THE MEDICINAL CHEMISTRY OF BICYCLIC SYSTEMS CONTAINING A NNN BOND

by

GHOUSE UNISSA BAIG

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Department of Pharmaceutical Sciences
Aston University
Birmingham B4 7ET

The medicinal chemistry of bicyclic systems containing NNN bond

Ghouse Unissa Baig
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Summary

Synthetic routes to, and properties of, 4-amino and 4(3H)-imino-1,2,3-benzotriazines are reviewed in detail in the Introduction.

4-(4-Cyanoanilino)-1,2,3-benzotriazine, when boiled in morpholine, afforded 3-[2-amino-N-(4-cyanophenyl)-benzimidoyl]-4-(4-cyanophenylimino)-3,4-dihydro-1,2,3-benzotriazine in addition to the major product 2-amino-N^2-(4-cyanophenyl)-N^1N^1-oxydiethylenebenzamidine. The yield of the former product increased when high boiling non-nucleophilic solvents were employed as the thermolysis medium.

Decomposition of 3-[2-amino-N-(4-cyanophenyl)-benzimidoyl]-4-(4-cyanophenylimino)-3,4-dihydro-1,2,3-benzotriazine in hot acid alone yielded 4-(4-cyanoanilino)-2-phenylquinazoline, whereas incorporation of nucleophiles in to the hot acidic media afforded a series of 4-(4-cyanoanilino)-2-(2-substituted phenyl) quinazolines. These quinazolines were prepared by an unambiguous route from the appropriate 2-(2-substituted phenyl)-4-chloroquinazolines and 4-cyanoaniline.

The tetracyclic triazine, 8-(4-cyanophenylimino)-8H-quinazolino[3,2-c]-1,2,3-benzotriazine, was prepared by the diazocyclisation of 2-(2-aminophenyl)-4-(4-cyanoanilino)quinazoline.

4-Anilino-1,2,3-benzotriazines and their precursor acyclic triazenes yielded 3-arylquinazolin-4(3H)-ones when heated in formamide.

The chemistry of the antitumour 3-alkylimidazo[5,1-d]-1,2,3,5-tetrazin-4(3H)-ones is comparable to that of benzotriazin-4(3H)-ones. The former series yielded 5-(3-alkyltriazen-1-yl)-imidazole-4-carboxamides when treated with cold aqueous sodium carbonate and afforded 5-aminoimidazole-4-carboxamide when boiled in water.

Nitration of 8-carbamoyl-3-alkylimidazotetrazinones yielded the corresponding 8-nitrocarboxamides and reaction with sodium nitrite in concentrated sulphuric acid yielded the corresponding 8-carboxylic acids.

5-Diazoimidazole-4-carboxamide generated by thermolysis of 8-carbamoyl-3-(2-chloroethyl)imidazo[5,1,-d]-1,2,3,5-tetrazin-4(3H)-one in acetic acid or pyridine was trapped by reactive methylenic ketones, nitriles or esters to afford imidazo[5,1-c]-1,2,4-triazines.

Thermolytic decomposition of the 3-alkylimidazotetrazinones in methanol and ethanol yielded 2-azahypoxanthine and 5-amino-1-carboalkoxyimidazole-4-carboxamide. In hydrazine hydrate, 3-(2-chloroethyl)imidazotetrazinone yielded 5-azidoimidazole-4-carboxamide, whereas the 3-alkyl analogues formed 5-amino-4-carbamoylimidazole-1-carbohydrazide.

Key words: Triazene
Triazine
Imidazotetrazinone
Diazotisation
Cyclisation

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To ABID and MUZAFFER

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PART I

INTRODUCTION

Part 1

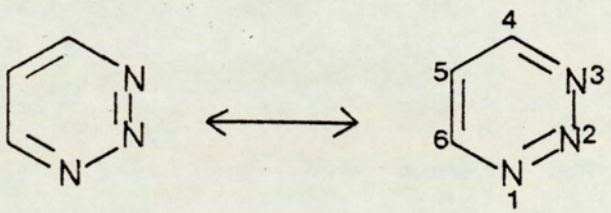
Introduction

Chemistry of 4-amino-1,2,3-benzotriazines and 4(3H)-imino-1,2,3-benzotriazines

Chapter 1

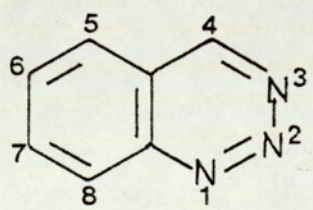
1.1 Nomenclature and definitions

The parent compound of the 1,2,3-triazine series, which is not known, has structure (1.1) and is numbered as indicated. In "Chemical Abstracts" the ring-system is called 1,2,3-triazine or v-triazine [1], where v denotes the vicinal arrangement of the three nitrogen atoms in the six membered ring.



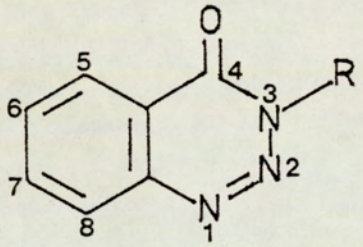
(1.1)

This ring-system, when annealed to the benzene ring, is called 1,2,3-benzotriazine (1.2). In the old literature, the name β-triazine is also used.



(1.2)

This designation was apparently based on the fact that two of the nitrogen atoms - N(2) and N(3) - are in a β position to the benzene ring[2]. An equally trivial name, benzazimide or benzazoimide[3], can be found in the old literature for 1,2,3-benzotriazin-4(3H)-ones (1.3) which are the most intensively studied class of 1,2,3-benzotriazine derivatives.



(1.3)

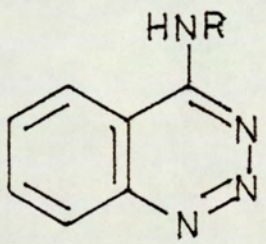
In the Introduction to this thesis, all known syntheses and properties of 4-amino and 4-imino(3H) benzotriazines are reviewed in detail (Chapter 2). The thermolytic decomposition of 4-anilino-1,2,3-benzotriazines and degradations of imidazo[5,1-d]-1,2,3,5-tetrazin-4(3H)-ones form the substance of the Discussion section of the thesis (Chapters 3 - 9).

Chapter 2

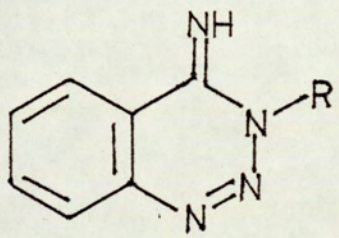
Chemistry of 4-amino-1,2,3-benzotriazines and 4(3H)-imino-1,2,3 benzotriazines

2.1 Syntheses of 4-amino- and 4(3H)-imino-1,2,3-benzotriazines

There are very few 4-amino-1,2,3-benzotriazines (2.1) or their isomeric 4(3H)-imino-1,2,3-benzotriazines (2.2) described in the literature and these are listed in Tables (2.1 and 2.2). The procedures which have been applied for their synthesis can be



(2.1)



(2.2)

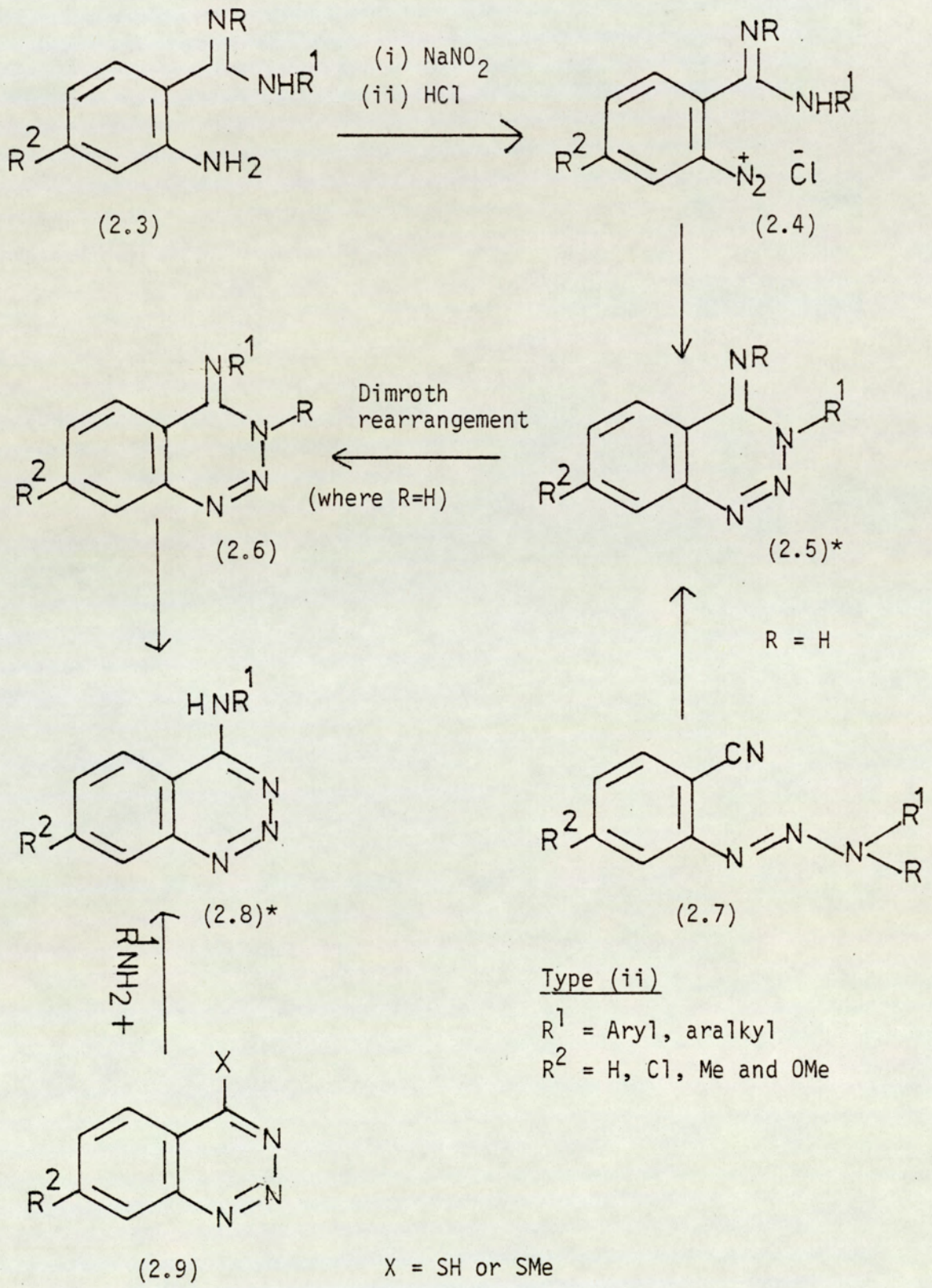
conveniently divided into the following three types (i)-(iii) and are summarised by scheme (2.1).

(i) from diazotisation of 2-aminobenzamidines

(ii) from cyclisation of 1-aryl-3-(2-cyanophenyl)triazenes

(iii) from 1,2,3-benzotriazin-4(3H)thiones

Type (i)



Type (ii)

R^1 = Aryl, aralkyl

R^2 = H, Cl, Me and OMe

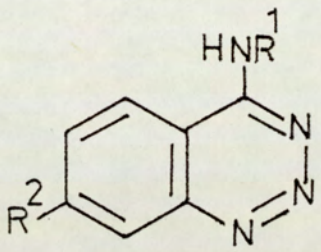
Type (iii)

* See Table 2.1 and 2.2 for the substituents

SCHEME (2.1)

Table 2.1

4-Amino-1,2,3-benzotriazines



Compound	R^1	R^2	mp (°C)	Reference(s)
2.10	H	H	266 (dec)	4
			284-285	5
	H (hydrochloride)	H	160-163 (dec)	5
	H (picrate)	H	237-238 (dec)	4
	H (methiodide)	H	216-217 (dec)	4
2.11	$MeO(CH_2)_2$	H	158-159 (dec)	6
2.12	$PhCH_2$	H	207-209 (dec)	7,8
2.13	$Ph(CH_2)_2$	H	202-204 (dec)	7,8
2.14	Ph	H	200-201 (dec)	7,8
			201 (dec)	9
2.15	$2\text{-}MeC_6H_4$	H	163-164 (dec)	7,8
2.16	$4\text{-}MeC_6H_4$	H	207-208 (dec)	8
2.17	$4\text{-}EtC_6H_4$	H	176-177 (dec)	8
2.18	$2\text{-}ClC_6H_4$	H	168-169 (dec)	7,8
2.19	$2\text{-}O_2NC_6H_4$	H	207-209 (dec)	7
2.20	$3\text{-}O_2NC_6H_4$	H	244-245 (dec)	7,8
2.21	$4\text{-}O_2NC_6H_4$	H	237-238 (dec)	7,8
2.22	$3\text{-}NCC_6H_4$	H	242-243 (dec)	7,8
2.23	$4\text{-}NCC_6H_4$	H	229-230 (dec)	7,8
2.24	$2\text{-}NH_2C_6H_4$	H	194-195 (dec)	7,8
2.25	$3\text{-}NH_2C_6H_4$	H	206-207 (dec)	7
2.26	$4\text{-}NH_2C_6H_4$	H	216-218 (dec)	7

Table 2.1 (continued)

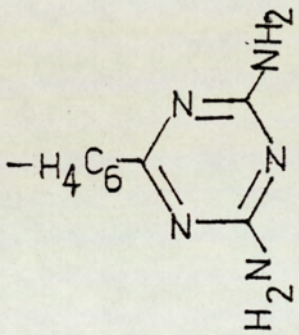
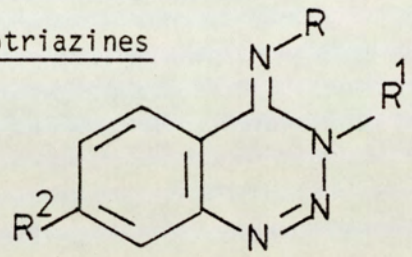
Compound	R^1	R^2	mp (°C)	Reference(s)
2.27	2-Pyridylmethyl	H	180-181	6
2.28	3-Pyridylmethyl	H	213-214	6
2.29		H	259-260 (dec)	8,10

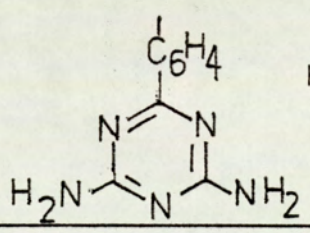
Table 2.2

4(3H)-Imino-1,2,3-benzotriazines



Compound	R	R^1	R^2	mp (°C)	Reference(s)
2.30[a]	n-Bu	H	H	153-155	6,11
2.31	n-Bu	H	Cl	198	11
2.32	$Et_2N(CH_2)_2$	H	H	130-132	6,11
2.33	$Et_2N(CH_2)_2$	H	Cl	181-182	11
2.34	$EtO(CH_2)_3$	H	H	112-114	11
2.35	n-$HexO(CH_2)_3$	H	H	95-97	11
2.36	$PhCH_2$	H	H	201-204	11
2.37	$Ph(CH_2)_2$	H	Cl	204-205	11
2.38	4-$MeOC_6H_4CH_2$	H	H	205-207	11
2.39	4-$ClC_6H_4CH_2$	H	H	213-214	11
2.40	$Ph(CH_2)_2$	H	H	200-202	11
2.41	$Ph(CH_2)_3$	H	H	145-147	11
2.42[b]	Ph	Me	H	130-131	12
2.42	Ph	Me	H	131-132[a]	9
2.43	2-ClC_6H_4	Me	H	99-101	9,12
2.44	2-$O_2NC_6H_4$	Me	H	147-148	12
2.45[b]	Ph	Et	H	83-84[a]	9,12
2.46	2-ClC_6H_4	Et	H	112-114	12
2.47	2-$O_2NC_6H_4$	Et	H	123-124	12
2.48[b]	Ph	i-Pr	H	76-77[a]	9,12
2.49[b]	Ph	C_6H_{11}	H	94-95[a]	9

Table 2.2 (continued)

Compound	R	R^1	R^2	mp (°C)	Reference(s)
2.50	H	$PhCH_2$	H	119-120	7,9
2.51	H	$Ph(CH_2)_2$	H	c	7
2.52	H	Ph	H	112-114	7,9
2.53	Ph	Ph	H	139-140	13
2.54	Ph	$PhCH_2$	H	112-113	8
2.55	H	2-MeC_6H_4	H	100-101	7
2.56	H	4-MeC_6H_4	H	103-104	13
2.57	4-MeC_6H_4	4-MeC_6H_4	Me	145	8
2.58	H	4-EtC_6H_4	H	112-113	7
2.59	H	2-ClC_6H_4	H	137-138	7
2.60	H	3-$O_2NC_6H_4$	H	230-231 (dec)	7
2.61	H	3-NCC_6H_4	H	224-226 (dec)	7
2.62	4-$MeOC_6H_4$	4-$MeOC_6H_4$	H	123-124	9
2.63	4-$MeOC_6H_4$	4-$MeOC_6H_4$	MeO	122-123	13
2.64	H		H	214-215 (dec)	10

a Later reported as 4-n-butylamino-1,2,3-benzotriazines[6]

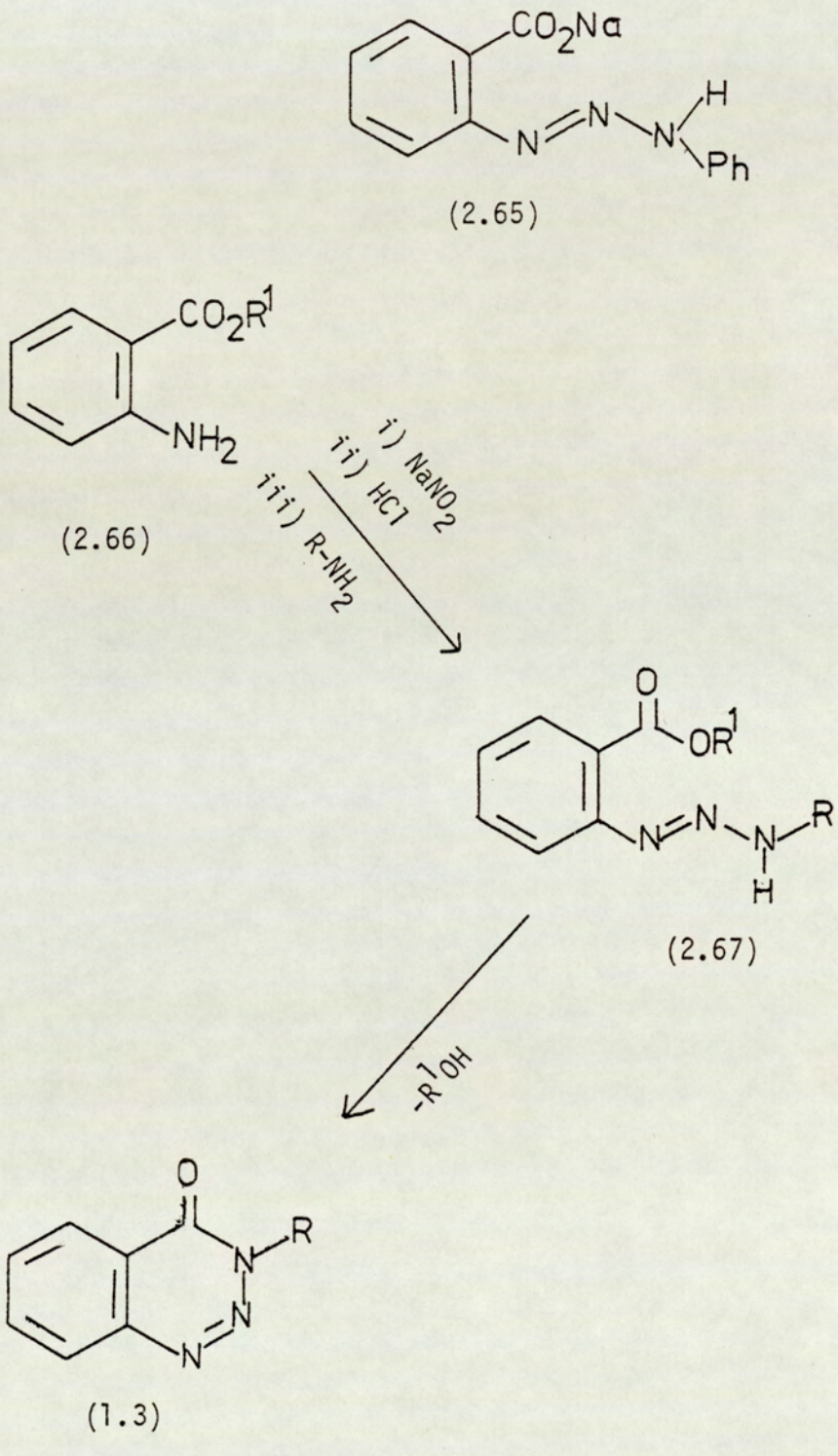
b Previously recorded as 3,4-dihydro-4-alkylimino-3-phenyl-1,2,3-benzotriazines[9]: later corrected as 3,4-dihydro-4-phenylimino-3-alkyl-1,2,3-benzotriazines[12].

c Melting point was not recorded[7].

2.1.1 Syntheses of Type (i)

From diazotisation of 2-aminobenzamidines

Shah[13], in 1924, prepared the 1,2,3-benzotriazine (2.53; Table 2.2) by the treatment of N,N^1-diphenyl-2-aminobenzamidine (2.3; $R=R^1=Ph$, $R^2=H$) (Scheme 2.1) with nitrous acid. Such amidines and their N-alkyl-N^1-aryl-substituted analogues were prepared efficiently by reduction of the corresponding 2-nitrobenzamidines, produced from 2-nitrobenzamides[14]. Partridge and Stevens[9] also adopted this method and obtained 3,4-dihydro-3-phenyl-4-imino-1,2,3-benzotriazine (2.52) and the isomeric 4-anilino-1,2,3-benzotriazine (2.14; Table 2.1). 2-Amino-N-phenylbenzamidine (2.3; $R=R^2=H$, $R^1=Ph$), when similarly treated with nitrous acid at 0 °C, afforded yellow flakes of compound (2.52) upon neutralisation with ammonia. The isomeric 4-anilino-1,2,3-benzotriazine (2.14) was obtained from the concentrated filtrate of the same reaction. Apparently the yields of the two isomers were variable. The structure of iminotriazine (2.52) was deduced from the identity of its alkaline hydrolysis product, the triazene carboxylate (2.65) with that of the product obtained from sodium hydroxide hydrolysis of 3-phenyl-1,2,3-benzotriazin-4(3H)-one (1.3; R=Ph)[15]. In contrast, the isomer 4-anilino-1,2,3-benzotriazine (2.14) lost nitrogen to yield 2-aminobenzanilide; 1,2,3-benzotriazin-4(3H)-one (1.3; R=H) behaves similarly[16]. The structure of imino triazine (2.52) was confirmed by the unambiguous cyclisation of 1-(2-cyanophenyl)-3-phenyltriazene (2.7; $R=R^2=H$, $R^1=Ph$) in aqueous ethanol (Scheme 2.1). The benzyl homologue (2.50) was similarly formed from benzylamine and 2-cyanobenzenediazonium chloride. Partridge and Stevens[9] also showed that 4-iminobenzotriazines (2.6; R=H) could be converted smoothly to 4-anilinobenzotriazines in aqueous acid by the Dimroth rearrangement (Scheme 2.1).


CO2Na
N
N
H
N
Ph
(2.65)
CO2R1
NH2
(2.66)
i) NaNO2
ii) HCl
iii) R-NH2
O
OR1
N
N
N
R
H
(2.67)
-R1OH
O
N
R
N
N
(1.3)

Scheme (2.2)

Parnell[4] prepared 4-amino-1,2,3-benzotriazine (2.10) by diazotisation of 2-aminobenzamidine dihydrochloride[17]; he further derivatised this aminotriazine to yield picrate and methiodide salts (Table 2.1).

2.1.2 Syntheses of Type (ii)
From cyclisation of 1-aryl-3-(2-cyanophenyl) triazenes

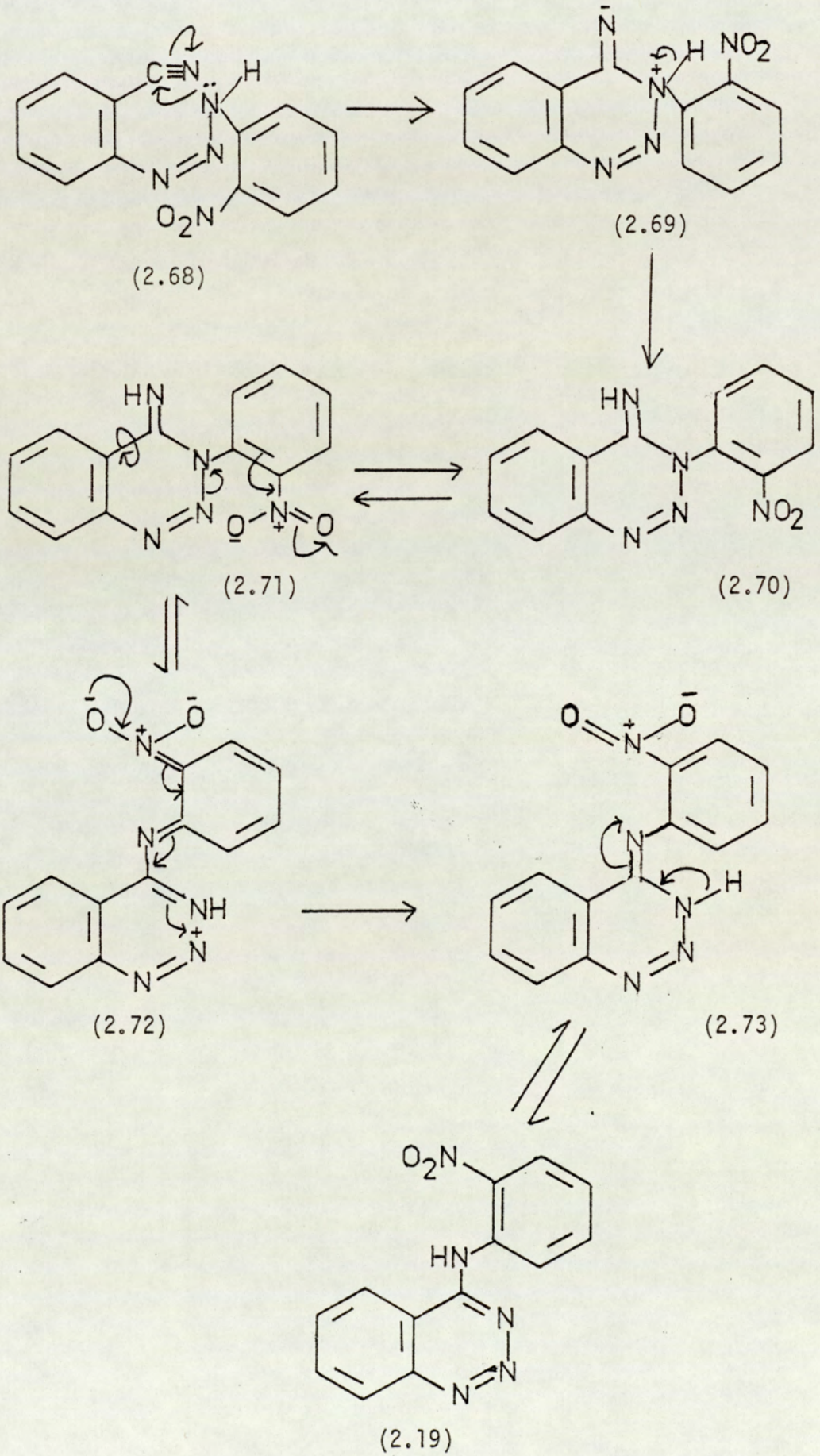
Mehner[15] first utilised triazenes in the synthesis of 1,2,3-benzotriazin-4(3H)-ones. He effected cyclisation of the triazene esters (2.67) to the benzotriazinones (1.3) in boiling ethanol containing substantial amounts of water (Scheme 2.2).

Similar cyclisations of 1-aryl-3-(2-cyanophenyl)triazenes (2.7; R=H, R^1=aryl, R^2=H) to 3,4-dihydro-3-aryl-4-imino-1,2,3-benzotriazines (2.5; R^1=aryl, R=H; Scheme 2.1) and the Dimroth rearrangements to isomeric 4-anilino-1,2,3-benzotriazines (2.8; R^1=Ph, R^2=H) have been studied in detail by Stevens and Stevens[7]. The propensity to rearrangement in the triazine series (2.5; R=H, R^1=aryl) is markedly influenced by the nature of the rearranging groups[18]. Compounds in which the aryl substituents are electron-donating or mildly electron-withdrawing, for example (2.7; R=R^2=H, R^1=Ph, 2-MeC_6H_4 or 2-ClC_6H_4), undergo smooth ring closure to yield 4-imino-1,2,3-benzotriazines (2.52, 2.55 or 2.59 respectively) when heated in either 70% aqueous ethanol or in 95% ethanol containing 2% piperidine[7,8,12] or in acetic acid[8].

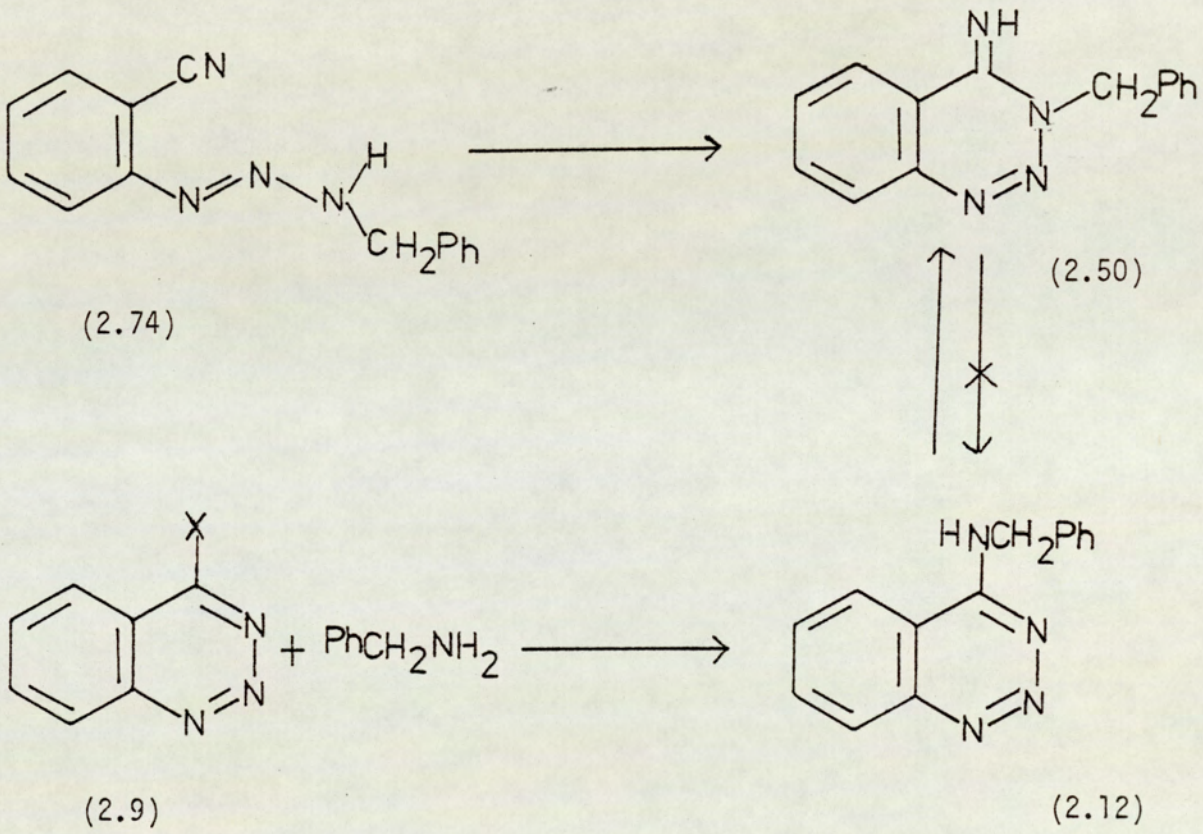
Compounds with a powerful electron-withdrawing group (2.7; R=R^2=H, R^1=2-$O_2NC_6H_4$, 4-$O_2NC_6H_4$ or 4-NCC_6H_4) on the other hand, undergo both ring closure and Dimroth rearrangement to give the 4-anilino-1,2,3-benzotriazines (2.19, 2.21, 2.23 respectively). For

example, the imine (2.70) (Scheme 2.3) formed by cyclisation of 1-(2-cyanophenyl)-3-(2-nitrophenyl)triazene (2.68) is so unstable that it cannot be isolated; it rearranges spontaneously to the isomer, 4-(2-nitroanilino)-1,2,3-benzotriazine (2.19)[8]. This behaviour is attributed to the electronic influence of the 2-nitro-substituent, which, by delocalising the negative charge developed on the amidine nitrogen atom formed following heterolytic fission of the N(2)-N(3) bond, disturbs the equilibrium (2.70) $\rightleftharpoons$ (2.71) in favour of the acyclic species. Bond rotation followed by recyclisation at the more nucleophilic (unsubstituted) amidine nitrogen atom leads to the rearranged product (2.19). Similar rate-enhancing effects operate in 4-nitro and 4-cyanoanilinotriazines (2.21 and 2.23). The 3-nitro and 3-cyano derivatives show intermediate behaviour and the imino intermediates (2.60 and 2.61) can be isolated and subsequently isomerised to 4-anilinotriazines (2.20 and 2.22).

The iminotriazines (2.52, 2.55 and 2.59) also undergo rearrangement to arylamino isomers (2.14, 2.15 and 2.18 respectively) when heated in either 95% ethanol or in 2N-hydrochloric acid, or better still in acetic acid[8]. These observations are fully consistent with known substituent effects in other Dimroth rearrangements[19]. The factors influencing cyclisation and rearrangement of 1-(2-cyanophenyl)-3-aralkyltriazenes (2.74) are not clear: the benzyltriazene (2.74) cyclises to the 4-imino-1,2,3-benzotriazine (2.50) in 70% aqueous ethanol and, more efficiently, in ethanol containing 20% piperidine. As expected, the iminotriazines (2.50 or 2.51) did not isomerise to aminotriazines (2.12 or 2.13) in boiling ethanol (120 hours) or in 2N-hydrochloric acid. On the other hand, 4-benzylaminotriazine (2.12), prepared unambiguously from benzylamine and 4-methylthio-1,2,3-benzotriazine[20,21,22] (2.9; X=SMe), (see the



Scheme (2.3)



X = SMe

Scheme (2.4)

following section) underwent the reverse rearrangement[7,9] to 4-imino-benzotriazine (2.50) in 2N-hydrochloric acid (10 days). The authors[9] consider that this could be due to a steric effect of the bulky aralkyl group which prefers to be located on the exocyclic nitrogen.

Siddiqui and Stevens[8] reinvestigated the rearrangement of imino-triazines in hot acetic acid and reported that acetic acid is the best solvent to promote rearrangement of the iminotriazines (2.50 and 2.51) to their isomers (2.12 and 2.13).

2.1.3 Syntheses of Type (iii) From 1,2,3-benzotriazin-4(3H)-thiones

This synthetic route was first developed as part of a search for biological activities in the 4-amino-1,2,3-benzotriazine series[5], as the analogous 4-amino-1,2,4-benzotriazines showed some remarkable pharmacological properties[23,24]. The aminotriazine (2.10) could not be obtained by the first two routes described earlier, since 2-aminobenzamidine and 2-aminobenzamidrazones were not available. [In a later report, Parnell[5] has succeeded in synthesising this aminotriazine (2.10) from diazotisation of 2-aminobenzamidine dihydrochloride].

A promising approach to the unsubstituted aminotriazine (2.10) appeared to involve the aminolysis of the easily accessible 4-methylthio-1,2,3-benzotriazine (2.9; X=S-Me)[9] (Scheme 2.1). Leonard and Curtin[25] prepared related N-substituted 4-aminoquinazolines by aminolysis of 4-mercaptoquinazolines[26]. Gilbert et al[11] prepared a number of alkylamino-1,2,3-benzotriazines by heating 1,2,3-benzotriazin-4(3H)-thiones with amines in suitable solvents and reported that aniline failed to react at 135 °C in

2-ethoxyethanol. Even the more basic aromatic amine p-anisidine behaved likewise and these authors[11] claimed that strongly basic aliphatic amines are required for this type of reaction to occur at modest temperatures. They did not pursue this reaction any further to optimise the conditions with respect to aromatic amines.

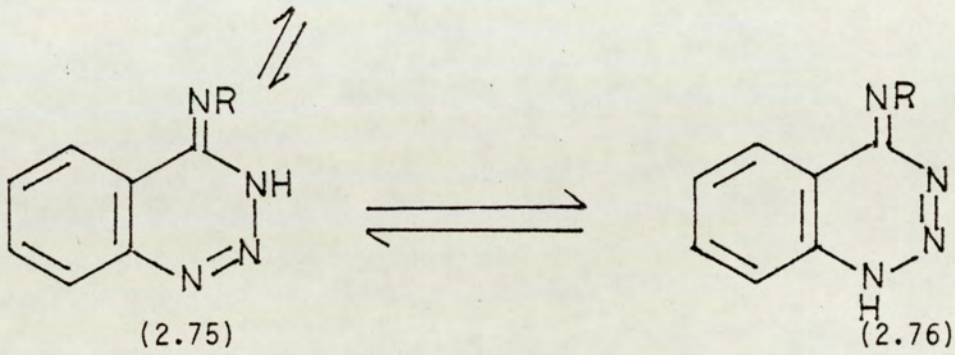
Stevens and Stevens[12] extended the above reaction to obtain substituted 4-anilino-1,2,3-benzotriazines (2.17) in reasonable yields by the interaction of 4-methylthio-1,2,3-benzotriazine (2.9; X=SMe) and o-chloroaniline in boiling ethanol for 66 hours.

2.2 Physical and spectral properties of 4-amino or 4(3H)-imino-1,2,3-benzotriazines

All 4-amino (2.1) or 4-imino-1,2,3-benzotriazines (2.2) are crystalline coloured compounds (yellow, brown or ochre). Infrared[4,7,8,9], ultraviolet[4,7,8,9], ^{1}Hnmr[6,28,29] and mass spectra[7,12,33] for these compounds are relatively unremarkable and found in the quoted literature. Melting points of compounds (2.1 and 2.2) are recorded (Tables 2.1 and 2.2).

Stevens and Stevens[7] reported that these isomeric compounds (2.1 or 2.2; R=aryl) can be distinguished by their distinctive uv and ir spectra: uv spectra of 4-imino-1,2,3-benzotriazines (2.2; R=aryl) showed characteristic double peaks in the ranges of 260-270 and 305-320 nm, whereas the 4-anilino-1,2,3-benzotriazines (2.1; R=aryl) exhibited only one band in each region. Similarly, the ir spectra of all 4-anilino-1,2,3-benzotriazines (2.1; R=aryl) showed a strong absorption at 1145 ± 10 cm^{-1} which was absent in the 3-aryl-4-iminobenzotriazines[7] (2.2; R=aryl) and this represents a very useful aid in distinguishing between the two series of isomers.

(2.1)



(2.75) (2.76)

4-Amino-1,2,3-benzotriazine (2.1) may exist in any of the above tautomeric forms (2.1, 2.75 and 2.76) but to date there is insufficient evidence to show which one of these is preferred[27]. In one report[11] the uv spectra of 2.32, 2.38 and 2.39 were shown to be nearly identical and this suggested that these compounds must have the same chromophoric system in solution.

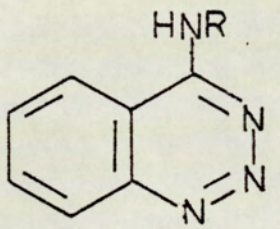
Recent studies[28,29] by ^{1}H nmr and ^{15}N nmr spectroscopy on 4-alkyl- and 4-arylamino-1,2,3-benzotriazines suggested that several 4-substituted-1,2,3-benzotriazines adopt the amino structural form (2.1) in solution. The results of these nmr studies are tabulated (Table 2.3 and 2.4). As indicated by Table 2.3 the signals for δ-CH_2 protons for the compound (2.11 and 2.30) appeared as double triplets. The compounds (2.27 and 2.28) showed doublets or two singlets. However, after shaking with D_2O the absorptions collapsed to triplets and singlets respectively[28]. Therefore, the authors[28] concluded that such signals could only arise from coupling with the adjacent N-H protons and not from the CH_2 group in a mixture of two imino forms (2.75 and 2.76).

These observations were further supported by ^{15}N nmr spectroscopy[28,29]. Only one example of a 4-arylamino-1,2,3-benzotriazine (2.16) was examined by this technique[28,29] (Table 2.4).

There were close similarities in the chemical shifts of the four nitrogen atoms for all compounds examined. This suggested that the arylamino-1,2,3-benzotriazine (2.16) also adopts the same amino-form in solution.

Table 2.3

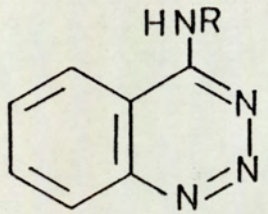
The δ and J values for α-methylene protons of 4-alkylamino-1,2,3-benzotriazines



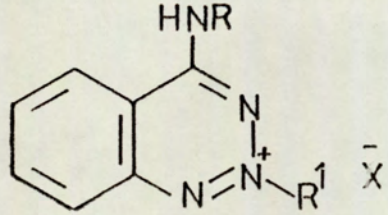
Compound	R	Solvent	J_{NH-CH_2} (Hz)	$J_{CH_2-CH_2}$ (Hz)	δ (ppm)
2.11	$MeO(CH_2)_2$	a	6.0	7.0	dt 3.82
2.11		b		7.0	t 3.82
2.30	n-Bu[c]	a	6.0	7.5	dt 3.61
2.30		b		7.5	t 3.61
2.27	2-Pyridylmethyl	a	6.0		d 5.0
2.27		b			s 5.0
2.28	3-Pyridylmethyl	a	6.0		d 4.90
		b			s 4.90

a Solvent DMSO-d_6; b as (a) but after shaking with D_2O; c same pattern observed in $CDCl_3$; d doublet; dt double triplet; s singlet.

Table 2.4 ^{15}N Chemical shifts (ppm) for 4-amino-1,2,3-benzotriazines and 4-amino-1,2,3-benzotriazinium betaines[a,b]



(2.1)



(2.77)

	R	R^1	X^-	N-2	N-1 or N_3		N-H
2.30	n-Bu			67.3	-15.4,	-67.7	-292.0
2.11	$MeO(CH_2)$			67.6	-13.7,	-67.3	-295.5
2.27	2-pyridylmethyl			69.9	-9.6, [-64.3][c],	-67.0	-293.5
2.28	3-pyridylmethyl			68.5	-10.6, [-63.4][c],	-66.3	e
2.16	$4\text{-}MeC_6H_4$			66.3	-7.6,	-65.1	-278.7
2.77a	n-Bu	n-Pr	I	-84.6	-40.5,	-91.8	-269.3
2.77b	$MeO(CH_2)_2$	n-Pr	I	-84.4	-39.6,	-91.6	-272.6
2.77c	$4\text{-}MeC_6H_4$	n-Pr	I	-85.5	-36.6,	-90.4	-261.4
2.77d	$4\text{-}MeC_6H_4$	n-Pr	Br	-85.1	-36.8,	-90.2	-259.4
2.77e	$4\text{-}MeC_6H_4$	n-Pr	$4\text{-}MeC_6H_4SO_3$	-85.0	-36.8,	-90.2	-261.4
2.77f	$4\text{-}MeC_6H_4$	Me	$MeSO_3$	-88.6	-35.8,	-93.5	-269.1
2.77g	$4\text{-}MeC_6H_4$	Me	$4\text{-}MeC_6H_4SO_3$	-88.6	-35.3,	-93.4	-261.4

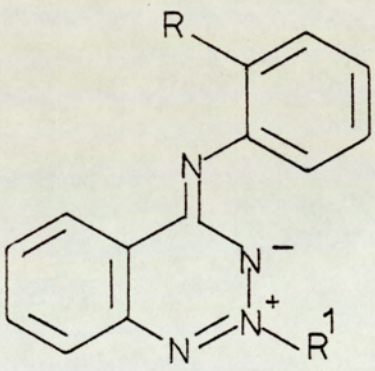
Table 2.4 (continued)

a Nitromethane used as external standard

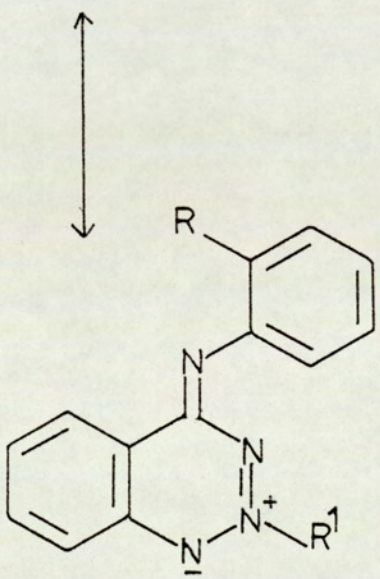
b Solvent DMSO-d_6

c Nitrogen shifts for the pyridyl nitrogens are shown in parentheses

e Chemical shift was not observed.



(2.78)



(2.79)

R = H, R^1 = n-Pr

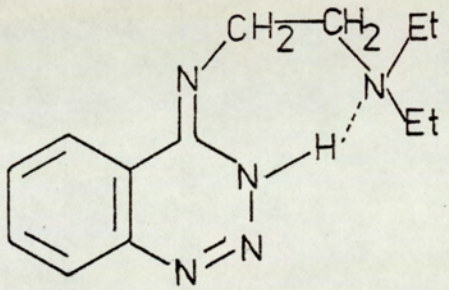
R = NO_2, R^1 = Me

It was also confirmed by ^{15}N nmr spectroscopy that 4-alkylamino or 4-arylamino-1,2,3-benzotriazines undergo regioselective N-quaternisation at the N(2) position. This nitrogen showed a pronounced up-field shift of ca 150 ppm on quaternisation. The chemical shift assignment for N(2) of the ring was further confirmed by ^{15}N nmr studies of the ^{15}N enriched (95%) N(2) isotopomer of compound (2.16) before and after N-alkylation. This compound showed an intense downfield resonance corresponding to labelled $^{15}N(2)$, which experienced an upfield shift ca 150 ppm after alkylation[30].

The ^{1}H nmr spectra of the quaternised benzotriazinium betaines (2.77a and 2.77b) showed double-triplet splitting patterns for the CH_2 protons adjacent to the side-chain NH group as in their precursors (see Table 2.3). This verifies that these benzotriazinium salts (2.77a and 2.77b) also adopt the amino form in solution.

The information obtained by x-ray crystallography of the benzotriazinium betaines (2.78)[31,32] agrees with the above nmr results and confirms that N-alkylation occurs at N(2). The anhydro bases can only exist in their imino forms (2.78 or 2.79).

The crystallographic results of Schwalbe[31] are in close agreement with Gilbert and Veldhuis'[11] findings in the alkyltriazine series. These latter authors[11] used infrared spectroscopy to distinguish between 4-(alkylamino) and 4-(alkylimino)-1,2,3-benzotriazines (2.1 and 2.75 or 2.76; R=alkyl) and came to the conclusion that most of their compounds have imino structures (2.72 or 2.73; R=alkyl). For example, in the case of compounds (2.1; R=H or alkyl) in the solid state, ir bands were noted at 3320 and 3100 cm^{-1}. The former band suggested the presence of an imino group and the latter peak was in the range for the NH mode in indazole derivatives. These observations were consistent with imino



(2.80)

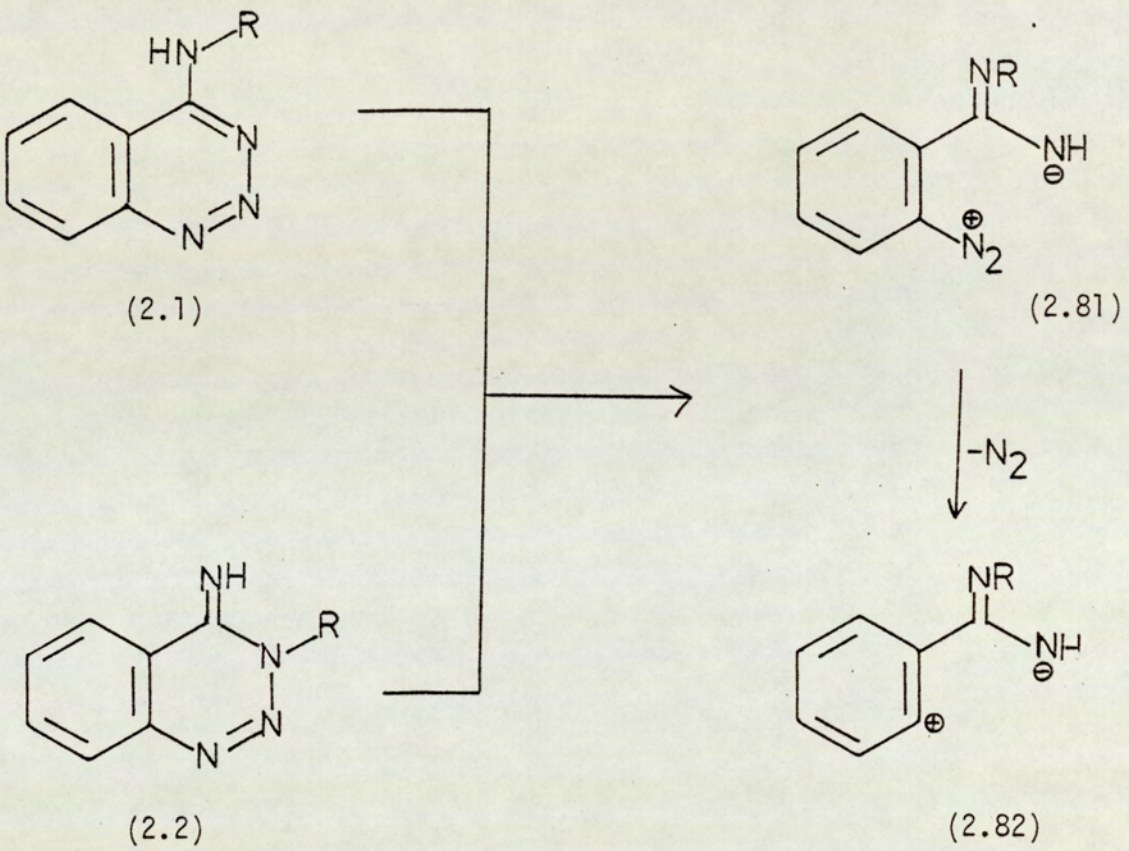
structures (2.75 or 2.76). The compound (2.1; R=4.$MeOC_6H_4CH_2$) was an exception. The solid state ir spectra of this compound contained a peak at 3345 cm^{-1} which favours the amino tautomer. The solution ir spectrum of only one of the above compounds (2.1; R=$(Et)_2N(CH_2)_2$) was mentioned in the same report. This compound showed a broad ir peak at 3370 cm^{-1} suggesting strong hydrogen bonding in the imino form (2.80).

Stevens et al[33] made intensive studies on the electron-impact promoted mass spectrum of 4-anilino (2.1; R=aryl) and 3-aryl-4-imino-1,2,3-benzotriazines (2.2; R=aryl). All these compounds showed molecular ions in low abundance and the fragmentation starts with the loss of a nitrogen molecule. The 4-arylaminotriazines in addition to $(M-N_2)^+$ peaks also showed intense peaks corresponding to loss of arylamino groups.

2.3 Chemical properties of 4-amino-1,2,3-benzotriazines and 4(3H)-imino-1,2,3-benzotriazines

4-Amino and 4(3H)-imino-1,2,3-benzotriazines (2.1 and 2.2), like most other 1,2,3-benzotriazines, behave as masked diazonium compounds. These compounds undergo ring fission at N(2)-N(3) bond and react with substrates that ordinarily react with diazonium compounds. The products obtained from hydrolysis or thermolysis can be explained by transient formation of the diazonium species (2.81) which cannot be isolated but can be trapped by coupling with 2-naphthol[7,8].

The decomposition also takes place by heterocyclic cleavage of the C-N bond leading to an aryl cation (2.82) which rapidly reacts with available nucleophiles. The ease with which the diazo-fragment

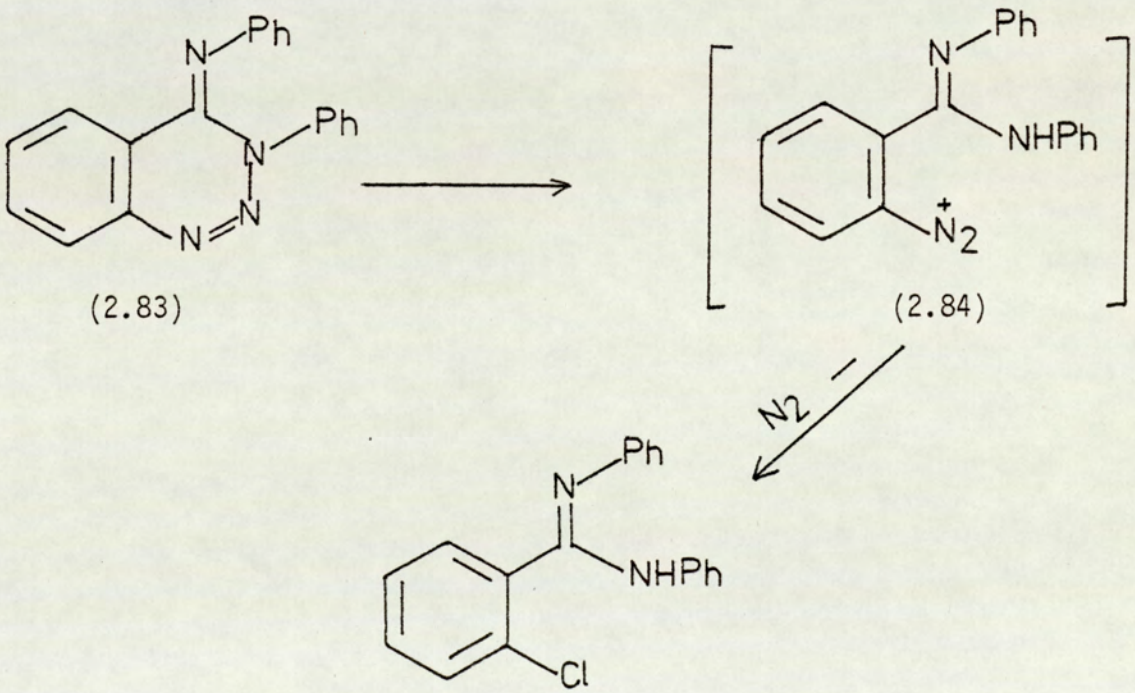


Scheme (2.5)

of 1,2,3-benzotriazines is replaced in both heterolytic and homolytic processes makes these compounds versatile intermediates in the synthesis of ortho-disubstituted arenes[34-36].

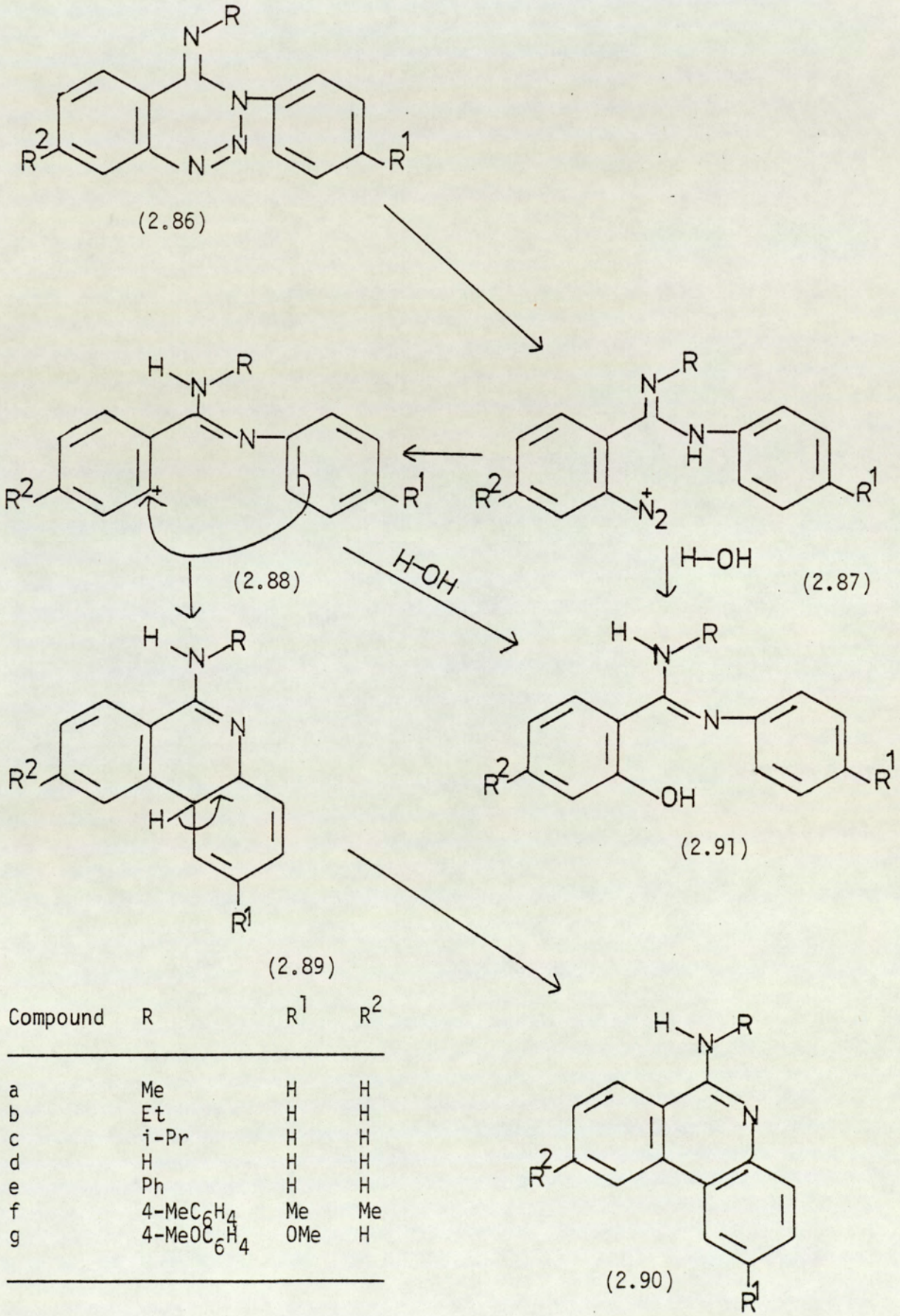
2.3.1 Decomposition of 4(3H)-imino-1,2,3-benzotriazines in acids

Shah[13] noted that when 3-phenyl-4-phenylimino-1,2,3-benzotriazine (2.83) was treated with dilute hydrochloric acid in the presence of copper bronze a compound with a formula $C_9H_{15}N_2Cl$ was isolated. This compound was formulated as a 2-chlorobenzamidine (2.85). During the reaction course the iminotriazine (2.83) apparently undergoes ring fission at the N(2)-N(3) bond to yield the diazonium intermediate (2.84) which then, by the Sandmeyer reaction yields the chloro compound (2.85).



(2.83) (2.84) (2.85)

Scheme (2.6)



Compound	R	R^1	R^2
a	Me	H	H
b	Et	H	H
c	i-Pr	H	H
d	H	H	H
e	Ph	H	H
f	4-MeC_6H_4	Me	Me
g	4-$MeOC_6H_4$	OMe	H

Scheme (2.7)

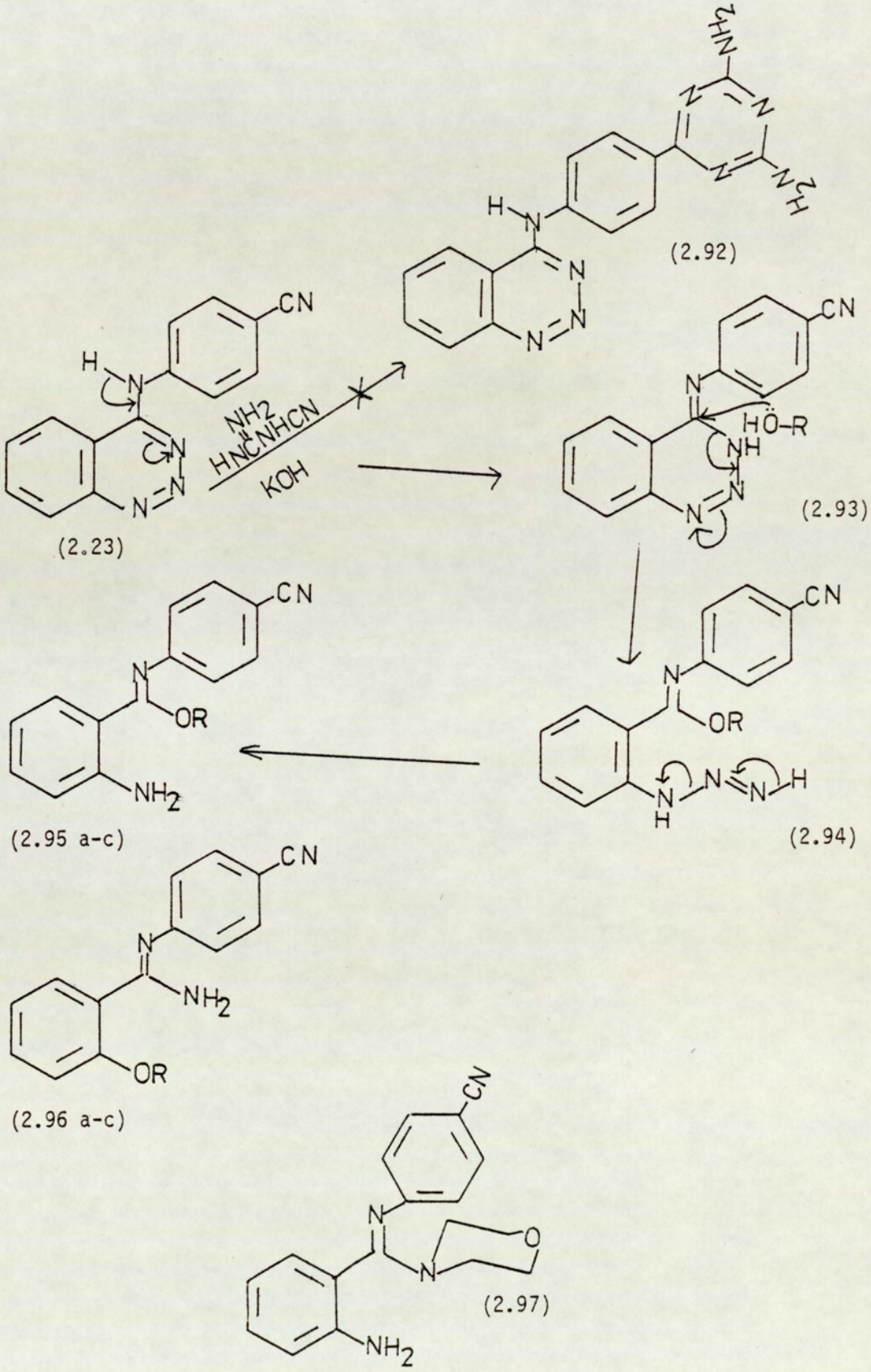
Similarly, the decomposition of 1,2,3-benzotriazines (2.42, 2.45, 2.48, 2.52, 2.53, 2.57 and 2.62; see Table 2.2) proceeds rapidly in 30% sulphuric acid containing copper powder or in 100% phosphoric acid[9]. As indicated by scheme (2.7), the loss of nitrogen forms an aryl cation (2.88), which undergoes intramolecular cyclisation from nucleophilic attack of the adjacent aryl group to yield substituted 6-aminophenanthridines (2.90a-g).

In one instance Partridge and Stevens[9] isolated 2-hydroxy-NN'-diphenylbenzamidine[39] (2.91; R=Ph, R^1=R^2=H) (44%) from the reaction of iminotriazine (2.53). This hydroxyamidine (2.91; R=Ph, R^1=R^2=H) probably formed by a "Sandmeyer-type" nucleophilic displacement of the diazonium cation.

The same 6-aminophenanthridine (2.90e) was obtained when the isomeric 4-anilino-1,2,3-benzotriazine (2.14) was used[9].

2.3.2 Decomposition of 4-amino or 4(3H)-imino-1,2,3-benzotriazines in alcohols

MacKenzie and Stevens[10] reacted 4-(4-cyanoanilino)-1,2,3-benzotriazine (2.23) and cyanoguanidine in boiling 2-methoxyethanol in an attempt to obtain 4-substituted arylamino-1,3,5-triazino-1,2,3-benzotriazines (2.92) for examination of their tumour inhibitory properties[40]. (Certain 2,4-diaminopyrimidines[41-43] and 2,6-diamino-1,3,5-triazines are known to exhibit such properties[44]). From the above reaction a colourless product was obtained with the evolution of nitrogen. The same product, in much improved yield, was obtained when the cyanoguanidine was omitted. Further analogues of this colourless compound were obtained from the reaction of the triazine (2.23) with various high boiling alcohols[10]. These compounds were

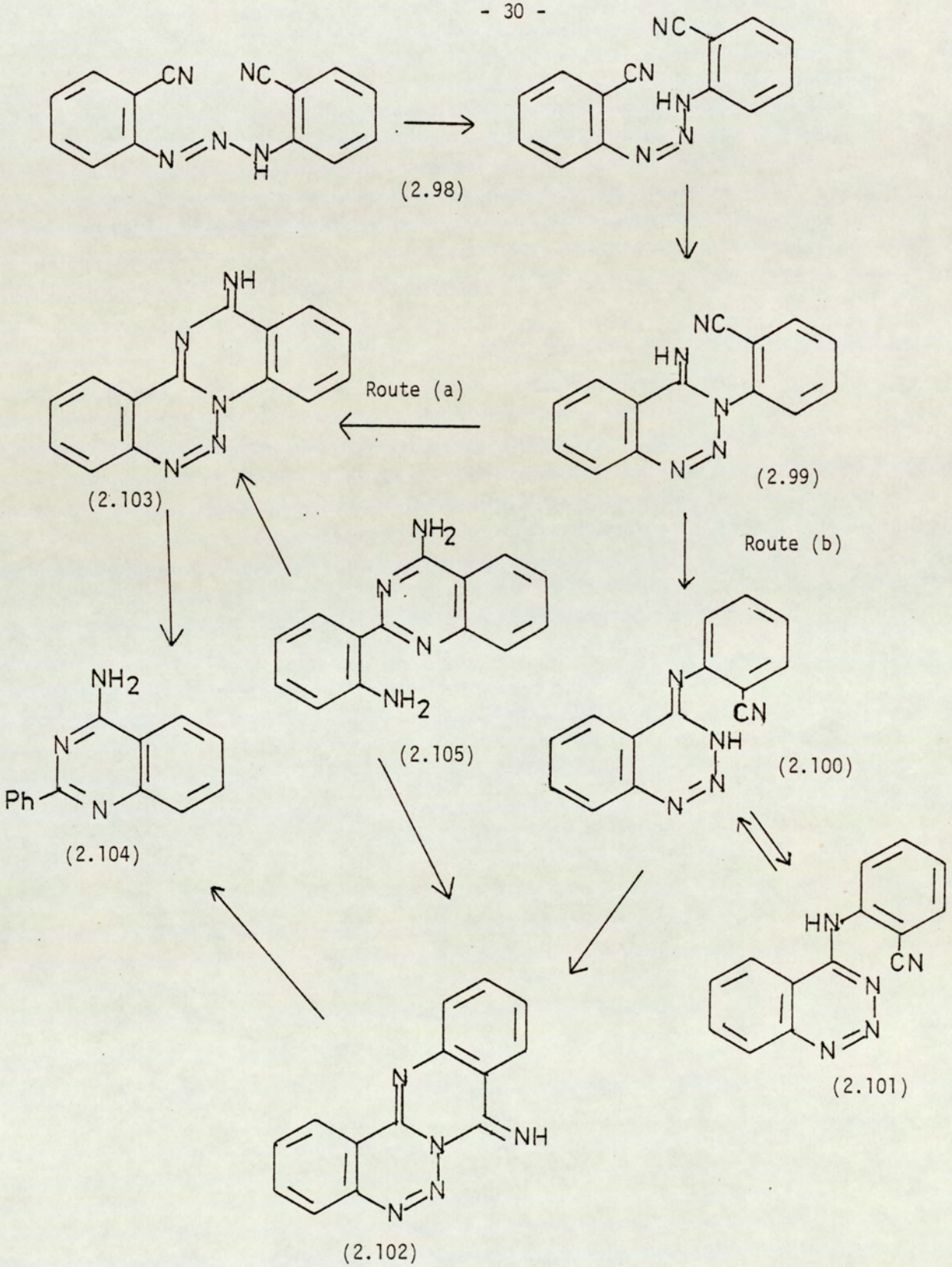


Scheme (2.8)

originally formulated as N-(4-cyanophenyl)-2-(2-alkoxy)benzamidines (2.96a-c, Scheme 2.8), on the basis of their spectroscopic properties[10]. It was discovered later[45] that these compounds also contained a diazotisable amino group; they were reformulated therefore as N-(4-cyanophenyl)-2-aminobenzimidates (2.95a-c). These imidates, unlike most imidates[46,47], are fairly stable in amines. For example, the imidate (2.95a) did not form the corresponding amidine (2.97) in boiling morpholine.

4-(2-Cyanoanilino)-1,2,3-benzotriazine (2.101) [although never isolated from the cyclisation of its acyclic precursor (2.98)] in hot ethanol afforded 4-amino-2-phenylquinazoline (2.104). It was suggested[9,12] that this triazine (2.101) further cyclises by amine-nitrile addition of both conveniently positioned cyano groups to yield the tetracyclic compound (2.102 or 2.103). This tetracyclic intermediate behaves as a masked diazonium compound and undergoes reductive elimination of nitrogen to yield 4-amino-2-phenylquinazoline (2.104). The same tetracyclic compound (2.102 or 2.103) was obtained from the diazotisation of 4-amino-2-aminophenylquinazoline (2.105). The tetracyclic compound obtained from this unambiguous route also decomposed in boiling ethanol to furnish 4-amino-2-phenylquinazoline (2.104) (Scheme 2.9).

Although decomposition of the triazene (2.98) might logically be expected to proceed by route (a), route (b) was also suggested by the authors[7] because of the ready rearrangement of 4-aminobenzotriazine (2.99), with the electron withdrawing cyano group, to 4-arylamino isomer (2.101).



Scheme (2.9)

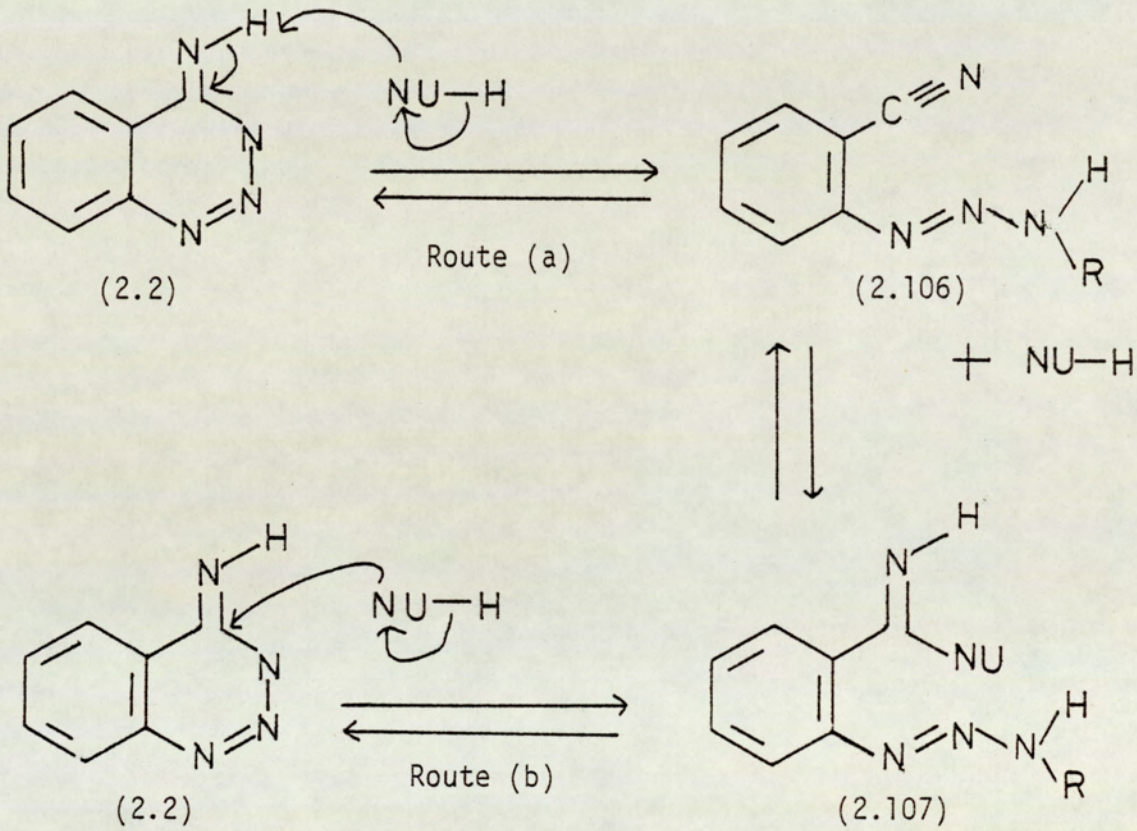
2.3.3 Decomposition of 4-amino or 4(3H)-imino-1,2,3-benzotriazines in secondary amines

When 3-substituted-3,4-dihydro-4-imino-1,2,3-benzotriazines (2.2) were boiled in piperidine for 15 minutes, the acyclic triazenes (2.106) were formed.

In boiling piperidine, the iminotriazines (2.2) underwent ring fission initiated by nucleophilic attack by the base[48]. Two possible mechanisms to account for the formation of triazene (2.100) could be envisaged for this reaction (Scheme 2.10): in route (a) the base (NU-H) abstracts the acidic imino proton; in route (b) initial attack by the nucleophile occurs at C-4. Preference was given to route (a) since it explains the stability of the 3-benzyltriazine (2.50) and the 3,4-disubstituted benzotriazines (2.42, 2.43, 2.48, 2.53 and 2.54) (see Table 2.2) towards boiling piperidine. In the benzyltriazine (2.50), the +I substituent would decrease the acidity of the imino proton to render its removal by base more difficult; the disubstituted triazines possess no abstractable imino proton. This mechanism by route (a) was further supported when 4-amino-1,2,3-benzotriazine-3-oxide (2.108)[49] afforded 2-azidobenzonitrile (2.110)[50] in both boiling piperidine and pyrrolidine[48].

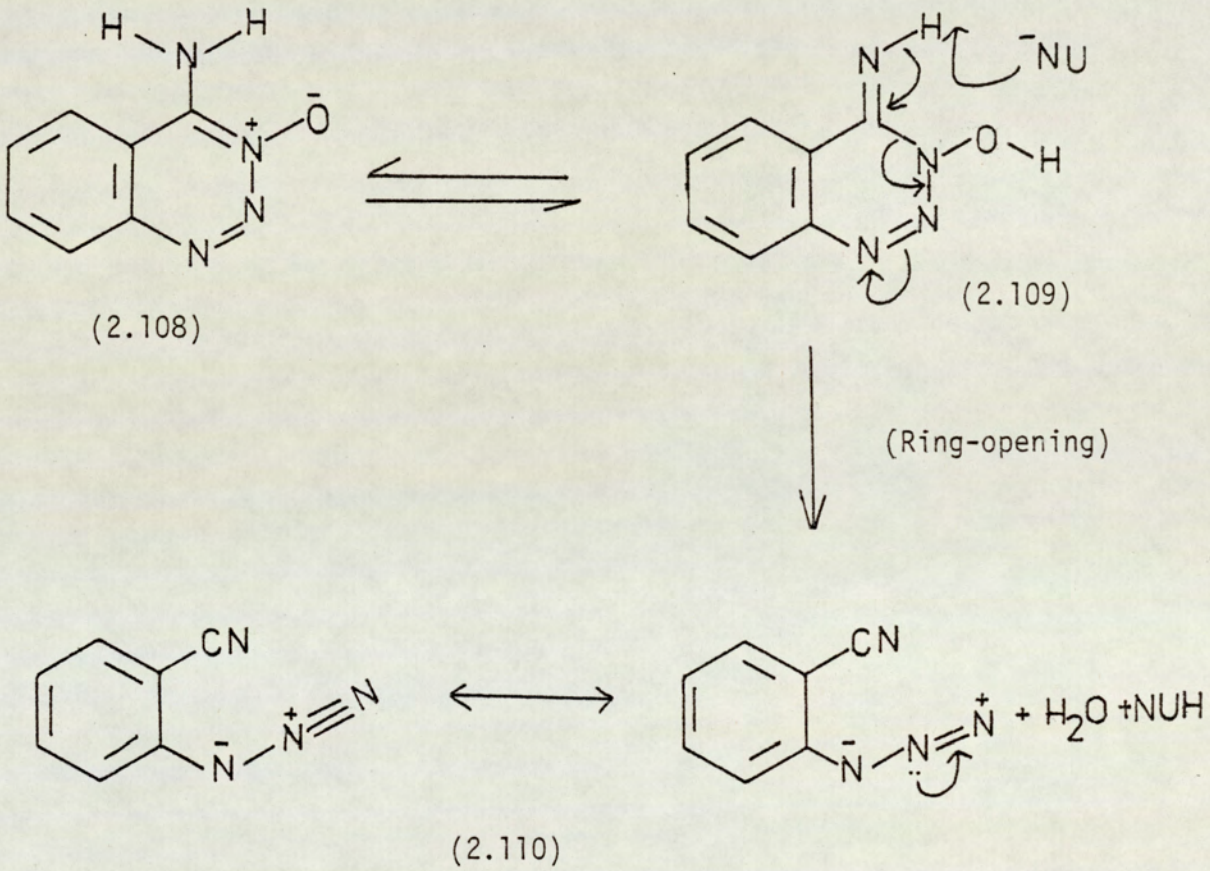
The ring cleavage was initiated probably by proton abstraction from the 4-imino group of the N-hydroxy tautomer (2.109) (Scheme 2.11). Route (b) depicted in scheme 2.10 was further discounted as the attack by nucleophile at C-4 would form intermediate amidines (2.107) and there is no evidence in the literature that such amidines should be particularly unstable and be degraded to nitriles[47].

The behaviour of 1-(2-cyanophenyl)-3-aryltriazenes (2.106, R=aryl) towards piperidine and its analogues illustrated the multiple



R = Ph, CH_2Ph, 2-(or 4)MeC_6H_4, $4EtC_6H_4$, $3NCC_6H_4$,
$2\text{-}ClC_6H_4$ and
$4\text{-}[N = C\text{-}(NH_2)\text{-}N = C\text{-}(NH_2)\text{-}N{=}C]\text{-}C_6H_4$

Scheme (2.10)

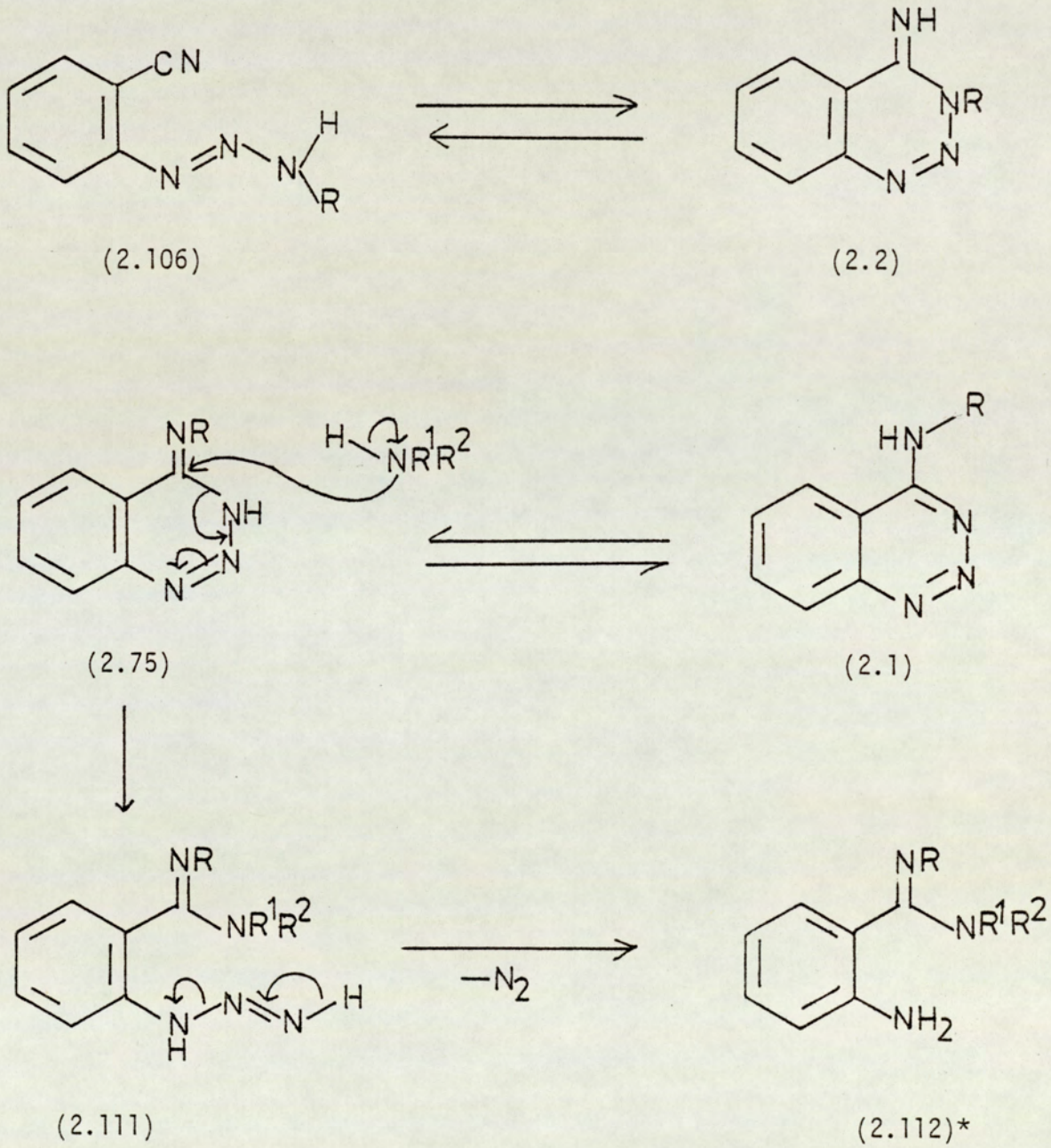


Scheme (2.11)

role of the nucleophile as catalyst and reactant. These reactions results were consistent with the above findings of Siddiqui and Stevens[48]. That is, at low concentrations the amines behave as a catalyst for the cyclisation of triazene (2.106) to its cyclic imino isomer (2.2). In high concentrations the amines act as a reactant nucleophile[45]. For example, the triazene (2.106; R=3-NCC_6H_4) was recovered unchanged from boiling in excess pyridine as the equilibrium acyclic $\rightleftharpoons$ cyclic species was overwhelmingly towards the acyclic isomer (2.106; R=3-NCC_6H_4)[45].

1-(2-Cyanophenyl)-3-(4-cyanophenyl)triazene (2.106; R=4-NCC_6H_4) behaved differently. The influence of a powerful electron withdrawing 4-cyano substituent in the iminotriazine counters the tendency for this imine to revert to starting acyclic triazene by encouraging the competing Dimroth rearrangement[45]. The 4-cyano-anilino-1,2,3-benzotriazine (2.1; R=4-NCC_6H_4) thus formed reacted with piperidine to afford a NNN'-tri-substituted 2-aminobenzamidine (2.112a). The same amidine (2.112a) with much improved yield was obtained when pre-formed benzotriazine (2.23) was heated with piperidine[45] (Scheme 2.12).

Further analogues of the amidine series (Table 2.5) were made by heating the 4-anilinobenzotriazines (2.1; R=aryl) with various secondary amines. The reaction takes place by ring fission via acyclic triazenes (2.111) with the loss of nitrogen (Scheme 2.13). An alternative but less likely route (Scheme 2.13) (because of the low temperatures involved) was also suggested by Stevens[45] who proposed that amidines (2.112) could arise by the reactions of amines with either benzazet-2(1H)-imine (2.114) or its valence tautomer (2.115) formed by the thermal extrusion of nitrogen from the starting benzotriazine (2.1).



(2.106) (2.2)

(2.75) (2.1)

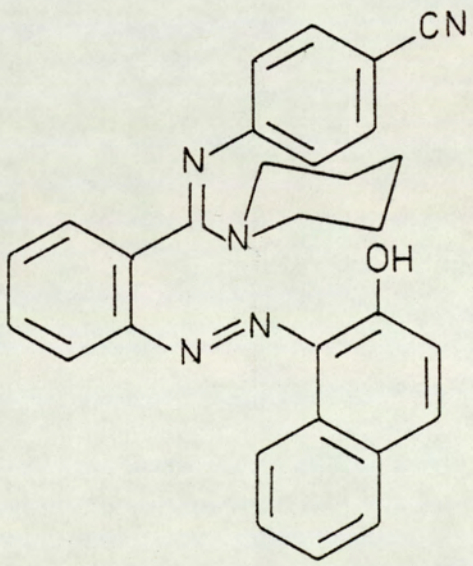
(2.111) (2.112)*

*(see Table 2.5)

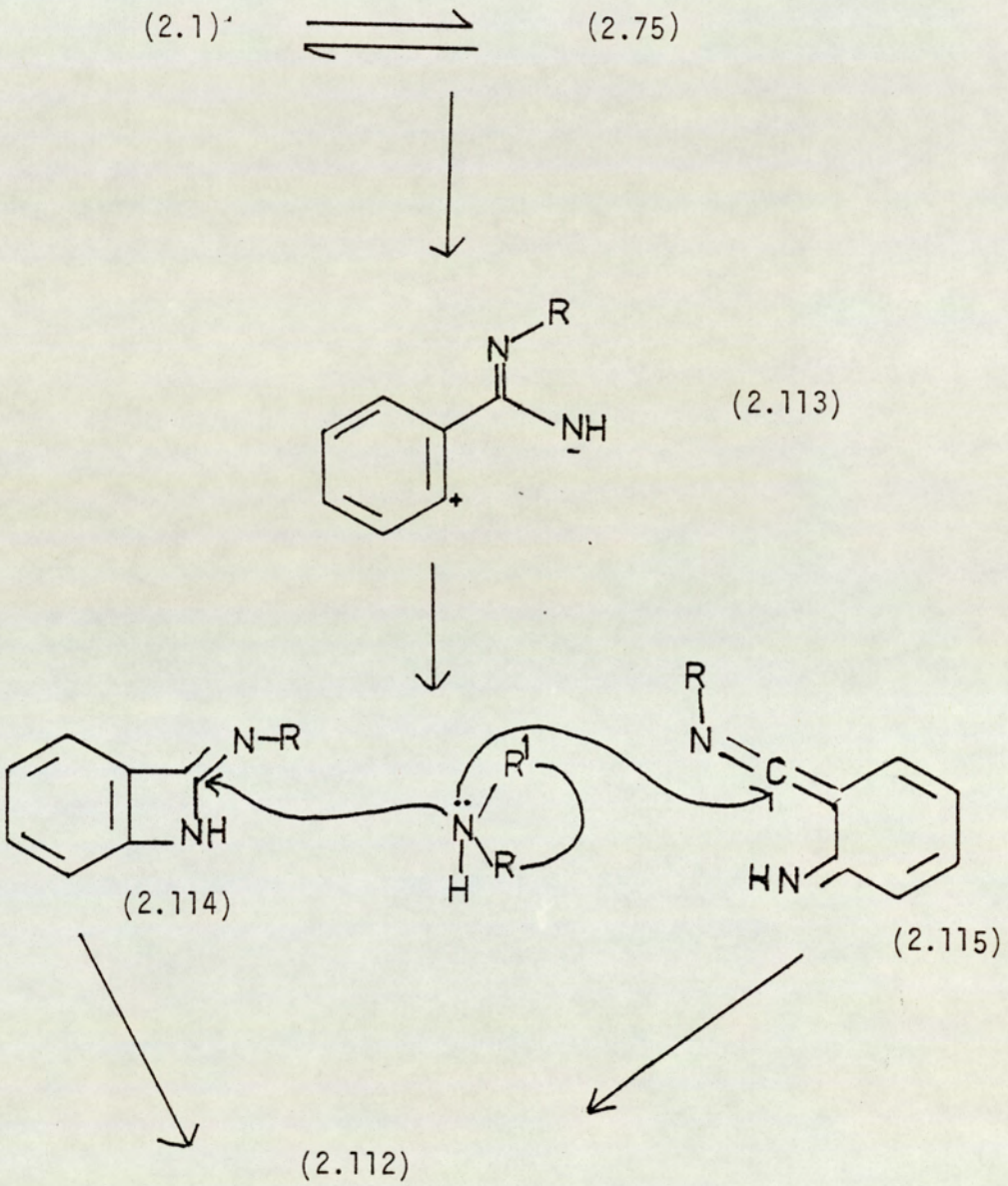
Scheme (2.12)

Table 2.5

Compound 2.112	R	R^1 - R^2	mp (°C)	% Yield	Refs
a	Ph	$(CH_2)_4$	116-117	70	45
b	Ph	$(CH_2)_5$	97	80	45
c	Ph	$(CH_2)_2$-O-$(CH_2)_2$	124-125	85	45
d	4-NCC_6H_4	$(CH_2)_5$	118-119	88	45
e	4-NCC_6H_4	$(CH_2)_2$-O-$(CH_2)_2$	117-118	70	45
f	2-$O_2NC_6H_4$	$(CH_2)_5$	127-128	82	45
g	3-$O_2NC_6H_4$	$(CH_2)_5$	118-119	80	45
h	4-$O_2NC_6H_4$	$(CH_2)_5$	66-67	90	45
i	3-$H_2NC_6H_4$	$(CH_2)_5$	116-117	82	45
j	4-$H_2NC_6H_4$	$(CH_2)_5$	173-174	80	45
k	4-MeC_6H_4	$(CH_2)_5$	130-131	85	45



(2.116)



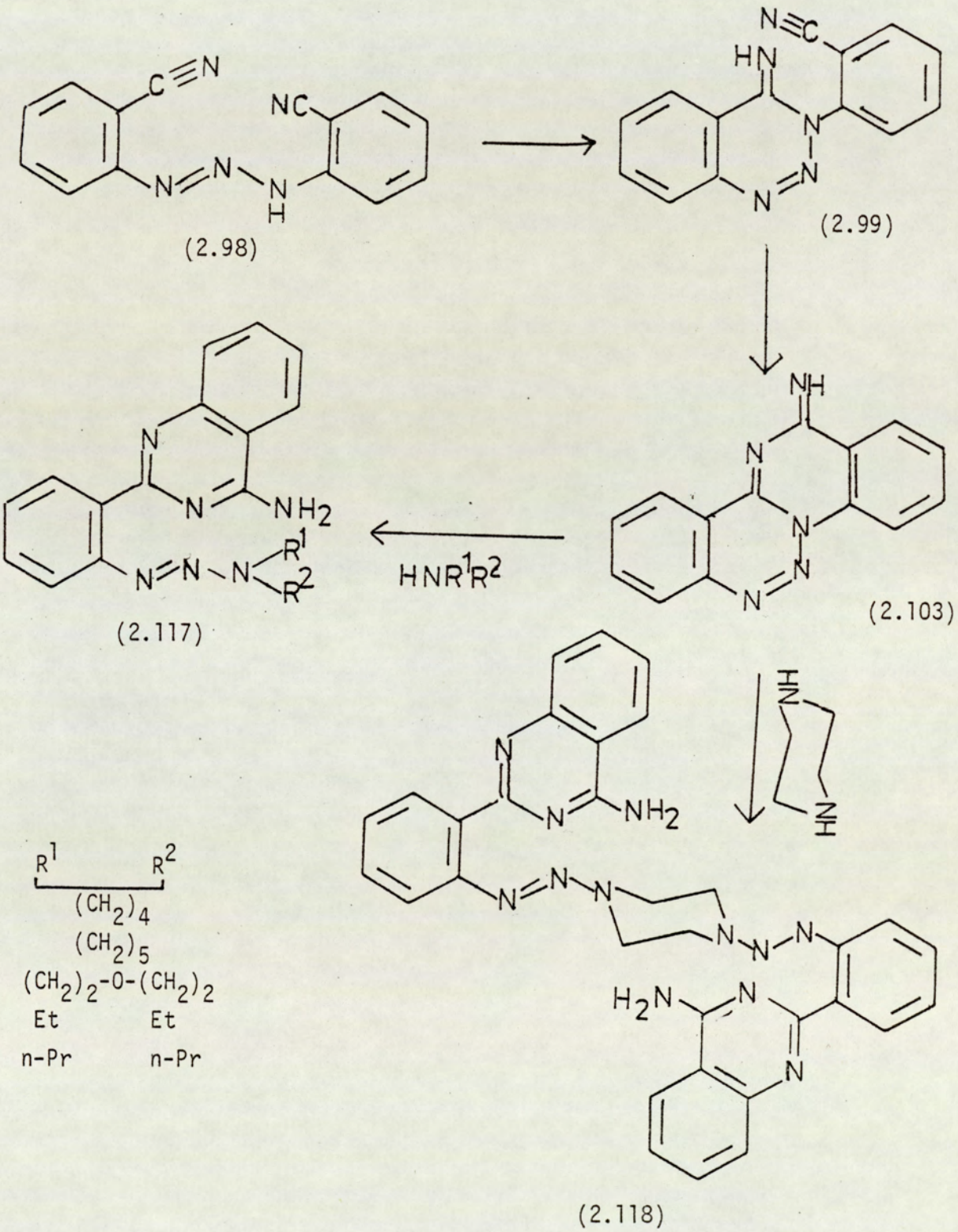
Scheme (2.13)

In one instance, a deep yellow high-melting solid was isolated from the reation between 4-(4-cyanoanilino)-1,2,3-benzotriazine (2.23) and morpholine. The structure of this substantial by-product will be discussed in Chapter 3 in the Discussion section of this thesis.

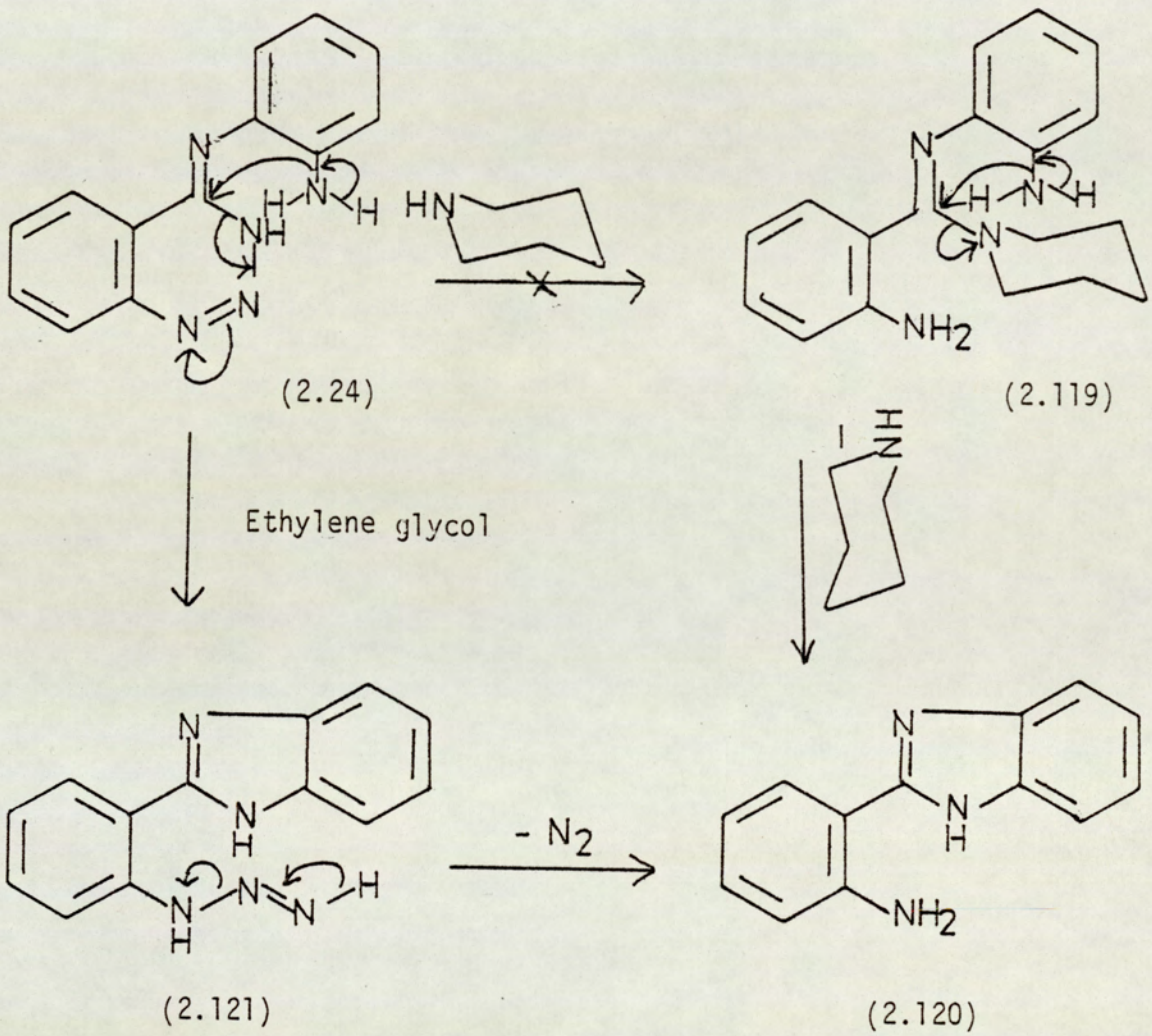
All the amidines (2.112a-k) have a diazotisable amino group. One such amidine (2.112d) was converted into an azonaphthol derivative (2.116)[45].

The reaction of 1,3-bis-2-cyanophenyltriazene (2.98) with secondary amines is yet more complicated since both cyano groups participate in the reaction (see page 30). This triazene, in boiling piperidine, pyrrolidine, morpholine, diethylamine or di-n-propylamine, with or without benzene as co-solvent, afforded triazenylquinazolines (2.117, Scheme 2.14) in excellent yields. When the triazene (2.98) was heated in piperazine the bis-triazenylquinazoline (2.118) was isolated[45]. In these cases the 4-(2-cyanoanilino)-1,2,3-benzotriazine (2.103) formed in the reaction sequence was not diverted to amidine (2.112; R=2-NCC_6H_4), but instead further cyclised to tetracyclic compound (2.103) which only then reacted with the corresponding amines[45].

Another anomalous result was reported[45]. When 2-aminoanilinobenzotriazine (2.24) was heated in secondary amines, the benzimidazole (2.120) was formed. The same compound (2.120) was obtained directly by heating benzotriazine (2.24) in ethylene glycol (Scheme 2.15). Therefore, it was suggested[45] that the amidine (2.119) was not an intermediate in this case, as the aminophenyl substituent is favourably aligned for intramolecular attack at C-4 leading to the benzimidazole <u>via</u> an unstable triazene (2.121).


(2.98)
(2.99)
(2.103)
(2.117)
(2.118)
HNR1R2
R1 R2
(CH2)4
(CH2)5
(CH2)2-O-(CH2)2
Et Et
n-Pr n-Pr

Scheme (2.14)



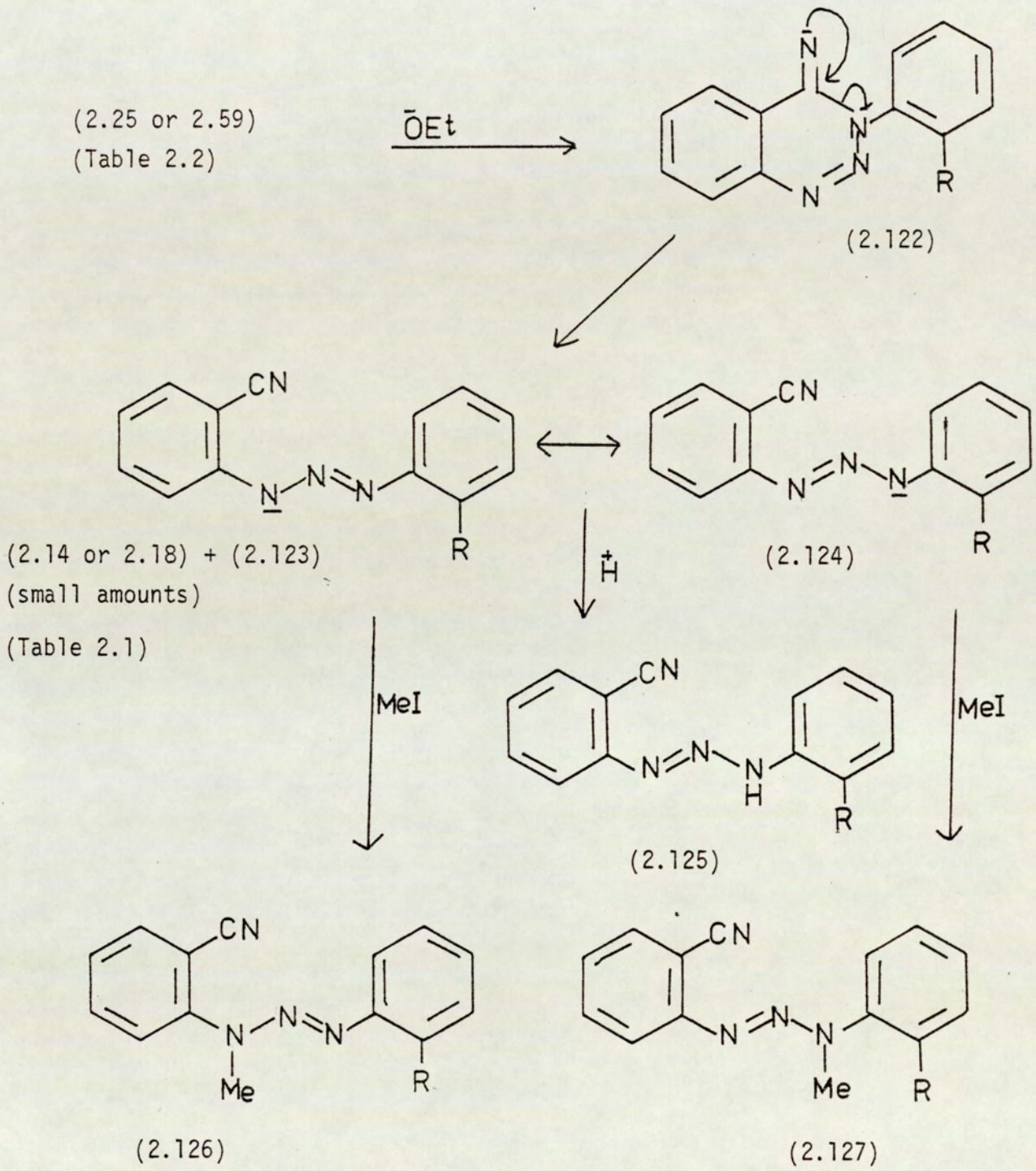
Scheme (2.15)

2.3.4 Decomposition of 4-amino or 4(3H)-imino-1,2,3-benzotriazines in sodium ethoxide

3,4-Dihydro-3-aryl-4-imino-1,2,3-benzotriazines (2.52 or 2.59, Table 2.2) undergo ring-fission in sodium ethoxide[51] solution to form red solutions of triazene anions (2.123 and 2.124; R=H or Cl) from which the free triazene (2.125; R=H, Cl) can be recovered upon acidification. [Small amounts of isomeric anilinotriazines (2.14 and 2.18) were also isolated and are formed presumably by rearrangement of the 4-iminotriazines (2.52 and 2.59)]. Interaction of the resonance-stabilised amibident anions (2.123 and 2.124; R=H or Cl) with methyl iodide afforded a mixture of mono-alkyltriazenes (2.126 or 2.127; R=H or Cl)[51] (Scheme 2.16). It is well documented in the literature that the alkylation of unsymmetrically substituted diaryltriazenes are known to give a mixture of 1 or 3-monoalkyl-triazenes[52]. The same triazene (2.127, R=H) was unambiguously prepared by interaction of diazotised anthranilonitrile and N-methyl-aniline. Attempted coupling reaction of diazotised aniline and N-methylanthranilonitrile to obtain the methyl triazene (2.126; R=H) were unsuccessful[51].

2.3.5 Alkylation of 4-amino-1,2,3-benzotriazines

4-Anilino-1,2,3-benzotriazines (2.1; R=aryl) upon alkylation with alkyl iodides in ethanol yielded the corresponding benzotriazinium iodides (2.77)[12] which upon basification afforded the deep red anhydro benzotriazinium betaines (2.128). In contrast, alkylation of 4-anilino-1,2,3-benzotriazines (2.1; R=aryl) with alkyl iodides in sodium ethoxide yielded upon basification a mixture of red

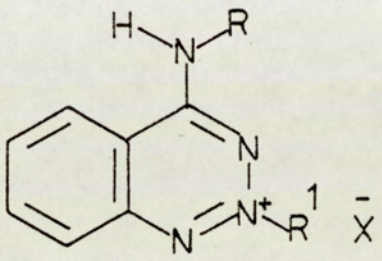


R = H, Cl

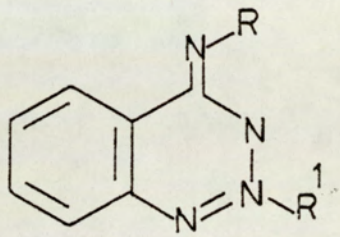
Scheme (2.16)

Table 2.6

2-Alkyl-4-imino-1,2,3-benzotriazinium salts and their anhydro bases



(2.77)



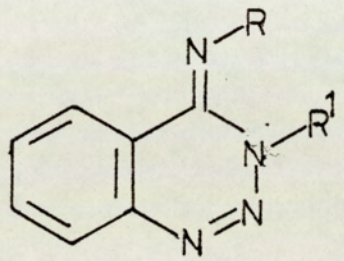
(2.128)

Compound	R	R^1	X	mp (°C)	Refs
1.128	Ph	Me		130-131	12
2.77	Ph	Me	I	219-220 (dec)	12, 53
2.77	4-MeC_6H_4	Me	I	a	53
2.128	2-ClC_6H_4	Me		157-158	12
2.77	2-ClC_6H_4	Me	I	196-198	12
2.128	2-$O_2NC_6H_4$	Me		199-200	12
2.77	2-$O_2NC_6H_4$	Me	Cl	202-203	12
2.128	2-NCC_6H_4	Me		180-181	12
2.128	4-NCC_6H_4	Me		202-203	12
2.77	4-NCC_6H_4	Me	I	212-214	12
2.77	$C_6H_5CH_2$	Me	I	a	12
2.77	$C_6H_5CH_2CH_2$	Me	I	a	12
2.128	Ph	Et		64-65	
2.77	Ph	Et	I	199-200	12
2.128	4-MeC_6H_4	Et		a	12
2.128	2-ClC_6H4	Et		a	12
2.128	2-ClC_6H_4	Et		197-198 (dec)	12

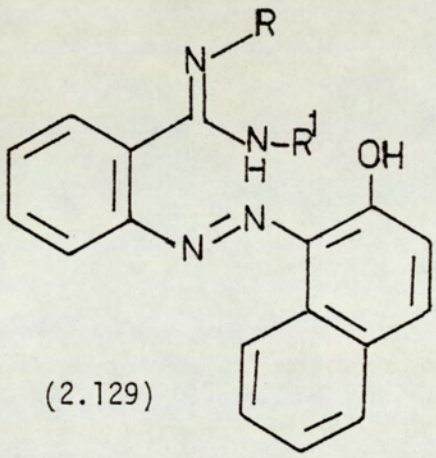
Table 2.6 (continued)

Compound	R	R^1	X	mp (°C)	Refs
2.128	2-$O_2NC_6H_4$	Et		125-126	12
2.77	2-$O_2NC_6H_4$	Et	I	184-186 (dec)	12
2.77	2-$O_2NC_6H_4$	Et	Cl.H_2O	172-173 (dec)	12
2.128	2-NCC_6H_4	Et		134-135	12
2.77	$C_6H_5CH_2$	Et	I	a	53
2.77	$C_6H_5CH_2CH_2$	Et	I	a	53
2.77	Ph	n-Pr	I	a	53
2.77	4-MeC_6H_4	n-Pr	I	a	53
2.128	2-$O_2NC_6H_4$	n-Pr		112-113	12
2.77	$C_6H_5CH_2$	n-Pr	I	a	53
2.77	$C_6H_5CH_2CH_2$	n-Pr	I	a	53
2.77	4-MeC_6H_4	i-Pr	I	a	53
2.77	Ph	i-Pr	I	a	53
2.128	2-$O_2NC_6H_4$	i-Pr		86-87	12
2.77	$C_6H_5CH_2$	i-Pr	I	a	53
2.77	$C_6H_5CH_2CH_2$	i-Pr	I	a	53
2.77	Ph	n-Bu	I	a	53
2.77	4-MeC_6H_4	n-Bu	I	a	53
2.77	$C_6H_5CH_2$	n-Bu	I	a	53
2.77	$C_6H_5CH_2CH_2$	n-Bu	I	a	53
2.77	Ph	n-Pen	I	a	53
2.77	4-MeC_6H_4	n-Pen	I	a	53
2.77	$C_6H_5CH_2$	n-Pen	I	a	53
2.77	$C_6H_5CH_2CH_2$	n-Pen	I	a	53

a = melting points were not recorded



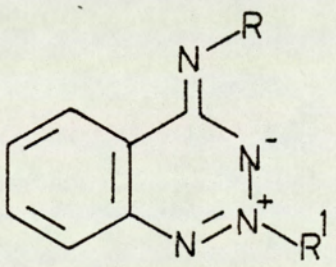
(2.48 or 2.53)



(2.129)

R = Ph

R^1 = Ph or i-Pr



(2.128)

Scheme (2.17)

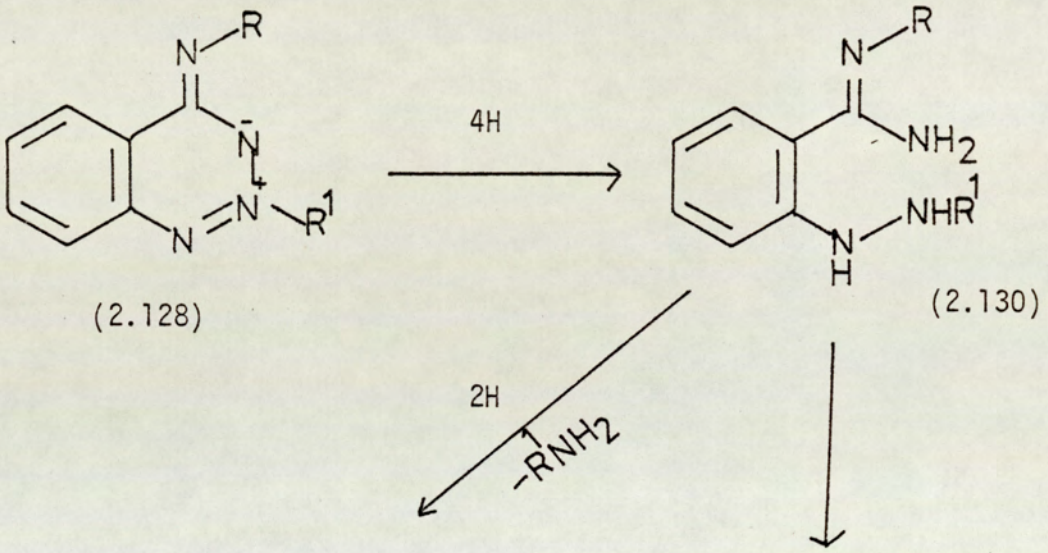
benzotriazinium betaines (2.128) and yellow isomeric N-alkylbenzotriazines (2.5; R=aryl, R^1=alkyl) (previously recorded as 3,4-dihydro-3-phenyl-4-alkylimino-1,2,3-benzotriazines[9]).

The 3,4-disubstituted benzotriazines (2.5) can be differentiated from the benzotriazinium betaines (2.128). The latter compounds (2.128) are very stable to ring fission whereas the former compounds (2.5) undergo irreversible cleavage of the triazine ring. For example, the triazines (2.48 or 2.53) when heated with 2-naphthol in acetic acid or butanol yielded the azo-naphthol derivatives (2.129; R=i.Pr or Ph)[12].

The red 2-alkyl-4(3H)-imino-1,2,3-benzotriazinium salts (2.77) and their anhydro bases (2.128) are listed in Table 2.6.

Stevens and Stevens[12] located the site of alkylation in alkyl-4(3H)-imino-1,2,3-benzotriazinium betaines (2.128) using a reductive technique.

For example, anilinobenzotriazinium betaine (2.128; R=Ph, R^1=Me) when heated in ethanol at 60-65 °C with hydrazine-Raney nickel yielded 2-aminophenylbenzamidine (2.131; R=Ph) and 3-anilino-2-methylindazole (2.132; R^1=Me, R=Ph). These two products are (2.131 and 2.132) probably derived from the same intermediate methyl-hydrazinobenzamide (2.130; R^1=Me, R=Ph) formed by the hydrogenolysis of the N(2)-N(3) bond of the triazinium betaines (2.128; R=Ph, R^1=Me). This intermediate hydrazine (2.130; R^1=Me, R=Ph) evidently decomposes by two routes; either by further hydrogenolysis to the 2-aminophenylbenzamidine (2.131; R=Ph) with the loss of alkylamine or by cyclo-deamination to form the indazole (2.132; R^1=Me, R=Ph).



(2.128) (2.130) (2.131) (2.132)

Scheme (2.18)

The benzotriazinium betaine (2.128; R^1=Me or Et, R=2-NCC_6H_4) upon hydrazine-Raney nickel reduction yielded 4-amino(2-aminophenyl)-quinazoline (2.105) via the amine-nitrile addition of the amidine intermediate (2.131; R=2-NCC_6H_4) under the basic conditions[54,55].

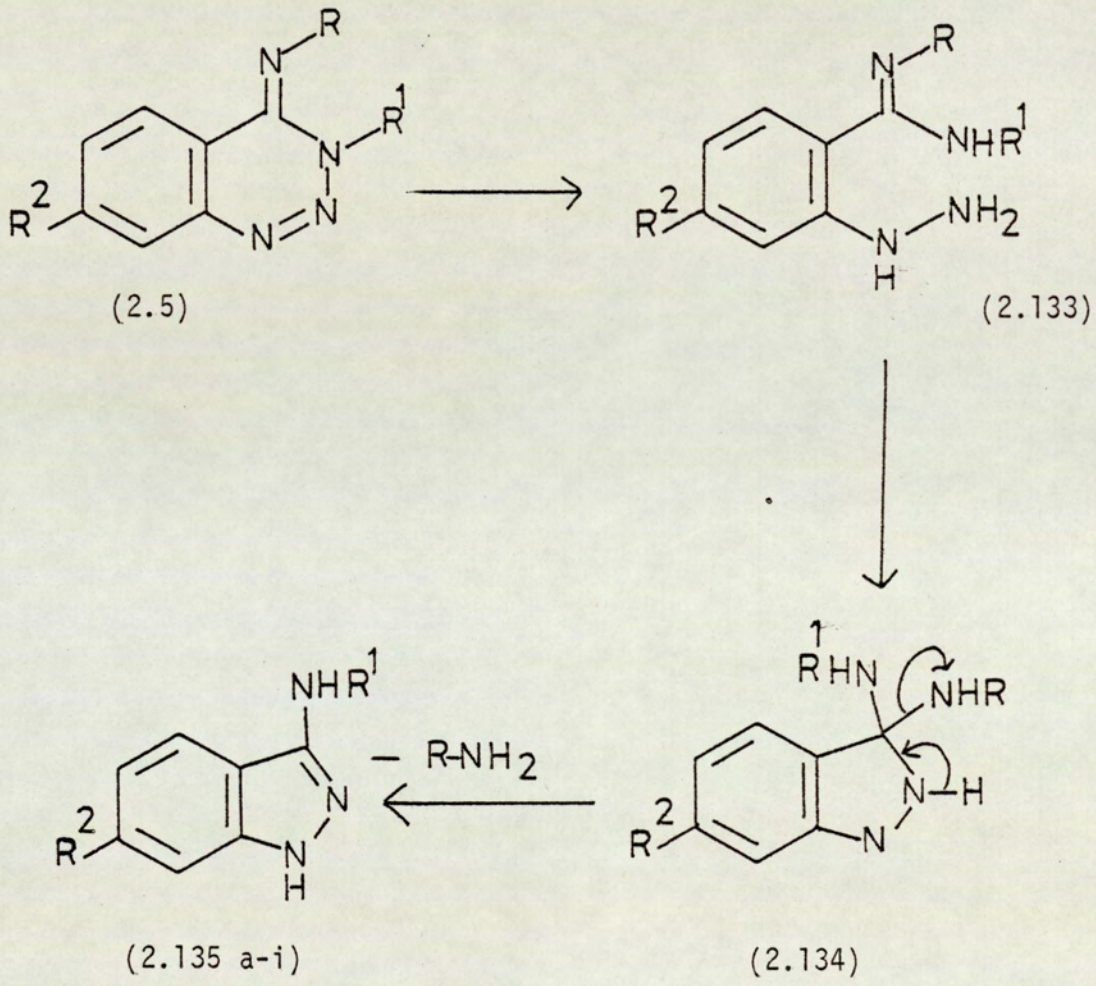
2.3.6 Reduction of 4-amino or 4(3H)-imino-1,2,3-benzotriazines

The well established diazonium character[34] of 1,2,3-benzotriazines implies a sensitivity towards reducing agents. Two types of reducing agents have been employed for the reductive degradations of this ring system.

(i) Stannous chloride

The reduction of 4-imino-1,2,3-benzotriazines (2.5) with stannous chloride and ethanol by heat yielded various 3-aminoindazoles (2.135 a-i)[9]. The reduction takes place by fission of the N(2)-N(3) bond of triazine ring to give 2-hydrazinobenzamidines (2.133 a-i) (Scheme 2.19), which then cyclise to 3-aminoindazoles (2.135 a-i) with the elimination of the weaker base, ie the aromatic amine[9].

Various analogues of 3-substituted aminoindazoles (2.135 a-i) made by Partridge and Stevens[9] from the corresponding iminotriazines are listed in Table 2.7. The indazole (2.135a) was obtained by the reduction of both isomeric 3-phenylimino (2.52) or 4-anilino-1,2,3-benzotriazine (2.14).


R
N
R1
N
N
N
R2
(2.5)
NHR1
NH2
N
H
(2.133)
R1HN
NHR
N
H
N
R2
(2.134)
NHR1
N
N
H
R2
(2.135 a-i)
– R-NH2

Scheme (2.19)

Table 2.7

Indazoles prepared from iminotriazines

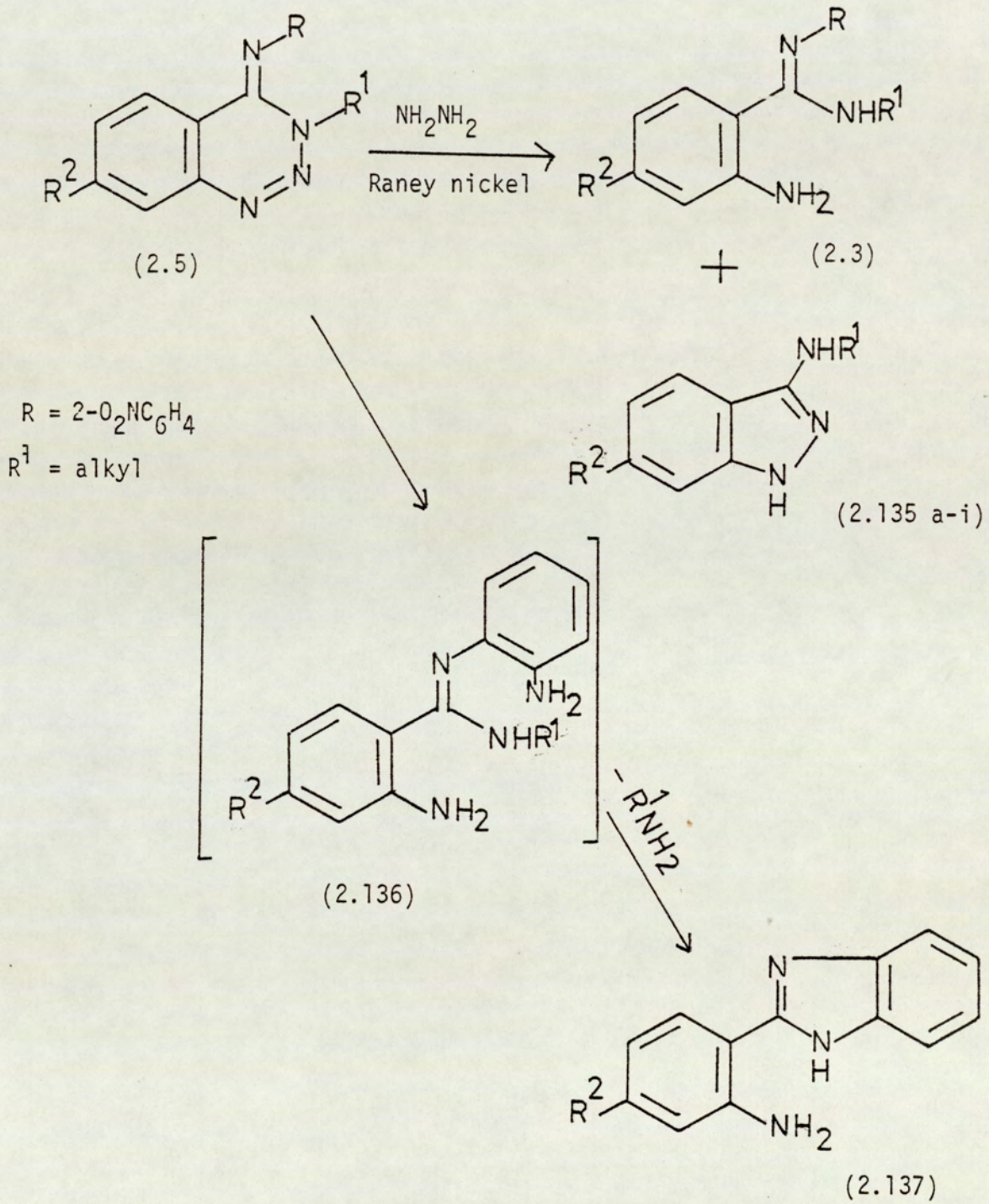
Iminotriazines*	Indazoles (2.135)	R^1	R^2	mp (°C)	Refs
2.52 or (2.14)	a	H	H	154-155	9
2.42	b	Me	H	147-148	9
2.45	c	Et	H	95-96	9
2.48	d	i-Pr	H	87-88	9
2.49	e	cyclo-Hex	H	123-124	9
2.50	f	CH_2Ph	H	123-124	9
2.53	g	Ph	H	170-171	9
2.57	h	4-MeC_6H_4	Me	161-162	9
2.62	i	4-$MeOC_6H_4$	H	169-170	9

*see Table 2.2

(ii) Hydrazine-Raney nickel

The reducing agent hydrazine-Raney nickel has been successfully applied in degradative studies of this ring system[9,12,35,56] notably as a means of locating the site of alkylation on the triazine ring[12].

Reduction of 4(3H)-imino-1,2,3-benzotriazines (2.5) with stannous chloride in ethanol yielded only 3-substituted aminoindazoles (2.135 a-i)[9] (see above). Reduction of the same triazines (2.5) with hydrazine-Raney nickel furnished a mixture of aminoindazoles (2.135) and 2-aminobenzamidines


R = 2-O2NC6H4
R1 = alkyl
NH2NH2
Raney nickel
(2.5)
(2.3)
(2.135 a-i)
(2.136)
- R1NH2
(2.137)

Scheme (2.20)

(2.3) as the major products.

The 4-aryl and 4-aralkylamino-1,2,3-benzotriazines (2.1; R=aryl or aralkyl) are reported to be more resistant to hydrazine-Raney nickel reduction than their isomeric 4-imino forms[51]. However, aminotriazine (2.12) like its 4-imino-3-benzyl isomer (2.50) afforded the aminobenzamidine (2.3; $R=R^2=H$, $R^1=CH_2Ph$) with hydrazine Raney nickel, but the phenethyl analogue (2.51) was recovered unchanged even after prolonged treatment. This triazine (2.51) also showed resistance towards other reducing agents such as lithium aluminium hydride[56].

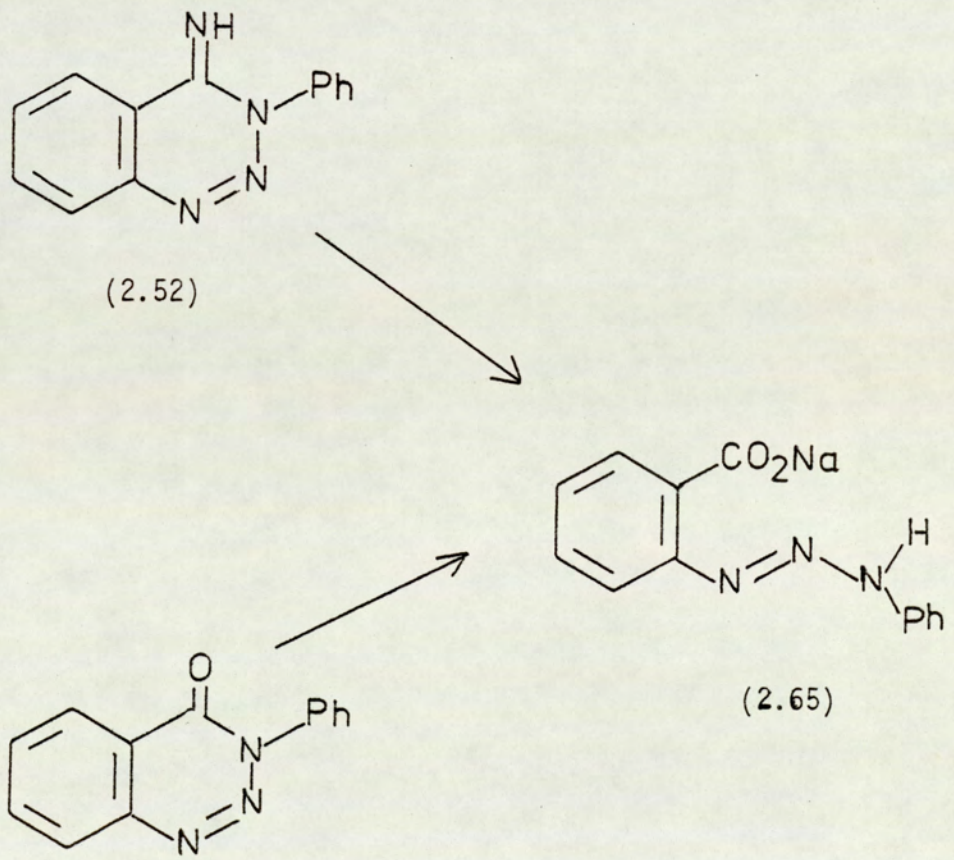
4-Anilino-1,2,3-benzotriazine (2.14) yielded small amounts of 2-aminobenzamidine (2.3; $R^2=H$, $R^1=Ph$) together with unchanged starting material (30%) from the hydrazine reduction. No fission of the triazine ring occurred in the reduction of 4-(2-chloroanilino)benzotriazine (2.18) or 4-(3-nitroanilino)benzotriazine (2.20). In the latter case the only product was the corresponding 4-(3-aminoanilino)-benzotriazine (2.25)[56].

The hydrazine-Raney nickel reductions of 3,4-disubstituted benzotriazines are summarised in Scheme 2.20. These triazines (2.5) afforded a mixture of 2-amino-benzamidines (2.3) and 3-anilinoindazoles (2.135)[56].

3-methyl-4-(2-nitroanilino)-1,2,3-benzotriazine (2.44) was an exception and gave 2-(2-aminophenyl)benzimidazole (2.137; $R^2=H$)[9] alone *via* the diaminobenzamidine (2.136; $R^1=Me$, $R^2=H$), formed from the reduction of the nitro group and the ring fission of the triazine.

2.3.7 Decomposition of 4(3H)-imino-1,2,3-benzotriazines in aqueous alkali

Although there are many examples of alkaline degradations of 1,2,3-benzotriazin-4(3H)-ones (1.3) in the literature[16,57-61] there is only one example of such decomposition reported in 4(3H)-imino-1,2,3-benzotriazine series[9]. Partridge and Stevens[9] isolated the triazene carboxylate (2.65) from the reaction of 3-phenyl-4-imino-1,2,3-benzotriazine (2.52) in aqueous sodium hydroxide. The identical triazene carboxylate (2.65) was also obtained from the alkaline hydrolysis of 3-phenyl-1,2,3-benzotriazin-4(3H)-one (2.160).



(2.52) (2.65) (2.160)

Scheme (2.21)

PART II

DISCUSSION OF THE EXPERIMENTAL RESULTS

Part II

Discussion of Experimental Results

Chapter 3

This chapter describes the synthesis and the thermolytic degradations of 4-anilino-1,2,3-benotriazines.

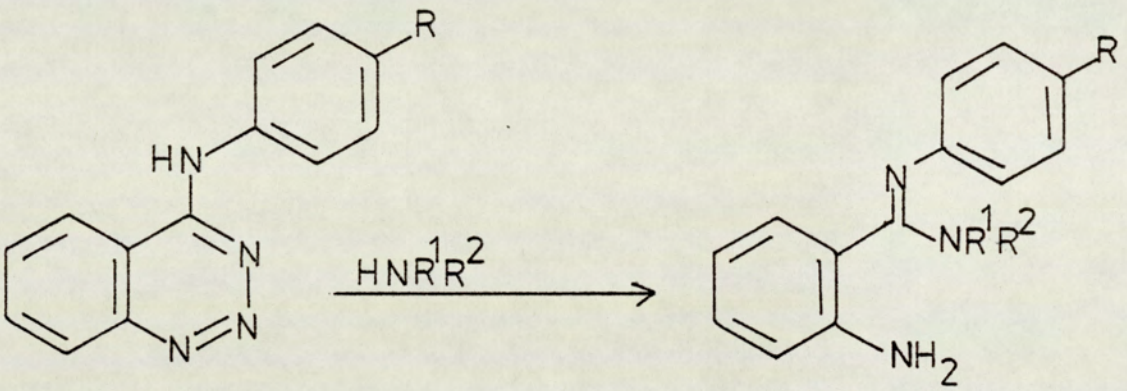
3.1 Synthesis of 4-anilino-1,2,3-benzotriazines

The 4-anilino-1,2,3-benzotriazines (3.1; R=CN, NO_2) were prepared by the method described in the literature[7,8] (see Type ii syntheses page 11).

3.2 Thermolytic decomposition of 4-anilino-1,2,3-benzotriazines

Interaction of a series of substituted 4-anilino-1,2,3-benzotriazines and boiling heteroalicyclic secondary amines such as pyrrolidine, piperidine and morpholine afforded high yields of 2-amino-N^2-aryl-N^1N^1-disubstituted benzamidines (3.2 a-d)[45]. In one instance, from the reaction of 4-(4-cyanoanilino)-1,2,3-benzotriazine (3.1; R=CN)[7,8] and morpholine, a deep yellow high-melting solid was obtained in addition to the expected amidine (3.2c)[45]. This chapter is concerned with the determination of the structure of this by-product and the mechanism of its formation.

When 4-(4-cyanoanilino)-1,2,3-benzotriazine (3.1; R=CN)[7,8] was boiled in pyrrolidine or piperidine, no coloured by-products were isolated: the only products were the amidines (3.2a or 3.2b) in 70



(3.1) (3.2 a-d)

	R	R^1 — R^2
a	CN	$-[CH_2]_4-$
b	CN	$-[CH_2]_5-$
c	CN	$-[CH_2]_2-O-[CH_2]_2-$
d	NO_2	$-[CH_2]_2-O-[CH_2]_2-$

Scheme (3.1)

and 88% yield respectively. The amidine (3.2c) was not a precursor of the deep yellow product since this amidine was recovered unchanged after prolonged boiling in morpholine.

The ir spectrum of the by-product showed NH and C≡N absorptions and when heated in 2N-hydrochloric acid with 2-naphthol, a deep red azo-dye was formed. This indicated that the by-product had an intact NNN linkage[62]. The yellow product melted at 246-248 °C with effervescence again indicating the presence of NNN linkage.

The elemental analysis of the yellow solid confirmed a molecular formula of $C_{28}H_{18}N_8$, corresponding to two molecules of 4-cyano-anilinobenzotriazine (3.1; R=CN) minus one molecule of nitrogen. The electron-impact-promoted mass spectrum showed a highest mass ion at m/z 438 ($C_{28}H_{18}N_6$) corresponding to ($M^{+\cdot}-N_2$).

Taking all the evidence into account, structure (3.8a) is proposed for the yellow product obtained from (3.1; R=CN) and morpholine. Similarly, when 4-(4-nitroanilino)-1,2,3-benzotriazine (3.1; R=NO_2) was boiled in morpholine a mixture of amidine (3.2d) and an orange high melting solid was obtained. The orange solid showed an intact NNN linkage and produced a red colour when heated with 2-naphthol in glacial acetic acid or 2N-hydrochloric acid. On the basis of mass spectral evidence and elemental analysis, the orange compound obtained from 4-nitroanilinobenzotriazine (3.1; R=NO_2) was assigned the structure (3.8b).

These two thermolysis products (3.8a,b) were insoluble in all conventional deuterated nmr solvents and trifluoracetic acid.

It was rather surprising that products of type (3.8a,b) were formed in the reactions of 4-anilinobenzotriazines (3.1; R=CN, NO_2) in morpholine since this nucleophilic reactant is present in large excess. Only those substrates bearing -M substituents in the anilino

moiety yield unusual products[45]; this structural feature evidently increases the electron deficiency at C-4, allowing the weakly nucleophilic anilinobenzotriazine to compete effectively with the morpholine.

It was noticed that, in the absence of nucleophilic solvent, the yield of thermolysis product (3.8a) from (3.1; R=CN) was markedly increased. For example, when compound (3.1; R=CN) was refluxed in diethyleneglycol dimethyl ether, it dissolved with effervescence and the deep yellow solid deposited from the hot solution in remarkably high yield (70%) (Table 3.1). Thermolysis of compound (3.1; R=CN) was also carried out in a range of high boiling solvents. The reaction time and % yield is tabulated in Table 3.1.

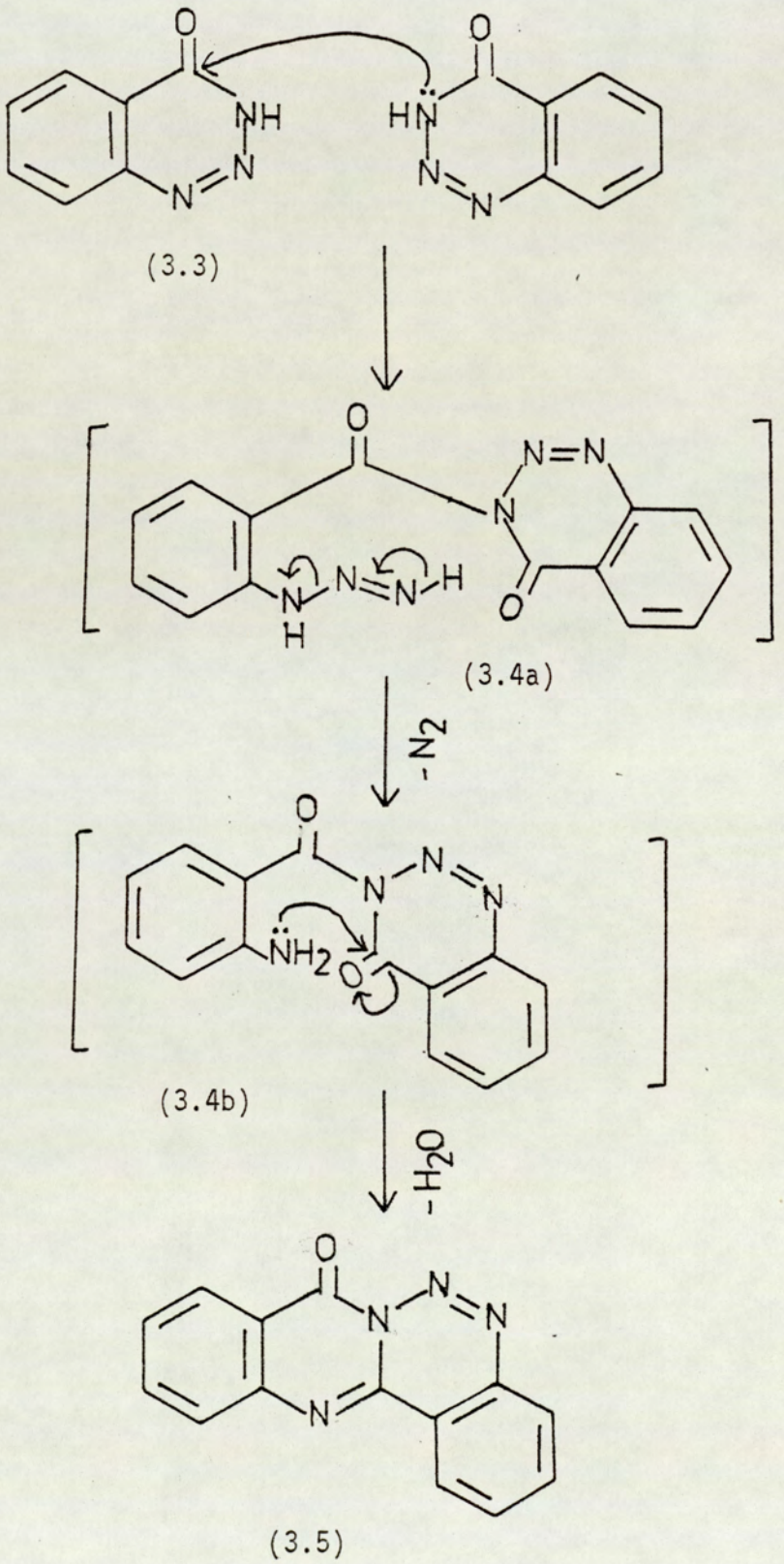
The mechanism of this decomposition is probably analogous to that proposed to account for the thermal conversions of 1,2,3-benzotriazin-4(3H)-one (3.3) to the tetracyclic triazine (3.5) via the anthraniloylbenzotriazinone (3.4)[63,64]. Thus attack by a molecule of anilinobenzotriazine (3.1; R=CN, NO_2) at C-4 of the arylimino tautomer (3.6; R=CN, NO_2) of another molecule leads to ring-opening and the formation of an unstable 3-aryltriazene (3.7a,b) which loses a nitrogen molecule to yield the observed products (3.8a,b) (Scheme 3.3).

Because degradation of anilinobenzotriazines (3.1; R=CN, NO_2) takes place substantially below their thermal decomposition points (229 and 237 °C respectively)[7] it is unlikely that the initial event is heterolysis of the NNN linkage leading to a benzazetimine (3.9a-b) or its valence tautomer (3.10a-b), although this possibility cannot be excluded: these labile species would react with amines to form amidines (3.2a-d) and with undecomposed substrate to afford by-products (3.8a-b).

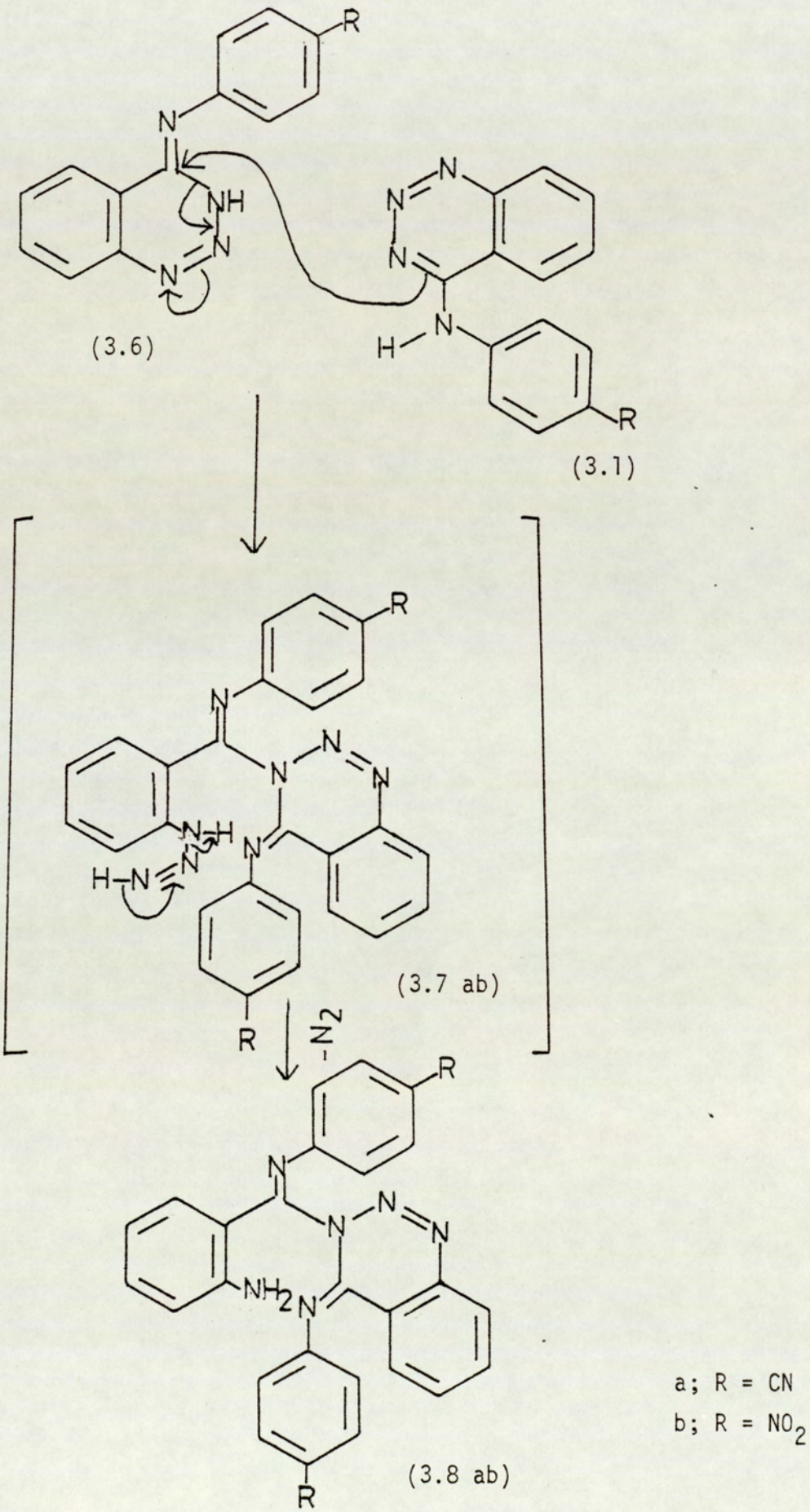
Table 3.1

Products formed from 4-(4-cyanoanilino)-1,2,3-benzotriazine (3.1; R=CN) in high boiling solvents

Solvents	Bp °C	Reaction Time (hrs)	Product	Yield %
Pyrrolidine	86-87	8	3.2a	70
Piperidine	104-107	7	3.2b	80
Morpholine	124-128	7	3.2c	80
			3.9a	17
N-Methylpiperazine	137-140	8	3.9a	30
2,6-Dimethyl-morpholine	147	8	3.9a	50
Diethylene glycol dimethyl ether	159-162	2	3.9a	70
2,4,6-Collidine	170-175	1.5	3.9a	70
Aniline	184	0.5	3.9a	60



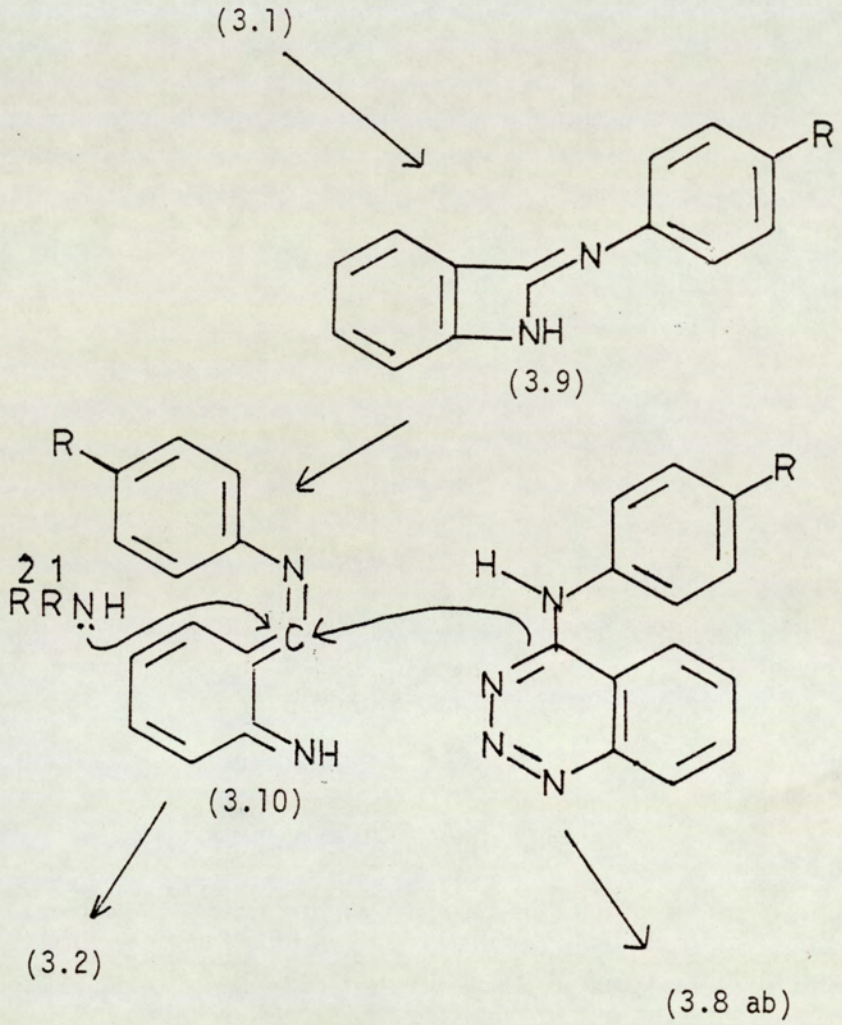
Scheme (3.2)



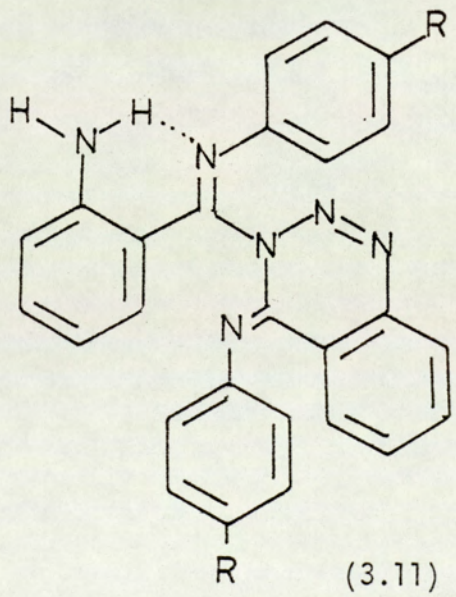
a; R = CN

b; R = NO_2

Scheme (3.3)



Scheme (3.4)



(3.11)

a; R = CN

b; R = NO_2

It is also surprising that compounds (3.8a,b) do not undergo further cyclisation to tetracycles with the liberation of 4-cyano- or 4-nitroaniline respectively under the thermolytic conditions. Possibly, the 1,2,3-benzotriazines (3.8a,b) exist as H-bonded rotamers (3.11a,b) because of the steric crowding imposed by the two arylimino groups and this restrains the free amino group from orthogonal attack at C-4 of the triazine ring. Even more surprisingly, compound (3.3a) was recovered unchanged after being boiled in 2M aqueous sodium hydroxide for 6 hours.

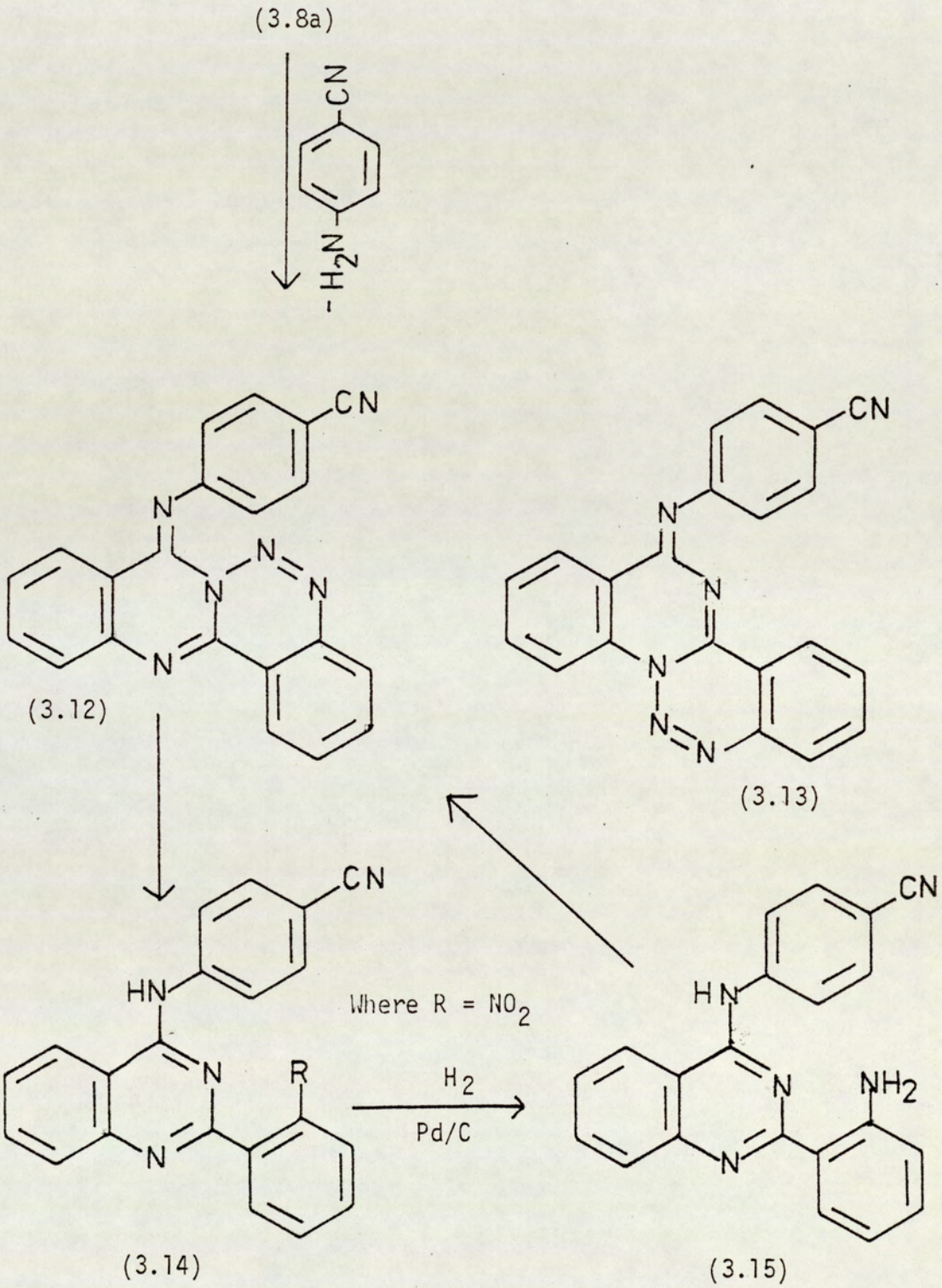
Cyclisation of 3-[2-amino-N-(4-cyanophenyl)benzimidoyl]-4-(4-cyanophenylimino)-3,4-dihydro-1,2,3-benzotriazine (3.8a) was achieved eventually under acidic conditions.

3.3 Decomposition of 3-[2-amino-N-(4-cyanophenyl)benzimidoyl]-4-(4-cyanophenylimino)-3,4-dihydro-1,2,3-benzotriazine in hot acetic acid

The yellow triazine (3.8a) dissolved slowly in hot acetic acid. After refluxing for six hours, it decomposed to yield a yellowish brown solid. 4-Cyanoaniline was detected in the reaction mixture by tlc (silica gel: acetic acid - EtOH 1:4). The solid, when heated in glacial acetic acid with 2-naphthol, produced no red colouration. This indicated that the compound no longer contained an NNN linkage.

The ir spectrum of the brown solid showed NH and C≡N absorptions. The electron-impact-promoted mass spectrum showed a molecular ion at m/z 322 and the major ion at 118 corresponded to 4-cyanoaniline.

On the basis of the above evidence the structure (3.14; R=H) was tentatively assigned for the brown solid. This structure assignment


(3.8a)
CN
-H2N
CN
N
N
N
N
N
(3.12)
CN
N
N
N
N
N
(3.13)
CN
HN
N
R
N
Where R = NO2
H2
Pd/C
(3.14)
CN
HN
N
NH2
N
(3.15)

Scheme (3.5)

was supported by elemental analysis and confirmed by its identity with 4-(4-cyanoanilino)-2-phenylquinazoline (3.14; R=H) synthesised by an unambiguous route (see the following section).

The same quinazoline (3.14; R=H) was also obtained by prolonged boiling of the triazine (3.8a) for 48 hours in acetic acid ethanol (4:1). Analogously when the compound (3.8a) was heated in 2N-sulphuric acid the product was 4-(4-cyanoanilino)-2-(2-hydroxyphenyl)quinazoline (3.14; R=OH).

Decomposition of (3.8a) in boiling acetic acid containing either sodium iodide or aqueous hydriodic acid gave the 2-(2-iodophenyl)-quinazoline (3.14; R=I) in high yield. When hydrobromic acid (45% solution in acetic acid) was used as solvent and reactant for this reaction, the triazine (3.8a) yielded the dihydrobromide salt of 2-(2-bromophenyl)-4-(4-cyanoanilino)quinazoline (3.14; R=Br). The free base (3.14; R=Br) was obtained upon basification of the dihydrobromide salt with aqueous dilute ammonia.

Degradation of (3.8a) in acetic acid and sodium azide yielded a crude sample of 2-(2-azidophenyl)quinazoline (3.14; $R=N_3$). The sample of 2-azidophenylquinazoline (3.14; $R=N_3$) was characterised by spectroscopy. The ir spectrum of this compound was consistent with the structural formula and showed absorptions at 2250 (C=N) and 2140 and 2160 cm^{-1} (N_3). Attempted recrystallisation of the 2-azidophenylquinazoline (3.14; $R=N_3$) from acetic acid to obtain an analytically pure sample, led to the isolation of the 2-aminophenylquinazoline (3.15).

A large scale synthesis of the 2-aminophenylquinazoline (3.15) was achieved by the catalytic hydrogenation of the 2-nitrophenylquinazoline (3.14; $R=NO_2$). The 2-aminophenylquinazoline (3.15) when diazotised in 10N-sulphuric acid with sodium nitrite at

0 °C, gave a reddish brown solid after basification with aqueous ammonia. This compound was characterised by spectroscopy. The elemental analysis suggested the molecular formula $C_{21}H_{12}N_6$ corresponding to the structure (3.12). Although an alternative cyclisation at N-1 of the quinazoline ring leading to an isomeric tetracyclic triazine (3.13) cannot be excluded, it is reported in the literature that N-3, exclusively, of the quinazoline is the site for related ring closures[73].

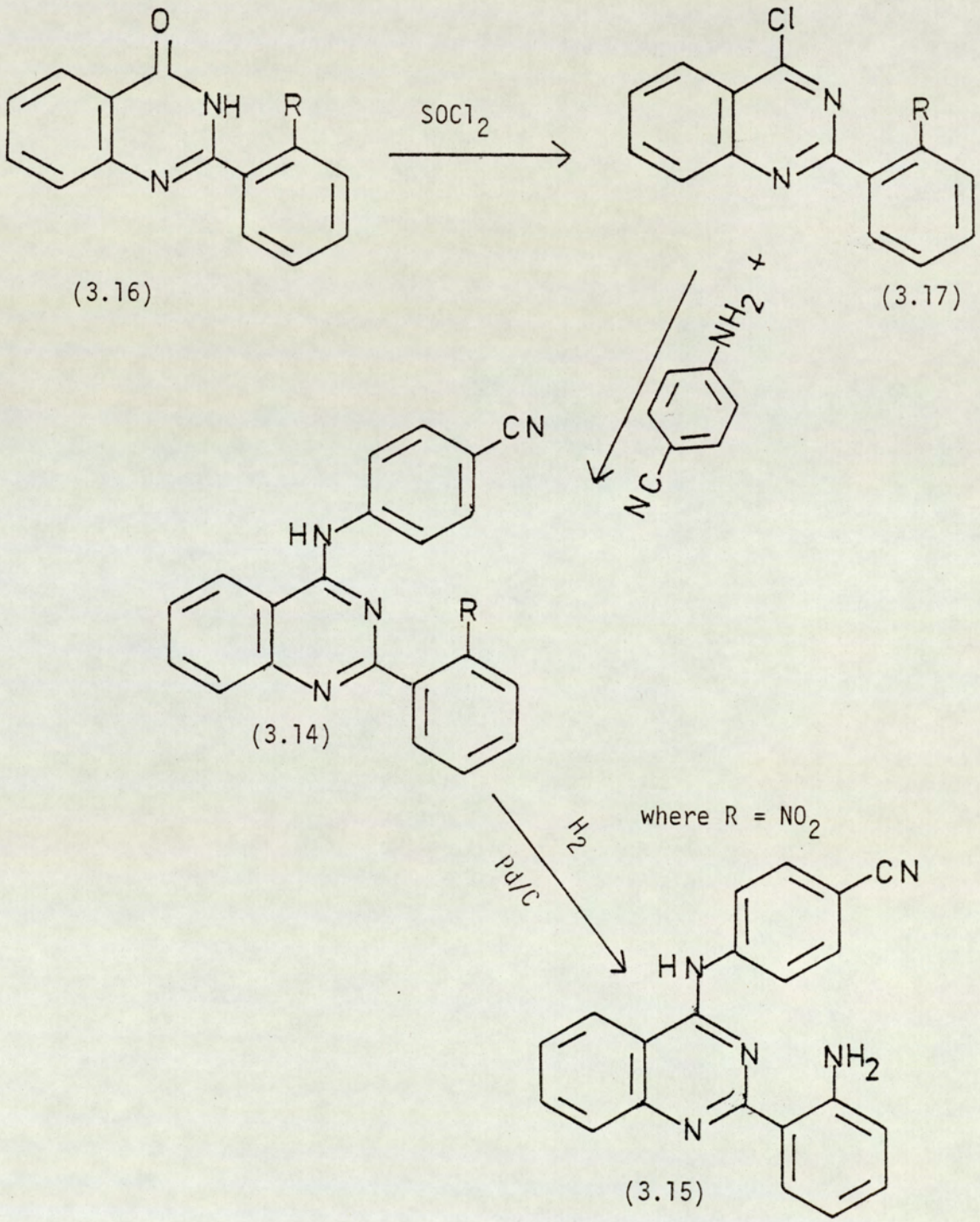
The degradations of the triazine (3.15a) to quinazolines (3.14; R=H, OH, I, Br, N_3) described above probably involve the quinazolinobenzotriazine (3.12) although the exact timing of cyclisation and diazo-displacement is not certain.

The tetracyclic intermediate (3.12) has never been isolated from any of the above degradations. However, the probable intermediacy of the tetracycle was confirmed by heating the triazine (3.8a) with 2-naphthol in acetic acid. The orange compound isolated from this reaction was identified as the azonaphthylphenylquinazoline (3.14; R=2-hydroxy-1-naphthylazo). The same azonaphthylquinazoline (3.14; R=2-hydroxy-1-naphthylazo) was prepared from the authentic sample of the tetracyclic compound (3.12) prepared from the diazotisation of 2-aminophenylquinazoline (3.15).

3.4 Synthesis of 4-(4-cyanoanilino)-2-phenylquinazolines

A series of 2-phenyl-4(3H)quinazolinones (3.16; R=H, OH, NO_2) was prepared by the methods described in the literature[65-73] (see Experimental).

The quinazolines (3.16; R=H, OH, NO_2) were converted to the 4-chloro-2-phenylquinazolines (3.17; R=H, OH, NO_2) by refluxing in


O
NH
R
N
SOCl2
Cl
N
R
N
(3.16)
(3.17)
NH2 +
NC
CN
HN
N
R
N
(3.14)
where R = NO2
H2
Pd/C
CN
HN
N
NH2
N
(3.15)

Scheme (3.6)

phosphorus oxychloride[70,71]. The chloroquinazolines (3.17; R=H, OH, NO_2) thus formed were then reacted with 4-cyanoaniline by heating under reflux in acetone containing 10M-hydrochloric acid (0.2 ml). The structures (3.14; R=H, OH, NO_2) of the products were confirmed by elemental analysis and spectroscopy. Thus the ir spectra of these yellow compounds showed a prominent absorption at 2225 cm^{-1} for the C≡N group and their uv spectra showed absorptions consistent with their chemical structures. These are recorded in the Experimental Section.

4-(4-Cyanoanilino)-2-(2-nitrophenyl)quinazoline (3.14; R=NO_2) underwent smooth catalytic hydrogenation in acetic acid over 10% palladium-charcoal catalyst at 2 atmospheres pressure to afford 2-(2-aminophenyl)-4-(4-cyanoanilino)quinazoline (3.15).

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Chapter 4

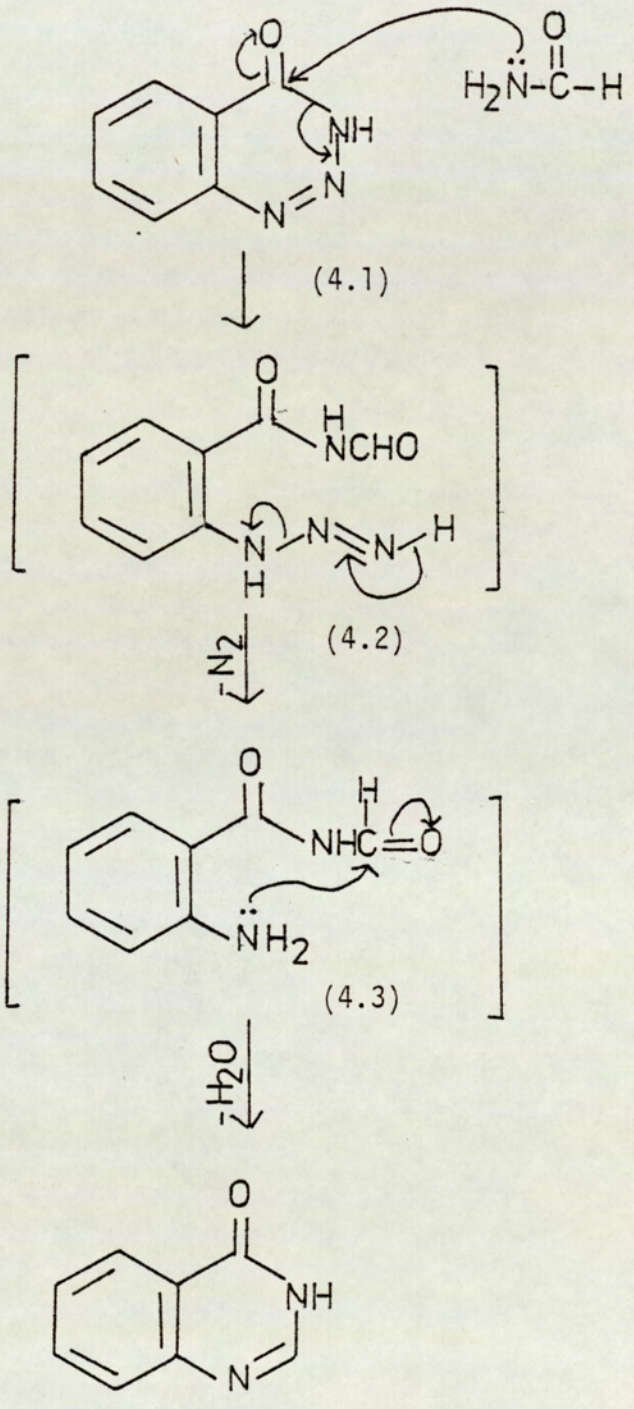
Reactions of 4-anilino-1,2,3-benzotriazines with formamides

Murray and Vaughan[63] obtained quinazolin-4(3H)-one (4.4) from the reaction of 1,2,3-benzotriazin-4(3H)-one (4.1) with formamide at 200 °C. These authors suggested that the compound (4.4) probably arises by cyclisation of the intermediate N-formylanthranilamide (4.3). Formamide in this reaction may be acting as a weak nucleophile initiating ring fission of the triazinone (4.1) to yield the acyclic triazene (4.2) (Scheme 4.1).

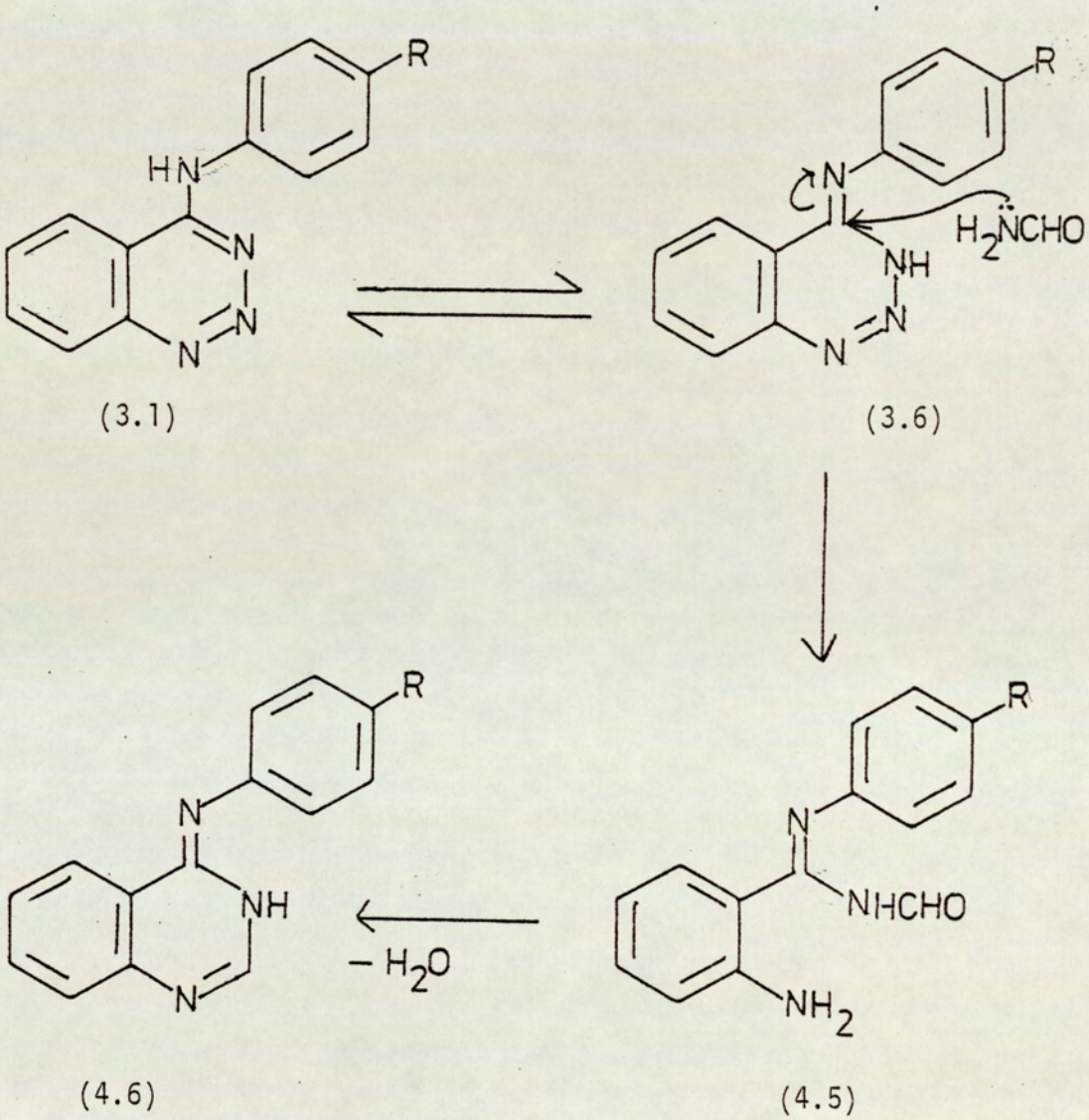
4-(4-Nitroanilino)-1,2,3-benzotriazine (3.1; R=NO_2) was dissolved in refluxing formamide. When the reaction mixture was cooled and diluted with water, a white crystalline solid was obtained. 4-Anilino-1,2,3-benzotriazines (3.1; R=H, CN) similarly yielded white solids when refluxed in formamide. These white crystalline solids were originally tentatively assigned structures (4.6; R=H, NO_2, CN) by analogy with the quinazolinone prepared by Murray and Vaughan[63].

However, the ^1H nmr spectra of these products showed one proton singlet at 9.5 ppm and appropriate aromatic resonances but showed no resonances for N-H protons. Also the electron-impact-promoted mass spectra of these compounds showed one mass unit in excess of their expected molecular ions.

On the basis of ^1H nmr mass spectral and analytical data the structures of compounds were reassigned (4.14; R=H, NO_2, CN). Two of these compounds (4.14; R=H, CN) proved to be identical to specimens prepared by reacting the corresponding anthraniloylanilines (4.10; R=H[74], CN) in warm formamide.


O
NH
N
N
$H_2\ddot{N}$-C-H
O
(4.1)
O
H
NCHO
N
N
H
N
H
(4.2)
$-N_2$
O
H
NHC
O
$\ddot{N}H_2$
(4.3)
$-H_2O$
O
NH
N
(4.4)

Scheme (4.1)



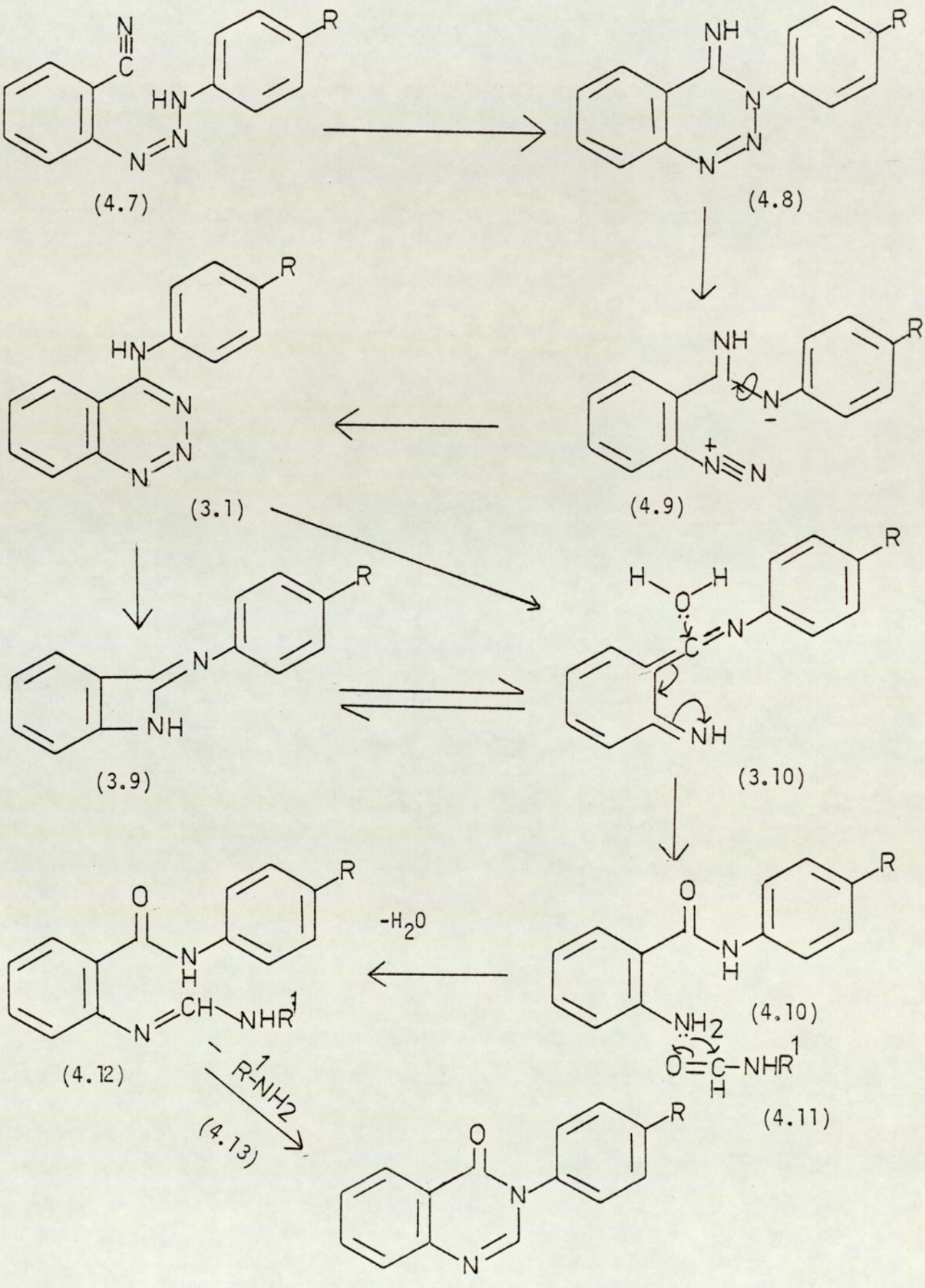
Scheme (4.2)

Subsequently, it was also found that 3-(2-cyanophenyl)-1-(4-cyanophenyl)triazene (4.7; R=CN) and the nitro analogue (4.7; R=NO_2) afforded the same quinazolinones (4.14; R=CN, NO_2) in very respectable yields when heated in formamide; whereas 3-(2-cyanophenyl)-1-phenyltriazene (4.7 R=H), when heated in formamide, decomposed to yield a black tarry intractable mixture of products.

The overall transformation (4.7)$\longrightarrow$(4.14) probably involves nine discrete steps in a highly efficient 'one-pot' process. As depicted in the Scheme (4.3), the transformation of (4.7) $\longrightarrow$ (4.14) is initiated by thermolytic degradation of 2-cyanophenyltriazene (4.7) to keteneimine (3.10). This intermediate (3.10) probably reacts with traces of water in the formamide yielding the anthranilamides (4.10) which subsequently react with formamide to generate formamidines (4.12). Finally cyclisation occurs to the corresponding quinazolinones (4.14) with loss of ammonia. The water liberated in the conversion of compounds (4.10) into (4.12) can be recycled to react with the ketenimines (3.10). Only a catalytic amount of water, therefore, is required to bring the reaction to completion.

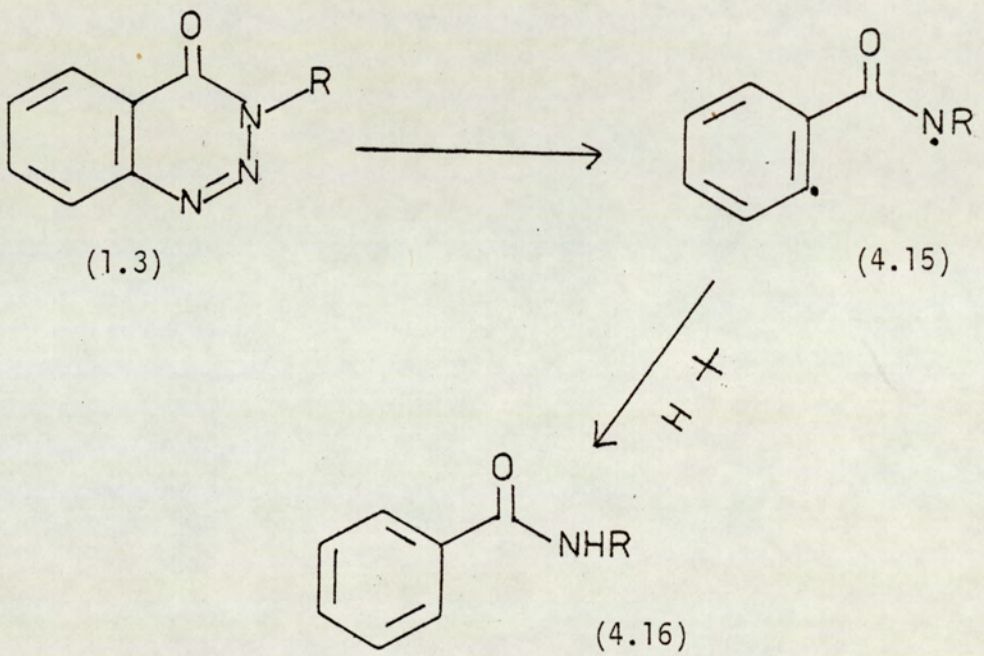
3-Aryl-1,2,3-benzotriazin-4(3H)-ones (3.1; R=Ph, NCC_6H_4, $O_2NC_6H_4$) are definitely not the intermediates in the pathway (4.7)$\longrightarrow$(4.14) as the 3-phenylbenzotriazinone (3.1; R=Ph), for example, decomposed slowly in boiling formamide to yield benzanilide (4.16; R=Ph) in 80% yield, presumably formed by hydrogen abstraction from the solvent by the intermediate biradical (4.15; R=Ph).

Anilinobenzotriazines (3.1; R=CN, NO_2) yielded the same 3-aryl-quinazolinones (4.14; R=CN, NO_2) respectively when heated in N-methylformamide. The reaction takes place via the inisolable formamidines (4.12; R=CN, NO_2, R^1=Me) with the elimination of methylamine. Efforts to extend the synthesis to simple 2-alkyl-3-aryl-



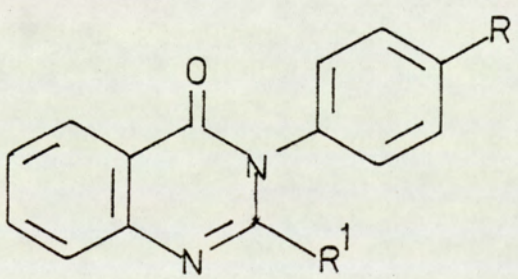
Scheme (4.3)

quinazolinones (4.17; R=CN, NO_2, R^1=Me) were not successful. Thus the 4-anilinobenzotriazines (3.1; R=CN, NO_2) in boiling N-methylacetamide instead gave the product (3.8; R=CN, NO_2) previously identified in the thermolysis reactions of the same triazines (see Chapter 3). In fact, a combination of boiling diglyme and acetamide was the most effective medium for the optimum formation (95%) of compound (3.8; R=CN, NO_2).



(1.3) (4.15) (4.16)

Scheme (4.5)



(4.17)

R = CN, NO_2

R^1 = Me

Chapter 5

5.1 Chemistry of imidazo[5,1-d]-1,2,3,5-tetrazin-4(3H)-ones

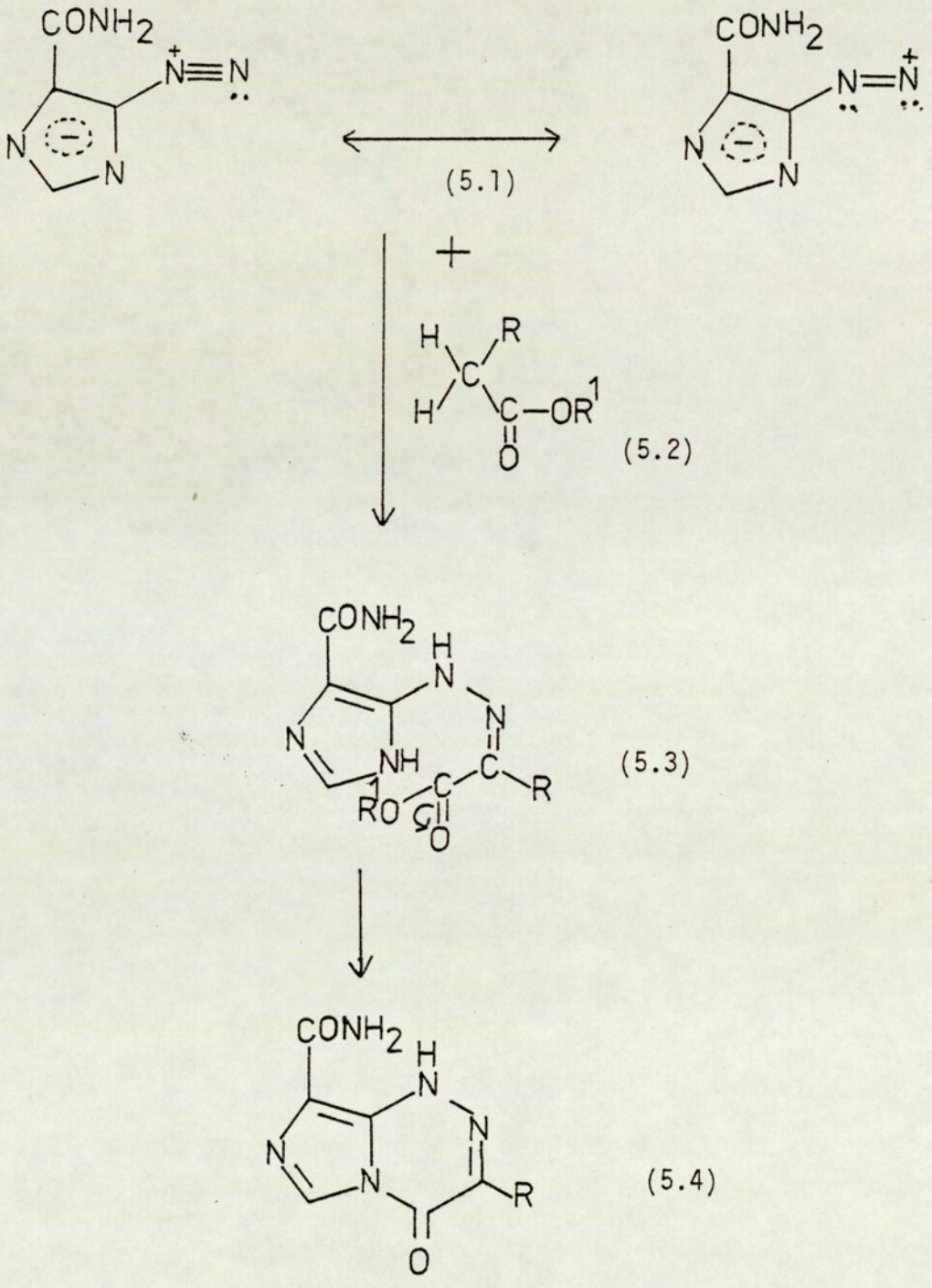
5.1.1 Introduction and Synthesis

Imidazo[5,1-d]-1,2,3,5-tetrazin-4(3H)-ones are represented by the structural formula (5.8) and numbered as indicated.

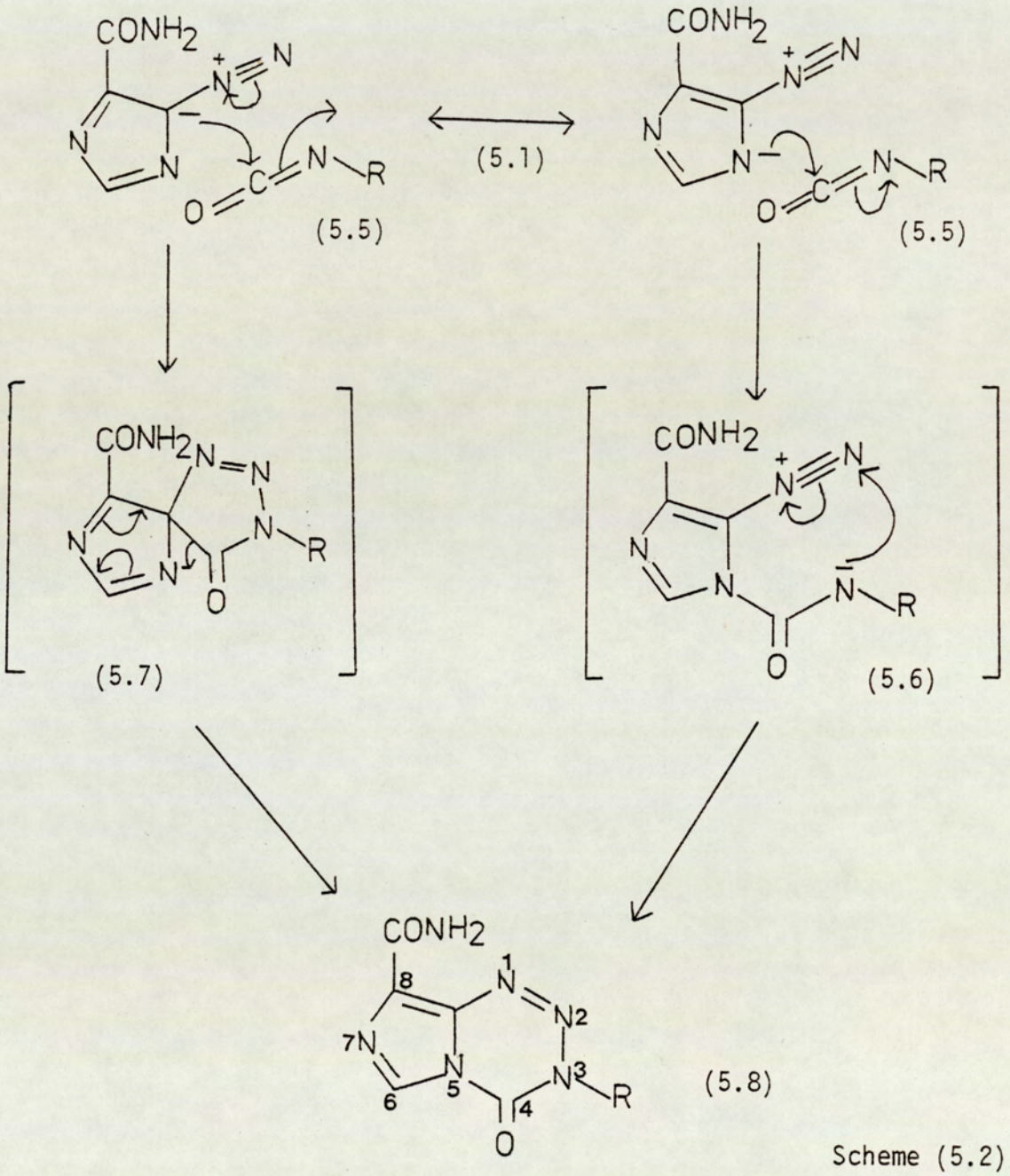
Stevens et al [76] synthesised imidazo[5,1-c]-1,2,4-triazin-4(3H)-ones (5.4) by coupling 5-diazoimidazole-4-carboxamide (5.1)[77] with reactive methylenic compounds (5.2) via intermediate isolable hydrazones (5.3) (Scheme 5.1) and further exploited the reactivity of diazoimidazole (5.1) and obtained imidazo[5,1-d]-1,2,3,5-tetrazin-4(3H)-ones (5.8)[78,79] by interaction with alkyl or aryl isocyanates (5.5) in either dichloromethane, ethyl acetate or acetonitrile. The 1,2,3,5-tetrazine system fused to an azole ring was first reported by Ege and Gilbert[80], who prepared a number of substituted pyrazolo[5,1-d]-1,2,3,5-tetrazin-4(3H)-ones by the interaction of diazopyrazoles with isocyanates.

The mechanism of these transformations possibly involves an initial [3+2] cycloaddition[81] to form unstable spirobicycles (5.7), which rearrange spontaneously by a [1,5] sigmatropic shift to the imidazotetrazinones (5.8) (Scheme 5.2). Alternatively, a two-step ionic mechanism involving initial nucleophilic attack by the imidazole ring nitrogen at the electrophilic carbonyl group of the isocyanate to yield the dipolar intermediate (5.6), followed by ring closure, is possible. The latter mechanism is preferred as the rate of reaction is greatly enhanced in polar solvents.

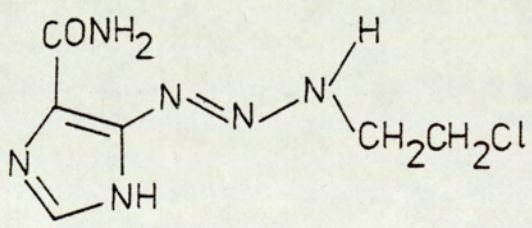
8-Carbamoyl-3-(2-chloroethyl)-imidazo[5,1-d]-1,2,3,5-tetrazin-



Scheme (5.1)



Scheme (5.2)



(MCTIC) (5.9)

4(3H)-one (5.8; R = CH_2CH_2Cl) was first synthesised by Stone in 1980[78] at Aston University. This compound showed potent antitumour properties[82-85] and may act as a prodrug of the acyclic triazene 5-[3-(2-chloroethyl)triazen-1-yl]imidazole-4-carboxamide (MCTIC) (5.9)[86], since it ring opens to form this triazene in aqueous sodium carbonate (see page 83).

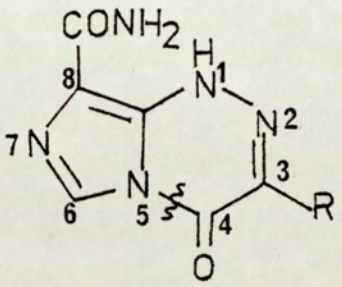
The name "mitozolomide" has been proposed by May & Baker Ltd, who are promoting this compound (5.8; R = CH_2CH_2Cl) for clinical use as an antitumour agent. The drug is presently in a multi-centred Phase II clinical trial.

The methyl analogue (5.8; R = Me) which also showed antitumour activity is at present undergoing toxicological evaluation prior to Phase I clinical trial.

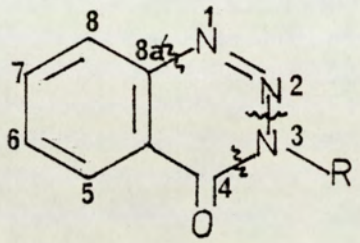
5.2 Chemical properties of imidazotetrazinones

5.2.1 **Aims and objectives of the work**

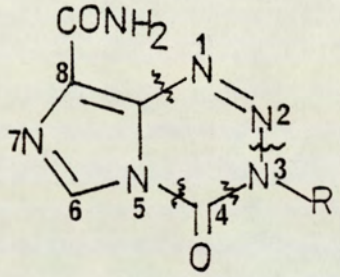
All molecules bearing NNN linkages possess versatile chemical reactivities. The chemistry of the NNN linkage in both cyclic (1,2,3 triazine) and acyclic (triazene) systems has been studied in an attempt to elucidate the potential of this reactive moiety in an antitumour setting[87-89]. A comparison may be made between the chemical properties of 3-substituted imidazotetrazinones (5.8), 3-substituted 1,2,3-benzotriazinones (1.3) and 3-substituted imidazo-1,2,4-triazinones (5.4). Benzotriazinones (1.3) undergo fission at the 1,8a-, 2,3- or 3,4-bonds depending on the conditions and the ionic or radical species thereby generated can be diverted to form a range of products.



(5.4)



(1.3)



(5.8)

~~~ = position of bond cleavage

Imidazotriazinones (5.4), although very stable towards acidic or basic conditions, suffer ring-fission in the presence of hydrazines and the reaction proceeds by cleavage of the 4,5-bond (only) to afford imidazol-5-ylazopyrazoles. For example, 3-acetylimidazotriazinone (5.10) yielded 3-methyl-4-(4-carbamoylimidazol-5-ylazo)-pyrazolin-5-one (5.12) from the reaction with hydrazine hydrate[90] (Scheme 5.3). 3-Substituted imidazotetrazinones (5.8) could be considered as a 'hybrid' of (3.1) and (5.4).

It was decided to investigate the effects elicited by the bridgehead nitrogen atom in compounds (5.8) and to explore the possibilities of cleavages of four different bonds (1,8a-, 2,3-, 3,4- and 4,5-). The prospect that these decompositions might generate a cascade of reactive molecules, some of which, although too unstable to isolate, could have antitumour significance was of interest.

The chemical reactions of the imidazotetrazinones are described in chapters 6-9.
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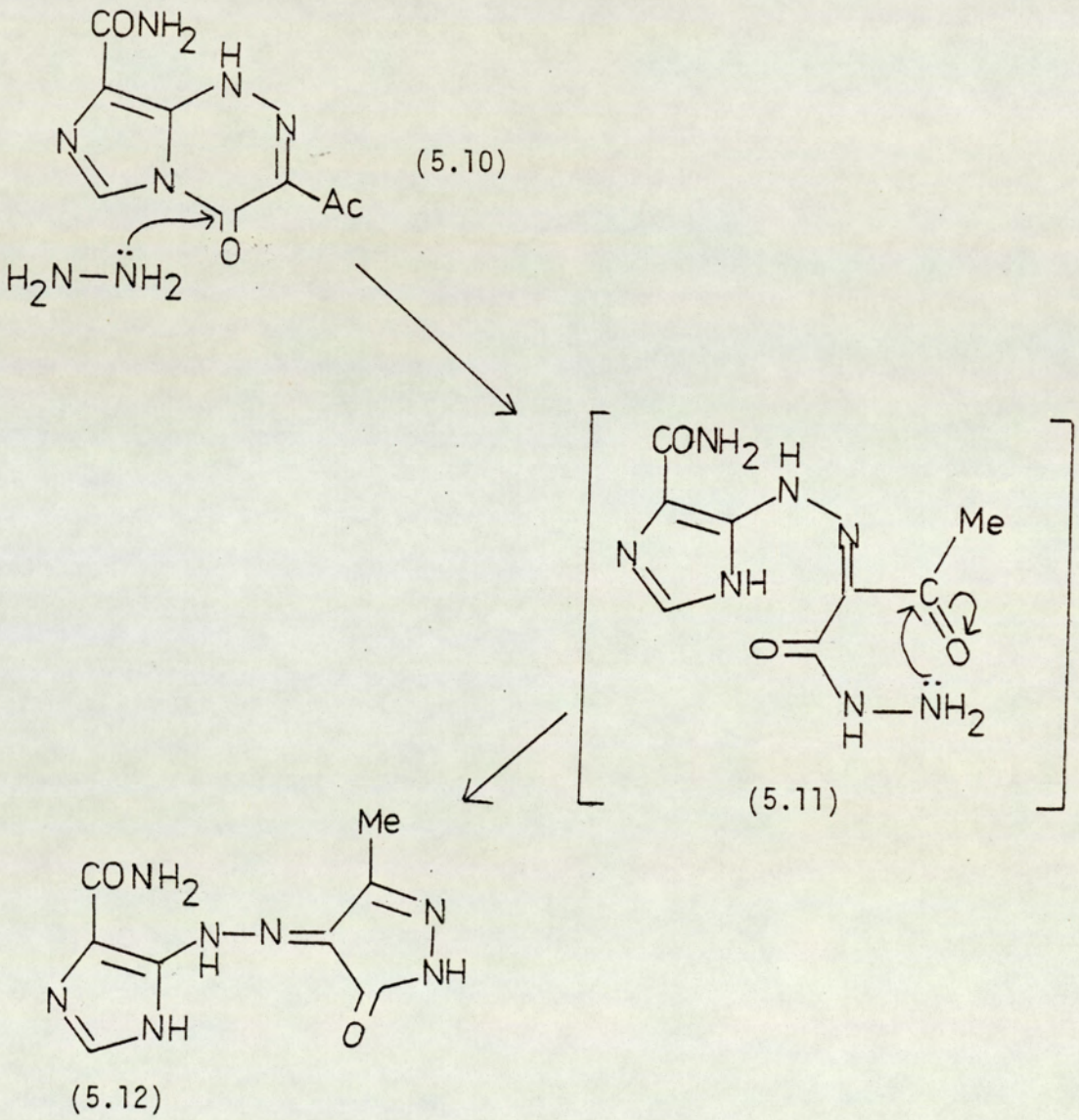

CONH2
H
N
N
N
N
Ac
O
H2N—NH2
(5.10)
CONH2
H
N
N
Me
N
NH
C
O
O
N
H
NH2
(5.11)
Me
CONH2
N
N
H
N—N
NH
N
NH
O
(5.12)

Scheme (5.3)

Chapter 6

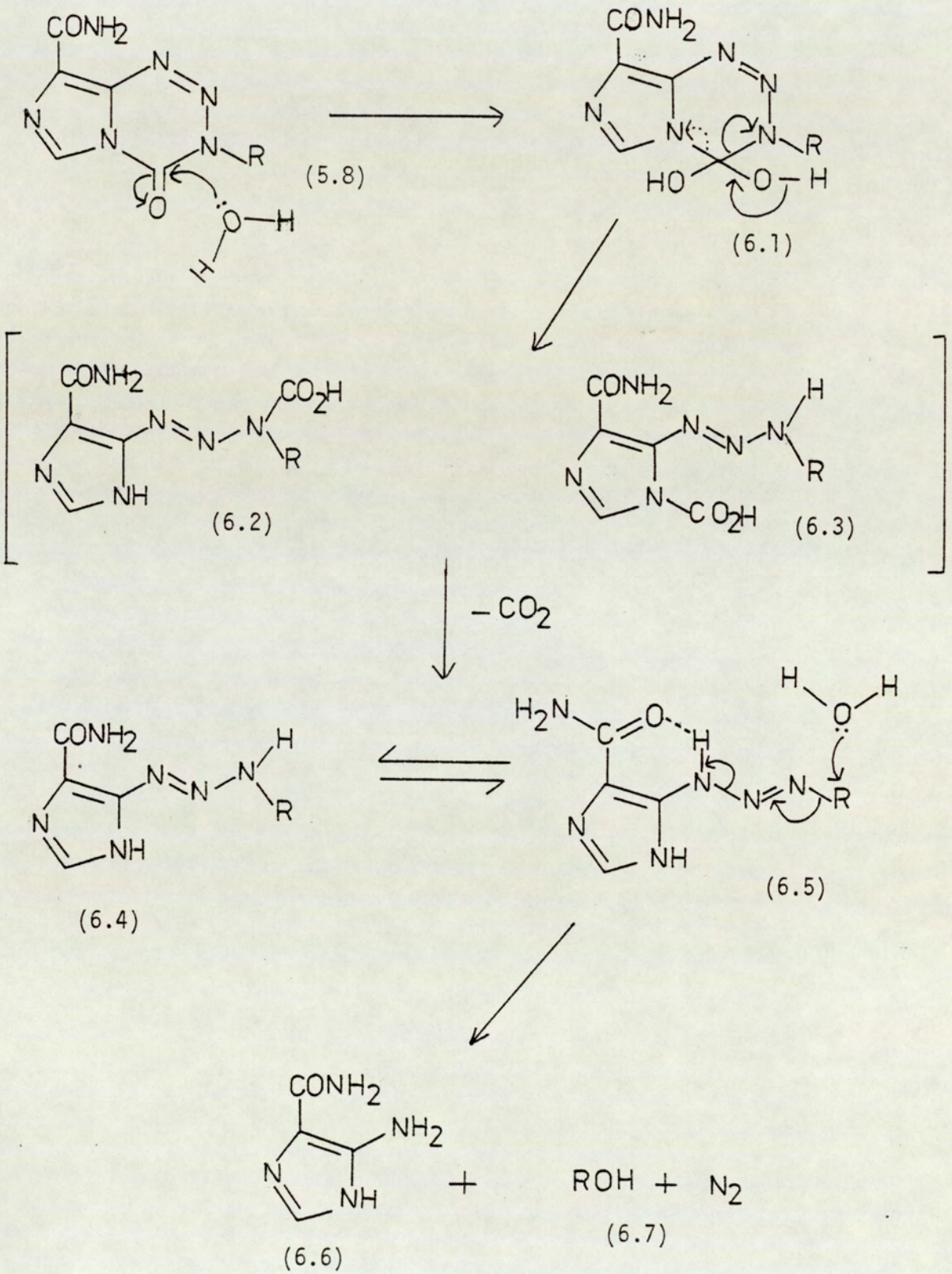
Decomposition of imidazotetrazinones in aqueous conditions

6.1 Decomposition in water

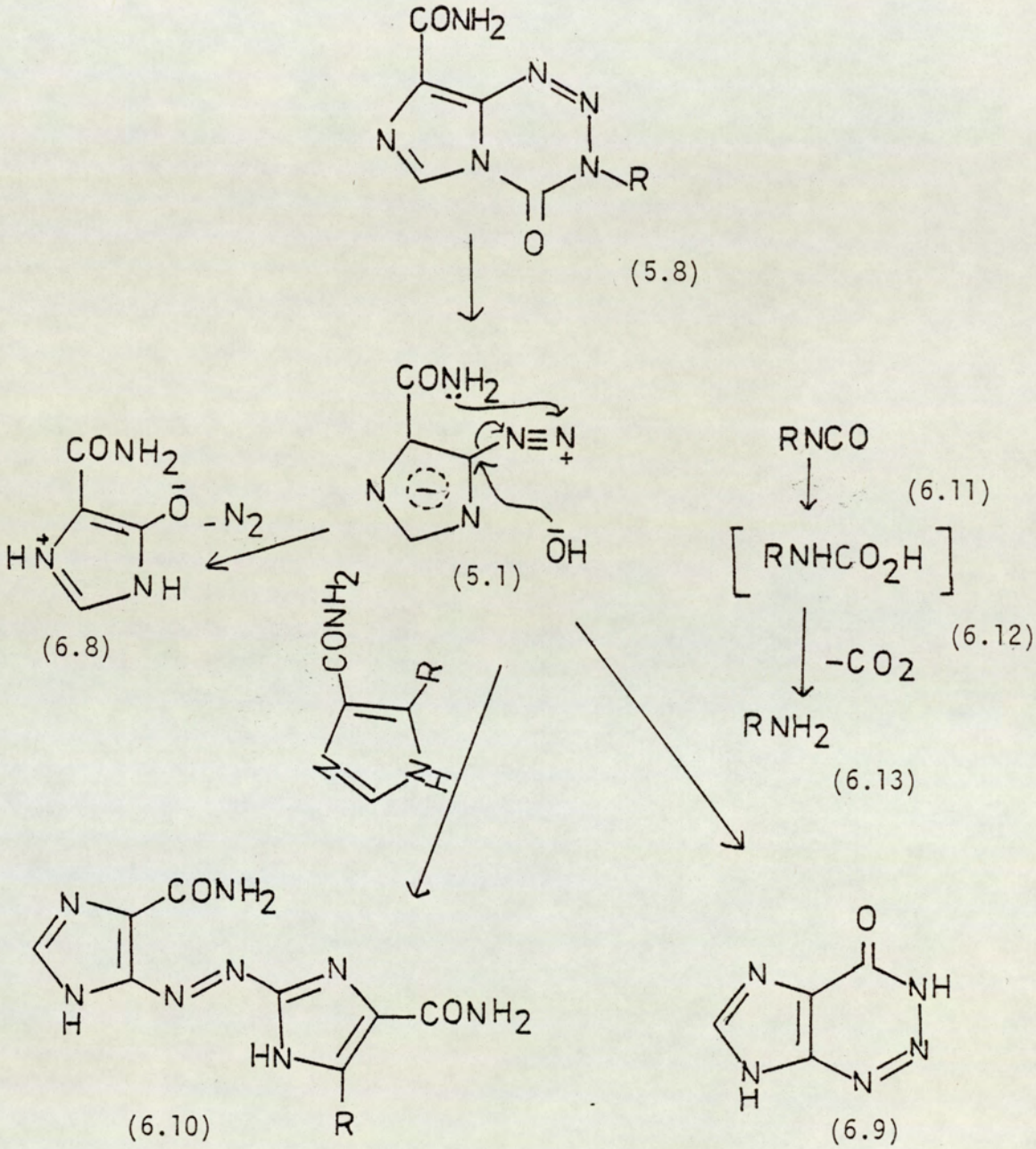
The 3-alkyltetrazinones (5.8; R = Me, Et) were stable in cold water (5-6 days) but decomposed rapidly with effervescence in boiling water to yield 5-aminoimidazole-4-carboxamide (6.6) as the major product. Traces of azahypoxanthine (6.9)[91] and the imidazolylazoimidazoles (6.10; R = NH_2, OH)[92-94] were detected by tlc (silica/EtOH/toluene 3:1). These azo compounds imparted a pink colouration to 5-aminoimidazole-4-carboxamide (6.6).

In contrast, mitozolomide (5.8; R = CH_2CH_2Cl) decomposed in boiling water to yield an insoluble maroon solid. This highly coloured solid showed uv absorption at λ_{max} 550 nm and was identified principally as the imidazolylazoimidazole (6.10; R = OH)[91-93] by its spectral properties. The filtrate from the reaction mixture, when evaporated to dryness under vacuum, afforded 2-azahypoxanthine (6.9)[91] and 5-aminoimidazole-4-carboxamide (6.6) with traces of the imidazolylazoimidazoles (6.10; R = OH, NH_2). All the above degradation products were confirmed from their identity with known samples.

The mechanisms for the above reactions are summarised in Schemes 6.1 and 6.2. Attack by water (Scheme 6.1) at C-4 of the imidazotetrazinone (5.8) yielded an unstable carbamic acid (6.2 or 6.3) depending on whether the 3-4 or the 4-5 bond, respectively, was broken initially. [The analogous reaction of 3-alkyltetrazinones (5.8; R = Me, Et) in boiling ethanol or methanol suggested that the


CONH2
N
N
N
N
R
O
O
H
H
(5.8)
CONH2
HO
O
H
(6.1)
CONH2
CO2H
NH
(6.2)
CONH2
H
R
CO2H
(6.3)
− CO2
CONH2
H
R
NH
(6.4)
H2N
O
H
H
O
H
R
NH
(6.5)
CONH2
NH2
NH
(6.6)
+
ROH + N2
(6.7)

Scheme (6.1)



R = OH, NH_2

Scheme (6.2)

3-4 bond is also likely to break (see page 108)]. Decarboxylation of the intermediate carbamic acids (6.2 or 6.3) would yield the triazenes (6.4 or 6.5). The triazene 6.5, by further attack of water, would yield 5-aminoimidazole-4-carboxamide (6.6) and the respective alcohols (6.7). It is possible that intramolecular hydrogen bonding controls the chemistry of the triazenes (6.4, 6.5) by stabilising the aminoimidazole tautomer (6.5) at the expense of azoimidazole tautomer (6.4), thus activating the alkyl group of triazene (6.5) to attack by nucleophile (water).

Formation of the imidazolylazoimidazoles (6.10; R = OH) and 2-azahypoxanthine (6.9) from the reaction of mitozolomide (5.8; R = CH_2CH_2Cl) in water can be explained by invoking an electrocyclic $6\overline{\pi}$ ring opening, leading to generation of 5-diazoimidazole-4-carboxamide (5.1) and 2-chloroethyl isocyanate (6.11; R = CH_2CH_2Cl), which subsequently would yield the observed products by the multiple reactions shown in Scheme 6.2.

It can be envisaged from the above reactions that mitozolomide (5.8; R = CH_2CH_2Cl) undergoes thermal degradation by retrocycloaddition in addition to hydrolytic decomposition which is observed in the 3-alkyltetrazinones (5.8; R = Me, Et).

6.2 Decomposition in aqueous sodium carbonate

The 3-methyltetrazinone dissolved with effervescence in cold 5% sodium carbonate solution. A buff coloured solid was isolated from the clear solution upon standing for a few minutes. The ir spectrum of the freshly isolated damp solid showed broad peaks between 3000-3500 cm^{-1} for NH and OH and also contained two carbonyl absorptions at 1730 and 1680 cm^{-1} indicating an acid and an amide carbonyl. This

unstable intermediate is possibly an acid (6.2 or 6.3; R = Me). When this acid was left to dry on the filter paper at room temperature, it further decomposed with effervescence yielding a pale pink solid. The ir spectrum of the solid showed a considerable change in the finger-print region and also the disappearance of the carbonyl band at 1730 cm^{-1}. The decomposed solid was characterised on the basis of the spectral evidence as 5-[3-methyltriazen-1-yl]imidazole-4-carboxamide (MTIC) (6.4; R = Me)[95].

The 3-ethyltetrazinone (5.8; R = Et), which is biologically inactive, behaved similarly and underwent hydrolysis to yield a buff coloured solid which was identified spectroscopically as being 5-[3-ethyltriazen-1-yl]imidazole-4-carboxamide (6.4; R = Et). The structure of the compounds (6.4; R = Me, Et) were confirmed from their identity with the known triazenes prepared by an unambiguous route from the reaction of 5-diazoimidazole-4-carboxamide (5.1) with the corresponding alkylamines[95].

8-Carbethoxy-3-(2-chloroethyl)imidazo[5,1-d]-1,2,3-5-tetrazin-4(3H)-one (5.8; R = CH_2CH_2Cl; $CONH_2$ = CO_2Et) reacted similarly in 5% aqueous sodium carbonate and decomposed with effervescence to yield a small amount of white solid (10%). The ir spectrum of the solid showed broad peaks between 2900 - 3500 cm^{-1} for carboxylic OH and NH respectively. A peak at 1718 cm^{-1} is attributed to a carboxylic acid. The electron-impact promoted mass spectrum of this compound did not show a molecular ion at m/z 245 (247) for the corresponding triazene (6.4; R = CH_2CH_2Cl; $CONH_2$ = CO_2Et) but instead showed M^+ at m/z 219, 217 (3:1) with major ions at 181, 168 and m/z 122. On the basis of the spectral evidence the structure (6.4; R = CH_2CH_2Cl; $CONH_2$ = COOH) was assigned to this triazene. The same triazene was also obtained in very low yield directly from the decomposition of

the 8-carboxylic acid analogue (5.8, R = CH_2CH_2Cl; $CONH_2$ = COOH) in 5% sodium carbonate solution.

Attempts to effect a similar decomposition of 8-sulphamoyl-3-(2-chloroethyl)imidazo[5,1-d]-1,2,3,5-tetrazin-4(3H)-one (5.8; R = CH_2CH_2Cl; $CONH_2$ = SO_2NH_2) and the N-methyl or NN-dimethylsulphamoyl-imidazotetrazinones (5.8; R = CH_2CH_2Cl; $CONH_2$ = SO_2NHMe, SO_2NMe_2) in 5% sodium carbonate solution were not successful. These compounds were extremely soluble in sodium carbonate solution and underwent smooth degradation yielding water soluble end products. These hydrolysis decompositions were followed by uv spectroscopy. The disappearance of a peak at λ_{max} 325 nm is indicative of degradation of tetrazinones (5.8) and triazenes (6.4) to the corresponding end products.

A mechanism that accounts for the salient features of these transformations in aqueous sodium carbonate is summarised in Scheme 6.1. Brief treatment of tetrazinones (5.8) with aqueous sodium carbonate leads to the isolation of acyclic triazenes (6.4), whereas prolonged treatment resulted in the formation of 5-aminoimidazole-4-carboxamide (5.1) and the respective alcohols (6.7) with the liberation of nitrogen. Shealy et al[86] have observed previously that the triazenylimidazole MCTIC (6.4; R = CH_2CH_2Cl) decomposes to 5-aminoimidazole-4-carboxamide (6.6) and other minor products.

The aforementioned chemistry lends weight to the hypothesis that the bicyclic tetrazinone (5.8; R = CH_2CH_2Cl) could be stable prodrug of MCTIC (6.4; R = CH_2CH_2Cl) and this view is supported by a comparison of the biochemical effects of both agents against tumour cells[96,97]. The bicyclic derivatives mitozolomide (5.8; R = CH_2CH_2Cl) and the 3-methyltetrazinone (5.8; R = Me) are more attractive clinical candidates than MCTIC (6.4; R = CH_2CH_2Cl) which is an unstable moiety engendering insuperable formulation problems.

Chapter 7

Reactions of imidazotetrazinones in mineral acids

7.1 In dilute mineral acids

Suspensions of the imidazotetrazinones (5.8; R = Me, Et, n-Pr, CH_2CH_2Cl) in dilute mineral acids remained unchanged for ca 1-2 days and degraded slowly as indicated by the disappearance of the peak at λ_{max} 325 nm in the uv spectra.

7.2 In concentrated acid

Imidazotetrazinones (5.8) are soluble in cold concentrated mineral acids such as nitric, sulphuric or chromic acid and were found to be stable in warm concentrated sulphuric acid. This stability is surprising in view of the chemical structures of these compounds and contrast with the acid sensitivity of the structurally related imidazoyltriazenes, eg. DTIC (6.3; R = H = Me)[92-94].

Attempted oxidation of the methyl group of the 3-methyltetrazinone (5.8; R = Me) to a carboxylic acid group with chromic acid yielded a new white polymorph of the tetrazinone upon dilution with water. The ^1H nmr, uv and mass spectral properties were identical to the starting material (5.8; R = Me). The ir spectrum of this polymorph, however, was different from the starting material and showed only two carbonyl absorptions at 1738 and 1672 cm^{-1} (Fig 7.2). The same white polymorph with two carbonyl peaks (Fig 7.2) was isolated on two occasions directly from the reaction of 5-diazoimidazole-4-carboxamide (5.1) and methyl isocyanate. This

Infrared spectra of the Polymorphic forms of 8-carbamoyl-3-methylimidazo[5,1-d] -1,2,3,5-tetrazin-4(3H)-one

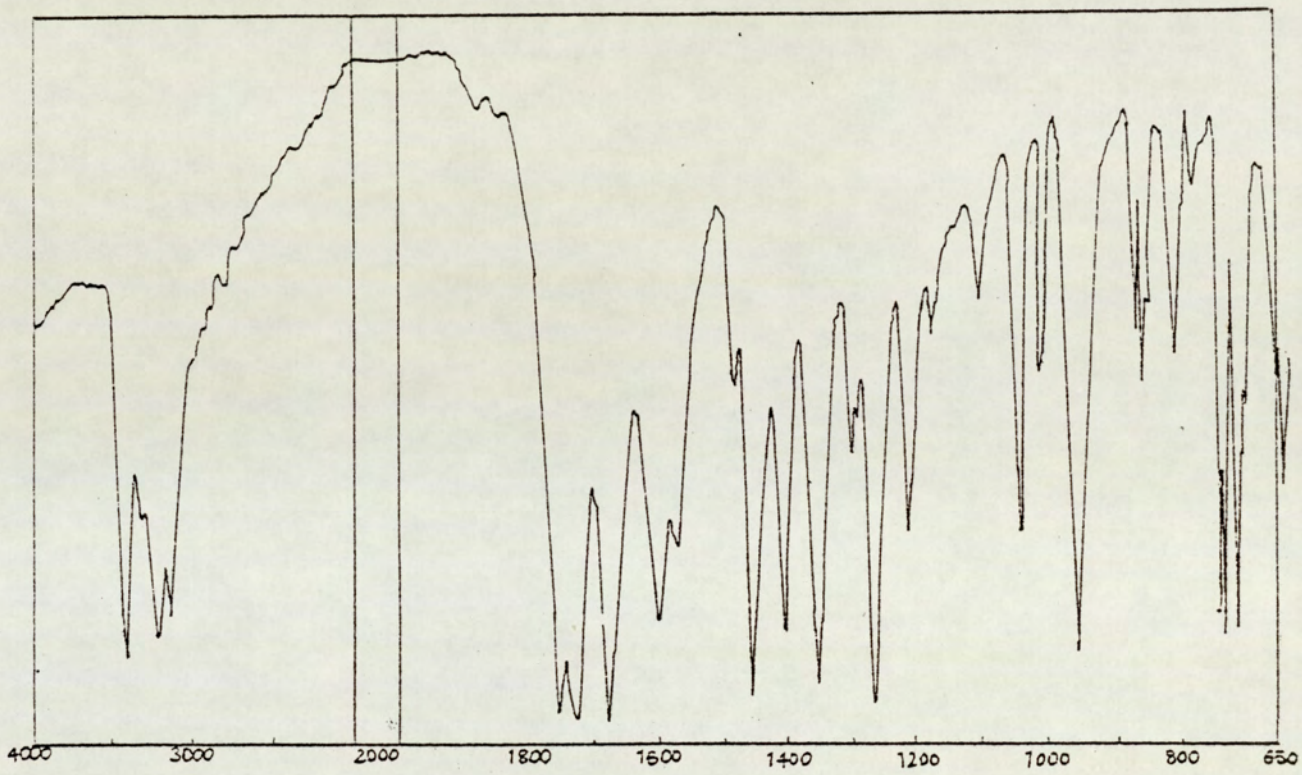


Figure (7.1)

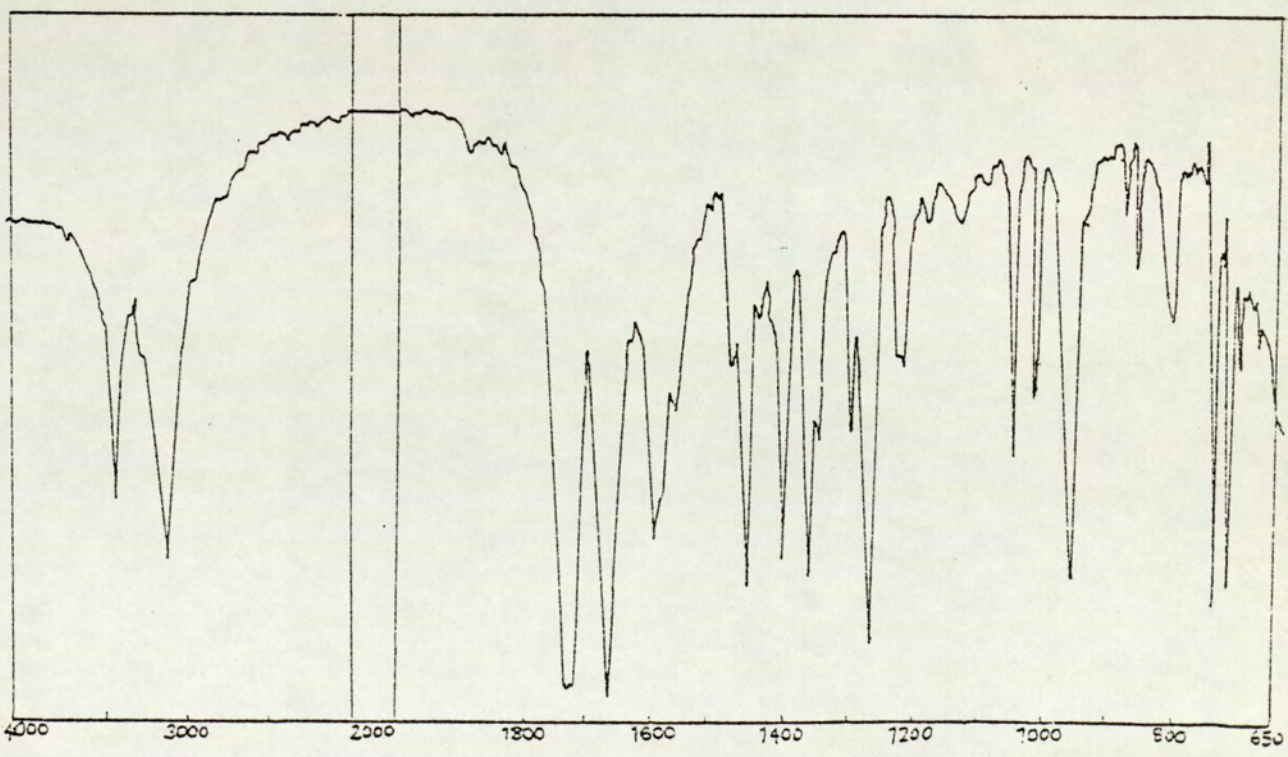


Figure (7.2)

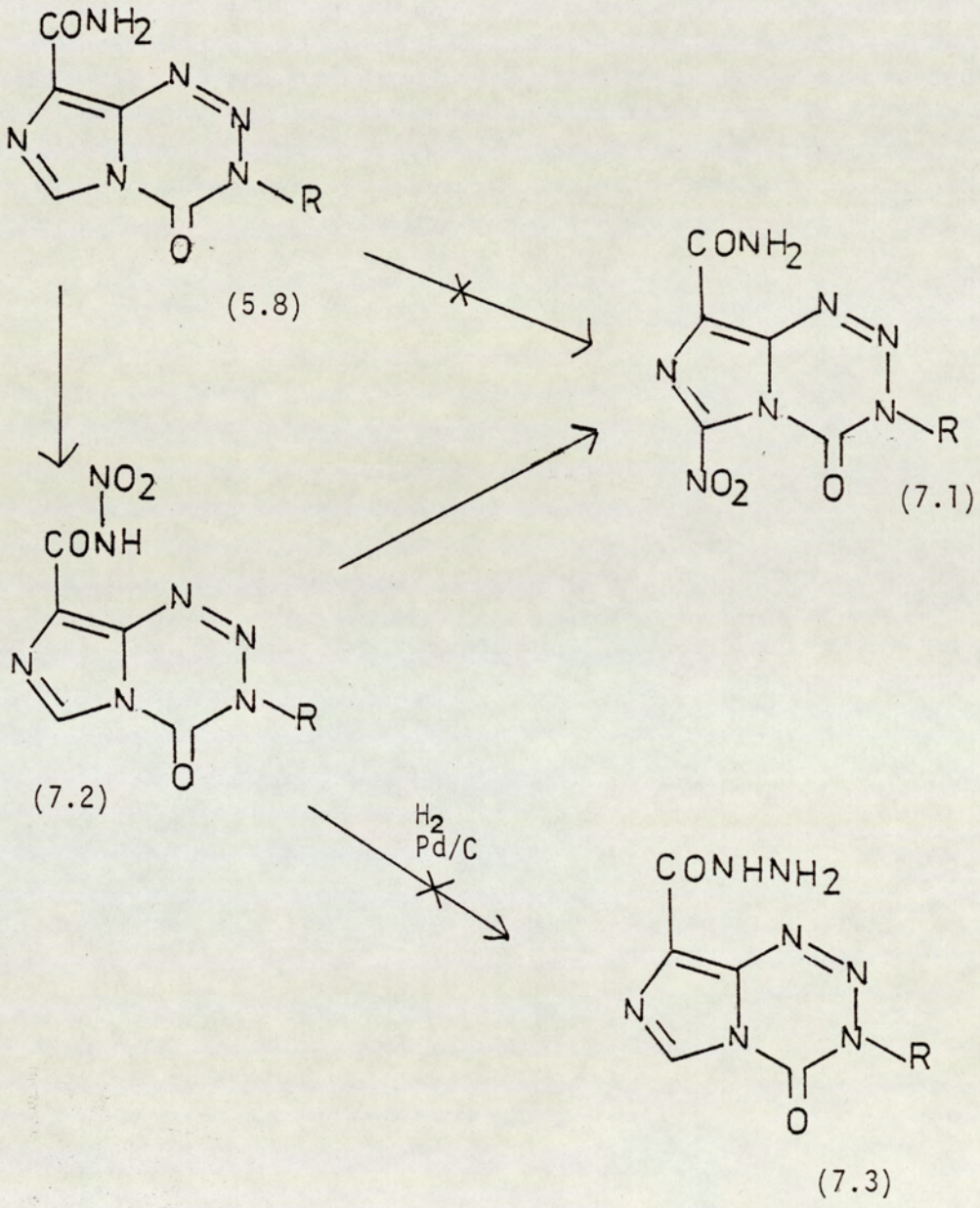
polymorph reverted to the original pink crystalline solid, ν_{max} 1742, 1718 and 1662 cm^{-1} (Fig 7.1) upon recrystallisation from acetone-water (3:1)

7.2.1 Nitration in concentrated nitric acid - sulphuric acid

The nitration of mitozolomide (5.8; R = CH_2CH_2Cl) was carried out in an attempt to obtain 8-carbamoyl-3-(2-chloroethyl)-6-nitroimidazo[5,1,-d]-1,2,3,5-tetrazin-4(3H)-one (7.1; R = CH_2CH_2Cl). A solution of mitozolomide in concentrated sulphuric acid was stirred at 0 °C with concentrated nitric acid (d = 1.42). When the clear solution of the reaction mixture was poured on to crushed ice, a white solid was obtained.

The uv spectrum of this compound in 95% ethanol showed peaks at λ_{max} 327, 255 and 213 nm corresponding to imidazotetrazinones. The ir spectrum showed two small NH absorptions at 3480 and 3200 cm^{-1} and a sharp prominent peak at 3120 cm^{-1} with two carbonyl frequencies at 1742 and 1710 cm^{-1}. The mass spectrum showed the expected molecular ion corresponding to the compound (7.1; R = CH_2CH_2Cl) at m/z 287 (289) in low abundance and ions at m/z 241 (243) and a base peak at m/z 182 and m/z 105(107). The ^1H nmr spectrum of this compound showed a sharp singlet for the imidazole (H-6) proton and a broad one proton NH resonance which disappeared on D_2O exchange. This spectral evidence excludes the possibility of the structure (7.1; R = CH_2CH_2Cl). On the basis of the spectroscopic and analytical data this product was assigned the structure (7.2; R = CH_2CH_2Cl).

When the methyltetrazinone (5.8; R = Me) was nitrated using a mild nitrating agent such as potassium nitrate and 2N-sulphuric acid



R = Me, CH_2CH_2Cl

Scheme (7.1)

the starting material (5.8; R = Me) was recovered unchanged. However, when this compound (5.8, R = Me) was nitrated under the identical conditions described for the nitration of mitozolomide (5.8; R = CH_2CH_2Cl), a mixture of the nitroamide (7.2; R = Me) and the 8-carboxylic acid (7.6; R = Me) (see page 92) was obtained. The mass spectrum of the mixture showed a prominent peak at m/z 239 (molecular ion for the compound [7.2; R = Me]) and m/z 182 corresponding to 5-diazoimidazole-4-nitrocarboxamide (5.1; $CONH_2$ = $CONHNO_2$) obtained by the retro cyclo-addition of the tetrazinone (7.2; R = Me). The spectrum also showed the presence of a peak at m/z 195 (M^+ acid) and m/z 138 for the corresponding 5-diazoimidazole-4-carboxylic acid (5.1; $CONH_2$= COOH).

Attempts were made to obtain the nitroamide (7.3; R = Me) as a sole product. The reaction was repeated several times under various conditions by alternating the concentrations of the nitrating mixture, each time compounds (7.3 and 7.6; R = Me) were isolated.

The nitroamide (7.2; R = CH_2CH_2Cl) could not be rearranged under a range of conditions to yield the 6-nitro analogue (7.1; R = CH_2CH_2Cl). For example, when stirred with concentrated sulphuric acid, the unchanged starting material (7.2; R = CH_2CH_2Cl) (90%) was recovered. When the same reaction was carried out at elevated temperature (45 °C), mitozolomide (5.8; R = CH_2CH_2Cl) was isolated.

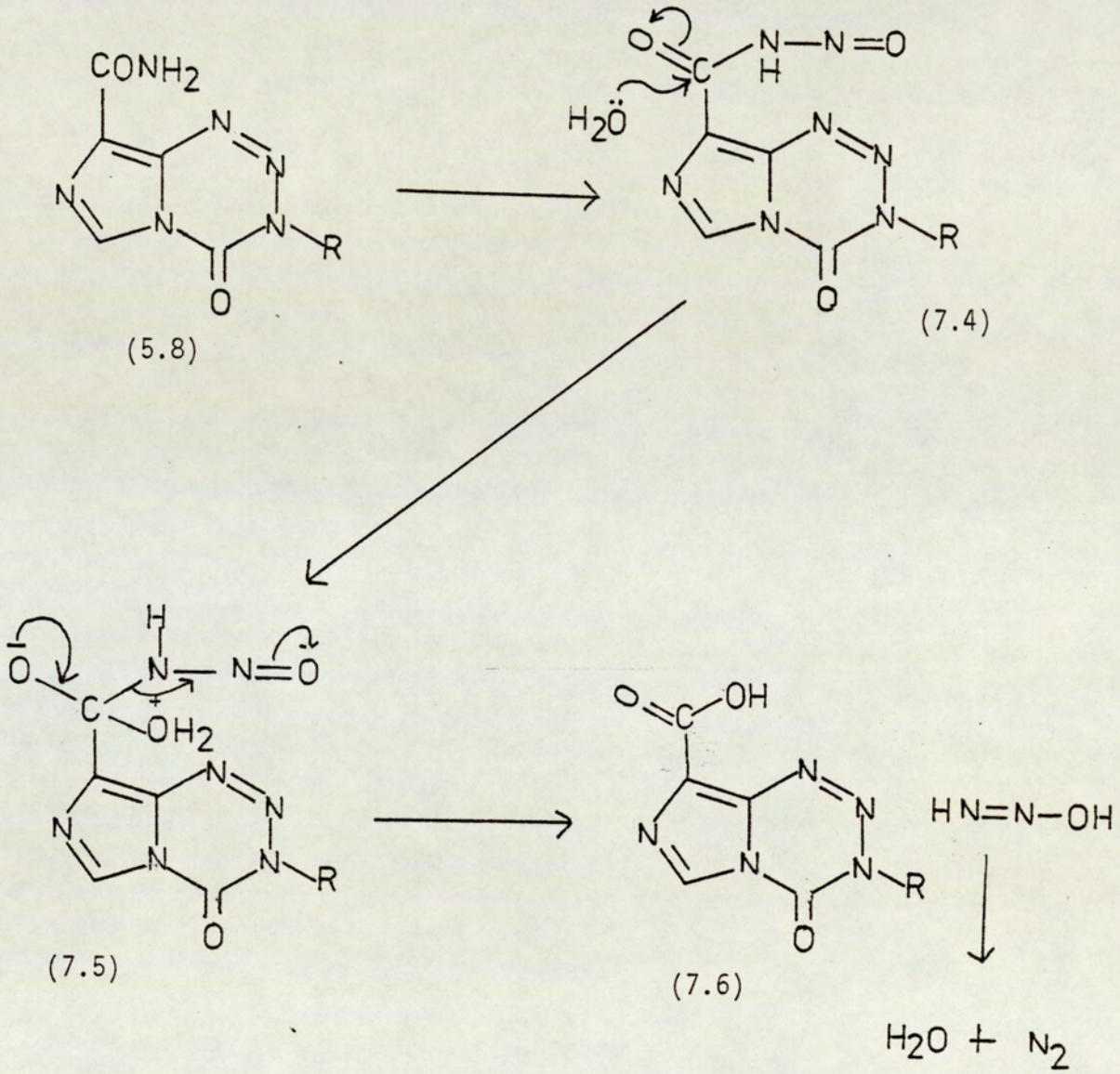
Attempted catalytic reduction of the nitroamide (7.2; R = CH_2CH_2Cl) at atmospheric pressure using 10% Pd/C yielded mitozolomide (5.8; R = CH_2CH_2Cl) possibly <u>via</u> the intermediate unisolable carbohydrazide (7.3; R = CH_2CH_2Cl) which probably underwent further reduction.

7.2.2 Deamination in concentrated sulphuric acid

There are certain limitations in obtaining 8-carboxylic analogues (7.6; R = Me, CH_2CH_2Cl) of the imidazotetrazinones from the corresponding nitriles (5.8; R = Me, CH_2CH_2Cl; $CONH_2$ = CN). The imidazotetrazinones are fairly stable in concentrated mineral acids; they can withstand temperatures up to 55-60 °C but decompose readily upon boiling. Such conversions of nitrile by hydrolysis would require excessive heating in concentrated mineral acids. The other alternative widely-used method, alkaline hydrolysis, was certainly excluded as this ring system does not survive the alkaline conditions.

May & Baker Ltd devised a method to dispose of the mutagenic mitozolomide (5.8; R = CH_2CH_2Cl) excreted in patients' urine during clinical usage[98]. They treated the drug with sodium hypochlorite solution. On a preparative scale they isolated the 8-carboxylic acid (7.6; R = CH_2CH_2Cl) in low yield. This method is quite unique but has its own limitations: sodium hypochlorite solution is very alkaline and has a pH of 11.0. The imidazotetrazine ring-system is very unstable in alkaline conditions.

Bouveaults and others[99-102] obtained acids from amides by treating solutions of amides in concentrated sulphuric acid with sodium nitrite. Accordingly, mitozolomide (5.8; R = CH_2CH_2Cl) was successfully converted to 3-(2-chloroethyl)-2,3,-dihydro-4-oxo-imidazo[5,1-d]-1,2,3,5-tetrazine-8-carboxylic acid (7.6; R = CH_2CH_2Cl) by an analogous method. A solution of mitozolomide in concentrated sulphuric acid was treated with solid sodium nitrite with a few drops of water at 35 °C. When the reaction mixture was poured onto crushed ice, a white solid was obtained.



R = Me, $CHCH_2Cl$

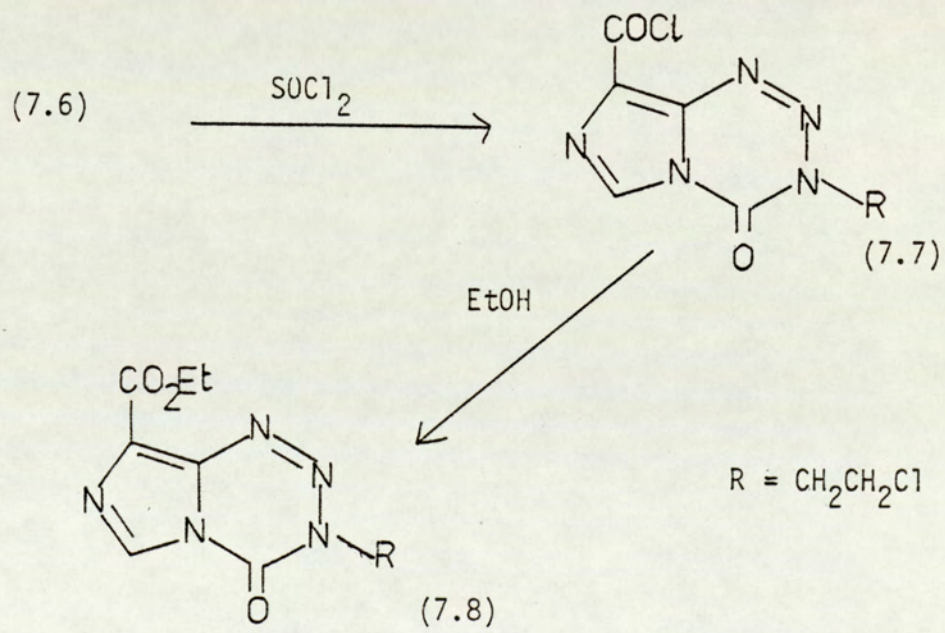
Scheme (7.2)

The product (7.6; R = CH_2CH_2Cl) was characterised by spectroscopy and was found to be identical to the compound obtained from the hydrolysis of mitozolomide (5.8; R = CH_2CH_2Cl) by sodium hypochlorite.

The methyltetrazinone (5.8; R = Me) responded similarly to these reagents and smoothly underwent conversion to the corresponding 8-carboxylic acid (7.6; R = Me). The same acid (7.6; R = Me) was isolated in a small amount as a by-product from the nitration of (5.8; R = Me) (see page 90).

As shown by the Scheme (7.2), the nitrosating mixture probably forms an intermediate N-nitrosoamide (7.4; R = Me, CH_2CH_2Cl) which undergoes hydrolysis to yield the corresponding carboxylic acid (7.6; R = Me, CH_2CH_2Cl).

The acid (7.6; R = CH_2CH_2Cl) was smoothly converted to the acid chloride (7.7; R = CH_2CH_2Cl) by heating in thionyl chloride. The acid chloride (7.7; R = CH_2CH_2Cl), upon stirring with absolute ethanol at room temperature, yielded the ethyl ester (7.8; R = CH_2CH_2Cl).



Scheme (7.3)

Chapter 8

Decomposition of imidazotetrazinones in acetic acid

3-Methylimidazotetrazinone (5.8; R = Me) dissolves in warm glacial acetic acid and no decomposition was noted when the solution was boiled for one hour; 70% of the starting material was recovered unchanged upon cooling. The reaction was monitored by ir and uv spectroscopy.

Mitozolomide (5.8; R = CH_2CH_2Cl), on the other hand, was only stable for a short period. Recrystallisation from warm acetic acid (50-60 °C) yielded a yellow solid. These crystals showed three carbonyl absorptions at 1742, 1705 and 1655 cm^{-1} in the ir spectrum (KBr disc). This polymorphic form of mitozolomide (5.8; R = CH_2CH_2Cl) was different from the polymorphs previously examined[79]. When recrystallised from aqueous acetone it reverted to the original form which showed NH absorptions at 3450, 3350, 3230 (broad) and 3120 cm^{-1} and carbonyl stretching frequencies at 1673 and 1748 cm^{-1}.

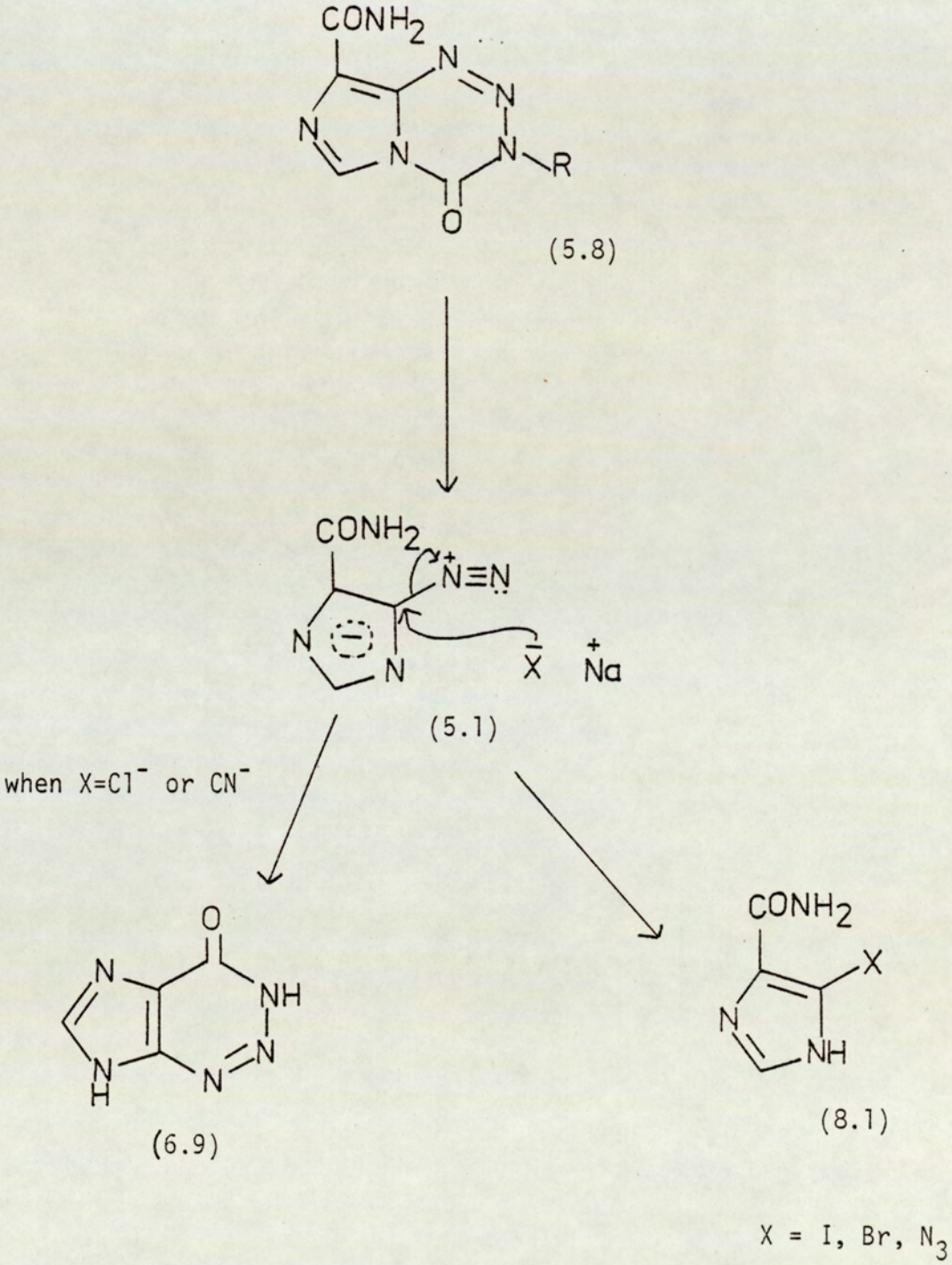
When mitozolomide (5.8; R = CH_2CH_2Cl) was heated under reflux in glacial acetic acid it slowly decomposed to yield 5-diazoimidazole-4-carboxamide (5.1) since the ir spectrum of the recovered material showed contaminating bands at 2198 and 1148 cm^{-1} characteristic of the presence of diazoimidazole carboxamide. This thermolytic generation of Diazo-IC (5.1) was exploited to furnish a "one-pot" conversion of the imidazo[5,1-d]-1,2,3,5-tetrazine ring system (5.8) to the de-aza system imidazo[5,1-c]-1,2,4-triazine (5.4)[76] (Scheme 5.1) and to obtain various 5-substituted imidazole carboxamide (8.1, X = I, Br) by nucleophilic substitution. These reactions will be discussed in the following sections.

8.1 Reactions with nucleophiles

Mitozolomide (5.8; R = CH_2CH_2Cl) acts as a "masked diazo compound" and can be regarded as a precursor of a reactive diazo species. This character is revealed prominently in warm acetic acid and the reaction proceeds with N2-N3 bond cleavage. Acetic acid proved to be an excellent medium for promoting "Sandmeyer-type" displacements of the diazo group with various nucleophiles. For example, 5-diazoimidazole-4-carboxamide (5.1), generated by heating mitozolomide (5.8; R = CH_2CH_2Cl) in acetic acid, underwent displacement of the diazo group with iodide, bromide and (possibly) azide groups. Cyanide and chloride anions were insufficiently nucleophilic to promote this displacement and failed to compete with the intramolecular cyclisation reaction to azahypoxanthine (6.9). These Sandmeyer-type reactions recall the related degradations of 1,2,3-benzotriazin-4(3H)-one to 2-iodo or 2-azidobenzamides in boiling acetic acid with sodium iodide or sodium azide[103] or to 2-chlorobenzanilides in boiling hydrochloric acid[15,16,104,105] or to salicylic acid derivatives in boiling acetic acid with copper bronze[106].

Mitozolomide (5.8; R =CH_2CH_2Cl), when heated in glacial acetic acid with sodium iodide, yielded 5-iodoimidazole-4-carboxamide (8.1; X = I) as a golden yellow solid. The iodoimidazole carboxamide (8.1; X = I) was characterised by spectroscopy and confirmed by elemental analysis.

A similar reaction of mitozolomide with sodium bromide in hot acetic acid furnished 5-bromoimidazole-4-carboxamide (8.1; X = Br). The same 5-bromoimidazole-4-carboxamide was also obtained in very low yield by stirring together 5-diazoimidazole-4-carboxamide (5.1) and


CONH2
N
N
N
N
N
R
O
(5.8)
CONH2
N≡N
N
N
X
Na
(5.1)
when X=Cl⁻ or CN⁻
O
NH
N
N
N
H
(6.9)
CONH2
X
N
NH
(8.1)
X = I, Br, N3

Scheme (8.1)

HBr (45% solution in acetic acid) at room temperature. Mitozolomide (5.8; R = CH_2CH_2Cl), when heated with sodium azide and glacial acetic acid, yielded a black tar. Chromatographic examination (tlc, silica/toluene/ethanol 1:3) of the solid showed the presence of a mixture; 5-aminoimidazole-4-carboxamide was identified as one component.

5-Azidoimidazole-4-carboxamide (8.1; X = N_3) obtained from the reaction of hydrazine with mitozolomide (see page 112) or 5-diazoimidazole-4-carboxamide[77] (5.1) was also found to be unstable. When subjected to heat in ethanol, it decomposed to yield an intractable black tar. Based on this observation, it could be assumed that the reaction of mitozolomide (5.8; R = CH_2CH_2Cl) with sodium azide in hot acetic acid probably yielded the unstable 5-azidoimidazole-4-carboxamide (8.1; X = N_3) which failed to survive under the reaction conditions.

8.2 Reactions with reactive methylenic compounds

Mitozolomide (5.8; R = CH_2CH_2Cl) was heated under reflux with reactive methylenic compounds in glacial acetic acid. The generated 5-diazoimidazole-4-carboxamide (5.1) was trapped by the reactive methylenic compounds (8.2) to yield hydrazones (8.3). These reaction changes could be demonstrated by uv spectroscopy. A peak at λ_{max} 325 nm (characteristic for mitozolomide) slowly disappeared with the emergence of a new peak at λ_{max} 350-400 nm; this indicated the initiation of the coupling reaction of diazo-IC (5.1) and reactive methylenic compounds (8.2; R = CO_2Me, CO_2Et, Ac) to form the corresponding hydrazones (8.3; R = CO_2Me, CO_2Et, Ac).[76] No attempts were made to isolate the intermediate hydrazones which cyclised to imidazotriazines (8.7; R = CO_2Me, CO_2Et, Ac) on further heating.

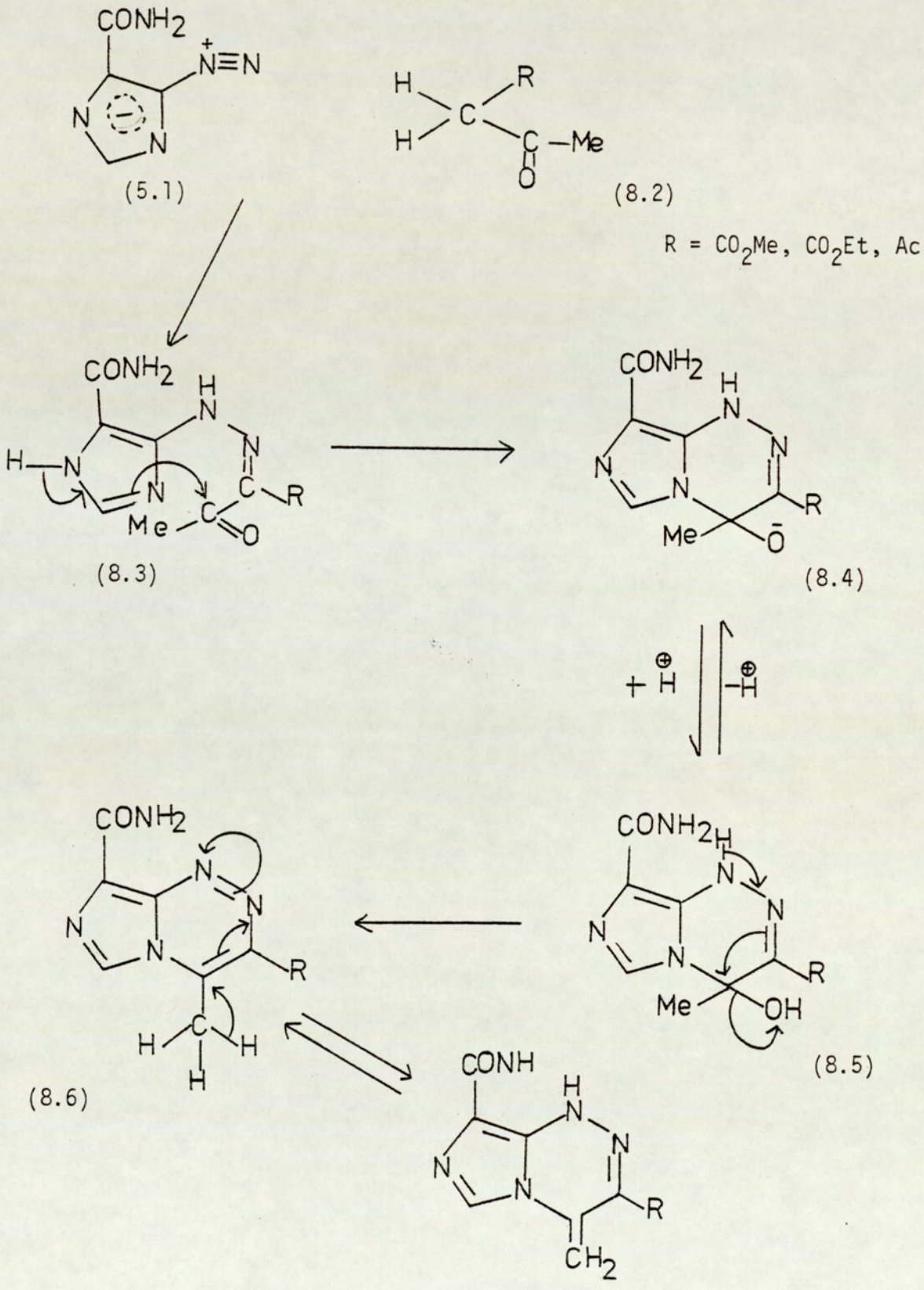
These cyclisation reactions were monitored by uv spectroscopy. Thus, when mitozolomide (5.8; R = CH_2CH_2Cl) was boiled in acetic acid with methyl acetoacetate for 0.5 hour, the golden yellow crystals of methyl 8-carbamoyl-1,4-dihydro-4-methyleneimidazo[5,1-c]-1,2,4-triazine-3-carboxylate (8.7; R = CO_2Me) were deposited from the cooled reaction mixture in the form of an acetic acid solvate.

The reaction of mitozolomide (5.8; R = CH_2CH_2Cl) in hot acetic acid with ethyl acetoacetate or acetylacetone (8.2; R = CO_2Et, Ac) yielded 4-methyleneimidazotriazine (8.7; R = CO_2Et, Ac) respectively. The structures of the imidazotriazines (8.7; R = CO_2Me, CO_2Et, Ac) were confirmed from their identity with the products obtained from the cyclisation of the purified hydrazones (8.3; R = CO_2Me, CO_2Et, Ac). These hydrazones were previously prepared by the author[76] from the coupling reactions of 5-diazoimidazole-4-carboxamide (5.1) with methyl or ethyl acetoacetate and with acetylacetone.

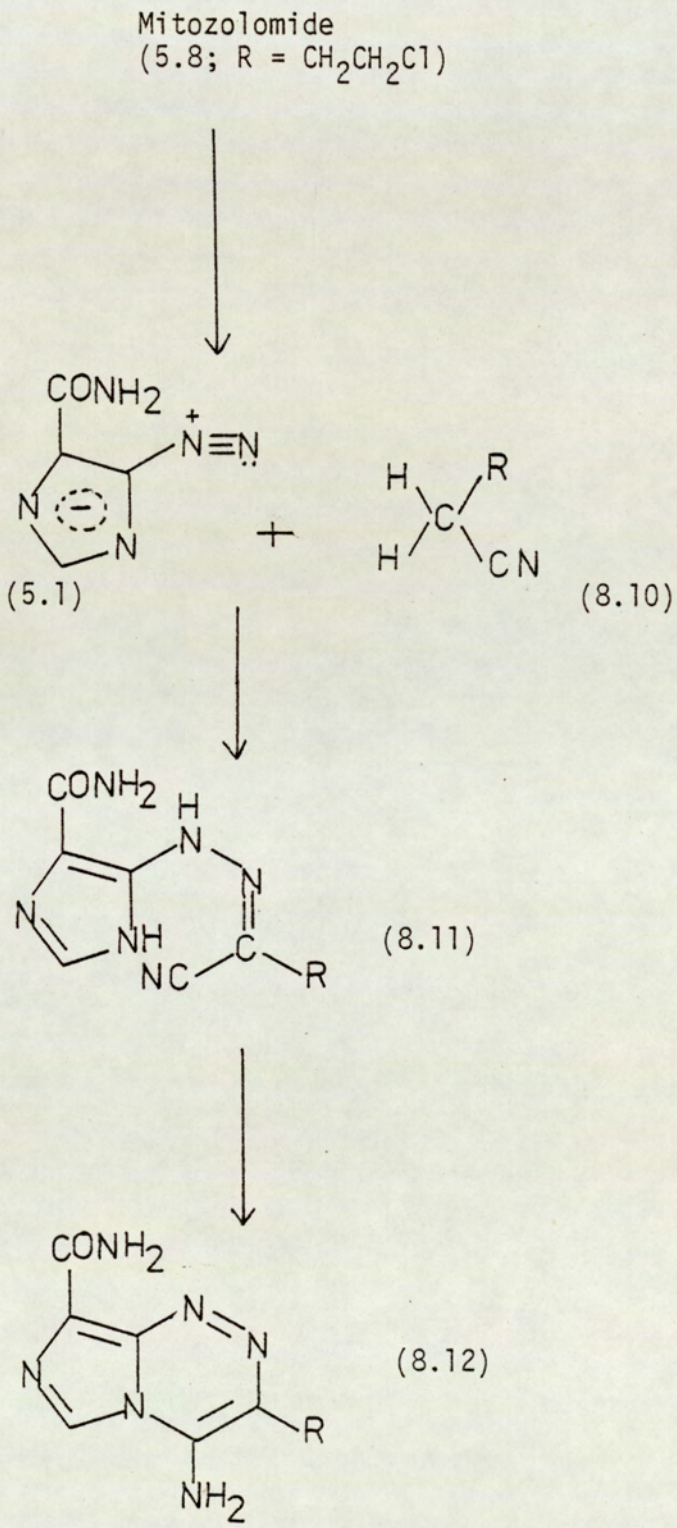
Similarly, when ethyl cyanoacetate or malonitrile were employed as the reactive methylenic substrates, the products were the 4-amino-imidazotriazines (8.12; R = CO_2Et and CN). The amino-ester (8.12; R = CO_2Et) was isolated as an acetic acid solvate. The aminoimidazo-triazines (8.12; R = CO_2Et, CN) were characterised by spectroscopic analysis and their structures confirmed by their identity with the authentic 4-aminotriazines (8.12; R = CO_2Et, CN) previously prepared from the hydrazones (8.11; R = CO_2Et, CN) obtained by coupling diazo-imidazole with ethyl cyanoacetate and malonitrile[76].

Comparative studies with the 3-methyl or 3-ethylimidazotetraz-inones (5.8; R = Me, Et) were not successful as these compounds reacted only very slowly in acetic acid with methylenic compounds to yield traces of imidazotriazines (8.7; 8.12).

Mitozolomide
(5.8; R = CH_2CH_2Cl)



Scheme (8.2)


Mitozolomide
(5.8; R = CH2CH2Cl)
CONH2
N≡N
N
N
(5.1)
H
R
C
H
CN
(8.10)
CONH2
H
N
N
N
NH
NC
C
R
(8.11)
CONH2
N
N
N
R
NH2
(8.12)
R = CO2Et, CN

Scheme (8.3)

8.3 Decomposition of mitozolomide in pyridine

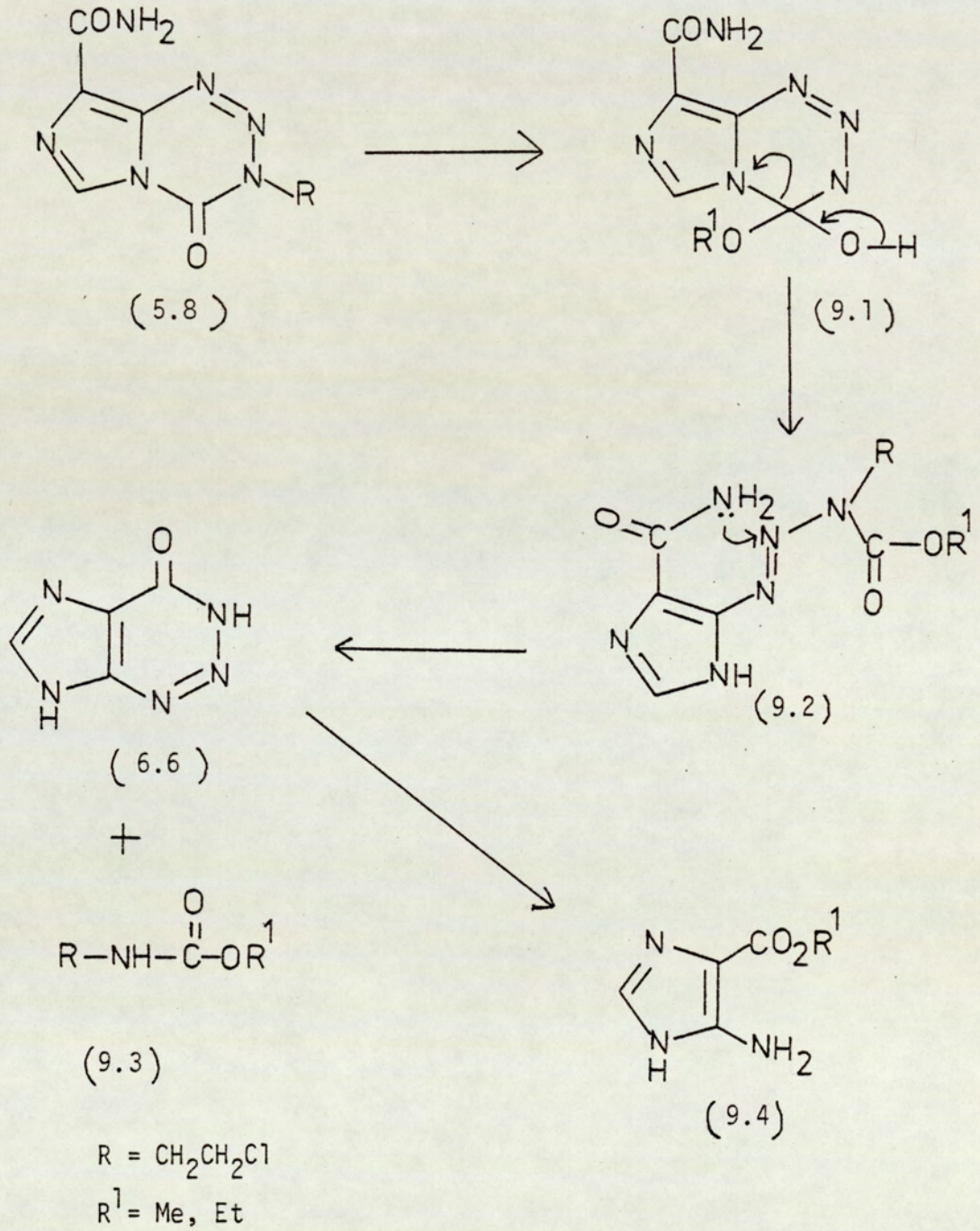
Thermal degradation of the tetrazinone ring to 5-diazoimidazole-4-carboxamide (5.1) was also noted in pyridine. When mitozolomide (5.8; R = CH_2CH_2Cl) was heated in pyridine-ethanol (1:1) with ethyl acetoacetate or ethyl cyanoacetate; the imidazotetrazinones (5.4; R = AC, CN) were formed via the intermediate hydrazones (5.4; Ac, CN). These hydrazones have been obtained independently from the coupling reaction of diazoimidazole carboxamide (5.1) and the respective methylene compound[76] and shown to cyclise to imidazotriazinones in bases (see page 75).

Chapter 9

9.1 Decomposition of imidazotetrazinones in alcohols

Mitozolomide (5.8; R = CH_2CH_2Cl) has been shown[79] to afford 2-azahypoxanthine (6.9), N-(2-chloroethyl)carbamates (9.3; R = CH_2CH_2Cl; R^1 = Me, Et) and traces of 5-aminoimidazole-4-carboxylates (9.4; R^1 = Me, Et) in hot methanol or ethanol; the reaction is greatly accelerated by the presence of aqueous ammonia. The mechanism is believed to involve initial attack by the nucleophiles at C-4 to generate hemiacetals (9.1) which ring-open to unstable triazenes (9.2) and thence undergo intramolecular cyclisation to 2-azahypoxanthine (6.9) with loss of alkyl carbamates (9.3) (Scheme 9.1). Subsequent ring-opening of azahypoxanthine is a reaction with many precedents in the chemistry of 1,2,3-benzotriazin-4(3H)-ones[15,16,104].

The 3-alkyltetrazinones (5.8; R = Me, Et) respectively were stable in boiling methanol for 60 h, 95% remained unreacted. Further decomposition occurred after prolonged heating (10 days). The small amount of unreacted material was destroyed by the addition of aqueous ammonia. The products isolated from both compounds were 2-azahypoxanthine (80%) and a colourless solid. This colourless solid was not observed when the decomposition of the tetrazinones (5.8; R = Me, Et) was carried out in methanolic ammonia at room temperature. The only solid isolated was 2-azahypoxanthine. The colourless solid analysed for $C_6H_8N_4O_3$; in agreement the mass spectrum showed a molecular ion at m/z 184. The ir spectrum indicated the presence of ester and amide carbonyl groups (ν C=O 1758 and 1665 cm^{-1}) together with NH absorptions at 3498, 3398 and 3250 cm^{-1}. The ^1H nmr spectrum

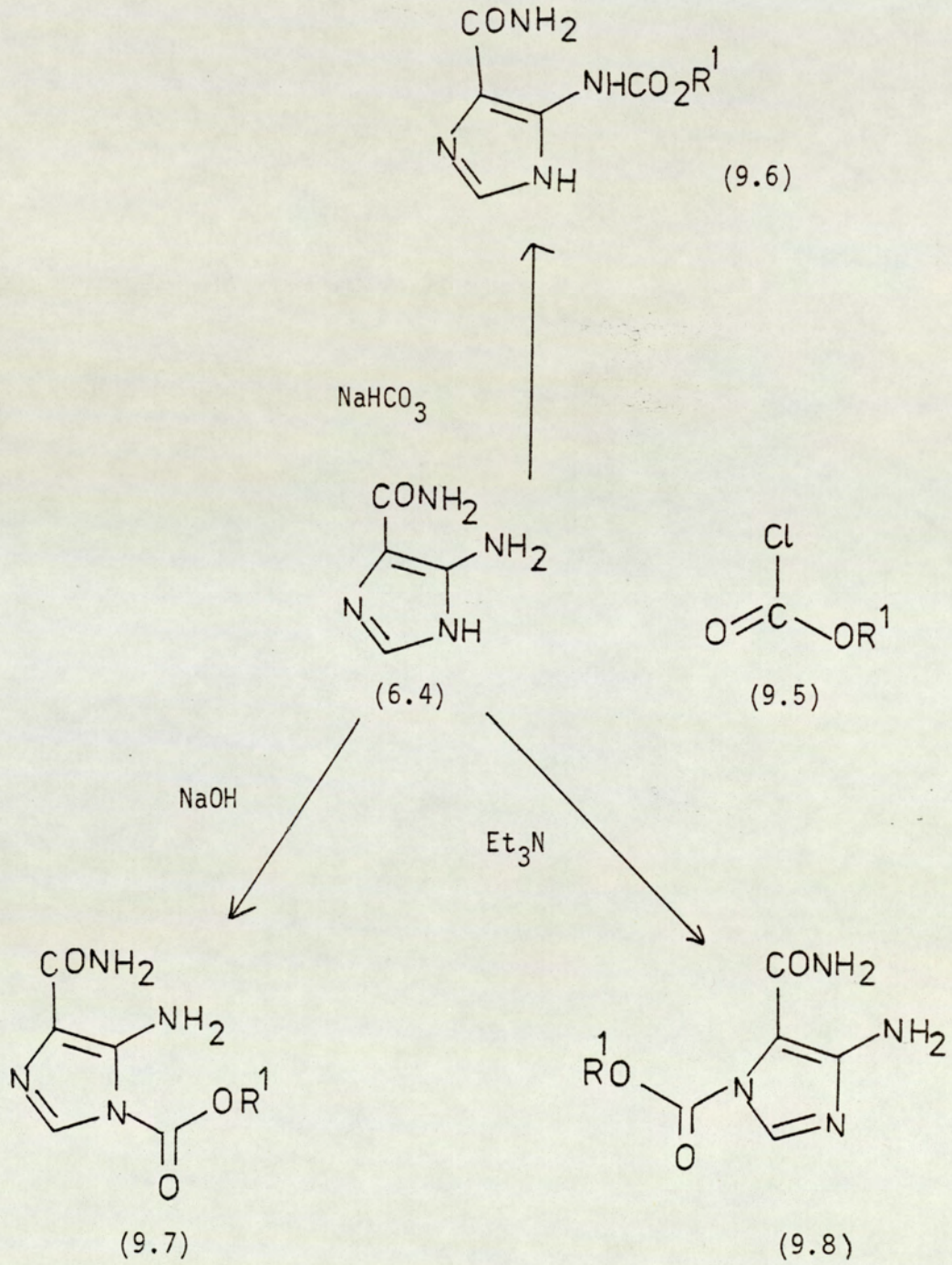

CONH2
R
O
(5.8)
(9.1)
NH2
OR1
(9.2)
(6.6)
+
R—NH—C—OR1
(9.3)
CO2R1
NH2
(9.4)
R = CH2CH2Cl
R1 = Me, Et

Scheme (9.1)

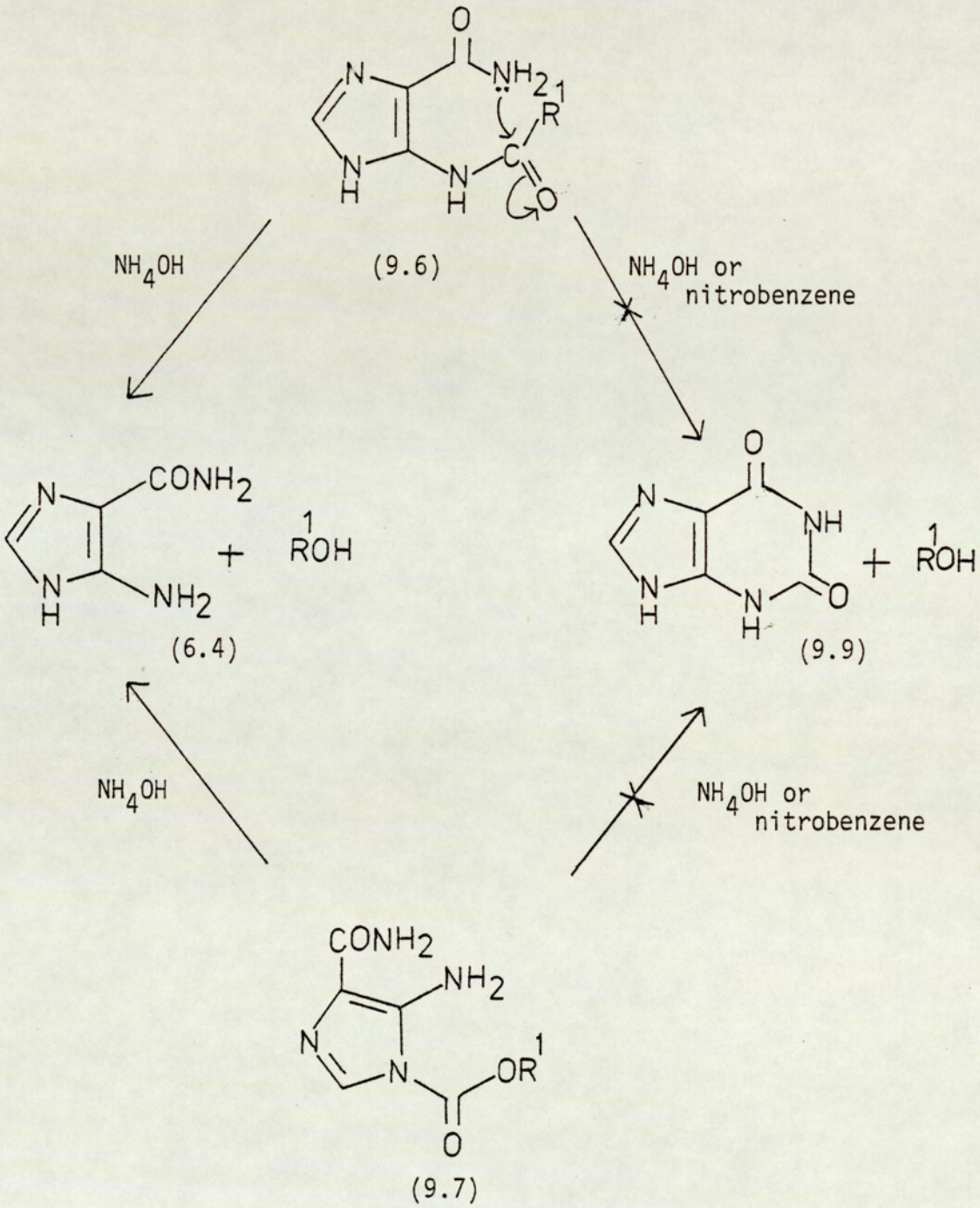
confirmed the presence of a methyl ester resonance at δ 3.92 ppm. Reactions between the tetrazinones (5.8; R = Me, Et) and hot ethanol followed an identical course to give 2-azahypoxanthine (6.9) (80%) and a product $C_7H_{10}N_4O_3$ (m/z 198) with ethyl ester absorptions in its ir and ^1H nmr spectra. The latter ester was identical to the product formed by reacting 5-aminoimidazole-4-carboxamide (6.6) with ethyl chloroformate (9.5) in tetrahydrofuran-aqueous sodium hydroxide and claimed[107] to have structure (9.7; R^1 = Et). Puzzlingly, it was also identical to the product formed between 5-aminoimidazole-4-carboxamide (6.6) and ethyl chloroformate (9.5; R^1 = Et) in aqueous potassium carbonate[108] or in ethanol and triethylamine[109] respectively. Furthermore, the same ethyl ester (9.7; R^1 = Et) could be obtained from the transesterification of the methyl ester (9.5; R^1 = Me) in hot ethanol. It is not possible for the isomeric structure described in the Russian patent[109] (9.8; R^1 = Et) to be formed by simple ring-opening of the imidazotetrazinones (5.8; R = Me, Et) in ethanol.

Structure (9.6; R^1 = Et) was proposed by Shaw for the product synthesised from 5-aminoimidazole-4-carboxamide (6.6) and ethyl chloroformate (9.5; R^1 = Et) in aqueous potassium bicarbonate on the grounds that it cyclised to xanthine (9.9) on melting, in hot nitrobenzene or by heating in concentrated aqueous ammonia at 100 °C. No evidence for structure identification was advanced except that the identity of the xanthine (9.9) was checked by "paper chromatography and spectroscopy".

Contrary to Shaw's observations[108], when the above reactions were re-examined under identical conditions, it was found that the so-called ethyl ester (9.6; R^1 = Et) failed to cyclise to xanthine (9.9) when boiled in nitrobenzene for 10 minutes; crystals of the


CONH2
NHCO2R1
N
NH
(9.6)
NaHCO3
CONH2
NH2
N
NH
(6.4)
Cl
O
C
OR1
(9.5)
NaOH
Et3N
CONH2
NH2
N
N
OR1
O
(9.7)
CONH2
NH2
R1O
N
O
N
(9.8)
R1 = Me, Et

Scheme (9.2)

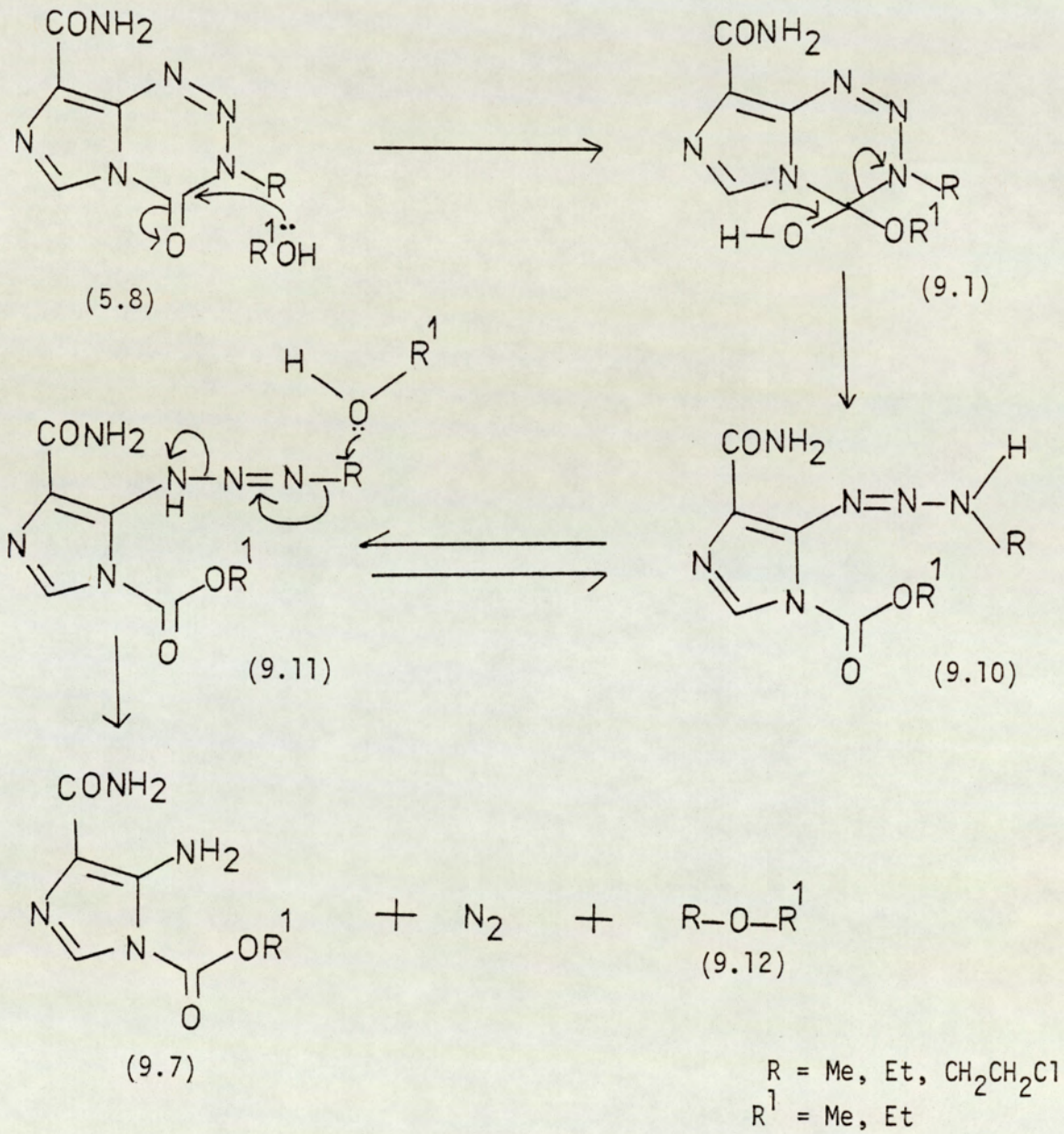

NH4OH
(9.6)
NH4OH or
nitrobenzene
CONH2
NH2
+ R1OH
(6.4)
NH
+ R1OH
(9.9)
NH4OH
NH4OH or
nitrobenzene
CONH2
NH2
OR1
(9.7)

Scheme (9.3)

starting material were recovered unchanged from the cold nitrobenzene solution. The methyl ester behaved similarly and some impure brownish black starting material was recovered from the hot nitrobenzene.

It could be envisaged that nucleophilic attack at C-4 of the tetrazinones (5.8; R = Me, Et) would yield the tetrahedral adducts (9.1; R = R^1 = Me, Et), which then fragment by cleavage of the 3,4-bond to yield unstable 3-alkyltriazenes (9.10; R = R^1 = Me, Et), which suffer further breakdown by nucleophilic attack at the electrophilic alkyl group (Scheme 9.4). The dialkyl ethers (9.12) proposed as by-products have not been isolated but the transformation (9.11)$\rightarrow$(9.7) is fully consistent with the known chemistry of monoalkyltriazenes[86,95].

Thus, breakdown of hemiacetals (9.1) involves exclusively fission of the 4,5-bond when group R is 2-chloroethyl (Scheme 9.1) leading to 2-azahypoxanthine (6.9), whereas cleavage of the 3,4-bond intervenes when R = Me or Et and the 1,4,5-trisubstituted imidazoles (9.7) are formed to a minor extent (Scheme 9.4). It is interesting to note that a crystallographic study has revealed that the C-4 position in a series of imidazotetrazinones is the most electron-deficient nucleus in the ring-system and presumably most vulnerable to nucleophilic attack. Furthermore, the 4,5-bond is the longest and the weakest bond[110]. Definitive bond lengths in hemiacetals (9.1) are not available since these unstable intermediates cannot be isolated for crystallographic analysis. The propensity for heterolysis of the 3,4- or 4,5-bonds is presumably controlled by the steric or electronic effects of substituents R and R^1 and the competitive leaving group affinities of the imidazolyl and triazenyl fragments.


CONH2
N
N
N
N
N
R
O
R1OH
(5.8)
CONH2
N
N
N
N
R
H
O
OR1
(9.1)
H
R1
O
CONH2
N
N
N
N
R
OR1
O
(9.11)
CONH2
N
N
N
H
R
N
N
OR1
O
(9.10)
CONH2
NH2
N
N
OR1
O
(9.7)
+ N2 +
R–O–R1
(9.12)
R = Me, Et, CH2CH2Cl
R1 = Me, Et

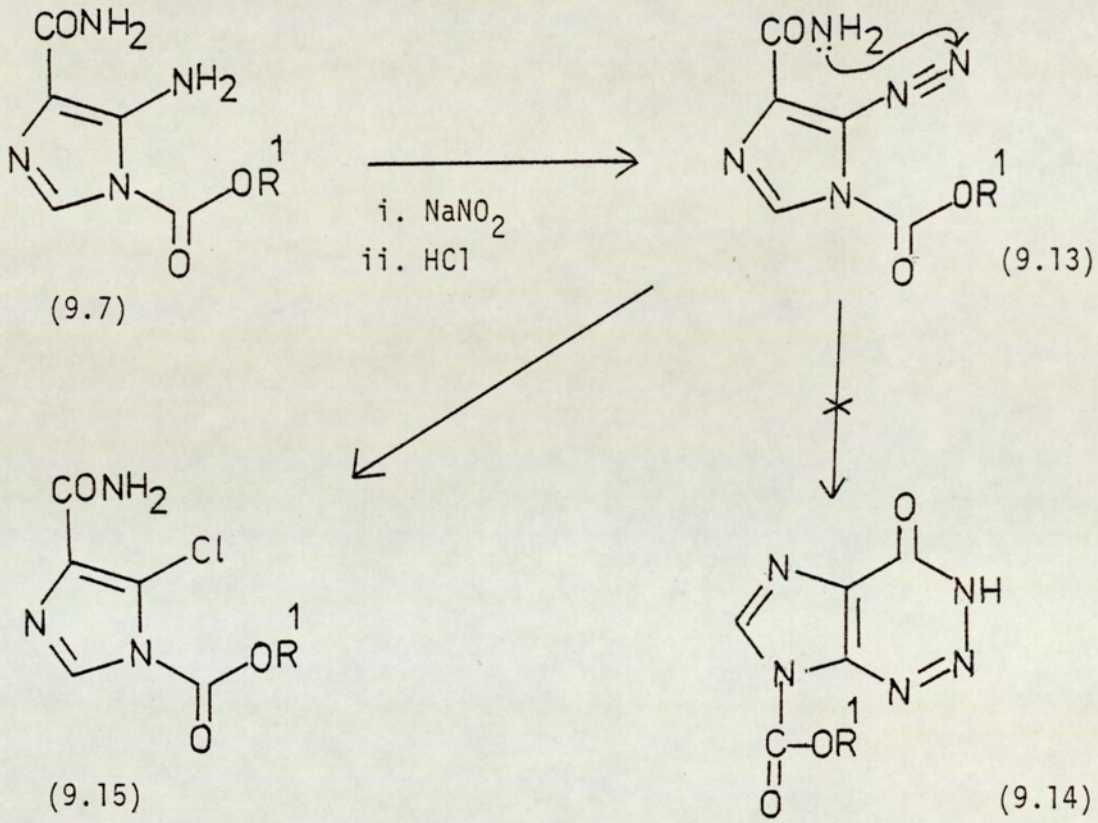
Scheme (9.4)

The presence of a free amino group in the compounds (9.7; R^1 = Me, Et) was confirmed by diazotisation reactions. When a suspension of these compounds in 2N-hydrochloric acid at 0 °C was treated with aqueous sodium nitrite, a bright purple colour was formed followed by deposition of a solid. Ir spectra of the creamy solids showed NH absorptions at 3420, 3305 and 3180 cm^{-1} together with carbonyl absorptions for ester and amide at 1762 and 1678 cm^{-1} respectively. The ^1H nmr spectra showed the corresponding resonances for methyl and ethyl esters. The mass spectra of the compounds derived from the methyl and ethyl esters showed a molecular ion at 203 (205) and 217 (219), respectively, in a ratio 3:1 indicating the presence of chlorine. These compounds analysed correctly as $C_6H_6ClN_3O_3$ and $C_7H_8ClN_3O_3$ and were assigned the structures (9.15; R^1 = Me, Et) respectively.

Diazotisation of 5-aminoimidazole-4-carboxamide (6.6) gives a diazo derivative (5.1) stabilised as a zwitterion by deprotonation of the imidazole ring NH[77]. This possibility is not open to the coloured diazonium species (9.13) formed on diazotisation of the 1-carboalkoxyimidazoles (9.7, R^1 = Me, Et). Whereas 5-diazoimidazole-4-carboxamide (5.1) cyclises readily to 2-aza-hypoxanthine (6.9) in acid or basic conditions[77,91] intramolecular cyclisation of the diazonium salts (9.13; R^1 = Me, Et) to 1-substituted azahypoxanthine (9.14; R^1 = Me, Et) is not observed. Instead, the products (9.15; R^1 = Me, Et) are formed by Sandmeyer-type displacement of the diazonium group by chloride anion (Scheme 9.5).

NB.

While this work was in progress, Russian workers[111] corrected their previous report[109] and confirmed the structure (9.7; R^1 =



R^1 = Me, Et

Scheme (9.5)

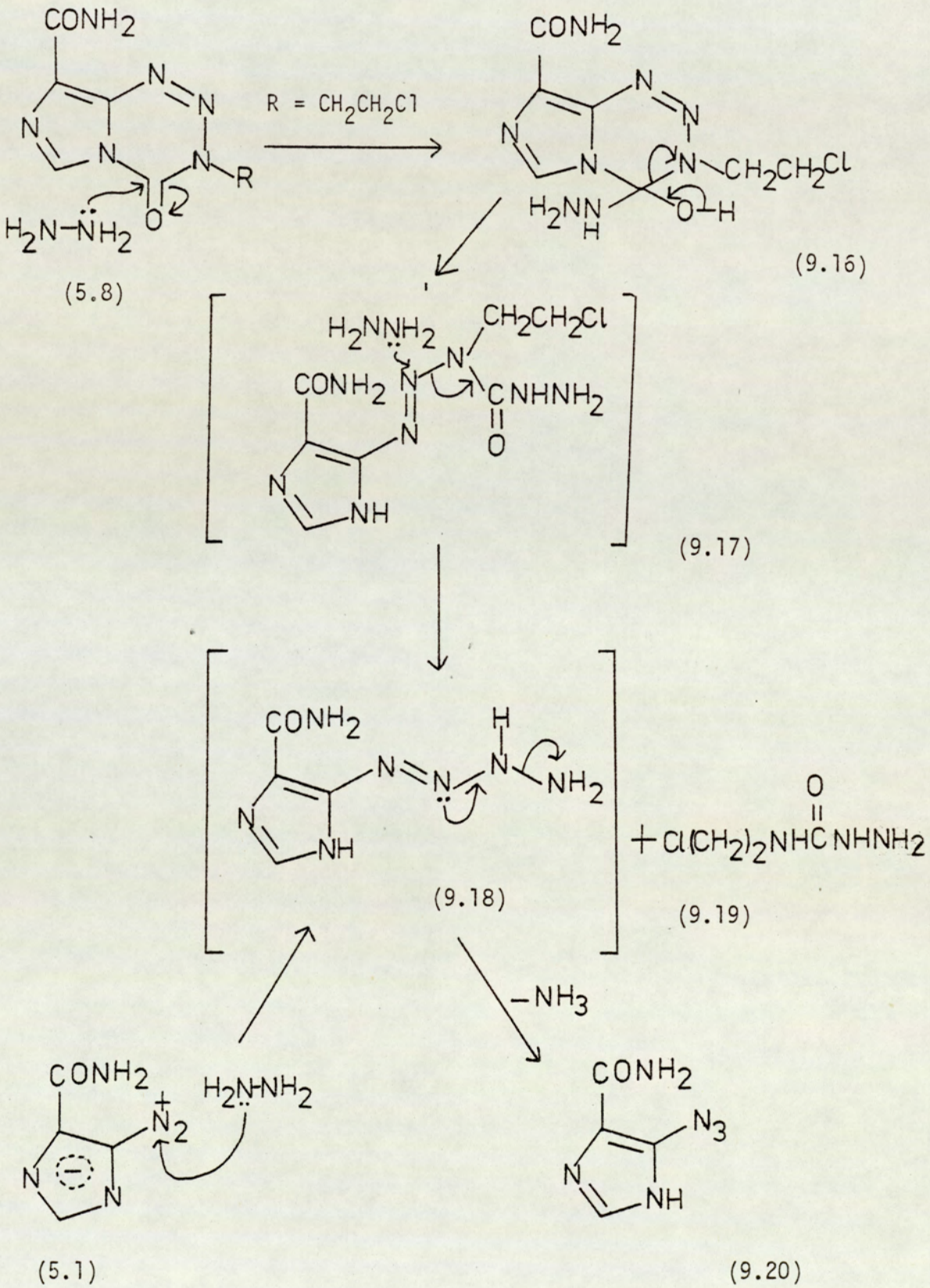
CO_2Me) and excluded the structure (9.8; R^1 = CO_2Me) for the compound obtained from the reaction of 5-aminoimidazole-4-carboxamide and methyl chloroformate.

9.2 Decomposition of imidazotetrazinones in hydrazine

Mitozolomide (5.8; R = CH_2CH_2Cl) effervesced vigorously when treated with hydrazine hydrate at room temperature and produced 5-azidoimidazole-4-carboxamide (9.20)[112,113]. This compound was characterised by a strong absorption at 2148 cm^{-1} in its ir spectrum. An identical compound (9.20) was obtained from the reaction of pure 5-diazoimidazole-4-carboxamide (5.1) and hydrazine hydrate[112].

The reaction is probably initiated by the addition of hydrazine at C-4 of mitozolomide (5.8; R = CH_2CH_2Cl) to form an adduct (9.16) which ring-opens with cleavage of the 3,4-bond leading to triazene (9.17) (Scheme 9.6). Whereas in the presence of weaker nucleophiles an unstable triazene of this type undergoes intramolecular cyclisation to 2-azahypoxanthine (6.9) (Scheme 9.1), hydrazine can intercede to divert the reaction to an unstable tetrazene (9.18) and semicarbazide (9.19). The tetrazene[114] is a feasible intermediate on the pathway to 5-azidoimidazole-4-carboxamide (9.20) since the azide is known to be formed from 5-diazoimidazole-4-carboxamide (5.1) and hydrazine[112].

A similar reaction of hydrazine hydrate with 3-alkyl analogues (5.8; R = Me, Et, n-Pr and CH_2CH_2OMe) produced a gentle effervescence and led to the isolation of an identical white product from each alkyltetrazinone. This product was not 5-azidoimidazole-4-carboxamide since an ir spectroscopic examination of the white compound revealed the absence of the frequency at 2148 cm^{-1}



(5.8) (9.16) (9.17) (9.18) (9.19) (5.1) (9.20)

Scheme (9.6)

corresponding to an azide. The spectrum showed NH absorptions at 3500, 3395, 3320 and 3195 cm^{-1} with carbonyl stretching frequencies at 1710, 1662 and 1638 cm^{-1}.

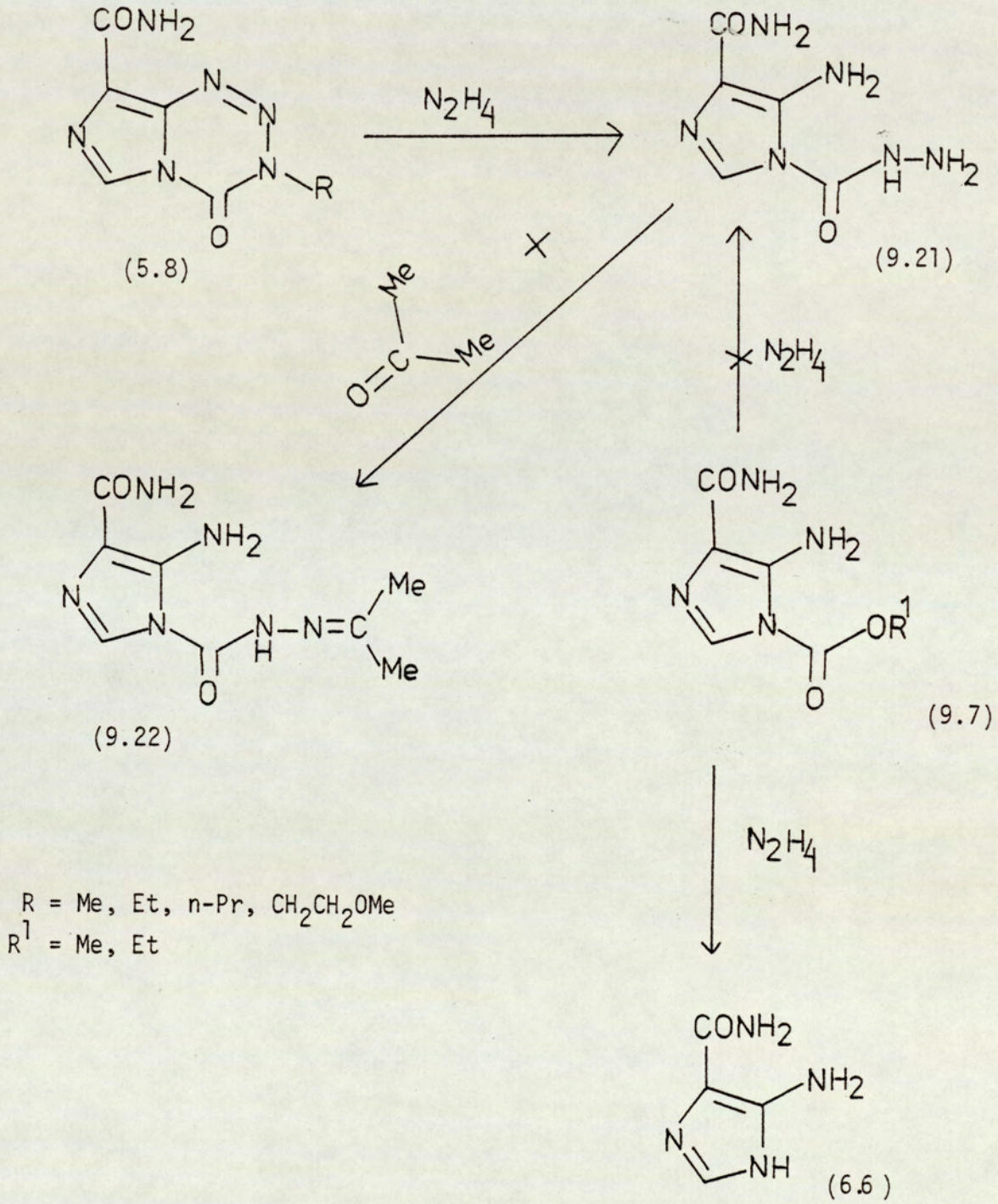
The ^1H nmr spectrum of this compound showed a one proton sharp singlet of imidazole ring proton at δ 7.66 ppm and also showed resonances for seven more protons corresponding to different NH groups at 6.86 and 6.30 ppm; these two broad singlets collapsed after D_2O exchange.

The mass spectrum of this compound showed a molecular ion peak at m/z 184 and substantial ions at m/z 153 and 126 derived from the loss of (M^+-31; NH_2-NH) and a fragment CH_2N_2O (with rearrangement).

On the basis of the above spectral evidence, structure (9.21) was tentatively assigned to the white compound. This assignment was further confirmed by elemental analysis which confirmed a molecular formula $C_5H_8N_6O_2$. Attempts were made to prepare this hydrazide (9.21) from the reaction of imidazole esters (9.7; R^1 = Me, Et) and hydrazine. When these esters were heated in diluted aqueous hydrazine (10%), fusion of the ring-carbamate bond occurred and the product was 5-aminoimidazole-4-carboxamide (6.6).

The structure (9.21) was, however, confirmed by its conversion to a hydrazone (9.22) on treatment with acetone. Surprisingly, reaction with benzaldehyde did not afford a related hydrazone (9.23); instead the product was the benzylidene derivative of 5-amino-imidazole-4-carboxamide (9.24). This structure was corroborated by an independent synthesis between 5-aminoimidazole-4-carboxamide (6.6) and benzaldehyde.

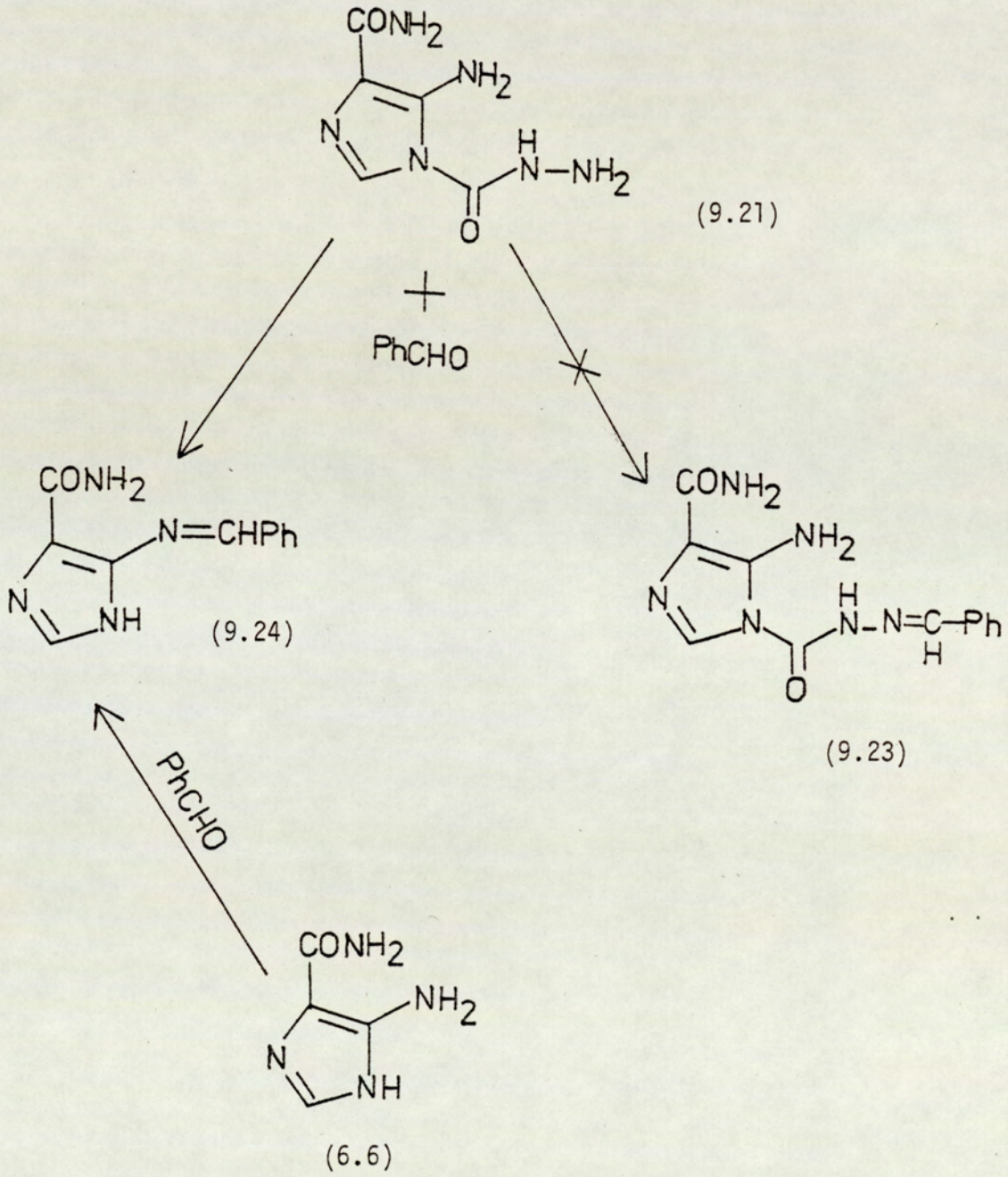
The mechanism for the reaction of 3-alkyltetrazinone (5.8; R = Me, Et, n-Pr and CH_2CH_2OMe) with hydrazine is probably identical to that outlined in Scheme(9.1) except that the attacking nucleophile is hydrazine and not an alcohol (Scheme 9.9).



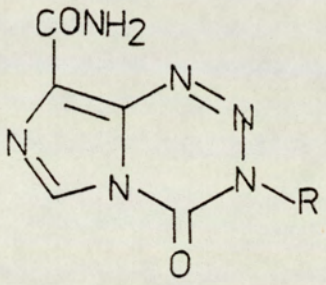
R = Me, Et, n-Pr, CH_2CH_2OMe

R^1 = Me, Et

Scheme (9.7)

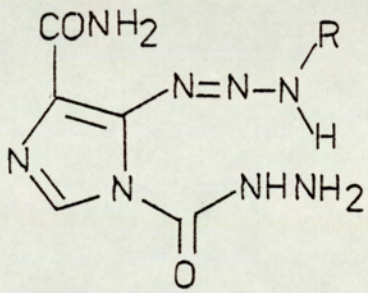

CONH2
NH2
N
N
H
N–NH2
O
(9.21)
PhCHO
CONH2
N=CHPh
N
NH
(9.24)
CONH2
NH2
N
N
H
N–N=C–Ph
H
O
(9.23)
PhCHO
CONH2
NH2
N
NH
(6.6)

Scheme (9.8)

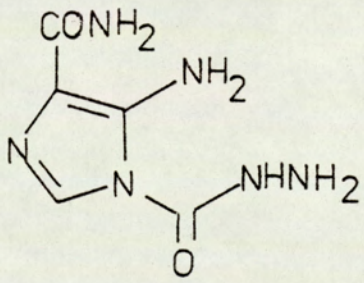


(5.8)

N_2H_4



(9.25)



+ $RNHNH_2 + N_2$

(9.21)

R = Me, Et, n-Pr

CH_2CH_2OMe

Scheme (9.9)

Chapter 10

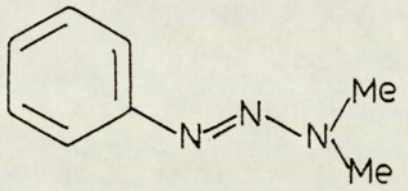
Conclusions

The number of compounds of biological importance with NNN linkages has increased over recent years.

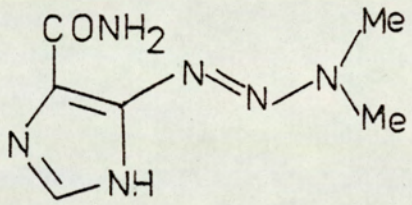
Aryldialkyltriazenes were first reported in the literature by Baeyer[115] as early as 1875, but their tumour inhibitory activity was not discovered until 1955 when Clarke[116] showed that 3,3-dimethyl-1-phenyltriazene (10.1) inhibited the growth of sarcoma 180 in mice.

The clinical use of triazenes, however, stemmed from attempts to design antagonists of 5-aminoimidazole-4-carboxamide (6.6) whose riboside-5'-phosphate derivative is an intermediate in de novo purine synthesis. Shealy[95] synthesised 5-(3,3-dimethyltriazen-1-yl)imidazole-4-carboxamide DTIC (10.2) as a potential antitumour agent. The rationale behind the synthesis of DTIC was to provide a stable "carrier" form of 5-diazoimidazole-4-carboxamide (5.1). DTIC was subsequently shown to be active against many experimental tumours. Again, in spite of a broad spectrum of biological properties, this compound had its own drawbacks mainly owing to its instability. Not surprisingly therefore, further derivatives of triazenes were synthesised and evaluated in attempts to establish structure-activity patterns.

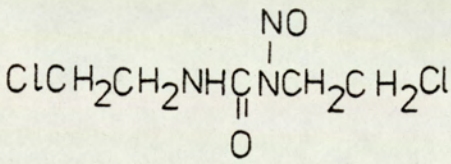
8-Carbamoyl-3-alkylimidazo[5,1-d]-1,2,3,5-tetrazin-4(3H)-ones (5.8; R = Alkyl) were synthesised as cyclic stable variants of the triazenes (6.4). Chemical studies revealed that mitozolomide (5.8; R = CH_2CH_2Cl) can potentially decompose, like the nitrosourea BCNU (10.3), to give 2-chloroethyl isocyanate. Alternatively, mitozolomide can be regarded as a pro-drug of 5-[3-(2-chloroethyl)triazen-1-



(10.1)



(10.2)



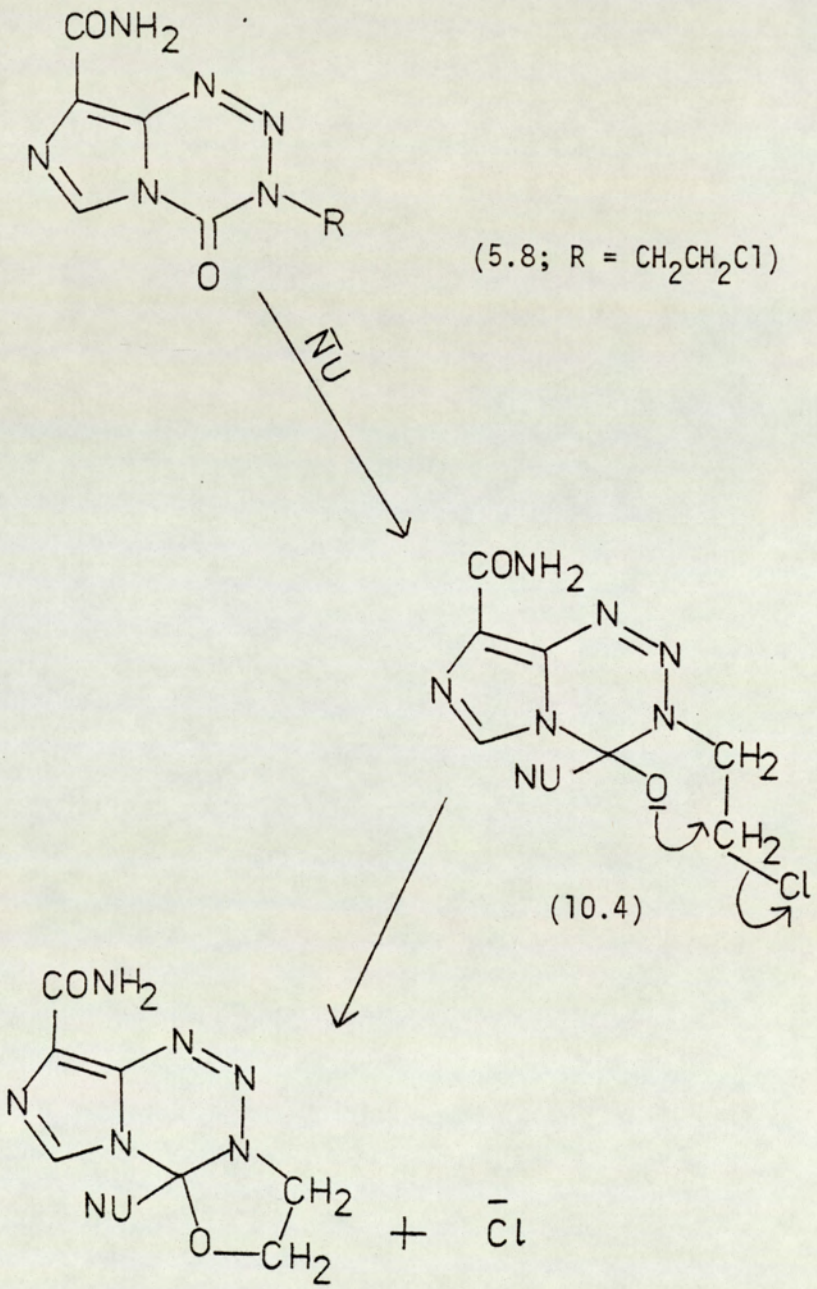
(10.3)

yl]imidazole-4-carboxamide (MCTIC) (6.4; R = CH_2CH_2Cl) into which it decomposes at physiological pH. This observation led Horgan et al[96,97] to investigate the mechanism of the antitumour activity of mitozolomide (5.8; R = CH_2CH_2Cl) in comparison with BCNU (10.3) and MCTIC (6.4; R = CH_2CH_2Cl). Their results confirmed that the most likely metabonate produced from mitozolomide is MCTIC (6.4; R = CH_2CH_2Cl), and it has been shown that this unstable triazene can cross-link DNA[83,85,96]. However, the 3-methylimidazotetrazinone (5.8; R = Me) also has pronounced antitumour properties, but with a different spectrum of activity[117] and it is implausible that this derivative could lead to a species capable of cross-linking DNA.

Comparative chemical studies on mitozolomide and the 3-alkyl analogues (5.8; R = Me, Et, n-Pr and CH_2CH_2OMe) showed some anomalies. Under conditions of basic pH, these tetrazinones yielded the corresponding alkyltriazenes (6.4); however, they reacted differently with hydrazine and alcohols. Mitozolomide appears to cleave in the presence of oxygen and nitrogen nucleophiles exclusively at the C(4)-N(5) bond, whereas the 3-alkyl analogues also fragment at the N(3)-C(4) bond (althogh only to a minor extent in alcohols).

8-Carbamoyl-3-(2-methoxyethyl)imidazo[5,1-d]-1,2,3,5-tetrazin-4(3H)-one (5.8; R = CH_2CH_2OMe), although resembling mitozolomide (5.8; R = CH_2CH_2Cl) in electronic properties and possessing a similar chain length at N(3), behaves more like the 3-alkyl analogues in undergoing cleavage at the N(3)-C(4) bond, at least in the presence of nitrogen nucleophiles (Chapter 9).

The tetrahedral adduct (10.4) formed by the attack of nucleophiles at C(4) in mitozolomide might undergo intramolecular cyclisation to form an oxazolidine intermediate (10.5). Such a



(10.5)

Scheme (10.1)

species can only be formed from the chloroethyl-substituted tetrazine (Scheme 10.1). Possibly, this intermediate might cleave exclusively at the observed bond.

At present, no convincing explanation can be provided for such variations in the mode of reaction of 3-alkylimidazotetrazinones. The behaviour of further 2-haloethyl analogues (5.8; R = CH_2CH_2F, CH_2CH_2Br, CH_2CH_2I) should be investigated to clear up these anomalies.

The studies reported in this thesis were carried out in the expectation that results might provide an insight into a chemical explanation for the different antitumour activities of the structurally-related compounds.

Three other alkyl tetrazinones (5.8; R = Et, n-Pr and CH_2CH_2OMe), which have proved to be inactive in some antitumour tests[118], showed the same chemical properties as 3-methyl tetrazinone (5.8; R = Me). Similarly, 3-(2-chloroethyl)-2,3-dihydro-4-oxoimidazo[5,1-d]-1,2,3,5-tetrazine-8-carboxylic acid (7.6; R = CH_2CH_2Cl) with marginal biological activity, ring-opens in aqueous sodium carbonate solution to yield the corresponding triazene (6.4; R = CH_2CH_2Cl; $CONH_2$ = CO_2H). Further studies of different imidazotetrazinones with variations at the N(3) sidechain and C(8) positions are required to derive a satisfactory structure-activity relationship pattern.

PART III

EXPERIMENTAL RESULTS

Part III

Experimental

1. Ir spectra were recorded as potassium bromide discs on a Unicam SP 200 spectrophotometer.

2. Uv spectra were recorded on a Unicam SP 8000 spectrophotometer (in 95% EtOH).

3. ^{1}H nmr spectra were recorded on a Varian EM360A spectrometer, with tetramethyl silane as internal standard. All the peaks are assigned in terms of δ values. Abbreviations used in the interpretation of the nmr spectra are:
s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet; J = coupling constant (Hz).

4. Mass spectra were measured at 70 eV on a VG Micromass 12B single focusing spectrometer with a source temperature in the range 300 ^{o}C; M^{+} signifies the molecular ion peak observed.

5. Melting points are uncorrected.

Chapter 11

Experimental

Synthesis of 4-anilino-1,2,3-benzotriazines

1-(2-Cyanophenyl)-3-(4-cyanophenyl)triazene (4.7; R = CN)

Sodium nitrite (0.69 g) in water (5 ml) was added dropwise to a stirred suspension of 2-aminobenzonitrile (1.18 g) in concentrated hydrochloric acid (10 ml) at 0 °C over 30 minutes and the mixture was stirred at 0 °C for a further 1 hour. The solution was neutralised to pH 7 with a saturated aqueous solution of sodium acetate. To the buffered solution, 4-aminobenzonitrile (1.18 g) was added and the reaction mixture was stirred for 2 h. The dicyanotriazene (4.7; R=CN) (2.1 g, 85%) was collected by filtration, washed with water and recrystallised from benzene as yellow plates; mp 175-180 °C (efferv.) (lit[7] 175-180 °C (efferv.)).

1-(2-Cyanophenyl)-3-(4-nitrophenyl)triazene (4.7; R = NO_2).

2-Aminobenzonitrile (1.18 g) was diazotised and coupled with 4-nitroaniline (1.38 g) as described above. 1-(2-cyanophenyl)-3-(4-nitrophenyl)triazene (2.4 g, 90%) was recrystallised from benzene as pale yellow plates; mp 240 °C (efferv.) (lit[7] 240-241 °C (efferv.)).

4-(4-Cyanoanilino)-1,2,3-benzotriazine (3.1; R = CN)

Cyanophenyltriazene (4.7; R = CN) (2.0 g) in 70% aqueous ethanol (40 ml) was boiled under reflux for six hours. The solution was cooled and 4-(4-cyanoanilino)-1,2,3-benzotriazine (1.8 g, 90%) was filtered off. Recrystallisation from butanol afforded yellow

needles; mp 237-240 °C (efferv.) (lit[7] 238 °C (efferv.)).

4-(4-Nitroanilino)-1,2,3-benzotriazine (3.1; R = NO_2)

1-(2-Cyanophenyl)-3-(4-nitrophenyl)triazene (2.0 g) in ethanol (40 ml) was heated under reflux for 10 hours. The solution was cooled and 4-(4-nitroanilino-1,2,3-benzotriazine (1.8 g, 90%) was filtered off. Recrystallisation from butanol afforded orange rosettes, mp 237-238 °C (efferv.) (lit[7] 237-238 °C (efferv.)).

Thermolysis of 4-(4-cyanoanilino)-1,2,3-benzotriazines

3-[2-Amino-N-(4-cyanophenyl)benzimidoyl]-4-(4-cyanophenylimino)-3,4-dihydro-1,2,3-benzotriazine (3.8a)

4-(4-Cyanoanilino)-1,2,3-benzotriazine (3.1; R = CN) (4.8 g) was boiled under reflux (7 h) in morpholine (50 ml) and the clear solution was kept at 4 °C for 10 days. The precipitated yellow benzotriazine (3.8a) (1.2 g, 26%) was collected by filtration and washed with acetone and hot toluene and recrystallised from dimethylformamide, mp 246-248 °C (efferv.). (Found: C, 71.84; H, 3.7; N, 23.8; m/z 438.1592 [M^+-N_2]. $C_{28}H_{18}N_8$ requires C, 72.1; H, 3.9; N, 24.0%; m/z 438.1596 [M^+-N_2]); ν_{max} (KBr) 3370 (NH), 2228 cm^{-1} (C≡N).

The filtrate was evaporated to dryness under reduced pressure and a brown viscous residue was obtained which was dissolved in benzene. To the benzene solution light petroleum was added to afford 2-amino-N^2-(4-cyanophenyl)-$NN^{1,1}$-oxydiethylenebenzamidine (3.2c) (3.6 g, 73%) which was identical (mp and ir) to an authentic sample[45]. The NN-oxydiethylenebenzamidine (3.2c) was recovered unchanged after being boiled in morpholine for 3 h.

The same benzotriazine (3.8 a) was also prepared in good yield

by boiling benzotriazine (1.3; R = CN) under reflux in various organic solvents. The reaction conditions and percentage yield are recorded in Table 3.1.

3-[2-Amino-N-(4-nitrophenyl)benzimidoyl]-4(4-nitrophenylimino)-3,4-dihydro-1,2,3-benzotriazine (3.8b)

4-(4-Nitroanilino)-1,2,3-benzotriazine (3.1; R = NO_2) (4.5 g) was boiled under reflux for 1 h in morpholine (20 ml); the benzotriazine slowly dissolved to give a red solution. The cooled solution was shaken with toluene (50 ml) and water (50 ml) and a brown insoluble material (0.1 g) was collected by filtration, washed with acetone and water, and crystallised from dimethylformamide to furnish dihydrobenzotriazine dimethylformamide solvate (3.8b) (0.85, 16%) as ochre rosettes, mp 275 °C (efferv.) (Found: C, 60.2; H, 4.4; N, 22.0; m/z 478.1389 [M^+-N_2]. $C_{26}H_{18}N_8O_4.C_3H_7NO$ requires C, 60.1; H, 4.3; N, 21.8%; m/z 478.1371[$M-N_2$]); ν_{max} (KBr) 3320 (NH), 1665 (C=O), 1530 and 1355 cm^{-1} (NO_2).

2-Amino-N^2-(4-nitrophenyl)-N^1N^1-oxydiethylenebenzamidine (3.2d)

The toluene-water mixture from the above reaction was separated. The aqueous layer was further extracted with toluene (2 x 50 ml). The combined toluene extracts were dried over anhydrous sodium sulphate and evaporated to dryness under reduced pressure. The crude viscous solid was recrystallised from toluene-light petroleum (bp 60-80 °C) to afford 2-amino-N^2-(4-nitrophenyl)-N^1N^1-oxydiethylenebenzamidine (3.2d) (3.6 g, 66%) as yellow leaflets, mp 170-171 °C (Found: C, 62.5; H, 5.7; N, 17.0; M^+, 326. $C_{17}H_{18}N_4O_3$ requires C, 62.6; H, 5.5; N, 17.2%; M^+, 326); ν_{max} (KBr) 3500, 3350 (NH), 1525 and 1350 cm^{-1} (NO_2).

2-Amino-N^2-(4-cyanophenyl)-N^1N^1-tetramethylenebenzamidine (3.2a)

A solution of 4-(4-cyanoanilino)-1,2,3-benzotriazine (3.1; R=CN)[7] (0.50 g) in pyrrolidine (5 ml) was boiled for 8 h. The solution was cooled and triturated with benzene-light petroleum (bp 60-80 °C) to afford the 2-aminobenzamidine (3.2a) (0.4 g, 70%). Crystallisation from toluene-light petroleum (bp 60-80 °C) yielded light feathery crystals, mp 95-97 °C (Found: C, 72.8; H, 6.4; N, 18.7%; M^+, 290. $C_{18}H_{18}N_4$ 0.25 H_2O requires C, 73.1; H, 6.5; N, 19.1%; M^+, 290); ν_{max} (KBr) 3480, 3350 (NH), 2213 cm^{-1} (C≡N).

Synthesis of 4-(4-cyanophenyl)-2-phenylquinazolines

4-(4-Cyanoanilino)-2-(2-nitrophenyl)quinazoline (3.14; R = NO_2)

4-Chloro-2-(2-nitrophenyl)quinazoline (3.17; R = NO_2) (2.85 g) and 4-aminobenzonitrile (1.18 g) were heated under reflux (2 h) in acetone (150 ml) with 10N-hydrochloric acid (0.2 ml). The product, 4-(4-cyanoanilino)-2-(2-nitrophenyl)quinazoline (3.14; R = NO_2) (3.2 g, 87%), was collected by filtration and recrystallised from ethanol-acetic acid (3:1) to yield white crystals, mp 234-235 °C (Found: C, 68.6; H, 3.5; N, 19.1; M^+, 367. $C_{21}H_{13}N_5O_2$ requires C, 68.7; H, 3.5; N, 19.1%; M^+, 367); ν_{max} (KBr) 2240 cm^{-1}. (C≡N); λ_{max} (95% EtOH) 230, 250 (infl.), 275 (infl.), 325 (infl.), 337 and 350 nm.

4-(4-Cyanoanilino)-2-(2-nitrophenyl)quinazoline dihydrochloride (3.14; R = NO_2)

The free base (3.14; R = NO_2) (1.0 g) was stirred at room temperature in 10N-hydrochloric acid (10 ml) to afford the dihydrochloride salt (3.14; R = NO_2) (1.0 g, 71%) which was collected

by filtration, mp 262-265 °C (Found: C, 55.3; H, 3.45; N, 15.3; M^+, 367 as the molecular ion of the base $C_{21}H_{13}N_5O_2$. $2HCl.H_2O$ requires C, 55.1; H, 3.7; N, 15.3%; M^+, 367).

2-(2-Aminophenyl)-4-(4-cyanoanilino)quinazoline (3.15)

4-(4-Cyanoanilino)-2-(2-nitrophenyl)quinazoline free base (3.14; R = NO_2) (0.4 g) was dissolved in acetic acid (120 ml) and subjected to catalytic hydrogenation over 10% palladium/charcoal (0.15 g) at 2-atmospheres. The reaction mixture was filtered through celite and washed with acetic acid (10 ml). The combined filtrate and washings were evaporated to dryness under reduced pressure to afford 2-aminophenylquinazoline (3.15) (0.35 g, 97%), which was crystallised from aqueous acetic acid as a monohydrate; mp 220-225 °C (decomp.) (Found: C, 71.0; H, 4.35; N, 19.9; M^+, 337. $C_{21}H_{15}N_5.H_2O$ requires C, 71.0; H, 4.7; N, 19.75%; M^+, 237); ν_{max} (KBr) 3480, 3350 (NH), 2230 cm^{-1} (C≡N); λ_{max} (95% EtOH) 235, 257 (infl.) 288, 329, 340 (infl.) 353 nm.

8-(4-Cyanophenylimino)-7,8-dihydroquinazolino[3,2-c]-1,2,3-benzotriazine (3.12)

A solution of the aminophenylquinazoline (3.15) (0.17 g) in 5N-sulphuric acid (5 ml) was diazotised with a solution of sodium nitrite (0.07 g) in water (3 ml) at 0 °C. The mixture was stirred for 2 h at 0 °C then basified with concentrated aqueous ammonia. After 1 h, the quinazolinobenzotriazine (3.12) (0.16 g, 90%) was collected by filtration and crystallised from ethanol and had mp 205-210 °C (efferv.) (Found: C, 69.8; H, 3.5; N, 22.7; M^+, 348. $C_{21}H_{12}N_6.0.75H_2O$ requires C, 69.8; H, 3.7; N, 23.2%; M^+, 348); ν_{max} (KBr) 2220 cm^{-1} (C≡N); λ_{max} (95% EtOH) 250, 278 (infl.), 340 (infl.), 352 nm.

4-(4-Cyanoanilino)-2-phenylquinazoline (3.14; R = H)

The quinazoline (3.14; R = H) was synthesised by the following methods:-

(i) 4-Chloro-2-phenylquinazoline (3.17; R = H) (1.2 g) and 4-aminobenzonitrile (1.18 g) were boiled under reflux in acetone (150 ml) containing 10N-hydrochloric acid (0.2 ml) for 1 h. The precipitated buff solid (1.6 g, 95%) was collected by filtration, washed and recrystallised from acetic acid to afford the phenylquinazoline (3.14; R = H), mp 206-207 °C (Found: C, 76.4; H, 4.65; N, 16.5; M^+, 322.12184. $C_{21}H_{14}N_4$. 0.5 H_2O requires C, 76.1; H, 4.5; N, 16.9%; M^+, 322.121199); ν_{max} (KBr) 2225 cm^{-1} (C≡N); λ_{max} (95% EtOH) 256, 279, 326, 340 and 352 nm.

(ii) The same 4-(4-cyanoanilino)-2-phenylquinazoline (3.14; R=H) (90%) was formed when compound (3.8a) (0.3 g) was boiled under reflux in acetic acid (6 ml) for 6 h or in a mixture of acetic acid-ethanol (4:1) for 48 h.

(iii) The identical (mp, ir and uv) quinazoline (3.14; R = H) (90%) was also obtained when 8-(4-cyanophenylimino)-7,8-dihydroquinazolino[3,2-c]-1,2,3-benzotriazine (3.12) (0.3 g) was heated under reflux in ethanol (20 ml) for 24 h.

4-(4-Cyanoanilino)-2-phenylquinazoline dihydrochloride

The quinazoline free base (3.14; R = H) (0.15 g) was suspended in 10N-hydrochloric acid (1.0 ml) and stirred for 10 minutes at room temperature. The dihydrochloride (3.14.2HCl; R = H) (0.15 g, 79%)

was collected by filtration and had mp 270-272 °C (Found: C, 64.0; H, 4.1; N, 14.3; M^+, 322 as the molecular ion of the free base. $C_{21}H_{14}N_4.2HCl$ requires C, 63.95; H, 4.06; N, 14.2%; M^+, 322); ν_{max} 2220 cm^{-1} (C≡N); λ_{max} (95% EtOH) 250, 270, 324, 336 and 342 nm.

4-(4-Cyanoanilino)-2-(2-hydroxyphenyl)quinazoline (3.14; R = OH)

This compound (3.14, R = OH) was prepared by the following methods:-

(i) 4-Chloro-2-(2-hydroxyphenyl)quinazoline (3.17; R = OH) (1.20 g) and 4-aminobenzonitrile (1.18 g) were boiled under reflux in acetone (150 ml) containing 10N-hydrochloric acid (0.2 ml) for 1 h. The reaction mixture was cooled to afford the hydroxyphenylquinazoline (3.14; R = OH) (1.6 g, 90%) which was collected by filtration and washed with aqueous ammonia and recrystallised from aqueous acetic acid as a monohydrate mp 212-215 °C (decomp.) (Found: C, 70.65; H, 4.4; N, 15.9; M^+, 338. $C_{21}H_{14}N_4O.H_2O$ requires C, 70.8; H, 4.5; N, 15.7%; M^+, 338); ν_{max} (KBr) 3450, br(OH), 2230 cm^{-1} (C≡N); λ_{max} (95% EtOH) 258, 278 (infl.), 325 (infl.), 339, 354 nm.

(ii) The same hydroxyphenylquinazoline (3.14; R = OH) (0.25 g and 0.20 g respectively) was formed when benzotriazine (3.8a) (0.5 g) was boiled under reflux in 2N-hydrochloric acid or in 1N-sulphuric acid (10 ml) for 1 h and had mp 212-215 °C (from aqueous acetic acid).

(iii) An identical (ir, uv and mp) sample of 2-hydroxyphenyl-

quinazoline (3.14; R = OH) (0.28 g) was obtained when 8-(4-cyanophenylimino)-7,8-dihydroquinazolino[3,2-c]-1,2,3-benzotriazine (3.12) (0.5 g) was heated under reflux in 2N-sulphuric acid (10 ml) for 1 h.

4-(4-Cyanoanilino)-2-(2-iodophenyl)quinazoline (3.14; R = I)

The benzotriazine (3.8a) (0.26 g) was heated under reflux with sodium iodide (0.7 g) in acetic acid (10 ml) for 2.5 h. The cooled reaction mixture deposited a violet solid, which was collected by filtration. The violet solid yielded the yellow iodophenylquinazoline (0.18 g, 75%) when rinsed with acetone. Recrystallisation from 95% ethanol gave golden crystals of the monohydrate, mp 230-232 °C (Found: C, 53.6; H, 3.3; M^+, 447. $C_{21}H_{13}IN_4.H_2O$ requires C, 54.0; H, 3.2%; M^+, 447); ν_{max} (KBr) 2220 cm^{-1} (C≡N); λ_{max} (95% EtOH) 260, 288, 330, 340 and 355 nm (infl.).

The same (ir and uv) iodophenylquinazoline (0.18 g, 75%) (mp 230- 232 °C) was formed when the benzotriazine (3.8a) (0.26 g) was heated under reflux in acetic acid (10 ml) containing hydroiodic acid (57% aqueous solution; 1 ml) for 20 minutes.

8-(4-Cyanophenylimino)-7,8-dihydroquinazolino[3,2-c]-1,2,3-benzotriazine (3.12) (0.3 g) was heated under reflux with hydriodic acid (1 ml) in acetic acid (10 ml) for 20 minutes. The iodophenylquinazoline (0.22 g, 72%) was isolated and had mp 230-232 °C with identical ir and uv.

2-(2-Bromophenyl)-4-(4-cyanoanilino)quinazoline (3.14; R = Br)

(i) The benzotriazine (3.8a) (0.4 g) was heated under reflux in acetic acid (5 ml) and 45% hydrogen bromide in acetic acid

(1 ml) for 1 h. The crude bromophenylquinazoline dihydrobromide (0.32 g, 90%) was collected by filtration and was purified by acetone reprecipitation from an aqueous solution, mp 260 °C (decomp.) (Found: C, 44.7; H, 2.6; M^+, 399 (401). $C_{21}H_{13}BrN_4.2HBr$ requires C, 44.7; H, 2.6%; M^+, 399 (401) as the molecular ion of the base); ν_{max} (KBr) 2220 cm^{-1} (C≡N); λ_{max} (95% EtOH) 244, 260 (infl.), 280 (infl.) 325 (infl.), 341 and 355 nm (infl.).

(ii) The same (ir and uv) bromophenylquinazoline dihydrobromide (0.2 g, 71%) was isolated when the quinazolinobenzotriazine (2.12) (0.25 g) was heated in hydrobromic-acetic acid under the conditions described above, and had mp 260 °C (decomp.).

The free base, mp 166-168 (from ethanol), was formed when the dihydrobromide salt was neutralised with dilute aqueous ammonia (Found: C, 59.3; H, 3.7; N, 13.7; M^+, 399 (401). $C_{21}H_{13}BrN_4.1.5\ H_2O$ requires C, 58.9; H, 3.7; N, 13.1%; M^+, 399 (401)); ν_{max} (KBr) 2210 cm^{-1} (C≡N); λ_{max} (95% EtOH) 240, 256, 280 (infl.) 320 (infl.), 340 and 350 nm.

2-(2-Azidophenyl)-4-(4-cyanoanilino)quinazoline (3.14; R = N_3)

(i) A mixture of the benzotriazine (3.8a) (0.26 g) and sodium azide (0.065 g) was boiled under reflux in acetic acid (10 ml) for 3 h. The orange product was collected by filtration and washed with water and dried at room temperature. The crude 2-azidophenylquinazoline (0.15 g, 58%) had mp 210-212 °C (efferv.) (Found: M^+, 363. $C_{21}H_{13}N_7$ requires M^+, 363); ν_{max} (KBr) 2250 (C≡N), 2140 and

2160 cm^{-1}) (N_3); λ_{max} (95% EtOH) 235, 256 (infl.) 287, 325 (infl.), 338, 353 nm (infl.).

Repeated crystallisation of the azide from aqeuous acetic acid afforded a pure sample of 2-(2-aminophenyl)-4-(4-cyanoanilino)quinazoline hydrate (3.15), mp 220-225 °C (decomp.) identical to a sample described above (page 127).

(ii) When the quinazolinobenzotriazine (3.12) (0.20 g) was heated under reflux with sodium azide (0.08 g) in acetic acid (10 ml) it yielded an orange azidoquinazoline (0.16 g, 80%). This was transformed to the corresponding amine (3.15) on recrystallisation from aqueous acetic acid.

4-(4-Cyanoanilino)-2-[2-(2-naphthol-2-ylazo)]phenylquinazoline (3.14; R = 2-hydroxynaphth-1-ylazo)

(i) The benzotriazine (3.8a) (0.35 g) and 2-naphthol (0.15 g) were boiled under reflux in acetic acid (10 ml) for 2 h. The orange azophenylquinazoline (0.3 g, 83%) was collected by filtration, crystallised from ethanol and had mp 320° (decomp.) (Found: C, 73.3; H, 3.9; N, 17.0. $C_{31}H_{20}N_6O.H_2O$ requires C, 73.0; H, 4.3; N, 16.5%); ν_{max} (KBr) 2240 cm^{-1} (C≡N).

(ii) The same (ir and uv) azonaphthylquinazoline (0.20 g, 58%) was formed when the quinazolinobenzotriazine (3.12) (0.25 g) was heated under reflux with 2-naphthol (0.15 g) in acetic acid (10 ml) as above.

4-Hydroxy-2-phenylquinazoline (3.16; R = H)

N-(2-cyanophenyl)benzamide (5.0 g) in dioxan (15 ml) and 20% aqueous sodium hydroxide (100 ml) was refluxed for 1 h with 30% hydrogen peroxide (60 ml). Further hydrogen peroxide (25 ml) was added, and the refluxing was continued for 30 minutes. Water (500 ml) was added and the solution was neutralised with acetic acid and was made alkaline with aqueous ammonia. The precipitated 4-hydroxy-2-phenylquinazoline (3.45 g, 70%) was recrystallised from toluene and had mp 135 °C (Lit[66] 135-136 °C).

Similarly prepared 4-hydroxy-2(2-nitrophenyl)quinazoline (3.16; R = NO_2) (85%) had mp 227-228 °C (lit[67] 227-228) and 4-hydroxy-2-(2-hydroxyphenyl)quinazoline (3.16; R = OH) (80%) had mp 297-299 °C (lit[66] 297-298 °C).

4-Chloro-2-phenylquinazoline (3.17; R = H)

4-Hydroxyphenylquinazoline (3.16; R = H) (1.0 g) was boiled under reflux in phosphorus oxychloride (20 ml) for 3 h. The phosphorus oxychloride was removed by distillation under reduced pressure. The brown oil was poured on to crushed ice. The solid was crystallised from toluene to afford white crystals (0.08 g, 68%) of 4-chloro-2-phenylquinazoline mp 127-128 °C (lit[71] 127.5-128.5 °C). 4-Hydroxy-2-(2-nitrophenyl)quinazoline (3.17; R = NO_2) when boiled in phosphorus oxychloride yielded 4-chloro-2-(2-nitrophenyl)quinazoline (31.7; R = NO_2) (0.75 g, 65%); mp 180 °C (lit[67] 179-181 °C).

4-Hydroxy-2-(2-hydroxyphenyl)quinazoline (31.6; R = OH) when refluxed as above in phosphorus oxychloride afforded 4-chloro-2-(2-hydroxyphenyl)quinazoline (3.17; R = OH) (0.68 g, 65%); mp 230-232 °C (lit[67] 230-232 °C).

Reactions of 4-anilino-1,2,3-benzotriazines with formamides

3-(4-Cyanophenyl)quinazolin-4(3H)-one (4.14; R = CN)

(i) A mixture of 4-(4-cyanoanilino)-1,2,3-benzotriazine[7] (3.1; R = CN) (2.0 g) and formamide (20 ml) was boiled under reflux (0.5 h). The benzotriazine dissolved with effervescence to yield a brown solution. The solution was cooled, diluted with water and the white crystalline solid was collected (1.80 g, 85%). The quinazoline was recrystallised from aqueous dimethylformamide and had mp 258-260 °C (Found: C, 72.6; H, 3.7; N, 16.95; M^+, 247. $C_{15}H_9N_3O$ requires C, 72.9; H, 3.6; N, 17.0%; M^+, 247); ν_{max} (KBr) 2220 (C≡N), 1690 cm^{-1} (C=O); δ (trifluoroacetic acid) 9.50 (1H, s, H-2), 8.6-7.7 ppm (8H, m, aromatic).

(ii) The same (ir and ^1H nmr) quinazolinone (85%) was formed when the benzotriazine (3.1; R = CN) (1.0 g) was boiled (0.5 h) in N-methylformamide (10 ml); mp 258-260 °C.

(iii) 2-Amino-N-(4-cyanophenyl)benzamide (4.10; R = CN) (1.0 g) was boiled in formamide (10 ml) for 0.5 h. The reaction mixture was cooled and diluted with water to yield the same quinazoline (90%) (ir and ^1H nmr); mp 258-260 °C.

3-(4-Nitrophenyl)quinazolin-4(3H)-one (4.14; R = NO_2)

A mixture of 4-(4-nitroanilino)-1,2,3-benzotriazine[7] (3.1; R = NO_2) (2.0 g) and formamide (20 ml) (or N-methylformamide [20 ml]) was boiled under reflux (0.5 h). The reaction mixture was cooled and

diluted with water to afford a white crystalline solid (1.85 g, 80%). The resulting quinazoline had mp 265-167 °C (Found: C, 62.8; H, 3.2; N, 15.7; M^+, 267. $C_{14}H_9N_3O_3$ requires C, 62.9; H, 3.4; N, 15.7%; M^+, 267); ν_{max} (KBr) 1690 (C=O), 1525 and 1355 cm^{-1} (NO_2); δ (trifluoroacetic acid) 9.52 (1H, s, H-2), 8.7-7.9 ppm (8H, m, aromatic).

3-Phenylquinazolin-4(3H)-one (4.14; R = H)

4-Anilino-1,2,3-benzotriazine (3.1; R = H) in hot formamide similarly yielded the quinazolinone (50%); mp 138-140 °C (lit[74] 139 °C).

2-Amino-N-(4-cyanophenyl)benzamide (4.10; R = CN)

To a solution of N-(4-cyanophenyl)-2-nitrobenzamide (1.30 g) in ethanol (50 ml), 10% palladium/charcoal (0.30 g) was added. The reaction mixture was shaken (1 h) with hydrogen at atmospheric pressure. The filtrate, after evaporation to dryness under reduced pressure yielded a cream solid (0.92 g, 80%); mp 194-196 °C (from ethanol). (Found: C, 70.7; H, 4.7; N, 17.6; M^+, 237. $C_{14}H_{11}N_3O$ requires C, 70.9; H, 4.6; N, 17.7%; M, 237); ν_{max} (KBr) 2225 (C≡N), 1660 and 1662 cm^{-1} (C=O).

Decomposition of imidazotetrazinones in water

The 3-alkyltetrazinones (5.8; R = Me, Et) (0.2 g) were boiled under reflux (0.5 h) in water (10 ml). The tetrazinones dissolved with effervescence and pink solutions were obtained. The solutions, when evaporated to dryness under reduced pressure yielded 5-amino-imidazole-4-carboxamide (6.6) (0.09 g, 70%), mp 120 °C; ν_{max} (KBr) 3450, 3250 br (bonded NH), 1682 cm^{-1} (C=O); δ (DMSO-d_6) 7.2 (1H, s,

H-2), 6.68 ppm (2H, brs, NH_2). These spectra were identical with the spectra of an authentic sample obtained by the neutralisation of the hydrochloride salt of 5-aminoimidazole-4-carboxamide (6.6) with concentrated ammonia.

3-(2-Chloroethyl)imidazo[5,1-d]-1,2,3,5-tetrazin-4(3H)-one (5.8; R = CH_2CH_2Cl) (0.24 g) dissolved with effervescence when boiled under reflux (0.5 h) in water (10 ml) and yielded an insoluble maroon solid, 4-carbamoyl-2-(4-carbamoylimidazol-5-ylazo)imidazolium-5-olate (6.10; R = OH) (0.01 g, 8%), mp >350 °C (decomp.) (lit[93] >350 °C (decomp.); ν_{max} (KBr) 3375 br (bonded NH, OH), 1658 cm^{-1} (C=O); λ_{max} (dimethylformamide) 550 nm. The filtrate, after evaporation to dryness under reduced pressure followed by washing with ether, yielded 5-aminoimidazole-4-carboxamide (6.6) (0.07 g, 58%) which was identical (ir and uv) with authentic sample (6.6).

Traces of 2-azahypoxanthine (6.9) were detected by tlc (silica/ toluene-acetone).

Decomposition of imidazotetrazinones in aqueous sodium carbonate solution

5-(3-Methyltriazen-1-yl)imidazole-4-carboxamide (6.4; R = Me)

A mixture of 3-methylimidazo[5,1-d]-1,2,3,5-tetrazin-4(3H)-one (5.8; R = Me) (0.20 g) and 5% aqueous sodium carbonate (10 ml) was stirred at room temperature for 20 minutes. A buff solid (0.09 g, 52%) was collected by filtration and washed with cold water. The triazene (6.4; R = Me); mp 178 °C (explodes!); ν_{max} (KBr) 3398, 3250 (NH) and 1670 cm^{-1} (C=O); λ_{max} (95% EtOH) 326, 232 nm.; δ (DMSO-d_6) 7.68 (1H, s, H-2) 7.5 (br 2H, s, NH_2) and 3.0 ppm (3H, s, CH_3). This compound was identical with a sample prepared independently by coupling 5-diazoimidazole-4-carboxamide (5.1) with methylamine in

ethylacetate at room temperature in the dark, mp 176-178 °C (lit mp 175-180 °C)[95].

5-(3-Ethyltriazen-1-yl)imidazole-4-carboxamide (6.4; R = Et)

The triazene (6.4; R = Et) (0.1 g, 58%) was obtained similarly from the reaction of 3-ethylimidazo[5,1-d]-1,2,3,5-tetrazin-4(3H)-one (5.8; R = Et) (0.20 g) and 5% aqueous sodium carbonate solution (10 ml). The identical triazene (85%) was prepared by coupling 5-diazoimidazole-4-carboxamide (5.1) with ethylamine in ethyl acetate, mp 156 °C (explodes!) (lit 154-158 °C)[95]; ν_{max} (KBr) 3500, 3295 (NH), 2650 cm^{-1} (C=O); λ_{max} (95% EtOH) 325 and 234 nm; δ (DMSO-d_6) 8.6 (1H, s, H-2), 7.5 (2H, brs, NH_2), 4.5 (2H, q, J=7Hz, $\underline{CH_2}$) and 1.3 ppm (3H, t, J=6Hz, $\underline{CH_3}$).

5-[3-(2-Chloroethyl)triazen-1-yl]imidazole-4-carboxylic acid (6.4; R = CH_2CH_2Cl; $CONH_2$ = COOH)

The triazene carboxylic acid (6.4; R = CH_2CH_2Cl; $CONH_2$ = COOH) (0.02 g, 11%) was obtained from the reaction of ethyl 3-(2-chloroethyl)-2,3-dihydro-4-oxoimidazo[5,1-d]-1,2,3,5-tetrazine-8-carboxylate (7.8; R = CH_2CH_2Cl) (0.20 g) and 5% aqueous sodium carbonate solution (10 ml) and had mp 148-151 °C (explodes!); ν_{max} (KBr) 3400 cm^{-1} br (bonded OH), 1720 cm^{-1} (C=O); λ_{max} (95% EtOH) 326 and 234 nm; M^+, m/z 217 (219), 181 (M^+ - HCl), 168 (M^+ - CH_2Cl) and m/z 122.

Nitration of imidazo[5,1-d]-1,2,3,5-tetrazin-4(3H)-ones

3-(2-Chloroethyl)-8-(N-nitrocarbamoyl)imidazo[5,1-d]-1,2,3,5-tetrazin-4(3H)-one (7.2; R = CH_2CH_2Cl)

To a stirred solution of mitozolomide (5.8; R = CH_2CH_2Cl) (0.24 g) in concentrated sulphuric acid (2.5 ml) at 0 °C, concentrated nitric acid (d = 1.42; 1 ml) was added dropwise. The solution was maintained at 0 °C for 1 h and then was poured on to crushed ice. The precipitated solid was collected by filtration, washed with cold water and recrystallised from aqueous acetone, to give the nitroamide (0.26 g, 93%) as colourless crystals, mp 160-161 °C (efferv.) (Found: C, 28.6; H, 1.99; Cl, 12.0; N, 33.6; M^+, 287 (289). $C_7H_6ClN_7O_4$. 0.25 H_2O requires C, 28.8; H, 2.1; Cl, 12.1; N, 33.6%; M^+, 287 (289); ν_{max} (KBr) 3200 (NH), 1750, 1720 and 1620 cm^{-1} (C=O); λ_{max} (95% EtOH) 326 nm; δ (DMSO-d_6) 9.05 (1H, s, H-6), 8.25 (1H, brs, NH); 4.70 (2H, t, J=6Hz, $CH_2\underline{CH_2}Cl$) and 4.05 ppm (2H, t, J=6Hz, $\underline{CH_2}CH_2Cl$).

3-Methyl-8-(N-nitrocarbamoyl)-imidazo[5,1-d]-1,2,3,5-tetrazin-4(3H)-one (7.2; R = Me)

To a stirred solution of the methyltetrazinone (5.8; R = Me) (0.20 g) in concentrated sulphuric acid (2 ml) at 0 °C, concentrated nitric acid (d = 1.42; 1 ml) was added dropwise. The solution was maintained at 0 °C for 15 mins and poured on to crushed ice. The precipitated white solid (0.20 g) which was a mixture of two compounds (tlc: silica/toluene-acetone/3:1) was collected by filtration. The mixture was stirred in cold acetone (40 ml) for 0.5 h and the suspension was filtered. The collected white solid was identified as 3-methyl-2,3-dihydro-4-oxoimidazo[5,1-d]-1,2,3,5-

tetrazine-8-carboxylic acid (7.6; R = Me) (0.15 g, 63%) (identical ir, mp, ms, see later). The filtrate after evaporation of acetone at room temperature yielded the nitroamide (7.2; R = Me) (0.06 g, 25%); mp 208 °C (efferv.). (Found: C, 29.2; H, 2.5; M^+, 239. $C_6H_5N_7O_4.0.25\ H_2O$ requires C, 29.5; H, 2.3; M^+, 239); ν_{max} (KBr) 3250 (NH), 1738 and 1705 cm^{-1}. (C=O); λ_{max} (95% EtOH) 325 nm; δ(DMSO-d_6) 8.90 (1H, s, H-6), 8.26 (1H, brs, NH) and 3.90 ppm (3H, s, Me).

NB. Separation of the mixture of nitroamide (7.2; R = Me) and the carboxylic acid (7.6; R = Me) by column chromatography (silica-toluene-acetone) was not successful due to the instability of the ring-system.

Deamination of imidazo[5,1-d]-1,2,3,5-tetrazin-4(3H)-ones in concentrated sulphuric acid

3-(2-Chloroethyl)-2,3,-dihydro-4-oxoimidazo[5,1-d]-1,2,3,5-tetrazine-8-carboxylic acid (7.6; R = CH_2CH_2Cl)

To a solution of 3-(2-chloroethyl)imidazotetrazinone (5.8; R = CH_2CH_2Cl) (1.0 g) in concentrated sulphuric acid (8.0 ml), sodium nitrite (0.8 g) was added with stirring at room temperature. To the reaction mixture, five drops of water were added and the mixture was heated at 35 °C for 1.5 h. The reaction mixture was poured onto crushed ice. The white microcrystals of the carboxylic acid (0.93 g, 93%) were collected by filtration and washed with cold water; mp 160-162°C (efferv.) (from aqueous acetone). (Found: C, 33.6; H, 2.3; Cl, 14.1; N, 27.9; M^+, 243 (245). $C_7H_6ClN_5O_3.\ 0.5\ H_2O$ requires C, 33.4; H, 2.7; Cl, 14.05; N, 27.8%; M^+, 243 (245)); ν_{max} (KBr) 3539, 3490 and 3110 (OH), 1730 and 1640 cm^{-1} (C=O); λ_{max} (95% EtOH) 326 nm;

δ (DMSO-d_6) 8.78 (1H, s, H-2), 4.60 (2H, t, J=6Hz, $CH_2\underline{CH_2}Cl$) and 4.0 ppm (2H, t, J=6Hz, $\underline{CH_2}CH_2Cl$).

The carboxylic acid (7.6; R = CH_2CH_2Cl) (0.95 g, 95%) was also obtained in a different polymorphic form as a white crystalline solid from the above reaction. Recrystallisation from aqueous acetone yielded colourless needles; mp 162-163 °C (efferv.); ν_{max} (KBr) 3450, 3100 (OH), 1740 and 1698 cm^{-1} (C=O); with identical uv, ^1H nmr and ms characteristics to the above sample. The structure of this polymorph was confirmed by X-ray crystallography[119].

3-Methyl-2,3,-dihydro-4-oxoimidazo[5,1-d]tetrazine-8-carboxylic acid (7.6; R = Me)

To a solution of 3-methylimidazotetrazinone (5.8; R = Me) (1.0 g) in concentrated sulphuric acid (8.0 ml) sodium nitrite (0.5 g) was added with stirring at room temperature. To the reaction mixture, five drops of water were added. The reaction mixture was stirred for 15 mins and then poured onto crushed ice to afford white micro crystals of the carboxylic acid (6.7; R = Me) (0.9 g, 90%) which were collected by filtration. Recrystallisation from aqueous acetone yielded colourless crystals; mp 178-180 °C (efferv.). (Found: C, 35.9; H, 2.75; N, 35.5; M^+, 195. $C_6H_5N_5O_3.0.25\ H_2O$ requires C, 36.08; H, 2.75; N, 35.1%; M^+, 195); ν_{max} (KBr) 3640, 3350, 3160 (OH), 1745 and 1670 cm^{-1} (C=O); λ_{max} (95% EtOH) 325 nm; δ (DMSO-d_6) 8.62 (1H, s, H-6), 3.90 ppm (3H, s, Me).

8-Ethoxycarbonyl-3-(2-chloroethyl)imidazo[5,1-d]-1,2,3,5-tetrazin-4(3H)-one (7.8; R = CH_2CH_2Cl)

3-(2-Chloroethyl)oxoimidazotetrazine carboxylic acid (7.6; R = CH_2CH_2Cl) (1.0 g) was boiled in thionyl chloride (20 ml) for 2 h.

Thionyl chloride was removed by distillation under reduced pressure. The residue was dissolved in toluene (20 ml) and evaporated to dryness under reduced pressure to afford crude 8-chlorocarbonyl-3-(2-chloroethyl)imidazo[5,1-d]-1,2,3,5-tetrazin-4(3H)-one (7.7; R = CH_2CH_2Cl) (0.8 g). The crude acid chloride was stirred in ethanol (10 ml) at room temperature for 2 h. A creamy crystalline solid of 8-ethoxycarbonylimidazotetrazinone (7.8; R = CH_2CH_2Cl) (0.75 g, 90%) was collected by filtration and had mp 112 °C (efferv.). (Found: C, 39.6; H, 3.65; N, 25.9; M^+, 271 (273). $C_9H_{10}ClN_5O_3$ requires C, 39.79; H, 3.71; N, 25.78%; M^+, 271 (273)); ν_{max} (KBr) 1780 and 1740 cm^{-1} (C=O); λ_{max} (95% EtOH) 326 nm; δ (DMSO-d_6) 8.9 (1H, s, H-6) 4.65 (2H, t, J=6Hz, $CH_2\underline{CH_2}Cl$), 4.35 (2H, t, J=6Hz, $\underline{CH_2}CH_2Cl$), 4.0 (2H, q, J=6Hz, $\underline{CH_2}CH_3$) and 1.35 ppm (3H, t, J=6Hz, $CH_2\underline{CH_3}$).

Reactions of imidazo[5,1-d]-1,2,3,5-tetrazin-4(3H)-ones in acetic acid

5-Bromoimidazole-4-carboxamide (8.1; X = Br)[120]

(i) Mitozolomide (5.8; R = CH_2CH_2Cl) (0.24 g) was boiled under reflux in acetic acid (10 ml) containing sodium bromide (0.5 g) for 3.0 hrs. The cooled reaction mixture deposited a deep brown solid of 5-bromoimidazole-4-carboxamide hydrobromide (0.06 g, 33%), which was collected by filtration and purified by acetone reprecipitation from an aqueous solution and had mp 210 °C. (Found: C, 19.6; H, 1.9; N, 17.0; M^+, 189 (191). $C_4H_4BrN_3O$. HBr requires C, 19.2; H, 2.0; N, 16.8%; M^+, 189 (191) as the molecular ion of the free base); ν_{max} (KBr) 3420, 3190 (NH) and 1680 cm^{-1} (C=O); δ (DMSO-d_6) 8.6

(1H, s, H-2), 7.36 ppm (2H, brs, NH_2).

(ii) The same (identical mp and ir) bromoimidazole hydrobromide (8.1; X = Br) (0.08 g) was formed when mitozolomide (5.8; R = CH_2CH_2Cl) (0.24 g) was heated under reflux in acetic acid (10 ml) and 45% hydrogen bromide in acetic acid (3 ml) for one hour.

(iii) The same (identical ir and mp) bromoimidazole hydrobromide (0.05 g, 14%) was obtained when 5-diazoimidazole-4-carboxamide (5.1) (0.26 g) was stirred in acetic acid containing 45% hydrogen bromide (5 ml) at room temperature in the dark.

The filtrate from the above reaction (i - iii) yielded azahypoxanthine (6.9) (0.09 g, 65%) when evaporated to dryness under reduced pressure and was identical (uv and ir) with the authentic sample (6.9) obtained by the intramolecular cyclisation of 5-diazoimidazole-4-carboxamide in aqueous ammonia[91].

5-Iodoimidazole-4-carboxamide (8.1; X = I)[120]

Mitozolomide (5.8; R = CH_2CH_2Cl) (0.24 g) was heated under reflux in acetic acid (10 ml) containing sodium iodide (0.5 g) for 2.5 h. The cooled mixture deposited a violet solid, which was collected by filtration and rinsed with acetone to yield golden yellow crystals of the iodoimidazole hydriodide (0.05 g, 21%) mp 285-287 °C (Found: C, 12.7; H, 1.5; N, 12.0%, M^+, 237. $C_4H_4IN_3O.HI$ requires C, 13.15; H, 1.36; N, 11.5%; M^+, 237 (as the molecular ion of the free base); ν_{max} (KBr) 3420, 3190 (NH), 1675 cm^{-1}

(C=O); δ (DMSO-d_6) 8.62 (1H, s, H-2), 7.36 ppm (2H, brs, NH_2).

Methyl 8-carbamoyl-1,4-dihydro-4-methyleneimidazo[5,1-c]-1,2,4-triazine-3-carboxylate (8.7; R = CO_2Me)

Mitozolomide (5.8; R = CH_2CH_2Cl) (0.24 g), methyl acetoacetate (1.5 ml) and acetic acid (6 ml) were boiled under reflux for 0.5 h. The cooled solution deposited golden yellow crystals of the methyl triazine-carboxylate (8.7; R = CO_2Me) as an acetic acid solvate (0.18 g, 78%), which was collected by filtration and had mp 250 °C (decomp.) (lit[76] 250 °C (decomp.)); ν_{max} (KBr) 3370, 3300 (NH), 3200 br (NH), 1710 and 1695 cm^{-1} (C=O); λ_{max} (95% EtOH) 367, 296 (infl.), 278 (infl.) 267 and 232 nm; δ (TFA) 9.1 (1H, s, H-6) 6.48 (2H, d, J=4Hz, $\underline{CH_2}$), 5.92 (2H, d, J=4Hz, $\underline{CH_2}$), 4.1 (3H, s, $CO\underline{CH_3}$), 2.23 ppm (3H, s, $\underline{CH_3}COOH$).

Ethyl 8-carbamoyl-1,4-dihydro-4-methyleneimidazo[5,1-c]-1,2,4-triazine-3-carboxylate (8.7; R = CO_2Et)

Mitozolomide (5.8; R = CH_2CH_2Cl) (0.24 g), ethyl acetoacetate (1.5 ml) and acetic acid (6 ml) were heated under reflux for 0.5 h. The cooled solution deposited brownish yellow solid (0.16 g, 66%) of ethyl triazine-carboxylate (8.7; R = CO_2Et) which was collected by filtration and recrystallised from ethanol-acetic acid and had mp 270 °C (decomp.) (lit[76]) 270 °C (decomp.); ν_{max} (KBr) 3330 (NH) 3180 br (NH), 1720 and 1699 cm^{-1} (C=O); λ_{max} (95% EtOH) 367, 298, 278 (infl.), 267 and 235 nm; δ (TFA) 9.12 (1H, s, H-6), 6.5 (2H, d, J=4Hz, $\underline{CH_2}$), 5.9 (2H, d, J=4Hz, $\underline{CH_2}$) 4.5 (2H, q, J=6Hz, $\underline{CH_2}$-CH_3) and 1.5 ppm (3H, t, J=6Hz, CH_2-$\underline{CH_3}$).

3-Acetyl-8-carbamoyl-1,4-dihydro-4-methyleneimidazo[5,1-c]-1,2,4-triazine (8.7; R = Ac)

Mitozolomide (5.8; R = CH_2CH_2Cl) (0.24 g) was heated under reflux with acetylacetone (1.5 ml) in acetic acid (6 ml) for 0.5 h. The reaction mixture was cooled to yield a brown solid (0.1 g, 48%) of acetyltriazine (8.7; R = Ac), which was collected by filtration and recrystallised from acetic acid-ethanol and had mp 250 °C (decomp.) (lit[76] mp 250 °C (decomp.); ν_{max} (KBr) 3460 (NH), 3220-3120 br (NH), 1670 cm^{-1} br (C=O); λ_{max} (95% EtOH) 366, 279 (infl.), 270 (infl.), 253, 242 nm; δ (TFA) 9.14 (1H, s, H-6), 6.62 (2H, d, J=4Hz, $\underline{CH_2}$), 5.9 (2H, d, J=4Hz, $\underline{CH_2}$) and 2.7 ppm (3H, s, $CO\underline{CH_3}$).

Ethyl 4-amino-8-carbamoylimidazo[5,1-c]-1,2,4-triazine-8-carboxylate (8.12; R = CO_2Et)

Mitozolomide (0.24 g) was heated (0.5 h) under reflux with ethyl cyanoacetate (1.5 ml) in acetic acid (6 ml). Pink crystals of amino-imidazotriazine acetic acid solvate (0.16 g, 64%) (8.12, R = CO_2Et) were deposited from hot solution and had mp 360 °C (decomp.) (lit[76] 360 °C (decomp.)); ν_{max} (KBr) 3398 (NH) 3180, 3020 br (NH), 1695 and 1630 cm^{-1} (C=O); λ_{max} (95% EtOH) 382, 330 (infl.), 316, 307 (infl.), 244 and 206 nm; δ (TFA) 9.22 (1H, s, H-6), 4.56 (2H, q, J=7Hz, $CH_3\underline{CH_2}$), 2.23 (3H, s, $CO_2\underline{CH_3}$) and 1.5 ppm (3H, t, J=7Hz, $\underline{CH_3}$-CH_2).

4-Amino-8-carbamoyl-3-cyanoimidazo[5,1-c]-1,2,4-triazine (8.12; R = CN)

Mitozolomide (0.24 g) and malononitrile (0.2 g) were heated under reflux (0.5 h) in acetic acid (6 ml). Cooled reaction mixture deposited a brownish yellow solid (0.11 g, 55%) which was collected by filtration. Recrystallised from ethanol-acetic acid, it had mp

360°C (decomp.) (lit[76] 350-360 (decomp.); ν_{max} (KBr) 3440, 3330, 3260 and 3130 br (NH), 2250 (CN) and 1660 cm^{-1} (C=O); δ (TFA) 9.12 (1H, s, H-6).

8-Carbamoyl-3-cyanoimidazo[5,1-]-1,2,4-triazin-4(1H)-one (5.4; R = CN)

Mitozolomide (5.8; R = CH_2CH_2Cl) (0.24 g) was boiled under reflux with ethyl cyanoacetate (1.5 ml) in pyridine (10 ml) and ethanol (15 ml) for 8 h. The reaction mixture was cooled and the precipitated maroon solid (0.1 g, 50%) was collected by filtration. It was recrystallised from dimethylformamide-ethanol to afford a yellowish brown solid, mp >300 °C (decomp.) (lit[76] 300 °C (decomp.); ν_{max} (KBr) 3410 (NH), 2220 (CN) and 1673 cm^{-1} (C=O).

8-Carbamoyl-3-acetylimidazo[5,1-c]-1,2,4-triazin-4(1H)-one (5.4; R = Ac)

Mitozolomide (5.8; R = CH_2CH_2Cl) (0.24 g) was heated under reflux with methyl (or ethyl) acetoacetate (1.5 ml) in pyridine (5 ml) and ethanol (10 ml) for 8 h. The reaction mixture was cooled and the deposited maroon solid (.08 g, 35%) was collected by filtration. It was recrystallised from dimethylformamide-ethanol to yield brown solid of acetyltriazinone (5.4; R = Ac), mp > 300 °C (decomp.); (Lit[76] 300 °C (decomp.)); ν_{max} (KBr) 3500, 3450, 3350 (NH), 3150 br (NH), 1710 (Ac), 1665 cm^{-1} (C=O); λ_{max} (95% EtOH) 376, 289, 250 (infl.) and 207 nm; δ (TFA) 9.02 (1H, s, H-6) and 2.82 ppm (3H, s, CH_3CO).

Decomposition of 3-alkylimidazotetrazinones in alcohols

1-Methoxycarbonyl-5-aminoimidazole-4-carboxamide (9.7; R^1 = Me)

(i) 3-Methylimidazo[5,1-d]-1,2,3,5-tetrazin-4(3H)-one (5.8; R = Me) (0.5 g) was heated under reflux in methanol (20 ml) for 10 days. The solution was evaporated to dryness under reduced pressure. The isolated pink solid was a mixture of two compounds, which were separated by column chromatography (silica; 95% ethanol-toluene; 4:1). The first fraction, when evaporated to dryness under reduced pressure, yielded a buff solid (0.06 g, 13%) which was identified as 1-methoxycarbonyl-5-aminoimidazole-4-carboxamide (9.7; R^1 = Me). Recrystallisation from water afforded white needles, mp 178-180 °C (Found: C, 39.25; H, 4.3; N, 30.3; M^+, 184. $C_6H_8N_4O_3$ requires C, 39.2; H, 4.4; N, 30.4%; M^+, 184); ν_{max} (KBr) 3450, 3390 (NH), 1765 and 1665 cm^{-1} (C=O); λ_{max} (95% EtOH), 265 nm; δ (DMSO-d_6) 7.5 (1H, s, H-2), 6.9 (2H, brs, NH), 6.4 (2H, brs, NH) and 3.98 ppm (1H, s, $\underline{CH_3}$).

The second fraction from the above separation yielded a pink solid (0.25 g, 71%) after evaporation under reduced pressure and was identified as 2-azahypoxanthine (6.9), and was identical (ir and uv) to a sample prepared by the cyclisation of 5-diazoimidazole-4-carboxamide (5.1) in concentrated aqueous ammonia[91].

(ii) The same methyl ester (9.7; R^1 = Me) was prepared by the method described in a Japanese patent[107]. To a suspension of 5-aminoimidazole-4-carboxamide hydrochloride (4.3 g) in

tetrahydrofuran (30 ml) at 12 °C, a solution of methyl chloroformate (4.95 g) in tetrahydrofuran (20 ml) was added during 15 minutes. A solution of sodium hydroxide (2.24 g) in water (10 ml) was added and the reaction mixture was stirred for one hour at 25 °C. The white crystalline solid (3.8 g, 79%) was collected by filtration, recrystallised from water and had mp 178-180 °C.

(iii) To a suspension of 5-aminoimidazole-4-carboxamide hydrochloride (1.0 g) in ethanol (150 ml) and triethylamine (1.25 g) at 0 °C, methyl chloroformate (1.16 g) was added. The reaction mixture was stirred at 25 °C for one hour and boiled for 20 mins. A mixture of 1-alkoxycarbonyl-5-aminoimidazole-4-carboxamide (9.7; R^1 = Me and Et) (0.95 g, 84%) was deposited (M^+, 184 and 198 respectively 3:1).

(iv) 5-Aminoimidazole-4-carboxamide hydrochloride (0.82 g) was stirred at 0 °C with potassium hydrogen carbonate (0.60 g) in water (15 ml). At 10 minute intervals, three portions of methyl chloroformate (0.2 ml) were added with additional potassium hydrogen carbonate (0.6 g) in divided portions of 0.2 g. Stirring was continued for one hour after the last addition. The solid (0.70 g, 75%) was collected by filtration, recrystallised from water and had mp 175-180 °C.

(v) 5-Aminoimidazole-4-carboxamide hydrochloride (1.0 g) was dissolved in 15% aqueous sodium carbonate (30 ml). Methyl chloroformate (2 ml) was added with stirring at room temperature. A white crystalline solid was formed

instantaneously. The reaction mixture was stirred for a further 20-30 minutes and the white crystals (0.90 g, 96%) were collected by filtration and recrystallised from water to afford a white solid mp 175-180 °C.

(vi) 3-Ethylimidazo[5,1-d]-1,2,3,5-tetrazin-4(3H)-one (5.8; R^1 = Et) (0.5 g) was heated under reflux in ethanol (20 ml) for 10 days. The reaction mixture, when evaporated under reduced pressure, yielded a pink mixture of two compounds which were separated by column chromatography (see above). The first fraction yielded the same (ir and uv) 1-methoxycarbonyl-5-aminoimidazole-4-carboxamide (0.07 g, 15%). The second fraction yielded azahypoxanthine (6.9) (0.24 g, 75%), and was identical (ir and uv) to previous samples (see above).

1-Ethoxycarbonyl-5-aminoimidazole-4-carboxamide (9.7; R^1 = Et)

(i) 3-Methylimidazo[5,1-d]-1,2,3,5-tetrazin-4(3H)-one (5.8; R = Me) (0.5 g) was heated under reflux in ethanol (20 ml) for 10 days. The solution was evaporated under reduced pressure to afford a pink mixture of two components. These compounds were separated by column chromatography (silica; 95% ethanol-toluene; 4:1). The first fraction, when evaporated to dryness under reduced pressure, yielded a white solid (0.095 g, 19%) which was identified as 1-ethoxycarbonyl-5-aminoimidazole-4-carboxamide (9.7; R^1 = Et). Recrystallisation from water afforded white micro needles, mp 180-182 °C (resolidified ca 200 °C and decomposed >350 °C)

(Found: C, 42.35; H, 4.95; N, 28.2; M^+, 198. $C_7H_{10}N_4O_3$ requires C, 42.4; H, 5.05; N, 28.3%; M^+, 198); ν_{max} (KBr) 3440, 3325, 3145 (NH), 1750 and 1665 cm^{-1} (C=O); λ_{max} (95% EtOH) 267 nm; δ (DMSO-d_6) 7.6 (1H, s, H-2), 6.92 (2H, brs, NH_2), 6.42 (2H, brs, NH_2), 4.42 (2H, q, J=6Hz, $\underline{CH_2}CH_3$), 1.32 ppm (3H, t, J=6Hz, $CH_2\underline{CH_3}$).

The second fraction from the above separation after evaporation under reduced pressure yielded a pink solid (0.28 g, 80%) and was identified as 2-azahypoxanthine (6.9), and was identical (ir and uv) to a sample prepared by the cyclisation of 5-diazoimidazole-4-carboxamide (5.1) in concentrated aqueous ammonia[91].

(ii) The same (ir and uv) <u>ethyl ester</u> (9.7; R^1 = Et) [originally named as carboethoxyaminoimidazole-4-carboxamide (9.6; R^1 = Et)][108] was prepared by the methods described by Shaw[108]. 5-Aminoimidazole-4-carboxamide hydrochloride (0.82 g) was stirred at 0 °C with potassium bicarbonate (0.60 g) in water (15 ml). At intervals of 10 minutes three portions of ethyl chloroformate (0.2 ml) were added with additional potassium bicarbonate (0.6 g) in divided portions of 0.2 g. Stirring was continued for one hour after the last addition. The crystals (0.78 g, 78%) were collected by filtration, recrystallised from water, and had mp 180-182 °C (resolidified over 200 °C then decomposed >350 °C).

(iii) To a suspension of 5-aminoimidazole-4-carboxamide hydrochloride (3.34 g) in tetrahydrofuran (30 ml) at 12 °C a solution of ethyl chloroformate (4.5 g) in tetrahydrofuran

(20 ml) was added during 15 minutes. A solution of sodium hydroxide (2.24 g) in water (10 ml) was added and the reaction mixture was stirred for one hour at 25 °C. The white crystalline solid (2.8 g, 70%) was collected by filtration, recrystallised from water and had mp 180-182 °C (solidified at 200 °C and then decomposed >350 °C).

(iv) To a suspension of 5-aminoimidazole-4-carboxamide hydrochloride (1.0 g) in ethanol (50 ml) and triethylamine (4.5 g) at 0 °C, ethyl chloroformate (1.9 g) was added and the reaction mixture was stirred for 0.5 h. The precipitated ethyl ester (9.7; R^1 = Et) (0.95 g) was collected by filtration and had mp 180-182 °C (solidified at 200 °C and decomposed >350 °C).

(v) 5-Aminoimidazole-4-carboxamide hydrochloride (1.0 g) was dissolved in 15% aqueous potassium carbonate (30 ml). Ethyl chloroformate (2 ml) was added with stirring at room temperature. A white crystalline solid was formed instantaneously. The reaction mixture was stirred for a further 20-30 mins and the white crystals (0.95 g, 79%) were collected by filtration and recrystallised from water to afford white microneedles which had mp 180-182 °C, solidified at 200 °C and decomposed >350 °C.

(vi) 3-Ethylimidazo[5,1-d]-1,2,3,5-tetrazin-4(3H)-one (5.8; R = Et) (0.2 g) was heated under reflux in ethanol (20 ml) for 10 days. The reaction mixture, when evaporated to dryness under reduced pressure, yielded a pink mixture of two

compounds which were separated by column chromatography (see above). The first fraction yielded the same (ir and uv) ethyl ester (9.7; R^1 = Et) (0.035 g, 19.5%). The second fraction yielded azahypoxanthine (6.9) (0.095 g, 73%) and was identical (ir and uv) to previous samples (see above).

1-Methoxycarbonyl-5-chloroimidazole-4-carboxamide (9.15; R^1 = Me)

A saturated solution of sodium nitrite (0.05 g) in water was added dropwise to the cooled (0 °C) suspension of the methyl ester (9.7; R^1 = Me) (0.20 g) in 2N hydrochloric acid (10 ml). The resulting purple solution was stirred for 4 h at 0 °C. The solution decolourised to yield a cream coloured solid (0.13 g, 59%) which was collected by filtration and recrystallised from aqueous ethanol and had mp 150-152 °C. (Found: C, 35.1; H, 2.8; N, 20.6; M^+, 203 (205). $C_6H_6N_3O_3Cl$ requires C, 35.38; H, 2.94; N, 20.6%; M^+, 203 (205)); ν_{max} (KBr) 3450, 3350, 3150 (NH), 1778 and 1660 cm^{-1} (C=O); λ_{max} (95% EtOH) 267 nm; δ (DMSO-d_6) 7.76 (1H, s, H-2), 6.62 (2H, brs, NH_2) and 3.3 ppm (3H, s, $CO_2\underline{Me}$).

1-Ethoxycarbonyl-5-chloroimidazole-4-carboxamide (9.15; R^1 = Et)

A cooled (0 °C) suspension of 1-carboethoxy-5-aminoimidazole-4-carboxamide (9.7; R^1 = Et) (0.20 g) in 2N-hydrochloric acid (10 ml) was treated with saturated aqueous sodium nitrite (0.05 g) as described above. 1-Ethoxycarbonyl-5-chloroimidazole-4-carboxamide (0.14 g, 63%) was collected by filtration. Recrystallisation from aqueous ethanol yielded pale yellow crystals, mp 158-159 °C. (Found: C, 38.45; H, 3.62; N, 19.3; M^+, 217 (219). $C_7H_8N_3O_3Cl$ requires C, 38.62, H, 3.67; N, 19.3%; M^+, 217 (219); ν_{max} (KBr) 3320, 3190 (NH), 1762 and 1665 cm^{-1} (C=O); λ_{max} (95% EtOH) 267 nm; δ (DMSO-d_6) 7.76

(1H, s, H-2), 6.7 (2H, brs, NH_2), 4.24 (2H, q, J=6Hz, $\underline{CH_2}CH_3$) and 1.3 ppm (3H, t, J=6Hz, $CH_2\underline{CH_3}$).

Decomposition of imidazotetrazinones in hydrazine

5-Azidoimidazole-4-carboxamide (9.20)

(i) To a stirred suspension of mitozolomide (5.8; R = CH_2CH_2Cl) (0.24 g) in ethanol (3 ml) at room temperature, a solution of hydrazine hydrate-ethanol (1:1, 2 ml) was added dropwise. A vigorous effervescence ensued and a solution was formed. After 0.5 h the precipitated white crystals of 5-azidoimidazole-4-carboxamide (0.08 g, 54%) were collected by filtration mp 147-148 °C (lit 145-147[112] and 146 °C[113]); ν_{max} (KBr) 3450, 3195 (NH), 2150, 2130 (N_3) and 1662 cm^{-1} (C=O); λ_{max} (95% EtOH) 267 nm; δ (DMSO-d_6) 7.64 (1H, s, H-2) and 7.5 ppm (2H, brs, NH_2).

(ii) To a stirred suspension of 5-diazoimidazole-4-carboxamide (5.1) (0.14 g) in ethanol (3 ml) at room temperature, a 50% solution of hydrazine in ethanol (2 ml) was added dropwise. The 5-diazoimidazole-4-carboxamide dissolved and, after 20-30 mins, white crystals of 5-azidoimidazole-4-carboxamide (9.20) (0.12 g, 92%) were formed, which were collected by filtration and had mp 147 °C (lit[112] 145-147 °C).

5-Amino-4-carbamoylimidazole-1-carbohydrazide (9.21)

(i) A suspension of 3-methyltetrazinone (5.8; R = Me) (0.25 g) in ethanol (3 ml) was stirred with 50% ethanolic hydrazine hydrate (2 ml) at room temperature. The precipitated buff coloured carbohydrazide (0.17 g, 74%) was collected by filtration and washed with water and had mp 183-185 °C (Found: C, 32.6; H, 4.2; N, 46.0; M^+, 184. $C_5H_8N_6O_2$ requires C, 32.6; H, 4.34; N, 45.65%; M^+, 184); ν_{max} (KBr) 3500, 3390 (NH), 1715 and 1660 cm^{-1}; λ_{max} (95% EtOH) 267 nm; δ (DMSO-d_6) 7.52 (1H, s, H-2), 6.74 and 6.34 ppm (2H, brs, NH_2).

(ii) A suspension of 3-ethyltetrazinone (5.8; R = Et) (0.25 g) in ethanol (3 ml) when similarly treated with 50% ethanolic hydrazine (2 ml) yielded the same (ir and uv) carbohydrazide (9.21) (0.15 g, 68%).

(iii) 3-Propyltetrazinone (5.8; R = n-Pr) (0.25 g) yielded the same (ir and uv) carbohydrazide (9.21) (0.12 g, 60%) when treated with hydrazine as above.

(iv) 3-(2-Methoxyethyl)tetrazinone (5.8; R = CH_2CH_2OMe) (0.25 g) yielded the identical (ir and uv) carbohydrazide (9.21) (0.1 g, 51%) when treated with 50% ethanolic hydrazine by the method described above.

8-Carbamoyl-3-(2-methoxyethyl)imidazo[5,1-d]-1,2,3,5-tetrazin-4(3H)-one (5.8; R = CH_2CH_2OMe).

A suspension of 5-diazoimidazole-4-carboxamide (5.1) (0.3 g) in acetonitrile (5 ml) was treated with 2-methoxyethyl isocyanate

(0.5 g) and the mixture was stirred at 45 °C in the dark for 24 hours. The resulting solid was filtered off and washed with diethyl ether to give crude 8-carbamoyl-3-(2-methoxyethyl)imidazo-[5,1-d]-1,2,3,5-tetrazin-4(3H)-one (0.45 g). The product was recrystallised from acetone to give pink rosettes, or from aqueous dimethylsulphoxide to give colourless needles, mp 164-165 °C (decomp.) (Found: C, 40.4; H, 4.2; N, 35.2%; M^+, 238. $C_8H_{10}N_6O_3$ requires C, 40.34; H, 4.2; N, 35.2%; M^+, 238); ν_{max} (KBr) 3420, 3300 (NH), 1720 and 1665 cm^{-1} (C=O); λ_{max} (95% EtOH) 326, 250 and 215 nm; δ (DMSO-d_6) 8.86 (1H, s, H-6), 7.7 (2H, brd, NH), 4.56 (2H, t, J=6Hz, CH_2CH_2OMe), 3.72 (2H, t, J=6Hz, CH_2CH_2OMe) and 3.3 ppm (3H, s, OMe).

Reactions of 5-amino-4-carbamoyl-imidazole-1-carbohydrazide

(i) A solution of carbohydrazide (9.21) (0.10 g), in a mixture of acetone-ethanol (3:1, 30 ml), was stirred at room temperature for 5 days. The solvent was removed by distillation under reduced pressure. A viscous residue was obtained, which was redissolved in cold ethanol and left overnight. The pink crystalline semicarbazone (9.22) (0.09 g, 75%) was collected by filtration, mp 220 °C (decomp.) (Found: C, 43.1; H, 5.4; N, 37.8%; M^+, 224. $C_8H_{12}N_6O_2$ requires C, 42.86; H, 5.36; N, 37.5%; M^+, 224); ν_{max} (KBr) 3120, 3300 (NH) and 1645 cm^{-1} (C=O); λ_{max} (95% EtOH) 265 nm; δ (DMSO-d_6) 9.14 (1H, brs, NH), 7.1 (1H, s, H-2), 6.69 (4H, brs, NH), 1.9 and 1.82 ppm (6H, 2xs, $2xCH_3$).

(ii) The carbohydrazide (9.21) (0.1 g) was heated for 0.5 h under reflux with benzaldehyde (0.5 ml) in ethanol (20 ml). The solution was left overnight at room temperature. A buff crystalline deposit of the benzylidene-derivative (9.24) (0.10 g, 86%) was collected by filtration and recrystallised from ethanol and had mp 242-245 °C (Found: C, 61.6; H, 4.6; N, 26.3%; M^+, 214. $C_{11}H_{10}N_4O$ requires C, 61.68; H, 4.67; N, 26.17%; M^+, 214); ν_{max} (KBr) 3400, 3160 (NH) and 1658 cm^{-1} (C=O); δ (DMSO-d_6) 9.24 (H, s, CHPh), 7.72 (1H, s, H-2), 7.82-7.48 (8H, m, Ph and NH).

(iii) The same (ir and nmr) benzylidene derivative (9.24) (0.15 g, 68%) was obtained when mitozolomide (5.8; R = CH_2CH_2Cl) (0.24 g) was heated under reflux (1 h) in water (10 ml) containing benzaldehyde (0.5 ml).

(iv) The same (ir and nmr) benzylidene derivative (9.24) (0.16 g, 76%) was also obtained as a pale pink solid when methyltetrazinone (5.8; R = Me) (0.19 g) was boiled under reflux (1 h) in water (10 ml) with benzaldehyde (0.5 ml).

(v) The same (ir and nmr) benzylidene derivative (9.24) (1.85 g, 97%) was obtained by heating 5-aminoimidazole-4-carboxamide (6.6) (free base) (1.30 g) and benzaldehyde (2.0 g) in ethanol (50 ml).

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