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Cyclic and Acyclic Modifications

of

5 — Aminoimidazole — 4 — carboxamide

by

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the degree of

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Cyclic and Acyclic Modifications of 5-Aminoimidazole-4-carboxamideSUMMARY

The Introduction gives a brief resumé of the biologically important aspects of 5-aminoimidazole-4-carboxamide (1) and explores, in depth, the synthetic routes to this imidazole. All documented reactions of 5-aminoimidazole-4-carboxamide are reviewed in detail, with particular emphasis on the preparation and subsequent coupling reactions of 5-diazoimidazole-4-carboxamide (6).

A series of thirteen novel 5-amino-2-arylaazoimidazole-4-carboxamide derivatives (117-129) were prepared by the coupling of aryldiazonium salts with 5-aminoimidazole-4-carboxamide. Chemical modification of these azo-dyes resulted in the preparation of eight previously unknown acyl derivatives (136-143). Interaction of 5-amino-2-arylaazoimidazole-4-carboxamides with ethyl formate in sodium ethoxide effected pyrimidine ring closure to the novel 8-arylaazohypoxanthines (144 and 145).

Several reductive techniques were employed in an effort to obtain the elusive 2,5-diaminoimidazole-4-carboxamide (71), a candidate chemotherapeutic agent, from the arylaazoimidazoles. No success can be reported although 5-amino-2-(3-aminoimidazol-2-yl)imidazole-4-carboxamide (151) was isolated due to a partial reduction and intramolecular cyclisation of 5-amino-2-(2-cyanophenylazo)imidazole-4-carboxamide (122). Further possible synthetic approaches to the diaminoimidazole are discussed in Chapter 4.

An interesting degradation of a known unstable nitrohydrazone is described in Chapter 5. This resulted in formation of 1,1-bis(pyrazol-3-ylazo)-1-nitroethane (164) instead of the expected cyclisation to a bicyclic tetrazine N-oxide.

An improved preparation of 5-diazoimidazole-4-carboxamide has been achieved, and the diazoazole formed cycloadducts with isocyanates to yield the hitherto unknown imidazo[5,1-d][1,2,3,5]tetrazin-7(6H)-ones. Eleven derivatives (167-177) of this new ring-system were prepared and characterised. Chemical and spectroscopic investigation showed this ring-system to be unstable under certain conditions, and a comparative study of stability within the group has been made.

"Retro-cycloaddition" under protic and photolytic conditions was an unexpected property of 6-substituted imidazo[5,1-d][1,2,3,5]tetrazin-7(6H)-ones. Selected examples of the imidazotetrazinone ring-system were tested for antitumour activity. The results of biological evaluation are given in Chapter 7, and have culminated in a Patent application by the collaborating body, May and Baker Ltd. One compound, 3-carbamoyl-6-(2-chloroethyl)imidazo[5,1-d][1,2,3,5]tetrazin-7(6H)-one (175), shows striking antitumour activity in rodent test systems.

Submitted by Robert Stone for the Degree of Doctor of Philosophy, 1981.

Key words: 5-Aminoimidazole-4-carboxamide ; Antitumour ;
Cycloaddition ; 5-Diazoimidazole-4-carboxamide ;
Imidazo[5,1-d][1,2,3,5]tetrazin-7(6H)-one.

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To my wife and parents

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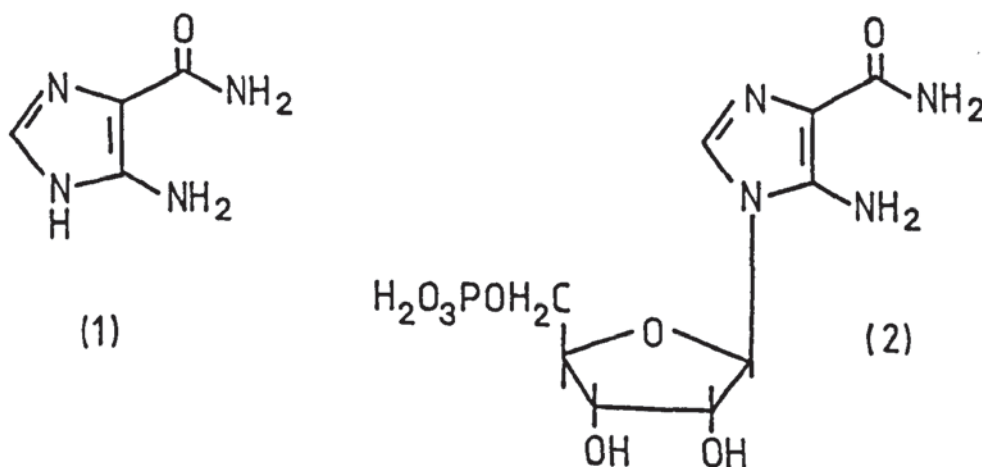
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Introduction

In the early 1940's the mechanism of antibacterial activity demonstrated by sulphonamides was intensively investigated, and an amine was found to accumulate in bacterial cultures^{1,2} when bacteriostatic concentrations of these drugs were present. Although isolated by Statton and Fox¹ in 1945, the structure of the amine was not correctly assigned until two years later, when Shive and co-workers³ showed it to be 5-aminoimidazole-4-carboxamide* (1, AIC). This imidazole, which had first been synthesised some twenty four years earlier by Windaus and Langenbeck,⁴ now became of considerable interest to both chemists and biochemists. It was generally believed that this amine was one of the

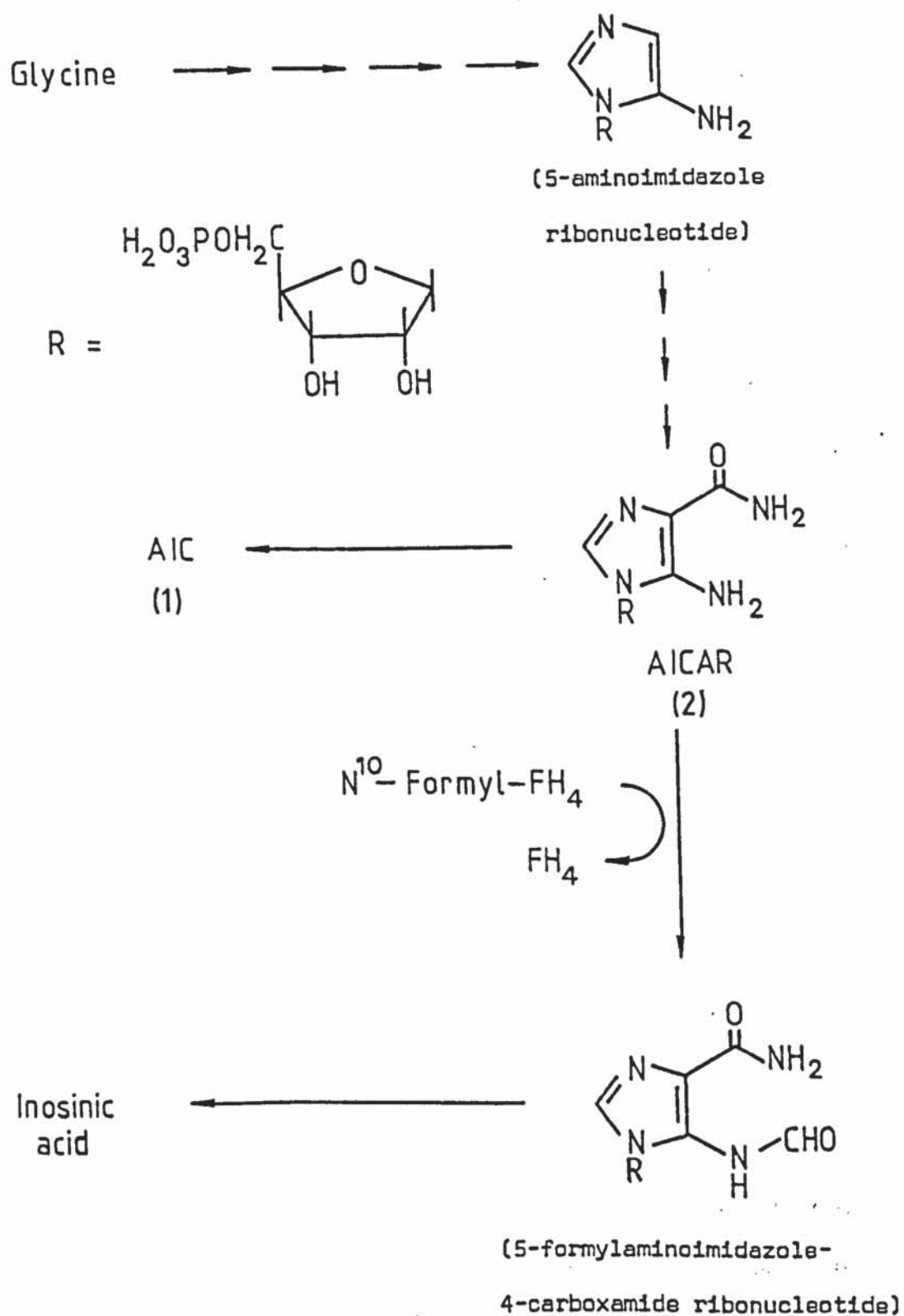


*When the nitrogen atom of the imidazole ring has no exocyclic substituent then there are two possible tautomeric forms. Therefore, compound (1) may be written as 5(4)-aminoimidazole-4(5)-carboxamide. However, if there is an exocyclic substituent then this ring nitrogen must be assigned as position 1, and this would give rise to specific isomeric forms. Throughout this work the more recent nomenclature, i.e. that which excludes alternative numbering in parentheses, has been adopted. The letter designation "AIC" for compound (1) has also been used extensively, and is employed here. It should be noted that "AICA" is also commonly used in the literature as an abbreviation for this compound. "AICAR" designates the ribotide of AIC (2).

precursors of purine bases, and was now available from both synthetic and biosynthetic routes.

However, it was soon shown that AIC did not play a direct role^{5,6} in the purine biosynthetic pathways, but that its ribonucleotide (2, AICAR) was of importance. Subsequently this molecule was detected, and isolated⁷, from the bacterial culture media. The central role of this ribonucleotide in *de novo* purine biosynthesis is summarised in Scheme 1.1, which has been adapted from the literature.⁸⁻¹⁰ The sulphonamides act as antimetabolites of 4-aminobenzoic acid and their action in bacterial cells, and other cells which require this acid for metabolism, precludes the synthesis of folic acid. This in turn prevents the donation of a single carbon unit by N¹⁰-formyltetrahydrofolic acid (N¹⁰-formyl-FH₄) to 5-aminoimidazole-4-carboxamide ribonucleotide (2, AICAR). Thus this purine precursor accumulates, and hydrolysis gives the free base (1) and a nucleoside.

The occurrence of AIC in human urine was first reported by Vilenkina¹¹ and later, values for its excretion levels were published.^{8,12,13} Several methods for the detection of AIC in urine have been developed;^{8,13,14} the latter reference describes a procedure also applicable for blood determinations. These show the level of AIC excreted to be constant and within the range of 0.6 - 1.5 mg/day for adults. It has been suggested that this level may reflect the level of purine synthesis in the body.⁸ Indeed it has been demonstrated that surgical stress increases excretion of AIC,¹³ while administration of 1,3-bis(2-chloroethyl)-1-nitrosourea, which is thought to have an inhibitory effect on purine biosynthesis, causes a decrease in AIC excretion.¹⁵ It is, therefore, not surprising that the pharmacological activity of AIC has been extensively researched. This work can be divided into three major areas: liver involvement, general enzyme effects, and finally cancer chemotherapy.



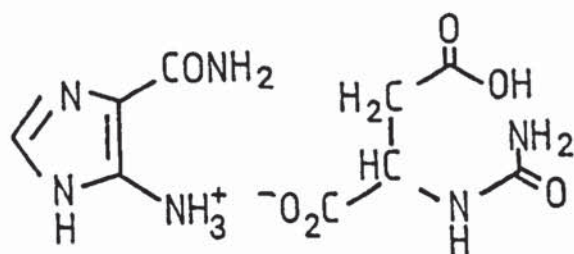
Scheme 1.1

1.1.1 Treatment of liver necrosis.

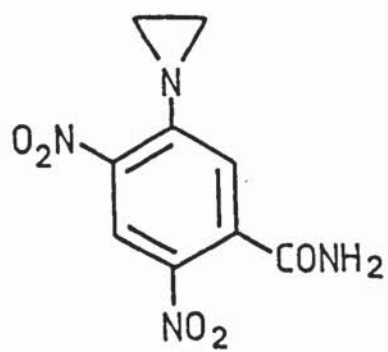
The beneficial effects of AIC to counter liver cell necrosis due to carbon tetrachloride toxicity were noted,^{16,17} and more recent studies have shown the amine to be beneficial in protecting against carbon tetrachloride induced acute, sub-acute and chronic liver damage.¹⁸ The orotate salt of AIC¹⁹ was also shown to have this protective effect as well as displaying an ability to rectify "fatty liver" in rats which had been fed a protein-deficient diet or amino acid imbalance.²⁰ Ethionine was found to deplete the liver of purine nucleotides and increase total lipid content. Both effects were reversed by simultaneous or subsequent administration of AIC.²¹ Recently Carminati²² has shown AIC ureidosuccinate (3) to confer protection against experimental hepatic steatoses induced by ethionine, galactosamine, thioacetamide or carbon tetrachloride. It is claimed that stimulation of liver regeneration is also observed. This activity is explained by the drug supplying precursors for the synthesis of purine and pyrimidine nucleotides, and thereby stimulating cellular regeneration.

1.1.2 Enzyme studies.

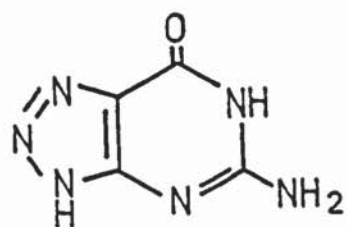
There has been a great deal of interest shown in the interaction of AIC with various enzyme systems,²³⁻²⁵ both as a substrate and as an inhibitor. It was postulated that the presence of this amine could be indicative of folate deficiency in humans.²⁶ It has been claimed that an increase in AIC urinary excretion is associated with folic acid deficiency and (or) vitamin B₁₂ deficiency in both experimental animals, and man.²⁷⁻³⁰ However, Harrison *et al*³¹ found no evidence to suggest such a relationship, and quite correctly pointed out that folic acid co-enzymes are important at two sites of the purine biosynthetic pathway. In which case no increase in AIC excretion would be expected.



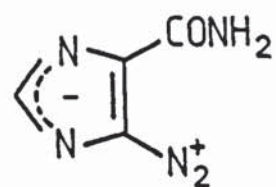
(3)



(5)



(4)



(6)

Inhibition of the enzyme responsible for deamination of 8-azaguanine (4) to the relatively non-toxic 8-azaxanthine by AIC has received much attention.³² Potentiation of the carcinostatic effect of 8-azaguanine, which results from this enzyme inhibition, has been demonstrated both *in vitro* and *in vivo*.^{33,34} Subsequently, structure-activity studies have shown that a proton on the ring nitrogen, and the oxygen atom of the 4-carboxamide group are necessary for activity.^{35,36}

1.1.3 Cancer chemotherapy.

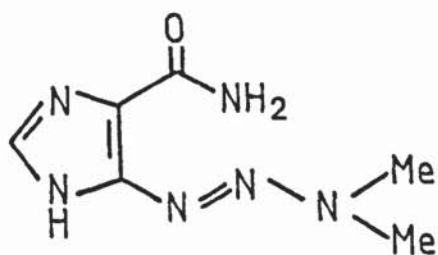
It is with respect to antitumour studies involving AIC that the majority of the literature is devoted. Relatively, only a small fraction of the published work is concerned with possible direct effects of AIC in this area. During the 1950's it was still thought that AIC was a purine precursor, and studies were designed to show incorporation of ¹⁴C labelled AIC into purines. Both *in vitro* and *in vivo* experiments seemed to indicate this to be the case.³⁷⁻³⁹ Several workers, using similar techniques, investigated the distribution of AIC in various tumour systems, and radiolabelled ¹⁴C-AIC was found to be incorporated to a greater extent in tumour bearing mouse livers.⁴⁰ Later studies have also shown tumours to have increased utilisation of this imidazole.⁴¹⁻⁴³ Bennett⁴⁴ found ¹⁴C-guanine incorporation into polynucleotides of sarcomas and adenocarcinomas in rodents to be stimulated by AIC; and Beal,⁴⁵ working with Novikoff hepatoma cells in culture, has noted that both DNA and RNA synthesis were enhanced by the addition of AIC.

The monofunctional alkylating agent CB 1954, 5-(1-aziridinyl)-2,4-dinitrobenzamide (5), is a potent, selective inhibitor of Walker tumour. AIC will protect against the selective effects of this agent upon DNA synthesis.^{46,47} However, this has not been found to be the case with melphalan,⁴⁸ a difunctional alkylating agent.

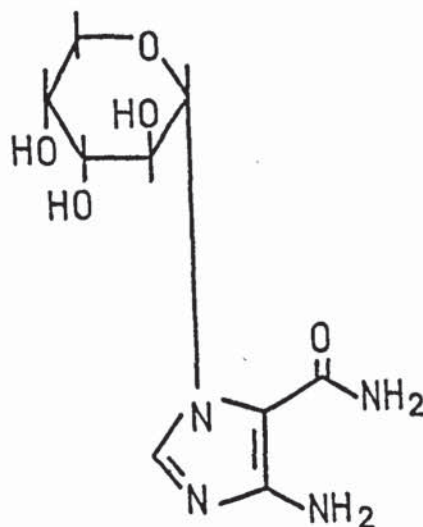
Although AIC does not possess antitumour activity of its own, there have been many attempts to synthesise compounds which may prove to be antimetabolites for this biologically important base. This line of thought has been pursued because of the previously mentioned work which shows that AIC is taken up by both tumour, and healthy tissue, and can be utilised, *via* salvage pathways,⁴⁹ in the cell. It was Shealy⁵⁰ who showed that 5-diazoimidazole-4-carboxamide (DIAZO-IC, 6) was the product of diazotisation of AIC. This internally-stabilised diazoimidazole was shown to inhibit the growth of human epidermoid carcinoma (H.Ep-2) cells *in vitro*, the Ehrlich ascites carcinoma in mice and Walker 256 carcinoma in rats. Further information on the biological activity of DIAZO-IC can be found in a review by Stevens.⁵¹

Because of the chemical instability of DIAZO-IC many derivatives have been prepared and the triazenes, formed by coupling with amines,⁵² proved to be a particularly fruitful area. The dimethyl-triazene, 5-(3,3-dimethyltriazene-1-yl)imidazole-4-carboxamide, (DTIC, Dacarbazine[®], 7) is used clinically in malignant melanoma,⁵³ and shows activity against several tumour systems. Initially it was thought that this compound acted as a "carrier" of the active DIAZO-IC.^{43,52} Other studies showed AIC to be the major metabolite of DTIC⁵⁴⁻⁵⁶ and *in vitro* studies confirmed an enhanced antitumour effect against Chinese hamster ovary cells and malignant human melanoma cells, when the drug was exposed to light.⁵⁷⁻⁵⁹ These conditions are known to continuously generate DIAZO-IC.⁶⁰

However, a correlation could not be demonstrated between rate of formation of DIAZO-IC, from a group of dialkyltriazenoimidazoles, and antitumour effect.⁵² This information, coupled with the fact that with ¹⁴C-2 labelled DTIC radioactivity is incorporated into RNA and DNA even



(7)



(8)

in the dark,⁵⁷ strongly suggests that the activity of DTIC is not entirely due to the release of Diazo-IC. Although the mode of action of DTIC is still not confirmed it is currently thought to be linked with an N-demethylation activation pathway.^{61,62}

A sugar derivative of AIC (4-amino-1- α -D-arabinopyranosyl-imidazole-5-carboxamide, 8) has been shown to inhibit growth of Ehrlich acites carcinoma in mice:⁶³ several other sugar derivatives which were also prepared, showed no activity.

Antitumour compounds can generally be placed into one of several major categories - the alkylating agents, antibiotics, antimetabolites, hormonal agents, mitotic inhibitors and a group of miscellaneous natural products and synthetic compounds. We can now assign many of our clinically important drugs to one of these groups. However Dacarbazine[®], the injectable form of DTIC, must still be considered as a random synthetic,⁶⁴ although it clearly seems to have been conceived as an antimetabolite or

purine antagonist. When approaching the design of synthetic antitumour agents it is advantageous to have a biochemical target which is specific for one type, or all types, of tumour cells. Unfortunately, this long-sought information is not yet available and so we must take the next, most logical, course of action. That is, to use known biochemical events to predict a possible active compound, or group of compounds.

Therefore, with extensive work readily available on the distribution, detection and pharmacology of AIC, coupled with knowledge of *de novo* purine biosynthesis, this imidazole seemed to represent an ideal basis upon which to elaborate potential new agents.

1.2 Synthetic Routes to 5-Aminoimidazole-4-carboxamide.

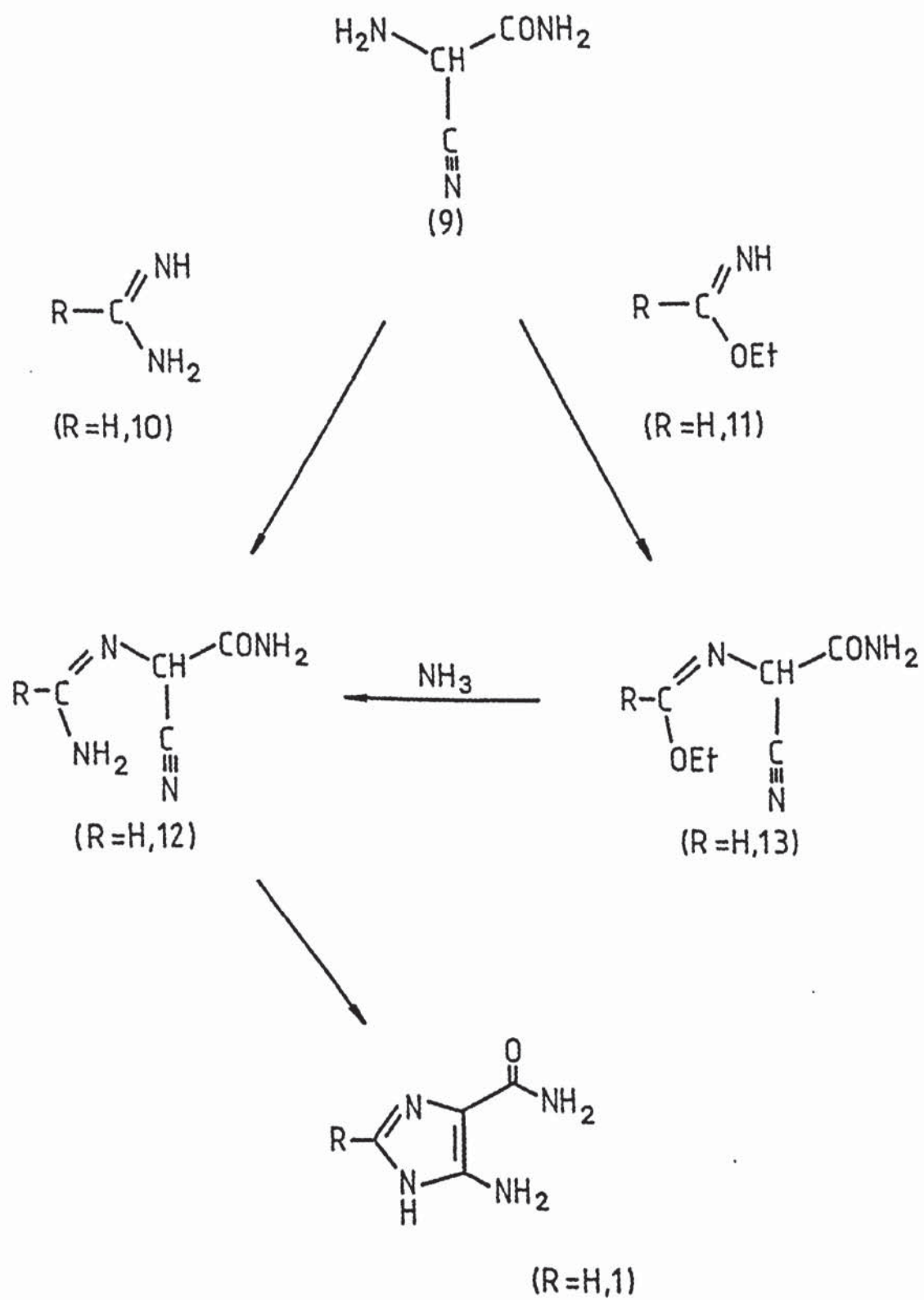
In this section I intend to review only those syntheses which can be considered of interest to the synthetic organic chemist, and so the previously reported isolation of AIC from bacterial cultures or from the riboside *via* enzymatic or chemical routes will not be covered. The synthetic routes may be considered to belong to one of three major categories: ring formation, ring modification, and finally, ring cleavage.

1.2.1 Ring formation.

1.2.1.1 From 2-amino-2-cyanoacetamide.

This involves the interaction of 2-amino-2-cyanoacetamide (9) with the appropriate amidine (10) or imidate (11) as shown in Scheme 1.2. The reaction may be thought of as proceeding *via* an intermediate amine (12) when formamidine (10) is utilised.⁴⁹ The aminonitrile, in all cases, provides the carbon atoms 4 and 5 of the imidazole ring and hence the substituents at these positions - namely the carboxamide and amino functions. Cook, Heilbron and Smith⁶⁵ pioneered this synthetic method but found they were unable to isolate AIC as the free base, obtaining only a brown, diazotisable oil. However, treatment of crude base with picric acid allowed them to isolate and characterise the imidazole as a salt. Schwartz⁶⁶ patented this route in 1970 using the acetate salt of formamidine under similar conditions but isolating the product as the hydrochloride. A later patent⁶⁷ describes preparation of the sulphate, phosphate, perchlorate, arylsulphonate and orotate salts of AIC.

Thioimidates are considered to give more satisfactory results in this type of synthesis:⁴⁹ however, the title compound has not been

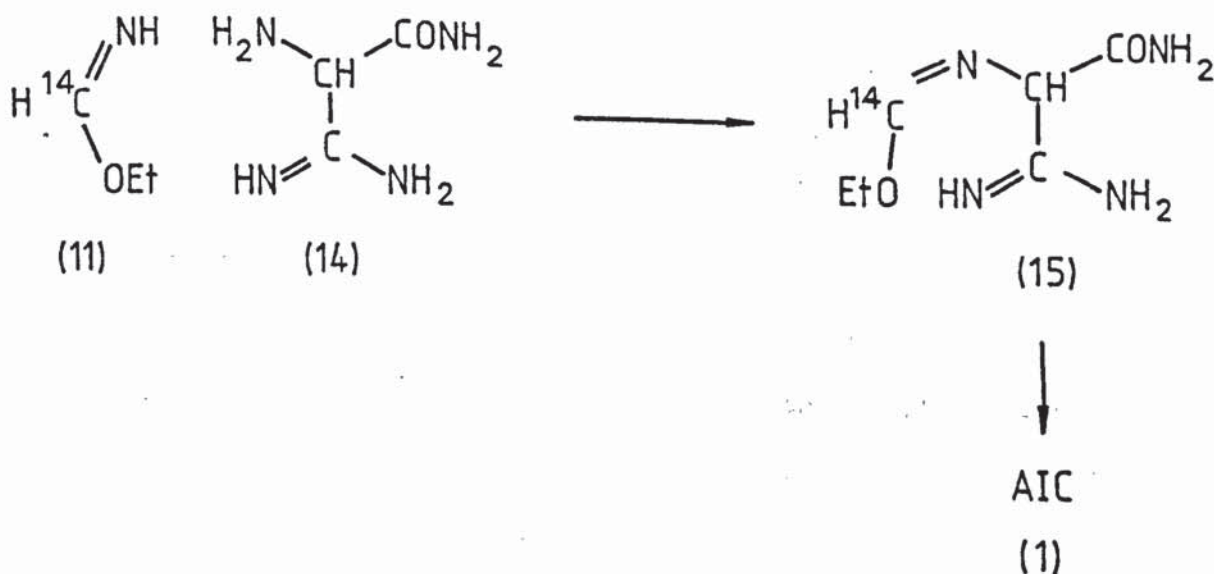


Scheme 1.2

prepared in this manner although Rose⁶⁸ has prepared many 2-substituted derivatives of AIC in this way.

Soon after the biochemical importance of AIC had been recognised synthesis of ^{14}C -AIC labelled at position 5 was successfully attained in 30% yield^{69,70} by using ethyl formimidate (11) and aminocynoacetamide labelled in the nitrile function. Shaw and co-workers⁷¹ showed that the linear imidate (13) could be isolated from the reaction mixture, and that an amine was required to effect cyclisation, presumably *via* the amidine (12). In the case of AIC, ammonia is used to furnish the nitrogen atom at the 1-position. This method allowed the free base to be obtained directly from the reaction mixture.

A variation on this method was recently employed⁷² in order to obtain ^{14}C -2 labelled AIC. This involved the interaction of ^{14}C -ethyl formimidate (11) and 2-amino-2-amidinoacetamide (14) in hydrochloric acid (Scheme 1.3). Treatment with sodium bicarbonate liberated AIC free base which was converted to the phosphate salt by phosphoric acid.



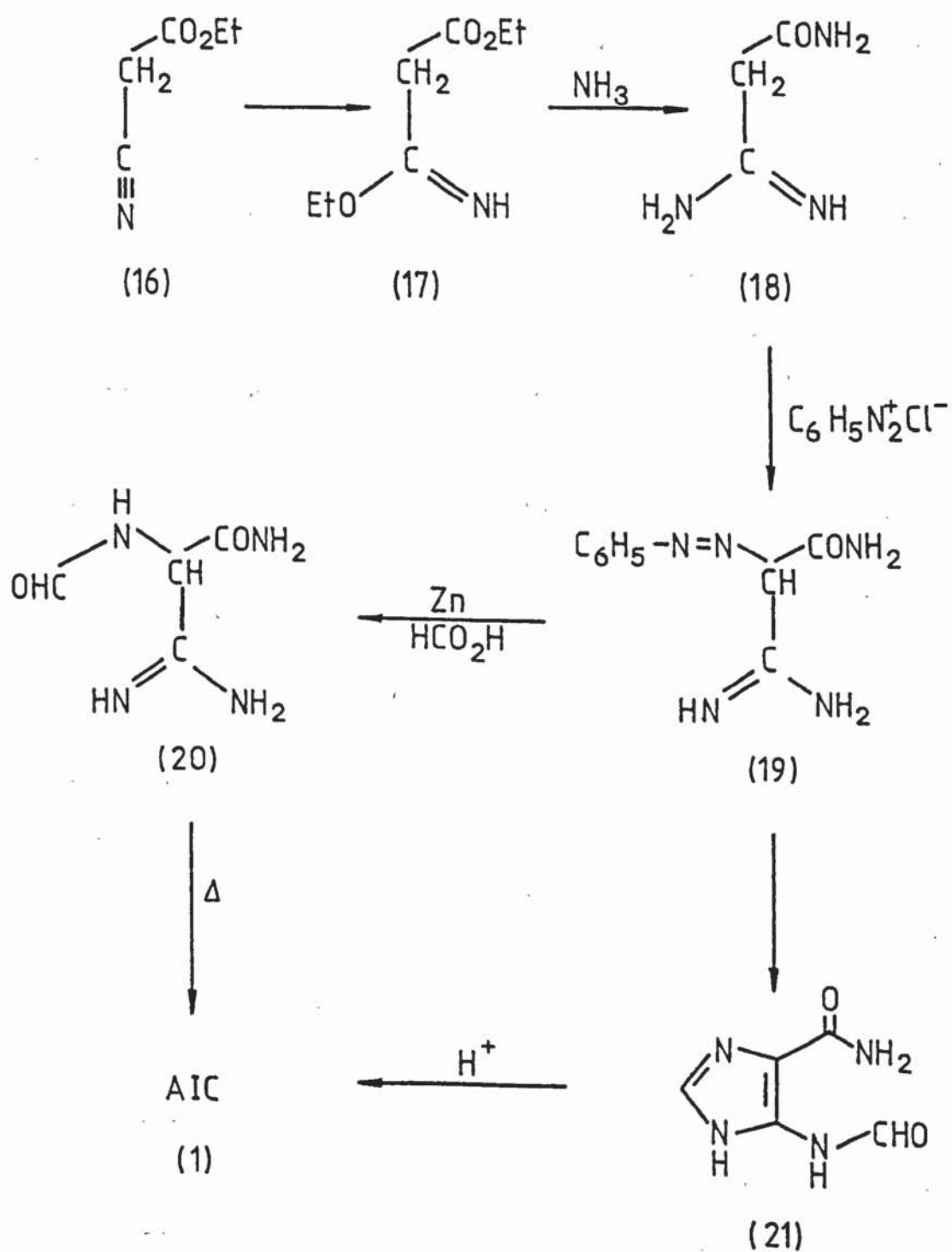
Scheme 1.3

The synthetic routes from aminonitriles have proved to be very adaptable, and a variety of 2-substituted AIC derivatives can be easily prepared by choice of the appropriate amidine, imidic acid ester or imidic acid thioester⁶⁵ (Scheme 1.2, R=alkyl, etc.). It is also possible to incorporate an aryl or alkyl group at the 4-position in place of the carboxamide function by use of the corresponding aryl- or alkyl-amino-nitrile.⁷³⁻⁷⁵ Aminoimidazole carboxylates are also prepared by this route. The Shaw method, which allows isolation of the imidate intermediate, can be used to vary the substituent at the 1-position of the imidazole ring. This is simply achieved by use of the appropriate amine to effect cyclisation: hence aniline instead of ammonia would result in formation of 1-phenyl-AIC.

1.2.1.2 From aminomalonic acid.

Shaw and Woolley⁷⁶ devised a new route (Scheme 1.4) which is also applicable to the synthesis of imidazoles related to AIC. Ethylcyanoacetate (16) represented the starting material which was converted to the imidate (17) by a process described by Glickman and Cope.⁷⁷ Malonamidine (18) was obtained in high yield from the imidate and ammonia. The second ring nitrogen is introduced by coupling of an aryl diazonium salt at the reactive methylene group, and subsequent reduction of the azo bond with zinc dust in formic acid yields the formamido derivative (20). Ring closure to AIC is simply achieved by melting the formamido compound at 170° until the imidazole crystallises from the melt. Noguchi⁷⁸ also used this route to synthesise ¹⁴C-labelled AIC in 23% yield: starting with ethylcyanoacetate labelled in the nitrile function the resultant imidazole was labelled at C-5 of the ring.

Montgomery,⁷⁹ while investigating this method for large scale

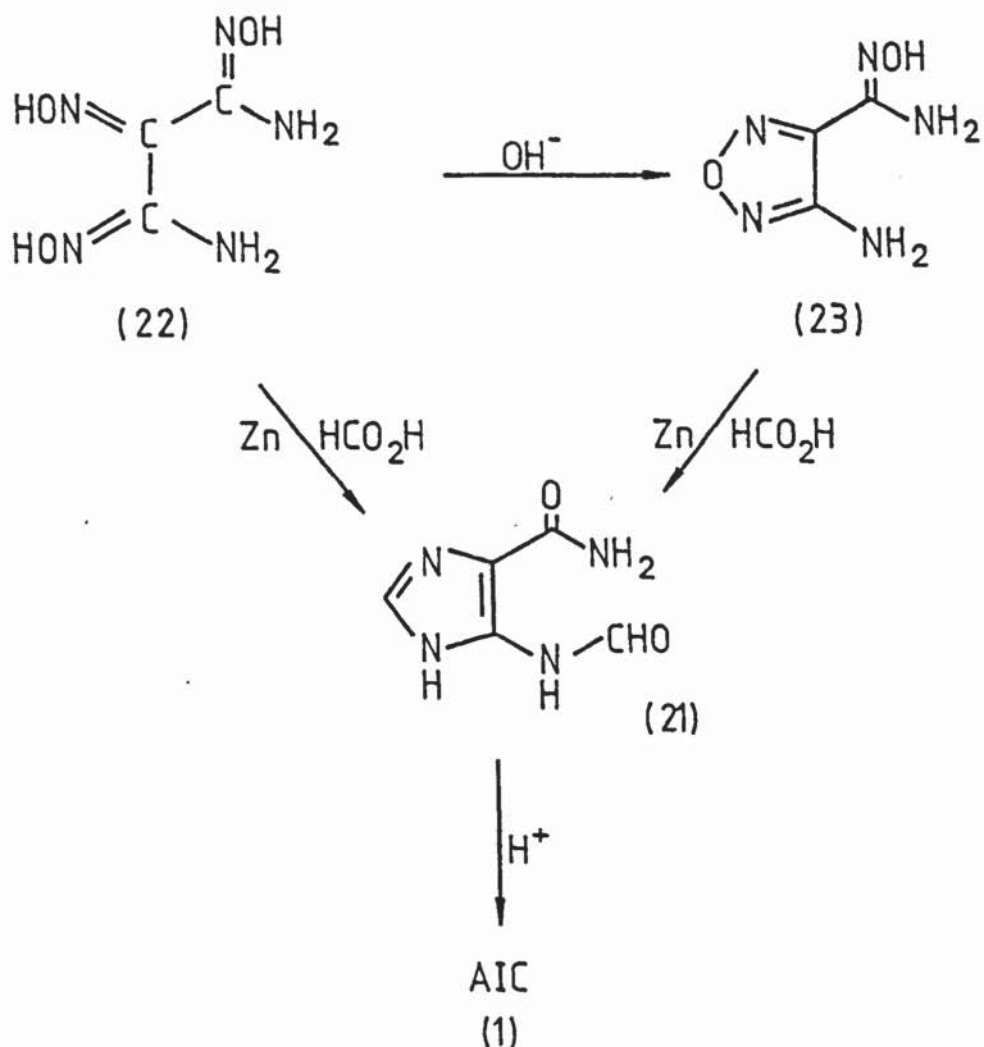


Scheme 1.4

synthesis of AIC, noted that after reduction of the arylazo intermediate (19) cyclisation of the formamido compound had already partially taken place. Furthermore, he discovered that complete cyclisation could be effected by simply refluxing the filtrate after removal of zinc dust. This process yields the formylamino derivative of AIC (21) which may be easily hydrolysed to the aminoimidazole in dilute acid.⁸⁰ The reductive step may also be successfully completed by employing palladium as a catalyst in formic acid. The Montgomery modification generally results in a purer product, free from the green pigment so characteristic of the Shaw and Woolley method. Cyclisation after reduction has also been conveniently achieved by the use of a molar equivalent of ethyl orthoformate^{81,82} which gives a high yield conversion to the imidazole under milder conditions.

The versatility of this route is demonstrated by the introduction of other substituents which is, of course, entirely dependent upon the chosen starting materials. Hence N-benzylcyanoacetamide will result in the formation of N-benzyl-AIC,⁸³ whereas the use of acetic acid for the reductive step will result in 2-methyl-AIC.⁸⁴ Choice of the appropriate orthoester⁸¹ to effect cyclisation will also result in modification of the C-2 position.

There is a variation of this method which uses malononitrile as the starting material,⁸⁵ and nitrosation in aqueous solution, rather than azo coupling, provides the precursor group at the 2-position. Subsequent reaction with hydroxylamine results in formation of the required three carbon unit, 1,3-diamino-1,2,3-trioximinopropane (22) as outlined in Scheme 1.5. Treatment of the trioxime with a reducing agent in formic acid results in formation of 5-formylaminoimidazole-4-carboxamide⁸⁶ (21), from which AIC may be obtained by acid hydrolysis as previously stated.

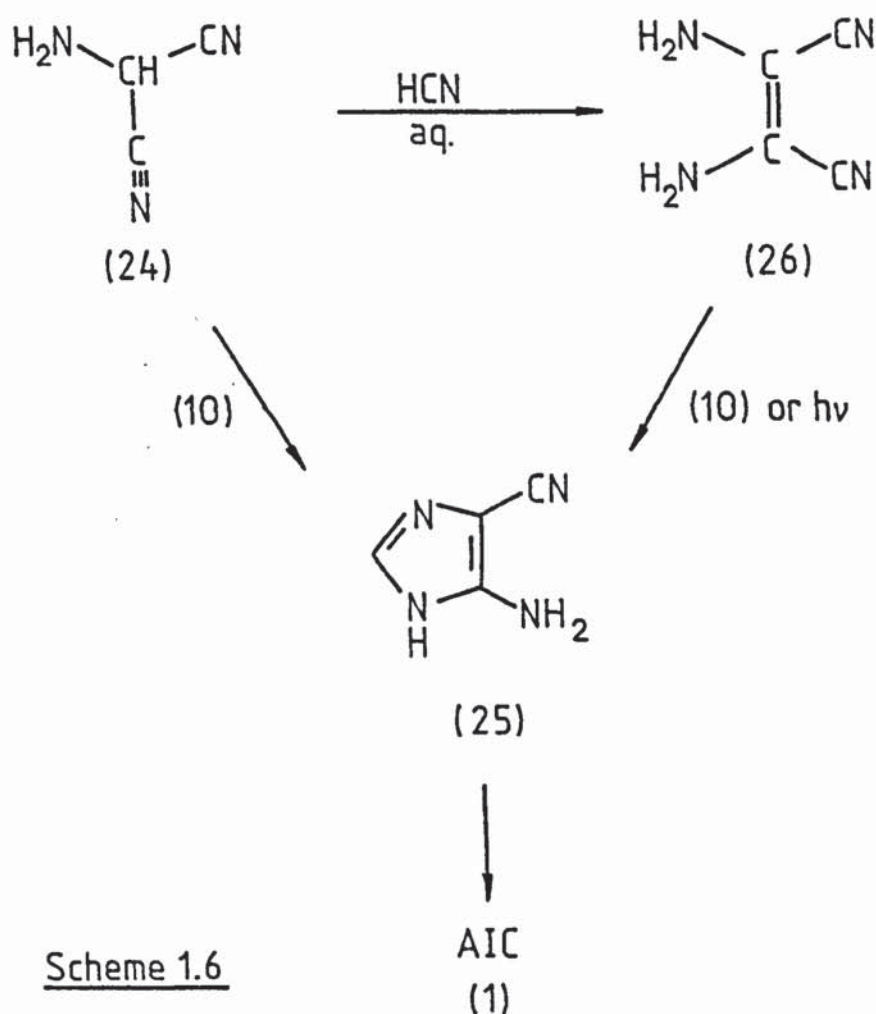


Scheme 1.5

Alternatively the furazan (23) may be prepared by the addition of base to the intermediate trioxime,^{85,87} or directly from malononitrile. Treatment of the furazan with formic acid and reduction provides another route to the formylamino derivative of AIC.

1.2.1.3 Prebiotic pathways.

For obvious reasons there has been considerable interest shown in the prebiotic synthesis of purines. Consequently, the simpler precursor



molecules which provide the synthetic "stepping stones" to the biologically important purine bases have also been investigated. Oro and Kimball⁸⁸ proved that AIC was one of the products formed in the base-catalysed condensation of hydrogen cyanide in aqueous ammonia. This was later confirmed by Ferris *et al.*⁸⁹ These reducing conditions are considered to be similar to those which existed on primitive earth. A multistep route to these compounds was proposed which included the intermediate 2-aminomalononitrile (24) and this dinitrile was subsequently prepared under such conditions.^{90,91} Reaction of the dinitrile with formamidine (10),

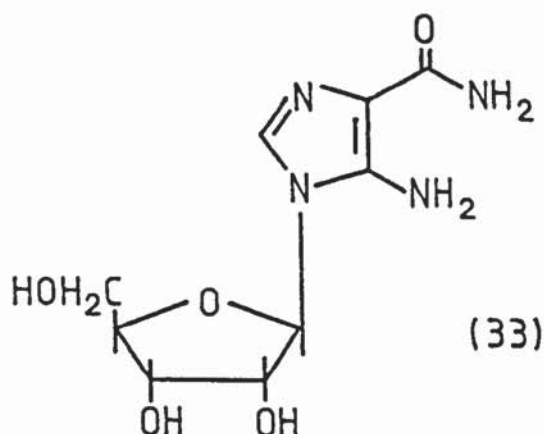
another compound isolated from simulated primitive earth conditions, results in formation of 5-aminoimidazole-4-carbonitrile (25) which can be isolated in 35% yield (Scheme 1.6). However, a more stable hydrogen cyanide tetramer is also formed under aqueous conditions, diaminomalononitrile (26); this will also form the aforementioned carbonitrile in the presence of formamidine⁹² (10). Indeed it has been demonstrated that the tetramer can be photochemically converted to the carbonitrile, *via* the *trans* form, under aqueous conditions in yields as high as 80%.⁹³ Alkaline hydrolysis of the carbonitrile to AIC is easily achieved and will be discussed more fully in a following section (1.2.2.3). It is perhaps a sobering thought that Sagan⁹⁴ claims spectroscopic evidence for the presence of AIC in the Jovian atmosphere.

1.2.2 Ring modifications.

1.2.2.1 Reduction.

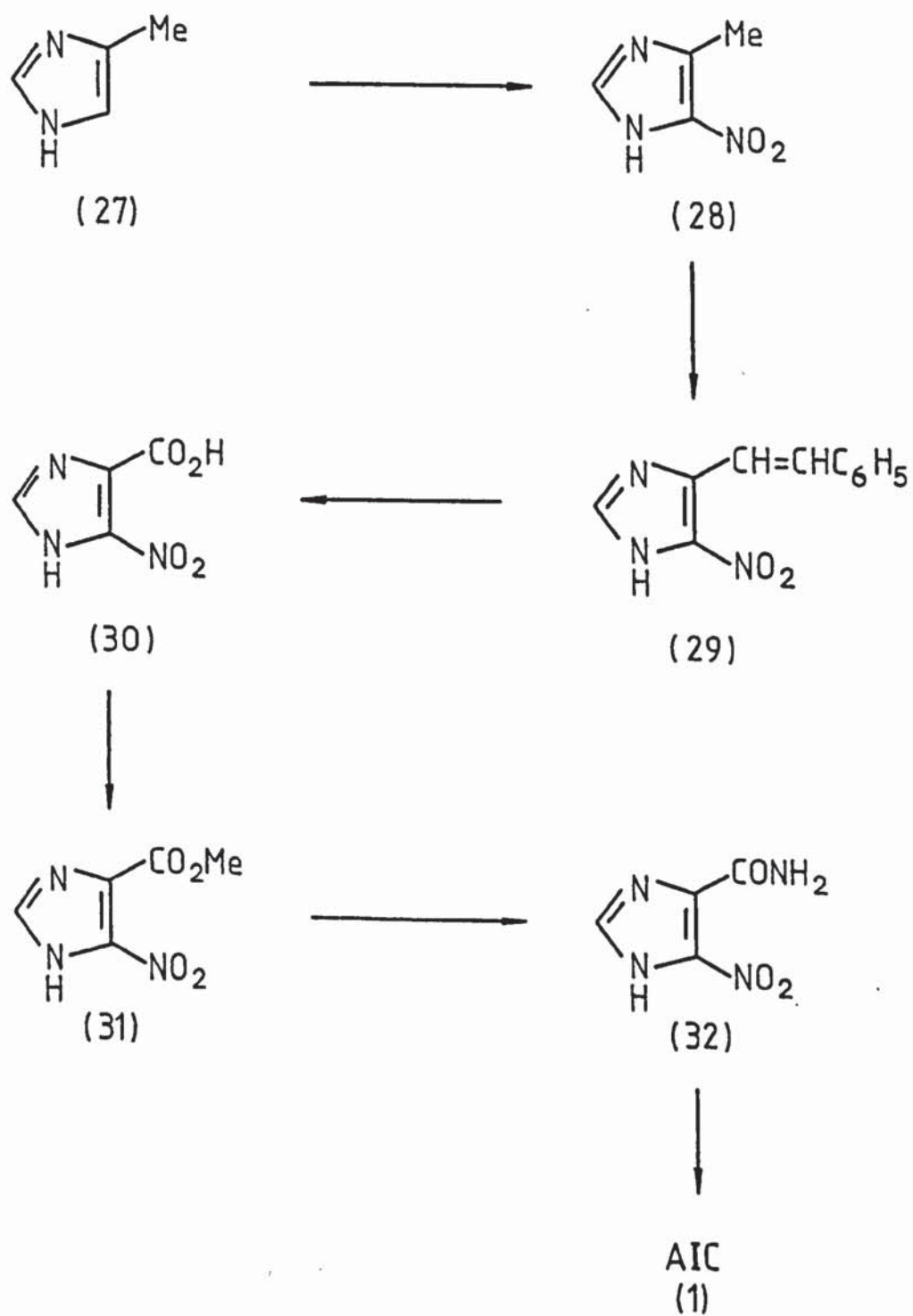
Inevitably the reduction of a nitro group to yield an aminoimidazole received initial attention, and this was the method by which AIC was first synthesised in the laboratory by Windaus and Langenbeck.⁴ Nitration of 4-methylimidazole (27) furnished the nitroimidazole (28) which was converted to AIC by the sequence outlined in Scheme 1.7.

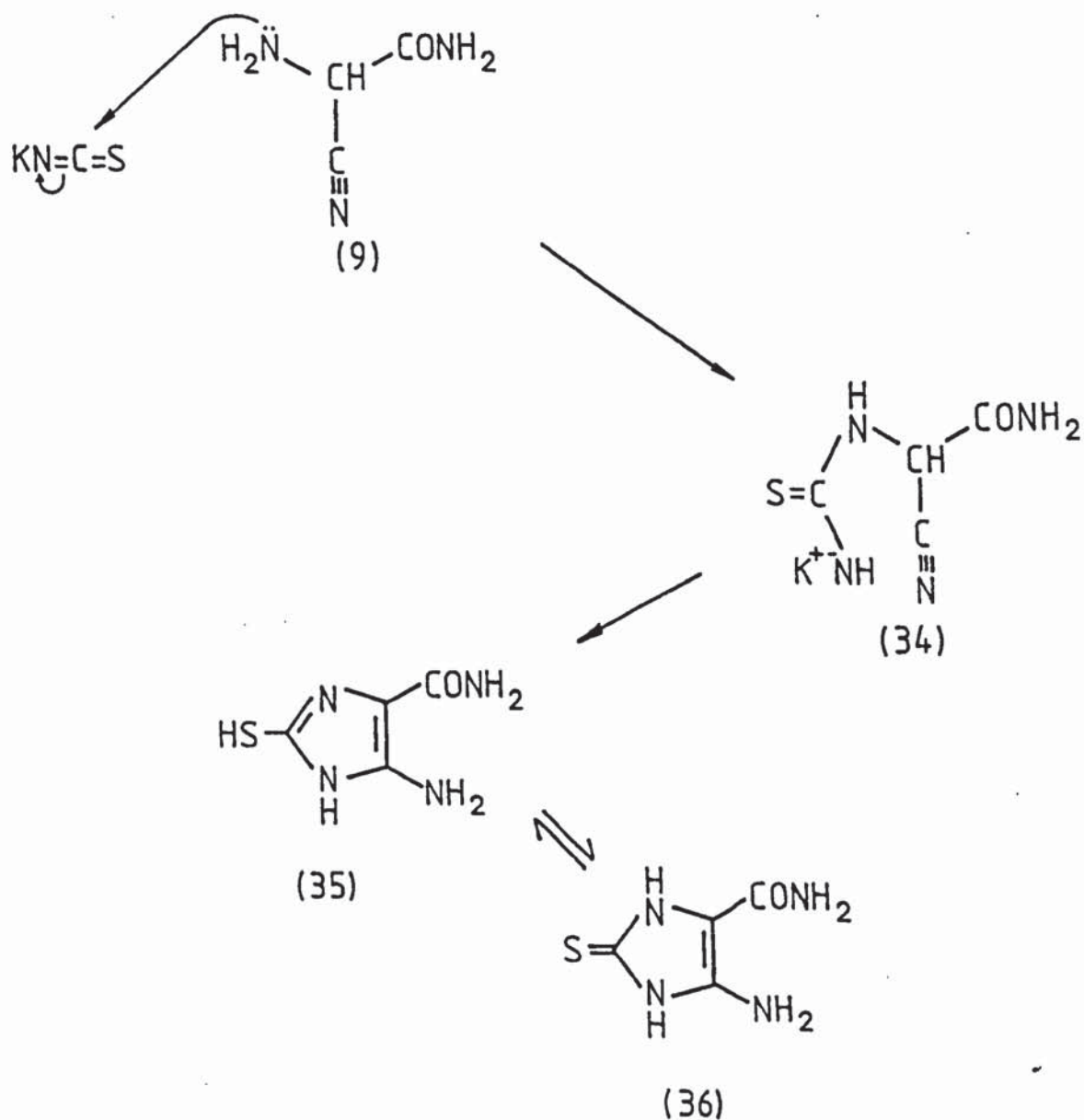
While there have been modifications to this method published in the literature, notably to improve the yield of the nitrocarboxylic acid intermediate (30), the reductive process has not been improved. The ribonucleoside (33) of AIC has also been prepared⁹⁵ by hydrogenation, over



Adams platinum catalyst, of the corresponding nitro precursor.

Another possible reductive route would be the final step cleavage of an arylazoimidazole to yield an amino group in the required position. In early studies⁹⁶ exploring this pathway it was found that use of stannous chloride would effectively reduce such compounds: however, to date, AIC has not been prepared in this manner, although the riboside of AIC has been obtained by catalytic cleavage of an -N=N- bond.⁹⁷

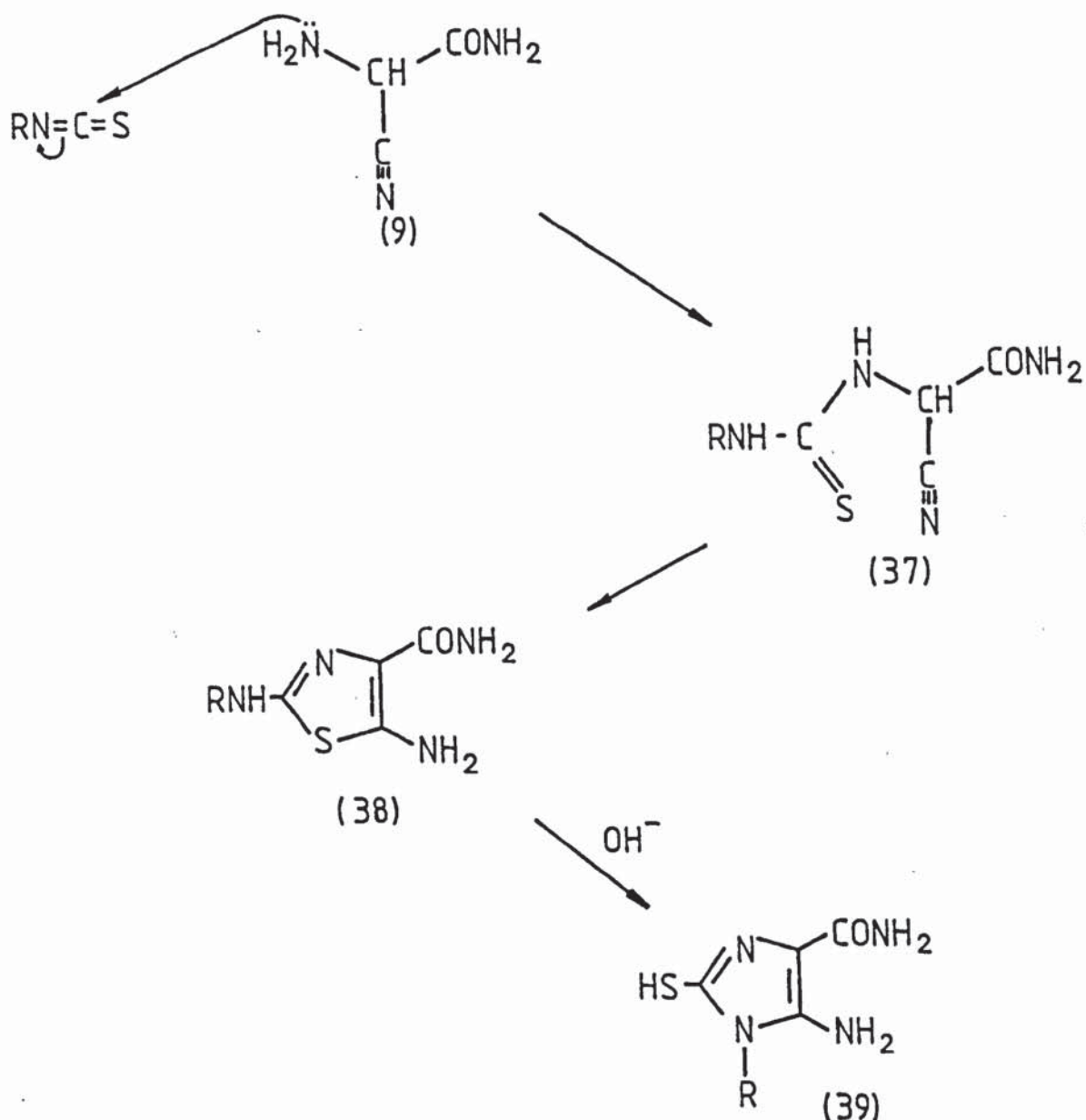
Scheme 1.7



Scheme 1.8

1.2.2.2. Desulphurisation.

A modification upon the synthetic route to imidazole ring formation from aminonitriles which has been previously outlined (1.2.1.1) was utilised to prepare the 2-thione of AIC.⁹⁸ Potassium thiocyanate reacted under acidic conditions with aminocyanacetamide (9) to give 5-amino-2-



Scheme 1.9

mercaptoimidazole-4-carboxamide (35) or the tautomeric 5-amino-2-thioxoimidazoline-4-carboxamide (36). Although the intermediates were not isolated, the reaction would be expected to proceed *via* the thiourea (34) as outlined in Scheme 1.8. The group at N-1, in this case a proton, is derived from the thiocyanate, and therefore, use of aryl- or alkyl-isothiocyanates would yield 1-substituted imidazoles. An interesting variation of this route was discovered when isothiocyanates were used for synthesis of imidazoles

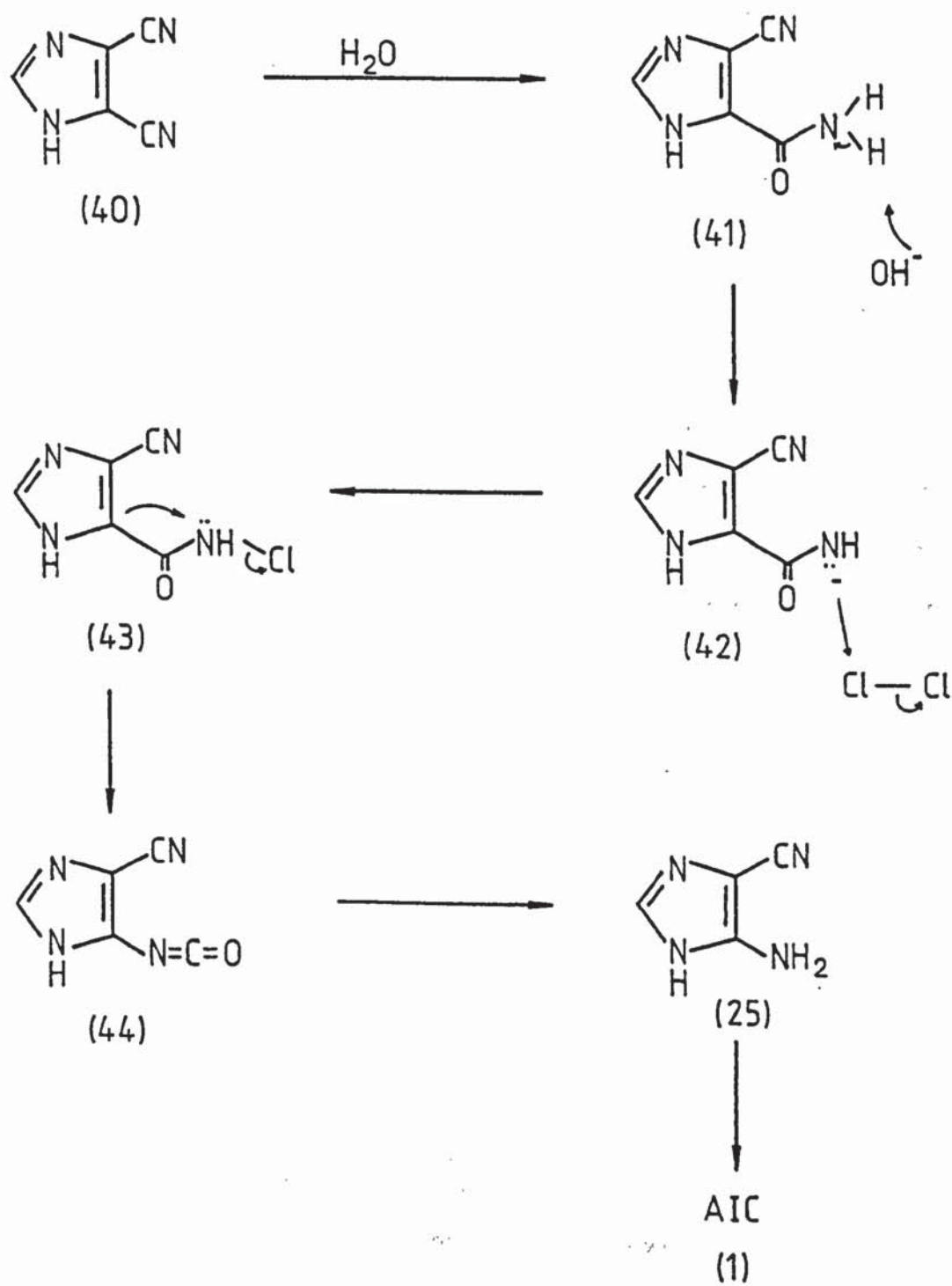
(Scheme 1.9). The reaction proceeds, as with potassium thiocyanate, *via* the thiourea; however, the product of the reaction is a 2,5-diaminothiazole ^{99,100} (38) which can be isolated in some cases. Under the influence of mild aqueous base these 5-aminothiazoles give 5-amino-2-mercaptoimidazoles (39) or the tautomeric amino-2-thioxoimidazolines *via* a Dimroth rearrangement.

The mercapto or thione group at the 2-position can be removed by Raney nickel desulphurisation ^{65,101,102} resulting in an imidazole unsubstituted at the 2-position. There is no literature reference to the preparation of AIC by desulphurisation of the mercapto derivative.

1.2.2.3 Hydrolysis.

Among the products obtained from experiments investigating reactions under primitive earth conditions are the imidazole carbonitriles. It may be considered that this route to AIC is of little synthetic importance; however, it has been noted ¹⁰³⁻¹⁰⁵ that interaction of hydrogen cyanide with ammonia (or sodium cyanide and ammonium chloride) under anhydrous conditions yields 20% of the dinitrile (40).

The imidazole carbonitriles are ideal precursors of AIC, and the pathway from 4,5-dicyanoimidazole (40) has been investigated by Yamada, ¹⁰⁶ this is summarised in Scheme 1.10. Partial hydrolysis in dilute sodium hydroxide ¹⁰⁷ yields the monoamide (41), which undergoes the Hofmann rearrangement reaction ^{108,109} in the presence of hypochlorite to yield an intermediate isocyanate (44). 5-Aminoimidazole-4-carbonitrile (25) is obtained following hydrolysis and decarboxylation of the isocyanate group. Finally hydrolysis of the carbonitrile to AIC can be achieved efficiently without isolation of the carbonitrile if preferred. ^{91,92,110,111}

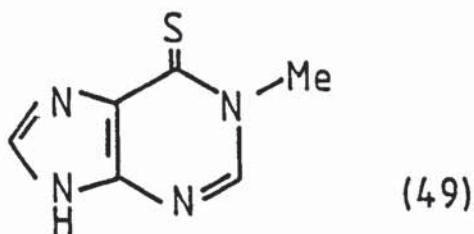


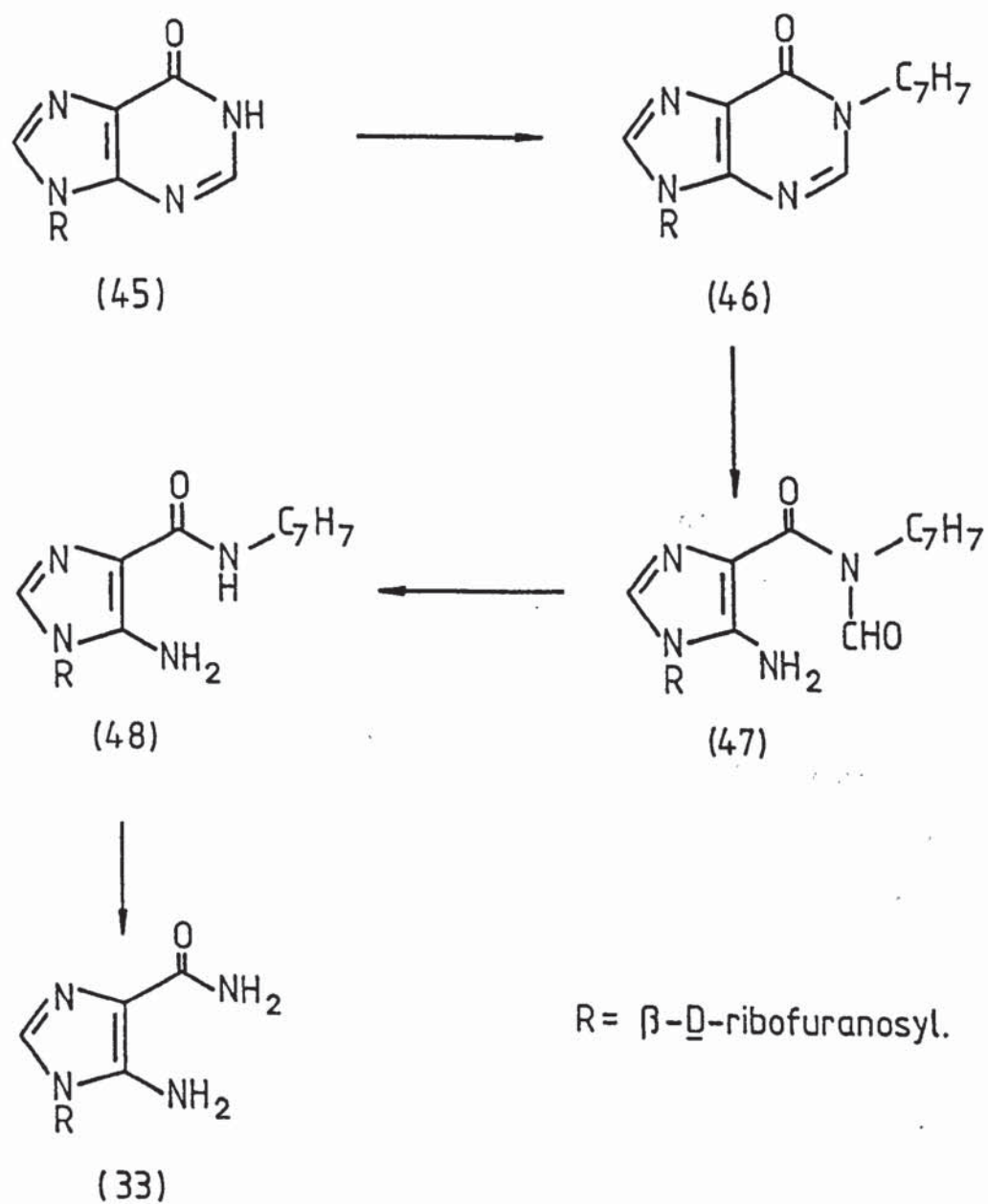
Scheme 1.10

1.2.3 Ring cleavage of purines.

Fischer¹¹² observed that purines methylated at ring nitrogen were more susceptible to alkaline hydrolysis than their non-alkylated counterparts. Shaw¹¹³ also noted that purines, alkylated at ring nitrogen, undergo hydrolytic attack directed preferentially in the pyrimidine ring. Thus Shaw synthesised the ribonucleoside of AIC starting from inosine (45) by first labilizing the pyrimidine ring by forming the 1-benzyl derivative (46). This then formed N-benzyl-AIC (48) upon alkaline ring cleavage: the benzyl group was subsequently removed with sodium and liquid ammonia (Scheme 1.11). Problems with the debenzylation step reduce the yields: this method is, therefore, not ideal for the synthesis of AIC.

Suzuki¹¹⁴ found that inosine would ring open at C-2 in aqueous dilute sodium hydroxide at pH 10 without any modification of the ring nitrogens. He also discovered that, under similar conditions, hypoxanthine would undergo base-catalysed ring opening to AIC. The conditions required are rather drastic (150° in sealed tube) and hence the yield of 30%. Mercaptopurines also ring open at C-2 in the pyrimidine ring when previously alkylated at ring nitrogen. For example 1-methylpurine-6-thione (49) gave AIC when treated with aqueous ammonia at 145° for 24 hours.^{115, 116} Presumably N-demethylation occurs before or during the ring cleavage.

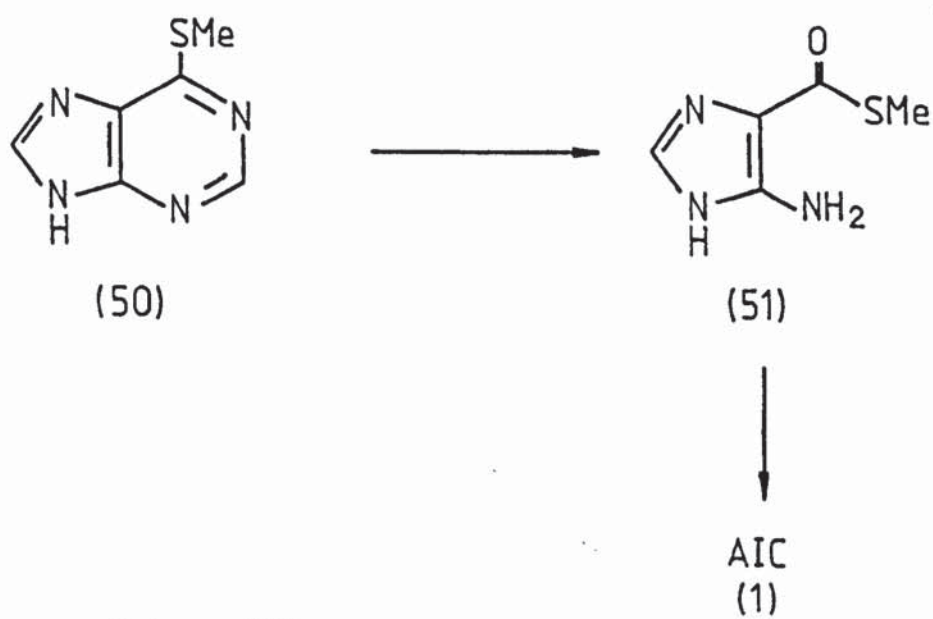


Scheme 1.11

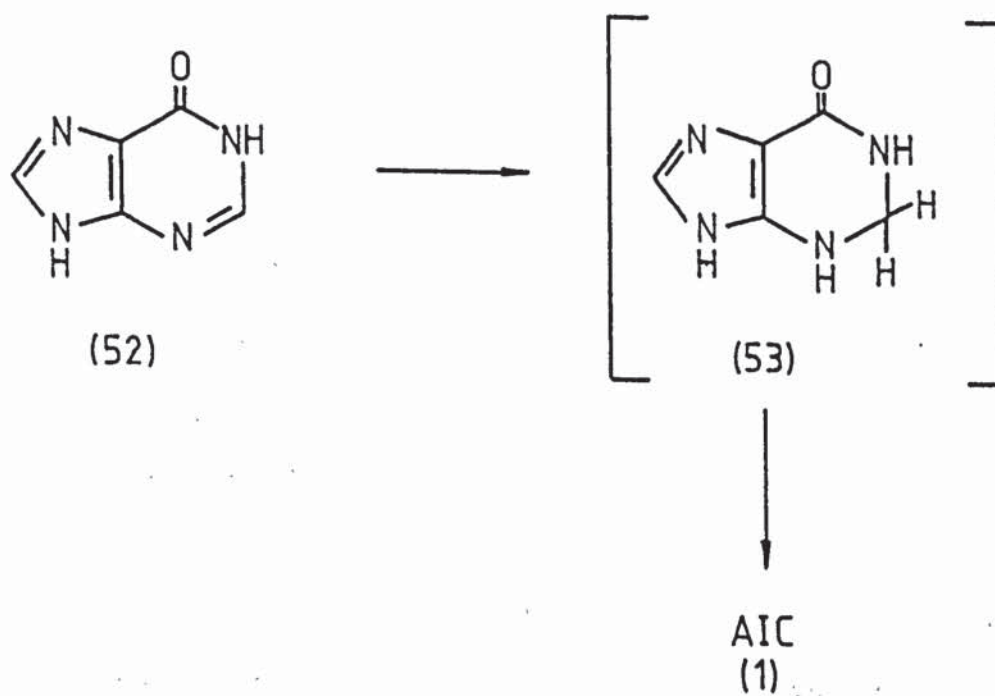
Acidic ring cleavage of purines unsubstituted on ring nitrogen generally results in extensive degradation,^{117,118} however, there are still several examples of AIC syntheses successfully achieved under such conditions. Albert¹¹⁹ achieved the acid-catalysed ring opening of mercaptopurines which had been methylated on sulphur. He found that 6-methylthiopurine (50) ring opened to yield a thioester (51) which, upon ammonolysis, gave AIC [Scheme 1.12].

Hydrogen generated *in situ* by amalgams or metals in acid solution effects hydrogenolysis of the pyrimidine ring to yield the appropriate aminoimidazole. Examples of such reductive degradation of purines can be found with hypoxanthine (52) and sodium amalgam¹²⁰ or zinc dust^{121,122} in dilute acid [Scheme 1.13]. Both reactions result in the formation of AIC in yields of 20-30%, presumably by way of reduction at C-2 / N-3 (53), and hydrolysis of the aminal function.

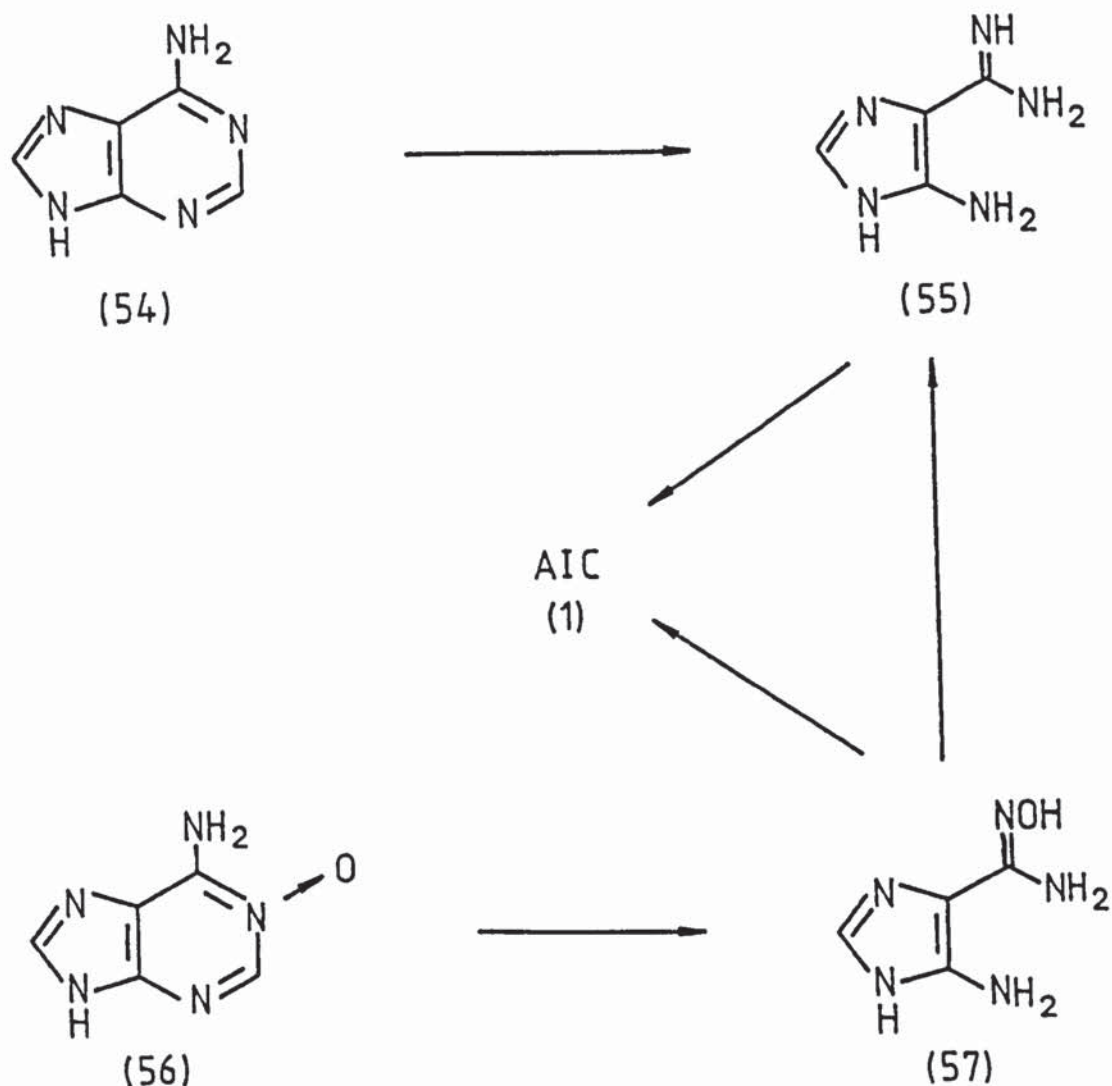
Using milder conditions 5-aminoimidazole-4-carboxamide (55) can be isolated in low yield by the acidic hydrolysis¹¹⁷ of adenine (54). Alternatively the N-oxide of adenine (56), as prepared by Stevens and Brown,¹²³ is susceptible to acidic ring cleavage under even milder conditions¹²⁴ to give 5-aminoimidazole-4-carboxamidoxime (57) in high yield (> 75%). This can then be converted to the carboxamide by hydrogenation over Raney nickel, with subsequent hydrolysis to AIC. Direct synthesis of AIC from the carboxamidoxime can also be achieved by extended acidic hydrolysis [Scheme 1.14]. Formation of the amidoxime by acidic ring opening has also been demonstrated from 2-methyladenine-1-oxide,¹²⁵ and from the phosphate of adenosine-1-oxide¹²⁶ with the accompanying loss of the ribofuranosyl group. AIC has also been recorded as a minor product in the acid hydrolysate of 1-hydroxyisoguanine.¹²⁷



Scheme 1.12



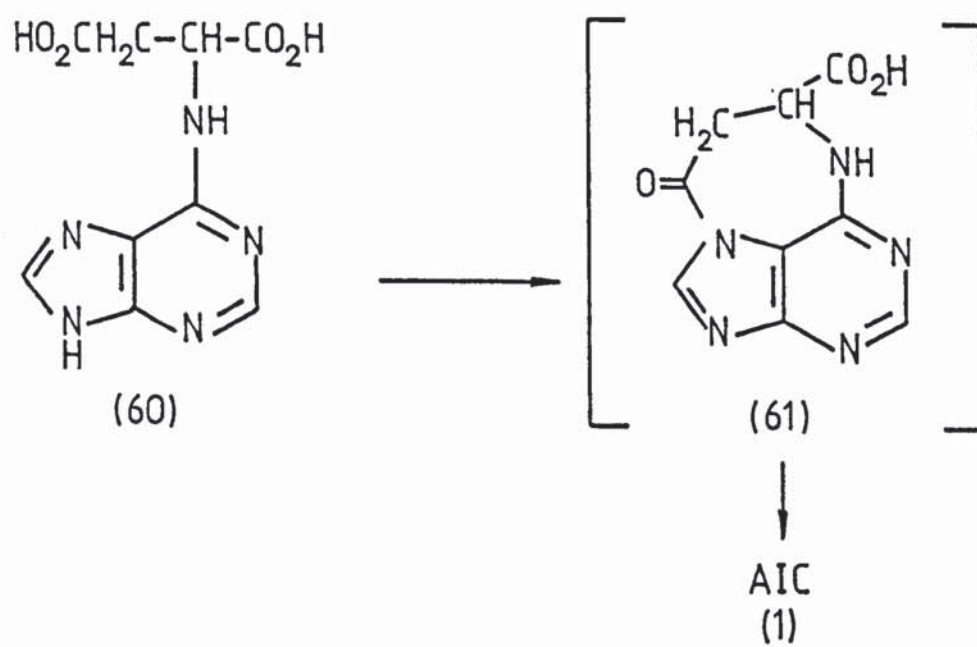
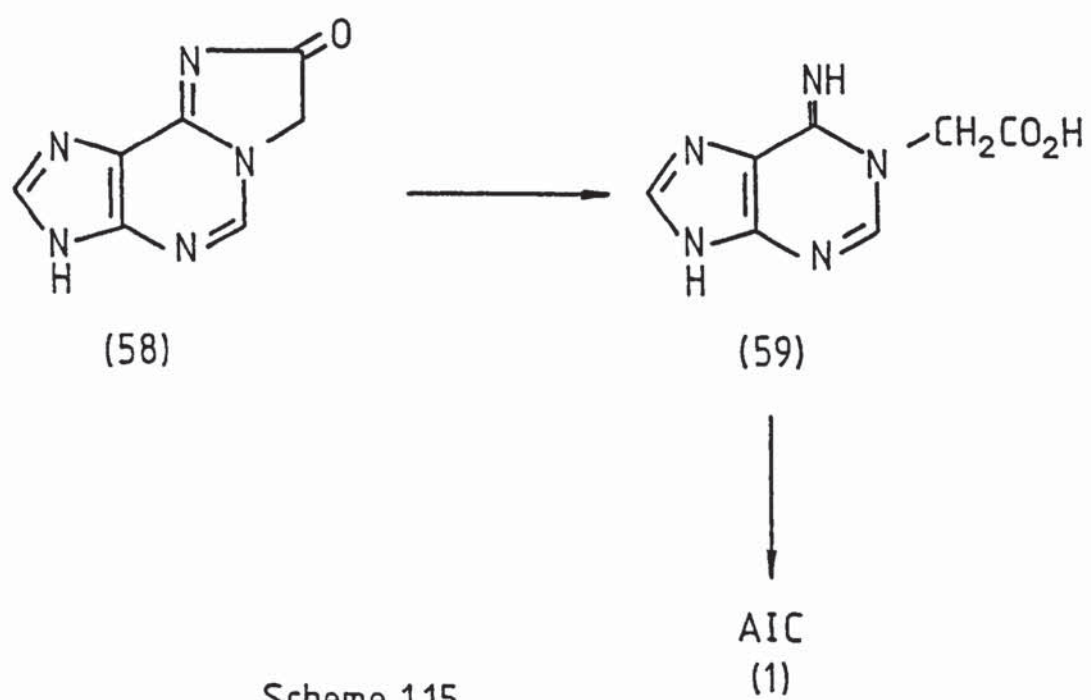
Scheme 1.13



Scheme 1.14

There are two other cases of AIC formation by acidic ring opening which should be mentioned. First is cleavage of the pyrimidine ring of glycyadenine (59) which is formed from the imidazopurine^{128,129} (58) (Scheme 1.15). The second route is the acidic cleavage of N-(purin-6-yl)aspartic acid (60) to AIC, which has been postulated to proceed *via* an intermediate lactam¹³⁰ (61) due to internal condensation at the purine N-7 (Scheme 1.16).

We must therefore conclude that the earlier methods for the



synthesis of AIC pioneered by Shaw and Cook, which involve synthesis of the imidazole ring, are probably the methods of choice.

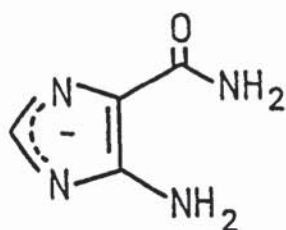
1.3 Reactions of 5-Aminoimidazole-4-carboxamide.

1.3.1 General reactivity.

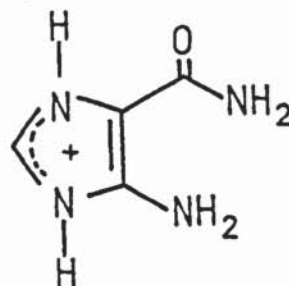
Due to the electron releasing character of the sp^2 hybridised nitrogen (N-1) which donates a lone pair of electrons to the aromatic sextet, AIC can be described as a Π - excessive heterocycle. This concept was first enunciated by Albert.¹³¹ The Π - excessive nature of AIC therefore determines the reactions one would expect of this ring system. The other ring azomethine-type nitrogen, has an electron-attracting influence and to some extent reduces the Π - excessive nature of the compound. This also increases its stability to acids. AIC, like many other imidazoles, is acid stable and forms crystalline salts with a variety of organic,^{69,132} and mineral acids.^{72,76} These salts generally have high melting-points (eg. AIC hydrochloride, m.p. 255°). The free base may be easily obtained from these acid salts by neutralisation with hydroxide,¹³³ or bicarbonate, to afford the crystalline monohydrate⁷² which is reasonably stable (m.p. 168°). The crystal structure of the monohydrate free base and also the phosphate salt have been determined by Simon.¹³⁴ It is often the phosphate salt which is used for treatment of liver dysfunction, although the salt formed with ureidosuccinic acid²² is also of importance. The lone pair of electrons on the azomethine nitrogen also increases water solubility and with two hydrogen bonding groups at C-4 and C-5 AIC shows high water solubility.

The imidazoles must be considered as amphoteric substances due to the two types of ring nitrogen which exert both acidic ($-NH-$) and basic ($=N-$) properties. The nitrogen at the 3-position ($=N-$) increases the acid strength of the system because of its electron-attracting character, which

is comparable in power to a nitro group. N-deprotonation will give the resonance-stabilised imidazolyl anion (62). Other evidence of this pseudo-



(62)



(63)

acidic nature of imidazoles in general is their conversion to organo-metallic salts. However, no reference can be found to the formation of such salts with AIC and this may be due to the amino group at C-5 which will oppose the electron-withdrawing effect of the azomethine nitrogen. The cation (63) may also be formed in strong acids, with protonation occurring at N-3.

Electrophilic substitution is generally a characteristic reaction of Π - excessive substances. Unsubstituted imidazole tends to undergo electrophilic attack predominantly at C-5 which is the carbon atom least deactivated by N-3. However, in AIC, C-2 will also be activated to some extent by the 5-amino group. One would therefore expect electrophilic substitution of the ring to take place at C-2.

Reactions with nucleophiles would not be expected to occur with AIC; indeed there are very few examples of nucleophilic substitution in the whole of 1,3-azole chemistry. Other predicted reactions of AIC are those associated with the two functional groups at C-4 and C-5.

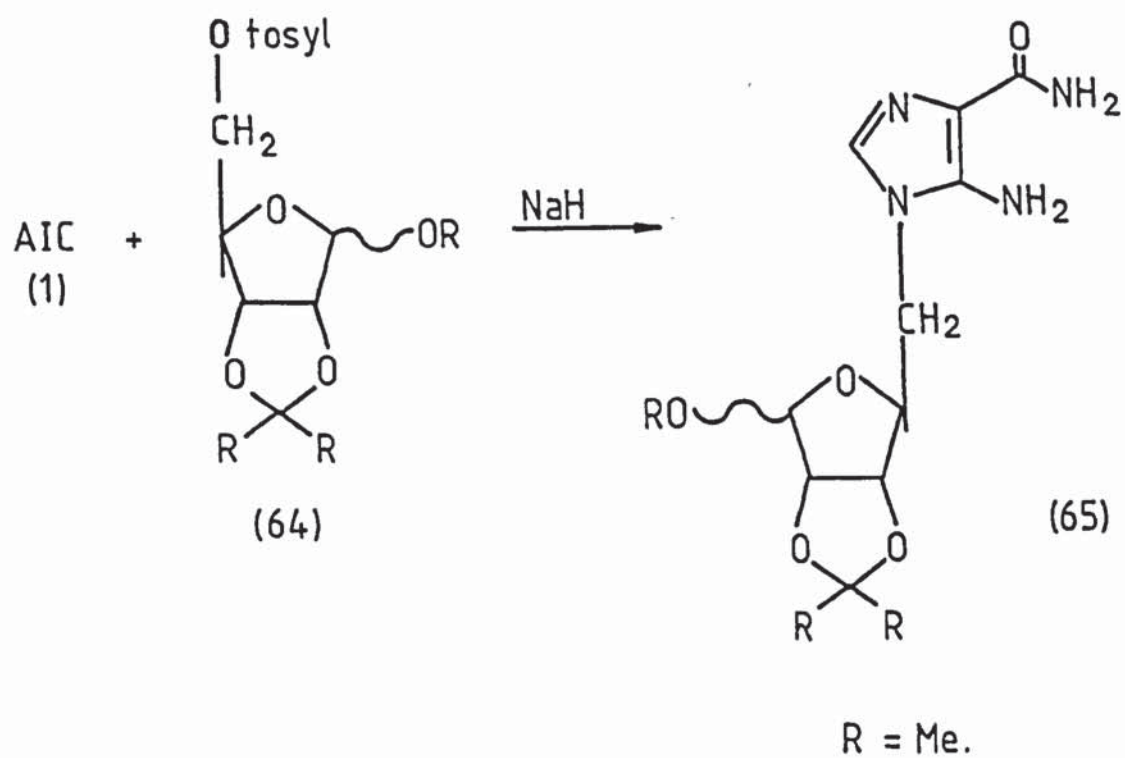
1.3.2 Reactions at ring nitrogen.

Although there are many examples of 1-substituted AIC deriv-

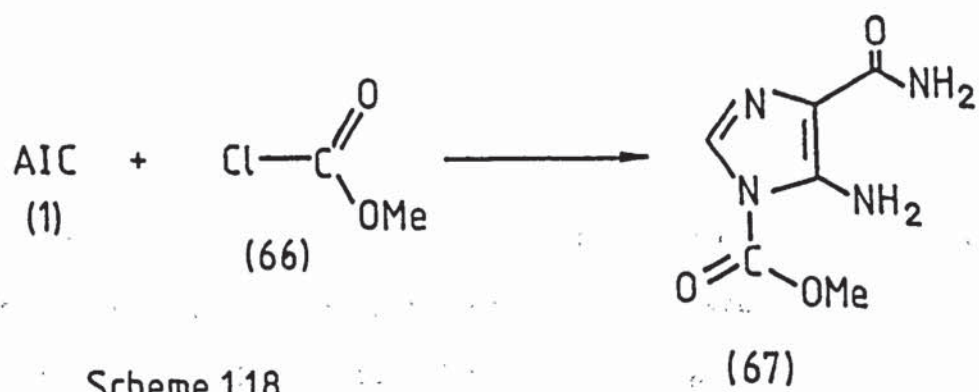
atives in the literature (eg. ribosides and deoxyribosides) the usual route to such compounds is not *via* AIC. The majority of these derivatives are formed by ring closure^{135,136} after the requisite substituent has already been incorporated into the carbon-nitrogen backbone. Another method utilised for such compounds is the condensation of a sugar group with the corresponding silver or chloromercury salt⁹⁵ of 5-nitroimidazole-4-carboxamide, which can then be catalytically reduced to the required 1-substituted AIC derivative. 1-Methyl-AIC may also be prepared in this manner by the use of methyl iodide.

It has already been stated that AIC will form an anion or cation under certain conditions by deprotonation or protonation of ring nitrogen. However, there are very few literature references to reactions which take place exclusively at ring nitrogen. Inushiro¹³⁷ has patented such a reaction (Scheme 1.17). This Japanese patent describes the preparation of imidazole nucleosides by the treatment of AIC with protected riboses. An example cited is the reaction with methyl 2,3-O-isopropylidene-5-O-tosyl-*D*-ribofuranoside (64, R=Me) which condenses with AIC in the presence of sodium hydride to yield the ribofuranoside (65).

The only other reference to AIC reacting exclusively at ring nitrogen to form a 1-substituted derivative is also described by a Japanese patent. Enoki¹³⁸ outlines a general preparative procedure for the introduction of an alkylester group at N-1 of AIC. The patent quotes the reaction between methylchloroformate (66) and AIC in tetrahydrofuran at low temperature, followed by treatment with hydroxide to give the imidazolylformate (67) in 90% yield as summarised in Scheme 1.18.



Scheme 1.17

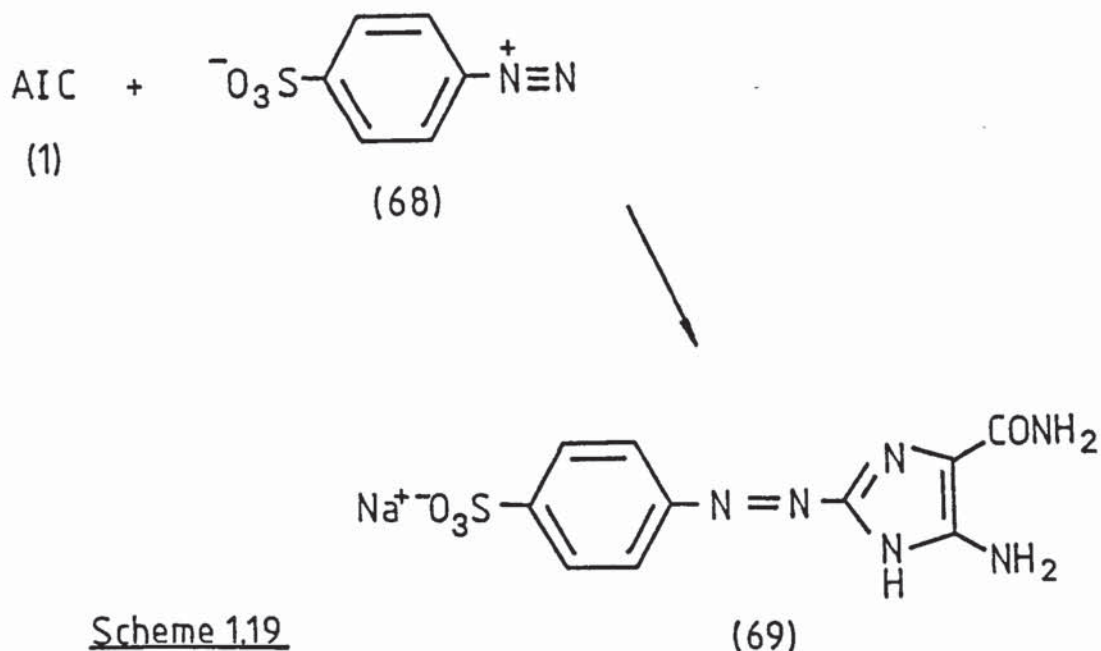


Scheme 1.18

1.3.3 Reactions involving C-2 of the imidazole ring.

The carbon atom at C-2 of AIC is deactivated to electrophilic attack by the azomethine nitrogen: however, this effect is somewhat counteracted by the other ring nitrogen and this, in conjunction with the amino group at C-5, results in an overall activation of C-2 to electrophilic substitution. AIC will couple with aryl diazonium salts to give 2-substituted arylazo-dyes, whereas the corresponding 5-nitroimidazole will not couple with the weakly electrophilic diazonium species. Diazo coupling will only occur in appropriately buffered solutions and it has been suggested that the reaction proceeds *via* electrophilic attack on low equilibrium concentrations of the N-deprotonated imidazolyl anion!¹³⁹ the reaction follows second order kinetics.¹⁴⁰

Burian¹⁴¹ had earlier suggested that such azoimidazoles must be N-azo derivatives because he found it was necessary to have a free imino group to demonstrate coupling. However, Pauly¹⁴² had independently elucidated the structure of such compounds as C-azo derivatives. The use of diazotised sulphanilic acid (68) to form deeply coloured azo-dyes is now known as the "Pauly diazo test" and it provides a basis for the identification, and in some cases, the quantitative estimation of imidazoles (Scheme 1.19). Although the majority of these azo products are red/orange in colour, AIC, when coupled, gives a unique dark blue colour when it is developed on tlc. plates with sodium carbonate solution.¹⁴³ It was suggested by Ames¹⁴⁴ that such a distinct colour could be used for quantitative estimation of this biochemically important base. This reaction has as yet, only been used qualitatively and no preparative procedure for isolation of the dye (69) has been recorded. Brondeau¹⁴⁵ has also coupled a diazonium salt, Fast Blue B, to AIC while investigating the various 2-azo

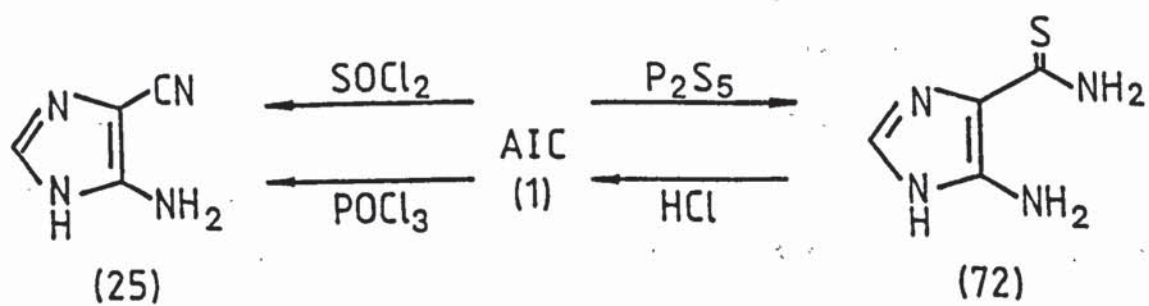
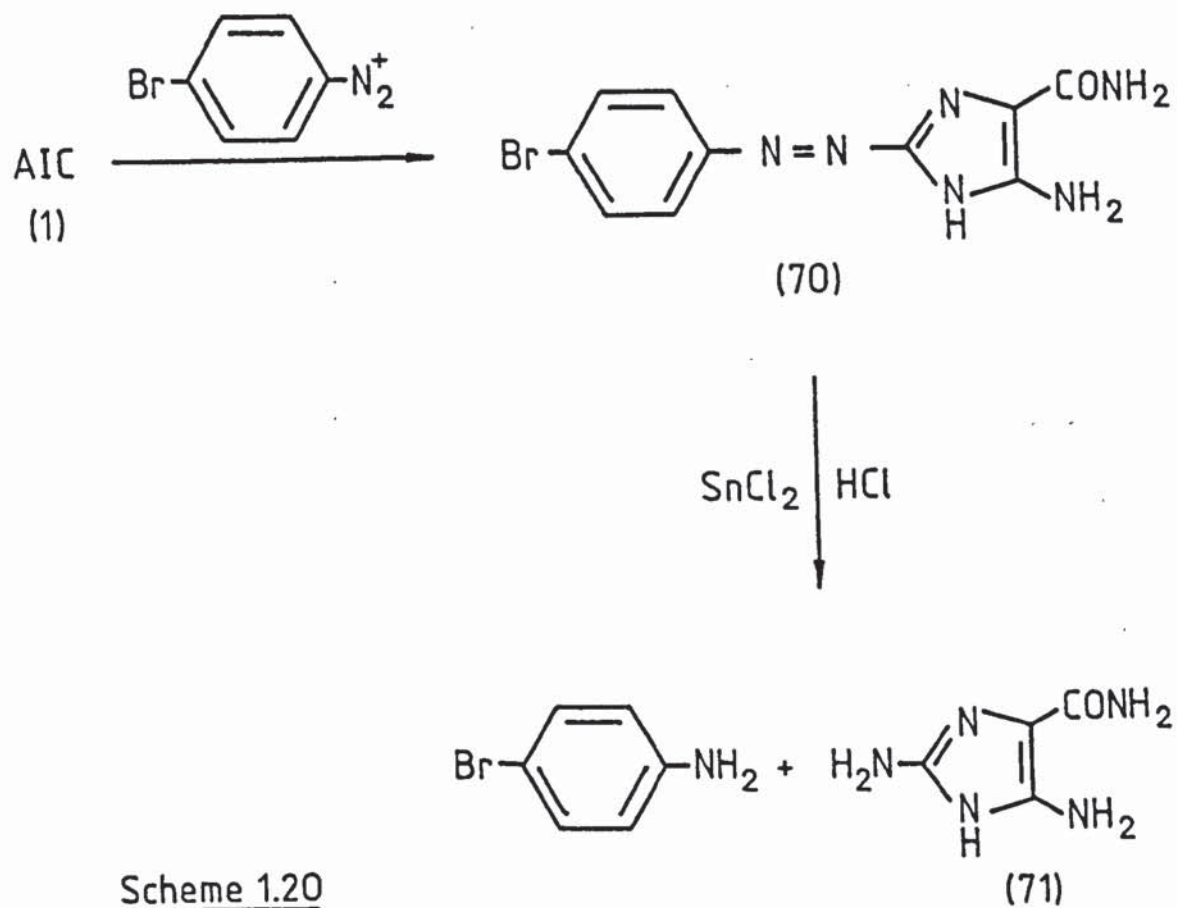


derivatives of histamine, another biologically important imidazole. The resultant brown azo-dye was not isolated, but its electronic absorption spectrum displayed an azo chromophore at 535 nm.

The only reported isolation of a 2-azo-AIC derivative is by Hirata *et al*⁸⁴ in 1955. They isolated, and purified the hydrochloride salt of a red azo-dye (70) after the diazotisation and subsequent coupling of 4-bromoaniline (Scheme 1.20). Furthermore, they then claim to have reduced the azo bond with stannous chloride in hydrochloric acid following a procedure devised by Farger and Pyman.⁹⁶ The resultant amine, 2,5-diaminoimidazole-4-carboxamide (71) was allegedly isolated as the dihydrochloride salt (25-30%).

1.3.4 Modification of the 4-carboxamide function.

Dehydration of the carboxamide group was successfully achieved by Ferris and Orgel^{90,91} when they were investigating the synthesis of



Scheme 1.21

AIC from the aminonitriles (1.2.1.3). They found that thionyl chloride in pyridine would dehydrate the carbamoyl compound back to starting material, 5-aminoimidazole-4-carbonitrile (25) (Scheme 1.21). Phosphorus oxychloride¹⁴⁶ is an alternative dehydrating agent which will effect this transformation.

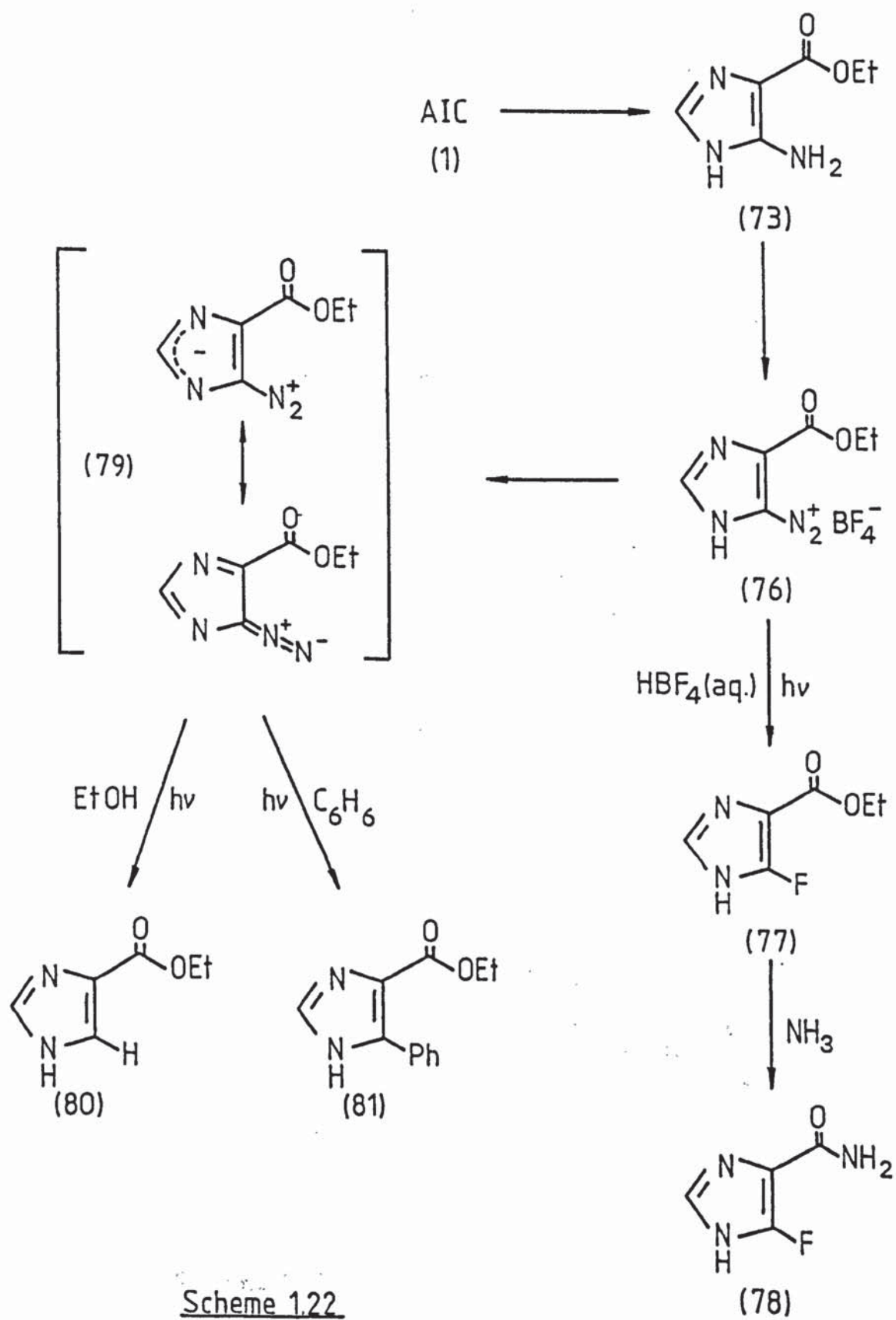
The sulphur analogue of AIC, 5-aminoimidazole-4-thiocarboxamide (72) has been prepared directly from the carboxamide¹⁴⁷ (Scheme 1.21) with phosphorus pentasulphide in refluxing pyridine. This thiocarboxamide reverts to the oxygen analogue when treated with dilute hydrochloric acid on a steam bath.

Kirk¹⁴⁸ has converted AIC to the ester (73) (50-60%) by exposure of AIC to 5-10 equivalents of anhydrous methane sulphonic acid in refluxing ethanol over a period of 2-3 weeks (Scheme 1.22).

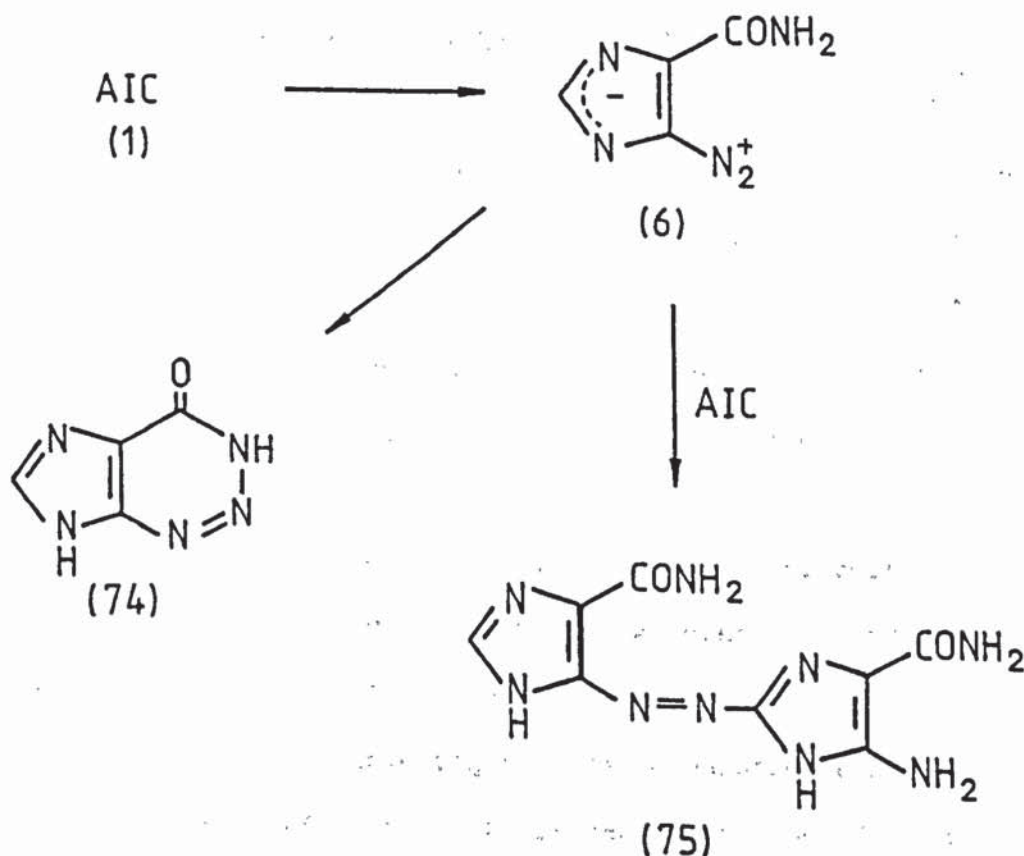
1.3.5 Reactions of the 5-amino group.

AIC has been detected in bacterial culture media and biological fluids by a test system which is dependent upon diazotisation of the 5-amino function to form a stable diazonium salt. This test, known as the Bratton-Marshall¹⁴⁹ test, involves coupling of the diazonium salt to N-(1-naphthyl)ethylenediamine to develop a deep red colour for colorimetric estimation. In order to ensure other amines, such as the sulphonamides in bacterial culture media, were not interfering it was first necessary to acetylate all other amines. AIC is known not to acetylate under neutral conditions,^{7,150} and so an accurate estimation of AIC can be obtained by this method.^{13,41}

The reaction of AIC with sodium nitrite in hydrochloric acid had, at first, been thought to furnish 2-azahypoxanthine (imidazo [4,5-d]-[1,2,3]triazin-7(6H)-one, 74) directly in high yield.¹⁵¹ This azapurinone



had first been isolated as the monohydrate by Stetten and Fox¹ when they diazotised AIC obtained from biological sources. However, Shealy and co-workers⁵⁰ proved that the initial product of diazotisation was the diazo derivative (6, Diazo-IC) which is best represented by structure (6) (Scheme 1.23) because it exists as an internally resonance-stabilised diazo compound. It is obtained as stable, buff coloured needles which, if carefully dried in the dark, can be stored for a period of years. It should be noted here that care is necessary to ensure that no local concentration of the amine occurs during diazotisation, otherwise coupling

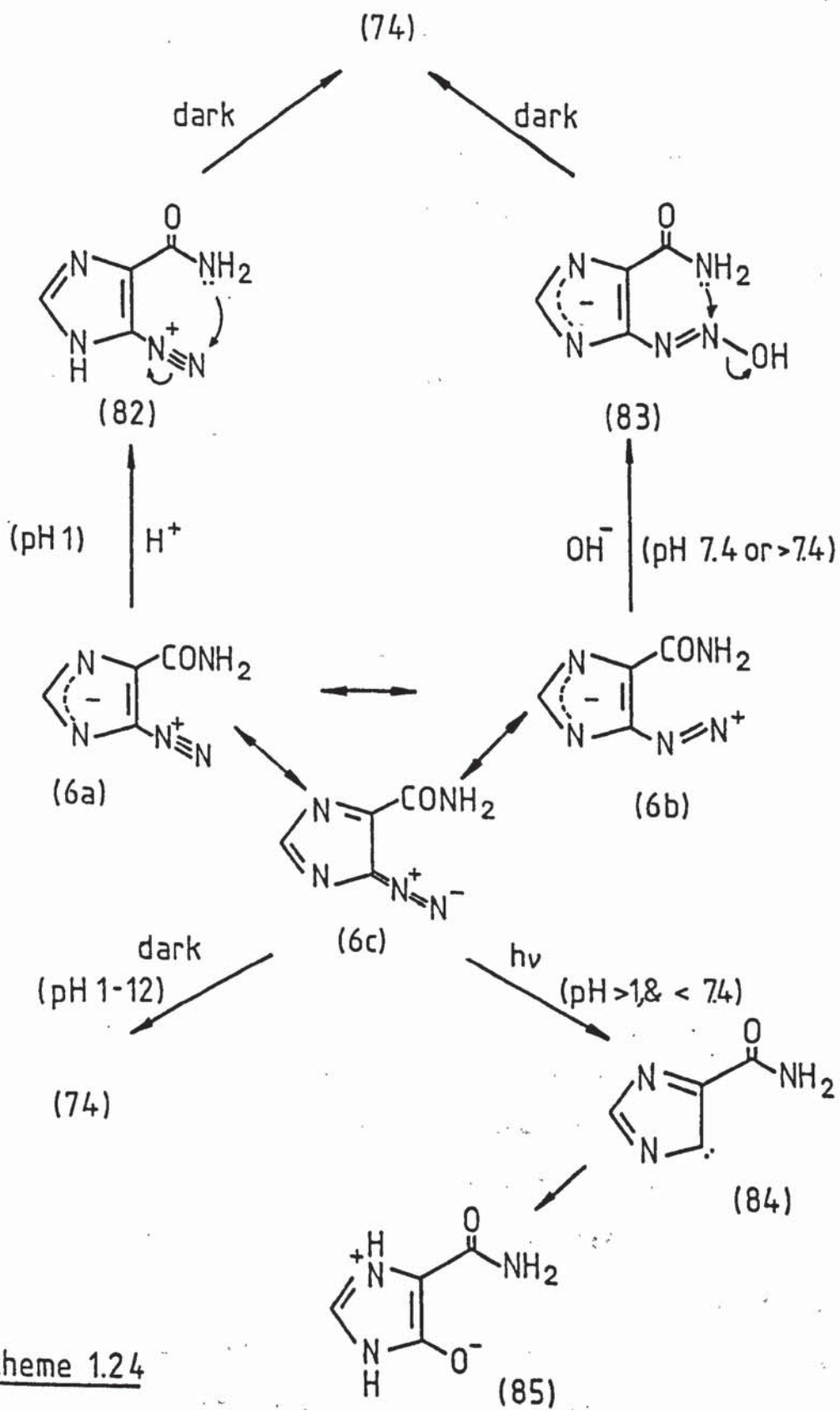


Scheme 1.23

occurs at the activated C-2 of AIC (1.3.2) resulting in a deep purple azo-dye (75).

Kirk¹⁴⁸ was interested in the diazo chemistry of AIC as a route to the halogenoimidazoles but found that conventional Sandmeyer-type routes to these compounds were not applicable. This was because of the intramolecular cyclisation of Diazo-IC to 2-azahypoxanthine, which proceeds at a faster rate than photofluorination. Therefore he diazotised the amino ester (73) to yield the stable diazonium fluoroborate (76) without intramolecular cyclisation occurring. Since diazonium species are known to undergo facile photoextrusion of nitrogen,¹⁵² Kirk trapped the reactive intermediate (cation) by irradiation in aqueous tetrafluoroboric acid to yield ethyl 5-fluoroimidazole-4-carboxylate (77). The fluorine atom is not subject to nucleophilic displacement and so the carboxamide (78) may be readily obtained with ammonia. It was also found that photoextrusion of nitrogen could be achieved in other solvents to give a variety of 5-substituted derivatives of the ester: for example ethyl imidazole-4-carboxylate (80) and ethyl 5-phenylimidazole-4-carboxylate (81) are the products obtained in ethanol and benzene respectively (Scheme 1.22), possibly *via* a radical process.

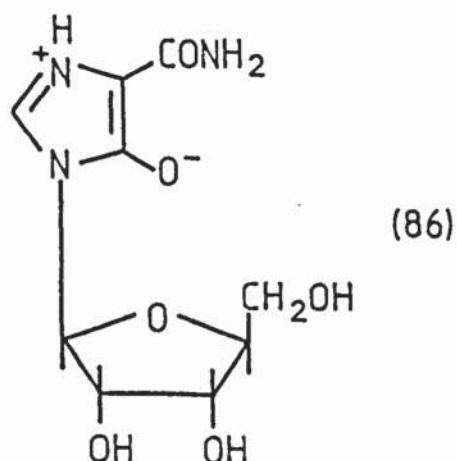
Shealy proposed a series of dipolar structures or zwitterions to represent Diazo-IC (6 a-c), and it has been suggested by Horton and Stevens¹⁵³ that these forms are important in the explanation of reactions concerning Diazo-IC. They have investigated the pH dependence of the intramolecular cyclisation of Diazo-IC to 2-azahypoxanthine (74), and conclude that in the dark this reaction occurs over a pH range of 1-12, to furnish the azapurinone. This is in agreement with previous reports.⁵⁰ Also, as expected, at pH 1 and pH > 7.4, 2-azahypoxanthine was obtained in



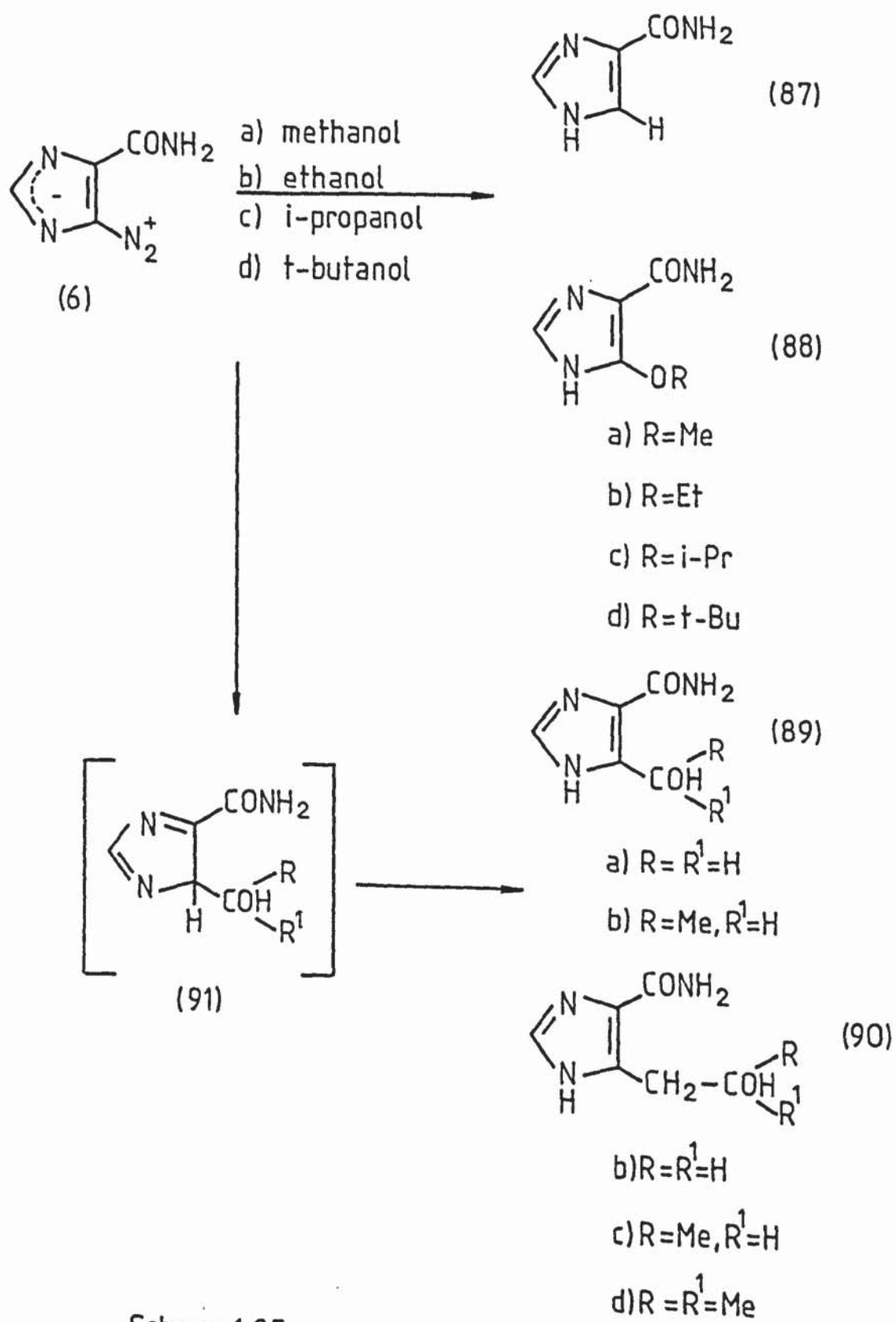
Scheme 1.24

the light, presumably *via* the protonated imidazole diazonium ion (82) and the imidazole diazohydroxide (83) respectively (Scheme 1.24). This is a light catalysed reaction, and the rate of 2-azapurinone formation was found to increase with increasing pH.

Strangely within the intermediate pH range (> 1 and < 7.4) another product was obtained, 4-carbamoylimidazolium-5-olate (85).^{72,154} This product was formed in an exclusively photochemical process and the immediacy of a reactive carbene (84) was proposed. This imidazolium olate is of considerable interest in the field of cancer chemotherapy because it is the aglycone component of the antitumour antibiotic bredinin (86).^{155,156}



Kang¹⁵⁷ has also investigated the photolysis of Diazo-IC; at 20° in alcohols he noted a rapid evolution of nitrogen and the formation of C-H bond insertion products, as well as the expected imidazole-4-carboxamide (87), and the 5-alkoxyimidazole-4-carboxamides (88) (Scheme 1.25). 5-(Hydroxyalkyl)imidazole-4-carboxamides (89 and 90) can be isolated in yields up to 80%. The products are probably formed by interaction of the carbene (84) with the appropriate alcohol, and proceed *via* an intermedi-



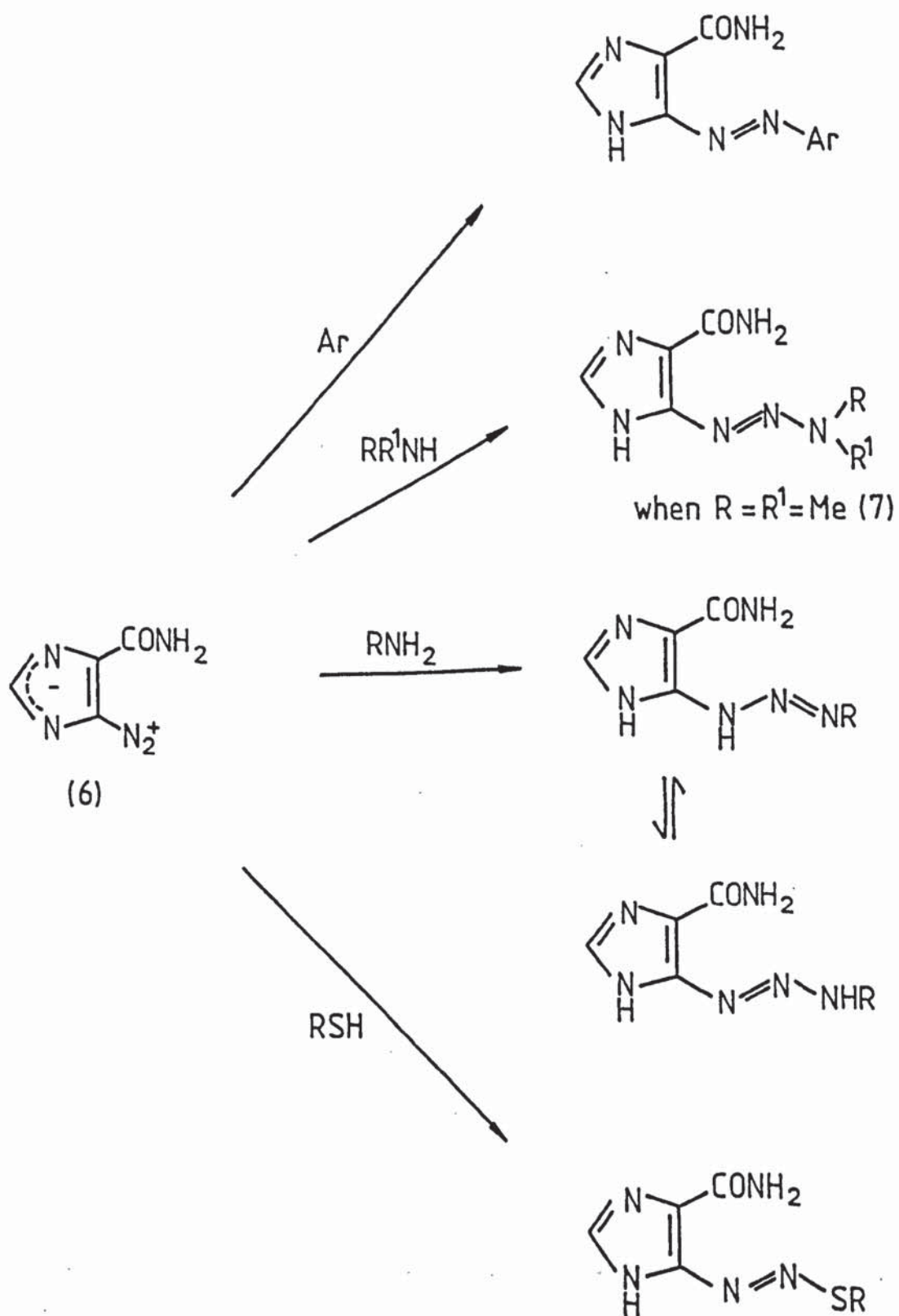
Scheme 1.25

ate (91), with resultant [1,5] sigmatropic hydrogen rearrangement.

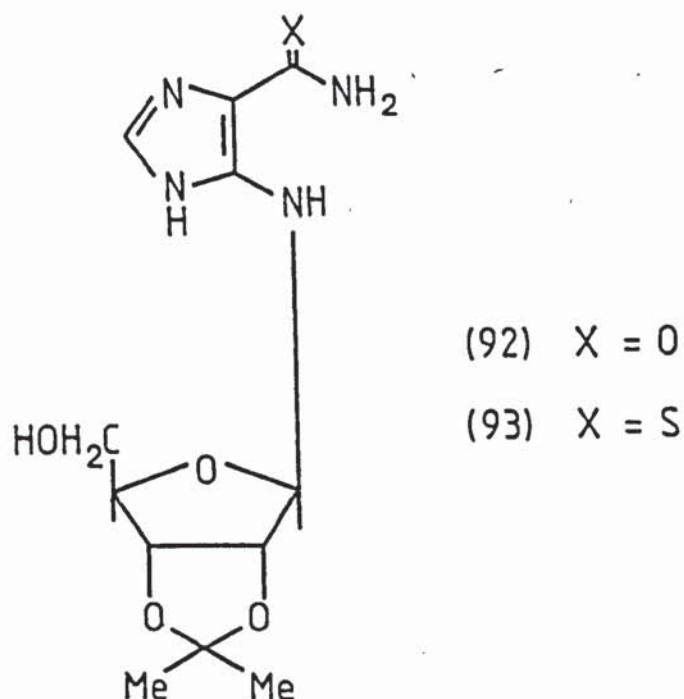
The thermolytic behaviour of Diazo-IC was also studied by Kang and shown to occur efficiently (~100%) in both primary and secondary alcohols at 60°. Again loss of nitrogen was observed with the concomitant formation of imidazole-5-carboxamide (87) and to a lesser extent the alkoxyimidazole carboxamides (88).

Diazo-IC will couple readily with activated aromatic substrates, amines, thiols and other nucleophiles (Scheme 1.26), and the products of such reactions have been reviewed by Shealy⁴⁹ and Stevens.⁵¹ Coupling with amines has been of considerable interest because of the antitumour activity demonstrated by 5-(3,3-dimethyltriazene-1-yl)imidazole-4-carboxamide (DTIC, 7). Many other triazenoimidazoles have been synthesised,^{52, 158-163} and evaluated for antitumour activity. Stability studies of the triazenoimidazoles have confirmed some to be photolabile, and in particular DTIC will release Diazo-IC in the light.^{60, 158} The liberated Diazo-IC can then cyclise to 2-azahypoxanthine (74) or form the imidazolium olate (84) depending on the conditions.

Other reactions involving the amino group at C-5 are generally involved in the cyclisation to hypoxanthine and its derivatives, and are covered in the following section (1.3.6). However, De Gourcy¹⁶⁴ has recently prepared a sugar derivative of AIC by condensation of this imidazole with 2,3-O-isopropylidene-D-ribofuranose to yield the glycosylamine (92). The thiocarboxamide (93) has also been prepared.



Scheme 1.26

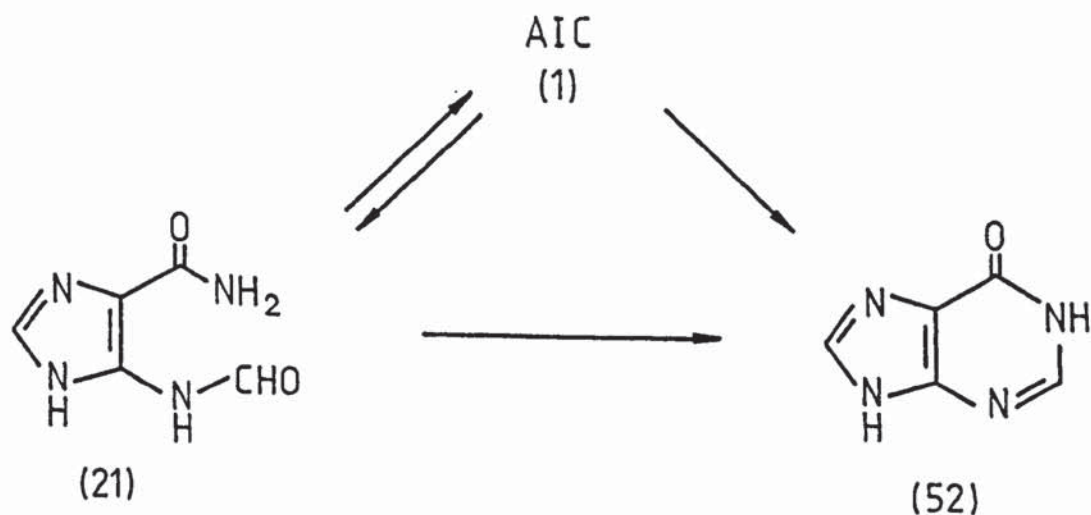


1.3.6 Conversion to purines.

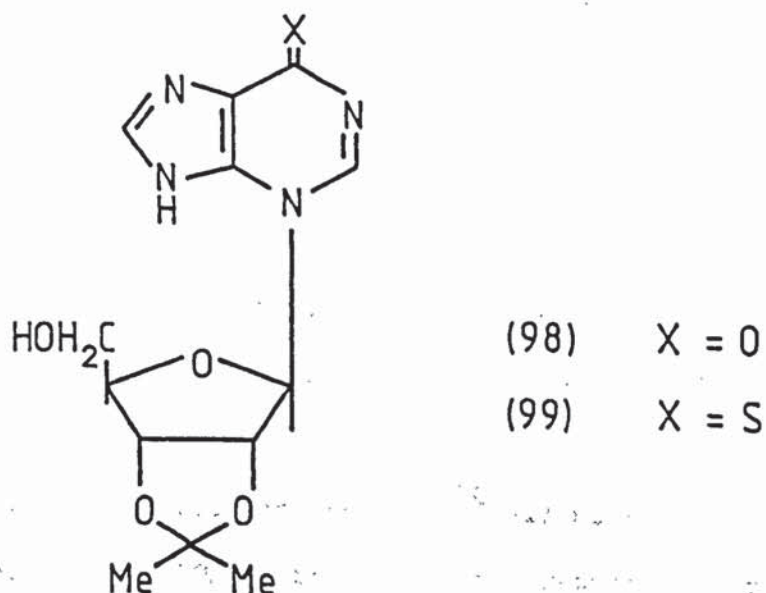
It has been previously stated that one reason for the widespread interest in the synthesis of AIC derivatives was their conversion, by pyrimidine ring closure, to purines. Initially it had been noted by Shaw⁸⁰ that AIC would form hypoxanthine (52) when heated in formamide at 185°. The stepwise course of the reaction was indicated by isolation of the intermediate 5-formylaminoimidazole-4-carboxamide (21) when the reaction was conducted at the lower temperature of 150° (Scheme 1.27). The formylamino intermediate may also be prepared by the action of formic acid in acetic anhydride on the parent amine. Cyclisation may be easily effected by weakly basic conditions, even bicarbonate solutions will suffice.⁸⁵ Ring closure also occurs in non-aqueous media in the presence of ethanolic sodium ethoxide. Dilute acids, on the other hand, induce hyd-

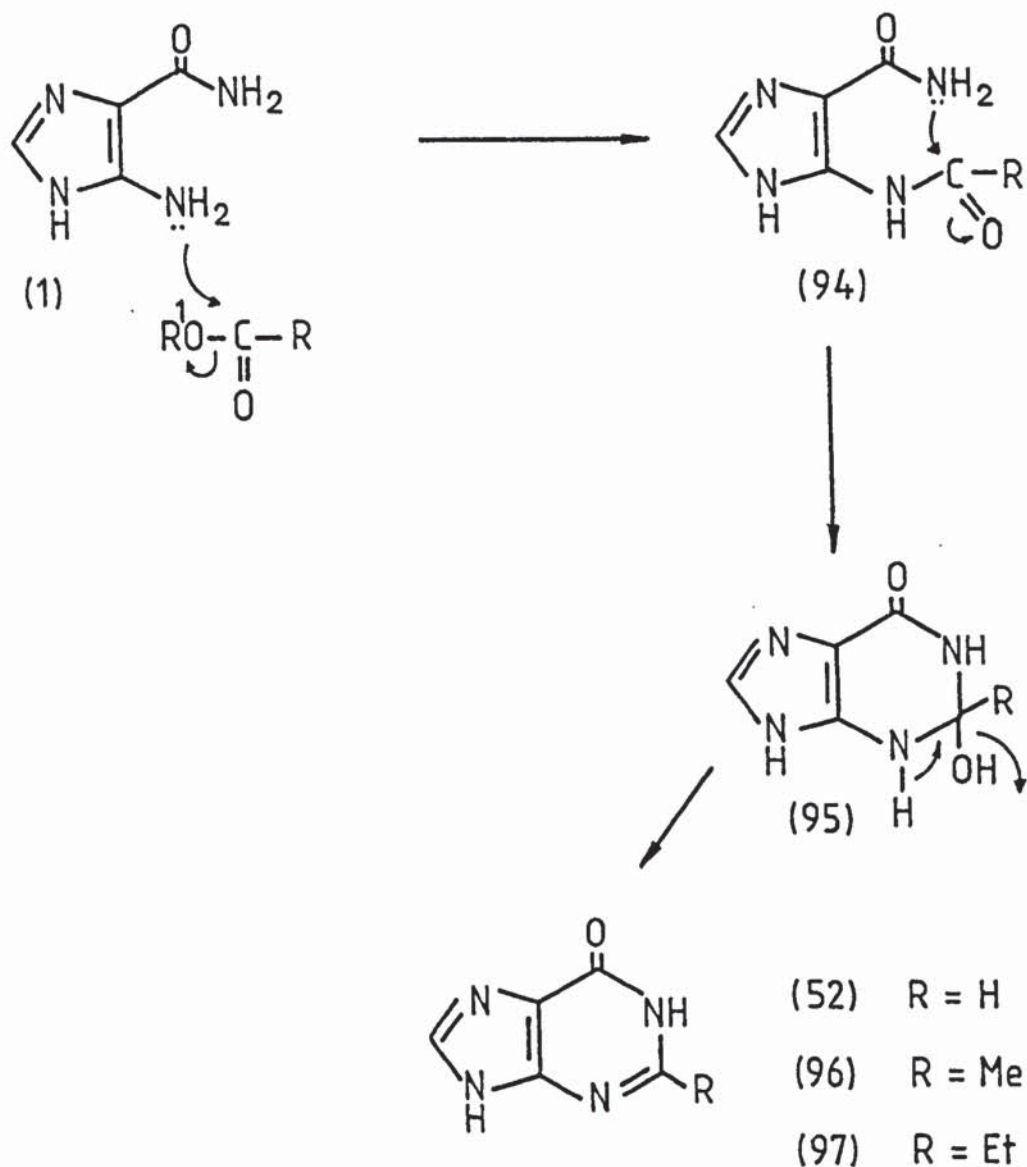
rolysis⁷⁹ to the amine.

Alkylesters and orthoesters (trialkyl) are commonly used as a way of introducing a one carbon unit between two nitrogen atoms (essentially a Traube synthesis) to give the purines. Both hypoxanthine⁸¹ and 2-alkylhypoxanthines¹⁶⁵ (96,97) have been prepared in this manner (Scheme 1.28).



Scheme 1.27

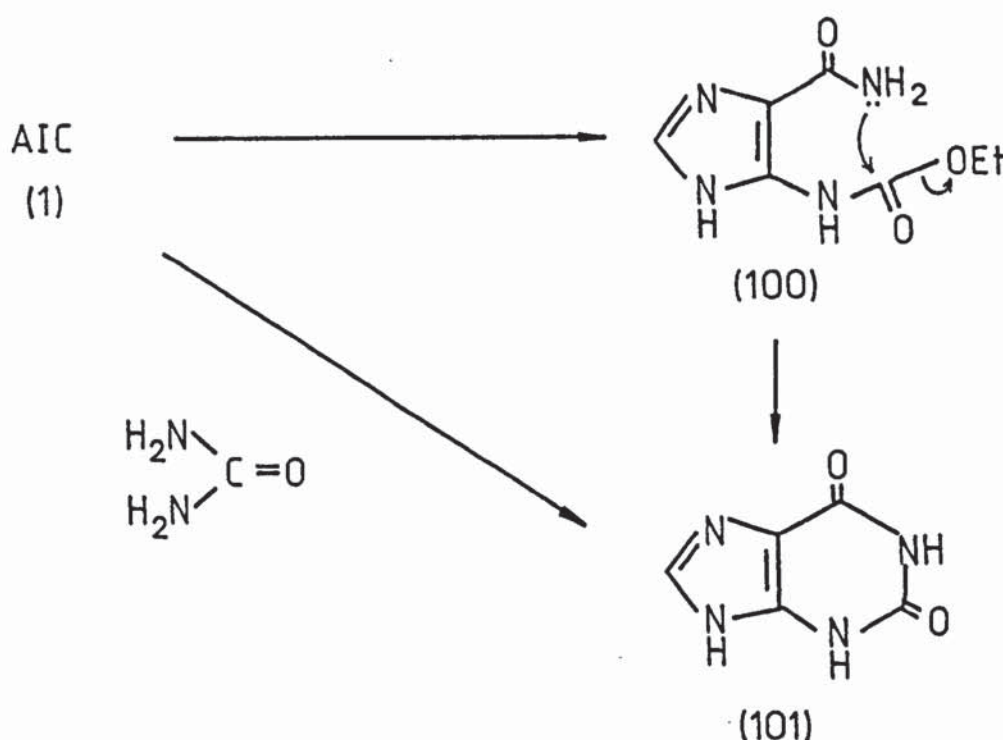




Scheme 1.28

De Gourcy¹⁶⁴ has also used this route to cyclise his previously prepared glycosylamines (92,93) to the hypoxanthosine (98) and hypoxanthiosine (99) derivatives.

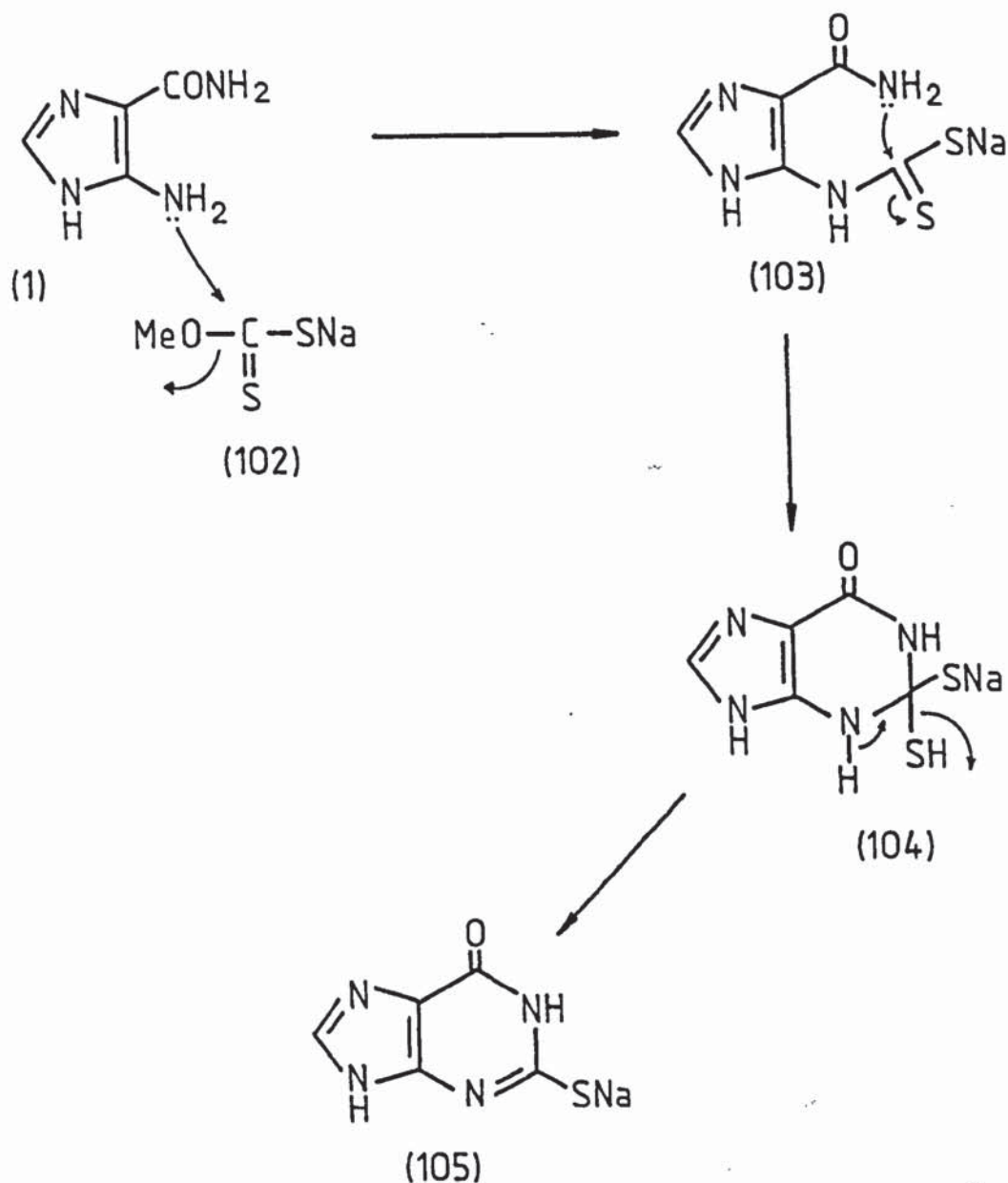
The choice of ethylchloroformate⁸⁰ allows a low temperature condensation reaction with AIC to take place, the resultant carbethoxy-amidoimidazole (100) may then be ring closed to xanthine (101) by heat or alkali (Scheme 1.29). Fusion of AIC with urea will also yield xanthine



Scheme 1.29

(75%). The latter reaction was first performed by Statton and Fox¹ on the base they had isolated from bacterial cultures before its chemical nature had been determined.

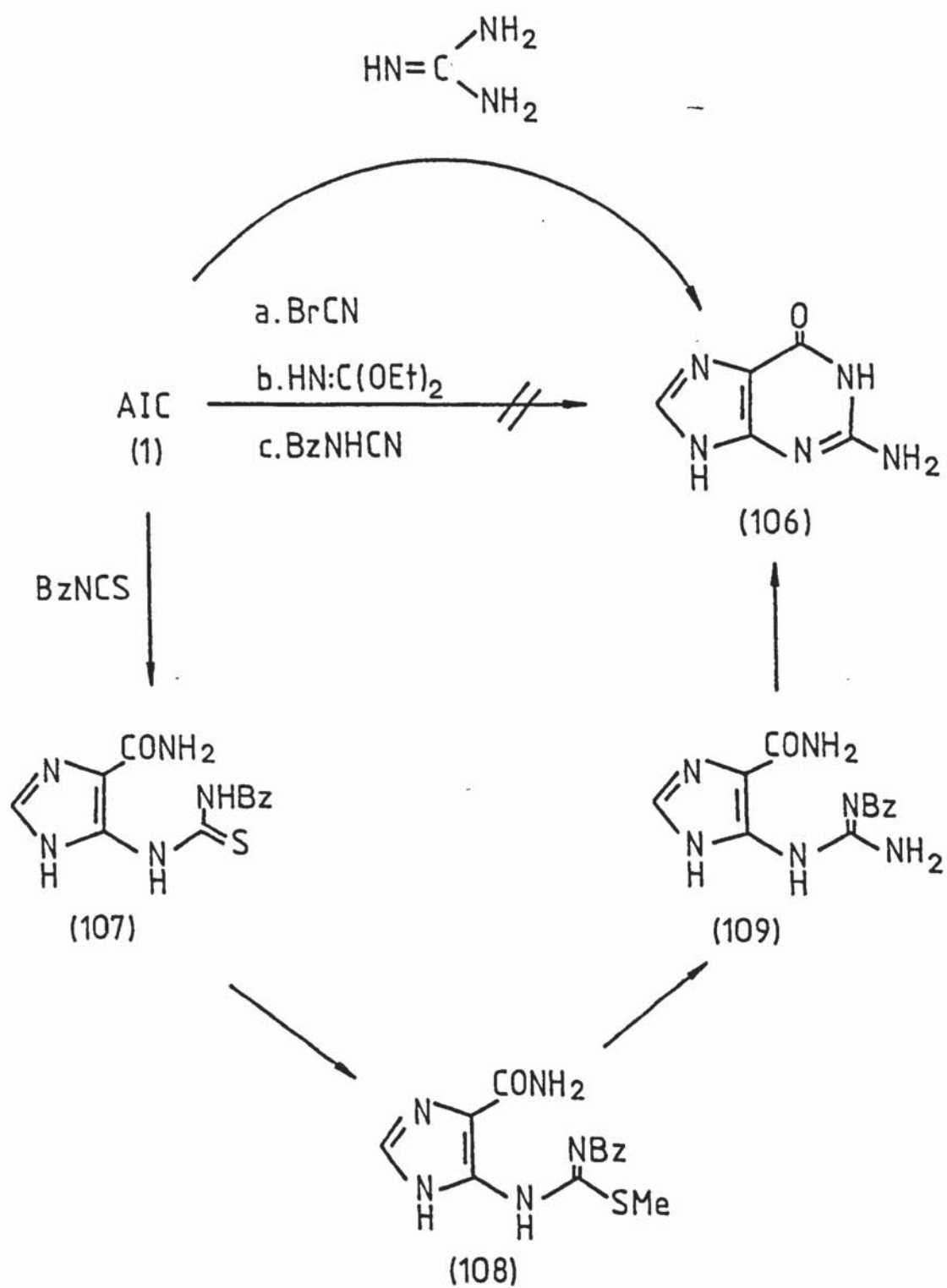
The insertion of a carbon unit in this manner obviously has wide practicability to introduce a group at the 2-position of the purine ring system. We have already seen that with esters a 2-alkyl group may be introduced. Similarly, 2-trifluoromethylhypoxanthine¹⁶⁶ is obtained from trifluoroacetamide and AIC. A workable route to the 6-oxo-2-thiopurines (105) can be followed by the treatment of AIC with carbon disulphide in alcoholic base.¹⁶⁷ The xanthate ester (102) is generated *in situ* and presumably the reaction proceeds by way of the intermediates (103 and 104) to yield the sodium salt of 2-thiohypoxanthine (105). This is also the product when crystalline sodium methylxanthate is used in



Scheme 1.30

DMF¹⁶⁸ (Scheme 1.30). The 2-thio derivative can be methylated on sulphur with methyl iodide or desulphurised to hypoxanthine with Raney nickel.

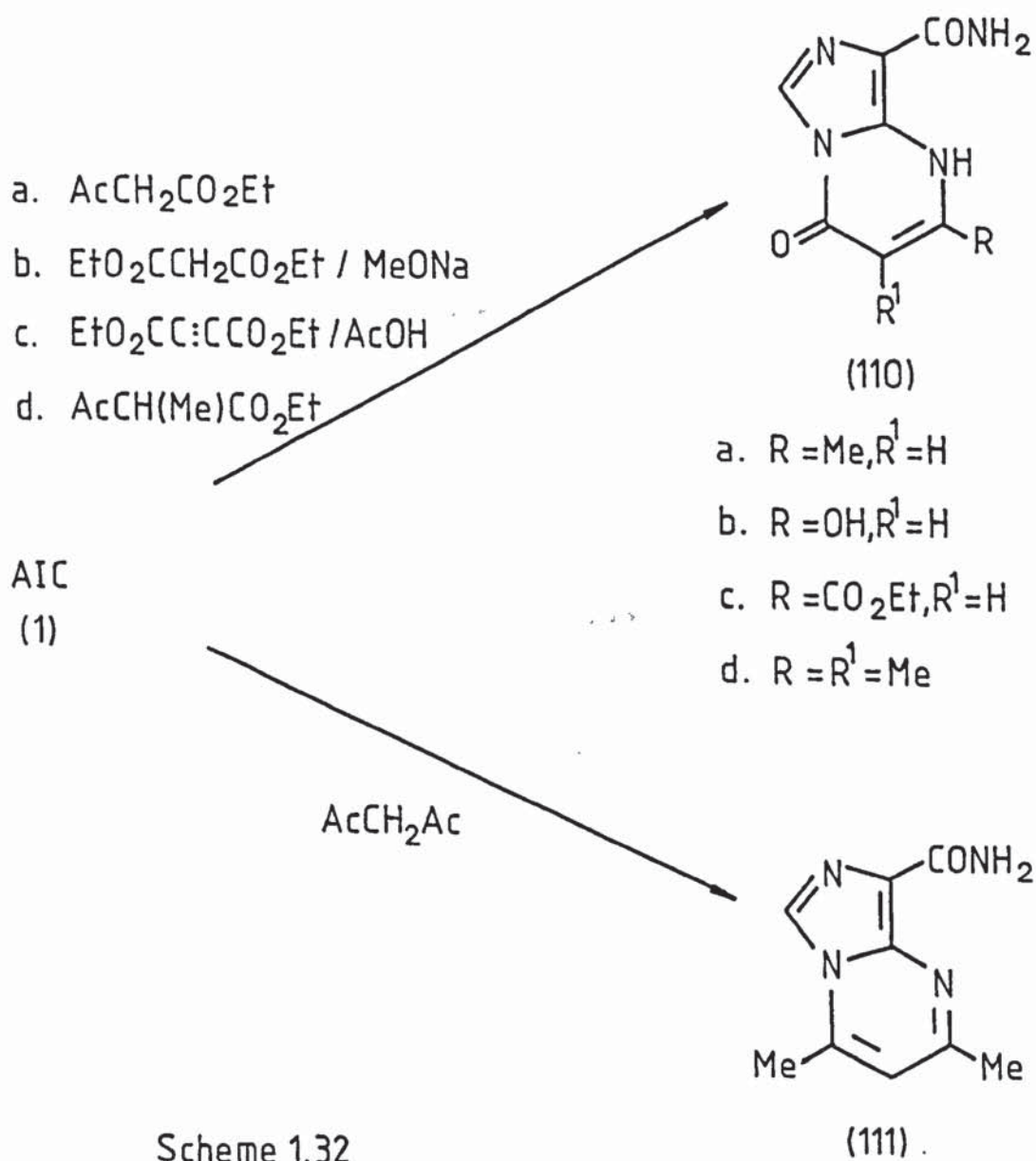
Isocyanates have been used to effect ring closure of AIC. Stanovnik¹⁶⁹ has used this as a route to the xanthines, while Okutsu¹⁷⁰ utilised isothiocyanates to introduce a substituted amino group in position 2. Later Yamazaki,¹⁷¹ who found he could not synthesise guanine



Scheme 1.31

(106) by direct ring closure with reagents such as cyanogen bromide, ethylimidocarbonate or benzoyl cyanamide, also used isothiocyanate to introduce the 2-amino group (Scheme 1.31). After initial formation of the benzoylthiourea (107), methylation with methyl iodide afforded the S-methyl derivative (108) from which the guanidinyll compound (109) could be easily prepared with ammonia. Treatment of the product with alkali yielded the 2-aminopurine. A more direct route involves fusion of guanidine with AIC which, it is claimed, yields 80% guanine hydrochloride¹⁷² after treatment with hydrochloric acid.

Finally an interesting reaction of AIC with β -dicarbonyl compounds has been investigated by Novinson.¹⁷³ The products isolated were the imidazo[1,5- α]pyrimidine-3-carboxamides (110), ring closure occurring in this case through the 5-amino group and the imidazole ring nitrogen. Thus the product obtained from condensation with ethylacetoacetate is 3-carbamoyl-5-methylimidazo[1,5- α]pyrimidin-7(4H)-one (110a). Condensation with the β -diketone, acetylacetone, yielded the corresponding dimethylimidazo[1,5- α]pyrimidine (111). These reactions are summarised as Scheme 1.32.



Scheme 1.32

Discussion of Experimental Results

Legend

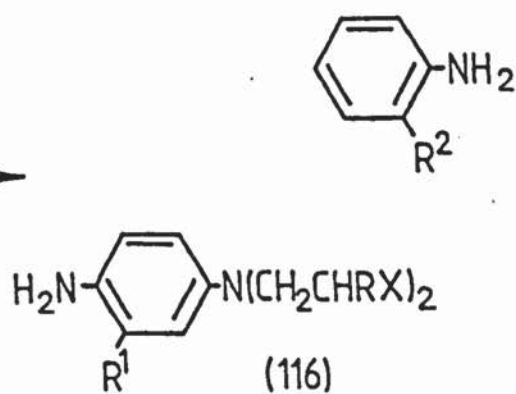
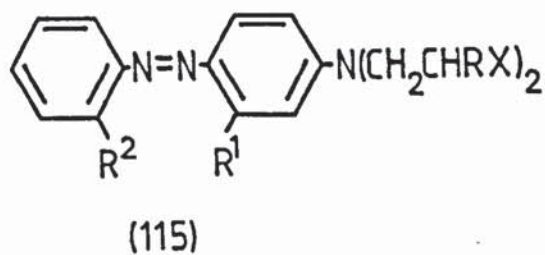
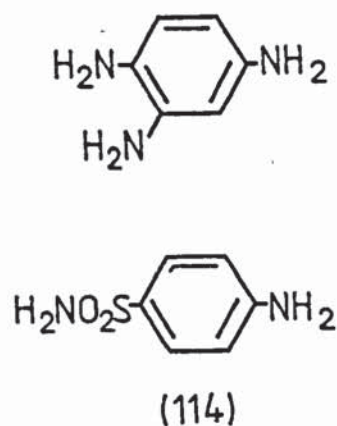
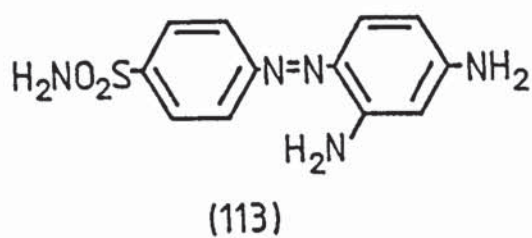
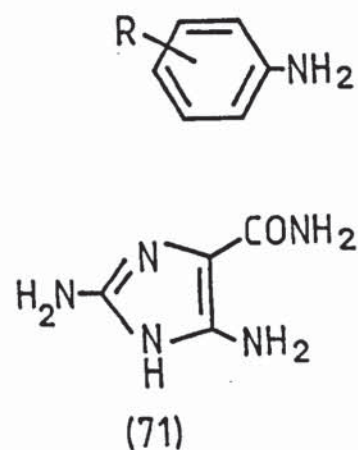
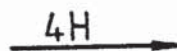
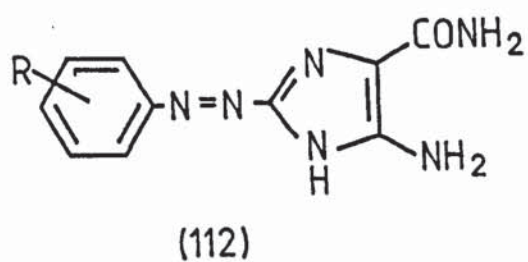
AIC	5-Aminoimidazole-4-carboxamide.
AICAR	AIC ribotide.
DMF	Dimethylformamide.
DMSO	Dimethylsulphoxide.
DNA	Deoxyribose nucleic acid.
DTIC	5-(3,3-Dimethyltriazene-1-yl)imidazole-4-carboxamide.
i.m.	Intramuscular.
i.p.	Intraperitoneal.
i.r.	Infrared.
%I.S.T.	Percentage increase in survival time.
L1210(CR)	Lymphocytic leukaemia, resistant to cyclophosphamide.
L1210(R)	Lymphocytic leukaemia, resistant to nitrosoureas.
L1210(S)	Lymphocytic leukaemia, sensitive to nitrosoureas.
M5076	Reticulum cell sarcoma.
m.p.	Melting-point.
m.s.	Mass spectrometry.
n.m.r	¹ H nuclear magnetic resonance.
P388	Lymphocytic leukaemia.
p.p.m.	Parts per million.
RNA	Ribose nucleic acid.
s.c.	Subcutaneous.
t _{1/2}	Half-life.
t.l.c.	Thin layer chromatography.
TLX5(R)	Thymus derived lymphoma, resistant to nitrosoureas.
TLX5(S)	Thymus derived lymphoma, sensitive to nitrosoureas.
TMS	Tetramethylsilane.
u.v.	Ultraviolet.

2.1 Synthesis and Characterisation of 5-Amino-2-arylazoimidazole-4-carboxamide Derivatives.

2.1.1 Rationale and synthesis.

AIC is known to couple with diazonium salts in alkaline medium to form highly coloured solutions.¹⁴³⁻¹⁴⁵ This reaction has been the basis for detection and estimation of this biologically important imidazole. However, there has been only one published report concerning the isolation and characterisation of the azo-dye produced.⁸⁴ Subsequently, it was claimed that cleavage of the azo bond of the arylazoimidazole (112; R=Br) was effected by a reductive process to yield the novel 2,5-diaminoimidazole-4-carboxamide (71) along with the substituted aniline (Section 1.3.3). Considering the importance of AIC ribotide in *de novo* purine biosynthesis it was thought that the diaminoimidazole carboxamide could be taken up and utilised by cells, and could be a candidate chemotherapeutic agent.

The biological reduction of azo derivatives occurs mainly in the liver and intestine, with the gut microflora, intestinal tissue and contents all implicated in the process. However, lipophilic azo derivatives of low water solubility are predominantly reduced by microsomal azoreductase in the liver.¹⁷⁴ The reaction pathway has been shown to proceed *via* the hydrazo intermediate. The action of hepatic azoreductase to yield the corresponding amines has been the basis for development of several biologically active compounds. The antibacterial effect of prontosil (113) was demonstrated to be due to the reductive cleavage of an azo bond to release sulphanilamide (114).^{175,176} The principle of "latent activity" has also been applied in the field of cancer chemotherapy. The mustard fragment in the azo compound (115) is chemically unreactive due



Scheme 2.1

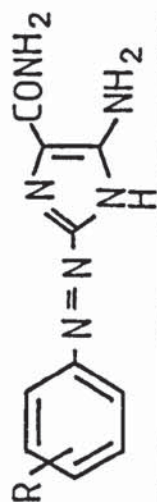
$R, R^1 = H, Me$; $R^2 = H, CO_2H$; $X = \text{Halogen}$.

to the deactivating influence of the azo bond. Reductive cleavage of the N=N bond releases short lived alkylating agents^{177,178} of the general structure (116) (Scheme 2.1) which may be of value in treating tumours at the site of activation (ie. the liver).

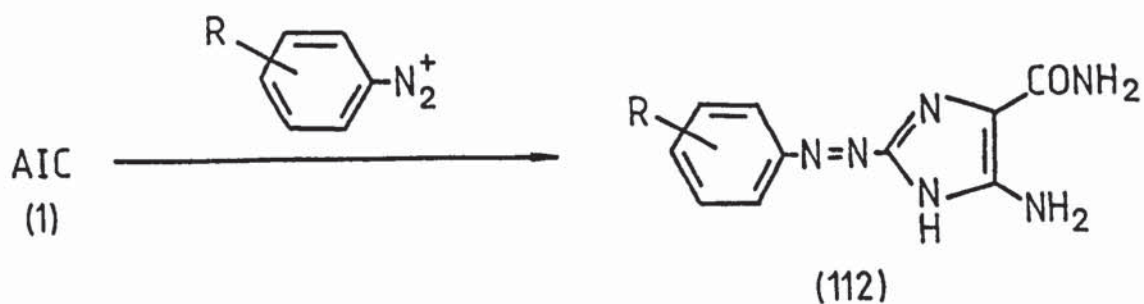
It was envisaged that the azo derivatives formed by coupling aryldiazonium salts at the C-2 position of AIC would, upon hepatic azo reduction, release the potentially active diaminoimidazole carboxamide. Therefore a series of such azo derivatives, of general structure (112), were prepared by coupling aryldiazonium salts with AIC (Scheme 2.2). Sodium acetate was used as a buffer because diazo-coupling will not occur in strongly acidic solution. Physical characteristics of these dyes are compiled in Table 2.1. The dried products were generally deep orange-red solids. Purification by crystallisation from organic solvents proved difficult unless water was present, but from aqueous methanol most dyes formed bright orange-brown needles or plates of metallic appearance. Karl-Fischer microanalysis confirmed the presence of variable amounts of water in the crystalline state. This was also the case when aqueous acetone was employed for crystallisation. The purple crystalline products generally analysed as monohydrates. Removal of water of crystallisation was achieved by prolonged heating at 100°. The remaining derivatives were purified by recrystallisation from DMSO or DMF to yield red-purple products. The sulph-onamide (128) crystallised from DMF with one mole of solvent of crystallisation.

One exception remained, the 4-methoxy derivative (125), which could only be recrystallised from aqueous ethanol (yellow needles). The yellow colour was quite distinct from the orange-red colour range of the other derivatives. However, the u.v.-visible spectrum showed that this dye

Table 2.1 Physical characteristics of arylazoimidazoles (112)



Compound number.	R	Yield (%)	M.p. ^o	Crystallisation solvent.	Appearance.
(70)	4-Br	58	282(decomp.)	Methanol(aq.)	orange needles.
(117)	H	93	175(decomp.)	Methanol(aq.)	orange plates.
(118)	4-Cl	75	283(decomp.)	Methanol(aq.)	orange needles.
(119)	2-NO ₂	50	310(decomp.)	DMF	red microcrystals.
(120)	3-NO ₂	83	278(decomp.)	DMF	red powder.
(121)	4-NO ₂	94	>330	DMF	purple powder.
(122)	2-CN	57	254(decomp.)	Acetone(aq.)	purple needles.
(123)	4-Me	61	261(decomp.)	Methanol(aq.)	orange plates.
(124)	4-Ac	66	244(decomp.)	DMF	purple microcrystals.
(125)	4-OMe	63	248(decomp.)	Ethanol(aq.)	yellow needles.
(126)	4-SO ₂ NH ₂	57	259(decomp.)	DMSO	purple microcrystals.
(127)	4-SO ₂ NHAc	71	218(decomp.)	DMF	pink prisms.
(128)		52	288(decomp.)	DMF	orange microcrystals.
(129)		71	271(decomp.)	Acetone(aq.)	orange needles.



(70) R = 4-Br

(122) R = 2-CN

(117) R = H

(123) R = 4-Me

(118) R = 4-Cl

(124) R = 4-Ac

(119) R = 2-NO₂

(125) R = 4-OMe

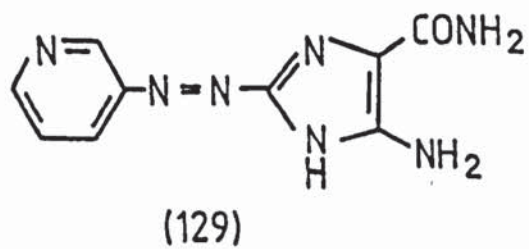
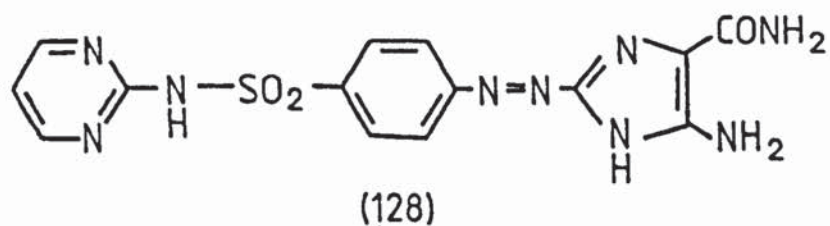
(120) R = 3-NO₂

(126) R = 4-SO₂NH₂

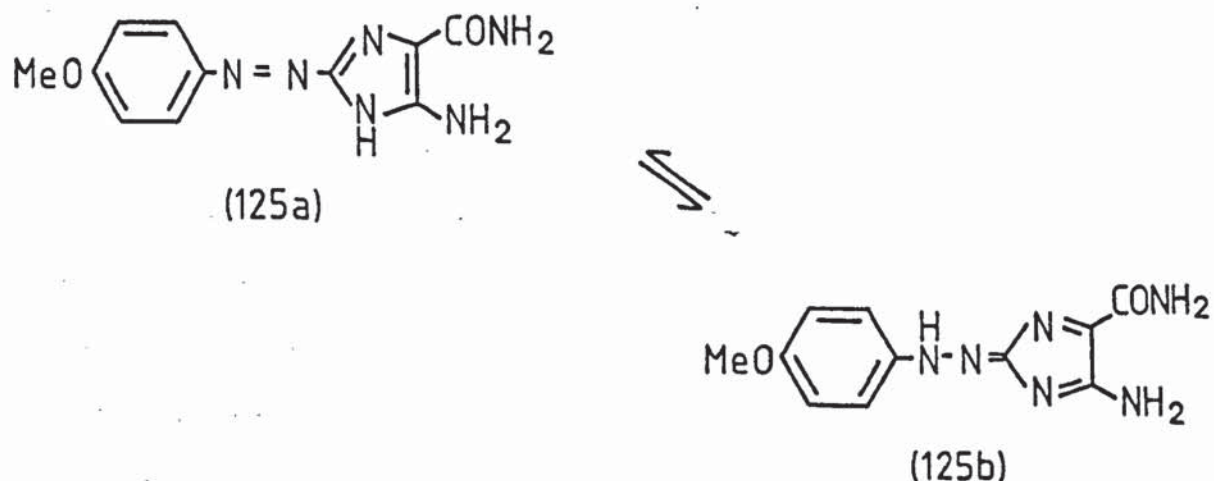
(121) R = 4-NO₂

(127) R = 4-SO₂NHAc

Scheme 2.2



had a peak of maximal absorption at 462 nm, well within the expected range for an azo bond conjugated with two aromatic systems, and comparable to the other derivatives. Possibly this methoxy derivative exists in the hydrazone tautomeric state (125b) in the crystal form, with the azo modification (125a) preferred in solution. This yellow colour was also noted



with the product formed from diazotisation and coupling of sulphanilamide (126). However, attempts to repeat this experiment were not successful, and the only result was formation of the red azo-dye. It may well be that solvent conditions (ie. degree of solvation) are important in this case.

All the dyes decomposed at their melting-points. Values quoted (Table 2.1) refer to the point at which decomposition begins; this is seen as a rapid darkening of the compound with deposition of charred particles on the walls of the capillary tube. Often the result is a charred mass and no definite melting-point can be discerned.

The azo-dyes were generally soluble in polar organic solvents but only sparingly soluble in water; the pyridin-3-yl derivative (129) showed greatest solubility in water. The compounds were insoluble in non-polar organic solvents such as ether and chloroform. The arylazoimidazoles dissolved to some extent in sodium hydroxide solution yielding red-purple

solutions, and most of the azo-dyes dissolved readily in hydrochloric acid to give stable solutions from which the hydrochloride salt could be isolated. However, the 2-cyano derivative [122] proved to be unstable under such conditions and discharge of the red colour was noted over a period of one to two hours at room temperature. Subsequent t.l.c. studies on the pale yellow solution indicated a minimum of five unidentified products, all of which fluoresced under u.v. light at 254 nm.

A striking feature of these derivatives is their behaviour in concentrated sulphuric acid—they form brightly coloured magenta solutions, presumably due to protonation of the molecule (see Section 2.1.2.2). The formation of such highly coloured solutions is a characteristic feature of all arylazoimidazoles and has been referred to as "halochromism".¹⁷⁹

2.1.2 Spectral properties.

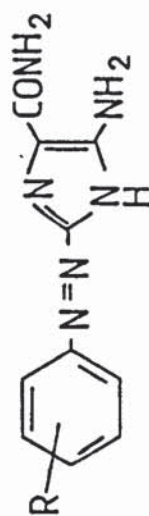
2.1.2.1 Infrared spectroscopy.

The i.r. spectra (KBr discs) of arylazoimidazoles (Table 2.2) show absorption in the $3500\text{--}3200\text{ cm}^{-1}$ region indicative of N-H stretching. These peaks tend to be part of a much larger, broader band which is the result of intermolecular hydrogen bonding. The most prominent band of the spectrum is due to carbonyl stretching (Amide I) and is found in the $1700\text{--}1650\text{ cm}^{-1}$ region, often associated with another strong absorption band at $1640\text{--}1600\text{ cm}^{-1}$. This is the N-H bending frequency of the amide (Amide II). There are also other strong bands in the 1600 cm^{-1} region indicative of N-H bending.

2.1.2.2 Ultraviolet - visible spectroscopy.

The u.v.- visible spectra (Table 2.2) of these compounds all show maxima in the range 454-490 nm which are sensitive to substituent and solvent shifts. There are also other, less intense, bands located

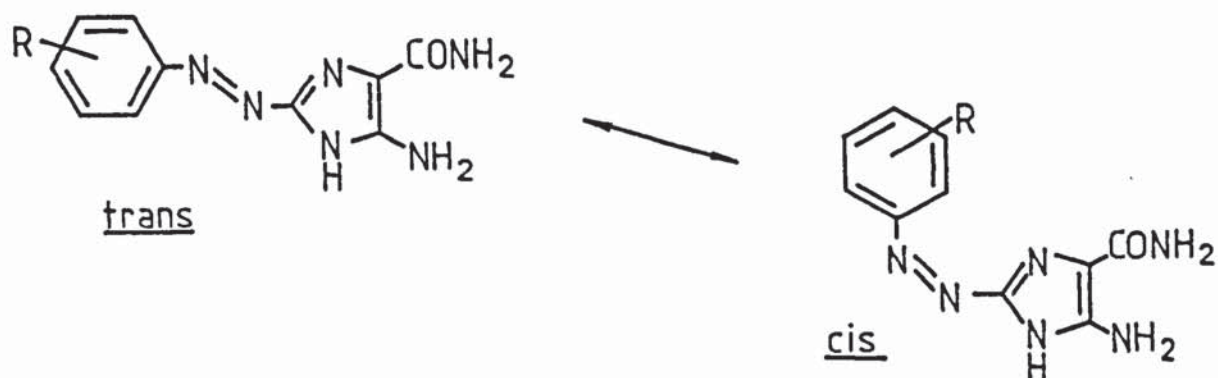
Table 2.2 U.v-visible and i.r. spectral characteristics of arylazomidazoles.



Compound number.	R	$\nu_{\max.}$ (cm^{-1}) in KBr.	$\lambda_{\max.}$ (nm).		
			A	B	C
(70)	4-Br	3450-3200(bonded NH), 1680, 1640(CONH ₂).	464	-	-
(117)	H	3400-3150(bonded NH), 1680, 1640(CONH ₂).	454	485	462
(118)	4-Cl	3450-3200(bonded NH), 1680, 1640(CONH ₂).	466	498	480
(119)	2-NO ₂	3450-3190(bonded NH), 1690, 1650(CONH ₂), 1500, 1390(NO ₂).	490	-	-
(120)	3-NO ₂	3450-3150(bonded NH), 1665, 1630(CONH ₂), 1530, 1360(NO ₂).	474	492	496
(121)	4-NO ₂	3450-3200(bonded NH), 1700, 1640(CONH ₂), 1480, 1340(NO ₂).	484	-	-
(122)	2-CN	3450-3200(bonded NH), 2250(CN), 1660, 1595(CONH ₂).	486	-	-
(123)	4-Me	3400-3150(bonded NH), 1670, 1630(CONH ₂).	458	495	468
(124)	4-Ac	3500-3250(bonded NH), 1650, 1640(CONH ₂ , Ac).	490	-	-
(125)	4-OMe	3400-3200(bonded NH), 1650, 1610(CONH ₂).	462	482	470
(126)	4-SO ₂ NH ₂	3400-3200(bonded NH), 1690, 1640(CONH ₂), 1340, 1160(SO ₂).	470	-	-
(127)	4-SO ₂ NHAc	3450-3200(bonded NH), 1710(Ac), 1660, 1620(CONH ₂), 1160(SO ₂).	473	-	-
(128)		3450-3050(bonded NH), 1700, 1650(CONH ₂), 1160(SO ₂).	470	-	-
(129)		3450-3150(bonded NH), 1700, 1650(CONH ₂).	464	-	-

A, 95% ethanol; B, 95% ethanol + 6N-hydrochloric acid; C, 95% ethanol + concentrated aqueous ammonia.

between 254 and 275 nm. The presence of an electron-withdrawing substituent in the benzene ring leads to increased conjugation and so an absorption maximum at longer wavelength (117, $\lambda_{\text{max.}}$ 454 nm; 121, $\lambda_{\text{max.}}$ 484 nm). A methyl or methoxy group *para* to the azo bond however, only induces a bathochromic shift of 4 or 8 nm respectively, relative to that of the unsubstituted analogue. The presence of a carbonyl group adjacent to the benzene ring (124) produces a bathochromic shift to 490 nm. It must be remembered that the sp^2 hybridised nitrogen atoms of the azo bond result in a coplanar, but not linear molecule. Therefore, these compounds may exist in the *cis* or *trans* forms, although the lowest energy, or most stable form, (i.e. *trans*) would be expected to predominate.

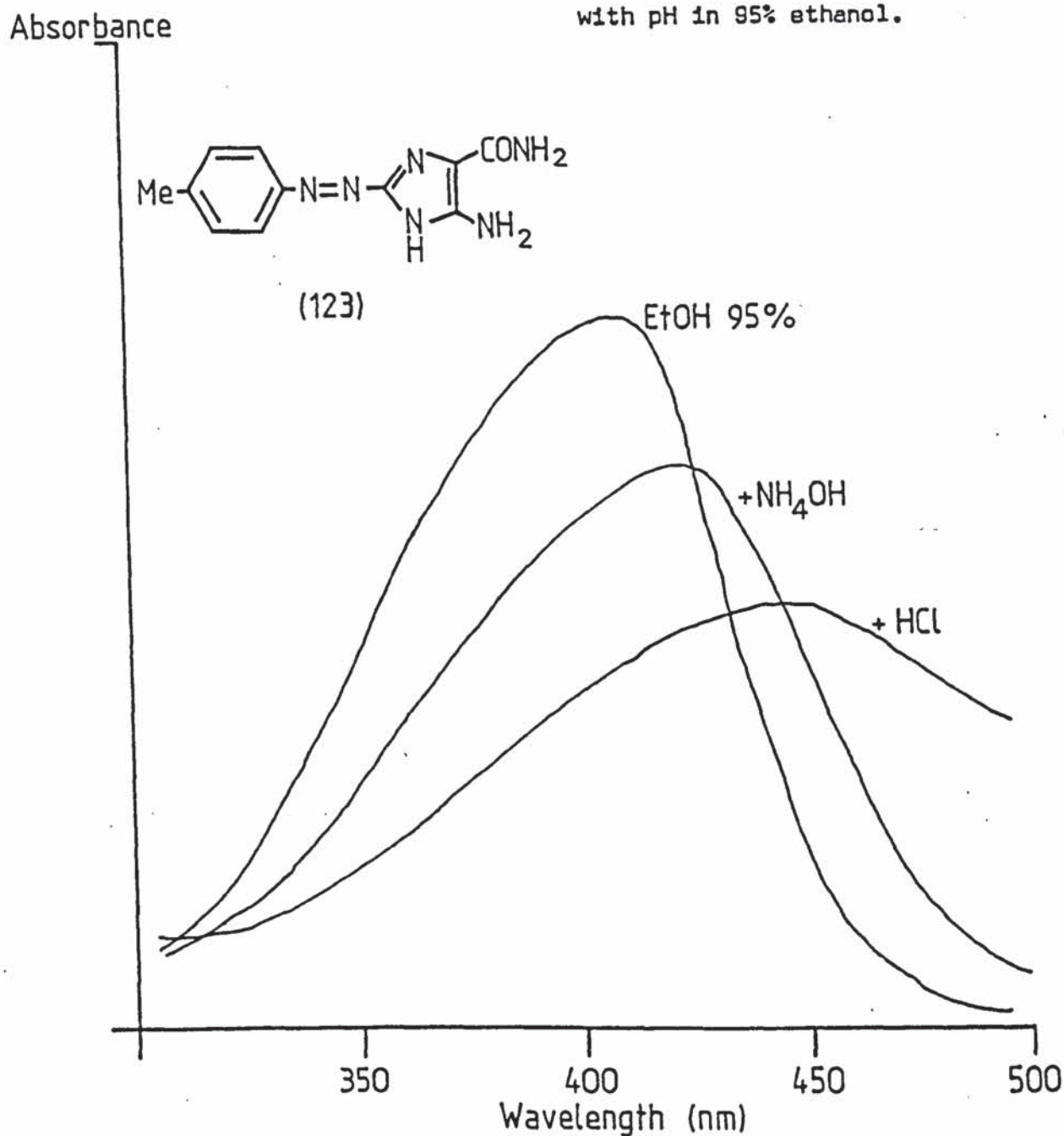


The effects of pH on the electronic absorption spectra of arylazimidazoles are summarised in Table 2.2 and the spectra of compound (123) are illustrated in Figure 2.1. It is helpful to regard this arylazoimidazole system as a type of donor-acceptor chromogen.¹⁸⁰⁻¹⁸² This may be interpreted by envisaging the arylazo group as the electron-acceptor for the electron-donating aminoimidazole function. On addition of acid the lone pair of electrons ($\ddot{N}H_2$) would be removed from the conjugated system due to protonation, and so a hypsochromic shift would be expected. No doubt protonation of imidazole ring nitrogen would also occur (130). However, under such strongly acidic conditions the weakly basic azo group would also become



Figure 2.1

U.v.-visible spectral changes
with pH in 95% ethanol.



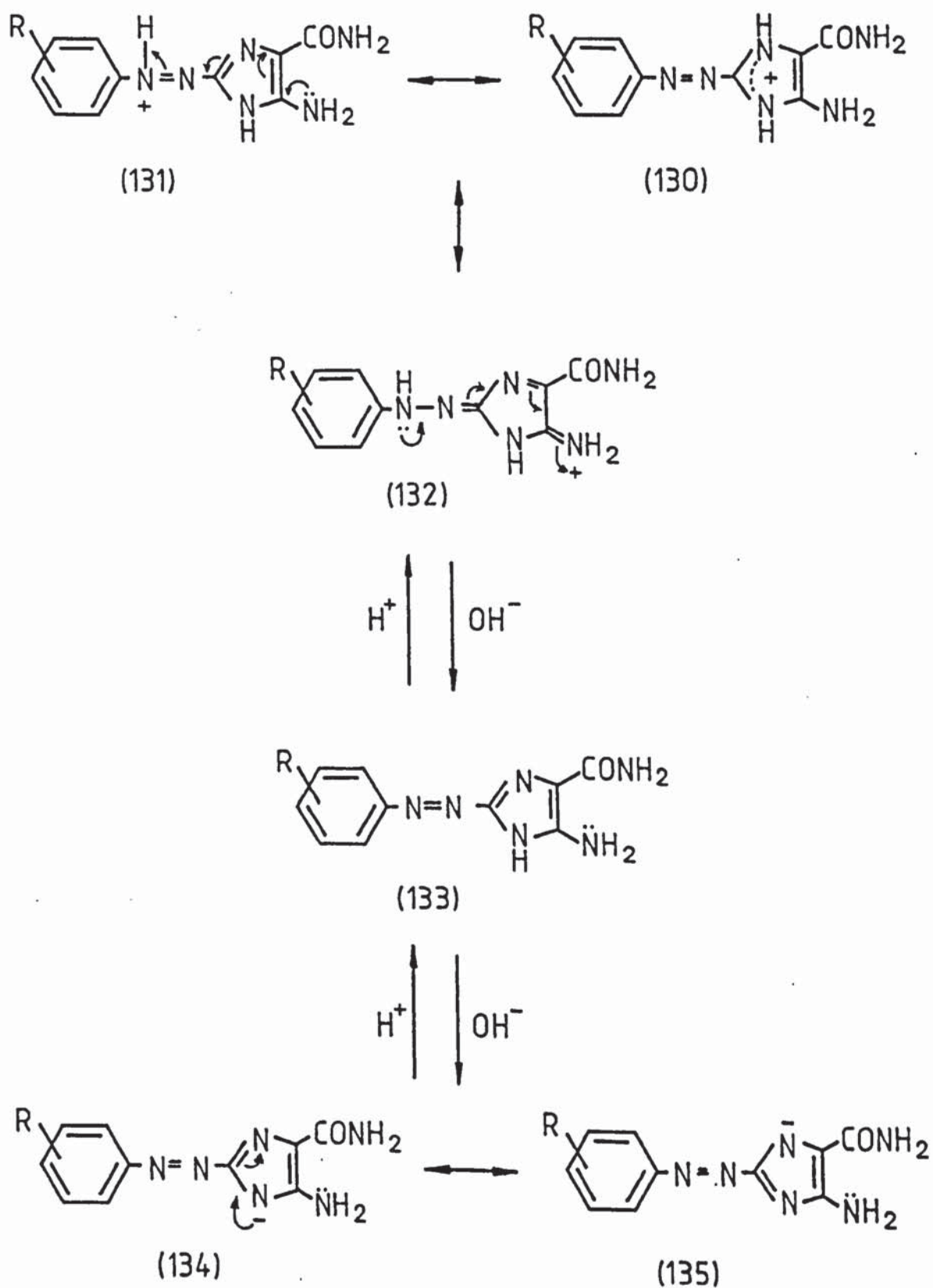
protonated¹⁸³ (131). As a functional group the azonium cation bears a certain resemblance to the diazonium cation and it can exert a powerful electron-attracting effect within the molecule. Therefore, protonation of the azo group would result in a greater electron-attracting or accepting

ability and hence an increased electron density migration occurs. The fundamental chromogen of the azonium cation is, in fact, best represented by the resonance structure (132) rather than (131) and the charge migration in the protonated species occurs in the direction as indicated in (132). This may be equated with a lower transitional energy for the long-wavelength band of the chromogen (ie. a red shift). If this is true then an electron attracting substituent on the benzene ring would tend to reduce the overall migration and result in a reduced bathochromic effect upon protonation: conversely the bathochromic shift should increase with increasing electron-donating strength of the substituent. This trend is certainly observed in the derivatives studied.

This is, of course, the opposite effect to that occurring in the neutral dye (133) and the anionic species (134, 135). The lone pair of electrons of the amino group would be available for donation under neutral conditions, and the presence of base would, in addition, result in formation of the resonance-stabilised imidazolyl anion causing a bathochromic effect. However, this time the charge migration is in the opposite direction to that shown by the protonated species. Therefore, the bathochromic shift is greater for derivatives with electron-withdrawing substituents in the benzene ring. Presence of electron-donating substituents reduces the shift. We may conclude that any factor which facilitates the displacement of electron density (ie. acids and bases in the present context) will exert a bathochromic effect (Scheme 2.3).

2.1.2.3 Mass spectrometry of arylazoimidazoles.

All the arylazoimidazoles with the exception of the sulphonamide derivatives (127) and (128) showed abundant molecular ions. In general one would expect rupture of the azo linkage to generate the four



Scheme 2.3

indicated ions (Figure 2.2).

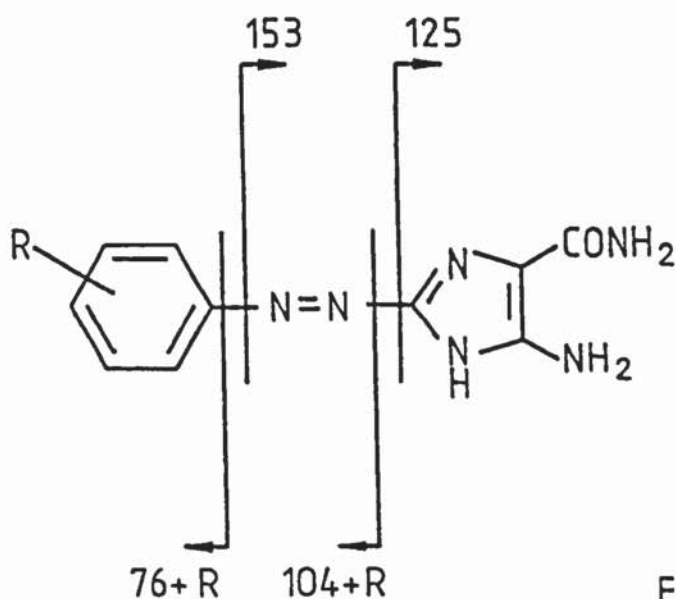


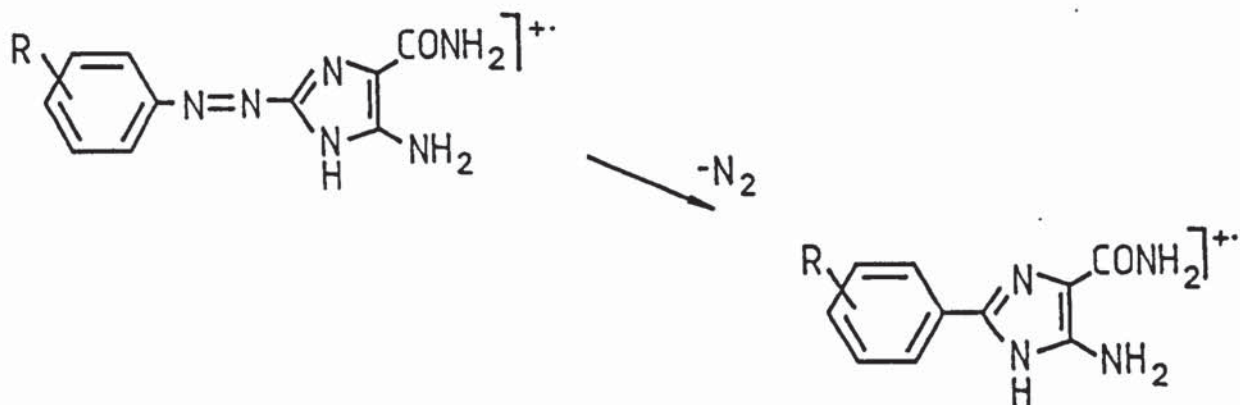
Figure 2.2

Surprisingly there was no evidence of ions at m/e 125 and m/e 153. Indeed the abundance of certain fragmentation ions indicated imidazole ring opening to be the major pathway of disintegration. Only after this event does rupture of the C-N bond between the remainder of the imidazole ring and the azo linkage occur.

One of the well established processes of skeletal rearrangement in mass spectrometry is the expulsion of a bridging group in the form of a neutral molecule, and this is most common when both A and C are aromatic systems. This fragmentation (loss of N_2) was detected in many of the mass spectra of the arylazoimidazoles. It was conspicuously ab-



sent from the 2-cyano derivative (122) however, because it is well estab-



lished that substituents *ortho* to the putative expelled group may significantly affect the rearrangement.¹⁸⁴ The mass spectral data is tabulated in the Appendix.

Fragmentation of the imidazole ring seemed to be preceded by loss of NH_2 probably from the carboxamide function to yield the acylium ion. In all cases this was followed by two successive losses of 28 mass units, presumably one represented the neutral CO species. A further loss of mass 52 generated the arylazo fragment. Fragments corresponding to the aniline or substituted aniline were also noted; possibly these arise from the hydrazo tautomer.

The sulphonamide derivatives did not show a molecular ion in their mass spectra but had a fragmentation pattern which corresponded to all other derivatives after loss of the imidazole ring.

2.1.2.4 ^1H nuclear magnetic resonance spectra of arylazoimidazoles.

^1H n.m.r. spectroscopy reveals little of interest in this group of azo-dyes due to the paucity of protons. However, in deuterated DMSO the amino group absorbs between $\delta 6.20$ and 6.75 . The carboxamide protons absorb as a broad singlet within the range $\delta 7.00$ and 7.35 , with the aromatic protons generally observed in the $\delta 7.50 - 8.50$ region. One of the sulphonamide derivatives (128) crystallised from dimethylformamide apparently

as a dimethylformamide solvate. Its n.m.r. spectrum showed two peaks at δ 2.75 and 2.95 each integrating for three protons, confirming presence of the solvent.

2.2 Chemical Modification of 5-Amino-2-arylazoimidazole-4-carboxamide Derivatives.

2.2.1 Acetylamino derivatives.

2.2.1.1 Synthesis and characterisation.

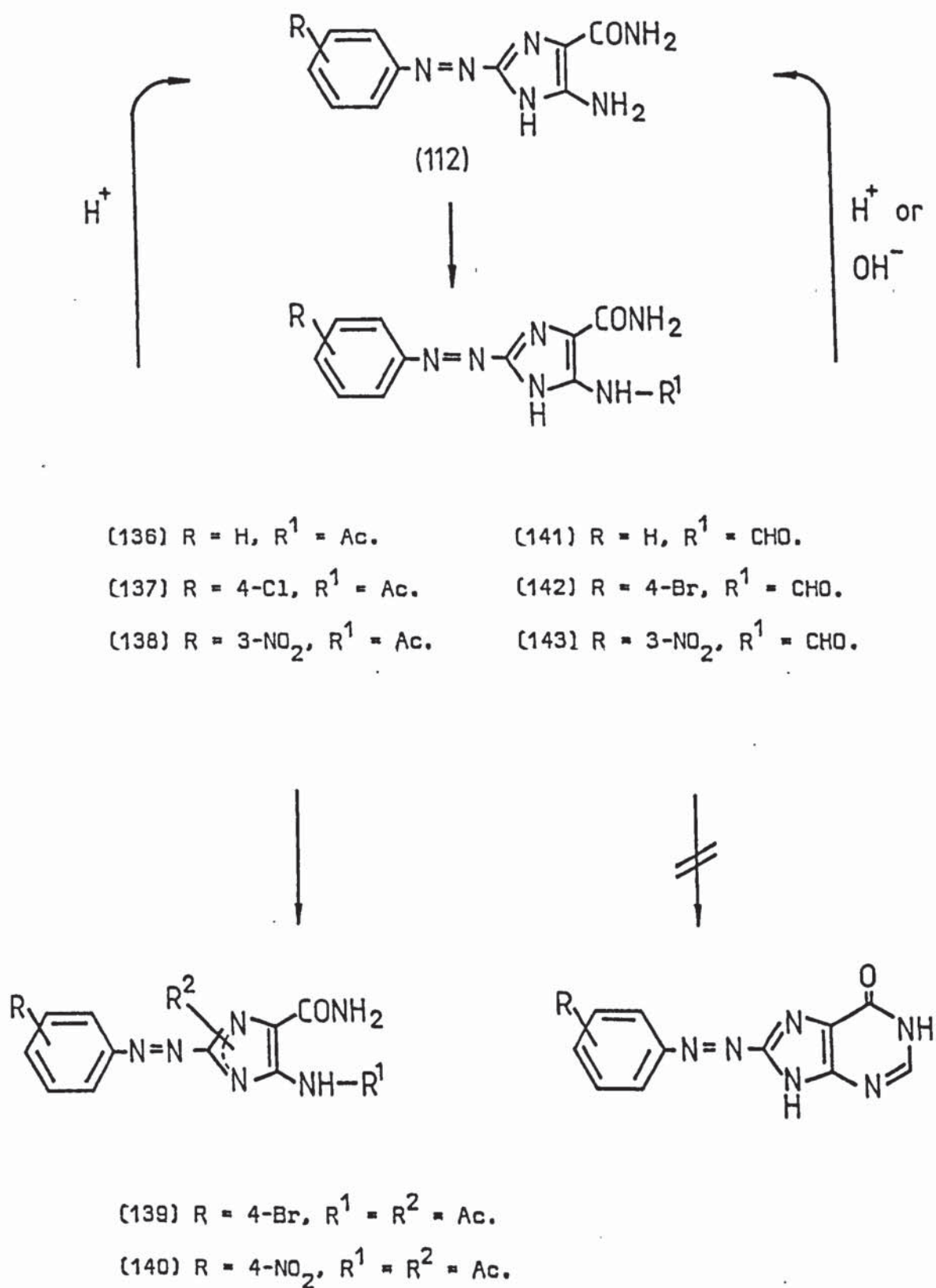
The azo-dyes were easily acetylated at the 5-amino group by treatment with acetic anhydride in glacial acetic acid (Scheme 2.4). The monoacetylated products (136-138) were obtained in satisfactory yield after a short reaction time. These yellow-brown crystalline compounds had lower melting-points than the parent amino compounds although, once again, the melting-points were indistinct and associated with decomposition.

Presence of the acetyl group elicited a hypsochromic shift (50-60 nm) of the longest wavelength absorption in the visible spectra of the dyes. The carbonyl group was readily identified by i.r. spectroscopy (1700 cm^{-1}). Mass spectrometry indicated these derivatives to be mono-acetyl compounds with all three derivatives having abundant molecular ions (Appendix). Initial loss of the characteristic fragment of mass 42 (ketene) was observed.

The non-diazotisable mono-acetylamino derivatives were stable in cold dilute hydrochloric acid. De-acetylation occurred in hot acid.

Extensive refluxing of two of the amino derivatives in a glacial acetic acid - acetic anhydride mixture resulted in formation of diacetyl compounds (139,140).

The electronic absorption spectra of the diacetyl compounds showed a hypsochromic shift ($\sim 50\text{ nm}$) of the longest wavelength absorption. Two carbonyl groups in addition to the carboxamide were apparent in the i.r. spectra, with two intense bands around 1700 cm^{-1} . Both diacetyl



derivatives showed an abundant molecular ion in the mass spectrum and two successive losses of mass 42 were noted which indicated the presence of two acetyl groups.

The site of the second acetylation is a point of some discussion although ring nitrogen seems a likely target. Normally ring-acetylated imidazoles are unstable.¹³⁹ However, the introduction of the first acetyl group to form the acetylamino function at C-5 may stabilise the product formed from ring nitrogen acetylation. This, of course, introduces the possibility of isomers dependent upon which ring nitrogen becomes acetylated.

2.2.1.2 ¹H n.m.r. studies.

Application of ¹H n.m.r. spectroscopy to the elucidation of the structure of the acetylamino derivatives proved to be most enlightening. The free amino group in the aminoimidazoles normally absorbs between δ 6.20 and 6.75. The N-H absorption (broad singlet) in the acetylamino derivatives was deshielded by the proximity of the acetyl group: this resulted in a pronounced shift to lower field (δ 10.35-10.45). Introduction of the second (ring) acetyl group resulted in a reduction of this deshielding to some extent and the N-H proton was now observed at δ 10.00-10.10. The carboxamide NH₂ appears as a broad singlet in the region of δ 7.35-7.50, slightly downfield when compared with the parent aminoimidazole carboxamides.

The methyl protons of the acetylamino group at C-5 absorb as a singlet at δ 2.25 for all acetylated azo-dyes. From the n.m.r. spectra of the diacetyl derivatives (139) and (140) it seems that two isomers are present. In addition to the absorption at δ 2.25 there are two other singlets evident in the spectra at δ 1.90, 2.35 and δ 2.26, 2.40 respectively. Due to the tautomeric nature of the starting material the acetyl group

may be introduced at N-1 or N-3 of the imidazole ring. Both peaks integrate for ~ 1.5 protons suggesting that the two isomers are present in approximately equal quantities.

2.2.2 Formylamino derivatives.

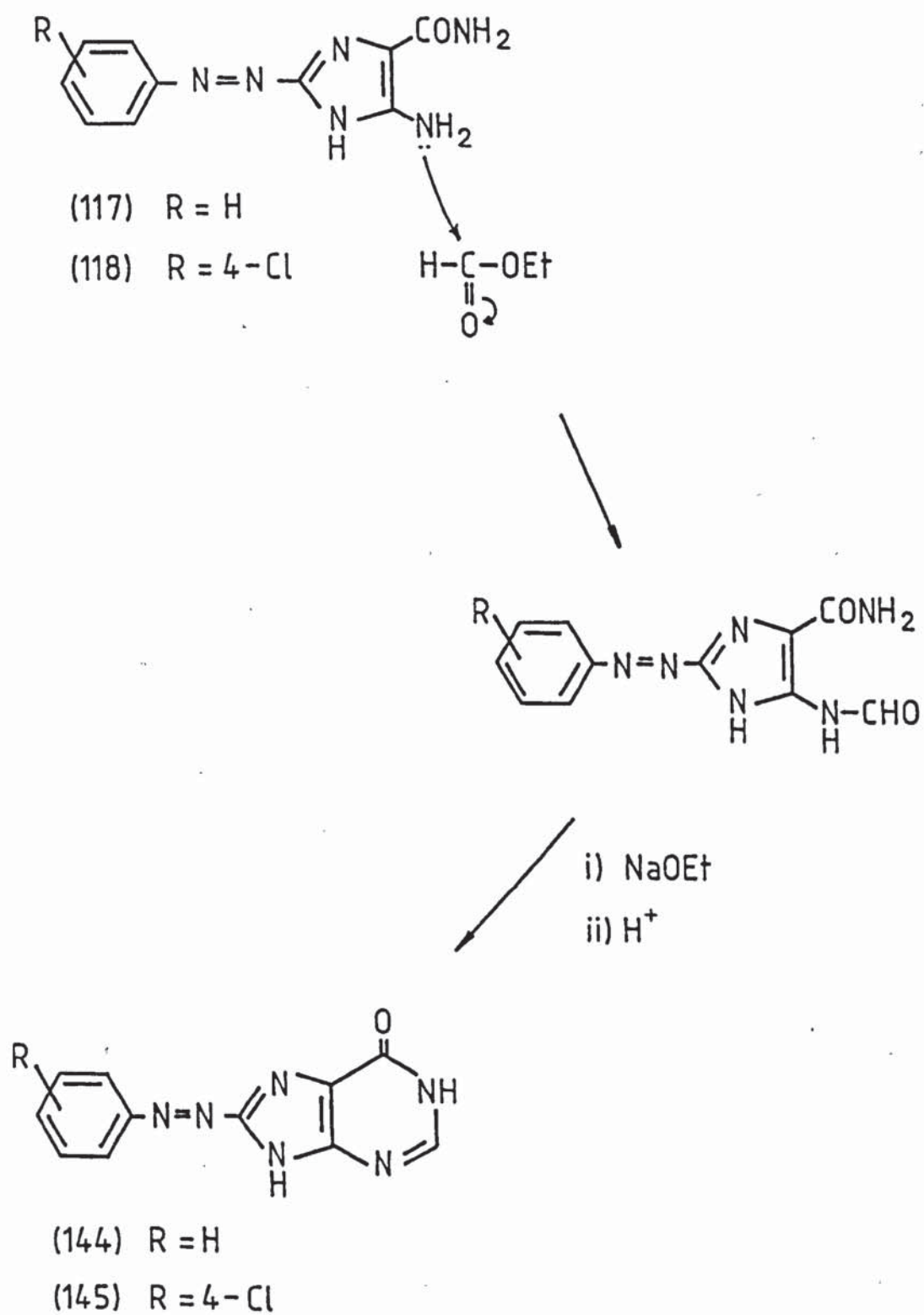
Interaction of derivatives (70,117 and 120) with boiling 98% formic acid resulted in formylation of the amino group. The formylamino derivatives (141-143) rapidly crystallised from the reaction mixture and no further reaction (ie. cyclisation to the arylazohypoxanthines) was detected.

Although 5-formylaminoimidazole-4-carboxamide (21) readily cyclised to hypoxanthine (52) in base⁸⁰ the presence of the arylazo function inhibits the comparable reaction in the present series of compounds. Deformylation resulted when compound (141) was treated with aqueous sodium bicarbonate solution or aqueous sodium hydroxide. Only a trace of the corresponding 8-arylazohypoxanthine (144) (Section 2.2.3) was detected (t.l.c.). Treatment of (141) with ethanolic sodium ethoxide dramatically enhanced conversion to (144), but this was still associated with detectable deformylation. Hydrolysis of the formylamino derivatives in boiling dilute hydrochloric acid regenerated the aminoimidazoles (Scheme 2.4).

The electronic absorption spectra of the formyl- and acetyl-amino- derivatives were very similar and the formyl carbonyl group in (70,117 and 120) absorbed at 1700 cm^{-1} . These derivatives produced abundant molecular ions in their mass spectra, with an initial loss of mass 28 (CO) evident in all cases (Appendix).

2.2.3 8-Arylazohypoxanthines.

After attempts to cyclise arylazo-AIC derivatives by N-formylation and ring closure had failed another route to the 8-substituted



Scheme 2.5

hypoxanthines was investigated. This involved the use of ethyl formate in the presence of sodium ethoxide which is known to condense with AIC¹⁶⁵ to yield hypoxanthine. Using similar conditions the 8-arylazohypoxanthines (144) and (145) were obtained as water soluble sodium salts (Scheme 2.5). Treatment of the salts with hydrochloric acid liberated the free acids.

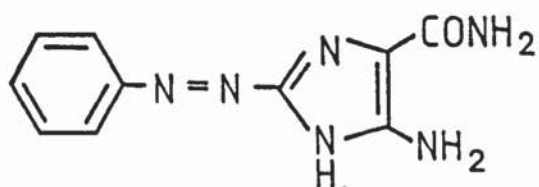
All attempts to prepare such derivatives by a coupling interaction of a diazonium salt and hypoxanthine met with failure. There is an early, dubious claim that an 8-arylazohypoxanthine could be prepared by coupling of diazotised 4-aminobenzenesulphonic acid with hypoxanthine which was supported by correct analytical data.¹⁴¹ However, this was later disputed by both Cavalieri¹⁸⁵ and Robins.¹⁸⁶ Therefore, cyclisation of aryl-azo-AIC derivatives constitutes the only route by which the 8-arylazohypoxanthines have been prepared.

A strong absorption band ($1700-1690\text{ cm}^{-1}$) is observed in the i.r. spectra of these cyclic derivatives (144) and (145) which suggests a predominant existence as lactam tautomers. These derivatives absorb strongly at λ_{max} , 377 and 386 nm respectively (cf. λ_{max} , 454 and 466 nm in the aryl-azo-AIC precursors).

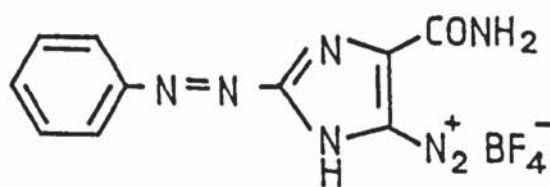
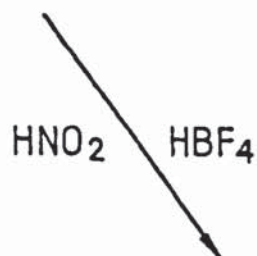
2.2.4 Diazotisation of 2-arylazo-AIC derivatives.

The 5-amino group of 2-arylazo-AIC derivatives could be diazotised in dilute hydrochloric acid by the addition of sodium nitrite. The deep magenta colours were discharged and yellow solutions formed which coupled with β -naphthol to form orange azo-dyes: these dyes were not isolated for identification. However, diazotisation of 2-phenylazo-AIC (117) in tetrafluoroboric acid (10%) resulted in precipitation of a golden tetrafluoroborate salt which was crystallised from ethereal 1-methyl-2-pyrrolidinone. The i.r. spectrum of this diazonium salt was most distinctive

with the diazo group absorbing strongly at 2200 cm^{-1} and B-F bonds in the 1245 cm^{-1} region. Predictably the presence of the diazo group resulted in a considerable blue shift in the visible spectrum (from 454 nm to 366 nm) for this derivative (146).



(117)



(146)

Scheme 2.6

3.1

Reduction of Arylazoimidazoles.

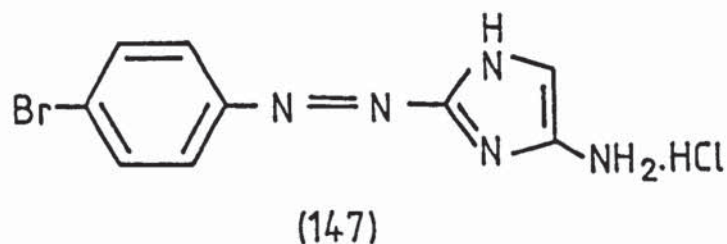
Chemical and catalytic reduction of the arylazoimidazoles should liberate 2,5-diaminoimidazole-4-carboxamide (71) and an arylamine. If this were the case the arylazoimidazoles could act as potential "pro-drugs" for the diaminoimidazole carboxamide in a biological environment.

3.1.1 Stannous chloride and hydrochloric acid.

Reduction of 4-bromophenylazo-AIC (70) by stannous chloride in hydrochloric acid has already been investigated⁸⁴ and a yield of 25-30% of the target diaminoimidazole carboxamide (71) has been claimed. Unexpected difficulty was experienced when this work was repeated following the vague experimental detail in the research report. Additionally it soon became clear that under basic conditions the product was unstable. This was indicated by a rapid darkening of the reaction mixture at the neutralisation step.

A yellow powder which precipitated early in the reductive procedure was isolated and freed from tin residues with hydrogen sulphide. A product (m.p. 233⁰) crystallised from the aqueous solution and corresponded to that described by the Japanese workers. Spectral analysis of the products showed that this was not 2,5-diaminoimidazole-4-carboxamide since its mass spectrum showed an abundant molecular ion at mass 265 and an isotope peak at M+2 of equal intensity. Loss of carbonyl absorption was also noted in the i.r. spectrum. The product was tentatively identified as 4-amino-2-(4-bromophenylazo)imidazole hydrochloride (147) although this compound should be highly coloured and the product was obtained as colourless needles.

It should be noted that the carbon content of (147) is similar



to that of the proposed 2,5-diaminoimidazole-4-carboxamide dihydrochloride⁸⁴ (35.7% and 35.8% respectively). Application of n.m.r. spectroscopy indicated loss of the carboxamide function with the appearance of a singlet at δ 9.8 (H-5 on the imidazole ring) integrating for one proton. The carboxamide NH_2 absorbance was notably absent.

It is, therefore, not surprising that further reduction resulted in degradation of the liberated imidazole. Loss of the stabilising carboxamide function would undoubtedly result in an unstable diaminoimidazole.⁹⁶

Attempts to reduce other arylazoimidazoles (117, 122 and 129) by this method resulted only in isolation of the arylamine moiety. The acetylamino derivative (137) was also reduced in the hope that an acetylated diaminoimidazole would be more stable: satisfactory reduction was not achieved however.

3.1.2 Sodium dithionite.

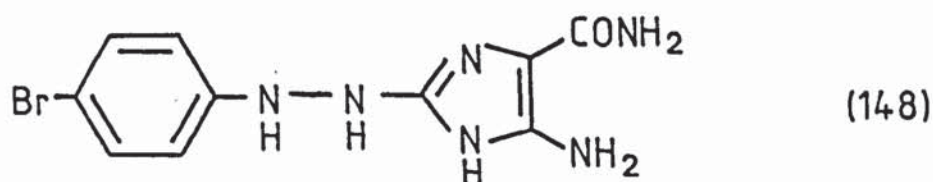
Reduction of compounds (117) and (118) with sodium dithionite resulted in rapid discharge of the red azo colour. However, isolation of aniline and 4-chloroaniline respectively from the arylazo component proved to be the only tangible result of such experiments.

Attempted reduction of 2-cyanophenylazo-AIC (122) gave an interesting cyclisation to form an indazole (see Section 3.1.3).

The acetylamino derivatives [136] and [137] could not be successfully cleaved by this reductive method. T.l.c. indicated both starting materials and the hydrolysis products (ie. aminoimidazoles) were present in the reduction mixture.

3.1.3 Catalytic hydrogenation.

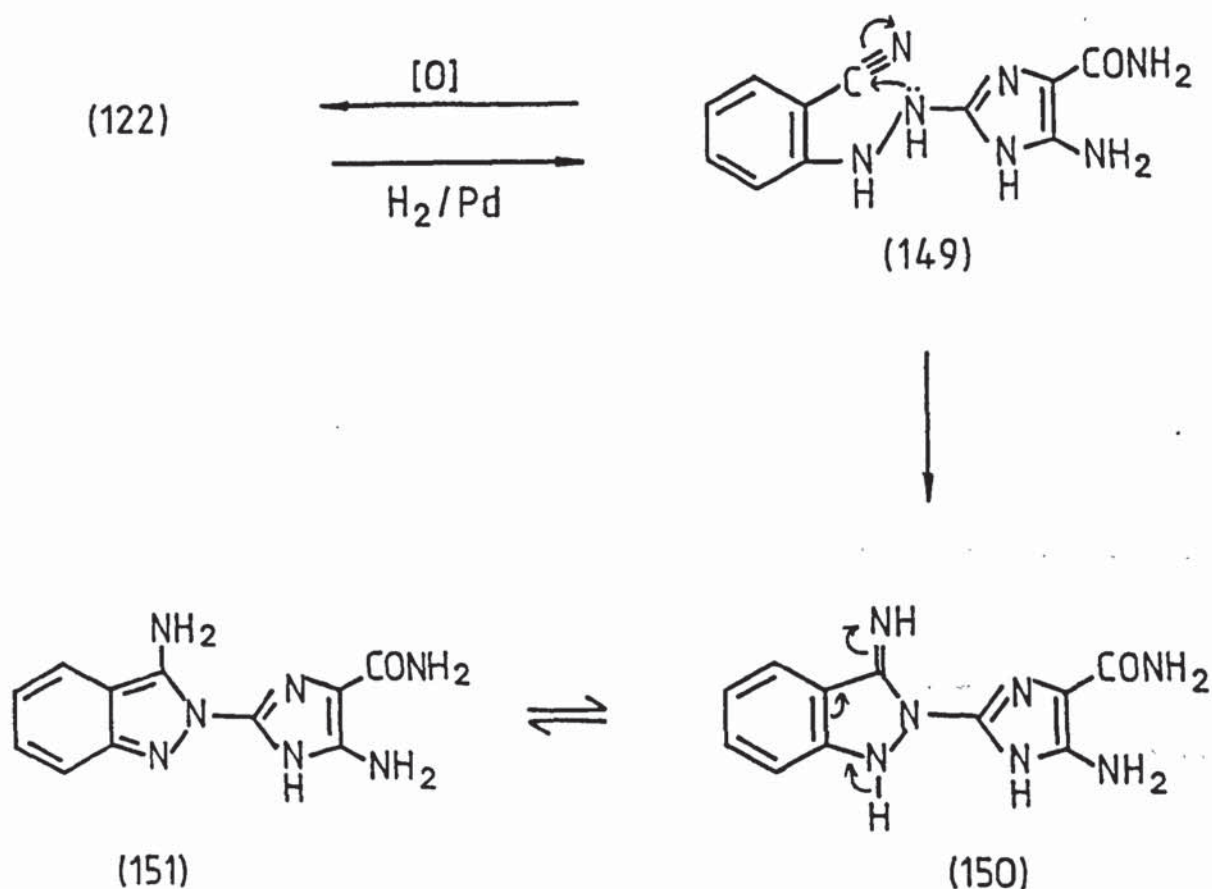
Hydrogenation employing palladium on charcoal as a catalyst resulted in rapid discharge of the red azo colour of 4-bromophenylazo-AIC (70). This was accompanied by precipitation of a cream solid: this solid rapidly reoxidised in air to regenerate the starting material. At this stage only half of the calculated volume of hydrogen had been absorbed, and it was assumed that the precipitate was the hydrazo compound [148].



Cleavage of the azo bond was not observed.

Addition of hydrochloric acid and further hydrogenation resulted in precipitation of a white powder. Purification from water yielded a product which charred above 150° but did not melt below 300°. N.m.r. spectroscopy (D₂O) revealed no protons absorbing δ0-10 (downfield of TMS) while electronic absorption spectroscopy revealed no extensive chromophore (λ_{max.} 280 nm). The i.r. spectrum showed N-H stretching (3400-3100 cm⁻¹, hydrogen bonded), with intense bands at 1700, 1680 and 1640 cm⁻¹ suggestive of two carbonyl groups. Peaks in the mass spectrum at mass 192 and 177 were also evident. This compound has not yet been identified.

Catalytic hydrogenation of 2-cyanophenylazo-AIC (122) also resulted in precipitation of the hydrazo compound (149) which reoxidised



Scheme 3.1

to starting material on contact with air. Extended hydrogenation with gentle warming ($35-40^\circ$) followed by addition of hydrochloric acid yielded 5-amino-2-(3-aminoindazol-2-yl)imidazole-4-carboxamide (151) (Scheme 3.1). This was identical to the product obtained by reduction of (122) with sodium dithionite.

Catalytic hydrogenation of an acetamino derivative (136) in a mixture of acetic acid and acetic anhydride was unsuccessful in providing 2,5-diacetylaminoimidazole-4-carboxamide. Surprisingly t.l.c. examin-

ation of the reduction products indicated the presence of acetanilide as the only identified product.

3.1.4 Raney nickel and hydrazine hydrate.

Precipitation of the hydrazo compounds was noted when Raney nickel and hydrazine hydrate were employed for the reduction of the azo-dyes [70] and [117] in ethanol. Reversion to starting material was observed in the presence of air. Further reduction to cleave the hydrazo bond could not be achieved at room temperature. Increase in temperature (45°) resulted in dissolution of the precipitates and a rapid darkening of the solutions. 4-Bromoaniline and aniline were the only products isolated from the reduction of [70] and [117]. However, stability of the required diaminoimidazole carboxamide would be suspect under the basic conditions generated by this method.

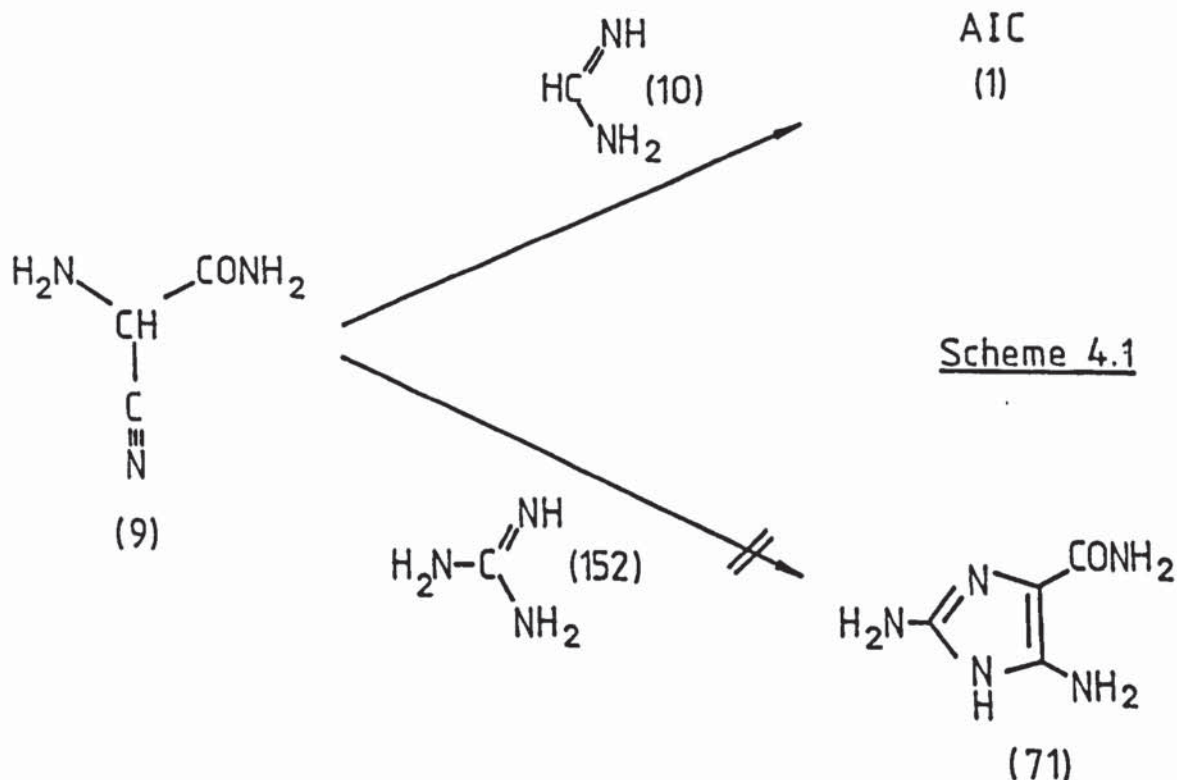
We must therefore conclude that a reductive approach to the synthesis of 2,5-diaminoimidazole-4-carboxamide is not practicable. Other routes to this elusive imidazole are discussed in the next chapter.

4.1 Approaches to Synthesis of 2,5-Diaminoimidazole-4-carboxamide.

Nitration of imidazoles normally involves the 4(5)-position and very few examples of 2-nitroimidazoles exist. AIC cannot be nitrated at C-2 and therefore this route, involving a subsequent reductive step, could not be considered.

4.1.1 Direct synthesis of the imidazole ring.

A modification of the route described by Cook *et al*⁶⁵ (Section 1.2.1.1) to prepare AIC from 2-amino-2-cyanoacetamide (9) was next considered. This entailed substitution of guanidine (152) for formamidine (10) in the preparative procedure in order to introduce the extra amino group at position 2. Aminocynoacetamide was prepared by the literature



methods in reported yield,^{69,70,187} and initial experiments were conducted

under conditions described by Schwartz.⁶⁶ These conditions were those known to give an acceptable yield of AIC when formamidine was utilised (Scheme 4.1). However, formation of the diaminoimidazole (71) was not observed even though reaction conditions were varied considerably (Table 4.1) in an attempt to obtain the elusive diamino compound.

Table 4.1 Attempted direct synthesis of 2,5-diaminoimidazole-4-carboxamide:
reaction conditions.

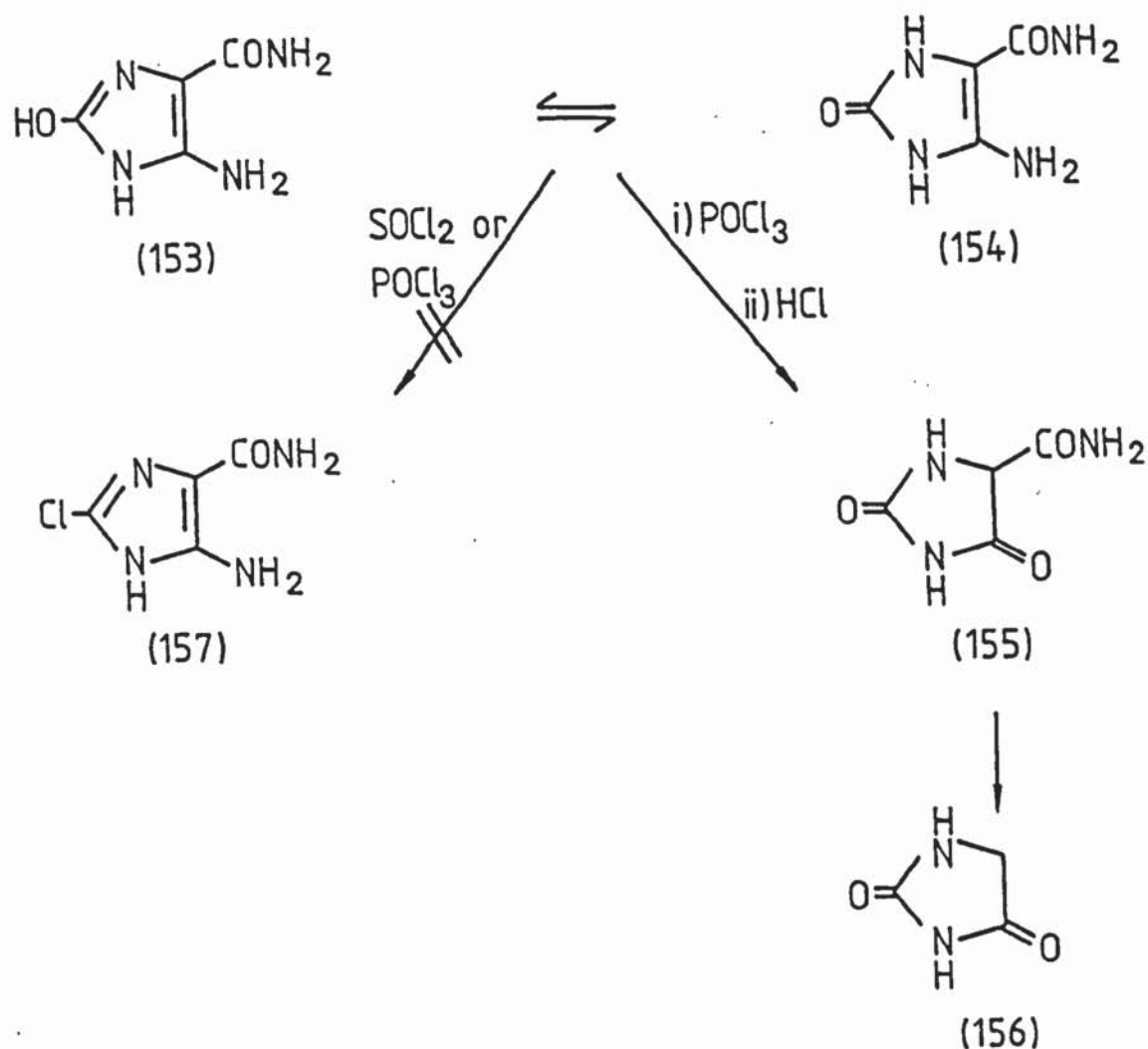
Solvent	Temperature ^o	Guanidine	Atmosphere	Duration(h)
Methanol*	70	acetate	H ₂	0.5
Methanol	70	hydrochloride	H ₂	0.5-1.5
Methanol	70	carbonate	H ₂	0.5-2.0
Methanol	70	free base	H ₂	0.5
Methanol	100	acetate	H ₂	1.0
Ethanol + NaOH	50	free base	H ₂	0.5
Ethanol + EtONa	20	free base	N ₂	2.0
Ethanol + EtONa	50	free base	N ₂	2.0
Dry fusion	150	free base	Air	0.25
Dry fusion	150	free base	N ₂	0.25-1.0

* Conditions described by Schwartz.⁶⁶

4.1.2 From 5-amino-2-hydroxyimidazole-4-carboxamide.

Preparation of 2-hydroxy-AIC (153) was successfully achieved by the literature method¹⁸⁸ (75%). It was intended to substitute the 2-hydroxy [or 2-oxo group of the tautomer (154)] with chlorine. Replacement of

chlorine by ammonia should then furnish the amino group required at the 2-position.



Scheme 4.2

Treatment of (153) with thionyl chloride under reflux resulted in recovery of the starting material. However, phosphorus oxychloride treatment at 100° yielded a white crystalline solid; this proved to be the acid hydrolysis product 2,5-dioxoimidazoline-4-carboxamide (155). This material was sub-

4.1.4 Ring opening of 8-aminohypoxanthine.

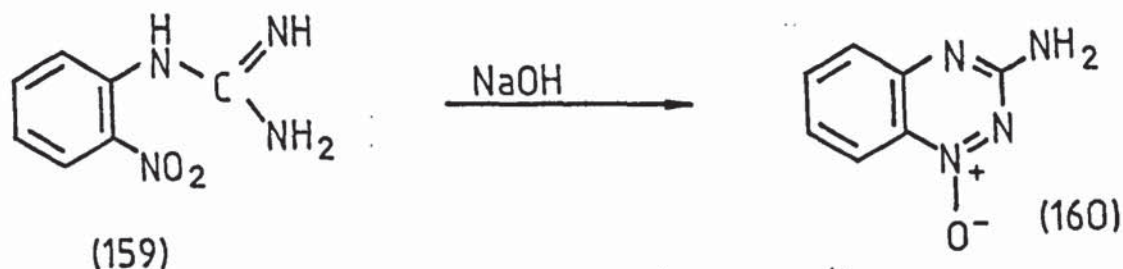
8-Aminohypoxanthine^{191,192} failed to ring open when treated with zinc dust in dilute sulphuric acid, conditions which are known to ring open hypoxanthine at C-2 and yield AIC.^{121,122}

4.1.5 Direct amination.

Introduction of an amino group directly into the 2-position of AIC was attempted with hydroxylamine-O-sulphonic acid. This reaction may be considered to be an electrophilic substitution and directly comparable with the electrophilic coupling of aryldiazonium salts to AIC at C-2 (Section 2.1.1). In aqueous media (70°) a reaction was indicated by t.l.c. and purification of product from starting material was attempted by application of ion-exchange chromatography. When products were eluted from the column (Amberlite IR 120) with 3N-ammonium hydroxide immediate darkening of the ion-exchange resin was noted. This was assumed to be indicative of product deterioration. Unchanged AIC could still be detected in the eluate.

This result indicates that amination could be occurring but that the product formed is unstable under alkaline conditions.

Cyclisation of *o*-nitrophenylguanidines (159) in alkali is a general route to the 3-amino-1,2,4-benzotriazine-1-oxides¹⁹³ (160).



An investigation was conducted of the reverse situation when the nitro group was present in the side chain and nitrogen part of a ring (161).



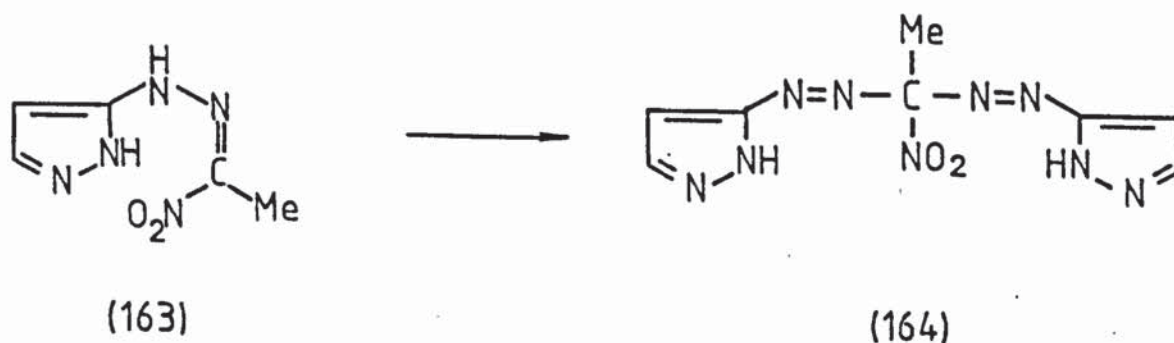
It has been reported that the coupling product of 3-diazopyrazole and nitroethane was an unstable azo derivative which could not be purified. The structure was later deduced as being a nitrohydrazone (163) by its conversion to a pyrazolotriazine.¹⁹⁴

Interaction of 3-diazopyrazole¹⁹⁵ and nitroethane in the presence of sodium hydroxide resulted in a yellow solution. The product was precipitated from solution by the addition of excess sodium acetate. It was discovered that when very dilute coupling conditions were employed the product was a stable mustard coloured powder, however, more concentrated

conditions resulted in formation of a brown gum.

The crystalline nitrohydrazone (ethanol) could be stored at 0° for several months as a yellow powder (m.p. 137° efferv.) without noticeable deterioration. The i.r. spectrum showed a broad band (3400-3200 cm^{-1}) indicative of bonded N-H, and the nitro group was observed as two bands at 1520 and 1380 cm^{-1} . The compound absorbed at λ_{max} 363 nm in the u.v.-visible spectrum which is typical for a hydrazone. An abundant molecular ion at mass 169 was observed in the mass spectrum.

The stability of the nitrohydrazone (163) was examined in ethanolic solution under acidic, neutral and basic conditions. In all cases a darkening of the ethanolic solutions was noted from orange to red-brown within five days at room temperature. The products from both the ethanolic (neutral) and ethanol-pyridine solutions were isolated as fawn coloured needles and shown to be identical. The ethanol-acetic acid mixture yielded a white microcrystalline solid.



Scheme 5.1

It is proposed that formation of (164) occurs under all conditions due to partial disproportionation of the hydrazone so generating the diazo species. This may then couple to an undissociated molecule of the hydrazone.

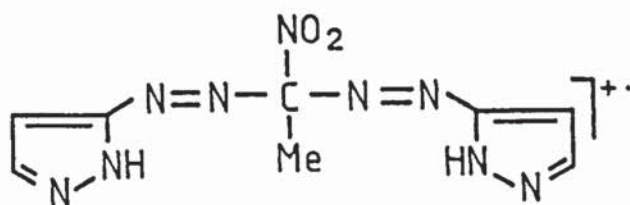
The n.m.r. spectrum (D_2O) of the product from (163) and ethanol

supports structure (164) and shows two doublets for the pyrazole protons at δ 8.05 and 6.85, each integrating for two protons. The methyl protons absorb as a singlet at δ 3.00. The relatively downfield location of the methyl protons may be explained by the presence of the geminal nitro group and the deshielding pyrazolylazo groups. The white microcrystalline solid isolated from acetic acid and ethanol was formulated as the acetic acid solvate of (164). A singlet at δ 1.95 integrating for three protons confirmed the presence of acetic acid.

The mass spectral fragmentation of unsolvated (164) is shown in Scheme 5.2; speculative structures for the major ions are indicated.

Maximum absorption at 285 nm was observed in the electronic absorption spectra for compound (164) and its acetic acid solvate, and addition of base resulted in a bathochromic shift (λ_{max} , 358 nm) in both cases. The i.r. spectrum of (164) provided further evidence for correct assignment of structure with the nitro group evident as two bands at 1510 and 1350 cm^{-1} . The acetic acid solvate, in addition, shows the characteristic bands for O-H stretching (3000 - 2500 cm^{-1}).

Both products were unstable in hydrochloric acid with rapid effervescence of nitrogen dioxide being observed. An unidentified white crystalline material resulted from such treatment. The i.r. spectrum of this compound indicated loss of the nitro group which was corroborated by the upfield chemical shift of the methyl protons to δ 2.90. The pyrazole protons were still evident as doublets at δ 8.15 and 6.80. The mass spectrum of the product showed abundant peaks at mass 188 and 122.

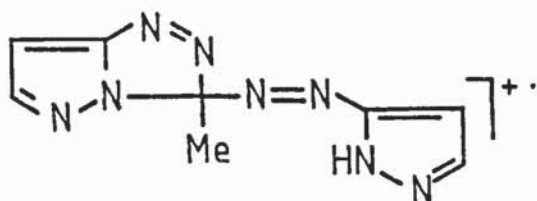


calc. 263.08879

given. 263.08926

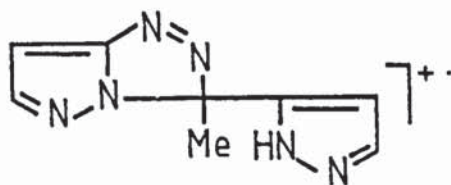
(error 1.7 ppm)

↓ -47 (HNO₂)



m/e 216

↓ -28 (N₂)

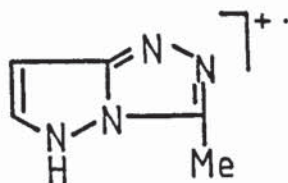


calc. 188.08133

given. 188.08104

(error 1.5 ppm)

↓ -C₃H₃N₂⁺
+H⁺

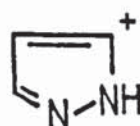


calc. 122.05899

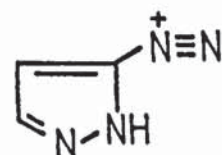
given. 122.05924

(error 2.0 ppm)

Other characteristic ions occur at :



m/e 67



m/e 95

Scheme 5.2

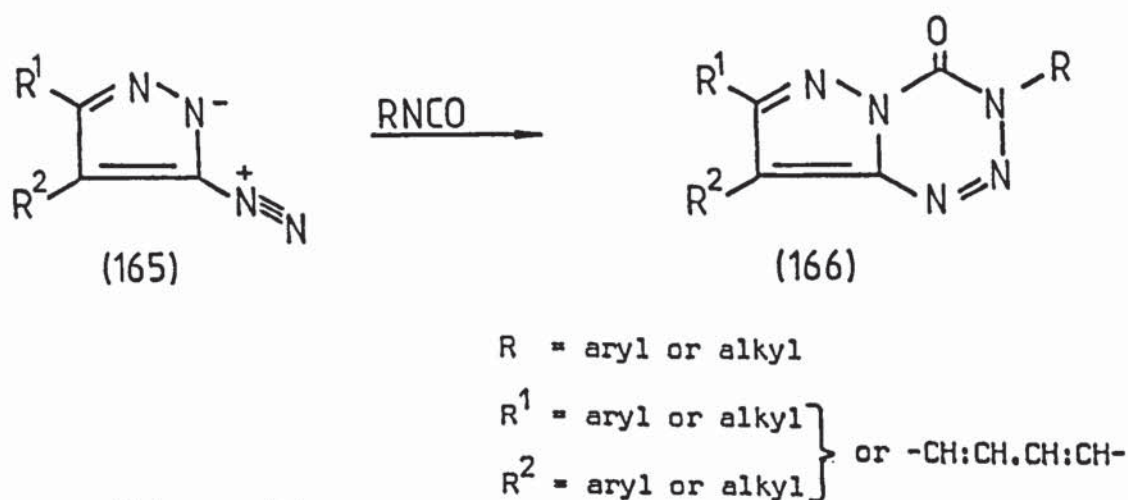
6.1

Synthesis and Characterisation of
Imidazo[5,1-d] [1,2,3,5]tetrazin-7(6H)-ones.

6.1.1 Rationale and synthesis.

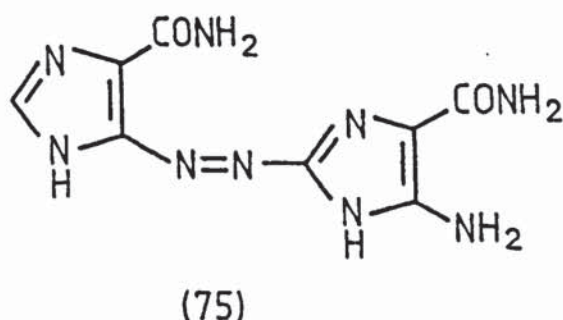
Diazo-IC (6) is known to have antitumour properties⁵¹ but due to its chemical reactivity and tendency to cyclise to 2-azahypoxanthine (74) it has never received a clinical trial. Reaction of Diazo-IC with amines has provided a host of triazenes, one of which, DTIC (7), is used clinically (Section 1.1.3). Although it was proposed to incorporate Diazo-IC in a molecule which would act as a "carrier" or pro-drug form of this reactive diazo-azole, there is no evidence that this is the mechanism of action of DTIC.^{52,57,61,62}

The basis for the work described in this Chapter was a paper by Ege and Gilbert¹⁹⁶ in which they described a cycloaddition reaction between diazo-azoles and isocyanates (Scheme 6.1) which yielded the hitherto unknown pyrazolo[5,1-d] [1,2,3,5]tetrazin-7(6H)-ones (166). These compounds have recently been patented.¹⁹⁷



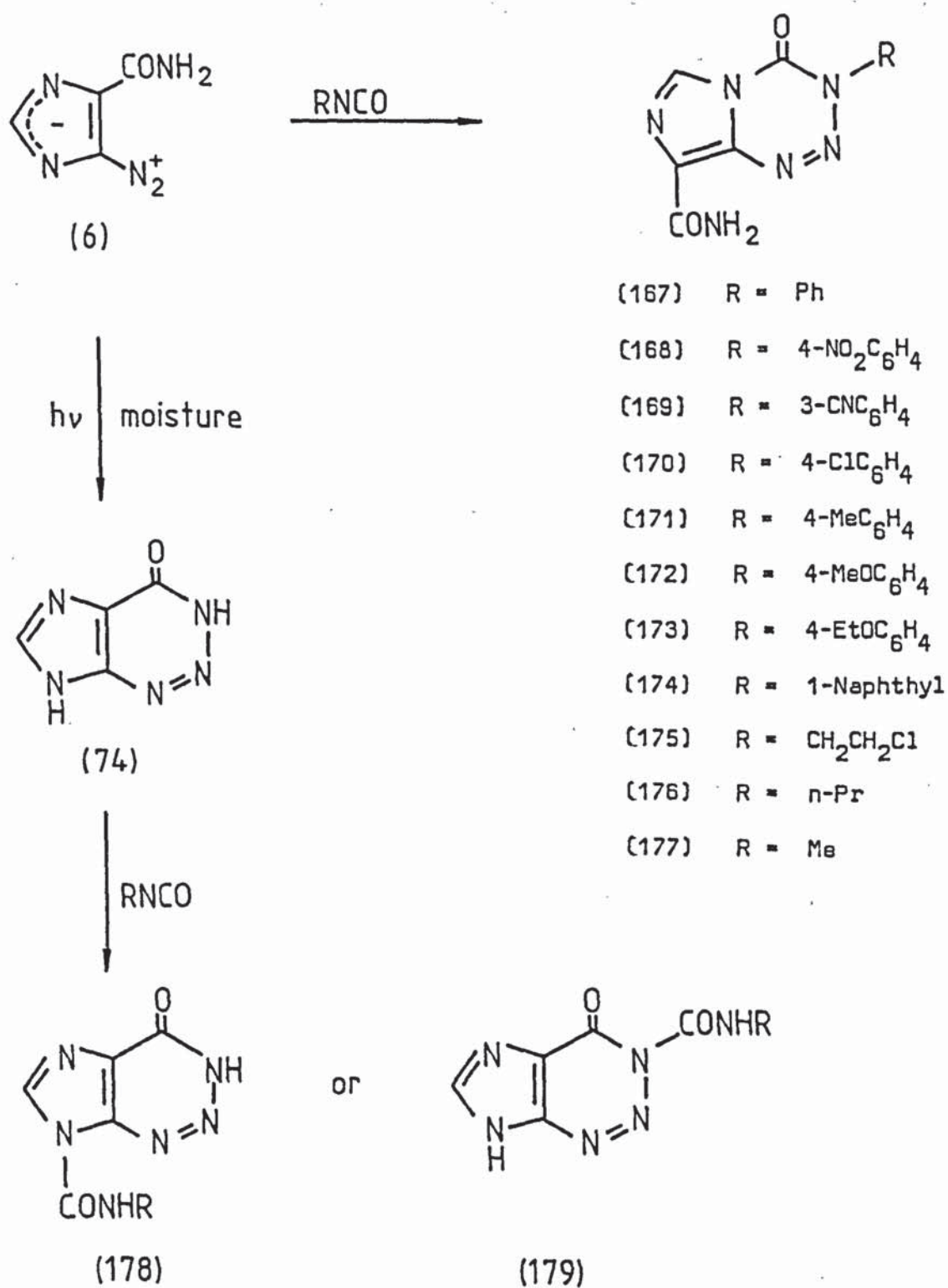
Scheme 6.1

Until 1979 it was generally believed that the 1,2,3,5-tetrazine ring-system was inherently unstable,^{198,199} although Kubo²⁰⁰ claims to have prepared several derivatives by electrochemical oxidation of cyanamide. It was envisaged that Diazo-IC would furnish a series of imidazo-[5,1-*d*][1,2,3,5]tetrazin-7-ones by interaction with aryl- and alkyl-isocyanates. An improved synthesis of Diazo-IC based upon the original Shealy method⁵⁰ was developed which did not result in formation of the reported purple byproduct which has been identified as (75).



In view of the reactive nature of Diazo-IC all reactions were carried out in dry dichloromethane with excess isocyanate in the dark. Diazo-IC is only sparingly soluble in dichloromethane and reaction progress was monitored by the gradual disappearance of the buff coloured diazo compound (ν_{max} , 2190 cm^{-1}). After 30 days the reaction was generally complete. Considering the extended reaction time it was essential to ensure anhydrous, dark conditions, otherwise 2-azahypoxanthine and perhaps the carbamoylated azapurinones (178) and (179) could have become prominent impurities (Scheme 6.2).

Isolation of the imidazotetrazinones (167-177) in high yield was conveniently achieved by dilution of the reaction mixture with ether. The products were cream or pastel coloured powders which were soluble in DMF, DMSO, 1-methyl-2-pyrrolidinone and sparingly soluble in alcohols.



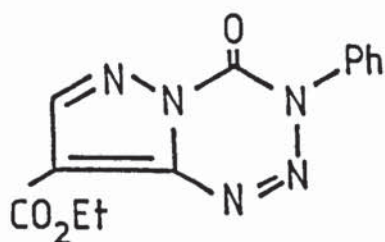
Scheme 6.2

Melting-points ranged between 138 and 210^o (Table 6.1) and were associated with violent decomposition or effervescence. Attempted purification of the products from hot methanol yielded Diazo-IC and it was discovered that the imidazotetrazinone ring-system was unstable in the protic solvents normally used for crystallisation. Further purification of these compounds from ethereal 1-methyl-2-pyrrolidinone has been demonstrated by successive microanalytical determinations. Compound (175) showed changes in the N-H stretching region of the i.r. spectrum and below 1400 cm⁻¹ (KBr disc) after purification. This was considered to be due to polymorphism because solution i.r. spectra (CH₂Cl₂) of the two forms were identical.

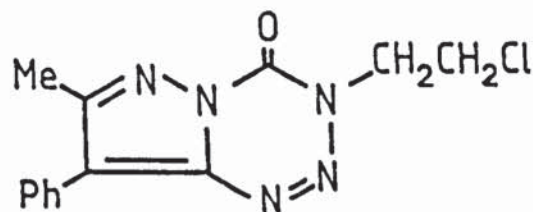
Compounds in this series were stored under dry, dark conditions for several months without detectable deterioration, although a slight darkening in colour was noted.

Occasionally an unidentified red pigment was obtained in very small quantity which did not significantly affect elemental microanalysis figures but resulted in a pink coloured product. This is presumably a coupling product of Diazo-IC.^{60,153}

For comparative purposes two pyrazolotetrazinones were also prepared. Addition of ethyl 3-diazopyrazole-4-carboxylate²⁰¹ to phenyl isocyanate yielded the cycloadduct (180). The pyrazolotetrazinone (181) derived from 3-diazo-5-methyl-4-phenylpyrazole²⁰² and 2-chloroethyl isocyanate was obtained after a reaction time of twelve hours.

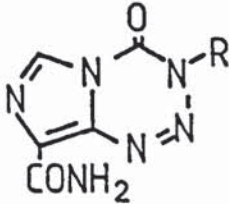


(180)



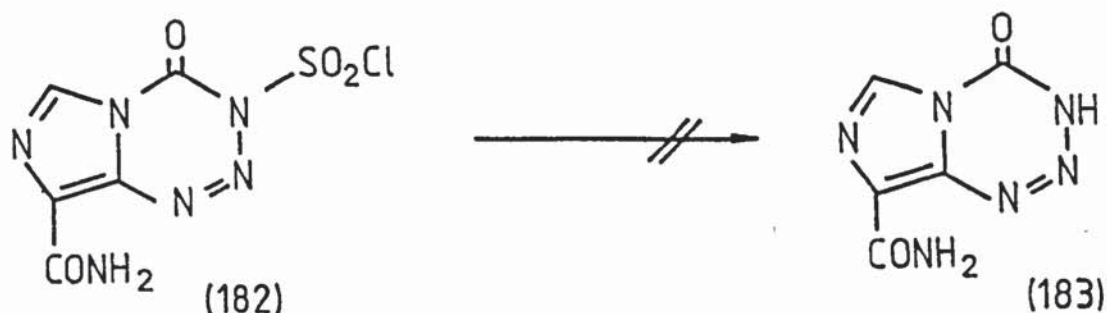
(181)

Table 6.1 Physical characteristics of imidazotetrazinones.

Compound Number.		Yield (%)	M.p. ⁰	Appearance.
167	Ph	76	145(decomp.)	cream powder.
168	4-NO ₂ C ₆ H ₄	89	140-145(decomp.)	yellow powder.
169	3-CNC ₆ H ₄	61	135-138(efferv.)	cream powder.
170	4-ClC ₆ H ₄	80	138-141(decomp.)	orange powder.
171	4-MeC ₆ H ₄	84	142-144(decomp.)	white powder.
172	4-MeOC ₆ H ₄	77	155-160(decomp.)	cream powder.
173	4-EtOC ₆ H ₄	94	153(decomp.)	cream powder.
174	1-Naphthyl	55	144-146(decomp.)	mauve powder.
175	CH ₂ CH ₂ Cl	91	158(efferv.)	cream powder.
176	n-Pr	21	167(efferv.)	pink powder.
177	Me	28	210(efferv.)	fawn microcrystals

6.1.2 Limitations of the cycloaddition pathway.

Preparation of the unsubstituted imidazotetrazinone (183) was a desirable objective and its synthesis was attempted *via* the chlorosulphonyl derivative (182) (Scheme 6.3).



Scheme 6.3

The reaction between N-chlorosulphonyl isocyanate²⁰³ and Diazo-IC proceeded relatively quickly and a light orange powder was deposited from the dichloromethane mixture. This was assumed to be the chlorosulphonyl derivative (182) and was isolated in the usual manner and stored in a desiccator for several days without visible deterioration. However, exposure to moist air initiated rapid darkening in colour and a brown gum was formed after a few minutes.

Mild reduction of a freshly prepared sample of (182) was attempted with sodium bisulphite and sodium bicarbonate. However, although this method for removing chlorosulphonyl groups is a standard procedure^{204,205} immediate degradation of the compound was evident and this route was not investigated further. The importance of the unsubstituted derivative (183) as a precursor to further examples of this ring-system should ensure that further attempts at synthesis are carried out.

Diazo-IC would not form cycloadducts with cyclohexyl-, n-butyl-, t-butyl-, n-tridecyl- and n-pentadecyl-isocyanates under the described

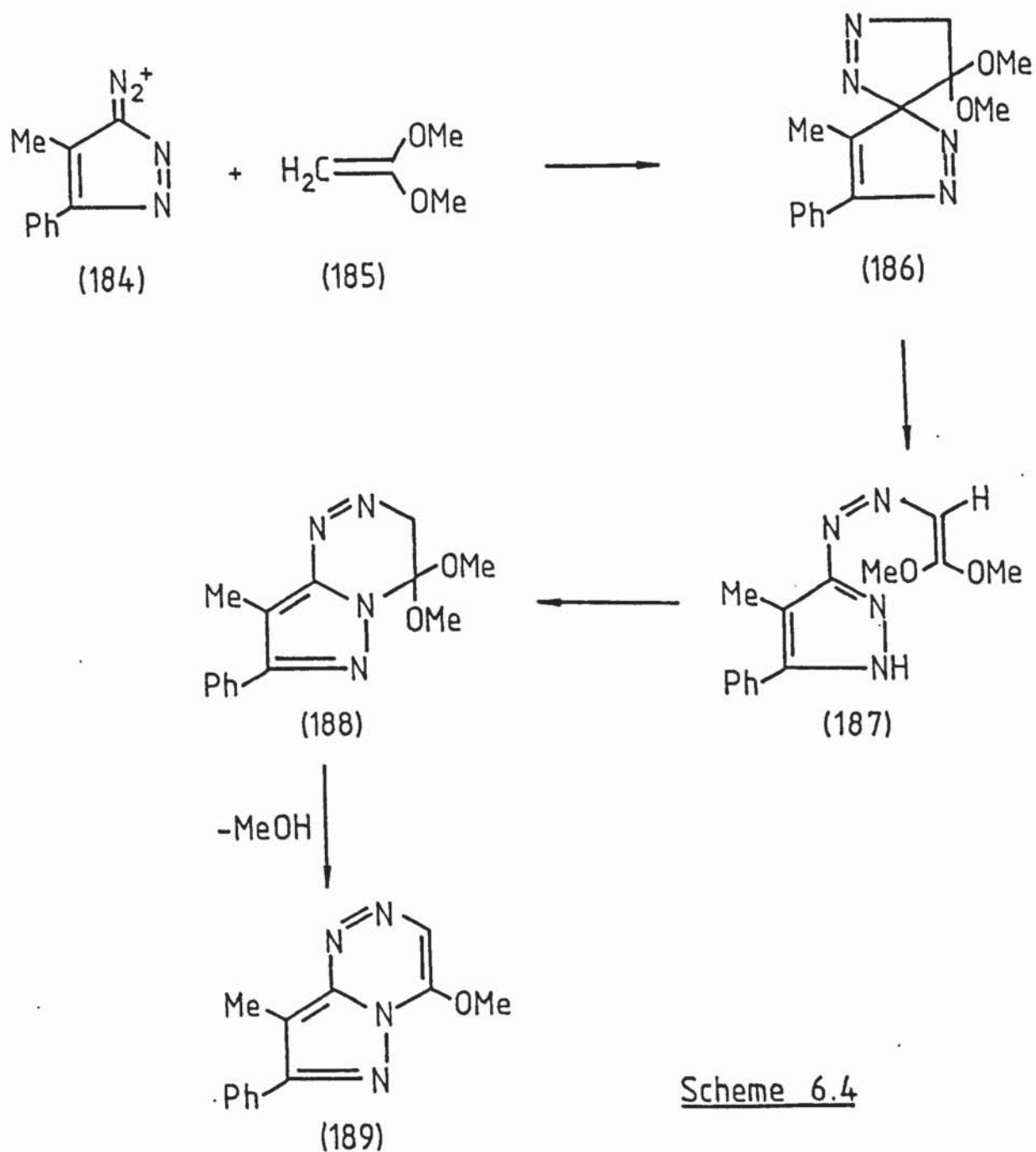
conditions. Similarly phenyl- and *p*-tolyl-isothiocyanates and *N,N'*-diphenyl-, *N,N'*-di-*p*-tolyl- and *N,N'*-dicyclohexyl-carbodiimides failed to participate in these reactions. In all these attempts Diazo-IC was recovered from the reaction mixture. There are of course other heterocumulenes which may be considered for further investigation: nitrile oxides, nitrile imines and nitrile sulphides constitute just three attractive possibilities.

6.1.3 Mechanism.

1,3-Dipolar cycloadditions have been extensively investigated as a route to heterocycles,²⁰⁶⁻²⁰⁹ with the addition of diazo-alkanes to olefins among the most thoroughly studied.²¹⁰ The cycloadditions of simple diazo-alkanes are regarded as concerted reactions.²¹¹⁻²¹³ However, the application of heterocyclic diazo compounds to such reactions has received only minimal attention, although this constitutes a route of immense practicability to other heterocyclic ring-systems.^{196,214-218}

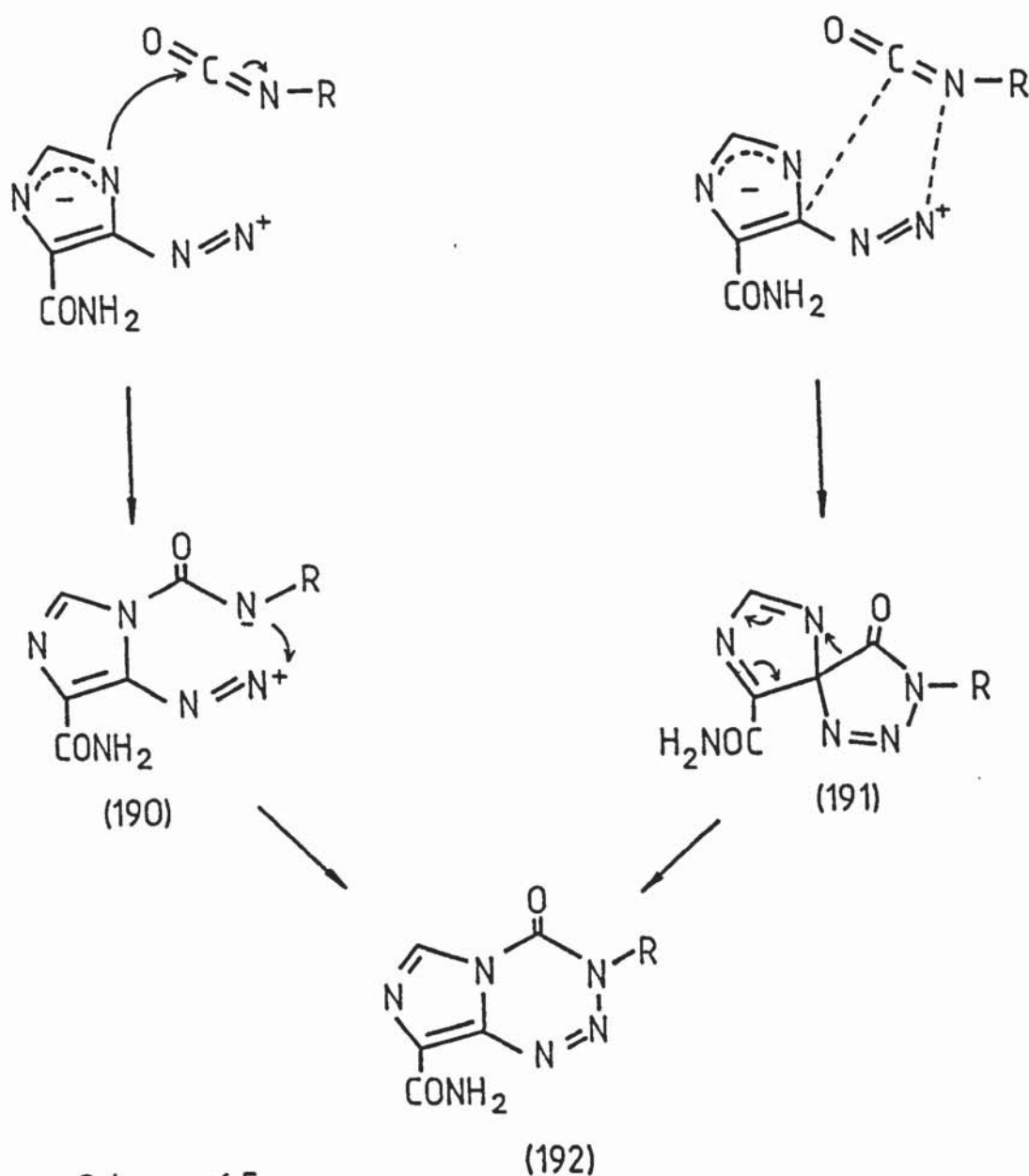
Padwa and Kumagai²¹⁹ have investigated the cycloaddition of 3-diazo-4-methyl-5-phenylpyrazole (184) and electron-rich olefins to yield 1,7-cycloadducts. Scheme 6.4 outlines the reaction with 1,1-dimethoxyethylene (185) to form the pyrazolotriazine (189). Examination of product formation as a function of time indicated the presence of intermediates (186-188). The synthesis can be rationalised as involving a concerted 1,3-dipolar addition to form (186) with subsequent rearrangement to the pyrazolotriazine (189).

The mechanism of cycloaddition between diazo-azoles and electron-deficient heterocumulenes has not yet been ascertained. Two alternatives may be considered — the stepwise or concerted pathways. Ege and Gilbert¹⁹⁶ proposed two possible alternatives for a stepwise mechanism: ring nitrogen acylation (190) followed by intramolecular cyclisation: or initial [3+2]



Scheme 6.4

cycloaddition (191) followed by a [1,5] sigmatropic shift (Scheme 6.5). The latter may be favourably compared with the mechanism described by Padwa and Kumagai²¹⁹ and, in view of their work, a stepwise mechanism seems likely, with initial [3+2] cycloaddition and subsequent molecular rearrange-



Scheme 6.5

ment an attractive prospect. Gilchrist and Storr²²⁰ also considered a concerted mechanism to be unlikely when heterocumulenes are involved.

"Heterocumulenes are usually efficient 1,3-dipolarophiles. Whether these cycloadditions are concerted is very much open to question since ionic intermediates would often be highly stabilised."

6.1.4 Characterisation of imidazo [5,1-d] [1,2,3,5] tetrazin-7(6H)-ones.

6.1.4.1 Spectroscopic properties.

The i.r. spectra of the imidazotetrazinones showed bands in the $3400\text{--}3150\text{ cm}^{-1}$ region indicative of N-H stretching of the carboxamide group. Absence of bands at 2250 and 2190 cm^{-1} indicated that the cumulative double bond systems isocyanate and diazo, present in the starting ingredients, were not present. The carboxamide carbonyl was observed between 1700 and 1650 cm^{-1} (Table 6.2) with the lactam carbonyl absorbing between 1750 and 1730 cm^{-1} . This latter value is comparable to the carbonyl stretching frequency recorded for the pyrazolotetrazinones.¹⁹⁶ When considering the two isomeric alternative structures (178) and (179) (Scheme 6.2) the lactam carbonyl would be expected to absorb as a broad band ($\sim 1700\text{ cm}^{-1}$). The carbonyl band of the secondary amide in these structures would also be expected below 1700 cm^{-1} .

All derivatives showed maximal absorption in their electronic absorption spectra around $325\text{--}340\text{ nm}$. Table 6.2 lists appropriate data (see also Section 6.2.1).

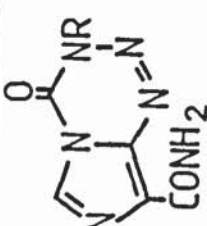
6.1.4.2 ^1H n.m.r. spectra.

The proton at position 1 in the imidazotetrazinones absorbed as a singlet at $\delta 8.9\text{--}9.0$ and integrated for one proton. [See Figure 6.1 for the spectrum of the 2-chloroethyl derivative (175).] The carboxamide NH_2 was observed as a broad peak ($\delta 7.7\text{--}7.9$) integrating for two protons. Correct assignment of structure was confirmed by absorptions corresponding to substituents at position 6 (Table 6.3).

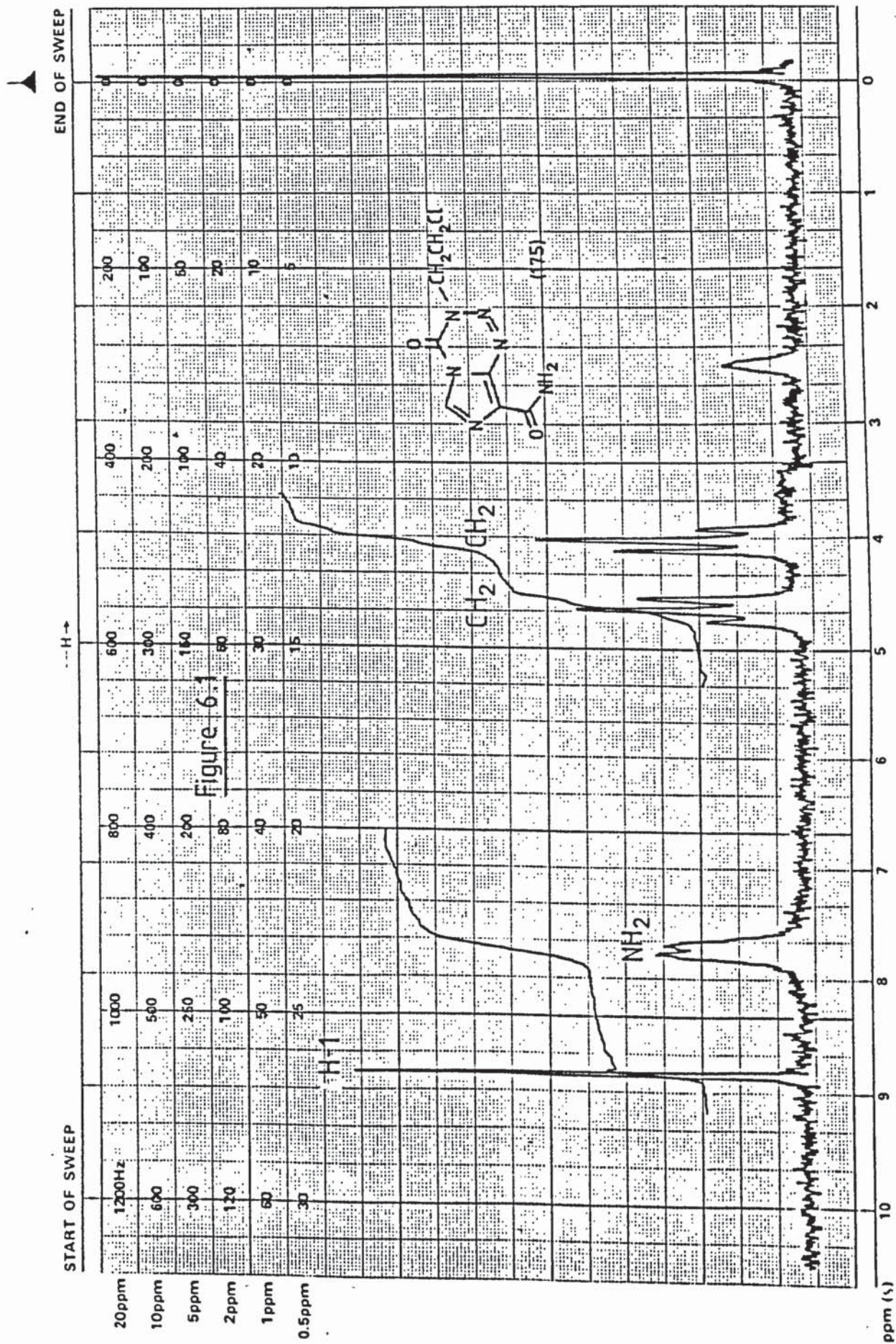
6.1.4.3 Mass spectra.

The 6-aryl derivatives of the imidazotetrazinones did not show a molecular ion in their mass spectra, however, peaks were present at mass

Table 6.2 Summary of u.v. and i.r. spectra of the imidazotetrazinones.

Compound Number.		$\lambda_{\text{max.}}$ (nm) ^a	$\nu_{\text{max.}}$ (cm ⁻¹) ^b	
167	Ph	331	3400, 3100(NH), 1730(lactam C=O), 1695, 1605(CONH ₂).	
168	4-NO ₂ C ₆ H ₄	330	3450, 3150(NH), 1750(lactam C=O), 1680, 1600(CONH ₂), 1525, 1350(NO ₂).	
169	3-CNC ₆ H ₄	328	3400, 3150(NH), 2210(C≡N), 1750(lactam C=O), 1690, 1610(CONH ₂).	
170	4-ClC ₆ H ₄	334	3450, 3100(NH), 1730(lactam C=O), 1700, 1610(CONH ₂).	
171	4-MeC ₆ H ₄	335	3400, 3170(NH), 1735(lactam C=O), 1700, 1610(CONH ₂).	
172	4-MeOC ₆ H ₄	337	3420, 3080(NH), 1730(lactam C=O), 1660, 1640(CONH ₂).	
173	4-EtOC ₆ H ₄	338	3420, 3080(NH), 1730(lactam C=O), 1650, 1640(CONH ₂).	
174	1-Naphthyl	300 ^c	3350, 3100(NH), 1750(lactam C=O), 1680, 1610(CONH ₂).	
175	CH ₂ CH ₂ Cl	325	3500, 3200(NH), 1740(lactam C=O), 1680, 1600(CONH ₂).	
176	n-Pr	328	3400, 3100(NH), 1720(lactam C=O), 1690, 1610(CONH ₂).	
177	Me	327	3390, 3100(NH), 1750(lactam C=O), 1680, 1600(CONH ₂).	

a 95% ethanol; b KBr disc; c very broad peak.



OPERATOR ROBERT STONE

REMARKS: TMS, std.

DATE 7.10.1980

SPECTRUM NO. RYS168.2

SAMPLE RYS 168

min

5

SWEEP TIME

2 x 100

SPECTRUM 1:1PL

SWEEP WIDTH 10 ppm

sec

FILTER 0.05

END OF SWEEP 0 min

mG

RF POWER 0.05

SOLVENT DMSO-d6

Table 6.3 ^1H n.m.r. spectra of imidazotetrazinones recorded in $\text{DMSO}-d_6$

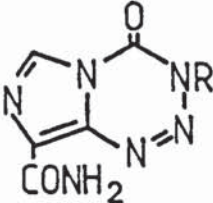
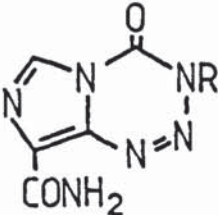
Compound Number.		Chemical Shifts (δ)	
167	Ph	8.95	(1H, s, H-1)
		7.85	(2H, br. s, NH_2)
		7.60	(5H, m, Ph)
169	3- CNC_6H_4	9.00	(1H, s, H-1)
		7.85	(2H, br. s, NH_2)
		7.45	(4H, m, Ar)
170	4- ClC_6H_4	8.95	(1H, s, H-1)
		7.70	(6H, br. m, Ar and NH_2)
171	4- MeC_6H_4	8.95	(1H, s, H-1)
		7.80	(2H, br. s, NH_2)
		7.50	(4H, m, Ar)
		2.40	(3H, s, Me)
172	4- MeOC_6H_4	9.05	(1H, s, H-1)
		7.90	(2H, br. s, NH_2)
		7.40	(4H, q, Ar)
		3.90	(3H, s, Me)
173	4- EtOC_6H_4	8.95	(1H, s, H-1)
		7.85	(2H, br. s, NH_2)
		7.35	(4H, q, Ar)
		4.10	(2H, q, CH_2)
		1.35	(3H, t, Me)

Table 6.3 (continued).

Compound Number.		Chemical Shifts (δ)
174	1-Naphthyl	9.05 (1H,s,H-1) 8.25-7.65 (9H,br.m,Naphthyl and NH ₂)
175	CH ₂ CH ₂ Cl	8.85 (1H,s,H-1) 7.70 (2H,br.s,NH ₂) 4.60 (2H,t,CH ₂ Cl) 4.05 (2H,t,CH ₂ CH ₂ Cl)
176	n-Pr	8.80 (1H,s,H-1) 7.75 (2H,br.s,NH ₂) 4.25 (2H,t,CH ₂ CH ₂ CH ₃) 1.80 (2H,m,CH ₂ CH ₂ CH ₃) 0.95 (3H,t,Me)
177	Me	8.85 (1H,s,H-1) 7.75 (2H,br.s,NH ₂) 3.90 (3H,s,Me)

values corresponding to Diazo-IC (m/e 137) and the appropriate isocyanate. The equal abundance of these peaks implied that retro-cycloaddition in the spectrometer probe was occurring.

The alkyl derivatives in contrast all showed abundant molecular ions together with peaks corresponding to Diazo-IC and the appropriate isocyanate.

6.2 Investigation of Physical Properties of Imidazotetrazinones.

6.2.1 Estimation of half-life ($t_{1/2}$).

In order to compare the stability of substituted imidazotetrazinones in 95% ethanol $t_{1/2}$ for each compound was estimated by u.v.-visible spectroscopy. The spectra after total degradation ($t = \infty$) of the imidazotetrazinones resembled a spectrum of 2-azahypoxanthine.

Changes in absorbance with time were monitored for each compound at λ_{max} of the long-wavelength absorption band (eg. Figure 6.2) and exponential decay was observed. Therefore, first order kinetics applied.

$$\text{First order rate equation} \quad -\frac{\delta C}{\delta t} = kC$$

$$\text{Therefore} \quad kt = \ln \frac{(C_0 - C_\infty)}{(C - C_\infty)}$$

When C = initial concentration

C_0 = concentration at time t

C_∞ = concentration at infinity

k = first order velocity constant

A plot of $\ln \frac{(C_0 - C_\infty)}{(C - C_\infty)}$ verses time allowed graphical estimation of $t_{1/2}$ (eg. Figure 6.3) while linear regression indicated correlation coefficients (r) in the order of 0.9999 for this data. Table 6.4 details the $t_{1/2}$ values for the imidazotetrazinones.

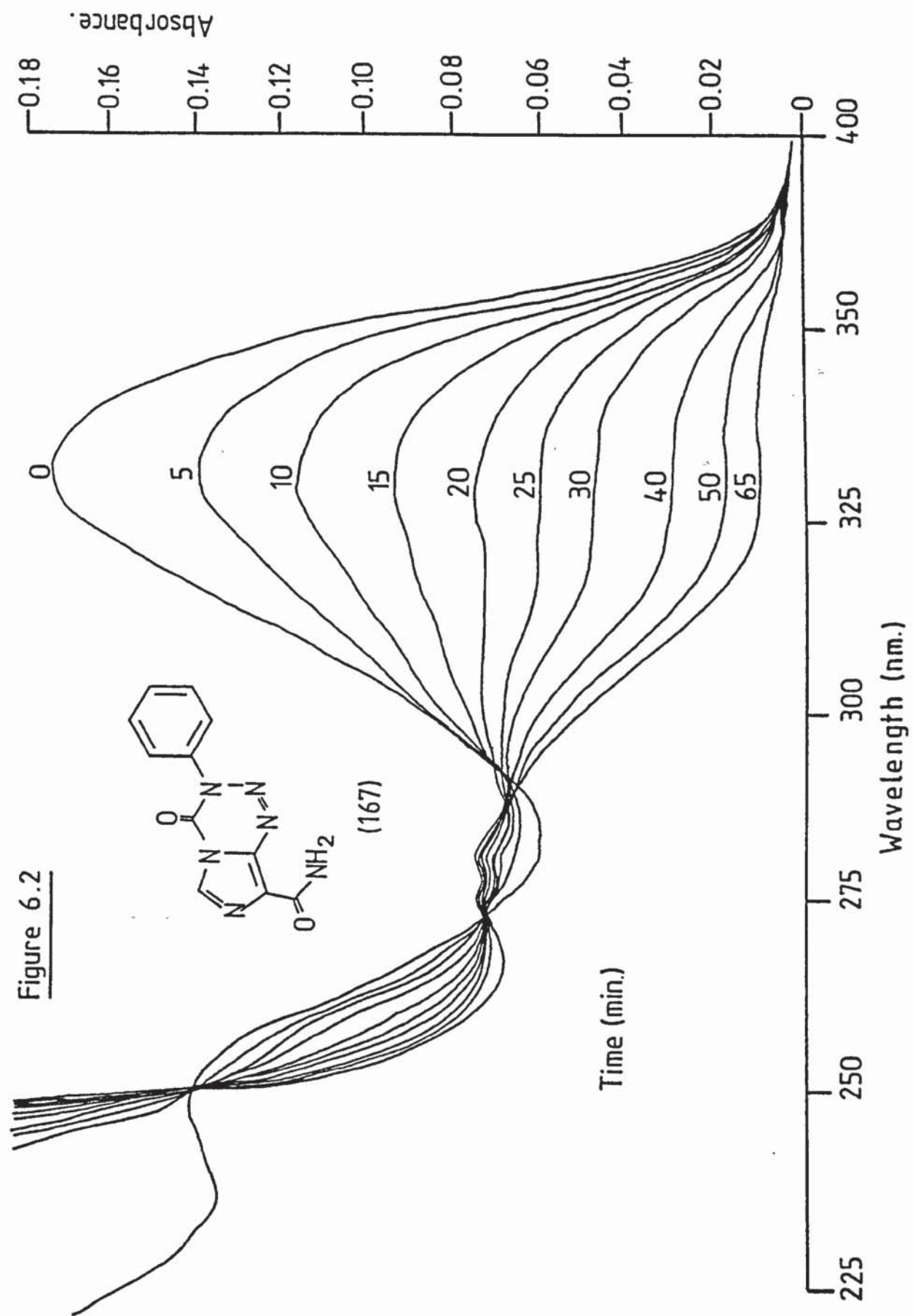


Figure 6.3

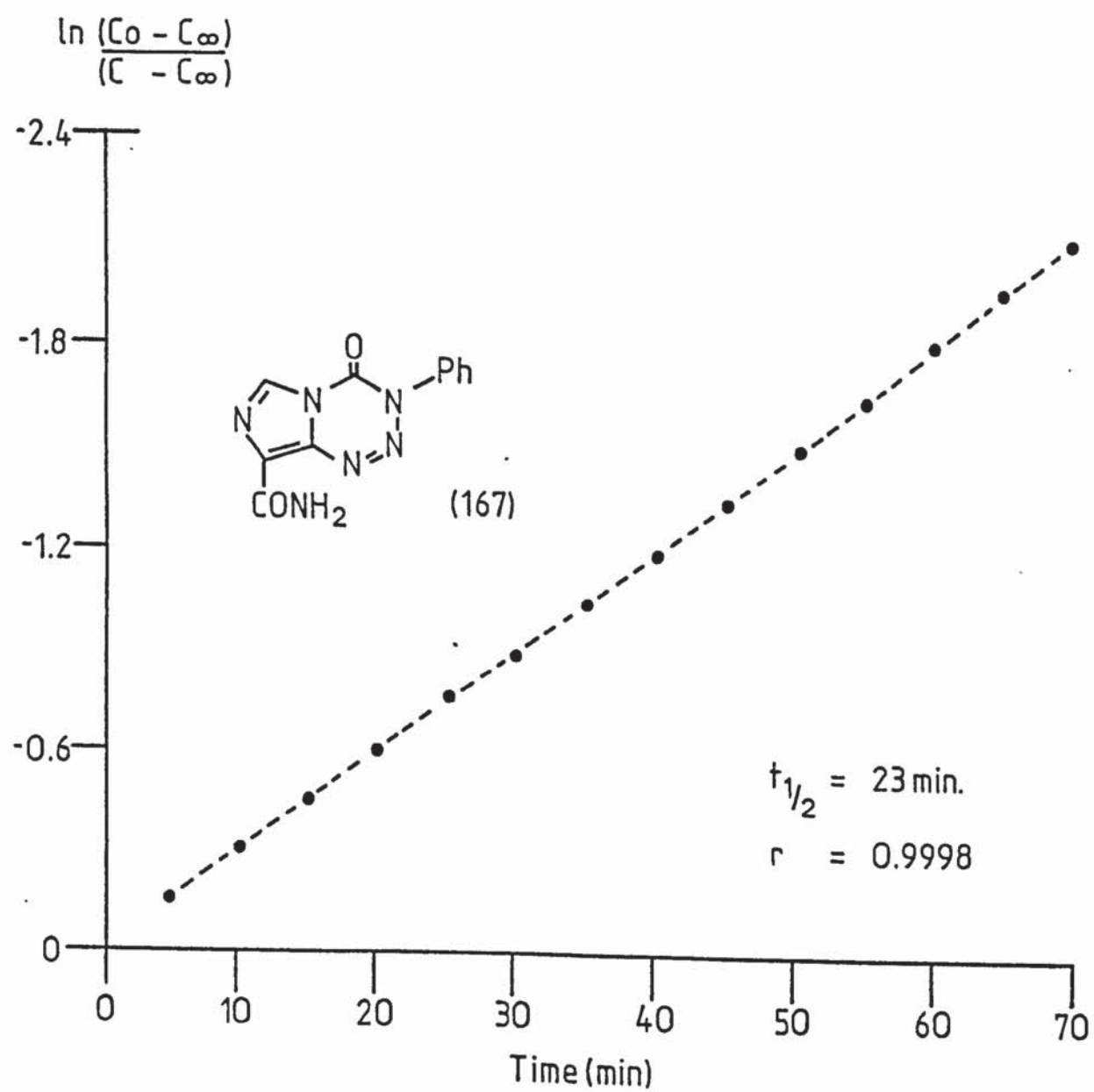
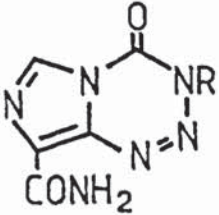


Table 6.4 Stability of the imidazotetrazinones in solution.

Compound Number.		$t_{1/2}$ (min)		
		A	B	C
167	Ph	23.0	9.0	-
169	3-CNC ₆ H ₄	2.6	-	-
170	4-ClC ₆ H ₄	6.3	8.2	2.9
171	4-MeC ₆ H ₄	40.0	7.5	-
172	4-MeOC ₆ H ₄	69.0	8.5	155.0
173	4-EtOC ₆ H ₄	78.0	8.0	-
174	1-Naphthyl	4.6	-	-
175	CH ₂ CH ₂ Cl	72.0	70.0	98.0
176	n-Pr	155.0	155.0	-
177	Me	495.0	495.0	-

A: 95% ethanol, dark, 28°.

B: 95% ethanol, light, 18°.

C: Phosphate buffer pH 7.4, dark, 28°

6.2.1.1 Aryl derivatives.

Electron-withdrawing substituents in the phenyl ring were found to reduce the stability of the ring-system in ethanol, whereas electron-releasing groups stabilised the imidazotetrazinone ring-system. The 4-nitro derivative (168) decomposed too rapidly for an accurate determination of $t_{1/2}$ to be measured. A Hammett plot of substituent constant (σ) against $t_{1/2}$ with respect to the unsubstituted phenyl derivative (167) confirmed this relationship (Figure 6.4).

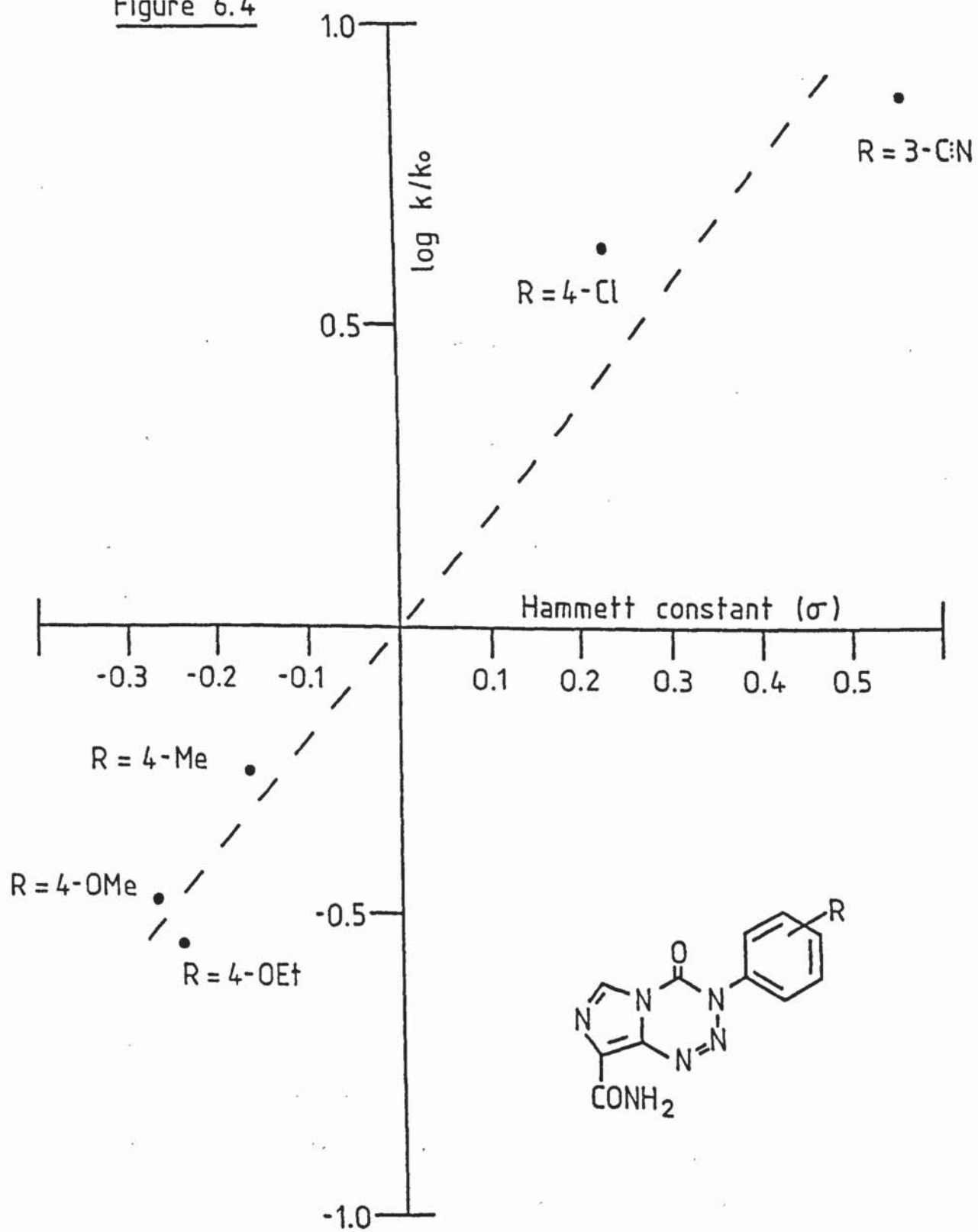
Exposure of ethanolic solutions of the 6-arylimidazotetrazinones to light was found to result in faster degradation (Table 6.4) and the nature of the substituent in the phenyl ring did not have a significant effect on stability under such conditions. This would seem to indicate a different mechanism (possibly radical in nature) in photodegradation. Because of the possible biological importance of compounds in this series (see Section 7.2) estimations of $t_{1/2}$ for compounds (170) and (172) in phosphate buffer at physiological pH were also made.

Further investigation of the phenyl derivative (167) showed rapid degradation in 95% ethanol after the addition of aqueous ammonium hydroxide ($t_{1/2} \sim 3$ min.), while the presence of hydrochloric acid had no significant effect on $t_{1/2}$. This phenyl derivative proved to be stable in dry dichloromethane at 28° in the dark; however, exposure to white light resulted in immediate degradation which followed first order kinetics. Removal of the light source resulted in a stable solution containing a lower concentration of the substrate. Half-life under such conditions was 17.9 minutes.

6.2.1.2 Alkyl derivatives.

The alkylimidazotetrazinones were more stable in 95% ethanol

Figure 6.4



than the aryl derivatives, with the methyl derivative [177] having a $t_{\frac{1}{2}}$ of 495 minutes at 28° (Table 6.4). Increase in carbon chain length, and introduction of halogen were both associated with reduced stability. These N-alkyl compounds were light stable, and formed stable solutions in dry dichloromethane. The 2-chloroethyl derivative [175] was reasonably soluble in phosphate buffer (pH 7.4) and a value of 98 minutes was obtained for $t_{\frac{1}{2}}$.

Addition of aqueous ammonium hydroxide resulted in rapid degradation in ethanolic solutions of N-alkyl compounds ($t_{\frac{1}{2}} \sim 3$ min.), while hydrochloric acid produced no significant change in stability.

6.2.1.3 Investigation of known pyrazolotetrazinones.

Compound [180] was found to be unstable in 95% ethanol at 28° in the dark. This has not been previously reported. Values of 152 and 13.2 minutes were obtained for $t_{\frac{1}{2}}$ in dark and light conditions respectively. The greater stability when compared with [167] may be explained by the ester group which is incapable of cyclisation to a 2-azapurinone. This is considered to be a component of the "driving-force" to tetrazine ring-opening in the imidazotetrazinones.

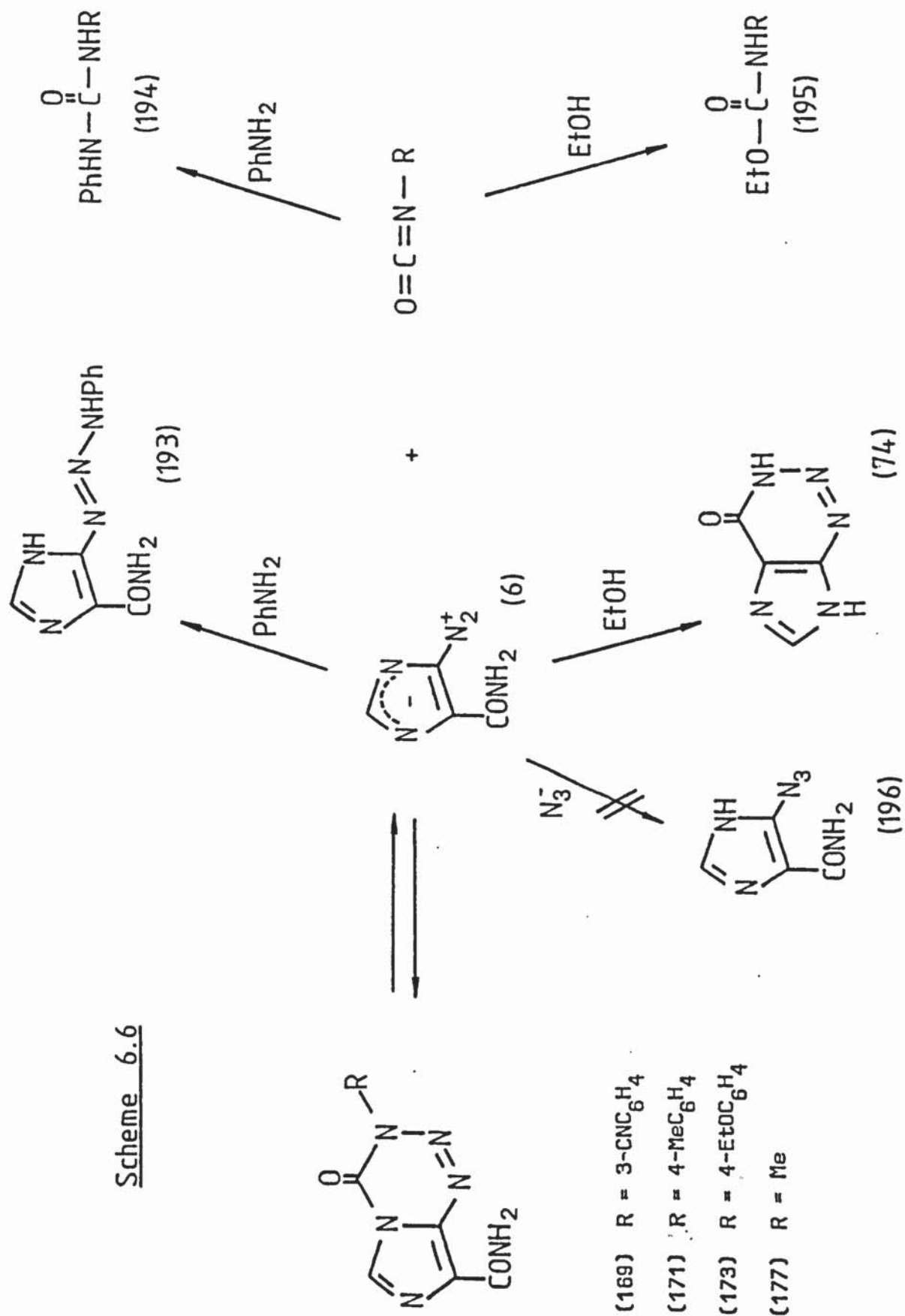
The pyrazolotetrazinone [180] was stable in dry dichloromethane in the dark, but exposure to white light resulted in degradation ($t_{\frac{1}{2}} = 19.2$ min.). Removal of the light source gave a stable solution containing a lower concentration of the pyrazolotetrazinone. When this experiment was repeated at 0-5°, an increase in absorbance at λ_{max} was noted after the light source was removed. Presumably reassociation of the degradation product(s) was occurring in the dark.

The other pyrazolotetrazinone [181] was stable in all solvents tested under both light and dark conditions.

Interaction of compounds (169,171,173 and 177) with aniline resulted in formation of a triazene (193) and the corresponding ureas (194) (Scheme 6.6). This was confirmed by t.l.c. examination of the reaction mixture against standard compounds on silica plates using chloroform / methanol mixtures as developing solvents. The triazene (193) was independently prepared from Diazo-IC and aniline.¹⁶³

T.l.c. analysis of the mixtures formed by degradation of compounds (171) and (177) in 95% ethanol confirmed the presence of 2-azahypoxanthine (74) and the corresponding carbamates (195) (Scheme 6.6).

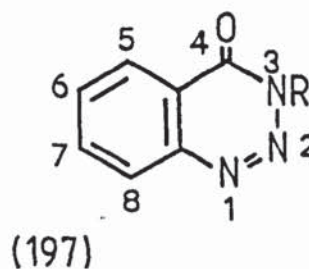
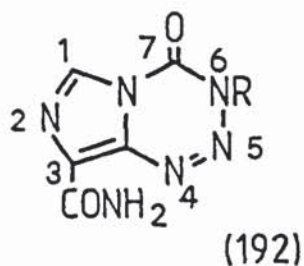
A Sandmeyer-type reaction was attempted in order to trap any diazo intermediate in the degradative pathway. The 4-methoxyphenyl compound (172) was decomposed in the presence of sodium azide in glacial acetic acid. However, formation of the azide (196) following cleavage of the tetrazine ring was not observed although 2-azahypoxanthine could be detected as a result of Diazo-IC release.



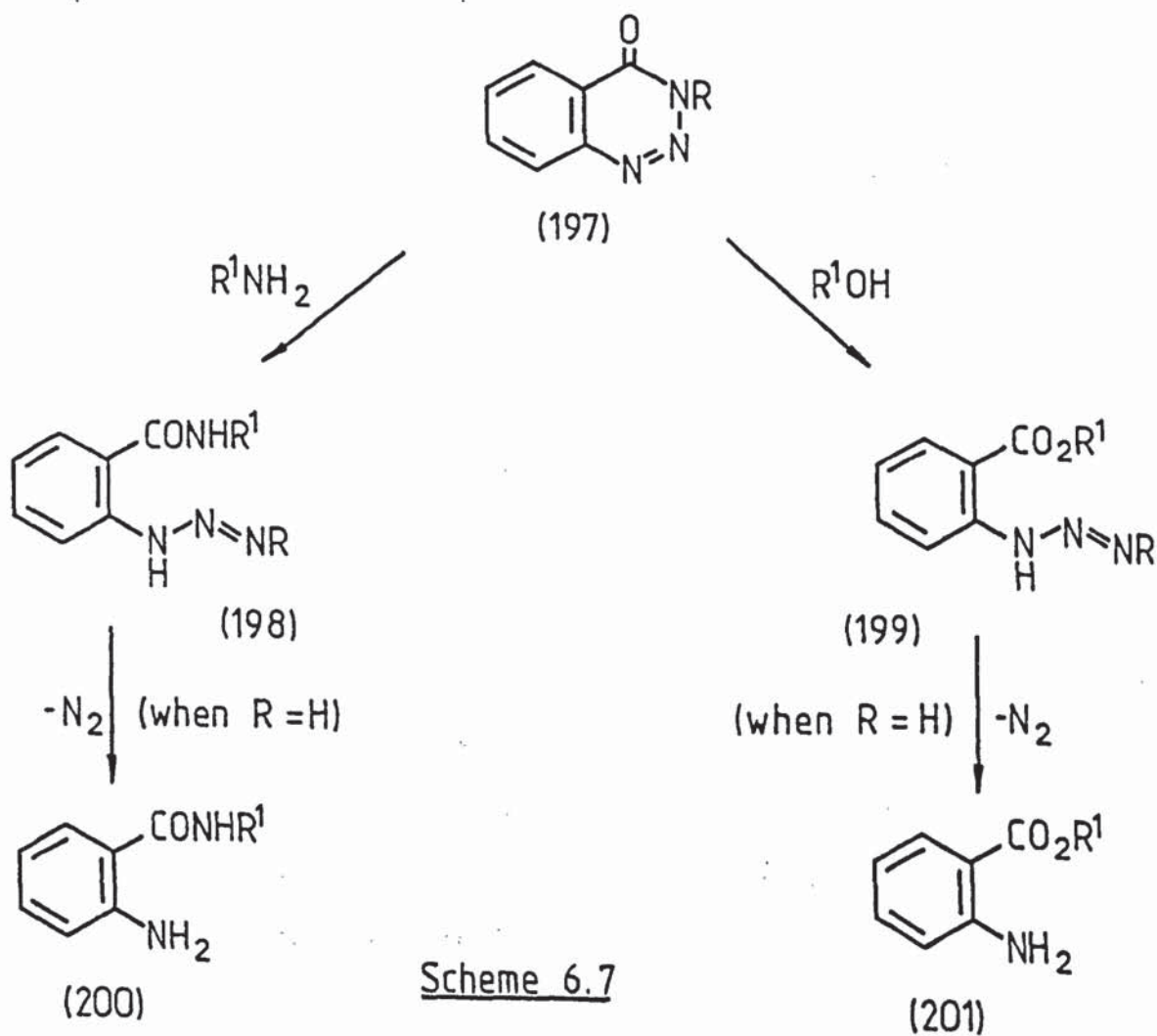
6.4

Comparison between Imidazotetrazinones and1,2,3-Benzotriazin-4-(3H)-ones.

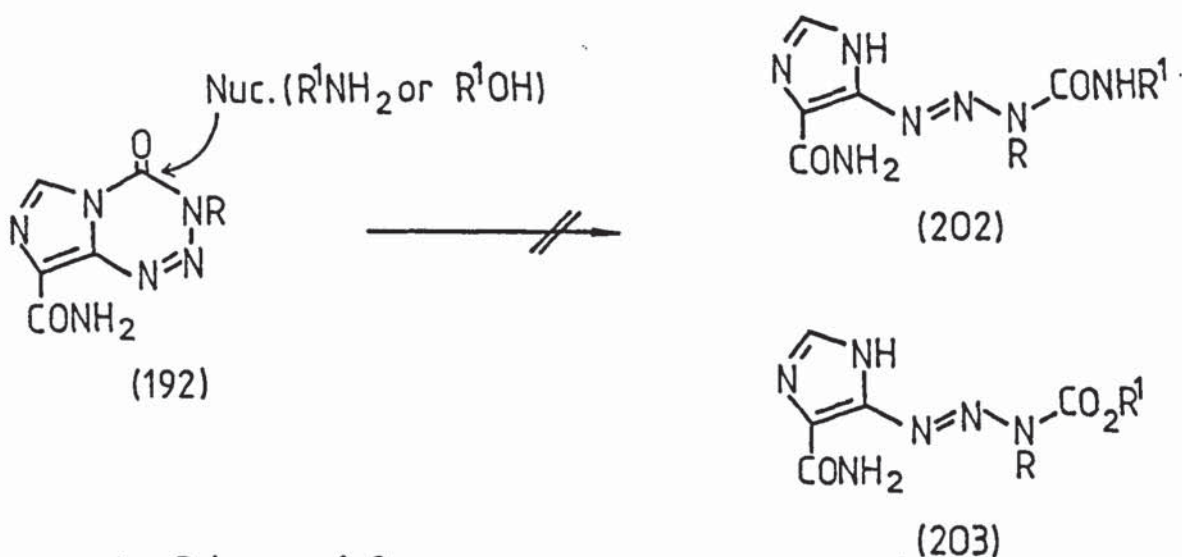
A comparison may be made between the chemical reactions of 6-substituted imidazotetrazinones and 3-substituted 1,2,3-benzotriazinones [197].



The benzotriazinones are known to undergo nucleophilic attack at C-4^{121,122} resulting in ring-opening to yield triazenes, (198) and



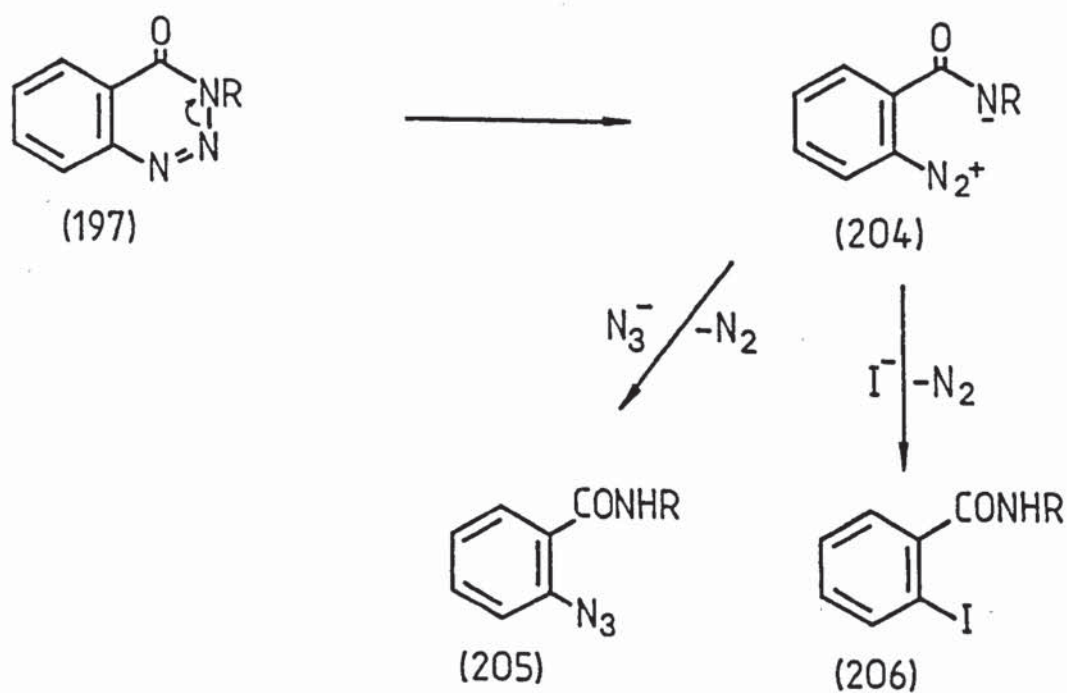
(199), with amines and alcohols respectively (Scheme 6.7). The parent benzotriazinone (197; R = H) subsequently loses nitrogen to afford anthranilamide derivatives, (200) and (201). There is no evidence to indicate that imidazotetrazinones undergo a comparable attack at C-7. Such a pathway initiated by reaction with amines or alcohols (Scheme 6.8) would result in the formation of triazenes (202) and (203). Thiones of the



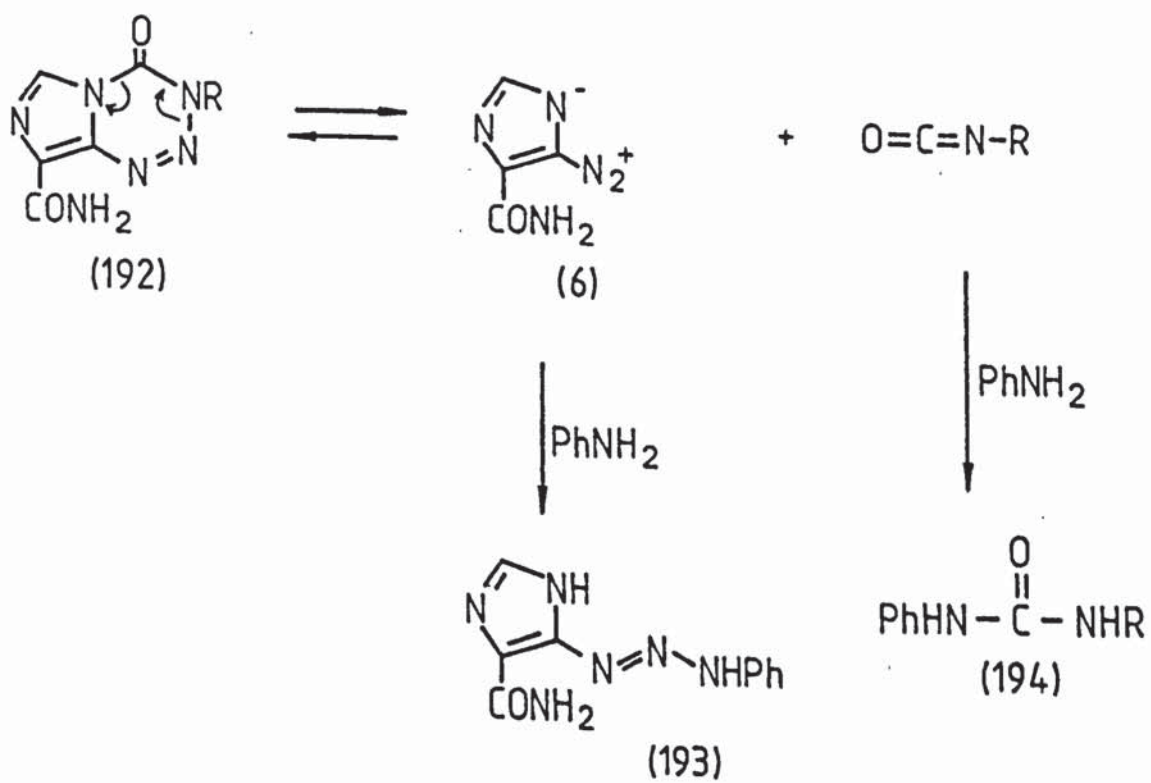
Scheme 6.8

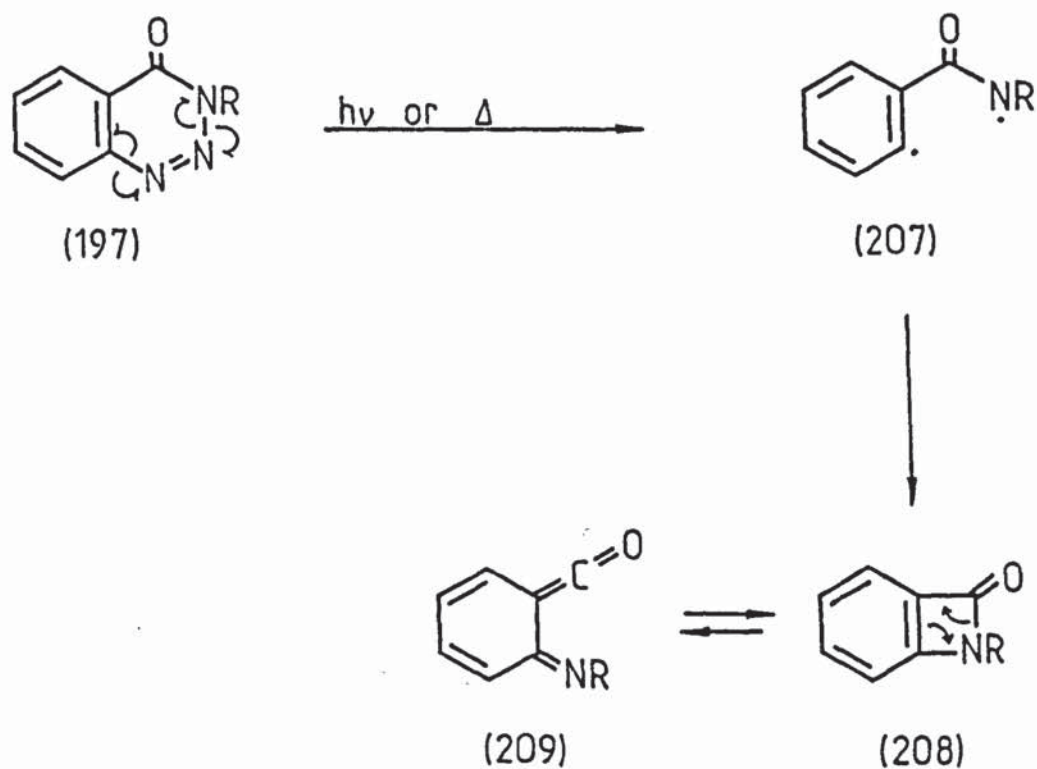
triazenes (202; aryl instead of imidazocarboxamide) are known and can be prepared from mono-substituted triazenes and isothiocyanates (when R = aryl).²²³ These triazenes have not been detected as degradation products of imidazotetrazinones.

Benzotriazinones undergo heterolytic fission²²⁴ to generate the betaine (204) which may be trapped with a nucleophile (i.e. iodide or azide).²²⁵ Heterolysis also explains the observed degradation of imidazotetrazinones in protic solvents. Presence of aniline in solution intercepts the diazo intermediate (Diazo-IC), generated by release of isocyan-



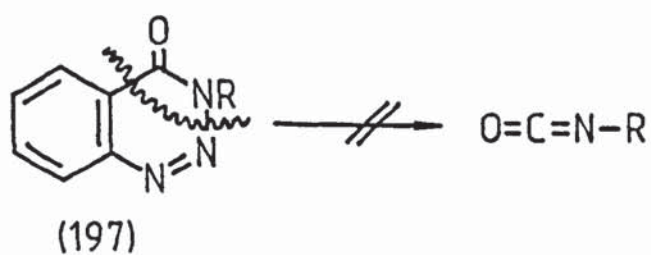
Scheme 6.9





Scheme 6.11

No evidence for the liberation of an isocyanate fragment has ever been adduced in the extensive literature covering the fragmentation of benzotriazinones.²³⁰



Biological Evaluation

7.1 Biological Evaluation of Arylazoimidazoles.

7.1.1 Antitumour activity.

A representative sample of arylazoimidazoles was screened by the National Cancer Institute (U.S.A.) for antitumour activity. The mouse lymphocytic leukaemia P388 system²³¹ was used for a primary screen.

Result

Compound number	Maximum daily dose (mg/kg)	Result
117	400	inactive
118	200	inactive
122	400	inactive
125	400	inactive
126	400	inactive

7.1.2 General screening.

All arylazoimidazoles were submitted for general screening. This involved testing for activity in the following areas :

Antiarthritic activity	Antiviral activity
Antibacterial activity	Fungicidal activity
Antimalarial activity	Herbicidal activity
Antitrichomonal activity	Insecticidal activity

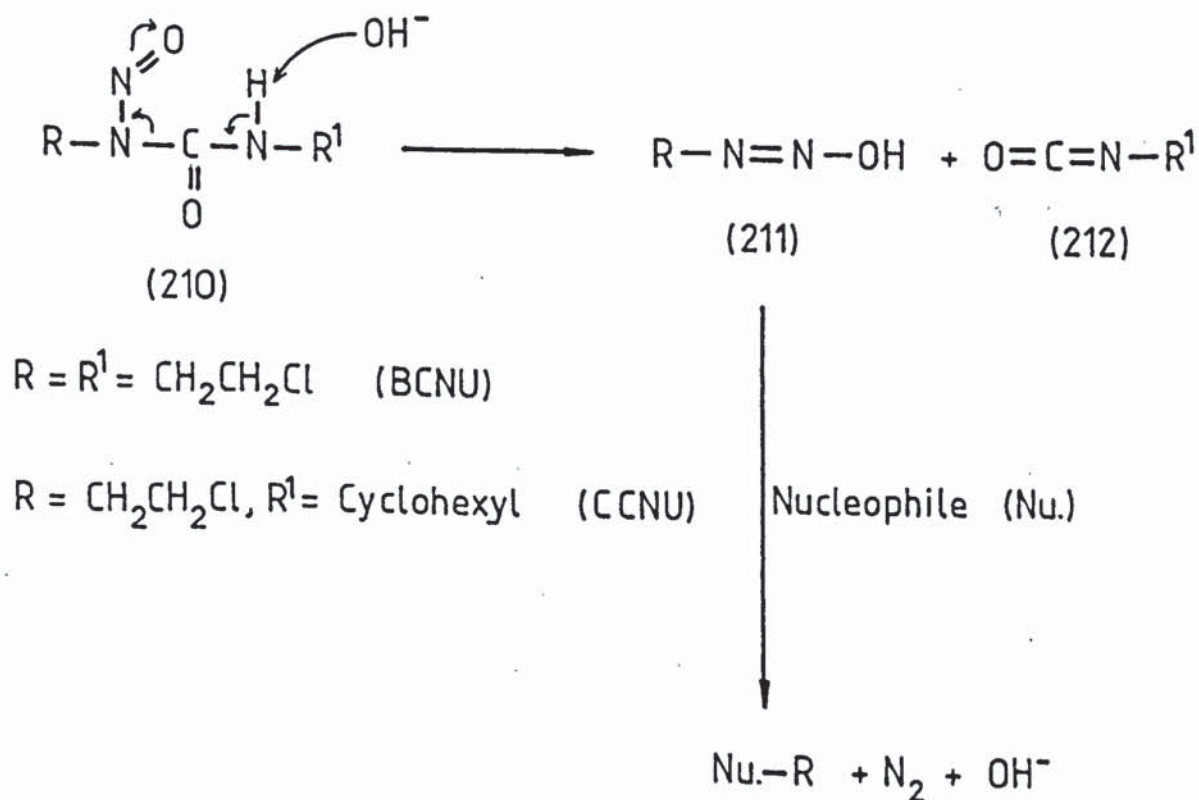
Result

Five derivatives (121,123,137,138 and 142) demonstrated antibacterial activity against anaerobes. No other activity was noted.

7.2 Biological Evaluation of Imidazotetrazinones.

7.2.1 Antitumour activity.

Nitrosoureas (210) are known antitumour agents and several of them have undergone clinical trials. They are known to hydrolyse in aqueous media²³² (Scheme 7.1), releasing an isocyanate (212) and an incipient alkylating species (211). Although the mechanism of antitumour action of the nitrosoureas is unclear, it is currently thought that both the incipient alkylating action and the carbamoylating activity of the isocyanate may be important.^{233,234}



Scheme 7.1

Investigation of the chemical properties of the imidazotetrazinones (Section 6.3) indicated release of Diazo-IC and the appropriate isocyanate under certain conditions. The similarity between the degradative products of nitrosoureas and 6-substituted imidazotetrazinones prompted the use of TLX5(S)²³⁵ as an initial screen for antitumour activity of imidazotetrazinones. This mouse tumour is particularly responsive to nitrosoureas but inherently resistant to alkylating agents of the β -chloroethyl type.

Table 7.1 summarises the data obtained from antitumour evaluation of imidazotetrazinones and related compounds. The 4-chlorophenyl (170) and 4-methoxyphenyl (172) imidazotetrazinones proved to be inactive against the initial screen [TLX5(S)] at daily doses of 64 and 32 mg/kg respectively. However, two 6-alkyl derivatives, 2-chloroethyl (175) and methyl (177), were found to be active against TLX5(S). Activity of (175) against TLX5(S) is graphically illustrated in Figure 7.1.

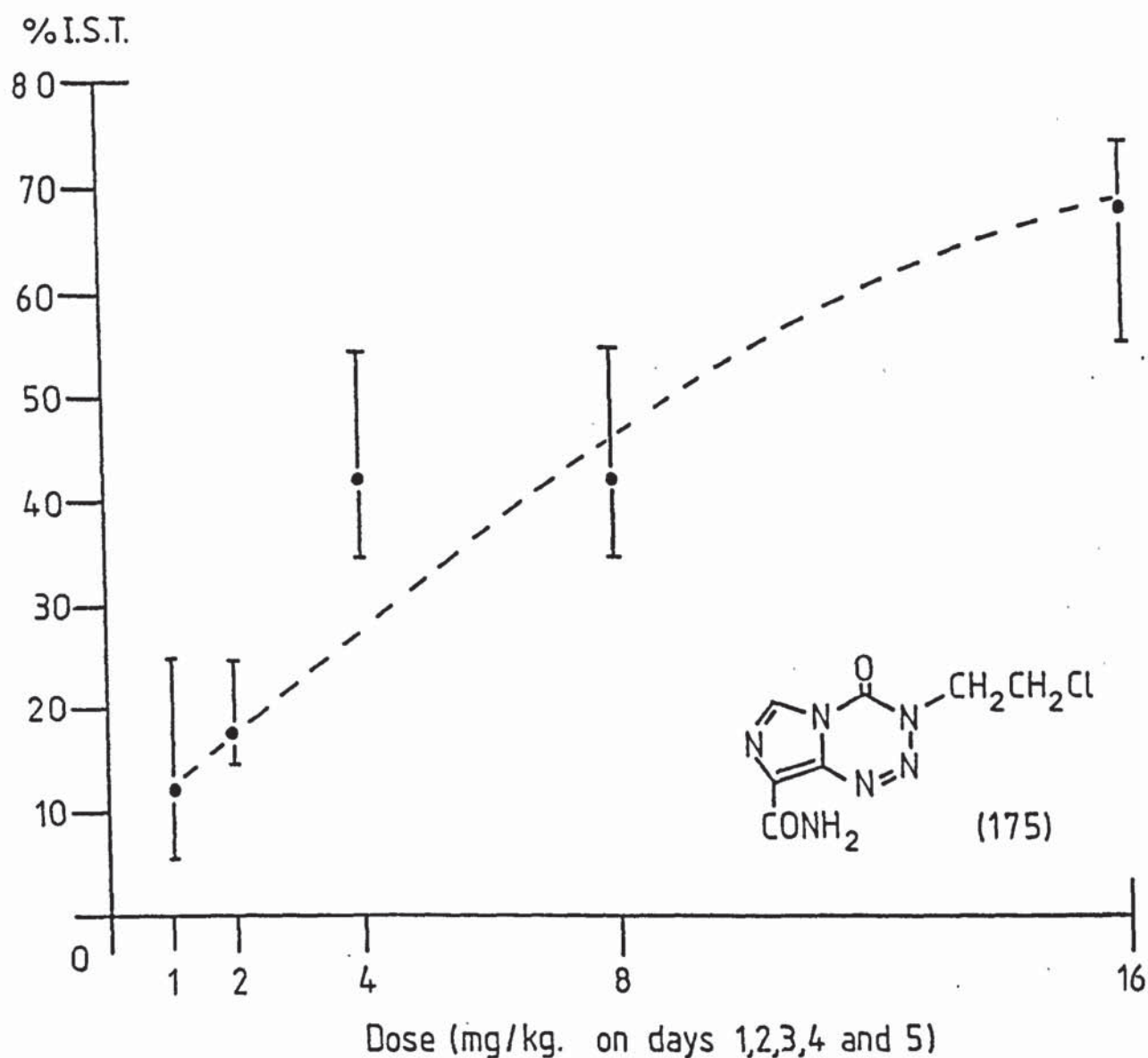
It can be seen from Table 7.1 that Diazo-IC, one of the fragments which might be liberated from (175) or (177) *in vivo*, was inactive against TLX5(S). It must be remembered, however, that gradual release of Diazo-IC from a compound such as (175), within susceptible cells, could considerably alter its bioavailability. DTIC was found to give a comparable maximum %I.S.T. to compound (175) against TLX5(S) although an acceptable dose-response curve could not be obtained for the triazene.

The 2-chloroethyl derivative (175) showed activity against L1210²³¹ (both sensitive and resistant to nitrosoureas) solid and ascites tumours. Independent screening²³⁶ of this compound at Rhone--Poulenc, France has also shown a significant level of activity against L1210(S) with 10/10 survivors on day 35 (391.0 %I.S.T.) after administration of

Table 7.1 A summary of antitumour testing of imidazotetrazinones and related compounds.

Compound	Tumour	Site of implantation	Histology	No. of ^a Doses	Toxic dose (mg/kg/day)	Maximum %I.S.T.	Dose (mg/kg/day)	No. of Cures
170	TLX5(S)	s.c.	solid	5	A	1.9	64.0	0/5
172	TLX5(S)	s.c.	solid	5	A	-3.7	32.0	0/5
175	TLX5(S)	s.c.	solid	5	32	67.3	16.0	0/5
175	TLX5(R)	s.c.	solid	5	32	17.7	32.0	0/5
175	L1210(S)	s.c.	solid	5	32	>500.0	16.0	5/5 ^d
175	L1210(R)	s.c.	solid	5	32	52.0	32.0	0/5
175	L1210(S)	i.p.	ascites	6	32	230.0	16.0	3/6 ^d
175	L1210(S) ^b	i.p.	ascites	4	20	391.0	10.0	10/10 ^c
175	L1210(CR) ^b	i.p.	ascites	4	20	>360.0	10.0	9/10 ^c
175	P388 ^b	i.p.	ascites	4	15	663.0	7.5	5/5 ^d
Diazo-IC	TLX5(S)	s.c.	solid	5	16	4.0	4.0	0/5
DTIC ^e	TLX5(S)	s.c.	solid	5	A	98.0	6.25	0/5
DTIC	L1210(S) ^b	i.p.	ascites	4	A	133.0	200.0	0/10
DTIC	L1210(CR) ^b	i.p.	ascites	4	200	132.0	100.0	0/10
DTIC	P388 ^b	i.p.	ascites	4	A	181.0	100.0	0/5

Figure 7.1 Dose - response curve : Compound (175) vs TLX5(S)



10 mg/kg/day on days 1,2,3 and 4. DTIC (200 mg/kg/day) gave a 133.0 %I.S.T. with no survivors on day 35.

Activity was also observed against a cyclophosphamide - resistant L1210 tumour, with 10 mg/kg/day of compound (175) producing a >360.0 %I.S.T. (9/10 survivors). A lower level of antitumour activity was observed

with DTIC (100 mg/kg/day) against this tumour (132.0%I.S.T., no survivors).

It should be noted that the cyclophosphamide - resistant L1210 and the TLX5(S) are both tumours resistant to β -chloroethyl type alkylating agents, but normally sensitive to nitrosoureas. The activity of compound (175) against these tumours was paralleled by an *in vitro* study²³⁷ which demonstrated a similar level of growth inhibition by (175) against Walker tumour cells, both sensitive and resistant to β -chloroethyl type alkylating agents.

Independent screening²³⁶ of the 2-chloroethyl compound (175) against the P388 tumour at a dose of 7.5 mg/kg/day gave >663.0%I.S.T. with all test mice surviving on day 60. This compares with 181.0%I.S.T. (no survivors) when mice bearing P388 were treated with DTIC.

Screening of (175) against M5076* ovarian sarcoma in mice indicated an optimum dose of 8 mg/kg/day which resulted in 5/5 cures on day 70. DTIC (25 mg/kg on days 1,5,9,13 and 17) and a nitrosourea, N¹-(2-chloroethyl)-N³-cyclohexyl-1-nitrosourea (40 mg single dose on day 1), also gave 100% cures.

The 2-chloroethyl derivative (175) was found to be active against Lewis lung carcinoma in mice²³⁶; thus 20 mg/kg/day for four days resulted in 85% inhibition of primary tumour growth and 100% inhibition of metastases. (cf. DTIC 100 mg/kg/day; 27% inhibition of primary tumour, 61% inhibition of metastases). Remarkably, oral administration of (175) (instead of i.p.) gave a comparable level of activity against both Lewis lung carcinoma and the P388 tumour in mice.²³⁶

* I.m. injection of 10^6 M5076 ovarian sarcoma cells per mouse (volume 0.1 ml) on day 0. Drug administered daily, i.p., on days 1 - 17. Evaluation of antitumour activity by measurement of tumour volume on day 70.

The spectrum of antitumour activity demonstrated by 3-carbamoyl-6-(2-chloroethyl)imidazo [5,1-*d*][1,2,3,5] tetrazin-7(6*H*)-one on the tumour test systems examined is notably similar to that of the nitroso-ureas, although, it is not possible at the present time to draw any firm conclusions regarding the mode of action of this imidazotetrazinone.

The methyl derivative (177) was also found to be active against L1210(S) (Table 7.1) with minimal weight loss occurring at 80 mg/kg/day. It appears that the methyl compound may be less toxic than the 2-chloroethyl derivative. Further antitumour evaluation of the methyl compound is in progress at the time of writing.

Experimental Detail

1. I.r. spectra were recorded as KBr discs on a Unicam SP 200 spectrophotometer.
2. U.v. spectra were recorded on a Unicam SP 8000 spectrophotometer in 95% ethanol unless otherwise specified.
3. ^1H n.m.r. spectra were recorded on a Varian EM 360A NMR spectrometer in $\text{DMSO}-d_6$ unless otherwise stated. TMS was incorporated as an internal standard.
4. Mass spectra were measured at 70 eV on a V.G. Micromass 12B single focusing spectrometer. Relative intensities (I%) are given in parentheses.
* denotes most abundant ion. ie. (100).
5. All melting-points are recorded uncorrected.

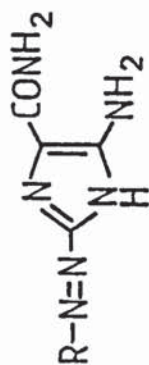
5-Amino-2-phenylazoimidazole-4-carboxamide (117)

Aniline (3.72g; 0.04 mol) was diazotised in 2N-hydrochloric acid (80 ml) by the dropwise addition of sodium nitrite (2.76g; 0.04 mol) in water (10 ml) at low temperature (0-5°). After addition was completed the reaction mixture was stirred for a further 0.25 h.

A solution of AIC hydrochloride (6.48g; 0.04 mol) in water (200 ml) which incorporated excess sodium acetate trihydrate to ensure adequate buffering of pH (~ 6.5) was chilled to 0-5°. The previously prepared diazonium salt solution was added in portions with stirring to yield a red precipitate. The product was collected and washed with water. Crystallisation from aqueous methanol afforded *5-amino-2-phenylazoimidazole-4-carboxamide monohydrate* (93%) as orange plates, m.p. 175° (decomp.) (Found : C, 48.8; H, 4.64; N, 34.4%. $C_{10}H_{10}N_6O \cdot H_2O$ requires C, 48.4; H, 4.84; N, 33.9%.); λ_{\max} . 454 nm; ν_{\max} . 3400-3150 (bonded NH), 1680, 1640 cm^{-1} (CONH₂); δ (DMSO- d_6) 6.20 (2H, br.s, NH₂), 7.00 (2H, br.s, CONH₂), 7.55 (5H, m, Ph).

Experimental details of the synthesis of other 5-amino-2-arylazoimidazole-4-carboxamides are summarised as Table 8.1. Physical characteristics and spectroscopic (i.r. and u.v.) data may be found in Tables 2.1 and 2.2 respectively in Section 2.1.

Table 8.1 Synthesis and physical properties of arylazoimidazoles.




Compound Number.	R	Reaction Conditions	M.p. ^o	Yield (%)	Molecular Formula	Elemental analysis (%) (Required %)			¹ H n.m.r. (δ) (DMSO-d ₆)
						C	H	N	
70	4-BrC ₆ H ₄	A	282 ^α	58	C ₁₀ H ₉ BrN ₃ O	38.6 (38.8)	3.0 (2.9)	27.6 (27.2)	6.40(2H, br.s, NH ₂) 7.10(2H, br.s, CONH ₂) 7.65(4H, s, Ar)
118	4-ClC ₆ H ₄	A	283 ^α	75	C ₁₀ H ₉ ClN ₃ O	45.3 (45.4)	3.4 (3.4)	32.0 (31.8)	6.35(2H, br.s, NH ₂) 7.05(2H, br.s, CONH ₂) 7.60(4H, q, Ar)
119	2-NO ₂ C ₆ H ₄	B	310 ^α	50	C ₁₀ H ₉ N ₃ O ₃	43.6 (43.6)	3.16 (3.30)	36.0 (35.6)	
120	3-NO ₂ C ₆ H ₄	B	278 ^α	83	C ₁₀ H ₉ N ₃ O ₃	44.0 (43.6)	3.16 (3.30)	35.6 (35.6)	6.75(2H, br.s, NH ₂) 7.35(2H, br.s, CONH ₂) 8.00(4H, m, Ar)
121	4-NO ₂ C ₆ H ₄	B	>300	94	C ₁₀ H ₉ N ₃ O ₃	44.0 (43.6)	3.19 (3.30)	35.6 (35.6)	7.30(2H, br.s, CONH ₂) 7.60-8.25(6H, br.m, NH ₂ and Ar)

Table 8.1 (continued).

Compound Number.	R	Reaction Conditions	M.p. ^o	Yield (%)	Molecular Formula	Elemental analysis (%) (Required %)			¹ H n.m.r. (δ) (DMSO-d ₆)
						C	H	N	
122	2-CNC ₆ H ₄	C	254 ^a	57	C ₁₁ H ₉ N ₃ O ·H ₂ O	48.3 (48.3)	3.9 (4.0)	35.3 (35.8)	
123	4-MeC ₆ H ₄	A	261 ^a	61	C ₁₁ H ₁₂ N ₆ O · $\frac{1}{2}$ H ₂ O	52.1 (52.1)	5.26 (5.13)	33.7 (33.2)	
124	4-AcC ₆ H ₄	A	244 ^a	66	C ₁₂ H ₁₂ N ₆ O ₂	52.7 (52.9)	4.33 (4.41)	30.9 (30.9)	
125	4-MeOCC ₆ H ₄	A	248 ^a	63	C ₁₁ H ₁₂ N ₆ O ₂	51.0 (50.8)	4.86 (4.61)		
126	4-NH ₂ SO ₂ C ₆ H ₄	A	259 ^a	57	C ₁₀ H ₁₁ N ₃ O ₃ S	38.4 (38.8)	3.62 (3.60)	31.4 (31.7)	
127	4-AcNH ₂ SO ₂ C ₆ H ₄	A	218 ^a	71	C ₁₂ H ₁₃ N ₃ O ₃ S ·H ₂ O	39.5 (39.0)	4.02 (4.06)	26.7 (26.6)	

Table 8.1 (continued).

Compound Number.	R	Reaction Conditions	M.p. ^o	Yield (%)	Molecular Formula	Elemental analysis (%) (Required %)	¹ H n.m.r. (δ) (DMSO-d ₆)
128	4- 	A	288 ^a	52	C ₁₄ H ₁₃ N ₃ O ₃ S ·Me ₂ NCHO	44.6 (44.3) 4.25 (4.34) 29.8 (30.4)	2.70(3H, s, Me) 2.90(3H, s, Me)
129	3-Pyridinyl	A	271 ^a	71	C ₉ H ₉ N ₇ O ·H ₂ O	43.4 (43.4) 4.3 (4.4) 39.3 (39.3)	

A See detail for compound (117).

B 5N-hydrochloric acid used for diazotisation step.

C 8N-hydrochloric acid used for diazotisation step.

^a Melts with decomposition.

8.2 Synthesis of Acyl Derivatives of Arylazoidimidazoles.*5-Acetylamino-2-phenylazoidimidazole-4-carboxamide (136)*

The azo-dye (117) (1.0g) was heated under reflux in a mixture of glacial acetic acid (10 ml) and acetic anhydride (10 ml) for 2 h. The resultant dark brown solution when diluted with water (20 ml) yielded the *acetylamino derivative* which crystallised from aqueous acetone as brown needles (59%), m.p. 258^o (decomp.), (Found : C, 52.6; H, 4.2; N, 31.0%.

$C_{12}H_{12}N_6O_2$ requires C, 52.9; H, 4.4; N, 30.9%.), λ_{max} 397 nm; ν_{max} 3350-3200 (bonded NH), 1700 (Ac), 1680, 1650 cm^{-1} (CONH₂); δ (DMSO-*d*₆) 2.25 (3H, s, Me), 7.45 (2H, br. s, CONH₂), 7.75 (5H, m, Ph), 10.40 (1H, br. s, NH).

Experimental data of other acyl derivatives are compiled as Tables 8.2 and 8.3.

The acetylamino derivative (136) was stable in 2N-hydrochloric acid at ambient temperature. However, heating on a steam bath induced rapid hydrolysis to the parent amine. This was identified by t.l.c. analysis of the mixture against the 2-phenylazoidimidazole (117) as a reference standard, on silica plates using methanol/chloroform mixtures as developing solvents.

The formylamino derivative (141) was found to be unstable in 2N-hydrochloric acid at ambient temperature. T.l.c. analysis of the mixture after 0.5 h against the parent amino compound (117), on silica plates with methanol/chloroform mixtures as developing solvents, indicated hydrolysis of the formyl group to yield the parent amine. This was also the result of compound (141) heated on a steam bath for 0.5 h in aqueous solutions of sodium bicarbonate and sodium hydroxide. Ethanolic sodium ethoxide was found to effect ring closure of (141) to the *8-substituted hypoxanthine* (144), although hydrolysis to the parent amine (117) was also evident (t.l.c.).

8.2 Synthesis of Acyl Derivatives of Arylazoimidazoles.*5-Acetylamino-2-phenylazoimidazole-4-carboxamide (136)*

The azo-dye (117) (1.0g) was heated under reflux in a mixture of glacial acetic acid (10 ml) and acetic anhydride (10 ml) for 2 h. The resultant dark brown solution when diluted with water (20 ml) yielded the *acetylamino derivative* which crystallised from aqueous acetone as brown needles (59%), m.p. 258⁰(decomp.), (Found : C, 52.6; H, 4.2; N, 31.0%.

$C_{12}H_{12}N_6O_2$ requires C, 52.9; H, 4.4; N, 30.9%.), λ_{max} . 397 nm; ν_{max} . 3350 - 3200 (bonded NH), 1700 (Ac), 1680, 1650 cm^{-1} (CONH₂); δ (DMSO-*d*₆) 2.25 (3H, s, Me), 7.45 (2H, br. s, CONH₂), 7.75 (5H, m, Ph), 10.40 (1H, br. s, NH).

Experimental data of other acyl derivatives are compiled as Tables 8.2 and 8.3.

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The formylamino derivative (141) was found to be unstable in 2N-hydrochloric acid at ambient temperature. T.l.c. analysis of the mixture after 0.5 h against the parent amino compound (117), on silica plates with methanol/chloroform mixtures as developing solvents, indicated hydrolysis of the formyl group to yield the parent amine. This was also the result of compound (141) heated on a steam bath for 0.5 h in aqueous solutions of sodium bicarbonate and sodium hydroxide. Ethanolic sodium ethoxide was found to effect ring closure of (141) to the *8-substituted hypoxanthine (144)*, although hydrolysis to the parent amine (117) was also evident (t.l.c.).

Table 8.2 Synthesis and physical characteristics of acyl derivatives



of arylazoimidazoles.

Compound Number	R	R ¹	R ²	Reaction Time (h)	Reaction Conditions	Crystallisation Solvent	M.p. ^o	Yield (%)	Appearance
137	4-Cl	H	Ac	2.5	A	Ethanol	257-260 ^α	69	light brown needles.
138	3-NO ₂	H	Ac	2.0	A	Acetone(aq.)	232 ^α	69	mustard powder.
139	4-Br	Ac	Ac	24.0	A	Acetic acid	245-247 ^α	35	brown plates.
140	4-NO ₂	Ac	Ac	24.0	A	Acetone(aq.)	288 ^α	43	orange needles.
141	H	H	CHO	1.0	B	Acetic acid	269 ^α	54	yellow needles.
142	4-Br	H	CHO	1.0	B	Acetic acid	247-250 ^α	73	mustard needles.
143	3-NO ₂	H	CHO	2.0	B	Acetic acid	258 ^α	90	orange needles.

A Glacial acetic acid - acetic anhydride (1:1).

B 98 - 100% formic acid.

^α Melts with decomposition.

Table 8.3 Analytical and spectral characteristics of acyl derivatives
of arylazoimidazoles.



Compound Number	R	R ¹	R ²	Molecular Formula	Elemental analysis (%) (Required %)			$\lambda_{\text{max.}}$ (nm)	$\nu_{\text{max.}}$ (cm ⁻¹)	¹ H n.m.r. (δ) (DMSO-d ₆)
137	4-Cl	H	Ac	C ₁₂ H ₁₁ ClN ₆ O ₂	47.3 (47.0)	3.7 (3.6)	28.0 (28.0)	410	3400-3200 (bonded NH), 1710 (Ac), 1690, 1660 (CONH ₂).	2.25 (3H, s, Me) 7.45 (2H, br. s, CONH ₂) 7.75 (4H, q, Ar) 10.35 (1H, br. s, NH)
138	3-NO ₂	H	Ac	C ₁₂ H ₁₁ N ₇ O ₄	45.3 (45.4)	3.6 (3.5)	30.8 (30.9)	408	3450-3250 (bonded NH), 1700* (Ac), 1660, 1595 (CONH ₂).	2.25 (3H, s, Me) 7.50 (2H, br. s, CONH ₂) 8.20 (4H, m, Ar) 10.45 (1H, br. s, NH)
139	4-Br	Ac	Ac	C ₁₄ H ₁₃ BrN ₆ O ₃	42.5 (42.8)	3.3 (3.3)	20.9 (21.4)	400	3500-3300 (bonded NH), 1750, 1695 (2 x Ac), 1660, 1580 (CONH ₂).	1.90 (1½H, s, ½Me) 2.25 (3H, s, Me) 2.35 (1½H, s, ½Me) 7.80 (4H, s, Ar) 10.00 (1H, br. s, NH)

* Inflection.

Table 8.3 (continued).

Compound Number	R	R ¹	R ²	Molecular Formula	Elemental analysis (%) (Required %)			λ_{max} (nm)	ν_{max} (cm ⁻¹)	¹ H n.m.r. (δ) (DMSO-d ₆)
140	4-NO ₂	Ac	Ac	C ₁₄ H ₁₃ N ₂ O ₅	46.3 (46.8)	3.7 (3.6)	27.2 (27.3)	429	3500-3250 (bonded NH), 1710, 1695* (2 x Ac), 1660, 1600 (CONH ₂), 1530, 1350 (NO ₂).	2.25 (3H, s, Me) 2.26 (1½H, s, ½Me) 2.40 (1½H, s, ½Me) 7.50 (2H, br. s, CONH ₂) 8.20 (4H, q, Ar) 10.10 (1H, br. s, NH)
141	H	H	CHO	C ₁₁ H ₁₀ N ₂ O ₂	51.4 (51.2)	3.8 (3.9)	32.8 (32.6)	398	3350-3300 (bonded NH), 1690 (CHO), 1660, 1620 (CONH ₂).	7.50-8.00 (8H, br. m, CONH ₂ , CHO and Ph) 8.65 (1H, br. s, NH)
142	4-Br	H	CHO	C ₁₁ H ₉ BrN ₂ O ₂	39.2 (39.2)	2.52 (2.67)	24.9 (24.9)	410	3450-3300 (bonded NH), 1720 (CHO), 1680, 1635 (CONH ₂).	7.50 (2H, br. s, CONH ₂) 7.80 (5H, s, CHO and Ar) 8.65 (1H, br. s, NH)
143	3-NO ₂	H	CHO	C ₁₁ H ₉ N ₂ O ₄ ·H ₂ O	41.5 (41.1)	3.37 (3.42)	30.0 (30.5)	438	3400-3100 (bonded NH), 1700 (CHO), 1670, 1595 (CONH ₂).	7.55 (2H, br. s, CONH ₂) 8.25 (6H, br. m, NH, CHO and Ar)

* Inflection.

8-Phenylazohypoxanthine (144)

2-Phenylazo-AIC (117) (1.0g) was partially dissolved in ethanol (25 ml) and sodium ethoxide [prepared from sodium (1.0g) in ethanol (25 ml)] was added. Ethyl formate (2 ml) was then added and the mixture refluxed gently for 3 h. On cooling the sodium salt of *8-phenylazohypoxanthine* separated as a yellow powder. Treatment with dilute hydrochloric acid yielded the free acid which crystallised from acetic acid as the hemihydrate (67%), m.p. $> 300^{\circ}$ (Found : C, 52.4; H, 3.32; N, 33.4; H_2O , 4.4%. $C_{11}H_8N_6O \cdot \frac{1}{2}H_2O$ requires C, 53.0; H, 3.62; N, 33.7; H_2O , 3.6%.); λ_{max} . 377 nm; ν_{max} . 3200 (br., bonded NH), 1690 cm^{-1} (lactam C=O).

8-(4-Chlorophenylazo)hypoxanthine (145)

Treatment of (118) (1.0g) by the procedure described for the preparation of (144) yielded the hemihydrate as a yellow microcrystalline solid from acetic acid (75%), m.p. $> 300^{\circ}$, (Found : C, 46.6; H, 2.62; Cl, 12.5; N, 29.7; H_2O , 3.7%. $C_{11}H_7ClN_6O \cdot \frac{1}{2}H_2O$ requires C, 46.6; H, 2.82; Cl, 12.5; N, 29.6; H_2O , 3.2%.); λ_{max} . 386 nm; ν_{max} . 3500-3100 (bonded NH), 1680 cm^{-1} (lactam C=O).

8.4 Diazotisation of 5-Amino-2-phenylazoimidazole-
-4-carboxamide (117).

4-Carbamoyl-2-phenylazoimidazole-5-diazonium tetrafluoroborate (146)

A deep purple solution of 2-phenylazo-AIC (117) (0.23g) in 10% tetrafluoroboric acid (5 ml) was chilled with an ice/salt bath to 0°. Dropwise addition of sodium nitrite (0.69g) in water (2 ml) produced a colour change to light orange and precipitation of the *diazonium tetrafluoroborate* as an orange microcrystalline solid. Purification of the unstable diazonium salt was achieved from ethereal 1-methyl-2-pyrrolidinone, m.p. 147° (decomp.); λ_{max} . 366 nm; ν_{max} . 3500-3250 (bonded NH), 2200 (N≡N), 1695, 1660 (CONH₂), 1250 cm⁻¹ (B-F); analysis pending.

8.5

Reduction of Arylazoimidazoles.

All reductions are summarised as Table 8.4.

8.5.1 Stannous chloride and hydrochloric acid.

Stannous chloride reductions in hydrochloric acid were conducted by the literature method.^{84,96}

Reduction of 2-(4-bromophenylazo)AIC (70)

The azo-dye (70) (5.0g) was partially dissolved in ethanol (50 ml) and a solution of stannous chloride (6.0g) in 35% hydrochloric acid (30 ml) was added with stirring. Immediate discharge of the red azo colour was noted with precipitation of a yellow solid. After filtration the residue was treated with H_2S in water to free the precipitate from tin residues, and yielded 4-amino-2-(4-bromophenylazo)imidazole hydrochloride (147) as white needles from water, m.p. 233° (decomp.);

λ_{max} . 263 nm; δ (DMSO- d_6) 7.95(4H,s,Ar), 9.80(1H,s,H-5); m/e 267 (46), 265 (M^+ , 46), 251 (94), 250 (100), 249 (94), 248 (96), 223 (32), 221 (22), 184 (34), 182 (32), 171 (31), 169 (31), 157 (13), 155 (28), 143 (14), 90 (62), 76 (26), 75 (26), 63 (42).

The filtrate was evaporated to dryness *in vacuo*, and dissolved in water (50 ml). Ether extraction yielded 4-bromoaniline after the aqueous solution had been neutralised with ammonium hydroxide. Rapid darkening of the mixture was observed at the neutralisation step. No further products were obtained after reacidification with hydrochloric acid.

8.5.2 Sodium dithionite

Azo-dyes reduced by sodium dithionite were first dissolved in aqueous ethanol and sufficient sodium dithionite was added, with stirring

and warming, to effect discharge of the azo colour. Heating on a steam bath for 0.5 h ensured total cleavage of the azo bond. After evaporation to dryness various extraction procedures were carried out (ether, ethyl acetate, alcohol).

8.5.3 Catalytic hydrogenation.

Catalytic hydrogenation was performed at 1 atmosphere in methanol or acetic acid / acetic anhydride mixtures with palladium 10% on charcoal. Hydrogenation was continued until total discharge of the azo colour was observed.

Catalytic hydrogenation of 5-amino-2-(4-bromophenylazo)AIC (70)

2-(4-Bromophenylazo)AIC (70) (2.2g) was partially dissolved in methanol (40 ml) and, after addition of palladium 10% on charcoal, hydrogenated at one atmosphere. A cream coloured precipitate formed, and after 5 h no further uptake of hydrogen was noted (180 ml absorbed; 320 ml required). Removal of the precipitate from solution resulted in rapid conversion to the original azo-dye (70) upon contact with air and it was therefore concluded that this precipitate was the *hydrazo compound (148)*.

Addition of concentrated hydrochloric acid (1 ml) and rehydrogenation resulted in the expected uptake of hydrogen within 3 h. The alcoholic solution was filtered to isolate catalyst and a white powder which had formed, and the residue extracted with water to yield an *unidentified white crystalline solid* which darkened in colour between 150-200° but did not melt below 300°; λ_{max} . 280 nm; ν_{max} . 3400-3100, 1700, 1680, 1640 cm^{-1} , m/e 192 (66), 177 (63), 136 (100), 135 (87), 124 (42).

The filtrate was examined by t.l.c. and found to contain the unknown white compound and *4-bromoaniline hydrochloride*. The latter compound was isolated by neutralisation with ammonium hydroxide and ether extraction of the free base.

5-Amino-2-(3-aminoindazol-2-yl)imidazole-4-carboxamide (151)

2-(2-Cyanophenylazo)AIC (122) (2.5g) was partially dissolved in methanol (100 ml) and, after addition of 10% palladium on charcoal, hydrogenated at one atmosphere. After 14 h a cream precipitate had formed and only half of the required volume of hydrogen (200 ml) had been absorbed. The cream precipitate reverted to the starting azo-dye (122) on contact with air and was assumed to be the *hydrazo compound* (149).

Rehydrogenation with warming (30-35°) for 48 h resulted in disappearance of the cream precipitate and formation of a yellow precipitate. Filtration of the hydrogenation mixture and extraction of the residue with aqueous ethanol yielded an unstable light brown crystalline solid (48%), however, treatment with dilute hydrochloric acid yielded the *indazole hydrochloride* as a stable yellow powder, m.p. 214-216° (decomp.), (Found : C, 41.3; H, 4.5; N, 31.2; H₂O, 7.1%. C₁₁H₁₁N₇O.HCl1½H₂O requires C, 41.8; H, 4.5; N, 31.0; H₂O, 7.1%); λ_{max} . 273, 328 nm; ν_{max} . 3450-3150 (bonded NH), 1660, 1630 cm⁻¹ (CONH₂); m/e 257 (M⁺, 22), 240 (19), 214 (24), 213 (19), 187 (8), 186 (8), 171 (5), 160 (5), 145 (48), 133 (73), 118 (100), 81 (51), 77 (19), 64 (22), 43 (95).

Treatment of the filtrate with hydrochloric acid before concentration *in vacuo* yielded a further quantity of the indazole.

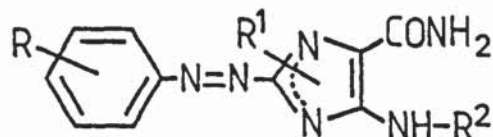
8.5.4 Raney nickel and hydrazine hydrate.

Reduction of compounds (70, 117 and 139) with Raney nickel and hydrazine hydrate was attempted in absolute ethanol at ambient temperature. Hydrazine hydrate was added dropwise to a stirred solution of azo-dye and catalyst. Compounds (70) and (117) both formed a white precipitate which reverted to starting material on contact with air. This was assumed to be the *hydrazo compound*. Further addition of

hydrazine hydrate could not induce total reduction of the azo bond.

Upon increasing the temperature to 45° solutions of (70) and (117) rapidly darkened and *4-bromoaniline* and *aniline* respectively, were the only products isolated from the dark brown solutions.

Table 8.4 Reduction of arylazoimidazoles.



Compound Number	R	R ¹	R ²	Reductive Process	Result
117	H	H	H	A,B,C.	Aniline only, isolated
117	H	H	H	D.	Precipitation of hydrazo compound.
118	4-Cl	H	H	B.	4-Chloroaniline only, isolated.
70	4-Br	H	H	A.	4-Bromoaniline and 5-amino-2-(4-bromophenylazo)imidazole (147) isolated.
70	4-Br	H	H	D,E.	Precipitation of hydrazo compound (148).
70	4-Br	H	H	F.	Unidentified white compound and 4-Bromoaniline isolated.
70	4-Br	H	H	C.	4-Bromoaniline only, isolated.
122	2-CN	H	H	A.	Anthranilonitrile only, isolated.
122	2-CN	H	H	B,E.	Cyclisation to indazole (151).
136	H	H	Ac	B.	No reduction.
136	H	H	Ac	G.	Acetanilide only, identified.
137	4-Cl	H	Ac	A.	4-Chloroaniline only, identified.
139	4-Br	Ac	Ac	B,C,D.	No reduction.

A Stannous chloride and hydrochloric acid.

B Sodium dithionite.

C Raney nickel and hydrazine hydrate, 45°.

D Raney nickel and hydrazine hydrate, 20°.

E Catalytic hydrogenation in methanol.

F Catalytic hydrogenation in methanol and hydrochloric acid.

G Catalytic hydrogenation in acetic acid / acetic anhydride.

Approaches to Synthesis of2,5-Diaminoimidazole-4-carboxamide (71).8.5.1 Direct synthesis of imidazole ring.

Initial experiments were conducted by the method described by Schwartz *et al.*⁶⁶ Variation of reaction conditions and results are compiled as Table 4.1 (Section 4.1.1).

8.5.2 From 2-hydroxy-AIC (153).

2-Hydroxy-AIC¹⁸⁸ (153) (0.5g) was heated under reflux in thionyl chloride (1.0 ml) for 1 h. Evaporation of thionyl chloride resulted in total recovery of starting material.

Treatment of 2-hydroxy-AIC (0.2g) with phosphorus oxychloride (5 ml) at 100° for 3 h yielded a white crystalline solid on cooling, m.p. 253° (decomp.) from water. This compound was shown to be identical with a sample of 2,5-dioxoimidazoline-4-carboxamide (155) prepared by action of dilute hydrochloric acid on (153).¹⁸⁸

Continued treatment of (153) with phosphorus oxychloride at 100° (48 h) yielded a dark brown solid which afforded a white crystalline solid (needles) from methanol/charcoal boil, m.p. 220°. This was assumed to be *hydantoin* (156); lit.m.p. 222°.

8.5.3 From 2-methylmercapto-AIC (158).

2-Methylmercapto-AIC¹⁸⁹ (0.2g) was heated with aniline (0.5 ml) in a sealed glass tube at 100° for 8 h. Total recovery of the imidazole was possible. After resealing the tube heating was continued at 150° for 48 h. Although darkening of the tube contents was observed the imidazole (158) was unchanged and recovered from the reaction mixture.

This experiment was repeated with aqueous ammonium hydroxide

and diethylamine and in both cases the methylmercapto derivative (158) was recovered, unchanged.

8.5.4 Ring opening of 8-aminohypoxanthine.

Ring opening of 8-aminohypoxanthine^{191,192} was attempted by the literature method^{121,122} without success. The purine was recovered from the reaction mixture unchanged.

8.5.5 Direct amination.

AIC (1.6g) in water (15 ml) at ambient temperature was stirred and hydroxylamine-O-sulphonic acid (1.13g) was gradually added. The reaction mixture was then heated on a steam bath for 0.5 h and allowed to cool to room temperature. T.l.c. examination indicated the presence of an additional compound. Further heating could not induce total conversion of AIC (t.l.c.).

Potassium carbonate (1.73g) was added to the reaction mixture and the resultant solution was passed down an ion-exchange column (Amberlite IR 120, H⁺) for purification.

Elution with 3N-ammonium hydroxide resulted in immediate darkening of the column and collection of a dark brown eluate. T.l.c. of the eluate indicated the presence of AIC but not of the formerly observed product.

3-Aminopyrazole²³⁸ (8.3g) was diazotised in 5N-hydrochloric acid (50 ml) by the dropwise addition of sodium nitrite (8.5g) in water (20 ml) at constant low temperature (0-5°). The resultant diazonium solution was added, in portions, with stirring to a chilled solution of nitroethane (7.5 ml) and sodium hydroxide (3.6g) in water (1.5 L). Introduction of excess sodium acetate trihydrate with vigorous stirring resulted in precipitation of a mustard coloured powder which was collected and washed with ice-water before drying. Crystallisation from ethanol afforded the crystalline *hydrazone* (163) (58%), m.p. 137° (efferv.); λ_{max} . 363 nm; ν_{max} . 3400, 3200 (NH), 1520, 1380 cm^{-1} (NO_2); m/e 169 (M^+ , 100), 123 (74), 122 (91), 98 (54), 82 (86), 81 (87), 66 (73).

Treatment of the hydrazone (163) (2.5g) with ethanol (50 ml) for 7 days at ambient temperature gave a dark brown solution. Concentration *in vacuo*, and addition of ether afforded 1,1-bis(pyrazol-3-ylazo)-1-nitroethane (164) as fawn coloured needles (45%) from ethanol, m.p. 185-7° (decomp.) (Found : C, 36.5; H, 3.45; N, 48.5%; M^+ , 263.08926.

$\text{C}_8\text{H}_9\text{N}_9\text{O}_2$ requires C, 36.5; H, 3.42; N, 47.9%; M , 263.08879.), λ_{max} . 285 nm; ν_{max} . 3100 (NH), 1510, 1350 cm^{-1} (NO_2); δ (DMSO- d_6) 3.00 (3H, s, Me), 6.85 (2H, d, 2 x pyrazole C-4), 8.05 (2H, d, 2 x pyrazole C-5); m/e 263 (M^+ , low abundance), 188 (9), 173 (8), 122 (100), 108 (10), 105 (8), 95 (11), 93 (35), 83 (95), 81 (80), 68 (98), 67 (75), 66 (97).

Addition of pyridine (5 ml) to the ethanolic solution of the nitrohydrazone (163) and stirring for 7 days at ambient temperature gave an identical product (164), in similar yield (41%).

Inclusion of glacial acetic acid (5 ml) instead of pyridine

resulted in isolation of the *acetic acid solvate of (164)* as a white powder (28%) from ethanol, m.p. 175-180⁰(decomp.); $\lambda_{\text{max.}}$ 285 nm; $\nu_{\text{max.}}$ 3100(NH), 2900-2400(bonded OH), 1520, 1340 cm^{-1} (NO₂); δ (DMSO-*d*₆) 1.95(3H,s,Me), 3.00(3H,s,Me), 6.85(2H,d,2 x pyrazole C-4), 8.05(2H,d, 2 x pyrazole C-5).

Both (164) and the acetic acid solvate were unstable in 6N-hydrochloric acid with rapid effervescence of nitrogen dioxide observed. Evaporation to dryness and crystallisation from ethanol afforded an unidentified white crystalline material (needles) m.p. 184⁰(efferv.); $\lambda_{\text{max.}}$ 285 nm; $\nu_{\text{max.}}$ 3150, 2950, 1505, 1410, 1390, 1310 cm^{-1} ; δ (DMSO-*d*₆) 2.90(3H,s) 6.80(2H,d) 8.15(2H,d).

8.7 Synthesis of Imidazotetrazinones.*5-Diazoimidazole-4-carboxamide (6)*

AIC hydrochloride (6.48g) dissolved in 1N-hydrochloric acid (100 ml) and cooled to 0° was slowly added (over 5 min) to a mixture of sodium nitrite (3.3g, 20% excess) and crushed ice (50g) in water (50 ml). The reaction vessel was externally cooled by an ice/salt bath and the contents were vigorously stirred throughout diazotisation. The mixture was stirred for an additional 0.25 h and filtered to isolate the *diazo-azole* as buff coloured needles (60%). After washing thoroughly with ice water the product was dried at ambient temperature in the dark, m.p. 210° (decomp.) Lit. 210° (decomp.).

No purple byproduct⁵⁰ (75) was formed during diazotisation.

3-Carbamoyl-6-(2-chloroethyl)imidazo [5,1-d] [1,2,3,5] tetrazin-7(6H)-one (175)

Diazo-IC (0.3g) was suspended in anhydrous dichloromethane (10 ml) and excess 2-chloroethyl isocyanate (1.0 ml) was added. After stirring in the dark at ambient temperature for 20 days the reaction mixture was diluted with anhydrous ether (30 ml) and filtered. The cream coloured residue was thoroughly washed with anhydrous ether and then dried at ambient temperature in air to afford the *imidazo [5,1-d] [1,2,3,5] tetrazin-7-one* as a cream coloured microcrystalline solid (90%) m.p. 158° (efferv.) (Found : C, 35.1; H, 2.72; N, 34.8%. $C_7H_7ClN_6O_2$ requires C, 34.7; H, 2.89; N, 34.7%.); λ_{max} 325 nm; ν_{max} 3500, 3200(NH), 1740 (lactam C=O), 1680, 1660 cm^{-1} (CONH₂).

Purification of the imidazotetrazinone by dissolution in

anhydrous 1-methyl-2-pyrrolidinone at room temperature, and precipitation by the addition of five volumes of anhydrous ether afforded the *imidazotetrazinone* in another, probably polymorphic form, m.p. 164-165° (efferv.) (Found : C, 34.7; H, 3.01; N, 34.9%. $C_7H_7ClN_6O_2$ requires C, 34.7; H, 2.89; N, 34.7%.); λ_{\max} . 325 nm; ν_{\max} . 3500, 3400(NH), 1750(lactam C=O), 1680 cm^{-1} (CONH₂).

Solution i.r. spectra (CH_2Cl_2) of both forms proved to be identical.

Experimental details of other imidazotetrazinones are compiled as Table 8.5. A summary of physical characteristics, u.v., i.r., and n.m.r. data may be found in Tables 6.1, 6.2 and 6.3 (see Section 6.1) and mass spectral data in the appendix.

Decomposition of imidazotetrazinones in presence of aniline.

The 3-cyanophenyl-, 4-tolyl-, 4-ethoxyphenyl- and methyl-imidazotetrazinones (compound numbers 169, 171, 173 and 177 respectively) were dissolved in acetonitrile and excess aniline was added. After stirring in the dark at ambient temperature for 12 h the mixtures were examined by t.l.c. on silica plates using chloroform/methanol mixtures as developing solvents. The three spots present, in all cases, were identified as starting material, 5-(3-phenyltriazene-1-yl)imidazole-4-carboxamide (193) and the respective ureas.

Decomposition of imidazotetrazinones in ethanol.

Ethanolic solutions of the 4-tolyl (171) and methyl (177) derivatives were stirred for 12 h at ambient temperature. T.l.c. exam-

ination of the mixtures on silica plates showed the presence of two compounds in addition to starting material. These were shown to be 2-azahypoxanthine and the respective carbamates by comparison with standard compounds using chloroform/ethanol mixtures and ether as developing solvents.

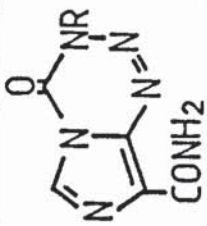
Decomposition of an imidazotetrazinone in presence of sodium azide.

The 4-methoxy derivative (172) (0.3g) was suspended in acetic acid (10 ml) and sodium azide (0.1g) was added. The mixture was stirred at ambient temperature in the dark and the mixture was examined by t.l.c. at various time intervals. Development of the silica plates with chloroform/methanol mixtures indicated degradation by the presence of 2-azahypoxanthine (chromatographed against a sample of the 2-azapurinone). The solid in suspension was isolated after 24 hours, and i.r. spectroscopy showed it to be starting material. No evidence of azide formation was noted.

Half-life studies of imidazotetrazinones

Solutions of the imidazotetrazinones were prepared in 95% ethanol, dry dichloromethane or phosphate buffer (pH 7.4). The change in intensity with time of absorption at λ_{max} of the longest wavelength was recorded. Half-life determinations were made under dark and light (white light) conditions. Temperature control was achieved by an external water bath linked to the u.v. spectrophotometer.

Table 8.5 Experimental detail of Imidazo [5,1-d] [1,2,3,5] tetrazin-7(6H)-ones.

Compound Number		Reaction Conditions	Reaction Duration (days)	Molecular Formula	Elemental analysis (%) (Required %)		
					C	H	N
167	Ph	A,B	25	C ₁₁ H ₈ N ₆ O ₂	51.0 (51.4)	3.4 (3.1)	33.4 (32.8)
168	4-NO ₂ C ₆ H ₄	A	7	C ₁₁ H ₇ N ₇ O ₄	42.1 (43.8)	2.3 (2.3)	30.7 (32.5)
169	3-CNC ₆ H ₄	A	7	C ₁₂ H ₇ N ₇ O ₂	52.4 (51.2)	2.37 (2.51)	35.5 (34.9)
170	4-ClC ₆ H ₄	A,D	11	C ₁₁ H ₇ ClN ₆ O ₂	45.8 (45.4)	2.28 (2.43)	
171	4-MeC ₆ H ₄	A	4	C ₁₂ H ₁₀ N ₆ O ₂	52.8 (53.3)	3.51 (3.73)	31.3 (31.1)
172	4-MeOC ₆ H ₄	A	7	C ₁₂ H ₁₀ N ₆ O ₃	49.9 (50.3)	3.5 (3.5)	29.0 (29.3)
173	4-EtOC ₆ H ₄	A	10	C ₁₃ H ₁₂ N ₆ O ₃	51.1 (51.9)	3.8 (4.0)	27.9 (27.9)
174	1-Naphthyl	A	15	C ₁₅ H ₁₀ N ₆ O ₂	57.4 (58.8)	3.34 (3.29)	28.3 (27.4)
176	n-Pr	A	30	C ₈ H ₁₀ N ₆ O ₂	43.4 (43.2)	4.57 (4.53)	38.0 (37.8)
177	Me	C,D	21	C ₆ H ₆ N ₆ O ₂	36.8 (37.1)	3.10 (3.09)	

A See detail for compound (175).

B Purified from DMSO by addition of acetone.

C Diazo-IC suspended in excess methyl isocyanate, no solvent (CH₂Cl₂) incorporated.

D Product slurried with anhydrous methanol for purification, then washed with ether.

5-(3-Phenyltriazene-1-yl)imidazole-4-carboxamide (193)

Diazo-IC (0.2g) was suspended in ethanol (3.0 ml) and aniline (0.2g) was added. After stirring in the dark at ambient temperature for 17 h the yellow precipitate was isolated and washed thoroughly with anhydrous ether to afford the *triazene* (72%), m.p. 159-160° (decomp.), (Found : C, 51.6; H, 4.3; N, 36.8%. $C_{10}H_{10}N_6O$ requires C, 52.1; H, 4.4; N, 36.5%); λ_{\max} 372 nm; ν_{\max} 3400-3200 (bonded NH), 1650, 1600 cm^{-1} (CONH₂); m/e 230 (M^+ , 20), 202 (15), 185 (15), 169 (20), 168 (13), 167 (10), 126 (33), 109 (45), 104 (40), 93 (66), 77 (100).

Appendix

A.1 Mass spectra of novel arylazoimidazoles.

Compound Number.										
<u>117</u>	m/e :	230	213	212	202	201	185	157	141	124
	I% :	71	7	8	5	5	21	11	6	8
	m/e :	105	93	85	77*	66	43			
	I% :	28	59	19	100	35	42			
<u>118</u>	m/e :	266	264	249	248	247	239	237	221	219
	I% :	17	52	2	2	5	3	8	5	11
	m/e :	193	191	167	141	139	138	129	128	127*
	I% :	3	7	3	5	13	6	36	9	100
<u>119</u>	m/e :	275*	258	247	245	230	217	202	201	200
	I% :	100	11	19	32	38	14	16	16	27
	m/e :	184	172	150	149	138	122	108	92	73
	I% :	14	16	14	24	43	57	59	95	76
<u>120</u>	m/e :	275*	259	258	245	230	227	202	201	200
	I% :	100	9	9	13	27	13	16	11	14
	m/e :	184	172	150	149	138	122	108	92	73
	I% :	14	9	18	36	18	68	30	59	52
<u>121</u>	m/e :	275	243	231	230	229	213	212	200	172
	I% :	68	4	11	6	13	6	8	14	12
	m/e :	171	170	159	143	141	136	133	119*	91
	I% :	9	9	11	22	14	15	33	100	99
<u>122</u>	m/e :	255	210	182	149	145	133	118	104	102
	I% :	23	8	5	7	7	28	58	11	19
	m/e :	91	69	65	64	60	45*			
	I% :	31	18	19	16	54	100			
<u>123</u>	m/e :	244	226	201	200	199	171	158	157	156
	I% :	52	11	11	11	16	13	11	16	19
	m/e :	150	119	118	107	106	92	91*	77	65
	I% :	8	16	18	29	39	15	100	69	35
<u>123</u>	m/e :	44	43							
	I% :	32	35							

Compound
Number.

<u>124</u>	m/e :	272	257	229	228	227	212	199	186	185
	I% :	46	8	6	6	12	6	5	5	5
	m/e :	141	135	120	119	92	73	65	44*	43
	I% :	14	60	90	36	66	88	56	100	90
<u>125</u>	m/e :	260	243	242	217	215	200	199	187	174
	I% :	78	12	23	20	20	13	8	18	8
	m/e :	172	135	134	123	122	121	108	107*	92
	I% :	8	35	20	43	20	25	65	100	33
	m/e :	77	57	55	45	43				
	I% :	58	45	40	55	50				
<u>126</u>	m/e :	309	264	244	230	212	200	198	185	184
	I% :	9	3	2	2	3	4	3	4	3
	m/e :	182	174	173	172	171	158	157	156*	141
	I% :	5	6	9	89	5	6	10	100	7
	m/e :	124	108	92	73	65	64	45		
	I% :	10	64	87	72	85	64	89		
<u>127</u>	m/e :	214	198	182	174	173	172	158	157	156*
	I% :	90	40	36	59	95	91	90	90	100
	m/e :	151	150	141	140	135	128	125	118	109
	I% :	22	24	50	46	45	64	45	30	93
	m/e :	108	94	93	92	91	90	86	80	
	I% :	90	70	80	70	70	55	75	80	
<u>128</u>	m/e :	277	264	263	262	211	185	184*	183*	170
	I% :	2	2	2	3	3	35	100	100	35
	m/e :	156	140	136	108	95	93	92	80	79
	I% :	25	14	14	96	80	50	95	25	20
	m/e :	78	44							
	I% :	33	50							
<u>129</u>	m/e :	231*	214	213	202	186	158	106	105	94
	I% :	100	26	17	5	26	24	20	35	33
	m/e :	93	92	79	78	66	65			
	I% :	21	15	32	40	27	23			

A.2 Mass spectra of novel 5-acetylamino-2-arylaazoimidazoles.

Compound
Number.

<u>136</u>	m/e :	272	230	213	185	175	174	157	155	150
	I% :	53	76	14	26	12	37	14	12	75
	m/e :	141	135	129	113	111	105	97	93*	85
	I% :	19	26	32	28	32	33	61	100	88
	m/e :	77	73	69	57					
	I% :	86	66	82	98					
<u>137</u>	m/e :	308	306	266	264*	236	221	219	193	191
	I% :	24	58	35	100	9	10	26	6	11
	m/e :	183	165	141	139	129	127	113	111	75
	I% :	4	7	13	32	20	47	30	82	20
	m/e :	65								
	I% :	16								
<u>138</u>	m/e :	317	276	275*	230	202	200	184	150	149
	I% :	48	18	100	13	7	7	9	12	25
	m/e :	122	108	92	81	80	76	75	71	65
	I% :	37	28	29	15	15	12	12	15	19
	m/e :	57								
	I% :	22								
<u>139</u>	m/e :	394	392	352	350	310	308	282	280	266
	I% :	24	22	16	16	18	18	9	11	9
	m/e :	265	263	254	252	230	228	215	213	188
	I% :	13	11	33	31	20	20	18	27	16
	m/e :	186	185	183	173*	171*	157	155	92	65
	I% :	18	14	14	100	100	38	38	69	51
	m/e :	59								
	I% :	45								
<u>140</u>	m/e :	359	317	275*	257	241	240	230	207	202
	I% :	25	56	100	22	14	14	22	22	8
	m/e :	201	200	199	150	149	122	118	108	92
	I% :	8	14	14	21	26	44	48	66	40
	m/e :	91	81	69	65	57				
	I% :	38	48	56	44	78				

A.3 Mass spectra of novel 5-formylamino-2-arylaazoimidazoles.

Compound
Number.

<u>141</u>	m/e :	258	230	213	212	202	201	187	186	185
	I% :	34	63	7	11	5	4	6	13	18
	m/e :	157	151	129	105	93	92	77	66*	65
	I% :	11	25	9	32	89	38	93	100	54
<u>142</u>	m/e :	338	336	310*	308*	293	292	291	290	282
	I% :	17	17	100	100	10	9	10	10	8
	m/e :	281	280	279	265	263	237	235	185	184
	I% :	8	8	8	44	44	25	23	35	27
	m/e :	183	182	173	171	157	155	109	108	92
	I% :	37	25	37	42	88	88	17	12	35
	m/e :	91	90	76						
	I% :	37	42	85						
<u>143</u>	m/e :	303	275	258	245	230	202	201	200	167
	I% :	29	67	4	4	8	5	4	4	8
	m/e :	155	149	122	108	92	91	81	80	73*
	I% :	9	71	37	92	37	21	50	46	100

A.4 Mass spectra of novel 8-arylazohypoxanthines.

Compound Number.										
<u>144</u>	m/e :	240	239	238	213	212	211	185	184	157
	I% :	7	8	9	4	26	8	7	10	17
	m/e :	151	130	118	105	104	103	94	93	92
	I% :	10	7	7	6	8	11	22	66	34
	m/e :	91	78	77	66*	65	51			
	I% :	13	18	77	100	60	40			
<u>145</u>	m/e :	276	274	248	246	221	220	219	218	193
	I% :	3	10	10	33	3	5	9	12	9
	m/e :	191	141	139	129	127	113	111*	102	101
	I% :	25	2	7	27	62	35	100	4	6
	m/e :	99	92	77	73	65				
	I% :	7	18	4	8	26				

A.5 Mass spectra of novel imidazo[5,1-d] [1,2,3,5] tetrazin-7(6H)-ones.

Compound
Number.

<u>167</u>	m/e :	137	120	119*	111	95	94	93	92	91
	I% :	25	50	100	12	12	8	12	26	90
	m/e :	90	78	77	76	75	74	66	65	64
	I% :	18	15	14	9	9	13	25	45	75
	m/e :	63	59	52	51	50				
	I% :	78	28	32	48	39				
<u>168</u>	m/e :	164*	137	134	118	111	106	94	90	78
	I% :	100	56	45	17	4	16	14	77	10
	m/e :	66	64	63	54					
	I% :	26	17	28	21					
<u>169</u>	m/e :	144*	137*	116	111	102	94	90	89	88
	I% :	100	100	95	6	3	42	8	82	18
	m/e :	76	75	72	66	65	64	63	62	54
	I% :	17	19	20	59	31	28	29	52	44
<u>170</u>	m/e :	155	153*	137	127	125	90	77	66	64
	I% :	36	100	36	21	57	36	7	14	11
	m/e :	63	54							
	I% :	18	11							
<u>171</u>	m/e :	137	133*	132	111	105	104	94	91	78
	I% :	36	100	50	6	33	56	19	28	39
	m/e :	77	76	66	65	64	63	54	52	51
	I% :	31	11	42	19	11	19	42	42	56
<u>172</u>	m/e :	149*	137	134	121	106	94	91	90	80
	I% :	100	25	54	6	21	4	5	5	4
	m/e :	78	66	54	53	52	51			
	I% :	15	10	10	4	10	8			
<u>173</u>	m/e :	163	149	137	135*	111	109	107	96	90
	I% :	65	5	22	100	3	3	48	7	4
	m/e :	79	66	54	52					
	I% :	13	9	7	17					
<u>174</u>	m/e :	306 ^a	169*	141	140	137	115	114	113	111
	I% :		100	41	38	28	6	27	12	3
	m/e :	109	94	85	71	66	63	54		
	I% :	5	8	13	14	17	12	14		
<u>175</u>	m/e :	244	242	193	137	111	107	105	95	94
	I% :	2	6	25	37	38	13	34	35	18
	m/e :	93	79	77	69	68	66	57	56*	49
	I% :	12	22	58	24	36	28	16	100	26

a - very low abundance.

Compound
Number.

<u>176</u>	m/e :	222	193*	137	119	109	94	85	84	81
	I% :	27	100	64	9	10	27	9	10	9
	m/e :	66	56	54						
	I% :	32	36	18						

<u>177</u>	m/e :	194	137*	121	111	94	82	81	66	65
	I% :	31	100	4	4	8	4	4	35	10
	m/e :	58	57	54						
	I% :	6	9	14						

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