Triazene Drug Metabolites. Part 14.[1] Kinetics and Mechanism of the Acid-catalysed Hydrolysis of 3-Alkoxymethyl-3-alkyl-1-aryltriazenes

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Alkoxymethyltriazenes undergo an acid-catalysed hydrolysis reaction to form the parent aniline. The reaction is specific-acid-catalysed, with a solvent deuterium isotope effect of k_H/k_D = 0.4–0.5; there are no pH-independent or base-catalysed pathways. Electron-donating substituents in the aryl ring of the triazene moiety enhance the rate of decomposition, giving rise to a Hammett ρ value of *ca.* −1.6. The sign and magnitude of this ρ value is interpreted in terms of a mechanism which involves protonation of the substrate at the ether oxygen atom followed by cleavage of the O–CH_2 bond of the alkoxymethyl group to form a triazenyliminium ion intermediate. This intermediate can be trapped by the inclusion of ethanol in the hydrolysis medium. A Taft plot of log k_H *vs.* σ^* for the alkyl group of the alkoxy moiety is curved, reflecting the fact that electron-donating alkyl groups enhance substrate protonation but reduce the leaving-group ability of the alcohol in the iminium-ion-forming process whereas electron-withdrawing groups will have precisely the opposite effects in both these steps. For the range of substrates studied, electron-donating alkyl groups provide the more reactive substrates. Moreover, the second-order rate constants for acid catalysis, k_H, of the ethyl, allyl and benzyl ethers are of similar magnitude, precluding processes involving the formation of carbocations derived from the ether alkyl groups.

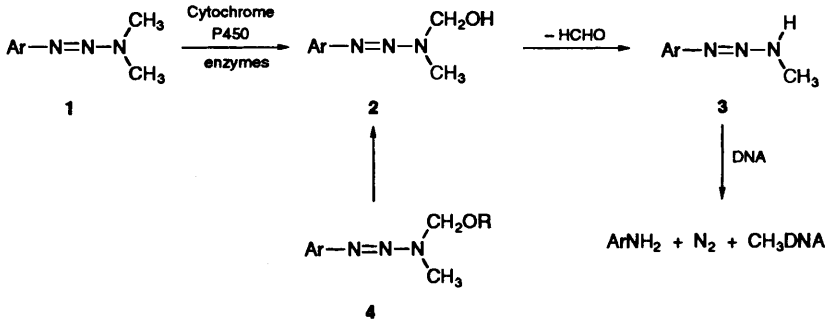
1-Aryl-3,3-dimethyltriazenes **1** are antitumour compounds used to treat malignant melanoma.[2] They suffer metabolic oxidation by cytochrome P450 enzymes to give hydroxymethyltriazenes **2**, which, by loss of formaldehyde, generate the cytotoxic monomethyltriazenes **3**. These are known alkylating agents, capable of alkylating DNA and RNA (Scheme 1).[3,4] While the conversion of **2** to **3** in non-aqueous solvents is slow ($t_{\frac{1}{2}} \approx 45$ min),[5] in aqueous media it appears to be instantaneous. Therefore, as part of our continuing interest in the metabolism of dimethyltriazenes, and especially in the development of potential prodrugs of the triazene moiety, we have directed our attention to the alkoxymethyltriazenes **4**. These compounds are the ether derivatives of the hydroxymethyltriazenes **2**, with the potential to act as prodrugs.

Methoxymethyltriazenes were first synthesised by Vaughan and co-workers, who also reported their antitumour activity.[6] They are active on some tumours *in vivo*, but not *in vitro*, suggesting the need for metabolic activation to generate an active species. Subsequently, we reported a more direct and general synthesis of 3-alkoxymethyl- and 3-alkylthiomethyl-1-aryl-3-alkyltriazenes,[7] which involved the reaction of hydroxymethyltriazenes with alcohols or thiols in the presence of anhydrous HCl. We have now improved this synthetic method by using *N*,*N*-dimethylformamide (DMF), rather than the parent alcohols themselves, as the solvent thereby extending the range of compounds available. Since methoxymethyltriazenes were also found to be stable in aqueous pH 7.4 buffer, an observation that has been highlighted as the reason for their inactivity *in vitro*,[8] we were interested in investigating the range of reactivity of substituted **4** that can be achieved by changing the structure of the groups X and R. Herein we report our results for the hydrolysis of compounds **4a–t** in aqueous acidic media.

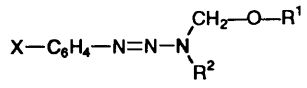
Experimental

Substrates and Reagents.—The 3-alkoxymethyl-3-alkyl-1-aryltriazenes were either available from earlier work or were prepared as reported previously.[7] For compounds **4a**, **b**, **f–i**, **q–s** the solvent employed was DMF rather than the alcohol itself. New compounds had spectroscopic and analytical data consistent with their proposed molecular structures.

Kinetics.—The decomposition of the alkoxymethyltriazenes **4** was followed by monitoring the decrease in UV absorbance of the substrate, at an appropriate wavelength, using a Perkin–Elmer Lambda 2 spectrophotometer. For solubility reasons, acetonitrile (10%) was necessary as a cosolvent. Reaction solutions were monitored continuously in cells thermostatted to ±0.1 °C. Initial substrate concentrations were *ca.* 5 × 10^{-5} mol dm^{-3}. The ionic strength was maintained at 0.5 mol dm^{-3} by the addition of $NaClO_4$. At the conclusion of each experiment the pH of the reaction solution was measured. In deuteriated



Scheme 1



4

	R^1	R^2	X		R^1	R^2	X
a	$-CH_2CF_3$	Me	-CN	**k**	$-CMe_3$	Me	$-CO_2Et$
b	$-CH_2-CH=CH_2$	Me	-CN	**l**	-I $-CMe_3$	Me	$-NO_2$
c	-Et	Me	-CN	**m**	-Et	Me	$-CONH_2$
d	$-CHMe_2$	Me	-CN	**n**	-Et	Me	$-CO_2Me$
e	$-CMe_3$	Me	-CN	**o**	-Et	Me	$-CO_2Et$
f	$-CH_2-C_6H_4-NO_2$	Me	-CN	**p**	-Et	Me	$-NO_2$
g	$-CH_2-C_6H_5$	Me	-CN	**q**	$-CH_2CF_3$	Me	$-CONH_2$
h	$-CH_2-C_6H_4-Me$	Me	-CN	**r**	$-CH_2CF_3$	Me	$-CO_2Et$
i	$-CH_2-C_6H_4-OMe$	Me	-CN	**s**	$-CH_2CF_3$	Me	$-NO_2$
j	$-CMe_3$	Me	$-CONH_2$	**t**	$-CMe_3$	Et	-CN

solvents values were calculated from the expression pD = pH + 0.4.[9]

Pseudo-first-order rate constants (reproducible to ±10%) were obtained from the slopes of plots of $\ln(A_t - A_\infty)$ *vs.* time, here A_t and A_∞ are the absorbance at time t and infinity, respectively.

Product Analysis.—The UV spectra of the reaction solutions at the conclusion of the reaction were identical with those of the corresponding anilines. In selected cases the anilines were isolated from larger scale reactions.

From a large scale hydrolysis of **4e** in a mixed solvent comprising formate buffer of pH 3.6 and ethanol (1 : 1), it proved possible to isolate **4c** as the major product.

Results and Discussion

Alkoxymethyltriazenes are stable in neutral and basic aqueous media. In acidic solutions, however, they decompose to the corresponding aniline. Pseudo-first-order rate constants for the hydrolysis of **4a–t** were determined in aqueous buffers at several acidity values ranging from pH 2–7, using several buffer concentrations at each pH. The results obtained for compound **4a** (Table 1) demonstrate clearly that k_o is dependent upon the proton concentration but independent of buffer concentration, indicative of specific-acid catalysis. Similar results were obtained for the other substrates. Second-order rate constants for this acid-catalysed process were obtained for the slopes of plots of k_o *vs.* $[H^+]$ (Fig. 1). These are straight lines which pass through the origin, verifying the absence of a non-catalysed process. Values of the second-order catalytic constants, k_H, are presented in Table 2.

For **4a**, k_o values were determined using formate buffers in both H_2O and D_2O, allowing a solvent deuterium isotope effect of $k_H/k_D = 0.49$ to be determined. A similar solvent deuterium isotope effect, $k_H/k_D = 0.38$, was determined for **4o**. These values are those expected for specific acid catalysis, and are consistent with a pre-equilibrium protonation of the substrate.[10] The effect of temperature on the pseudo-first-order rate constants for the hydrolysis of **4e** and **4o** was studied and the results are shown in Table 3. The data enable values for the entropy of activation to be calculated, and for both **4e** and **4o** $\Delta S^\ddagger \approx -35\ (\pm 20)$ J mol^{-1} K^{-1}. These values, though negative, are close to zero and more characteristic of a unimolecular than a bimolecular process.

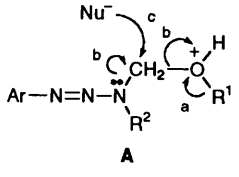
The two most probable sites for protonation of the substrate are the ether oxygen and the triazene N(1) nitrogen atom. Protonation of the ether oxygen would give **A**, while protonation of the triazene system would give **B**.

It is possible for species **A** to decompose to the observed

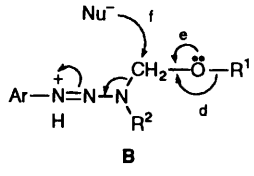
Table 1 Pseudo-first-order rate constants, k_o, for the hydrolysis of **4a** in aqueous buffers at 25 °C

Buffer	[Buffer]/mol dm^{-3}	pH	$k_o/10^{-6}$ s^{-1}
CH_2ClCO_2H	0.001	2.53	162
	0.005	2.55	129
	0.008	2.40	158
	0.002	2.29	196
	0.003	2.27	195
	0.080	2.27	203
	0.20	2.26	170
HCO_2H	0.075	2.76	78
	0.10	3.07	43
	0.05	3.18	40
	0.08	3.14	41
	0.10	3.13	56
	0.20	3.15	40
	0.30	3.18	39
	0.07	3.505[a]	38.5
	0.08	3.638[a]	31.9
	0.1	3.826[a]	23.0
	0.125	4.026[a]	16.8
	0.15	4.176[a]	14.4
$MeCO_2H$	0.075	4.07	7.4
	0.0375	4.15	7.61
	0.075	4.12	7.5
	0.150	4.20	7.43
	0.225	4.18	7.71
	0.300	4.17	7.74
	0.350	5.43	0.896

[a] In D_2O value quoted is meter pH reading +0.4.



A



B

products by one of three processes (shown by the arrows on the structure) (a) cleavage of the O–R^1 bond to form an hydroxymethyltriazene and a carbocation, (b) formation of an iminium ion with cleavage of the CH_2–O bond, and (c) nucleophile-assisted cleavage of the CH_2–O bond. Species **B** also has three potential decomposition pathways available, (d) cleavage of the O–R^1 bond with concerted formation of formaldehyde and the monoalkyltriazene, (e) formation of the monoalkyltriazene and R^1–O^+=CH_2 ion, and (f) formation of the monoalkyltriazene *via* nucleophilic attack at the NCH_2 carbon atom. Intuitively, one might expect the more electron rich triazene nitrogen atom system to be the site of protonation, but we believe the data point to **A** being the protonated triazene

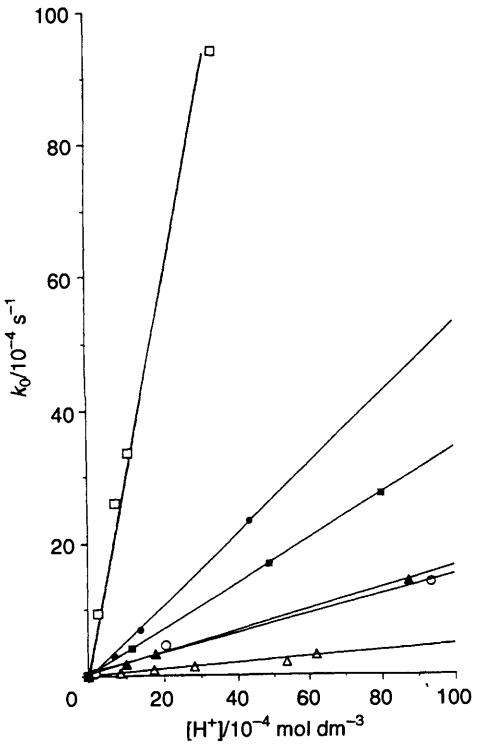


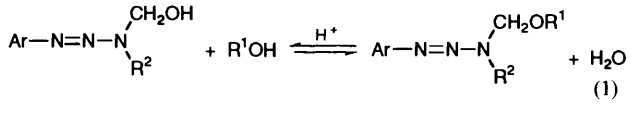
Fig. 1 Plots of k_o *vs.* [H$^+$] for the hydrolysis of **4a–e, g**: **4a** □, **4b** ○, **4c** △, **4d** ■, **4e** ●, **4g** ▲ at 25 °C

Table 2 Values of the second-order rate constants k_H for the acid-catalysed hydrolysis of **4a–t** at 25 °C

Compound	$k_H/10^{-2}$ dm^3 mol^{-1} s^{-1}	Compound	$k_H/10^{-2}$ dm^3 mol^{-1} s^{-1}
4a	4.8	**4k**	576
	9.8[a]	**4l**	146
4b	15	**4m**	201
4c	34	**4n**	141
4d	53	**4o**	150
4e	273		398[a]
4f	21	**4p**	29
4g	16	**4q**	17.8
4h	21	**4r**	13.9
4i	32	**4s**	3.4
4j	778	**4t**	23 100

[a] Value for catalysis by D$^+$ in D_2O.

species that leads to product formation and that it does so *via* an iminium ion, route (b). What are the arguments in favour of the formation of **A** and the route (b)? First, the acid-catalysed formation of alkoxymethyltriazenes from the corresponding hydroxymethyltriazenes and alcohols[7] [eqn. (1)] demands that it is the oxygen atom of the hydroxymethyltriazene that is protonated. The principle of microscopic reversibility therefore implies that the hydrolysis reaction must also proceed *via* the ether oxygen protonated form **A**.



(1)

Furthermore, we have shown elsewhere that hydroxymethyl- and alkoxymethyl-triazenes bind to lanthanide metal ions through the oxygen atom rather than the triazene system.[11] Second, the lack of buffer catalysis, whether the buffer species be chloroacetate, formate, acetate or phosphate, implies that there

Table 3 Effect of temperature on the pseudo-first-order rate constants for the hydrolysis of **4e** in pH 3.22 formate buffer and **4o** in pH 2.71 monochloroacetate buffer

4e		**4o**	
T/°C	$k_o/10^{-3}$ s^{-1}	T/°C	$k_o/10^{-3}$ s^{-1}
20	0.77	15.6	1.4
25	1.57	23.9	3.2
30	1.84	34.4	9.1
35	3.83	47.9	30.0
40	5.35		
E_a = 76 (± 5) kJ mol^{-1}		E_a = 77 (± 5) kJ mol	
$\Delta S^\ddagger$ = −34 (± 20) J mol^{-1} K^{-1}		$\Delta S^\ddagger$ = −35 (± 20) J mol^{-1} K^{-1}	

is no nucleophile-assisted pathway ruling out paths (c) and (f). The low, almost zero, values for the entropy activation are also not consistent with an S_N2 attack at an sp^3 carbon. Third, while the value of k_H for the *tert*-butyl ether **4e** is considerably larger than for other ether alkyl groups (Table 2), which might imply formation of an alkyl carbonium ion *via* cleavage of the O–R^1 bond [species **A**, path (a) and species **B**, path (d)], comparison of the k_H value of the ethyl ether **4c** with those of the allyl, **4b**, and 4-substituted benzyl, **4f–i**, ethers clearly demonstrates that formation of a carbocation from the R^1 group is precluded. Thus, pathways (a) and (d) can be discounted. Fourth, a large scale solvolysis of the *tert*-butyl ether **4e** at pH 3.6 in a mixed aqueous–ethanol (1 : 1) solvent yielded the corresponding ethyl ether **4c**. Under analogous conditions the parent hydroxymethyltriazene decomposes to the corresponding aniline. Since other evidence precludes the bimolecular pathways (c) and (f) as the route by which **4e** may be converted into **4c**, this observation allows a choice to be made between the triazenyliminium ion formed from **A** *via* path (b) and the R$^1\overset{+}{O}$=CH$_2$ ion formed from **B** *via* path (d); formation of **4e** can only be accounted for by trapping of the triazenyliminium ion with solvent ethanol. It also provides further evidence for discounting path (a).

Thus, acid-catalysed hydrolysis of alkoxymethyltriazenes appears to proceed *via* protonation of the ether oxygen atom followed by cleavage of the CH$_2$–O bond to form a triazenyliminium ion and the appropriate alcohol. Substituent effects in the aryl ring certainly seem to be consistent with this interpretation. Fig. 2 presents Hammett plots for the ethyl ethers **4c**, **m–p**, the *tert*-butyl ethers **4e**, **j–l** and the 2,2,2-trifluoroethyl ethers **4a**, **q–s** which give rise to ρ values of −1.9, −1.6 and −1.6 respectively. The negative sign of ρ identifies positive charge development in the triazene moiety in the transition state: while cleavage of the *O*-protonated form **A** will certainly involve development of greater positive charge as the transition state is reached, cleavage of the *N*-protonated form **B** would involve a diminution of such positive charge. However, given that the data point to a two-step process, protonation of the substrate followed by decomposition of the so-formed protonated substrate, the value of ρ is a composite of the ρ for protonation and the ρ for decomposition. The magnitude of ρ is therefore of mechanistic significance. Substituents in the aryl ring would be expected to exert little influence on the protonation of the ether oxygen atom to form **A**, in the same way that complex formation between hydroxymethyltriazenes and lanthanide metal ions (a process that occurs through the oxygen atom of the hydroxymethyltriazene) exhibits almost zero correlation with the Hammett σ value.[11] Cleavage of **A** *via* paths (a) and (c) would also be little affected by aryl substituents, whereas path (b), iminium ion formation, involves a non-bonding pair of electrons that is in conjugation with the ring. Therefore electron-donating substituents would be expected to increase the rate. Indeed, the process in path (b) is strictly

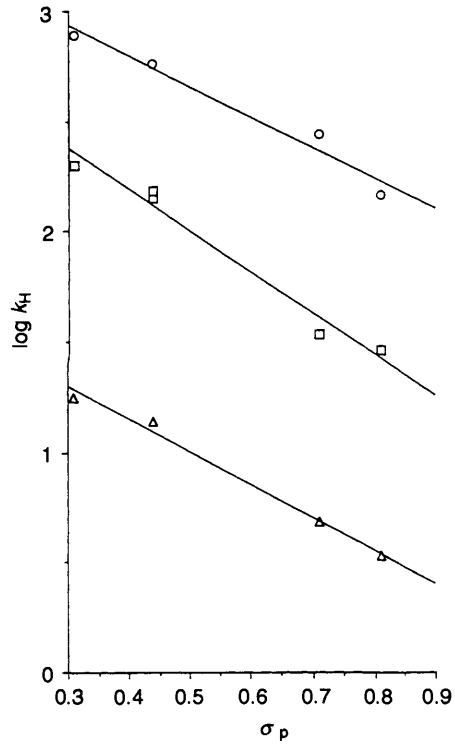


Fig. 2 Hammett plots for the hydrolysis of **4e**, **j–l** ○; **4c**, **m–p** □; **4a**, **q–s** △

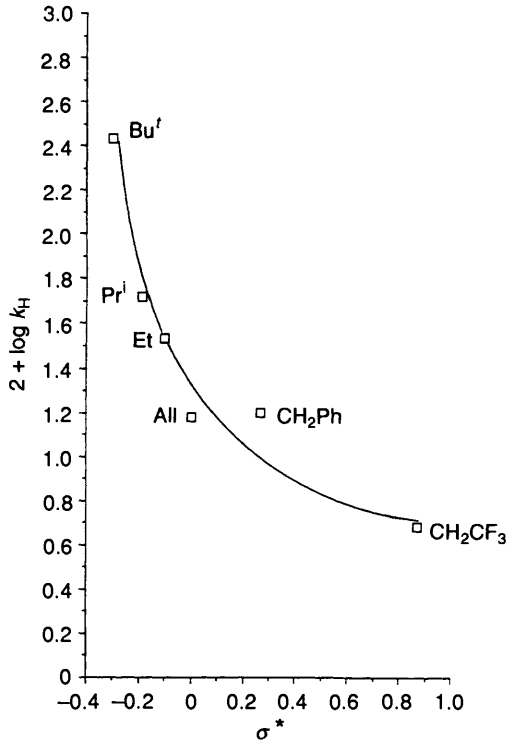
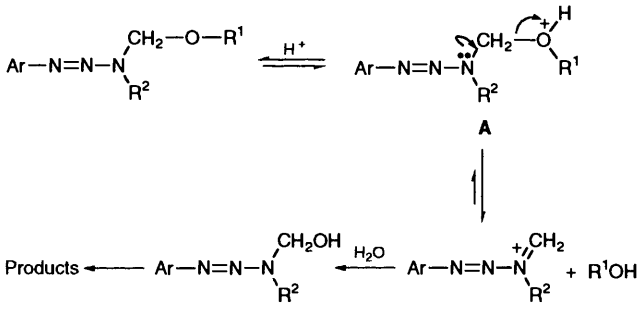


Fig. 3 Taft plot for the hydrolysis of **4a–e**, **g**



Scheme 2 Mechanism of the acid-catalysed hydrolysis of alkoxymethyltriazenes

analogous to iminium ion formation from acyloxymethyl triazenes (in which a carboxylate ion acts as the leaving group rather than alcohol in the present case) for which a ρ value of -2 was obtained.[12] This is remarkably similar to the values determined here for the ethers. In contrast, electron-donating substituents would be expected to enhance the formation of the protonated form **B** (giving rise to a negative ρ value) whereas cleavage by any of paths (d), (e) and (f) would be enhanced by electron-withdrawing substituents (giving a positive ρ value). We can gain an idea of the likely magnitude of the ρ value for the protonation process from two related reports involving monomethyltriazenes: reaction of monomethyltriazenes with benzoic acids gives a ρ value of -0.92,[13] and complex formation between monomethyltriazenes and Cu^{2+} and Zn^{2+} ions a ρ value of -1.[14] These values are somewhat less negative than the values obtained in the present work. Moreover, we have recently reported that the acid-catalysed hydrolysis of acyltriazenes, a process involving nucleophile-assisted cleavage of an *N*-protonated intermediate analogous to **B** [i.e. path (f)], displays a ρ value of -0.8.[1] Thus, we are led to conclude that the reaction involves formation of a triazenyliminium ion from an ether oxygen-protonated form **A** (Scheme 2). The enhanced rate for the *N*-ethyl compound **4t** over the corresponding *N*-methyl compound **4e** is consistent with a stabilisation of the positive charge in the iminium ion.

All three series of alkoxymethyltriazenes, *viz.* ethoxy, *tert*-butoxy and 2,2,2,-trifluoroethoxy, display similar Hammett ρ values, implying a similar mechanism in each case. However, while the data in Table 2 demonstrate that an alkyl carbocation is not formed, the role of the ether alkyl group is not so easy to establish.

A Taft plot of log k_H *vs.* σ^* (Fig. 3) is decidedly curved and presumably reflects the opposing influence of the R^1 group on the first two steps in Scheme 2. Thus, electron-donating R^1 groups enhance the degree of protonation of the substrate, step 1 (which would give rise to a negative ρ), while electron-withdrawing groups will enhance the leaving group ability of the alcohol in step 2 (which would give rise to a positive ρ). The form of the curve in Fig. 3 implies that, for the range of compounds studied, the former effect dominates, though the enhanced reactivity of compound **4a** over that expected from such an interpretation suggests that the nucleofugacity of the CF_3CH_2OH group becomes important. Nevertheless, the ρ values for the ethyl and 2,2,2-trifluoroethyl series of ethers, together with the inverse solvent deuterium isotope effect, k_H/k_D, in both cases, implies that for **4a** protonation of the substrate is not the rate limiting process.

It is therefore of interest to compare the results we have reported here with those obtained from the corresponding aryloxymethyltriazenes,[15] where the leaving group R^1O is a phenol. For such compounds, hydrolysis proceeds *via* parallel acid-catalysed, buffer-catalysed and spontaneous (non-catalysed) processes. Only for the 4-nitrophenoxy derivative was the spontaneous loss of the phenolate ion of any importance. Ethers derived from phenols of higher pK_a values do not exhibit a non-catalysed expulsion of the phenolate ion. Unfortunately, the incomplete nature of the data precludes a definitive assessment of the type of acid- and buffer-catalysed processes that are operating: the authors favour specific-acid catalysis, with the buffer anion acting as a nucleophile to displace either the phenolate ion from the unprotonated substrate or the phenol from the protonated substrate.[15] However, the solvent

deuterium isotope effect reported, $1.6 < k_H/k_D < 2.28$, implies general, rather than specific, acid catalysis. If that is the case, then buffer catalysis almost certainly arises from the buffer anion assisting general-acid-catalysed cleavage of the phenolate. However, such analysis must remain tentative until more substantive data are available; the reported solvent deuterium isotope effect relates to one pH/pD and buffer value only, which means that it is a composite of the isotope effect on the acid-catalysed and buffer-catalysed processes. Nevertheless, there is a clear distinction between aryloxymethyl- and alkoxymethyl-triazenes. While both undergo hydrolysis *via* formation of a triazenyliminium ion, the former contain the better leaving group and proceed *via* spontaneous or general-acid-catalysed liberation of the phenolate ion whereas the latter contain a poor-leaving group and require full protonation of the ether oxygen prior to cleavage of the $O-CH_2$ bond. As far as acting as triazene prodrugs is concerned, the alkoxymethyltriazenes hydrolyse too slowly at physiological pHs. Moreover, the formation of the very reactive electrophilic iminium ion species may well give rise to unwanted toxic effects by reacting with biological nucleophiles.

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