Triazene Drug Metabolites. Part 12.† Base Catalysed Formation of 3-Alkyl-1-aryltriazenes from 3-Alkyl-3-hydroxymethyl-1-aryltriazenes

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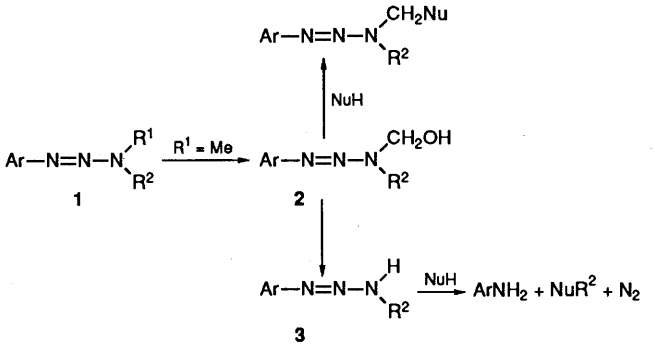
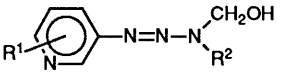
3-Alkyl-3-hydroxymethyl-1-aryltriazenes are stable in aprotic solvents, but decompose in non-aqueous protic solvents to the corresponding 3-alkyl-1-aryltriazenes. The reactions are catalysed by the presence of bases, and the catalytic activity of the base depends upon its aqueous pK_a. A Brønsted plot of the catalytic rate constant, k_B, *versus* base pK_a gives rise to a Brønsted β value of 1.1. Tertiary bases have no catalytic effect. The deuterium isotope effect for the piperidine catalysed reaction is *ca.* 1.4. The catalytic rate constants k_B depend upon the triazene structures, increasing with the size of the 3-alkyl group (Pr > Et > Me) and with electron-withdrawing ability of the substituent in the 1-aryl group (Cl > H > MeO). The observations are rationalised in terms of a six-membered transition state involving the N–CH_2–OH group of the triazene and the N–H of the base and in which proton transfer from the hydroxy group to the nitrogen atom of the catalyst is extensive. In mixed aqueous–ethanol solvents tertiary bases also have a catalytic effect. A Brønsted β value of 0.67 and a solvent deuterium isotope effect of 1.18 identify the proton as *ca.* 70% transferred to the base in the transition state. In no reaction was aminomethylation of the base observed.

1-Aryl-3,3-dialkyltriazenes **1** are cytotoxic compounds that require oxidative metabolism to express biological activity.[1] The dimethyl derivatives **1**; $R^1 = R^2 = Me$ have useful anti-cancer properties and are used clinically to treat malignant melanoma.[2] The ultimate cytotoxic metabolite is generally considered to be the monoalkyltriazene **3**; $R^2 = Me$,[3] which is known to be able to alkylate nucleic acids.[4] Implicit in this process in the α-hydroxylated triazene **2**[5] (Scheme 1) and one

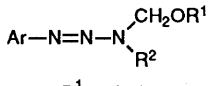


Scheme 1 Potential metabolic pathways for 1-aryl-3,3-dialkyltriazenes (1)

such derivative has been identified in blood[6] and rat urine.[7] The hydroxymethyltriazene **2** can generate the corresponding monoalkyltriazene by loss of formaldehyde, and there has been much current interest in the chemistry and biology of **2** and its derivatives *e.g.* **4** and **5**.[8–13] Alternatively, it has been proposed that the hydroxymethyltriazene **2** itself reacts directly with biological nucleophiles[14] (Scheme 1). However, it has been reported that the hydroxymethyltriazenes, HMTs, **2** are unstable in aqueous solutions, decomposing essentially instantaneously to the monoalkyl derivatives **3**.[12] Fortunately, the HMTs are stable in aprotic organic solvents and decompose only slowly in non-aqueous protic ones. We therefore set out to examine the reaction between the compounds **2** and various nucleophiles in such solvents in an attempt to discover those processes likely to be of relevance to the biological chemistry of these compounds. The HMTs used in the present work were **2a–f**. We were constrained to the use of these 3-pyridyl derivatives because of the need for HPLC analysis of reaction mixtures. The hydroxymethyltriazenes, **2**, and alkyltriazenes, **3**, have essentially identical UV characteristics, and whereas these two compounds may be separated chromatographically when Ar = 3-pyridyl, the former suffer decomposition when Ar = phenyl and substituted phenyl and cannot be separated chromatographically from **3**.



2a; R^1 = 2-MeO R^2 = Me
b; R^1 = 2-Cl R^2 = Me
c; R^1 = 6-Cl R^2 = Me
d; R^1 = H R^2 = Me
e; R^1 = H R^2 = Et
f; R^1 = H R^2 = Pr



4; R^1 = alkyl
5; R^1 = acyl

Experimental

Substrates and Products.—All HMTs, **2**, and corresponding monoalkyltriazenes **3** were synthesised by procedures developed previously.[15,16] Coupling constant values J are given in Hz.

2a: m.p. 99–100 °C; ν_{max}/cm^{-1} 3180, 1618, 1420, 1350, 1040 and 1020; $\delta_H([^2H_6]DMSO)$ 8.26 (1 H, d, J = 2), 7.82 (1 H, dd, J = 9, 2), 6.83 (1 H, d, J = 9), 6.20 (1 H, t, J = 7, exch.), 5.14 (2 H, d, J = 7) and 3.88 (3 H, s) 3.16 (3 H, s) (Found: C, 48.9; H, 6.4; N, 28.7. Calc. for $C_8H_{12}N_4O_2$: C, 48.97; H, 6.16; N, 28.55%).

2b: m.p. 120–122 °C; ν_{max}/cm^{-1} 3225, 1360, 1200, 1110 and 1035; $\delta_H([^2H_6]DMSO)$ 8.37 (1 H, d, J = 2), 7.82 (1 H, dd,

† For Part 11, see *J. Chem. Soc., Perkin Trans. 1*, 1991, 3241.

Table 1 Pseudo-first-order constants, k_s, for the decomposition of **2a–f** in various solvents

Triazene	Solvent	T/°C	$k_s/10^{-4}$ s^{-1}
2a	EtOH	37	3.1
2b	EtOH	37	3.0
2c	EtOH	37	5.85
2d	EtOH	37	3.09
		30	1.28
		25	0.88
		20	0.54
	EtOD	37	2.98
	MeOH	37	6.58
	Pr^{i}OH	37	2.8
	MeCN	37	0
	Dioxane	37	0
2e	EtOH	25	3.91
2f	EtOH	25	4.75

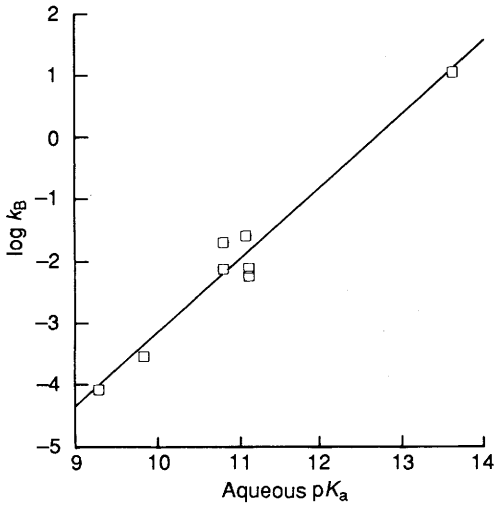


Fig. 1 Brønsted plot for the base catalysed decomposition of **2d**

J = 9, 2), 7.46 (1 H, d, J = 9), 6.39 (1 H, t, J = 7, exch.) and 5.12 (2 H, d, J = 7), 3.15 (3 H, s) (Found: C, 39.7; H, 4.4; N, 26.8. Calc. for $C_7H_9ClN_4O$: C, 39.92; H, 4.31; N, 26.60%).

2c: m.p. 86–87 °C; ν_{max}/cm^{-1} 3200, 1235, 1192, 1060, 1030 and 1010; δ_H([$^2H_6$]DMSO) 8.12 (1 H, dd, J = 5, 2), 7.76 (1 H, dd, J = 7.5, 2), 7.28 (1 H, dd, J 7.5, 5), 6.26 (1 H, t, J = 7, exch.) and 5.12 (2 H, d, J = 7), 3.26 (3 H, s) (Found: C, 40.1; H, 4.5; N, 26.5. Calc. for $C_7H_9ClN_4O$: C, 39.92; H, 4.31; N, 26.6%).

2e: m.p. 83–84 °C; ν_{max}/cm^{-1} 3165, 1415, 1235, 1195 and 1042; δ_H([$^2H_6$]DMSO) 8.58 (1 H, d, J = 2), 8.37 (1 H, d, J = 5), 7.74 (1 H, dt, J = 8, 2), 7.36 (1 H, dd, J = 8, 5), 6.30 (1 H, t, J = 7, exch.), 5.13 (2 H, d, J = 7) 3.77 (2 H, q, J = 7) 3.38 (3 H, s) and 1.21 (3 H, t, J = 7) (Found: C, 53.3; H, 6.9; N, 30.7. Calc. for $C_9H_{12}N_4O$: C, 53.32; H, 6.90; N, 31.09%).

2f: m.p. 80–82 °C; ν_{max}/cm^{-1} 3180, 1510, 1232, 1185, 1095 and 1047; δ_H([$^2H_6$]DMSO) 8.62 (1 H, d, J = 2), 8.29 (1 H, dd, J = 5, 2), 7.60 (1 H, dt, J = 8, 2) 7.20 (1 H, dd, J = 8, 5), 6.30 (1 H, t, J = 7, exch.), 5.12 (2 H, d, J = 7), 3.68 (2 H, d, J = 7), 3.38 (3 H, s), 1.68 (2 H, sex, J = 7) and 0.89 (3 H, t, J = 7) (Found: C, 55.6; H, 7.5; N, 28.8. Calc. for $C_9H_{14}N_4O$: C, 55.65; H, 7.26; N, 28.84%).

Catalysts and Solvents.—All amines were redistilled or recrystallised before use. Solvents were dried by known methods and deionised water was used throughout this study.

Kinetic Method.—Reactions were initiated by adding the hydroxymethyltriazene to a solution of the appropriate nucleophile (10^{-4}–1.0 mol dm^{-3}) in the solvent of study such that the initial concentration of hydroxymethyltriazene was *ca.* 10^{-3} mol dm^{-3}. The reaction solution was sampled at timed intervals by HPLC analysis. Chromatographic separation of the HMT and corresponding alkyltriazene **3** was achieved using a 25 cm × 5 mm i.d. column containing spherisorb S5-ODS2 packing employing pH 6.5 0.02 mol dm^{-3} ammonium acetate in acetonitrile–methanol–water (2:3:5) as eluting solvent. Samples were detected using a Cecil Instruments CE2112 UV monitor and sample peaks were quantified using a Shimadzu C-R1B chromatopac integrator. Reactions followed first-order kinetics for at least four half-lives. Rate constants obtained by this method were reproducible to ±10%.

Results and Discussion

In aprotic solvents the hydroxymethyltriazenes **2** are stable, but in alcohol solvents the HMTs decompose cleanly to the corresponding monoalkyltriazene **3**. The pseudo-first-order rate constants, k_s, for these reactions are contained in Table 1, and these show moderate variations with both triazene and solvent structure. The conversion of **2** to **3** is, however, catalysed by the presence of some nitrogen nucleophiles. These catalysed reactions are first-order in the concentration of both substrate and the added nucleophile (Table 2). The reactions are thus governed by eqn. (1), where k_B is the second-order rate constant

$$\text{rate} = \{k_s + k_B[B]\}[\mathbf{2}] \quad (1)$$

for catalysed reaction, and [B] the concentration of the added nucleophile. Values of k_B were determined from the slope of plots of the observed pseudo-first-order rate constants, k_0, *versus* [B]. Table 3 contains k_B values for the decomposition of compound **2d** catalysed by a variety of added nucleophiles.

Two observations are immediately apparent. First, the tertiary bases *N*-methylpiperidine, pyridine, *N*-methylimidazole, triethylamine and tributylamine display no catalytic activity. Second, the catalytic activity of the nucleophiles broadly follows the order of the aqueous pK_a. This dependence is shown in the Brønsted plot (Fig. 1); bases with p$K_a \geq 9.5$ are efficient catalysts for the conversion of **2d** to the corresponding **3** whereas those with pK_a = 9.5 are unable to catalyse the reaction.* The slope of the line is 1.1. Interestingly, steric hindrance in the added nucleophile appears to have little effect on the rate of the reaction. Thus 2,2,6,6-tetramethylpiperidine is slightly more reactive towards **2d** than piperidine itself, whereas *N*-methylpiperidine has no catalytic activity.

The catalytic behaviour of *N*-methylcyclohexylamine in acetonitrile proved somewhat different to that in alcohol solvents. A plot of k_0 *versus* [*N*-methylcyclohexylamine] for compound **2d** is curved [Fig. 2(*a*)], the rate appearing to approach a maximal value at high concentrations. We rationalise this effect as follows. In acetonitrile the base is associated:

$$B_n \overset{K_D}{\rightleftharpoons} nB_f$$

from which it follows that

$$[B]_t = n[B]_f^n/K_D + [B]_f \quad (2)$$

where $[B]_t$ = the total concentration of base, $[B]_f$ = the concentration of free base present in solution, B_n = the associated

* In our preliminary communication (S. C. Cheng, L. Fernandes, J. Iley and E. Rosa, *Tetrahedron Lett.*, 1985, **26**, 1557) we identified bases with pK_a < 9.5 as having a catalytic, albeit weak, effect. On re-examination of the data we believe the extremely small changes observed in k_0 with increasing [B] are due rather to a medium effect brought about by the large amounts of nucleophile required.

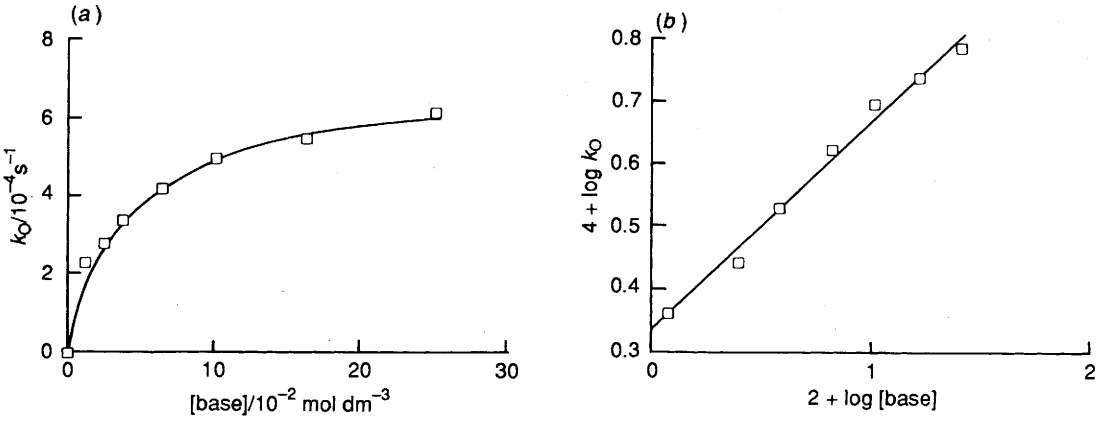


Fig. 2 (*a*) Dependence of k_0 *versus* [base] for decomposition of **2d** in CH_3CN; (*b*) corresponding plot of log k_0 *versus* log [base]

Table 2 Observed first-order rate coefficients, k_0, for decomposition of **2d** in the presence of piperidine and piperazine in EtOH at 37 °C

[Piperidine]/mol dm^{-3}	$k_0/10^{-4}$ s^{-1}	[Piperazine]/mol dm^{-3}	$k_0/10^{-4}$ s^{-1}
0.01	3.50	0.005	3.43
0.033	5.83	0.01	3.69
0.058	7.94	0.03	4.23
0.088	9.32	0.05	4.83
0.10	10.20	0.07	5.27

Table 3 Second-order rate coefficients, k_B, for the conversion of **2d** to the corresponding monomethyltriazene

Added nucleophile	T/°C	$k_B/10^{-2}$ dm^3 mol^{-1} s^{-1} [a]
1,1,3,3-Tetramethylguanidine	37	1040
Piperidine	19.5	0.0596
	25	0.105
	30	0.241
	37	0.718
		0.528 [b]
2,2,6,6-Tetramethylpiperidine	19.8	0.29
	25	0.714
	30	1.41
	37	2.46
N-Methylpiperidine	37	0
Butylamine	37	1.951
Dibutylamine	37	0.592
Tributylamine	37	0
Triethylamine	37	0
N-Methylcyclohexylamine	37	0.705
		3.27 [c]
		0.032 [d]
		0.65 [e]
Piperazine	37	0.028
Diallylamine	37	0.008
Pyridine	37	0
N-Methylimidazole	37	0

[a] In EtOH unless otherwise stated. [b] In EtOD. [c] In MeOH. [d] In Pr^{i}OH. [e] In DMSO.

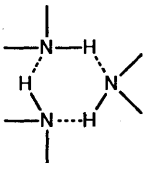
base, K_D the dissociation constant of the associated base and n = the degree of association. Assuming only the monomeric base species catalyses the reaction, then

$$k_0 = k_B[B]_f \quad (3)$$

Now, there are two possible extremes of eqn. (2); either $[B]_f > n[B]_f^n/K_D$ in which case $[B]_t \approx [B]_f$ and k_0 *versus* $[B]_t$ should be linear which it clearly is not, or $n[B]_f^n/K_D > [B]_f$ in which case

$$k_0 = \left(\frac{K_D}{n}\right)^{1/n} k_B[B]_t^{1/n} \quad (4)$$

Thus, a plot of log k_0 *versus* log $[B]_t$ should give a slope of $1/n$ and intercept log $\{k_B (K_D/n)^{1/n}\}$. Such a plot is shown in Fig. 2(*b*), from which the slope is 0.33. Thus the degree of association is 3, which is perfectly reasonable and readily explicable by the formation of the non-catalytic cyclic trimer **6**.



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Without K_D an accurate value of k_B cannot be adduced. However, a minimum value is obtainable since the inequality $n[B]_f^n/K_D > [B]_f$ only holds if the maximum value of K_D is 10^{-8} mol^2 dm^{-6}. Thus, the minimum value of k_B for *N*-methylcyclohexylamine in acetonitrile is 66.3 × 10^{-2} dm^3 mol^{-1} s^{-1}, which is almost one hundred fold greater than the value of k_B in ethanol.

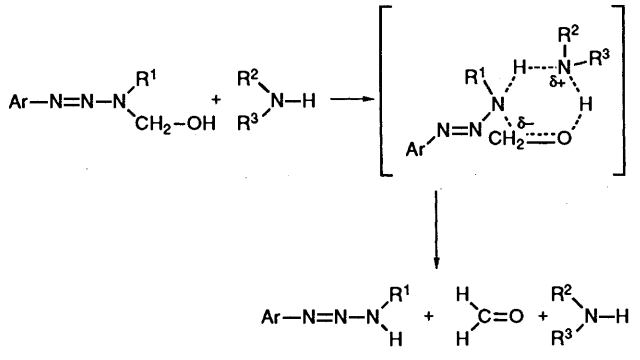
Values of the catalytic rate constants, k_B, for other HMTs in ethanol using 1,1,3,3-tetramethylguanidine, piperidine or *N*-methylcyclohexylamine catalysts are contained in Table 4. These show that HMT structure has a significant affect on the rate of decomposition. Thus, increasing the size of the N-3 alkyl substituent increases k_B. Moreover, the aryl ring substituent has a large effect, electron-withdrawing groups increasing k_B. Even though the data set is limited by the compounds that were available, it is clear that the rate diminishes in the order Cl > H > MeO. Indeed, *N*-methylcyclohexylamine is an efficient catalyst for **2b–d**, but has no catalytic activity for **2a**. Thus, HMTs with electron-donating substituents in the aryl ring have Brönsted plots displaced to the right of that shown in Fig. 1, whereas those with electron-withdrawing substituents are displaced to the left.

The first-order dependence on [substrate] and [catalyst] and

Table 4 Second-order rate coefficients, k_B, for the catalysed decomposition of **2a–f** in ethanol

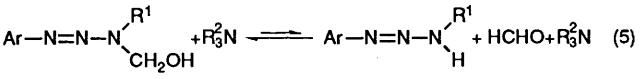
	$k_B/10^{-2}$ dm^3 mol^{-1} s^{-1}					
Nucleophile	**2a**	**2b**	**2c**	**2d**	**2e**	**2f**
1,1,3,3-Me$_4$ guanidine[a]	33.5	12 410		1 040		
Piperidine[b]				0.105	0.376	0.519
N-Methylcyclohexylamine[a]	0	2.08	3.75	0.705		

[a] At 37 °C. [b] At 25 °C.



Scheme 2 Proposed mechanism of the base-catalysed decomposition of 3-alkyl-3-hydroxymethyltriazenes to 3-alkyltriazenes

the observation that the catalyst requires an X–H bond for activity points to a mechanism involving a six-membered cyclic transition state (Scheme 2). The alternative mechanism involving EtOH as a proton donor appears to be precluded by the lack of catalysis by tertiary bases. Moreover, the possibility that the tertiary bases fail to catalyse the reaction due to an unfavourable equilibrium, eqn. (5), is unlikely, since no formation



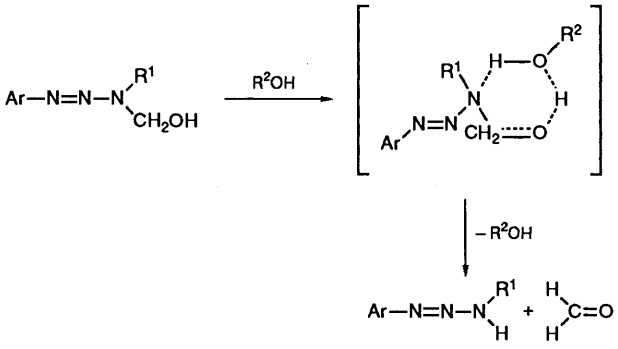
(5)

of an HMT could be observed when triethylamine was added to an equimolar solution of monomethytriazene and formaldehyde under conditions identical to those used for the decomposition reaction.

It is apparent from the Brönsted β value and the solvent isotope effect that the transition state is a late one, resembling products. Thus, the β of 1.1 is clear evidence that the O–H proton is extensively transferred. Moreover, the solvent deuterium isotope effect for the piperidine catalysed decomposition reaction, k_B^H/k_B^D, is 1.36. Since $k^H/k^D = \varphi_R/\varphi_T$ and $\varphi_T = (\varphi_R)^{1-x}(\varphi_P)^x$, where φ_R, φ_P and φ_T are the proton fractionation factors for the reactants, products and transition state, respectively, and α is the extent of proton transfer in the transition state,[17] it follows that α may be calculated using the observed kinetic deuterium isotope effect and the known values of φ_R and φ_P. Using values of $\varphi_{HMT} = 1.25$, $\varphi_{MMT} = 0.92$ and $\varphi_{base} = 0.92$,[17] a value for α of 1 is obtained.

Such a mechanism is consistent with significant charge build up at the N-3 nitrogen if proton transfer to this centre from the amine is not well advanced. Certainly, the effect of the substituent in the aryl ring is consistent with stabilising a significant increase in negative electron density in the triazene group. Indeed, this type of stabilising effect has been observed for the hydroxide ion catalysed decomposition of *N*-hydroxymethylbenzamides, in which there is an increase of negative charge on the amide nitrogen.[18] The magnitude of the effect for the amides is somewhat attenuated, however, presumably because the negative charge is not in direct conjugation with the ring. As would be expected for charge separation in the transition state, the rate of the reaction increases roughly in line with the polarity of the solvent. The role of the alkyl group is less certain, the rate increasing with either the steric bulk of the group or its electron-donating ability. We favour the former explanation for two reasons. First, increasing the rate by increasing the electron density at the N-3 nitrogen atom runs counter to the effect seen for the substituents in the aryl group. Second, it is known from other compounds, *viz.* the amides, that *N*-hydroxymethylamides are more stable than the corresponding *N*-hydroxymethyl-*N*-methylamides[19] and that *N*-hydroxyalkylamides are much less stable than their *N*-hydroxymethyl counterparts.[20]

The mechanism of the solvent promoted reaction is less clear. It is obvious that a protic solvent is required, but what is the role of this proton? We favour a mechanism involving a six-membered transition state, as for the catalysed reaction, with the solvent taking the place of the catalyst (Scheme 3). The lack



Scheme 3 Proposed mechanism for the decomposition of 3-hydroxymethyltriazenes in protic solvents

of a substantial solvent deuterium isotope effect implies there is not extensive proton transfer in the transition state. The dependence on the alkyl group of the alcohol (Me > Et > Pr) may reflect steric differences, though this is unlikely since 1,1,6,6-tetramethylpiperidine is a better catalyst than piperidine, or it may reflect the greater acidity of MeOH as opposed to EtOH or PrOH. Neither proton is therefore available for undergoing general base catalysis as observed experimentally with the tertiary bases.

Since HMTs decompose extremely rapidly in aqueous media, we were interested in examining the effect of added water on the decomposition of **2d** in ethanol. Table 5 shows that the observed pseudo-first-order rate constants increase with increasing water content. This is probably a medium effect, although it may be a true catalytic effect in which a water molecule replaces an ethanol molecule (see structure **7**). Assuming no medium effect, a plot of k_0 *versus* $[H_2O]$ is linear giving a catalytic rate

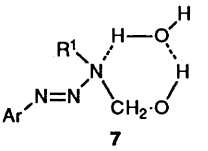


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Table 5 Observed pseudo-first-order rate constants from the decomposition of **2d** in ethanol–water mixtures

[Water]/mol dm^{-3}	$k_0/10^{-4}$ s^{-1}
0	3.09
2.78	6.62
5.6	7.86
8.3	10.35
11.1	14.7

Table 6 Observed-pseudo-first-order rate constants for the decomposition of **2d** in the presence of Et_3N in mixed ethanol–water solvent. $[H_2O]$ = 2.7 mol dm^{-3}

$[Et_3N]/10^{-2}$ mol dm^{-3}	$k_0/10^{-4}$ s^{-1}
0	6.62
3.2	8.31
5.3	8.91
6.3	9.89
11.9	12.2
17.1	14.1
22.9	16.00

Table 7 Values of the catalytic rate constants, $k_{B/W}$, for the base catalysed decomposition of **2d** in ethanol–water mixtures at 37 °C

Catalyst	$k_{B/W}/10^{-5}$ dm^6 mol^{-2} s^{-1}	pK_a
Morpholine	7.48,[a] 7.60[b]	8.36
Diallylamine	57.2[a]	9.29
Triethylamine	160,[a] 200,[b] 153[a,c]	10.65
N-Methylcyclohexylamine	468,[a] 568[b]	10.80

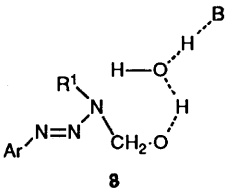
[a] Fixed $[H_2O]$, variable [B]. [b] Fixed [B], variable $[H_2O]$. [c] In $EtOD/D_2O$.

constant for water, k_W, of 10.0 × 10^{-5} dm^3 mol^{-1} s^{-1}. This value lies well away from the Brönsted plot in Fig. 1.

A structure such as **7** reveals that a proton is potentially available for general base catalysis, in which case a tertiary base such as triethylamine may exhibit a catalytic effect. This is indeed observed (Table 6). The catalysis due to the presence of the base in the presence of water may be analysed using eqn. (6), where $k_{B/W}$ is the catalytic rate constant for the base/water reaction. Eqn. (6) assumes that the overall rate of decomposition of **2d** is the sum of the spontaneous reaction in ethanol

$$k_0 = k_S + k_W[H_2O] + k_B[B] + k_{B/W}[B][H_2O] \quad (6)$$

solvent, the water catalysed reaction, the base catalysed reaction and the base/water catalysed reaction. If the base concentration is varied, at fixed water content then a plot of k_0 *versus* [B] will give an intercept of $\{k_S + k_W[H_2O]\}$ and a slope of $\{k_B + k_{B/W}[H_2O]\}$. Alternatively, if the water concentration is varied at a fixed base concentration a plot of k_0 *versus* $[H_2O]$ will give rise to a straight line of slope $\{k_W + k_{B/W}[B]\}$ and intercept $\{k_S + k_B[B]\}$. Since k_B, k_W, $[H_2O]$ and [B] are known, values of $k_{B/W}$ may be obtained from the slopes of the straight lines. Values of $k_{B/W}$ so obtained for four bases are contained in Table 7. A Brönsted plot for these data gives a line of slope 0.67. The solvent isotope effect for the triethylamine catalysed reaction, $k^H_{B/W}/k^D_{B/W}$, is 1.18. Using the observed kinetic deuterium isotope effect and the known values of φ_R and φ_P (1.25 for the HMT, 1.0 for water and 0.97 for Et_3NH^+),[17] a value of α = 0.65 is obtained. Therefore, both the Brönsted plot and the kinetic



8

deuterium isotope effect identify the proton as *ca.* 70% transferred to the base in the transition state, which probably resembles **8**.

Thus, in solvents which contain water tertiary bases also become effective catalysts for the conversion of HMTs to the corresponding monoalkyltriazenes in a manner dependent on their pK_a. Consequently, it would appear that HMT derivatives of dimethyltriazenes are unsuitable candidates both as prodrugs and for the transport form of the triazene moiety *in vivo*. Moreover, the proposal that HMTs are aminomethylating agents rather than alkylating agents finds no substantiation in the current work.

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