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Potential Antitumour Pro-Drugs – 1-Aryl-3-arylthiomethyl-3-methyltriazenes and 1-Aryl-3-aryloxymethyl-3-methyltriazenes

By

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Submitted in partial fulfilment of the requirements
for the degree of Doctor of Philosophy

at

Dalhousie University
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Dedication

This thesis is dedicated to Peter Hill whose blend of enthusiasm and cynicism put me on the right road.

TABLE OF CONTENTS

ILLUSTRATIONS

TABLES

ABSTRACT

The literature relating to antitumour triazenes has been reviewed with particular regard to the 3,3-dimethyltriazenes and their α-substituted derivatives. Their metabolism and mechanism of action in biological systems has been examined and used as a rationale for the experimental efforts of the work.

A series of novel 1-aryl-3-arylthiomethyl-3-methyltriazenes have been synthesized and extensively characterized by a variety of techniques. These compounds have been tested *in vivo* for antitumour response and do show a degree of activity.

A further series of 1-aryl-3-aryloxymethyl-3-methyltriazenes were prepared and characterized. In addition, a novel series of 1-aryl-3-arylmethyl-3-methyltriazenes were characterized as a side product of the synthesis. The aryloxymethyl compounds were subjected to extensive experiments to determine their behaviour with respect to hydrolysis in aqueous media, an important consideration for a potential pro-drug that depends upon decomposition for its efficacy. As part of this work we were able, for the first time to measure the rate of initial decay of a hydroxymethyltriazene derivative by following the rate of formation of the nitrophenolate ion liberated in this first step.

The N(2)-N(3) rotation of hydroxymethyltriazenes and derivatives have been examined by high field NMR and for the first time rotational barriers have been measured for these compounds and some conclusions drawn about the preferred conformations of these species.

ABBREVIATIONS AND SYMBOLS USED

cm	Centimetres
DMF	N,N-Dimethylformamide
DNA	Deoxyribonucleic acid
DTIC	5-(3,3-Dimethyl-1-triazeno)imidazole-4-carboxamide
g	Grams (also acceleration in centrifugation 1g=9.8 m s^{-2})
HPLC	High Performance Liquid Chromatography
Hz	Hertz
IR	Infrared Spectroscopy
K	Kelvin
Kcal	Kilocalories
MHz	Megahertz
min	Minutes
mL	Millilitres
MTIC	5-(3-monomethyl-1-triazeno)imidazole-4-carboxamide
NMR	Nuclear Magnetic Resonance Spectroscopy
nm	Nanometres
ppm	Parts Per Million
RNA	Ribonucleic Acid
TLC	Thin Layer Chromatography
TMS	Tetramethylsilane
UV	Ultraviolet Spectroscopy

Acknowledgements

I gratefully acknowledge the invaluable support and guidance of my supervisors Dr. Don Hooper and Dr. Keith Vaughan who have given most generously of their time and talents to bring this work to its conclusion.

Working in two chemistry departments has made me indebted to twice as many individuals, and I am grateful to them all. I would particularly mention Dr. D.R. Arnold, and Dr.T.P. Forrest (Dalhousie). In addition I acknowledge the financial support of Dalhousie University in the form of a Graduate Fellowship, and the technical support of the Atlantic Regional Magnetic Resonance Centre, the master glassblower Mr. Jürgen Müller, and the Chemistry Department of Saint Mary's University.

Last, and most importantly, my sincerest thanks go to my friends and colleagues Kevin McMahon and Dr. Marco Gruwel for "useful discussions" unrelated to anything in particular.

Chapter 1

Introduction

1,1 Introduction

In a review article entitled "The development of a second-generation triazene", Wilman [1] has stated what amounts to the medicinal chemist's credo as follows: "The general approach in the search for a second-generation analogue suitable for clinical investigation is usually twofold. One is determination of the mechanism of action of the parent drug and any metabolism this might involve, and the second a structure-activity study aimed at establishing the basic structural requirements of this molecular type for the biological activity of interest." Capitalizing on this knowledge, it is possible to plan the search for a better therapeutic agent with some sense of direction. The "shot in the dark" approach has been successful, but is a most inefficient method of attaining a specific goal.

These comments, of course apply only in a perfect world. It is plain that the search for a better drug cannot be delayed for want of an absolutely perfect understanding of the underlying biochemistry. In the case of the antitumour triazenes enough is known of their mode of action and structural requirements to give further research direction, but there are still many puzzling features of these agents which require explanation. It is to be hoped that the synthesis and testing (both chemical and biological) of new analogues may reveal an underlying pattern which will cast some light on the Stygian

complexities of cancer biochemistry.

1,2 1-Aryl-3-alkyltriazenes

1,2,1 Introduction

1-Aryl-3-alkyltriazenes (1), sometimes referred to as "monoalkyltriazenes", are important not only as a large class of compounds with diverse and interesting chemistry, but also because of their biological properties since it is thought that they may be the active metabolite of dialkyltriazenes (11, 12), which are known to have antitumour activity.

Ar-N=N-NH-R (R= Alkyl)

(1)

1,2,2 Synthesis

There are two general methods for the synthesis of monoalkyltriazenes, the Grignard method and the diazonium coupling method. The Grignard method, discovered by Dimroth [2], involves the reaction of a Grignard reagent with an aryl azide (Scheme 1,1).

$$Ar\text{-}N_3 + RMgX \longrightarrow Ar\text{-}N(MgX)N{=}N\text{-}R \xrightarrow{H_2O} Ar\text{-}NHN{=}N\text{-}R \rightleftharpoons Ar\text{-}N{=}N\text{-}NHR$$

(Scheme 1,1)

The method is versatile with regard to variations in the aryl group and has been applied to the synthesis of novel analogues such as triazenes labeled with ^{14}C in the alkyl group [3], Ferrocenylmethyltriazene [4] and ^{14}C labeled triphenylvinyltriazenes [5]. A related synthesis

involves the reaction of azides with a sulphur ylide to give vinyltriazenes [6] (Scheme 1,2).

$$R\text{-}N_3 + 2\ CH_2{=}S(CH_3)_2 \longrightarrow R\text{-}N{=}N\text{-}NHCH{=}CH_2 + 2\ (CH_3)_2SO$$

(Scheme 1,2)

This method however is only useful where R is p-O_2N-C_6H_4- or C_6H_5-CH_2-, other azides yielding dihydrotriazoles. The Grignard method (which should perhaps be called the Dimroth method) has the advantage that unlike the other available methods, the product is not contaminated with dimeric or polymeric nitrogen chain compounds, but of course cannot be used where the aryl ring contains a substituent which reacts with the Grignard reagent.

Coupling of an aryldiazonium salt with a primary amine is a seemingly simple means of generating monoalkyltriazenes, but this method [7] does suffer from the drawback that the nascent monoalkyltriazene can react with a further equivalent of diazonium ion (Scheme 1,3) to form the pentaazadiene (2).

$$Ar\text{-}\overset{\oplus}{N}_2 + R\text{-}NH_2 \xrightarrow{-H^{\oplus}} Ar\text{-}N{=}N\text{-}NHR \xrightarrow[-H^{\oplus}]{Ar\overset{\oplus}{N}_2} Ar\text{-}N{=}N\text{-}\underset{\displaystyle R}{\underset{|}{N}}\text{-}N{=}N\text{-}Ar$$

(2)

(Scheme 1,3)

The facility with which this second coupling occurs has been found [8] to be dependent on the nature of the substituents in the aryl group. A strongly electron-

withdrawing group on the aryl ring reduces electron density at the N-3 position and thus prevents further coupling. The method has been used successfully with various aryl substituents [9] and can be employed with a variety of alkyl substituents [10,11,12,13].

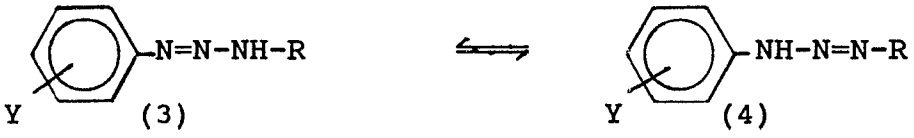
One other synthesis of interest is the specific reaction of an arylhydrazine with nitrosotrifluoromethane to give 1-aryl-3-trifluoromethyltriazenes [14] (Scheme 1,4).

$$Ar\text{-}NH\text{-}NH_2 + CF_3NO \longrightarrow Ar\text{-}NH\text{-}N{=}N\text{-}CF_3 \rightleftharpoons Ar\text{-}N{=}N\text{-}NH\text{-}CF_3$$

(Scheme 1,4)

1,2,3 Structure

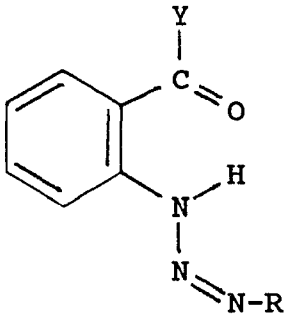
Due to the potentially mobile hydrogen atom on N-3, monoalkyltriazenes frequently exhibit tautomerism (Scheme 1,5).



(Scheme 1,5)

These structures can be detected by NMR spectroscopy of solutions [15]. At low temperatures two separate signals are observed for the N-CH_3 protons of the two tautomers, one at low field, a singlet assigned to N-CH_3 in the unconjugated tautomer (4, R=CH_3) and a high field doublet due to (3, R=CH_3), the methyl protons predictably being split by the adjacent N-H in this tautomer. The intensities of the

signals give an indication of the relative populations of (3) and (4) and reveals the influence of the nature of the aryl substituent on the equilibrium. Triazenes with an electron-donating substituent on the aryl ring favour the conjugated tautomer whilst those with electron-withdrawing substituents show more or less equal parts of the two tautomers. Solvent effects on this tautomerism have been investigated [16] and confirm this conclusion. The X-Ray crystal structure of 1-*p*-tolyl-3-methyltriazene has been determined [17]. This shows that in the solid state the conjugated form (3) is preferred as it is in solution. Triazenes with an *ortho*- substituent on the aryl ring capable of hydrogen-bonding tend to favour the unconjugated form (5) [15].

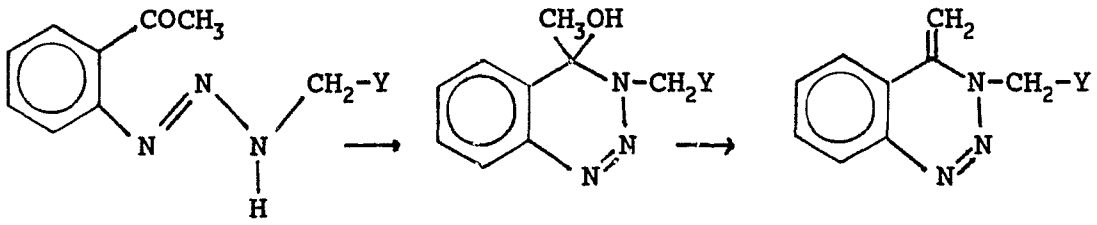


(5)

1,2,4 Chemistry

1-aryl-3-alkyltriazenes with appropriate substituents have been employed [18] as precursors for a variety of heterocyclic systems. When there is a suitably reactive *ortho* substituent on the aryl ring, interaction with N-3 of the triazene moiety gives 1,2,3-triazine derivatives, e.g.

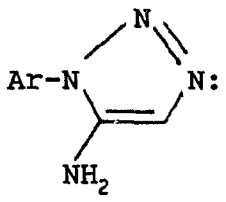
(Scheme 1,6).



(6) (7) (8)
(Not isolable)

(Scheme 1,6)

In this case the intermediate hydroxytriazine (7) is not isolable and undergoes spontaneous dehydration to the methylenetriazine. However if the *o*-substituent contains no α-hydrogen, the dehydration cannot take place and the hydroxytriazine can be isolated. For example, if *o*-aminobenzaldehyde is carefully diazotized and coupled with an aqueous solution of a primary amine, the stable 4-hydroxy-3-alkyl-1,2,3-benzotriazine is formed. If the alkyl group at N-3 bears a reactive substituent Y, then it is possible to obtain 5-(arylamino)-, and by a Dimroth type rearrangement, 5-amino-1-aryl-1,2,3-triazoles (9) and (10) [19].



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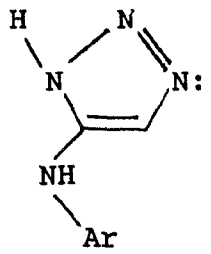


(10)

Perhaps more interesting from the medicinal chemist's point of view are the degradative pathways of the monoalkyltriazenes since these may throw some light upon the *in vivo* activity of these compounds. A study of the thermolysis of 3-methyl-1-*p*-tolyltriazene in tetrachloroethene [20] showed a variety of products that could be explained by a scheme involving homolytic scission in both tautomers (Schemes 1,7 & 1,8).

$$Ar\text{-}NH\text{-}N{=}N\text{-}CH_3 \longrightarrow [ArNH^{\bullet} \quad {}^{\bullet}N{=}N\text{-}CH_3] \longrightarrow \underset{5\%}{ArNHCH_3} + N_2$$

$$\underset{42\%}{ArNH_2} + N_2 + {}^{\bullet}CH_3$$

(Scheme 1,7)

$$Ar\text{-}N{=}N\text{-}NH\text{-}CH_3 \longrightarrow [Ar\text{-}N{=}N^{\bullet} \quad {}^{\bullet}NH\text{-}CH_3] \longrightarrow \underset{\underset{10\%}{Ar\text{-}Cl}}{Ar^{\bullet}} + N_2 + {}^{\bullet}NH\text{-}CH_3$$

(Scheme 1,8)

It is interesting to note that the major products arise from the decomposition of the less favoured unconjugated tautomer which may therefore be assumed to be the more reactive species.

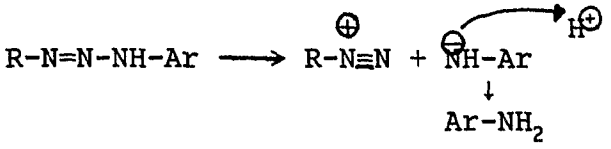
The stability of the monomethyltriazenes in aqueous

media is central to the elucidation of the pharmacology of these compounds, and due to their key role as supposed antitumour metabolites, this area has naturally attracted a great deal of interest. A protolysis mechanism is generally accepted (Scheme 1,9).

$$Ar\text{-}NH\text{-}N{=}N\text{-}R \xrightarrow{H^{\oplus}} Ar\text{-}NH_2 + N_2 + R^{\oplus}$$

(Scheme 1,9)

A correlation has been established between the rate of protolysis and the σ-value of the substituent in the aryl group [21]. The kinetic data in this study should however be treated with caution since these workers based their treatment on the premise that the only catalyzing species is $H_3O^{\oplus}$. Others have shown good correlation with the acid dissociation constant of $R\text{-}N^{\oplus}H_3$ regardless of the ease of generation of $R^{\oplus}$ [22]. In this study, no N-alkylated anilines were formed and the $H_3O^{\oplus}$ reaction of $Ph\text{-}CD_2\text{-}NH\text{-}N{=}N\text{-}Ph$ showed an isotope effect (k_H/k_D) of 1.1 i.e., a secondary isotope effect. This evidence suggests that the deamination pathway catalyzed by a variety of species must involve the alkanediazonium ion as a free solvent-equilibrated intermediate (Scheme 1,10),



(Scheme 1,10)

i.e., a unimolecular heterolysis of the N-N bond.

1,2,5 Biological Activity

The presence of surfactants which might mimic the environment of cellular lipids can have a variety of effects on hydrolysis. Cationic surfactants cause a rate decrease at all pH values studied whilst anionic surfactants enhance acid catalyzed hydrolysis but decrease the observed rate constants in the pH independent region [23].

This decomposition can be used to generate "hot" carbocations which can act as irreversible active-site inhibitors of some *E. coli* enzymes [24]. If for example a ß-D-galactopyranosylmethyl group is incorporated into a triazene as the R-group, the cation generated specifically inhibits ß-galactosidase by alkylation of methionine 500 in *Lac*-Z-ß-galactosidase [25]. Again the mechanisms have been studied for these decompositions [22] and seem to involve unimolecular heterolysis of the unconjugated tautomer.

The key hypothesis in the discussion of the role of monomethyltriazenes in the bioactivation of 3,3-dimethyltriazenes is that DNA methylation is the source of antitumour activity. One piece of evidence is that monomethyltriazenes are direct-acting (i.e., without

metabolic activation) mutagens in *Salmonella typhimurium* [26]. More convincing is the *in vitro* incubation of monomethyltriazene with DNA which results in the methylation of several heterocyclic bases [27], mainly guanine and adenine, but for this agent (MTIC) more than 99% of the methyl group is recovered as methanol.

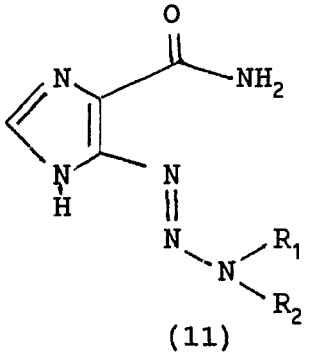
MTIC has been studied *in vivo* on human melanoma cells with the intention of elucidating to what extent the effects of MTIC might be epigenetic, e.g., RNA alkylation. In one study [28] MTIC was shown to increase the thymidine and deoxycytidine pools but not deoxyguanidine, but this did not clarify the actual effect responsible for activity. More useful results were obtained in a study [29] on BE and HT29 cell lines which have the phenotypes Mer^+ and Mer^- i.e. HT29 has the ability to repair O^6-methylguanine lesions whilst the BE cell line does not. Thus preferential toxicity of MTIC towards the BE cell line indicates specifically DNA alkylation at O^6-guanine positions. It is noteworthy that the analogous monoethyltriazene is not toxic to the Mer^- line which may be due to the inability of the monoethyltriazene to cause lesions, or DNA ethylation may be more easily repaired. In a more detailed study using a modified form of a standard DNA sequencing technique, Hartley *et al.* in an elegant study, [30] showed that unlike monoethylating analogues which showed no sequence preference, monomethyl- and monochloroethyltriazenes

alkylate guanine bases extensively at the N^7 position with a preference for runs of contiguous guanines. It is interesting to note that some oncogenes are rich in triplets of guanidine bases and may be responsible for the differential cytotoxicity of triazene-type alkylating agents.

1,3 1-Aryl-3,3-dialkyltriazenes

1,3,1 Introduction

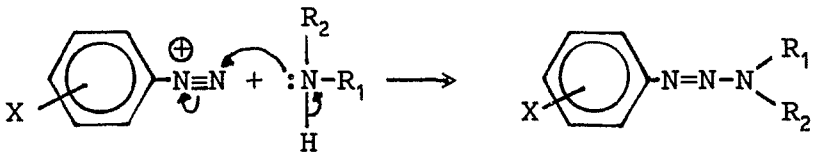
The 1-aryl-, and 1-heteroaryl-3,3-dialkyltriazenes are biologically active triazenes and include one of the few triazene antitumour agents currently in clinical use, 5-(3,3-Dimethyl-1-triazeno)imidazole-4-carboxamide, more conveniently called DTIC and sold under the trade name Dacarbazine (11), $R_1 = R_2 = CH_3$.



(11)

1.3.2 Synthesis

The dialkyltriazenes are easily prepared in good yield by coupling aqueous secondary amines with an aqueous solution of an aromatic diazonium ion in the presence of sodium carbonate (Scheme 1,11)[31]. The diazonium salt is prepared in solution and treated *in situ* with the amine.

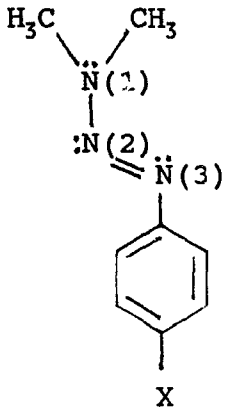


(Scheme 1,11)

A wide variety of such compounds can be prepared, the only limitation being the availability of relatively stable aryl and heteroaryl diazonium salts and secondary amines. Some aryl- and heteroarylamines undergo side reactions on diazotization, and serious difficulties can arise if the arylamine contains a base-sensitive functional group e.g., phenolic hydroxyls. In these sensitive cases where the presence of nitrous acid or other reagents interferes with the coupling reaction, it has been found [32] that the diazonium tetrafluoroborate or hexafluorophosphate can be isolated and purified and subsequently coupled with the secondary amine in an appropriate solvent.

1,3,3 Structure

Variable temperature ^{1}H-NMR studies [33] have shown that there is considerable restriction of rotation about the N(2)-N(3) "single" bond, which suggests some contribution from dipolar structures such as (13) and (14)



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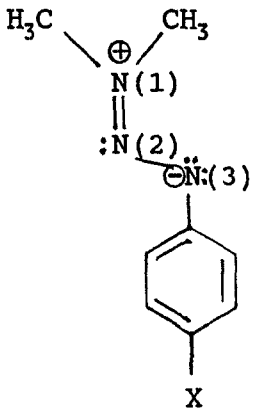

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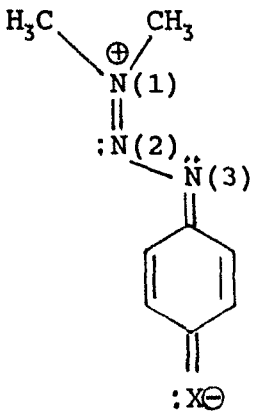
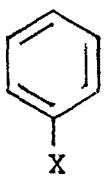


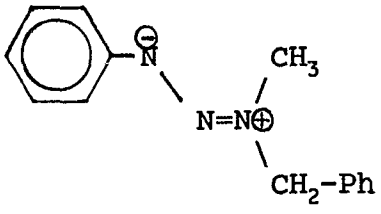
(14)

Natural-abundance ^{13}C and enriched ^{15}N studies [34] provide strong evidence that structure (13) at least is a major contributor to the structure. The ^{15}N chemical shifts of N(1) and N(3) are very sensitive to the nature of X, the greater the electron withdrawing ability of X, the more deshielded is N(3) and the more shielded N(1), conformable with the structure (13). These changes in shifts correlate reasonably with Hammett σ_p constants. From the ^{13}C investigation into substituent effects on the aromatic chemical shifts, it was concluded that structure (14) might be a small contributor, but this conclusion was based on comparisons with substituent effects in the simple benzene analogue (15),



(15)

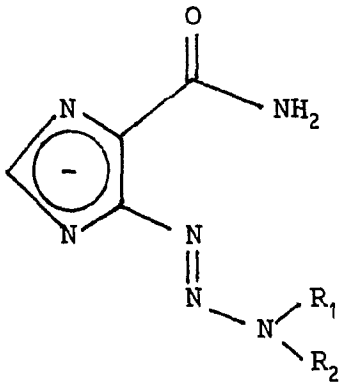
using the assumption that substituent effects on shifts are strictly additive which is of course not strictly true. Since the differences between the triazene and the benzene analogue shifts were barely beyond the limit of detectability, any conclusion regarding the charge distribution on the aromatic ring should be regarded as speculative. A more elaborate study [35] in which the low temperature ^{13}C-NMR spectra of 1-aryl-3,3-dialkyl- and 1,3,3-trialkyltriazenes were analyzed, gave $\Delta G^{\ddagger}$ barriers to N(2)-N(3) rotation of around 13 kcal mole^{-1} for the dialkyl- and 10 kcal mole^{-1} for the trialkyltriazenes. This suggests that the aryl group stabilization (at least for the unsubstituted ring) of the 1,3-dipolar contribution is only about one quarter of the total barrier. This study also showed that for unsymmetrical 3,3-dialkyltriazenes where one of the R groups was methyl, the methyl group was shifted upfield at low temperatures, the bulkier alkyl group being shifted downfield. This corresponds to the more sterically demanding group orienting itself *trans* to the nitrogen atom bearing the negative charge (16).



(16)

In an interesting study [36] conducted on the heteroaryl compound DTIC (11), the pH dependence of the

N(3)-N(2) rotation barrier was measured, demonstrating the conjugation between the heteroaryl ring and the N(3) via N(2)=N(1). Depending upon pH, the ring may be deprotonated (17a), singly (18a) or doubly (19a) protonated, and each state has a differing degree of charge delocalization and hence a different N(3)-N(2) rotational barrier.



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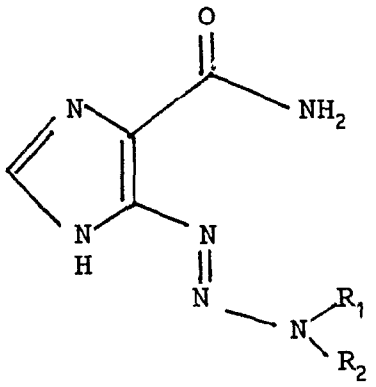

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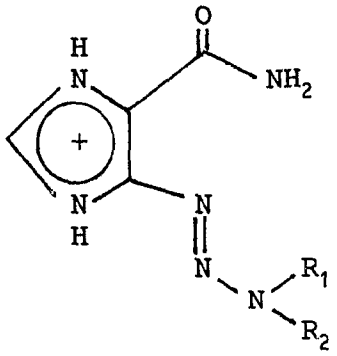


a; $R_1=R_2=Me$
b; $R_1=Me$, $R_2=CH_2OH$

(19)

It was also concluded that rotation would be significantly hindered at physiological temperature and pH, and that biological oxidation to the hydroxymethyl species (17b, 18b, 19b) would probably occur preferentially at one of the diastereotopic methyl groups. The motivation for

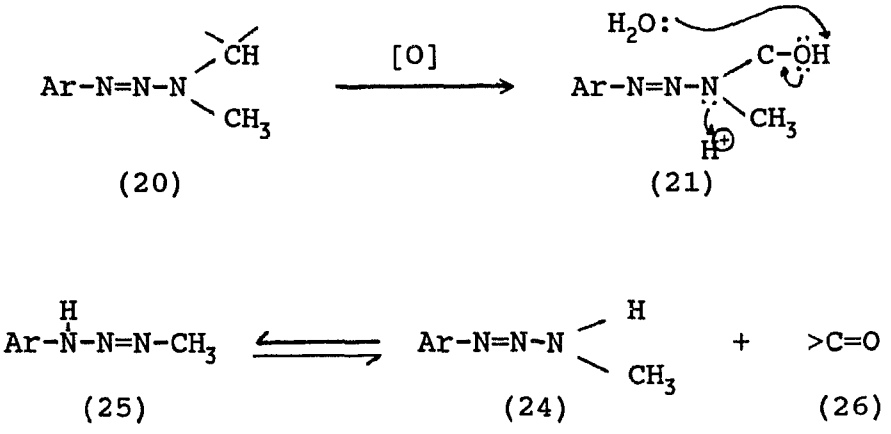
this study was that whilst such biological oxidation might yield only one diastereomer with a particular activity, a purely chemical synthesis could give a mixture consisting of the "biological" and the other diastereomer which could have quite different anti-tumor properties. If this were the case, it might shed some light on the confusion surrounding the selective/non-selective antitumour properties already mentioned under monomethyltriazenes. This group, however, concluded (seemingly without experimental evidence) that the hydroxymethyl compound would not have a diastereomeric interconversion barrier sufficient for this effect to have any bearing in structure/activity studies.

MNDO molecular orbital calculations [37] confirm that the most stable conformation should be that having the two methyl groups in the same plane as the three nitrogen atoms and that the rotational barrier should increase with increasing electron withdrawing power of the X-substituent.

1,3,4 Antitumour Activity and Metabolism

Clarke *et al.* [38] showed in 1959 that 3,3-dimethyl-1-phenyltriazene could inhibit *in vivo* growth of the mouse sarcoma S-180. Since this tumour was already known to be highly sensitive to alkylating agents such as mustard gases, they proposed that the triazene was acting as an alkylating agent too, possibly as a source of reactive diazonium ions which could then "react with elements essential for cell proliferation".

Structure-activity relationship studies quickly showed [39] that at least one methyl group was necessary at N-3 for activity. More refined studies [39] established that in addition to the requirement that one of the N-3 alkyl groups be a methyl group, the other group had to have an α-hydrogen for antitumour activity. The aryl group does not directly affect the antitumour activity of these compounds, but is merely a carrying group for the triazene and may be a heterocycle as it is in DTIC. The generally accepted scheme for the metabolic activation of 3,3-dialkyltriazenes is shown in Scheme 1,12.



(Scheme 1,12)

The 3,3-dialkyl-1-aryltriazene (20) undergoes oxidative metabolism in the liver to the α-hydroxymethyltriazene (21) [40], this being in keeping with the requirement for an α-hydrogen on one alkyl group. Chemical loss of the α-hydroxyl group as the corresponding carbonyl compound (26), gives the monoalkyltriazene (24) which is tautomeric

with the unstable 3-aryl-1-alkyltriazene (25) [15] which has been shown [41] to be capable of alkylating DNA. The interaction of the methylating intermediate in the metabolic decomposition of DTIC with cellular macromolecules has been studied by ^{14}C radiolabeling. The fraction of the labeled methyl groups that was not excreted was recovered mainly as 7-[^{14}C]-methylguanine predominantly from the liver but also from lung and kidney tissue in rats.

The evidence strongly suggests that the most likely active metabolites of dialkyltriazenes are the corresponding monoalkyltriazenes. It is, however, not at all clear why a monomethyltriazene should be active, whilst all other monoalkyltriazenes are not. An attempt was made to correlate the biological activity of dimethyltriazenes with electronic and conformational structure [37] and this study suggested that a charge transfer mechanism might be an important step in the metabolism since there was some correlation of activity with LUMO orbital energy. Also it was suggested that demethylation should occur preferentially from a planar conformation of N(2)-N(3)-(CH_3).

It is reasonably well established that the active metabolite of the dialkyltriazenes is the corresponding monoalkyltriazene, but why this monoalkyltriazene should only be active when R=CH_3 remains unclear since it would seem that any monoalkyltriazene should have the potential to alkylate DNA. The kinetics of the enzymic demethylation of

3,3-dimethyltriazenes have been studied [42] by following the rate of formaldehyde production, and the electron withdrawing power of the aryl substituent does not affect the rate, showing that the electron density in the triazene chain is not relevant to metabolic oxidation. This is suprising since the stability of the monomethyltriazene is markedly affected by the aryl substituent [9,43]. This kinetic study also confirmed that the monomethyltriazene produced no formaldehyde, i.e., did not undergo any appreciable demethylation by liver microsomes. It has been shown by HPLC studies [44], however, that monomethyltriazenes rapidly disappear when incubated with a mouse liver homogenate or isolated mouse hepatocytes, but the products of the decomposition have not been identified. The dimethyltriazene used in this study was found to be extensively metabolized *in vitro* to yield the monomethyltriazene, the corresponding arylamine and possibly other products. The products were found to be selectively toxic to TLX5 lymphoma cells, but the cytotoxicity was much greater than could be accounted for by the amount of monomethyltriazene formed.

The TLX5 lymphoma can be made sensitive (TLX5(S)) or resistant (TLX5(R)) to dimethyltriazenes and other alkylating agents and a study exploiting this difference [44] has shown that the monomethyltriazenes are non-selectively cytotoxic without any metabolic activation,

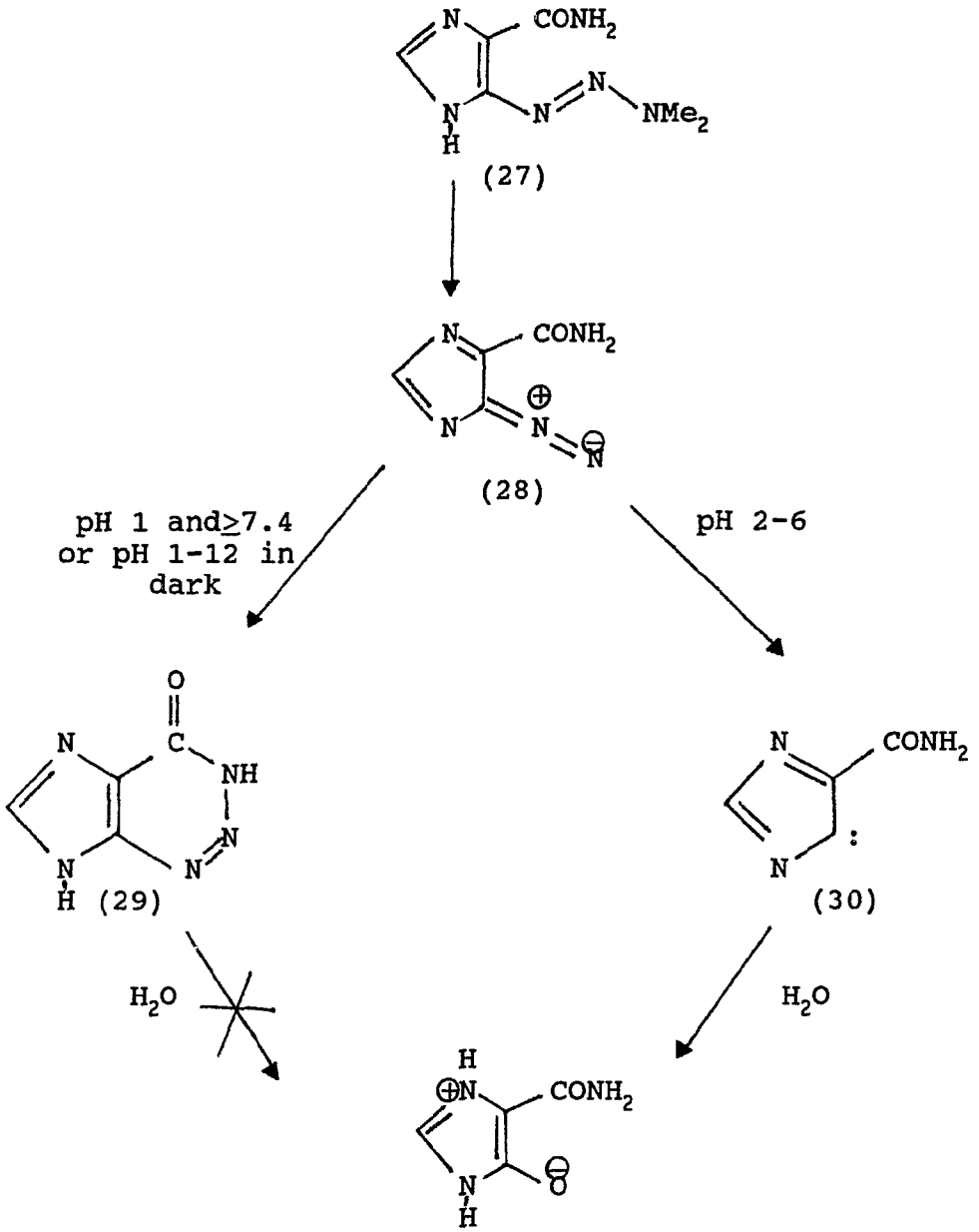
i.e., equitoxic to TLX5(S) and TLX5(R). A non-selective cytotoxic species can also be generated from 3,3-diethyltriazenes which have been shown in other studies [45] to be inactive *in vivo*. It is therefore supposed that metabolism of dimethyltriazenes yields both selective and non-selective products, and that the selective species are not monomethyltriazenes. In an attempt to explain these puzzling differences, Farina and co-workers [46] performed a comparative study of the metabolism and activity of an aryldimethyltriazene with those of an aryldiethyltriazene. It was found that 27% of the diethyltriazene was converted to the monoethyltriazene compared with 79% for the dimethyltriazene. Although the diethyltriazene was only partially metabolized to the monoethyltriazene, it nevertheless disappeared rapidly from reaction mixtures, presumably following a metabolic pathway different from that of the dimethyltriazene. It was also shown that the better antitumoral activity of the dimethyltriazene was accompanied by less, not greater toxicity which suggests that the toxicity is not mainly due to the monoalkyl derivative which is supposed to be responsible for the antineoplastic properties of dimethyltriazenes.

Metabolic dealkylation has been employed [47] as a yardstick by which to gauge the best combination of N(3) methyl and alkyl groups for antitumour activity. The extent of oxidative metabolism and the *in vivo* antitumour activity

are dependent upon an optimum hydrophobicity of the alkyl group obtained with a propyl substituent.

1,3,5 Chemistry

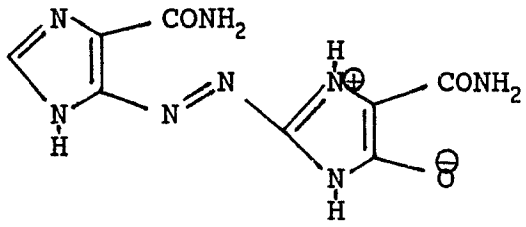
The drug DTIC was originally conceived as an active site inhibitor with a view to disruption of DNA synthesis. This provides an explanation for the incorporation of the imidazole ring in the molecule. DTIC was found to be active, but not as an inhibitor of DNA synthesis, and was subsequently licensed for clinical use. The drug, however has some serious drawbacks as a useful chemotheraputic agent, not least of these being its photosensitivity. It was noted [48] that venous pain at the injection site and other side-effects such as nausea, vomiting and hepatic dysfunction could be reduced by scrupulously shielding the drug from light, and administering it under photographic safe-light conditions. The photodecomposition has been studied under a variety of pH conditions [49] and the following conclusions were drawn (Scheme 1,13).



(Scheme 1,13)

The drug DTIC (27) was found to be stable in the dark, but in diffuse light at pH 1 or greater than 7.4, 2-azahypoxanthine (29) was the final product of photolysis.

In the intervening pH range (pH 2-6) the product was 4-carbamoylimidazolium-5-olate (31). Interestinglv this compound is the aglycone of the antibiotic bredinin which inhibits leukemia cell proliferation in mice. This compound is colourless, and therefore does not account for the pink colouration noted in DTIC solutions exposed to light. This colouration was found to be due to 4-carbamoyl-2-(4-carbamoylimidazol-5-ylazo)imidazolium-5-olate (32).



(32)

The usual method for the synthesis of aryl fluorides involves the diazotization of arylamines. This has the disadvantage that homolytic cleavage of the N-aryl bond gives a variety of unwanted products. A useful method which overcomes this problem [50] uses the dialkyltriazene as a source of diazonium ion, thus providing a diazonium ion free from oxides of nitrogen. The triazene is treated with 70% HF in pyridine. The method is mild, nearly quantitative and fast, all-important for ^{18}F labeling of compounds for medicinal use (Scheme 1,14).

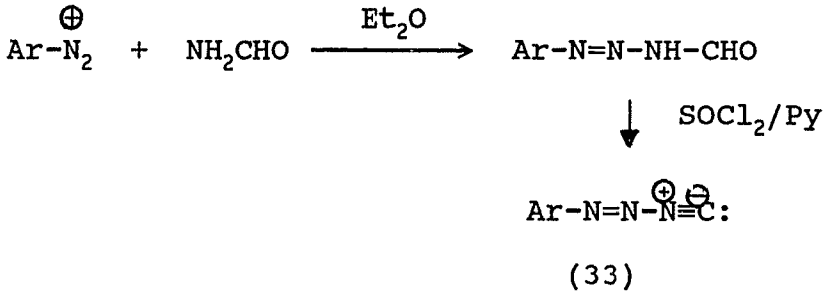
$$ArN{=}N{-}NR_2 \xrightarrow{HF} Ar{-}F + R_2NH + N_2$$

(Scheme 1,14)

An extension of this method [51] using an H^+-form ion exchange resin as a catalyst in the presence of iodide ion provides a regiospecific synthesis for aryl iodides.

A proposed group of possible oxidative metabolites of 3,3-dimethyltriazenes are the 3-formyltriazenes which can be prepared by oxidation of dimethyltriazenes with t-butylhydroperoxide in the presence of V_2O_5 [52]. There is as yet no evidence for the metabolic formation of 3-formyltriazenes *in vivo* or *in vitro*, but this reaction is interesting in that it represents the only successful chemical oxidation of a dimethyltriazene leaving the triazene moiety intact.

It has been claimed [53] that stable diazoisocyanides (33) could be made by coupling formamide with diazonium ions in ethereal solution, followed by dehydration with thionyl chloride in pyridine (Scheme 1,15).



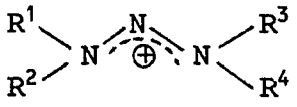
(33)

(Scheme 1,15)

Other workers have not been able to repeat these results [54], rather showing the product of these reactions to be the crude aryl azide.

The class of compounds known as triazenium salts of

type (34) can be obtained [55] by alkylation, generally intramolecularly, of triazenes trisubstituted by alkyl and/or aryl groups. The cations can be isolated as stable tetrafluoroborates, perchlorates or iodides.

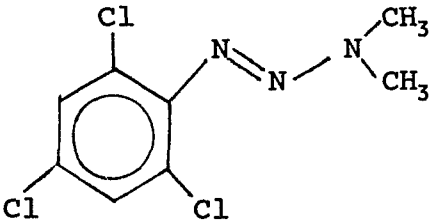


(34)

1,4 1-Aryl-3-hydroxymethyl-3-methyltriazenes and Derivatives

1,4,1 Introduction

The 1-aryl-3-hydroxymethyltriazenes, commonly refered to as "hydroxymethyltriazenes", whilst being invoked as important intermediates in the metabolism and antitumour activation of dimethyltriazenes, had long been regarded as highly unstable transient species. Efforts to identify the urinary metabolites of the tumour inhibitory substance 1-(2,4,6-trichlorophenyl)-3,3-dimethyltriazene (35) [56] led to the recovery of the corresponding 1-O-(triazenylmethyl)-glucuronic acid (36).



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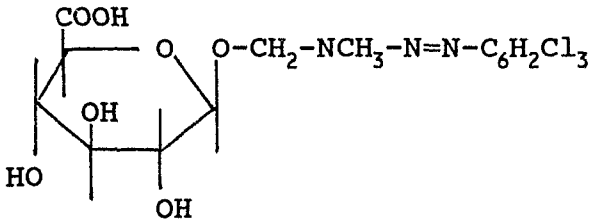


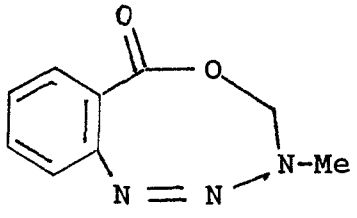
(36)

The nature of this metabolite was elucidated by degradative methods (acid hydrolysis followed by TLC of products). NMR

studies in D_2O and accurate field desorption mass spectrometry confirmed this structure. This first formal evidence of the metabolic oxidation of a dimethyltriazene led to the suggestion that such O-conjugated substances might be the transport forms of the active metabolites since enzymic removal of the glucuronic acid moeity followed by the loss of formaldehyde would yield the direct-acting monomethyltriazene.

1,4,2 Synthesis

Hydroxymethyltriazenes may be prepared [57] as stable, crystalline solids by the diazotization of an arylamine with sodium nitrite and subsequent coupling with a pre-mixed solution of 40% formaldehyde and 25% methylamine (10:1) at -5°C. Successful syntheses of hydroxymethyltriazenes by this procedure were only achieved from diazonium salts bearing -M substituents in the *para*-position. Efforts to prepare a "masked" hydroxymethyltriazene (37) led to the formation of the triazinone (38), probably by cyclization of a monomethyltriazene liberated by decomposition of the corresponding hydroxymethyltriazene.



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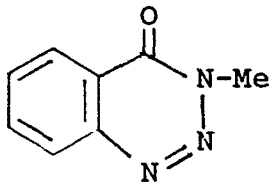
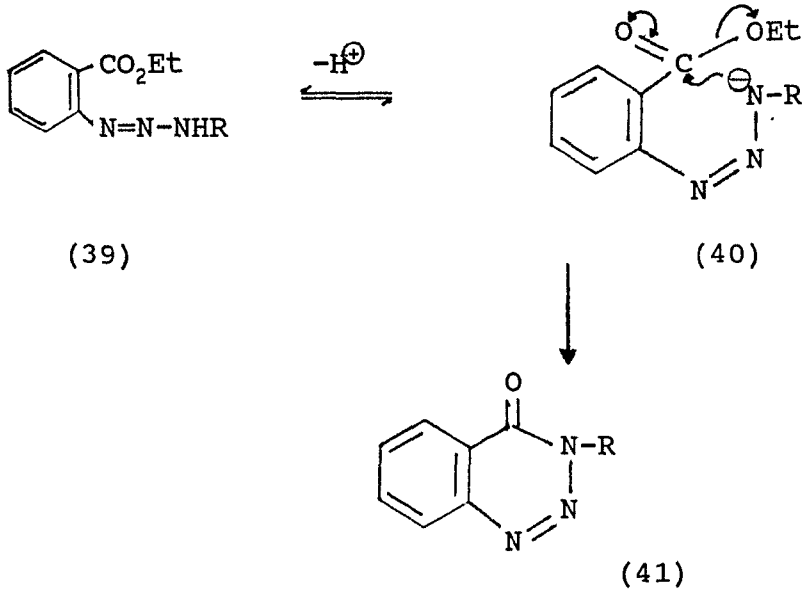


(38)

It has been shown [58] that in acetate buffer, the monomethyltriazene (39) formed by diazotizing an anthranilate ester and coupling with a primary amine, cyclizes to give a benzotriazine-4-one (41), (Scheme 1,16), the reaction being almost quantitative when catalyzed by alumina.



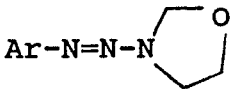
(39) (40) (41)

(Scheme 1,16)

Kolar [59] has prepared 5-(-3-hydroxymethyl-3-methyl-1-triazeno)imidazole-4-carboxamide (the hydroxymethyl derivative of DTIC) by condensing the monomethyltriazene

with excess formaldehyde in anhydrous methanol.

The "masked" hydroxymethyltriazenes, the 3-(arylazo)--1,3-oxazolidines (42) and 3-(arylazo)tetrahydro- -1,3-oxazines (43)



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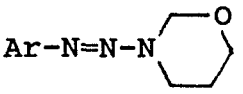
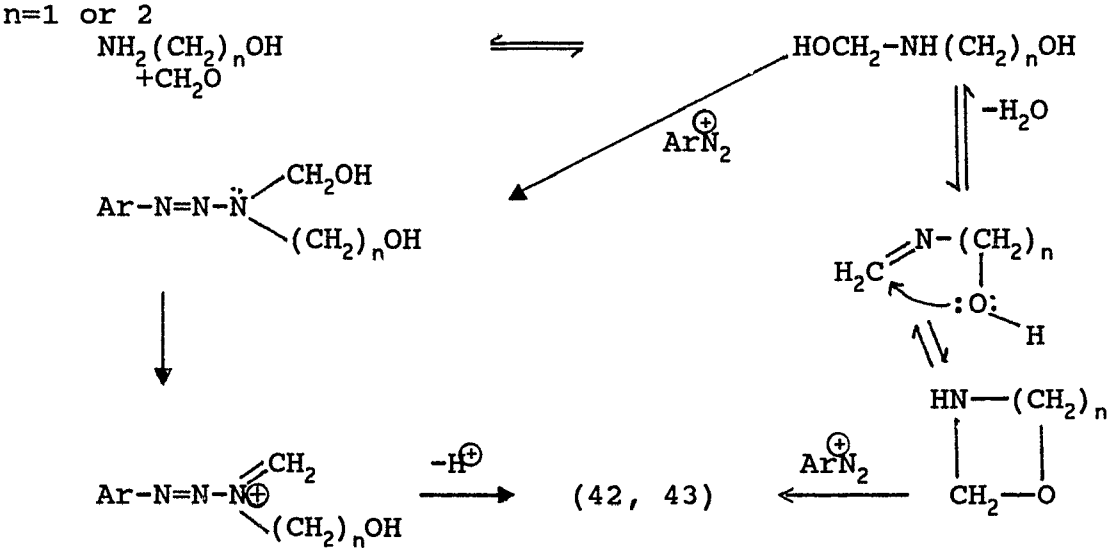


(43)

have been prepared [60] by coupling the diazonium salt of an aromatic amine with a mixture of the appropriate carbinolamine in water and aqueous formaldehyde (scheme 1,17).



(Scheme 1,17)

The corresponding thiazolidines cannot be made in this way, the products being bright orange substances that decompose rapidly to give intractible tars. The thiazolidine derivatives can however be made using the

diazonium tetrafluoroborate.

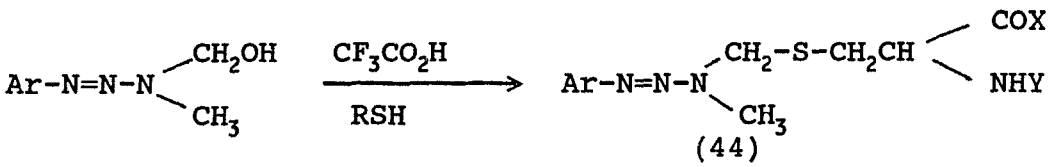
Derivatives of the hydroxymethyltriazenes are readily made [61], the acetoxymethyl derivatives by treatment with anhydrous acetic anhydride in pyridine, and the benzoyloxymethyltriazenes by reacting the appropriate hydroxymethyltriazene with benzoyl chloride in pyridine. In both cases the pyridine was found to be neccessary to remove the acid formed, which would otherwise have caused hydrolysis of the product. These derivatives provide an activated form of the hydroxymethyltriazene which can be reacted with methanol or ethanol to give the corresponding methoxy- or ethoxymethyltriazenes.

The acetoxymethyl derivatives of hydroxymethyltriazenes are also useful intermediates in the synthesis of 3-arylthiomethyltriazenes [62]. In this case, unlike the reaction with alcohols, the thiols do not react directly, not being strong enough nucleophiles, but react smoothly in dimethylformamide when converted to the thiophenolate ion using sodium hydride.

Similarly, the 3-aryloxymethyltriazenes can be obtained by reacting the acetoxymethyltriazene with a phenol. Here the conversion to the phenolate is not always neccessary, but the choice of solvent is critical and the reaction seems to work only in chloroform [63].

Iley and co-workers [64] have synthesized S-cysteinyl (44a) S-(N-acetylcysteinyl) (44b) and S-glutathionyl

conjugates, (Scheme 1,18).



(44)

a) X=OH Y=H
b) X=OH Y=$COCH_3$
c) X=$NHCH_2CO_2H$ Y=$COCH_2CH_2(CO_2H)NH_2$

(Scheme 1,18)

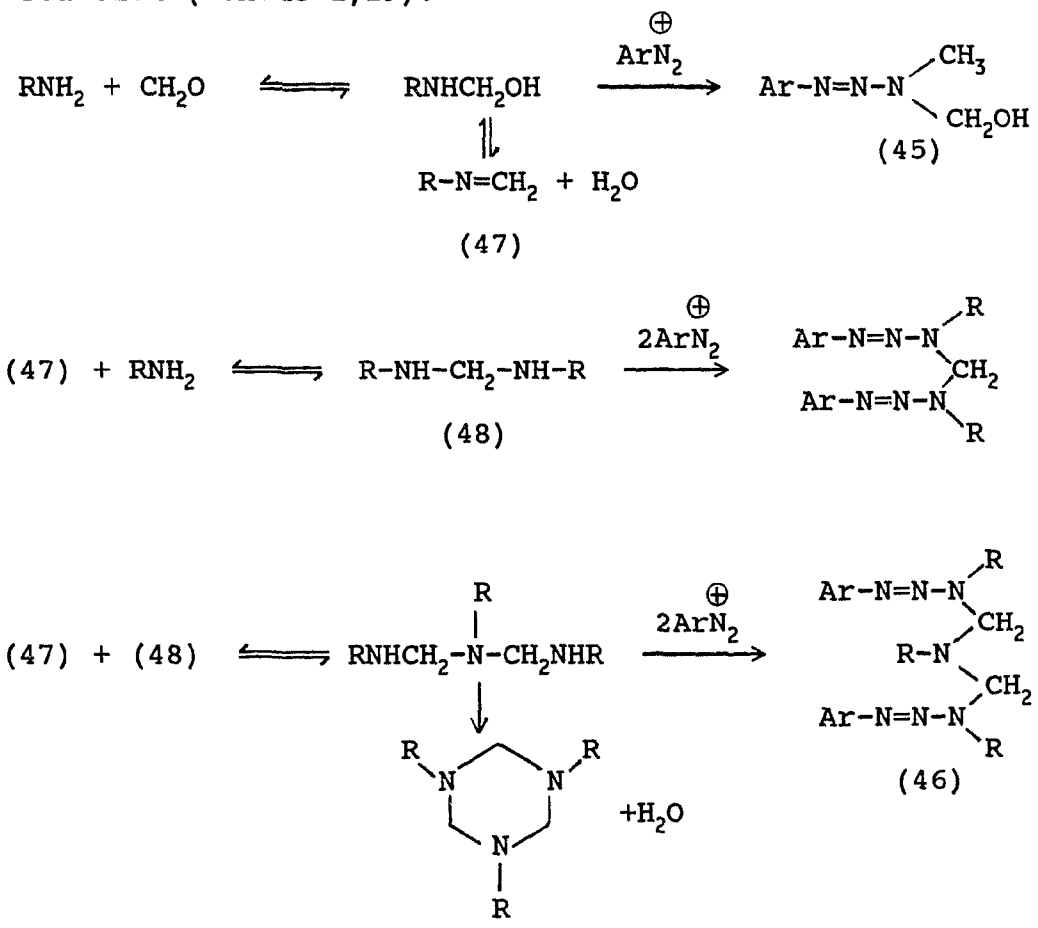
The hydroxymethyltriazene (Ar=3'pyridyl) was added to a cooled solution of the amino acid in trifluoroacetic acid. the method is limited however to the 1-(3'pyridyl)- -3-hydroxymethyl-3-methyltriazenes.

Working along similar lines, the same group [65] have prepared 1-aryl-3-alkoxymethyl- and 1-aryl-3- -alkylthiomethyl-3-methyltriazenes by reacting the corresponding hydroxymethyltriazene with an alcohol or thiol in the presence of hydrogen chloride under anhydrous conditions.

1,4,3 Chemistry

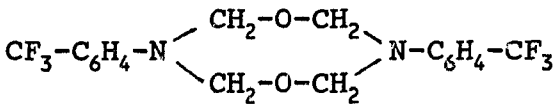
The synthesis of hydroxymethyltriazenes by coupling diazonium ions with mixtures of aqueous solutions of formaldehyde and methylamine is not as straightforward as it may seem. Attempts to produce these compounds where the aryl substituent is other than -M have proved unsuccessful. It was claimed by Juliard and co-workers [66] that modifying the coupling procedure by using the diazonium tetrafluoroborate afforded hydroxymethyltriazenes with other

than -M substituents (e.g., Br, Cl). These compounds were shown by Vaughan *et al.* [67] to be the novel bis-(1-aryl-3-methyltriazen-3-ylmethyl)methylamines (46). This reaction was studied by the same group [68], particularly the effect of changes in the ratio of formaldehyde to methylamine on the product ratio. It was found that the lower this ratio, the greater the proportion of the bis-triazene in the product mixture. This was used with other evidence to elucidate a plausible mechanism for the reaction (Scheme 1,19).



(Scheme 1,19)

A further intresting, if limited variation on this theme was the reaction between 4-trifluoromethylbenzenediazonium chloride and methylamine/formaldehyde mixtures [69], which affords a cyclic product identified as 3,7-bis-(4-trifluoromethylphenyl)-1,5,3,7-dioxadiazocine (49).

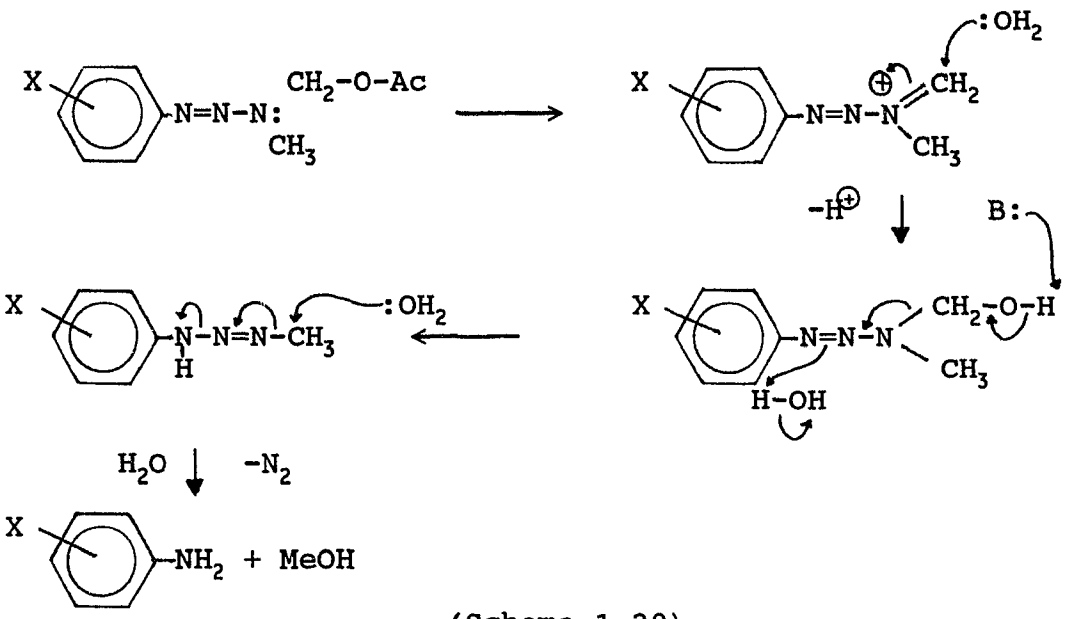


(49)

It is likely that the ratio of methylamine to formaldehyde (9:40) was responsible for this specific reaction which has not been explained to date.

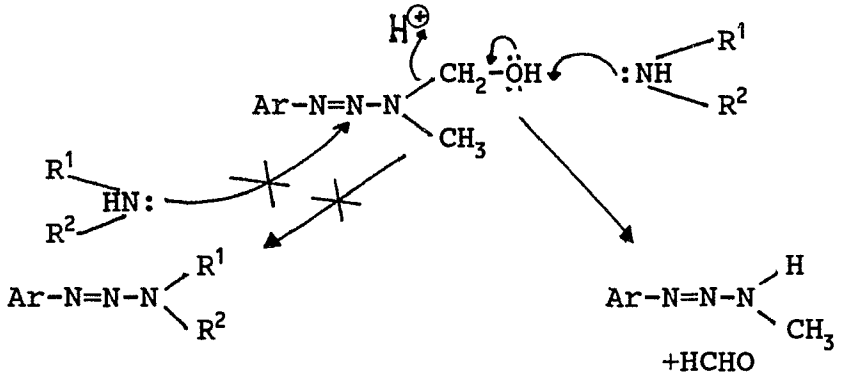
An important and controversial feature of the chemistry of hydroxymethyltriazenes is the involvement or otherwise of an iminium ion in the hydrolysis of these compounds, and a number of studies have been done in an attempt to answer this question.

Limited kinetic work shortly after the first successful synthesis of hydroxymethyltriazenes led to the proposal of the following scheme of decomposition for hydroxymethyl- and acetoxymethyltriazenes (Scheme 1,20) [57].



(Scheme 1,20)

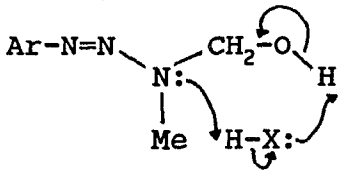
Iley and Cheng [70] used the base-promoted decomposition of hydroxymethyltriazenes to prepare monomethyltriazenes and showed that amine exchange was not responsible for this outcome (Scheme 1,21).



(Scheme 1,21)

Further work by the same group [71] in developing an HPLC method for following these reactions, led to the proposal that, at least for the reactions considered in ethanol, that a cyclic transition state might be involved

since structural requirements for the base included both a lone pair and an X-H bond (50).



(50)

This group has also studied the interaction of hydroxymethyltriazenes with Lewis acids [72]. With Lewis acids, Hammett ρ-values for the catalyzed decomposition together with lanthanide shift reagent NMR experiments suggest that species such as Fe^{3+}, Fe^{2+}, Zn^{2+} and Cu^{2+} (ρ-values -3.4, -2.5, -2.7 and -1.4 respectively) act by binding to the hydroxyl oxygen atom.

Vaughan *et al.* [73] have adduced considerable evidence for the generation of iminium ions from acetoxymethyltriazenes, but not from hydroxymethyltriazenes. Correlation of the rate of hydrolysis of the acetoxymethyltriazenes with the Grunwald-Winstein parameter (Y) for solvent ionizing power, is good, supporting an S_N1 Mechanism. Also a non-common-ion effect is observed with lithium chloride. It was stated that: "..hydroxymethyltriazenes do not react via iminium ions and that functionalization to a derivative such as the acetate is neccessary for iminium ion generation". Iley [74] subsequently arrived at similar results for the benzoyloxymethyltriazenes. It was found [65] that under

more severe reaction conditions, it was possible to prepare ether derivatives by reacting an alcohol directly with the hydroxymethyltriazene in aprotic medium with HCl. This indicated that under extreme conditions (which might have been expected to decompose the triazene moiety completely) an iminium ion may be generated from the hydroxymethyltriazene.

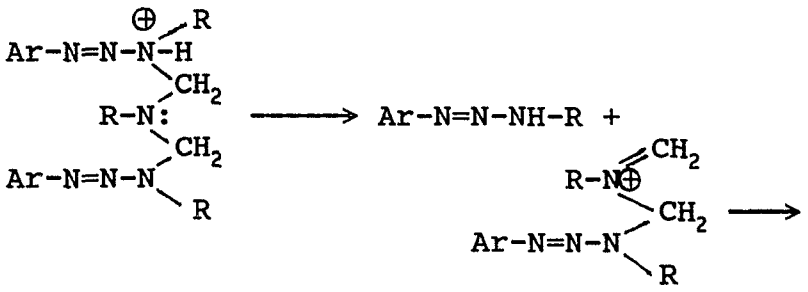
1,4,4 Biological Activity

1-Aryl-3-hydroxymethyl-3-methyltriazenes were shown by Vaughan *et al.* to have pronounced activity against the TLX5(S) lymphoma *in vivo*, being at least as active as the corresponding dimethyltriazenes. This activity, as expected, is not shared by the 3-hydroxymethyl-3-ethyl compounds, suggesting that they exert their activity via the monomethyltriazene degradation product. The hydroxymethyltriazenes were also active against the TLX5(R) resistant strain, which calls into question the hypothesis that these compounds may be the selective species responsible for the activity of dimethyltriazenes. The benzoates have low antitumour activity against TLX5, presumably due to the speedy alternative degradation pathway via loss of the benzoate ion. A comparative study on the human melanoma M21 cell line *in vitro* [76] confirmed that the benzoate was a poor inhibitor of cell growth, but interestingly the ethylhydroxymethyl- as well as the methylhydroxymethyltriazenes were toxic and this toxicity

was shown not to be due to the final breakdown products of the triazene (formaldehyde, arylamine etc.).

A series of hydroxymethyl-, acetoxymethyl-, methoxymethyl- and dimethyltriazenes have been investigated [77] for activity. The acetoxymethyltriazenes were active against TLX5, P388 and PC6 tumours in mice, and inhibit growth of TLX5, Np and Li cells *in vitro* without metabolic activation, with a comparable spectrum of activity to that of the hydroxymethyl- and monomethyltriazenes. Methoxymethyltriazenes were active on TLX5 tumour *in vivo* but not otherwise, consistent with the chemical stability of these compounds and the requirement for O-demethylation to generate an active species.

The N,N-Bis(1-aryl-3-methyltriazen-3-yl)-methylamines have shown activity *in vivo* [78] and inhibit cell growth *in vitro* without prior metabolism probably due to a facile fragmentation in which the bistriazene behaves as an 'aminal' ($N-CH_2-N$) and undergoes anchimerically assisted fragmentation via an iminium ion intermediate (Scheme 1,22).



(Scheme 1,22)

1,5 Objectives

Hydroxymethyltriazenes possess significant antitumour activity and are stable in the solid state. However they are not stable in solution at physiological pH and therefore they may not be useful as therapeutic agents by themselves. Nevertheless, there is a real possibility that a derivative of the hydroxymethyltriazene might have the appropriate properties to act as a prodrug, *i*.e. decompose at a slow but reasonable rate under physiological conditions to afford an active species. None of the derivatives prepared to date have the required characteristics, and the objective of this project is to develop some new derivatives of the hydroxymethyltriazenes and to devise the optimum molecular structure for best prodrug characteristics.

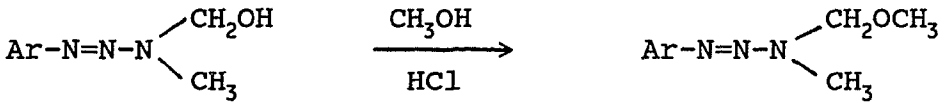
For a recent review article on the subject of antitumour triazenes, see K. Vaughan, pp 159-183 in Chemistry of Antitumour Agents, Edited by D.E.V. Wilman, Chapman & Hall, New York, (1990).

Chapter 2

Synthesis of 1-Aryl-3-arylthiomethyl-3-methyltriazenes

2,1 Introduction

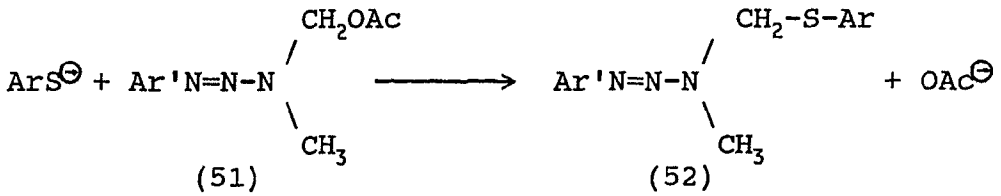
Iley *et al.* [64] has previously shown that it is possible to convert hydroxymethyltriazenes directly tc methyl ether by reaction in methanol using anhydrous hydrogen chloride as a catalyst, (Scheme 2,1).



(Scheme 2,1)

This acid treatment, which surprisingly does not decompose the triazene moiety, may be used with an alkanethiol to prepare the corresponding alkanethiomethyltriazene.

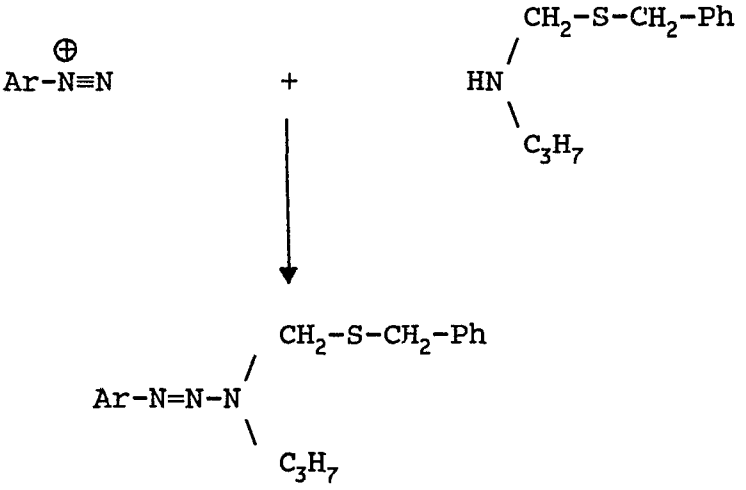
Acetoxymethyltriazenes react smoothly with methanol to give methyl ethers [72], but not at all with pure thiols. This lack of reactivity is attributable to the poor ionizing ability of the thiol as a solvent. In the present study, the acetate methodology, displacing an acetate leaving-group with a nucleophile, was modified to overcome this difficulty by converting the thiol to the corresponding thiophenolate ion using sodium hydride (Scheme 2,2).



(Scheme 2,2)

A completely different approach to the synthesis of α-

substituted dimethyltriazenes is to couple the aryldiazonium ion with a suitably substituted amine, (Scheme 3,3)



(Scheme 2,3)

This approach was examined briefly, but due to the difficulties in preparing the starting amines, their powerful stench and the unpromising results of the preliminary investigation, the method was not pursued further.

2,2 Experimental

Except where specified, all reagents were reagent grade materials purchased from the Aldrich Chemical Co. Ltd., and were used without further purification.

All solvents were purchased from BDH Chemicals Ltd. and were dried over molecular sieves type 4A except for acetone which was dried over anhydrous sodium sulphate. In some instances the solvents were distilled prior to use. For some reactions chloroform was washed repeatedly with distilled water, dried overnight over anhydrous sodium sulphate, distilled and stored in the dark [78].

1-Aryl-3-arylthiomethyl-3-methyltriazenes (52)

2,2,1 Method A

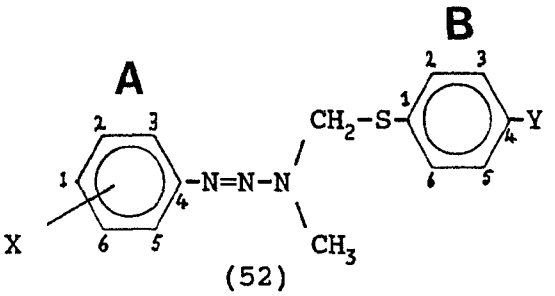
Thiophenol (or *p*-thiocresol) was treated with one equivalent of sodium hydride in anhydrous DMF (Aldrich Gold Label) to give a solution of the sodium thiophenolate (or thiocresolate) with concentration 2 M. The appropriate acetoxymethyltriazene (51) (0.004 moles) prepared by the method of Vaughan *et al*. [61] was dissolved in anhydrous DMF (6.0 mL) and treated with a twofold excess of the sodium thiophenolate (or thiocresolate) solution (4.0 mL). After stirring at room temperature for 2 hours, the red reaction mixture was poured into cold distilled water (25 mL), extracted with ether (3X20 mL) and these ether extracts washed with water, aqueous sodium bicarbonate and again water (25 mL each).

The ether extracts were dried over anhydrous calcium chloride and evaporated. The oily residue was purified by column chromatography on silica gel with 1:1:1 hexane/ethyl acetate/carbon tetrachloride as eluent, followed by recrystallization from a hexane/chloroform mixture. It was important that the thiophenolate (or thiocresolate) solution be prepared freshly as on standing there was a tendency to decomposition giving an ammoniacal smell and intractable reaction products which defied efforts at purification. This procedure gives moderate yields of the 3-arylthiomethyltriazenes (52,Figure 2,1) which with one exception are crystalline solids.

Pertinent physical data and yields are given in table 2,1. Melting points were determined using a Reichert hot-stage microscope and are uncorrected.

Infrared spectra were obtained using a Perkin-Elmer 299 Infrared Spectrophotometer, calibrated against polystyrene film. Samples were prepared in $CHCl_3$ solution and IRs run using matched KBr cells (Perkin-Elmer) with a $CHCl_3$ reference.

UV absorption maxima and extinction coefficients of chloroform solutions were measured using a Cary 219 UV-Visible spectrophotometer (Varian).



(52)

Compound	X	Y
52a	*p*-CH_3O_2C-	H
52b	*p*-$C_2H_5O_2C$-	H
52c	*p*-O_2N-	H
52d	*p*-NC-	H
52e	*p*-CH_3CO-	H
52f	*p*-CH_3O_2C-	CH_3
52g	*p*-$C_2H_5O_2C$-	CH_3
52h	*p*-O_2N	CH_3
52i	*p*-NC-	CH_3
52j	*o*-CF_3	H

(Figure 2,1)

Table 2,1 Physical Data for the 1-Aryl-3-arylthiomethyl-3-methyltriazenes (52)

Cmpnd.	X[g]	Y	Yield (%)	m.p. (°C)	M^+ Found	M^+ Calc.	IR (cm^{-1})	max.[a] (nm.)	ε[b]
52a	CH_3O_2C	H	84	111-114	315.1033	315.1041	1710[c]	337	19700
52b	EtO_2C	H	7	83-84	329.1200	329.1193	1705[c]	322	19190
52c	O_2N	H	33	110-111	302.0839	302.0837	850[d]	363	21800
52d	NC	H	43	96-98	-	-	2210[e]	342	20800
52e	CH_3CO	H	6	100-103	-	-	1670[c]	328	19700
52f	CH_3O_2C	CH_3	17	108-110	329.1202	329.1193	1715[c]	333	20900
52g	EtO_2C	CH_3	23	79-81	-	-	1710[c]	324	19780
52h	O_2N	CH_3	15	108-110	316.1002	316.0990	855[d]	334	23450
52i	CN	CH_3	32	91-94	-	-	2210[e]	343	20930
52j	*o*-CF_3	H	34	Oil	325.0864	325.0853	740/ 760[f]	320	18500

[a] Measured in $CHCl_3$ at 25°C
[b] Units: $1000cm^2.mole^{-1}$
[c] C=O
[d] NO_2
[e] CN
[f] 1,2-disubstituted aromatic
[g] All X substituents are *para* except for compound 52j

Accurate molecular ion measurements were carried out at the Mass Spectrometry Laboratory of the Department of Chemistry, University of Alberta. A Kratos MS 50 instrument was used in the EI mode. Direct probe temperature 100-140°C, Ionization Voltage 70 eV, Scan rate 10 sec.dec.$^{-1}$
^{13}C and ^{1}H NMR spectra were acquired on the Nicolet 360 NB spectrometer of the Atlantic Region Magnetic Resonance Centre at Dalhousie University. Chemical shifts were measured in $CDCl_3$ solutions at 20°C, and are quoted relative to TMS (1%) internal standard (Table 2,2 and 2,3).

Mass spectra were obtained for representative samples from the MS service facility of Dalhousie University using a Consolidated Electrodynamics Corporation CEC 21-104 mass spectrometer and the operating parameters were as follows. Source temperature 130-145°C, Direct probe temperature 75-130°C, Accelerating voltage 800 V, Ionization voltage 70 eV, Scan rate 6 sec.dec.$^{-1}$. The relative intensities of the mass spectral peaks for these compounds are shown in table 2,4.

Table 2,2 ^{1}H NMR Data for the 1-Aryl-3-arylthiomethyl-3-methyltriazenes (52)

Cmpnd.	Arom. A (AA'BB')	Arom. B	Multiplet	NCH_2	NCH_3	X	Y
52a	8.02-7.17	7.48-7.23	(AA'BB'C)	5.21	3.23	3.88 (s,OMe)	-
52b	7.93-7.13	7.46-7.22	(AA'BB'C)	5.22	3.23	1.37[a],4,33[c]	-
52c	8.10-7.16	7.46-7.21	(AA'BB'C)	5.22	3.26	-	-
52d	7.54-7.15	7.47-7.26	(AA'BB'C)	5.22	3.25	-	-
52e	7.54-7.16	7.47-7.19	(AA'BB'C)	5.24	3.24	2.58 (s,Ac)	-
52f	7.95-7.08	7.33-7.14	(AA'BB')	5.19	3.25	3.91 (s,OMe)	2.29
52g	7.93-7.04	7.34-7.12	(AA'BB')	5.16	3.22	1.37[a],4.33[b]	2.26
52h	8.13-7.06	7.35-7.16	(AA'BB')	5.18	3.27	-	2.27
52j	7.60-6.93 (ABXY)	7.50-7.15	(AA'BB'C)	5.22	3.24	-	-

[a] Triplet, J=7Hz
[b] Quartet, J=7Hz

Table 2,3 ^{13}C NMR Data for 1-Aryl-3-arylthiomethyl-3-methyltriazenes (52) in $CDCl_3$ at 20°C.

Compd.	Arom. A[a] 1	2,6	3,5	4	NCH_3	NCH_2
52a	127.2	133.5	120.6	153.6	33.7	62.5
52b	127.3	133.4	120.5	153.4	33.6	62.4
52c	145.1	124.5	121.0	155.0	33.9	62.6
52d	119.2	133.5	121.3	153.2	33.6	62.5
52e	133.5	133.2	120.8	153.6	33.6	62.4
52f	126.7	133.5	120.2	153.3	33.3	62.5
52g	127.3	134.0	120.5	153.6	33.6	62.8
52h	145.3	122.7	120.9	155.8	33.9	62.5
52i	119.5	132.7	121.4	153.3	33.9	63.0
52j	1	2	3	4	5	6
	125.1	127.9	125.9	147.0	125.9	132.8	33.6	62.2

[a] Aromatic peak assignments based upon empirical shift parameters.

Table 2,3 ^{13}C NMR Data for 1-Aryl-3-arylthiomethyl-3-methyltriazenes (52) in $CDCl_3$ at 20°C (Continued)

Arom. B[a]					
1	2,6	3,5	4	X	Y
130.6	129.1	130.3	128.0	167.0s 52.0q	-
132.7	130.2	129.0	127.9	166.2s 60.8t 14.7q	-
132.5	129.1	133.7	128.3	-	-
133.7	129.0	132.7	128.1	108.6s	-
133.2	129.0	129.3	128.8	197.3s 26.5q	-
137.9	129.9	129.4	128.7	166.5s 51.5q	20.7
138.3	129.8	130.2	129.9	166.4s 60.7t 14.3q	21.0
138.0	129.7	134.0	128.9	-	21.3
138.0	129.9	134.2	129.0	108.5s	21.1
136.8	129.0	129.0	129.3	117.9	-

Table 2,4 Mass Spectral Data for the 1-Aryl-3-arylthiomethyl-3-methyltriazenes (52)

	Ion Abundance (% of Base Peak)								
Compd.	$M^{+\cdot}$	ArN_2^+	Ar^+	$RSCH_2^+$[a]	RSH^+	RS^+	R^+	[M-102][c]	$[M-CH_3(CH_2)_nO]^+$
52a	1	66	100	2	4	8	9	6	2 n=0
52b	7	70	100	2	6	9	5	(6)[c]	3 n=1
52c	2	100	85	9	7	20	4	-	-
52d[d]	6	93	100	3	5	23	4	-	-
52f	3	66	100	3	4	11	6	5	1 n=0
52h	2	65	100	5	3	9	3	-	-
52j	8	70	100	2	5	13	3	-	-

[a] R represents the aryl ring of the arylthiomethyl group
Inlet temp. 100-180°C, EI Ionization Voltage 70eV, Scan Rate 10s/dec.
[c] See text, page 57
[d] Inlet Temp. 180°C, EI Ionization Voltage 70eV, Scan Rate 6s/dec.

Three representative samples were submitted for elemental analysis to Canadian Microanalytical Service Ltd., Delta, British Columbia, and the results are shown in table 2,5.

Table 2,5 Elemental analyses for some 1-Aryl-3-arylthiomethyl-3-methyltriazenes (52)

Compd.		C	H	N	O[a]	S
52b	Calc.	61.98	5.81	12.76	9.71	9.73
	Found	61.92	5.80	12.74	9.62	9.92
52c	Calc.	55.62	4.67	18.53	10.58	10.58
	Found	55.38	4.64	18.38	11.18	10.42
52f	Calc.	61.98	5.81	12.76	9.71	9.73
	Found	61.91	5.80	12.72	9.91	9.66

[a]By Difference

Two compounds (52a and 52f) were submitted to The Institute for Cancer Research, Royal Marsden Hospital, Sutton, U.K. for *in vivo* testing of antitumour activity against the ADJ/PC6 model tumour system, and the relevant data are presented in table 2,6.

All products were examined by TLC for purity and gave only one spot in each case.

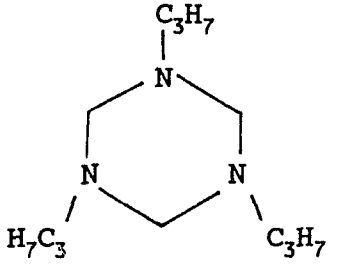
Table 2,6 Antitumour Activity of Two 1-Aryl-3-aryl-thiomethyl-3-methyltriazenes against the ADJ/PC6 Tumour *in vivo*

Cmpnd.	ID_{90}[a] (mg/kg)	LD_{50}[b] (mg/kg)	TI[c]	Optimal Dose (mg/kg)	% Inhibition of tumour growth
52a	325	>800	2.46	800	97.5
52f	50	>400	>8	400	96.7
51[d]	16.5	140	8.5	25	98.2

[a] 90% Inhibitory Dose
[b] 50% Lethal Dose
[c] TI= Therapeutic Index, LD_{50}/ID_{90}
[d] For comparison, Acetoxymethyltriazene Ar = *p*-AcC_6H_4 [5]

2,2,2 Method B

2,2,2a Synthesis of 1,3,5-Tri-n-propylhexahydro-1,3,5-triazine (53)

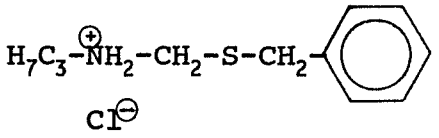


(53)

The method of Reynolds and Cossar [79] was modified as follows. Distilled water (200 mL) and sodium hydroxide (30 g) were added to a 2 litre three-necked flask equipped with a stirrer, thermometer and a dropping funnel. After the sodium hydroxide had dissolved, benzene (200 mL) was introduced. With external cooling (salt/ice bath),

propylamine (4 moles, 184 g) was added and the mixture cooled to 20-25°C. 40% aqueous formaldehyde solution (360 mL) was added slowly with stirring while maintaining the temperature below 25°C. After the formalin addition the benzene layer was separated, dried over anhydrous sodium sulphate followed by anhydrous magnesium sulphate and the benzene removed under vacuum. The product was distilled under reduced pressure through a column packed with glass helices to give 1,3,5-tri-n-propylhexahydro-1,3,5-triazine, b.p. 110°C @ 14 mmHg, n_D^{25}=1.4572, 134g (47%). ^{1}H NMR data, ($CDCl_3$) relative to 1% TMS: δ3.3, 6H,s(ring CH_2); δ2.5, 6H,m(Propyl N-C(1)H_2); δ1.45, 6H,m(Propyl C(2)H_2); δ0.9, 9H,t(propyl CH_3). IR data (thin film NaCl plates, reference air: 1460,1380 cm^{-1} (C-H bend); 1200,1110 cm^{-1} (C-N stretch); 1000,920 cm^{-1} (C-H out of plane bend).

2,2,2b Synthesis of thiobenzylmethyl-n-propylamine Hydrochloride (54)



(54)

Anhydrous hydrogen chloride (Matheson Ltd.) was absorbed in an anhydrous acetonitrile (100 mL) solution of 1,3,5-tri-n-propylhexahydro-1,3,5-triazine (0.003 moles, 7.03 g) which was maintained at *ca.* -30°C (dry ice/acetone).

Benzyl mercaptan (0.1 mole, 12.4 g. d=1.058 hence 11.74 mL) was dissolved in acetonitrile (50 mL) and added slowly to the above solution. The reaction mixture was allowed to remain at room temperature for 15 hours, cooled (ice) and poured into cold ether (400 mL). The crystalline salt was filtered off, washed and dried under vacuum. A sample was recrystallized from acetonitrile. M.p. 136-138 °C, 16.4g (72%). ^{1}H NMR Data ($CDCl_3$) relative to (1%) TMS: δ7.49-7.26, 5H,m(Aromatic); δ4.2, 2H,s((N-CH_2-S); δ3.9, 2H,s(S-CH_2-Ar); δ3.0, 2H,br.s(NH_2); δ1.9, 2H,m(propyl N-C(1)H_2); δ1.8, 2H,m(propyl C(2)H_2); δ0.98, 3H,t,J=9Hz (propyl CH_3).

2,2,2c Reaction of Thiobenzylmethyl-n-propylamine Hydrochloride (54) with *p*-Cyanobenzenediazonium ion

4-Aminobenzonitrile (0.016 moles, 1.95 g) was dissolved in concentrated hydrochloric acid (5 mL) and distilled water (65 mL). Sodium nitrite (0.016 mole, 1.25 g) was dissolved in water (10 mL). The arylamine was diazotized at 0°C for 1 hour with this solution. The diazonium solution was tested with starch iodide paper and sulphamic acid added until there was no evidence of nitrous acid, and the salt solution then carefully neutralized with sodium bicarbonate. An excess of the aminosulphide (4.2 g) was dissolved in water (100 mL). Methanol was added to give a clear solution which was then cooled to 0°C and added slowly with stirring to the diazonium salt solution. The mixture was stirred for 1 hour and the resulting yellow oil extracted into

chloroform (3X25 mL), dried with anhydrous sodium sulphate and the chloroform removed under vacuum. The resulting oil decomposed rapidly to give an intractable tar.

2,3 Results and Discussion

Identification of the 1-aryl-3-arylthiomethyl-3-methyltriazenes was based on IR and NMR spectroscopic analysis, molecular weight (by accurate mass MS), electron impact mass spectrometry and, in selected cases, elemental analysis. Physical data and yields are shown in table 2,1.

The infra-red spectra show no notable features apart from the bands due to the aryl X-substituent, and there were no detectable bands from hydroxy- or acetoxy- groups. The ^1H NMR spectra (Table 2,2) show the overlapping pattern of two aromatic nuclei, an $N-CH_3$ resonance at δ 3.22-3.27, the $Ar-S-CH_2$ resonance at δ 5.16-5.24 and the expected resonances from protons, where present in the X or Y substituents.

The $S-CH_2$ resonances were much further downfield than anticipated and occur at approximately the same shift as the parent hydroxymethyltriazene. We had predicted that replacing OH by S-Ph would cause a slight upfield shift. The S-alkyl resonances occur at *ca.* δ 4.95 [65], and additive chemical shift parameters for methylene protons [81] predict a downfield shift of 0.29 ppm for CH_2-S-Ar

compared with CH_2-S-R. These arylthiomethyl shifts are thus consistent with the observations for alkylthiomethyl compounds, but these are also anomalous by comparison with the hydroxymethyltriazenes. Further NMR work was carried out in an effort to explain these anomalous shifts and this investigation is discussed in chapter 5.

The ^{13}C data are given in table 2,3 and show no surprising features. Empirical substituent effect parameters [82] predict an upfield shift of approximately 28 ppm for the S-CH_2 relative to HO-CH_2 and the experimental values were around 17 ppm which is reasonable considering the approximations made in this comparison. No parameter was listed for S-Ar, so the value for S-R was used. The aromatic signals were assigned by consideration of the multiplicities of the fully coupled spectra, peak heights and predicted shifts from standard correlation tables [82]. To obtain good predictions for the A ring carbon shifts, it was necessary to calculate a set of substituent shift parameters for the triazene moiety -N=N-N-R_2 . These shifts (A_i) are as follows: *ipso*=+20.7, *ortho*=-8.2, *meta*=+2.4, *para*=-3.8 and whilst based upon a rather small data set (10 compounds) gave a substantially better prediction in the empirical formula:

$$\delta_c(k) = 128.5 + \Sigma A_i(R)$$

than any of the available alternatives such as -$N(CH_3)_2$.

The mass spectra of these compounds are dominated by

the loss of the terminal nitrogen of the triazene moiety with its associated substituents, giving the diazonium ion, and by further loss of N_2 to give Ar^+ (table 2,4), (Fig. 2,2). The peak at M-102 is of particular interest, representing an unusual intramolecular rearrangement, and a full discussion of this phenomenon is included in Chapter 3.

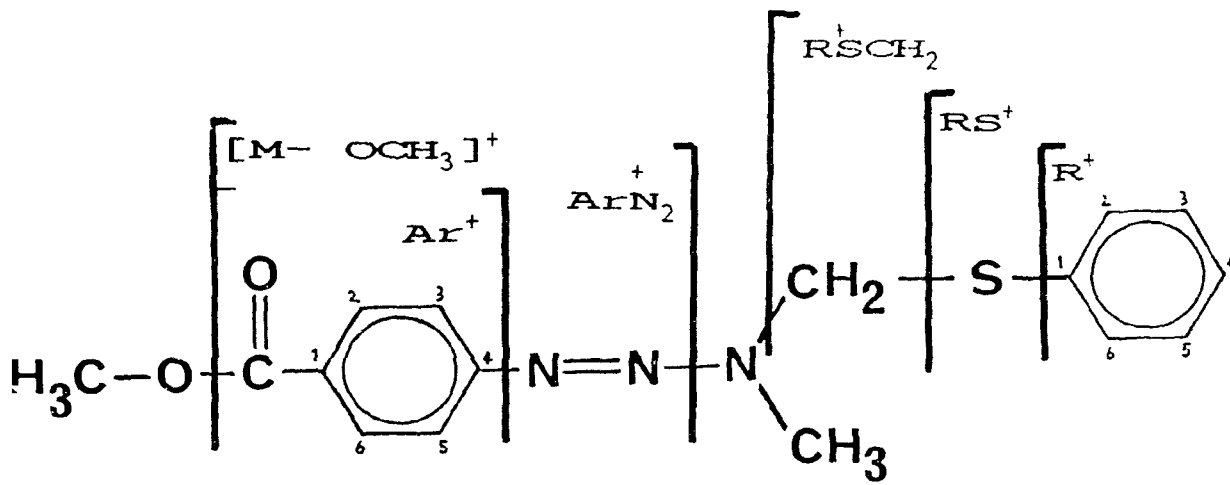


Figure 2,2

Typical Mass Spectral Fragmentation of an

Arylthiomethyltriazene

The 1-aryl-3-arylthiomethyl-3-methyltriazenes were prepared in the hope that they would have half-lives under physiological conditions intermediate between those of the acetoxymethyltriazenes which decay too quickly and the methoxymethyltriazenes which do not decay at all due to the poor leaving group character of the methoxide ion. These methyl ethers are consequently not cytotoxic *in vitro* [77]. Interestingly the 3-alkanethiomethyltriazenes are also stable in aqueous buffer, showing no tendency to decompose over a 24 hour period [83]. In contrast, initial kinetic data on the arylthiomethyltriazenes described here appeared to indicate that they were very labile in aqueous buffer (pH 7.2, 0.05 M sodium phosphate), with half-lives in the range 4-16 minutes. Subsequent experiments however, showed that this apparent decay was due, at least in part, to the poor solubility of the compounds in buffer. Compound 52f was subsequently tested in the pH 3.3 formate buffer/isopropanol system used in chapter 4, and was found to have a $t_{1/2}$ = 2.43 ± 0.13 min. ($k = 5.46 \times 10^{-3} \pm 4.0 \times 10^{-4}$). This is substantially longer than for the corresponding hydroxymethyltriazene(1-[4-carbomethoxy]phenyl-3-hydroxymethyl-3-methyltriazene), $t_{1/2}$ = 0.66 ± 0.04 min. ($k = 1.95 \times 10^{-2}$). The stability of these compounds was sufficiently encouraging, being substantially more stable than the hydroxymethyltriazene, for samples to be sent for antitumour testing (table 2,6). Compounds 52a

and 52f were tested against the ADJ/PC6 solid tumour [62] and do have activity comparable with other α-substituted alkyltriazenes. However, although a comparable percentage of tumour inhibition was achieved, this required a dose much larger than for the analogous acetoxymethyltriazene, and this is reflected in the lower therapeutic index.

2,4 Conclusions

The successful syntheses of the arylthiomethyltriazenes illustrates the usefulness of the acetoxymethyltriazenes as synthetic intermediates for the preparation of hydroxymethyltriazene derivatives. The alternative method which was briefly examined has not been shown to be unusable, but the obnoxious nature of the intermediates (Figure 2,3) (they have a nauseating odour and pass freely through vinyl gloves and skin) demand special handling techniques and extremely efficient containment. Since an alternative method was found, there appeared to be no necessity for persevering with this approach.

With regard to the somewhat discouraging results of the tumour tests, it should, however be pointed out that the results for *in vivo* tests in mice may have little bearing on the pharmacokinetics of these substances in man, and the low therapeutic indices may well be offset by favourable metabolic kinetics. The compounds were shown to have some activity in a limited number of tumour cell lines. It is possible that additional antitumour activity may be found

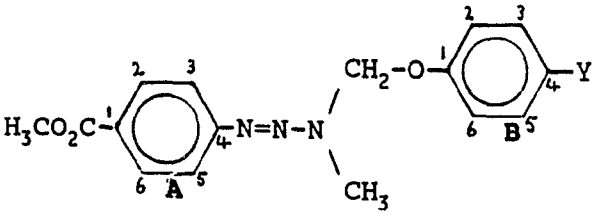
when a larger tumour panel is used.

Chapter 3

Synthesis of 1-Aryl-3-aryloxymethyl-3-methyltriazenes and 1-Aryl-3-arylmethyl-3-methyltriazenes

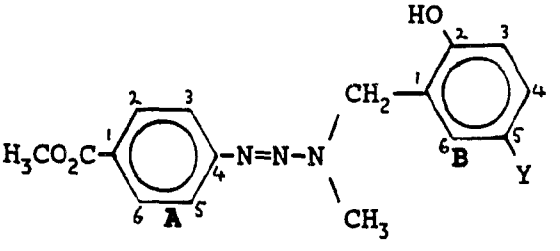
3.1 Introduction

After our successful syntheses of S-aryl substituted 1-aryl-3,3-dimethyltriazenes, it was regarded as important to prepare the O-aryl analogues. Our objective was to develop a triazene prodrug which, like hydroxymethyltriazene, did not require oxidative metabolism, but which had a longer half-life. The S-aryl derivatives were less reactive than the hydroxymethyltriazenes under similar conditions. If the stability of these compounds depended to some extent on the leaving-group ability of -S-Ar, then comparison of the pK_a's of substituted phenols (pK_a = 7.15 for *p*-NO_2, 10.21 for *p*-OMe and 9.98 for phenol itself) and thiophenol (7.8) suggests that an -O-Ar substituent should give triazenes with hydrolysis profiles ranging from somewhat faster to substantially slower than that of the S-Aryl compounds. The appropriate substituted phenols are more readily available than the corresponding thiophenols, enabling us to examine a range of compounds to determine how leaving group ability affects the hydrolysis rates. Simple replacement of a thiophenol with a phenol, in the methodology outlined in chapter 2, was unsuccessful, the desired product being undetectable in the reaction mixtures. It turned out that a change of solvent was required to effect the desired substitution, and in some cases, the initial generation of a phenolate ion with sodium hydride was found to be unnecessary.



Number	Y
55a	OCH_3
55b	CH_3
55c	H
55d	Cl
55e	Br
55f	CO_2CH_3
55g	CN
55h	NO_2

(Figure 3,1)



Number	Y
56a	H
56b	CH_3
56c	Br

(Figure 3.2)

3.2 Experimental

All solvents were purchased from BDH Chemicals Ltd. and were dried over molecular sieves type 4A except for acetone which was dried over anhydrous sodium sulphate. In some instances the solvents were distilled prior to use. For some reactions chloroform was washed repeatedly with distilled water, dried overnight over anhydrous sodium sulphate, distilled and stored in the dark [79].

Melting points were determined using a Reichert hot-stage microscope and are uncorrected.

Infrared spectra were obtained using a Perkin-Elmer 299 Infrared Spectrophotometer, calibrated against polystyrene film. Samples were prepared in $CHCl_3$ solution and IRs run using matched KBr cells (Perkin-Elmer) with a $CHCl_3$ reference.

1-Aryl-3-aryloxymethyl-3-methyltriazenes (55)

3.2.1 Method A

The appropriate 1-aryl-3-acetoxymethyl-3-methyltriazene (51),(1.3 mmoles) prepared by the method of Vaughan *et al.* [61] was dissolved in dry chloroform (10 mL). A 50% excess of the phenol (1.9 mmoles) was added and the mixture refluxed for 2-18 hours depending on the desired product. Where the phenol substituent was H, the mixture was not heated, but stirred for 24 hours. The chloroform was removed under vacuum and the resulting oil washed with anhydrous ether. This usually resulted in the precipitation

of the product which was filtered off, dried and recrystallized from a suitable solvent. Where the product was an oil, it was chromatographed on silica gel with hexane/ether prior to recrystallization.

3.2.2 Method B

As in method A the 1-aryl-3-acetoxymethyl-3-methyltriazene was dissolved in chloroform. Sodium hydride 60% oily suspension (0.08 g) was placed in a round bottomed flask fitted with a rubber septum and washed with dry petroleum ether (3 X 10 mL) to remove the oil, affording clean sodium hydride (2.0 mmoles). The appropriate phenol (1.9 mmoles) was dissolved in anhydrous chloroform (20 mL) and added carefully to the sodium hydride, provision being made for the safe egress of the hydrogen generated by the reaction. The resulting sodium phenolate solution was filtered to remove unreacted sodium hydride and added to the acetoxymethyltriazene solution and refluxed for 2-2 1/2 hours. Subsequent work up was the same as for Method A. Column chromatography of the reaction products of method B yielded a second product [85], identified as 56a-c, in addition to the main product (55c,b and e).

In both methods the progress of the reaction was followed by T.L.C. Using 3:3:1 Petroleum ether/Ether/ Chloroform as solvent. Except where specified, all reagents were reagent grade materials purchased from Aldrich Chemical Co. Ltd., Montreal and were used without further

purification.

The physical data and yields for the compounds 55a-h are shown in table 3,1, and those for compounds 56a-c in table 3,2. UV Maxima were determined in a 2:1 mixture of 0.1 M phosphate buffer (pH 7.5) and isopropanol.

^{13}C (at 90 MHz) and ^{1}H NMR (at 361 MHz) spectra were acquired on the Nicolet 360 NB spectrometer of the Atlantic Region Magnetic Resonance Centre at Dalhousie University. Sweep widths for ^{13}C were 20 000 Hz and for ^{1}H 4000 Hz. The spectra were obtained at 20°C in deuterochloroform. Chemical shifts in ppm. were measured relative to tetramethylsilane (1% internal standard). These shifts are presented in tables 3,3 - 3,6.

Positive ion mass spectra were obtained from the MS service facility of Dalhousie University using a Consolidated Electrodynamics Corporation CEC 21-104 mass spectrometer and the operating parameters were as follows. Source temperature 130-145°C, Direct probe temperature 75-130°C, Accelerating voltage 800 V, Ionization voltage 70 eV, Scan rate 6 s dec^{-1}. The relative intensities of the mass spectral peaks for the compounds 55a-h are shown in table 3,7.

Elemental analyses were performed on three of the compounds by Canadian microanalytical Service Ltd., Delta, British Columbia, and these assays are shown in table 3,8.

Table 3,1 Physical Data for 1-Aryl-3-aryloxymethyl-3-methyltriazenes (55)

No.	Y	Method	Yield (%)	m.p. (°C)	Solvent	UV λmax (nm)	Infra-Red (cm^{-1})	
55a	OCH_3	A	36	127-129	Ether	316	1705 (C=O)	1600 (R.St)
55b	CH_3	B	8.5	78-79	Hexane/Ether	307	1705 (C=O)	1600 (R.St)
55c	H	A	57	46-47	Hexane/Ether	320	1710 (C=O)	1590 (R.St)
55d	Cl	A	52	95-98	Ether	303	1710 (C=O)	1600 (R.St)
55e	Br	A	78	99-102	Hexane/Ether	322	1710 (C=O)	1600 (R.St)
55f	MeO_2C	A	36	95-96	Ether/$CHCl_3$	303	1720 (C=O)	1600 (R.St)
55g	CN	A	34	136-139	Hexane/Ether	314	1710 (C=O)	2225 (C≡N)
55h	NO_2	B	45	152-153	Ether/$CHCl_3$	312	1710 (C=O)	1605 (R.St)

Table 3,2 Physical Data for 1-Aryl-3-arylmethyl-3-methyltriazenes (56)

No.	Y	Method	Yield (%)	m.p. (°C)	Solvent	UV λmax (nm)	IR (cm^{-1})	
56a	H	B	22	102-104	Hexane/Ether	-	1720 (C=O)	3400 (OH)
56b	CH_3	B	11	118-119	Ether	-	1710 (C=O)	3400 (OH)
56c	Br	B	tr	84-86	Ether/Hexane	344	1705 (C=O)	3410 (OH)

Table 3,3 ^{1}H NMR Data for 1-Aryl-3-aryloxymethyl-3-methyltriazenes (55) in $CDCl_3$ at 20°C

Compd.	CH_3O	Arom. A (AA'BB')	NCH_3	NCH_2	Arom. B	Y
55a	3.91	8.03-7.41	3.29	5.66	6.95-6.80	3.75 (OCH_3)
55b	3.91	8.04-7.43	3.29	5.69	7.07-6.83	2.28 (CH_3)
55c	3.91	8.04-7.45	3.30	5.72	7.31-6.82	
55d	3.91	8.05-7.45	3.29	5.70	7.26-6.93	
55e	3.92	8.04-7.46	3.29	5.70	7.40-6.89	
55f	3.92	8.06-7.41	3.31	5.79	7.98-7.03	3.88 (OCH_3)
55g	3.92	8.07-7 49	3.31	5.79	7.61-7.07	
55h	3.92	8.22-7.50	3.32	5.84	8.08-7.08	

Table 3,4 ^{1}H NMR Data for 1-Aryl-3-arylmethyl-3-methyltriazenes (56) in $CDCl_3$ at 20°C

No.	CH_3O	Arom. A AA'BB'	NCH_3	NCH_2	Arom. B.	Y	OH
56a	3.92	8.06-7.52	3.68	4.83	7.27-6.87		10.2 (Br.)
56b	3.91	8.05-7.50	3.67	4.78	7.23-6.63	2.28 (CH_3)	9.95 (Br.)
56c	3.91	8.05-7.50	3.68	4.75	7.36-6.82		10.3 (Br.)

Table 3,5 ^{13}C NMR Data for 1-Aryl-3-aryloxymethyl-3-methyltriazenes (55) in $CDCl_3$ at 20°C

No.	CH_2O	C=O	Arom. A[a]				NCH_3	NCH_2
			C_1	$C_{2,6}$	$C_{3,5}$	C_4		
55a	52.00	166.90	127.81	130.54	116.53	153.51	33.72	83.39
55b	51.92	166.89	127.90	131.80	116.72	153.67	33.67	83.78
55c	52.02	166.90	127.81	130.54	116.53	153.51	33.72	83.39
55d	52.05	166.83	127.96	130.57	117.79	153.29	33.76	83.50
55e	52.07	166.84	127.97	130.57	118.19	153.27	33.73	83.35
55f	51.96	166.82	128.11	131.59	120.95	153.20	33.77	82.84
55g	52.01	166.82	128.31	130.63	116.48	152.98	33.81	82.83
55h	52.01	166.72	128.35	130.78	115.68	152.88	33.85	83.00

[a] Peak assignments based upon emperical shift parameters

Table 3.5 ^{13}C NMR Data for 1-Aryl-3-aryloxymethyl-3-methyltriazenes (55) in $CDCl_3$ at 20°C (Continued)

	Arom. B[a]				
No	C_1	$C_{2,6}$	$C_{3,5}$	C_4	Y
55a	150.55	120.89	118.30	155.08	55.66 (OCH_3)
55b	154.67	120.94	130.53	131.80	20.52 (CH_3)
55c	156.70	120.91	129.59	122.28	
55d	155.24	120.90	129.08	127.27	
55e	155.75	120.90	132.43	114.61	
55f	160.45	115.47	130.59	123.88	52.96 (OCH_3), 166.54 (C=O)
55g	160.03	120.96	134.03	112.18	118.78 (C≡N)
55h	161.69	120.96	125.85	142.24	

Table 3,6 ^{13}C **NMR Data for 1-Aryl-3-arylmethyl-3-methyltriazenes (56) in** $CDCl_3$ **at 20°C**

No.	CH_3O	C=O	Arom. A[a] C_1	$C_{2,6}$	$C_{3,5}$	C_4	NCH_3	NCH_2
56a	52.01	166.81	130.62	130.78	119.78	152.10	42.22	50.02
56b	52.00	166.77	129.14	130.79	119.81	153.44	42.37	50.00
56c	52.05	166.74	127.60	130.82	119.80	151.76	42.34	49.52

[a] Peak Assignments based upon empirical shift parameters

Table 3,6 ^{13}C **NMR Data for 1-Aryl-3-arylmethyl-3-methyltriazenes (56) in** $CDCl_3$ **at 20°C (Continued)**

No.	Arom. B[a] C_1	C_2	C_3	C_4	C_5	C_6	Y
56a	127.35	155.70	118.11	130.52	119.92	131.24	
56b	130.79	145.65	120.34	117.92	131.55	131.08	20.42 (CH_3)
56c	131.05	155.00	122.62	120.04	111.42	133.31	

Table 3,7 Mass Spectral Intensities for the 1-Aryl-3-aryloxymethyl-3-methyltriazenes (55)

Ion	Y					
	OCH_3	CH_3	Br	CO_2CH_3	CN	NO_2
$[M]^{+\cdot}$	1	1	0.1	3	0.4	1
$[M-CH_3O]^+$	2	3	1	4	3	3
$[M-CH_3C=O]$	12	11	4	7	1	1
$[M-102]^+$ [a]	4	22	8	21	2	2
$[ArN_2]^+$	55	57	64	45	49	61
$[ArH]^+$	12	10	10	9	10	12
$[Ar]^+$	100	100	100	100	100	100
$[O_2CC_6H_4]^+$	10	11	10	18	14	39
$[Y-C_6H_4-OCH_2]^+$	2	5	1	1	-	-[b]
$[Y-C_6H_4-OH]^+$	24	15	6	4	1.5	11
$[Y-C_6H_4-O]^+$	38	14	3	3	2	1
$[Y-C_6H_4]^+$	3	8	2	-[c]	5	4
$[Y-C_5H_4]^+$	9	7	5	10	7	1
$[C_7H_7]^+$	1	-[d]	1	2	4	5

[a] See discussion, page 76
[b] Coincident with $[ArH]^+$ (M/e 136)
[c] Coincident with $[Ar]^+$ (M/e 135)
[d] Coincident with $[Y-C_6H_4]$ (M/e 91)

Table 3,8 Elemental Analyses for some 1-Aryl-3-aryloxymethyl-3-methyltriazenes

No.		C	H	N	O[a]	Br
55a	Calc.	61.99	5.81	12.76	19.44	-
	Found	61.95	5.62	12.74	19.69	-
55e	Calc.	50.80	4.26	11.11	12.70	21.13
	Found	51.02	4.45	11.18	12.30	21.05
55h	Calc.	61.99	5.81	12.76	19.44	-
	Found	61.95	5.62	12.74	19.69	-

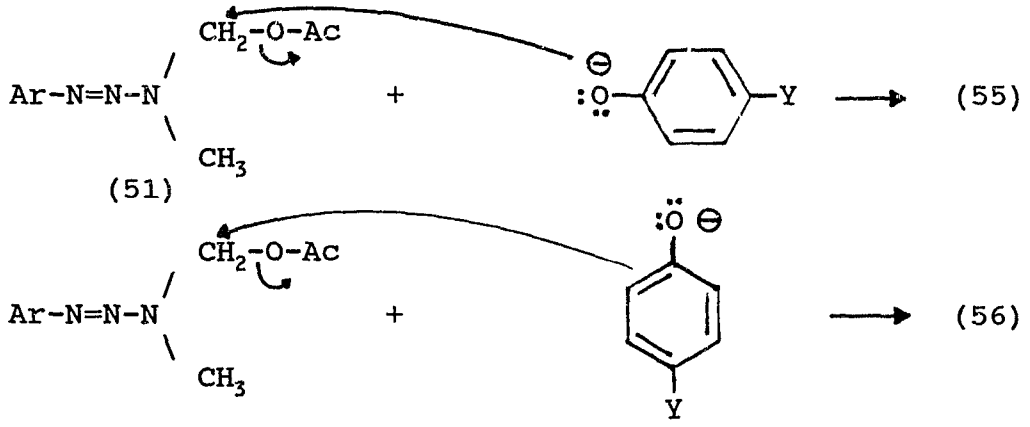
[a] Calculated by difference

All compounds were examined by TLC for purity and showed only one spot in each case.

3.3 Results and discussion

Using chloroform as a solvent instead of dimethylformamide enabled us to prepare the desired compounds in yields ranging from 8.5% to 78%. Whilst the lower yield reactions might be considered to be of little synthetic usefulness, they did afford us sufficient material for our further investigations and afford materials not available by any other synthetic route attempted so far. In two cases the generation of the phenolate ion with sodium hydride prior to reaction with the acetoxymethyltriazene (51, Ar = $H_3CO_2C-C_6H_4-$) gave the best yields of the 1-aryl-3-aryloxymethyl-3-methyltriazenes (55b and 55h).

It is noteworthy that in the reactions using sodium hydride, where the reaction time was extended from two to eighteen hours, a second product, the 1-aryl-3-arylmethyl-3-methyltriazene (56a - c) was formed. These compounds are thought to arise in the following way (Scheme 3,1).



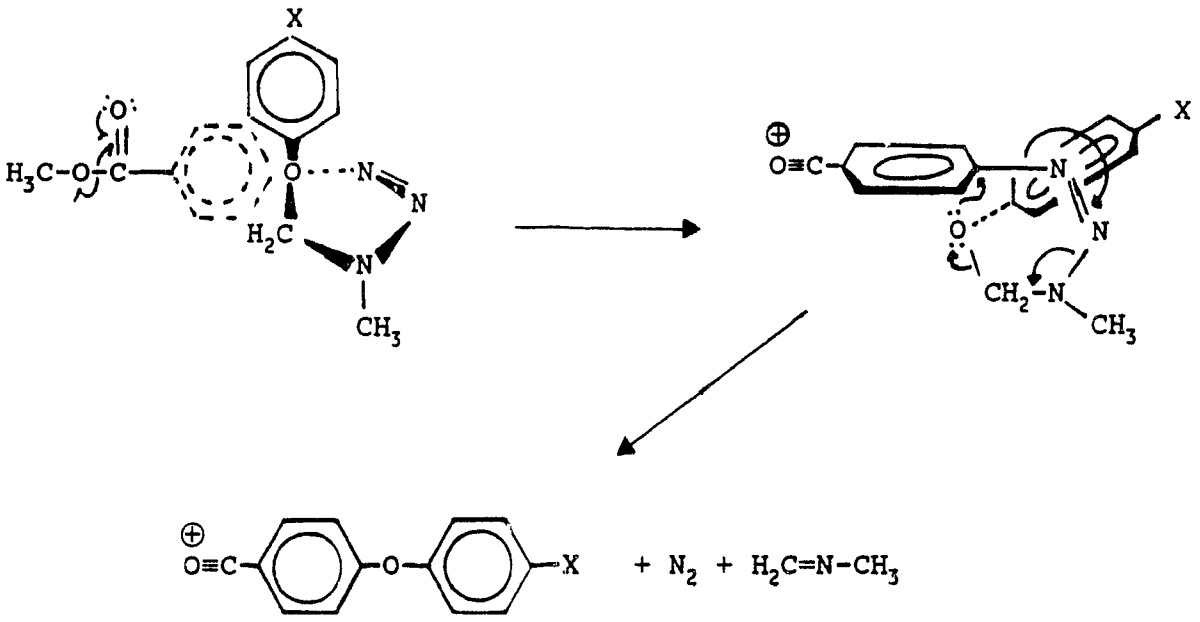
(Scheme 3,1)

The formation of (56) under these conditions could be

due to a rearrangement of (55), but so far we have been unable to produce (56) from pure (55) under the conditions of the reaction so that the possibility of this rearrangement remains a matter for speculation.

The ^{13}C and ^{1}H NMR data show no unusual features, and are in complete agreement with the proposed structures. The ^{13}C aromatic peaks were assigned by consideration of their decoupled multiplicities, peak heights and additive substituent shift correlation calculations. For further remarks on the NMR behavior of these compounds, see Chapter 5.

The mass spectra of all the 1-aryl-3-aryloxymethyl-3-methyltriazenes (55) exhibited a fairly intense peak at M-102 which caused some puzzlement. Comparison with the mass spectra of the S-aryl analogues (52a-h) indicated that the structural requirement for this feature was an ester function on the 1-aryl end of the molecule. Re-examination of the accurate-mass measurements for the S-aryl analogues showed that the mass lost in this reaction was 102.0668 corresponding to the empirical formula $C_3H_8N_3O$ for the methyl ester, and 116.0825 corresponding to $C_4H_{10}N_3O$ for the ethyl ester. These cannot be lost as single fragments, and the remaining charged species must arise from some kind of intramolecular rearrangement. One possibility is shown in scheme 3,2.



(Scheme 3,2)

3,4 Conclusions

The 1-aryl-3-aryloxymethyl-3-methyltriazenes (55a-h) were synthesised by what appears to be a quite general method, and were extensively characterised by a wide variety of techniques. In addition, the novel 1-aryl-3-arylmethyl-3-methyltriazenes (56a-c) were detected in modest yields from some reactions.

The effects of varying the solvent upon the reaction products and also that of the presence or absence of sodium hydride have been noted, but this is an area for further investigation since the precise nature of these effects is still unknown. Of further interest was the novel mass spectral rearrangement common to both the O-aryl and the S-

aryl compounds where the 1-aryl group contains an ester function. Whilst at this point the rearrangement scheme must be regarded as speculative, it is hoped that we will soon have corroboration in the form of tandem-MS data.

Chapter 4

Hydrolysis of 1-Aryl-3-aryloxymethyl-3-methyltriazenes

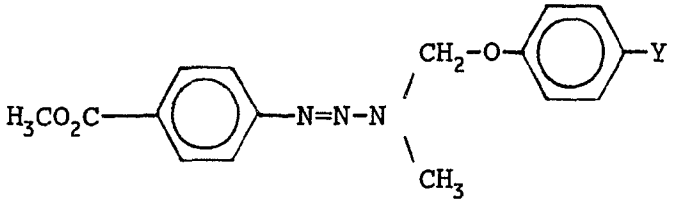
4,1 Introduction

Preliminary investigations of the hydrolysis of the 1-aryl-3-arylthiomethyl-3-methyltriazenes (52) indicated that these compounds were considerably more labile in aqueous buffer than the corresponding O- or S-methyl compounds, and yet with substantially longer half-lives than hydroxymethyltriazenes. As already mentioned, it was hoped that the 1-aryl-3-aryloxymethyl-3-methyltriazenes (55) would have half-lives that would render them useful as antitumour pro-drugs. The wide range of readily available substituted phenols enabled us to prepare a selection of compounds whose stabilities in aqueous buffers were expected to yield a broad range of half-lives. The measurement of these half-lives will provide the necessary information to enable us to choose compounds for antitumour testing.

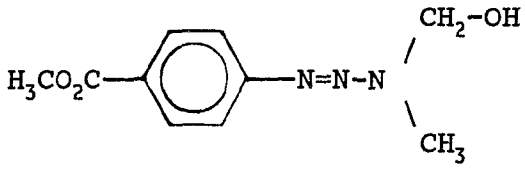
4,2 Experimental

4,2,1 Materials

The compounds used in this study (Fig. 4,1) were prepared by established procedures from the literature [75] with the exception of the 3-aryloxymethyl-3-methyltriazenes (55) whose synthesis and characterization are reported in chapter 3.



Compound	Y
55a	OCH_3
55b	CH_3
55c	H
55d	Cl
55e	Br
55f	CO_2CH_3
55g	CN
55h	NO_2



(57)

(Figure 4,1)

All compounds used were recrystallized to constant melting point. Approximately 1.5 mg. of each test compound was dissolved in 1 ml. of dried, distilled dimethylsulfoxide. These solutions were stored under refrigeration (-10° C) and thawed for use as required. Buffers were prepared as follows.

MES buffer

2[N-morpholino]ethanesulfonic acid (Sigma M8250) (4.88g) was dissolved in 250 ml. distilled water to give a 0.1 M solution. This was adjusted to pH 5.5 using 0.2 M sodium hydroxide.

Formate buffers (pH 3.3)

0.1 molar: 88% formic acid solution (3.206 g) and sodium formate (2.630 g) made up to 1 L.

0.5 molar: 88% formic acid solution (16.03 g) and sodium formate (13.17 g) made up to 1 L.

0.01 molar: 88% formic acid solution (0.321 g) and sodium formate (0.236 g) made up to 1 L.

HCl pH 2.0 (Not a true buffer)

0.2 M hydrochloric acid (6.5 mL) and 0.2 M potassium chloride (43.5 mL) made up to 100 mL.

NaOH pH 12.0 (Not a true buffer)

0.2 M Sodium hydroxide (6.0 mL) and 0.2 M potassium chloride (44 mL) made up to 100 mL.

Phosphate pH 7.5

0.2 M monobasic sodium phosphate (16 mL) and 0.2 M dibasic sodium phosphate (84 mL) made up to 200 mL.

The pH of the buffer solutions was checked periodically using an Accumet model 810 pH meter (Fisher Scientific) standardized with commercial standard buffers at pH 7.00 and 4.00 (Fisher Scientific).

4,2,2 UV Analysis

UV-visible absorption measurements were made using a Cary 219 spectrophotometer (Varian) with repeat scan and timed delay facilities. The cell chamber was thermostatted at 38±1° C. These measurements were made in one of three modes. For fast hydrolyses ($t_{1/2}$<10 min.) the spectrophotometer was operated at fixed wavelength (320 nm, close to the triazene maximum) with the chart set for time drive to give a continuous trace of decay with time at this wavelength. For intermediate rates (10 min. < $t_{1/2}$ < 60 min.), the spectrophotometer was set to scan from 450 to 230 nm. at 2 nm s^{-1} repeatedly with a 4 min. cycletime in overlay mode so that isosbestic points could be observed as a check on spectrophotometer stability. Very slow hydrolyses ($t_{1/2}$ > 60 min.) were measured at a fixed wavelength (320 nm) with a timed delay so that a measurement was made for 10 seconds every hour or every 90 min. as appropriate.

Hydrolyses were carried out in stoppered Spectrosil quartz cells (Fisher Scientific) in neat buffer (3 mL) or in a 2:1 buffer-isopropanol mixture (2 mL buffer: 1 mL isopropanol). An aliquot (50 μL) of the DMSO solution of the compound under investigation was added to the cell which was then quickly stoppered, inverted once to mix, inserted in the spectrophotometer and the appropriate program started. Volumes were measured with Eppendorf pipettes.

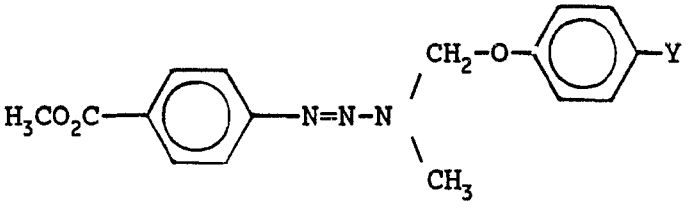
After each run, absorbances were measured manually and values for ln A calculated according to:

$$\ln A = \ln (A_t - A_\infty)/(A_0 - A_\infty) \qquad \text{(Eqn. 4,1)}$$

where A_t is the absorbance after time t, A_∞ is the absorbance after complete hydrolysis and A_0 is the initial absorbance. All experiments were carried out in duplicate. If the corresponding values of ln A for the two runs differed by more than 0.05 , then the data were discarded and the experiment repeated. A simple linear least-squares fit program was used to generate plots of these results. The results for the 1-aryl-3-aryloxymethyl-3-methyltriazenes (55) are presented in figures 4,2 - 4,4. Plots for the corresponding hydroxymethyltriazene (57) are given for the buffer/isopropanol system (Fig. 4,5) and also for neat buffer (Fig. 4,6) for comparison. The slope of each plot represents the rate constant k_{obs}. Half-lives were calculated using:

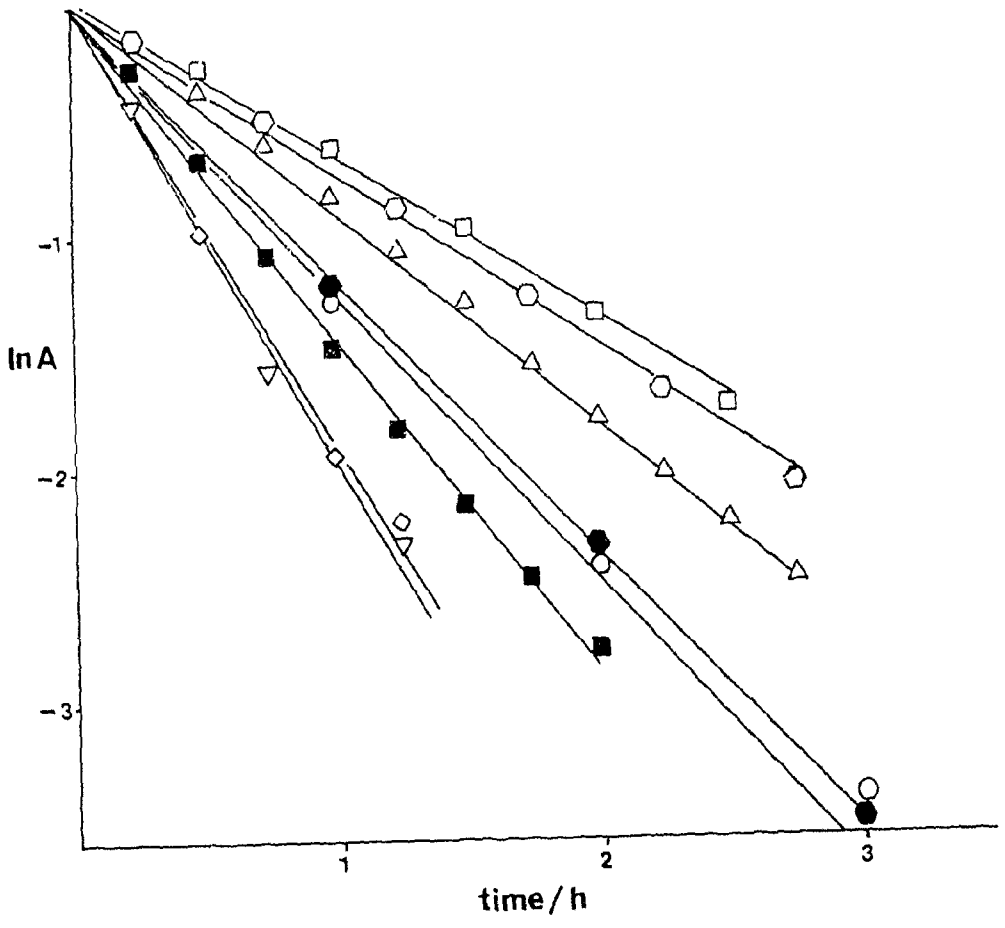
$$t_{1/2} = \{60(\ln 0.5) - A\}/B \qquad \text{(Eqn. 4,2)}$$

Where A is the y-intercept and B the slope in minutes^{-1}. These parameters are presented in tables 4,1 - 4,3.



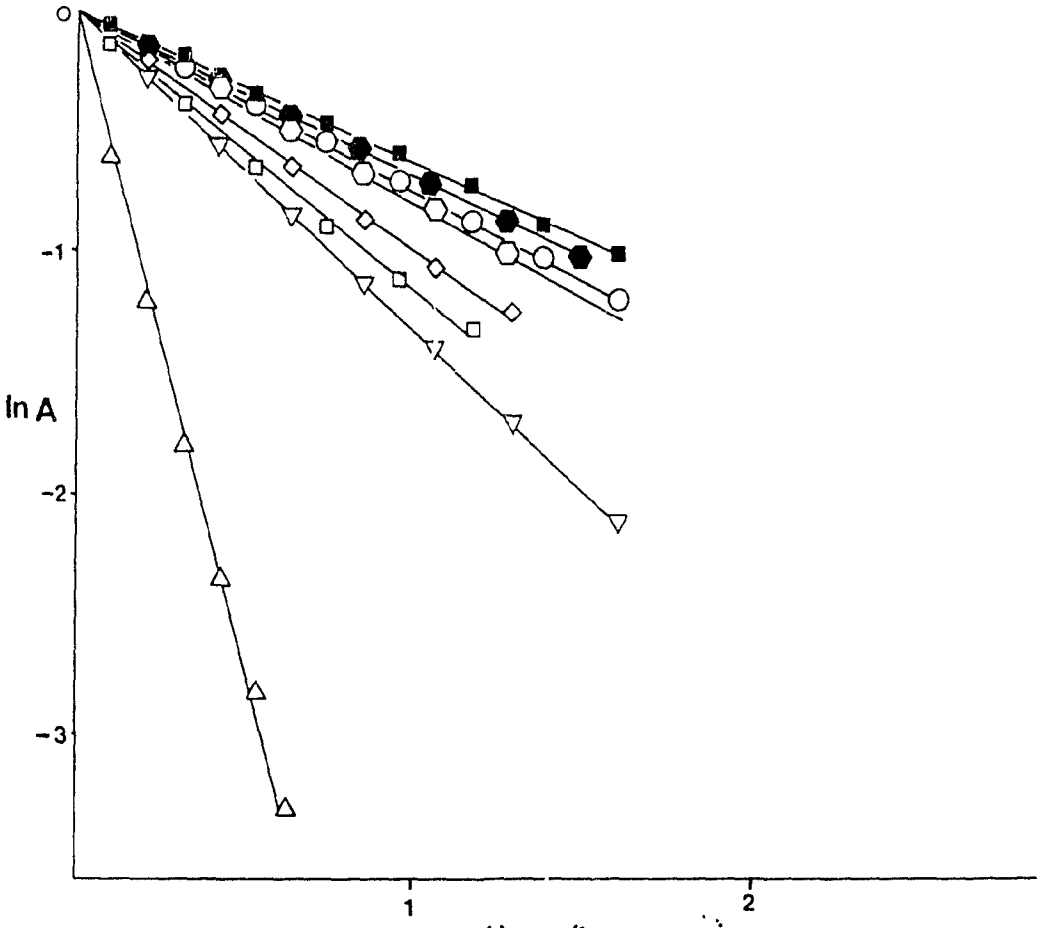
LEGEND FOR FIGURES 4,2 - 4,4

SYMBOL	COMPOUND	Y-GROUP
▽	55a	OCH_3
□	55b	CH_3
⬡	55c	H
○	55d	Cl
⬢	55e	Br
■	55f	CO_2CH_3
◇	55g	CN
△	55h	NO_2



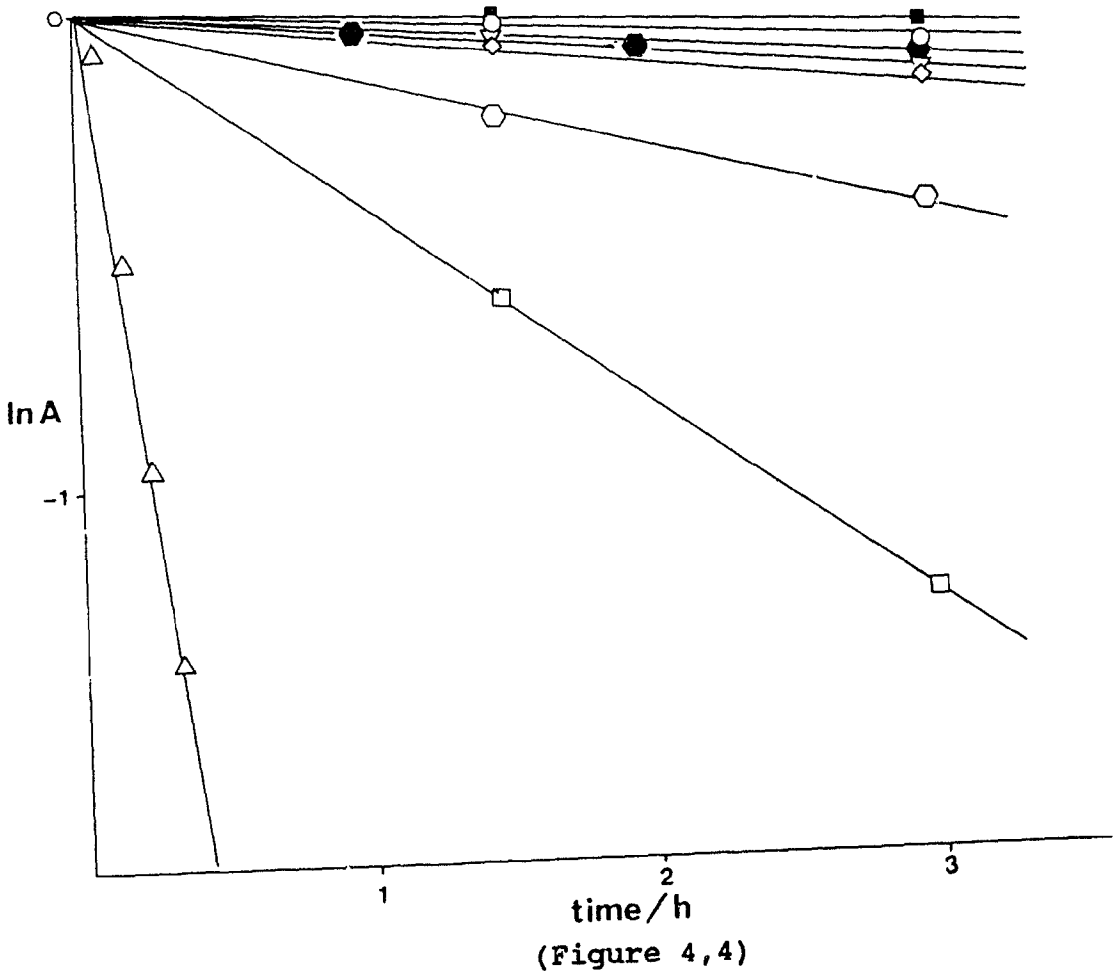
(Figure 4,2)

Hydrolysis of 1-Aryl-3-aryloxymethyl-3-methyltriazenes in pH 2.0 Buffer/Isopropanol System



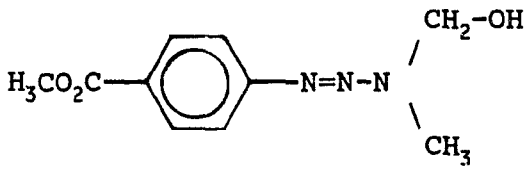
(Figure 4,3)

Hydrolysis of 1-Aryl-3-aryloxymethyl-3-methyltriazenes in pH 3.3 Buffer/Isopropanol System



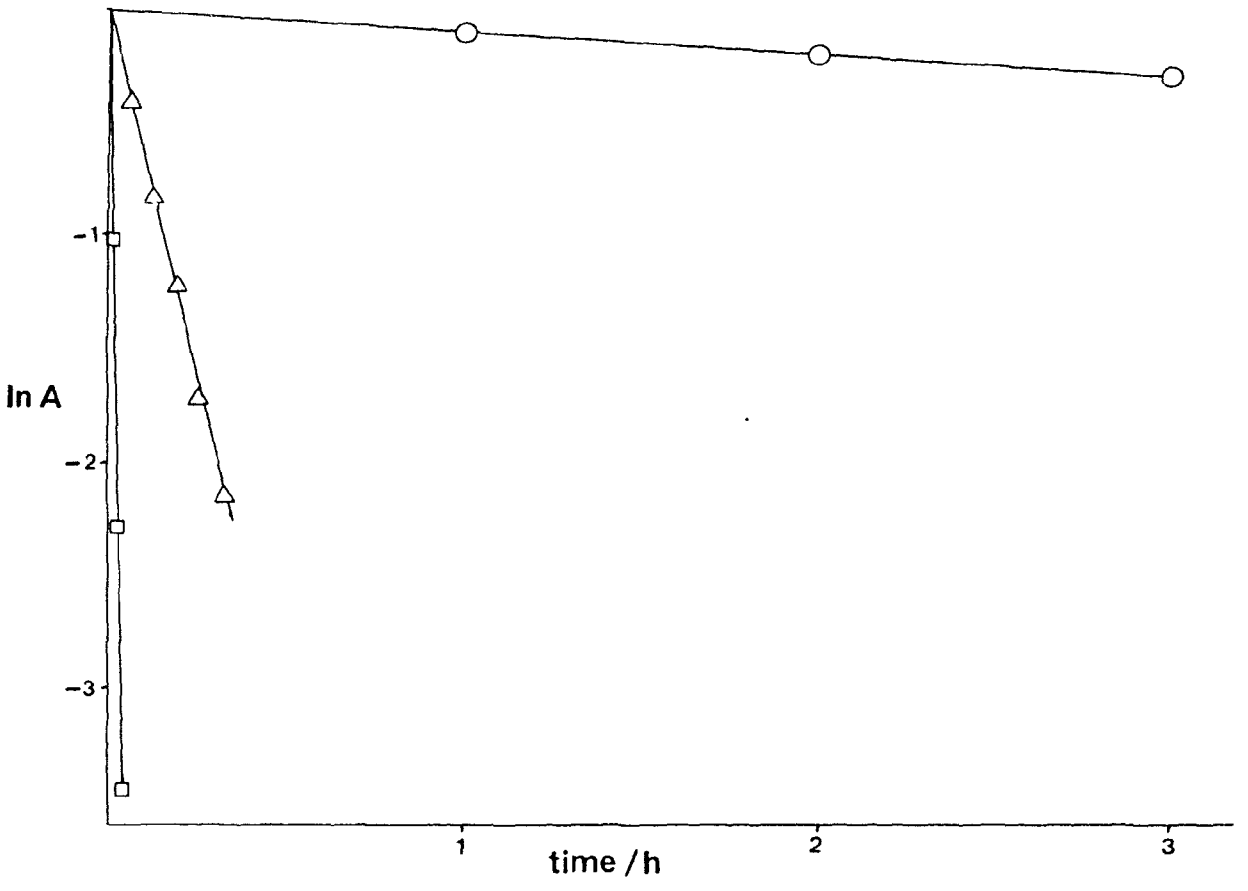
(Figure 4,4)

Hydrolysis of 1-Aryl-3-aryloxymethyl-3-methyltriazenes in pH 5.5 Buffer/Isopropanol System



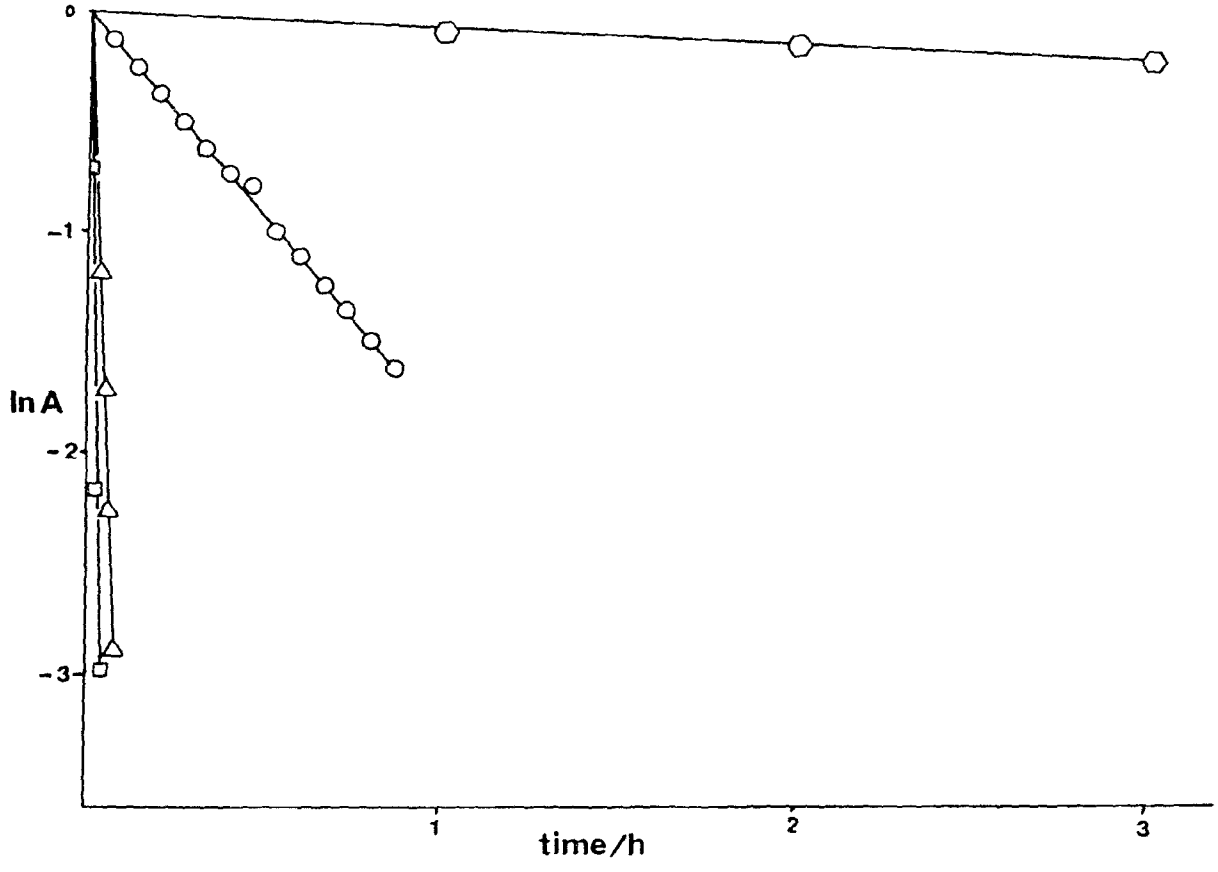
LEGEND FOR FIGURFS 4,5 & 4,6

SYMBOL	BUFFER
□	Formate pH 3.3
△	MES pH 5.5
○	Phosphate pH 7.5
⬡	NaOH/KCl pH 12.0



(Figure 4,5)

Hydrolysis of 1-(4-Methoxycarbonylphenyl)-3-hydroxymethyl-3-methyltriazenes in Various Buffer/Isopropanol Systems



(Figure 4,6)

Hydrolysis of 1-(4-Methoxycarbonylphenyl)-3-hydroxymethyl-3-methyltriazenes in Various Neat Buffer Systems

Table 4,1 Kinetic Data for Aryloxymethyltriazenes (55) in pH 2.0 Buffer/Isopropanol

Cpnd	Intrcpt	Slope	$-k_{obs}$ /10^{-3}	Log -k	$t_{1/2}$ (min)	r
55a	-0.329	-0.367	6.12	-2.213	1.9	.983
55b	0.264	-0.365	6.08	-2.216	1.2	.988
55c	0.016	-0.356	5.95	-2.226	2.0	.998
55d	-0.054	-0.288	4.80	-2.318	2.2	.999
55e	-0.234	-0.268	4.47	-2.349	1.7	.999
55f	0.083	-0.195	3.25	-2.2[illegible]3	1.9	.999
55g	0.071	-0.177	2.95	-2.530	4.3	.999
55h	0.114	-0.237	3.96	-2.403	3.4	.999
57	-0.082	-1.093	0.018	-0.174	0.56	.998

Table 4,2 Kinetic Data for Aryloxymethyltriazenes (55) in pH 3.3 Buffer/ Isopropanol

Cpnd	Intrcpt	Slope /10^{-2}	$-k_{obs}$ /10^{-3}	Log-k	$t_{1/2}$ (min)	r
55a	0.0073	-3.41	0.567	-3.246	20.6	.999
55b	-0.087	-2.32	0.387	-3.413	26.1	.998
55c	0.017	-1.75	0.291	-3.536	40.6	.999
55d	-0.049	-1.79	0.298	-3.525	36.0	.999
55e	-0.0063	-1.95	0.325	-3.487	35.2	.999
55f	-0.0007	-2.05	0.342	-3.466	33.8	.999
55g	-0.041	-2.90	0.491	-3.309	22.5	.999
55h	-0.120	-13.51	2.25	-2.648	4.24	.999
57	0.079	-117.25	19.54	-1.709	0.66	.999
52a	-0.075	-32.75	5.458	-2.264	2.43	.999

Table 4,3 Kinetic Data for Aryloxymethyltriazenes (55) in pH 5.5 Buffer/ Isopropanol

Cpnd	Intrcpt	Slope /10^{-3}	$-k_{obs}$ /10^{-5}	Log-k	$t_{1/2}$ (hrs.)	r
55a	0.029	-1.122	1.869	-4.728	10.7	.999
55b	0.022	-1.065	1.776	-4.753	11.2	.999
55c	0.012	-0.448	0.746	-5.127	26.2	.999
55d	0.028	-0.575	0.958	-5.018	20.9	.992
55e	0.0008	-0.557	0.928	-5.032	20.8	2.999
55f	-0.046	-2.170	3.620	-4.441	4.97	.999
55g	0.029	-7.127	10.87	-3.925	1.69	.999
55h	0.359	-87.60	146.0	-2.836	0.20	.999
57	0.023	-0.108	179.0	-2.745	0.11	.999

The logarithms of these rate constants plotted against the Hammett σ_p values are shown in figure 4,7.

Changes in rate constant k_{obs} with ionic strength at constant pH were examined by measuring the hydrolysis rate for each compound in formate buffer (pH 3.3) at three ionic strengths. The original buffers were 0.1, 0.01 and 0.5 M which on dilution with isopropanol became 0.06, 0.006 and 0.33 M. These rates are shown in figure 4,8 plotted against Hammett σ_p values. From the rate values at three pH values and at three ionic strengths at constant pH, plots of log k *versus* [H] and log k *versus* [buffer] gave straight lines

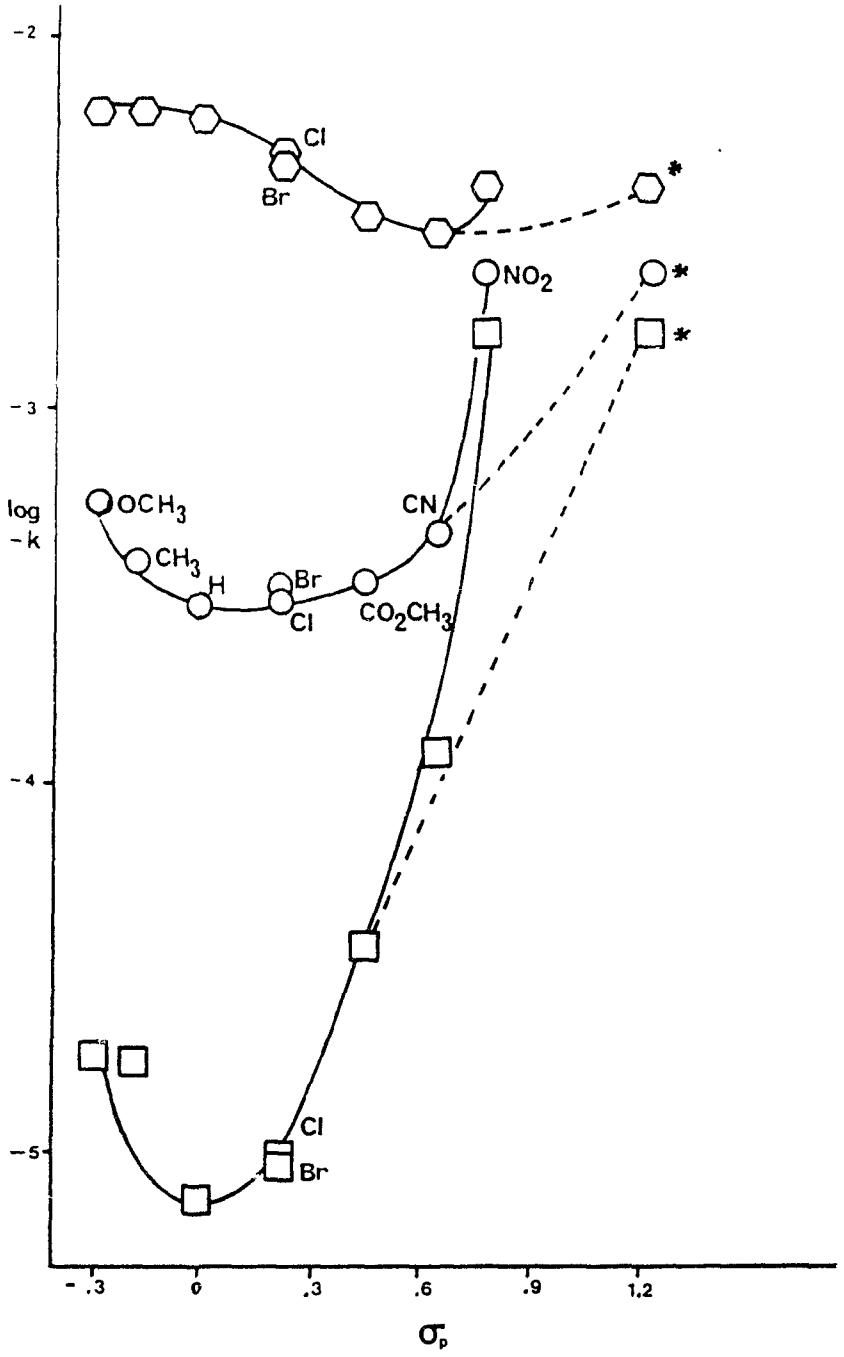
FIGURE 4,7

HAMMETT PLOTS FOR ARYLOXYMETHYLTRIAZENE (55) HYDROLYSIS AT VARIOUS pH VALUES

LEGEND

SYMBOL	pH
⬡	2.0
○	3.3
□	5.5

* Corresponding point for NO_2 using σ_p^-



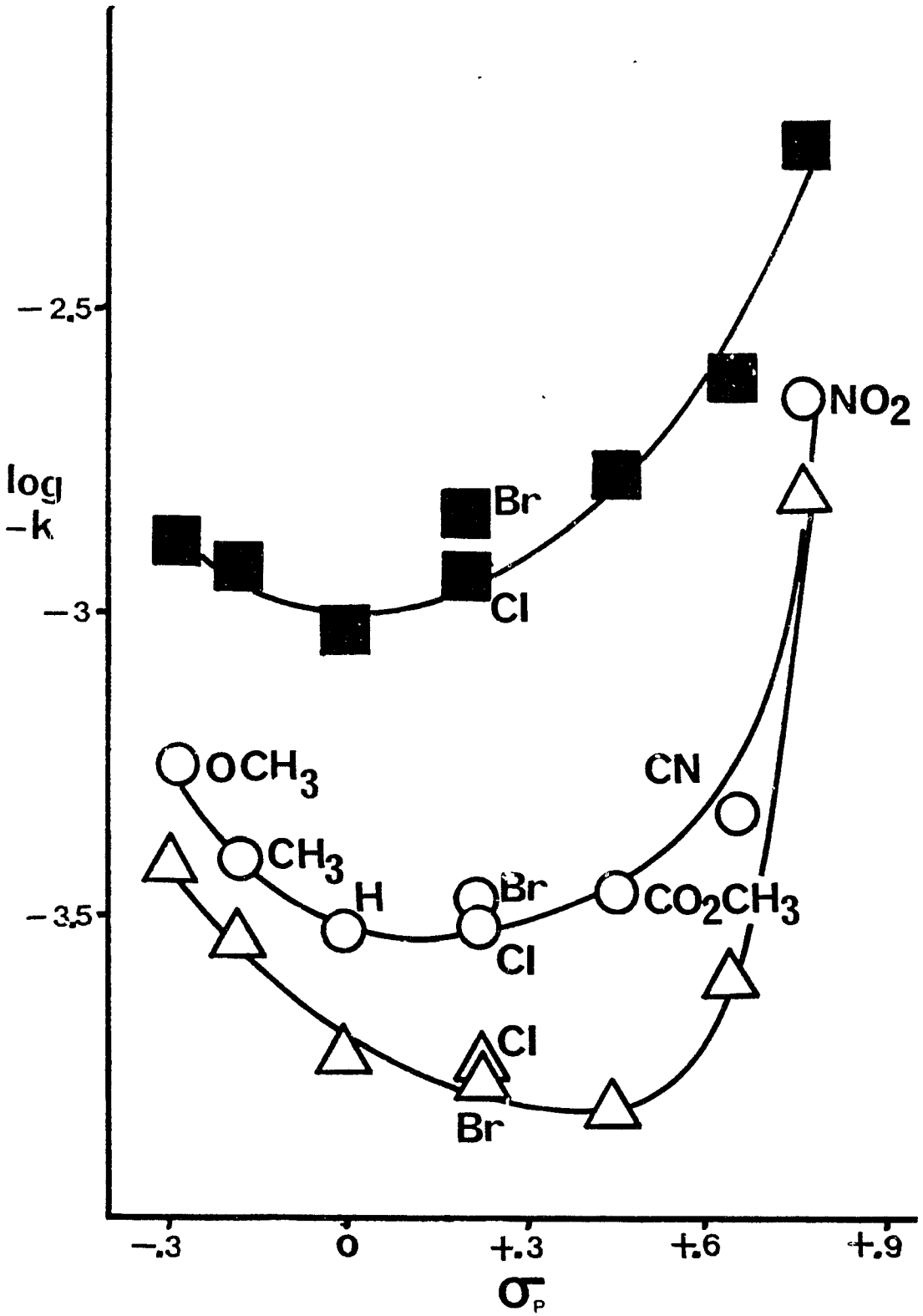
(Figure 4,7)

FIGURE 4,8

HAMMETT PLOTS FOR ARYLOXYMETHYLTRIAZENE (55) HYDROLYSIS AT pH 3.3 AND VARIOUS BUFFER CONCENTRATIONS

LEGEND

SYMBOL	BUFFER CONCENTRATION
■	0.33 M
○	0.06 M
△	0.006 M



(Figure 4,8)

whose slopes represented the sensitivity of each compound to changes in pH or ionic strength. These sensitivities are shown graphically in figure 4,9.

Compound 55h (Y = NO_2) was hydrolysed in neat phosphate buffer at pH 7.5, and under these conditions, in addition to the triazene peak at 320 nm, it was possible to observe the growth of the nitrophenolate peak at 400 nm (Figure 4,10). The kinetic data for this experiment are shown below.

k_{max} (nm)	k (s^{-1})	$t_{1/2}$ (min.)
320	1.05×10^{-3}	11
400	1.15×10^{-2}	1.0

4,2,3 HPLC Analyses

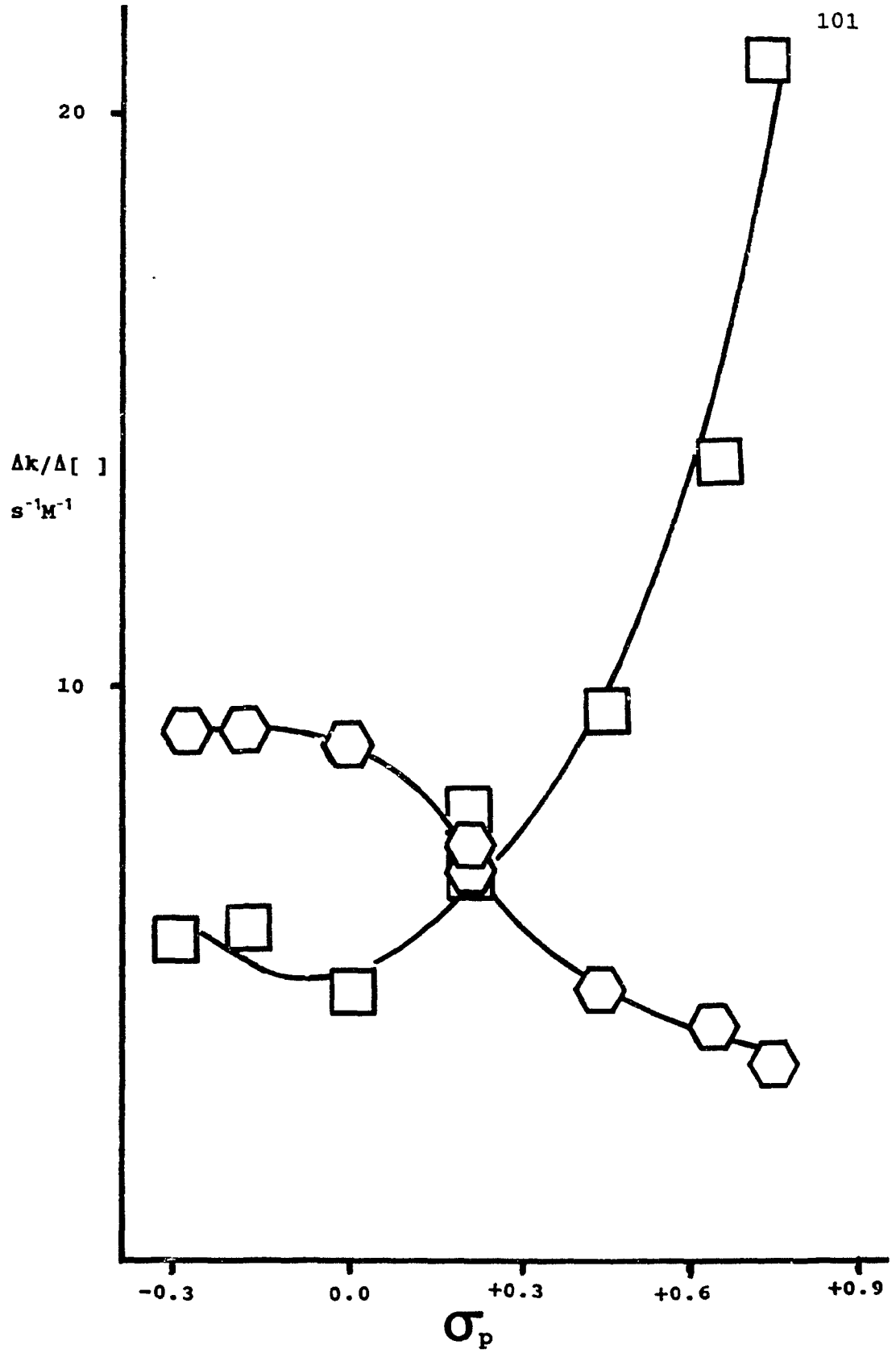
HPLC analyses were performed on a Varian model 5000 liquid chromatograph equipped with a microprocessor controlled binary solvent delivery system. Data were collected with a Varian model 4290 integrator. A 250 X 4 mm, 10 μm Lichrosorb NH_2 (Hibar) column was used with a 3 mm amino guard column (Brownlee Labs). Isocratic elution with an isopropanol/n-hexane (60:40) at a flow of 1.5 mL min^{-1} gave the best separations.

FIGURE 4,9

PLOT OF RATE SENSITIVITY TO IONIC STRENGTH AND HYDRONIUM ION CONCENTRATION AS A FUNCTION OF HAMMETT σ_p VALUE

LEGEND

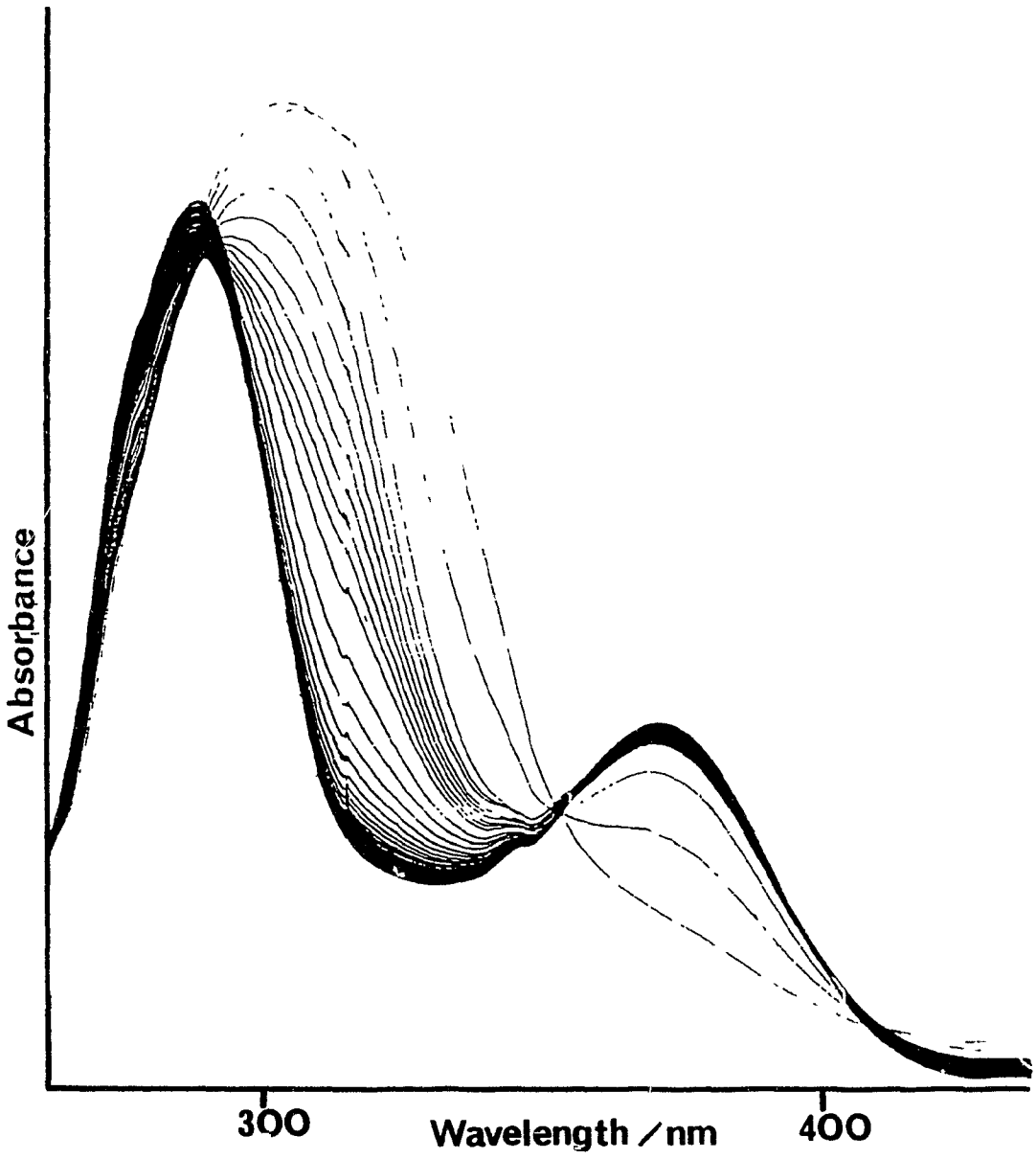
SYMBOL	Y-AXIS
⬡	$\Delta k/\Delta[H_3O^+]$ X 20
□	$\Delta k/\Delta$[buffer] X 2000



(Figure 4,9)

FIGURE 4,10

REPEAT-SCAN (CYCLE TIME 1 MIN.) UV SPECTRUM FOR 1-(4-CARBOMETHOXY)PHENYL-3-(4-NITRO)PHENYLOXYMETHYLTRIAZENE (55h) IN pH 7.5 PHOSPHATE BUFFER



(Figure 4,10)

External standards were prepared by dissolving a weighed mass of the desired compound in 25 mL isopropanol as follows.

Compound 55e	4.9 mg.
4-Bromophenol (HOC_4H_4Br)	14.7 mg.
Methyl-4-aminobenzoate (M4AB)	2.2 mg
Methyl-4-hydroxybenzoate (M4HB)	0.74 mg

Standards, with the exception of compound 55e, were reagent grade (Aldrich) used without further purification and were found to be of acceptable purity (>98%). The standards were run in triplicate and their retention times and response factors are given in table 4,4.

Table 4,4 Retention Times and Response Factors for Reference Standards.

Compd.	Ret. Time (min)	Response Factor ($mg^{-1}/10^7$)[a]	Molar Response ($mmole^{-1}/10^9$)
55e	2.41±0.05	1.16±0.02	4.38±0.08
M4HB	4.71±0.02	9.1±0.3	13.83±0.5
M4AB	2.86±0.02	2.351±0.002	3.555±0.003
HOC_4H_4Br	3.18±0.02	0.272±0.009	0.471±0.002

[a] Response in integrator units

Hydrolysis of the 4-bromophenyloxymethyltriazene (55e) was carried out as follows. pH 3.3 formate buffer (25 mL.) and isopropanol (12.5 mL.) were equilibrated in a water bath. The triazene was dissolved in the isopropanol which

was then added to the buffer. Immediately, and then at *ca* 30 min. intervals, a 5 mL. aliquot was pipetted into a separatory funnel. This aqueous phase was extracted with dichloromethane (3x10 mL.), the organic layers combined, dried with anhydrous sodium sulphate, filtered and evaporated under vacuum. This material was then dissolved in isopropanol, filtered through a 0.2 μm membrane (Metricel GA-8) and made up to 10 mL.

A test mixture was prepared containing suitable amounts of the three likely products and subjected to the extraction procedure outlined above. The analysis of this extract was compared with the standards to establish the efficiency of the extraction process for each component. For the starting material, the t_0 concentration was established by comparison with the standard and this value then related to the known amount of substrate in the system. The recoveries were: Comp. 55e,76%; M4HB,97.8%; M4AB,61.4%; HOC_4H_4Br,78.5%.

The chromatograms for the hydrolysis experiment are shown in figure 4,11. The results of this experiment are given in table 4,5.

Table 4,5 Results of Analysis of the Hydrolysis of 1-(4-Carbomethoxy)phenyl-3-(4-bromo)phenyloxymethyl-3-methyltriazene (55e) Measured by Disapearance of Substrate

Time (min.)	Amt in Sample ($g/10^{-3}$)	Corr.for Recovery ($g/10^{-3}$)	Moles ($/10^{-5}$)	$\ln C^a$
0	11.7	15.4	4.08	0
32	6.17	8.11	2.15	-0.641
65	3.49	4.59	1.22	-1.210
92	1.88	2.47	0.654	-1.830
1080	1.03	1.35	0.358	-2.433

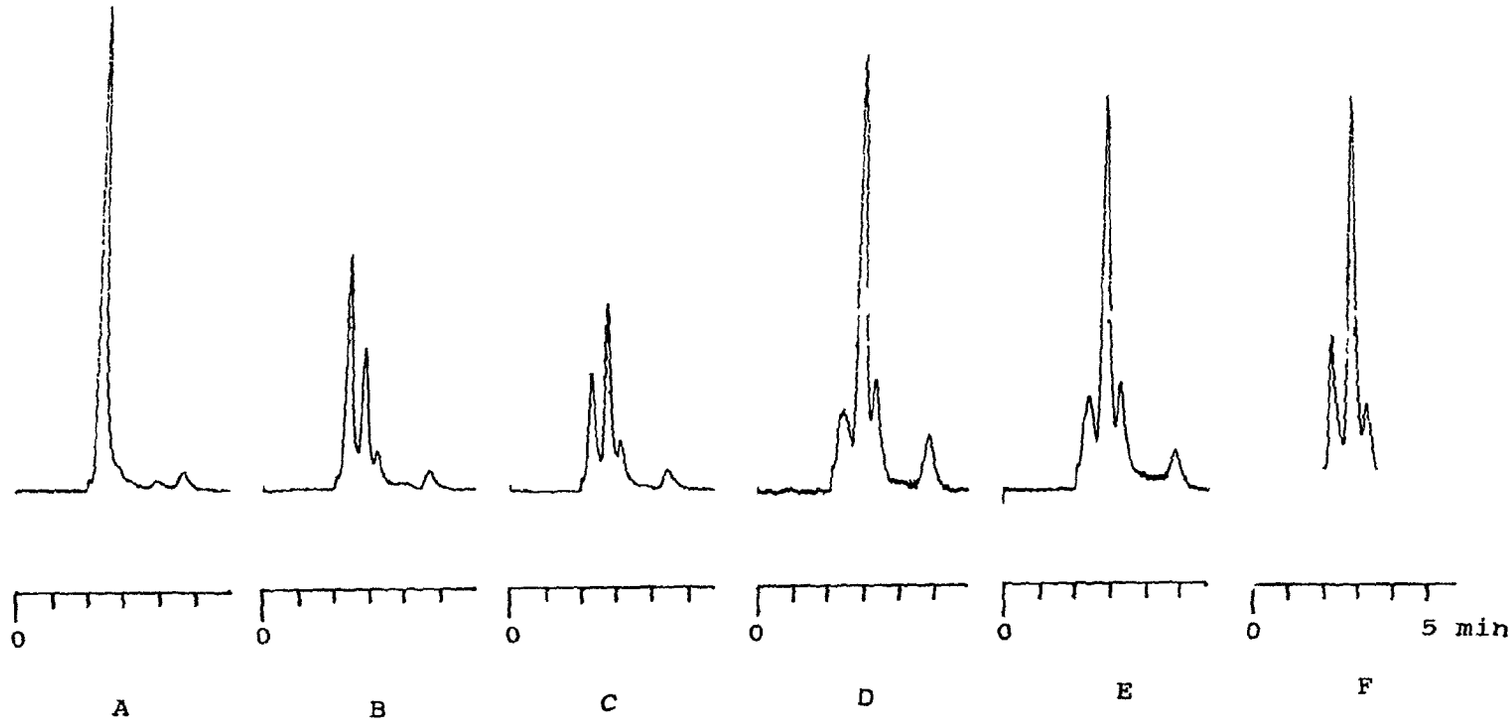
[a] $\ln (M_t/M_o)$ where M_t is the number of moles present at time t and M_o is the number of moles present at the start of the experiment.

FIGURE 4,11

HYDROLYSIS OF 1-(4-CARBOMETHOXY)PHENYL-3-(4-BROMO)PHENYLOXYMETHYLTRIAZENE (55e) FOLLOWED BY HPLC

LEGEND

A	0 minutes
B	32 minutes
C	56 minutes
D	92 minutes
E	18 hours
F	6 days



(Figure 4,11)

Assays for the products are shown in table 4,6.

Table 4,6 Assay of Products of Hydrolysis Experiment.

	Assay in Moles $/10^{-5}$		
Time (min.)	M4AB	Bromo-phenol	M4HB
0	-	-	-
32	2.19	2.13	C.051
65	2.55	2.86	0.057
92	3.13	3.43	0.055
1080	3.17	3.58	0.053

4.3 Results and Discussion

Attempts to examine the hydrolysis of the 1-aryl-3-aryloxymethyl-3-methyltriazenes (55) in neat aqueous buffer met with little success since, with the exception of compound 55h (Y = NO_2), concentrations suitable for UV spectrophotometric analysis were unobtainable due to the low solubility of these compounds. For this reason it was decided to use a mixed buffer/isopropanol system. It has been shown that the presence of alcohols in aqueous systems leads to disruption of hydrogen bonding which in turn leads to a greater number of free water molecules being available to solvate protons and hence a lowering of the effective hydrogen ion concentration [84]. It was felt that accurate measurements of acidity functions for these systems was beyond the scope of this study since the values obtained were primarily for comparison with other related compounds. The pH values quoted are those obtained from a normal laboratory pH meter and are thus only approximate indications of acidity.

The 1-aryl-3-aryloxymethyltriazenes (55) decomposed extremely slowly, if at all, in the pH 7.5 buffer-isopropanol system. For example, compound 55e showed no appreciable decomposition after 2 days. The only member of the series that showed any tendency to decay under these conditions was compound 55h, and its behavior was erratic to the extent that reliable kinetic data could not be obtained.

Thus it became desirable to establish the kinetic parameters of the parent hydroxymethyltriazene (compound 57) at different pH values and in the mixed buffer/isopropanol solvent system. Table 4,7 shows the kinetic data pertaining to the graphical decays in figures 4,5 and 4,6.

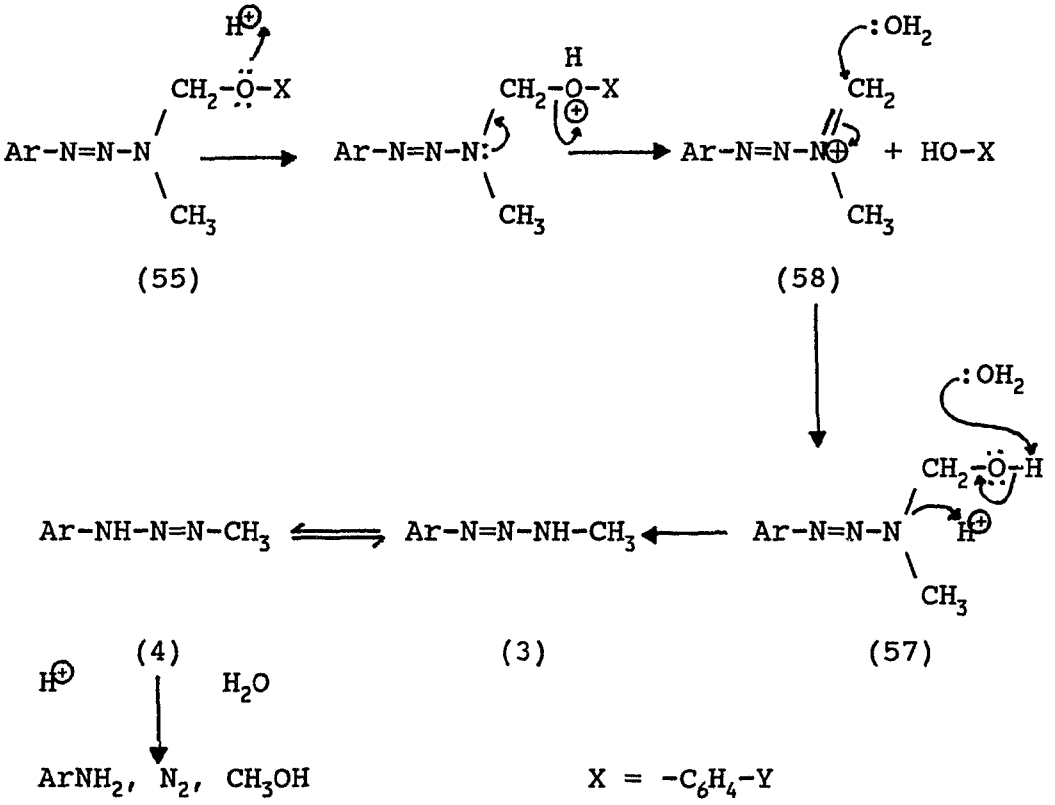
Table 4,7 Kinetic Data for the Hydrolysis of HMT (57) in Various Buffers at Different pH Values.

pH	Neat Buffer		Buffer-Isopropanol	
	$t_{1/2}$	$k_{obs}/10^{-3}$	$t_{1/2}$	$k_{obs}/10^{-3}$
3.3	18.2s	44.2	40s	19.5
5.5	1.59m	11.9	6.7m	1.78
7.5	9.81m	0.52	4.2h	0.046
12.0	14.7h	0.012	-	-

Thus in an alkaline medium at pH 12.0 (Figure 4,6) the hydroxymethyltriazene is surprisingly stable with a half-life of 14.7 hours. In phosphate buffer at pH 7.5, the rate of hydrolysis is 43 times faster and at acid pH 5.5, the rate of hydrolysis of the HMT (57) is increased by a further factor of 23. Lowering the pH further to pH 3.3 has a much smaller effect on the very fast rate of hydrolysis.

Adding isopropanol to the buffer system (Figure 4,6) slows the hydrolysis of the HMT (57) by a factor of 11.3 at pH 7.5 and by a factor of 6.7 at pH 5.5. The rate of hydrolysis of (57) at pH 3.3 is much less affected by the presence of the alcohol. These observations are consistent

with the presence of isopropanol causing a decrease in the effective hydrogen ion concentration. Furthermore, it can be adduced from this evidence that protonation plays a significant role in the acid catalysed decomposition of the hydroxymethyltriazenes - see Scheme 4,1.

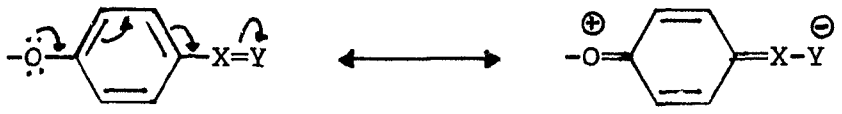


(Scheme 4,1)

Tables 4,1, 4,2 and 4,3 show the results for the measurement of kinetic parameters of the aryloxymethyltriazenes (55) in buffer systems of pH 2.0, 3.3 and 5.5 obtained from the plots of ln A versus time shown for all of these compounds in figures 4,2, 4,3 and 4,4 respectively. In all cases the aryloxymethyltriazenes

decompose at a significantly lower rate than the parent hydroxymethyltriazene (57). For example, the *p*-bromophenyloxymethyltriazene (55e) decomposes from 3 to 190 times more slowly than 57 depending upon the pH. All sets of data show varying half-life values for the decomposition of the aryloxymethyltriazenes (55a-h) as a function of the substitution X in the aryl moiety. It is logical, therefore to look for a Hammett correlation for these various substituents.

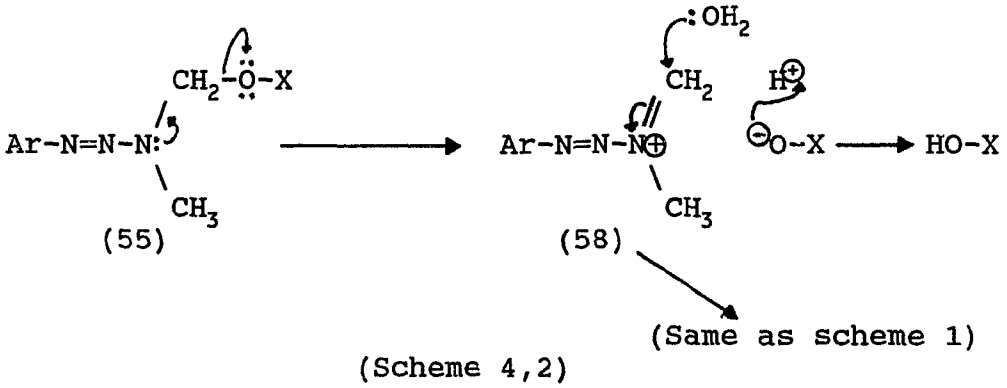
A general observation of figures 4,2, 4,3 and 4,4 suggest immediately that under strongly acidic conditions (pH 2.0) the presence of an electron withdrawing group slows the reaction down. This observation supports the suggestion in Scheme 4,1 that protonation of the ether oxygen is important in the rate determining step. Resonance interaction of the ether oxygen with the para substituent X, will decrease the electron density at O and thus lower the rate of protonation (Figure 4,12).



(Figure 4,12)

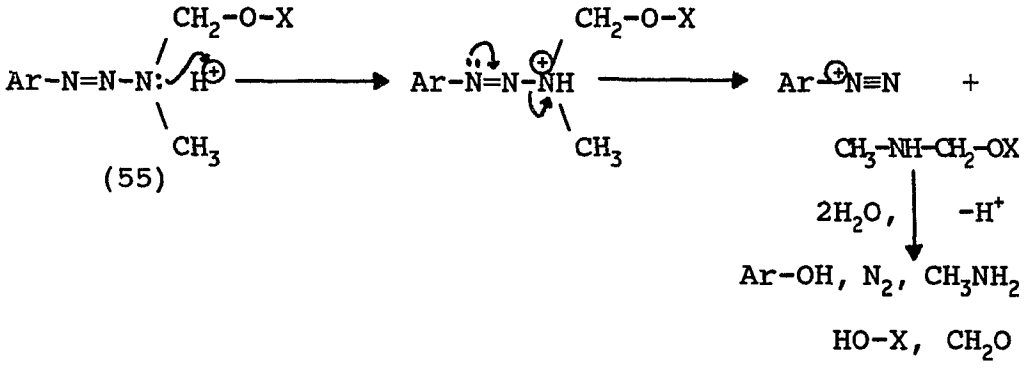
However at pH 3.3, it is evident that both strongly electron donating (OCH_3) and electron withdrawing (NO_2) groups promote the hydrolysis, whereas at pH 5.5 a conventional pattern emerges where the electron withdrawing

ability favours the hydrolysis. This observation suggests that at close to neutral conditions, protonation is less important and that the stability of the leaving group is the overriding factor. This leads further to the suggestion that the hydrolysis at pH 5.5 proceeds mainly by an S_N1 pathway via an iminium ion intermediate (58) (Scheme 4,2).



(Scheme 4,2)

There is a third possible pathway by which the aryloxymethyltriazenes can decompose in aqueous conditions, and this is outlined in scheme 4,3.



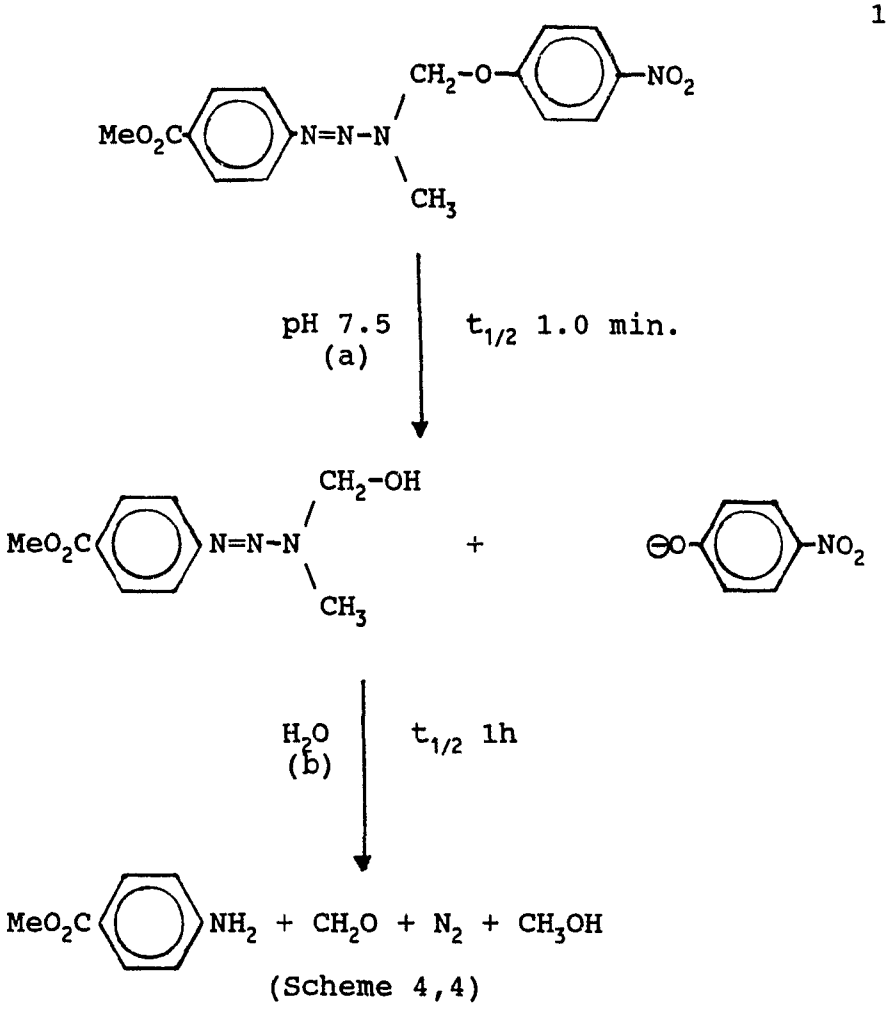
(Scheme 4,3)

In Scheme 4,1 the phenyl ether linkage is first protonated leading to loss of the corresponding phenol and the generation of an iminium ion (58). This ion is rapidly attacked by water to give the hydroxymethyltriazene (57). The decay of this species has been investigated by Vaughan *et al* [76]. The hydroxymethyltriazene loses formaldehyde to yield a monomethyltriazene (3) which is tautomeric with a highly unstable 1-methyl-3-aryltriazene (4) which is hydrolysed to the amine, nitrogen and methanol. Scheme 4,2 is essentially the same as scheme 4,1 except that the initial step involving the scission of the ether linkage proceeds without protonation to give an ion pair. Scheme 4,3 proceeds by N-protonation resulting in the formation of an aryldiazonium ion and an α-phenoxydimethylamine. In an aqueous environment these species would yield a phenol (methyl-4-hydroxybenzoate in the compounds studied), methylamine, methanol, nitrogen and the phenol derived from the scission of the aryl ether linkage.

The HPLC study provided evidence that the mechanism depicted in scheme 4,3 was not operative. Although traces of methyl-4-hydroxybenzoate were detected in the hydrolysis mixture, its level was low (>2 mole%) and constant. This compound was subsequently found to be a more or less ubiquitous contaminant of α-hydroxymethyltriazene derivatives at these levels. This finding was confirmed by the presence of significant amounts of the products expected

from the first two reaction schemes. The HPLC method did have one shortcoming in that over long periods of time a degree of transesterification took place between the solvent isopropanol and the methyl-4-aminobenzoate product. This ester had a retention time almost identical with that of the substrate and was not resolvable by the method in use. This transesterification was however slow compared with the hydrolysis of interest, isopropyl-4-aminobenzoate only becoming detectable after some 18 hours of hydrolysis. The $t_{1/2}$ value obtained for compound 55e was 36.4 minutes by HPLC compared with 35.2 minutes by UV spectrophotometry, so the methods are in agreement to within 3.5 %.

Whilst the HPLC method eliminates the possibility that the mechanism shown in Scheme 4,3 is in operation, it cannot distinguish between the mechanisms of Schemes 4,1 and 4,2. The hydrolysis of compound 55h in neat buffer is interesting in the sense that it clearly shows the initial generation of a phenolate ion which is fast ($t_{1/2}$ = 1.0 min.), followed by a slower ($t_{1/2}$ = 11 min.) decomposition of the triazene moiety, (Scheme 4,4). The decomposition of this compound under these conditions is unique in our experience in that it represents the first absolute measurement of the fast rate of initial decomposition of a hydroxymethyltriazene derivative. It is tempting to conclude that the phenolate ion formed indicates that the ion-pair mechanism (Scheme 4,2) is effective in this case, but at pH 7.5 the phenol

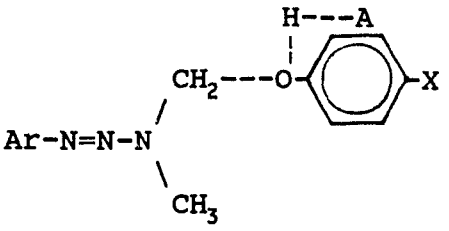


(Scheme 4,4)

itself would be appreciably ionized as it is generated.

The hydrolyses of compounds with a range of substituents give a better indication of the mechanism. The shape of the Hammett plots in figure 4,7 clearly indicate that a change of mechanism occurs as the phenyl substituent becomes more electron-withdrawing. For electron donating substituents ρ is negative indicating that the phenyl ring has a lower electron density in the transition state than in the starting material. Conversely for electron withdrawing substituents ρ is positive indicating that the aromatic ring

has a higher electron density in the transition state than in the starting material. For electron donating groups it seems that protonation is the rate limiting step (Scheme 4,1) and that the increased electron density on the ether oxygen promotes this protonation. In the case of the electron withdrawing substituents, the decreased electron density at the ether oxygen would tend to stabilise the nascent phenolate ion (Scheme 4,2). Varying the strength of the buffer at constant pH showed that for all substituents there was a linear rate dependence on buffer concentration, but that the extent of this dependence varied with the substituent (see figure 4,9). This behavior is consistent with competing reactions, i.e the mechanisms shown in schemes 4,1 and 4,2 with the proportion of each mechanism being determined by the phenyl substituent. Thus for electron donating species there is a marked degree of specific acid catalysis as shown by the small sensitivity of the rate to changes in ionic strength and the larger sensitivity to pH consistent with proton transfer taking place in a rapid pre-equilibrium. For the electron withdrawing groups the rate limiting step is characterised by general acid catalysis consistent with proton transfer assisting the departure of the phenolate ion (Figure 4,12).



(Figure 4,13)

4,4 Conclusions

The results of this investigation indicate that the 1-aryl-3-aryloxymethyl-3-methyltriazenes (55) do indeed have a range of stability in aqueous buffer intermediate between the labile hydroxymethyltriazenes and the inert methoxymethyltriazenes. For example in aqueous buffer at pH 3.3 the corresponding hydroxymethyltriazene (57) has a half-life of 0.66 minutes, the phenylthiomethyltriazene (52a) 2.43 minutes and the phenyloxymethyltriazene (55c) 40.6 minutes. The series (55a-h) has a range of half-lives from 4.2 to 40.6 minutes.

These results were encouraging enough to prompt us to send two samples (55e and 55h) to the National Cancer Institute, Bethesda, Maryland for testing against their new investigational *in vitro* disease oriented primary antitumour screen. This screen consists of over 60 tumour cultures and each tumour type is tested with five tenfold dilutions of the drug and the activity parameters interpolated from these results. Residual viable cell populations were estimated colorimetrically after staining the cell colonies. Both compounds were tested twice and the results compared for statistically significant differential sensitivities at three standard concentrations: GI_{50}, the concentration required for 50% growth inhibition; TGI, the concentration required to give total growth inhibition and LC_{50}, the concentration necessary for 50% lethality. A software

routine is used to grade the differential sensitivity, and values less than unity represent low, whilst values higher than 3 represent high differential sensitivity. These results are shown in table 4,8

Table 4,8 NCI *In Vitro* Screening Data

Parameter	Compd. 55e		Compd. 55h	
	Trial 1	Trial 2	Trial 1	Trial 2
Mean Response				
$\log_{10}GI_{50}$	-4.1	-4.3	-4.1	-4.2
$\log_{10}TGI$	-4.0	-4.0	-4.0	-4.0
$\log_{10}LC_{50}$	-4.0	-4.0	-4.0	-4.0
Differential Sensitivity				
GI_{50}	0.5[a]	0.5[b]	0.6	1.4
TGI	0.1	0.3[a]	0.2	0.4
LC_{50}	0.0[c]	0.0[d]	0.0	0.0

Statistically significant differential sensitivity in the following cell lines:
[a] Leukemia subpanel
[b] Leukemia, Miscellaneous and Renal subpanels
[c] Colon subpanel
[d] Non- small cell lung subpanel

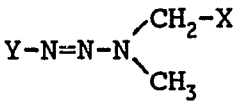
The NCI policy for this new screen is not to accept for testing any compound that they have previously screened in other experimental protocols. This unfortunately renders the mean response data impossible to interpret since we have no reference compound with which to compare the concentrations. Are these concentrations to be deemed high or low? The differential sensitivity data are more

has not (mer$^-$) the ability to repair DNA O^6-guanine lesions, so it is possible to determine if this form of lesion is important for these compounds.

Chapter 5

NMR Studies of the Rotational Properties of Some Triazenes

5,1 Introduction



52f) X = $-S-C_6H_4-CH_3$ Y = $p-CH_3CO_2-C_6H_4-$

58a) X = C_6H_5 Y = $p-CH_3(CH_2)_3-$

58b) X = $(CH_2)_2CH_3$ Y = $pC_6H_5CH_2-$

58c) X = $(CH_2)_2CH_3$ Y = $p-CH_3-$

57a) X = OH Y = $p-O_2N-C_6H_4-$

57b) Y = $p-NC-C_6H_4-$

57c) Y = $p-CH_3CO-C_6H_4$

57d) Y = $p-CH_3O_2C-C_6H_4$

57e) Y = $o-CF_3-C_6H_4$

51) X = OAc Y = $p-CH_3CO_2-C_6H_4-$

(Figure 5,1)

The 1-aryl-3-arylthiomethyl-3-methyltriazenes (52) prepared previously [62] were surprising in that the ^1H chemical shifts for the $N-CH_2-S-$ groups were almost identical with those of $N-CH_2-O$ in hydroxymethyltriazenes (57). This was contrary to our expectations based on the empirical shift parameters [81] commonly used for such predictions. One purpose of this study was to attempt to explain these deviations from prediction. A possible explanation of this irregularity is that the empirical parameters, which are assumed to be simply additive, do not allow for the possibility of synergistic effects. Indeed the triazene moiety is, to the best of our knowledge, not

listed in any compilations of such parameters, and a suitable parameter had to be deduced. In order to fully test the suitability of this parameter and to assess the degree of interaction of the two substituent groups would require the synthesis of a large number of hitherto unknown model compounds and measurement of the shifts of these and many more reference materials. Such a task may be undertaken at some time in the future as more data become available but was not deemed appropriate at this stage.

A second hypothesis and one which is more amenable to experimental testing is that at least part of the deviant behavior of these compounds may be due to hindered rotation or other conformational effects. In addition, whilst many NMR rotational barrier studies have been done on groups of compounds where the two substituents on N(3) are identical, [34,35,36,86,87], it was possible to find only one study where the N(3) substitution was unsymmetrical [35] and this only included a brief coverage of three compounds (58a,58b,58c, fig. 5,1). There are severe limitations on the methods available for probing the rotational behavior of these unsymmetrical compounds, but since this class includes the important hydroxymethyltriazenes, and their derivatives and analogues, it was felt to be worthwhile to attempt to obtain at least some broad idea of the rotational characteristics of these species.

5,2 Experimental

All variable temperature NMR experiments were performed on the Nicolet 360NB spectrometer of the Atlantic Region Magnetic Resonance Centre at Dalhousie University. ^{1}H (at 361 MHz) and ^{13}C (at 90 MHz) spectra were collected with sweep widths of 4000 Hz. and 20 000 Hz. respectively. Temperatures are accurate to 1°C. The NMR probes were calibrated at intervals during the time of these experiments. Deuterated solvents were obtained from MSD Isotopes Ltd. and frequencies and line widths were measured relative to tetramethylsilane 1% or 0.3% as internal standard. Spectral line widths were measured using the Nicolet LF routine which calculates a best-fit Lorentzian peak for each data set. Each data set was zero-filled to 32k, the peak rephased and the baseline fixed prior to calculation.

5,2,1 Revision of Additive Chemical Shift Parameters

A number of chemical shifts were obtained experimentally and others from literature sources for comparison. These data are presented in table 5,1. Compound types were chosen for comparison between O- and S-substituted triazenes and it can be readily seen that variations in the aryl substituents have little influence on these chemical shifts. Hence, for convenience the shifts for each compound type have been averaged and these figures used in the discussion which follows.

Table 5,1 NMR Chemical Shifts for a Variety of Triazenes in $CDCl_3$ at 20°C

X	Y	δ^H NCH_2	δ^H NCH_3	δ^C NCH_2	δ^C NCH_3
S-Ph[a]	*p*-MeO_2C	5.21	3.23	62.5	33.7
	p-EtO_2C	5.22	3.23	62.4	33.6
	p-O_2N	5.22	3.26	62.6	33.9
	p-NC	5.22	3.25	62.5	33.6
	p-MeO	5.24	3.24	62.4	33.6
	o-CF_3	5.22	3.24	62.2	33.6
S-Tolyl[a]	*p*-MeO_2C	5.19	3.25	62.5	33.3
	p-EtO_2C	5.16	3.22	62.8	33.6
	p-O_2N	5.18	3.27	62.5	33.9
Average		**5.21**	**3.24**	**62.5**	**33.6**
OAc[b]	*p*-MeO_2C	5.83	3.30	78.9	34.6
	p-CN	5.84	3.30	78.7	34.6
	p-O_2N	5.75	3.29	78.7	34.7
	p-MeOC	5.79	3.24	78.7	34.6
Average		**5.80**	**3.28**	**78.8**	**34.6**
OH[b]	*p*-O_2N	5.18	3.23	78.5	33.4
	p-Br	5.20	3.30	78.0	33.0
	o-CF_3	5.30	3.29	79.5	33.2
	p-MeO_2C	5.28	3.29	78.6	33.2
Average		**5.24**	**3.28**	**78.7**	**33.2**
OMe[b]	*p*-CN	5.15	3.30	86.6	33.4
	p-MeOC	5.10	3.30	86.5	33.3
Average		**5.13**	**3.30**	**86.6**	**33.4**
S-Et[c]	*p*-CN	4.90	3.27	-	-
S-Bu^t [c]	*p*-CN	5.02	3.25	-	-
O-Ar					
Ar=C_6H_5	*p*-MeO_2C	5.72	3.30	83.4	33.7
p-C_6H_4-CH_3		5.69	3.29	83.8	33.7
p-C_6H_4Cl		5.70	3.29	83.5	33.8
p-C_6H_4Br		5.70	3.29	83.4	33.7
p-$C_6H_4O_2N$		5.84	3.32	83.0	33.9
p-C_6H_4-OMe		5.66	3.29	84.6	33.8
p-C_6H_4CN		5.79	3.31	82.8	33.8
Average		**5.73**	**3.30**	**83.5**	**33.8**

[a] Reference [62] [b] Reference [61] [c] Reference [35]

5,2,2 Variable Temperature NMR Study of Hydroxymethyltriazenes (57)

Chemical shifts were measured for a number of hydroxymethyltriazenes at temperatures ranging from +80°C to -60°C in chloroform and toluene, and, where minor and major conformers could be distinguished, the coalescence temperature determined. A fine example of the sort of behaviour exhibited by the hydroxymethyltriazenes is shown in figure 5,2. The shifts, the shift differences between conformers and the relative populations of the two conformational states are shown in table 5,2.

IR spectra were obtained of solutions of hydroxymethyltriazene 57d in dry chloroform at three concentrations using Perkin-Elmer NaCl sealed solution cells. The ratios of hydrogen-bonded (3420 cm^{-1}) to free (3610 cm^{-1}) band itensities were measured and are as follows: 0.09 M, 0.56; 0.045 M, 0.66; 0.007 M, 0.70.

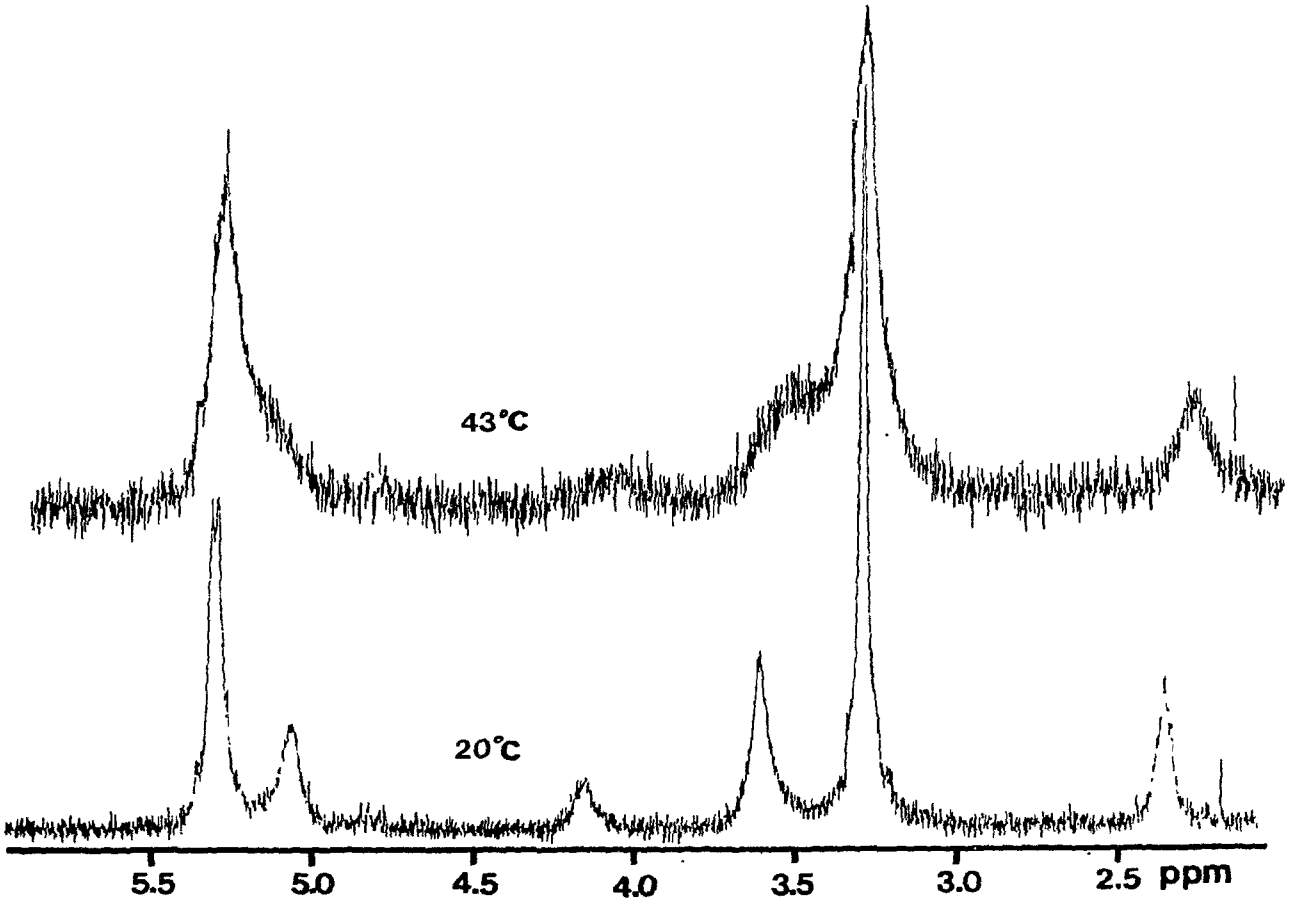


Figure 5,2
^{1}H NMR Temperature Dependant Behavior of 1-(2-Trifluoromethyl)phenyl-3-hydroxymethyl-3-methyltriazene (57e)

Table 5,2 Shift and Population Differences in Hydroxymethyltriazenes

Cmpnd.	CH_2[a]				CH_3[a]			
	Maj.[b]	Min.[c]	Diff.[d]	Pop.[e]	Min.	Maj.	Diff.	Pop.
57a	-	-	-	-	1339.24	1204.38	134.86	0.082
57b	1925.44	1846.48	78.96	0.081	1326.45	1197.55	128.90	0.076
57c	-	-	-	-	1324.61	1193.44	131.17	0.057
57d	1919.24	1839.32	79.87	0.133	1315.11	1194.03	121.08	0.102
57e	1920.69	1836.63	84.06	0.042	1314.77	1190.52	124.25	0.452

[a] Shifts in Hz. Relative to TMS
[b] Shift of Signal due to major Conformer
[c] Shift of Minor Conformer
[d] Shift Difference between Conformers
[e] Population of Minor Conformer Relative to that of the Major Conformer From Peak Integrations.

5,2,3 Variable Temperature Studies on 1-(4-methoxycarbonyl)phenyl-3-(4-methyl)phenylthiomethyl- (52), 1-(4-methoxycarbonyl)phenyl-3-(4-bromo)phenyloxymethyl- (55e) and 1-(4-methoxycarbonyl)phenyl-3-acetoxymethyl-3-methyltriazene (51).

The above named compounds were dissolved in toluene-d_8 or chloroform-d_3 and the ^1H and ^{13}C NMR spectra obtained at a variety of temperatures. The precise chemical shifts and line widths were measured for the $N-CH_2$ and $N-CH_3$ lines. The rate process of interest was detected by observing the variation of chemical shift with temperature. It was observed that in general the ^1H spectra gave clearer variations in line widths, and hence these were used exclusively in the study. These data are recorded in table 5,3 and shown graphically in figures 5,3 and 5,4.

Table 5,3 Chemical Shift and Linewidth measurements for an arylthiomethyltriazene (52f) in toluene-d_8

Temp. °C	Linewidths[a] (Hz)		Shifts[b] (Hz)	
	$N-CH_3$	$N-CH_2$	$N-CH_3$	$N-CH_2$
-30	0.15	0.24	1019.92	1620.30
-20	0.20	0.36	1023.08	1630.25
-10	0.29	0.60	1027.58	1642.73
0	0.38	0.69	1031.10	1651.82
+10	0.90	0.26	1034.64	1660.61
+19	2.24	2.74	1039.56	1671.44
+40	3.09	5.27	1049.20	1690.66
+60	1.76	1.94	1057.79	1706.06
+80	0.82	0.60	1066.48	1720.22

[a] The linewidth of TMS has been subtracted from these values
[b] Relative to TMS

FIGURE 5,3

^{1}H CHEMICAL SHIFT VARIATION WITH TEMPERATURE FOR 1-(4-METHOXYCARBONYL)PHENYL-3-(4-METHYL)PHENYLTHIOMETHYL-3-METHYLTRIAZENE (52) IN TOLUENE-d_8, SHIFTS MEASURED RELATIVE TO ROOM TEMPERATURE (25°C) SPECTRUM

□ = N-CH_2

▽ = N-CH_3

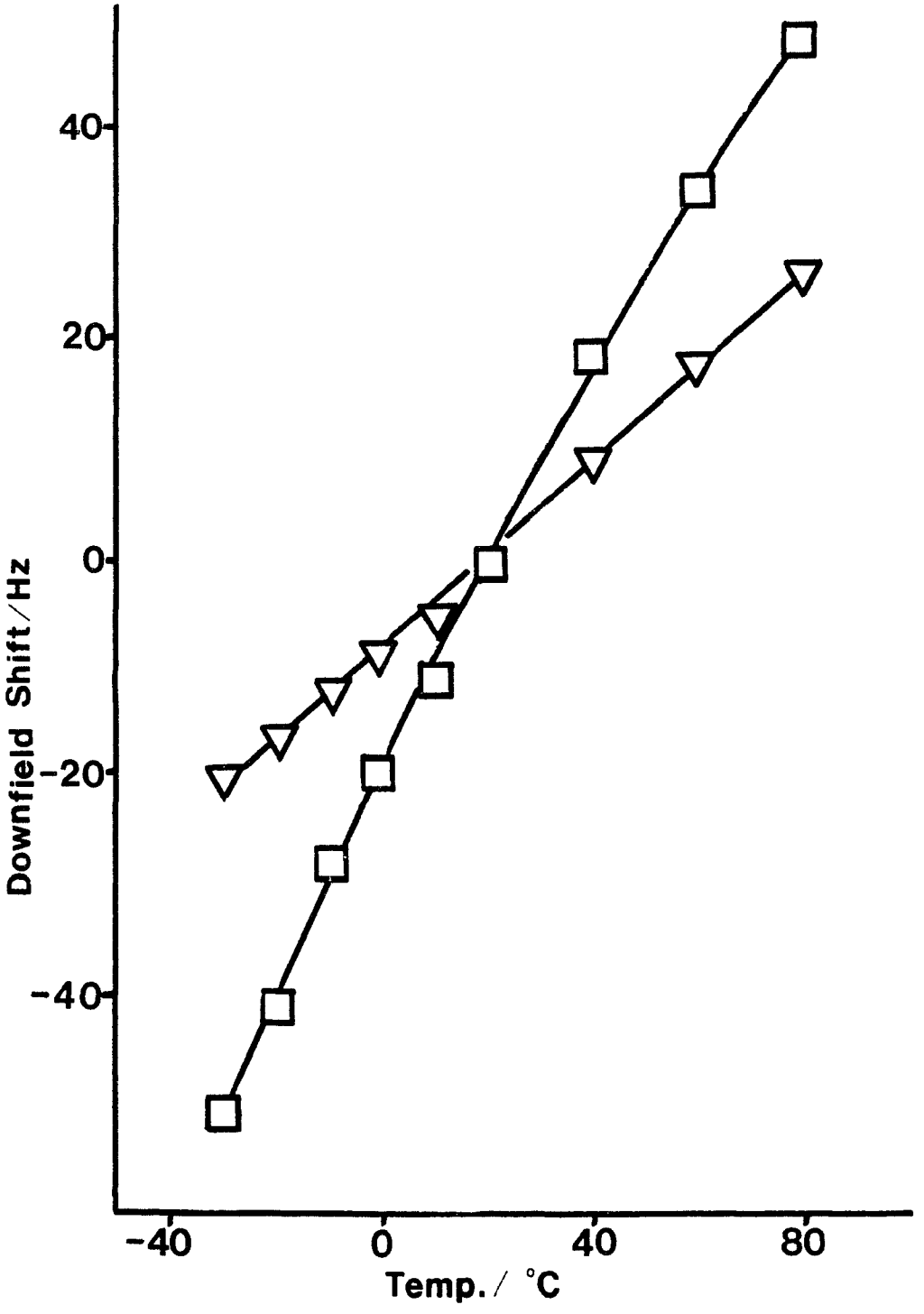


Figure 5,3

FIGURE 5,4

^{1}H NMR LINEWIDTH VARIATION WITH TEMPERATURE FOR 1-(4-METHOXYCARBONYL)PHENYL-3-(4-METHYL)PHENYLTHIOMETHYL-3-METHYLTRIAZENE (52) IN TOLUENE-d_8

□ = N-CH_2-

▽ = N-CH_3

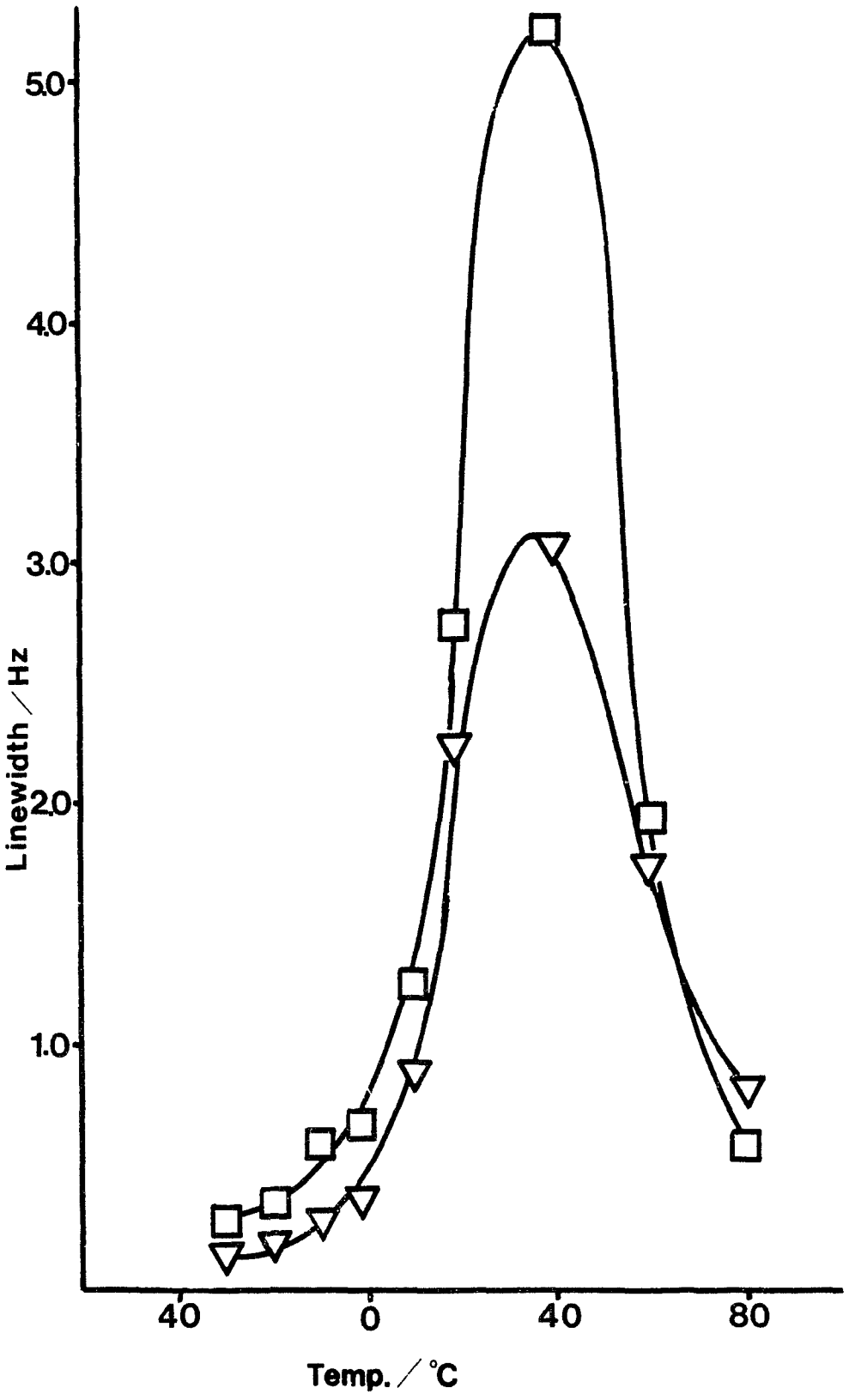


Figure 5,4

The coresponding data for the aryloxymethyl- and acetoxymethyltriazenes are shown in tables 5,4 and 5,5, and graphs of these relationships are given in figures 5,5 - 5,8.

Table 5,4 Chemical Shifts and Linewidth Measurements for an Aryloxymethyltriazene (55e) in Toluene-d_8

Temp. °C	Linewidth[a] (Hz)		Shift[b] (Hz)	
	$N-CH_3$	$N-CH_2$	$N-CH_3$	$N-CH_2$
-40	0.16	0.62	1003.30	1734.39
-30	0.15	0.60	1008.22	1748.48
-20	0.20	0.55	1012.52	1759.64
-10	0.25	0.48	1017.86	1772.55
-1	0.43	0.54	1022.51	1783.33
+11	0.49	0.60	1029.64	1797.98
+20	0.93	0.65	1033.90	1806.08
+30	0.94	0.65	1038.50	1814.92
+40	0.60	0.44	1043.31	1823.86
+50	0.42	0.41	1047.97	1832.27
+60	0.41	0.52	1052.05	1839.51
+70	0.50	0.55	1056.15	1846.50
+80	0.15	0.29	1060.50	1853.87

[a] The linewidth of TMS has been subtracted from these values
[b] Relative to TMS

Table 5,5 Chemical Shifts and Linewid ...s for an Acetoxymethyltriazene (51) in Toluene-d_6

Temp. °C	Linewidth[a] (Hz) N-CH_3	Linewidth[a] (Hz) N-CH_2	Shift[b] (Hz) N-CH_3	Shift[b] (Hz) N-CH_2
-30	0.25	0.73	1032.27	1907.03
-20	0.44	0.73	1036.36	1915.10
-10	0.23	0.39	1039.48	1921.33
-1	0.49	0.60	1042.29	1926.36
+10	1.11	0.19	1047.67	1934.14
+24	2.37	0.37	1052.44	1940.36
+30	1.57	0.32	1055.13	1943.86
+40	1.33	0.41	1058.75	1948.85
+50	0.67	0.26	1065.31	1956.45
+60	0.56	0.32	1065.35	1957.36
+70	0.46	0.44	1068.65	1961.82
+80	0.33	0.38	1072.59	1966.71

[a] The linewidth of TMS has been subtracted from these values
[b] Relative to TMS

FIGURE 5,5

^{1}H CHEMICAL SHIFT VARIATION WITH TEMPERATURE FOR 1-(4-METHOXYCARBONYL)PHENYL-3-(4-BROMO)PHENYLOXYMETHYL-3-METHYLTRIAZENE (55e) IN TOLUENE-d_8, SHIFTS MEASURED RELATIVE TO ROOM TEMPERATURE (25°C) SPECTRUM

□ = N-CH_2

▽ = N-CH_3

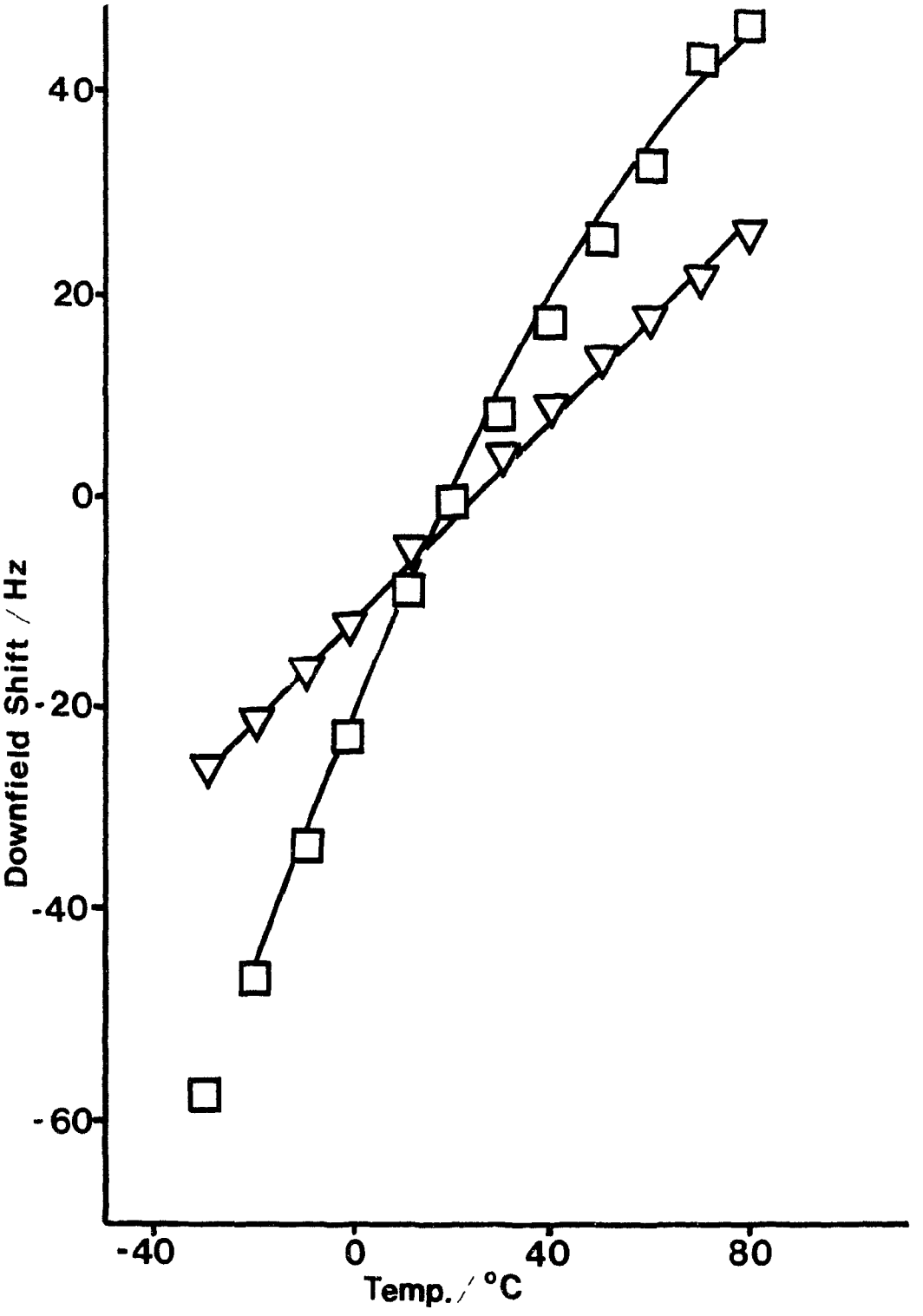


Figure 5,5

FIGURE 5,6

^{1}H NMR LINEWIDTH VARIATION WITH TEMPERATURE FOR 1-(4-METHOXYCARBONYL)PHENYL-3-(4-BROMO)PHENYLOXYMETHYL-3-METHYLTRIAZENE (55e) IN TOLUENE-d_8

□ = N-CH_2-

▽ = N-CH_3

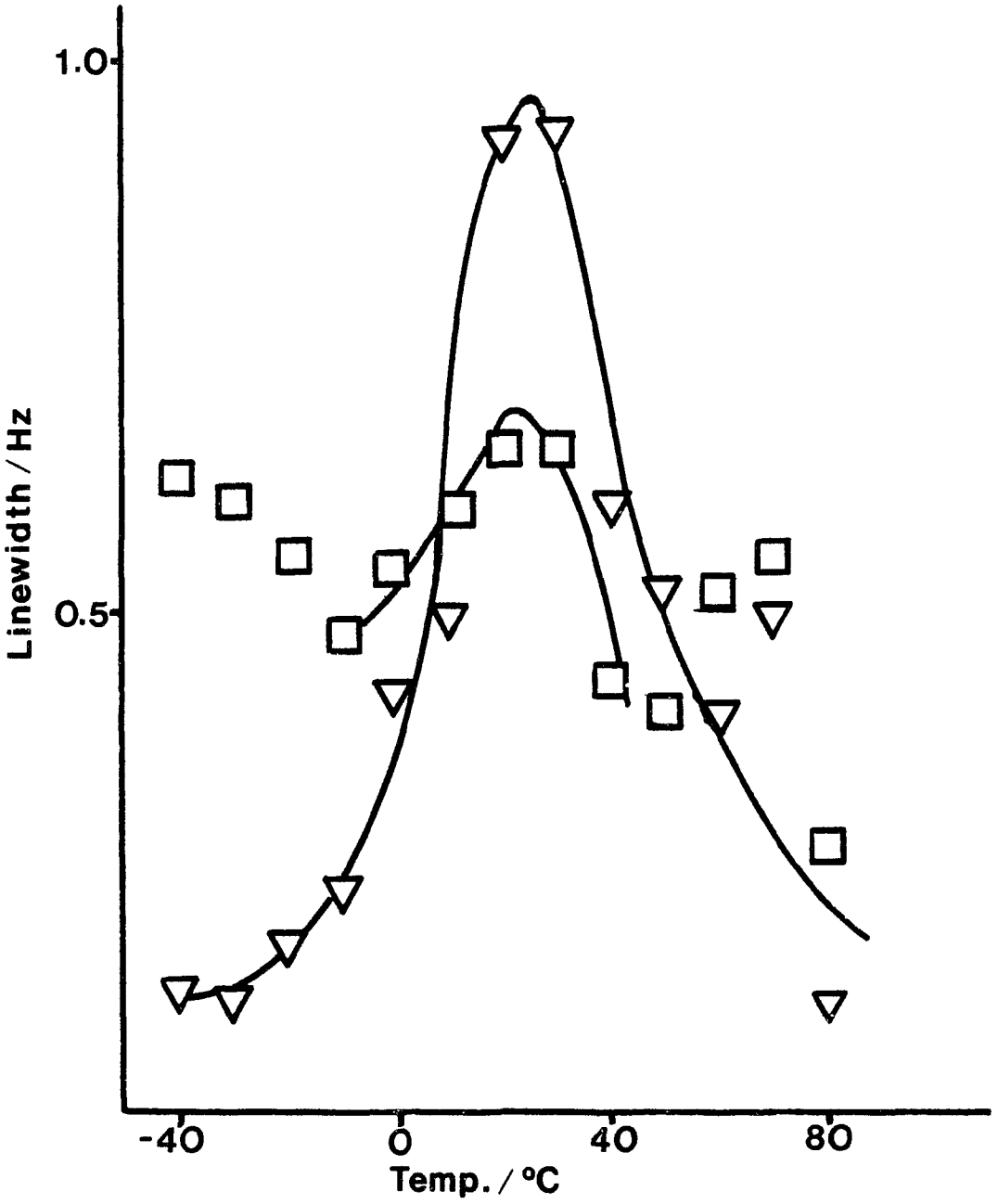


Figure 5,6

FIGURE 5,7

^1H CHEMICAL SHIFT VARIATION WITH TEMPERATURE FOR 1-(4-METHOXYCARBONYL)PHENYL-3-ACETOXYMETHYL-3-METHYLTRIAZENE (51) IN TOLUENE-d_8, SHIFTS MEASURED RELATIVE TO ROOM TEMPERATURE (25°C) SPECTRUM

□ = $N-CH_2$

▽ = $N-CH_3$

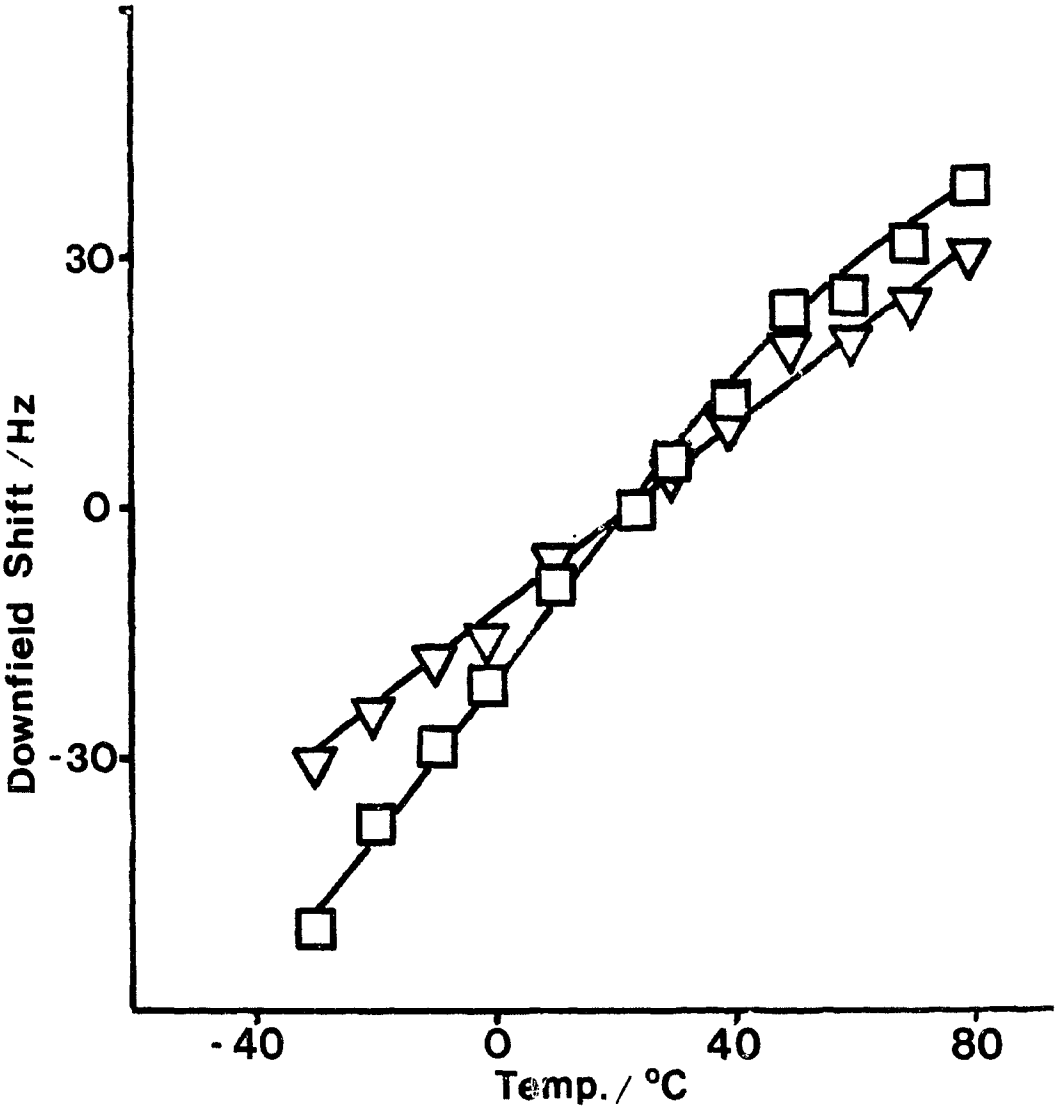


Figure 5,7

FIGURE 5,8

^{1}H NMR LINEWIDTH VARIATION WITH TEMPERATURE FOR 1-(4-METHOXYCARBONYL)PHENYL-3-ACETOXYMETHYL-3-METHYLTRIAZENE (51) IN TOLUENE-d_8

□ = N-CH_2-

▽ = N-CH_3

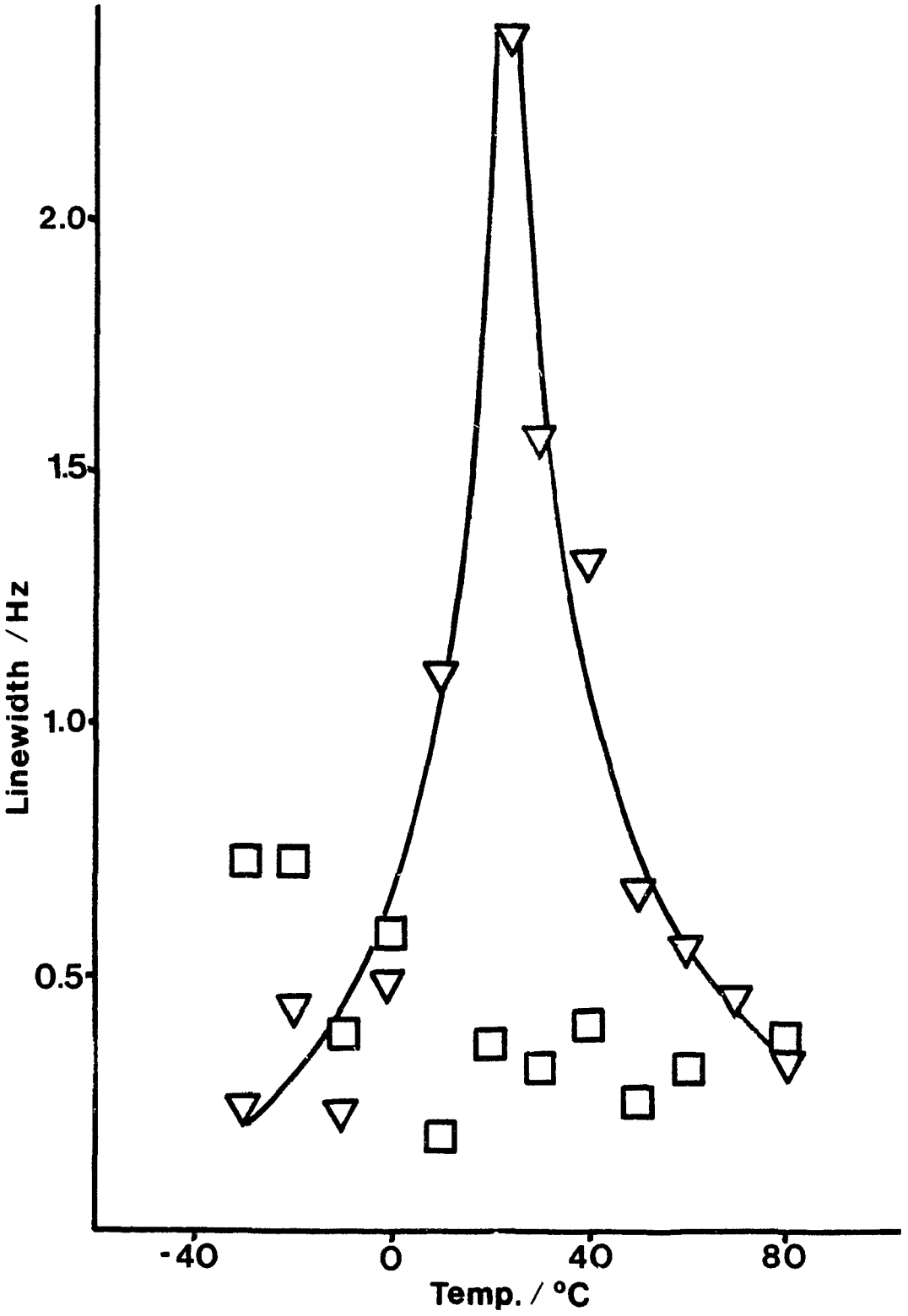


Figure 5,8

5,3 Results and discussion

5,3,1 Revision of Additive Chemical Shift Parameters

Chemical shifts for the N-CH_2 group were calculated using literature empirical shift parameters for ^1H [81] and ^{13}C [82]. This was hampered by the fact that parameters were not available for some groups. For example, for the initial estimate, the NR_2 parameter was used because none was available for the triazene moiety. The calculated shifts were compared with the experimental values and where the deviation was found to be constant for a variety of N-CH_2-X substituents, it was assumed that the difference was due to the difference between NR_2 and the triazene group, and the parameter altered accordingly. This new parameter should give better predictions for the N-CH_3 group also. For ^1H the NR_2 parameter α in the formula $\delta(k)= 0.23+\Sigma\alpha$ is 1.64 and the revised "triazene" parameter is 2.6 giving an improvement in estimate from 1.64 ppm (50% error) to 2.83 ppm (14% error), the actual average shift being 3.29 ppm. This new parameter does not more accurately predict these shifts because it does not take into account the ß-effect of the N-CH_2 group. Similarly for ^{13}C the new parameter in the expression $\delta(k) = -2.6+\Sigma\alpha$ is 31.6 against 42.0 giving a change in prediction from 39.4 ppm (17% error) to 30.0 ppm (13% error). One might conjecture that the poor improvement for ^{13}C is due to a greater contribution from the ß-substituents. The predicted and averaged actual shifts for

shifts for N-CH_2 are shown in tables 5,6 and 5,7.

Table 5,6 Comparison of Predicted[a] and Actual ^1H NMR Chemical Shifts δ (ppm) for Selected Triazene N-CH_2 Groups

X	Pred. Shift	Error	Refined[b] Pred.	Error	Actual Shift
S-Ar	3.56	1.65	4.75	0.46	5.21
O-Ar	4.53	1.20	5.72	0.01	5.73
OH	4.10	1.14	5.29	-0.05	5.24
OAc	4.62	1.18	5.81	-0.01	5.80
OMe	3.91	1.22	5.10	0.03	5.13
SR	3.27	1.69	4.46	0.50	4.96

[a] Reference [81]
[b] See text for explanation

Table 5,7 Comparison of Predicted[a] and Actual ^{13}C NMR Shifts δ (ppm) for selected Triazene N-CH_2 Groups

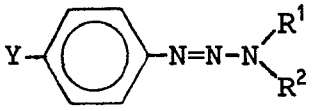
X	Pred. Shift	Error	Refined Pred.	Error	Actual Shift
S-Ar	59.4	-3.1	49.0	-13.5	62.5
O-Ar	93.4	9.9	83.0	0.5	83.5
OH	87.4	8.7	77.0	-1.7	78.7
O-Ac	90.4	11.6	80.0	1.2	78.8
O-Me	97.4	10.8	87.0	0.4	86.6

[a] Reference [82]

It can be seen from the ^{1}H data that regardless of the overall validity of the new parameter, there is a definite difference between the S- and O-substituted groups and that their shifts cannot be adequately predicted by the same parameter in this simple treatment. The opposite trend is detectable in the ^{13}C data which is in itself a useful piece of information. It is very probable that the "odd" behavior of the S-substituted compounds compared with the O- ones is due to a magnetic anisotropy effect or an electric field effect since these are important contributors to ^{1}H but not to ^{13}C shifts. Of course with a shift in perspective, we might conjecture that the S-substituted compounds exhibit "normal" behavior, and that all others are aberrant. These effects may be complicated by hindered internal rotation. This study attempts to elucidate these effects.

5,3,2 Variable Temperature NMR Study of Hydroxymethyltriazenes (57)

In triazenes, the rotational barrier is much smaller for N(2)-N(3) than for N(1)-N(2) [34]. If rotation about N(2)-N(3) is free, then the observed proton signal for N-CH_2 will be a single line with an averaged shift, with a negligible change in δ with temperature. If the rotation about N(2)-N(3) is completely hindered, i.e., a locked conformation, then again little temperature dependence should be observed in the N-CH_2 shift. A variable temperature ^{13}C study mainly, on triazenes where $R^1 = R^2$,

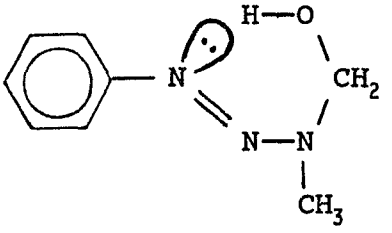


(Figure 5,9)

concluded by correlation of $\Delta G^{\ddagger}$ with Hammett σ values, that the restricted rotation observed was due to π-overlap between the amino and azo nitrogens and that this overlap is lost when the amino group rotates 90° from planarity. For 1-aryltriazenes, this barrier was found to be *ca.*13.8 kcal.mole^{-1} compared with a value of 13.7 kcal.mole^{-1} for the ^1H determination [34]. As anticipated this barrier was lowered to about 10.7 kcal.mole^{-1} when the aryl group was replaced with a benzyl group. The same workers examined three compounds where $R^1 \neq R^2$ and noted the same temperature dependence, but the calculation of $\Delta G^{\ddagger}$ was quite inexact. The appropriate carbons broaden at reduced temperature and

sharpen again at even lower temperatures at a different chemical shift. Since the alkyl substituents on the amino nitrogen were not the same, they would not coalesce. The "coalescence temperature" (T_c) was redefined as the temperature of maximum broadening and minimum height. T_c was estimated visually, and yielded barriers of *ca.* 10.5-11.5 kcal.mole^{-1} where the N(1) substituent was benzyl. In all three examples, the amino methyl was shifted upfield at reduced temperature. This requires that the methyl group be *cis* to N(1). The remaining alkyl group is shifted downfield and thus it was concluded that the orientation of the alkyl groups depends on steric requirements, i.e., the more sterically demanding substituent will orient itself *trans* to N(1).

For the hydroxymethyltriazenes (57), we can observe that for the N-CH_2 protons, the resonance of the minor conformer is shifted upfield from that of the major, and the reverse is true for the N-CH_3 group. On the basis of the arguments above, we can conclude that for the hydroxymethyltriazenes the CH_2- (the more sterically demanding) group is *cis* to N(1) in the preferred conformation contrary to our expectations. With hindsight it was evident that this phenomenon could easily be explained in terms of intramolecular hydrogen bonding between the OH and N(1) (Figure 5,4).



(Figure 5,10)

This is supported by the IR data which show a significant degree of hydrogen-bonding of the OH which is not sensitive to changes in concentration. This is only consistent with intramolecular hydrogen bonding.

According to Shanan-Atidi and Bar-Eli [88] if p_A and p_B are the populations of conformers A and B, A being the major one, then:

$$p_A - p_B = \Delta p = \left(\frac{X^2 - 2}{3} \right)^{3/2} \frac{1}{X} \qquad \text{(Eqn. 5,1)}$$

$$X = 2\pi\tau_c\delta\nu$$

Where τ_c is the lifetime (k^{-1}) at coalescence and $\delta\nu$ is the "frozen-out" shift difference between the conformers. Egan and Mislow [89] transformed the above equation into a polynomial and solved it for selected values of Δp. Using these values it is possible to calculate the free energy of activation for the processes A $\rightarrow$ B ($\Delta G_A^{\ddagger}$) and B $\rightarrow$ A ($\Delta G_B^{\ddagger}$) from Eyring's equations [90]:

$$\Delta G_A^{\ddagger} = RT_c \ln \left\{ \frac{k}{h\pi} \left(\frac{T_c}{\delta\nu} \right) \left(\frac{X}{1 - \Delta p} \right) \right\} \qquad \text{(Eqn. 5,2)}$$

$$\Delta G_B^{\ddagger} = RT_c \ln \left\{ \frac{k}{h\pi} \left(\frac{T_c}{\delta\nu} \right) \left(\frac{X}{1 + \Delta p} \right) \right\} \qquad \text{(Eqn. 5,3)}$$

The difference between equations 5,2 and 5,3 being the free energy difference between the two species. These activation parameters are shown for a variety of hydroxymethyltriazenes in table 5,8.

Table 5,8 Activation Parameters for the N(2)-N(3) Bond Rotation in Hydroxymethyltriazenes

Cmpnd.		$\Delta G_A^{\ddagger}$	$\Delta G_B^{\ddagger}$	ΔG
57a	$N-CH_3$	19.51	11.89	7.61
57b	$N-CH_3$	21.51	13.12	8.40
	$N-CH_2$	21.76	14.76	7.01
Average		**21.64±0.18**	**13.94±1.16**	**7.71±0.98**
57c	$N-CH_3$	20.70	12.07	8.63
57d	$N-CH_3$	19.40	12.39	7.01
	$N-CH_2$	19.92	13.71	6.21
Average		**19.66±0.37**	**13.05±0.93**	**6.61±0.57**
57e	$N-CH_3$	16.80	13.78	3.02
	$N-CH_2$	18.01	15.10	2.91
Average		**17.41±0.85**	**14.44±0.93**	**2.97±0.07**

We could observe no clear relationship between these values and the corresponding Hammett σ parameters using this approximate method. However it is interesting to note that for compound 57e where the aryl substituent is o-CF_3, the barriers to interconversion of the rotamers are significantly lower as is the energy difference between the two favoured states. There are two possible explanations for this behavior one of which is that the inductive effect

of the *ortho* CF_3 group is strong enough to significantly reduce the electron density on N(1), hence reducing the lone pair /OH interaction and thus lessening the energy difference between conformers. Another possible explanation is that the CF_3 group is close enough to the OH group in the favoured conformer to interfere sterically. X-Ray crystallographic data on this compound in the solid state should enable us to distinguish between these possibilities, but unfortunately such information was not available at the time of writing.

5,3,3 Variable Temperature Studies on 1-(4-methoxycarbonyl)phenyl-3-(4-methyl)phenylthiomethyl- (52), 1-(4-methoxycarbonyl)phenyl-3-(4-bromo)phenyloxymethyl- (55e) and 1-(4-methoxycarbonyl)phenyl-3-acetoxymethyl-3-methyltriazene (51).

The problem of extracting meaningful thermodynamic data from systems where one of the exchange partners is undetectably small has been addressed by several workers, including Grindley [91], Okazawa and Sorensen [92] and Anet and Basus [93]. The method of Sorensen will be used here. There are two types of process which can be studied, the degenerate case, e.g. A ⇌ A' and reactions A ⇌ B where the equilibrium constant is not necessarily unity, and the minor component may indeed be undetectable. The most effective method for dealing with both these cases is by computer simulation and matching. For our purposes these methods are

perhaps over-refined, and a simple procedure is preferred. A simple procedure for the case A ⇌ A' is the coalescence line shape relationship [94]

$$k = \pi\delta\nu/\sqrt{2} \qquad \text{(Equation 5,4)}$$

where $\delta\nu$ is the chemical shift separation of the two sites under conditions of slow exchange ("frozen out"), and k is the rate constant for the process. In cases of unequal populations it is common to use total line shape analysis to obtain useful thermodynamic data. If the population of the minor constituent is so small as to be unobservable, it is possible to detect the presence of exchange phenomena by simply observing the changes in the observable major peak. Sorenson's treatment included the development of simple empirical equations from ^{13}C maximum line widths (MLWs) using Fortran programs to generate graphical data from the standard exchange equation of Gutowsky and Holm [94]. For the process A ⇌ B' we define the forward rate constant as k_A. Fractional populations are expressed as p_B where $p_B < p_A$. Plots of k_A against $\delta\nu$ give straight lines passing through the origin, except for cases where p_B = 0.35-0.40 because in this region the line width measurements are perturbed at coalescence as the shoulder caused by the minor conformer passes from below to above the point where the width is measured. This correlation yields

$$k_A = c''_{(pB)}\ \delta\nu \qquad \text{(Equation 5,5)}$$

where $c''_{(pB)}$ is a constant for a fixed value of p_B. For the case A ⇌ A' where $p_B = 0.5$, $c'' = 2.19$, a deviation of only 1.4% from $\pi/\sqrt{2}$ in equation 5,4. p_B Can be incorporated into this equation giving

$$k_A = c'_{(pB)}\ p_B\ \delta\nu \qquad \text{(Equation 5,6)}$$

where $c'_{(pB)}$ is a new constant dependant on p_B. Plotting this parameter against p_B gives the relationship

$$k_A = c_o' p_B \delta\nu - c p_B^2 \delta\nu \qquad \text{(Equation 5,7)}$$

where $c_o' = 6.32$ and $c = 3.90$ when k_A is expressed in s^{-1}. The plot deviates from linearity for large values of p_B but in the cases under discussion, this is not important.

Plots of W_{max}, the maximum line width of the observable peak, against $\delta\nu$ are linear with intercept W_o, the natural line width in the absence of exchange which, for the purpose of this study, was taken to be the line width of TMS since this undergoes no exchange processes at the temperatures studied. These plots give

$$W_{max} = b_{(pB)}\delta\nu \qquad \text{(Equation 5,8)}$$

where $b_{(pB)}$ is a constant for a given value of p_B. Again,

plotting this constant as a function of p_B gives the following approximate formula

$$W_{max} = \delta\nu(p_B + p_B^2/2p_A^2) + W_o \qquad \text{(Equation 5,9)}$$

where p_A is $1-p_B$. Where p_B is small, this reduces to

$$W_{max} = \delta\nu p_B + W_o \qquad \text{(Equation 5,10)}$$

The above relationships were confirmed by *ab initio* derivations [93].

Turning now to the compounds under investigation, it is found that indeed for the most part, the minor conformer is undetectable. In order to establish a value for $\delta\nu$ it was necessary to resort to an approximate value from a model compound where the two conformers could be measured. The hydroxymethyltriazenes (Table 5,2) were the only available model, the assumption being that the shift difference between the two rotamers will be similar for the other species where the difference is caused by the same effect. For the hydroxymethyltriazenes the shift difference did not vary considerably with variations in the aryl substituent, and for this part of the study the value for 1-(4-methoxycarbonyl)phenyl-3-hydroxymethyl-3-methyltriazene (57d) was used as the model. The conformer shift differences $\delta\nu$ in this compound are: $N-CH_2$ = 79.87 Hz,

$N-CH_3$ = 121.08. The temperatures of maximum broadening T_c and the maximum line width $W_{max}-W_o$ were estimated graphically. These and the other necessary parameters for use in the Eyring equations (Equations 5,2 and 5,3) are given in table 5,9 and the activation parameters in table 5,10.

Table 5,9 Line Broadening, Coalescence Temperatures and Calculated Populations for an Arylthio-, an Aryloxy-, an Acetoxy and a Hydroxymethyltriazene in Toluene d_8

Cmpnd.		W_{max} (Hz)	T_c (K)	p_B	Δp	X[a]	$\delta\nu$ (Hz)
52f	$N-CH_3$	3.09	313	0.0255	0.9490	2.7808	121.08
	$N-CH_2$	5.27	313	0.0659	0.8680	2.6928	79.87
55e	$N-CH_3$	1.0	298	8.259×10^{-3}	0.9835	2.8095	121.08
	$N-CH_2$	0.65	298	8.138×10^{-3}	0.9837	2.8095	79.87
51	$N-CH_3$	2.37	293	0.0196	0.9609	2.7904	121.08
57e	$N-CH_3$	3.2	305	0.0412	0.9180	2.7518	121.08
	$N-CH_2$	3.0	305	0.0376	0.9249	2.7518	79.87

[a] See Equation 5,1

Table 5,10 Activation Energies (kcal/mole) for N(2)-N(3) rotation in an Arylthio- (52f), an Aryloxy- (55e) and an Acetoxymethyltriazene (51)

Cmpnd.		$\Delta G_A^{\ddagger}$	$\Delta G_B^{\ddagger}$	ΔG
52f	$N-CH_3$	25.43	13.44	12.01
	$N-CH_2$	23.57	14.85	8.73
	Average:	**24.52±1.3**	**14.2±1.0**	**10.4±2.3**
55e	$N-CH_3$	27.65	12.62	15.03
	$N-CH_2$	28.95	13.93	15.07
	Average:	**28.3±0.9**	**13.3±0.9**	**15.05±0.03**
51	$N-CH_3$	24.45	12.37	12.08
57e	$N-CH_3$	24.48	14.36	10.12
	$N-CH_2$	24.76	14.35	10.41
	Average:	**24.62±0.2**	**14.36±0.007**	**10.27±0.21**

It was unfortunate that the compounds 52f, 55e and 51 did not give useful results in $CDCl_3$ for direct comparison with the results for the hydroxymethyltriazenes (57). The broadening for these compounds in $CDCl_3$ occurred at low temperatures (below -40°C) at which their solubilities were poor. The hydroxymethyltriazene 57e was examined in toluene-d_8 for comparison and like the other species had no detectable minor conformer in this solvent but did show a line broadening maximum which was used to calculate activation parameters by the same method. It is immediately obvious that in toluene the barrier to N(2)-N(3) rotation is considerably higher than in chloroform. It is likely that this is due to strong interactions between the π-system of toluene and the conjugated nitrogen chain of the triazene.

Of further interest is the fact that for the hydroxymethyltriazene studied in both solvents (57e), The differences in activation energies is due to a marked increase in $\Delta G_A^{\ddagger}$ in changing from chloroform to toluene, $\Delta G_B^{\ddagger}$ being unaltered. In other words, the energy barrier going from the more to the less favoured conformer is raised in toluene, but the barrier going from the less to the more favoured conformer is unaltered. This is consistent with the more stable conformer being further stabilized by the toluene-triazene interaction.

As an estimate of the errors in the method, the values used for the ΔG calculations of compound 52f were varied and the activation parameters recalculated. The variation was based upon the maximum realistic error in each variable, though the 50% error in $\delta\nu$ is a rather pessimistic estimate. The results of these calculations are shown in table 5,11.

Table 5,11 Variation Caused by Estimated Errors in the Observed Values on the Calculation of ΔG in kcal/mole

Variable	Variation	Variation in $\Delta G_A^{\ddagger}$	Variation in $\Delta G_B^{\ddagger}$	Variation in ΔG
T_c	±5°C	±0.46	±0.30	±0.20
W_{max}	±0.5 Hz	±0.50	±0.01	±0.52
$\delta\nu$	±50%	±0.05	-1.35 +2.31	+1.36 -3.10

An observation for which we have as yet no explanation is that for all species studied, the shift variation with temperature is greater for the $N-CH_2$ than the $N-CH_3$ group, but the line broadening is greatest for $N-CH_2$ in the arylthio- and hydroxymethyltriazenes and greatest for $N-CH_3$ in the aryloxy- and acetoxymethyltriazenes. In the case of the aryloxynethyltriazene, the broadening is very small in both cases and the difference may not be significant, but for the acetoxymethyltriazene the $N-CH_2$ group did not appear to broaden at all.

5,4 Conclusions

This study has provided us with chemical shift parameters that give improved predictions for triazene groups. The N(2)-N(3) rotation barriers in hydroxymethyltriazenes have been measured in chloroform for the first time and indicate that at room temperature the conformers should not be isolable. Measurements on arylthio- aryloxy- and acetoxymethyltriazenes in toluene indicate that in this solvent the barriers are higher, but still not high enough to yield isolable rotamers. Whilst the difference in behavior of the arylthiomethyltriazene has been confirmed, its source remains an enigma.

Chapter 6

Conclusions

The arylthiomethyl and aryloxymethyltriazenes, both novel analogues of the hydroxymethyltriazenes, have been prepared and their properties studied. The arylthiomethyl compounds were tested on a very limited scale for antitumour activity, and whilst these results were promising, a far wider range of tumours might well reveal better selectivities for these compounds.

The hydrolysis of the aryloxymethyltriazenes was found to be exquisitely sensitive to the nature of the substituent on the aryl ring. In the course of these hydrolysis experiments we were able, for the first time, to measure the rate of initial decomposition of an α-oxidized dimethyltriazene. More extensive pH profiles for the hydrolysis of the compounds studied would be useful, as would more detailed work on the dependence of hydrolysis rates on ionic strength. Antitumour tests for these compounds show significant selectivity and further testing is in progress.

Conformational studies on a variety of HMT derivatives have revealed rotational barriers comparable with the results of previous studies, and interesting conformational preferences particularly for the hydroxymethyltriazenes themselves. We hope shortly to confirm and perhaps provide a fuller understanding of these findings by ^{15}NMR and X-ray crystallography.

Chapter 7

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Chapter 1

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