The Mechanism of Hydrolysis of Aryl Ether Derivatives of 3-Hydroxymethyltriazenes

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1-Aryl-3-aryloxymethyl-3-methyltriazenes hydrolyse to the corresponding anilines and phenols by specific-acid-catalysed, general-acid-catalysed and pH-independent mechanisms. All compounds studied exhibit specific- and general acid catalysis, though for **5a** general acid catalysis was not observed below a pH of approximately 4, while for compounds **5e,f**, such catalysis was absent above a pH of approximately 5. The pH-independent pathway is observed only for those compounds, **5d–f**, that contain good aryloxy nucleofugic groups. The specific-acid-catalysed pathway is supported by a solvent deuterium isotope effect (SDIE) of 0.64, consistent with a mechanism involving protonation of the substrate followed by rate-determining unimolecular decomposition of the protonated species. The k_{H+} values gave rise to a Hammett ρ value of –0.93, reflecting the competing effect of the substituents on the protonation of the substrate and the cleavage of the aryl ether. Correlation of k_{H}^{+} with the pK_a of the phenol leaving group affords a β_{lg} of 0.3. Decomposition of the protonated intermediate proceeds via a triazenyliminium ion that can be trapped by methanol. The general-acid-catalysed process exhibits an SDIE of 1.43 and Hammett ρ values of 0.49, 0.84 and 1.0 for reactions catalysed by chloroacetic, formic and acetic acids, respectively. Correlation of k_A with the pK_a of the acid gave Brønsted α values that diminish from 0.6 for *O*-aryl systems that are poor nucleofuges (**5a,b**) to 0.2 for the best nucleofuge (**5f**), reflecting the different extents of proton transfer required to expel each phenol. Compounds containing powerful nucleofuges exhibit a pH-independent reaction that has an SDIE of 1.1, a Hammett ρ value of 3.4 and a Brønsted β_{lg} value of 1.4. These imply a mechanism involving displacement of the aryloxide leaving group to form a triazenyliminium ion intermediate that again was trapped as a methyl ether.

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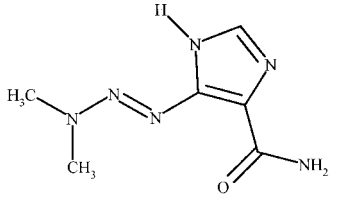
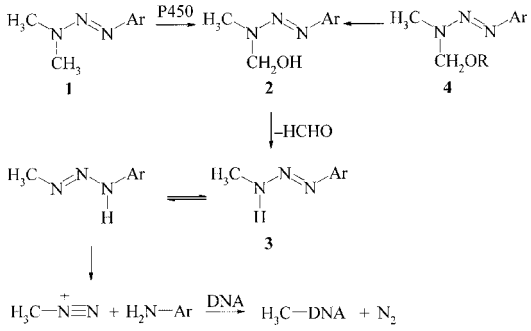
Introduction

The synthetic and biological utility of the triazene group is well-documented.[1] In particular, the biological action of the anticancer 1-aryl-3,3-dimethyltriazenes **1**, including a clinically used drug for malignant melanoma, dacarbazine (**1a**), is a consequence of their capacity to alkylate DNA.[2] These compounds suffer metabolic oxidation by cytochrome P450 enzymes to give hydroxymethyltriazenes **2**, which, by loss of formaldehyde, generate the cytotoxic monomethyltriazenes **3** (Scheme 1).[3] These are known alkylating agents, capable of methylating DNA and RNA.[4,5]

Prodrugs of the 3-hydroxymethyl-3-methyltriazene **2** are especially attractive as they bypass the requirement for metabolic activation, an inefficient process in humans.[6] Among the derivatives of **2** studied are esters,[7,8] particularly more recently the "cascade release molecules" that concomitantly target DNA and epidermal growth factor receptor tyrosine kinase,[9] and alkyl ethers.[10,11] Our investigations of the hydrolysis of 3-alkyloxymethyl-3-alkyl-1-aryltriazenes **4** showed these compounds to behave like acetals of formaldehyde, hydrolysing in aqueous buffers by forma-



1a



Scheme 1. Metabolic pathway for dimethyltriazenes.

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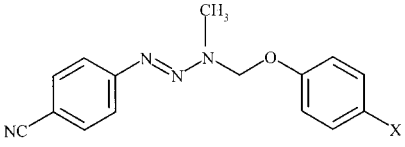
tion of a triazenyliminium ion intermediate by a specific-acid-catalysed pathway.[11] However, in general, the alkoxymethyltriazene derivatives **4** are too stable toward hydrolysis and metabolism[12] to be considered satisfactory prodrugs.

Acetals that contain a phenolic or other weakly basic leaving group undergo hydrolysis more readily; for such compounds, as well as the specific-acid-catalysed reaction observed for the alkyl analogues, general acid catalysis and pH-independent processes have been observed.[13] Thus, aryl, rather than alkyl, ether derivatives **5** of the hydroxymethyltriazene metabolite would appear more attractive candidates as prodrugs. Indeed, given the known cytotoxic activity of 4-methoxyphenol towards melanogenic cells,[14] compounds **5** offer the potential for developing mutual prodrug systems as antimelanoma agents.

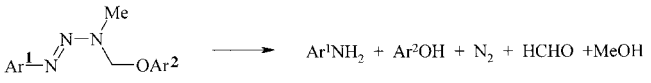
The aryloxymethyltriazenes **5** are known compounds.[15] Compounds **5** are specific examples of 1-aryl-3,3-dialkyltriazenes; as a class, these compounds are generally stable except when they are subjected to thermal or photochemical degradation, or to the action of acid.[16] Under the latter conditions, these compounds undergo protonation at the N^3 atom followed by scission of the N^2–N^3 bond to form a secondary amine and a diazonium ion.[17] Indeed, this is a reaction that has been used to release dialkylamines protected as a triazene functionality.[1] However, an investigation by Vaughan and coworkers[18] showed that aryloxymethyltriazenes behaved more like acetals than 3,3-dialkyltriazenes. Hydrolysis of these compounds was found to be specific acid and buffer catalysed and that there is also a spontaneous reaction for some compounds. Unfortunately, these investigators did not study the reaction over a sufficiently wide pH range, nor did they examine the nature of the buffer-catalysed process. Consequently, the reported structure–activity relationships and solvent isotope effect are composite values for all the reaction pathways involved. Therefore, we have synthesised the aryloxymethyltriazenes **5a**–**f** and have carried out an investigation of their hydrolysis in aqueous buffers to better understand the hydrolytic mechanisms that operate for these compounds.



5a X = OMe **5d** X = COMe
5b X = H **5e** X = CN
5c X = Cl **5f** X = NO_2

Results and Discussion

The aryloxymethyltriazenes **5** hydrolyze in aqueous solutions to the corresponding anilines and phenols [Equation (1)].



$$Ar^1NH_2 + Ar^2OH + N_2 + HCHO + MeOH \quad (1)$$

The reactions are easily monitored using changes in the UV spectra by following the loss of the starting material or formation of the product. However, in solutions of pH > 7 the intermediate formation of the monomethyltriazene **3** is observed; in these solutions the reactions were monitored using the wavelength at which there is an isosbestic point for the subsequent decomposition of **3** to the corresponding aniline. Pseudo-first-order rate constants, k_{obs}, for the hydrolysis of **5a**–**f** were determined in HCl and NaOH solutions and also in aqueous buffers at various pH values (using several buffer concentrations for each pH). Some examples of these results for compounds **5a** and **5f** are shown in Table 1, and these demonstrate that the dependence of k_{obs} upon proton and buffer concentration varies with the pH, the nature of the buffer material and the structure of the substrate. We shall discuss each in turn.

pH-Rate Profiles

Using the values of k_{obs} in HCl and NaOH solutions, together with the intercepts of plots of k_{obs} vs. [buffer], the pH-rate profiles shown in Figure 1 can be constructed.

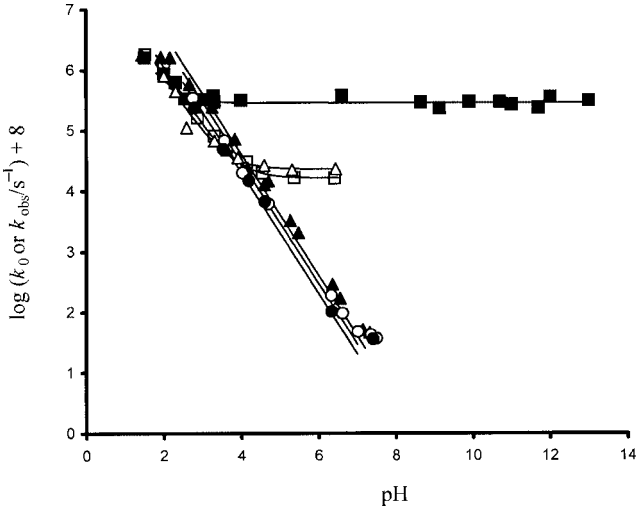


Figure 1. pH-Rate profiles for the hydrolysis of compounds **5a**–**f**: filled triangle, **5a**; circle, **5b**; filled circle, **5c**; square, **5d**; triangle, **5e**; filled square, **5f**.

Two different behaviours are observed. Compounds containing electron-donating substituents on the aryl ether ring, **5a,b**, or with no substituent, **5c**, display a specific-acid-catalysed reaction below pH of about 7.5; above this pH these compounds are stable, no decomposition being measurable after 60 days. In contrast, compounds with electron-attracting groups, **5d**–**f**, exhibit the specific-acid-catalysed reaction together with a pH-independent process; the pH

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Table 1. Pseudo-first-order rate constants, k_{obs}, for the hydrolysis of **5a** and **5f** in aqueous buffers at 25 °C.

5a				**5f**			
Buffer	[Buffer]/mol dm^{-3}	pH (pD)	$k_{obs}/10^{-5}$ s^{-1}	Buffer	[Buffer]/mol dm^{-3}	pH (pD)	$k_{obs}/10^{-5}$ s^{-1}
HCl	5×10^{-7}	6.30	0.3	HCl	0.0005	3.30[a]	294
	3.4×10^{-6}	5.47	2.03		0.001	3.00[a]	324
	5.6×10^{-6}	5.25	3.25		0.005	2.30[a]	616
	2.5×10^{-5}	4.60	12.6		0.01	2.00[a]	873
	6.92×10^{-5}	4.16	24.1		0.03	1.51[a]	1620
	0.000145	3.84	79.9		0.1	0.90[a]	1880
	0.000575	3.24	244		0.5	0.30[a]	1750
	0.001	3.00	587				
DCl	1.17×10^{-5}	5.33	14.4				
	7.14×10^{-5}	4.55	52.8				
	0.00035	3.86	311				
	0.000474	3.72	381				
	0.0008	3.50	700				
$ClCH_2CO_2H$	0.01	2.69	589	$ClCH_2CO_2H$	0.01	2.99	341
	0.05	2.78	448		0.05	2.87	404
	0.1	2.54	701		0.1	2.83	507
	0.2	2.58	671		0.2	2.84	618
	0.3	2.72	511		0.3	2.88	681
HCO_2H	0.075	3.26	251	HCO_2H	0.0375	3.29	402
	0.15	3.25	255		0.075	3.30	433
	0.225	3.23	241		0.15	3.29	552
	0.30	3.22	230		0.3	3.27	649
					0.4	3.25	804
CH_3COOH	0.0375	4.18	24.0				
	0.15	4.14	26.4				
	0.225	4.17	26.3				
	0.30	4.16	26.8				
	0.01	4.56	12.5				
	0.05	4.56	13.7				
	0.10	4.58	13.9				
	0.20	4.61	15.5				
	0.30	4.62	16.6				
	0.0875	5.43	2.58				
	0.175	5.45	3.31				
	0.28	5.51	4.05				
$H_2PO_4^-$	0.002	6.63	0.183		0.0125	6.53	377
	0.005	6.68	0.182		0.025	6.54	346
	0.01	6.69	0.203		0.05	6.58	422
	0.0025	7.25	0.051		0.1	6.63	325
	0.01	7.23	0.057		0.2	6.71	400
	0.0125	7.32	0.062				
	0.025	7.33	0.073				
imidazole	0.01	6.37	0.285	morpholine	0.05	8.63	265
	0.05	6.34	0.315		0.1	8.59	294
	0.1	6.38	0.338		0.2	8.66	314
	0.2	6.35	0.382	piperazine	0.025	9.85	297
	0.3	6.30	0.415		0.1	9.84	288
	0.01	7.11	0.049		0.15	9.88	285
	0.05	7.13	0.063	NaOH	0.0005	10.5[a]	293
	0.1	7.22	0.078		0.001	11.5[a]	264
	0.2	7.10	0.102		0.1	13[a]	311
	0.3	7.12	0.122				

[a] Calculated.

of incursion of the latter depends upon the substituent in the aryl ether, but occurs around pH 3–4.

The Specific-Acid-Catalysed Reaction

Observation of Figure 1 shows that all of the compounds suffer a proton-catalysed hydrolysis. Values of the second-order rate constants, k_H^+, for the proton-catalysed hydrolysis (Table 2) were obtained from plots of k_{obs} vs. [H$^+$]. For compound **5a**, the reaction was also studied in DCl and several buffers in D_2O, allowing determination of the corresponding k_D^+ value; the solvent deuterium isotope effect, k_H^+/k_D^+, is 0.64. A similar value, 0.62, has been reported for the 4-methoxyphenyl methyl acetal of benzaldehyde, a functionality similar to the aryloxymethyltriazenes.[19] For compound **5a**, the temperature effect enabled a determi-

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nation of $70 \pm 5\ kJ\,mol^{-1}$ for $\Delta H^{\ddagger}$ and $+4 \pm 10\ J\,K^{-1}\,mol^{-1}$ for $\Delta S^{\ddagger}$. These values are consistent with an A1 pathway.[20]

Table 2. Rate constants for the specific-acid-catalysed, k_H^+, and spontaneous, k_0, hydrolysis of **5a**–**f** at 25 °C.

Compound	$k_H^+/dm^3mol^{-1}s^{-1}$	$k_0/10^{-5}\ s^{-1}$
5a	5.54, 3.8[a], 7.53[b], 10.8[c], 17.6[d] 8.68[e]	
5b	1.93	
5c	1.44	
5d	1.12	16.4
5e	0.787	23
5f	0.397	285, 181[a], 349[b], 460[f], 763[g] 266[e]

[a] 293 K. [b] 300 K. [c] 304 K. [d] 309 K. [e] in D_2O. [f] 303 K. [g] 308 K.

Correlation of k_H^+ with σ_p (Figure 2) affords a ρ value of –0.93 ($r^2 = 0.93$) [correlation with σ^- gives a ρ of –0.61 ($r^2 = 0.89$)]. The sign of ρ reflects an increase in positive charge in the phenol moiety as the transition state is formed. However, the relatively low magnitude of ρ almost certainly reflects the fact that it is a composite of the ρ for substrate protonation and the ρ for subsequent decomposition. The solvent deuterium isotope effect is that expected for a pre-equilibrium protonation of the substrate, consistent with this interpretation. Thus, electron-donating substituents in the aryloxy ring will enhance protonation (negative ρ), but will retard the decomposition (positive ρ). The observed value implies that the former effect predominates, but the second effect lowers the magnitude of ρ.

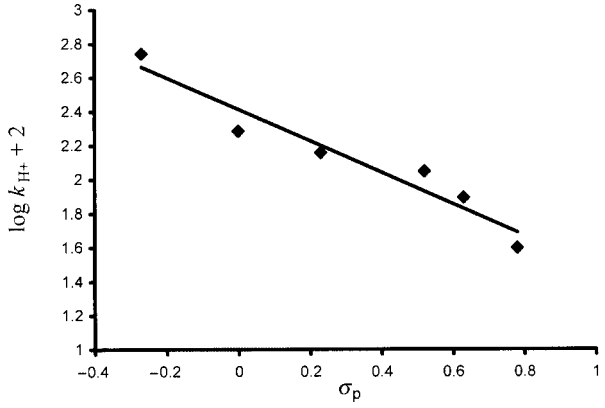
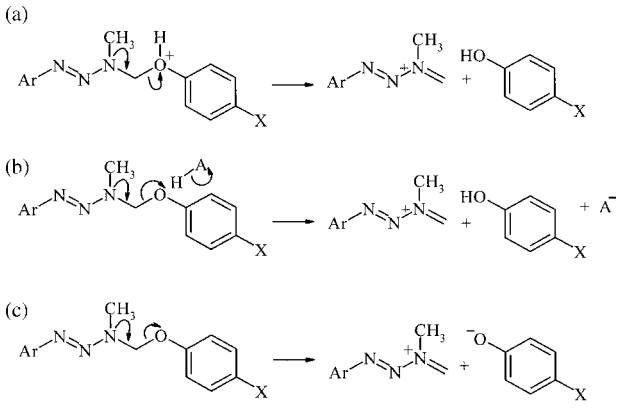
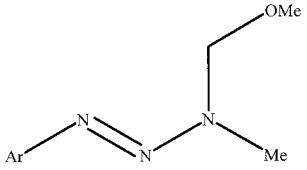


Figure 2. Hammett plot for the specific-acid-catalysed hydrolysis of compounds **5a**–**f**.

Taken together, these data are in agreement with a two-step, specific-acid-catalysed mechanism involving protonation of the substrate followed by rate-determining unimolecular decomposition of the protonated species (Scheme 2, process a). Of course, another mechanistic possibility is the protonation of the triazene, followed by rate-determining formation of an *O*-aryl formaldehyde cation with concomitant expulsion of a monomethyltriazene. However, we can rule this out, and confirm the proposed mechanism, by the following experiment. Compound **5a** was studied in 0.1 M formate buffer, for which there is no buffer catalysis for this compound, containing 50% methanol. The reaction was monitored over time by HPLC and, as well as the usual products of reaction, viz. the aniline and phenol, the methoxymethyltriazene **4** (Ar = 4-CNC_6H_4, R = Me) was also detected. This compound arises from the trapping of the triazenyliminium ion shown in Scheme 2, process a, and accounts for about 50% of the product yield before itself decomposing (Figure 3).



Scheme 2. (a) Specific-acid, (b) general-acid and (c) spontaneous pathways for the hydrolytic cleavage of aryloxymethyltriazenes.



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The General-Acid-Catalysed Reaction

The data in Table 1 demonstrate that the hydrolyses of compounds **5** are subject to buffer catalysis. For compound **5a**, buffer catalysis was observed only above pH of about 4; for compounds **5b,c**, buffer catalysis was observed across the pH range; for **5d**–**f**, buffer catalysis was observed only for chloroacetic, formic and acetic acid buffers.

Figure 4a shows the plots of k_{obs} vs. [total buffer] for the catalysis of **5a** by acetic acid buffers, and Figure 4b shows the corresponding plot of the slopes, k_{cat}, from Figure 4a against the mol fraction of the acid form of the buffer. The latter clearly demonstrates that the buffer-catalysed reaction involves only the general acid form of the buffer, and the bimolecular rate constant for general acid catalysis, k_A, is obtained from the intercept at a mol fraction of 1. For experimental expediency, more generally values of k_A were determined from the slopes of plots of k_{obs} vs. [general acid form of the buffer]; when determinations of k_A were made at two different pH values using the same buffer material such plots were parallel confirming the general-acid nature of the buffer catalysis. The values of k_A so obtained are listed in Table 3.

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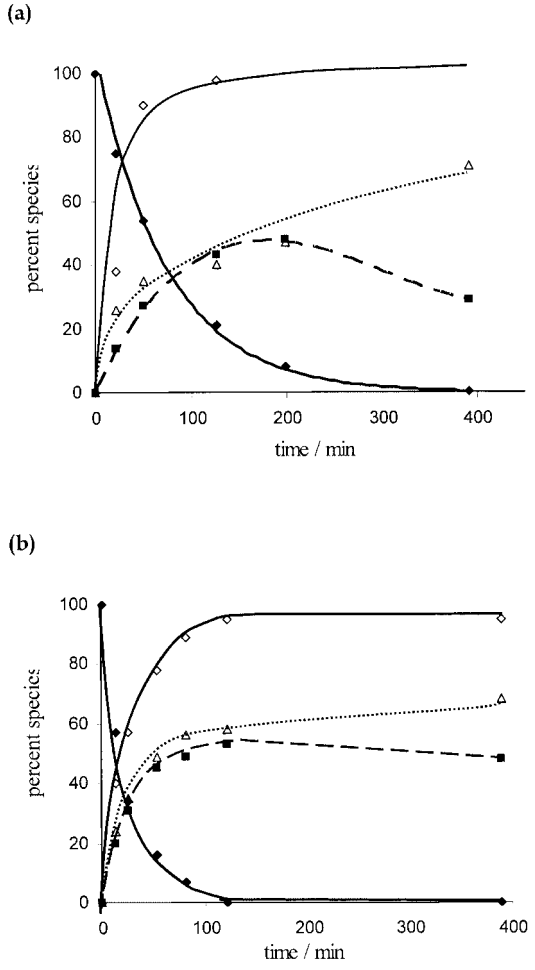

Figure 3. Time courses for the reactions of (a) **5a** and (b) **5f** in 0.1 M pH 4.1 formic acid buffer containing 50% aqueous methanol: filled diamond, **5**; filled square, **4**; diamond, corresponding phenol; triangle, corresponding aniline.

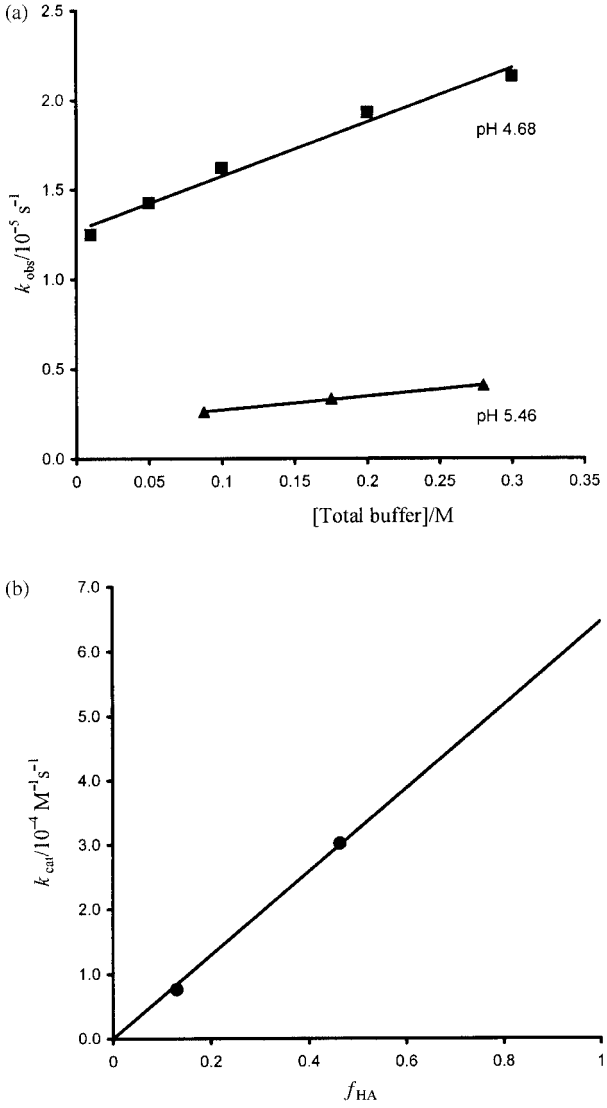

Figure 4. Plots of (a) k_{obs} vs. [total buffer] for the acetic acid catalysed hydrolysis of **5a** at 25 °C, and (b) the slopes of the lines in (a) vs. mol fraction of the acid form of the buffer.

Substituent effects for the general-acid-catalysed reaction were studied in chloroacetic, formic and acetic acid buffers. Values of $\log k_A$ were found to correlate better with σ^- than with σ_p, and the ρ values are 0.49, 0.86 and 1.0, respectively, for the three acids (Table 3). The corresponding correlations between $\log k_A$ and the pK_a of the phenol leaving group afford β_{lg} values of –0.23, –0.4 and –0.42 in the three acids, respectively. These positive ρ values contrast with the negative value obtained for specific acid catalysis and imply that, as might be expected, general acid catalysis is associated with an increase of negative charge in the phenol moiety as the transition state is formed. Moreover, the magnitude of ρ diminishes as the strength of the general acid increases. Again, such an effect was also observed with the benzaldehyde *O*-aryl acetals.[19] We interpret this as implying there is more extensive O–H bond making and less C–O bond breaking for a stronger acid than for a weaker acid. As described below, the uncatalysed reaction that involves C–O bond cleavage is highly sensitive to substituents and has a high positive value of ρ. Thus, the general-acid-catalysed reaction involving more extensive C–O bond cleavage will have the more positive value of ρ. Interestingly, the Hammett value for acetic acid catalysis quoted in Table 3 is obtained by omitting the rate constant for compound **5a**, which appears more reactive than such a plot would imply. A similar "up-turn" in reactivity was observed for electron-donating substituents in the *O*-aryl acetals of benzaldehyde.[19] This effect has been ascribed to varying amounts of proton transfer and C–O bond breaking in the transition states; C–O bond breaking runs ahead of O–H bond formation for electron-withdrawing substituents, the reverse being true for electron-donating substituents.[19]

The k_A values for compounds **5a–f** also correlate with the pK_a' of the acid catalysts (i.e. the pK_a values corrected for ionic strength[21]) and the derived Brønsted α values are listed in Table 3. These values are typical of general-acid-catalysed pathways, and it is clear that the Brønsted α value diminishes as the nucleofugacity of the phenol leaving group increases. Again, this behaviour parallels that of the

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Table 3. Second-order rate constants, k_A, for the general-acid-catalysed hydrolysis of **5a**–**f** at 25 °C.

Buffer	pK_a'[a]	$k_A/10^{-3}$ M^{-1} s^{-1}						Hammett ρ
		5a	**5b**	**5c**	**5d**	**5e**	**5f**	
$ClCH_2CO_2H$	2.72	[b]	7.97	12.2	27.5	20.5	33.0	0.49
	3.204[c]	22.8[c]						
HCO_2H	3.59	[b]	1.24	2.48	10.9	7.44	13.8	0.86
	4.074[c]	3.25[c]				5.20[c]		
$MeCO_2H$	4.60	0.65	0.490	0.865	3.73	3.85	9.22	1.0
	5.084[c]	0.23[c]	0.233[d]		2.36[d]			
			0.753[e]		4.85[g]			
			1.13[f]		6.72[e]			
					10.5[f]			
pyridine	5.07	0.032			[b]	[b]		
imidazole	6.72	0.0046	0.0078	0.0063	[b]	[b]	[b]	
phosphate	6.79	0.049	0.053				[b]	
Brønsted α		0.71	0.58	0.74	0.46	0.38	0.21	
(r^2)		(0.8)	(0.95)	(0.99)	(1)	(0.97)	(0.8)	

[a] The pK_a at 25 °C corrected for ionic strength effects; for work at other temperatures the pK_a was further corrected accordingly. [b] Buffer catalysis not observed. [c] In D_2O. [d] 293.5 K. [e] 303.3 K. [f] 308.5 K. [g] 300.1 K.

O-aryl acetals.[19] The data imply that proton transfer from the acid catalyst to the aryl ether oxygen is 60–70% complete in the transition state for those compounds containing relatively poor leaving groups, **5a-c**, diminishing to 20–40% complete for the compounds containing the better leaving groups. This is to be expected, as the better the leaving group the smaller the extent of proton transfer required for its expulsion. Consistent with this are the solvent kinetic deuterium isotope effects for compounds **5a**,**e**. For compound **5a** the reaction was studied using acetic acid buffers and a value for k_A^H/k_A^D of 2.8 was determined; a value of 2.14 has been reported for the acetic acid catalysed hydrolysis of benzaldehyde *O*-phenyl *O*-methyl acetal,[13] and a value between 2.65 and 3.4, depending on the composition of the solvent, for the formic acid catalysed hydrolysis of 2-(4-nitrophenoxy)tetrahydropyran.[22] For **5e** the reaction was studied by using formic acid buffers, and the corresponding isotope effect was calculated to be 1.4. Vaughan and coworkers reported[18] a value of $1.6 < k_H/k_D < 2.28$, but, as mentioned earlier, this includes components from both the proton and general acid pathways.

The effect of temperature on the buffer-catalysed pathway was examined for compounds **5b** and **5d** in acetic acid buffers (Table 3); for **5b** the values of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ are 70 ± 5 kJ mol^{-1} and -55 ± 10 J K^{-1} mol^{-1}, respectively, and for **5d** the corresponding values are 80 ± 5 kJ mol^{-1} and -43 ± 10 J K^{-1} mol^{-1}. These values, together with the Hammett ρ values, Brønsted α values and solvent isotope effects, are consistent[23] with the A-S_E2 mechanism shown in Scheme 2b, in which the expulsion of the phenol leaving group, to form a triazenyliminium ion, is aided by the general acid. The nature of the buffer catalysis described here contrasts with the role proposed by Vaughan,[18] who assumed the buffer to be acting as a nucleophile.

The pH-Independent Reaction

Above a pH of approximately 4, compounds **5d**–**f** exhibit a pH-independent reaction. For **5f**, the pH-rate profile (Figure 1) demonstrates that this reaction extends across the pH range up to a pH of about 13, with no incursion of a reaction involving HO^-. For these three compounds, a Hammett ρ value of 3.1 is obtained (Figure 5) when $\log k_{obs}$ is plotted against σ^-; the equivalent Brønsted β_{lg} value is 1.4. A Hammett ρ of 2.7 has been reported for 2-aryloxytetrahydropyrans.[24] The magnitude and sign of ρ, and the fact that the correlation is with σ^-, support a mechanism involving the unimolecular decomposition of the substrate, as depicted in Scheme 2c, involving expulsion of a phenoxide ion and the formation of the corresponding triazenyliminium ion. The high magnitudes of both ρ and β_{lg} are the result of the direct conjugation between the electron-attracting substituents on the phenol ring and the negative charge in the transition state.

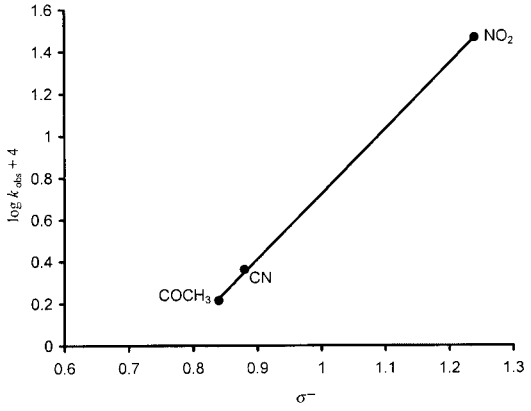

Figure 5. Hammett plot for the spontaneous hydrolysis of **5d**–**f** at 25 °C.

The spontaneous hydrolysis reaction was also studied for **5f** in D_2O solutions at pH 10–12 (Table 2), allowing the calculation of a solvent isotope effect of 1.1. Values of 1.1 and 1.13 have been reported for the analogous spontaneous hydrolysis of 2-(4-nitrophenoxy)tetrahydropyran[22] and *O*-ethyl *S*-phenyl benzaldehyde acetal,[25] respectively; again this is consistent with a process that involves unimolecular

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ionisation and does not involve proton transfer,[26] such as that proposed in Scheme 2c. To trap the triazenyliminium ion formed by this spontaneous process, the reaction of **5f** was carried out in a mixed solvent system containing 50% methanol at a pH at which the spontaneous reaction accounted for 90% of the reaction. Loss of the starting material, formation of the phenol and 4-cyanoaniline coproducts, and formation of the methoxymethyltriazene trapped product, were monitored by HPLC. The data in Figure 3 show that the starting aryloxymethyltriazene can be entirely accounted for by the aniline, phenol and trapped triazene products. Under the conditions of the experiments, approximately 50% of the iminium ion is trapped by the methanol cosolvent. Significantly, no methoxymethyl aryl ether was observed. Thus, compounds **5** undergo spontaneous hydrolysis to triazenyliminium and aryloxide ions rather than take the alternative pathway of forming a triazenyl anion and an *O*-aryl formaldehyde cation. The variation of rate constant with temperature affords $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values of 69 ± 5 kJ mol^{-1} and -60 ± 10 J K^{-1} mol^{-1}, respectively. The latter value is rather more negative than might be expected for a spontaneous ionisation process, even accounting for the presence of an organic cosolvent. However, a similar value (–41 J K^{-1} mol^{-1}) has been reported for the spontaneous hydrolysis of *O*-ethyl *S*-phenyl benzaldehyde acetal.[23] It has also been reported that, for formaldehyde derivatives, as we have here, the measured entropies of activation for spontaneous reactions are some 60–80 J K^{-1} mol^{-1} more negative than for the corresponding acid-catalysed process;[24] the corresponding difference here is 64 J K^{-1} mol^{-1}. This is probably due to the differing involvement of the solvent in the transition state; in the spontaneous process the reactant is a neutral species that ionises to form a cation and an anion, which will involve significant solvent organisation in the transition state, whereas the acid-catalysed process involves positively charged species throughout such that solvent reorganisation in the transition state is likely to be much less.

Conclusion

Like alkoxymethyltriazenes, aryloxymethyltriazenes decompose by cleavage of the *C*–*O* aminal bond to form a triazenyl iminium ion and a phenol. This distinguishes them from other 1-aryl-3,3-dialkyltriazenes which undergo cleavage of the N^2–N^3 triazene bond.[16] The aryloxymethyltriazenes are more reactive than their alkoxymethyl counterparts and undergo general-acid-catalysed and spontaneous hydrolysis pathways alongside the reaction catalysed by H^+. In this regard, they parallel the chemistry of *O*-aryl acetals, compounds to which they are structurally related. Unfortunately, the compound that contains both triazene and 4-methoxyphenol anticancer agents, **5a**, has a half-life of 270 h at physiological pH, making it far too stable to be considered a suitable mutual prodrug when considered alongside the clinically-used triazene prodrug temozolomide, a compound that has a half-life of 1.83 h in pH 7.4 phosphate buffer and a mean plasma half-life of 1.81 h.[27] Indeed, a recently reported carbamate derivative of a monomethyltriazene based on the O^6-benzylguanine scaffold, a compound that has in vitro antitumour activity comparable to temozolomide, has a hydrolysis half-life of 23 min.[28] However, the most reactive of the current compounds, **5f**, has a half-life of 1.5 min that is too short (leaving aside any toxicological issues related to 4-nitrophenol) and compounds **5d,e** have half-lives of around 25 min. The data we present in Figure 5 lead us to suggest that an aryloxymethyltriazene containing a biocompatible phenolic moiety that has a Hammett σ^- value of between 0.75 to 0.80 (or, what is equivalent, a pK_a of approximately 8.2) should have a half-life in the region of 2 h making it a potential candidate for a triazene prodrug.

Experimental Section

WARNING: All triazenes used in this study should be considered as mutagenic and carcinogenic and appropriate care should be taken to handle them safely.

Chemicals were reagent grade for the syntheses, LichroSolv (Merck) grade for HPLC analyses, and AnalaR grade for the kinetic investigations. ^{1}H NMR spectra were obtained as $CDCl_3$ solutions at 25 °C using a JEOL LA300 (300 MHz) spectrometer. Chemical shifts, δ, are given in ppm, and proton–proton coupling constants, *J*, are quoted in Hertz (Hz). Infrared spectra were recorded with a Perkin–Elmer 1310 spectrophotometer. Low resolution electrospray ionisation mass spectra were recorded with a VG Quattro mass spectrometer. Elemental analyses were performed by MEDAC Ltd., Brunel Science Centre, Surrey, TW20 0JZ, UK.

Substrates and Reagents: The 3-aryloxymethyl-3-methyl-1-(4-cyanophenyl)triazenes **5** were prepared as reported previously.[15,29] All are new compounds:

5a: 83 mg, 33%; m.p. 84–85 °C; IR: $\tilde{\nu}$ = 3054, 3007, 2954, 2221, 1600, 1504, 1443, 1034, 1013, 829 cm^{-1}. ^{1}H NMR: δ = 3.2 (s, 3 H, NMe), 3.76 (s, 3 H, OMe), 5.65 (s, 2 H, NCH_2O), 6.81–6.83 (d, ^3J = 9.03, 2 H, OAr), 6.92–6.94 (d, ^3J = 9.03, 2 H, OAr), 7.44–7.46 (d, ^3J = 8.55, 2 H, NAr), 7.61–7.63 (d, ^3J = 8.55, 2 H, NAr) ppm; *m*/*z* (%) = 297 [MH$^+$]. $C_{16}H_{16}N_4O_2$ (296): calcd. C 64.87, H 5.41, N 18.92; found C 65.0, H 5.50, N18.9.

5b: 145 mg, 64%; m.p. 76–78 °C; IR: $\tilde{\nu}$ = 3055, 2969, 2220, 1597, 1490, 1436, 1351, 1190, 1004, 987, 833 cm^{-1}. ^{1}H NMR: δ = 3.31 (s, 3 H, NMe), 5.73 (s, 2 H, NCH_2O), 6.98–7.00 (m, 3 H, OPh), 7.27–7.33 (m, 2 H, OPh), 7.47–7.50 (d, ^3J = 8.80, 2 H, NAr), 7.62–7.65 (d, ^3J = 8.80, 2 H, NAr) ppm; *m*/*z* (%) = 267 [MH$^+$]. $C_{15}H_{14}N_4O$ (266): calcd. C 67.65, H 5.30, N 21.03; found C 67.6, H 5.3, N 20.8.

5c: 192 mg, 75%; m.p. 89–91 °C; IR: $\tilde{\nu}$ = 3092, 3056, 2218, 1597, 1493, 1468, 1355, 1278, 978, 860, 820 cm^{-1}. ^{1}H NMR: δ = 3.30 (s, 3 H, NMe), 5.70 (s, 2 H, NCH_2O), 6.92–6.95 (2 H, ^3J = 8.98, OAr), 7.23–7.26 (2 H, ^3J = 8.26 Hz, OAr), 7.47–7.48 (2 H, ^3J = 8.62, NAr), 7.63–7.66 (2 H, ^3J = 8.62, NAr) ppm; *m*/*z* (%) = 301/303 [MH$^+$]. $C_{15}H_{13}N_4OCl$ (300.5): calcd. C 59.91, H 4.36, N 18.63; found C 59.9, H 4.30, N 18.45.

5d: 37 mg; 13%; m.p. 120–122 °C; IR: $\tilde{\nu}$ = 3064, 2223, 1664, 1600, 1576, 1474, 1360, 1236, 1206, 993 cm^{-1}. ^{1}H NMR: δ = 2.55 (s, 3 H, Ac), 3.33 (s, 3 H, NMe), 5.80 (s, 2 H, NCH_2O), 7.03–7.06 (2 H, ^3J = 8.98, OAr), 7.52–7.55 (2 H, ^3J = 8.80, NAr), 7.64–7.67 (2 H,

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^3J = 8.80, NAr), 7.92–7.95 (2 H, ^3J = 8.98, OAr) ppm; *m/z* (%) = 309 [MH^+]. $C_{17}H_{16}N_4O_2$ (308): calcd. C 66.22, H 5.23, N 18.17; found C 66.25, H 4.71, N 18.0.

5e: 40 mg; 15%; m.p. 154–6 °C; IR: $\tilde{\nu}$ = 3099, 3062, 2225, 1602, 1501, 1454, 1387, 1360, 1251, 994, 843 cm^{-1}. ^1H NMR: δ = 3.32 (s, 3 H, NMe), 5.79 (s, 2 H, NCH_2O), 7.05–7.08 (2 H, ^3J = 8.98, OAr), 7.52–7.55 (2 H, ^3J = 8.77, NAr), 7.59–7.62 (2 H, ^3J = 8.98, OAr), 7.65–7.68 (2 H, ^3J = 8.80, NAr) ppm; *m/z* (%) = 292 [MH^+]. $C_{16}H_{13}N_5O$ (291): calcd. C 65.97, H 4.50, N 24.03; found C 65.7, H 4.2, N 23.6.

5f: m.p. 184–186 °C; IR: $\tilde{\nu}$ = 3092, 3056, 2218, 1597, 1493, 1468, 1355, 1278, 978, 860, 820 cm^{-1}. ^1H NMR: δ = 3.33 (s, 3 H, NMe), 5.83 (s, 2 H, NCH_2O), 7.07–7.10 (2 H, ^3J = 9.16, OAr), 7.53–7.56 (2 H, ^3J = 8.80, NAr), 7.66–7.69 (2 H, ^3J = 8.80, NAr), 8.20–8.23 (2 H, ^3J = 9.37, OAr) ppm; *m/z* (%) = 312 [MH^+]. $C_{15}H_{13}N_5O_3$ (311): calcd. C 57.87, H 4.21, N 22.5; found C 57.7, H 4.0, N 22.1.

Kinetics: The decomposition of the triazenes **5** was followed by monitoring the decrease in the UV absorbance of the substrate at an appropriate wavelength with a Perkin–Elmer Lambda 2 spectrophotometer. For solubility reasons, acetonitrile (10%) was used as a cosolvent. The ionic strength of the reactions was maintained at 0.5 mol dm^{-3} by the addition of $NaClO_4$. In general, reaction solutions were monitored continuously in cells thermostatted to ±0.1 °C. Pseudo-first-order rate constants were obtained from slopes of plots of $\ln(A_t - A_\infty)$ vs. time, where A_t and A_∞ are the absorbances at time *t* and infinity, respectively. Rate constants determined by this method were reproducible to ±3%. For very slow reactions, a noncontinuous method was used, in which the reaction solution was kept in a thermostatted water bath and aliquots were taken at timed intervals and their absorbances measured in the spectrophotometer. For these reactions, only about 3 half-lives were followed and an initial rate method was used to calculate the rate constants. Rate constants determined by this method were reproducible to ±5%. The pH of the reaction solution was measured at the commencement and conclusion of each experiment. In deuterated solvents pD values were calculated from the expression pD = pH + 0.4.[30]

Product Analysis: The UV spectra of the reaction solutions at the conclusion of each experiment were identical with those of the corresponding anilines and phenols. In selected cases, the anilines and the phenols were identified and quantified by HPLC, and in some cases were isolated from large scale reactions. Hydrolyses of compounds **5b** and **5f** were also carried out in a pH 4.1 formate buffer containing 50% methanol. In these reactions, the formation of 1-(4-cyanophenyl)-3-methoxymethyl-3-methyltriazene, **4** (Ar = 4-NCC_6H_4, R = Me)[29] was observed by HPLC; quantification of the aryl ether starting material and methyl ether product was achieved with a system comprising a Lichrospher®100 5 µm RP-8 250 mm × 4 mm column and acetonitrile/water (65:35) as eluent at a flow rate of 1 mL min^{-1}, while the corresponding products of decomposition of the starting material, 4-cyanoaniline and phenol, were quantified by an eluent system comprising acetonitrile/water (30:70).

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