Tumour-inhibitory triazenes: search for a chemical basis for their mode of action.

by

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SUMMARY

The chemical and biological properties of the antitumour agent 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (DTIC) are reviewed in the introduction, which includes a discussion on the mode of action of DTIC, aryldialkyltriazenes and the carcinogenic N-nitrosamines.

4-Amino-2-[2-(piperidin-1-ylazo)phenyl]quinazoline(6a) and its related heteroalicyclic analogues (6b-i) were prepared by heating 1,3-di-o-cyanophenyltriazene in the respective secondary heteroalicyclic amines.

Half-diazotisation of 5-bromoanthranilonitrile in 2N-hydrochloric acid resulted in the formation of 1,3-di-(2-cyano-4-bromophenyl)triazene (25). 4-Amino-6-bromo-2-[5-bromo-2-(piperidin-1-ylazo) phenyl]quinazoline(20a) was obtained when the triazene (25) was heated in piperidine. The related derivatives (20b-f) were similarly obtained by heating the triazene (25) in a range of secondary amines. Treatment of anthranilonitrile and 5-bromoanthranilonitrile in dimethylsulphoxide with sodium hydride resulted in the formation of 4-amino-2-(2-aminophenyl)quinazoline(31) and 4-amino-6-bromo-2-(2-amino-5-bromophenyl)-quinazoline (27) respectively.

The diamine (27) was alternatively obtained by reduction of the triazene (25) in ethanol containing hydrazine and Raney nickel. Diazotisation of the diamine (27) led to the isolation of 2-(4-amino-6-bromoquinazolin-2-yl)-4-bromophenyldiazonium chloride dihydrochloride (35). 2, 10-Dibromoquinazolino-[3,2-c]-1,2,3-benzotriazin-8(7H)-imine(30) or its isomer (29) was formed when the diazonium salt (35) was recrystalised from methanol/ether. Treatment of the tetracyclic triazine (30) or isomer (29) with hot piperidine resulted in the formation of the quinazolinotriazene (20a). The triazenoquinazoline (20a) underwent decomposition in 6N-hydrochloric acid to the diazonium ion (35). Treatment of the triazenoquinazoline (6a) with 6N-hydrochloric acid or hot acetic acid, followed by basification resulted in the formation of quinazolino [3,2-c]-1,2,3-benzotriazin-8 (7H)-imine (24) or isomer (22).

The triazene (6a) underwent reductive elimination of the triazeno side-chain in acetic acid containing copper bronze, in boiling ethylene glycol, or by photolysis in ethanol or methanol to afford 4-amino-2-phenylquinazoline. Sandmeyer-type displacement of the triazeno side-chain occurred when the triazene (6a) was heated in acetic acid containing sodium iodide or sodium azide.

Triazene (6i) was hydrolysed in 50% alcoholic potassium hydroxide to yield 2-[2-(3,3-dimethyltriazen-1-yl)phenyl]quinazolin-4(3H)-one (19a).

Alkylation of the triazenoquinazoline (6a) with methyl iodide afforded a hydroiodide salt of the starting material and 1-methyl-2-[2-(piperidin-1-ylazo)phenyl]quinazolin-4(1H)-iminium iodide (68). The methylquinazolinium iodide (68) underwent hydrolysis in aqueous ammonia to 1-methyl-2-[2-piperidin-1-ylazo)phenyl]quinazolin-4(1H)-one (69). Treatment of the methyl quinazolinone (69) with acetic acid containing copper bronze resulted in the formation of 2-phenylquinazolin-4(3H)-one. Oxidation of the triazene (6a) with perbenzoic acid in benzene afforded a benzoate salt of the starting material. Oxidation of the triazene (6a) with potassium permanganate resulted in the formation of 1-[2-(4-aminoquinazolin-2-yl)phenylazo]piperidin-2-one, 4-amino-2-(2-aminophenyl)quinazoline and δ-valerolactone. Analagous oxidation of N-nitrosopiperidine afforded δ-valerolactone. Udenfriend oxidation of the triazene (6a) led to a mixture from which 4-amino-2-phenylquinazoline only was characterised.

5-Diazoimidazole-4-carbonitrile coupled with 5-aminoimidazole-4-carbonitrile to yield 5-amino-4-cyano-2-(4-cyanoimidazol-5-ylazo)imidazole. 1-(2-(cyanophenyl)-3-(4-cyanoimidazol-5-yl)triazene (102) was formed when 5-diazoimidazol-4-carbonitrile was coupled with anthranilonitrile. In boiling ethanol, the triazene (102) underwent cyclisation and decomposition to afford 2-phenyladenine.

8-(3,3-Dimethyltriazen-1-yl)guanine and 1-(guanin-8-ylazo) piperidine were obtained when 8-diazoguanine was coupled with dimethylamine and piperidine respectively. When 8-diazoguanine was coupled with methylamine, 8-aminoguanine was formed.

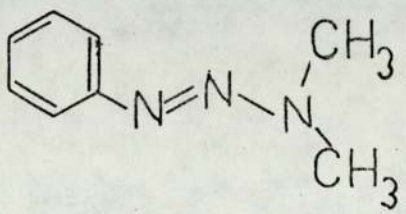
CONTENTS

Chapter 1 Introduction

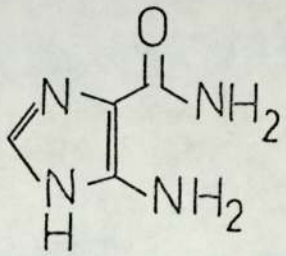
Historical introduction

Aryl-dialkyltriazenes were first reported in the literature by Baeyer[1] as early as 1875 but their tumour-inhibitory activity was not discovered until 1955, when Clarke [2] showed that 3,3-dimethyl-1-phenyltriazene [PDMT] (1) inhibited the growth of Sarcoma 180 in mice. This discovery was followed by reports from Dagg and Karnofsky,[3] who demonstrated the existence of teratogenic effects of PDMT on the chick embryo and confirmed the inhibitory activity against Sarcoma 180 and certain human tumours. The clinical use of triazenes however stemmed from attempts to design antagonists of 5-aminoimidazole-4-carboxamide [AIC] (2), whose riboside-5'-phosphate derivative is an intermediate in de novo purine synthesis. Shealy[4] synthesised 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide [DTIC] (3) as a potential antitumour agent. The rationale behind the synthesis of DTIC was to provide a stable "carrier" form of 5-diazoimidazole-4-carboxamide [diazo-ICA] (4) which was tumour-inhibitory[5] but chemically unstable, cyclising in solution to the relatively biologically inert 2-azahypoxanthine[5] (5). DTIC was subsequently shown to be active against many experimental tumours. It significantly increased the lifespan of mice bearing Leukemia L-1210[6] and is active against Sarcoma 180, Adenocarcinoma 755 and Ehrlich Ascites Carcinoma in mice.[7] Not surprisingly therefore, further derivatives of triazenes were synthesised and evaluated in attempts to establish structure-activity patterns. The triazenoquinazolines, represented here by the prototype 4-amino-[2-(2-piperidin-1-ylazo)phenyl]-quinazoline,[8] [NSC 163423] (6a) do not owe their discovery to any systematic study, but to the element of chance (not an uncommon occurrence in the field of science). In a futile attempt to recrystallize 1,3-di-o-cyanophenyl-triazene (7) from piperidine, Stevens[8] isolated a hydrate of the triazene (6a) in almost quantitative yield. This substance, and further alkyl

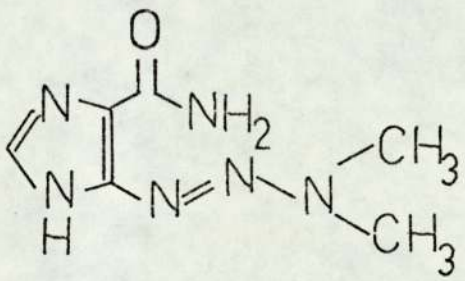
derivatives were routinely screened for tumour-inhibitory activity and were found to be approximately two hundred times more active than DTIC in inhibiting the growth of human epidermoid carcinoma of the nasopharynx in cell culture (H-Ep2).[9]



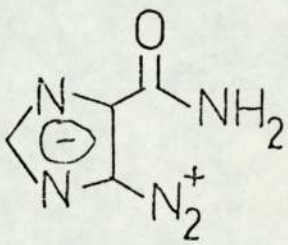
PDMT (1)



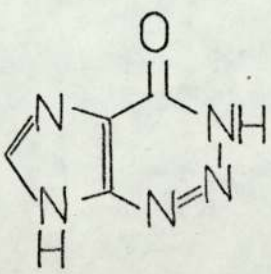
AIC (2)



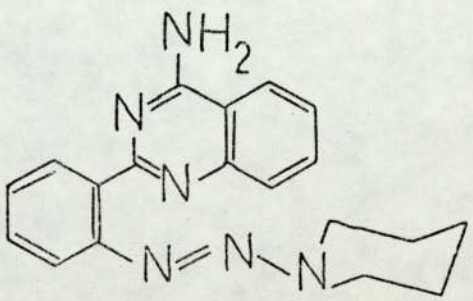
DTIC (3)



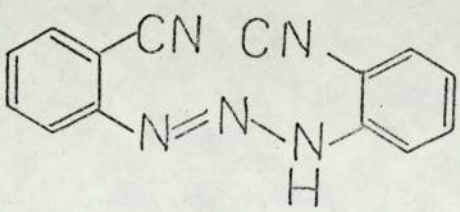
diazo- ICA (4)



(5)



(6a)



(7)

Clinical use and toxicity of DTIC

DTIC is a particularly useful agent in the treatment of the hitherto recalcitrant human malignant melanoma, where, following its use, regression rates of 20% have consistantly been obtained.[10] Evidence for activity against Hodgkin's disease and lymphosarcoma has been obtained for DTIC. This drug has recently become available for general clinical use (June 1975 marketed by Dome pharmaceuticals as Dacarbazine®) and is being evaluated in combination with other anti-neoplastic agents, as a single agent and as an adjunct to radiotherapy.

In addition to its desirable tumour-inhibitory activity, DTIC has a number of toxic effects in experimental animals and in humans. Carcinogenic,[11,12] teratogenic,[13 14] and immunosuppressive[14] activity have been demonstrated in rats, DTIC being distinguished by its ability to induce 100% incidence of tumours in the thymus of rats, a sinister feat not achieved by any other chemical carcinogen. In man, side effects from administering DTIC include nausea, vomiting, myelosuppression and hepatotoxicity.[15] These effects are common to many cytotoxic agents. C.N.S. complications have been reported in man after combination treatment with Adriamycin.[16] Most of these toxic properties manifested in experimental animals are elicited by many aryl-dialkyltriazenes and are not unique to DTIC.

Mode of action of DTIC

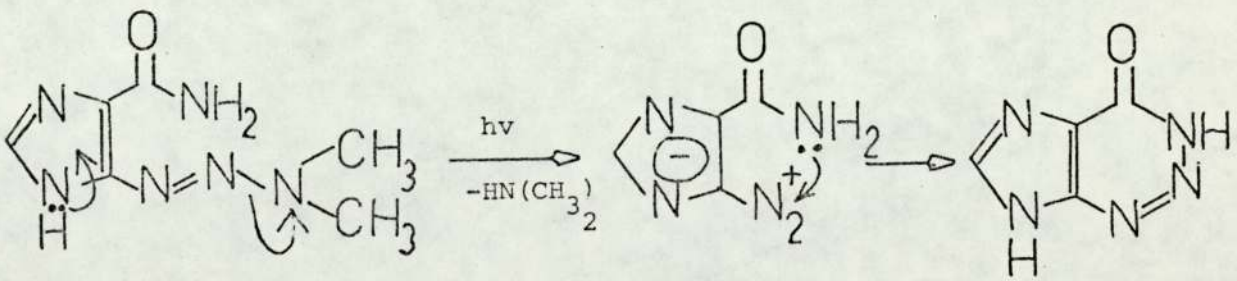
(i) Introduction

While the precise mode of action of DTIC and related triazenes is imperfectly understood, certain chemical and biochemical transformations of triazenes have been observed which may have important consequences when considering the mode of action of these substances in biological systems. In discussing the mode of action of DTIC, it is perhaps useful to consider certain aspects

separately - namely biochemical interactions and biological effects, since it has yet to be determined which, if any, of the biological effects (and in particular, the antitumour effect) are related to the known chemical and biochemical events. It is generally accepted that DTIC does not act as an antagonist of AIC (for which purpose it was originally synthesised) since it has been shown that triazenes not bearing the imidazole ring are just as effective as antitumour agents[17-19].

(ii) In vitro effects of DTIC and other imidazole triazenes

Shealy demonstrated that the exposure of DTIC and other imidazole triazenes to light in solution results in the formation of 2-azahypoxanthine.[4] This transformation is a consequence of a light-catalysed heterolytic dissociation of the triazene to diazo-ICA, which cyclises irreversibly to 2-azahypoxanthine (Scheme 1.1). The reaction is characterised by distinct spectral changes and may be conveniently followed by ultraviolet spectroscopy (Fig. 1.1.)



(3) (4) (5)

Scheme 1.1

The cyclisation product, 2-azahypoxanthine, is not considered to be involved in the tumour-inhibitory process although it is not devoid of biological activity being a potent inhibitor of the enzyme xanthine oxidase[20].

The relevance of the photodecomposition of DTIC to its mode of action in cell culture has been shown by several workers. Saunders and Schultz[21] found that the inhibitory effect of DTIC on Bacillus subtilis (B. subtilis)

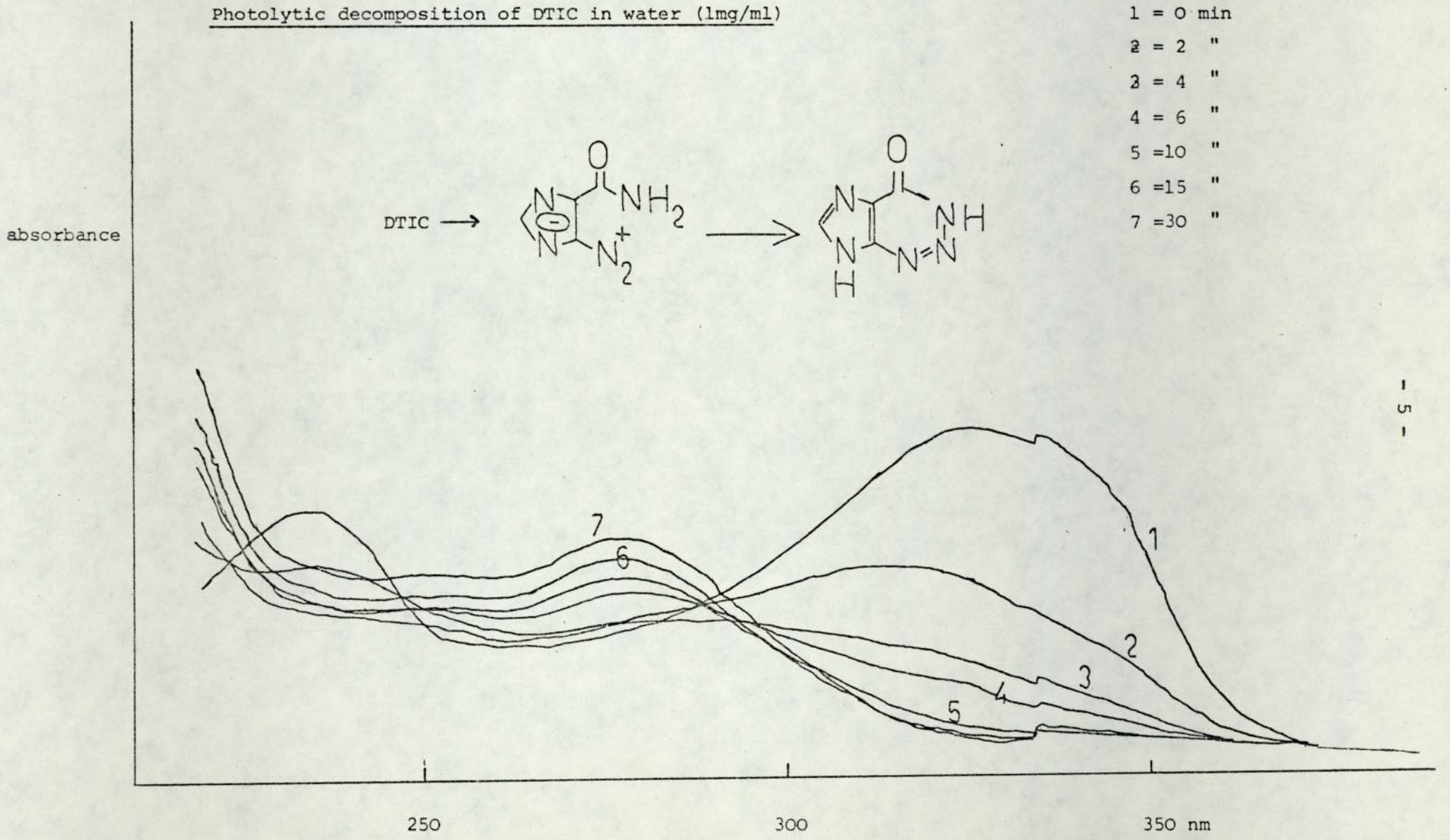

Photolytic decomposition of DTIC in water (1mg/ml)
1 = 0 min
2 = 2 "
3 = 4 "
4 = 6 "
5 =10 "
6 =15 "
7 =30 "
DTIC
absorbance
7
6
1
2
3
4
5
250
300
350 nm

Fig. 1.1.

was markedly increased by exposing cultures growing in the presence of DTIC to light, under which conditions diazo-ICA was continually being generated (Fig. 1.2). They also showed that a strain of B. subtilis resistant to DTIC was resistant to diazo-ICA. Yamamoto[22] reported that cysteine abolished the inhibitory activity of diazo-ICA in Escherichia coli (E. coli) and suggested that diazo-ICA might be the active form for inhibition of this organism by DTIC. Studies with bacteria indicated that DNA synthesis in E. coli is inhibited by diazo-ICA[22] and that DNA synthesis in B. subtilis is inhibited by DTIC.[21] Diazo-ICA has also been shown to interfere with RNA synthesis in E. coli.[22]

The light dependent lethality of DTIC to bacteria has been mimicked in mammalian cells. Gerulath and Loo[23] showed that DTIC was more lethal to Chinese hamster ovary cells and human malignant melanoma cells in culture in the presence than in the absence of light. Accordingly it is accepted that the growth inhibitory activity of DTIC in bacteria and mammalian cells in culture is due almost exclusively to diazo-ICA, formed by light-catalysed dissociation of DTIC. It has been proposed that diazo-ICA produces this action by interfering with macromolecular synthetic processes.[21,22]

In addition to its bacteriostatic and tumour-inhibitory properties diazo-ICA has been shown to be a potent inhibitor of xanthine oxidase.[24] Activation of monoamine oxidase,[25] release of 5-hydroxytryptamine from rabbit platelets[26] and multiple pharmacological effects in cats and rabbits [26] have been reported for diazo-ICA.

(iii) DTIC as a source of reactive diazonium ions

While the photodecomposition of DTIC to diazo-ICA is a property peculiar to imidazotriazenes, all triazenes may be considered as "masked" or "latent" diazonium ions[27]. They are highly unstable in acid conditions and readily undergo heterolytic fragmentation to diazonium ions. (Scheme 1.2)

Effect of DIC on *B. subtilis* 168

P. P. Saunders and G. A. Schultz, *Biochem.Pharmacol.*, 19, 911 (1970)

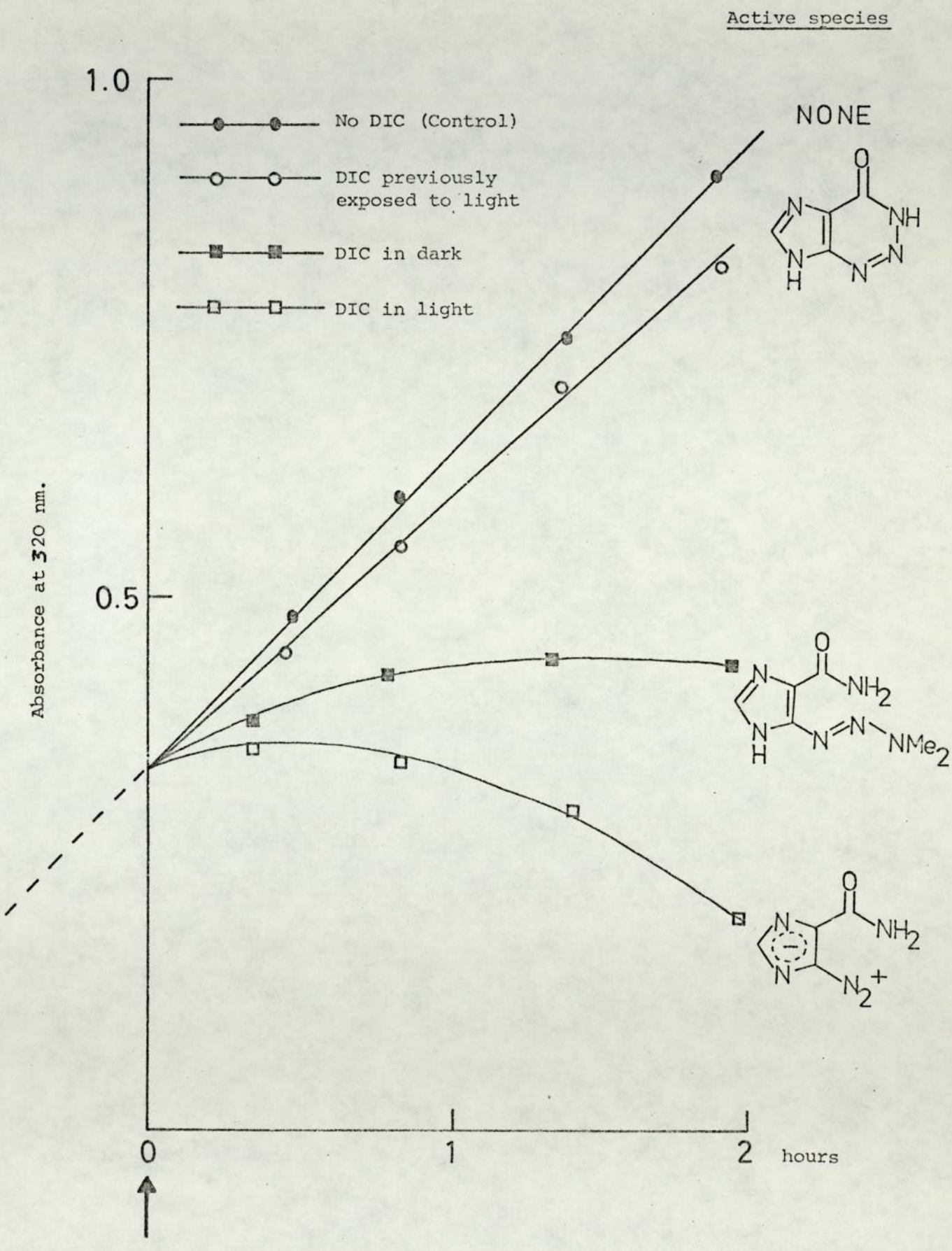
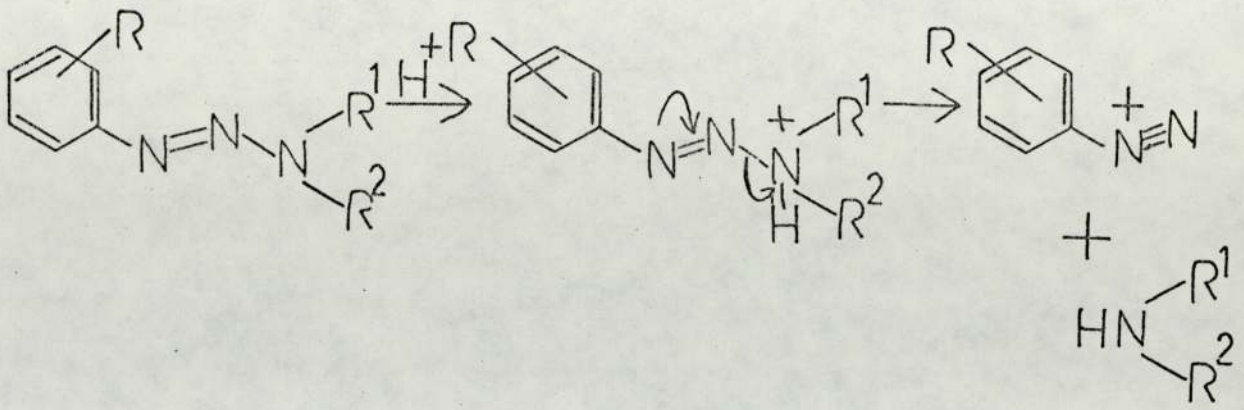
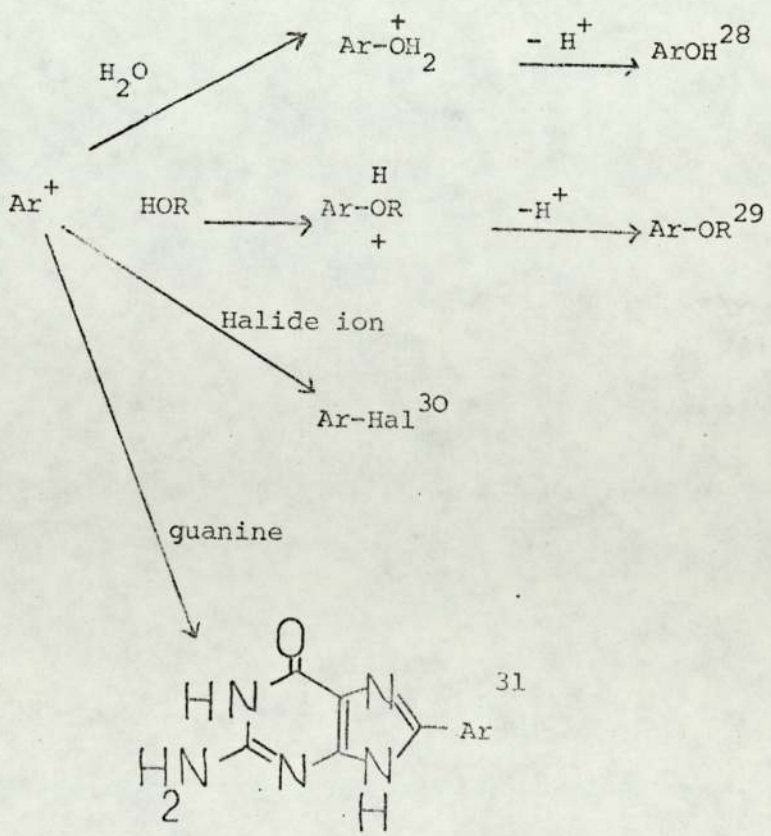


Fig. 1.2



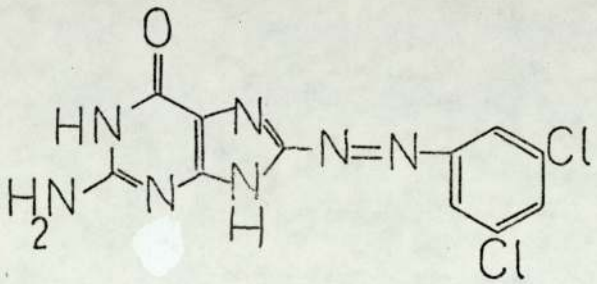
Scheme 1.2

Diazonium ions may react principally in two ways characterised by the nature of the products. This involves either loss or retention of the nitrogen atoms of the diazo group in the products. In the first instance, diazonium ions may decompose via S_N1 heterolytic cleavage of the C-N bond, to aryl cations (Ar^+) which react avidly with available nucleophiles (Scheme 1.3). Included in the first category are those reactions of diazonium compounds which proceed homolytically via aryldiazohydroxides to aryl radicals which subsequently react with nucleophiles. In either case the nitrogen atoms of the diazo group are lost in the products.



Scheme 1.3

Alternatively, diazonium ions may undergo coupling reactions to form products in which the nitrogen atoms of the diazo group are retained. Examples of possible biological relevance of this type of reaction include the coupling of diazotised 3,5-dichloroaniline with guanine to form the azo-derivative (8)[32] and the reaction of diazo-ICA with the thiol group of cysteine to form the azothioether (9)[24].



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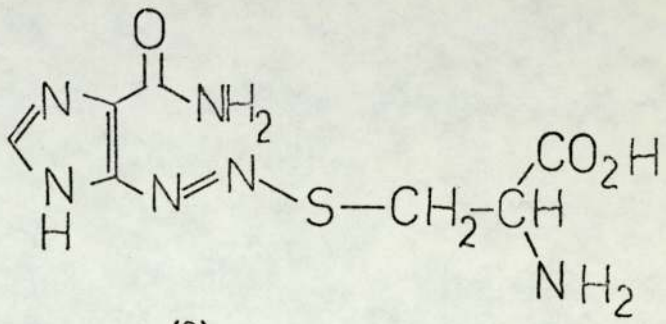


(9)

There are a large number of nucleophilic centres in a cell, each potentially capable of reaction with diazonium ions. These include the alcohol, thiol and amino groups of enzymes and structural proteins; the ionised phosphate groups and ring nitrogens of nucleotides and nucleic acids. In addition, free amino acids and peptides, vitamins, co-factors, steroids and lipids all possess nucleophilic centres capable of reaction with electrophiles. Therefore the introduction of a highly reactive electrophilic species into such an environment may be expected to have profound and potentially hazardous consequences for the well-being of the cell.

In recent years, the activity of aryl diazonium compounds has been exploited to elicit information about the structure of physiologically active proteins and the topography of the active sites of enzymes and the combining regions of antibodies. Some labelled macromolecules include insulin,[33] human erythrocyte membrane[34] and the enzymes trypsin[35] and chymotrypsinogen A and β-chymotrypsin.[36] The labelled amino acids have

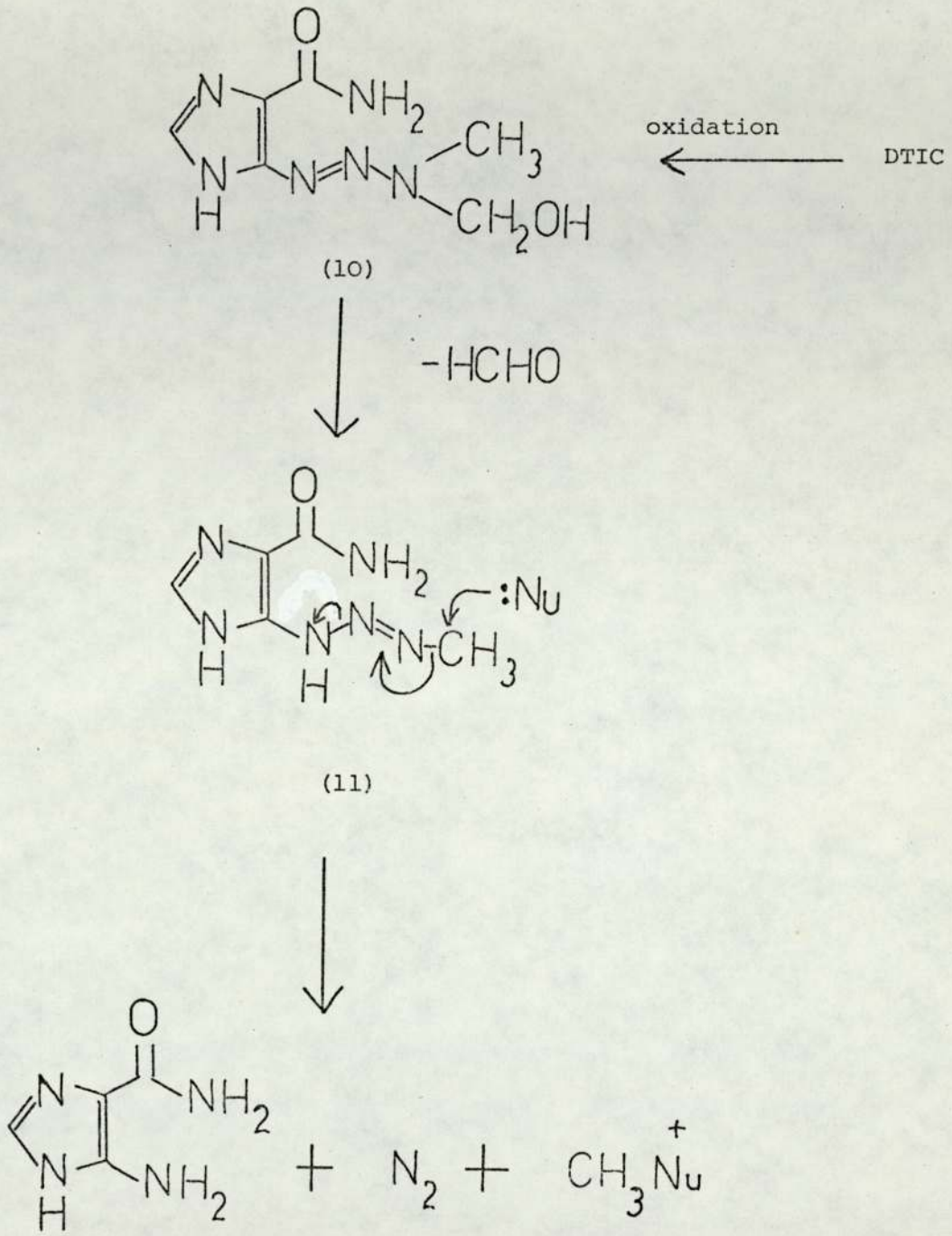
been tentativelyidentified in most cases as tyrosine, histidine and lysine.

The reversal of growth inhibition of B. subtilis and E. coli induced by diazo-ICA by thiols may be interpreted in a number of ways. Either diazo-ICA produces its biological effect as a consequence of a reaction with thiols in cellular molecules (eg. the coupling of diazo-ICA with cysteine residues); or alternatively, added thiols may quench the reactive electrophilic species thus removing it from the medium and preventing further reaction with significant cellular nucleophiles.

Whether the light-catalysed decomposition of DTIC has any counterpart in vivo, where there is, contrary to expectation, considerable light penetration or whether the molecule undergoes spontaneous heterolysis to diazo-ICA is a matter for conjecture. However. in attempts to associate tumour-inhibitory activity with chemical events,[19] it has been shown that the antitumour activity of triazenes is not related to the formation of diazonium ions but that the acute toxicity elicted by DTIC in man is due to diazo-ICA. Triazenes which do not undergo decomposition to diazonium ions under physiological conditions are nevertheless active against the TLX5 lymphoma.[19] Therefore it is considered that those triazenes which form a significant amount of diazonium ion may be unsuitable as antitumour agents since they may show an unfavourable Therapeutic Index.

(iv) In vivo effects of DTIC

DTIC is known to undergo a second transformation brought about by microsomal fractions containing the appropriate co-factors and in intact animals. This transformation involves oxidative N-demethylation to 5-(3-methyl-1-triazeno)-imidazole-4-carboxamide [MIC] (11). It is proposed that MIC undergoes spontaneous decomposition with loss of nitrogen and concurrent formation of AIC and methylation of nucleophiles. (Scheme 1.4)



(10)

(11)

(2)

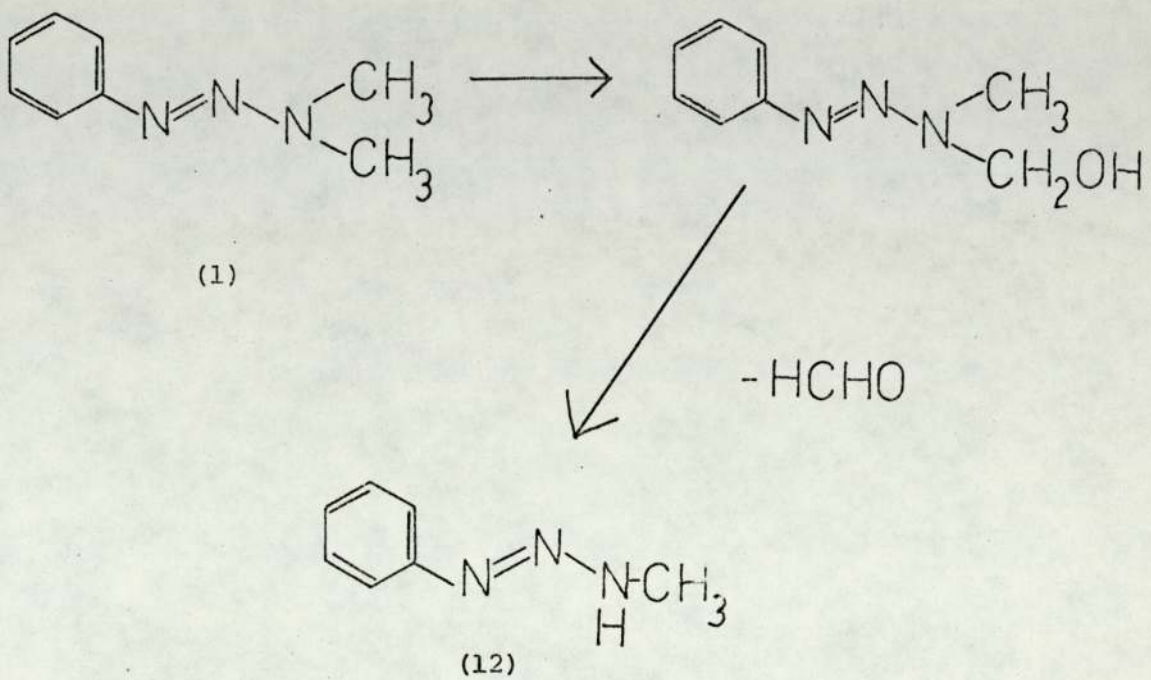
Scheme 1.4

Overwhelming evidence has been obtained for the operation of this pathway and is briefly summarised below.

Skibba, Beal, Ramirez and Bryan showed that DTIC was N-demethylated by rat liver microsomes to AIC and carbon dioxide[37]. The administration of DTIC (labelled in the methyl groups with ^{14}C) to rats and man was shown to result in the formation of AIC and the exhalation of $^{14}CO_2$.[37] ^{14}C-labelled 7-methylguanine was isolated from the urine of patients who received the labelled DTIC.[38] On the other hand, patients who were administered DTIC labelled in the imidazole 2-position with ^{14}C were found to excrete labelled AIC in the urine.[39] This clarified a previous observation of Householder and Loo,[40] who had reported that patients receiving DTIC excreted elevated amounts of AIC in the urine. They considered that this might be due either to interference by DTIC with AIC metabolism and consequent inhibition of purine synthesis, or, alternatively, to metabolism of DTIC to AIC as indicated above. The elevated levels of AIC in the urine of patients receiving DTIC therapy have been shown to be almost exclusively due to metabolism of DTIC.[39] In studies with ^{14}C-methyl-labelled DTIC, it has been shown that DTIC is also metabolised by human and animal tumour microsomes to ^{14}C-labelled formaldehyde.[41] Incubation of DTIC with tumour microsomes and nucleic acids resulted in the isolation of 7-methylguanine from the nucleic acid hydrolysates.[41] It has recently been shown by Hill[42] that DTIC is a substrate for microsomal enzymes of mouse liver and that the products of such metabolic oxidation of DTIC are AIC and formaldehyde.

Mechanism of carcinogenesis by aryl-dialkyltriazenes

After exhaustive studies on the mechanism of carcinogenesis by phenyl-triazenes it was reported[43] that aryl-dialkyltriazenes are dealkylated by microsomal fractions to form monoalkyltriazenes. (Scheme 1.5).



Scheme 1.5

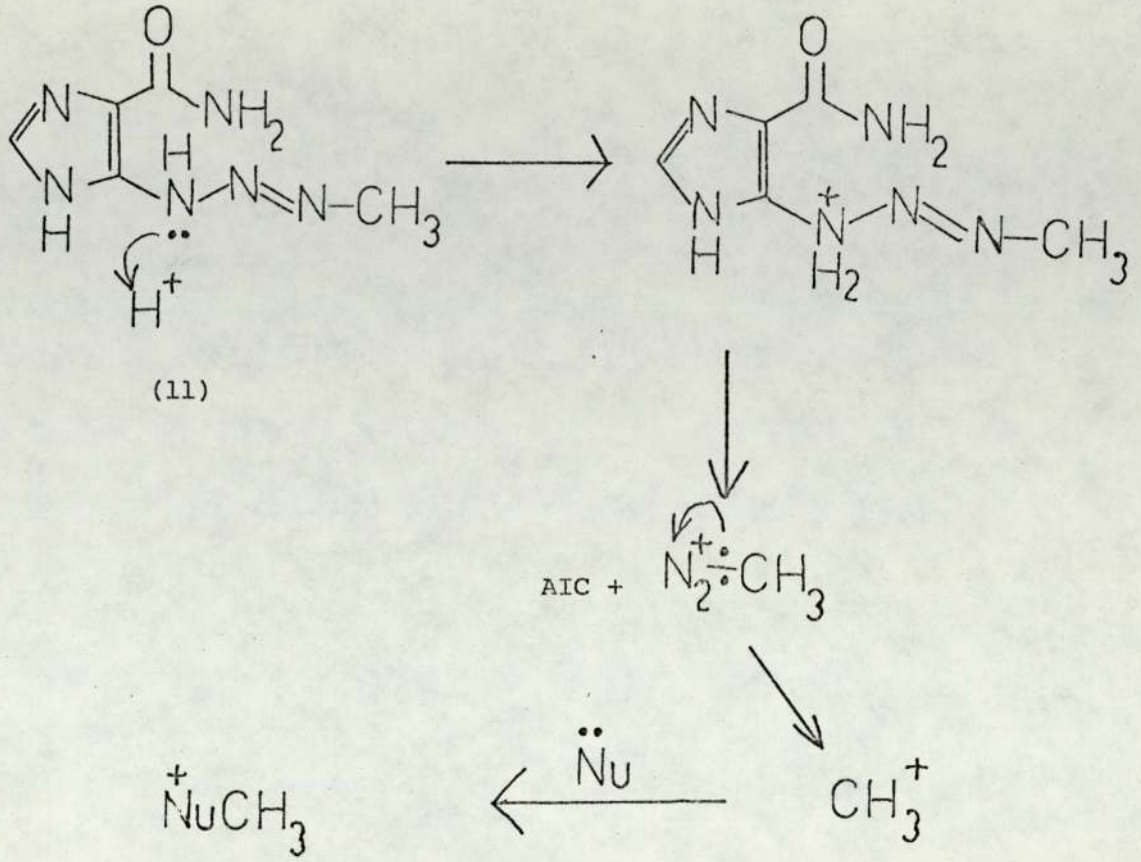
It was noted that phenyl-monomethyltriazene(12) is a potent proximate carcinogen, mainly producing tumours at the site of administration and it was proposed that carcinogenesis by phenyl-dimethyltriazenes is a consequence of metabolic N-demethylation to the corresponding monomethyltriazene which could methylate biopolymers. The methylating activity of monomethyltriazenes was first described by Dimroth[44] who found phenyl-monomethyltriazene a most efficient methylating agent, methylating phenols in high yields. It has been shown that injection of phenyl-dimethyltriazene into rats results in the formation of 7-methylguanine, isolated from RNA and DNA of the liver.[45] Finally Preussman and Von Hodenberg[46] showed that phenyl-monomethyltriazene will methylate N (7) of guanosine in vitro. It is appropriate to mention that MIC, the proposed active form of DTIC in tumour-inhibition, not only decomposes to AIC in solution, but is

tumour-inhibitory *per se*.[47] Indeed MIC has been shown to possess many of the properties of DTIC[48] and much of the toxicity to cells can be accounted for largely by MIC.

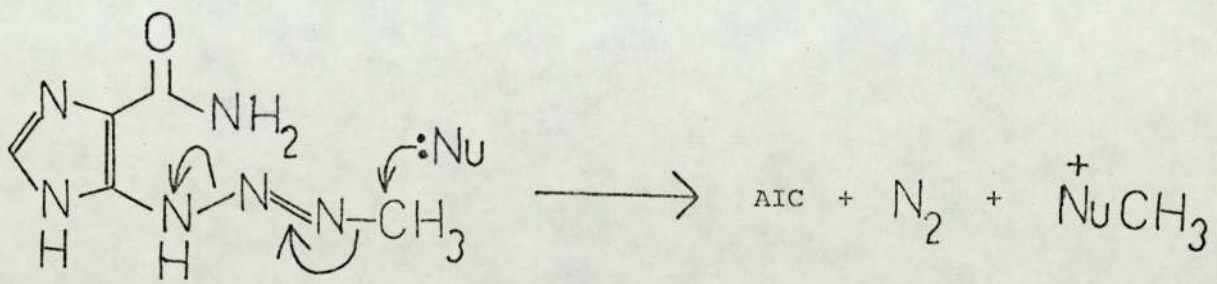
Mechanism of methylation by monomethyltriazenes

The nature of the methylating species derived from DTIC by metabolic transformation is the subject of some controversy. The intermediacy of diazomethane has been discounted by the observation that the methyl group of MIC is transferred intact to N(7) of guanine.[48a] One school of thought, however, favours the theory that MIC decomposes to form AIC and a "methyl carbonium ion" (*ie.* in kinetic terms, an S_N1 decomposition) which methylates biopolymers. Even the existence of such a reactive entity as a methyl carbenium ion is questionable. It therefore seems unlikely that such a promiscuous chemical species would exist in an aqueous medium long enough to react with any degree of specificity. Kinetic studies on the decomposition of 5-(3-*t*-butyl-1-triazeno)imidazole-4-carboxamide however, indicate that this triazene probably does decompose by an S_N1 process,[49] but in this case a stabilised *t*-butyl carbenium ion would be formed.

Methylation of nucleophiles by MIC and monomethyltriazenes may alternatively and perhaps more realistically, be considered in terms of an S_N2 reaction where MIC is acting as an "incipient" carbenium ion. (Scheme 1.6).



S_N1 decomposition



S_N2 decomposition

Scheme 1.6

Structure-activity relationships of tumour-inhibitory triazenes

Anticancer activity of aryl-dialkyltriazenes is associated with certain structural features. In a recent paper describing the structural requirements for an active metabolite of tumour-inhibitory triazenes, it was reported[19] that only those triazenes which could be metabolically N-dealkylated to monomethyltriazenes exhibited antitumour activity (in the TLX5 Lymphoma implanted into mice) (Table 1.1). It was also shown that there was no significant difference in antitumour activity in a series of triazenes containing varying substituents in the aromatic ring while maintaining the two methyl groups in the N(3) position (Table 1.2). The presence of electron-donating or electron-attracting groups in the aromatic ring greatly influences the half-life of hydrolysis of the triazenes to diazonium species. Therefore, since there was little difference in antitumour activity of these triazenes it was concluded that formation of diazonium ions plays little part in the antitumour action. It has also been shown that the diazonium ion derived from 5-(3,3-dimethyl-1-triazeno)-4-carbethoxy-2-phenylimidazole is extremely toxic to lymphoma cells *in vitro*, but it is not antitumour *in vivo* (mice) because it is also toxic to the host.[50] The parent triazene however is relatively non-toxic to the host and is highly active against the TlX5 tumour *in vivo*. Accordingly it is postulated that the antitumour activity of DTIC and related triazenes is a function of their capacity for transformation to monomethyltriazenes which may act as methylating agents, and that the acute toxicity may be due to formation of diazonium ions.[19]

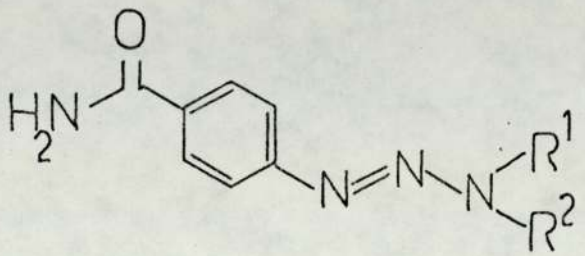
Significance of alkylating activity in tumour-inhibition and carcinogenesis.

These observations tend to place triazenes in the class of alkylating agents effective against neoplastic diseases. There are however, considerable uncertainties in this conclusion, amply illustrated by the following examples. The Rl and TLX5 Lymphomas do not respond to conventional alkylating agents (*eg.*Cyclophosphamide) but are extremely sensitive to dimethyltriazenes.[50]

Furthermore the plasma cell tumour, which is very sensitive to difunctional

Table 1:1 Antitumour activity of a series of 1-(p-carbamoylphenyl)-3,3-dialkyltriazenes against the TLX5 lymphoma.

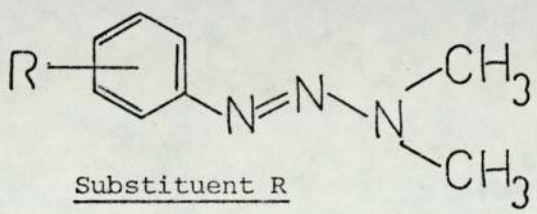
(adapted from Biochem. Pharmacol., 1975, 25,241)



R^1	R^2	Max % I.L.S.
CH_3	CH_3	55
C_2H_5	C_2H_5	inactive
$CH(CH_3)_2$	$CH(CH_3)_2$	inactive
CH_3	C_2H_5	46
CH_3	CH_2CH_2OH	42
CH_3	C_4H_9	78
CH_3	C_5H_{11}	102
CH_3	$CH(CH_3)_2$	52
CH_3	$CH_2\ C_6H_5$	86
CH_3	$C(CH_3)_3$	inactive
CH_3	OH	54
CH_3	H	43
C_2H_5	H	inactive

ILS = Increase in lifespan

Table 1.2 Anti-tumour activity of a series of 1-aryl-3,3-dimethyl-triazenes against the TLX5 Lymphoma (adapted from Biochem. Pharmacol.,1975,25, 241.)



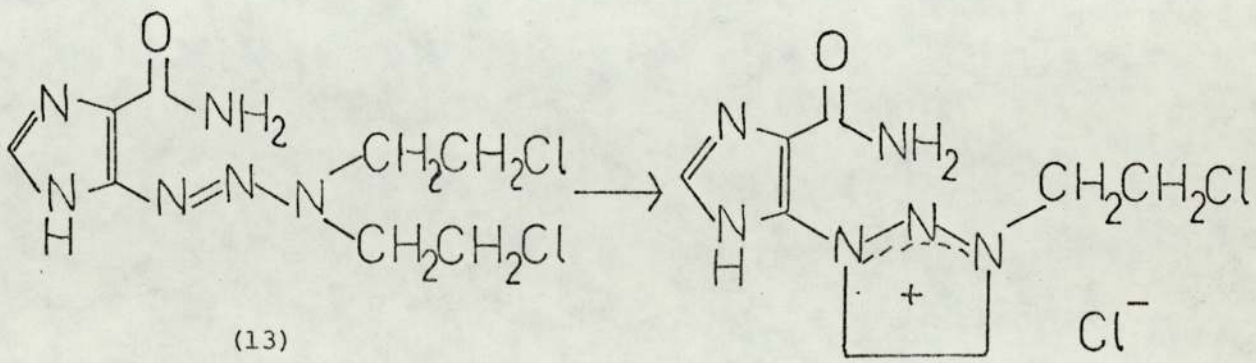
Substituent R	ILS
H	53
o-CO_2H	68
m-CO_2H	62
p-CO_2H	72
o-CO_2CH_3	53
m-CO_2CH_3	63
p-CO_2CH_3	58
p-$CO_2C_2H_5$	61
o-$CONH_2$	78
m-$CONH_2$	55
p-$CONH_2$	55
p-$CONHCH_2CO_2H$	46
p-OCH_3	41
p-NO_2	39
p-CF_3	61
p-SO_2CH_3	80

ILS = increase in lifespan. (%)

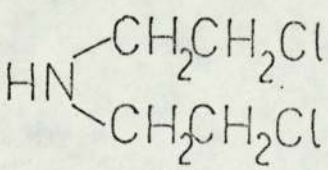
alkylating agents and insensitive to monofunctional alkylating agents responds to dimethyltriazenes (after metabolic N-demethylation). In addition to these observations it has been found that the most active triazene in the imidazole series, namely 5-[3,3-bis(2-chloroethyl)-1-triazeno]imidazole-4-carboxamide [BIC] (13) does not bear a methyl group.[51,52]

The reader will doubtless be aware at this stage of the difficulties inherent in interpreting the mode of action of DTIC. But DTIC presents a fairly clear picture compared to the dense haze surrounding BIC. It is interesting to contemplate some of the possibilities concerning the mode of action of BIC.

Firstly, it is considered that BIC is a carrier of the mustard (15) which it forms on acidic cleavage of the triazene linkage[53] and that the antitumour activity of BIC may be due in part to the release of this mustard. The other product of this cleavage is of course diazo-ICA. To add to the complexity of this situation evidence has been presented to the effect that BIC undergoes oxidative metabolism in a similar manner to DTIC.[42] In the case of BIC, such a transformation would result in the release of a chloroethylating species. BIC is also extremely unstable undergoing cyclisation in solution and even in the solid state to the isomeric triazolinium salt (14), but this species is inactive against L-1210.[51,52]



(13) (14)



(15)

Scheme 1.7

The methylation of nucleic acids by DTIC and dimethyltriazenes [eg.N(7) of guanine] is also of doubtful significance. Kruger[45] found that the carcinogen PDMT formed 7-methylguanine, isolated from DNA and RNA of rat liver, but tumours caused by administration of this carcinogen to rats have never been observed in the liver. Indeed the significance of alkylation of nucleic acid bases in tumour-inhibition and carcinogenesis is very much in question. Traditionally tumour-inhibitory alkylating agents were thought to owe their activity to a reaction with DNA[54] and attention has been focussed on such reactions as methylation of N(7) of guanine, O(6) of guanine, and to a lesser extent, on alkylation of N(1) or N(3) of adenine and N(3) of cytosine. There is evidence to suggest that carcinogenicity and mutagenicity (if not tumour-inhibitory activity) of certain alkylating agents correlates more closely with alkylation of phosphates and formation of phosphotriesters in DNA than it does with formation of 7-alkylguanine.[55] The carcinogenic cyclic nitrosamines (discussed in more detail later) also do not appear to alkylate nucleic acid bases, although there is evidence of some as yet unspecified interaction with RNA and DNA.[56]

Therefore at the risk of repetition, it is not yet possible to conclude which of the known chemical and biochemical events are related to the biological effects of triazenes and other cytotoxic compounds.

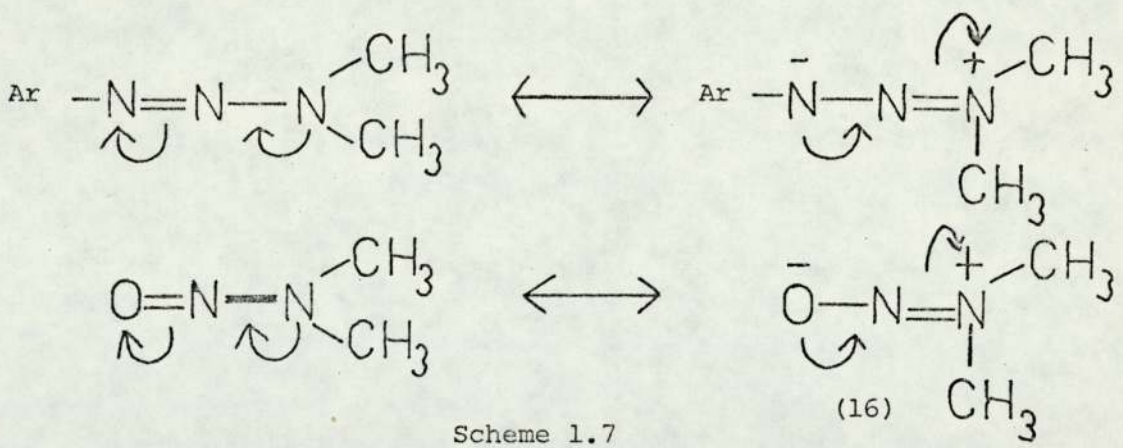
Immunogenic and antigenic effects of triazenes

To digress, an exciting and somewhat novel approach to the control of cancer was suggested by the observation of Clarke in 1955[2] that Sarcoma 180 failed to resume its usual rapid growth if pieces of the tumour treated with PDMT were implanted into mice. Strong antigenic changes in four Leukemia L-1210 lines have been demonstrated after treatment with DTIC over several transplant generations.[57] This change in antigenicity of the DTIC treated lines was accompanied by a decrease in oncogenic potential. Schmid and Hutchinson[58] have also shown that treatment of L-1210 with DTIC results in a decrease in oncogenic potential on transplantation, and noted the

ability of DTIC to induce antigenic cell change. The relationship between change in antigenicity and decrease in malignancy is not, however, understood. Following these studies, Campanile[59] showed that two lymphomas treated for sixteen transplant generations with DTIC in athymic mice became highly immunogenic. Mice which rejected the altered cell line were resistant to subsequent challenge with parenteral tumour cells. This suggests the possibility of obtaining immunogenic human neoplastic cells by drug treatment in athymic mice.

Carcinogenic N-nitrosamines

Aryl-dialkyltriazenes bear a close structural and electronic resemblance to the aliphatic N-nitrosamines whose carcinogenicity and toxicity are well documented.[60] (Scheme 1.7).



(16)

Scheme 1.7

Contribution from the 1,3-dipolar resonance structure (16) produces a planar structure with double-bond character between the two nitrogens resulting in hindered rotation about the N-N bond. Thus the n.m.r. spectrum of dimethylnitrosamine exhibits two aliphatic singlets; the trans-α-hydrogens resonate further downfield than the cis-α-hydrogens.[61] Restricted rotation about the N(2)-N(3) bond in aryl-dimethyltriazenes is likewise manifested in the splitting of the n.m.r. signal from the methyl protons.[62] Elucidation of the structure of the monohydrate hydrochloride salt of DTIC by X-ray crystallography showed that there is considerable double-bond character in the N(2)-N(3) bond as evidenced by the short bond distance of 1.305Å and by the planarity of bonding about N(3) indicating Sp^2 hybridisation.[63]

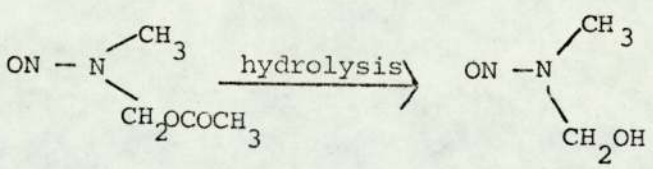
In view of these similarities between the N-nitrosamines and aryl-dialkyltriazenes, it is not surprising to note that the metabolism of dimethylnitrosamine (via oxidative N-demethylation to a methylating intermediate which can methylate guanine)[60 64,65] closely parallels the metabolic transformation of tumour-inhibitory and carcinogenic dimethyltriazenes. The carcinogenicity of dimethylnitrosamine is considered to be a direct result of its transformation to a methylating agent and subsequent reaction with cellular nucleophiles. Much less is known of the mechanism of carcinogenesis of the cyclic N-nitrosamines (eg. nitrosopiperidine, nitrosopyrrolidine and nitrosomorpholine), although it is suspected that these compounds are converted in vivo into alkylating agents. Lijinsky, Keefer, Loo and Ross,[56] however, failed to detect any alkylated bases derived from nucleic acids of livers of rats treated with cyclic nitrosamines. They did nevertheless concede that some type of chemical interaction between the nitrosamines and macromolecules may be involved in carcinogenesis since interaction with RNA and DNA was observed. In the light of the findings of Sun and Singer[55] on the correlation of alkylation of phosphate groups with carcinogenesis of certain alkylating carcinogens, these results need not be interpreted in such terms as to exclude an alkylating role in carcinogenesis for the cyclic nitrosamines.

According to a popular mechanism offered for the in vivo conversion of cyclic N-nitrosamines to alkylating agents, the key step is oxidative attack on the carbon alpha to the nitroso function. There are a number of theoretical ways in which the molecule could subsequently act in the capacity of a mono or difunctional alkylating agent. This aspect will be discussed in some detail in chapter 5.

Recent work by Lijinsky and Taylor[66,67] tends to confirm the importance of the α-carbon in metabolic activation. For example, in a study on the carcinogenicity of a series of methylated N-nitrosopiperidines[67] they found that there was no significant carcinogenic activity in 2,6-dimethyl-

-N-nitrosopiperidine and that carcinogenic activity was virtually absent in 2,2,6,6-tetramethyl-N-nitrosopiperidine. In the former case, oxidative attack at the α -carbon would be expected to be sterically hindered by the presence of the methyl groups, whereas in the latter case, in the absence of α-hydrogens, oxidative attack and subsequent activation of the molecule would be impossible. This view is further supported by the finding that a methyl group in the 3-position had only a modest dys-carcinogenic effect and that a methyl group in the 4-position did not reduce carcinogenic activity at all.

Powerful corroborative evidence for this theory has been supplied by the finding that the nitrosamine N-methyl-N-(2-acetoxymethyl)nitrosamine(17) does not require metabolic activation for mutagenic activity.[68] This compound would be expected to undergo facile non-enzymic hydrolysis to N-methyl-N-(2-hydroxymethyl)nitrosamine(18) (Scheme1.8) which is a suspected active metabolite of dimethylnitrosamine.



(17) (18)

Scheme 1.8

Compelling evidence was presented for analogous modes of action of dimethylnitrosamine and its α-acetoxyester at the molecular level in this study.[68] It would therefore appear to be highly plausible that N-methyl-N-(2-hydroxymethyl)nitrosamine could be a proximate metabolite of dimethylnitrosamine in carcinogenesis as well as in mutagenesis.[68] Similar studies on diethylnitrosamine and its α-acetoxyester[69] support the hypothesis that α-carbon hydroxylation may be the crucial first step in the metabolic activation of diethylnitrosamine and might therefore be of general applicability in the activation of all dialkylnitrosamines.

In contrast to these results it has been reported[70] that oxidation of N-nitrosopiperidine both in the Udenfriend system and by rat liver microsomes occurs predominantly in the 4-position, giving respectively N-nitrosopiperidin-4-one and N-nitrosopiperidin-4-ol. The possibility of concurrent oxidation at the α-carbon was not however excluded. The significance and consequences of oxidative attack on carbon atoms other than the α-carbon in nitrosamines will be discussed in chapter 5.

Triazenoquinazolines as tumour-inhibitory agents

The discussion has so far dealt with the properties of DTIC, related aryldialkyltriazenes and carcinogenic nitrosamines. It will be observed that the prototype of the triazenoquinazoline series, 4-amino-2-[2-(piperidin-1-ylazo)phenyl]quinazoline[NSC163423] (6a), active in cell culture against H-Ep2 is quite different in structure to DTIC and PDMT particularly in the important area of the alkyl substituent. While it is not difficult to envisage this compound acting through a diazonium mechanism, it is not obviously apparent that this compound could fulfil the structural requirements of tumour-inhibitory triazenes.[19] Metabolism of this compound to a monomethyltriazene seems unlikely. Preliminary work with the triazenoquinazolines[8] indicated that they are considerably more stable than DTIC and in particular are not prone to rapid photodecomposition; furthermore, antitumour activity was achieved at substantially lower doses than was observed for DTIC in the same test system. This is of paramount importance since the toxicity of triazenes has been shown to be dose related,[71] and in the case of DTIC the acute toxicity, due perhaps to formation of diazo-ICA, is dose limiting. Therefore for those reasons and because of the intriguing chemical analogies and anomalies, the triazenoquinazolines presented themselves as a promising new group of tumour-inhibitory agents worthy of further study. The chemistry of the cyclic nitrosamines is of obvious relevance to the chemistry of the triazenoquinazolines, both classes

of compound bearing a heterocyclic aliphatic function linked in the one case to a nitroso group and in the other to an arylazo group. The similarity in electronic distribution of these two systems has been noted (Scheme 1.7). A significant part of the practical work for this thesis concerned efforts to detect an alkylating capacity for the triazenoquinazolines and to establish a parallel with the carcinogen nitrosopiperidine.

Chapter 2

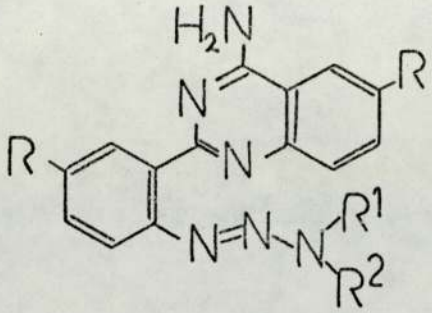
Synthesis of novel triazenoquinazolines

Introduction

The possibility that the tumour-inhibitory activity of the triazenoquinazolines may be a consequence of decomposition or conversion to any one of a variety of products makes a study of their mechanism of action important. Structure-activity studies are frequently useful in this context.

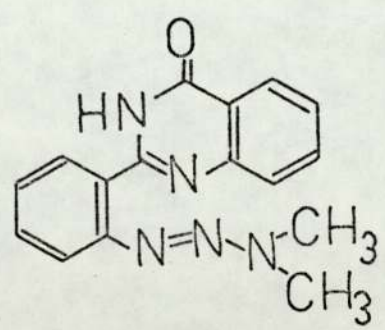
Structural variations on aryl-dialkyltriazenes may be considered to fall into two categories; those which alter the aryl function and those which modify the alkyl groups. Accordingly, further analogues of the triazene (6a) were prepared, in which the 2-,3- or 4-positions of the piperidine ring were substituted by a methyl group (6f-h). If the tumour-inhibitory activity of the triazenoquinazolines is dependent on such oxidative metabolism as was proposed for the cyclic nitrosamines, then the effects of these substitutions could conceivably be reflected in altered activity of the compounds. The dimethyl analogue (6i) was prepared for antitumour evaluation because of the established significance of the presence of methyl groups in related classes of tumour-inhibitory triazenes. In the course of this work, a facile method for hydrolysing 4-aminoquinazolines to the corresponding quinazolin-4(3H)-ones without degrading the triazene linkage was discovered. Thus the quinazolinone (19a) was synthesised and submitted for testing. Regrettably, biological results for many of these compounds are incomplete at the time of writing.

Variation in the aryl function was achieved by preparing the brominated triazenoquinazolines (20 a-f). The presence of hydrophobic bromine atoms in the molecule might be expected to affect lipid solubility, intracellular distribution and transport across membranes. Chemically, such substitution may influence the reactivity of the triazene linkage through participation of the non-bonding electrons of the bromine atom in conjugation (Scheme2.1). The bromine atom would similarly be expected to affect the stability and reactivity of the diazonium ion[72] formed by cleavage of the N-N linkage and might therefore be expected to affect the biological activity of the compounds, should this derive from such a diazonium species.

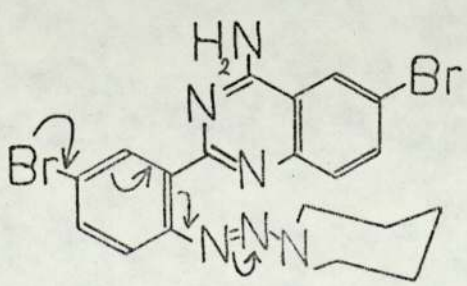


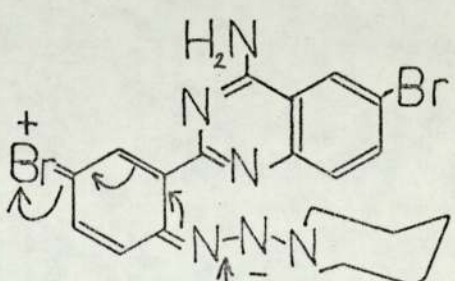
Compound	R	R^1	R^2
6 a	H	$-(CH_2)_5-$	
b	H	$-(CH_2)_4-$	
c	H	$-(CH_2)_2.O.(CH_2)_2-$	
d	H	n-Pr	n-Pr
e	H	Et	Et
f	H	$-CH(CH_3).[CH_2]_4-$	
g	H	$-CH_2-CH(CH_3)-[CH_2]_3-$	
h	H	$-[CH_2]_2-CH(CH_3).[CH_2]_2-$	
i	H	CH_3	CH_3

Compound	R	R^1	R^2
20 a	Br	$-(CH_2)_5-$	
b	Br	$-(CH_2)_4-$	
c	Br	$-[CH_2]_2.O.[CH_2]_2-$	
d	Br	$-CH(CH_3).[CH_2]_4-$	
e	Br	$-CH_2.CH(CH_3).[CH_2]_3-$	
f	Br	$-[CH_2]_2.CH(CH_3).[CH_2]_2$	



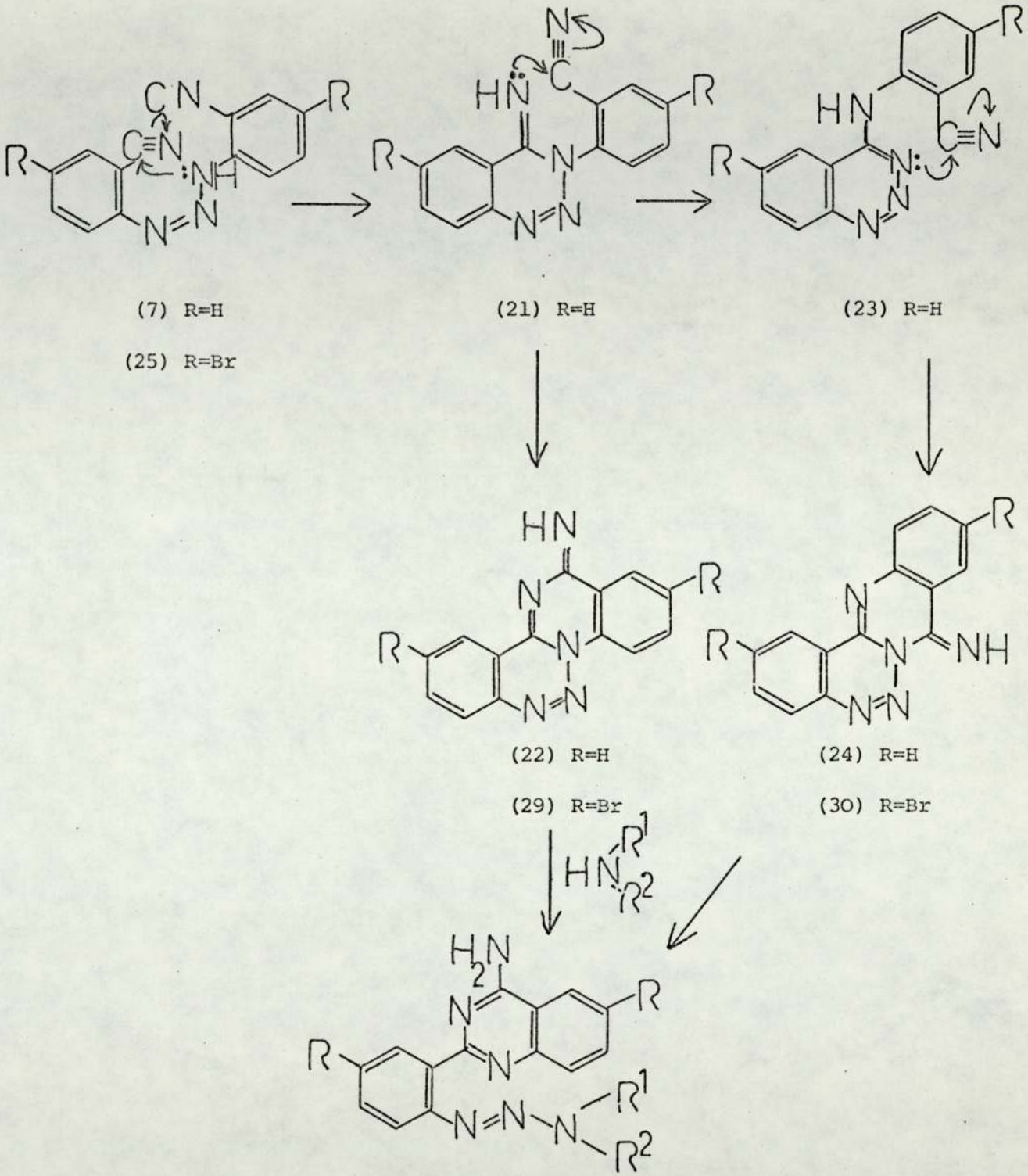
(19a)





Synthesis

The triazenoquinazolines may be conveniently prepared by either of two routes, both starting from anthranilonitriles. 1,3-Di-o-cyanophenyltriazene (7) (Scheme 2.2) is prepared by half diazotisation of o-aminobenzonitrile.[73] In the appropriate boiling secondary amines, this triazene is smoothly converted to the triazenoquinazolines (6a-e) respectively.[8] This conversion of the triazene(7) in boiling secondary amines involves formation of the 4-iminotriazine(21) by initial amine-nitrile addition. The 4-iminotriazine (21) may cyclise directly to the tetracyclic triazine (22) or, alternatively, may undergo Dimroth rearrangement to the 4-o-cyanoanilino-triazine (23)which subsequently cyclises to the isomeric tetracyclic triazine(24). In either case these tetracyclic triazines (which are not isolated), undergo ring-opening by the base to afford the required compounds. In the present work this synthesis was extended to the derivatives (6f-i). Similarly, the brominated triazene(25), prepared by half-diazotisation of 5-bromoanthranilonitrile, was converted in boiling secondary amines to the triazenoquinazolines (20a-f). The dibromotriazene(25) mimicked its unbrominated analogue (7) in all other chemical aspects examined[74] (Scheme 2.3). In boiling 70% ethanol the triazene (25) was smoothly decomposed to 4-amino-6-bromo-2-m-bromophenylquinazoline (26); in boiling ethanol containing hydrazine and Raney nickel (HRN), it gave the diamine (27) and in boiling ethanol containing 2-naphthol the azo-dye(28) was rapidly deposited. All these transformations probably proceed through the tetracyclic triazines (29) or (30) and in all likelihood could be more simply effected by employing these triazines as starting material.



(7) R=H

(25) R=Br

(21) R=H

(23) R=H

(22) R=H

(29) R=Br

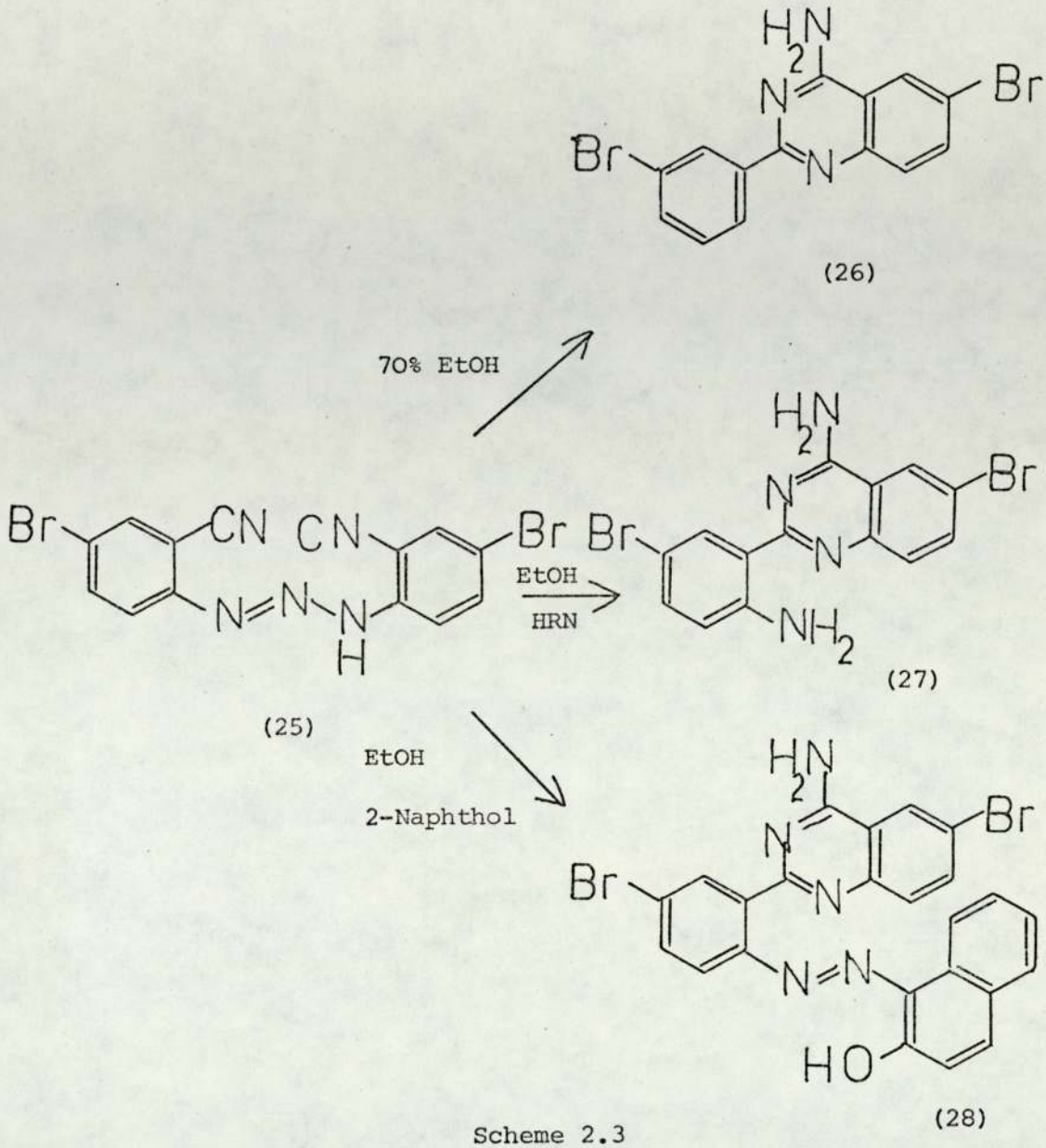
(24) R=H

(30) R=Br

(6) R=H

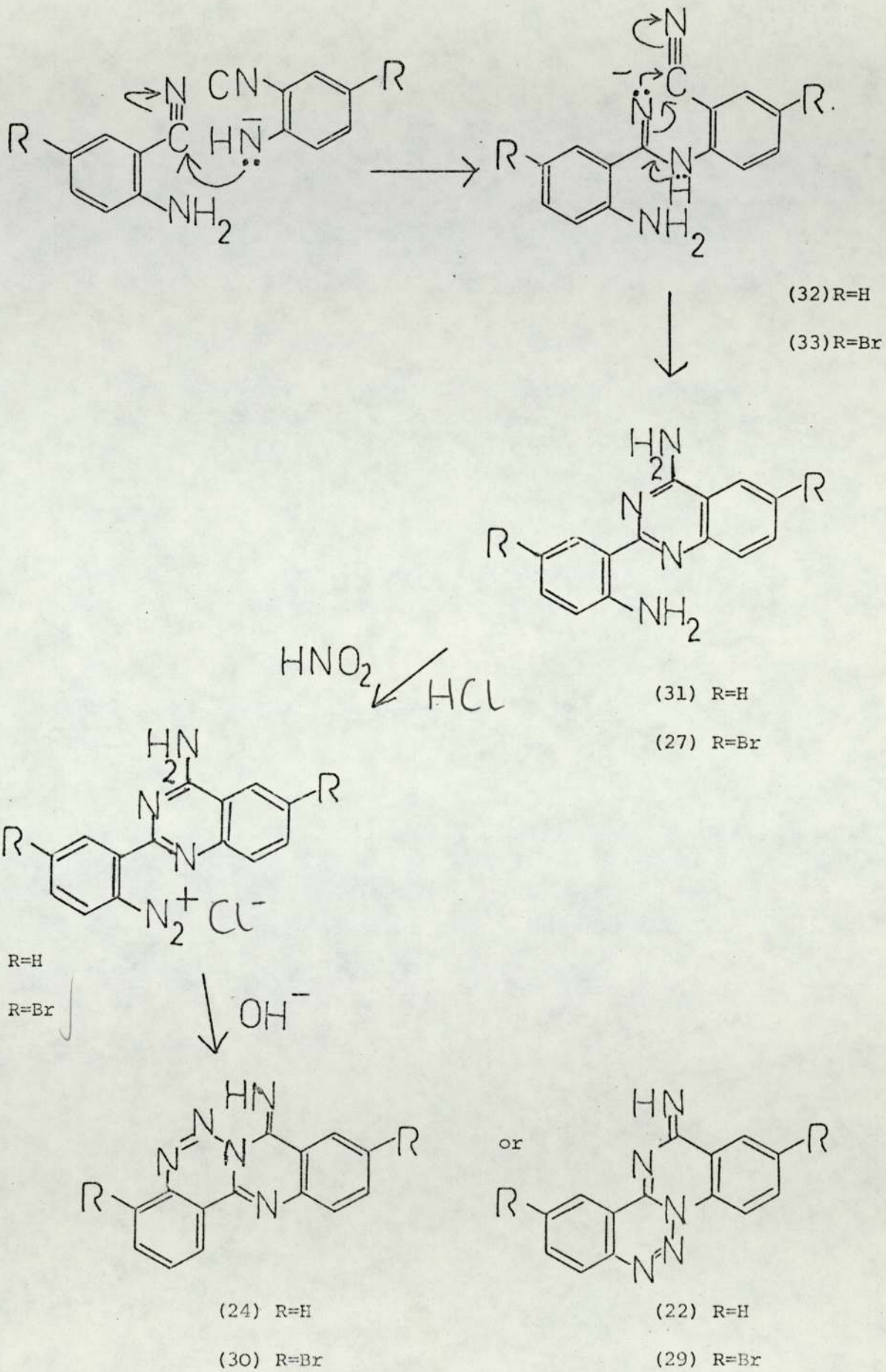
(20) R=Br

Scheme 2.2



Scheme 2.3

The tetracyclic triazines (22) and (29) or (24) and (30) may alternatively be obtained by diazotisation of the corresponding diamines (31)[75] and (27). (Scheme 2.4) This is the basis for the second synthetic approach to the triazenoquinazolines. A novel synthesis of the diamines (31) and (27) was suggested by a report[76] that reaction of nitriles with the anions of amines in dimethylsulphoxide afforded amidines in high yields. In the present case, treatment of anthranilonitrile or its 5-bromo analogue with sodium hydride in dimethylsulphoxide led to the isolation of the diamines (31) and (27) respectively, in near quantitative yields. The intermediate amidines (32) and (33) are not isolable in these cases, but undergo further amine-nitrile addition as indicated.

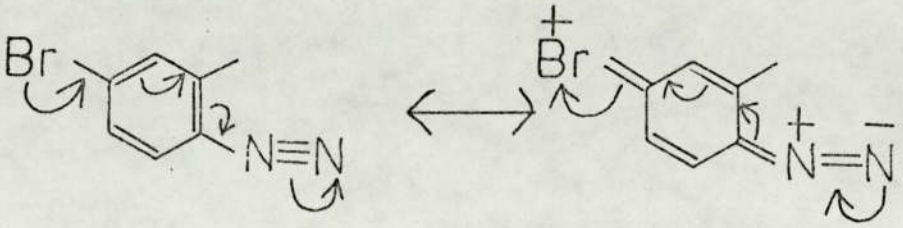

(32) R=H
(33) R=Br
HNO2
HCl
(31) R=H
(27) R=Br
(34) R=H
(35) R=Br
OH⁻
or
(24) R=H
(30) R=Br
(22) R=H
(29) R=Br

Scheme 2.4

Diazotisation of the diamines (31) and (27) in 2N and 6N - hydrochloric acid respectively, followed by basification, resulted in the formation of the tetracyclic triazines (22) and (29) or (24) and (30). Cyclisation of the diazonium ion (34) had previously been assumed to be on the quinazoline N(3) atom on the basis of a similar preference for cyclisation at the quinazoline N(3) atom in the reactions of other 2-o-aminophenylquinazolines with carbon inserting reagents.[77] The isomeric N(1) cyclised products (21) and (29) cannot however be excluded, since there seems no obvious way of distinguishing between these isomers. Furthermore, the quinazoline (6a) undergoes methylation at the N(1) atom (see chapter 4) - therefore the question of the structure of these tetracyclic triazines must remain open.

The triazenes (6a-e) have previously been prepared by reaction of the tetracyclic triazine (22) or (24) with the appropriate amines.[8] This synthesis was extended to selected members of the series (20), yields being almost quantitative.

Surprisingly, the diazonium ion (35) obtained by diazotisation of the diamine (27) proved remarkably stable and could be recrystallised unchanged from 6N-hydrochloric acid. Presumably this stability derives from contribution of the non-bonding bromine electrons (+M effect) in resonance interaction. (Scheme 2.5). This increases the double-bonded character of the C-N linkage and confers stability on the diazonium group.

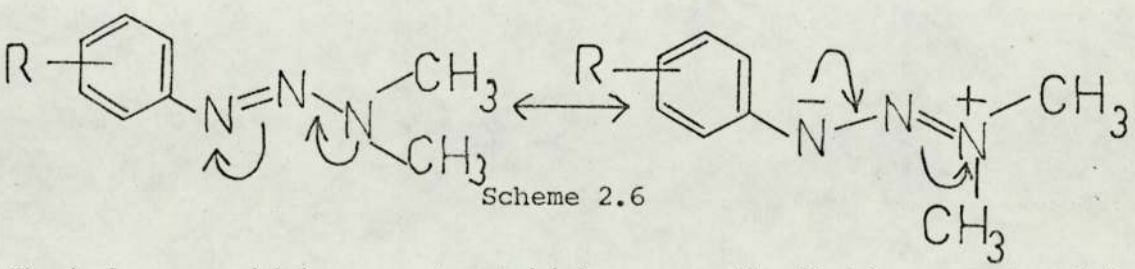


Scheme 2.5

Physical properties of triazenoquinazolines

The similarity in properties of the triazenes (6f-i) and (20a-f) to those of the triazene (6a) fully supports their assigned structures. Thus the electronic absorption spectra of the triazenes (6f-i) and (20a-f) exhibit the characteristic long wavelength absorption, showing considerable fine structure, present in the spectrum of the triazene (6a) (Table 2.1). Conversion of the triazene (6i) to (19a) by hydrolysis in 50% alcoholic potassium hydroxide was confirmed by the presence of strong sharp absorption at 1665cm^{-1} in the i.r. spectrum of the product, attributed to the carbonyl group. The remarkable stability of the triazene linkage under these conditions is noteworthy.

The 100 MHz ^{1}H n.m.r. spectrum of the dimethyltriazene (6i) in $CDCl_3$ shows a singlet for the methyl groups (T=6.95) whereas the signal from the methyl protons in the corresponding 4-quinazolinone (19a)is split into a doublet (at T=6.30 and T=6.50). Splitting of the signal from the methyl protons in 1-aryl-3,3-dimethyltriazenes has been reported[62] and was found to be temperature dependent. This splitting phenomenon has been interpreted in terms of restricted rotation about the N(2)-N(3) bond, arising from the partial double-bonded character of this linkage. (Scheme 2.6). Similar features are apparent in the n.m.r. spectrum of dimethylnitrosamine[61].



Scheme 2.6

The hydrogens which resonate at higher magnetic field are assigned to the cis-N-methyl group.[62] In the present case [triazene (19a)] it is conceivable that the effect of an oxo-group in the quinazoline 4-position is to increase the participation of the dipolar resonance hybrid so that the phenomenon

Electronic absorption spectra (λ_{max}/nm; log ε in parenthesis) of triazenoquinazolines (in 95% ethanol).

Compound								
	6a†	233	(4.45)	287	(4.27)	315*	(4.21)	329* (4.12)
	6f	233	(4.51)	287	(4.31)	315*	(4.26)	330* (4.23)
	6g	233	(4.48)	287	(4.28)	315*	(4.24)	
	6h	233	(4.49)	287	(4.29)	315*	(4.25)	330* (4.16)
	6i	233	(4.49)	288	(4.29)	315*	(4.24)	330* (4.11)
	20a	239*	(4.48)	295	(4.36)	325*	(4.28)	
	20b	239*	(4.46)	296	(4.32)	325*	(4.25)	
	20c	243*	(4.39)	297	(4.30)	325*	(4.23)	342* (4.06)
	20d	238*	(4.53)	295	(4.40)	325*	(4.33)	
	20e	240*	(4.49)	295	(4.39)	323*	(4.09)	
	20f	240*	(4.42)	296	(4.30)	325*	(4.24)	340* (4.12)

† with water of crystalisation.

* shoulder /or inflection.

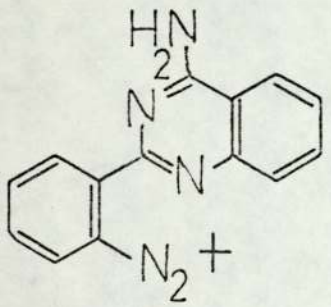
Table 2.1.

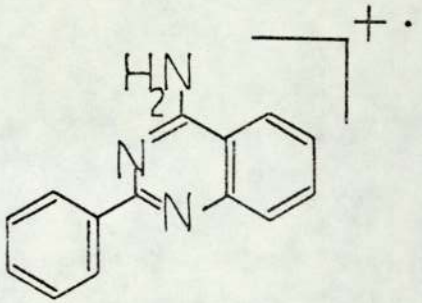
of splitting is observed at room temperature. Other long range effects may well operate and the quinazolinone (19a) would be a suitable candidate for structure elucidation by X-ray crystallography. In addition to the restricted rotation phenomenon it would be interesting to know the spatial dispositions of the two bulky substituents in the ortho disubstituted benzene (19a) and the trans or cis configuration of the triazene linkage. In 1,3-diaryltriazenes this is certainly trans.[78]

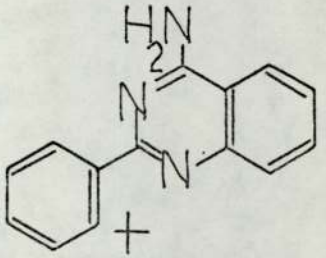
The assignment of structure to the triazenes (6f-h) is further supported by their mass spectra, all of which show the expected features in common with the spectrum of (6a) (Table 2.2). The molecular ions are not observed, but the spectra all show abundant ions at m/e 221 and m/e 220. The radical ion at m/e 221 is formed by loss of the triazeno side-chain accompanied by H-rearrangement. The ion at m/e 220 arises either from the radical ion by H-atom loss, or alternatively by fission of the entire triazeno fragment. The most abundant peaks in the spectra of the triazenes (6f-h) derive from heteroalicyclic fragments (m/e 99 and 98). Fragmentation of the heterocyclic radical from the intact molecule yields an unstable ion at m/e 248 which may be an acyclic diazonium ion (34) (Scheme 2.7) or a cyclic species (36): this loses nitrogen to form the arenium ion (37) at m/e 220.

The dimethyltriazene (6i) shows a very small molecular ion (<1%) and significant ions at m/e 248 (55%), 221 (100%) and 220 (86%).

The corresponding dimethyltriazenyl-quinazolinone (19a) also shows these features but in this case as minor pathways. The dominating cleavage is loss of neutral dimethylamine from the molecular ion to form a radical ion at m/e 248 (13%) which then expels carbon monoxide to form the radical ion at m/e 220 (100%) (Scheme 2.8). These ions are possibly cyclic species (38) and (39) respectively since subsequent fragmentations are identical to those of the molecular ion of benzimidazo[1,2-c]-1,2,3-benzotriazine (39)[79].



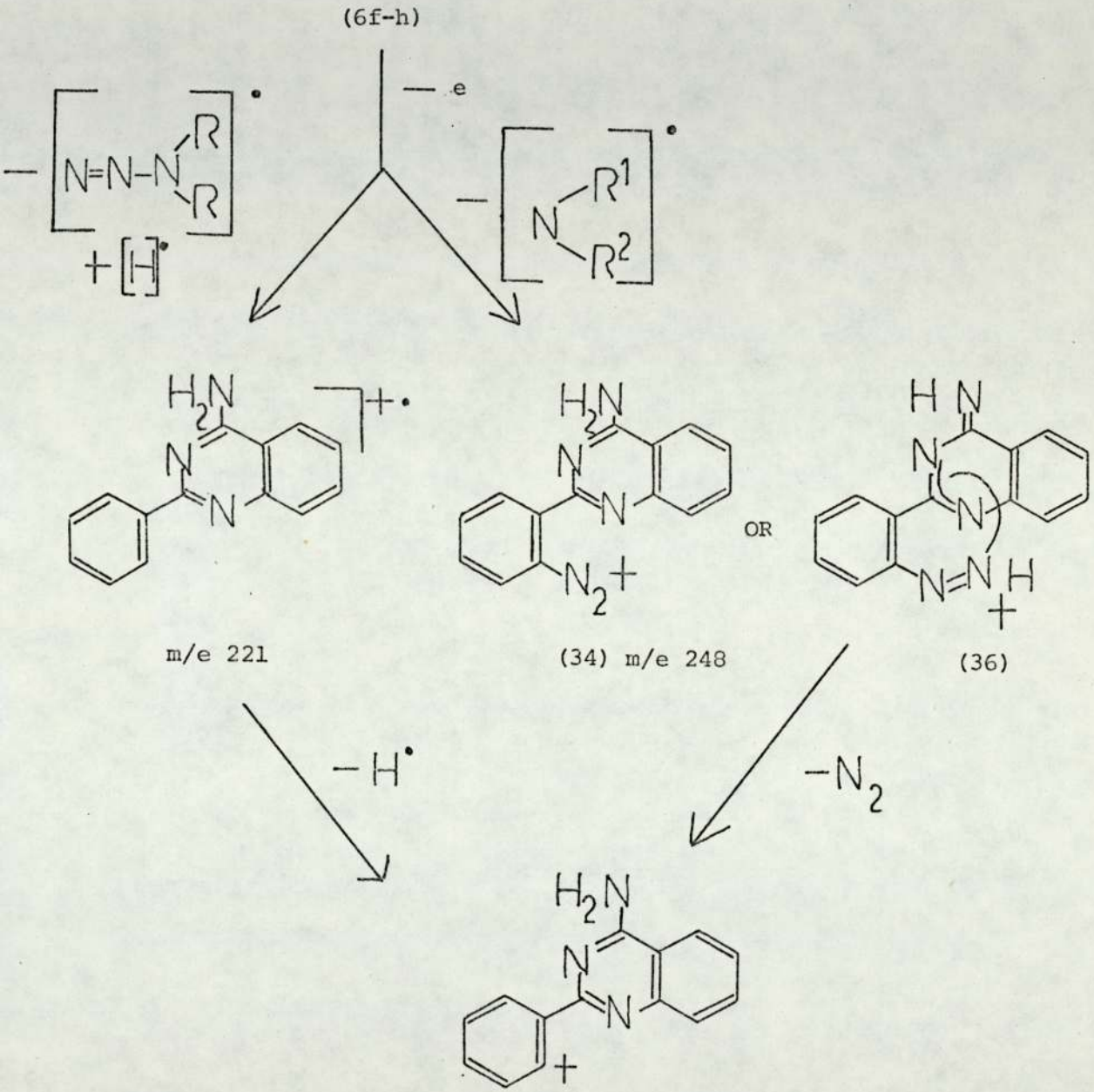




Compound	Molecular ion	m/e 248	m/e 221	m/e 220
6a	absent	5	100	46
6f	absent	5	100	57
6g	absent	5	100	18
6h	absent	1	100	23
6i	<1	55	100	86
PDMT	11.3	m/e 105 35.8	m/e 78 13.2	m/e 77 100

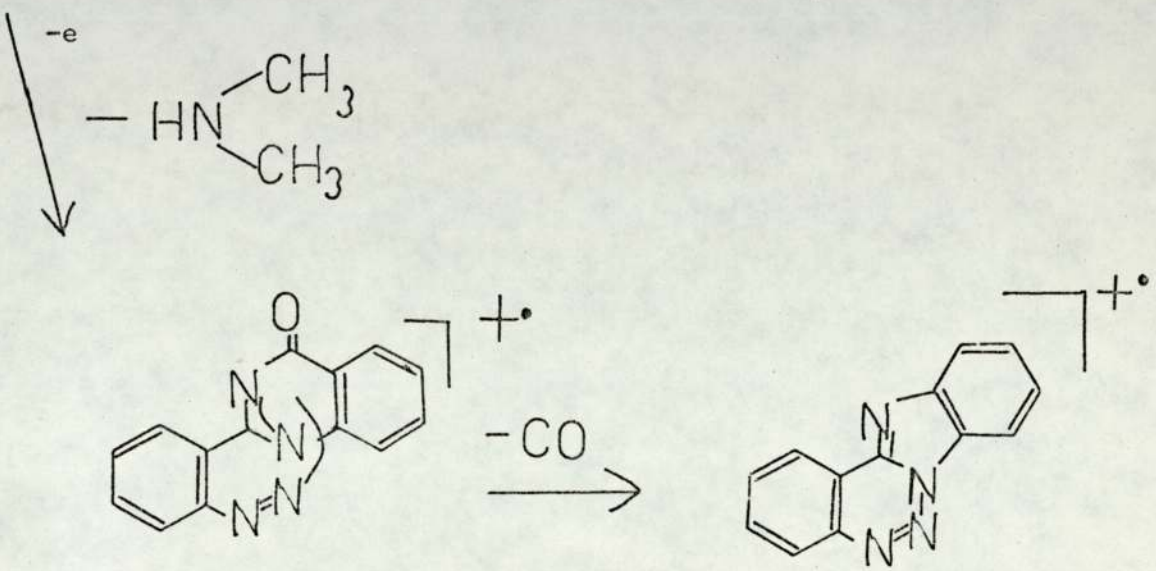
Table 2.2

Relative abundances (% of most abundant peaks) from mass spectra of triazenes recorded on an A.E.I. MS 9 spectrometer operating at 70eV.



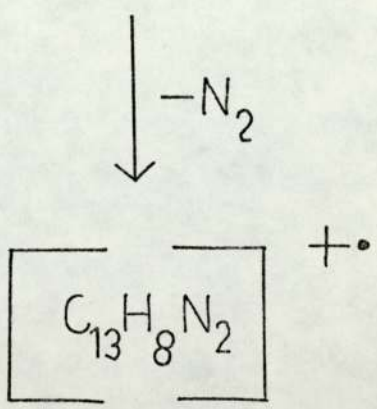
Scheme 2.7

(19a)



(38) m/e 248

(39) m/e 220



m/e 192

Scheme 2.8

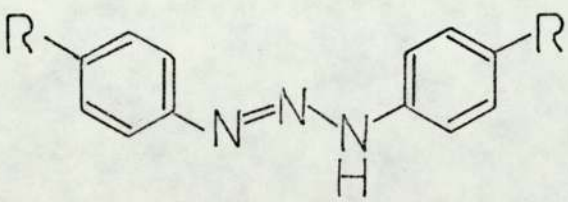
Solvate formation appears to be a general phenomenon in quinazolines[80] and was also observed in the triazenoquinazolines (6a), (6b)[8] and (20f) which crystallised as hydrates and in (6c), (a benzene solvate). Solvation in the quinazoline cation, unsubstituted in the 4-position is considered to be due to covalent hydration across the 3,4-double bond.[81,82] The presence of an amino group in the 4-position in the triazenes (6a) (6b) and (20f) excludes the possibility of solvation occurring by such covalent hydration. Hydration through hydrogen bonding also seems unlikely in triazenes (6a) and (6b) because of the presence of sharp absorption at 3605 cm^{-1} and 3615 cm^{-1} in the solid phase i.r. spectra of these compounds, usually attributed to an unassociated O-H stretching vibration. It is possible that the water molecules are sandwiched between the two bulky ortho substituents where intermolecular hydrogen bonding would be sterically prohibited.

Chapter 3

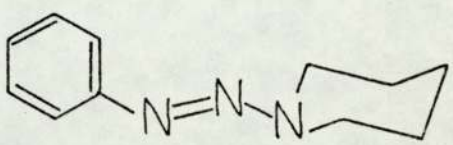
Properties of 4-amino-2-[2-(piperidin-1-ylazo)phenyl]quinazoline

Reactions with nucleophiles

Triazenes have been described as "masked" diazonium ions[27] and are precursors of electrophilic diazonium species. This character is, however, normally revealed only under acidic conditions. In view of the possibility that some electrophilic species may be responsible for the antitumour activity of triazenes, it seemed pertinent to examine the reactivity and stability of the intact triazene linkage in systems containing nucleophiles. The susceptibility of the triazene linkage to nucleophilic attack was examined in the reactions of the di-aryltriazenes (40) and the heteroalicyclic analogue (41) in amines. The triazenes (40) were recovered unchanged from prolonged boiling in piperidine or morpholine. Similarly no direct cleavage of the NNN linkage was observed in the reactions of the triazene (41) with the primary aromatic amines, p-nitroaniline or p-toluidine in ethanol.

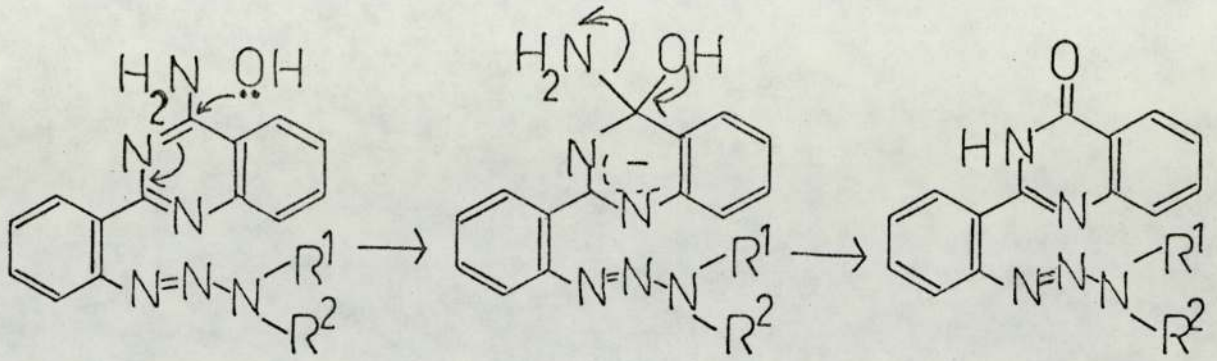


(40) R=CH_3, Cl, NO_2



(41)

The triazene linkage in (6a,b,i) proved remarkably stable in boiling 50% alcoholic potassium hydroxide, being quantitatively converted to the corresponding triazenoquinazolin-4(3H)-ones (19a-c) (Scheme 3.1) with retainment of the intact triazene linkage.



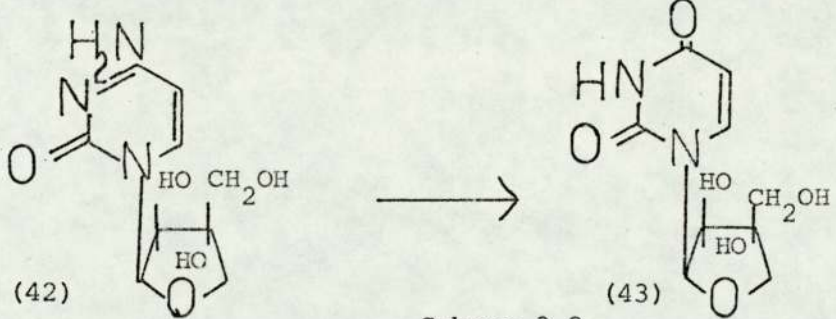
(6) (19)

	R^1	R^2
a	$-(CH_2)_5-$	
b	$-(CH_2)_4-$	
i	CH_3	CH_3

	R^1	R^2
a	CH_3	CH_3
b	$-(CH_2)-_4$	
c	$-(CH_2)_5$	

Scheme 3.1

An interesting although unrelated analogy to this reaction occurs in the intramolecular hydrolysis of the active anti-leukemic agent arabinosylcytosine (42) to arabinosyluracil (43) which is inactive[84] (Scheme 3.2).



(42) (43)

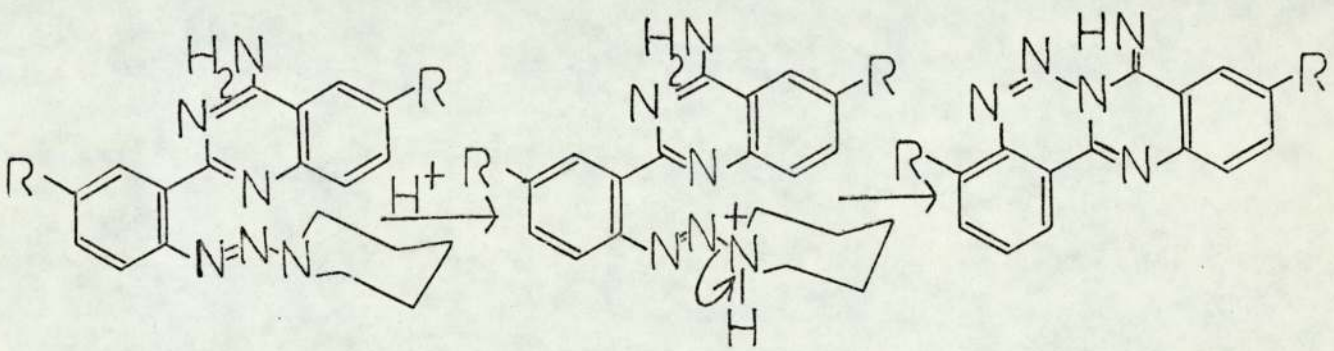
Scheme 3.2

No reaction was observed between cysteine and the triazene (6a) in aqueous dimethylformamide or in 1N-potassium hydroxide solution; nor was any coupling observed between the diazonium ion (34) derived from the triazene (6a) and cysteine in aqueous sodium hydroxide.

Acidic decompositions

The reactions of the triazene (6a) and its brominated analogue (20a) in acids clearly illustrate the latent diazonium character of these compounds. In all cases, reaction proceeds with N-N bond cleavage to afford the respective diazonium ions. Depending on the reaction conditions, a number of products may be derived from these species.

Treatment of the triazenes (6a) and (20a) with 6 N-hydrochloric acid at room temperature or acetic acid at 80°, followed by basification with sodium hydroxide led to the formation of the tetracyclic triazines (24) and (30) (Scheme 3.3) or the respective N(1) cyclised products. These conversions are clearly comparable to the photolytic decomposition of DTIC to diazo-ICA which cyclises to 2-azahypoxanthine. The tetracyclic triazine (24) or (22) has been shown to be susceptible to degradation in protic solvents [74] or in systems containing nucleophiles[8] and therefore should the acid promoted cyclisation of the triazene (6a) to the tetracycle (24) or (22) have any counterpart *in vivo* it seems unlikely that this triazine would be stable in such an environment.



(6a) R=H

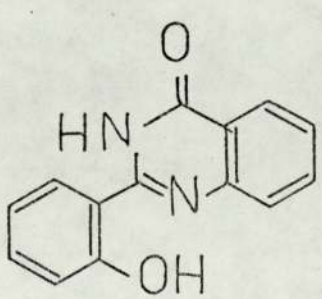
(20a) R=Br

(24) R=H

(30) R=Br

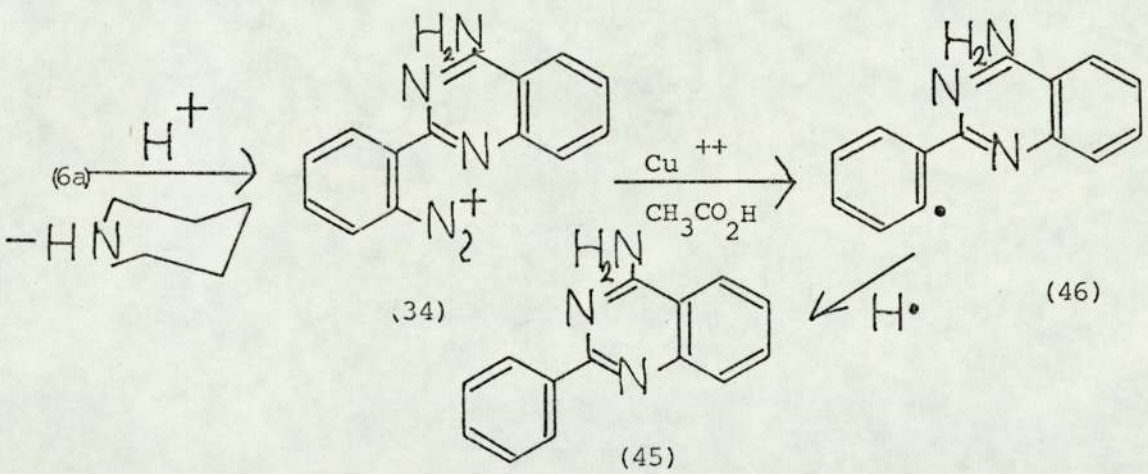
Scheme 3.3

In boiling 4N-sulphuric acid, the triazene (6a) underwent extensive decomposition with replacement of the triazeno side-chain and the 4-amino group by hydroxy groups. 2-(2-Hydroxyphenyl)quinazolin-4(3*H*)-one [85] (44) was isolated in 40% yield.



(44)

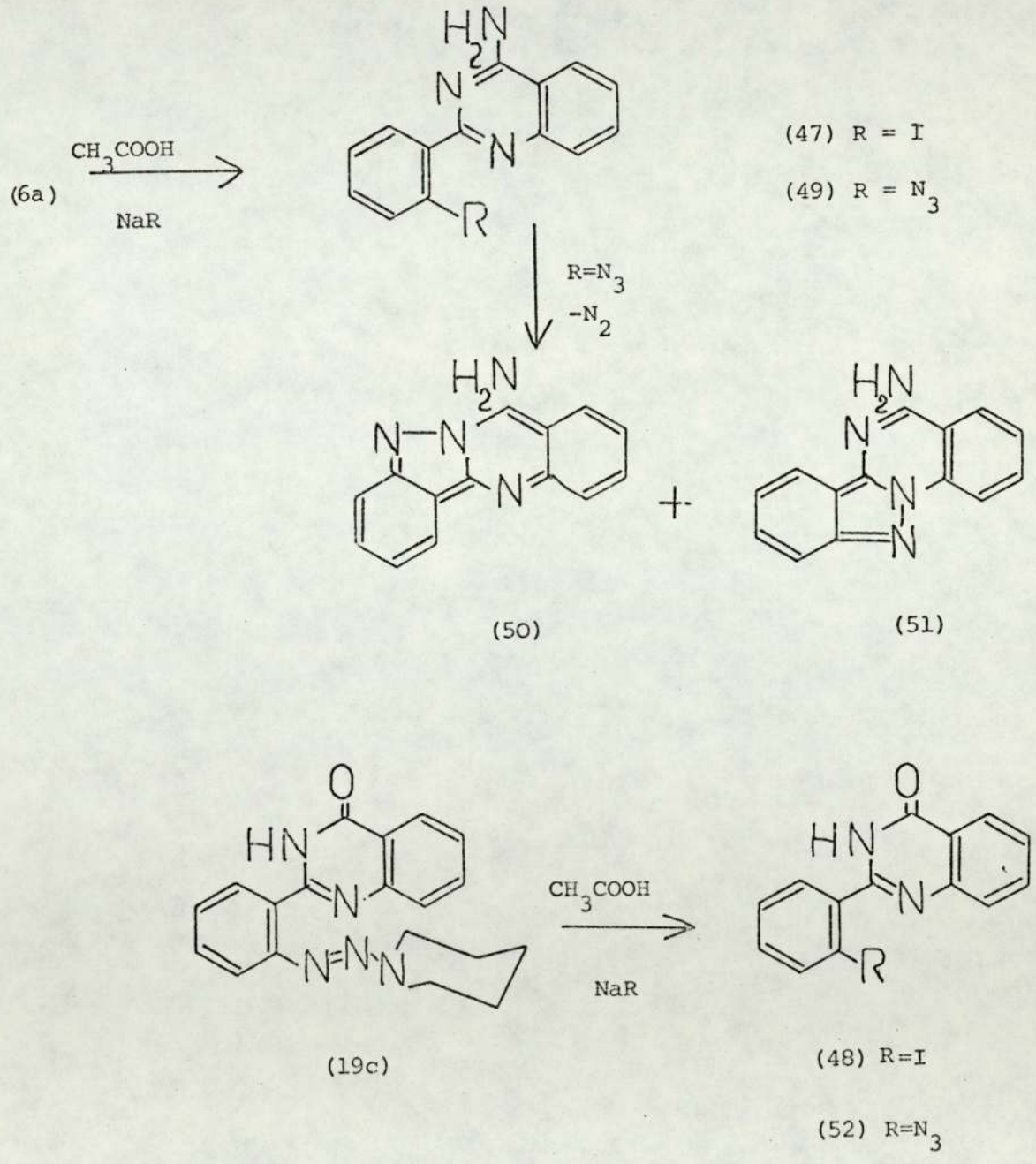
Incorporation of additional elements in the decompositions of the triazene (6a) in acetic acid resulted in some interesting conversions. In boiling acetic acid containing copper-bronze, the triazene (6a) underwent reductive elimination of the triazeno side-chain to afford 4-amino-2-phenylquinazoline (45) in excellent yield. Presumably this transformation proceeds *via* the diazonium ion (34) (Scheme 3.4). Subsequent steps in this conversion may be interpreted in terms of reductive elimination of nitrogen from the diazonium ion to form the radical (46) under the influence of cuprous ions. Reduction of aryl diazonium ions to aryl radicals by cuprous ions is considered to proceed *via* a complex formed between the diazonium ion, cuprous ion and related anion.[86] In the present case, abstraction of a hydrogen radical from the protic solvent yields the product (45).



Scheme 3.4

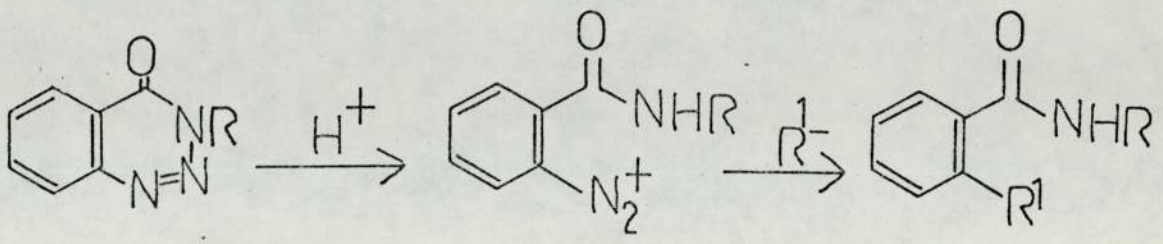
Acetic acid proved to be an excellent medium for promoting Sandmeyer-type displacements of the triazeno side-chain in the triazenes (6a) and (19c), and in related heterocyclic systems containing the NNN linkage.[87] The triazenes (6a) and 19c) (Scheme 3.5) formed the iodobenzenes (47) and (48) respectively in boiling acetic acid containing sodium iodide. When the triazene (6a) was boiled for a short time in acetic acid containing sodium azide, 4-amino-2-(2-azidophenyl)quinazoline (49) could be isolated albeit in low yield (≤20%). Prolonged boiling of the triazene (6a) in acetic acid containing sodium azide led to the formation of a mixture of the isomeric indazoles (50) and (51).

In this case, the N(3) and N(1) atoms of the quinazoline ring are sufficiently nucleophilic to promote nitrogen loss from the azide, probably in a concerted displacement (Scheme 3.5), forming the indazoles (50) and (51) respectively. The triazenoquinazolin-4-one (19c) formed the corresponding azide (52) under similar conditions, but this azide showed no tendency to cyclise, presumably because the N(1) and N(3) nitrogens of the quinazolin-4-one ring are less strongly nucleophilic due to the replacement of an amino group in the 4-position with an oxo-group.



Scheme 3.5

These substitutions present a useful and novel route to o-substituted arenes. The applicability of this reaction was examined in other heterocyclic systems containing the NNN linkage [87] and notably in derivatives of 1,2,3-benzotriazin-4(3H)-one. Thus, in the present work, 1,2,3-benzotriazin-4(3H)-one (53a) (Scheme 3.6) was converted in acetic acid containing sodium azide or sodium iodide to o-azidobenzamide and o-iodobenzamide respectively. The triazinone (53a) was, however, recovered unchanged from acetic acid containing sodium chloride, bromide, acetate, cyanide and sulphite. These anions are insufficiently nucleophilic to promote decomposition of the diazonium ion (54). The scope of this reaction is also limited by the nature of the N(3) substituent of the triazinone ring. Thus the N-alkyl- and N-aralkyl-triazinones (53b-d) and (e-f) respectively failed to decompose in acetic acid containing sodium azide or sodium iodide. The effect of such N(3) substitution would appear to be to stabilise the triazine ring to acid cleavage perhaps by an electron releasing effect. The N-aryl-triazinones (53g-k) on the other hand undergo efficient conversions to the respective o-substituted azides and iodides.[87] This represents a convenient synthesis of the azides (55g-k $R'=N_3$) which cannot be prepared by the conventional diazotisation-azidation route because of the dominating competitive intramolecular cyclisation to 3-aryl-1,2,3-benzotriazin-4 (3H)-ones.[88]



(53) (54) (55)

a R=H

b Me

c Et

d n-Pr

e Benzyl

f Phenethyl

g Ph

h o-Tolyl

i o-$NO_2 \cdot C_6H_4$

j m-$NO_2 \cdot C_6H_4$

k p-$NO_2 \cdot C_6H_4$

R^1 = I, N_3

Scheme 3.6

Reductions of 4-amino-2-[2-(piperidin-1-ylazo)phenyl] quinazoline.

Replacement of a primary aromatic amino group by hydrogen (ie. deamination) can be accomplished by diazotisation of the amine, and heating the resultant diazonium ion in a protic medium.[27] Analagous to this conversion is the decomposition of the triazene (6a) to 4-amino-2-phenylquinazoline in boiling ethylene glycol. This transformation was also accomplished by photolysis of the triazene (6a) in ethanol or methanol and was conveniently followed by U.V. spectroscopy (Fig. 3.1) and by thin layer chromatography (tlc). Photolysis

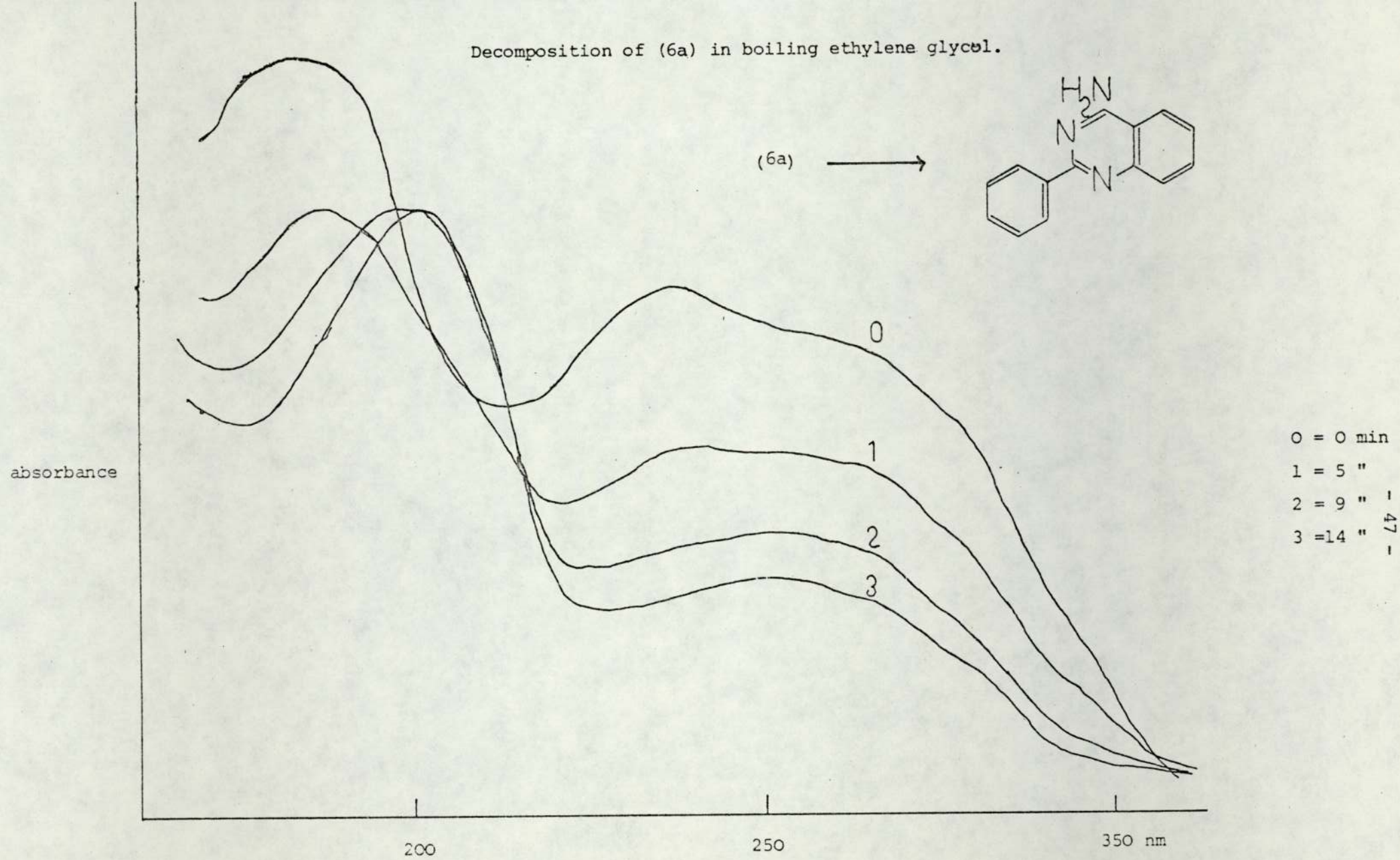

Decomposition of (6a) in boiling ethylene glycol.
(6a)
H2N
N
N
absorbance
0
1
2
3
0 = 0 min
1 = 5 "
2 = 9 "
3 =14 "
200
250
350 nm

Fig. 3.1

of DTIC, it will be recalled proceeds via diazo-ICA which is stabilised by loss of the acidic imidazo proton. Subsequent cyclisation to 2-azahypoxanthine occurs. In the photolytic decomposition of the triazene (6a) the intermediate diazonium ion cannot be stabilised in such a manner. Instead, reductive elimination of nitrogen occurs in the protic solvent.

A combination of hydrazine and Raney nickel (HRN) has been shown to effect N-N bond cleavage in a variety of heterocyclic systems.[74,89] The triazene (6a) also proved to be susceptible to this reagent and afforded the expected 4-amino-2-(2-aminophenyl)quinazoline (31).

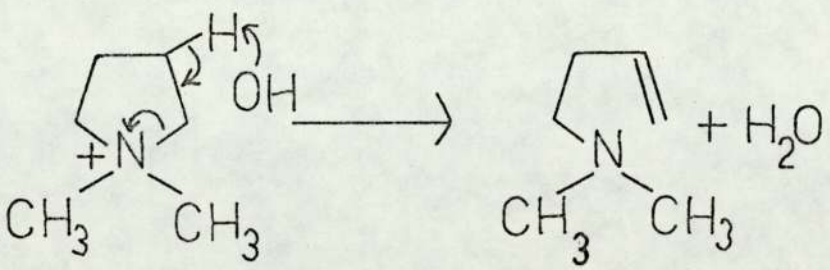
The products of the various decompositions described in this chapter were all submitted for antitumour evaluation and were found to be inactive. Since it has been shown that the capacity for transformation of tumour-inhibitory triazenes to alkylating agents is fundamental in their mode of action, (although subsequent events are unclear) efforts were next directed towards investigating the possibility of chemical transformation of the triazene (6a) to an alkylating entity.

Chapter 4

Alkylation of 4-amino-2-[2-(piperidin-1-ylazo)phenyl] quinazoline.

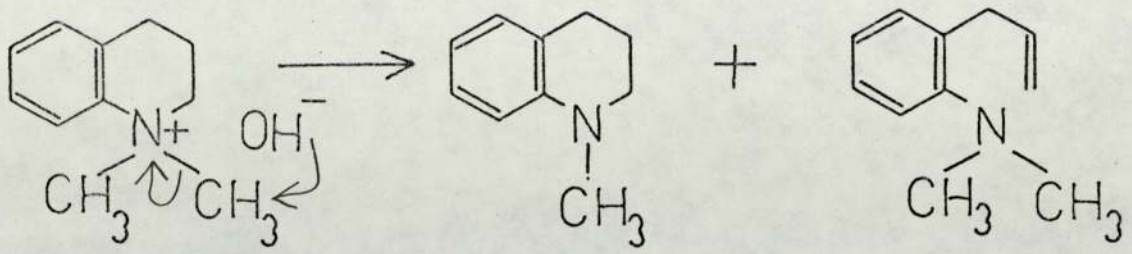
Introduction

The first working hypothesis behind attempts to develop an alkylating potential for the triazene (6a) concerned an exploitation of the Hofmann elimination reaction. Alkylation of a tertiary amine with eg. methyl iodide results in the formation of a quaternary ammonium iodide. Treatment of a quaternary ammonium iodide with an aqueous suspension of silver oxide affords the corresponding quaternary ammonium hydroxide. Such compounds decompose on heating ($\underline{n}125^{o}$) to form a tertiary amine, with elimination of an alkene. For example, N,N-dimethylpyrrolidinium hydroxide (56) is thermally decomposed to the tertiary amine[91] (57). (Scheme 4.1). This reaction, called the Hofmann elimination is analagous to the dehydrohalogenation of an alkylhalide. Hydroxide ion abstracts a hydrogen ion from the β-carbon; cleavage of the C-N bond occurs with retainment of the electrons on the nitrogen; and the double bond is generated. The reaction proceeds via an E_2 mechanism.



(56) Scheme 4.1 (57)

Hofmann elimination of a quaternary ammonium hydroxide by heating is not always effective for the opening of nitrogenous rings and its success is dependent on the conditions of the thermal decomposition.[91] For example, the quaternary hydroxide of N,N-dimethyl-1,2,3,4-tetrahydroquinoline (58) gives methanol and the original base (59) on heating as well as the ring-opened o-allyl-N,N-dimethylaniline[91] (60) (Scheme 4.2).

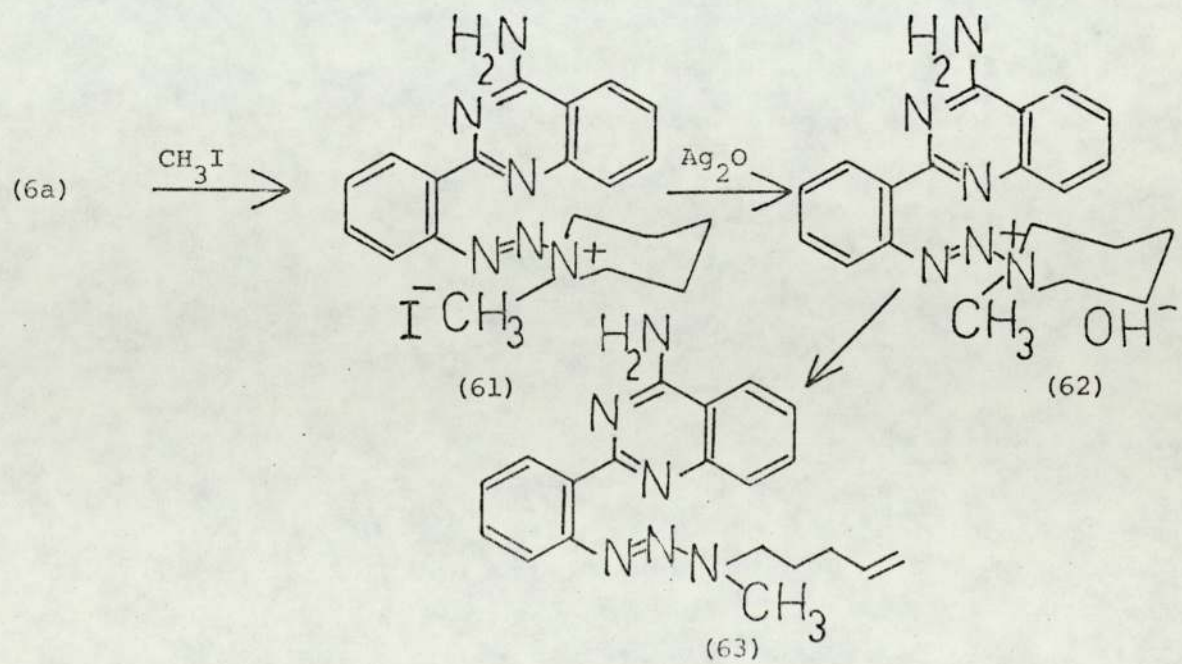


(58) (59) (60)

Scheme 4.2

In some of these cases, analagous ring-opening of the quaternary ammonium iodide may be achieved by reductive degradation with eg. sodium amalgam, or sodium in liquid ammonia (Birch reduction).[91]

In the present case, it was considered that methylation of the triazene (6a) with methyl iodide could have yielded the triazene (61), quaternised on the piperidine ring. (Scheme 4.3). Treatment of this salt with aqueous silver oxide would conceivably result in the formation of the corresponding quaternary ammonium hydroxide (62) which might be expected to undergo thermal decomposition to the ring-opened dialkyltriazene (63) by the Hofmann elimination route. It is significant that the possible product (63) of this transformation now fulfills the structural requirements for tumour-inhibitory triazenes,[19] in that oxidative dealkylation of this triazene would yield a monomethyltriazene. However, the thermal process would possibly cleave the triazene linkage.



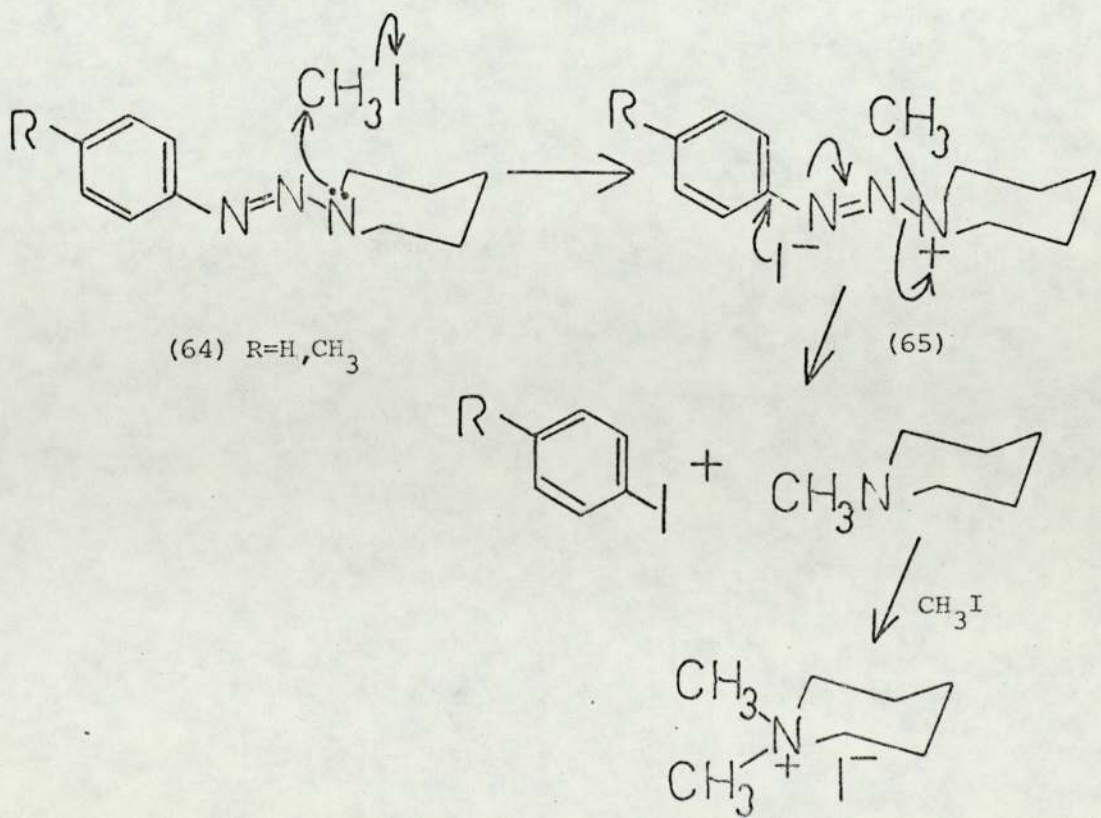
Scheme 4.3

Methylating agents eg. methionine, are present in biological systems and there are no theoretical objections to the quaternisation of the triazene (6a) in vivo. Subsequent steps in this proposed transformation could possibly be mediated by enzymes.

Methylation of model triazenes.

Methyl iodide has been shown to quaternise the N(2) atom of derivatives of 1,2,3-benzotriazine[92]. In view of the dipolar nature of the NNN linkage it was considered prudent to first examine the nucleophilicity of the triazeno N(3) atom in the reactions of the more simple aryl-dialkyltriazenes (64 R=H, NO_2, CH_3) with methyl iodide.

The triazene (64 R=H) (Scheme 4.4) undoubtedly underwent methylation on the N(3) atom with methyl iodide in methanol, or methyl iodide in benzene, since the quaternary ammonium salt($_{66}$) was eventually isolated. This observation was interpreted as follows : the intermediate (65 R=H) which could not be isolated, underwent iodide ion initiated displacement of the triazeno side-chain, with loss of nitrogen and formation of iodobenzene and N-methylpiperidine. The N-methylpiperidine thus formed reacted with a further molecule of methyl iodide to yield N,N-dimethylpiperidinium iodide (66).

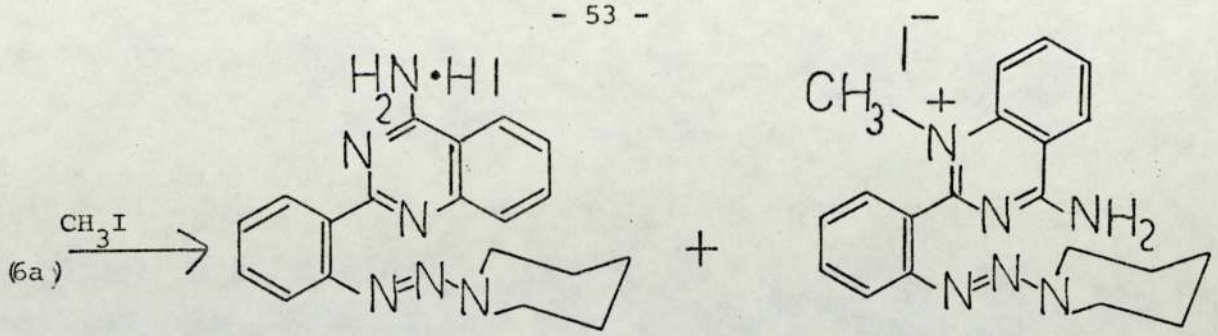


The reaction was influenced by the nature of the p-substituent. Thus an electron-attracting nitro group in the p-position (64 R=NO_2) reduced the basicity of the triazeno N(3) atom to the extent that this centre was insufficiently nucleophilic to participate in the S_N2 methylation reaction with methyl iodide. The triazene (64R=NO_2) was recovered unchanged from refluxing methyl iodide or refluxing methanol containing methyl iodide. The effect of an electron-releasing group in the p-position, ie, the triazene (64R=CH_3) was to eliminate the necessity for carrying out the reaction with methyl iodide in a methanolic medium ; N ,N-dimethylpiperidinium iodide was recovered from the reaction of the triazene (64 R=CH_3) in refluxing methyl iodide alone.

Should the quinazolinotriazene (6a) undergo methylation on the triazeno N(3) nitrogen, subsequent nucleophilic displacement of the triazeno side-chain by iodide ion might be sterically hindered by the bulky o-substituent. The proposed product (61) would then be a candidate for the Hofmann elimination or reductive degradation to the ring-opened triazene (63).

Methylation of 4-amino-2-[2-(piperidin-1-ylazo)phenyl]quinazoline

In the event, methylation of the triazene (6a) with methyl iodide or methyl iodide in tetrahydrofuran, took a different course. Two products were isolated neither of which was the desired N(3) methylated triazene (61). Both products contained the intact NNN linkage, as evidenced by the formation of azonaphthol derivatives when they were heated with acetic acid containing 2-naphthol(Bamberger-Goldberger test).[93] The first of these products (67) (15%) was a hydroiodide salt of the starting material. It was identified by conversion to the starting material on basification. The other product (80%) was assigned the structure (68) (Scheme 4.5) on the basis of the following chemical and spectroscopic evidence.

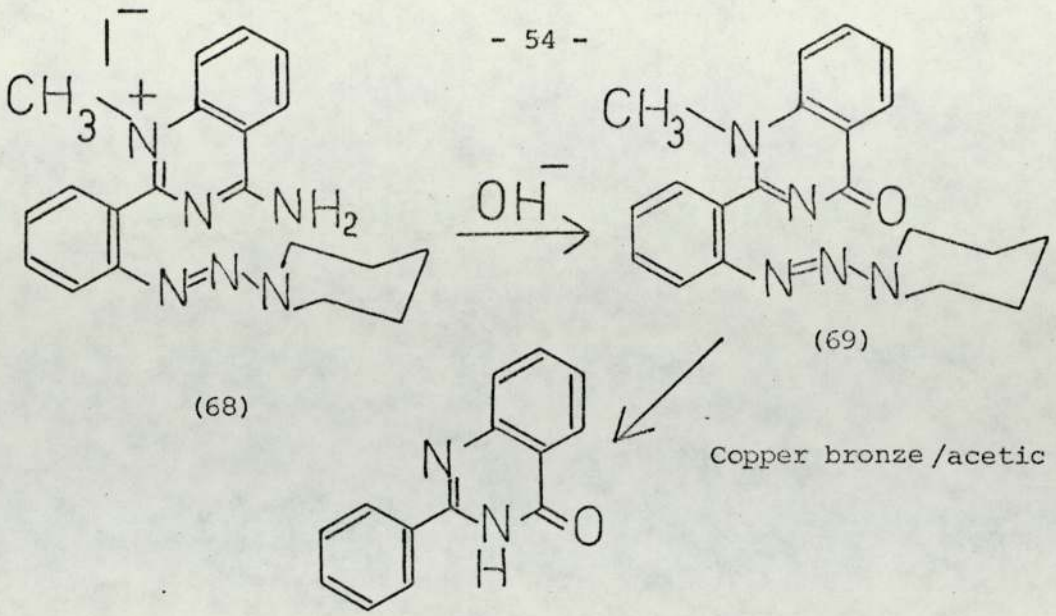


(67) (68)

Scheme 4.5

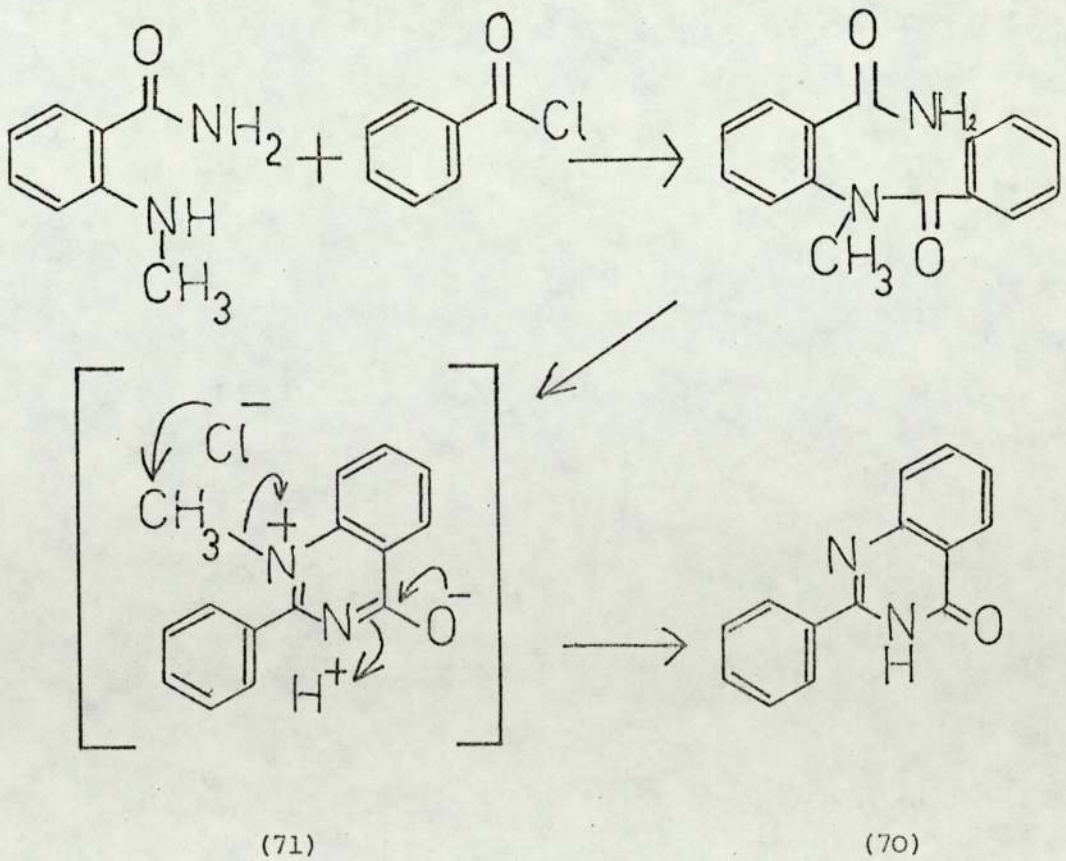
Basification of the methiodide (68) (which can also be considered as a hydroiodide salt of the 1-methylquinazolin-4(1H)-imine) led to hydrolysis and the isolation of a methylated quinazolone (69) (Scheme 4.6). This excluded methylation on the exocyclic 4-amino group of the triazene (6a). The mass spectrum of the methylated quinazolone (69) unequivocally established that the methyl group was attached to the quinazoline fragment and not to the triazeno side-chain; loss of the triazeno side-chain afforded an ion corresponding in mass number to 2-phenylquinazolinone plus 14 mass units (ie. + CH_3-H).

The methylated quinazolone (69) was recovered unchanged from ethanolic hydrazine containing Raney nickel and boiling ethylene glycol. The latter reagent might have been expected to effect reductive elimination of the triazeno side-chain to afford either of the known N(1) or N(3) methylated 2-phenylquinazolin-4-ones.[94] Reductive decomposition of (69) with hypophosphorous acid was likewise unsuccessful. In boiling acetic acid containing copper bronze however, the methylated quinazolone (69) underwent reductive elimination of the triazeno side-chain and demethylation to yield 2-phenylquinazolin-4(3H)-one (70) (Scheme 4.6) thus leaving the question of the site of methylation of the triazene (6a) unanswered.



Scheme 4.6

An explanation for this unexpected result was forthcoming when the properties of 1-methyl-2-phenylquinazolin-4(1H)-one and the N(3) methyl isomer were examined. Condensation of benzoyl chloride with 2-methylamino-benzamide in p-cymene gives 2-phenylquinazolin-4(3H)-one (70) and not 1-methyl-2-phenyl-quinazolin-4(1H)-one(71) as claimed.[95] (Scheme 4.7)



Scheme 4.7

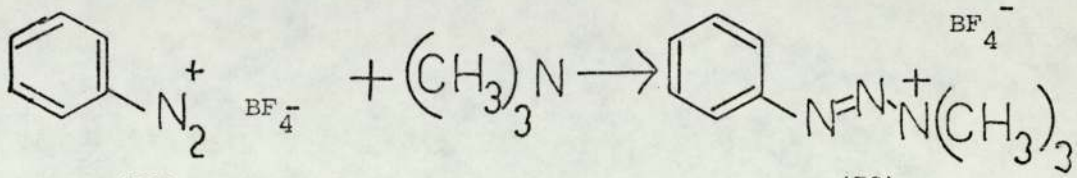
The dipolar character of the methylated quinazolin-4(1H)-one (71) makes it susceptible to chloride ion initiated demethylation. Other alkylated heterocycles with dipolar character readily undergo dealkylation under similar conditions.[96] In the case of the demethylation of the methylated triazenoquinazolone (69) in acetic acid containing copper bronze, acetate ion presumably initiates the demethylation.

The positioning of the methyl group on the quinazoline N(1) atom is corroborated by the observation that 2-phenyl-3-methyl-quinazolin-4(3H)-one was not only found to be stable under the conditions of its synthesis (prepared in the present work from benzoyl chloride and N-methylanthranilamide[97]) but was also recovered unchanged from boiling acetic acid containing copper bronze. The conclusion from these results is that 3-methylquinazolin-4-ones are stable and 1-methylquinazolin-4-ones are labile.

Two literature reports lend further support to the conclusion that the triazene (6a) undergoes methylation on the quinazoline N(1) atom. 4-Aminoquinazoline is methylated by methyl iodide at N(1) to afford the hydroiodide salt of 1,4-dihydro-4-imino-1-methylquinazoline,[98] and 1-methyl-2-phenylquinazolin-4(1H)-one is claimed to rearrange thermally to the thermodynamically more stable 3-methyl isomer, by a "1,3-alkyl" shift.[94] Unfortunately no reference was made in the latter publication to the preparation of the unstable 1-methyl isomer.

The position of the carbonyl absorption in the i.r. spectrum of the methylated quinazolone (69) also supports its assigned structure. α,β-unsaturated amides generally absorb in the region $1649cm^{-1}$ - $1639cm^{-1}$, whereas the β,γ-unsaturated counterparts show bands in the region $1679cm^{-1}$ - $1676cm^{-1}$.[94] The carbonyl absorption of the methylated quinazolone in question appears at $1642cm^{-1}$ which is nearer the region of the α,β - unsaturated compounds and thus tends to support the positioning of the methyl group on the quinazoline N(1) atom.

Quaternary triazenes of the type (73) were unreported in the literature at the time of this practical work. However Stevens[99] has recently prepared the analogue (73) by coupling the diazonium tetrafluoroborate (72) with trimethylamine in acetonitrile at -5°. (Scheme 4.8) Alkylation of phenyldimethyltriazene with dimethylsulphate led to a mixture of products which were not characterised.[99]



(72) Scheme 4.8 (73)

Since the site of alkylation of heterocyclic compounds is frequently determined by the reaction conditions,[100] methylation of the triazene (6a) was attempted with dimethylsulphate in sodium hydroxide but this also led to extensive decomposition and no products were characterised.

Chapter 5

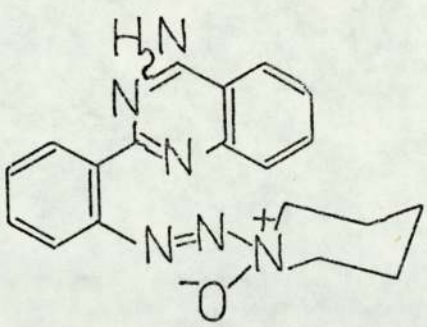
Oxidation of 4-amino-2-[2-(piperidin-1-ylazo)phenyl] quinazoline.

Introduction

The metabolic activation of DTIC, PDMT and the carcinogenic N-nitrosamines is apparently initiated by an oxidation reaction.[101] Therefore, any study on the chemistry of the triazenoquinazolines related to their mode of action, is incomplete without an examination of their behaviour on oxidation.

Oxidation with perbenzoic acid.

Quaternisation of the triazeno(N)3 atom of the triazene (6a) was attempted by oxidation with perbenzoic acid. This could have yielded the tertiary amine oxide (74). Pyrolysis of tertiary amine oxides yields an alkene and an N,N-dialkylhydroxylamine. The reaction (Cope elimination) is similar to the Hofmann elimination but differs in stereochemistry. The stereochemistry of the Cope elimination is cis whereas in the Hofmann elimination it is trans.[102]



(74)

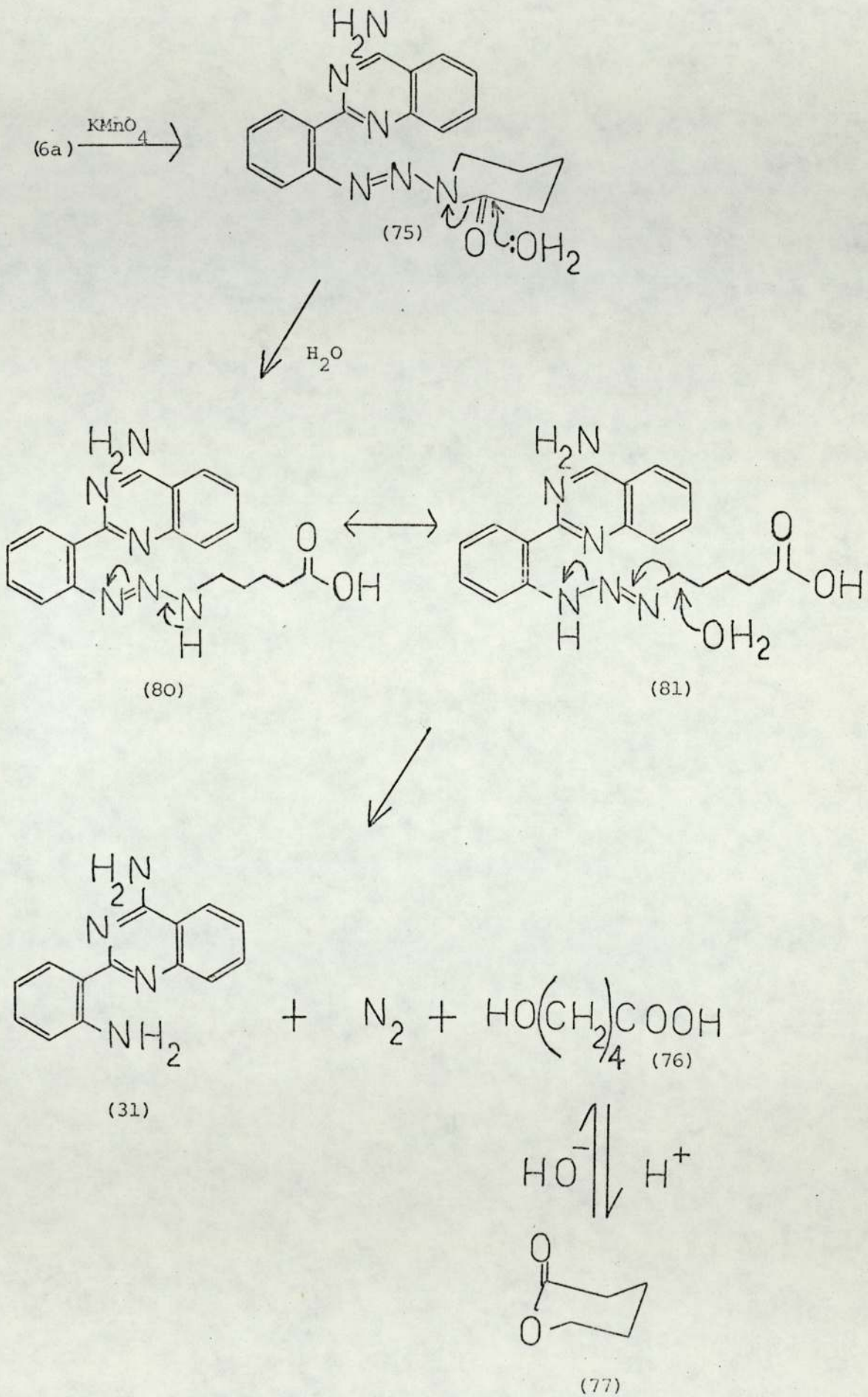
In fact, the quaternary triazene (74) would be unlikely to undergo a Cope elimination, since the reaction requires a planar intermediate which in this case is not possible because of the puckering of the piperidine ring. Oxidation of the triazene (6a) with perbenzoic acid in benzene afforded only a benzoate salt of the starting material. This result, along with the formation of the hydroiodide salt (67) (Chapter 4) confirms the strongly basic character of the triazene (6a).

Oxidation with potassium permanganate

Potassium permanganate was chosen as an oxidising agent for the triazene (6a) because not only is this reagent one of the most powerful of the oxidising agents in the chemist's armoury, but it is extremely versatile - its versatility being reflected in its ability to use different reaction paths depending on the structure of the substrate and on the reaction conditions.[103] Oxidation of the triazene (6a) with permanganate at 20° in aqueous acetone containing potassium bicarbonate, resulted in the formation of two new products, as determined by chromatographic analysis of a chloroform extract of the reaction mixture. The most polar of these products was identified as 4-amino-2-(2-aminophenyl)quinazoline. The identification of the diamine (31), an aromatic amine, as an oxidation product of a dialkyltriazene is highly significant. Formation of an aromatic amine by oxidation of a dialkyltriazene requires the intermediacy of a monoalkyltriazene. The triazene (6a) must therefore have undergone oxidative ring-opening to a monoalkyltriazene which decomposed to the diamine.

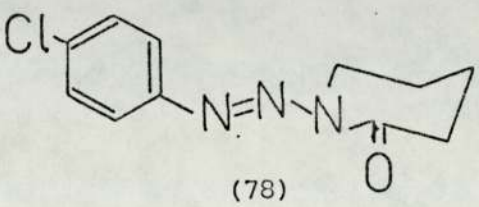
Fortuitously, the second oxidation product crystallised from a concentrated chloroform extract of the reaction mixture. This product, which contained the intact NNN linkage was assigned the structure (75) on the basis of the following observations. The mass spectrum of this product indicated that oxidation had not occurred on the quinazoline or phenyl fragments. In aqueous acetone (pH 9.0) in light or dark, the product (75) decomposed to form the diamine (31) and δ-hydroxyvaleric acid (76). δ-Hydroxyvaleric acid was isolated[104] as the lactone (77) and characterised by i.r. spectroscopy, gas chromatography and tlc of its hydroxamic acid derivative.

The assigment of the structure (75) to the oxidation product is further supported by its nmr spectrum which bears a striking resemblance in the aliphatic region to the nmr spectrum of 1-(4-chlorophenylazo)piperidin-2-one (78). This compound was formed on permanganate oxidation of 1-(4-chlorophenyl)-

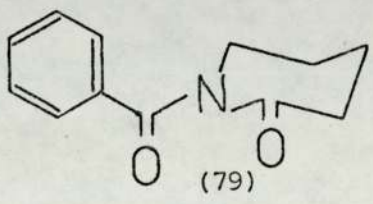


Scheme 5.1

piperidine[105] and its structure (78) confirmed partly on the basis of the similarity (in the aliphatic region) in nmr spectrum to N-benzoylpiperidin-2-one (79).



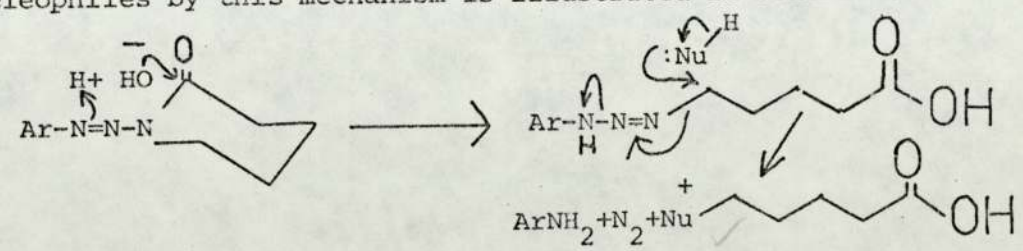
(78)



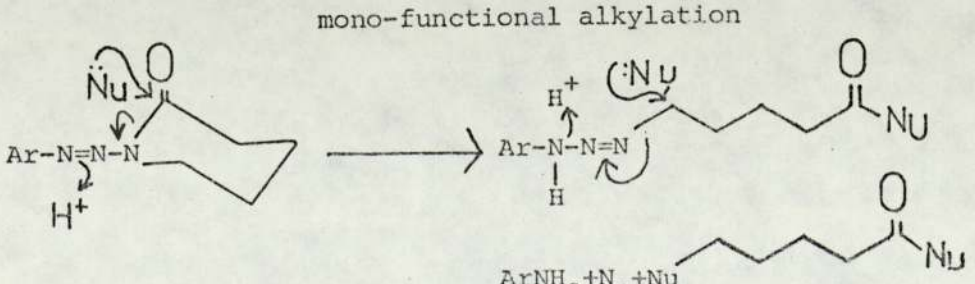
(79)

The reaction sequence (Scheme 5.1) may be interpreted as follows: initial α-hydroxylation of the triazene (6a) affords a hydroxylated intermediate which undergoes further oxidation to the isolable piperidin-2-one. In the basic medium, the piperidin-2-one undergoes nucleophilic attack by water to yield the unstable monoalkyltriazene (30) which is in equilibrium with the tautomer (81). The tautomer (81) alkylates a further molecule of water with formation of δ-hydroxyvaleric acid (76), the diamine (31) and loss of nitrogen.

α-Oxidation of the triazene (6a) by permanganate can therefore be seen to result in the formation of the intermediate (75) which has the capacity for alkylating nucleophiles. In this case the nucleophile was water. The capacity for both mono- or di-functional alkylation of biological nucleophiles by this mechanism is illustrated in Scheme 5.2



mono-functional alkylation



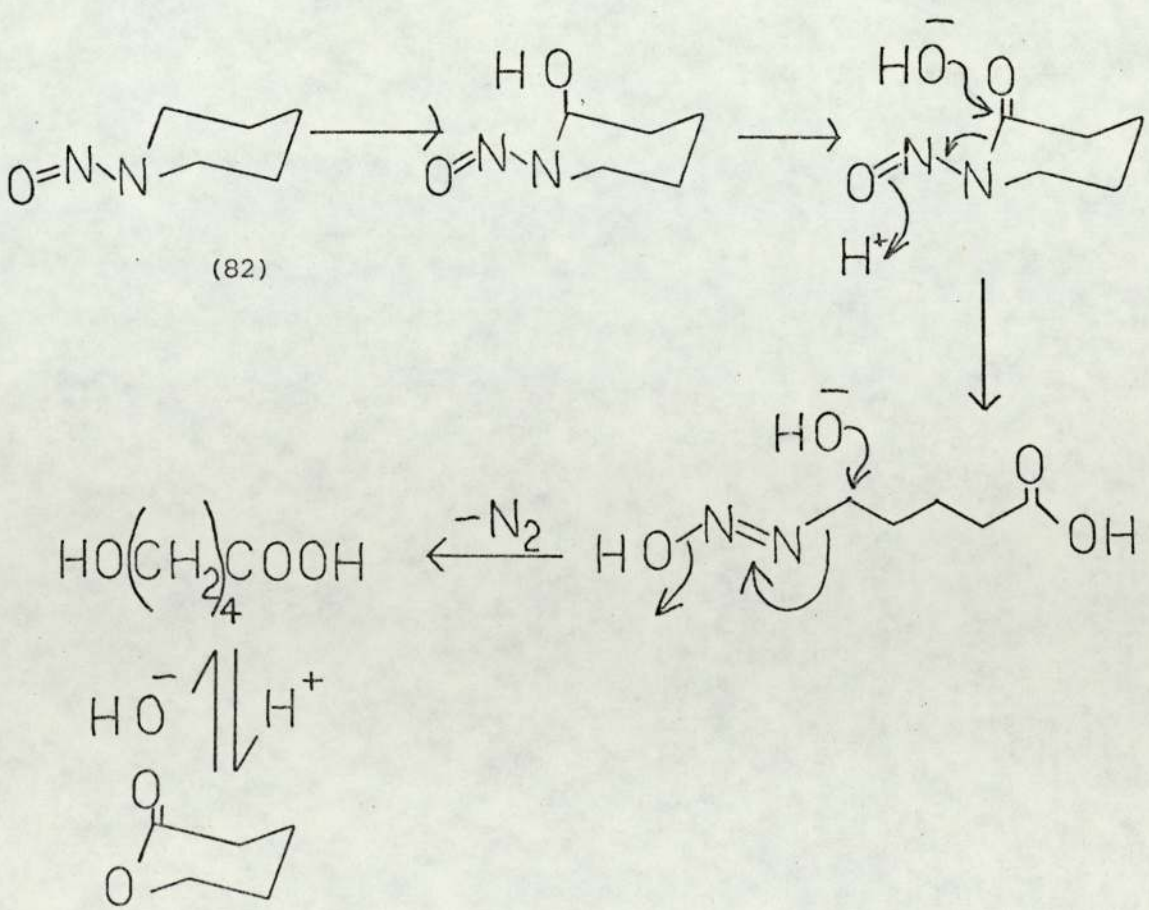
di-functional alkylation

Scheme 5.2

Di-functional alkylation of this nature could effectively crosslink two strands of DNA and thus inhibit DNA synthesis. The antitumour action of the nitrogen mustards is thought to be due in part to such a reaction with DNA.[106]

Further derivatives of the triazene (6a), [(6c), (6f), (6h) and (6i)] were subjected to analogous permanganate oxidation and in all cases the diamine (31) was formed indicating oxidative dealkylation to a monoalkyltriazene. The alkylating nature of these monoalkyltriazenes was inferred from the results of the oxidation of the triazene (6a).

In view of the similarity between the N-nitrosamines and aryl-dialkyltriazenes, nitrosopiperidine (82) was likewise oxidised by permanganate and was also shown to form the lactone (77). This sequence can be interpreted in terms of initial α-hydroxylation followed by analogous decomposition to that of the oxidised triazene (6a). (Scheme 5.3).



(82)

(77)

Scheme 5.3

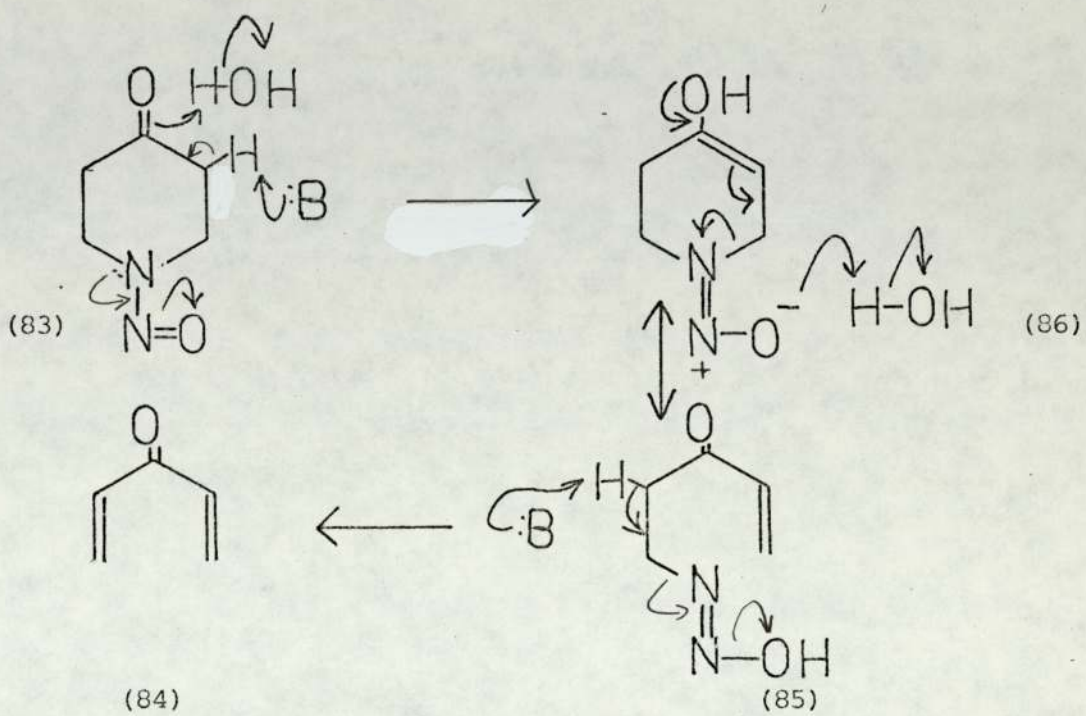
The significance of these results and the relevance to the mode of action of these two classes of compound are of course unanswered questions at present. It is quite possible that the two classes of compound exert their biological effects by a common mechanism, probably initiated by α-carbon oxidation. Should this be the case, then considering the toxicity of the nitrosamines, the future for the triazenoquinazolines as potential tumour-inhibitory agents may be less than promising. On the other hand, while both the triazene (6a) and nitrosopiperidine were observed to alkylate in the same way after permanganate oxidation,because of the substantial structural differences between these compounds it is more than likely that the two molecules will alkylate in different micro-environments; i.e. they will have affinities for different nucleophilic sites. Thus the consequences of alkylation at a particular site may be tumour formation; alkylation at a different specific site may result in tumour-inhibitory activity.

The similarity in tumorogenesis by certain aryl-dialkyltriazenes and the N-nitrosamines suggests that the two classes of compound may have a common mechanism of action.[107]

In addition to α-carbon oxidation, mechanisms of activation of the nitrosamines have been proposed which place importance on β- and γ-carbon oxidation. Nitrosopiperidine has been shown to undergo oxidation in the γ-position both by rat liver microsomes and in the Udenfriendoxidation system.[70] Recent results on the Udenfriend oxidation of nitrosopiperidine confirm these earlier results.[108]

However, it was found thatN-nitrosopiperidin-4-one was only mutagenic after further microsomal incubation.[108] This suggests that oxidation in the 4-position in nitrosamines may not contribute to the carcinogenicity and mutagenicity of these compounds. The derivative, N-nitrosotriacetonamine (83) (Scheme 5.4) decomposes in aqueous or alcoholic solution in the presence of a catalytic amount of base to yield 2,6-dimethyl-4-ketohepta -2,5-diene (84)

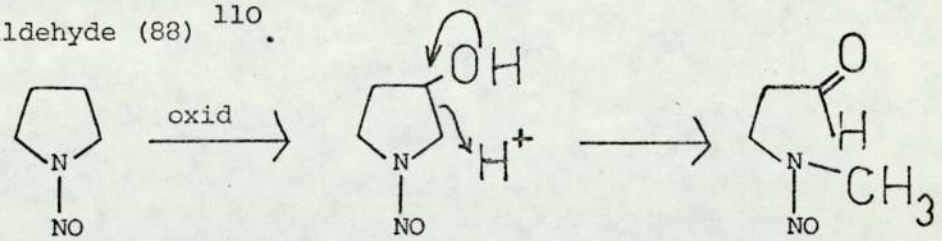
presumably through the primary alkyl diazonium hydroxide (86). Either (84) or (85) will alkylate nucleophiles.[109]



(83) (86) (84) (85)

Scheme 5.4

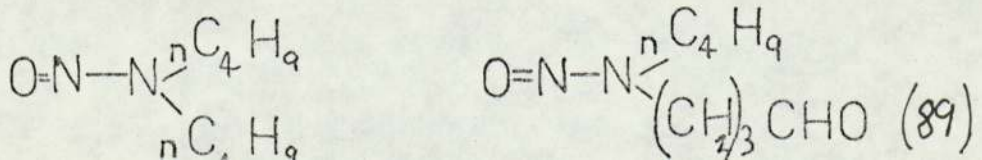
On the other hand, β-carbon hydroxylation of N-nitrosopyrrolidine (87) (Scheme 5.5), it has been claimed, could result in the formation of the nitrosoaminoaldehyde (88)[110].



(87) (88)

Scheme 5.5

N-Nitrosoaminoaldehydes are a class of compound which recent studies suggest may be metabolic intermediates in the activation of certain carcinogenic nitrosamines.[111] It has been proposed that 4-(N-butyl-N-nitrosoamino)-butanal (89) may be a critical intermediate in the induction of bladder tumours in rats by N-nitrosodi-n-butylamine (90).



In line with these proposals, it has been shown[105] that oxidation of 1-(4-chlorophenylazo)piperidine by permanganate, in the Udenfriend oxidation system, and by fortified rat liver microsomal preparations results in the formation of a variety of triazene products oxidised in the α, β, and γ-positions (Table 5.1) and in the formation of 4-chloroaniline, indicating some degree of oxidative dealkylation.

Oxidation of 4-amino-2-[2-(piperidin-1-ylazo)phenyl]quinazoline in the Udenfriend oxidation system.

The Udenfriend oxidation system closely approximates biological oxidation processes. It requires molecular oxygen, ferrous ion, and utilizes an electron-donor (ascorbic acid). The exact mechanism of oxidation is not known although it is considered that the Udenfriend system may effect epoxidation in the oxidation of aliphatic compounds through the generation of hydroxyl radicals. Hydroxylation may, on the other hand, be effected through complex formation between the substrate, metal ion electron donor and oxygen.[112]

The reaction products of the Udenfriend oxidation of the triazene (6a) in light or dark were subjected to chromatographic analysis on silica plates. The major reaction product in both cases was identified as 2-phenyl-4-aminoquinazoline. Neither the diamine (31) nor the piperidin-2-one (75), which are both products of permanganate oxidation of (6a) were detected. The quinazoline (45) has previously been isolated from triazene (6a) by decomposition in a protic medium (see chapter 3). The different pH of the permanganate oxidation (pH9.0) to that of the Udenfriend (pH6.5) may account for these differences.

Oxidation of 1-(4-chlorophenylazo)piperidine.[105]

Products	$KMnO_4$	Udenfriend	Incubation with fortified rat liver microsomal preparations
4-chloroaniline	+	+	+
1-(4-chlorophenylazo)piperidin -2-one	+	+	-
1-(4-chlorophenylazo)piperidin-4-one	+	+	-
1-(4-chlorophenylazo)piperidin-3-ol	-	+	-
1-(4-chlorophenylazo)piperidin-4-ol	+	+	+

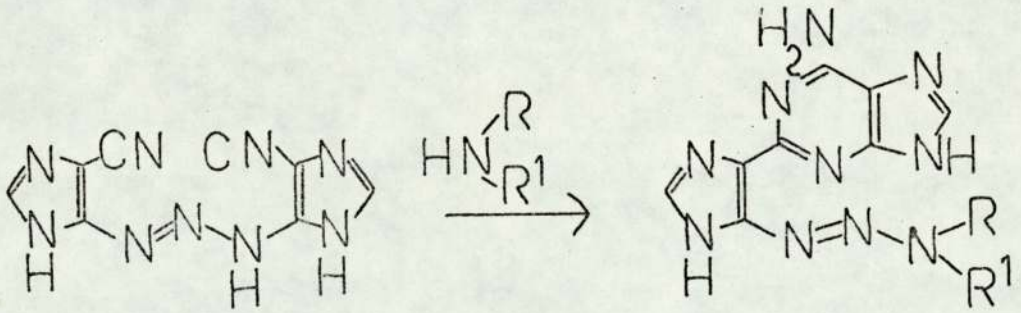
TABLE 5.1

Chapter 6

Synthesis and properties of triazene derivatives of purines

Adenine derivatives

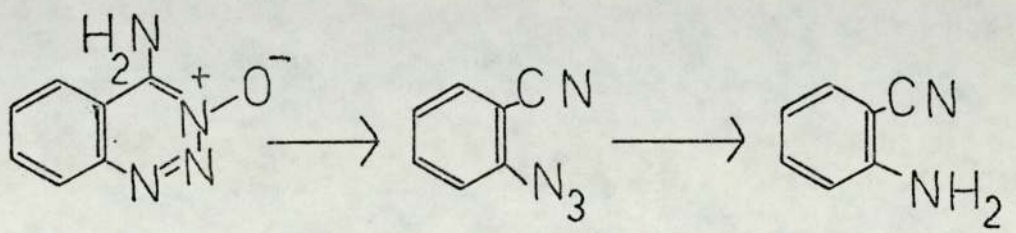
Analogues of both purines and pyrimidines (eg.6-mercaptopurine and 5-fluorouracil) can function as antimetabolites and have a useful application in cancer chemotherapy.[113] To recall the attention of the reader to the synthesis of the triazenoquinazolines, 1,3-di-o-cyanophenyltriazene(7) undergoes successive amine-nitrile addition in secondary amines to afford the triazenes (6a-i)[8]; this property was mimicked in the reactions of the brominated triazene (25) in secondary amines. Therefore it was anticipated that 1,3-di-(4-cyanoimidazol-5-yl)triazene(91) might undergo analogous transformation in secondary amines to yield the adenine derivatives (92) (Scheme 6.1).



(91) Scheme 6.1 (92)

The proposed products (92) of this sequence, as derivatives of adenine, might act as 'carriers' of the alkylating dialkyltriazeno function, which might be expected to confer specificity on the molecules. In theory, the synthesis of the triazene (91) might have been achieved by the addition of 5-diazoimidazole-4-carbonitrile to 5-aminoimidazole-4-carbonitrile (93). The first reported synthesis of the aminonitrile (93).was in 1966.[114] Ferris and Orgel condensed aminomalononitrile with formamidine acetate and isolated 5-aminoimidazole-4-carbonitrile as a tosylate salt. The method of preparation of aminomalonitrile given in this paper is exceedingly laborious and attempts to repeat it in the present work were unsuccessful.

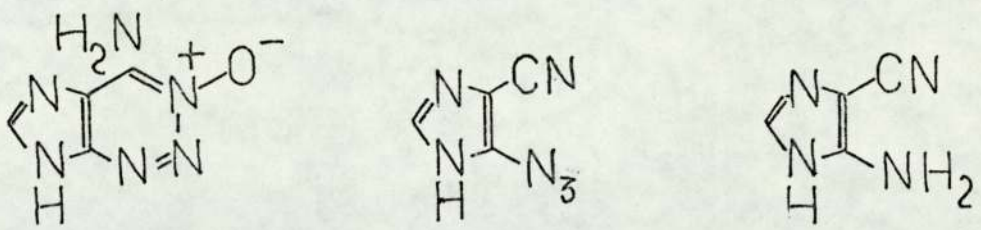
A novel synthesis of the o-aminonitrile (93) was suggested by a report[115] that 4-amino-1,2,3-benzotriazine-3-oxide (94) decomposes in piperidine to afford o-azidobenzonitrile (95) which may be conveniently reduced to o-aminobenzonitrile. (Scheme 6.2)



(94) (95)

Scheme 6.2

In the present context, it was considered that analogous decomposition of 2-azaadenine -1-oxide (96) would yield the azide (97) which might then be reduced to the required aminonitrile (93). (Scheme 6.3)

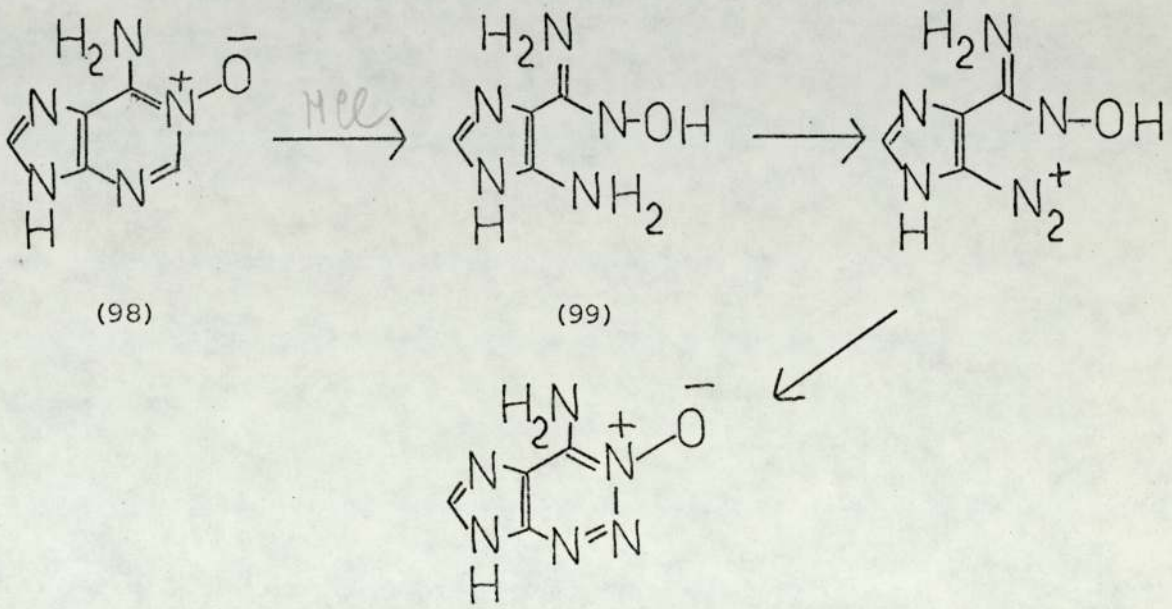


(96) (97) (93)

Scheme 6.3

2-Azaadenine-1-oxide was prepared according to the method of Stevens, Magrath, Smith & Brown[116]. Oxidation of adenine in peracetic acid yielded adenine-1-oxide (98) which decomposed in hot 3N-hydrochloric acid to the hydrochloride salt of 5-aminoimidazole-4-carboxamidoxime (99). Treatment of an aqueous solution of the salt (99) with nitrous acid led to the isolation of 2-azaadenine-1-oxide as described (Scheme 6.4). Decomposition of 2-azaadenine-1-oxide to the azide (97) was attempted in boiling piperidine

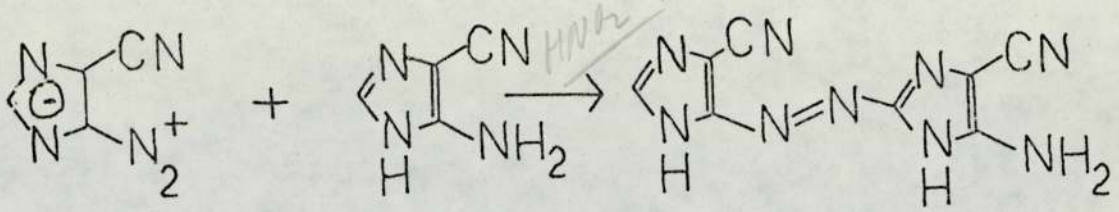
or collidine, or by fusion with ammonium acetate at 200°. In all cases the starting material was recovered.



(98) (99) (96)

Scheme 6.4

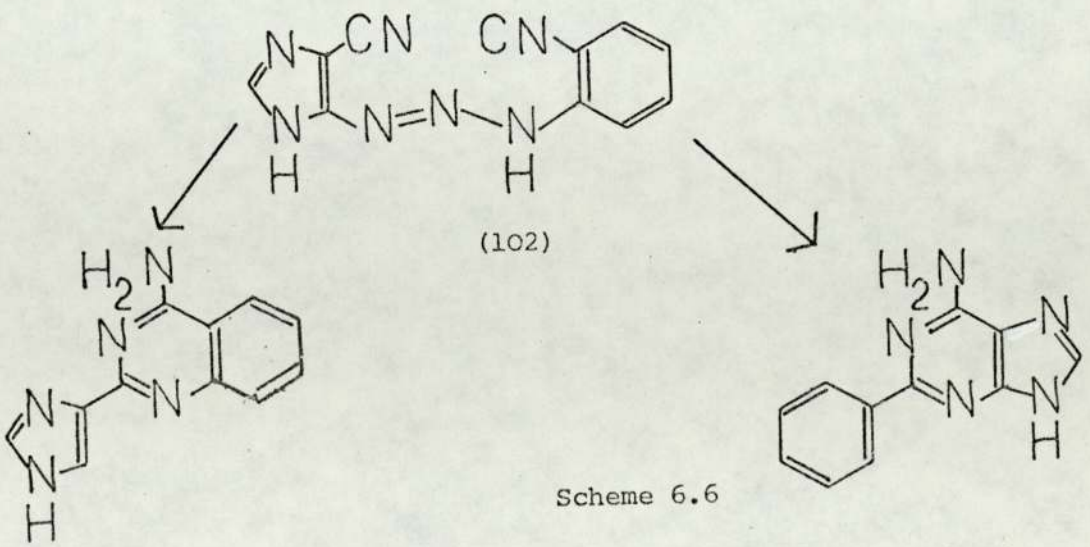
5-Aminoimidazole-4-carbonitrile (93) was finally obtained by the more conventional phosphorous oxychloride dehydration of 5-aminoimidazole-4-carboxamide, using an improved procedure by Shealy.[117] Diazotisation of 5-aminoimidazole-4-carbonitrile results in the formation of the stable 5-diazoimidazole-4-carbonitrile [117]. When this diazoderivative was added to a solution of 5-aminoimidazole-4-carbonitrile C- rather than N-coupling took place and the deep red azo-compound (101) was isolated. The structure of this product was deduced from its uv spectrum which exhibited long wavelength absorption (λ_{max} 512 nm characteristic of azo compounds) and from its CHN analysis. The 2-carbon is thus the most nucleophilic site in the π excessive imidazole ring, rather than the exocyclic amino group.



(93) (101)

Scheme 6.5

However, addition of the diazonium ion to an aqueous suspension of o-aminobenzonitrile afforded the N-coupled product (102). The unsymmetrical nature of this triazene permits two possible decomposition routes in protic solvents, and which are absolutely dependent on which cyano group undergoes initial nucleophilic attack. Cyclisation on the aryl cyano group followed by decomposition analogous to the decomposition of 1,3-di-o-cyano-phenyltriazene in ethanol, would yield 2-phenyladenine (104) (Scheme 6.6); alternative cyclisation on the imidazo cyano group would result in the formation of 4-amino-2-(imidazol-4-yl)quinazoline (103).

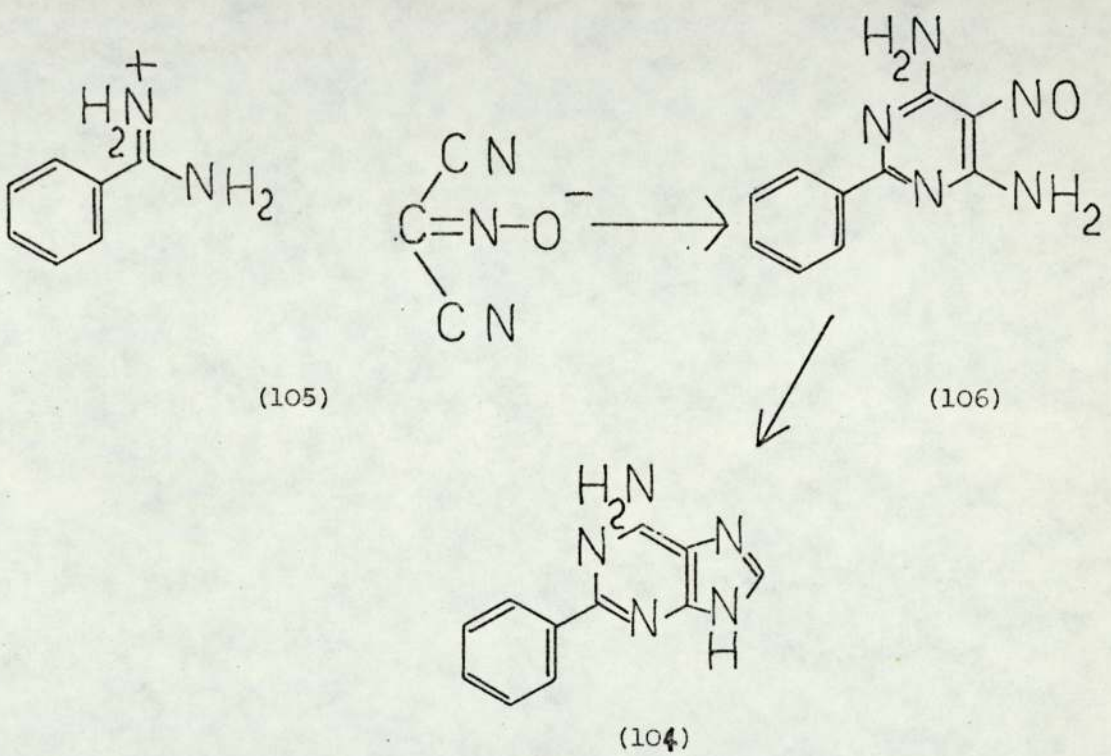


(102)

(103) (104)

Scheme 6.6

In boiling aqueous ethanol, the triazene (102) decomposed exclusively by the former route to afford 2-phenyladenine (104) in high yield. A sample of 2-phenyladenine was prepared unambiguously by the facile method of Taylor.[118] Thermal isomerisation of the benzamidinium salt of iso-nitrosomalononitrile (105) produced 2-phenyl-4,6-diamino-5-nitrosopyrimidine (106) which underwent a 'one pot' conversion to 2-phenyladenine when heated with formamidine, formic acid and sodium hydrosulphite.[118]



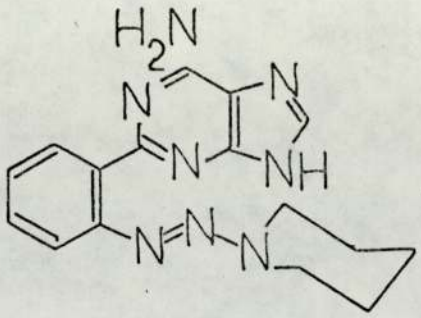
(105) (106) (104)

Scheme 6.7

The 2-phenyladenine prepared by this route was identical (uv, i.r., paper chromatography) to the product of decomposition of the triazene (102) in aqueous ethanol.

Thus a precedent was established for initial cyclisation on the aryl cyano group of the triazene (102). This is consistent with other observations on the unreactivity of cyanoazoles towards nucleophilic attack.[119]

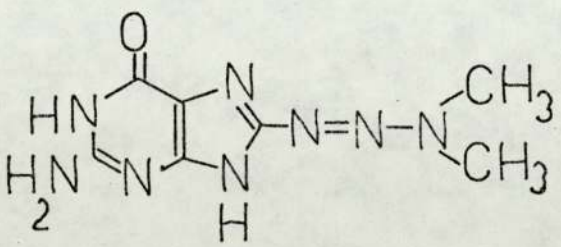
In boiling piperidine, the triazene (102) underwent extensive decomposition. At least nine products were detected (tlc and paper chromatography). The expected 2-[(2-piperidin-1-ylazo)phenyl]adenine (107) was not isolated.



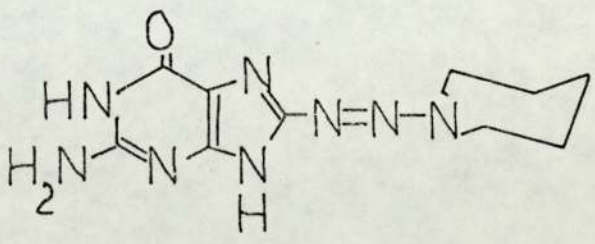
(107)

Synthesis of analogues of guanine

In 1961, Usbeck, Jones and Robins reported the synthesis of certain 8-triazenopurine nitrogen mustards.[120] To conclude the present work, the 8-triazenoguanine derivatives (108) and (109) were prepared for biological testing. The 8-aminoguanine required for this work was prepared by the method of Fischer.[32] Diazotised 3,5-dichloroaniline coupled with guanine in the 8-position; the resulting azo compound was reduced in alkaline sodium hydrosulphite to afford 8-aminoguanine. Diazotisation of 8-aminoguanine leads to the formation of the isolable solid 8-diazoguanine.[120] In the present work, addition of 8-diazoguanine to dimethylamine or piperidine in acetone, resulted in the formation of the triazenes (108) and (109) respectively.

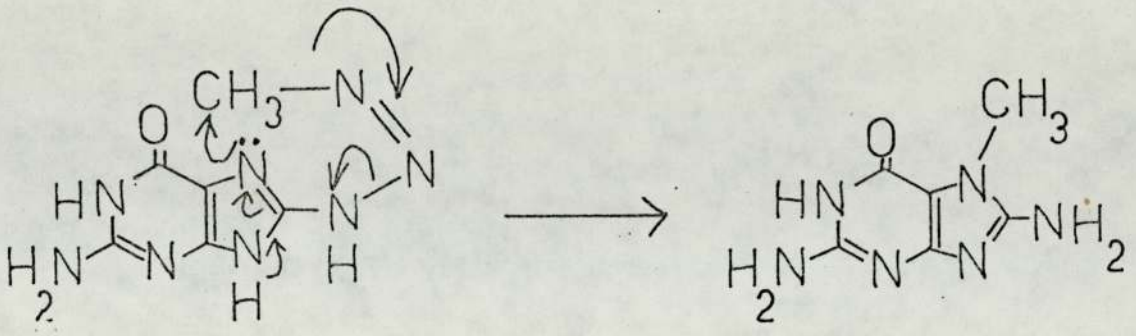


(108)



(109)

It was considered that the triazene (108) might be a candidate for oxidative demethylation in vivo and that the product of this transformation, the monomethyltriazene (110) could possibly decompose with intramolecular methylation of either N(7) or N(9) of the guanine fragment of the molecule. (Scheme 6.8).

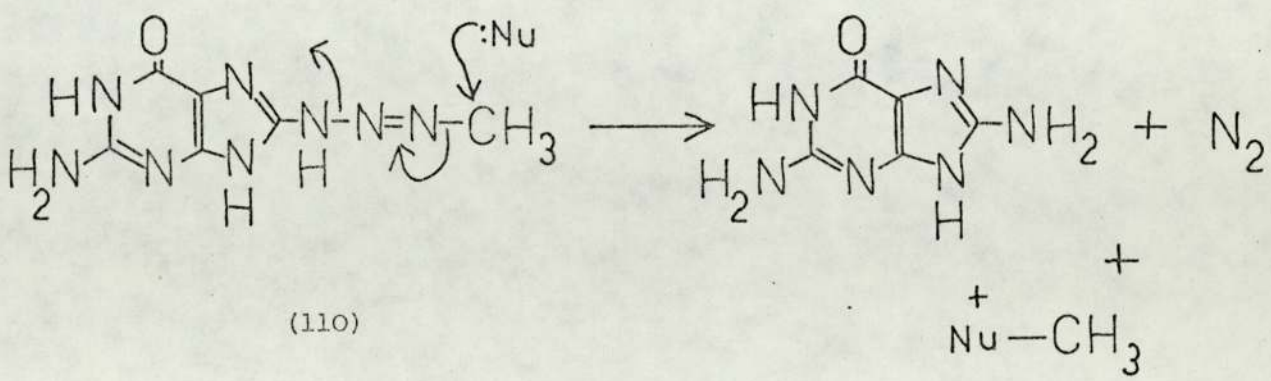


(110)

Scheme 6.8

To test this hypothesis, 8-diazoguanine was coupled with methylamine both in aqueous and in organic solvents. It was anticipated that the monomethyltriazene (110) would be formed and might decompose as indicated (Scheme 6.8). In the event, coupling took place, but decomposition took a different route, with methylation, presumably of solvent (Scheme 6.9). 8-Aminoguanine was isolated and the expected 8-amino-7(or 9)-methylguanine was not detected (tlc) in the reaction mixture.

The coupling of certain diazoimidazoles with primary amines has previously been shown to result in analogous regeneration of the amine from which the diazonium ion was derived.[121]



(110)

Scheme 6.9

Chapter 7

Experimental

(i) UV spectra were recorded on a Unicam SP 8000 spectrometer (in 95% ethanol) unless otherwise stated.

(ii) ^{1}H Nmr spectra were recorded on a Varian HA-100D spectrometer (Me_4Si as internal standard).

(iii) I.r. spectra were recorded (KBr discs) on a Perkin Elmer 157G spectrometer.

(iv) Mass spectra were measured at 70eV on an A.E.I. - G.E.C. MS 902 spectrometer with source temperature in the range 100-150°.

Experimental

4-Amino-2-(2-aminophenyl)quinazoline (3 2). —

A mixture of anthranilonitrile (3.0 g) and sodium hydride (0.6 g) in dimethylsulphoxide (13 ml) was stirred for 3 h at 0° and then for 21 h at 25°. Addition of 6N-hydrochloric acid (100 ml) to the mixture afforded the diaminoquinazoline dihydrochloride (3.0 g). It was identical (i.r. and mixed m.p.) to an authentic sample.[122]

Quinazolino[3,2-c]1,2,3-benzotriazin-8(7H)-imine (24)or[isomer(22)]. —

4-Amino-2-(2-aminophenyl)quinazoline dihydrochloride (1,39 g) in 2N-hydrochloric acid (30 ml) was diazotised at 0° for 30 mins with sodium nitrite (0.35 g) in water (3 ml). Basification of the suspension with ice-aqueous ammonia afforded the imine (0.55 g) identical (i.r) to an authentic sample.[74]

4-Amino-2-[2-(2-methylpiperidin-1-ylazo)phenyl]quinazoline(6f). —

1,3-Di-o-cyanophenyltriazene(7) (2.0 g) was boiled for 2 h in 2-methylpiperidine (5 ml). Trituration of the gum formed on evaporation of the solution with petrol-ether afforded a solid (81%) which crystallised from acetone as buff prisms, m.p. 181-183° (Found C, 69.1; H, 6.5; N, 24.4. $C_{20}H_{22}N_6$ requires (C, 69.4; H, 6.35; N, 24.3%).

4-Amino-2-[2-(3-methylpiperidin-1-ylazo)phenyl]quinazoline (6g). —

1,3-Di-o-cyanophenyltriazene(7) (2.0 g) was boiled in 3-methylpiperidine for 2 h. Addition of water afforded a solid (2.4 g) (88%) which crystallised from benzene-light petroleum, m.p. 158-160° (Found: C,69.3; H,6.4; N,24.55%).

Similarly prepared from 1,3-di-o-cyanophenyltriazene and 4-methylpiperidine was 4-amino-2-[2-(4-methylpiperidin-1-ylazo)phenyl]quinazoline (6 h) (90%), m.p. 146-147° (from toluene) (Found: C, 69.8; H, 6.2; N, 24.2%).

4-Amino-2-[2-(3,3-dimethyltriazen-1-yl)phenyl]quinazoline (6i). —
A solution of 1,3-di-o-cyanophenyltriazene(2.0 g) in anhydrous dimethylamine (10 ml) was kept at 4° for 30 days. The dimethyltriazenylphenylquinazoline (1.3 g) was slowly deposited on addition of water, and was recrystallised from hot water with m.p. 154-156° (Found: C, 65.8; H, 5.35; N, 29.0. $C_{16}H_{16}N_6$ requires C.65.75; H,5.5; N, 28.8%); T($CDCl_3$) 6.95 (S, 6H, 2xCH_3), 3.2 (br.s, 2H, NH_2) and 2.1 - 2.8(m,8H,Aryl-H).

2-[2-(3,3-Dimethyltriazen-1-yl)phenyl]quinazolin-4(3H)-one(19a). —
A mixture of the dimethyltriazene(6i) (1.5 g) and potassium hydroxide (10 g) in ethanol (20 ml) was boiled for 3.5 h, diluted with water and organic products extracted into ether (4 x 25 ml). The dried (sodium sulphate)ethereal extract was evaporated and the residue crystallised from ethanol. The dimethyltriazenylquinazolone (93%) had m.p. 212-213° (Found:C,65.1; H,5.15; N,24.1. $C_{16}H_{15}N_5O$ requires C,65.5;H,5.1;N,23.9%); γ_{max}(KBr) 1665 cm^{-1}(CO) T ($CDCl_3$) 6.30 and 6.50 (d, 6H, 2x CH_3).

Similarly prepared from the quinazolines (6b) and (6c) respectively. were the following: 2-[2-(pyrrolidin-1-ylazo)phenyl]quinazolin-4(3H)-one (65%); and 2[2-(morpholin-4-ylazo)phenyl]quinazolin-4(3H)-one (70%). These quinazolines were identical to authentic samples.[115]

1,3-Di-(2-cyano-4-bromophenyl)triazene(25). -
A suspension of 5-bromoanthranilonitrile[123] (3.94 g) in 2N-hydrochloric acid (25 ml) was treated at 0° with sodium nitrite (0.7 g) in water (5 ml) over 0.5 h. The mixture was diluted with ice-water (75 ml) and stirred at 0° for a further 2 h and kept at 4° overnight. The precipitated triazene (80%) crystallised from toluene as yellow needles, m.p. 185° (decomp.) (Found: C,41.7; H, 1.9; N, 17.2. $C_{14}H_7Br_2N_5$ requires C.41.5; H, 1.7; N, 17.3%); γ_{max}(KBr)3220(NH), 2239 and 2220 cm^{-1}(CN).

4-Amino-6-bromo-2-(2-amino-5-bromophenyl)quinazoline (27).—
(i) 1,3-Di-(2-cyano-4-bromophenyl)triazene(2.0 g) in ethanol (50ml)

containing Raney nickel (1.0 g) was treated at 60-65° over 1 h with hydrazine hydrate (5 x 1 ml). The hot solution was filtered through kieselguhr and the filtrate vacuum-evaporated to yield the diaminoquinazoline (1.8 g) which crystallised as yellow needles (from aqueous ethanol, or butanol) m.p. 285-287° (Found: C, 42.8; H, 2.5; N, 14.1. $C_{14}H_{10}Br_2N_4$ requires C,42.6; H, 2.5; N, 14.2%).

(ii) A mixture of finely powdered 5-bromoanthranilonitrile (9.8 g) and sodium hydride (0.6 g) in dimethylsulphoxide (13 ml) was stirred at 0° for 3 h and then at 25° for 21 h. Dilution of the mixture with water yielded the diaminoquinazoline (7.0 g) identical (i.r.)to the above sample.

4-Amino-6-bromo-2-(3-bromophenyl)quinazoline (26). — 1,3-Di-(2-cyano-4-bromophenyl)triazene (0.2 g) was boiled for 1 h in 75% aqueous ethanol (5 ml). Dilution of the solution with water afforded the quinazoline (53%) which was recrystallised from toluene, m.p. 257-258° (Found: C,44.7; H, 2.3; N, 10.9. $C_{14}H_9Br_2N_3$ requires C,44.4; H,2.1; N, 11.1%).

4-Amino-6-bromo-2-[5-bromo-2-(2-hydroxy-1-naphthylazo)phenyl]quinazoline (28).— An ethanolic solution (10 ml) of 2-naphthol (0.15 g) and 1,3-di-(2-cyano-4-bromophenyl)triazene (0.4 g) was boiled for 1 h. The precipitated naphthylazoquinazoline (89%) crystallised from dimethylformamide as red micro-rosettes, m.p. 316-318° (Found:C,52.8; H, 2.9; N, 12.85. $C_{24}H_{15}Br_2N_5O$ requires C,52.5; H,2.7; N, 12.75%).

2-(4-Amino-6-bromoquinazolin-2-yl)-4-bromophenyldiazonium chloride dihydrochoride (35). — 4-Amino-6-bromo-2-(2-amino-5-bromophenyl)quinazoline (2.0 g) was suspended in 6N-hydrochloric acid at 0° and treated with a solution of sodium nitrite (0.35 g) in water (3 ml). After 1.5 h the crude diazonium chloride dihydrochloride (70%) was collected and crystallised from 6N-hydrochloric acid as yellow prisms, m.p. 210° (decomp.) (Found: C, 33.3; H, 2.3; N, 13.7. $C_{14}H_8Br_2C_1N_5.2HCl$ requires C, 33.3; H, 1.9; N, 13.6%); γ_{max}(KBr) 2260cm^{-1} (N_2^+).

2,10-Dibromoquinazolin [3,2-c]-1,2,3-benzotriazin-8(7H)-imine (30) [or isomer (29)]. — Crystallisation of the diazonium chloride dihydrochloride (35) from ether-methanol (1:1) afforded the benzotriazin-imine monohydrochloride (95%) as pink needles, m.p. 213° (decomp.) (Found: C, 38.4; H, 2.3; N, 15.5. $C_{14}H_7Br_2N_5.HCl$ requires C, 38.05; H, 1.8; N, 15.9%).

4-Amino-6-bromo-2-[5-bromo-2-(piperidin-1-ylazo)phenyl] quinazoline (20a).— (i) 1,3-Di-(2-cyano-4-bromophenyl)triazene (0.8 g) was boiled for 2 h in piperidine and the solution diluted with water at 0°. The quinazoline (62%) crystallised from acetone as white micro-prisms, m.p. 198-200° (Found C, 46.7; H, 3.9; N,17.1. $C_{19}H_{18}Br_2N_6$ requires C, 46.5; H, 3.6; N, 17.2%).

(ii) 2, 10-Dibromoquinazolino [3,2-C]-1,2,3-benzotriazin-8(7H) imine hydrochloride (1.0 g) was boiled in piperidine (5 ml) for 1 h. The solution was diluted with ice-water (50 ml) to afford the quinazoline (20a) (0.65 g), identical (i.r.) to the above sample.

The following brominated triazenoquinazolines in the series (20) were prepared by reacting 1,3-di-(2-cyano-4-bromophenyl)triazene in the respective refluxing secondary amines for 2 h. and diluting the resultant solutions with water:

4-Amino-6-bromo-2-[5-bromo-2-(pyrrolidin-1-ylazo)phenyl] quinazoline (20b) (70%), m.p. 225-227° (from aqueous ethanol) (Found: C, 45.5; H, 3.2; N,17.7. $C_{18}H_{16}Br_2N_6$ requires C, 45.4; H, 3.4; N, 17.6%);

4-Amino-6-bromo-2-[5-bromo-2-(morpholin-4-ylazo)phenyl] quinazoline (20c) (78%), m.p. 203-205° (needles, from toluene) (Found: C, 43.8; H, 3.6; N, 17.5. $C_{18}H_{16}Br_2N_6O$ requires C, 43.9; H, 3.25; N, 17.1%);

4-Amino-6-bromo-2-[5-bromo-2-(2-methylpiperidin-1-ylazo)phenyl] quinazoline (20d) (85%), m.p. 225-227° (from aqueous dimethylformamide) (Found: C,47.8; H,4.2; N,16.6. $C_{20}H_{20}Br_2N_6$ requires C, 47.6; H, 4.0; N, 16.7%);

4-Amino-6-bromo-2-[5-bromo-2-(3-methylpiperidin-1-ylazo)phenyl]quinazoline (20e) (72%), m.p. 230-231° (from aqueous methanol) (Found: C, 47.6; H, 4.2; N, 16.9%);

4-Amino-6-bromo-2-[5-bromo-2-(4-methylpiperidin-1-ylazo)phenyl]quinazoline hydrate (20f) (84%), m.p. 198°-200° with sintering at 105° (prisms from aqueous methanol) (Found: C,46.1; H, 4.5; N, 15.8. $C_{20}H_{20}Br_2N_6.H_2O$ requires C, 45.9; H, 4.2; N, 16.1%).

Reactions of 1,3-diaryltriazenes in piperidine and morpholine.—

(i) 1,3-Di-p-tolytriazene and 1,3-di-p-chlorophenyltriazene were recovered in 90% yield from boiling piperidine or morpholine (3h). No new products were detected by tlc, and there was no change in the uv spectra of the solutions.

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(ii) 1,3-Di-p-nitrophenyltriazene was recovered in 95% yield from either boiling piperidine or morpholine (3h). There was no change in the uv spectra of the solutions during the reaction and no new products were detected (tlc). Similarly, no new products were detected (uv, tlc) when 1-phenylazopiperidine was boiled in 2-ethyoxyethanol with p-toluidine for 15 h or in ethanol containing p-nitroaniline for 10 h.

Reactions of 4-amino-2-[2-piperidin-1-ylazo)phenyl]quinazoline hydrate (6a). —

(i) The quinazoline hydrate (1g) was stirred in 6N-hydrochloric acid (20 ml) for 1 h at 20°. Basification of the solution with ice-aqueous sodium hydroxide afforded quinazolino[3,2-c]-1,2,3-benzotriazin-8(7H)-imine or isomer (0.3 g), identical (ir, m.p.) to an authentic sample.[74]

(ii) A solution of the quinazoline hydrate (2 g) in acetic acid (10 ml) was heated at 80° for 40 minutes. Basification of the mixture with ice-aqueous ammonia afforded the same tetracyclic triazine as in (i) above, identical (i.r.) to an authentic sample.

(iii) When the quinazoline hydrate (6a) was boiled in 4N – sulphuric acid (25 ml) for 2h, and cooled, 2-(2-hydroxyphenyl) quinazolin-4(3H)--one(45) (0.25g) was deposited and was identical (m.p. and i.r.) to an authentic sample.[125]

(iv) 4-Amino-2-(2-azidophenyl)quinazoline (49).–

The quinazoline hydrate (6a) (1.0 g) was boiled in acetic acid (10 ml)with sodium azide (4 mol. equiv.) for 15 min. The mixture was diluted with water, extracted into chloroform and the chloroform layer vacuum-evaporated. A benzene solution of the oily residue was chromatographically fractionated on an alumina column. The first yellow band, eluted with benzene, afforded the azidophenyl-quinazoline (27%), m.p. 143-145° (from ethanol) (Found: C.64.4; H, 3.9; N, 32.2. $C_{14}H_{10}N_6$ requires C,64.1; H, 3.8; N, 32.1%).

Cyclisation of the azidophenylquinazoline in boiling acetic acid was complete in 1 h. The products, identified as the isomeric indazoloquinazolines (50) and (51), were recognised as intensely fluorescent spots by tlc [on alumina (0.25 mm) with benzene as developing solvent] with identical r.f. values to authentic samples.[74].

(v) 4-Amino-2-(2-iodophenyl)quinazoline hydroiodide(47).–

The quinazoline hydrate (6a)(1 g) was boiled for 2 h in acetic acid (10 ml) containing sodium iodide (2 mol. equiv.). The solution was diluted with water to afford the brown hydroiodide (0.9 g) (66%), m.p. 254° indef. (from aqueous ethanol); (Found: C, 35.5; H, 2.3 ; N, 8.7. $C_{14}H_{10}N_3I \cdot HI$ requires C, 35.4; H, 2.3; N 8.8%).Basification of the salt with ice-aqueous ammonia afforded the free base, m.p. 191-193° (from ethanol) (Found: C,48.9; H, 3.05; N, 12.4. $C_{14}H_{10}N_3$ requires C48.9·H, 2.9; N, 12.1%).

(vi) The quinazoline hydrate (6a) (1g) was boiled in acetic acid (10 ml) containing copper bronze for 40 minutes. The solution was filtered through kieselguhr and the filtrate basified with ice-aqueous ammonia to afford 4-amino-2-phenylquinazoline (45) (90%), identical (i.r.) to an authentic sample.[122]

(vii) The quinazoline hydrate (1.0 g) was boiled in ethylene glycol (15 ml) for 1 h and the solution diluted with water. The chilled solution slowly deposited 4-amino-2-phenylquinazoline (40%) with identical i.r. to the sample above.

(viii) The quinazoline hydrate (0.1 g) was photolysed in methanol (1 litre) with a 100 W medium-pressure lamp in an Hanovia Photochemical Reactor equipped with a pyrex filter. After 24 h the UV spectrum of the photolysate was identical to that of a pure sample of 4-amino-2-phenylquinazoline (45) [λ_{max}(EtoH) 254, 285 (infl.), 304, 321(infl.) and 333 nm (infl.)[122]]. Tlc examination of the concentrated solution on silica gel employing ether: chloroform: methanol (10:2:1) as developing solvent confirmed the identification of the photo-product.

(ix) A sample of the quinazoline hydrate (0.1 g) in ethanol (25 ml) was exposed to laboratory light for 75 days. Tlc examination of the solution (as above) showed it to contain starting material and 4-amino-2-phenylquinazoline (45).

(x) A solution of the quinazoline hydrate (1.0 g) in ethanol (20 ml) containing Raney nickel (1.0 g) was treated over 1 h with hydrazine hydrate (5 x 1 ml). The mixture was filtered through Kieselguhr, evaporated and triturated with 6N-hydrochloric acid. The product 4-amino-2-(2-aminophenyl)quinazoline dihydrochloride (31) (92%) was identical to an authentic sample.[122]

Reactions of 2-[2-(piperidin-1-ylazo)phenyl]quinazolin-4(3H)-one(19c). —

(i) 2-(2-Azidophenyl)quinazolin-4(3H)-one(52)

The quinazolinone (19c)(1 g) was boiled for 1 h in acetic acid (10 ml) containing sodium azide (4 mol . equiv.). The solution was diluted with water to afford the quinazolinone (52) with m.p. > 300° (from methanol) (Found: C, 64.1; H, 3.5; N, 26.3. $C_{14}H_9H_5O$ requires C, 63.9; H, 3.4; N, 26.6%); γ_{max} (KBr) 3200-2750 (bonded NH, OH), 2140 and 2100 (N_3), 1680^{-1} (CO).

(ii) The quinazoline (19c) (1g) was boiled for 40 min. in acetic acid containing copper bronze and filtered through kieselguhr. Dilution of the solution with water afforded 2-phenylquinazolin -4(3H)-one (75%) identical (i.r.) to an authentic sample.[94]

Reactions of 4-amino-6-bromo-2-[5-bromo-2-(piperidin-1-ylazo)phenyl]-quinazoline (20a). —

(i) The quinazoline (20a) (0.6 g) was stirred at 25° in 6N-hydrochloric acid (15 ml) for 5 h to afford a yellow solid identical to 2-(4-amino-6-bromoquinazolin-2-yl)-4-bromophenyldiazonium chloride dihydrochloride (35).

Reaction of 4-amino-2-[2-(piperidin-1-ylazo)phenyl]quinazoline (6a) with cysteine.—

(i) The quinazoline (6a) was recovered in 95% yield after being stirred in aqueous dimethylformamide(1:1) containing cysteine (1 mol. equiv.) at 25° for 3 h.

(ii) Similarly the quinazoline was recovered in 95% yield from treatment with cysteine (1 mol. equiv.) in IN-potassium hydroxide solution for 3 h

(iii) The quinazoline (6a) (1 g) was treated with 6N-hydrochloric acid at 25° for 1 h. The crude 2-(4-aminoquinazolin-2-yl)phenyldiazonium chloride (34) was collected and added to a stirred solution of cysteine (0.3 g) in 1N-potassium hydroxide (10 ml). at 0°. The mixture was stirred at 0° for a further 2 h and diluted with water to afford quinazolino-[3,2-c]-1,2,3-benzotriazin-8(7H)-imine, identical (i.r.) to a previously prepared sample.

Reactions of 1,2,3-benzotriazin-4(3H)-one and derivatives in acetic acid.

(i) 2-Azidobenzamide (55 R=N_3). — 1,2,3-Benzotriazin-4(3H)-one (0.6 g) and sodium azide (4 mol. equiv.) were boiled in acetic acid (5 ml) for 1 h and the mixture diluted with water. The mixture was extracted into chloroform and the chloroform evaporated to afford a gum. Trituration of the gum with benzene yielded 2-azidobenzamide (95%) which crystallised from benzene as white needles, m.p. 135-136° (Lit.,[126] m.p. 135-136°); γ_{max} (KBr)

3378 and 3170 (NH), 2140 and 2110 (N_3), and 1653cm^{-1} (CO).

(ii) 2-Iodobenzamide (55;R=I). -- A solution of acetic acid (10 ml) containing 1,2,3-benzotriazin-4(3H)-one (1.0 g) and sodium iodide (2 mol. equiv.) was boiled(1 h)and diluted with water (20 ml). The precipitated iodobenzamide (85%) crystallised from aqueous ethanol as white crystals, m.p. 182-184° (Lit.,[127] m.p. 183°); γ_{max} 3350 and 3190 (NH), 1640cm^{-1} (CO).

(iii) When 1,2,3-benzotriazin-4(3H)-one (1 g) was boiled(1 h)in acetic acid (10 ml) containing 4 mol. equiv. of sodium bromide, chloride, cyanide, sulphite or acetate, dilution with water afforded the starting material in 90-95% yield.

(iv) Similarly, when derivatives of 1,2,3-benzotriazin-4(3H)-one substituted in the 3-position by, phenethyl, benzyl or ethyl groups were boiled in acetic acid containing sodium azide (4 mol. equiv.) no reaction occurred and starting materials (>95%) were recovered.

Reactions of aryl-dialkyltriazenes with methyl iodide

(i) 1-(Phenylazo)piperidine (1 g) was refluxed for 5 h in methanol (10 ml) containing methyl iodide (2 ml). Addition of excess ether to the cooled solution afforded N, N-dimethylpiperidinium iodide (0.3 g) identical (i.r. and m.p.) to a sample unambiguously prepared from N-methylpiperidine and methyl iodide. Gas chromatographic analysis of the reaction mixture was performed on a 2M Carbowax 20 m KoH column, run isothermally at 190° [carrier gas, N_2; 40ml/min; Perkin Elmer F11 gas chromatograph (FID)]. Authentic iodobenzene had the same retention time as a fraction present in the reaction mixture.

(ii) 1-(Phenylazo)piperidine (1 g) and methyl iodide (1 ml) were refluxed for 5 h in benzene (10 ml). The cooled solution deposited N, N-dimethylpiperidinium iodide, identical (i.r.) to the above sample.

(iii) 1-(Phenylazo)piperidine was recovered in 100% yield from refluxing methyl iodide alone (5 h).

(iv) 1-(p-Tolylazo)piperidine (1 g) was refluxed in methyl iodide for

11 h to afford N, N-dimethylpiperidinium iodide, identical (i.r.) to the previous samples.

(v) 1-(p-Nitrophenylazo)piperidine was recovered in 95% yield from refluxing methanol or ethanol containing methyl iodide (24 h).

4-Amino-1-methyl-2-[2-(piperidin-1-ylazo)phenyl]quinazolinium iodide (68). — 4-Amino-2-[2-(piperidin-1-ylazo)phenyl]quinazoline hydrate (6a) (3.0 g) and methyl iodide (3 ml) were boiled in tetrahydrofuran (20 ml) for 40 min. The precipitated yellow solid was collected and fractionally crystallised from ethanol. The least soluble fraction was 4-amino-2-[(piperidin-1-ylazo)-phenyl]quinazoline hydroiodide (67) (15%), m.p. 177° (yellow needles) (Found: C, 50.0; H, 4.8; N, 17.95. $C_{19}H_{20}N_6HI$ requires C,49.6; H, 4.6; N, 18.3%). A solution of the hydrciodide in methanol was treated with ice-aqueous ammonia to afford the free base (6a).

The second crystalline fraction (prisms) was the methylquinazolinium iodide (68) (80%), m.p. 234-236° (Found: C, 50.4; H, 4.9; N, 17.6. $C_{20}H_{23}IN_6$ requires C, 50.6; H, 4.85; N, 17.7%). 1-Methyl-2-[2-(piperidin-1-ylazo)phenyl]quinazolin-4(1H)-one (69). — An ethanolic solution (20 ml) of the methylquinazolinium iodide (68) (3 g) was poured onto ice-aqueous ammonia and the suspension slowly deposited the methylquinazolinone (69) (90%), m.p. 213-215° (white needles from aqueous ethanol); (Found: C, 69.2; H, 6.2; N, 20.5. $C_{20}H_{21}N_5O$ requires C, 69.2; H, 6.05; N, 20.2%). ν_{max} (KBr) 1642cm^{-1} (CO).

Properties of 1-methyl-2-[2-(piperidin-1-ylazo)phenyl]quinazolin-4(1H)-one

(i) The methylquinazolinone (0.6 g) in ethanol (10 ml) containing Raney nickel (1 g) was treated at 60-65° with hydrazine hydrate (5 x 1 ml) for 1 h and filtered through kieselguhr. The evaporated solution was triturated with ethanol to afford the starting material in 90% yield.

(ii) The methylquinazolinone (0.7 g) was boiled in ethylene glycol (5 ml) for 6 h and poured onto ice-water (50 ml), to afford the starting material (75%).

(iii) The methylquinazolinone (0.9 g) was stirred at 0° in 6N-hydrochloric acid (7 ml) containing hypophosphorous acid (50%) (4 ml) for 1 h, then kept at 4° for 12 h. The solution was brought to pH 10 with dilute sodium hydroxide solution. The white solid (80%) was shown to be starting material (i.r.).

(iv) 2-Phenylquinazolin-4(3H)-one (70). —

A solution of the methylquinazolinone (0.5 g) in acetic acid (5 ml) containing copper bronze (0.3 g) was boiled for 2 h. The filtered concentrated solution afforded 2-phenylquinazolin-4(3H)-one (63%) with identical i.r. to an authentic sample.[94] A further sample of 2-phenylquinazolin-4(3H)-one was prepared by the following route:

2-methylbenzamide (0.4 g) was dissolved in boiling p-cymene and benzoyl chloride (0.6 ml) was added dropwise over 5 minutes.

The white product which immediately formed was identical (i.r.,m.p.) to an authentic sample of 2-phenylquinazolin-4(3H)-one.

3- Methyl-2-phenylquinazolin-4(3H)-one

3- Methyl-2-phenylquinazolin-4(3H)-one was prepared by cyclisation of 2-benzamido-N methylbenzamide. It had m.p. 134 - 135° (Lit.,[97] m.p. 136-138°). The quinazolone (0.3 g) was boiled in acetic acid (5 ml) containing copper bronze (0.1 g) for 1 h. Dilution of the filtered solution with excess sodium hydroxide afforded a precipitate (0.28 g) identical (i.r.) to the starting material.

4-Amino-2-[2-(piperidin-1-ylazo)phenyl]quinazolinium benzoate.—

A solution of 4-amino-2-[2-(piperidin-1-ylazo)phenyl]quinazoline hydrate (3.32 g) in tetrahydrofuran (60 ml) containing perbenzoic acid[128] (1.38 g) in benzene (30 ml) was kept at 4° for 12 days. The evaporated solution afforded a gum which was triturated with acetone to produce the benzoate

salt of the starting material, m.p. 139-141° (from acetone). (Found: (C,68.3; H, 5.6; N, 17.3. $C_{26}H_{26}N_6O_2$ requires C,68.7; H, 5.7; N, 17.5%).

A solution of the salt in methanol treated with ice-aqueous ammonia afforded the starting material.

Permanganate oxidation of 4-amino-2-[2-(piperidin-1-ylazo)phenyl]-quinazoline hydrate (6a). — The quinazoline hydrate (0.8 g) was dissolved in acetone (60 ml). To this solution was added potassium bicarbonate (0.6 g) and potassium permanganate (0.9 g) in water (60 ml) and the mixture was stirred at 25° for 10 h. The suspension was filtered through Kieselguhr and extracted into chloroform (3 x 80 ml). The chloroform extracts were concentrated to 5 ml) and kept at 4° for 12 h. 1-[2-(4-Aminoquinazolin-2-yl)phenylazo]piperidin-2-one (25%) was collected with m.p. 202-204° (from chloroform) (Found 65.8; H,5.1; N, 24.0. $C_{19}H_{18}N_6$ requires C,65.9; H, 5.2; N, 24.3%);

γ_{max} (KBr) 3460, 3320, 3210 (NH), 1650 cm^{-1} (CO); τ(CD_3OD) 6.45m, 7.40m, 8.25m (2H, 2H, 4H) [N.B. 1(4-Chlorophenylazo)piperidin-2-one[105] has τ($CDCl_3$) 6.22m, 7.38m 8.20m(2H, 2H, 4H)];

N-benzoylpiperidin-2-one[105] has τ($CDCl_3$) 6.21m, 7.43m, 8.09m (2H, 2H, 4H).

The residual chloroform extract was subjected to chromatographic analysis on silica plates (1 mm) using ether: chloroform: methanol (10:2:1) as developing solvent. The band which co-chromatographed with authentic 4-amino-2-(2-aminophenyl) quinazoline was removed and dissolved in ethanol. A uv spectrum of this diluted solution was identical to that of the authentic diamine [λ_{max} (EtoH) 330 (infl),300 (infl), 262(sh), 234 nm].

The triazenoquinazolines (6c) (6f) (6h) and (6i) were oxidised as above: in all cases 4-amino-2-(2-aminophenyl) quinazoline was identified as described.

δ-Valerolactone

The reaction mixture from permanganate oxidation of the quinazoline hydrate

(6a) was filtered through Kieselguhr and stood for 2 h at 25° with 1% Sodium bicarbonate solution (100 ml). The solution was shaken with chloroform (2 x 100 ml) and the aqueous layer acidifed (pH) with hydrochloric acid. The acid solution was extracted into chloroform(2 x 50 ml) and the chloroform solution reduced to 5 ml. The i.r. spectrum (film) of this concentrate was identical to the i.r. spectrum of a solution of authentic δ-valerolactone in chloroform. Gas chromatographic analysis, performed isothermally at 150° on a 1MSE30 column with nitrogen gas (40 ml/min) on a Perkin Elmer F11 gas chromatograph (F.I.D.) confirmed the presence of δ-valerolactone in the concentrate above. The concentrate was treated with hydroxylamine hydrochloride to convert lactones to hydroxamic acid derivatives[129], and chromatographed on silica plates (0.25 nm) (ethanol as developing solvent) with a similarly treated sample of authentic δ-valerolactone in chloroform. The plates were sprayed with 5% ferric chloride solution. The two magenta spots had identical rf values.

Permanganate oxidation of N-nitrosopiperidine

A solution of nitrosopiperidine (0.28 g)[130] in acetone 60 (ml) was treated with potassium permanganate (0.9 g) and potassium bicarbonate (0.6 g) in water (60 ml) and stirred at 25° for 20 h. The filtered reaction mixture was purified as described for the isolation of lactones and the resulting chloroform concentrate reacted with hydroxylamine hydrochloride to convert any lactones present to the hydroxamic acid derivatives. The resulting solution was shown to contain the hydroxamic acid derivative of δ-valerolactone since it co-chromatographed (t.l.c.) with a similarly treated solution of authentic lactone in chloroform.

Udenfriend oxidation of 4-amino-2-[2-(piperidin-1-ylazo)phenyl] quinazoline:

To a solution containing sodium hydrogen phosphate (4.5 g) and potassium di-hydrogen phosphate (3.5g) in water (200 ml) was added ascorbic acid (0.82 g in water), ethylenediaminetetracetic acid (0.8 g in min. warm water) and ferrous

ammonium sulphate hexahydrate (0.17 g). The resulting solution was made up to 250 ml with water and added to a solution of the quinazoline hydrate (0.15 g) in acetone (100 ml). The flask was protected from light and shaken at 37° for 14 h. A chloroform extract (3 x 100 ml) of the solution was chromatographed on silica employing ether: chloroform: methanol (10:2:1) as developing solvent. The band corresponding to 4-amino-2-phenylquinazoline was removed and washed with ethanol. A uv spectrum of this diluted solution was identical to authentic 4-amino-2-phenylquinazoline.

Reactions of 2-azaadenine-1-oxide. —

(i) 2-Azaadenine-1-oxide (200 mg) was boiled for 2 h in piperidine (20 ml). Trituration of the evaporated solution with water afforded the starting material in 90% yield.

(ii) 2-Azaadenine-1-oxide (300 mg) was boiled in collidine (5 ml) for 5 h. Starting material (90%) was recovered from the cooled solution.

(iii) A mixture of 2-azaadenine-1-oxide (0.5 g) and ammonium acetate (2 g) was fused at 200° for 15 min. Starting material (80%) was recovered from the cooled mixture.

5-Amino-4-cyano-2-(4-cyanoimidazol-5-ylazo)imidazole (101). —

A solution of 5-aminoimidazole-4-carbonitrile (0.4 g) in 2N-hydrochloric acid (4 ml) was added dropwise to a solution of sodium nitrite (0.26g) in water (4 ml) at 0° over 45 min. After addition of excess urea, the resulting suspension was added in portions to a solution of 5-aminoimidazole-4-carbonitrile (0.4 g) in water (5 ml) containing sodium acetate (3 g). Stirring was continued for 1 hr. The azoimidazole (101) (70%) crystallised from aqueous dimethylformamide with m.p. >300° (Found: C, 40.3; H, 2.3; N, 57.0; $C_8H_5N_9$ requires C, 40.7; H, 2.1; N, 57.2%): γ_{max} (KBr) 2210 (CN), 3180 and 3320cm^{-1} (NH); λ_{max} (EtOH) 512 nm.

1-(2-Cyanophenyl) -3-(4-cyanoimidazol-5-yl)triazene (102). -

5 - Aminoimidazole-4-carbonitrile (0.4 g) in 2N-hydrochloric acid (4 ml) was treated at 0° with a solution of sodium nitrite (0.26 g) in water (4 ml) over 45 min. Excess urea was added followed by o-aminobenzonitrile (0.4 g) and ice-water (15 ml). Stirring was continued for 2 h. The precipitated triazene (102) (80%) which was collected and dried in a vacuum desiccator had m.p. 187° decomp. (from acetone) (Found:C,55.3; H, 3.1; N, 41.2. $C_{11}H_7N_7$ requires C,55.7; H, 2.95; N, 41.1%);

γ_{max} (KBr) 2223(CN) 3200cm^{-1} (NH).

Properties of 1-(2-cyanophenyl)-3-(4-cyanoimidazol-5-yl)triazene (102). -

(i) 1-(2-Cyanophenyl)-3-(4-cyanoimidazol-5-yl)triazene (102) (0.5 g) was boiled for 1 h in 90% aqueous ethanol (10 ml) and the solvent evaporated. 2-Phenyladenine (75%) crystallised from water with m.p. 325° (Lit., 321°), and i.r. & u.v. identical to an authentic sample [λ_{max} (water) 237, 268 nm]. The 2-phenyladenine prepared by this route co-chromatographed with an authentic sample [paper chromatography (Whatman No. 1) employing 3% ammonium chloride: isopropanol (2:1) as developing solvent; rf = 0.53].

(ii) The triazene (0.5 g) and piperidine (7 ml) were boiled for 1 hr. Tlc [silica (0.25 mn) employing ether: chloroform: methanol (10:2:1) as developing solvent] performed on the reaction mixture showed at least nine products. Paper chromatography (as above) showed the presence of 2-phenyladenine. No products were isolated.

1-(Guanin-8-ylazo)piperidine(107). -

8-Aminoguanine (0.9 g) was dissolved in 5% potassium hydroxide (10 ml) containing sodium nitrite (0.38g) and the solution added dropwise to hydrochloric acid (14 ml) over 10 min with stirring, at 10°. 8-Diazoguanine (0.6 g), [γ_{max} nujol 2240 cm^{-1} (N_2^+)] was collected, washed with methanol, dried in a vacuum desiccator ($CaCl_2$), and suspended in acetone (10 ml) containing piperidine (0.5 g). The suspension was stirred at 20° for 1.5 h to afford the guaninylazopiperidine hydrate, (lime-green rosettes, from

aqueous dimethylformamide) (Found:C, 43.1; H, 5.9; N, 39.8. $C_{10}H_{14}N_8O$. H_2O requires C 42.75; H, 5.7; N40.0%).

8 - (3,3-Dimethyltriazen-1-yl)guanine (108). —

8-Diazoguanine (0.6 g) was suspended in acetone (30 ml) and stirred with anhydrous dimethylamine at 0° for 2 h. The triazenylguanine hydrate (60%) had m.p. 240° (lime-green rosettes from water). (Found:C, 34.8; H 5.2; N, 46.3. $C_7H_{10}N_8O$. H_2O requires C, 35.0; H, 5.0; N, 46.6%).

8-Aminoguanine.

8-Diazoguanine (0.5 g) was suspended in acetone (20 ml) and methylamine (generated from methylamine hydrochloride and 10 N - sodium hydroxide) was bubbled through the suspension for 1 h. The mixture was stirred for 15 h to afford 8-aminoguanine (75%) (as sulphate from 10% sulphuric acid) chromatographically pure [paper chromatography Whatman No 1; employing 3% ammonium chloride: isopropanol (2:1) as solvent (rf = 0.18)], with uv spectrum identical to an authentic sample [λmax (water) 287,247nm].

Biological Results

All tests were performed according to protocols laid down by the drug evaluation branch, National Cancer Institute, Bethesda, Maryland.[131]

Table 1

Activity v L1210 leukaemia in vivo, CDF_1 mice

Tests were performed by intraperitoneal innoculation of male CDF_1 mice with 10^5 L1210 cells. Drug treatment was initiated 24h later. Tumour-inhibitory activity was evaluated by comparing mean survival times of innoculated mice receiving drug treatment with survival time of untreated controls.

Compound	Activity (% increase in lifespan)	Dose mg/Kg
6a	inactive	400
6f	inactive	400
6g	inactive	400
6h	inactive	400
27	inactive	400
31	inactive	400
20a	inactive	400
20b	inactive	400
20c	inactive	400
20d	inactive	400
20e	inactive	400
20f	inactive	400
2o or isomer 30	inactive	400
35	inactive	400
22 or isomer 24	inactive	400
diazo-ICA	4	5
DTIC	93	480

Table 2

Activity v TLX5 lymphoma

Tests performed at Chester Beatty Research Institute, London, using female CBA/LAC mice innoculated with 10^5 TLX5 cells.

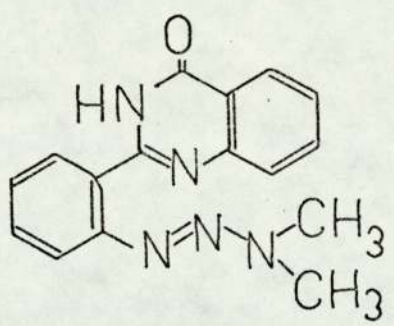
Compound	Max % ILS
19a	31.9
BCNU	39.2
6a	inactive
6i	inactive
6b	inactive
108	7.2

BCNU = bis-[2-chloroethyl]-N-nitrosourea.

ILS = increase in lifespan.

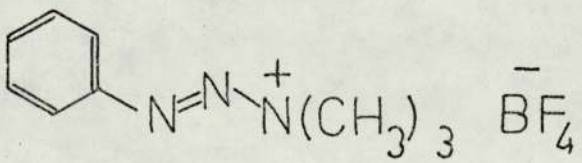
Discussion

None of the novel triazenes were active against H-Ep2 in cell culture. Surprisingly, 4-amino-2-[2-(piperidin-1-ylazo)phenyl] quinazoline (6a), which was active against HEp2 was inactive against the L1210 and TLX5 tumours *in vivo*. This lack of activity may be due to pharmacokinetic factors, eg. failure to gain access to the tumour cell because of the strongly basic character of the molecule. The quinazoline (6a) was shown to form benzoate and hydroiodide salts. The dimethylquinazolinone (19a) exhibited notable activity against the TLX5 lymphoma (*in vivo*), comparable to BCNU, whereas the corresponding 4-amino-2-[3,3-dimethyltriazen-1-yl)phenyl] - quinazoline (6i) was inactive against this tumour. These differences may also be due to the relative basicity of these molecules. Perhaps further quinazolinones should be examined for biological activity.



(19a)

Pharmacological studies were performed on 1-phenyl-3,3,3-trimethyltriazenium fluoroborate, (PTF), synthesised in collaboration with Stevens and Meredith.[99] Studies were conducted to determine if PTF has cholinergic or antagonistic activity, using freshly prepared solutions of PTF in 0.5% aqueous dimethylsulphoxide.



PTF

PTF caused a contraction of the guinea-pig ileum, and in concentrations from 10µg/ml to 320 µg/ml produced a graded dose/response curve. These contractions were antagonised by atropine (0.1 µg/ml) but not by mepyramine (10 µg/ml). These effects of PTF were shown to be similar to the responses of the tissue

to acetylcholine (ACh). In doses up to 1mg/ml PTF had no effect on rat hemi-diaphragm muscle contractions elicited by stimulation of the phrenic nerve at a frequency of 0.1 Hz.

Contractures of the frog rectus abdominis muscle were obtained in response to addition of PTF to the preparation in concentrations from 10 µg/ml to 500 µg/ml; these contractures were competitively antagonised by d-tubocurarine. The responses of this muscle to PTF were similar to the responses elicited by ACh. In each of these pharmacological preparations DMSO alone (0.5% in aqueous solution) had no effect.

The results of these preliminary pharmacological tests suggest that PTF has a cholinergic agonist action at nicotinic and muscarinic sites and that PTF and other chemically reactive triazenium salts may be of use in studies on the cholinergic receptor.

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Tumour-inhibitory triazenes: search for a chemical basis for their mode of action.

by

Colin P. Turnbull

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from the

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SUMMARY

The chemical and biological properties of the antitumour agent 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (DTIC) are reviewed in the introduction, which includes a discussion on the mode of action of DTIC, aryldialkyltriazenes and the carcinogenic N-nitrosamines.

4-Amino-2-[2-(piperidin-1-ylazo)phenyl]quinazoline(6a) and its related heteroalicyclic analogues (6b-i) were prepared by heating 1,3-di-o-cyanophenyltriazene in the respective secondary heteroalicyclic amines.

Half-diazotisation of 5-bromoanthranilonitrile in 2N-hydrochloric acid resulted in the formation of 1,3-di-(2-cyano-4-bromophenyl)triazene (25). 4-Amino-6-bromo-2-[5-bromo-2-(piperidin-1-ylazo) phenyl]quinazoline(20a) was obtained when the triazene (25) was heated in piperidine. The related derivatives (20b-f) were similarly obtained by heating the triazene (25) in a range of secondary amines. Treatment of anthranilonitrile and 5-bromoanthranilonitrile in dimethylsulphoxide with sodium hydride resulted in the formation of 4-amino-2-(2-aminophenyl)quinazoline(31) and 4-amino-6-bromo-2-(2-amino-5-bromophenyl)-quinazoline (27) respectively.

The diamine (27) was alternatively obtained by reduction of the triazene (25) in ethanol containing hydrazine and Raney nickel. Diazotisation of the diamine (27) led to the isolation of 2-(4-amino-6-bromoquinazolin-2-yl)-4-bromophenyldiazonium chloride dihydrochloride (35). 2, 10-Dibromoquinazolino-[3,2-c]-1,2,3-benzotriazin-8(7H)-imine(30) or its isomer (29) was formed when the diazonium salt (35) was recrystalised from methanol/ether. Treatment of the tetracyclic triazine (30) or isomer (29) with hot piperidine resulted in the formation of the quinazolinotriazene (20a). The triazenoquinazoline (20a) underwent decomposition in 6N-hydrochloric acid to the diazonium ion (35). Treatment of the triazenoquinazoline (6a) with 6N-hydrochloric acid or hot acetic acid, followed by basification resulted in the formation of quinazolino [3,2-c]-1,2,3-benzotriazin-8 (7H)-imine (24) or isomer (22).

The triazene (6a) underwent reductive elimination of the triazeno side-chain in acetic acid containing copper bronze, in boiling ethylene glycol, or by photolysis in ethanol or methanol to afford 4-amino-2-phenylquinazoline. Sandmeyer-type displacement of the triazeno side-chain occurred when the triazene (6a) was heated in acetic acid containing sodium iodide or sodium azide.

Triazene (6i) was hydrolysed in 50% alcoholic potassium hydroxide to yield 2-[2-(3,3-dimethyltriazen-1-yl)phenyl]quinazolin-4(3H)-one (19a).

Alkylation of the triazenoquinazoline (6a) with methyl iodide afforded a hydroiodide salt of the starting material and 1-methyl-2-[2-(piperidin-1-ylazo)phenyl]quinazolin-4(1H)-iminium iodide (68). The methylquinazolinium iodide (68) underwent hydrolysis in aqueous ammonia to 1-methyl-2-[2-piperidin-1-ylazo)phenyl]quinazolin-4(1H)-one (69). Treatment of the methyl quinazolinone (69) with acetic acid containing copper bronze resulted in the formation of 2-phenylquinazolin-4(3H)-one. Oxidation of the triazene (6a) with perbenzoic acid in benzene afforded a benzoate salt of the starting material. Oxidation of the triazene (6a) with potassium permanganate resulted in the formation of 1-[2-(4-aminoquinazolin-2-yl)phenylazo]piperidin-2-one, 4-amino-2-(2-aminophenyl)quinazoline and δ-valerolactone. Analagous oxidation of N-nitrosopiperidine afforded δ-valerolactone. Udenfriend oxidation of the triazene (6a) led to a mixture from which 4-amino-2-phenylquinazoline only was characterised.

5-Diazoimidazole-4-carbonitrile coupled with 5-aminoimidazole-4-carbonitrile to yield 5-amino-4-cyano-2-(4-cyanoimidazol-5-ylazo)imidazole. 1-(2-(cyanophenyl)-3-(4-cyanoimidazol-5-yl)triazene (102) was formed when 5-diazoimidazol-4-carbonitrile was coupled with anthranilonitrile. In boiling ethanol, the triazene (102) underwent cyclisation and decomposition to afford 2-phenyladenine.

8-(3,3-Dimethyltriazen-1-yl)guanine and 1-(guanin-8-ylazo) piperidine were obtained when 8-diazoguanine was coupled with dimethylamine and piperidine respectively. When 8-diazoguanine was coupled with methylamine, 8-aminoguanine was formed.

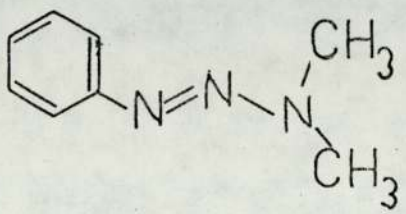
CONTENTS

Chapter 1 Introduction

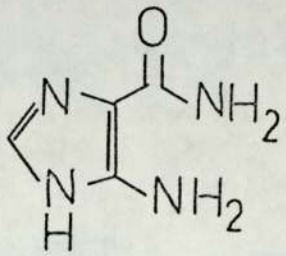
Historical introduction

Aryl-dialkyltriazenes were first reported in the literature by Baeyer[1] as early as 1875 but their tumour-inhibitory activity was not discovered until 1955, when Clarke [2] showed that 3,3-dimethyl-1-phenyltriazene [PDMT] (1) inhibited the growth of Sarcoma 180 in mice. This discovery was followed by reports from Dagg and Karnofsky,[3] who demonstrated the existence of teratogenic effects of PDMT on the chick embryo and confirmed the inhibitory activity against Sarcoma 180 and certain human tumours. The clinical use of triazenes however stemmed from attempts to design antagonists of 5-aminoimidazole-4-carboxamide [AIC] (2), whose riboside-5'-phosphate derivative is an intermediate in de novo purine synthesis. Shealy[4] synthesised 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide [DTIC] (3) as a potential antitumour agent. The rationale behind the synthesis of DTIC was to provide a stable "carrier" form of 5-diazoimidazole-4-carboxamide [diazo-ICA] (4) which was tumour-inhibitory[5] but chemically unstable, cyclising in solution to the relatively biologically inert 2-azahypoxanthine[5] (5). DTIC was subsequently shown to be active against many experimental tumours. It significantly increased the lifespan of mice bearing Leukemia L-1210[6] and is active against Sarcoma 180, Adenocarcinoma 755 and Ehrlich Ascites Carcinoma in mice.[7] Not surprisingly therefore, further derivatives of triazenes were synthesised and evaluated in attempts to establish structure-activity patterns. The triazenoquinazolines, represented here by the prototype 4-amino-[2-(2-piperidin-1-ylazo)phenyl]-quinazoline,[8] [NSC 163423] (6a) do not owe their discovery to any systematic study, but to the element of chance (not an uncommon occurrence in the field of science). In a futile attempt to recrystallize 1,3-di-o-cyanophenyl-triazene (7) from piperidine, Stevens[8] isolated a hydrate of the triazene (6a) in almost quantitative yield. This substance, and further alkyl

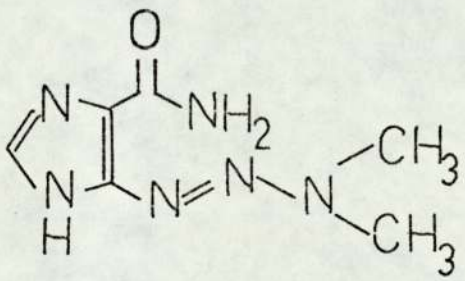
derivatives were routinely screened for tumour-inhibitory activity and were found to be approximately two hundred times more active than DTIC in inhibiting the growth of human epidermoid carcinoma of the nasopharynx in cell culture (H-Ep2).[9]



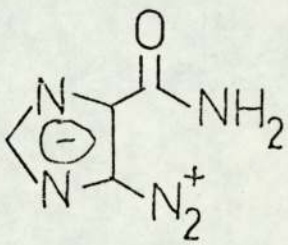
PDMT (1)



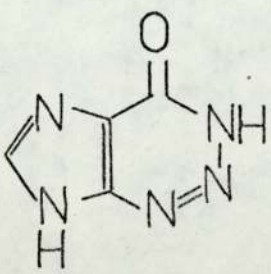
AIC (2)



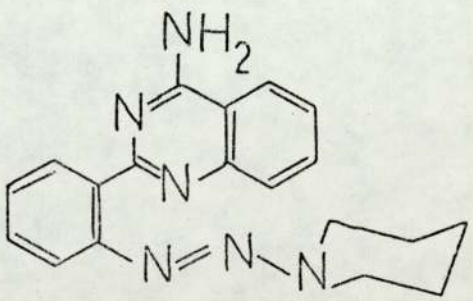
DTIC (3)



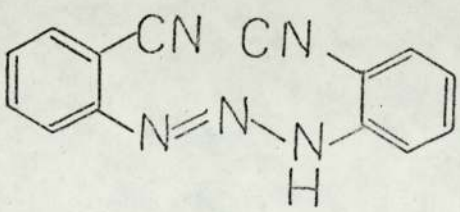
diazo- ICA (4)



(5)



(6a)



(7)

Clinical use and toxicity of DTIC

DTIC is a particularly useful agent in the treatment of the hitherto recalcitrant human malignant melanoma, where, following its use, regression rates of 20% have consistantly been obtained.[10] Evidence for activity against Hodgkin's disease and lymphosarcoma has been obtained for DTIC. This drug has recently become available for general clinical use (June 1975 marketed by Dome pharmaceuticals as Dacarbazine®) and is being evaluated in combination with other anti-neoplastic agents, as a single agent and as an adjunct to radiotherapy.

In addition to its desirable tumour-inhibitory activity, DTIC has a number of toxic effects in experimental animals and in humans. Carcinogenic,[11,12] teratogenic,[13 14] and immunosuppressive[14] activity have been demonstrated in rats, DTIC being distinguished by its ability to induce 100% incidence of tumours in the thymus of rats, a sinister feat not achieved by any other chemical carcinogen. In man, side effects from administering DTIC include nausea, vomiting, myelosuppression and hepatotoxicity.[15] These effects are common to many cytotoxic agents. C.N.S. complications have been reported in man after combination treatment with Adriamycin.[16] Most of these toxic properties manifested in experimental animals are elicited by many aryl-dialkyltriazenes and are not unique to DTIC.

Mode of action of DTIC

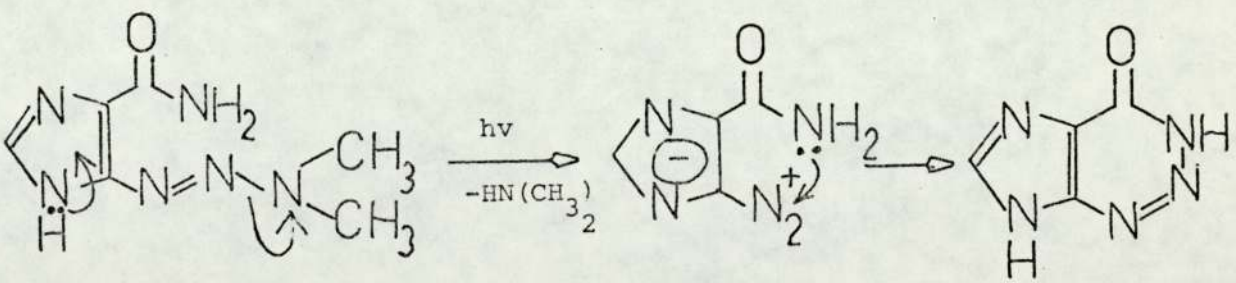
(i) Introduction

While the precise mode of action of DTIC and related triazenes is imperfectly understood, certain chemical and biochemical transformations of triazenes have been observed which may have important consequences when considering the mode of action of these substances in biological systems. In discussing the mode of action of DTIC, it is perhaps useful to consider certain aspects

separately - namely biochemical interactions and biological effects, since it has yet to be determined which, if any, of the biological effects (and in particular, the antitumour effect) are related to the known chemical and biochemical events. It is generally accepted that DTIC does not act as an antagonist of AIC (for which purpose it was originally synthesised) since it has been shown that triazenes not bearing the imidazole ring are just as effective as antitumour agents[17-19].

(ii) In vitro effects of DTIC and other imidazole triazenes

Shealy demonstrated that the exposure of DTIC and other imidazole triazenes to light in solution results in the formation of 2-azahypoxanthine.[4] This transformation is a consequence of a light-catalysed heterolytic dissociation of the triazene to diazo-ICA, which cyclises irreversibly to 2-azahypoxanthine (Scheme 1.1). The reaction is characterised by distinct spectral changes and may be conveniently followed by ultraviolet spectroscopy (Fig. 1.1.)



(3) (4) (5)

Scheme 1.1

The cyclisation product, 2-azahypoxanthine, is not considered to be involved in the tumour-inhibitory process although it is not devoid of biological activity being a potent inhibitor of the enzyme xanthine oxidase[20].

The relevance of the photodecomposition of DTIC to its mode of action in cell culture has been shown by several workers. Saunders and Schultz[21] found that the inhibitory effect of DTIC on Bacillus subtilis (B. subtilis)

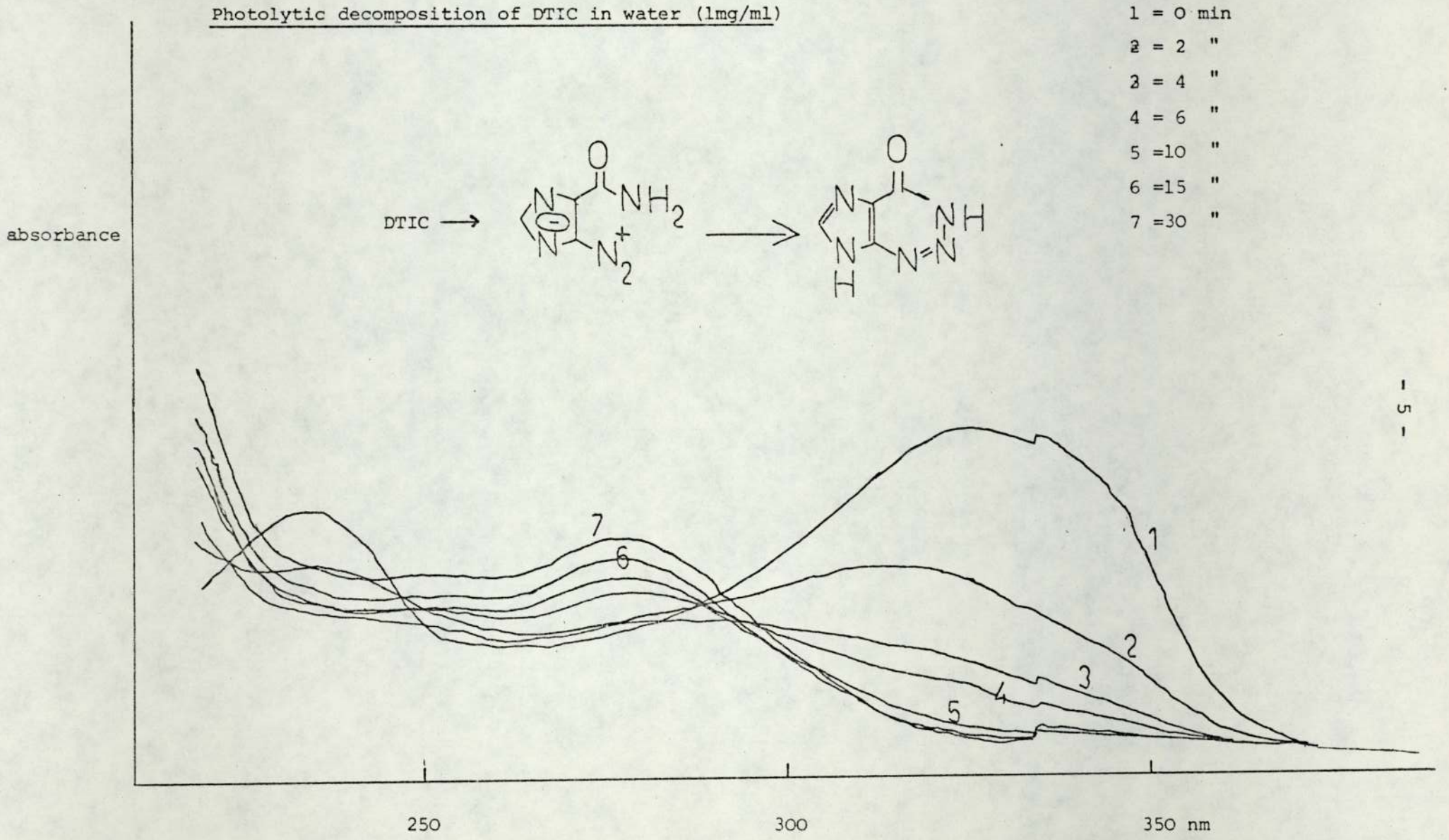

Photolytic decomposition of DTIC in water (1mg/ml)
1 = 0 min
2 = 2 "
3 = 4 "
4 = 6 "
5 =10 "
6 =15 "
7 =30 "
DTIC
absorbance
1
2
3
4
5
6
7
250
300
350 nm

Fig. 1.1.

was markedly increased by exposing cultures growing in the presence of DTIC to light, under which conditions diazo-ICA was continually being generated (Fig. 1.2). They also showed that a strain of B. subtilis resistant to DTIC was resistant to diazo-ICA. Yamamoto[22] reported that cysteine abolished the inhibitory activity of diazo-ICA in Escherichia coli (E. coli) and suggested that diazo-ICA might be the active form for inhibition of this organism by DTIC. Studies with bacteria indicated that DNA synthesis in E. coli is inhibited by diazo-ICA[22] and that DNA synthesis in B. subtilis is inhibited by DTIC.[21] Diazo-ICA has also been shown to interfere with RNA synthesis in E. coli.[22]

The light dependent lethality of DTIC to bacteria has been mimicked in mammalian cells. Gerulath and Loo[23] showed that DTIC was more lethal to Chinese hamster ovary cells and human malignant melanoma cells in culture in the presence than in the absence of light. Accordingly it is accepted that the growth inhibitory activity of DTIC in bacteria and mammalian cells in culture is due almost exclusively to diazo-ICA, formed by light-catalysed dissociation of DTIC. It has been proposed that diazo-ICA produces this action by interfering with macromolecular synthetic processes.[21,22]

In addition to its bacteriostatic and tumour-inhibitory properties diazo-ICA has been shown to be a potent inhibitor of xanthine oxidase.[24] Activation of monoamine oxidase,[25] release of 5-hydroxytryptamine from rabbit platelets[26] and multiple pharmacological effects in cats and rabbits [26] have been reported for diazo-ICA.

(iii) DTIC as a source of reactive diazonium ions

While the photodecomposition of DTIC to diazo-ICA is a property peculiar to imidazotriazenes, all triazenes may be considered as "masked" or "latent" diazonium ions[27]. They are highly unstable in acid conditions and readily undergo heterolytic fragmentation to diazonium ions. (Scheme 1.2)

Effect of DIC on *B. subtilis* 168

P. P. Saunders and G. A. Schultz, *Biochem.Pharmacol.*, 19, 911 (1970)

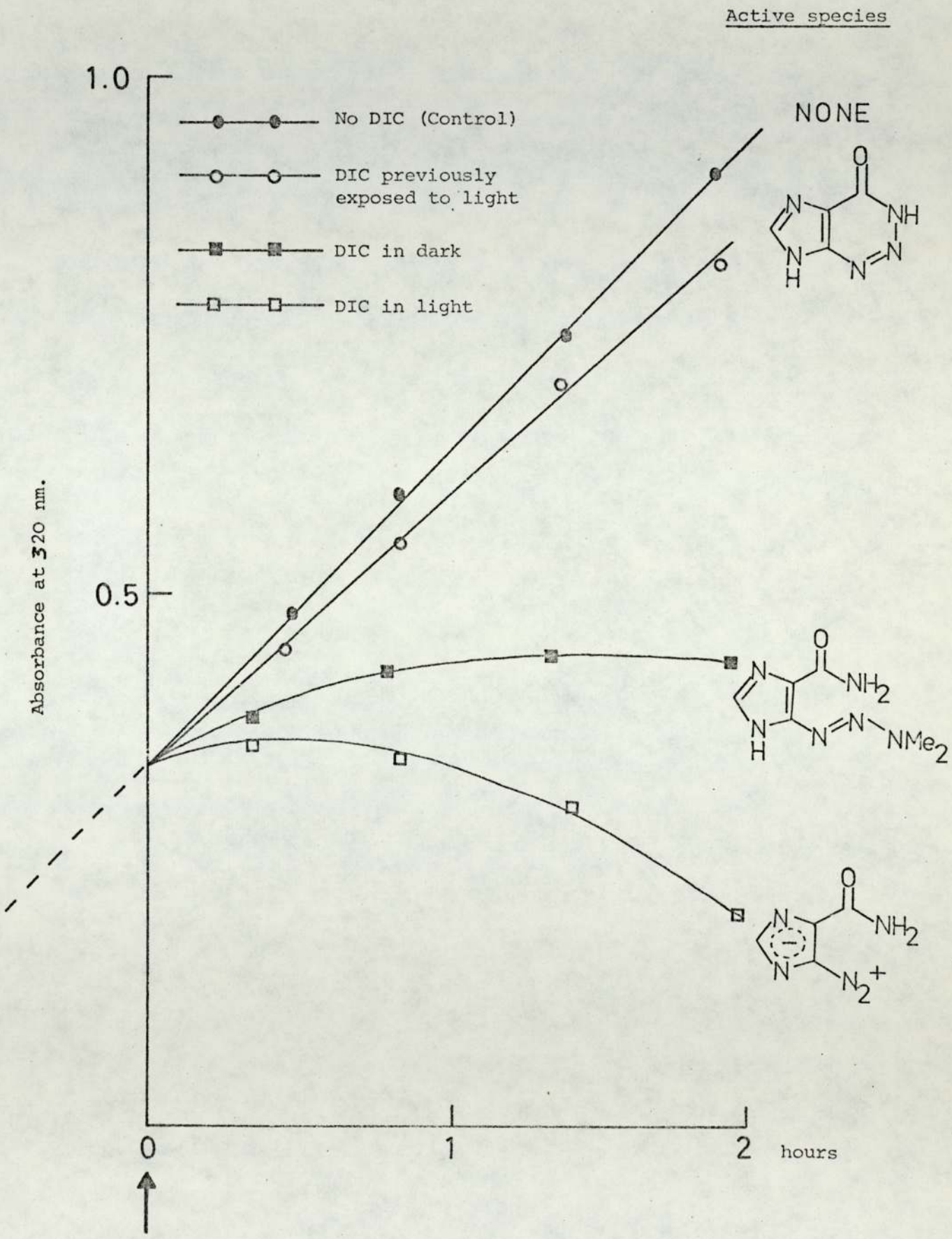
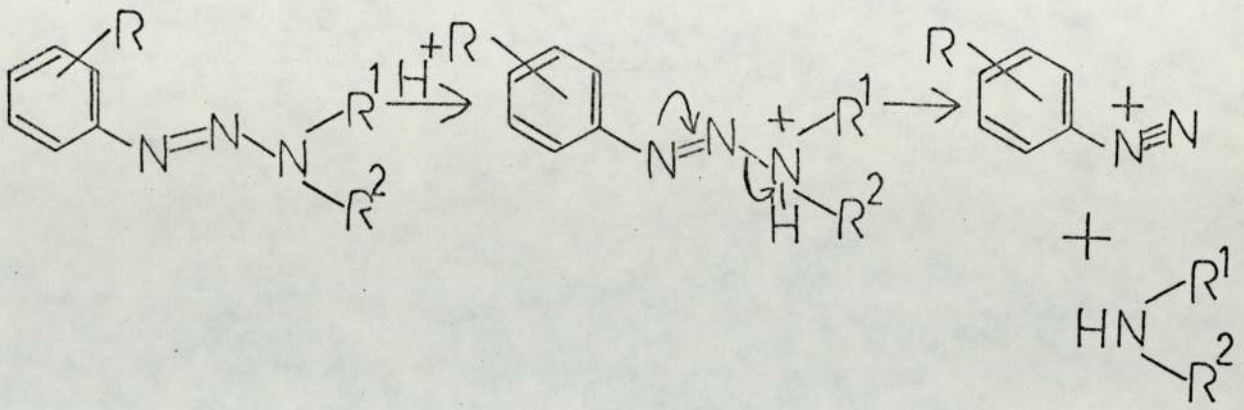
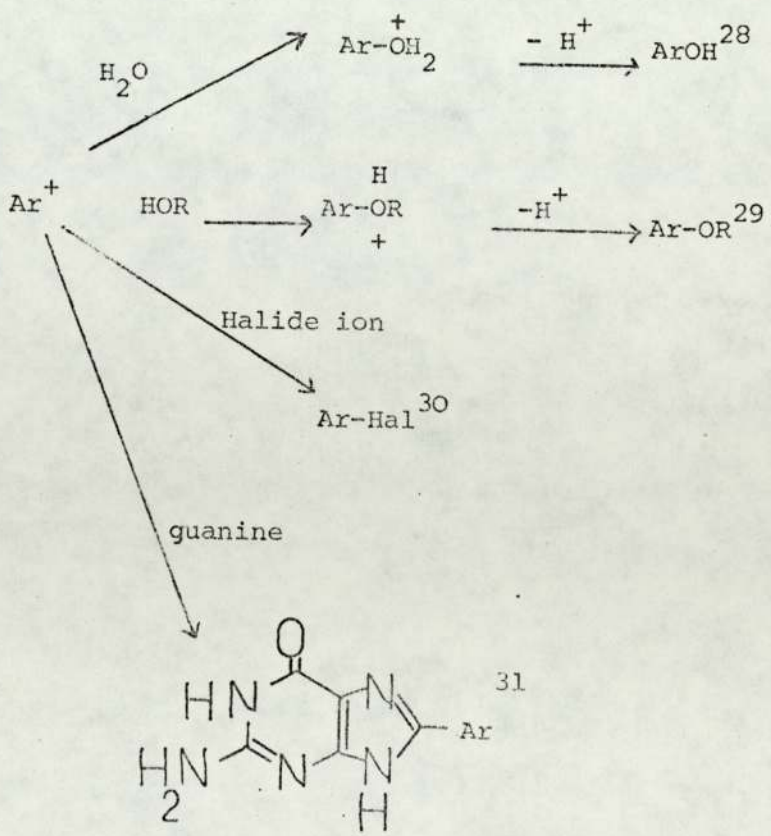


Fig. 1.2



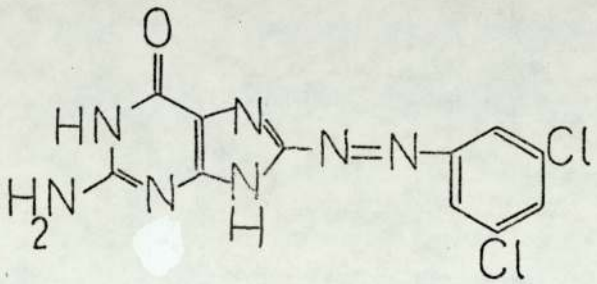
Scheme 1.2

Diazonium ions may react principally in two ways characterised by the nature of the products. This involves either loss or retention of the nitrogen atoms of the diazo group in the products. In the first instance, diazonium ions may decompose via S_N1 heterolytic cleavage of the C-N bond, to aryl cations (Ar^+) which react avidly with available nucleophiles (Scheme 1.3). Included in the first category are those reactions of diazonium compounds which proceed homolytically via aryldiazohydroxides to aryl radicals which subsequently react with nucleophiles. In either case the nitrogen atoms of the diazo group are lost in the products.

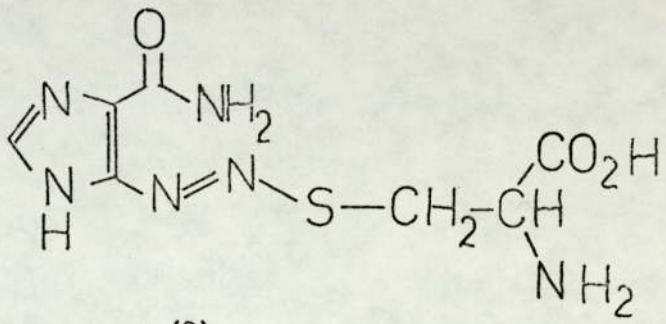


Scheme 1.3

Alternatively, diazonium ions may undergo coupling reactions to form products in which the nitrogen atoms of the diazo group are retained. Examples of possible biological relevance of this type of reaction include the coupling of diazotised 3,5-dichloroaniline with guanine to form the azo-derivative (8)[32] and the reaction of diazo-ICA with the thiol group of cysteine to form the azothioether (9)[24].



(8)



(9)

There are a large number of nucleophilic centres in a cell, each potentially capable of reaction with diazonium ions. These include the alcohol, thiol and amino groups of enzymes and structural proteins; the ionised phosphate groups and ring nitrogens of nucleotides and nucleic acids. In addition, free amino acids and peptides, vitamins, co-factors, steroids and lipids all possess nucleophilic centres capable of reaction with electrophiles. Therefore the introduction of a highly reactive electrophilic species into such an environment may be expected to have profound and potentially hazardous consequences for the well-being of the cell.

In recent years, the activity of aryl diazonium compounds has been exploited to elicit information about the structure of physiologically active proteins and the topography of the active sites of enzymes and the combining regions of antibodies. Some labelled macromolecules include insulin,[33] human erythrocyte membrane[34] and the enzymes trypsin[35] and chymotrypsinogen A and β-chymotrypsin.[36] The labelled amino acids have

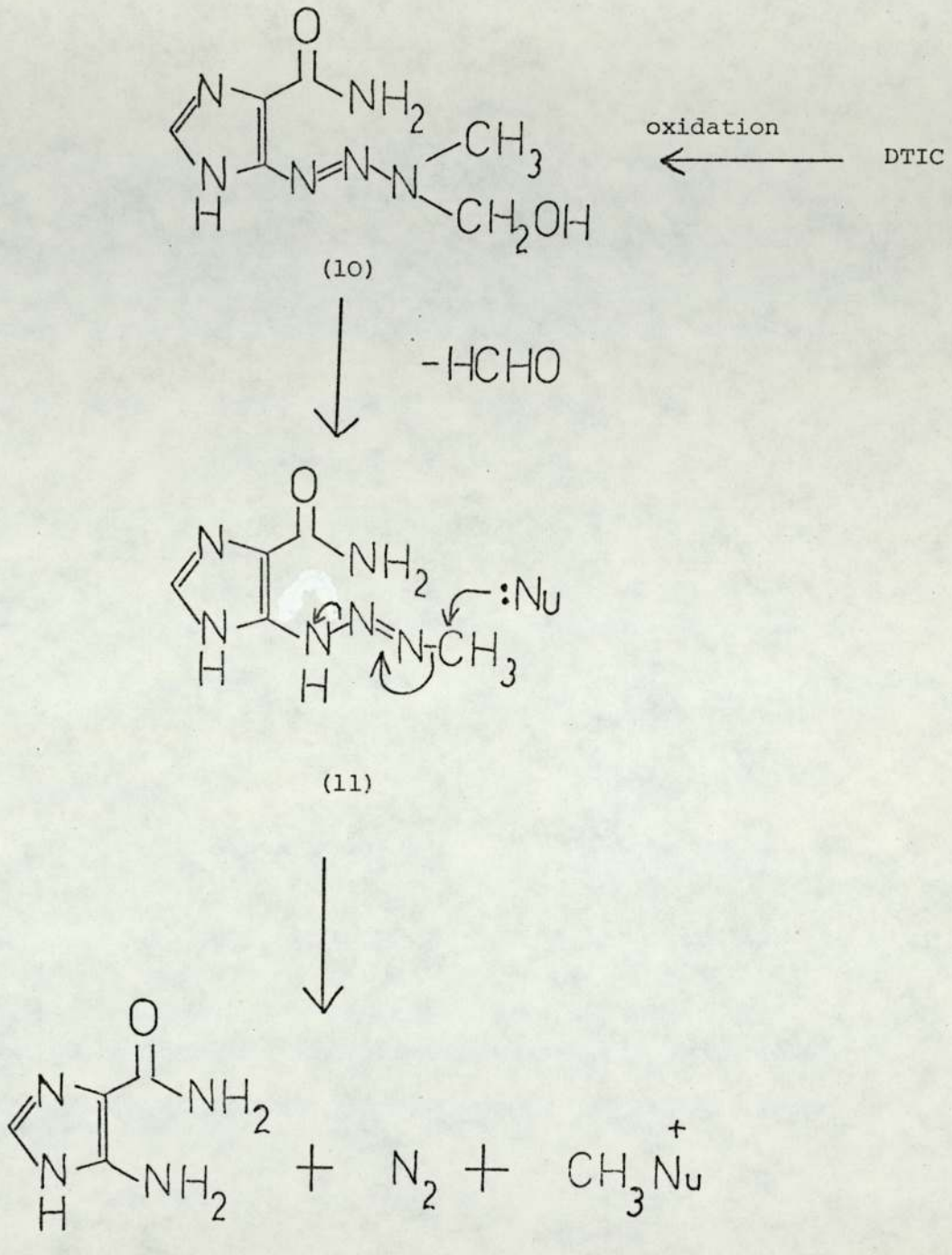
been tentativelyidentified in most cases as tyrosine, histidine and lysine.

The reversal of growth inhibition of B. subtilis and E. coli induced by diazo-ICA by thiols may be interpreted in a number of ways. Either diazo-ICA produces its biological effect as a consequence of a reaction with thiols in cellular molecules (eg. the coupling of diazo-ICA with cysteine residues); or alternatively, added thiols may quench the reactive electrophilic species thus removing it from the medium and preventing further reaction with significant cellular nucleophiles.

Whether the light-catalysed decomposition of DTIC has any counterpart in vivo, where there is, contrary to expectation, considerable light penetration or whether the molecule undergoes spontaneous heterolysis to diazo-ICA is a matter for conjecture. However. in attempts to associate tumour-inhibitory activity with chemical events,[19] it has been shown that the antitumour activity of triazenes is not related to the formation of diazonium ions but that the acute toxicity elicted by DTIC in man is due to diazo-ICA. Triazenes which do not undergo decomposition to diazonium ions under physiological conditions are nevertheless active against the TLX5 lymphoma.[19] Therefore it is considered that those triazenes which form a significant amount of diazonium ion may be unsuitable as antitumour agents since they may show an unfavourable Therapeutic Index.

(iv) In vivo effects of DTIC

DTIC is known to undergo a second transformation brought about by microsomal fractions containing the appropriate co-factors and in intact animals. This transformation involves oxidative N-demethylation to 5-(3-methyl-1-triazeno)-imidazole-4-carboxamide [MIC] (11). It is proposed that MIC undergoes spontaneous decomposition with loss of nitrogen and concurrent formation of AIC and methylation of nucleophiles. (Scheme 1.4)



(10)

(11)

(2)

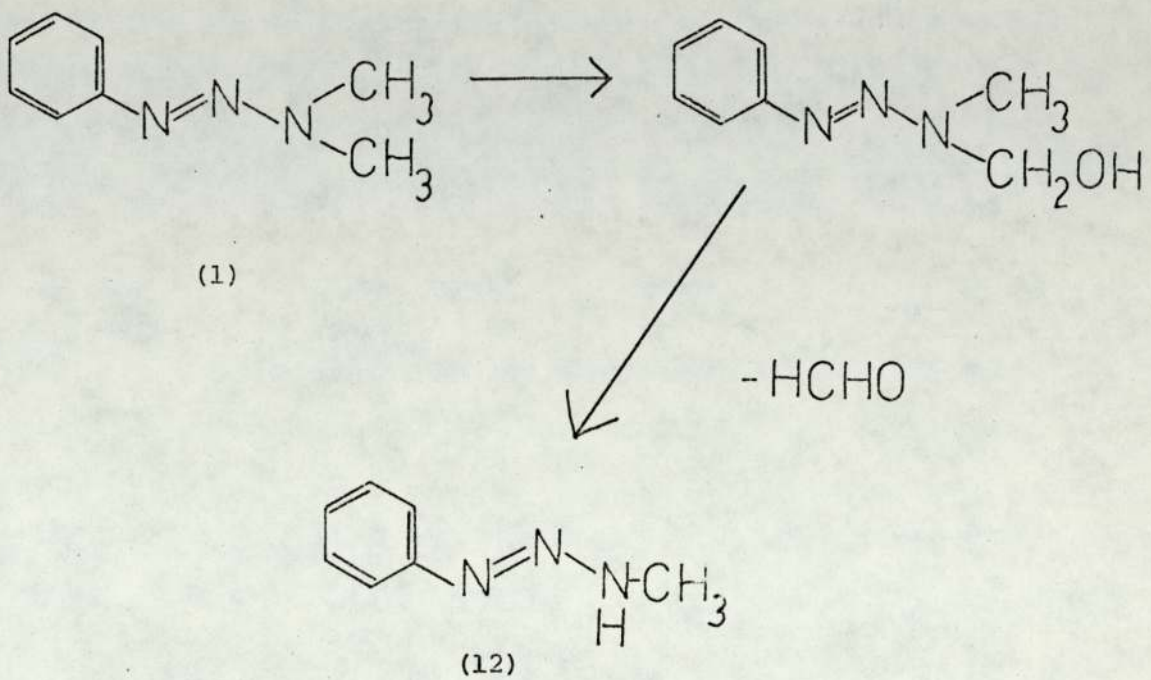
Scheme 1.4

Overwhelming evidence has been obtained for the operation of this pathway and is briefly summarised below.

Skibba, Beal, Ramirez and Bryan showed that DTIC was N-demethylated by rat liver microsomes to AIC and carbon dioxide[37]. The administration of DTIC (labelled in the methyl groups with ^{14}C) to rats and man was shown to result in the formation of AIC and the exhalation of $^{14}CO_2$.[37] ^{14}C-labelled 7-methylguanine was isolated from the urine of patients who received the labelled DTIC.[38] On the other hand, patients who were administered DTIC labelled in the imidazole 2-position with ^{14}C were found to excrete labelled AIC in the urine.[39] This clarified a previous observation of Householder and Loo,[40] who had reported that patients receiving DTIC excreted elevated amounts of AIC in the urine. They considered that this might be due either to interference by DTIC with AIC metabolism and consequent inhibition of purine synthesis, or, alternatively, to metabolism of DTIC to AIC as indicated above. The elevated levels of AIC in the urine of patients receiving DTIC therapy have been shown to be almost exclusively due to metabolism of DTIC.[39] In studies with ^{14}C-methyl-labelled DTIC, it has been shown that DTIC is also metabolised by human and animal tumour microsomes to ^{14}C-labelled formaldehyde.[41] Incubation of DTIC with tumour microsomes and nucleic acids resulted in the isolation of 7-methylguanine from the nucleic acid hydrolysates.[41] It has recently been shown by Hill[42] that DTIC is a substrate for microsomal enzymes of mouse liver and that the products of such metabolic oxidation of DTIC are AIC and formaldehyde.

Mechanism of carcinogenesis by aryl-dialkyltriazenes

After exhaustive studies on the mechanism of carcinogenesis by phenyl-triazenes it was reported[43] that aryl-dialkyltriazenes are dealkylated by microsomal fractions to form monoalkyltriazenes. (Scheme 1.5).



Scheme 1.5

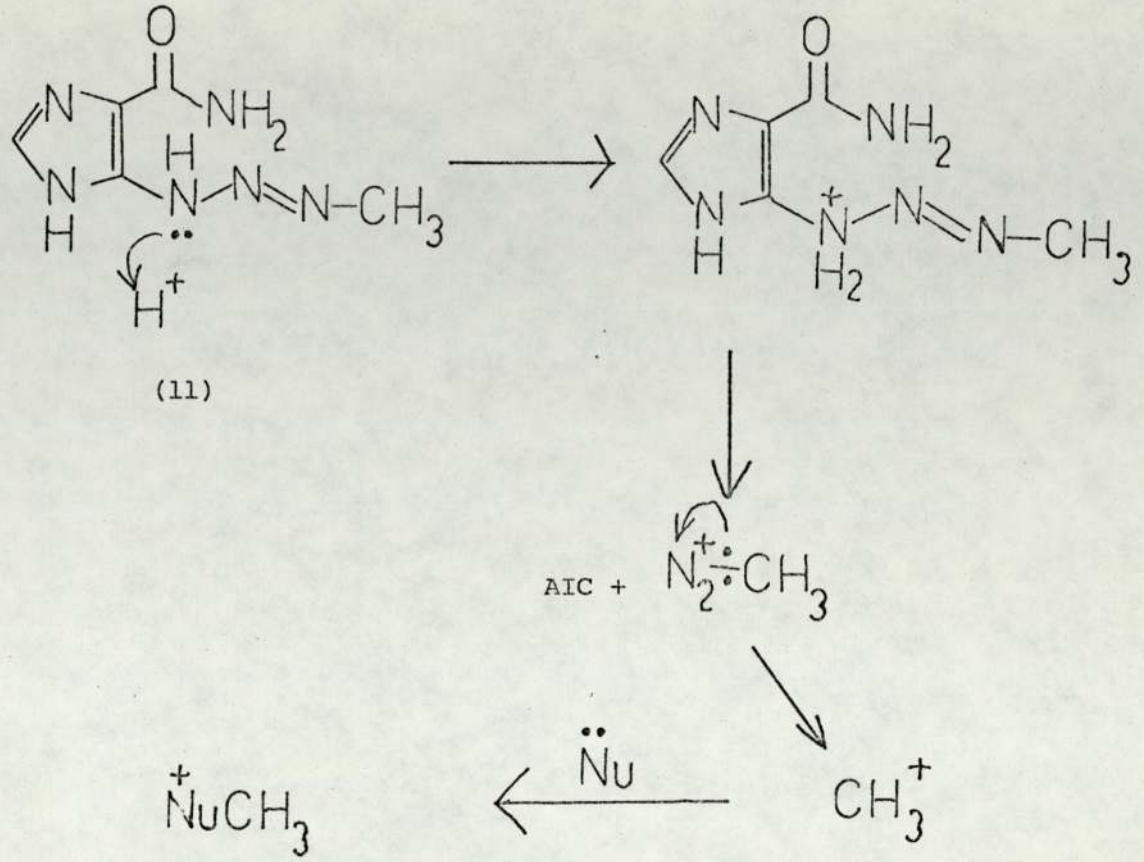
It was noted that phenyl-monomethyltriazene(12) is a potent proximate carcinogen, mainly producing tumours at the site of administration and it was proposed that carcinogenesis by phenyl-dimethyltriazenes is a consequence of metabolic N-demethylation to the corresponding monomethyltriazene which could methylate biopolymers. The methylating activity of monomethyltriazenes was first described by Dimroth[44] who found phenyl-monomethyltriazene a most efficient methylating agent, methylating phenols in high yields. It has been shown that injection of phenyl-dimethyltriazene into rats results in the formation of 7-methylguanine, isolated from RNA and DNA of the liver.[45] Finally Preussman and Von Hodenberg[46] showed that phenyl-monomethyltriazene will methylate N (7) of guanosine in vitro. It is appropriate to mention that MIC, the proposed active form of DTIC in tumour-inhibition, not only decomposes to AIC in solution, but is

tumour-inhibitory per se.[47] Indeed MIC has been shown to possess many of the properties of DTIC[48] and much of the toxicity to cells can be accounted for largely by MIC.

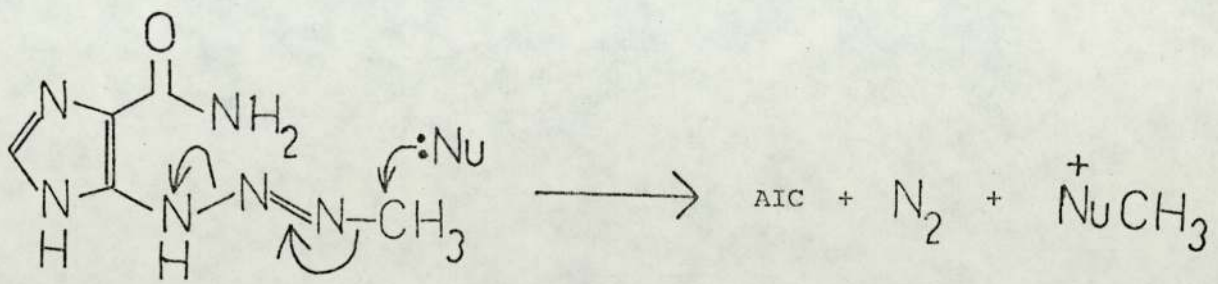
Mechanism of methylation by monomethyltriazenes

The nature of the methylating species derived from DTIC by metabolic transformation is the subject of some controversy. The intermediacy of diazomethane has been discounted by the observation that the methyl group of MIC is transferred intact to N(7) of guanine.[48a] One school of thought, however, favours the theory that MIC decomposes to form AIC and a "methyl carbonium ion" (ie. in kinetic terms, an S_N1 decomposition) which methylates biopolymers. Even the existence of such a reactive entity as a methyl carbenium ion is questionable. It therefore seems unlikely that such a promiscuous chemical species would exist in an aqueous medium long enough to react with any degree of specificity. Kinetic studies on the decomposition of 5-(3-t-butyl-1-triazeno)imidazole-4-carboxamide however, indicate that this triazene probably does decompose by an S_N1 process,[49] but in this case a stabilised t-butyl carbenium ion would be formed.

Methylation of nucleophiles by MIC and monomethyltriazenes may alternatively and perhaps more realistically, be considered in terms of an S_N2 reaction where MIC is acting as an "incipient" carbenium ion. (Scheme 1.6).



S_N1 decomposition



S_N2 decomposition

Scheme 1.6

Structure-activity relationships of tumour-inhibitory triazenes

Anticancer activity of aryl-dialkyltriazenes is associated with certain structural features. In a recent paper describing the structural requirements for an active metabolite of tumour-inhibitory triazenes, it was reported[19] that only those triazenes which could be metabolically N-dealkylated to monomethyltriazenes exhibited antitumour activity (in the TLX5 Lymphoma implanted into mice) (Table 1.1). It was also shown that there was no significant difference in antitumour activity in a series of triazenes containing varying substituents in the aromatic ring while maintaining the two methyl groups in the N(3) position (Table 1.2). The presence of electron-donating or electron-attracting groups in the aromatic ring greatly influences the half-life of hydrolysis of the triazenes to diazonium species. Therefore, since there was little difference in antitumour activity of these triazenes it was concluded that formation of diazonium ions plays little part in the antitumour action. It has also been shown that the diazonium ion derived from 5-(3,3-dimethyl-1-triazeno)-4-carbethoxy-2-phenylimidazole is extremely toxic to lymphoma cells *in vitro*, but it is not antitumour *in vivo* (mice) because it is also toxic to the host.[50] The parent triazene however is relatively non-toxic to the host and is highly active against the TlX5 tumour *in vivo*. Accordingly it is postulated that the antitumour activity of DTIC and related triazenes is a function of their capacity for transformation to monomethyltriazenes which may act as methylating agents, and that the acute toxicity may be due to formation of diazonium ions.[19]

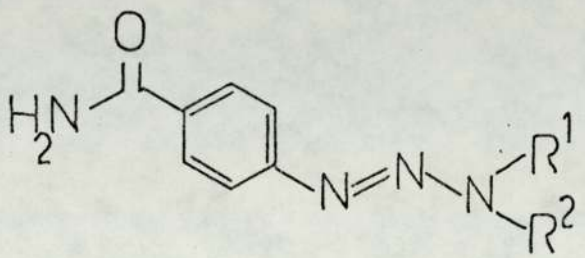
Significance of alkylating activity in tumour-inhibition and carcinogenesis.

These observations tend to place triazenes in the class of alkylating agents effective against neoplastic diseases. There are however, considerable uncertainties in this conclusion, amply illustrated by the following examples. The Rl and TLX5 Lymphomas do not respond to conventional alkylating agents (*eg.*Cyclophosphamide) but are extremely sensitive to dimethyltriazenes.[50]

Furthermore the plasma cell tumour, which is very sensitive to difunctional

Table 1:1 Antitumour activity of a series of 1-(p-carbamoylphenyl)-3,3-dialkyltriazenes against the TLX5 lymphoma.

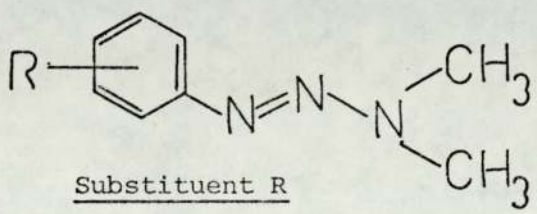
(adapted from Biochem. Pharmacol., 1975, 25,241)



R^1	R^2	Max % I.L.S.
CH_3	CH_3	55
C_2H_5	C_2H_5	inactive
$CH(CH_3)_2$	$CH(CH_3)_2$	inactive
CH_3	C_2H_5	46
CH_3	CH_2CH_2OH	42
CH_3	C_4H_9	78
CH_3	C_5H_{11}	102
CH_3	$CH(CH_3)_2$	52
CH_3	$CH_2\ C_6H_5$	86
CH_3	$C(CH_3)_3$	inactive
CH_3	OH	54
CH_3	H	43
C_2H_5	H	inactive

ILS = Increase in lifespan

Table 1.2 Anti-tumour activity of a series of 1-aryl-3,3-dimethyl-triazenes against the TLX5 Lymphoma (adapted from Biochem. Pharmacol.,1975,25, 241.)



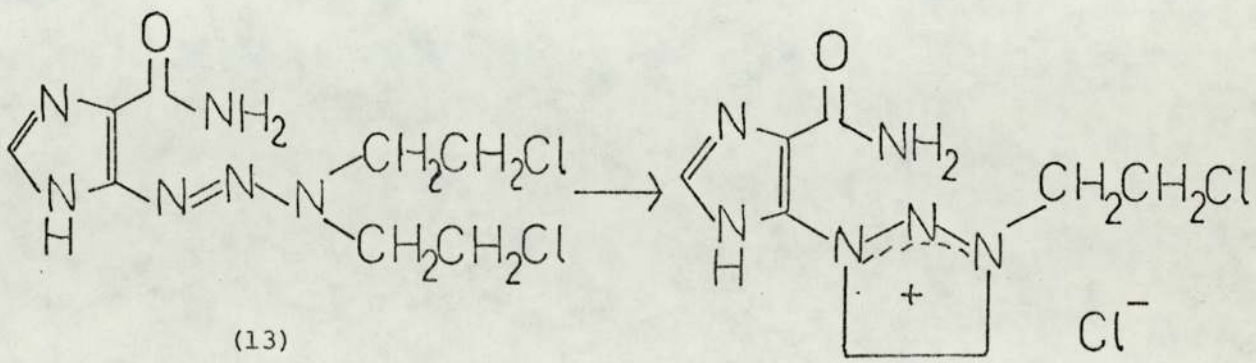
Substituent R	ILS
H	53
o-CO_2H	68
m-CO_2H	62
p-CO_2H	72
o-CO_2CH_3	53
m-CO_2CH_3	63
p-CO_2CH_3	58
p-$CO_2C_2H_5$	61
o-$CONH_2$	78
m-$CONH_2$	55
p-$CONH_2$	55
p-$CONHCH_2CO_2H$	46
p-OCH_3	41
p-NO_2	39
p-CF_3	61
p-SO_2CH_3	80

ILS = increase in lifespan. (%)

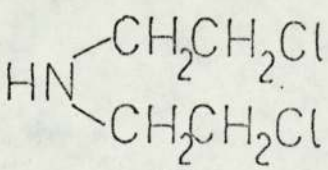
alkylating agents and insensitive to monofunctional alkylating agents responds to dimethyltriazenes (after metabolic N-demethylation). In addition to these observations it has been found that the most active triazene in the imidazole series, namely 5-[3,3-bis(2-chloroethyl)-1-triazeno]imidazole-4-carboxamide [BIC] (13) does not bear a methyl group.[51,52]

The reader will doubtless be aware at this stage of the difficulties inherent in interpreting the mode of action of DTIC. But DTIC presents a fairly clear picture compared to the dense haze surrounding BIC. It is interesting to contemplate some of the possibilities concerning the mode of action of BIC.

Firstly, it is considered that BIC is a carrier of the mustard (15) which it forms on acidic cleavage of the triazene linkage[53] and that the antitumour activity of BIC may be due in part to the release of this mustard. The other product of this cleavage is of course diazo-ICA. To add to the complexity of this situation evidence has been presented to the effect that BIC undergoes oxidative metabolism in a similar manner to DTIC.[42] In the case of BIC, such a transformation would result in the release of a chloroethylating species. BIC is also extremely unstable undergoing cyclisation in solution and even in the solid state to the isomeric triazolinium salt (14), but this species is inactive against L-1210.[51,52]



(13) (14)



(15)

Scheme 1.7

The methylation of nucleic acids by DTIC and dimethyltriazenes [eg.N(7) of guanine] is also of doubtful significance. Kruger[45] found that the carcinogen PDMT formed 7-methylguanine, isolated from DNA and RNA of rat liver, but tumours caused by administration of this carcinogen to rats have never been observed in the liver. Indeed the significance of alkylation of nucleic acid bases in tumour-inhibition and carcinogenesis is very much in question. Traditionally tumour-inhibitory alkylating agents were thought to owe their activity to a reaction with DNA[54] and attention has been focussed on such reactions as methylation of N(7) of guanine, O(6) of guanine, and to a lesser extent, on alkylation of N(1) or N(3) of adenine and N(3) of cytosine. There is evidence to suggest that carcinogenicity and mutagenicity (if not tumour-inhibitory activity) of certain alkylating agents correlates more closely with alkylation of phosphates and formation of phosphotriesters in DNA than it does with formation of 7-alkylguanine.[55] The carcinogenic cyclic nitrosamines (discussed in more detail later) also do not appear to alkylate nucleic acid bases, although there is evidence of some as yet unspecified interaction with RNA and DNA.[56]

Therefore at the risk of repetition, it is not yet possible to conclude which of the known chemical and biochemical events are related to the biological effects of triazenes and other cytotoxic compounds.

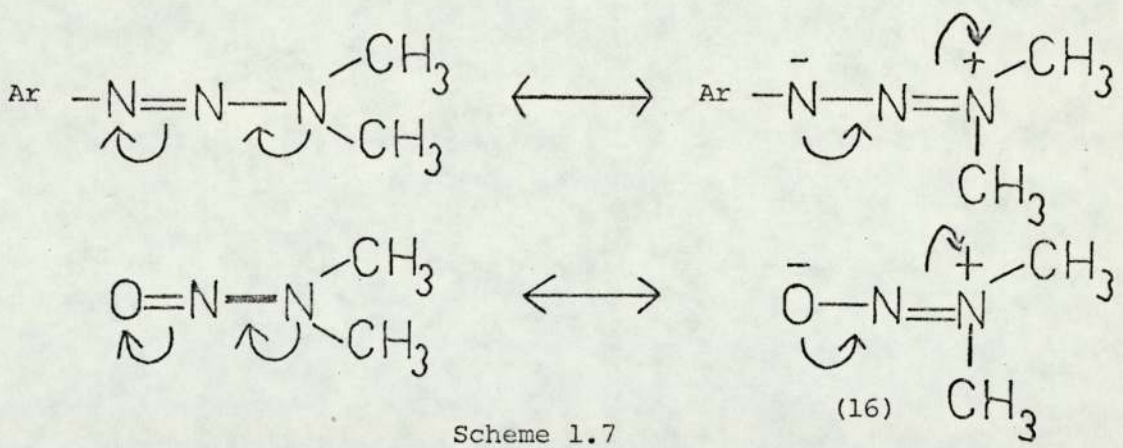
Immunogenic and antigenic effects of triazenes

To digress, an exciting and somewhat novel approach to the control of cancer was suggested by the observation of Clarke in 1955[2] that Sarcoma 180 failed to resume its usual rapid growth if pieces of the tumour treated with PDMT were implanted into mice. Strong antigenic changes in four Leukemia L-1210 lines have been demonstrated after treatment with DTIC over several transplant generations.[57] This change in antigenicity of the DTIC treated lines was accompanied by a decrease in oncogenic potential. Schmid and Hutchinson[58] have also shown that treatment of L-1210 with DTIC results in a decrease in oncogenic potential on transplantation, and noted the

ability of DTIC to induce antigenic cell change. The relationship between change in antigenicity and decrease in malignancy is not, however, understood. Following these studies, Campanile[59] showed that two lymphomas treated for sixteen transplant generations with DTIC in athymic mice became highly immunogenic. Mice which rejected the altered cell line were resistant to subsequent challenge with parenteral tumour cells. This suggests the possibility of obtaining immunogenic human neoplastic cells by drug treatment in athymic mice.

Carcinogenic N-nitrosamines

Aryl-dialkyltriazenes bear a close structural and electronic resemblance to the aliphatic N-nitrosamines whose carcinogenicity and toxicity are well documented.[60] (Scheme 1.7).



Scheme 1.7

Contribution from the 1,3-dipolar resonance structure (16) produces a planar structure with double-bond character between the two nitrogens resulting in hindered rotation about the N-N bond. Thus the n.m.r. spectrum of dimethylnitrosamine exhibits two aliphatic singlets; the trans-α-hydrogens resonate further downfield than the cis-α-hydrogens.[61] Restricted rotation about the N(2)-N(3) bond in aryl-dimethyltriazenes is likewise manifested in the splitting of the n.m.r. signal from the methyl protons.[62] Elucidation of the structure of the monohydrate hydrochloride salt of DTIC by X-ray crystallography showed that there is considerable double-bond character in the N(2)-N(3) bond as evidenced by the short bond distance of 1.305Å and by the planarity of bonding about N(3) indicating Sp^2 hybridisation.[63]

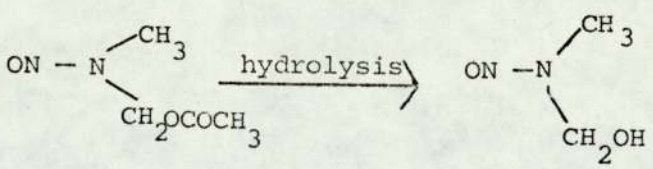
In view of these similarities between the N-nitrosamines and aryl-dialkyltriazenes, it is not surprising to note that the metabolism of dimethylnitrosamine (via oxidative N-demethylation to a methylating intermediate which can methylate guanine)[60 64,65] closely parallels the metabolic transformation of tumour-inhibitory and carcinogenic dimethyltriazenes. The carcinogenicity of dimethylnitrosamine is considered to be a direct result of its transformation to a methylating agent and subsequent reaction with cellular nucleophiles. Much less is known of the mechanism of carcinogenesis of the cyclic N-nitrosamines (eg. nitrosopiperidine, nitrosopyrrolidine and nitrosomorpholine), although it is suspected that these compounds are converted in vivo into alkylating agents. Lijinsky, Keefer, Loo and Ross,[56] however, failed to detect any alkylated bases derived from nucleic acids of livers of rats treated with cyclic nitrosamines. They did nevertheless concede that some type of chemical interaction between the nitrosamines and macromolecules may be involved in carcinogenesis since interaction with RNA and DNA was observed. In the light of the findings of Sun and Singer[55] on the correlation of alkylation of phosphate groups with carcinogenesis of certain alkylating carcinogens, these results need not be interpreted in such terms as to exclude an alkylating role in carcinogenesis for the cyclic nitrosamines.

According to a popular mechanism offered for the in vivo conversion of cyclic N-nitrosamines to alkylating agents, the key step is oxidative attack on the carbon alpha to the nitroso function. There are a number of theoretical ways in which the molecule could subsequently act in the capacity of a mono or difunctional alkylating agent. This aspect will be discussed in some detail in chapter 5.

Recent work by Lijinsky and Taylor[66,67] tends to confirm the importance of the α-carbon in metabolic activation. For example, in a study on the carcinogenicity of a series of methylated N-nitrosopiperidines[67] they found that there was no significant carcinogenic activity in 2,6-dimethyl-

-N-nitrosopiperidine and that carcinogenic activity was virtually absent in 2,2,6,6-tetramethyl-N-nitrosopiperidine. In the former case, oxidative attack at the α -carbon would be expected to be sterically hindered by the presence of the methyl groups, whereas in the latter case, in the absence of α-hydrogens, oxidative attack and subsequent activation of the molecule would be impossible. This view is further supported by the finding that a methyl group in the 3-position had only a modest dys-carcinogenic effect and that a methyl group in the 4-position did not reduce carcinogenic activity at all.

Powerful corroborative evidence for this theory has been supplied by the finding that the nitrosamine N-methyl-N-(2-acetoxymethyl)nitrosamine(17) does not require metabolic activation for mutagenic activity.[68] This compound would be expected to undergo facile non-enzymic hydrolysis to N-methyl-N-(2-hydroxymethyl)nitrosamine(18) (Scheme1.8) which is a suspected active metabolite of dimethylnitrosamine.



(17) (18)

Scheme 1.8

Compelling evidence was presented for analogous modes of action of dimethylnitrosamine and its α-acetoxyester at the molecular level in this study.[68] It would therefore appear to be highly plausible that N-methyl-N-(2-hydroxymethyl)nitrosamine could be a proximate metabolite of dimethylnitrosamine in carcinogenesis as well as in mutagenesis.[68] Similar studies on diethylnitrosamine and its α-acetoxyester[69] support the hypothesis that α-carbon hydroxylation may be the crucial first step in the metabolic activation of diethylnitrosamine and might therefore be of general applicability in the activation of all dialkylnitrosamines.

In contrast to these results it has been reported[70] that oxidation of N-nitrosopiperidine both in the Udenfriend system and by rat liver microsomes occurs predominantly in the 4-position, giving respectively N-nitrosopiperidin-4-one and N-nitrosopiperidin-4-ol. The possibility of concurrent oxidation at the α-carbon was not however excluded. The significance and consequences of oxidative attack on carbon atoms other than the α-carbon in nitrosamines will be discussed in chapter 5.

Triazenoquinazolines as tumour-inhibitory agents

The discussion has so far dealt with the properties of DTIC, related aryldialkyltriazenes and carcinogenic nitrosamines. It will be observed that the prototype of the triazenoquinazoline series, 4-amino-2[2-(piperidin-1-ylazo)phenyl]quinazoline[NSC163423] (6a), active in cell culture against H-Ep2 is quite different in structure to DTIC and PDMT particularly in the important area of the alkyl substituent. While it is not difficult to envisage this compound acting through a diazonium mechanism, it is not obviously apparent that this compound could fulfil the structural requirements of tumour-inhibitory triazenes.[19] Metabolism of this compound to a monomethyltriazene seems unlikely. Preliminary work with the triazenoquinazolines[8] indicated that they are considerably more stable than DTIC and in particular are not prone to rapid photodecomposition; furthermore, antitumour activity was achieved at substantially lower doses than was observed for DTIC in the same test system. This is of paramount importance since the toxicity of triazenes has been shown to be dose related,[71] and in the case of DTIC the acute toxicity, due perhaps to formation of diazo-ICA, is dose limiting. Therefore for those reasons and because of the intriguing chemical analogies and anomalies, the triazenoquinazolines presented themselves as a promising new group of tumour-inhibitory agents worthy of further study. The chemistry of the cyclic nitrosamines is of obvious relevance to the chemistry of the triazenoquinazolines, both classes

of compound bearing a heterocyclic aliphatic function linked in the one case to a nitroso group and in the other to an arylazo group. The similarity in electronic distribution of these two systems has been noted (Scheme 1.7). A significant part of the practical work for this thesis concerned efforts to detect an alkylating capacity for the triazenoquinazolines and to establish a parallel with the carcinogen nitrosopiperidine.

Chapter 2

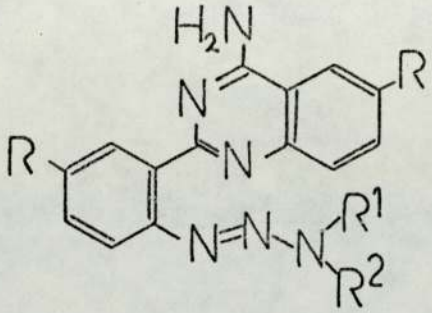
Synthesis of novel triazenoquinazolines

Introduction

The possibility that the tumour-inhibitory activity of the triazenoquinazolines may be a consequence of decomposition or conversion to any one of a variety of products makes a study of their mechanism of action important. Structure-activity studies are frequently useful in this context.

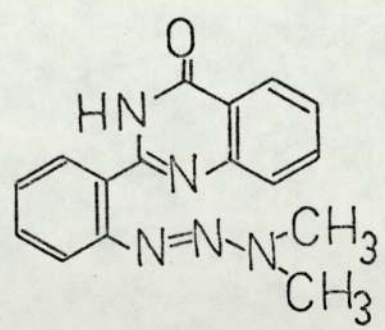
Structural variations on aryl-dialkyltriazenes may be considered to fall into two categories; those which alter the aryl function and those which modify the alkyl groups. Accordingly, further analogues of the triazene (6a) were prepared, in which the 2-,3- or 4-positions of the piperidine ring were substituted by a methyl group (6f-h). If the tumour-inhibitory activity of the triazenoquinazolines is dependent on such oxidative metabolism as was proposed for the cyclic nitrosamines, then the effects of these substitutions could conceivably be reflected in altered activity of the compounds. The dimethyl analogue (6i) was prepared for antitumour evaluation because of the established significance of the presence of methyl groups in related classes of tumour-inhibitory triazenes. In the course of this work, a facile method for hydrolysing 4-aminoquinazolines to the corresponding quinazolin-4(3H)-ones without degrading the triazene linkage was discovered. Thus the quinazolinone (19a) was synthesised and submitted for testing. Regrettably, biological results for many of these compounds are incomplete at the time of writing.

Variation in the aryl function was achieved by preparing the brominated triazenoquinazolines (20 a-f). The presence of hydrophobic bromine atoms in the molecule might be expected to affect lipid solubility, intracellular distribution and transport across membranes. Chemically, such substitution may influence the reactivity of the triazene linkage through participation of the non-bonding electrons of the bromine atom in conjugation (Scheme2.1). The bromine atom would similarly be expected to affect the stability and reactivity of the diazonium ion[72] formed by cleavage of the N-N linkage and might therefore be expected to affect the biological activity of the compounds, should this derive from such a diazonium species.

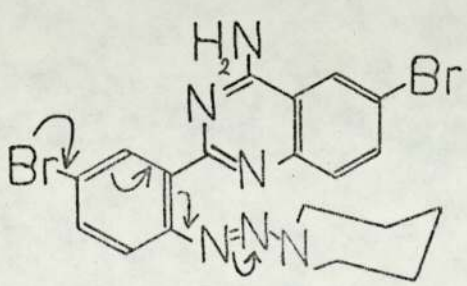


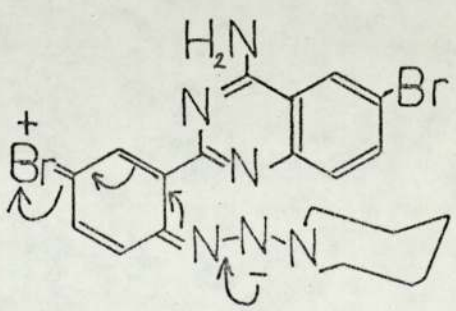
Compound	R	R^1	R^2
6 a	H	$-(CH_2)_5-$	
b	H	$-(CH_2)_4-$	
c	H	$-(CH_2)_2.O.(CH_2)_2-$	
d	H	n-Pr	n-Pr
e	H	Et	Et
f	H	$-CH(CH_3).[CH_2]_4-$	
g	H	$-CH_2-CH(CH_3)-[CH_2]_3-$	
h	H	$-[CH_2]_2-CH(CH_3).[CH_2]_2-$	
i	H	CH_3	CH_3

Compound	R	R^1	R^2
20 a	Br	$-(CH_2)_5-$	
b	Br	$-(CH_2)_4-$	
c	Br	$-[CH_2]_2.O.[CH_2]_2-$	
d	Br	$-CH(CH_3).[CH_2]_4-$	
e	Br	$-CH_2.CH(CH_3).[CH_2]_3-$	
f	Br	$-[CH_2]_2.CH(CH_3).[CH_2]_2$	



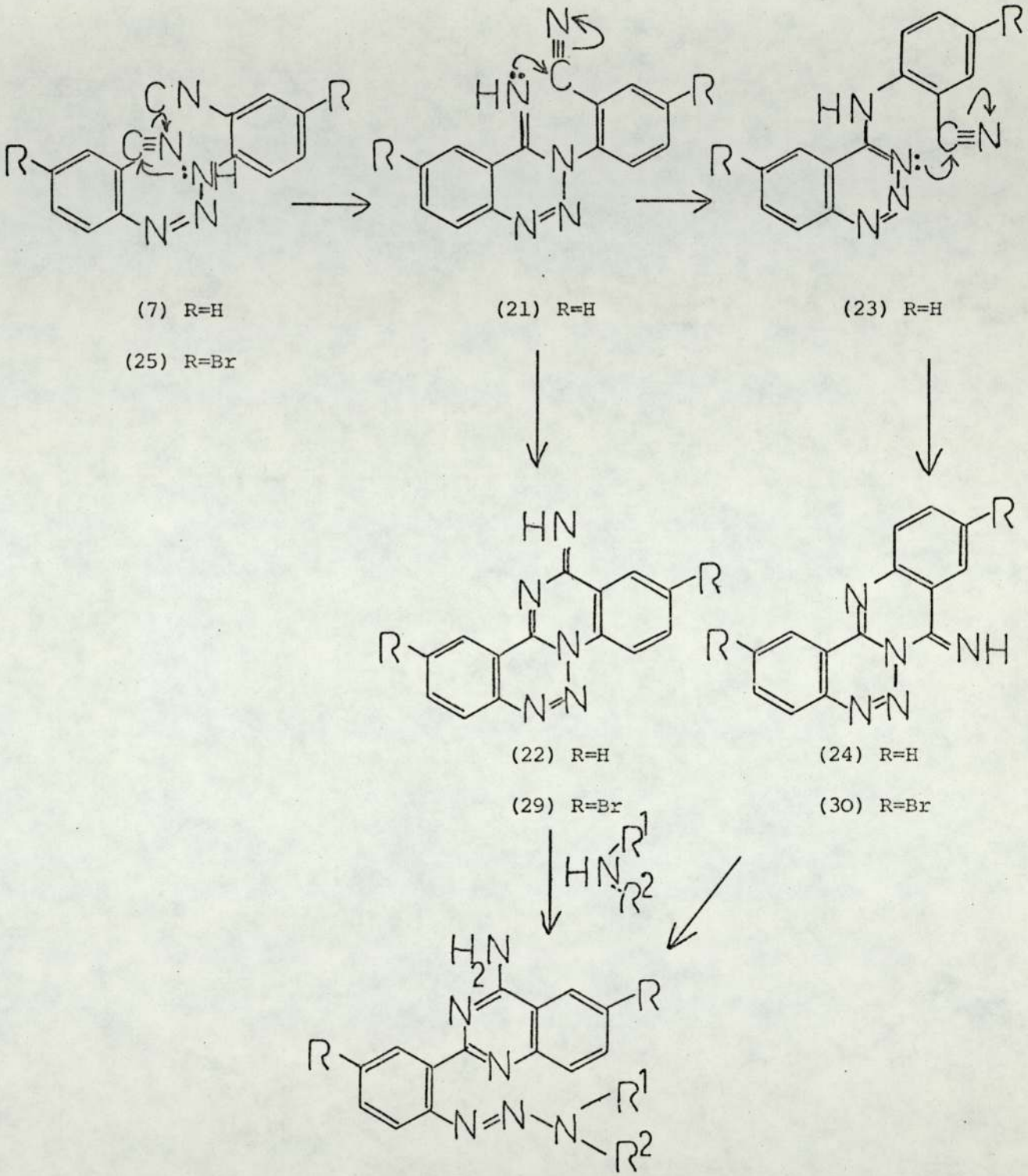
(19a)





Synthesis

The triazenoquinazolines may be conveniently prepared by either of two routes, both starting from anthranilonitriles. 1,3-Di-o-cyanophenyltriazene (7) (Scheme 2.2) is prepared by half diazotisation of o-aminobenzonitrile.[73] In the appropriate boiling secondary amines, this triazene is smoothly converted to the triazenoquinazolines (6a-e) respectively.[8] This conversion of the triazene(7) in boiling secondary amines involves formation of the 4-iminotriazine(21) by initial amine-nitrile addition. The 4-iminotriazine (21) may cyclise directly to the tetracyclic triazine (22) or, alternatively, may undergo Dimroth rearrangement to the 4-o-cyanoanilino-triazine (23)which subsequently cyclises to the isomeric tetracyclic triazine(24). In either case these tetracyclic triazines (which are not isolated), undergo ring-opening by the base to afford the required compounds. In the present work this synthesis was extended to the derivatives (6f-i). Similarly, the brominated triazene(25), prepared by half-diazotisation of 5-bromoanthranilonitrile, was converted in boiling secondary amines to the triazenoquinazolines (20a-f). The dibromotriazene(25) mimicked its unbrominated analogue (7) in all other chemical aspects examined[74] (Scheme 2.3). In boiling 70% ethanol the triazene (25) was smoothly decomposed to 4-amino-6-bromo-2-m-bromophenylquinazoline (26); in boiling ethanol containing hydrazine and Raney nickel (HRN), it gave the diamine (27) and in boiling ethanol containing 2-naphthol the azo-dye(28) was rapidly deposited. All these transformations probably proceed through the tetracyclic triazines (29) or (30) and in all likelihood could be more simply effected by employing these triazines as starting material.



(7) R=H

(25) R=Br

(21) R=H

(23) R=H

(22) R=H

(29) R=Br

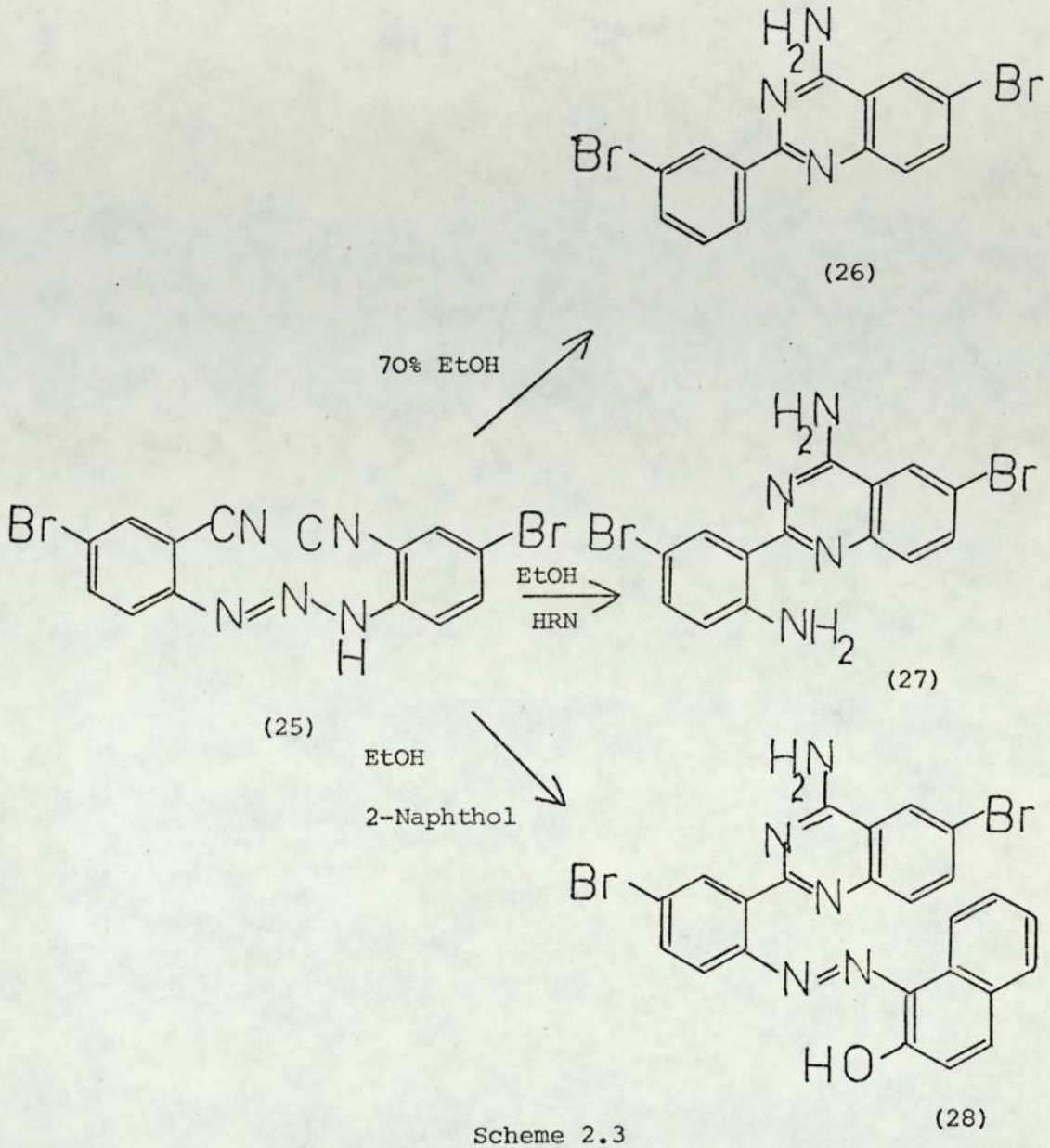
(24) R=H

(30) R=Br

(6) R=H

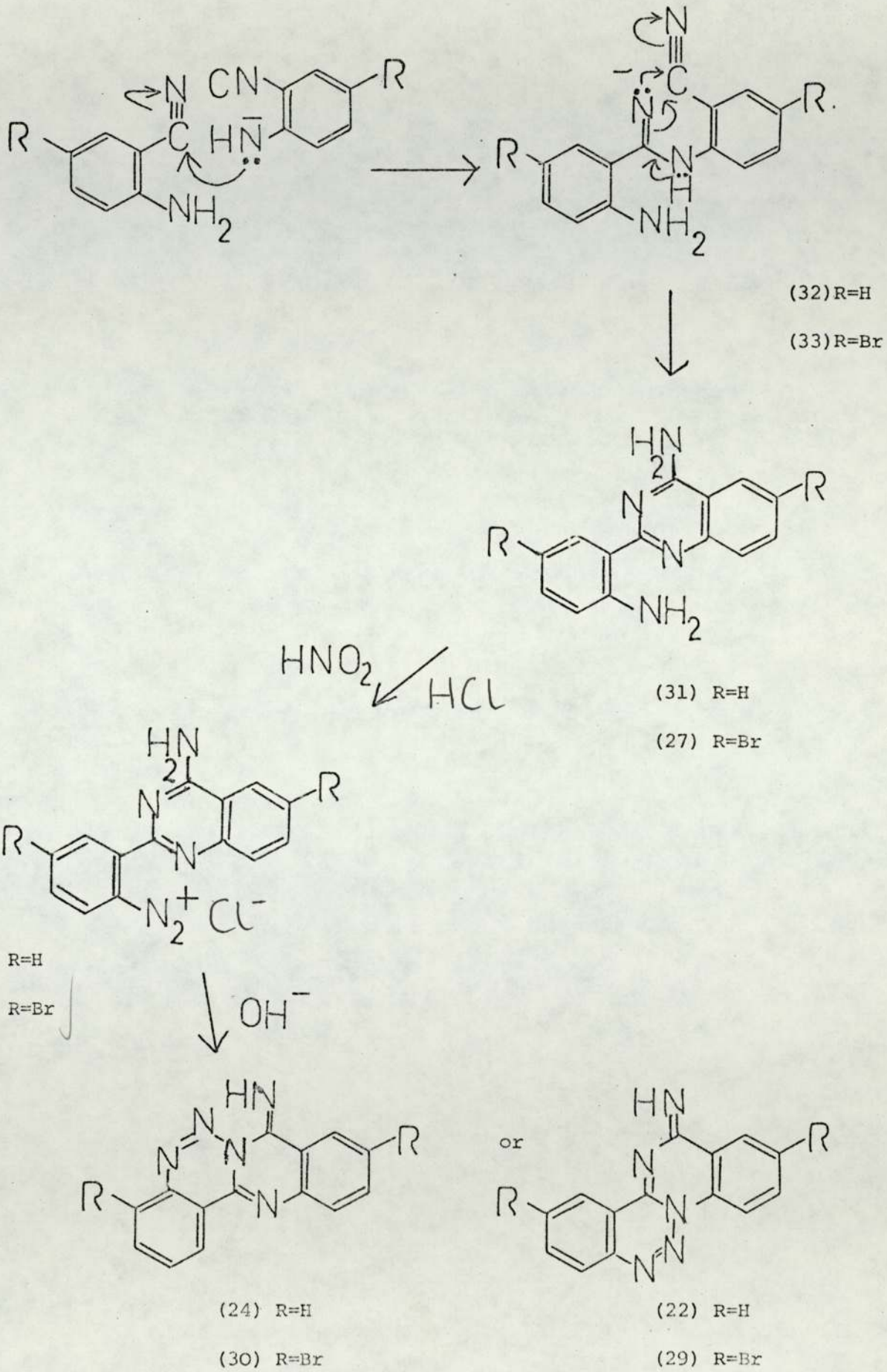
(20) R=Br

Scheme 2.2



Scheme 2.3

The tetracyclic triazines (22) and (29) or (24) and (30) may alternatively be obtained by diazotisation of the corresponding diamines (31)[75] and (27). (Scheme 2.4) This is the basis for the second synthetic approach to the triazenoquinazolines. A novel synthesis of the diamines (31) and (27) was suggested by a report[76] that reaction of nitriles with the anions of amines in dimethylsulphoxide afforded amidines in high yields. In the present case, treatment of anthranilonitrile or its 5-bromo analogue with sodium hydride in dimethylsulphoxide led to the isolation of the diamines (31) and (27) respectively, in near quantitative yields. The intermediate amidines (32) and (33) are not isolable in these cases, but undergo further amine-nitrile addition as indicated.

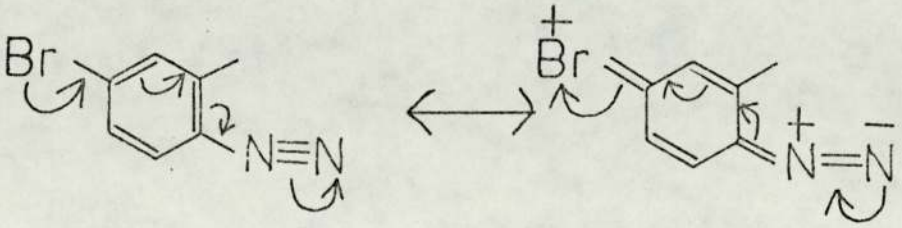

(32) R=H
(33) R=Br
HNO2
HCl
(31) R=H
(27) R=Br
(34) R=H
(35) R=Br
OH⁻
or
(24) R=H
(30) R=Br
(22) R=H
(29) R=Br

Scheme 2.4

Diazotisation of the diamines (31) and (27) in 2N and 6N - hydrochloric acid respectively, followed by basification, resulted in the formation of the tetracyclic triazines (22) and (29) or (24) and (30). Cyclisation of the diazonium ion (34) had previously been assumed to be on the quinazoline N(3) atom on the basis of a similar preference for cyclisation at the quinazoline N(3) atom in the reactions of other 2-o-aminophenylquinazolines with carbon inserting reagents.[77] The isomeric N(1) cyclised products (21) and (29) cannot however be excluded, since there seems no obvious way of distinguishing between these isomers. Furthermore, the quinazoline (6a) undergoes methylation at the N(1) atom (see chapter 4) - therefore the question of the structure of these tetracyclic triazines must remain open.

The triazenes (6a-e) have previously been prepared by reaction of the tetracyclic triazine (22) or (24) with the appropriate amines.[8] This synthesis was extended to selected members of the series (20), yields being almost quantitative.

Surprisingly, the diazonium ion (35) obtained by diazotisation of the diamine (27) proved remarkably stable and could be recrystallised unchanged from 6N-hydrochloric acid. Presumably this stability derives from contribution of the non-bonding bromine electrons (+M effect) in resonance interaction. (Scheme 2.5). This increases the double-bonded character of the C-N linkage and confers stability on the diazonium group.

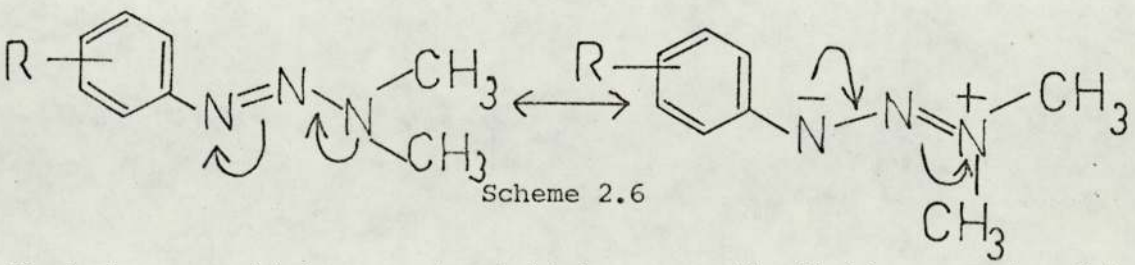


Scheme 2.5

Physical properties of triazenoquinazolines

The similarity in properties of the triazenes (6f-i) and (20a-f) to those of the triazene (6a) fully supports their assigned structures. Thus the electronic absorption spectra of the triazenes (6f-i) and (20a-f) exhibit the characteristic long wavelength absorption, showing considerable fine structure, present in the spectrum of the triazene (6a) (Table 2.1). Conversion of the triazene (6i) to (19a) by hydrolysis in 50% alcoholic potassium hydroxide was confirmed by the presence of strong sharp absorption at 1665cm^{-1} in the i.r. spectrum of the product, attributed to the carbonyl group. The remarkable stability of the triazene linkage under these conditions is noteworthy.

The 100 MHz ^{1}H n.m.r. spectrum of the dimethyltriazene (6i) in $CDCl_3$ shows a singlet for the methyl groups (T=6.95) whereas the signal from the methyl protons in the corresponding 4-quinazolinone (19a)is split into a doublet (at T=6.30 and T=6.50). Splitting of the signal from the methyl protons in 1-aryl-3,3-dimethyltriazenes has been reported[62] and was found to be temperature dependent. This splitting phenomenon has been interpreted in terms of restricted rotation about the N(2)-N(3) bond, arising from the partial double-bonded character of this linkage. (Scheme 2.6). Similar features are apparent in the n.m.r. spectrum of dimethylnitrosamine[61].



Scheme 2.6

The hydrogens which resonate at higher magnetic field are assigned to the cis-N-methyl group.[62] In the present case [triazene (19a)] it is conceivable that the effect of an oxo-group in the quinazoline 4-position is to increase the participation of the dipolar resonance hybrid so that the phenomenon

Electronic absorption spectra (λ_{max}/nm; log ε in parenthesis) of triazenoquinazolines (in 95% ethanol).

Compound								
	6a†	233	(4.45)	287 (4.27)	315*	(4.21)	329*	(4.12)
	6f	233	(4.51)	287 (4.31)	315*	(4.26)	330*	(4.23)
	6g	233	(4.48)	287 (4.28)	315*	(4.24)		
	6h	233	(4.49)	287 (4.29)	315*	(4.25)	330*	(4.16)
	6i	233	(4.49)	288 (4.29)	315*	(4.24)	330*	(4.11)
	20a	239*	(4.48)	295 (4.36)	325*	(4.28)		
	20b	239*	(4.46)	296 (4.32)	325*	(4.25)		
	20c	243*	(4.39)	297 (4.30)	325*	(4.23)	342*	(4.06)
	20d	238*	(4.53)	295 (4.40)	325*	(4.33)		
	20e	240*	(4.49)	295 (4.39)	323*	(4.09)		
	20f	240*	(4.42)	296 (4.30)	325*	(4.24)	340*	(4.12)

† with water of crystalisation.

* shoulder /or inflection.

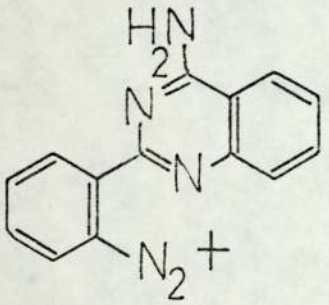
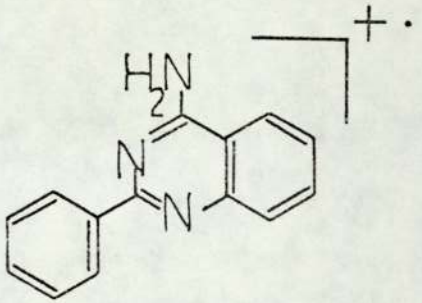
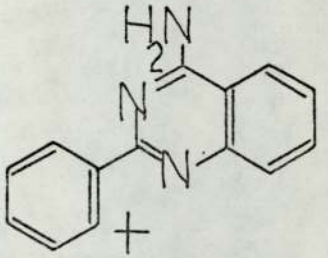
Table 2.1.

of splitting is observed at room temperature. Other long range effects may well operate and the quinazolinone (19a) would be a suitable candidate for structure elucidation by X-ray crystallography. In addition to the restricted rotation phenomenon it would be interesting to know the spatial dispositions of the two bulky substituents in the ortho disubstituted benzene (19a) and the trans or cis configuration of the triazene linkage. In 1,3-diaryltriazenes this is certainly trans.[78]

The assignment of structure to the triazenes (6f-h) is further supported by their mass spectra, all of which show the expected features in common with the spectrum of (6a) (Table 2.2). The molecular ions are not observed, but the spectra all show abundant ions at m/e 221 and m/e 220. The radical ion at m/e 221 is formed by loss of the triazeno side-chain accompanied by H-rearrangement. The ion at m/e 220 arises either from the radical ion by H-atom loss, or alternatively by fission of the entire triazeno fragment. The most abundant peaks in the spectra of the triazenes (6f-h) derive from heteroalicyclic fragments (m/e 99 and 98). Fragmentation of the heterocyclic radical from the intact molecule yields an unstable ion at m/e 248 which may be an acyclic diazonium ion (34) (Scheme 2.7) or a cyclic species (36): this loses nitrogen to form the arenium ion (37) at m/e 220.

The dimethyltriazene (6i) shows a very small molecular ion (<1%) and significant ions at m/e 248 (55%), 221 (100%) and 220 (86%).

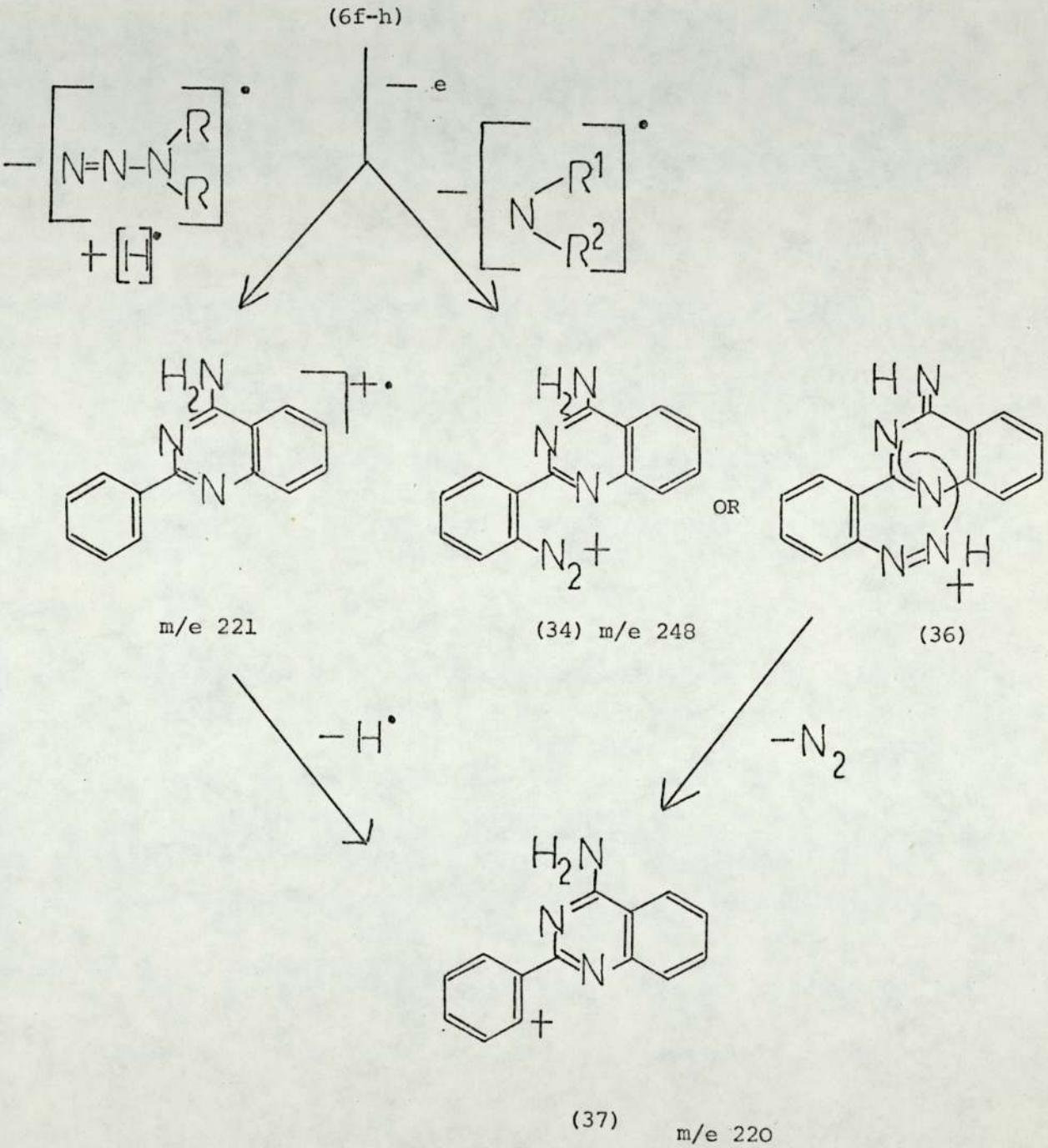
The corresponding dimethyltriazenyl-quinazolinone (19a) also shows these features but in this case as minor pathways. The dominating cleavage is loss of neutral dimethylamine from the molecular ion to form a radical ion at m/e 248 (13%) which then expels carbon monoxide to form the radical ion at m/e 220 (100%) (Scheme 2.8). These ions are possibly cyclic species (38) and (39) respectively since subsequent fragmentations are identical to those of the molecular ion of benzimidazo[1,2-c]-1,2,3-benzotriazine (39)[79].

Compound	Molecular ion	m/e 248	m/e 221	m/e 220
6a	absent	5	100	46
6f	absent	5	100	57
6g	absent	5	100	18
6h	absent	1	100	23
6i	<1	55	100	86
PDMT	11.3	m/e 105 35.8	m/e 78 13.2	m/e 77 100

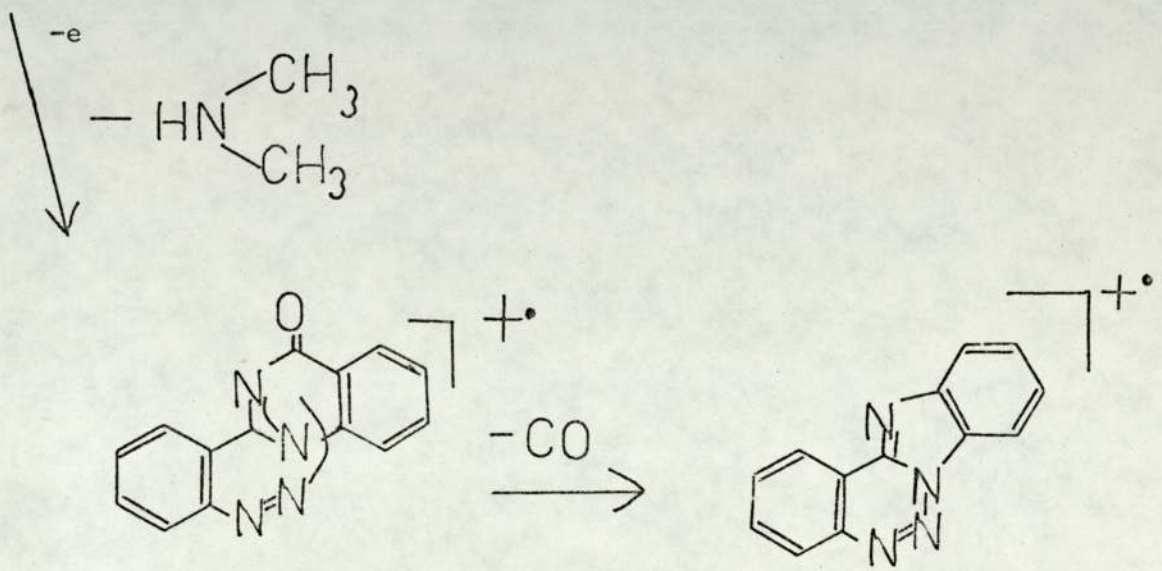
Table 2.2

Relative abundances (% of most abundant peaks) from mass spectra of triazenes recorded on an A.E.I. MS 9 spectrometer operating at 70eV.



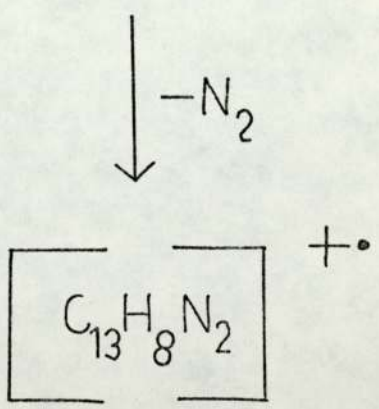
Scheme 2.7

(19a)



(38) m/e 248

(39) m/e 220



m/e 192

Scheme 2.8

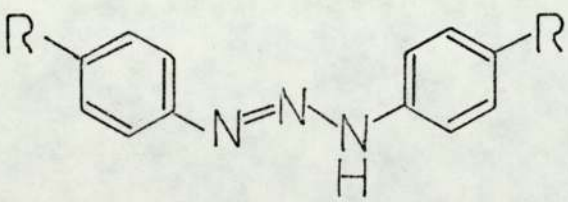
Solvate formation appears to be a general phenomenon in quinazolines[80] and was also observed in the triazenoquinazolines (6a), (6b)[8] and (20f) which crystallised as hydrates and in (6c), (a benzene solvate). Solvation in the quinazoline cation, unsubstituted in the 4-position is considered to be due to covalent hydration across the 3,4-double bond.[81,82] The presence of an amino group in the 4-position in the triazenes (6a) (6b) and (20f) excludes the possibility of solvation occurring by such covalent hydration. Hydration through hydrogen bonding also seems unlikely in triazenes (6a) and (6b) because of the presence of sharp absorption at 3605 cm^{-1} and 3615 cm^{-1} in the solid phase i.r. spectra of these compounds, usually attributed to an unassociated O-H stretching vibration. It is possible that the water molecules are sandwiched between the two bulky ortho substituents where intermolecular hydrogen bonding would be sterically prohibited.

Chapter 3

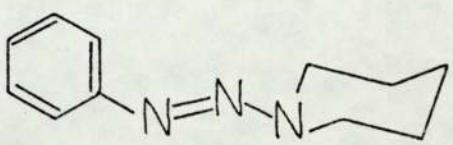
Properties of 4-amino-2-[2-(piperidin-1-ylazo)phenyl]quinazoline

Reactions with nucleophiles

Triazenes have been described as "masked" diazonium ions[27] and are precursors of electrophilic diazonium species. This character is, however, normally revealed only under acidic conditions. In view of the possibility that some electrophilic species may be responsible for the antitumour activity of triazenes, it seemed pertinent to examine the reactivity and stability of the intact triazene linkage in systems containing nucleophiles. The susceptibility of the triazene linkage to nucleophilic attack was examined in the reactions of the di-aryltriazenes (40) and the heteroalicyclic analogue (41) in amines. The triazenes (40) were recovered unchanged from prolonged boiling in piperidine or morpholine. Similarly no direct cleavage of the NNN linkage was observed in the reactions of the triazene (41) with the primary aromatic amines, p-nitroaniline or p-toluidine in ethanol.

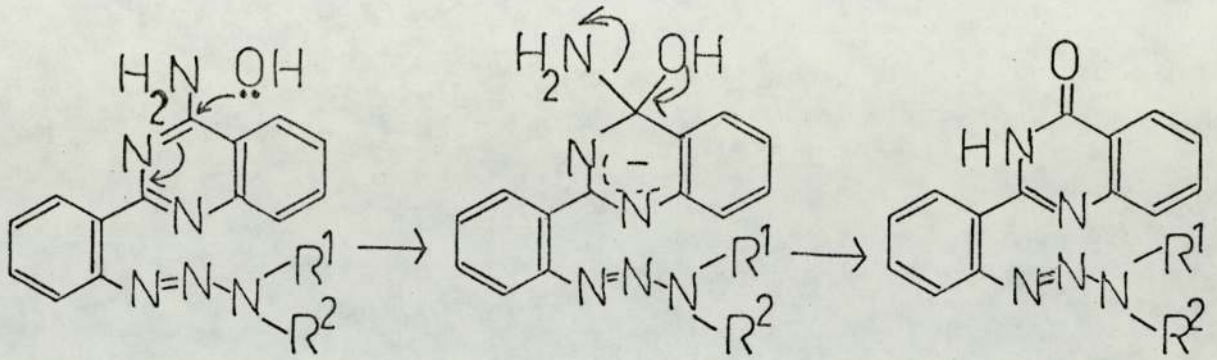


(40) R=CH_3, Cl, NO_2



(41)

The triazene linkage in (6a,b,i) proved remarkably stable in boiling 50% alcoholic potassium hydroxide, being quantitatively converted to the corresponding triazenoquinazolin-4(3H)-ones (19a-c) (Scheme 3.1) with retainment of the intact triazene linkage.



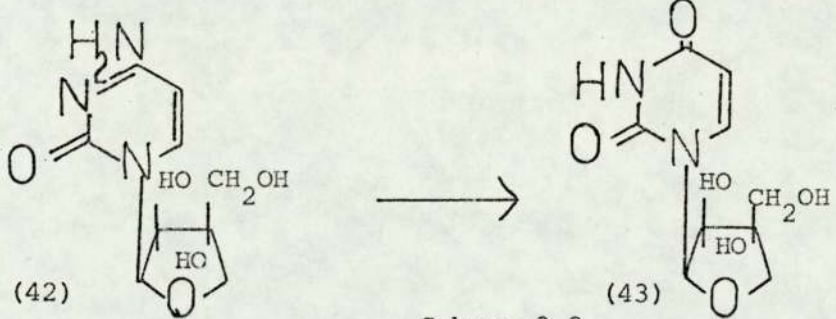
(6) (19)

	R^1	R^2
a	$-(CH_2)_5-$	
b	$-(CH_2)_4-$	
i	CH_3	CH_3

	R^1	R^2
a	CH_3	CH_3
b	$-(CH_2)-_4$	
c	$-(CH_2)_5$	

Scheme 3.1

An interesting although unrelated analogy to this reaction occurs in the intramolecular hydrolysis of the active anti-leukemic agent arabinosylcytosine (42) to arabinosyluracil (43) which is inactive[84] (Scheme 3.2).



(42) (43)

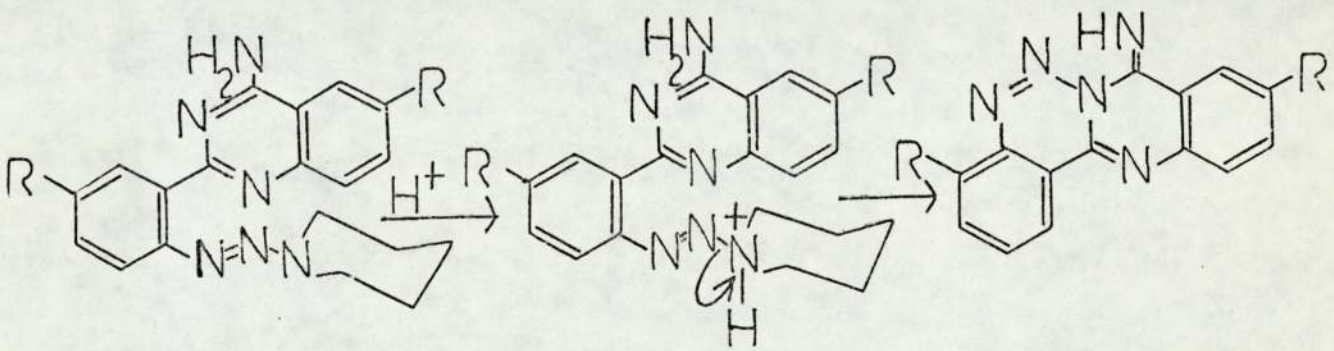
Scheme 3.2

No reaction was observed between cysteine and the triazene (6a) in aqueous dimethylformamide or in 1N-potassium hydroxide solution; nor was any coupling observed between the diazonium ion (34) derived from the triazene (6a) and cysteine in aqueous sodium hydroxide.

Acidic decompositions

The reactions of the triazene (6a) and its brominated analogue (20a) in acids clearly illustrate the latent diazonium character of these compounds. In all cases, reaction proceeds with N-N bond cleavage to afford the respective diazonium ions. Depending on the reaction conditions, a number of products may be derived from these species.

Treatment of the triazenes (6a) and (20a) with 6 N-hydrochloric acid at room temperature or acetic acid at 80°, followed by basification with sodium hydroxide led to the formation of the tetracyclic triazines (24) and (30) (Scheme 3.3) or the respective N(1) cyclised products. These conversions are clearly comparable to the photolytic decomposition of DTIC to diazo-ICA which cyclises to 2-azahypoxanthine. The tetracyclic triazine (24) or (22) has been shown to be susceptible to degradation in protic solvents [74] or in systems containing nucleophiles[8] and therefore should the acid promoted cyclisation of the triazene (6a) to the tetracycle (24) or (22) have any counterpart *in vivo* it seems unlikely that this triazine would be stable in such an environment.



(6a) R=H

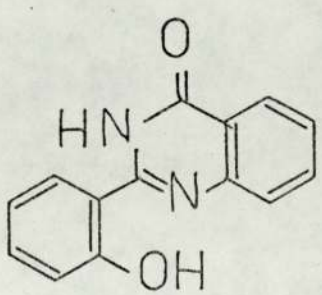
(20a) R=Br

(24) R=H

(30) R=Br

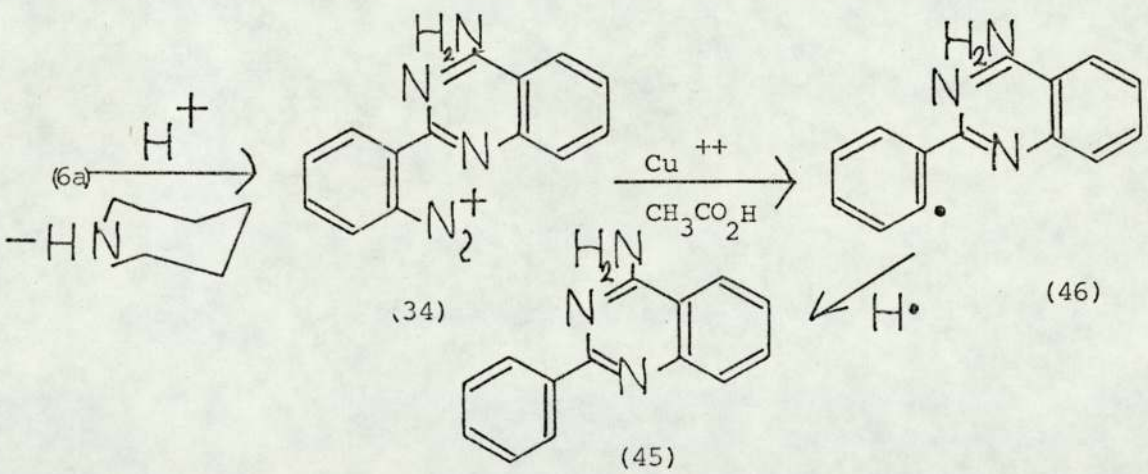
Scheme 3.3

In boiling 4N-sulphuric acid, the triazene (6a) underwent extensive decomposition with replacement of the triazeno side-chain and the 4-amino group by hydroxy groups. 2-(2-Hydroxyphenyl)quinazolin-4(3H)-one [85] (44) was isolated in 40% yield.



(44)

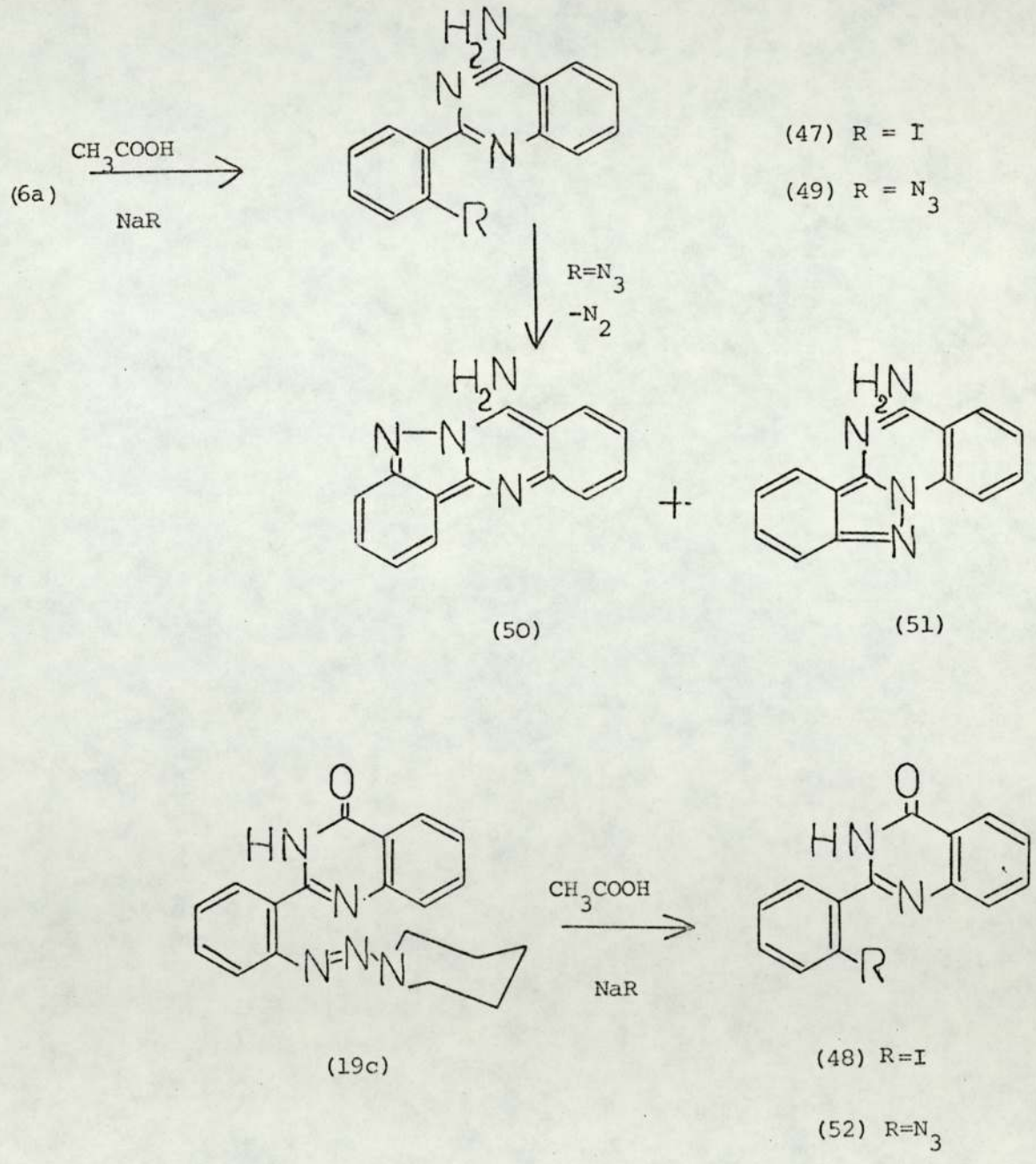
Incorporation of additional elements in the decompositions of the triazene (6a) in acetic acid resulted in some interesting conversions. In boiling acetic acid containing copper-bronze, the triazene (6a) underwent reductive elimination of the triazeno side-chain to afford 4-amino-2-phenylquinazoline (45) in excellent yield. Presumably this transformation proceeds *via* the diazonium ion (34) (Scheme 3.4). Subsequent steps in this conversion may be interpreted in terms of reductive elimination of nitrogen from the diazonium ion to form the radical (46) under the influence of cuprous ions. Reduction of aryl diazonium ions to aryl radicals by cuprous ions is considered to proceed *via* a complex formed between the diazonium ion, cuprous ion and related anion.[86] In the present case, abstraction of a hydrogen radical from the protic solvent yields the product (45).



Scheme 3.4

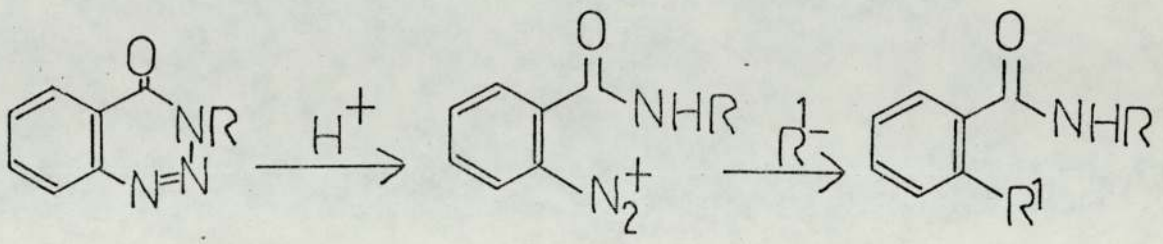
Acetic acid proved to be an excellent medium for promoting Sandmeyer-type displacements of the triazeno side-chain in the triazenes (6a) and (19c), and in related heterocyclic systems containing the NNN linkage.[87] The triazenes (6a) and 19c) (Scheme 3.5) formed the iodobenzenes (47) and (48) respectively in boiling acetic acid containing sodium iodide. When the triazene (6a) was boiled for a short time in acetic acid containing sodium azide, 4-amino-2-(2-azidophenyl)quinazoline (49) could be isolated albeit in low yield (≤20%). Prolonged boiling of the triazene (6a) in acetic acid containing sodium azide led to the formation of a mixture of the isomeric indazoles (50) and (51).

In this case, the N(3) and N(1) atoms of the quinazoline ring are sufficiently nucleophilic to promote nitrogen loss from the azide, probably in a concerted displacement (Scheme 3.5), forming the indazoles (50) and (51) respectively. The triazenoquinazolin-4-one (19c) formed the corresponding azide (52) under similar conditions, but this azide showed no tendency to cyclise, presumably because the N(1) and N(3) nitrogens of the quinazolin-4-one ring are less strongly nucleophilic due to the replacement of an amino group in the 4-position with an oxo-group.



Scheme 3.5

These substitutions present a useful and novel route to o-substituted arenes. The applicability of this reaction was examined in other heterocyclic systems containing the NNN linkage [87] and notably in derivatives of 1,2,3-benzotriazin-4(3H)-one. Thus, in the present work, 1,2,3-benzotriazin-4(3H)-one (53a) (Scheme 3.6) was converted in acetic acid containing sodium azide or sodium iodide to o-azidobenzamide and o-iodobenzamide respectively. The triazinone (53a) was, however, recovered unchanged from acetic acid containing sodium chloride, bromide, acetate, cyanide and sulphite. These anions are insufficiently nucleophilic to promote decomposition of the diazonium ion (54). The scope of this reaction is also limited by the nature of the N(3) substituent of the triazinone ring. Thus the N-alkyl- and N-aralkyl-triazinones (53b-d) and (e-f) respectively failed to decompose in acetic acid containing sodium azide or sodium iodide. The effect of such N(3) substitution would appear to be to stabilise the triazine ring to acid cleavage perhaps by an electron releasing effect. The N-aryl-triazinones (53g-k) on the other hand undergo efficient conversions to the respective o-substituted azides and iodides.[87] This represents a convenient synthesis of the azides (55g-k $R'=N_3$) which cannot be prepared by the conventional diazotisation-azidation route because of the dominating competitive intramolecular cyclisation to 3-aryl-1,2,3-benzotriazin-4 (3H)-ones.[88]



(53) (54) (55)

a R=H

b Me

c Et

d n-Pr

e Benzyl

f Phenethyl

g Ph

h o-Tolyl

i o-$NO_2 \cdot C_6H_4$

j m-$NO_2 \cdot C_6H_4$

k p-$NO_2 \cdot C_6H_4$

R^1 = I, N_3

Scheme 3.6

Reductions of 4-amino-2-[2-(piperidin-1-ylazo)phenyl] quinazoline.

Replacement of a primary aromatic amino group by hydrogen (ie. deamination) can be accomplished by diazotisation of the amine, and heating the resultant diazonium ion in a protic medium.[27] Analagous to this conversion is the decomposition of the triazene (6a) to 4-amino-2-phenylquinazoline in boiling ethylene glycol. This transformation was also accomplished by photolysis of the triazene (6a) in ethanol or methanol and was conveniently followed by U.V. spectroscopy (Fig. 3.1) and by thin layer chromatography (tlc). Photolysis

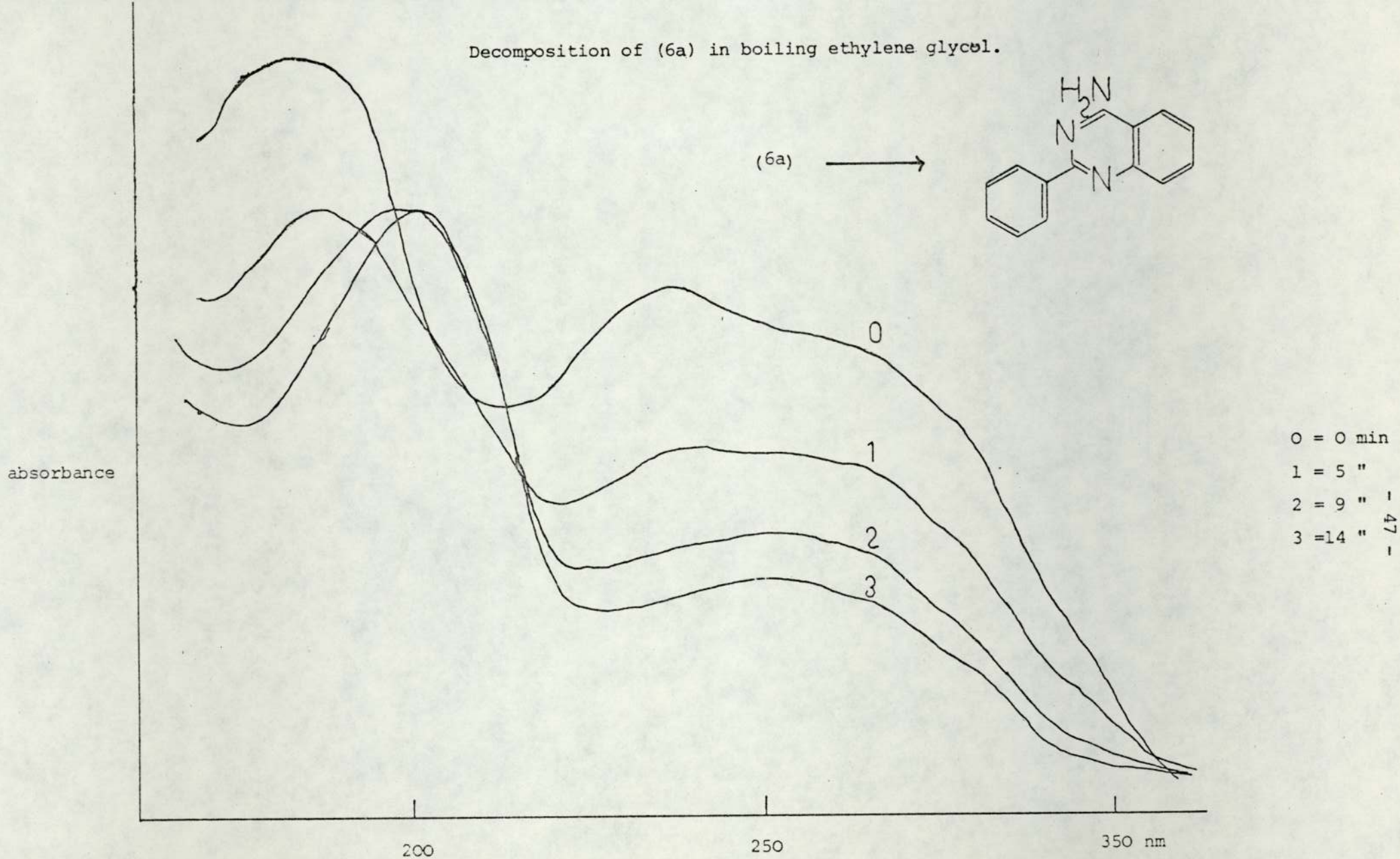

Decomposition of (6a) in boiling ethylene glycol.
(6a)
H2N
N
N
absorbance
0
1
2
3
200
250
350 nm
0 = 0 min
1 = 5 "
2 = 9 "
3 =14 "

Fig. 3.1

of DTIC, it will be recalled proceeds via diazo-ICA which is stabilised by loss of the acidic imidazo proton. Subsequent cyclisation to 2-azahypoxanthine occurs. In the photolytic decomposition of the triazene (6a) the intermediate diazonium ion cannot be stabilised in such a manner. Instead, reductive elimination of nitrogen occurs in the protic solvent.

A combination of hydrazine and Raney nickel (HRN) has been shown to effect N-N bond cleavage in a variety of heterocyclic systems.[74,89] The triazene (6a) also proved to be susceptible to this reagent and afforded the expected 4-amino-2-(2-aminophenyl)quinazoline (31).

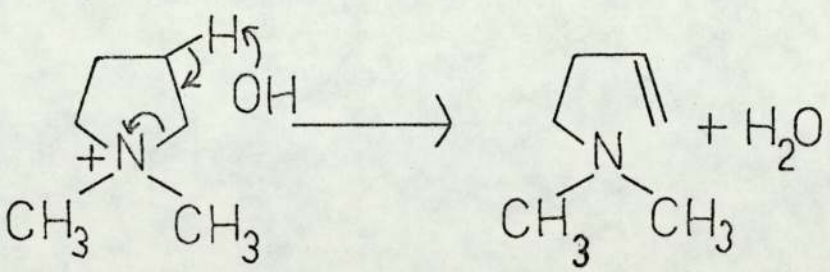
The products of the various decompositions described in this chapter were all submitted for antitumour evaluation and were found to be inactive. Since it has been shown that the capacity for transformation of tumour-inhibitory triazenes to alkylating agents is fundamental in their mode of action, (although subsequent events are unclear) efforts were next directed towards investigating the possibility of chemical transformation of the triazene (6a) to an alkylating entity.

Chapter 4

Alkylation of 4-amino-2-[2-(piperidin-1-ylazo)phenyl] quinazoline.

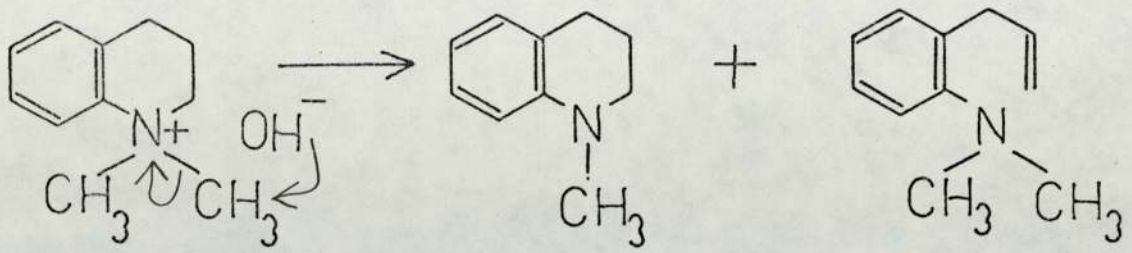
Introduction

The first working hypothesis behind attempts to develop an alkylating potential for the triazene (6a) concerned an exploitation of the Hofmann elimination reaction. Alkylation of a tertiary amine with eg. methyl iodide results in the formation of a quaternary ammonium iodide. Treatment of a quaternary ammonium iodide with an aqueous suspension of silver oxide affords the corresponding quaternary ammonium hydroxide. Such compounds decompose on heating (~125°) to form a tertiary amine, with elimination of an alkene. For example, N,N-dimethylpyrrolidinium hydroxide (56) is thermally decomposed to the tertiary amine[91] (57). (Scheme 4.1). This reaction, called the Hofmann elimination is analagous to the dehydrohalogenation of an alkylhalide. Hydroxide ion abstracts a hydrogen ion from the β-carbon; cleavage of the C-N bond occurs with retainment of the electrons on the nitrogen; and the double bond is generated. The reaction proceeds via an E_2 mechanism.



(56) Scheme 4.1 (57)

Hofmann elimination of a quaternary ammonium hydroxide by heating is not always effective for the opening of nitrogenous rings and its success is dependent on the conditions of the thermal decomposition.[91] For example, the quaternary hydroxide of N,N-dimethyl-1,2,3,4-tetrahydroquinoline (58) gives methanol and the original base (59) on heating as well as the ring-opened o-allyl-N,N-dimethylaniline[91] (60) (Scheme 4.2).

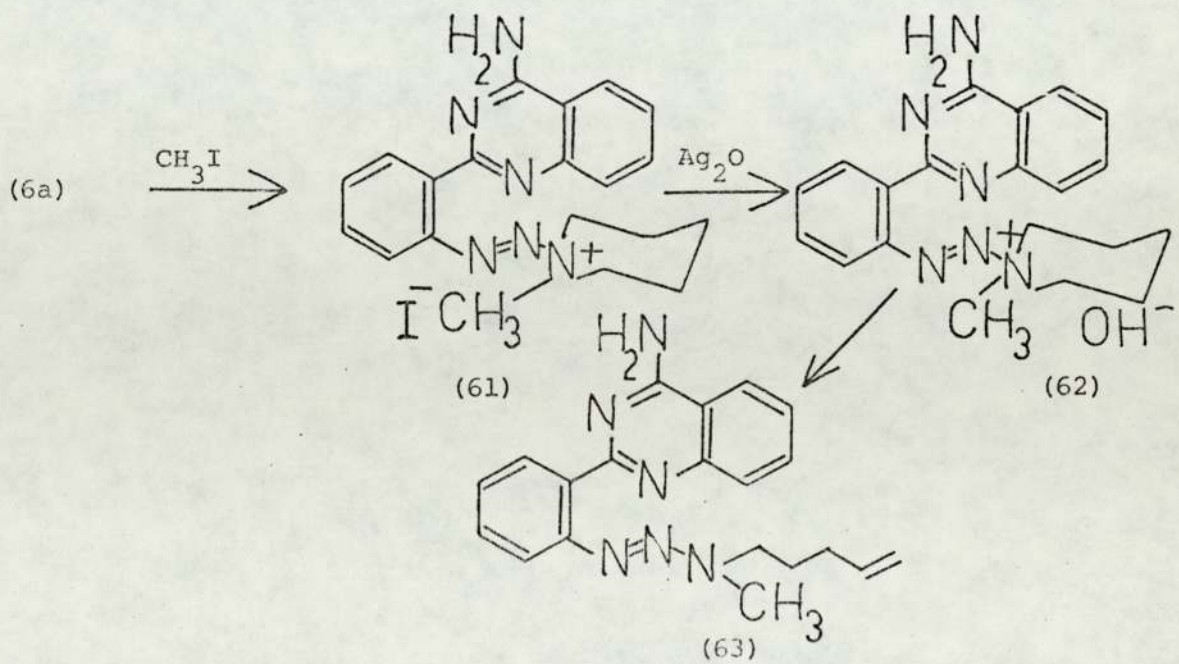


(58) (59) (60)

Scheme 4.2

In some of these cases, analagous ring-opening of the quaternary ammonium iodide may be achieved by reductive degradation with eg. sodium amalgam, or sodium in liquid ammonia (Birch reduction).[91]

In the present case, it was considered that methylation of the triazene (6a) with methyl iodide could have yielded the triazene (61), quaternised on the piperidine ring. (Scheme 4.3). Treatment of this salt with aqueous silver oxide would conceivably result in the formation of the corresponding quaternary ammonium hydroxide (62) which might be expected to undergo thermal decomposition to the ring-opened dialkyltriazene (63) by the Hofmann elimination route. It is significant that the possible product (63) of this transformation now fulfills the structural requirements for tumour-inhibitory triazenes,[19] in that oxidative dealkylation of this triazene would yield a monomethyltriazene. However, the thermal process would possibly cleave the triazene linkage.



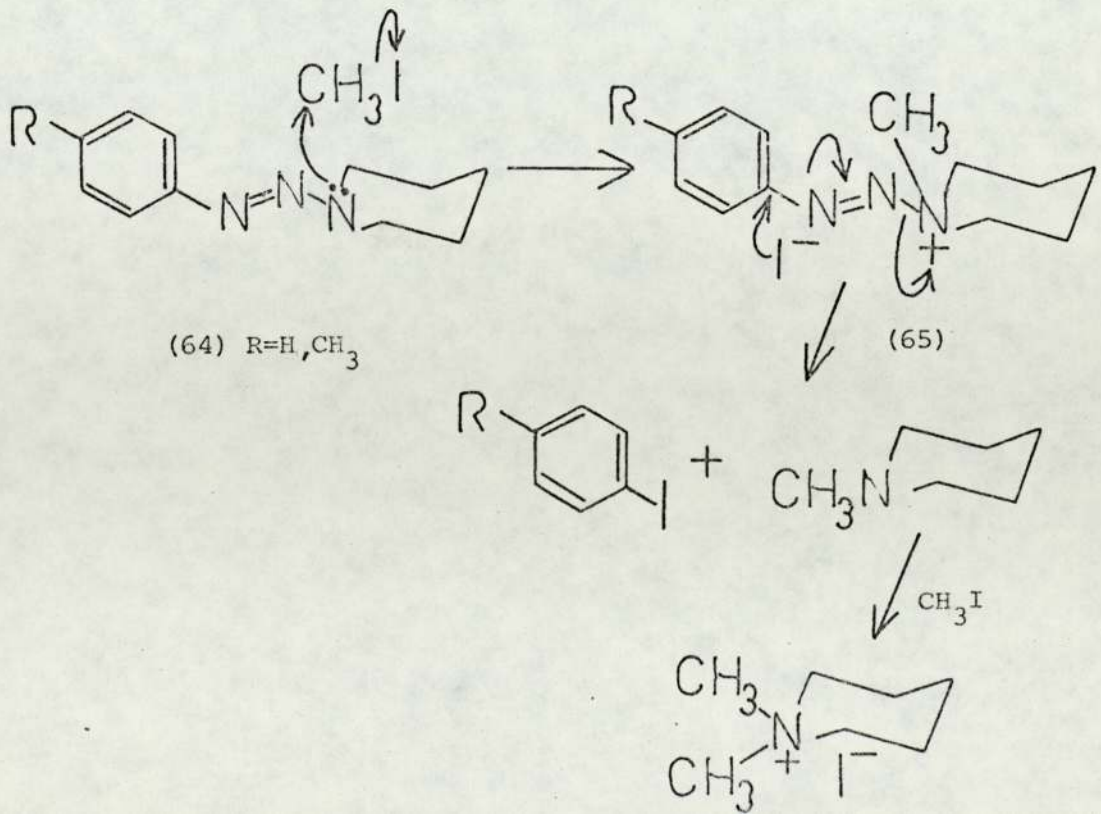
Scheme 4.3

Methylating agents eg. methionine, are present in biological systems and there are no theoretical objections to the quaternisation of the triazene (6a) in vivo. Subsequent steps in this proposed transformation could possibly be mediated by enzymes.

Methylation of model triazenes.

Methyl iodide has been shown to quaternise the N(2) atom of derivatives of 1,2,3-benzotriazine[92]. In view of the dipolar nature of the NNN linkage it was considered prudent to first examine the nucleophilicity of the triazeno N(3) atom in the reactions of the more simple aryl-dialkyltriazenes (64 R=H, NO_2, CH_3) with methyl iodide.

The triazene (64 R=H) (Scheme 4.4) undoubtedly underwent methylation on the N(3) atom with methyl iodide in methanol, or methyl iodide in benzene, since the quaternary ammonium salt($_{66}$) was eventually isolated. This observation was interpreted as follows : the intermediate (65 R=H) which could not be isolated, underwent iodide ion initiated displacement of the triazeno side-chain, with loss of nitrogen and formation of iodobenzene and N-methylpiperidine. The N-methylpiperidine thus formed reacted with a further molecule of methyl iodide to yield N,N-dimethylpiperidinium iodide (66).

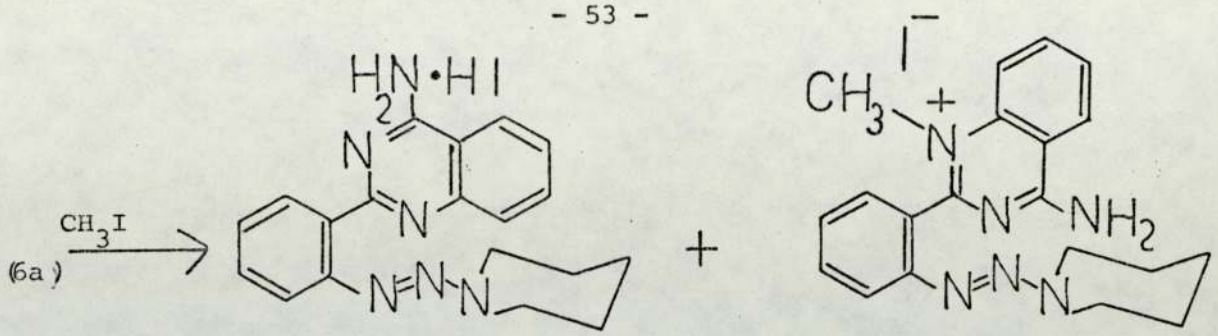


The reaction was influenced by the nature of the p-substituent. Thus an electron-attracting nitro group in the p-position (64 R=NO_2) reduced the basicity of the triazeno N(3) atom to the extent that this centre was insufficiently nucleophilic to participate in the S_N2 methylation reaction with methyl iodide. The triazene (64R=NO_2) was recovered unchanged from refluxing methyl iodide or refluxing methanol containing methyl iodide. The effect of an electron-releasing group in the p-position, ie, the triazene (64R=CH_3) was to eliminate the necessity for carrying out the reaction with methyl iodide in a methanolic medium ; N ,N-dimethylpiperidinium iodide was recovered from the reaction of the triazene (64 R=CH_3) in refluxing methyl iodide alone.

Should the quinazolinotriazene (6a) undergo methylation on the triazeno N(3) nitrogen, subsequent nucleophilic displacement of the triazeno side-chain by iodide ion might be sterically hindered by the bulky o-substituent. The proposed product (61) would then be a candidate for the Hofmann elimination or reductive degradation to the ring-opened triazene (63).

Methylation of 4-amino-2-[2-(piperidin-1-ylazo)phenyl]quinazoline

In the event, methylation of the triazene (6a) with methyl iodide or methyl iodide in tetrahydrofuran, took a different course. Two products were isolated neither of which was the desired N(3) methylated triazene (61). Both products contained the intact NNN linkage, as evidenced by the formation of azonaphthol derivatives when they were heated with acetic acid containing 2-naphthol(Bamberger-Goldberger test).[93] The first of these products (67) (15%) was a hydroiodide salt of the starting material. It was identified by conversion to the starting material on basification. The other product (80%) was assigned the structure (68) (Scheme 4.5) on the basis of the following chemical and spectroscopic evidence.

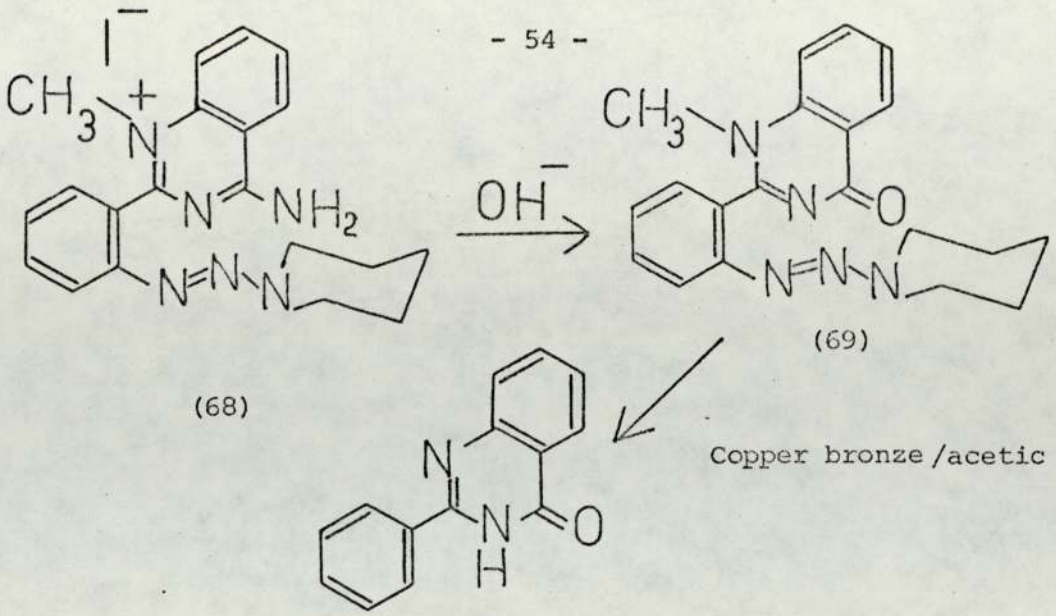


(67) (68)

Scheme 4.5

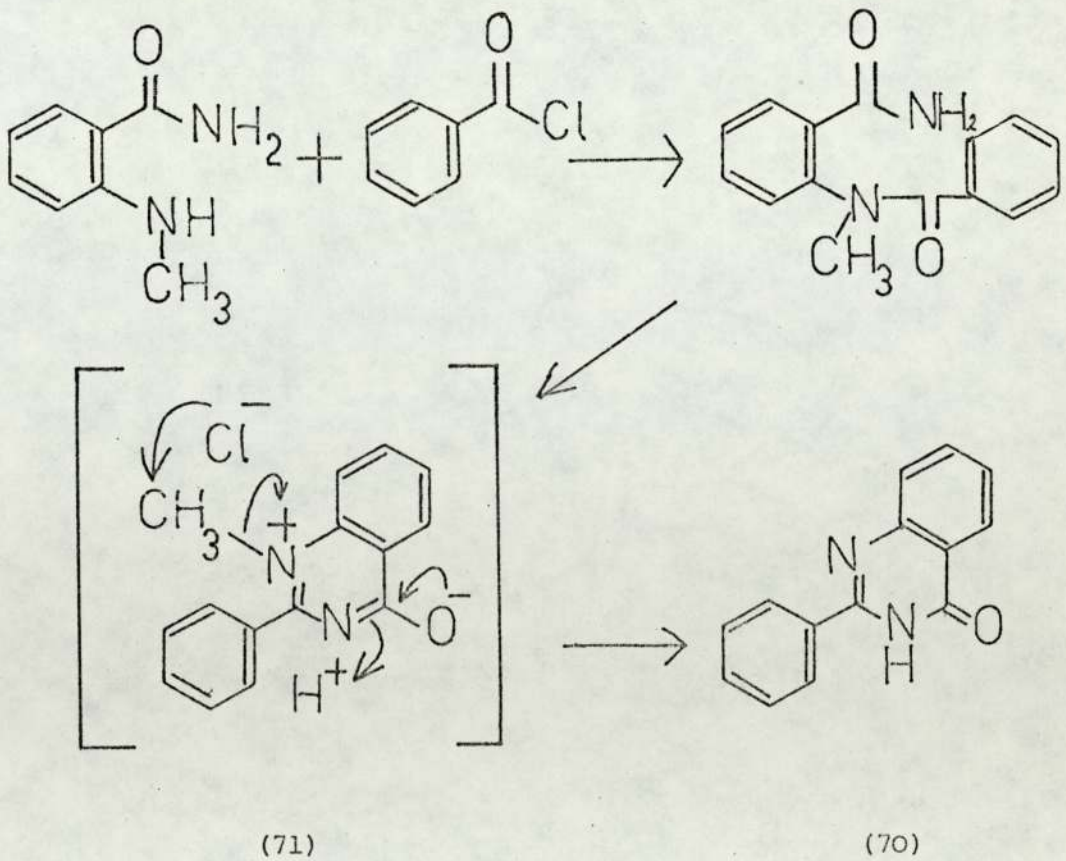
Basification of the methiodide (68) (which can also be considered as a hydroiodide salt of the 1-methylquinazolin-4(1H)-imine) led to hydrolysis and the isolation of a methylated quinazolone (69) (Scheme 4.6). This excluded methylation on the exocyclic 4-amino group of the triazene (6a). The mass spectrum of the methylated quinazolone (69) unequivocally established that the methyl group was attached to the quinazoline fragment and not to the triazeno side-chain; loss of the triazeno side-chain afforded an ion corresponding in mass number to 2-phenylquinazolinone plus 14 mass units (ie. + CH_3-H).

The methylated quinazolone (69) was recovered unchanged from ethanolic hydrazine containing Raney nickel and boiling ethylene glycol. The latter reagent might have been expected to effect reductive elimination of the triazeno side-chain to afford either of the known N(1) or N(3) methylated 2-phenylquinazolin-4-ones.[94] Reductive decomposition of (69) with hypophosphorous acid was likewise unsuccessful. In boiling acetic acid containing copper bronze however, the methylated quinazolone (69) underwent reductive elimination of the triazeno side-chain and demethylation to yield 2-phenylquinazolin-4(3H)-one (70) (Scheme 4.6) thus leaving the question of the site of methylation of the triazene (6a) unanswered.



Scheme 4.6

An explanation for this unexpected result was forthcoming when the properties of 1-methyl-2-phenylquinazolin-4(1H)-one and the N(3) methyl isomer were examined. Condensation of benzoyl chloride with 2-methylamino-benzamide in p-cymene gives 2-phenylquinazolin-4(3H)-one (70) and not 1-methyl-2-phenyl-quinazolin-4(1H)-one(71) as claimed.[95] (Scheme 4.7)



Scheme 4.7

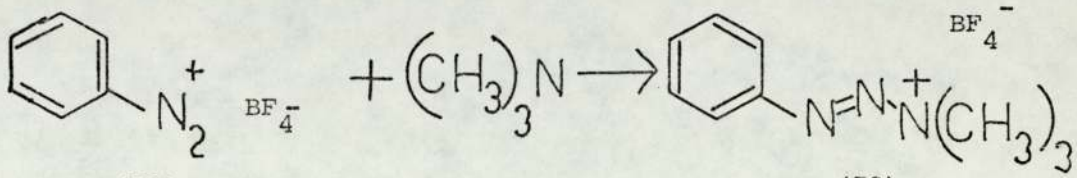
The dipolar character of the methylated quinazolin-4(1H)-one (71) makes it susceptible to chloride ion initiated demethylation. Other alkylated heterocycles with dipolar character readily undergo dealkylation under similar conditions.[96] In the case of the demethylation of the methylated triazenoquinazolone (69) in acetic acid containing copper bronze, acetate ion presumably initiates the demethylation.

The positioning of the methyl group on the quinazoline N(1) atom is corroborated by the observation that 2-phenyl-3-methyl-quinazolin-4(3H)-one was not only found to be stable under the conditions of its synthesis (prepared in the present work from benzoyl chloride and N-methylanthranilamide[97]) but was also recovered unchanged from boiling acetic acid containing copper bronze. The conclusion from these results is that 3-methylquinazolin-4-ones are stable and 1-methylquinazolin-4-ones are labile.

Two literature reports lend further support to the conclusion that the triazene (6a) undergoes methylation on the quinazoline N(1) atom. 4-Aminoquinazoline is methylated by methyl iodide at N(1) to afford the hydroiodide salt of 1,4-dihydro-4-imino-1-methylquinazoline,[98] and 1-methyl-2-phenylquinazolin-4(1H)-one is claimed to rearrange thermally to the thermodynamically more stable 3-methyl isomer, by a "1,3-alkyl" shift.[94] Unfortunately no reference was made in the latter publication to the preparation of the unstable 1-methyl isomer.

The position of the carbonyl absorption in the i.r. spectrum of the methylated quinazolone (69) also supports its assigned structure. α,β-unsaturated amides generally absorb in the region $1649cm^{-1}$ - $1639cm^{-1}$, whereas the β,γ-unsaturated counterparts show bands in the region $1679cm^{-1}$ - $1676cm^{-1}$.[94] The carbonyl absorption of the methylated quinazolone in question appears at $1642cm^{-1}$ which is nearer the region of the α,β - unsaturated compounds and thus tends to support the positioning of the methyl group on the quinazoline N(1) atom.

Quaternary triazenes of the type (73) were unreported in the literature at the time of this practical work. However Stevens[99] has recently prepared the analogue (73) by coupling the diazonium tetrafluoroborate (72) with trimethylamine in acetonitrile at -5°. (Scheme 4.8) Alkylation of phenyldimethyltriazene with dimethylsulphate led to a mixture of products which were not characterised.[99]



(72) Scheme 4.8 (73)

Since the site of alkylation of heterocyclic compounds is frequently determined by the reaction conditions,[100] methylation of the triazene (6a) was attempted with dimethylsulphate in sodium hydroxide but this also led to extensive decomposition and no products were characterised.

Chapter 5

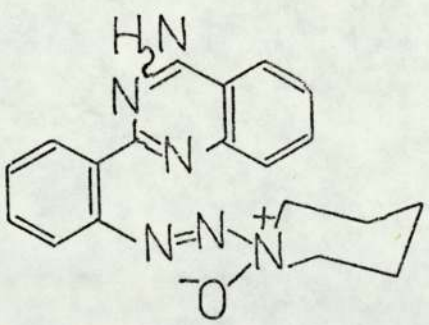
Oxidation of 4-amino-2-[2-(piperidin-1-ylazo)phenyl] quinazoline.

Introduction

The metabolic activation of DTIC, PDMT and the carcinogenic N-nitrosamines is apparently initiated by an oxidation reaction.[101] Therefore, any study on the chemistry of the triazenoquinazolines related to their mode of action, is incomplete without an examination of their behaviour on oxidation.

Oxidation with perbenzoic acid.

Quaternisation of the triazeno(N)3 atom of the triazene (6a) was attempted by oxidation with perbenzoic acid. This could have yielded the tertiary amine oxide (74). Pyrolysis of tertiary amine oxides yields an alkene and an N,N-dialkylhydroxylamine. The reaction (Cope elimination) is similar to the Hofmann elimination but differs in stereochemistry. The stereochemistry of the Cope elimination is cis whereas in the Hofmann elimination it is trans.[102]



(74)

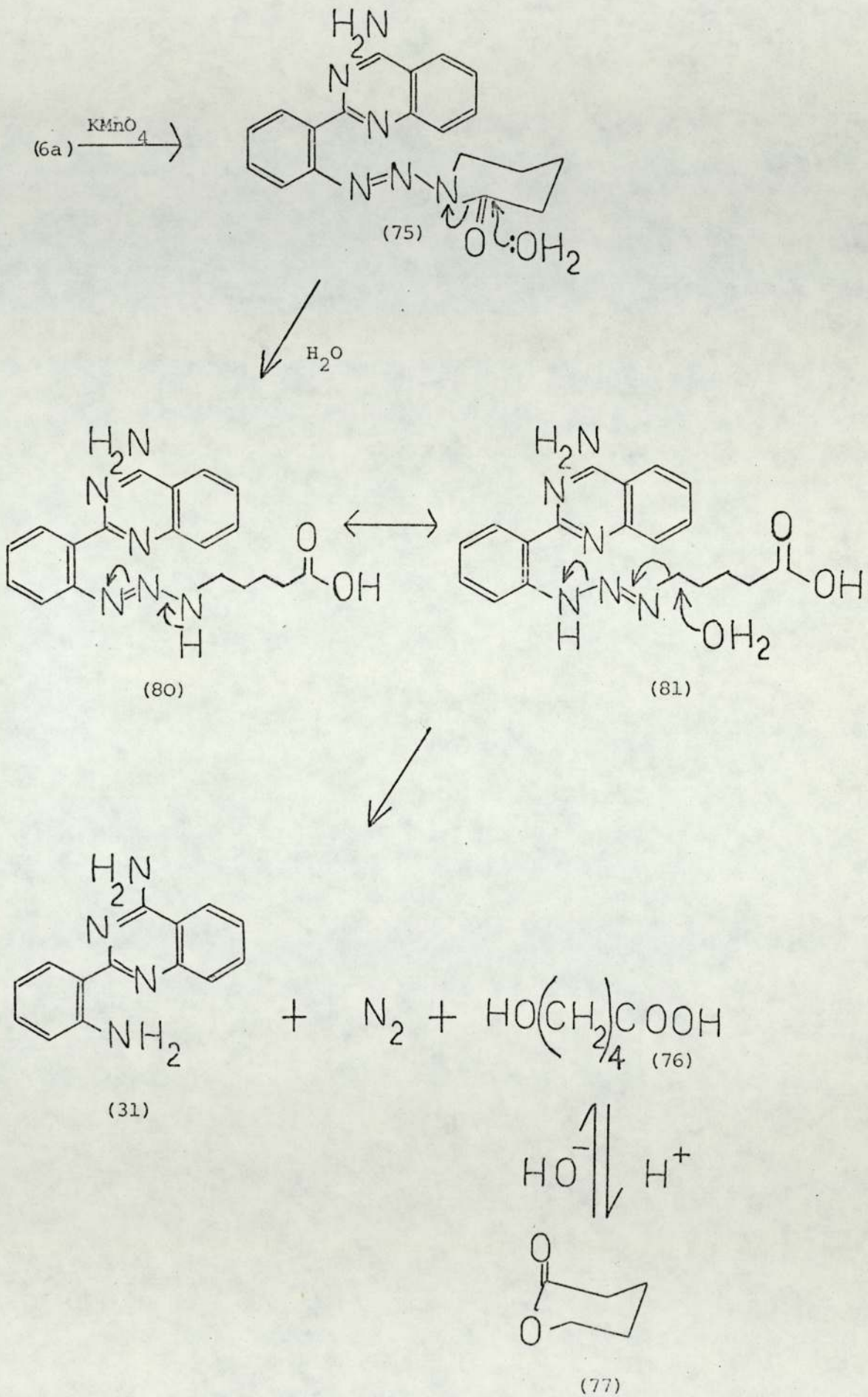
In fact, the quaternary triazene (74) would be unlikely to undergo a Cope elimination, since the reaction requires a planar intermediate which in this case is not possible because of the puckering of the piperidine ring. Oxidation of the triazene (6a) with perbenzoic acid in benzene afforded only a benzoate salt of the starting material. This result, along with the formation of the hydroiodide salt (67) (Chapter 4) confirms the strongly basic character of the triazene (6a).

Oxidation with potassium permanganate

Potassium permanganate was chosen as an oxidising agent for the triazene (6a) because not only is this reagent one of the most powerful of the oxidising agents in the chemist's armoury, but it is extremely versatile - its versatility being reflected in its ability to use different reaction paths depending on the structure of the substrate and on the reaction conditions.[103] Oxidation of the triazene (6a) with permanganate at 20° in aqueous acetone containing potassium bicarbonate, resulted in the formation of two new products, as determined by chromatographic analysis of a chloroform extract of the reaction mixture. The most polar of these products was identified as 4-amino-2-(2-aminophenyl)quinazoline. The identification of the diamine (31), an aromatic amine, as an oxidation product of a dialkyltriazene is highly significant. Formation of an aromatic amine by oxidation of a dialkyltriazene requires the intermediacy of a monoalkyltriazene. The triazene (6a) must therefore have undergone oxidative ring-opening to a monoalkyltriazene which decomposed to the diamine.

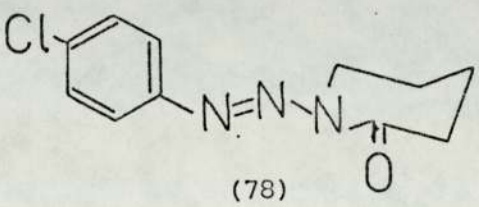
Fortuitously, the second oxidation product crystallised from a concentrated chloroform extract of the reaction mixture. This product, which contained the intact NNN linkage was assigned the structure (75) on the basis of the following observations. The mass spectrum of this product indicated that oxidation had not occurred on the quinazoline or phenyl fragments. In aqueous acetone (pH 9.0) in light or dark, the product (75) decomposed to form the diamine (31) and δ-hydroxyvaleric acid (76). δ-Hydroxyvaleric acid was isolated[104] as the lactone (77) and characterised by i.r. spectroscopy, gas chromatography and tlc of its hydroxamic acid derivative.

The assigment of the structure (75) to the oxidation product is further supported by its nmr spectrum which bears a striking resemblance in the aliphatic region to the nmr spectrum of 1-(4-chlorophenylazo)piperidin-2-one (78). This compound was formed on permanganate oxidation of 1-(4-chlorophenyl)-

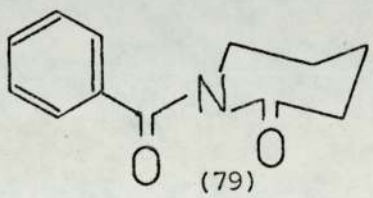


Scheme 5.1

piperidine[105] and its structure (78) confirmed partly on the basis of the similarity (in the aliphatic region) in nmr spectrum to N-benzoylpiperidin-2-one (79).



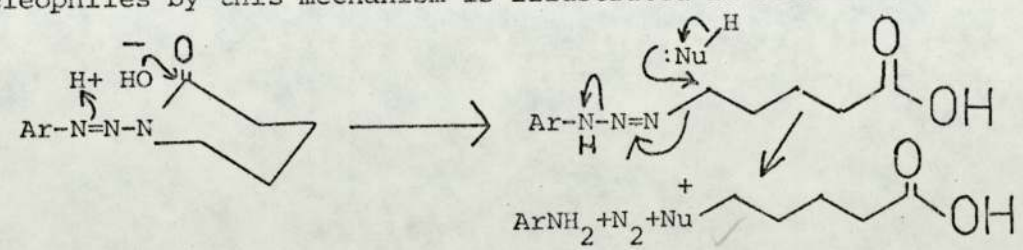
(78)



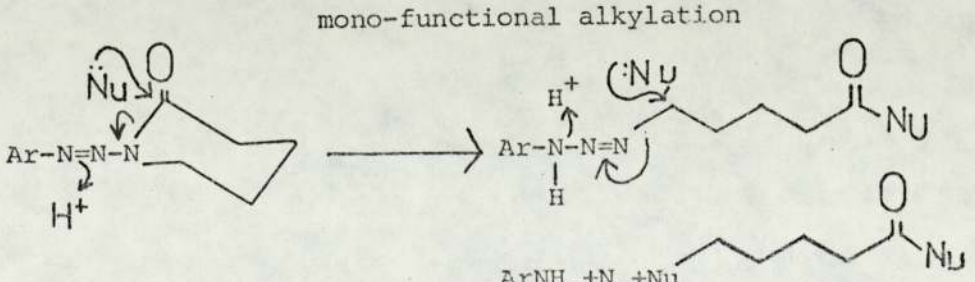
(79)

The reaction sequence (Scheme 5.1) may be interpreted as follows: initial α-hydroxylation of the triazene (6a) affords a hydroxylated intermediate which undergoes further oxidation to the isolable piperidin-2-one. In the basic medium, the piperidin-2-one undergoes nucleophilic attack by water to yield the unstable monoalkyltriazene (30) which is in equilibrium with the tautomer (81). The tautomer (81) alkylates a further molecule of water with formation of δ-hydroxyvaleric acid (76), the diamine (31) and loss of nitrogen.

α-Oxidation of the triazene (6a) by permanganate can therefore be seen to result in the formation of the intermediate (75) which has the capacity for alkylating nucleophiles. In this case the nucleophile was water. The capacity for both mono- or di-functional alkylation of biological nucleophiles by this mechanism is illustrated in Scheme 5.2



mono-functional alkylation



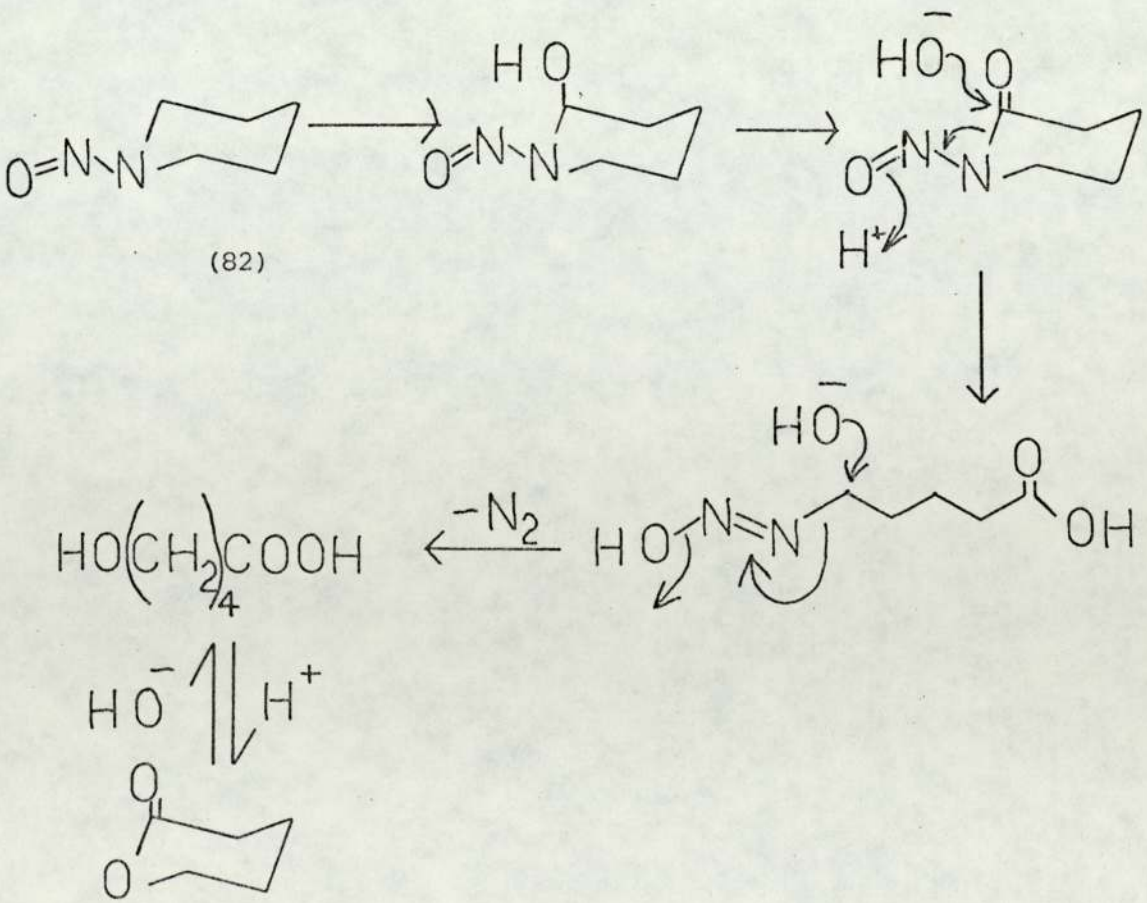
di-functional alkylation

Scheme 5.2

Di-functional alkylation of this nature could effectively crosslink two strands of DNA and thus inhibit DNA synthesis. The antitumour action of the nitrogen mustards is thought to be due in part to such a reaction with DNA.[106]

Further derivatives of the triazene (6a), [(6c), (6f), (6h) and (6i)] were subjected to analogous permanganate oxidation and in all cases the diamine (31) was formed indicating oxidative dealkylation to a monoalkyltriazene. The alkylating nature of these monoalkyltriazenes was inferred from the results of the oxidation of the triazene (6a).

In view of the similarity between the N-nitrosamines and aryl-dialkyltriazenes, nitrosopiperidine (82) was likewise oxidised by permanganate and was also shown to form the lactone (77). This sequence can be interpreted in terms of initial α-hydroxylation followed by analogous decomposition to that of the oxidised triazene (6a). (Scheme 5.3).



(82)

(77)

Scheme 5.3

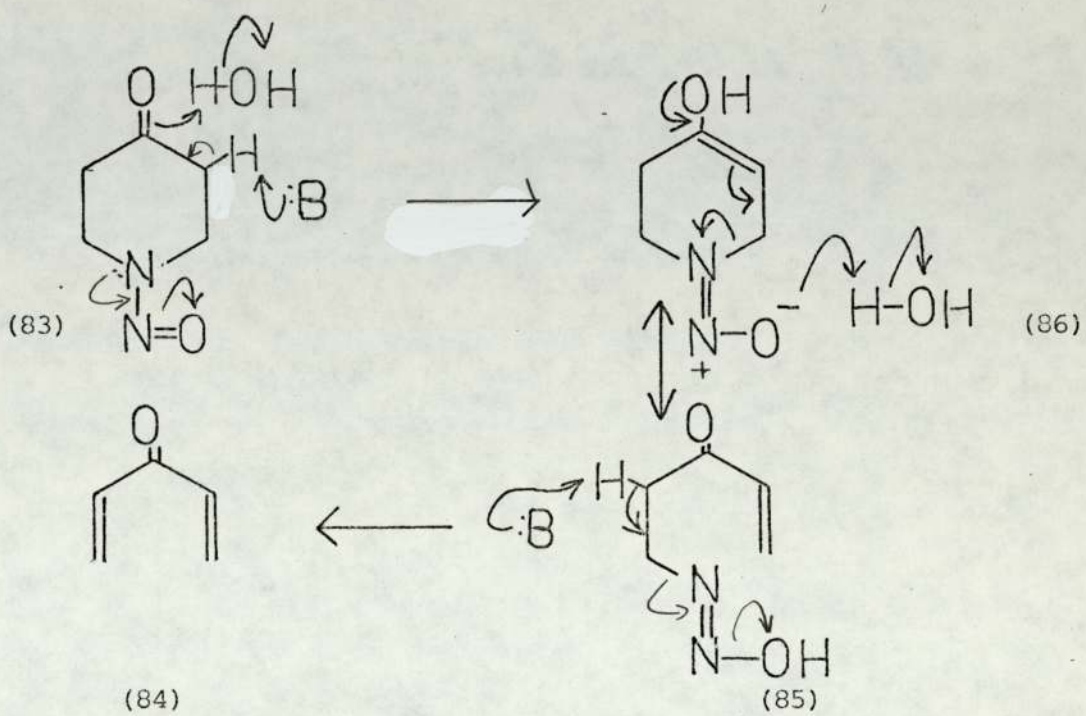
The significance of these results and the relevance to the mode of action of these two classes of compound are of course unanswered questions at present. It is quite possible that the two classes of compound exert their biological effects by a common mechanism, probably initiated by α-carbon oxidation. Should this be the case, then considering the toxicity of the nitrosamines, the future for the triazenoquinazolines as potential tumour-inhibitory agents may be less than promising. On the other hand, while both the triazene (6a) and nitrosopiperidine were observed to alkylate in the same way after permanganate oxidation,because of the substantial structural differences between these compounds it is more than likely that the two molecules will alkylate in different micro-environments; i.e. they will have affinities for different nucleophilic sites. Thus the consequences of alkylation at a particular site may be tumour formation; alkylation at a different specific site may result in tumour-inhibitory activity.

The similarity in tumorogenesis by certain aryl-dialkyltriazenes and the N-nitrosamines suggests that the two classes of compound may have a common mechanism of action.[107]

In addition to α-carbon oxidation, mechanisms of activation of the nitrosamines have been proposed which place importance on β- and γ-carbon oxidation. Nitrosopiperidine has been shown to undergo oxidation in the γ-position both by rat liver microsomes and in the Udenfriendoxidation system.[70] Recent results on the Udenfriend oxidation of nitrosopiperidine confirm these earlier results.[108]

However, it was found thatN-nitrosopiperidin-4-one was only mutagenic after further microsomal incubation.[108] This suggests that oxidation in the 4-position in nitrosamines may not contribute to the carcinogenicity and mutagenicity of these compounds. The derivative, N-nitrosotriacetonamine (83) (Scheme 5.4) decomposes in aqueous or alcoholic solution in the presence of a catalytic amount of base to yield 2,6-dimethyl-4-ketohepta -2,5-diene (84)

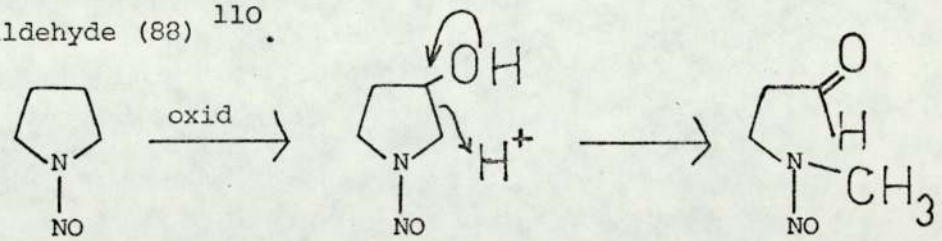
presumably through the primary alkyl diazonium hydroxide (86). Either (84) or (85) will alkylate nucleophiles.[109]



(83) (84) (85) (86)

Scheme 5.4

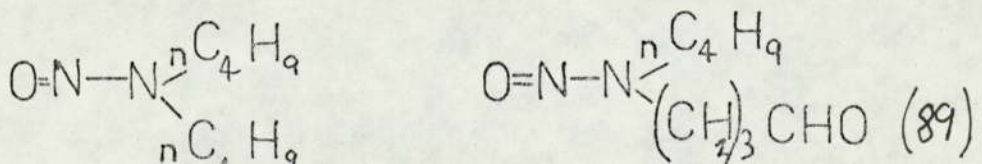
On the other hand, β-carbon hydroxylation of N-nitrosopyrrolidine (87) (Scheme 5.5), it has been claimed, could result in the formation of the nitrosoaminoaldehyde (88)[110].



(87) (88)

Scheme 5.5

N-Nitrosoaminoaldehydes are a class of compound which recent studies suggest may be metabolic intermediates in the activation of certain carcinogenic nitrosamines.[111] It has been proposed that 4-(N-butyl-N-nitrosoamino)-butanal (89) may be a critical intermediate in the induction of bladder tumours in rats by N-nitrosodi-n-butylamine (90).



In line with these proposals, it has been shown[105] that oxidation of 1-(4-chlorophenylazo)piperidine by permanganate, in the Udenfriend oxidation system, and by fortified rat liver microsomal preparations results in the formation of a variety of triazene products oxidised in the α, β, and γ-positions (Table 5.1) and in the formation of 4-chloroaniline, indicating some degree of oxidative dealkylation.

Oxidation of 4-amino-2-[2-(piperidin-1-ylazo)phenyl]quinazoline in the Udenfriend oxidation system.

The Udenfriend oxidation system closely approximates biological oxidation processes. It requires molecular oxygen, ferrous ion, and utilizes an electron-donor (ascorbic acid). The exact mechanism of oxidation is not known although it is considered that the Udenfriend system may effect epoxidation in the oxidation of aliphatic compounds through the generation of hydroxyl radicals. Hydroxylation may, on the other hand, be effected through complex formation between the substrate, metal ion electron donor and oxygen.[112]

The reaction products of the Udenfriend oxidation of the triazene (6a) in light or dark were subjected to chromatographic analysis on silica plates. The major reaction product in both cases was identified as 2-phenyl-4-aminoquinazoline. Neither the diamine (31) nor the piperidin-2-one (75), which are both products of permanganate oxidation of (6a) were detected. The quinazoline (45) has previously been isolated from triazene (6a) by decomposition in a protic medium (see chapter 3). The different pH of the permanganate oxidation (pH9.0) to that of the Udenfriend (pH6.5) may account for these differences.

Oxidation of 1-(4-chlorophenylazo)piperidine.[105]

Products	$KMnO_4$	Udenfriend	Incubation with fortified rat liver microsomal preparations
4-chloroaniline	+	+	+
1-(4-chlorophenylazo)piperidin -2-one	+	+	-
1-(4-chlorophenylazo)piperidin-4-one	+	+	-
1-(4-chlorophenylazo)piperidin-3-ol	-	+	-
1-(4-chlorophenylazo)piperidin-4-ol	+	+	+

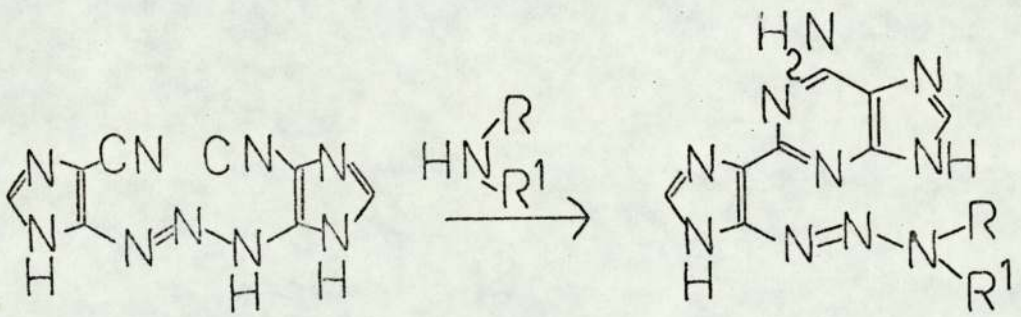
TABLE 5.1

Chapter 6

Synthesis and properties of triazene derivatives of purines

Adenine derivatives

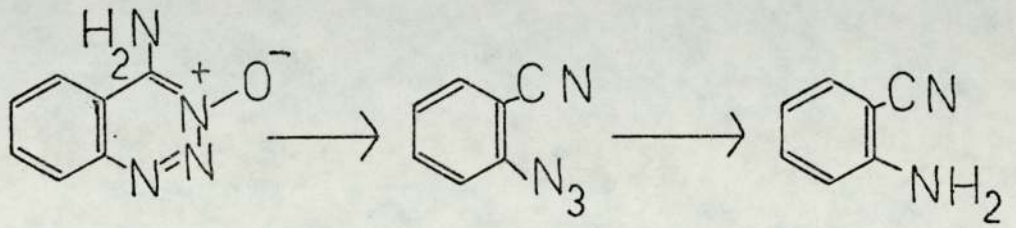
Analogues of both purines and pyrimidines (eg.6-mercaptopurine and 5-fluorouracil) can function as antimetabolites and have a useful application in cancer chemotherapy.[113] To recall the attention of the reader to the synthesis of the triazenoquinazolines, 1,3-di-o-cyanophenyltriazene(7) undergoes successive amine-nitrile addition in secondary amines to afford the triazenes (6a-i)[8]; this property was mimicked in the reactions of the brominated triazene (25) in secondary amines. Therefore it was anticipated that 1,3-di-(4-cyanoimidazol-5-yl)triazene(91) might undergo analogous transformation in secondary amines to yield the adenine derivatives (92) (Scheme 6.1).



(91) Scheme 6.1 (92)

The proposed products (92) of this sequence, as derivatives of adenine, might act as 'carriers' of the alkylating dialkyltriazeno function, which might be expected to confer specificity on the molecules. In theory, the synthesis of the triazene (91) might have been achieved by the addition of 5-diazoimidazole-4-carbonitrile to 5-aminoimidazole-4-carbonitrile (93). The first reported synthesis of the aminonitrile (93).was in 1966.[114] Ferris and Orgel condensed aminomalononitrile with formamidine acetate and isolated 5-aminoimidazole-4-carbonitrile as a tosylate salt. The method of preparation of aminomalonitrile given in this paper is exceedingly laborious and attempts to repeat it in the present work were unsuccessful.

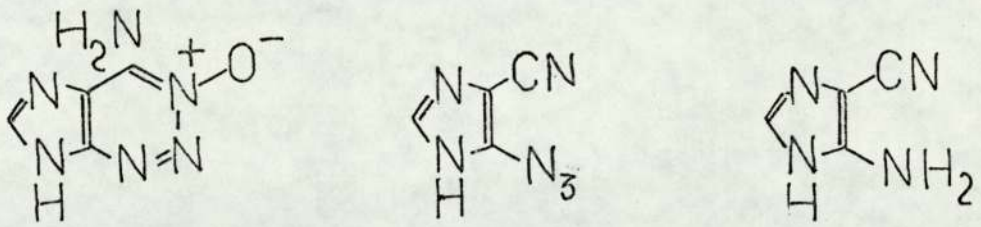
A novel synthesis of the o-aminonitrile (93) was suggested by a report[115] that 4-amino-1,2,3-benzotriazine-3-oxide (94) decomposes in piperidine to afford o-azidobenzonitrile (95) which may be conveniently reduced to o-aminobenzonitrile. (Scheme 6.2)



(94) (95)

Scheme 6.2

In the present context, it was considered that analogous decomposition of 2-azaadenine -1-oxide (96) would yield the azide (97) which might then be reduced to the required aminonitrile (93). (Scheme 6.3)

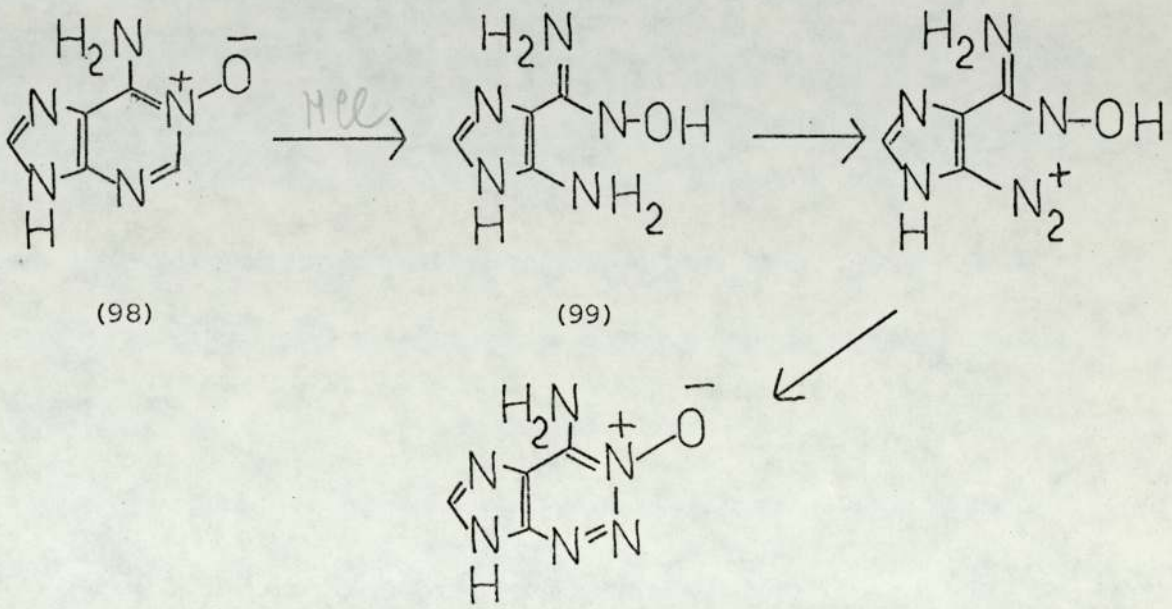


(96) (97) (93)

Scheme 6.3

2-Azaadenine-1-oxide was prepared according to the method of Stevens, Magrath, Smith & Brown[116]. Oxidation of adenine in peracetic acid yielded adenine-1-oxide (98) which decomposed in hot 3N-hydrochloric acid to the hydrochloride salt of 5-aminoimidazole-4-carboxamidoxime (99). Treatment of an aqueous solution of the salt (99) with nitrous acid led to the isolation of 2-azaadenine-1-oxide as described (Scheme 6.4). Decomposition of 2-azaadenine-1-oxide to the azide (97) was attempted in boiling piperidine

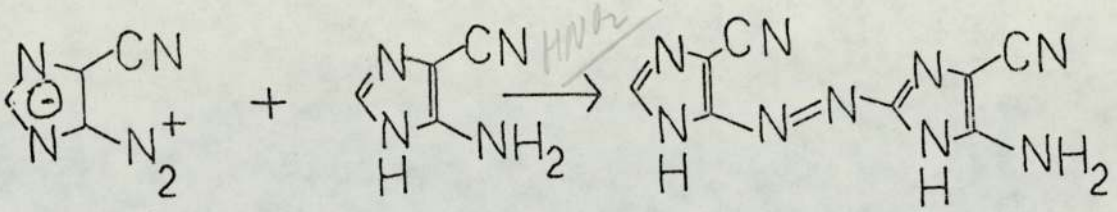
or collidine, or by fusion with ammonium acetate at 200°. In all cases the starting material was recovered.



(98) (99) (96)

Scheme 6.4

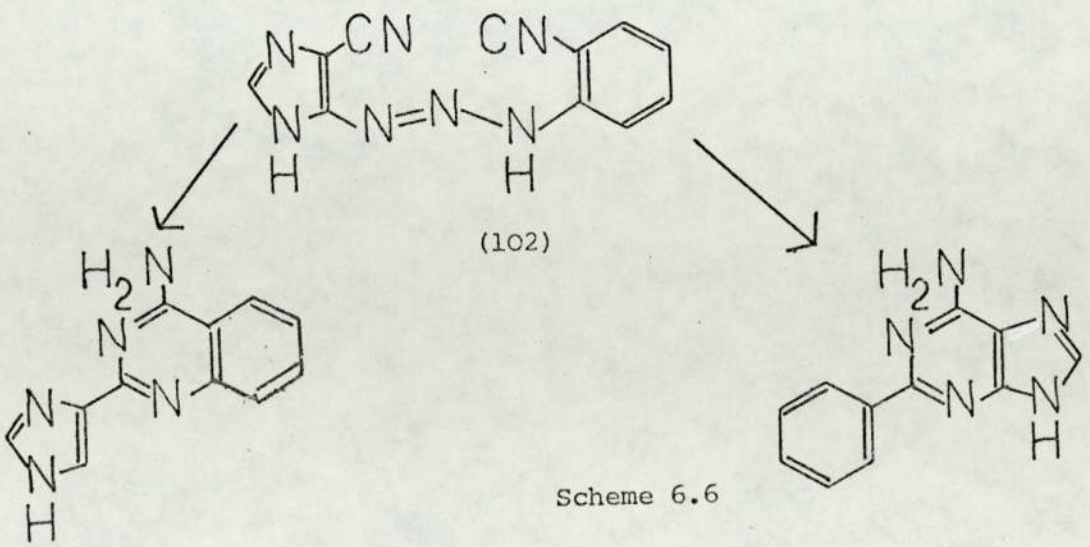
5-Aminoimidazole-4-carbonitrile (93) was finally obtained by the more conventional phosphorous oxychloride dehydration of 5-aminoimidazole-4-carboxamide, using an improved procedure by Shealy.[117] Diazotisation of 5-aminoimidazole-4-carbonitrile results in the formation of the stable 5-diazoimidazole-4-carbonitrile [117]. When this diazoderivative was added to a solution of 5-aminoimidazole-4-carbonitrile C- rather than N-coupling took place and the deep red azo-compound (101) was isolated. The structure of this product was deduced from its uv spectrum which exhibited long wavelength absorption (λ_{max} 512 nm characteristic of azo compounds) and from its CHN analysis. The 2-carbon is thus the most nucleophilic site in the π excessive imidazole ring, rather than the exocyclic amino group.



(93) (101)

Scheme 6.5

However, addition of the diazonium ion to an aqueous suspension of o-aminobenzonitrile afforded the N-coupled product (102). The unsymmetrical nature of this triazene permits two possible decomposition routes in protic solvents, and which are absolutely dependent on which cyano group undergoes initial nucleophilic attack. Cyclisation on the aryl cyano group followed by decomposition analogous to the decomposition of 1,3-di-o-cyano-phenyltriazene in ethanol, would yield 2-phenyladenine (104) (Scheme 6.6); alternative cyclisation on the imidazo cyano group would result in the formation of 4-amino-2-(imidazol-4-yl)quinazoline (103).

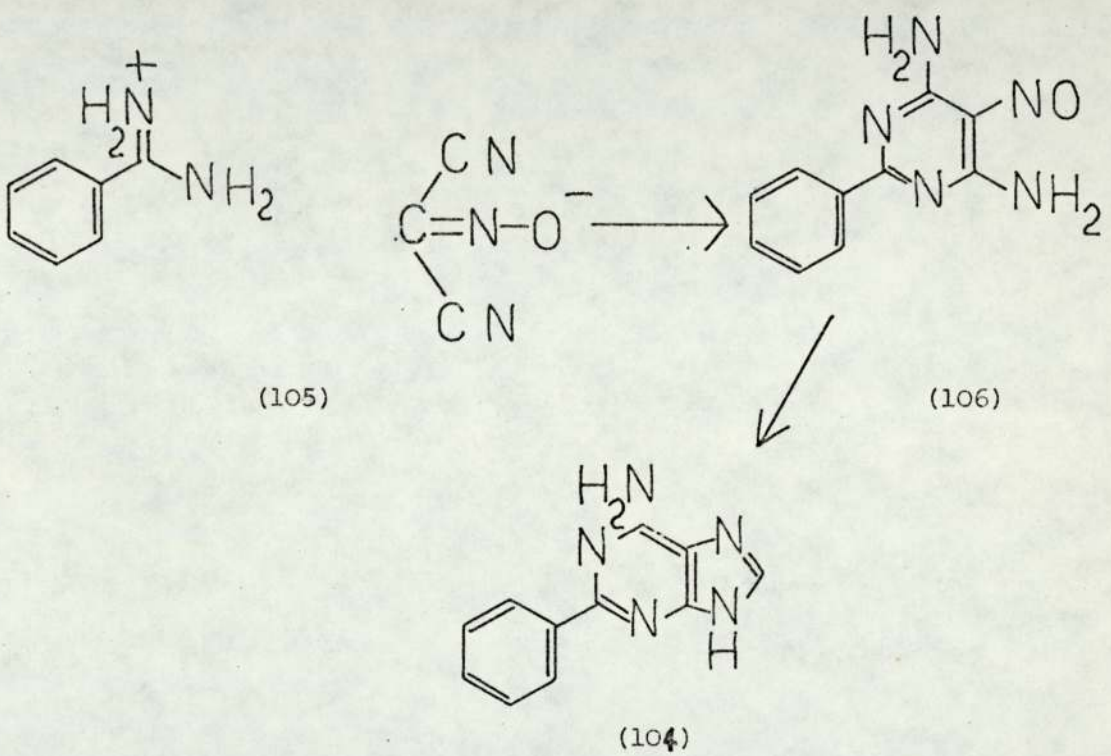


(102)

(103) (104)

Scheme 6.6

In boiling aqueous ethanol, the triazene (102) decomposed exclusively by the former route to afford 2-phenyladenine (104) in high yield. A sample of 2-phenyladenine was prepared unambiguously by the facile method of Taylor.[118] Thermal isomerisation of the benzamidinium salt of iso-nitrosomalononitrile (105) produced 2-phenyl-4,6-diamino-5-nitrosopyrimidine (106) which underwent a 'one pot' conversion to 2-phenyladenine when heated with formamidine, formic acid and sodium hydrosulphite.[118]



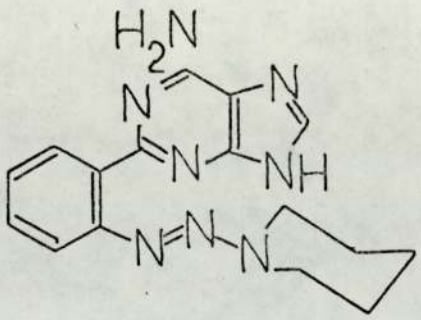
(105) (106) (104)

Scheme 6.7

The 2-phenyladenine prepared by this route was identical (uv, i.r., paper chromatography) to the product of decomposition of the triazene (102) in aqueous ethanol.

Thus a precedent was established for initial cyclisation on the aryl cyano group of the triazene (102). This is consistent with other observations on the unreactivity of cyanoazoles towards nucleophilic attack.[119]

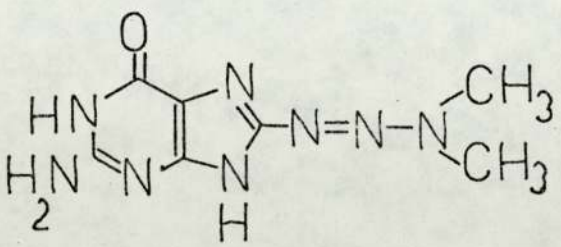
In boiling piperidine, the triazene (102) underwent extensive decomposition. At least nine products were detected (tlc and paper chromatography). The expected 2-[(2-piperidin-1-ylazo)phenyl]adenine (107) was not isolated.



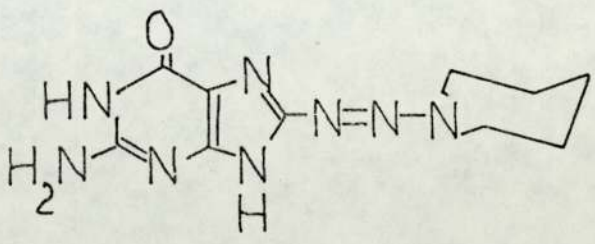
(107)

Synthesis of analogues of guanine

In 1961, Usbeck, Jones and Robins reported the synthesis of certain 8-triazenopurine nitrogen mustards.[120] To conclude the present work, the 8-triazenoguanine derivatives (108) and (109) were prepared for biological testing. The 8-aminoguanine required for this work was prepared by the method of Fischer.[32] Diazotised 3,5-dichloroaniline coupled with guanine in the 8-position; the resulting azo compound was reduced in alkaline sodium hydrosulphite to afford 8-aminoguanine. Diazotisation of 8-aminoguanine leads to the formation of the isolable solid 8-diazoguanine.[120] In the present work, addition of 8-diazoguanine to dimethylamine or piperidine in acetone, resulted in the formation of the triazenes (108) and (109) respectively.

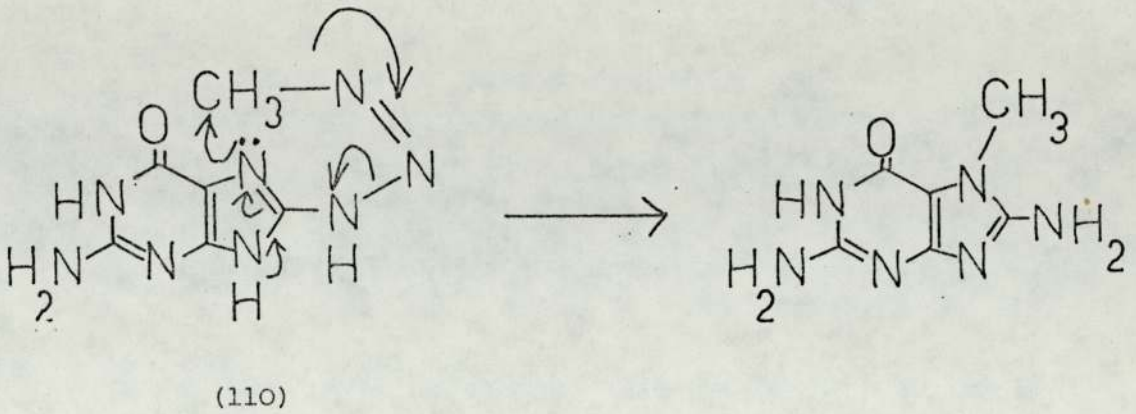


(108)



(109)

It was considered that the triazene (108) might be a candidate for oxidative demethylation in vivo and that the product of this transformation, the monomethyltriazene (110) could possibly decompose with intramolecular methylation of either N(7) or N(9) of the guanine fragment of the molecule. (Scheme 6.8).

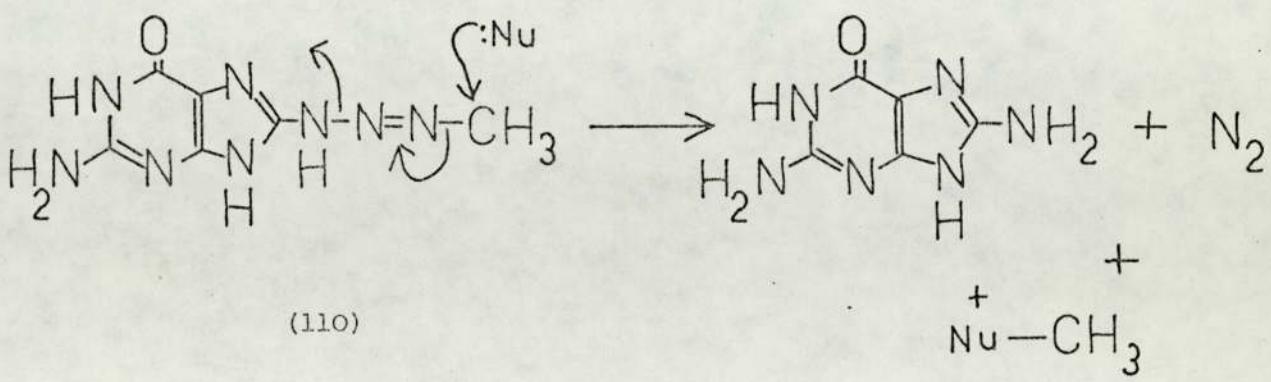


(110)

Scheme 6.8

To test this hypothesis, 8-diazoguanine was coupled with methylamine both in aqueous and in organic solvents. It was anticipated that the monomethyltriazene (110) would be formed and might decompose as indicated (Scheme 6.8). In the event, coupling took place, but decomposition took a different route, with methylation, presumably of solvent (Scheme 6.9). 8-Aminoguanine was isolated and the expected 8-amino-7(or 9)-methylguanine was not detected (tlc) in the reaction mixture.

The coupling of certain diazoimidazoles with primary amines has previously been shown to result in analogous regeneration of the amine from which the diazonium ion was derived.[121]



(110)

Scheme 6.9

Chapter 7

Experimental

(i) UV spectra were recorded on a Unicam SP 8000 spectrometer (in 95% ethanol) unless otherwise stated.

(ii) ^{1}H Nmr spectra were recorded on a Varian HA-100D spectrometer (Me_4Si as internal standard).

(iii) I.r. spectra were recorded (KBr discs) on a Perkin Elmer 157G spectrometer.

(iv) Mass spectra were measured at 70eV on an A.E.I. - G.E.C. MS 902 spectrometer with source temperature in the range 100-150°.

Experimental

4-Amino-2-(2-aminophenyl)quinazoline (3 2). —

A mixture of anthranilonitrile (3.0 g) and sodium hydride (0.6 g) in dimethylsulphoxide (13 ml) was stirred for 3 h at 0° and then for 21 h at 25°. Addition of 6N-hydrochloric acid (100 ml) to the mixture afforded the diaminoquinazoline dihydrochloride (3.0 g). It was identical (i.r. and mixed m.p.) to an authentic sample.[122]

Quinazolino[3,2-c]1,2,3-benzotriazin-8(7H)-imine (24)or[isomer(22)]. —

4-Amino-2-(2-aminophenyl)quinazoline dihydrochloride (1,39 g) in 2N-hydrochloric acid (30 ml) was diazotised at 0° for 30 mins with sodium nitrite (0.35 g) in water (3 ml). Basification of the suspension with ice-aqueous ammonia afforded the imine (0.55 g) identical (i.r) to an authentic sample.[74]

4-Amino-2-[2-(2-methylpiperidin-1-ylazo)phenyl]quinazoline(6f). —

1,3-Di-o-cyanophenyltriazene(7) (2.0 g) was boiled for 2 h in 2-methylpiperidine (5 ml). Trituration of the gum formed on evaporation of the solution with petrol-ether afforded a solid (81%) which crystallised from acetone as buff prisms, m.p. 181-183° (Found C, 69.1; H, 6.5; N, 24.4. $C_{20}H_{22}N_6$ requires (C, 69.4; H, 6.35; N, 24.3%).

4-Amino-2-[2-(3-methylpiperidin-1-ylazo)phenyl]quinazoline (6g). —

1,3-Di-o-cyanophenyltriazene(7) (2.0 g) was boiled in 3-methylpiperidine for 2 h. Addition of water afforded a solid (2.4 g) (88%) which crystallised from benzene-light petroleum, m.p. 158-160° (Found: C,69.3; H,6.4; N,24.55%).

Similarly prepared from 1,3-di-o-cyanophenyltriazene and 4-methylpiperidine was 4-amino-2-[2-(4-methylpiperidin-1-ylazo)phenyl]quinazoline (6 h) (90%), m.p. 146-147° (from toluene) (Found: C, 69.8; H, 6.2; N, 24.2%).

4-Amino-2-[2-(3,3-dimethyltriazen-1-yl)phenyl]quinazoline (6i). —
A solution of 1,3-di-o-cyanophenyltriazene (2.0 g) in anhydrous dimethylamine (10 ml) was kept at 4° for 30 days. The dimethyltriazenylphenylquinazoline (1.3 g) was slowly deposited on addition of water, and was recrystallised from hot water with m.p. 154-156° (Found: C, 65.8; H, 5.35; N, 29.0. $C_{16}H_{16}N_6$ requires C.65.75; H,5.5; N, 28.8%); T($CDCl_3$) 6.95 (S, 6H, 2xCH_3), 3.2 (br.s, 2H, NH_2) and 2.1 - 2.8(m,8H,Aryl-H).

2-[2-(3,3-Dimethyltriazen-1-yl)phenyl]quinazolin-4(3H)-one(19a). —
A mixture of the dimethyltriazene(6i) (1.5 g) and potassium hydroxide (10 g) in ethanol (20 ml) was boiled for 3.5 h, diluted with water and organic products extracted into ether (4 x 25 ml). The dried (sodium sulphate)ethereal extract was evaporated and the residue crystallised from ethanol. The dimethyltriazenylquinazolone (93%) had m.p. 212-213° (Found:C,65.1; H,5.15; N,24.1. $C_{16}H_{15}N_5O$ requires C,65.5;H,5.1;N,23.9%); γ_{max}(KBr) 1665 cm^{-1}(CO) T ($CDCl_3$) 6.30 and 6.50 (d, 6H, 2x CH_3).

Similarly prepared from the quinazolines (6b) and (6c) respectively. were the following: 2-[2-(pyrrolidin-1-ylazo)phenyl]quinazolin-4(3H)-one (65%); and 2[2-(morpholin-4-ylazo)phenyl]quinazolin-4(3H)-one (70%). These quinazolines were identical to authentic samples.[115]

1,3-Di-(2-cyano-4-bromophenyl)triazene(25). -
A suspension of 5-bromoanthranilonitrile[123] (3.94 g) in 2N-hydrochloric acid (25 ml) was treated at 0° with sodium nitrite (0.7 g) in water (5 ml) over 0.5 h. The mixture was diluted with ice-water (75 ml) and stirred at 0° for a further 2 h and kept at 4° overnight. The precipitated triazene (80%) crystallised from toluene as yellow needles, m.p. 185° (decomp.) (Found: C,41.7; H, 1.9; N, 17.2. $C_{14}H_7Br_2N_5$ requires C.41.5; H, 1.7; N, 17.3%); γ_{max}(KBr)3220(NH), 2239 and 2220 cm^{-1}(CN).

4-Amino-6-bromo-2-(2-amino-5-bromophenyl)quinazoline (27).—
(i) 1,3-Di-(2-cyano-4-bromophenyl)triazene(2.0 g) in ethanol (50ml)

containing Raney nickel (1.0 g) was treated at 60-65° over 1 h with hydrazine hydrate (5 x 1 ml). The hot solution was filtered through kieselguhr and the filtrate vacuum-evaporated to yield the diaminoquinazoline (1.8 g) which crystallised as yellow needles (from aqueous ethanol, or butanol) m.p. 285-287° (Found: C, 42.8; H, 2.5; N, 14.1. $C_{14}H_{10}Br_2N_4$ requires C,42.6; H, 2.5; N, 14.2%).

(ii) A mixture of finely powdered 5-bromoanthranilonitrile (9.8 g) and sodium hydride (0.6 g) in dimethylsulphoxide (13 ml) was stirred at 0° for 3 h and then at 25° for 21 h. Dilution of the mixture with water yielded the diaminoquinazoline (7.0 g) identical (i.r.)to the above sample.

4-Amino-6-bromo-2-(3-bromophenyl)quinazoline (26). — 1,3-Di-(2-cyano-4-bromophenyl)triazene (0.2 g) was boiled for 1 h in 75% aqueous ethanol (5 ml). Dilution of the solution with water afforded the quinazoline (53%) which was recrystallised from toluene, m.p. 257-258° (Found: C,44.7; H, 2.3; N, 10.9. $C_{14}H_9Br_2N_3$ requires C,44.4; H,2.1; N, 11.1%).

4-Amino-6-bromo-2-[5-bromo-2-(2-hydroxy-1-naphthylazo)phenyl]quinazoline (28).— An ethanolic solution (10 ml) of 2-naphthol (0.15 g) and 1,3-di-(2-cyano-4-bromophenyl)triazene (0.4 g) was boiled for 1 h. The precipitated naphthylazoquinazoline (89%) crystallised from dimethylformamide as red micro-rosettes, m.p. 316-318° (Found:C,52.8; H, 2.9; N, 12.85. $C_{24}H_{15}Br_2N_5O$ requires C,52.5; H,2.7; N, 12.75%).

2-(4-Amino-6-bromoquinazolin-2-yl)-4-bromophenyldiazonium chloride dihydrochoride (35). — 4-Amino-6-bromo-2-(2-amino-5-bromophenyl)quinazoline (2.0 g) was suspended in 6N-hydrochloric acid at 0° and treated with a solution of sodium nitrite (0.35 g) in water (3 ml). After 1.5 h the crude diazonium chloride dihydrochloride (70%) was collected and crystallised from 6N-hydrochloric acid as yellow prisms, m.p. 210° (decomp.) (Found: C, 33.3; H, 2.3; N, 13.7. $C_{14}H_8Br_2C_1N_5.2HCl$ requires C, 33.3; H, 1.9; N, 13.6%); γ_{max}(KBr) 2260cm^{-1} (N_2^+).

2,10-Dibromoquinazolin [3,2-c]-1,2,3-benzotriazin-8(7H)-imine (30) [or isomer (29)]. — Crystallisation of the diazonium chloride dihydrochloride (35) from ether-methanol (1:1) afforded the benzotriazin-imine monohydrochloride (95%) as pink needles, m.p. 213° (decomp.) (Found: C, 38.4; H, 2.3; N, 15.5. $C_{14}H_7Br_2N_5.HCl$ requires C, 38.05; H, 1.8; N, 15.9%).

4-Amino-6-bromo-2-[5-bromo-2-(piperidin-1-ylazo)phenyl] quinazoline (20a).— (i) 1,3-Di-(2-cyano-4-bromophenyl)triazene (0.8 g) was boiled for 2 h in piperidine and the solution diluted with water at 0°. The quinazoline (62%) crystallised from acetone as white micro-prisms, m.p. 198-200° (Found C, 46.7; H, 3.9; N,17.1. $C_{19}H_{18}Br_2N_6$ requires C, 46.5; H, 3.6; N, 17.2%).

(ii) 2, 10-Dibromoquinazolino [3,2-C]-1,2,3-benzotriazin-8(7H) imine hydrochloride (1.0 g) was boiled in piperidine (5 ml) for 1 h. The solution was diluted with ice-water (50 ml) to afford the quinazoline (20a) (0.65 g), identical (i.r.) to the above sample.

The following brominated triazenoquinazolines in the series (20) were prepared by reacting 1,3-di-(2-cyano-4-bromophenyl)triazene in the respective refluxing secondary amines for 2 h. and diluting the resultant solutions with water:

4-Amino-6-bromo-2-[5-bromo-2-(pyrrolidin-1-ylazo)phenyl] quinazoline (20b) (70%), m.p. 225-227° (from aqueous ethanol) (Found: C, 45.5; H, 3.2; N,17.7. $C_{18}H_{16}Br_2N_6$ requires C, 45.4; H, 3.4; N, 17.6%);

4-Amino-6-bromo-2-[5-bromo-2-(morpholin-4-ylazo)phenyl] quinazoline (20c) (78%), m.p. 203-205° (needles, from toluene) (Found: C, 43.8; H, 3.6; N, 17.5. $C_{18}H_{16}Br_2N_6O$ requires C, 43.9; H, 3.25; N, 17.1%);

4-Amino-6-bromo-2-[5-bromo-2-(2-methylpiperidin-1-ylazo)phenyl] quinazoline (20d) (85%), m.p. 225-227° (from aqueous dimethylformamide) (Found: C,47.8; H,4.2; N,16.6. $C_{20}H_{20}Br_2N_6$ requires C, 47.6; H, 4.0; N, 16.7%);

4-Amino-6-bromo-2-[5-bromo-2-(3-methylpiperidin-1-ylazo)phenyl]quinazoline (20e) (72%), m.p. 230-231° (from aqueous methanol) (Found: C, 47.6; H, 4.2; N, 16.9%);

4-Amino-6-bromo-2-[5-bromo-2-(4-methylpiperidin-1-ylazo)phenyl]quinazoline hydrate (20f) (84%), m.p. 198°-200° with sintering at 105° (prisms from aqueous methanol) (Found: C,46.1; H, 4.5; N, 15.8. $C_{20}H_{20}Br_2N_6.H_2O$ requires C, 45.9; H, 4.2; N, 16.1%).

Reactions of 1,3-diaryltriazenes in piperidine and morpholine.—

(i) 1,3-Di-p-tolytriazene and 1,3-di-p-chlorophenyltriazene were recovered in 90% yield from boiling piperidine or morpholine (3h). No new products were detected by tlc, and there was no change in the uv spectra of the solutions.

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(ii) 1,3-Di-p-nitrophenyltriazene was recovered in 95% yield from either boiling piperidine or morpholine (3h). There was no change in the uv spectra of the solutions during the reaction and no new products were detected (tlc). Similarly, no new products were detected (uv, tlc) when 1-phenylazopiperidine was boiled in 2-ethyoxyethanol with p-toluidine for 15 h or in ethanol containing p-nitroaniline for 10 h.

Reactions of 4-amino-2-[2-piperidin-1-ylazo)phenyl]quinazoline hydrate (6a). —

(i) The quinazoline hydrate (1g) was stirred in 6N-hydrochloric acid (20 ml) for 1 h at 20°. Basification of the solution with ice-aqueous sodium hydroxide afforded quinazolino[3,2-c]-1,2,3-benzotriazin-8(7H)-imine or isomer (0.3 g), identical (ir, m.p.) to an authentic sample.[74]

(ii) A solution of the quinazoline hydrate (2 g) in acetic acid (10 ml) was heated at 80° for 40 minutes. Basification of the mixture with ice-aqueous ammonia afforded the same tetracyclic triazine as in (i) above, identical (i.r.) to an authentic sample.

(iii) When the quinazoline hydrate (6a) was boiled in 4N – sulphuric acid (25 ml) for 2h, and cooled, 2-(2-hydroxyphenyl) quinazolin-4(3H)--one(45) (0.25g) was deposited and was identical (m.p. and i.r.) to an authentic sample.[125]

(iv) 4-Amino-2-(2-azidophenyl)quinazoline (49).–

The quinazoline hydrate (6a) (1.0 g) was boiled in acetic acid (10 ml)with sodium azide (4 mol. equiv.) for 15 min. The mixture was diluted with water, extracted into chloroform and the chloroform layer vacuum-evaporated. A benzene solution of the oily residue was chromatographically fractionated on an alumina column. The first yellow band, eluted with benzene, afforded the azidophenyl-quinazoline (27%), m.p. 143-145° (from ethanol) (Found: C.64.4; H, 3.9; N, 32.2. $C_{14}H_{10}N_6$ requires C,64.1; H, 3.8; N, 32.1%).

Cyclisation of the azidophenylquinazoline in boiling acetic acid was complete in 1 h. The products, identified as the isomeric indazoloquinazolines (50) and (51), were recognised as intensely fluorescent spots by tlc [on alumina (0.25 mm) with benzene as developing solvent] with identical r.f. values to authentic samples.[74].

(v) 4-Amino-2-(2-iodophenyl)quinazoline hydroiodide(47).–

The quinazoline hydrate (6a)(1 g) was boiled for 2 h in acetic acid (10 ml) containing sodium iodide (2 mol. equiv.). The solution was diluted with water to afford the brown hydroiodide (0.9 g) (66%), m.p. 254° indef. (from aqueous ethanol); (Found: C, 35.5; H, 2.3 ; N, 8.7. $C_{14}H_{10}N_3I \cdot HI$ requires C, 35.4; H, 2.3; N 8.8%).Basification of the salt with ice-aqueous ammonia afforded the free base, m.p. 191-193° (from ethanol) (Found: C,48.9; H, 3.05; N, 12.4. $C_{14}H_{10}N_3$ requires C48.9·H, 2.9; N, 12.1%).

(vi) The quinazoline hydrate (6a) (1g) was boiled in acetic acid (10 ml) containing copper bronze for 40 minutes. The solution was filtered through kieselguhr and the filtrate basified with ice-aqueous ammonia to afford 4-amino-2-phenylquinazoline (45) (90%), identical (i.r.) to an authentic sample.[122]

(vii) The quinazoline hydrate (1.0 g) was boiled in ethylene glycol (15 ml) for 1 h and the solution diluted with water. The chilled solution slowly deposited 4-amino-2-phenylquinazoline (40%) with identical i.r. to the sample above.

(viii) The quinazoline hydrate (0.1 g) was photolysed in methanol (1 litre) with a 100 W medium-pressure lamp in an Hanovia Photochemical Reactor equipped with a pyrex filter. After 24 h the UV spectrum of the photolysate was identical to that of a pure sample of 4-amino-2-phenylquinazoline (45) [λ_{max}(EtoH) 254, 285 (infl.), 304, 321(infl.) and 333 nm (infl.)[122]]. Tlc examination of the concentrated solution on silica gel employing ether: chloroform: methanol (10:2:1) as developing solvent confirmed the identification of the photo-product.

(ix) A sample of the quinazoline hydrate (0.1 g) in ethanol (25 ml) was exposed to laboratory light for 75 days. Tlc examination of the solution (as above) showed it to contain starting material and 4-amino-2-phenylquinazoline (45).

(x) A solution of the quinazoline hydrate (1.0 g) in ethanol (20 ml) containing Raney nickel (1.0 g) was treated over 1 h with hydrazine hydrate (5 x 1 ml). The mixture was filtered through Kieselguhr, evaporated and triturated with 6N-hydrochloric acid. The product 4-amino-2-(2-aminophenyl)quinazoline dihydrochloride (31) (92%) was identical to an authentic sample.[122]

Reactions of 2-[2-(piperidin-1-ylazo)phenyl]quinazolin-4(3H)-one(19c). —

(i) 2-(2-Azidophenyl)quinazolin-4(3H)-one(52)

The quinazolinone (19c)(1 g) was boiled for 1 h in acetic acid (10 ml) containing sodium azide (4 mol . equiv.). The solution was diluted with water to afford the quinazolinone (52) with m.p. > 300° (from methanol) (Found: C, 64.1; H, 3.5; N, 26.3. $C_{14}H_9H_5O$ requires C, 63.9; H, 3.4; N, 26.6%); γ_{max} (KBr) 3200-2750 (bonded NH, OH), 2140 and 2100 (N_3), 1680^{-1} (CO).

(ii) The quinazoline (19c) (1g) was boiled for 40 min. in acetic acid containing copper bronze and filtered through kieselguhr. Dilution of the solution with water afforded 2-phenylquinazolin -4(3H)-one (75%) identical (i.r.) to an authentic sample.[94]

Reactions of 4-amino-6-bromo-2-[5-bromo-2-(piperidin-1-ylazo)phenyl]-quinazoline (20a). —

(i) The quinazoline (20a) (0.6 g) was stirred at 25° in 6N-hydrochloric acid (15 ml) for 5 h to afford a yellow solid identical to 2-(4-amino-6-bromoquinazolin-2-yl)-4-bromophenyldiazonium chloride dihydrochloride (35).

Reaction of 4-amino-2-[2-(piperidin-1-ylazo)phenyl]quinazoline (6a) with cysteine.—

(i) The quinazoline (6a) was recovered in 95% yield after being stirred in aqueous dimethylformamide(1:1) containing cysteine (1 mol. equiv.) at 25° for 3 h.

(ii) Similarly the quinazoline was recovered in 95% yield from treatment with cysteine (1 mol. equiv.) in IN-potassium hydroxide solution for 3 h

(iii) The quinazoline (6a) (1 g) was treated with 6N-hydrochloric acid at 25° for 1 h. The crude 2-(4-aminoquinazolin-2-yl)phenyldiazonium chloride (34) was collected and added to a stirred solution of cysteine (0.3 g) in 1N-potassium hydroxide (10 ml). at 0°. The mixture was stirred at 0° for a further 2 h and diluted with water to afford quinazolino-[3,2-c]-1,2,3-benzotriazin-8(7H)-imine, identical (i.r.) to a previously prepared sample.

Reactions of 1,2,3-benzotriazin-4(3H)-one and derivatives in acetic acid.

(i) 2-Azidobenzamide (55 R=N_3). — 1,2,3-Benzotriazin-4(3H)-one (0.6 g) and sodium azide (4 mol. equiv.) were boiled in acetic acid (5 ml) for 1 h and the mixture diluted with water. The mixture was extracted into chloroform and the chloroform evaporated to afford a gum. Trituration of the gum with benzene yielded 2-azidobenzamide (95%) which crystallised from benzene as white needles, m.p. 135-136° (Lit.,[126] m.p. 135-136°); γ_{max} (KBr)

3378 and 3170 (NH), 2140 and 2110 (N_3), and 1653cm^{-1} (CO).

(ii) 2-Iodobenzamide (55;R=I). -- A solution of acetic acid (10 ml) containing 1,2,3-benzotriazin-4(3H)-one (1.0 g) and sodium iodide (2 mol. equiv.) was boiled(1 h)and diluted with water (20 ml). The precipitated iodobenzamide (85%) crystallised from aqueous ethanol as white crystals, m.p. 182-184° (Lit.,[127] m.p. 183°); γ_{max} 3350 and 3190 (NH), 1640cm^{-1} (CO).

(iii) When 1,2,3-benzotriazin-4(3H)-one (1 g) was boiled(1 h)in acetic acid (10 ml) containing 4 mol. equiv. of sodium bromide, chloride, cyanide, sulphite or acetate, dilution with water afforded the starting material in 90-95% yield.

(iv) Similarly, when derivatives of 1,2,3-benzotriazin-4(3H)-one substituted in the 3-position by, phenethyl, benzyl or ethyl groups were boiled in acetic acid containing sodium azide (4 mol. equiv.) no reaction occurred and starting materials (>95%) were recovered.

Reactions of aryl-dialkyltriazenes with methyl iodide

(i) 1-(Phenylazo)piperidine (1 g) was refluxed for 5 h in methanol (10 ml) containing methyl iodide (2 ml). Addition of excess ether to the cooled solution afforded N, N-dimethylpiperidinium iodide (0.3 g) identical (i.r. and m.p.) to a sample unambiguously prepared from N-methylpiperidine and methyl iodide. Gas chromatographic analysis of the reaction mixture was performed on a 2M Carbowax 20 m KoH column, run isothermally at 190° [carrier gas, N_2; 40ml/min; Perkin Elmer F11 gas chromatograph (FID)]. Authentic iodobenzene had the same retention time as a fraction present in the reaction mixture.

(ii) 1-(Phenylazo)piperidine (1 g) and methyl iodide (1 ml) were refluxed for 5 h in benzene (10 ml). The cooled solution deposited N, N -dimethylpiperidinium iodide, identical (i.r.) to the above sample.

(iii) 1-(Phenylazo)piperidine was recovered in 100% yield from refluxing methyl iodide alone (5 h).

(iv) 1-(p-Tolylazo)piperidine (1 g) was refluxed in methyl iodide for

11 h to afford N, N-dimethylpiperidinium iodide, identical (i.r.) to the previous samples.

(v) 1-(p-Nitrophenylazo)piperidine was recovered in 95% yield from refluxing methanol or ethanol containing methyl iodide (24 h).

4-Amino-1-methyl-2-[2-(piperidin-1-ylazo)phenyl]quinazolinium iodide (68). — 4-Amino-2-[2-(piperidin-1-ylazo)phenyl]quinazoline hydrate (6a) (3.0 g) and methyl iodide (3 ml) were boiled in tetrahydrofuran (20 ml) for 40 min. The precipitated yellow solid was collected and fractionally crystallised from ethanol. The least soluble fraction was 4-amino-2-[(piperidin-1-ylazo)-phenyl]quinazoline hydroiodide (67) (15%), m.p. 177° (yellow needles) (Found: C, 50.0; H, 4.8; N, 17.95. $C_{19}H_{20}N_6HI$ requires C,49.6; H, 4.6; N, 18.3%). A solution of the hydrciodide in methanol was treated with ice-aqueous ammonia to afford the free base (6a).

The second crystalline fraction (prisms) was the methylquinazolinium iodide (68) (80%), m.p. 234-236° (Found: C, 50.4; H, 4.9; N, 17.6. $C_{20}H_{23}IN_6$ requires C, 50.6; H, 4.85; N, 17.7%). 1-Methyl-2-[2-(piperidin-1-ylazo)phenyl]quinazolin-4(1H)-one (69). — An ethanolic solution (20 ml) of the methylquinazolinium iodide (68) (3 g) was poured onto ice-aqueous ammonia and the suspension slowly deposited the methylquinazolinone (69) (90%), m.p. 213-215° (white needles from aqueous ethanol); (Found: C, 69.2; H, 6.2; N, 20.5. $C_{20}H_{21}N_5O$ requires C, 69.2; H, 6.05; N, 20.2%). ν_{max} (KBr) 1642cm^{-1} (CO).

Properties of 1-methyl-2-[2-(piperidin-1-ylazo)phenyl]quinazolin-4(1H)-one

(i) The methylquinazolinone (0.6 g) in ethanol (10 ml) containing Raney nickel (1 g) was treated at 60-65° with hydrazine hydrate (5 x 1 ml) for 1 h and filtered through kieselguhr. The evaporated solution was triturated with ethanol to afford the starting material in 90% yield.

(ii) The methylquinazolinone (0.7 g) was boiled in ethylene glycol (5 ml) for 6 h and poured onto ice-water (50 ml), to afford the starting material (75%).

(iii) The methylquinazolinone (0.9 g) was stirred at 0° in 6N-hydrochloric acid (7 ml) containing hypophosphorous acid (50%) (4 ml) for 1 h, then kept at 4° for 12 h. The solution was brought to pH 10 with dilute sodium hydroxide solution. The white solid (80%) was shown to be starting material (i.r.).

(iv) 2-Phenylquinazolin-4(3H)-one (70). —

A solution of the methylquinazolinone (0.5 g) in acetic acid (5 ml) containing copper bronze (0.3 g) was boiled for 2 h. The filtered concentrated solution afforded 2-phenylquinazolin-4(3H)-one (63%) with identical i.r. to an authentic sample.[94] A further sample of 2-phenylquinazolin-4(3H)-one was prepared by the following route:

2-methylbenzamide (0.4 g) was dissolved in boiling p-cymene and benzoyl chloride (0.6 ml) was added dropwise over 5 minutes.

The white product which immediately formed was identical (i.r.,m.p.) to an authentic sample of 2-phenylquinazolin-4(3H)-one.

3- Methyl-2-phenylquinazolin-4(3H)-one

3- Methyl-2-phenylquinazolin-4(3H)-one was prepared by cyclisation of 2-benzamido-N methylbenzamide. It had m.p. 134 - 135° (Lit.,[97] m.p. 136-138°). The quinazolone (0.3 g) was boiled in acetic acid (5 ml) containing copper bronze (0.1 g) for 1 h. Dilution of the filtered solution with excess sodium hydroxide afforded a precipitate (0.28 g) identical (i.r.) to the starting material.

4-Amino-2-[2-(piperidin-1-ylazo)phenyl]quinazolinium benzoate.—

A solution of 4-amino-2-[2-(piperidin-1-ylazo)phenyl]quinazoline hydrate (3.32 g) in tetrahydrofuran (60 ml) containing perbenzoic acid[128] (1.38 g) in benzene (30 ml) was kept at 4° for 12 days. The evaporated solution afforded a gum which was triturated with acetone to produce the benzoate

salt of the starting material, m.p. 139-141° (from acetone). (Found: (C,68.3; H, 5.6; N, 17.3. $C_{26}H_{26}N_6O_2$ requires C,68.7; H, 5.7; N, 17.5%).

A solution of the salt in methanol treated with ice-aqueous ammonia afforded the starting material.

Permanganate oxidation of 4-amino-2-[2-(piperidin-1-ylazo)phenyl]-quinazoline hydrate (6a). — The quinazoline hydrate (0.8 g) was dissolved in acetone (60 ml). To this solution was added potassium bicarbonate (0.6 g) and potassium permanganate (0.9 g) in water (60 ml) and the mixture was stirred at 25° for 10 h. The suspension was filtered through Kieselguhr and extracted into chloroform (3 x 80 ml). The chloroform extracts were concentrated to 5 ml) and kept at 4° for 12 h. 1-[2-(4-Aminoquinazolin-2-yl)phenylazo]piperidin-2-one (25%) was collected with m.p. 202-204° (from chloroform) (Found 65.8; H,5.1; N, 24.?. $C_{19}H_{18}N_6$ requires C,65.9; H, 5.2; N, 24.3%);

γ_{max} (KBr) 3460, 3320, 3210 (NH), 1650 cm^{-1} (CO); τ(CD_3OD) 6.45m, 7.40m, 8.25$_m$ (2H, 2H, 4H) [N.B. 1(4-Chlorophenylazo)piperidin-2-one[105] has τ($CDCl_3$) 6.22m, 7.38m 8.20m(2H, 2H, 4H)];

N-benzoylpiperidin-2-one[105] has τ($CDCl_3$) 6.21m, 7.43m, 8.09m (2H, 2H, 4H).

The residual chloroform extract was subjected to chromatographic analysis on silica plates (1 mm) using ether: chloroform: methanol (10:2:1) as developing solvent. The band which co-chromatographed with authentic 4-amino-2-(2-aminophenyl) quinazoline was removed and dissolved in ethanol. A uv spectrum of this diluted solution was identical to that of the authentic diamine [λ_{max} (EtoH) 330 (infl),300 (infl), 262(sh), 234 nm].

The triazenoquinazolines (6c) (6f) (6h) and (6i) were oxidised as above: in all cases 4-amino-2-(2-aminophenyl) quinazoline was identified as described.

δ-Valerolactone

The reaction mixture from permanganate oxidation of the quinazoline hydrate

(6a) was filtered through Kieselguhr and stood for 2 h at 25° with 1% Sodium bicarbonate solution (100 ml). The solution was shaken with chloroform (2 x 100 ml) and the aqueous layer acidifed (pH) with hydrochloric acid. The acid solution was extracted into chloroform(2 x 50 ml) and the chloroform solution reduced to 5 ml. The i.r. spectrum (film) of this concentrate was identical to the i.r. spectrum of a solution of authentic δ-valerolactone in chloroform. Gas chromatographic analysis, performed isothermally at 150° on a 1MSE30 column with nitrogen gas (40 ml/min) on a Perkin Elmer F11 gas chromatograph (F.I.D.) confirmed the presence of δ-valerolactone in the concentrate above. The concentrate was treated with hydroxylamine hydrochloride to convert lactones to hydroxamic acid derivatives[129], and chromatographed on silica plates (0.25 nm) (ethanol as developing solvent) with a similarly treated sample of authentic δ-valerolactone in chloroform. The plates were sprayed with 5% ferric chloride solution. The two magenta spots had identical rf values.

Permanganate oxidation of N-nitrosopiperidine

A solution of nitrosopiperidine (0.28 g)[130] in acetone 60 (ml) was treated with potassium permanganate (0.9 g) and potassium bicarbonate (0.6 g) in water (60 ml) and stirred at 25° for 20 h. The filtered reaction mixture was purified as described for the isolation of lactones and the resulting chloroform concentrate reacted with hydroxylamine hydrochloride to convert any lactones present to the hydroxamic acid derivatives. The resulting solution was shown to contain the hydroxamic acid derivative of δ-valerolactone since it co-chromatographed (t.l.c.) with a similarly treated solution of authentic lactone in chloroform.

Udenfriend oxidation of 4-amino-2-[2-(piperidin-1-ylazo)phenyl] quinazoline:

To a solution containing sodium hydrogen phosphate (4.5 g) and potassium di-hydrogen phosphate (3.5g) in water (200 ml) was added ascorbic acid (0.82 g in water), ethylenediaminetetracetic acid (0.8 g in min. warm water) and ferrous

ammonium sulphate hexahydrate (0.17 g). The resulting solution was made up to 250 ml with water and added to a solution of the quinazoline hydrate (0.15 g) in acetone (100 ml). The flask was protected from light and shaken at 37° for 14 h. A chloroform extract (3 x 100 ml) of the solution was chromatographed on silica employing ether: chloroform: methanol (10:2:1) as developing solvent. The band corresponding to 4-amino-2-phenylquinazoline was removed and washed with ethanol. A uv spectrum of this diluted solution was identical to authentic 4-amino-2-phenylquinazoline.

Reactions of 2-azaadenine-1-oxide. —

(i) 2-Azaadenine-1-oxide (200 mg) was boiled for 2 h in piperidine (20 ml). Trituration of the evaporated solution with water afforded the starting material in 90% yield.

(ii) 2-Azaadenine-1-oxide (300 mg) was boiled in collidine (5 ml) for 5 h. Starting material (90%) was recovered from the cooled solution.

(iii) A mixture of 2-azaadenine-1-oxide (0.5 g) and ammonium acetate (2 g) was fused at 200° for 15 min. Starting material (80%) was recovered from the cooled mixture.

5-Amino-4-cyano-2-(4-cyanoimidazol-5-ylazo)imidazole (101). —

A solution of 5-aminoimidazole-4-carbonitrile (0.4 g) in 2N-hydrochloric acid (4 ml) was added dropwise to a solution of sodium nitrite (0.26g) in water (4 ml) at 0° over 45 min. After addition of excess urea, the resulting suspension was added in portions to a solution of 5-aminoimidazole-4-carbonitrile (0.4 g) in water (5 ml) containing sodium acetate (3 g). Stirring was continued for 1 hr. The azoimidazole (101) (70%) crystallised from aqueous dimethylformamide with m.p. >300° (Found: C, 40.3; H, 2.3; N, 57.0; $C_8H_5N_9$ requires C, 40.7; H, 2.1; N, 57.2%): γ_{max} (KBr) 2210 (CN), 3180 and 3320 cm^{-1} (NH); λ_{max} (EtOH) 512 nm.

1-(2-Cyanophenyl) -3-(4-cyanoimidazol-5-yl)triazene (102). -

5 - Aminoimidazole-4-carbonitrile (0.4 g) in 2N-hydrochloric acid (4 ml) was treated at 0° with a solution of sodium nitrite (0.26 g) in water (4 ml) over 45 min. Excess urea was added followed by o-aminobenzonitrile (0.4 g) and ice-water (15 ml). Stirring was continued for 2 h. The precipitated triazene (102) (80%) which was collected and dried in a vacuum desiccator had m.p. 187° decomp. (from acetone) (Found:C,55.3; H, 3.1; N, 41.2. $C_{11}H_7N_7$ requires C,55.7; H, 2.95; N, 41.1%);

γ_{max} (KBr) 2223(CN) 3200cm^{-1} (NH).

Properties of 1-(2-cyanophenyl)-3-(4-cyanoimidazol-5-yl)triazene (102). -

(i) 1-(2-Cyanophenyl)-3-(4-cyanoimidazol-5-yl)triazene (102) (0.5 g) was boiled for 1 h in 90% aqueous ethanol (10 ml) and the solvent evaporated. 2-Phenyladenine (75%) crystallised from water with m.p. 325° (Lit., 321°), and i.r. & u.v. identical to an authentic sample [λ_{max} (water) 237, 268 nm]. The 2-phenyladenine prepared by this route co-chromatographed with an authentic sample [paper chromatography (Whatman No. 1) employing 3% ammonium chloride: isopropanol (2:1) as developing solvent; rf = 0.53].

(ii) The triazene (0.5 g) and piperidine (7 ml) were boiled for 1 hr. Tlc [silica (0.25 mn) employing ether: chloroform: methanol (10:2:1) as developing solvent] performed on the reaction mixture showed at least nine products. Paper chromatography (as above) showed the presence of 2-phenyladenine. No products were isolated.

1-(Guanin-8-ylazo)piperidine(107). -

8-Aminoguanine (0.9 g) was dissolved in 5% potassium hydroxide (10 ml) containing sodium nitrite (0.38g) and the solution added dropwise to hydrochloric acid (14 ml) over 10 min with stirring, at 10°. 8-Diazoguanine (0.6 g), [γ_{max} nujol 2240 cm^{-1} (N_2^+)] was collected, washed with methanol, dried in a vacuum desiccator ($CaCl_2$), and suspended in acetone (10 ml) containing piperidine (0.5 g). The suspension was stirred at 20° for 1.5 h to afford the guaninylazopiperidine hydrate, (lime-green rosettes, from

aqueous dimethylformamide) (Found:C, 43.1; H, 5.9; N, 39.8. $C_{10}H_{14}N_8O$. H_2O requires C 42.75; H, 5.7; N40.0%).

8 - (3,3-Dimethyltriazen-1-yl)guanine (108). —

8-Diazoguanine (0.6 g) was suspended in acetone (30 ml) and stirred with anhydrous dimethylamine at 0° for 2 h. The triazenylguanine hydrate (60%) had m.p. 240° (lime-green rosettes from water). (Found:C, 34.8; H 5.2; N, 46.3. $C_7H_{10}N_8O$. H_2O requires C, 35.0; H, 5.0; N, 46.6%).

8-Aminoguanine.

8-Diazoguanine (0.5 g) was suspended in acetone (20 ml) and methylamine (generated from methylamine hydrochloride and 10 N - sodium hydroxide) was bubbled through the suspension for 1 h. The mixture was stirred for 15 h to afford 8-aminoguanine (75%) (as sulphate from 10% sulphuric acid) chromatographically pure [paper chromatography Whatman No 1; employing 3% ammonium chloride: isopropanol (2:1) as solvent (rf = 0.18)], with uv spectrum identical to an authentic sample [λmax (water) 287,247nm].

Biological Results

All tests were performed according to protocols laid down by the drug evaluation branch, National Cancer Institute, Bethesda, Maryland.[131]

Table 1

Activity v L1210 leukaemia in vivo, CDF_1 mice

Tests were performed by intraperitoneal innoculation of male CDF_1 mice with 10^5 L1210 cells. Drug treatment was initiated 24h later. Tumour-inhibitory activity was evaluated by comparing mean survival times of innoculated mice receiving drug treatment with survival time of untreated controls.

Compound	Activity (% increase in lifespan)	Dose mg/Kg
6a	inactive	400
6f	inactive	400
6g	inactive	400
6h	inactive	400
27	inactive	400
31	inactive	400
20a	inactive	400
20b	inactive	400
20c	inactive	400
20d	inactive	400
20e	inactive	400
20f	inactive	400
2o or isomer 30	inactive	400
35	inactive	400
22 or isomer 24	inactive	400
diazo-ICA	4	5
DTIC	93	480

Table 2

Activity v TLX5 lymphoma

Tests performed at Chester Beatty Research Institute, London, using female CBA/LAC mice innoculated with 10^5 TLX5 cells.

Compound	Max % ILS
19a	31.9
BCNU	39.2
6a	inactive
6i	inactive
6b	inactive
108	7.2

BCNU - bis-[2-chloroethyl]-N-nitrosourea.

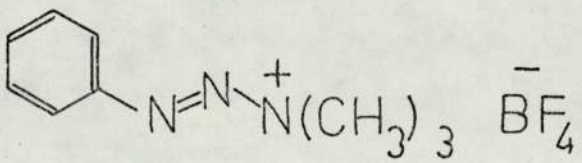
ILS - increase in lifespan.

Discussion

None of the novel triazenes were active against H-Ep2 in cell culture. Surprisingly, 4-amino-2-[2-(piperidin-1-ylazo)phenyl] quinazoline (6a), which was active against HEp2 was inactive against the L1210 and TLX5 tumours *in vivo*. This lack of activity may be due to pharmacokinetic factors, eg. failure to gain access to the tumour cell because of the strongly basic character of the molecule. The quinazoline (6a) was shown to form benzoate and hydroiodide salts. The dimethylquinazolinone (19a) exhibited notable activity against the TLX5 lymphoma (*in vivo*), comparable to BCNU, whereas the corresponding 4-amino-2-[3,3-dimethyltriazen-1-yl)phenyl] - quinazoline (6i) was inactive against this tumour. These differences may also be due to the relative basicity of these molecules. Perhaps further quinazolinones should be examined for biological activity.

(19a)

Pharmacological studies were performed on 1-phenyl-3,3,3-trimethyltriazenium fluoroborate, (PTF), synthesised in collaboration with Stevens and Meredith.[99] Studies were conducted to determine if PTF has cholinergic or antagonistic activity, using freshly prepared solutions of PTF in 0.5% aqueous dimethylsulphoxide.



PTF

PTF caused a contraction of the guinea-pig ileum, and in concentrations from 10µg/ml to 320 µg/ml produced a graded dose/response curve. These contractions were antagonised by atropine (0.1 µg/ml) but not by mepyramine (10 µg/ml). These effects of PTF were shown to be similar to the responses of the tissue

to acetylcholine (ACh). In doses up to 1mg/ml PTF had no effect on rat hemi-diaphragm muscle contractions elicited by stimulation of the phrenic nerve at a frequency of 0.1 Hz.

Contractures of the frog rectus abdominis muscle were obtained in response to addition of PTF to the preparation in concentrations from 10 µg/ml to 500 µg/ml; these contractures were competitively antagonised by d-tubocurarine. The responses of this muscle to PTF were similar to the responses elicited by ACh. In each of these pharmacological preparations DMSO alone (0.5% in aqueous solution) had no effect.

The results of these preliminary pharmacological tests suggest that PTF has a cholinergic agonist action at nicotinic and muscarinic sites and that PTF and other chemically reactive triazenium salts may be of use in studies on the cholinergic receptor.

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