Reactions of Substituted Arenediazonium Chlorides with Methylamine-Formaldehyde Premix Revisited: Reactivity and Transformations of Methylolamine Intermediates and Their Biological Significance

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Dedicated to Professor Erich Hecker on the occasion of his 60th birthday

Arenediazonium Ions, Methylolamine Intermediates

Modulation of the N-azo coupling between ring-substituted arenediazonium chlorides and premixed methylamine-formaldehyde leads not only to 1-aryl-3-hydroxymethyl-3-methyltriazenes and their dimers, but also to unexpected cyclic and complex products. The syntheses comprise reactions with arenediazonium chlorides bearing both −M and +M substituents at *para* and *ortho*/*para* positions of the phenyl ring. One of the major constituents isolated from a mixture of products is the O-acetate of 3-hydroxymethyl-3-methyl-1-(2,4,6-trichlorophenyl)triazene. This product was obtained from the reaction of 2,4,6-trichlorobenzenediazonium chloride and methylamine-formaldehyde mixture which was then stabilized by acetylation.

The structures of the isolated products could be derived from reactive methylolamines and electrophilic intermediates that possibly occur *in vivo* and thereby offer a plausible mechanistic explanation for the carcinogenic and tumour-inhibitory activity associated with the open-chain triazene compounds in the living cell.

3-Alkyl-1-aryl-3-(1-hydroxyalkyl)triazenes (Fig. 1) are metabolic intermediates in the oxidative cytochrome P-450 mediated activation of 1-aryl-3,3-dimethyltriazenes (Fig. 2) which have been known as an important class of tumour-inhibitory agents [1, 2] and indirect carcinogens [3, 4]. N-Hydroxymethyl-N-methylnitrosamine was proposed by Magee and associates in mid fifties as an unstable intermediate of diazomethane in the enzymatic activation of the hepatocarcinogenic dimethylnitrosamine [5, 6].

Although triazenyl alkylolamines have been regarded as transient molecular species, experimental evidence for their stability has been gradually surfacing. More than a decade ago a hypothesis, implicating both the phenyl and imidazole hydroxymethyltriazenes as cornerstone metabolites, began to emerge. The initial concepts were developed from metabolic [7–9], synthetic [9, 10], and therapeutic [11, 12] studies.

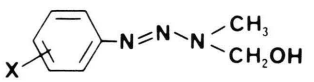

Fig. 1. 1-Aryl-3-hydroxymethyl-3-methyltriazene.

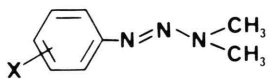

Fig. 2. 1-Aryl-3,3-dimethyltriazene.

Enzymatic demethylation of 1-aryl-3,3-dimethyltriazenes was recognized as a reversible process and utilized for the synthesis of the corresponding 3-hydroxymethyl derivative: 3-Methyl-1-phenyltriazene, obtained from phenylazide by Grignard reaction, readily added methanolic formaldehyde to give a single product of lower R_F. The compound was identified as 3-hydroxymethyl-3-methyl-1-phenyltriazene (Fig. 1, Ar = phenyl) by mass spectrometry [4]. More importantly, 5-(3-hydroxymethyl-3-methyl-1-triazeno)imidazole-4-carboxamide was isolated as a urinary metabolite of 5-(3,3-dimethyl-1-triazeno) imidazole-4-carboxamide (DTIC, DIC, NSC-45388) which is a clinically useful cancer chemotherapeutic drug. 5-(3-Hydroxymethyl-3-methyl-1-triazeno) imidazole-4-carboxamide was also synthesized by a condensation of 5-(3-methyl-1-triazeno)imidazole-4-carboxamide with methanolic formaldehyde [10].

A fundamental advance in hydroxymethyltriazene synthesis was reported by Vaughan [9] who introduced a one pot N-coupling reaction of *p*-methoxycarbonylbenzenediazonium chloride with premixed

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methylamine-formaldehyde. The method was modified by Julliard *et al.* [13] who used solid 4-X-arenediazonium fluoroborates, instead of diazonium chloride solutions. Cheng *et al.* [14] critically examined the products of this reaction by ^{1}H NMR spectroscopy and found that the described compounds were bis[(3-aryl-1-methyl-2-triazen-1-yl)-methyl]methylamines, rather than 1-aryl-3-hydroxymethyl-3-methyltriazenes. The results were confirmed by Lafrance *et al.* [15].

The structures and proportions of products derived from coupling of arenediazonium ions with methylamine-formaldehyde mixtures depend mainly on substituents in the phenyl ring, ratio of reactants, temperature, and pH. We report the synthesis of several classes novel compounds, including 3,7-bis(4-X-aryl)-1,5,3,7-dioxadiazocanes and products derived from 2,4,6-trichlorobenzenediazonium ion. We have identified N,N-bis-{[3-(4-tolyl)-1-methyl-2-triazen-1-yl]methyl}methylamine which we believe is the first dimeric compound bearing an electron-releasing methyl substituent at the 4-position of the phenyl ring.

Materials and Methods

General

All aromatic amines, reagents and other materials were of the purest grades available.

Melting points (m.p.): Tottoli capillary melting point apparatus (uncorrected).

Elemental analyses were performed by the Analytical Laboratory of the Max-Planck-Institute of Medical Research, Heidelberg.

UV spectra were measured on a Hitachi U-3200 spectrometer and IR (ν [cm^{-1}]) on a Perkin Elmer 580B instrument. ^{1}H and ^{13}C NMR (δ [ppm] relative to internal TMS; in $CDCl_3$ unless otherwise noted; J [Hz] = apparent coupling constant): HX90, WH90 or Bruker AM500 spectrometer at 70 eV. Mass spectra were determined at 100 eV with a Finnigan MAT711 mass spectrometer, Bremen, FRG.

All spectra were measured at the Spectroscopic Unit, Institute of Biochemistry, German Cancer Research Center, Heidelberg, and may be retrieved from their data bank.

Reagents, thin-layer and column chromatography

N-Ethyl-1-naphthylamine (Fluka, Buchs, Switzerland) NEN reagent was prepared by dissolving 700 mg of N-ethyl-1-naphthylamine hydrochloride in ethanol (285 ml) and making up the solution to 350 ml with conc. hydrochloric acid.

Precoated silica gel plates F-254 (Merck AG, Darmstadt, FRG) were used for thin-layer chromatography in the following solvents: (1) toluene; (2) toluene/acetone (3:1 v/v).

Silica gel (0.05–0.2 mm) and neutral or basic alumina (Merck AG, Darmstadt) were used as solid supports.

Synthetic methods

3,7-Bis(4-trifluoromethylphenyl)-1,5,3,7-dioxadiazocane

4-Trifluoromethylaniline (4.03 g, 0.025 mol) in HCl (37%, 10 ml) and water (20 ml) was diazotized with $NaNO_2$ (2.76 g, 0.04 mol) in water (6 ml) and the clarified diazonium solution was coupled over 20 min with a premixed solution of methylamine (9 ml, 40%) and formaldehyde (40 ml, 37%), cooled to −5 °C. After the addition had been completed, the reactants (pH 4–5) were mechanically stirred for 30 min and adjusted to pH 1 with HCl; stirring was continued for another 15 min after which the reaction mixture was placed in a refrigerator (4 °C) overnight. The separated solid was filtered, washed with water and the dried product crystallized from benzene (m.p. 201; yield 0.9 g, 18%).

3-Hydroxymethyl-3-methyl-1-(2,4,6-trichlorophenyl)-triazene derivatives

2,4,6-Trichloroaniline (9.82 g, 0.05 mol) in HCl (37%, 20 ml) and H_2O (40 ml) was diazotized with $NaNO_2$ (4.8 g, 0.07 mol). The cooled 2,4,6-trichlorobenzenediazonium solution was coupled (30 min) with a deeply cooled (−10 °C) mixture of formaldehyde (37%, 80 ml) and methylamine (40%, 18 ml). The reaction mixture was adjusted to pH 7 with sodium bicarbonate, and allowed to stand overnight. The aqueous phase was decanted and the separated viscous product was dissolved in ether (100 ml), the solution washed with water, and dried over sodium sulphate. Evaporation of the solvent left a yellow residue (5 g) which was chromatographed on a silica gel column (2.5 × 25 cm), eluted with diisopropyl ether. After collecting 10 ml of blank effluent, the product was eluted with 60 ml of the solvent; the residue was crystallized from benzene: *n*-hexane (1:3). The yield of 3,11-dimethyl-1,7,13-tris-

(2,4,6-trichlorophenyl)-5,9-dioxa-1,2,3,7,11,12,13-heptazatridecan-1,12-diene, m.p. 132 °C, was 0.35 g (7%); (Compound A).

A portion of the yellow product (2 g) was taken up in diethyl ether (8 ml) and the solution cooled to −10 °C. Acetic anhydride (1.6 g, 0.015 mol) was diluted with diethyl ether (7 ml) and added over 5 min. The reaction was stirred for an hour and allowed to stand overnight. The solvent was removed *in vacuo* and the residue chromatographed on a 2 × 20 cm silica gel column, packed in diisopropyl ether. The development was continued with tetrahydrofuran which eluted the product. Evaporation of the solvent left a residue which was crystallized from diisopropyl ether : *n*-hexane (1:2); m.p. 81 °C (0.78 g; 39%); (Compound B).

Another portion of crude product (2 g) was taken up in pyridine (5 ml), cooled to 0 °C, and acetylated by a gradual addition of acetic anhydride (1.6 g, 0.015 mol). The reaction was stirred for 30 min with cooling and for an additional hour at room temperature; thereafter the mixture was poured over ice (20 g), and the acetylated products were extracted with ether (2 × 15 ml). The constituents were chromatographed on a column of silica gel (1 × 20 cm) in diisopropyl ether. After collecting 40 ml of the blank effluent, {[3-(2,4,6-trichlorophenyl)-1-methyl-2-triazene-1-yl]methyl}acetate was eluted with 150 ml of the solvent. The residue was crystallized from *n*-hexane; m.p. 78 °C (0.54 g, 28%); (Compound C).

Further elution of the column with tetrahydrofuran removed N-methyl-N-{[3-(2,4,6,-trichlorophenyl)-1-methyl-2-triazen-1-yl]methyl}acetamide (Compound B) which was purified as above; m.p. 81 °C (0.70 g; 35%).

N,N-Bis{[3-(4-tolyl)-1-methyl-2-triazen-1-yl] methyl}methylamine; [3,5,7-trimethyl-1,9-bis(4-methylphenyl)-1,2,3,5,7,8,9-heptazanona-1,8-diene]

4-Toluidine (5.35 g, 0.05 mol) in HCl (37%, 15 ml) and water (15 ml) was diazotized with $NaNO_2$ (4.8 g, 0.07 mol) in water (10 ml) with cooling in an ice bath. The clarified diazonium solution (charcoal) was treated with a pinch of urea to destroy unreacted nitrite. The diazonium solution was slowly added (30 min) to a mixture of formaldehyde (37%, 90 ml) and methylamine (40%, 20 ml) which was cooled to −5 °C. The reaction mixture was neutralized to pH 7 with solid bicarbonate and allowed to stand overnight in a refrigerator (4 °C). The product was extracted with chloroform (3 × 30 ml), the washed organic phase dried over natrium sulphate, and the solvent removed in a rotary film evaporator. The residue of N,N-bis{[3-(4-tolyl)-1-methyl-2-triazen-1-yl] methyl}methylamine was recrystallised from diisopropyl ether : *n*-hexane (2:3); m.p. 82 °C; yield 2.7 g (41%).

Results and Discussion

N-Azo coupling of −M and +M substituted arenediazonium ions with methylamine-formaldehyde premix leads to a variety of compounds the structure of which is sensitive to a) the substituent in the phenyl ring, b) proportion of reactants, c) temperature, and d) pH of the reaction.

3,7-Bis-(4-X-aryl)-1,5,3,7,-dioxadiazocanes (Fig. 3) were isolated from coupling of ben-

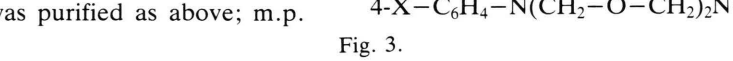

Fig. 3.

Table. Physical data of 3,7-bis(4-X-aryl)-1,5,3,7-dioxadiazocanes.

Compound	X	Yield [%]	M.p. [°C]	Arom. (AA'BB') (8 H, 2 d)	*J* [Hz]	δ (^{1}H NMR) in $CDCl_3$ CH_2 (8 H, s)	X	M^+ *m/z*	IR ν [cm^{-1}]
3a	4-F_3C	18	201	6.92, 7.23	9	5.24	–	406 (15%)	1030
3b	4-CH_3OOC	49	217	6.90, 7.70	9	5.22	3.83 (6H, s)	386 (66%)	1035
3c	4-NC	50	227	6.89, 7.25	8.5–9	5.21	–	320 (17%)	1040
3d	4-NO_2	53	258	6.93, 7.81	9	5.28 (in d_6-DMSO)		360 (17%)	1035

zenediazonium ion, substituted with a powerful electron-withdrawing group, in a reaction that was ultimately adjusted to pH 1. The structure of 3,7-bis(4-trifluoromethylphenyl)-1,5,3,7-dioxadiazocane (**3a**) and of related 4-methoxycarbonyl- (**3b**), 4-cyano- (**3c**) and 4-nitrophenyl- (**3d**) analogues (Table) was elucidated by spectroscopic and analytical methods.

The ^{1}H NMR spectra in $CDCl_3$ and d_6-DMSO revealed a typical AA'BB' pattern of *p*-disubstituted benzenes (8H, 2d), centered around δ 6.92 and δ 7.23, and a broad singlet of the methylene protons (8H) at δ 5.21–5.28, integrating to 1:1. Neither signal showed any exchange after deuteriation. The relatively broad resonance of the methylene protons may be due to slow conformational changes associated with the dioxadiazocane ring. Moreover, no signal could be seen in the ^{1}H NMR spectra at δ 3.13–3.31, characteristic for 3N methyl protons in triazenes [4]. As expected, none of the dioxadiazocanes – though sensitive to acid – showed diazo C-coupling with the NEN reagent. Mass spectra revealed the following molecular ions: (**3a**) $C_{18}H_{16}O_2N_2F_6$, *m/z* 406 (15%); (**3b**) $C_{20}H_{22}O_6N_2$, *m/z* 386 (66%); (**3c**) $C_{18}H_{16}O_2N_4$, *m/z* 320 (17%); (**3d**) $C_{16}H_{16}O_6N_4$, *m/z* 360 (17%), in complete agreement with the established structure.

The mechanism of 3,7-bis(4-trifluoromethylphenyl)-1,5,3,7-dioxadiazocane formation is proposed in the Scheme.

The kinetically relevant intermediate is 1-methyl-3-(4-trifluoromethylphenyl)triazene (**4a**), the unconjugated tautomer, favoured by the CF_3 substituent at the 4-position of the phenyl ring [16]. Condensation of **4a** with formaldehyde would lead to 3-hydroxymethyl-1-methyl-3-(4-trifluoromethylphenyl)-triazene (**4b**) which would subsequently cleave at low pH to methanediazonium ion and N-hydroxymethyl-4-trifluoromethylaniline (**4c**) which reacts with another molecule of formaldehyde to give N,N-dihydroxymethyl-4-trifluoromethylaniline (**4d**). Elimination of water from the protonated form of **4e** leads to N-hydroxymethyl-N-methylene-4-trifluoromethylanilinium ions (**4f**) which condense across the $-N=CH_2$ double bond to afford 3,7-bis(4-trifluoromethylphenyl)-1,5,3,7-dioxadiazocane. We became aware of previous publication reporting the structure of 1,5-bis(4-trifluoromethylphenyl)-3,7-dioxa-1,5-diazacyclooctane (**3a**) [18] first after our preliminary communication [17].

A major interest in our laboratory is that 3,3-dimethyl-1-(2,4,6-trichlorophenyl)triazene shows a pronounced tumour-inhibitory action against murine

Scheme

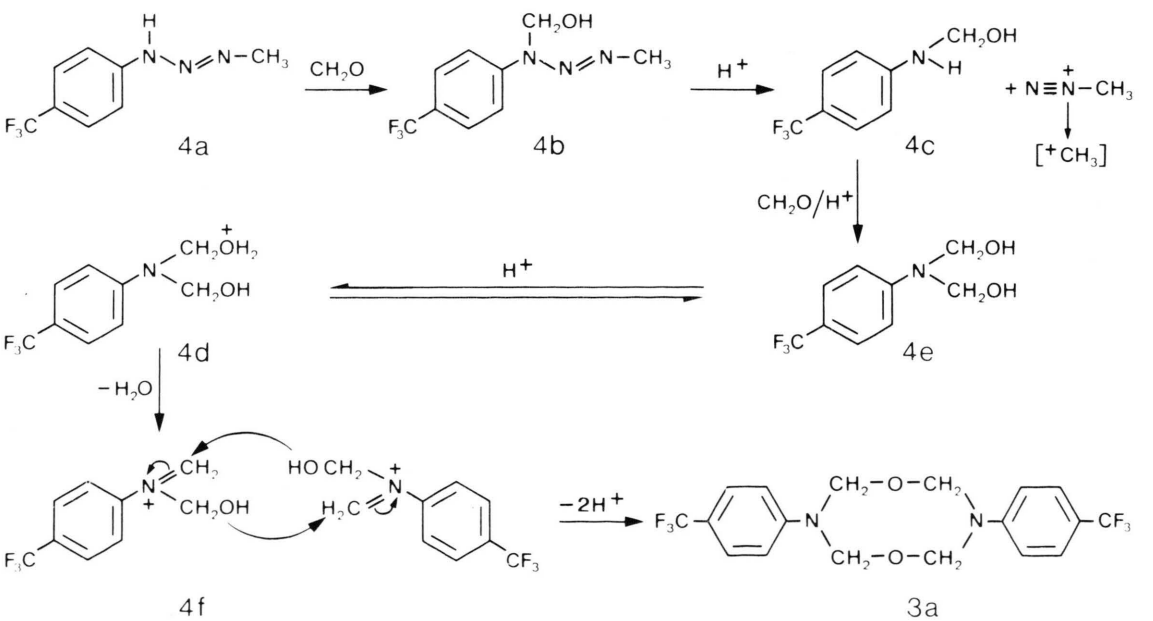

Fig. 4.

TLX5 lymphoma (IST 79%) and ADJ/PC6 plasmocytoma (therapeutic index 25). By virtue of its chlorination at the 2,4,6-positions of the phenyl ring and our previous metabolic experience [8], the compound is well suited for mechanistic chemical studies. In this vein we thus coupled 2,4,6-trichlorobenzenediazonium chloride with methylamine-formaldehyde mixture but the reaction yielded no single product but left a yellow viscous residue. Column chromatography enabled the isolation of 3,11-dimethyl-1,7,13-tris-(2,4,6-trichlorophenyl)-5,9-dioxa-1,2,3,7,11,12,13-heptazatrideca-1,12-diene (Fig. 5).

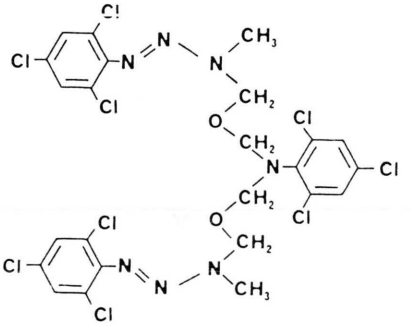

Fig. 5. 3,11-Dimethyl-1,7,13-tris-(2,4,6-trichlorophenyl)-5,9-dioxa-1,2,3,7,11,12,13-heptazatrideca-1,12-diene.

The elution of the column with diisopropyl ether removed the bulk of the products but an efficient fractionation of individual components could not be effected. The mixture was acetylated and fractionated on a silica gel column in diisopropyl ether. After collecting 40 ml of effluent, N-methyl-N-{[3-(2,4,6-trichlorophenyl)-1-methyl-2-triazen-1-yl]methyl} acetamide (Fig. 6) and [3-(2,4,6-trichlorophenyl)triazene-1-methyl-2-triazen-1-yl]methyl}acetate (Fig. 7) were isolated.

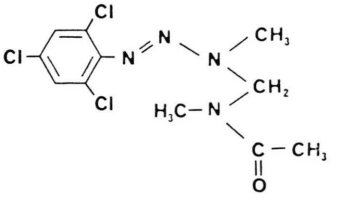

Fig. 6. N-Methyl-N-{[3-(2,4,6-trichlorophenyl)-1-methyl-2-triazen-1-yl]methyl}acetamide.

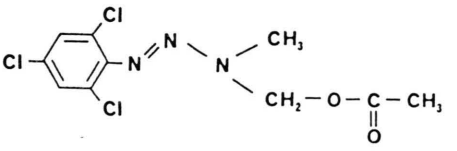

Fig. 7. {[3-(2,4,6-trichlorophenyl)-1-methyl-2-triazen-1-yl] methyl}acetate.

At this point it cannot be ascertained whether compound B (Fig. 6) was already present in the reaction mixture, or arose by cleavage of a more complex intermediary structure by acetylation. However, it appears that 3-acetoxymethyl-3-methyl-1-(2,4,6-trichlorophenyl)triazene (Fig. 7) could have arisen from the dimeric N,N-bis{[3-(2,4,6-trichlorophenyl)-1-methyl-2-triazenyl]methyl}methylamine (not shown) by an attack of electrophilic acetyl at the central nucleophilic N-atom.

We also report the isolation of dimeric triazenyl derivatives that were formed by N-diazo coupling of arenediazonium chlorides bearing electropositive (CH_3-) substituents. An example of such a compound is N,N-bis{[3-(4-tolyl)-1-methyl-2-triazen-1-yl]methyl}methylamine (Fig. 8).

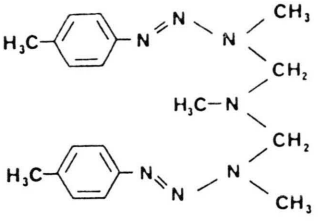

Fig. 8. N,N-Bis{[3-(4-tolyl)-1-methyl-2-triazen-1-yl]methyl}methylamine.

The structures of the isolated compounds [19] were formed from intermediary 1-aryl-3-hydroxymethyl-3-methyltriazenes by the following mechanisms: 1. Loss of formaldehyde to yield 1-aryl-3-methyltriazene, followed by a prototropic shift and subsequent release of methanediazonium ion, or 2. by formaldehyde retention and ionization of the methylolamine to the corresponding triazenium (iminium) ion. The released electrophilic species react with available nucleophilic N, O or C atoms present in the reaction mixture. It is conceivable that electrophilic intermediates, generated from 1-aryl-3,3-dimethyl-

triazenes *in vivo,* react in a similar manner with crucial nucleophilic sites in cellular biopolymers. These electrophiles may thus provide a molecular basis for the differentiation between the carcinogenic and/or tumour-inhibitory activity that is associated with the open chain triazene compounds [20–21].

We have began studies to characterize the biological effects of several of these compounds. Preliminary results with the 3,7-bis(4-X-aryl)-1,5,3,7-dioxadiazocanes, tested for mutagenic activity in the Ames test, suggest that compounds **3a**, **3b** and **3d** are weakly mutagenic in strains TA98 and TA100. Surprisingly, 3,7-bis(4-trifluoromethylphenyl)-1,5,3,7-dioxadiazocane shows a pronounced and protracted mitogenic effect (over 9 days) in the lymphatic organs of rates [23]. These *in vitro* and *in vivo* studies are being continued.

Acknowledgement

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