Open-chain nitrogen compounds. Part XV.[1] A kinetic study of the hydrolysis of 1-aryl-3-aryloxymethyl-3-methyltriazenes and related triazenes

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The kinetics of hydyrolysis of a series of 1-aryl-3-aryloxymethyl-3-methyltriazenes, Ar-N=N-NMe-CH_2OAr', was studied over the pH range 2–7.5. Reactions were followed by the change in UV absorbance spectra of the triazenes. The aryloxymethyltriazenes decompose more slowly at pH 7.5 than the hydroxymethyltriazenes, Ar-N=NMe-CH_2OH; the hydrolysis is favoured by the presence of an electron-withdrawing group in Ar'. A mixed isopropanol/buffer system was developed in order to improve solubility of the aryloxymethyl triazenes. Lowering the pH caused an increase in the rate of hydrolysis and under strongly acidic conditions an electron-withdrawing group in Ar' actually slows down the reaction. A Hammett plot of the pseudo-first-order rate constant, k_{obs}, is curved, indicating that two or more mechanisms operate simultaneously and that the contribution of each mechanism is substituent-dependent. A plot of k_{obs} vs. [buffer] is linear; the slope of the plot affords the rate constant, k_b, for the buffer-catalyzed reaction for each substituent. A Hammett plot of k_b vs. σ is linear with $\rho = +0.55$, suggesting that the buffer-catalyzed reaction involves nucleophilic displacement of the phenoxy group by the buffer anion. Further analysis afforded the specific acid-catalyzed rate constants, k_{H^+}, for each substituent; this component of the reaction has a negative ρ, consistent with a mechanism involving protonation at the ether oxygen. The postulation that specific acid catalysis is a component of the reaction mechanism was confirmed by the observation of a solvent deuterium isotope effect, $2.28 > k_H/k_D > 1.60$. Only the *p*-NO_2 and *p*-CN phenyloxymethyltriazenes showed any spontaneous decomposition.

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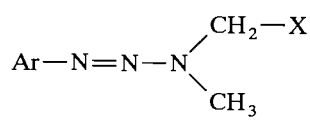
On a déterminé la cinétique de l'hydrolyse d'une série de 1-aryl-3-aryloxyméthyl-3-méthyltriazènes, Ar-N=N-NMe-CH_2OAr', à des pH allant de 2 à 7,5. On a suivi les réactions par le changement du spectre d'absorbance UV des triazènes. À un pH de 7,5, les aryloxyméthyltriazènes se décomposent plus lentement que les hydroxyméthyltriazènes, Ar-N=N-NMe-CH_2OH; l'hydrolyse est favorisée par la présence d'un groupe électro-attracteur dans la portion Ar'. On a développé un système mixte isopropanol/tampon pour améliorer la solubilité des aryloxyméthyltriazènes. Un abaissement du pH provoque une augmentation de la vitesse d'hydrolyse et, dans des conditions fortement acides, un groupe électro-attracteur dans la portion Ar' inhibe complètement la réaction. La courbe de Hammett de la constante de vitesse du pseudo-premier ordre, k_{obs}, n'est pas rectiligne; ceci indique que deux ou plusieurs mécanismes opèrent simultanément et que la contribution de chacun dépend du substituant. Une courbe de k_{obs} vs. [tampon] est linéaire; la pente de la courbe fournit la constante de vitesse, k_b, pour la réaction catalysée par le tampon pour chaque substituant. Une courbe de Hammett de k_b vs. σ est linéaire et $\rho = +0{,}55$, suggérant que la réaction catalysée par le tampon implique une substitution nucléophile du groupe phénoxyle par l'anion du tampon. Une analyse plus approfondie fournit les constantes de vitesse des réactions soumises à une catalyse acide spécifique, k_{H^+}, pour chaque substituant; la valeur de ρ pour cette composante de la réaction est négative et cette valeur est en accord avec un mécanisme impliquant une protonation de l'oxygène de l'éther. Le postulat à l'effet que la catalyse acide spécifique est une composante du mécanisme de la réaction a été confirmé par l'observation d'un effet isotopique du deutérium du solvant, $2{,}28 > k_H/k_D > 1{,}60$. Les seuls phényloxyméthyltriazènes à se décomposer spontanément sont les dérivés *p*-NO_2 et *p*-CN.

[Traduit par la rédaction]

Introduction

1-Aryl-3,3-dimethyltriazenes (**1***a*) have been known for many years (1) to have antitumour activity against murine tumours and one such triazene, DTIC (Dacarbazine, NSC 45388), has found clinical use against human malignant melanoma (2). The presumed mode of action of DTIC involves enzymatic oxidation to a hydroxymethyltriazene (**1***b*), followed by loss of formaldehyde to give the monomethyltriazene, Ar-N=N-NHMe, the putative cytotoxic metabolite. It has been suggested that one of the limitations to DTIC efficacy is the apparent inefficiency of oxidative metabolism in the human liver (3). Thus it would seem to be a worthwhile exercise to search for a triazene derivative of DTIC that would act as a prodrug and not require oxidative metabolism.



1 *a* X = H
b X = OH
c X = OMe
d X = OAc
e X = SAr'

The hydroxymethyltriazene (**1***b*) ("HMT") would seem to be an ideal candidate as a pro-drug, but all of the biological

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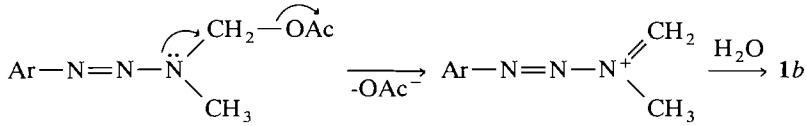
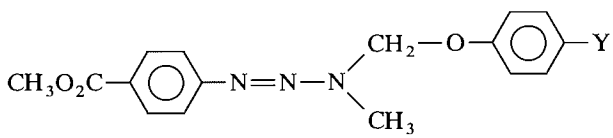


FIG. 1

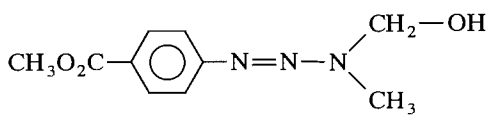
studies suggest that the hydroxymethyltriazene is a very transient species in vivo. Nevertheless, we were successful in achieving the synthesis of the first stable hydroxymethyltriazene (4), and although these compounds are stable in the crystalline state, they do indeed decompose rapidly in solution under physiological conditions (5). In developing the chemistry of these novel *N*-hydroxymethyl compounds, we were aware of the desirability of preparing a further derivative of HMT with appropriate stability for pro-drug use.

Thus we synthesized the methyl ether derivatives (**1***c*) and the acetoxymethyltriazenes (**1***d*) (6); the difference in chemical behavior of these two apparently similar derivatives is quite striking but can be rationalized by the difference in the facility of the leaving group X. The ether derivatives (**1***c*) with a poor leaving group, OMe, are comparable in stability at pH 7.5 to the dimethyltriazene (**1***a*), whereas the acetoxymethyltriazene (**1***d*) decomposes in solution as rapidly as the hydroxymethyltriazene (**1***b*). Apparently the relatively good leaving group, OAc, is displaced rapidly to afford an iminium ion, which hydrolyses to the HMT (See Fig. 1).

We previously extended the range of hydroxymethyltriazene derivatives to the 1-aryl-3-arylthiomethyl-3-methyltriazenes (**1***e*) (7) and more recently to the 1-aryl-3-aryloxymethyl-3-methyltriazenes (**2***a–h*) (8). The isolation of these compounds is an exciting development in the search for the ultimate triazene pro-drug, since the aryloxy group would be expected to fall in between the methoxy and acetoxy groups in leaving group ability. Furthermore, the extensive variation of the Y-group in **2** should provide for a "tailor-made"



2 *a* Y = OCH_3
b Y = CH_3
c Y = H
d Y = Cl
e Y = Br
f Y = CO_2CH_3
g Y = CN
h Y = NO_2



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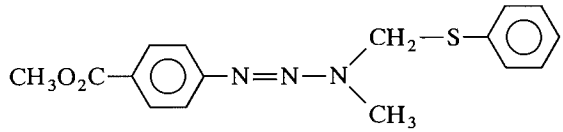

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leaving group with the appropriate reactivity required in the pro-drug. Accordingly we have undertaken a thorough study of the solution behavior of these new aryloxymethyltriazenes in order to assess their potential as triazene pro-drugs.

Experimental

The compounds used in this study were prepared by established procedures from the literature (5, 6) with the exception of the 3-aryloxymethyl-3-methyltriazenes (**2***a–h*) whose synthesis and characterization are reported in the previous paper in this series (8). 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 dimethyl sulfoxide. 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) (4.88 g) 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 M: 88% formic acid solution (3.206 g) and sodium formate (2.630 g) made up to 1 L. 0.5 M: 88% formic acid solution (16.03 g) and sodium formate (13.17 g) made up to 1 L. 0.01 M: 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). The pH measurements were carried out with solutions thermostatted at 38 ± 1°C, but there was no attempt to control the ionic strength of the medium.

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 cycle time 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 sec 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–isopro-

TABLE 1. Kinetic data for the hydrolysis of aryloxymethyltriazenes at pH 2.0 in 0.066 M isopropanol/chloride buffer mixture

Compound	Intercept	Slope	$k_{obs}/10^{-3}$ (s^{-1})	Log k	$t_{1/2}$ (min)	r
2*a*	−0.329	−0.367	6.12	−2.213	1.9	0.983
2*b*	0.264	−0.365	6.08	−2.216	1.2	0.988
2*c*	0.016	−0.356	5.95	−2.226	2.0	0.998
2*d*	−0.054	−0.288	4.80	−2.318	2.2	0.999
2*e*	−0.234	−0.268	4.47	−2.349	1.7	0.999
2*f*	0.083	−0.195	3.25	−2.213	1.9	0.999
2*g*	0.071	−0.177	2.95	−2.530	4.3	0.999
2*h*	0.114	−0.237	3.96	−2.403	3.4	0.999
3	−0.082	−1.093	0.018	−0.174	0.56	0.998

TABLE 2. Kinetic data for the hydrolysis of aryloxymethyltriazenes at pH 3.3 in isopropanol/formate buffer mixture of differing buffer concentration

	0.33 M[a]			0.066 M[a]			0.0066 M[a]		
Compound	$k_{obs}/10^{-3}$ (s^{-1})	Log k	$t_{1/2}$ (min)	$k_{obs}/10^{-3}$ (s^{-1})	Log k	$t_{1/2}$ (min)	$k_{obs}/10^{-3}$ (s^{-1})	Log k	$t_{1/2}$ (min)
2*a*	1.313	−2.882	8.99	0.567	−3.246	20.6	0.379	−3.421	30.6
2*b*	1.211	−2.917	9.42	0.387	−3.413	26.1	0.286	−3.543	36
2*c*	0.940	−3.027	12.6	0.291	−3.536	40.6	0.182	−3.741	63.8
2*d*	1.101	−2.958	10.6	0.298	−3.525	36.0	0.167	−3.776	54.1
2*e*	1.422	−2.847	8.37	0.325	−3.487	35.2	0.162	−3.788	68.7
2*f*	1.694	−2.771	6.86	0.342	−3.466	33.8	0.155	−3.81	75
2*g*	2.456	−2.609	4.8	0.491	−3.309	22.5	0.249	−3.603	45.4
2*h*	5.010	−2.299	2.6	2.25	−2.648	4.24	1.498	−2.825	7.4
3	—	—	—	19.54	−1.709	0.66	—	—	—

[a]Buffer concentration.

TABLE 3. Kinetic data for the hydrolysis of aryloxymethyltriazenes at pH 5.5 in 0.066 M isopropanol/MES buffer mixture

Compound	Intercept	Slope/10^{-3}	$k_{obs}/10^{-5}$ (s^{-1})	Log k	$t_{1/2}$ (h)	r
2*a*	−0.029	−1.122	1.869	−4.728	10.7	0.999
2*b*	0.022	−1.065	1.776	−4.753	11.2	0.999
2*c*	0.012	−0.448	0.746	−5.127	26.2	0.999
2*d*	0.028	−0.575	0.958	−5.018	20.9	0.992
2*e*	0.0008	−0.557	0.928	−5.032	20.8	0.999
2*f*	−0.046	−2.170	3.620	−4.441	4.97	0.999
2*g*	0.029	−7.127	10.87	−3.925	1.69	0.999
2*h*	0.359	−87.60	146.0	−2.836	0.20	0.999
3	0.023	−0.108	179.0	−2.745	0.11	0.999

panol 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:

$$[1] \quad \ln A = \ln (A_t - A_\infty)/(A_\infty - A_0)$$

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 slope of each plot represents the rate constant k_{obs} (s^{-1}). Half-lives were calculated using:

$$[2] \quad t_{1/2} = 60\{(\ln 0.5) - A\}/B$$

where A is the y-intercept and B the slope in min^{-1}. The results of this kinetic analysis are presented in Tables 1–3.

Changes in rate constant k_{obs} with buffer concentration at constant pH were examined by measuring the hydrolysis rate for each compound in formate buffer (pH 3.3) at three concentrations. 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 Fig. 8 plotted against Hammett σ_p values.

TABLE 4. Retention times and response factors for reference standards

Compound	Retention time (min)	Response factor ($mg^{-1}/10^7$)[a]	Molar response ($mmol^{-1}/10^9$)
2e	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.

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 × 4 mm, 10 μm Lichrosorb NH_2 (Hibar) column was used with a 3 mm amino guard column (Brownlee Labs). Isocratic elution with isopropanol/*n*-hexane (60:40) at a flow of 1.5 mL min^{-1} gave the best separations. External standards were prepared by dissolving a weighed mass of the desired compound in 25 mL isopropanol as follows.

Compound **2e**	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 **2e**, 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.

Hydrolysis of the 4-bromophenyloxymethyltriazene (**2e**) was carried out as follows. Formate buffer (pH 3.3, 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 (3 × 10 mL), and the organic layers were 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 containing suitable amounts of the three likely products was prepared 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 as follows: compound **2e**, 76%; M4HB, 97.8%; M4AB, 61.4%; HOC_4H_4Br, 78.5%.

The chromatograms for the hydrolysis experiment are shown in Fig. 2. The results of this analysis are given in Table 5, and the assay of the hydrolysis in Table 6.

Solvent deuterium isotope effect

The *p*-bromophenyloxymethyltriazene (**2e**) was dissolved in dimethyl sulfoxide (1.5 mg/mL) and 50 μL of this solution was added to the buffer/isopropanol mixture (0.1 M, pH/pD 3.07, 40 ± 1°C) in a cuvette. In one experiment, the buffer was made up in H_2O to give the rate constant (k_H); in a second experiment, the buffer was made up in D_2O to give the deuterium isotope rate constant (k_D). The observed k_H/k_D value was 1.60. Since the isopropanol was not deuterated, an exchange can take place, i.e.,

$$i\text{-PrOH} + D_3O^+ \rightleftharpoons i\text{-PrOD} + HD_2O^+$$

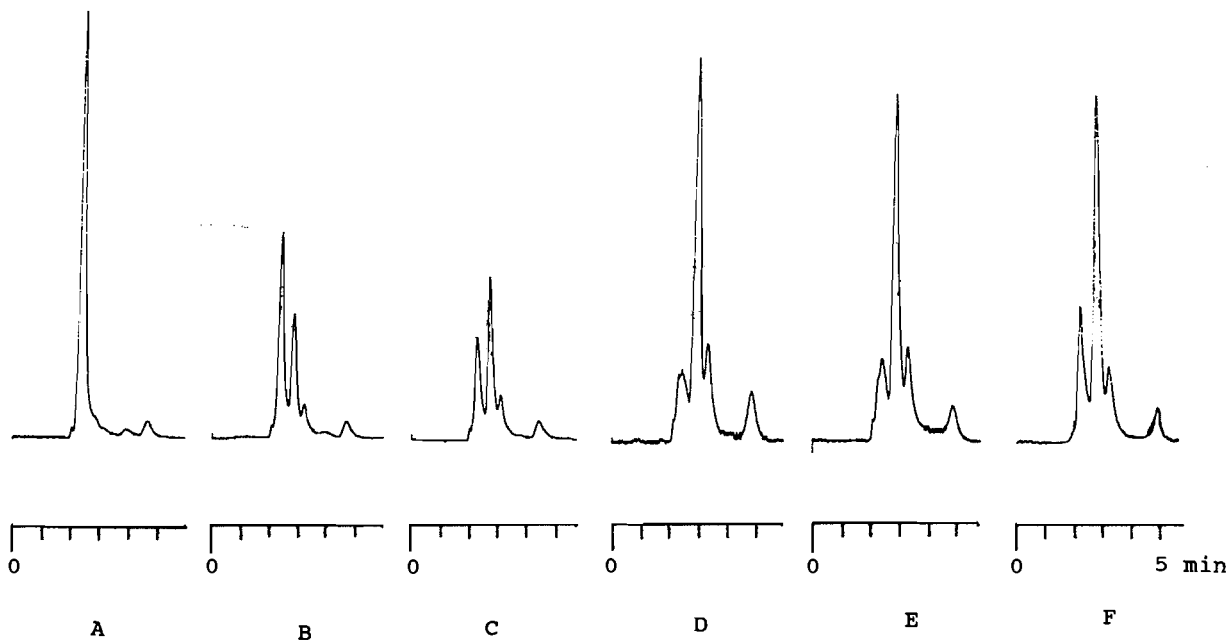


FIG. 2. Hydrolysis of 1-(4-carbomethoxyphenyl)-3-(4-bromophenyloxymethyl)-3-methyltriazene (**2e**) followed by HPLC. A, 0 min; B, 32 min; C, 56 min; D, 92 min; E, 18 h; F, 6 days.

TABLE 5. Results of HPLC analysis of the hydrolysis of compound **2e** measured by disappearance of substrate

Time (min)	Amount in sample ($g/10^{-3}$)	Corrected 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_0) where M_t is the number of moles present at time t and M_0 is the number of moles present at the start of the experiment.

TABLE 6. Assay of products of HPLC hydrolysis experiment

Time (min)	Assay in moles/10^{-5}		
	M4AB	Bromophenol	M4HB
0		—	—
32	2.19	2.13	0.051
65	2.55	2.86	0.057
92	3.13	3.43	0.055
1080	3.17	3.58	0.053

which implies that k_D(obs) could be expressed as:

$$1/3\ k_H + 2/3\ k_D\ (\text{true}) = k_D\ (\text{obs}) = 5.15 \times 10^{-4}\ s^{-1}$$

(since the reaction solvent is composed of 2/3 buffer and 1/3 isopropanol).

Thus the true k_D could be as low as $3.61 \times 10^{-4}\ s^{-1}$ and the upper limit of k_H/k_D would equal 2.28 (if all the isopropanol was deuterated). The conclusion of the experiment is that there is definitely a solvent deuterium isotope effect in the range $1.60 < k_H/k_D < 2.28$.

Results and discussion

The most reactive compound of the series of aryloxymethyl triazenes (**2a–h**) at physiological pH was predictably the *p*-nitrophenyloxymethyltriazene (**2h**), which decomposed in neat pH 7.5 phosphate buffer in almost exactly the same manner as its parent hydroxymethyltriazene (**3**). A close examination of the UV absorbance decay of the hydrolysis of **2h** (Fig. 3) reveals some subtle differences from the hydrolysis of **3**. Firstly, there is a short induction period at the start of the reaction during which the triazene absorbance at 320 nm does not decay; after this induction period, the UV absorbance decays in a first-order manner to give a pseudo-first-order rate constant, k_2, equal to $1.05 \times 10^{-3}\ s^{-1}$ corresponding to a half-life ($t_{1/2}$) of 11 min. This value is equal, within experimental error, to the half-life of the parent hydroxymethyltriazene (5).

The second feature of Fig. 3 is the observation of a second absorbance at ca. 400 nm that *grows in* during the early stage of reaction. This absorbance can be attributed to the

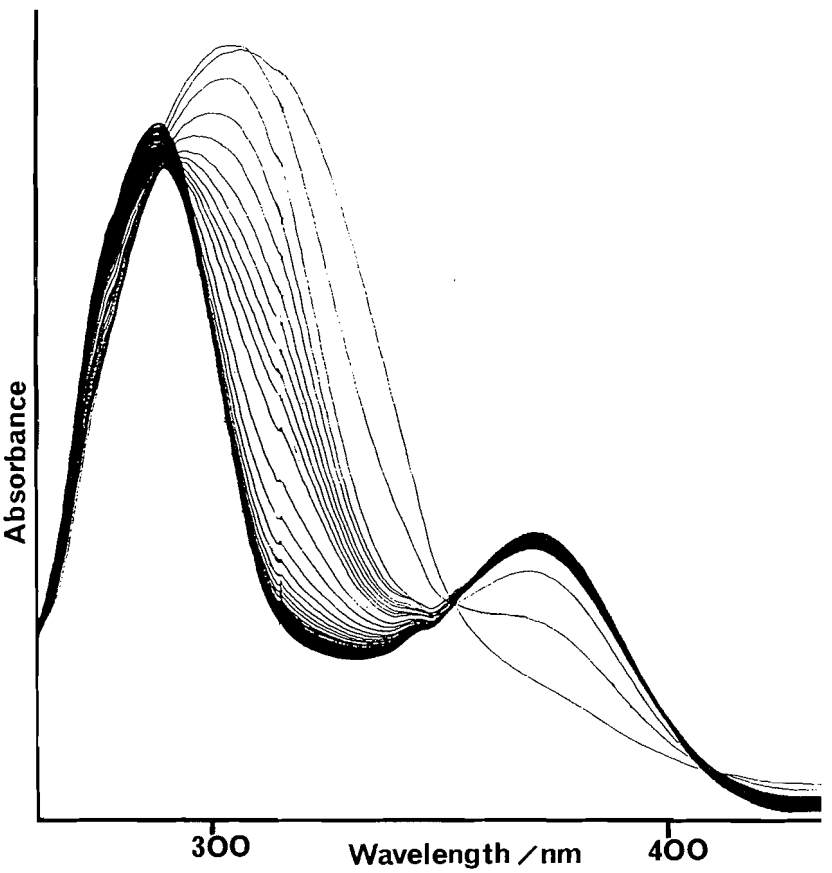


FIG. 3. Repeat-scan (cycle time 1 min) UV spectrum for 1-(4-carbomethoxyphenyl)-3-(4-nitrophenyloxymethyl)-3-methyl triazene (**2h**) in pH 7.5 phosphate buffer.

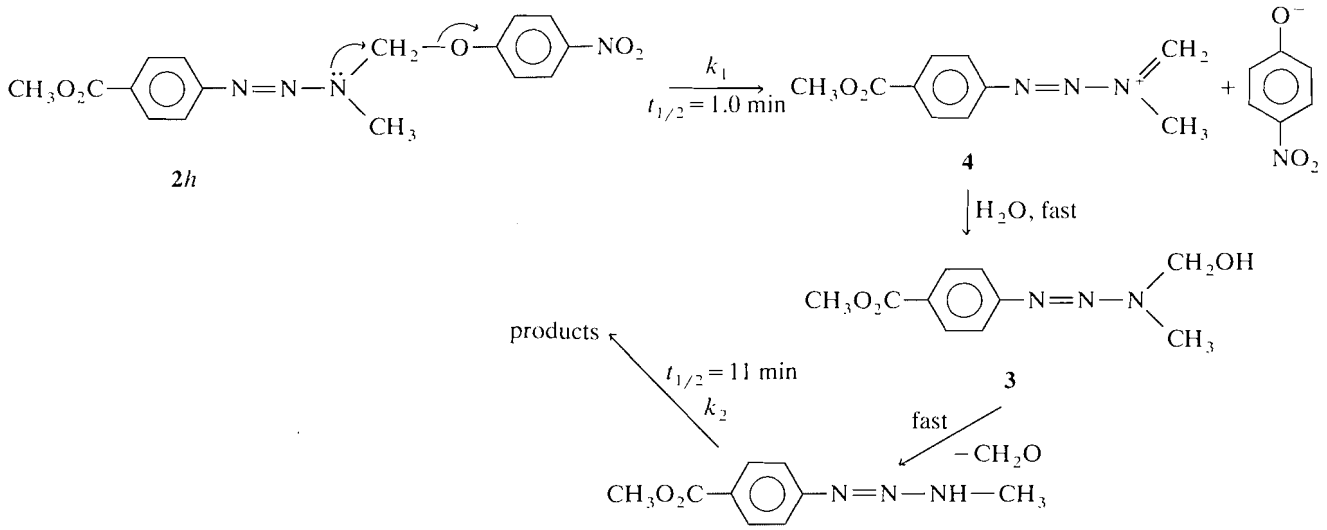


FIG. 4

formation and release of the *p*-nitrophenolate ion during the initial, rapid decomposition of the aryloxymethyltriazene (**2***h*). Kinetic analysis of the growth of the 400-nm peak gives a second pseudo-first-order rate constant, k_1, equal to 1.15 $\times 10^{-2}$ s^{-1}, which converts into a half-life of 1.0 min.

This result can be explained logically by the mechanism shown in Fig. 4, in which the triazene (**2***h*) undergoes spontaneous ionization (k_1) to afford the iminium ion intermediate (**4**) and the *p*-nitrophenolate ion. Fast hydrolysis of the iminium ion by water affords the hydroxymethyltriazene (**3**), followed by rapid loss of formaldehyde to afford the monomethyltriazene, Ar-N=N-NHMe, which then decomposes with the observed half-life (k_2) of 11 min (5). The decomposition of the *p*-nitrophenoxymethyltriazene (**2***h*) under these conditions is unique in our experience in that it represents the first absolute measurement of the fast rate of the initial decomposition of a hydroxymethyltriazene derivative.

The success of the hydrolysis experiment with the *p*-nitrophenoxymethyltriazene (**2***h*) in neat aqueous buffer was not, unfortunately, carried over to the other compounds in this series. With the exception of **2***h*, the solubility of the aryloxymethyltriazenes in the buffer was too low to give concentrations suitable for UV spectrophotometric analysis. The triazene simply precipitated out of solution before any measurable reaction occurred. For this reason, it was deemed desirable to develop a mixed buffer/isopropanol system. An important consideration of using such a system is the establishment of a pH scale for such a mixture. 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 (9). The pH values quoted are those obtained from a normal laboratory pH meter and are thus only an approximate indication of acidity.

To compare the reactivity of the new aryloxymethyltriazenes (**2**) with the parent hydroxymethytriazene (**3**) it was necessary to establish the kinetic parameters of **3** at different pH values and in the mixed buffer/isopropanol solvent system.

The decomposition of compound **3** was examined over the pH range 3.3–12.0 and was found to follow first-order behavior in all cases. The kinetic data from these experiments are shown in Table 7. Thus in an alkaline medium at pH 12.0 the hydroxymethyltriazene is surprisingly stable, with a half-life of 14.7 h. 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 (**3**) 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.

TABLE 7. Kinetic data for the hydrolysis of the hydroxymethyltriazene (**3**) for various buffers and pH values

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

Adding isopropanol to the buffer system slows the hydrolysis of the HMT (**3**) 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 **3** 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-catalyzed decomposition of the hydroxymethyltriazenes.

Tables 1, 2, and 3 show the results for the measurement of kinetic parameters of the aryloxymethyltriazenes (**2**) in isopropanol/buffer systems of pH 2.0, 3.3, and 5.5 obtained from the plots of ln *A* versus time for the loss of the triazene UV absorbance. In all cases the aryloxymethyltriazenes decompose at a significantly lower rate than the parent hydroxymethyltriazene (**3**). For example, the *p*-bromophenyloxymethyltriazene (**2***e*) decomposes from 3 to 190 times more slowly than **3**, depending upon the pH. All sets of data show varying half-life values for the decomposition of the aryloxymethyltriazenes (**2***a*–*h*) as a function of the substituent X in the aryl moiety. It is logical therefore to

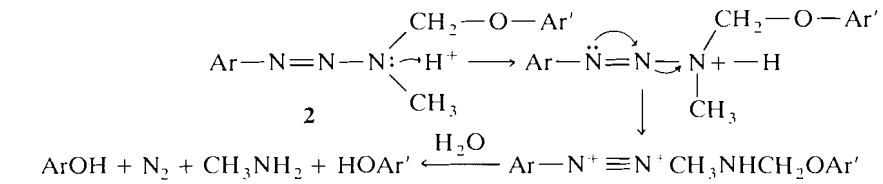


FIG. 5

look for a Hammett correlation for these various substituents.

A general observation from the data in Tables 1–3 is that, for each compound, the lower the pH the faster the reaction. This observation suggests that protonation of the ether oxygen is important in the rate-determining step.

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 simpler pattern emerges where the electron-withdrawing ability favours the hydrolysis.

A possible site of protonation in the aryloxymethyltriazenes (**2**) is the N-3 nitrogen, which would ultimately lead to fragmentation of the triazene at the N2—N3 bond as shown in Fig. 5. Subsequent decomposition of the arenediazonium ion leads to a phenol (methyl-4-hydroxybenzoate in the compounds studied) in addition to the phenol produced by the decomposition of the phenoxymethylamine fragment. The HPLC study of the decompositton of the *p*-bromophenoxymethyltriazene (**2*e***) provided evidence that the mechanism depicted in Fig. 5 is *not* operative. Although traces of methyl-4-hydroxybenzoate were detected in the hydrolysis mixture, its level was low (<2 mol%) and constant. This compound was subsequently found to be a ubiquitous contaminant of α-hydroxymethyltriazenes and their derivatives. The $t_{1/2}$ value obtained for compound **2*e*** was 36.4 min by HPLC compared to 35.2 min by UV spectrophotometry.

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 slow compared with the hydrolysis of interest, isopropyl 4-aminobenzoate only being detectable after 18 h of hydrolysis.

The actual products of hydrolysis of **2*e*** are principally methyl-4-aminobenzoate and *p*-bromophenol, which is consistent with a fragmentation of **2*e*** at the ether linkage to give *p*-bromophenol and the hydroxymethyltriazene (Fig. 6 and Tables 5 and 6). However, the ultimate question is how does this fragmentation occur, and to find an answer we turn our attention to a Hammett analysis.

Figure 7 is a Hammett plot for the hydrolysis rate constants, k_{obs}, of the aryloxymethyltriazenes (**2**) at the pH values 2.0, 3.3, and 5.5 using the data for k_{obs} in Tables 1, 2, and 3. The curved nature of the Hammett plots at all pH's suggests strongly that there is more than one pathway of decomposition. In addition to a spontaneous decomposition, there is clearly an acid-catalyzed component to the overall reaction, since for every substituent the rate of reaction goes up as the pH is lowered.

A second revealing Hammett plot is that shown in Fig. 8, which shows the effect of changing the buffer concentration while holding the pH constant at 3.3. It is clear from Fig. 8 that the rate of reaction increases for each substituent as the buffer concentration is increased, suggesting that there is a third, buffer-catalyzed component to the overall decomposition of **2**.

Thus it appears that the rate of decomposition of the aryloxymethyltriazenes (**2*a–h***) is the sum of the rates of three distinct processes: (*i*) the spontaneous decomposition (k_{sp}); (*ii*) an acid-catalyzed reaction (k_{H^+}); and (*iii*) a buffer-catalyzed reaction (k_B). The measured rate constant, k_{obs}, must itself have three components and may be expressed as follows:

$$[3] \quad k_{obs} = k_{sp} + k_{H^+}[H^+] + k_B[B]$$

The only uncertain quantity in this expression is [B], which could be assumed to be either the buffer anion concentration [A^-], or the buffer acid concentration [HA], or the total buffer concentration {[A^+] + [HA]}. As a first step we used the total buffer concentration, which was plotted against the k_{obs} at pH 3.3 for each substituent. The slope of each line affords k_B for the respective substituent and these values are shown in Table 8. A Hammett plot of log k_B versus σ_p gives a good straight line fit with $\rho = +0.55$. Since the ρ-value for

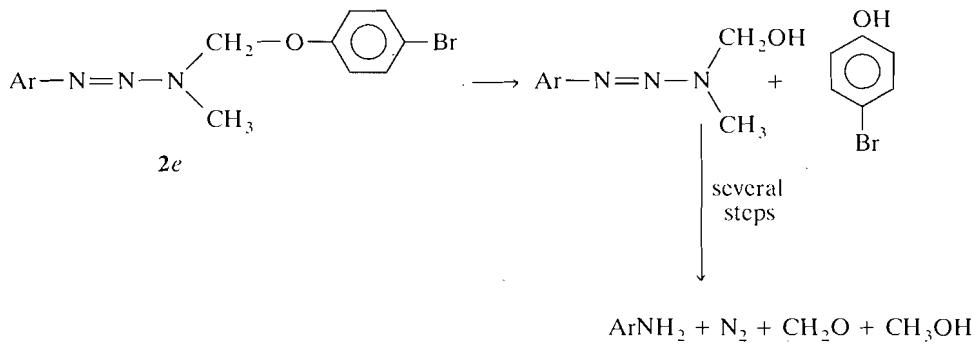


FIG. 6

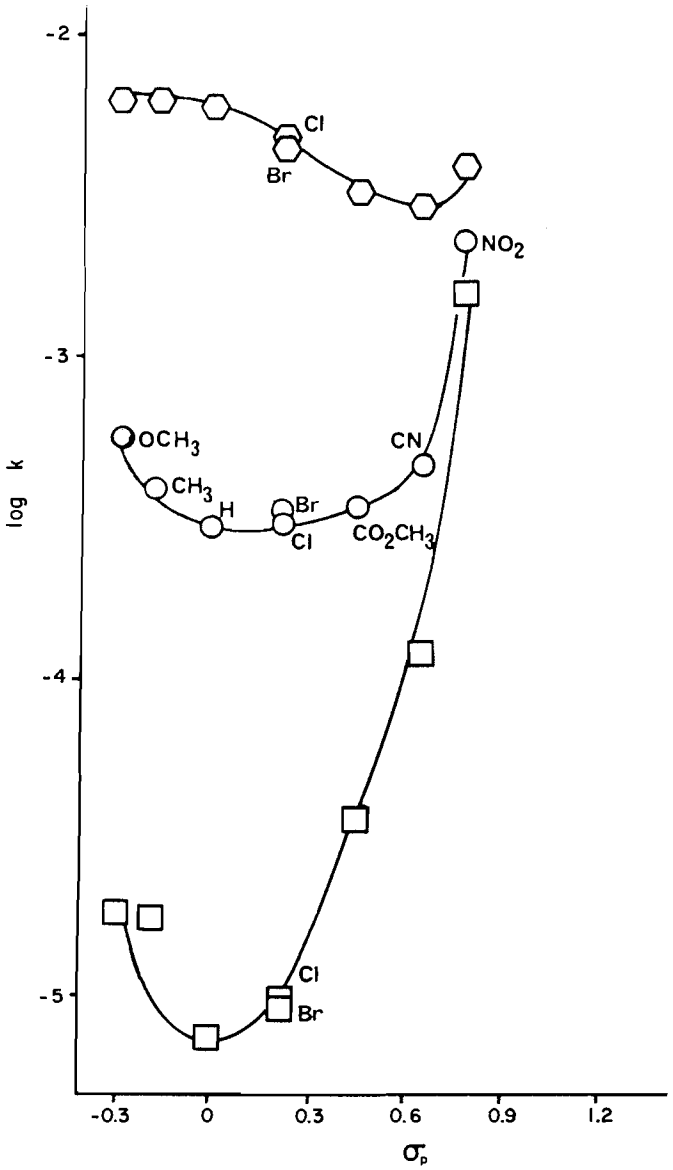


FIG. 7. Hammett plots for the hydrolysis of the aryloxy methyltriazenes (*2a–h*) at various pH values: -○-○- pH 2.0; -○-○- pH 3.3; -□-□- pH 5.5.

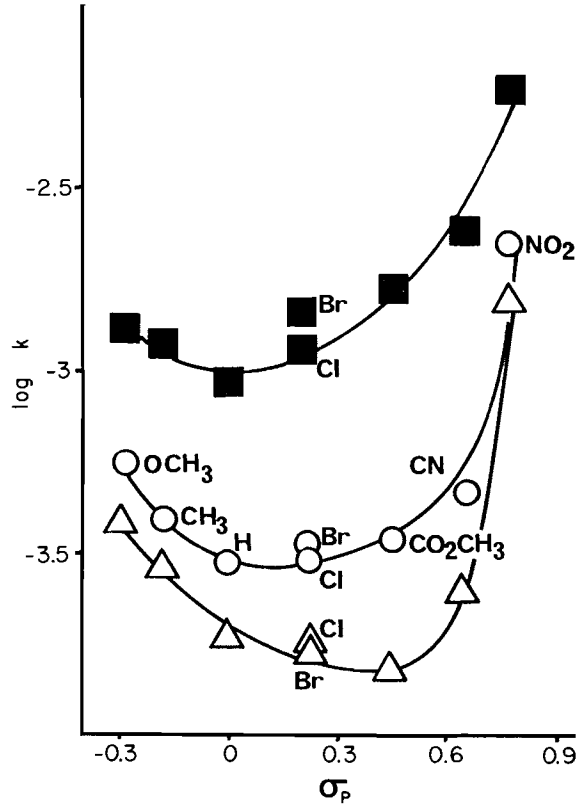


FIG. 8. Hammett plots for hydrolysis of aryloxymethyl triazenes (*2a–h*) at pH 3.3 with various buffer concentrations: -■-■- 0.33 M; -○-○- 0.066 M; -△-△- 0.0066 M.

TABLE 8. Kinetic parameters calculated for the hydrolysis of 1-aryl-3-aryloxymethyl-3-methyltriazenes in aqueous buffer according to

$$k_{obs} = k_{Sp} + k_{H^+}[H^+] + k_B[B]$$

where [B] is defined as [HA] + [NaA]

Substituent	σ	k_B[a] × 10^4 (s^{-1} mol^{-1})	k_{H^+}[b] (s^{-1} mol^{-1})	k_{Sp}[c] × 10^4 (s^{-1})
OCH_3	−0.27	18.9	0.58	0.778
CH_3	−0.17	19.4	0.60	−1.11
H	0.00	15.7	0.60	−2.05
Cl	0.23	19.4	0.50	−1.57
Br	0.23	26.2	0.44	−0.911
CO_2CH_3	0.32	32.1	0.31	−0.279
CN	0.66	46.3	0.26	1.64
NO_2	0.78	70.9	0.18	19.6
ρ-values		+0.55	−0.49	+8.98

[a]From plot of k_{obs} vs. [B] extrapolated to [B] = 0 pH 3.3.
[b]Slope of plots of $k_{obs} - k_B[B]$ vs. $[H^+]$.
[c]Intercept of $k_{obs} - k_B[B]$ vs. $[H^+]$.

the specific acid-catalyzed reaction has a negative value (see below), this observation is more consistent with a buffer anion catalyzed S_N2 displacement of the leaving group.

Using the k_B from the analysis above, the next step was to plot, for each substituent, the term $\{k_{obs} - k_B[B]\}$ versus $[H^+]$. The slope of this line gives the k_{H^+} for each substituent, and the intercept at $[H^+] = 0$ gives k_{sp}. The results are tabulated in Table 8, but it should be noted that these values represent an approximation due to the uncertainty in the pH value of the mixed solvent system and also because of the difficulty of relating one buffer system to another with respect to the k_B term in eq. [3].

The kinetic analysis in Table 8 is consistent with the three mechanisms shown in Fig. 9. The spontaneous rate constant turns out to be essentially zero for all but the nitro and cyano substituted compounds (**2***g* and **2***h*), which is consistent with a spontaneous dissociation mechanism, Fig. 9 (*i*), affording the iminium ion and the free phenolate ion. This pathway is evidently facilitated by the presence of a strongly electron-withdrawing substituent (X), which improves the leaving group character of the aryloxy group beyond the apparent threshold for reactivity.

The Hammett plot of k_{H^+} versus σ_p gives a negative value, consistent with the proton-catalyzed mechanism, Fig. 9 (*ii*). Protonation of the ether oxygen precedes the dissociation step so that this pathway is favoured by an increase in electron

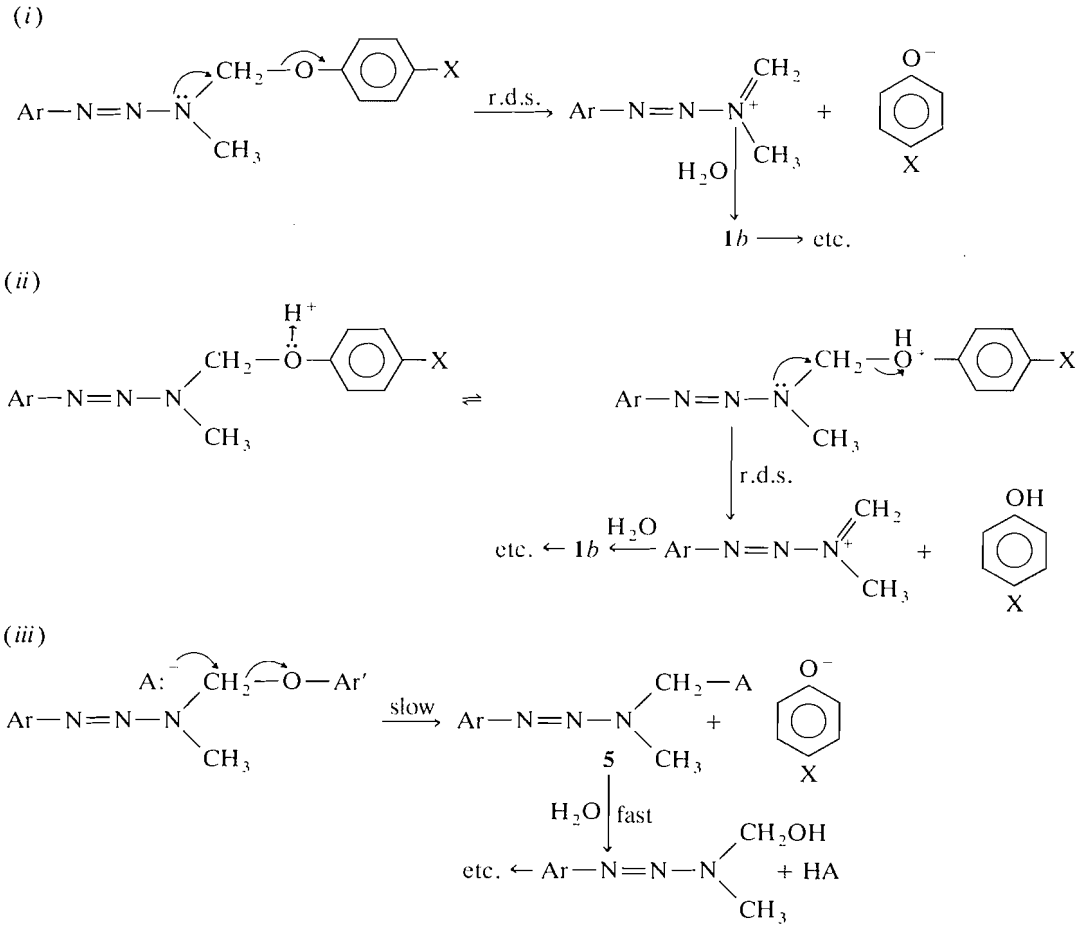


FIG. 9

density at the ether oxygen when an electron-releasing substituent is present at X.

The surprising result of this analysis is the positive ρ-value obtained from the Hammett plot for the buffer-catalyzed reaction. Significantly, the slope of this Hammett plot was still positive when we carried out the analysis using buffer acid concentration [HA] in place of [B] in eq. [4]. If the buffer catalysis was due to general acid catalysis by the buffer acid, HA, then the slope of the Hammett plot should be negative in the same manner as the specific acid catalysis. The positive ρ for the buffer-catalyzyed reaction is consistent with the nucleophilic catalysis mechanism, Fig. 9 (*iii*), in which the anion A^- causes a slow displacement of the aryloxy leaving group followed by fast displacement of A^- by water. When A^- is formate, the intermediate **5** is similar in structure to an acetoxymethyltriazene (**1***d*), which is known to hydyrolyse rapidly in buffer solution (6).

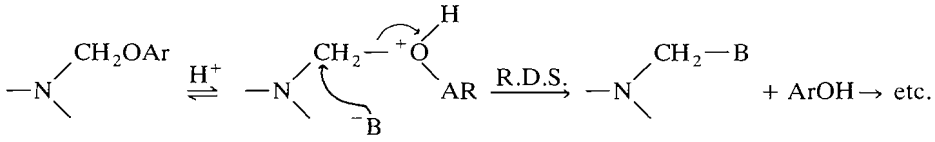
If the hypothesis of Fig. 9 (*iii*) is correct, then there should be a similar or possibly better fit of the data to eq. [3] using the buffer anion concentration $[A^-]$ in place of total buffer concentration. The results of such an analysis are shown in Table 9; k_A replaces k_B. The results are essentially the same as those in Table 8; the slope of the specific acid-catalysis Hammett plot turns out to be more negative, indicating a greater sensitivity to the electronic character of X. Thus the analysis presented in Table 9 provides added support to the mechanistic hypothesis in Fig. 9. However, an alternative mechanism involving the displacement of ArOH by S_N2 attack of the buffer anion on the protonated substrate, i.e.,

TABLE 9. Kinetic parameters calculated for the hydrolysis of 1-aryl-3-aryloxymethyl-3-methyltriazenes in aqueous buffer according to:

$$k_{obs} = k_{Sp} + k_{H^+}[H^+] + k_A[A^-]$$

Substituent	σ	k_A[a] × 10^4 (s^{-1} mol^{-1})	k_{H^+}[b] (s^{-1} mol^{-1})	k_{Sp}[c] × 10^4 (s^{-1})
OCH_3	−0.27	0.49	0.56	0.478
CH_3	−0.17	0.50	0.56	−0.429
H	0.00	0.41	0.56	−0.768
Cl	0.23	0.50	0.47	−0.221
Br	0.23	0.68	0.38	−0.209
CO_2CH_3	0.32	0.83	0.23	1.362
CN	0.66	1.20	0.17	0.246
NO_2	0.78	1.83	0.06	14.830
ρ-values		+0.55	−0.82	—

[a]From plot of k_{obs} vs. $[A^-]$ extrapolated to $[A^-] = 0$ at pH 3.3.
[b]Slope of plots of $k_{obs} - k_A[A^-]$ vs. $[H^+]$.
[c]Intercept of $k_{obs} - k_A[A^-]$ vs. $[H^+]$.



cannot entirely be ruled out.

To verify that there is specific acid catalysis in the hydrolysis of the aryloxymethyltriazenes, a solvent deuterium isotope experiment was performed with the *p*-bromophenyloxymethyltriazene (**2***e*). The rate of hydrolysis of **2***e* at pH 3.07 in buffer/D_2O (k_D) was compared with the rate of hydrolysis in buffer/H_2O (k_H) at 40°C, using nondeuterated isopropanol as the cosolvent. The observed ratio, k_H/k_D = 1.60, may only be an appparent value due to the possible exchange of deuterium with the solvent; the true value of k_H/k_D could be as high as 2.28. Nevertheless, it can be said categorically that there is a solvent deuterium isotope effect in the hydrolysis of **2***e* at pH 3.07, which supports the prior argument that the hydrolysis is subject to specific acid catalysis.

Conclusion

In conclusion, it is interesting to compare the results of this study with similar work of Iley *et al.* (10)[3] on the kinetics of hydrolysis of the 1-aryl-3-alkoxymethyl-3-methyl triazenes (**1***c*). These compounds hydrolyse in acidic solutions to give the corresponding anilines, and the kinetic study, over the pH range 1–5, showed that the reaction suffers specific acid catalysis. Apparently, the alkoxymethyltriazenes do not decompose spontaneously and do not react by an anion-catalyzed mechanism analogous to that shown in Fig. 9 (*iii*) for the aryloxymethyltriazenes. This behaviour is entirely consistent with the subtle, but significant, difference in structure between the alkoxy- and aryloxy-methyltriazenes, in particular with respect to the leaving group ability. Evidently, the methoxide ion, MeO^-, is not able to dissociate spontaneously, nor is it readily displaced by weak anions, whereas the aryloxide ion can suffer both types of reaction in addition to the specific acid-catalyzed reaction shared by both classes of compound.

Finally, is there any point in considering these aryloxy methyltriazenes as candidates for drug screening? The results reported in this paper clearly show that the stability of these compounds in aqueous media is exquisitely sensitive to the electronic character of the X substituent in the aryloxy moiety; the half-lives range from 1 min to several days! Thus, it is highly probably that a suitable aryloxymethyl triazene can be tailor-made with the perfect chemical stability and slow-release characteristics for use as a pro-drug. Preliminary investigations of the biological activity of these new compounds shows that some members of the series demonstrate stastically significant differential toxicity to several cell lines of the NCI tumour panel.

Acknowledgements

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