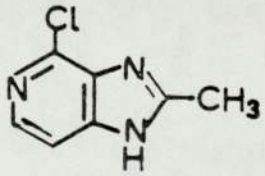


2HCl

(215)



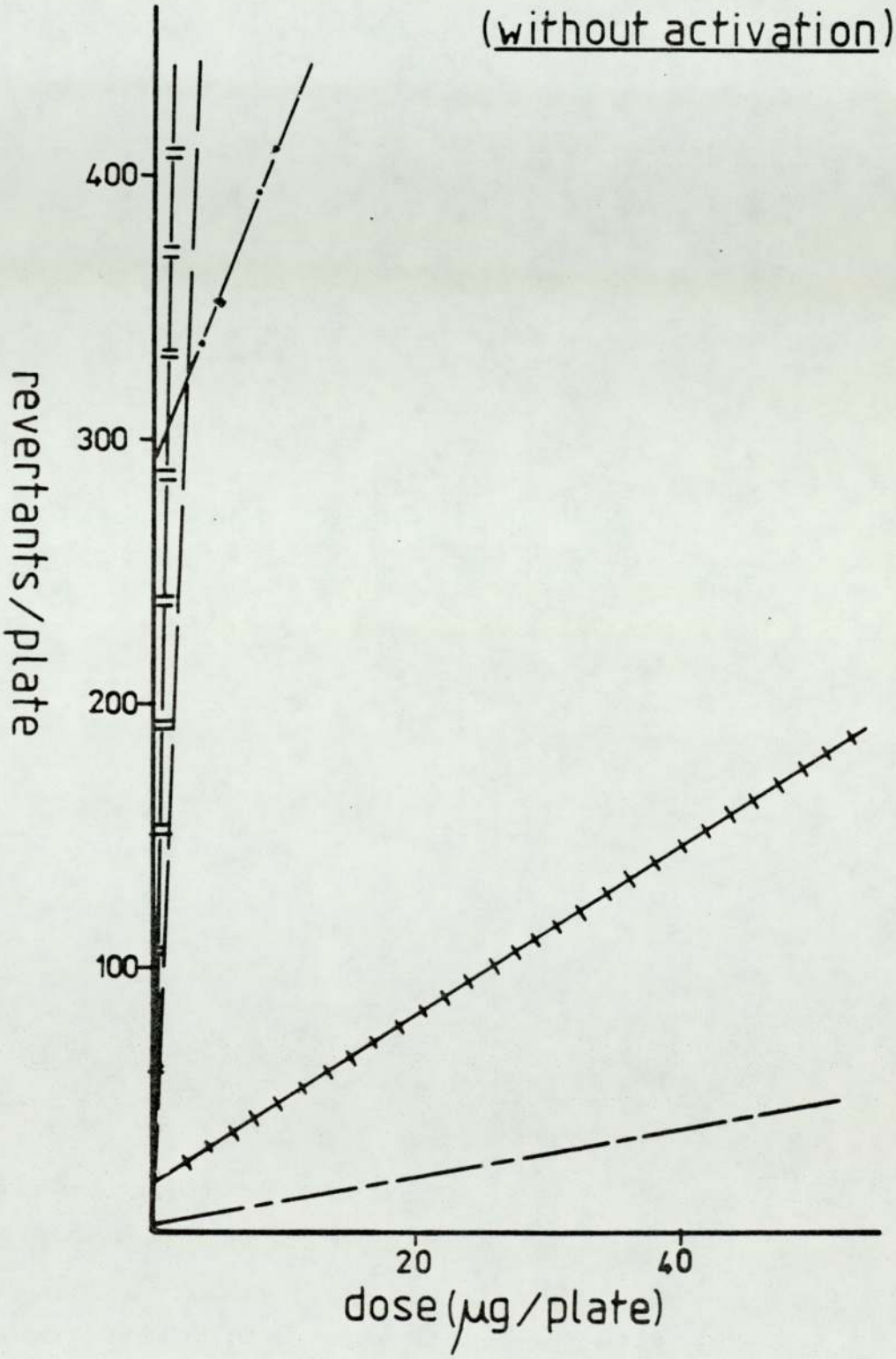
(217)



(211)

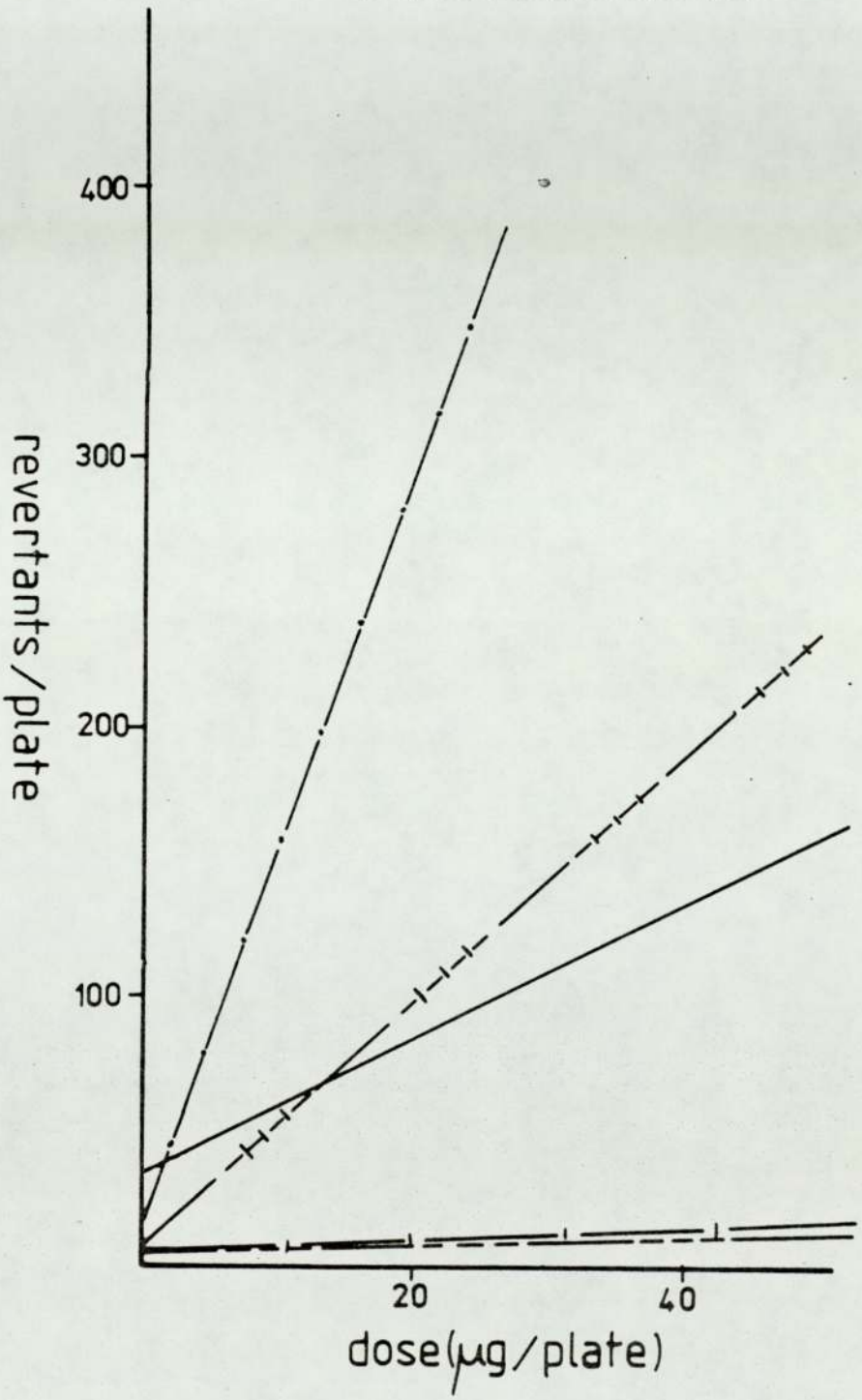
Figure 10

Comparative mutagenic activity in TA 1538. (without activation)



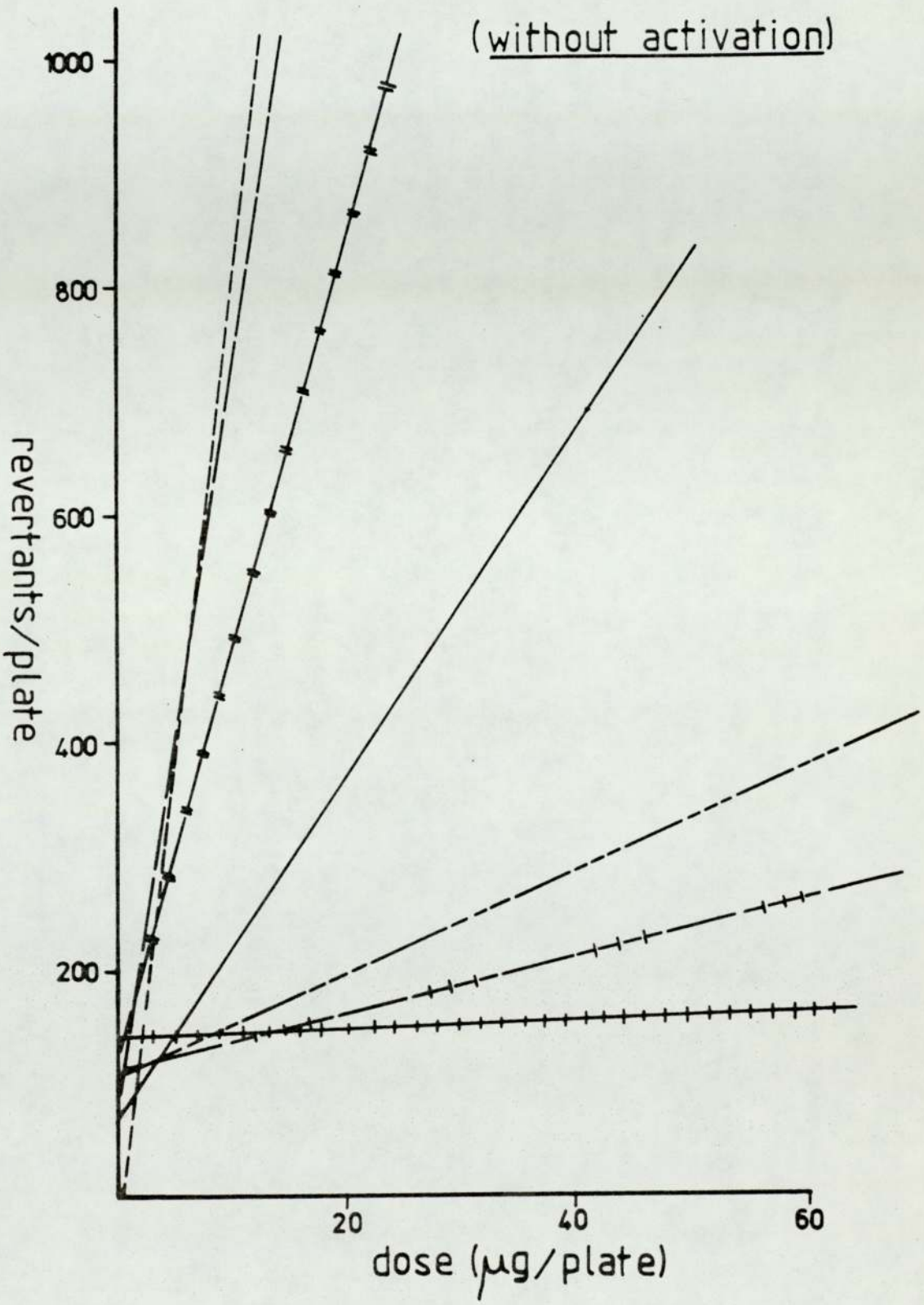
Graph 17

Comparative mutagenic activity in TA 1537.
(without activation)



Graph 18

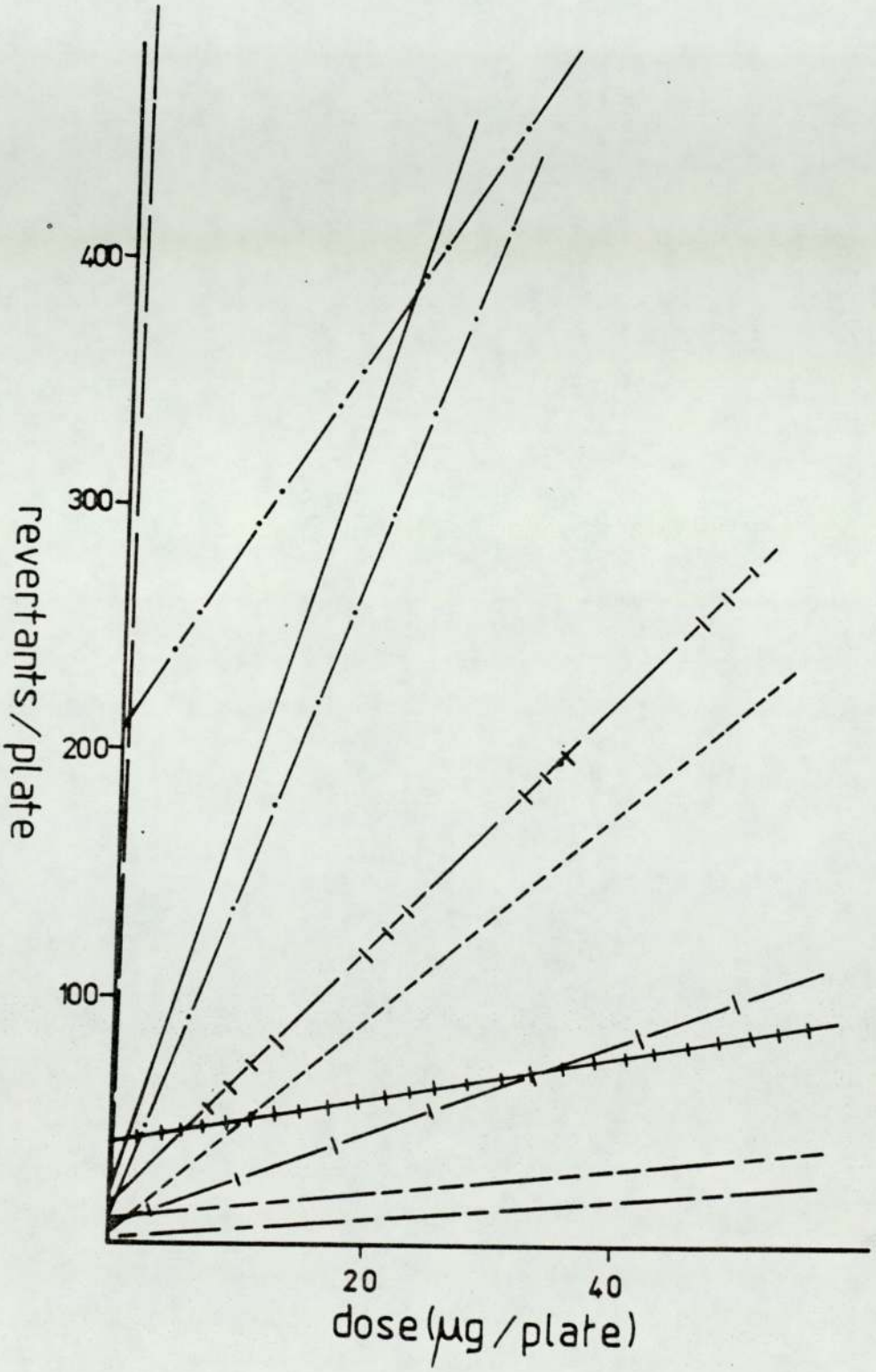
Comparative mutagenic activity
in TA 100.



Graph 19

Comparative mutagenic activity in TA 98.

(without activation)

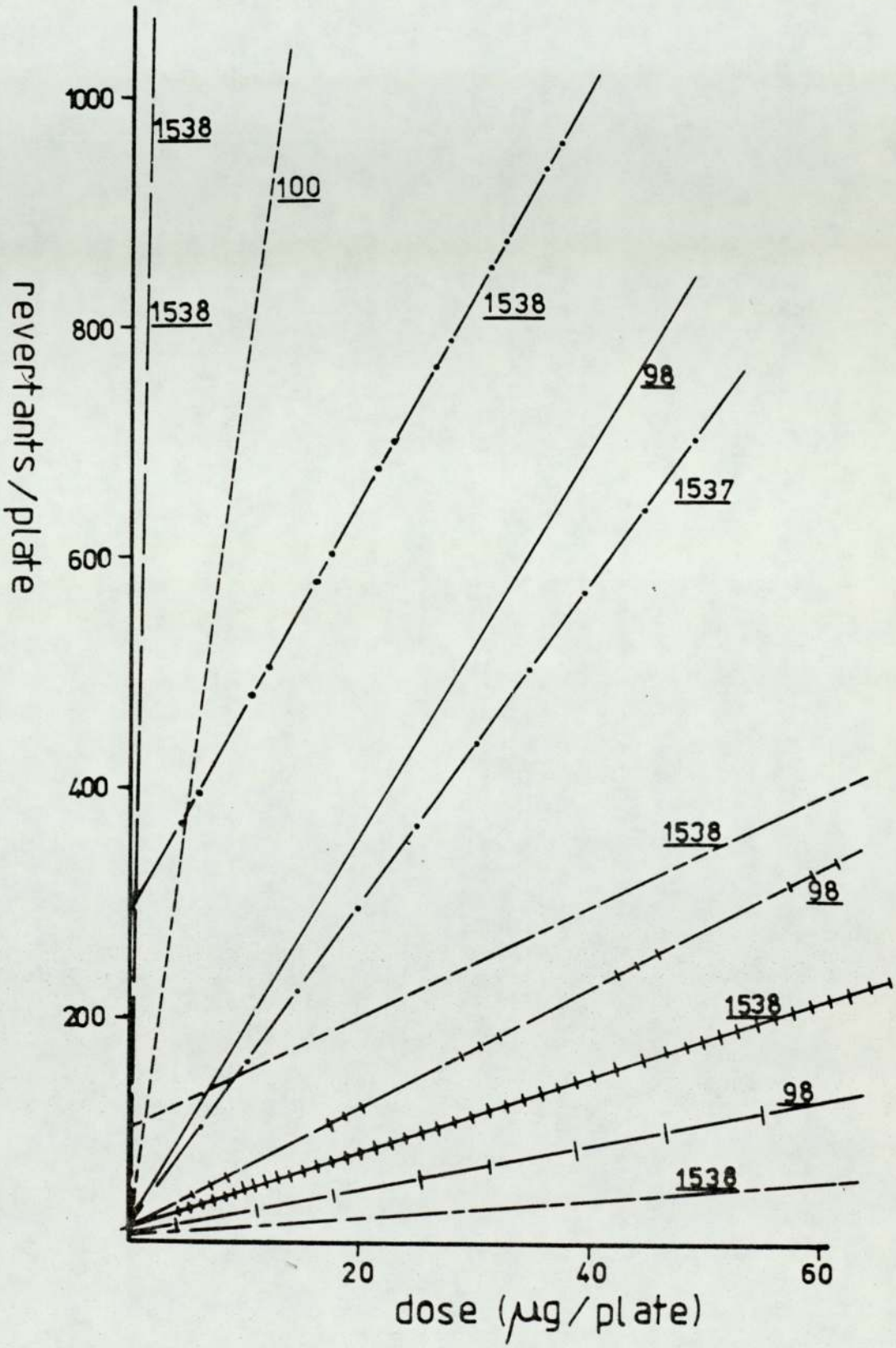


Graph 20

Maximal mutagenic activity

— variable strains.

(without activation)



Graph 21

Table 6

Reversion of Salmonellae mutants by compound 203

	(without activation)			
dose	TA100	TA1535	TA98	TA1537
5 µg	72 94	17 24	36 10	spot test negative
50 µg	98 136	28 18	13 27	
500 µg	113 106	18 21	29 30	
-ve control	110 102 98	18 13 10	30 23 38	

Table 6 (continued)

	(with S-9 activation)			
150 µl of S-9 mix per plate				
5 µg	152 141	25 19	24 20	29 35
50 µg	192 202	33 38	25 26	31 29
500 µg	187 196	36 28	33 35	40 28
100 µl of S-9 mix per plate				
5 µg	143 132	29 27	33 31	33 24
50 µg	243 236	35 43	24 32	34 33
500 µg	193 170	29 39	36 34	35 28
50 µl of S-9 mix per plate				
5 µg	102 106	19 28	25 20	C 26
50 µg	134 107	34 29	18 28	36 35
500 µg	102 80	22 21	27 25	37 31
strain DMSO	109 99 144	19 24 29	22 29 27	10 5 5
	TA100	TA1535	TA98	TA1537

Table 7

Reversion of Salmonellae mutants by compound 104

	(without activation)				
dose	TA1538	TA1537	TA98	TA100	TA1535
10 µg	8 4	7 6	20 41	92 100	71 43
100 µg	6 18	14 C	30 60	102 128	69 91
1000 µg	15 8	6 10	19 34	79 58	72 101
-ve control	18 17 15	9 9 7	57 35 39	134 136 128	30 33 21

Table 7 (continued)

	(with S-9 activation)			
150 µl of S-9 mix per plate				
5 µg	19 C	126 C	30 33	14 15
50 µg	21 27	144 128	24 29	15 13
500 µg	31 34	161 124	35 33	12 16
100 µl of S-9 mix per plate				
5 µg	28 24	87 82	22 28	10 9
50 µg	31 26	210 107	25 22	11 10
500 µg	29 33	110 163	27 29	12 11
50 µl of S-9 mix per plate				
5 µg	21 26	107 85	27 25	6 7
50 µg	23 20	108 93	10 22	7 6
500 µg	32 27	65 76	C 29	9 7
bug DMSO	31 26 24	130 105 124	33 30 21	11 7 6
	TA1535	TA100	TA98	TA1537

Table 8

Reversion of Salmonellae mutants by compound 7

	(without activation)				
dose	TA1535	TA1537	TA1538	TA98	TA100
100 µg	22 28	7 25	7 18	31 25	70 125
1000 µg	31 40	14 12	9 12	38 48	130 133
-ve control	18 18 25	13 11 6	5 11 8	32 31 19	139 120 123

Table 9

Reversion of Salmonellae mutants by compound 163

(without activation

dose	TA98		TA100		Ta1538		TA1537	TA1535
10 µg	22	-	100	-	4	-	-	-
	40	-	119	-	1	-	-	-
100 µg	35	24	78	134	15	14	48	11
	37	30	125	>1000	11	8	9	5
500 µg	-	>1000	-	91	-	13	6	10
	-	C	-	120	-	4	95	7
1000 µg	49	26	126	80	10	120	3	7
	26	38	175	200	18	>1000	2	8
-ve control	38	25	126	146	17	11	10	26
	36	20	102	132	14	8	12	20
	37	24	126	124	13	10	7	26

(without activation)

dose	TA98	TA100	TA1538	TA1537	TA1535
10 µg	38	124	spot test - negative	9	20
	29	108		4	27
50 µg	32	137		13	23
	23	113		11	31
500 µg	24	97		14	22
	14	76		23	17
-ve control	42	139		9	25
	40	120		7	22
	34	123		9	29

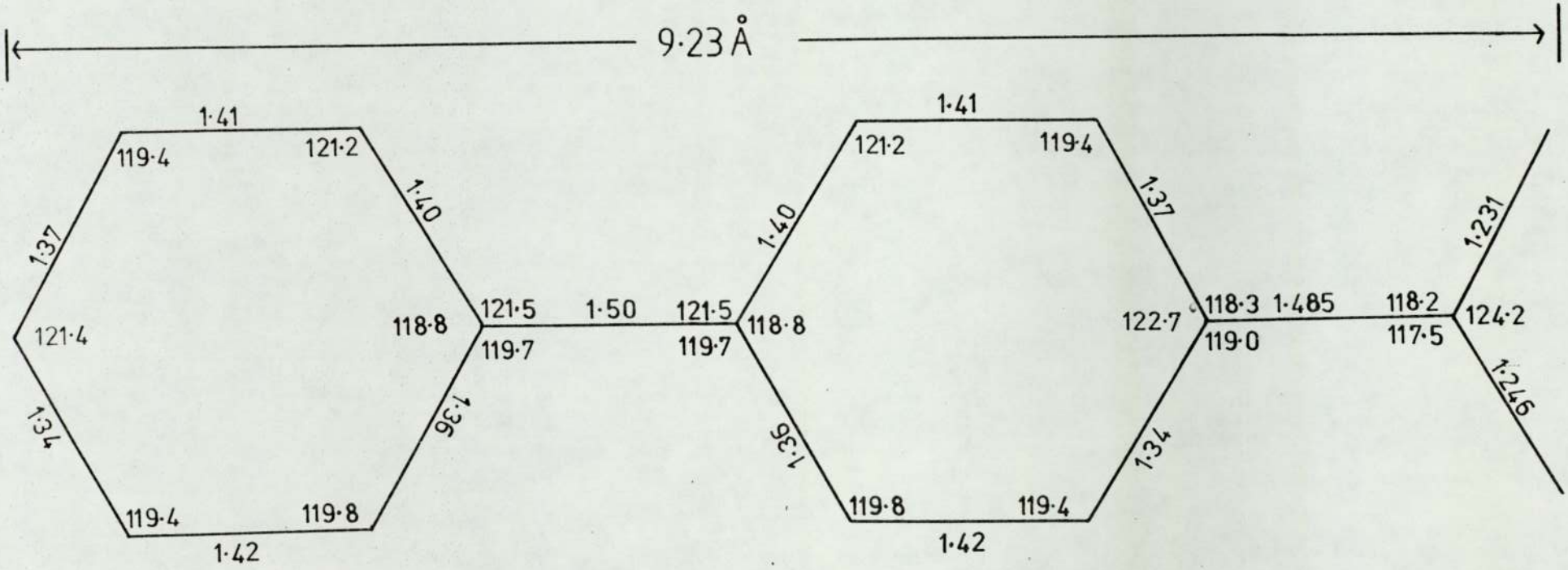
Table 8 (continued)

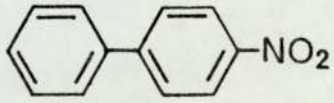
	(with S-9 activation)			
150 µl of S-9 mix per plate				
5 µg	C 123	35 26	40 45	22 10
50 µg	200 159	30 31	C 37	19 21
500 µg	110 157	43 43	44 54	22 25
100 µl of S-9 mix per plate				
5 µg	130 112	30 21	34 36	13 16
50 µg	138 143	29 45	40 35	14 11
500 µg	112 139	39 43	39 49	16 23
50 µl of S-9 mix per plate				
5 µg	123 144	23 29	25 33	4 3
50 µg	147 88	52 41	23 26	4 4
500 µg	97 71	29 36	21 24	3 5
strain DMSO	109 99 144	19 24 29	22 29 27	10 5 5
	TA100	TA1535	TA98	TA1537

Table 9 (continued)

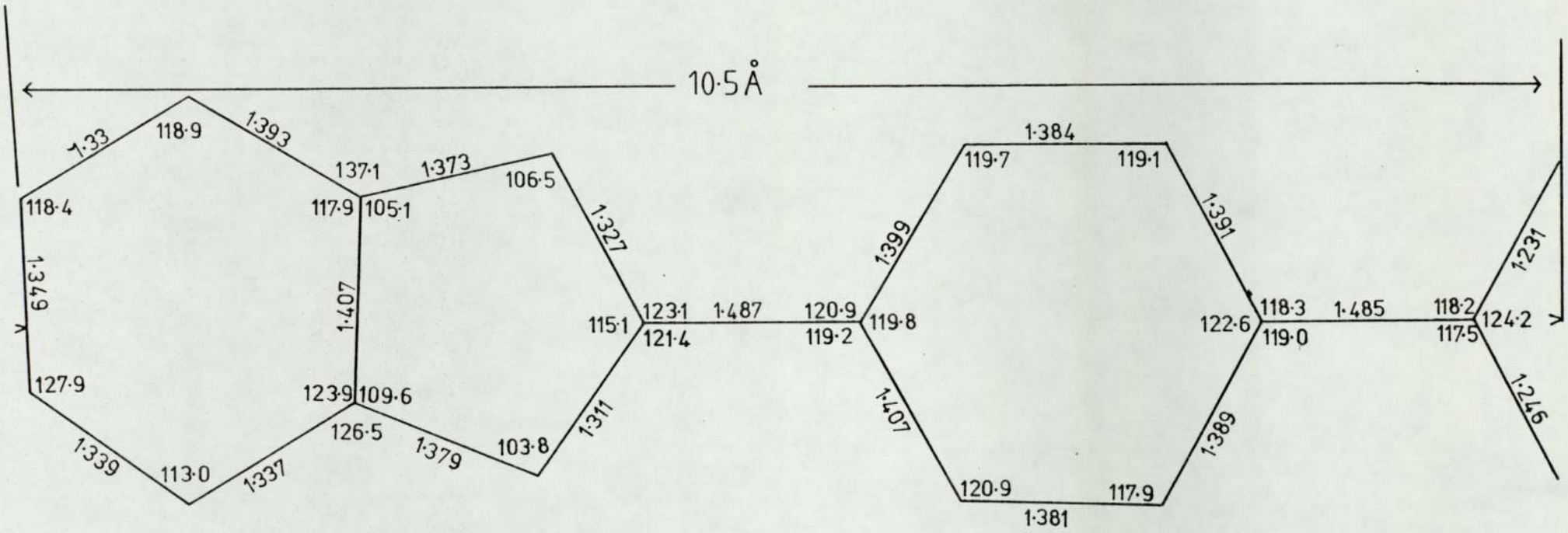
	(with S-9 activation)			
150 µl of S-9 mix per plate				
5 µg	61 34	119 138	40 59	15 C
50 µg	53 30	163 135	55 44	21 18
500 µg	63 41	148 138	56 49	14 20
100 µl of S-9 mix per plate				
5 µg	33 29	155 142	35 25	9 8
50 µg	28 38	C 185	38 41	4 8
500 µg	42 36	112 122	49 40	12 11
50 µl of S-9 mix per plate				
5 µg	33 25	133 98	39 31	8 6
50 µg	31 26	97 87	41 28	9 5
500 µg	19 22	81 116	32 33	6 8
bug DMSO	31 26 24	141 135 128	33 30 21	11 7 6
	TA1535	TA100	TA98	TA1537

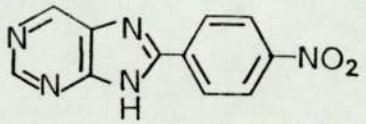
APPENDIX 1 [135,137]





APPENDIX 1 continued [135,136]





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