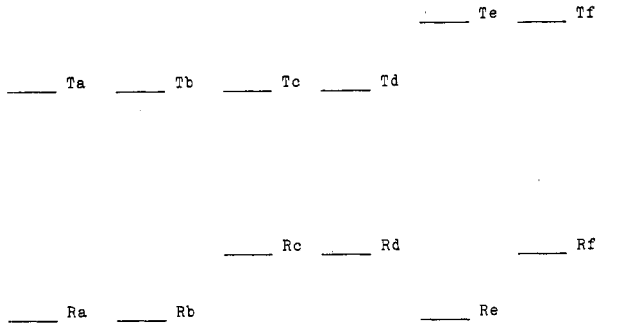
Scheme I



actually no restrictions as to how the reacting conformers get to the several transition states.

$$k = a(\exp[(S_{T1} - S_{av})/R] \exp[-(\Delta H_{T1} - \Delta H_{av})/RT] + ...) \quad (19)$$

$$k = a(k_1'' + k_2'' + k_3'' + ...) \quad (20)$$

$$D \cdot k = a(\exp[(S_{T1} - S_{av})/R] \exp[-(\Delta H_{T1} - \Delta H_{av})/RT] \exp[(S_{av} - S_{R1})/R] \exp[-(\Delta H_{av} - \Delta H_{R1})/RT] + ...) \quad (21)$$

To illustrate the consequences of the derivation, Scheme I shows several hypothetical reactant–transition state pairs. In Table I are shown the separate components of k, the k_i/f_1 values of eq 1; their sum k is the same k given by eq 8. Example HO gives the rate constant based on a single lowest energy conformer of reactant and a single lowest energy conformer of the transition state. (Other conformers are of high enough energy that they may be neglected.) Example H1 shows that the rate constant is unchanged for two (or any number of) entirely equivalent reaction paths. Example H2 shows that extra channels increase the rate constant, while example H3 shows that the presence of nonproductive reactant conformers decreases the rate constant. In all cases the HO rate constant is the reference for comparisons.

Table I. Dependence of the Overall Rate Constant on Conformer Energies[a]

conformers	ΔG_{Ri}	ΔG_{Ti}	f_i	$10^6 f_i k_i'$	$10^6 k$
HO Ra.Ta	0	8	1.0	1.37	1.37
H1 Ra.Ta	0	8	0.5	0.685	
Rb.Tb	0	8	0.5	0.685	1.37
H2 Ra.Ta	0	8	0.936	1.28	
Rc.Tc	2	8	0.032	1.28	
Rd.Td	2	8	0.032	1.28	3.83
H3 Ra.Ta	0	8	0.492	0.67	
Re.Te	0	10	0.492	0.02	
Rf.Tf	2	10	0.017	0.02	0.72

[a] Hypothetical data. For each example the value of k based on the global minima is 1.37×10^{-6}.

In applying eq 8 to esterification reactions the corrections to log k_{calc} ranged from roughly –0.25 to +0.25 where k_{calc} is based on just the global minima.[29] Although the correction is often unnecessary, the standard deviation for the ester study was 0.24 and omitting the correction would needlessly bias the results.

1,3-Dimethyl-3-acyltriazenes: Synthesis and Chemistry of a Novel Class of Biological Methylating Agents†

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The synthesis and hydrolytic decomposition of 1,3-dimethyl-3-(diethoxyphosphinyl)triazene (DMP), 1,3-dimethyl-3-carboethoxytriazene (DMC), 1,3-dimethyl-3-acetyltriazene (DMA), and 1,3-dimethyl-3-(*N*-methylcarbamoyl)triazene (DMM) are described. The kinetics of hydrolysis of DMP and DMC were investigated in aqueous buffers as a function of pH. DMP was found to be subject to acid catalysis up to pH 4.5 but then followed uncatalyzed kinetics up to pH 11.5. DMC, on the other hand, was catalyzed by acid at pH <4.5 and base catalyzed at pH >9.5. It exhibited uncatalyzed kinetics in the intervening pH region. DMA and DMM also appear to follow uncatalyzed kinetics in the vicinity of neutral pH. The order of reactivity of the four triazenes at pH 7.5 was found to be DMP > DMC > DMA > DMM. The mechanism of the hydrolytic decomposition in the uncatalyzed region is seen as a direct dissociation of the acyltriazenes to the methyldiazonium ion and the respective acylamidyl anions. The intermediacy of the methyldiazonium ion during the decomposition of DMC was established by deuterium exchange studies when the decomposition was carried out in deuterium oxide buffers. The four triazenes were tested in a bacterial mutagenesis assay by using the His$^-$ strains of *Salmonella typhimurium*. DMP, DMC, and DMA were found to be directly acting mutagens in strains that require a base substitution to revert to wild type. These results are consistent with the methyldiazonium ion acting as the ultimate mutagen. The mutagenicity of DMC was enhanced by porcine liver esterase, which suggested that this enzyme was capable of hydrolyzing the carboethoxy group to release the highly reactive dimethyltriazene.

The chemistry of 1-aryl-3,3-dialkyltriazenes has been the subject of active investigation for over 30 years.[1] Many members of that class have been shown to have mutagenic,[2] carcinogenic,[3] and antitumor[4] properties. One of these triazenes, 5-(*N*,*N*-dimethyltriazeno)imidazole-4-carboxamide (DTIC) is in use clinically as an agent against metastatic melanoma.[5] A few years ago, we reported on

† This paper is dedicated to Prof. Vladimir Prelog, ETH, Zürich, on the occasion of his 80th birthday.

(1) For a recent review of triazene chemistry and biology, see: Kolar, G. In *Chemical Carcinogens*, 2nd ed.; Searle, C. E., Ed.; ACS Monogr. 184; American Chemical Society: Washington, DC, 1984; pp 869–914.
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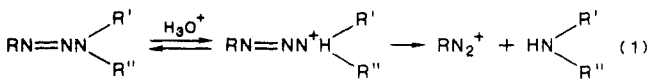
0022-3263/86/1951-3751$01.50/0

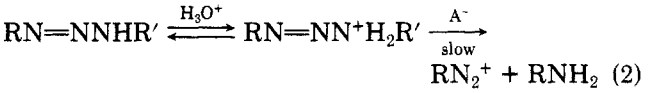
the first general synthesis of a new class of triazenes, 1,3,3-trialkyltriazenes.[6,7] These compounds were prepared by indirect alkylation of the previously known 1,3-dialkyltriazenes.[8] The earlier syntheses of the latter, however, were very cumbersome, and thus our modifications have provided a ready access to these interesting compounds.

The new triazenes are very sensitive to proteolytic decomposition. Most of them are stable in aprotic media or as pure compounds but are rapidly hydrolyzed in water. We have shown[9,10] that the trialkyltriazenes decompose in aqueous buffers by a specific acid catalyzed reaction to the corresponding alkyldiazonium ions and amines (eq 1).



(1)

The aqueous decomposition of 1,3-dialkyltriazenes also results in the formation of diazonium ions, although the mechanism of this decomposition is somewhat more complicated. The reaction is still catalyzed by specific acid, but in the presence of oxyacid buffers (e.g., phosphate, carbonate, arsonate) the slow step in the reaction is catalyzed by general base[11] (eq 2). The nature of the general



(2)

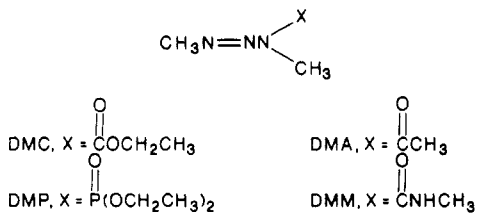
base catalysis is uncertain but appears to involve a nucleophilic attack on N-2. Thus, di- and trialkyltriazenes form alkyldiazonium ions under extraordinarily mild conditions and would be expected to be powerful biological alkylating agents. Indeed, we found various trialkyltriazenes to be potent, directly acting, bacterial mutagens.[12]

One important impediment to the study of the biological properties of triazenes is their instability in aqueous solutions close to neutral pH. Thus, we sought to attenuate this reactivity by substituting 1,3-dialkyltriazenes with acyl groups. We had previously noted that 1,3-dimethyl-3-acetyltriazene was much more stable in aqueous solution than either di- or trialkyltriazenes.[9] This paper reports on our studies of the chemistry of these acylated derivatives, together with some preliminary biochemical and biological data.

The compounds in the present study included 3-carboethoxy-1,3-dimethyltriazene (DMC), 3-(diethoxyphosphinyl)-1,3-dimethyltriazene (DMP), 3-(*N*-methylcarbamoyl)-1,3-dimethyltriazene (DMM), and 3-acetyl-1,3-dimethyltriazene (DMA).

Experimental Section

Safety Note. The preparation of these triazenes involves the use of the potentially hazardous methyl azide. This treacherous substance should be handled with great care. Its explosion propensity is similar to that of diazomethane and consequently no ground-glass joints or sharp edges ought to be present in the apparatus. The reaction vessels should be protected from strong light.[11]



Triazenes of all kinds are biological alkylating agents and should be considered to be toxic compounds and potentially carcinogenic. Adequate safeguards (efficient hoods, protective clothing, no exposure to vapors) should be enforced in these experiments.

Materials. The preparations of 1,3-dimethyltriazene (DMT) and 1,3-dimethyl-3-acetyltriazene (DMA) were described earlier.[7] All chemicals used in the syntheses were reagent grade (Aldrich) and were used without additional purification. Buffers were prepared as described previously[9] by using water that was distilled from $KMnO_4$. Measurements of pH were made with an Orion Model 710 digital pH meter using a Fisher (13-639270) high ionic strength combination electrode (calomel reference). IR spectra were obtained on a Perkin-Elmer Model 297 infrared spectrophotometer. UV–vis spectra were obtained on a Hewlett-Packard double beam diode array processor, Model 8450A, or a Cary, Model 14, spectrophotometer. NMR spectra were obtained on a Nicolet NT-300 spectrometer or a Varian XL-200 spectrometer. Mass spectra, including exact mass measurements, were carried out on a VG-Micromass ZAB-2F spectrometer equipped with a VG data system, Model 2035, or a VG-Micromass Model 7070 spectrometer.

3-Carboethoxy-1,3-dimethyltriazene (DMC). A solution of 1,3-dimethyltriazene (8.0 g, 0.11 mol) in anhydrous ether (30 mL) was added dropwise over 10 min to a stirred suspension of potassium hydride (~4.8 g, 0.12 mol) in anhydrous ether (50 mL) containing 50 mg of dicyclohexano-18-crown-6 ether at 25 °C under nitrogen. The suspension was stirred for 3 h and then cooled to 0 °C. A solution of ethyl chloroformate (13.0 g, 0.120 mol) in anhydrous ether (30 mL) was then added dropwise over 30 min. The reaction mixture was allowed to warm gradually to 25 °C, stirred overnight, and then hydrolyzed by the dropwise addition of 20 mL of 5% sodium bicarbonate at 0 °C. An additional 20 mL of cold water was added to dissolve remaining salts, and the ether layer was separated. The cold aqueous layer was reextracted with 40 mL of ether. The combined ether layers were dried with sodium sulfate, and the ether was removed by distillation through a 4-in. Vigreux column at atmospheric pressure. The residue was then fractionally distilled at reduced pressure to give 12.1 g (0.834 mol, 76.5%) of DMC as a colorless liquid: bp 68–69 °C (8 mm); IR (CCl_4) 2980, 2920, 1715, 1160 cm^{-1}; UV (CH_3CN) λ_{max} 232 nm (log ϵ 4.11); ^1H NMR ($CDCl_3$, Me_4Si) δ 1.38 (3 H, t, J = 7 Hz), 3.23 (3 H, s), 3.67 (3 H, s), 4.38 (2 H, q, J = 7 Hz); proton decoupled ^{13}C NMR ($CDCl_3$, Me_3Si) δ 14.32, 29.23, 48.89, 62.73; exact mass calcd m/z for $C_5H_{11}N_3O_2$ 145.0851, found 145.0851.

3-(Diethoxyphosphinyl)-1,3-dimethyltriazene (DMP). The reaction of 1,3-dimethyltriazene (4.0 g, 55 mmol) with diethyl chlorophosphate (9.7 g, 56 mmol) was carried out in a manner analogous to that used in the preparation of DMC. Following hydrolytic workup, drying of the ether extract with sodium sulfate, and concentration at atmospheric pressure, the residue was fractionally distilled at reduced pressure to give 5.8 g (28 mmol, 51%) of DMP as a colorless liquid: bp 57 °C (0.09 mm); IR (CCl_4) 2990, 2910, 1280, 1260, 1170, 1025, 960 cm^{-1}; UV (CH_3CN) λ_{max} 222 nm (log ϵ 3.90); ^1H NMR ($CDCl_3$, Me_4Si) δ 1.35 (6 H, t, J = 6.9 Hz), 3.08 (3 H, d, J = 7.0 Hz), 3.61 (3 H, s), 4.16 (4 H, m); proton decoupled ^{13}C NMR ($CDCl_3$, Me_3Si), δ 15.93 (d, J_{C-P} = 6.8 Hz), 30.66 (d, J_{C-P} = 6.9 Hz), 48.77, 63.51 (d, J_{C-P} = 5.1 Hz). DMP fails to give a molecular ion under normal EI conditions. However, under conditions of high sample pressure, autochemical ionization occurs and an acceptable M + 1 peak is observed: exact mass calcd m/z (M + 1) for $C_6H_{17}N_3O_3P$ 210.1008, found 210.0994.

1,3-Dimethyl-3-(*N*-methylcarbamoyl)triazene (DMM). A solution of 1.8 mL of methyl isocyanate (1.7 g, 30 mmol) in dry

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(7) Smith, R. H., Jr.; Michejda, C. J. *Synthesis* **1983**, 476–477.
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(12) Sieh, D. H.; Andrews, A. W.; Michejda, C. J. *Mutat. Res.* **1980**, *73*, 5714–5718.

petane (10 mL) was added dropwise to a solution of 1,3-dimethyltriazene in dry petane (20 mL) under nitrogen at a rate to just maintain a gentle reflux of the solvent. Cooling to room temperature produced a solid, white mass. The precipitate was filtered and washed with ice-cold pentane (30 mL). Recrystallization from hot pentane with cooling to −25 °C afforded 3.0 g (23 mmol, 77%) of pure DMM as colorless flat needles: mp 57.5–58 °C; IR (CCl_4) 3450, 2960, 2910, 1695, 1510, 1050 cm^{-1}; UV (CH_3CN) λ_{max} 244 (log ϵ 3.96); ^1H NMR ($CDCl_3$, Me_3Si) δ 2.94 (3 H, d, J = 5 Hz), 3.22 (3 H, s), 3.59 (3 H, s), 6.3 (1 H, br); proton decoupled ^{13}C NMR ($CDCl_3$, Me_4Si) δ 26.50, 27.06, 48.59, 155.2; exact mass calcd m/z for $C_4H_{10}N_4O$ 130.0841, found 130.0853.

Product Studies. A mixture of DMP (215 mg, 1.03 mmol) in 10 mL of water distilled from $KMnO_4$ (pH 5.7) was stirred at 25 °C for 2.5 h; slow evolution of gas was evident. The resulting solution was extracted with methylene chloride (2 × 25 mL). The methylene chloride extract was dried with sodium sulfate and concentrated on a rotary evaporator at 25 °C to give 160 mg (9.6 mmol, 93%) of diethyl *N*-methylphosphoramidate. The structure and purity of the product were confirmed by comparison of the ^1H NMR spectrum with that of an authentic sample prepared from the reaction of diethyl chlorophosphate with 2 equiv of aqueous methylamine: ^1H NMR ($CDCl_3$, Me_4Si) δ 1.34 (6 H, t, J = 7 Hz), 2.61 (4 H, d broadened, J = 11.7 Hz), 4.10 (4 H, m). D_2O shake sharpened δ 2.61 (3 H, d) and 4.8 (HOD).

A mixture of DMC (200 mg, 1.38 mmol) and 25 mL of water distilled from $KMnO_4$ (pH 7.0) was stirred at 25 °C for 18 h. A slow evolution of gas was observed. The resulting solution was extracted with methylene chloride (3 × 25 mL). The methylene chloride was dried with sodium sulfate and concentrated on a rotary evaporator at 25 °C to give 162 mg of a colorless oil. ^1H NMR analysis showed this oil to contain only unreacted DMC (0.18 mmol) and ethyl *N*-methylcarbamate (0.99 mmol, 83% based on unrecovered DMC). The structure of the latter compound was confirmed by ^1H NMR comparison with an authentic sample. A concurrent experiment with ethyl *N*-methylcarbamate revealed no significant hydrolytic decomposition of the carbamate under these conditions.

In a similar experiment using 1 mmol of DMC in 20 mL of pH 7.4, 1.0 M phosphate buffer (μ = 2.25 M maintained with $NaClO_4$) the yield of ethyl *N*-methylcarbamate was 89%. All of the DMC had decomposed during the reaction time (18 h).

Deuterium for Proton Exchange in the Methyldiazonium Ion. This experiment was carried out in the same manner as previously described.[10] DMC (0.145 g, 1 mmol) in 25 mL of pH 7.4, 1.0 M phosphate buffer (μ = 2.25 M ($NaClO_4$)) prepared in D_2O was allowed to decomose for 14 h at 20 °C. The methanol formed during the reaction was isolated by distillation in a microstill. The analysis of the deuterium content was carried out on a VG-Micromass ZAB-2F mass spectrometer operating in the electron impact mode at a resolution of 35 000. A peak matching unit, interfaced to a VG-2035 data system, was used to monitor CH_4O^+ (m/z 32.0262), CH_3DO^+ (m/z 33.0325), $CH_2D_2O^+$ (m/z 34.0388), CHD_3O^+ (m/z 35.0450), and CD_4O^+ (m/z 36.0613) and the reference N_2 (28.0061). Correction factors were applied for the differences in energy of the measured ions and the relative percentages of each species were calculated on the basis of the total amount of nitrogen present.

Kinetic Measurements. The rates were followed spectrophotmetrically with either a Cary Model 14 UV–vis spectrophotometer or a Hewlett-Packard Model 8450A diode array spectrophotometer. The reaction solutions were contained in thermostated 1-cm cells, and the temperature was held constant to within ±0.1 °C. The decomposition of each triazene was followed by monitoring the absorbance at its respective λ_{max} (see individual syntheses sections). The reaction cuvette was charged with the appropriate buffer (1.341 mL), and the reaction was initiated by the addition of 9 μL of a 3 × 10^{-3} M solution of the triazene in acetonitrile. The reference contained the same buffer (1.341 mL) and 9 μL of acetonitrile. A minimum of 100 absorbance vs. time points, collected over 3.5 half-lives, were used in determining the first-order rate constants from a computer program employing the Guggenheim approximation least-squares method.[13]

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The only exception involved treatment of the data for the decomposition of DMC at pH >9.5 where absorbance data indicated two consecutive decomposition steps. In this case an iterative curve fitting program[14] written by us for an Apple II+ computer was used to obtain the best value for k_1 (see Results section) by using an experimentally determined value of k_2 for the decomposition of DMT under identical conditions. Each rate constant represents an average of two determinations. When the deviation was >3% of the mean, three or more runs were averaged to obtain a more accurate value.

Activation parameters were obtained for the decomposition of DMP, DMC, and DMA in 0.10 M lysine buffer (μ = 0.25 M ($NaClO_4$)) at pH 7.5. Rate constants were obtained over the 25–50 °C range for DMP and DMC (six temperatures) and over the 35–50 °C range for DMA (four temperatures). No attempt was made to correct the pH for temperature since the reactions were pH independent in this range. The enthalpies and entropies of activation were obtained from the slopes and intercepts, respectively, of the graphs of log (k_{obsd}/T) vs. $1/T$. The lines were calculated by linear regression analysis of the data and the correlation coefficients were found to be better than 0.999.

Mutagenesis Experiments. The *Salmonella typhimurium* assay was used essentially as described by Ames et al.,[15] with modifications as described by Andrews et al.[16] The compounds were screened by using all five primary tester strains (TA 1535, TA 1537, TA 1538, TA 98, and TA 100), with or without added Aroclor 1254-stimulated rat liver postmitochondrial supernatant (S9 fraction). Dose–response data were obtained over the range of 0.01 μmol/plate of compound to 10 μmol/plate by using the TA 1535 strain, which proved to be the most sensitive strain for these compounds. In the case of DMC, additional dose–response data were obtained with added S9 fraction or with various amounts of added porcine liver esterase (Sigma Chemical Co., Type II, carboxyl esterase, EC 3.1.1.1, 120 units/mg protein). The experiments were carried out by adding an aliquot of 100 μL of an appropriate solution of each compound in dimethyl sulfoxide (Me_2SO) to each dish. The dishes contained 0.2 mL of the tester strain, 20 mL of VBE agar, and, if required, 75 μL of the S9 fraction or the requisite amount of esterase dissolved in sterile water. The plates were incubated at 37 °C for 48 h. The revertant colonies were counted using a hand-held tally.

Results

Synthesis of Acyl Triazenes. The introduction of the acyl substituent on 1,3-dimethyltriazene was a smooth reaction involving the reaction of the anion of the triazene with the corresponding acyl halide. The direct acylation of the triazene was very sluggish or would not proceed at all, in keeping with the previously observed[6] low nucleophilicity of dialkyltriazenes. The sole exception was the synthesis of the carbamoyl triazene DMM. In that case, 1,3-dimethyltriazene reacted smoothly with methyl isocyanate in pentane to produce a good yield of DMM.

pH Dependence of the Rate of Decomposition of DMP. The rate of decomposition of DMP was determined over the pH range 2.5–11.5 in lysine buffer. The observed rate constants, which were cleanly first-order, are listed in Table I. The graphic display of these data in Figure 1 clearly shows the biphasic nature of the dependence of rate on pH. In the pH range 2.5–3.5 the rate was inversely proportional to pH. Above pH 4.5 the rate was independent of pH.

pH Dependence of the Rate of Decomposition of DMC. Determination of the rate of decomposition of DMC was made over the pH range 1.8–11.5 in lysine buffer. The observed rate constants are listed in Table

(14) The program used the basic algorithm (with the P. Lepsa modification) presented in: Wiberg, K. B. *Physical Organic Chemistry*; Wiley: New York, 1964; pp 567–574.

(15) Ames, B. N.; McCann, J.; Yamasaki, E. *Mutat. Res.* **1975**, *31*, 347–364.

(16) Andrews, A. W.; Thibault, L. H.; Lijinsky, W. *Mutat. Res.* **1978**, *51*, 311–318.

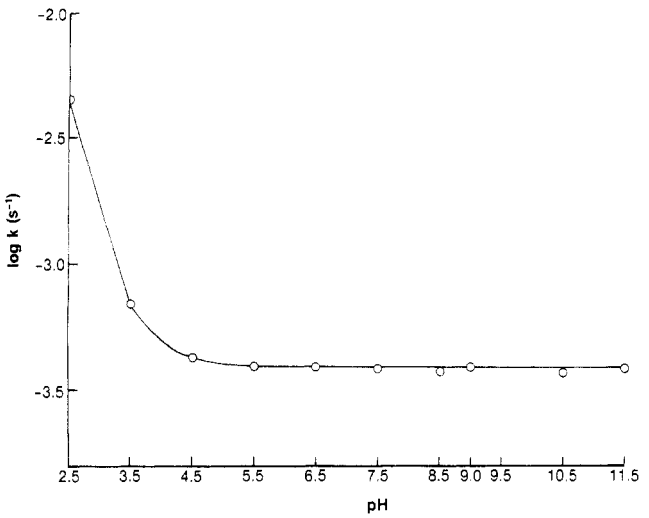


Figure 1. pH profile for the decomposition of 3-(diethoxyphosphinyl)-1,3-dimethyltriazene (DMP) in 0.10 M lysine buffer.

Table I. pH Profile for the Rate of Decomposition of DMP, DMC, and 1,3-Dimethyltriazene (DMT) in 0.10 M Lysine Buffer[a] at 25 °C

pH	DMP 10^4k_{obsd}, s^{-1}	DMC 10^5k_{obsd}, s^{-1}	DMT 10^5k_{obsd}, s^{-1}
1.8		146 ± 0.3	
2.5	44.3 ± 0.5	30.0 ± 1.1	
3.5	6.88 ± 0.04	3.13 ± 0.11	
4.5	4.26 ± 0.02	1.29 ± 0.11	
5.5	3.96 ± 0.02	1.32 ± 0.01	
6.5	3.86 ± 0.02		
7.5	3.87 ± 0.03	1.28 ± 0.03	1050
8.5	3.78 ± 0.05	1.47 ± 0.01	128.5
9.5	3.96 ± 0.07	1.35 ± 0.03	8.58
		(1.18 ± 0.10)[b]	
10.5	3.72 ± 0.15	1.48 ± 0.04	1.92
		(1.61 ± 0.20)[b]	
11.5	3.86 ± 0.06	9.73 ± 0.32	0.265
		(5.34 ± 0.15)[b]	

[a] The ionic strength was maintained constant at 0.25 M with $NaClO_4$. [b] Values for k_1 estimated by consecutive kinetics calculation using DMT data.

Table II. Comparative Rates of Proteolytic Decomposition of 3-Acyltriazenes at 50 °C and Activation Parameters for DMP, DMC, and DMA[a]

substrate	10^4k_{obsd}, s^{-1}	$\Delta H^{\ddagger}$, kcal mol^{-1}	$\Delta S^{\ddagger}$, eu
DMP	51.6 ± 0.2	18.4	−0.6
DMC	2.81 ± 0.08	23.0	−0.2
DMA	0.714 ± 0.009	22.1	−0.4
DMM	0.288 ± 0.011		

[a] pH 7.5, 0.10 M lysine buffer, ionic strength = 0.25 M maintained with $NaClO_4$.

I, and displayed graphically in Figure 2. The rate constants, calculated by the Guggenheim approximation program were first order in all but the highest pH buffer. In the latter case, the estimated A_∞ value, normally very close to 0 for all other pH values, was approximately one-half that of the initial absorbance in pH 11.5 buffer. Thus, a program employing an iterative method for calculating consecutive first order rate constants was used to obtain the values reported in Table II over the pH range 9.5–11.5, which span the pH region at which the rate of DMT decomposition (see Table I) approaches and then becomes slower than the observed rate of DMC disappearance.

Proton Exchange in the Methyldiazonium Ion. The mass spectrometric analysis of the extent of deuterium

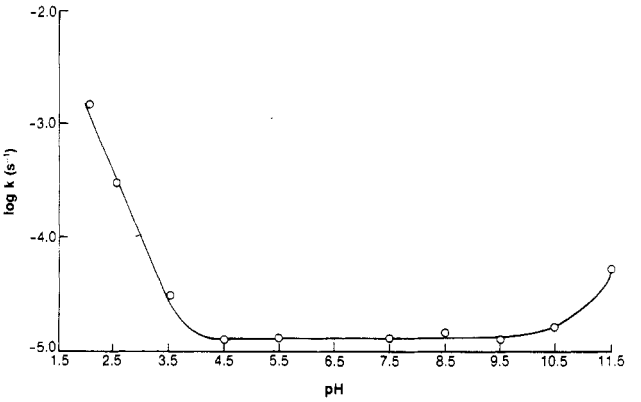


Figure 2. pH profile for the decomposition of 3-carboethoxy-1,3-dimethyltriazene (DMC) in 0.10 M lysine buffer.

incorporation into methanol produced by the decomposition of DMC in pH 7.4 phosphate buffer prepared in D_2O was as follows: CH_3DO, 52.1 ± 1.9%; CH_2D_2O, 34.0 ± 1.1%; CHD_3O, 11.7 ± 0.5%; CD_4O, 2.3 ± 0.1%. These results are for two runs, with each ion scanned at least five times.

The data for DMC may be compared with other sources of the methyldiazonium ion, analyzed in an identical fashion under the same conditions.[10] Thus, for example *N*-nitroso-*N*-methylcarbamate gave the following results: CH_3DO, 49.5 ± 4.5; CH_2D_2O, 35.4 ± 3.4; CHD_3O, 11.7 ± 5.7, CD_4O, 3.3 ± 2.2.

Comparative Rates of Decomposition of 3-Acyltriazenes at pH 7.5 and Temperature Dependence of the Rates of Decomposition of DMP, DMC, and DMA. The comparison of the rates of decomposition of the acylated triazenes DMP, DMC, DMA, and DMM was made at pH 7.5 at 50 °C. The solutions were buffered with lysine. It is clear from the data listed in Table II that there are considerable differences in the hydrolytic stability of the triazenes. The rate of hydrolytic decomposition of DMP is roughly 20 times faster than that of DMC, which is about 4 times faster than the acetyltriazene DMA. The (methylcarbamoyl)triazene DMM is, in turn, about 3.4 times slower than DMA. Thus, the differences between the four compounds span two orders of magnitude in the rates of hydrolytic decomposition. Activation parameters were determined for DMP, DMC, and DMA (Table III). Only DMP exhbited a significantly lower enthalpy of activation than the other two compounds. The difference in the activation parameters between DMC and DMA was not remarkable.

Mutagenesis. All four triazenes were screened against the five primary tester strains of *Salmonella typhimurium*, TA 1535, TA 1537, TA 1538, TA 98, and TA 100, with and without activation by rat liver S9. DMA, DMC, and DMP but not DMM were active in the base pair substitution strains, TA 1535 and TA 100, at a dose of 500 μg/plate of each compound but were not active in the frameshift strains. The active compounds did not require S9 activation. The TA 1535 strain, which appeared to be the most responsive in the preliminary trials, was used for a dose–response study. In the experiments in which DMC was tested, dose response data were obtained for bacterial cultures containing rat liver S9 and also for cultures which contained 10 units of porcine liver esterase. The dose–response data are shown in Table III. It is clear from these data that DMP, DMC, and DMA are directly acting mutagens and exhibit the mutagenic response in a dose-dependent manner. The mutagenic activity of DMC was enhanced by the addition of rat liver S9 or by esterase. DMM was not mutagenic under these conditions.

Table III. Dose-Response Mutagenesis Data for Acyl Triazenes in the *Salmonella typhimurium* Strain TA 1535[a]

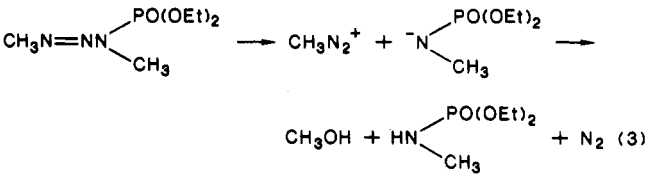
	revertant colonies per plate					
				DMC		
dose, μmol/plate	DMA[b]	DMP	DMM	-S9	+S9	+esterase[c]
0	27	27	22	16	11	11
Me_2SO	19	22	14	8	9	12
10	(1479)	(4423)	9	(1044)	(1900)	(2683)
5	(2552)	(5467)	24	(335)	(957)	(1044)
2.5	(1465)	(2857)	8	(75)	(139)	(141)
1	(1088)	(348)	21	10	(218)	(212)
0.5	31	20		12	21	21
0.01	10			16	20	9

[a] Controls were cells alone (0) and cells treated with dimethyl sulfoxide (Me_2SO). The criterion for positive activity (values in parentheses) was the number of revertant colonies per plate greater than twice the mean of the control plates. [b] Preliminary experiments indicated that inclusion of the S9 mix in the bacterial incubations did not affect the mutagenicity of DMA, DMP, and DMM. The values quoted here are without S9 activation. [c] 10 units of esterase per plate, where 1 unit is the amount of esterase required to hydrolyze 1 μmol of ethyl butyrate per minute at pH 8.0, 25 °C.

Discussion

Previous work on di- and trialkyltriazenes has established that these substances are convenient sources of alkyldiazonium ions.[9-11] These reactive ions are considered to be the ultimate carcinogenic metabolites from a variety of carcinogens, including *N*-nitroso compounds, alkylhydrazines, and 1-aryl-3,3-dialkyltriazenes.[17] The alkyl triazenes have provided a very useful tool for the study of the chemical properties of alkyldiazonium ions. Moreover, it would be expected that triazenes would exhibit potent biological activity without the necessity of enzymatic activation systems. We were able to show that trialkyltriazenes are potent bacterial mutagens[12] and that they are capable of alkylating DNA in vitro and in vivo.[18] The alkyltriazenes, however, are so readily decomposed in aqueous solutions that we sought ways of attenuating this reactivity. Since the decomposition of di- and trialkyltriazenes depends on protonation at N-3 (eq 1 and 2) and trialkyltriazenes are somewhat more reactive than dialkyltriazenes,[11] an obvious way to accomplish this goal was to substitute N-3 with strongly electron-attracting acyl groups. This strategy was successful, but the mechanism of the decomposition of the acyltriazenes proved to be very different from the alkyl analogues.

The 3-(diethoxyphosphinyl)triazene, DMP, decomposed in aqueous solutions with clean, first-order kinetics over a broad pH range. However, a plot of the rate constants vs pH (Figure 1) revealed a biphasic pH profile. The reaction was dependent on acid in the range of pH 2.5–4.5 but then became pH independent up to pH 11.5, the highest pH investigated. These data may be compared with those for the aqueous hydrolysis of diethoxyphosphinic *N*-methylamide, investigated by Kühne and co-workers.[19] The pH profile for this reaction, obtained at 80 °C, indicated that the reaction was acid-catalyzed in the region of pH 2–7 and then became base-catalyzed. The rates were generally several orders of magnitude slower than the triazene analogue. In the acid-catalyzed region, the reaction proceeded by a P–N bond scission, while in the base-catalyzed region, C–O cleavage predominated. Thus, it appears that, in the acid-catalyzed region, our phosphinyltriazene reacts to liberate 1,3-dimethyltriazene, which would be expected to be very short-lived under those conditions.[11] In the pH-independent region (pH 4.5–11.5), the reaction must take an entirely different course. It is significant that the only product that was found during the decomposition of DMP in the pH-independent region was diethoxyphosphinic *N*-methylamide, which was isolated in a 93% yield. The available data are best accounted for by assuming that DMP undergoes a heterolytic N–N bond cleavage to produce a methyldiazonium ion and the stabilized diethoxyphosphinic *N*-methylamide anion. The anion is then protonated by the solvent, and the diazonium ion is hydrolyzed to methanol (eq 3).



It could be argued that an alternative means of decomposition could be an S_N2-like reaction involving water as a nucleophile, which would displace molecular nitrogen and the phosphinic amide anion in a concerted process. Indeed, a reaction analogous to that was observed by Trost and Pearson[20] in the reactions of 1-[(phenylthio)-methyl]-3-alkyl-3-acyltriazenes with nucleophiles in polar, aprotic media. Such a reaction is less likely under our conditions, and, in support of that, direct evidence for the intermediate formation of the methyldiazonium ion was obtained during the decomposition of DMC (see below).

The pH profile for the carboethoxytriazene DMC (Figure 2) indicates that the reaction was acid catalyzed in the pH range of 1.8–4.5, became pH-independent in the range of pH 4.5–8.5, and then was based catalyzed at pH >8.5. In the absence of N–H bonds, simple carbamates undergo hydroxide ion catalyzed hydrolyses which proceed through tetrahedral intermediates to form carbonic acids. These are then readily decarboxylated.[21] Considerably more confusion exists about the detailed mechanism of acid-catalyzed hydrolysis of carbamates. Moodie and Towill[22] argued that hydrolysis of alkylcarbamates in strongly acidic solutions proceeds by N-protonation, followed by nucleophilic addition of water to the carbonyl carbon. If this mechanism operated in the case of DMC, the result would be the formation of ethylcarbonic acid and 1,3-dimethyltriazene. An alternative mechanism might involve an O-protonated intermediate, which would lose the elements of ethanol and result in the formation of 3-carboxy-1,3-

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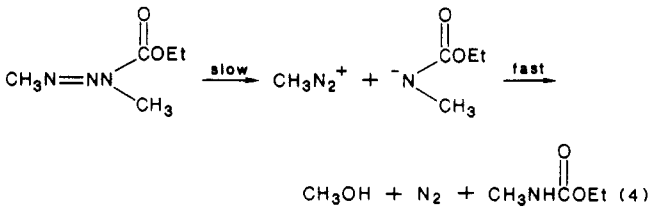
dimethyltriazene. Our data do not permit us to distinguish between these mechanisms. The results, however, would be the same: the dimethyltriazine would be liberated and would suffer rapid, acid-catalyzed decomposition. The pH-independent domain for DMC decomposition appears to involve direct dissociation to the methyldiazonium ion and the *O*-ethyl *N*-methylcarbamyl anion. In keeping with this hypothesis, ethyl *N*-methylcarbamate was isolated from the decomposition in high yield. It was found to be stable under our conditions. Thus, DMC decomposition (eq 4) is analogous to the decomposition of DMP in the uncatalyzed region.



It is interesting to note that in the base-catalyzed region, the reaction at the carboethoxy residue is more rapid than the acid-catalyzed decomposition of 1,3-dimethyltriazene. It was necessary to apply an iterative method, using independently obtained values for the rate of decomposition of 1,3-dimethyltriazene (Table I), to calculate the rate constants for the carbamate. The corrected numbers were used in Figure 2.

Support for the formation of the methyldiazonium ion was obtained by carrying out the hydrolysis in buffered D_2O. We had shown earlier[10] that deuterium incorporation into the product methanol can be used as a diagnostic criterion for the methyldiazonium ion, which exists in equilibrium with diazomethane in aqueous solution. It should be pointed out that this method allows one to distinguish between those reactions in which the diazonium ion is the first formed intermediate and those were the diazoalkane is formed first. In the first instance, the methanol would have a significant amount of unexchanged methanol and decreasing amounts of methanol-d_1, -d_2, and d_3. In the second case there should be little or no methanol-d_0 and methanol-d_1 would predominate. Clearly, our data favor the first of the two cases, as do the methanols derived from other methyldiazonium ion sources such as ethyl *N*-nitroso-*N*-methylcarbamate, *N*-nitroso-*N*-methylurea, (acetoxymethyl)methylnitrosamine and 1,3,3-trimethyltriazene.[10]

The kinetic data on DMP and DMC suggest that the dissociative mechanism may be a general reaction for acyltriazenes at near neutral pH. Although pH profiles were not determined for DMA and DMM, their behavior is entirely consistent with this mechanism. Comparison of the rates of decomposition for the four acyltriazenes at 50 °C at pH 7.5 (Table II) indicates the order of DMP > DMC > DMA > DMM. This order would be expected to be governed by the relative abilities of the four acyl substituents to stabilize a neighboring negative charge. The phosphorus substituent would be expected to be most efficient in that regard because of the well-known ability of the diethoxyphosphinyl group to stabilize a neighboring negative charge (e.g., the Wadsworth–Emmons intermediate phosphoramidate anions[23]). The order of CO(OEt) > $C(O)CH_3$ > $C(O)NHCH_3$ is reasonable since it mirrors the changes in electronegativity of the carbonyl carbon. Thus, the order of reactivity of the four acyltriazenes is seen to be the same as the expected order of stability of the respective anions.

The activation parameters were not particularly informative except that the entropy of activation values close to zero are consistent with a mechanism which resembles a unimolecular solvolysis.[24]

Uncatalyzed hydrolysis, similar to that proposed in this work, was observed by Pytela and co-workers[24] during their studies of the hydrolytic decomposition of 3-acyl-1,3-diphenyltriazenes. These workers performed detailed kinetic investigations of 3-acetyl-, 3-(*N*-methylcarbamoyl)-, and 3-(*N*-phenylcarbamoyl)-1,3-diphenyltriazenes, as well as on many symmetrically disubstituted 3-carbamoyl- and 3-acetyl-1,3-di(3- and 4-X)phenyltriazenes. They concluded that the hydrolysis in the acid-catalyzed region proceeded directly to the respective acylamides and the phenyldiazonium ions. The noncatalyzed region reaction was explained by the formation of the acylamide and the phenyldiazonium ion with water acting as the catalyst. The base-catalyzed reaction involved the initial formation of the 1,3-diphenyltriazenyl anion and the carboxylate ion. While these reactions are related to the processes observed in the present study, there is a considerable difference between the substrates. The aryltriazenes lead to the relatively stable aryldiazonium ions which allow reactions to proceed reversibly. In the present case, methyldiazonium ions, once formed, must proceed on to products. Thus, there is no a priori reason to assume that the two types of substrates would behave analogously. Indeed, in our earlier studies on the hydrolytic decomposition 1,3,3-trialkyltriazenes and 1,3-dialkyltriazenes, both of which involve specific acid catalysis, we noted the differences between those reactions and the general acid catalyzed reactions of 1-aryl-3-alkyltriazenes.

The dissociation of the acyltriazenes into methyldiazonium ions suggested that these materials ought to be biological alkylating agents. We had previously shown[12] that trialkyltriazenes, which dissociate into alkyldiazonium ions in an acid-catalyzed reaction, are potent, directly acting mutagens in the *Salmonella typhimurium* bacterial assay developed by Ames and co-workers.[15] It was of interest, therefore, to test the acyltriazenes in an analogous manner. It is clear from the data presented in Table III that DMP, DMC, and DMA are strongly mutagenic in this assay. Curiously, DMM is not mutagenic under these conditions, which, however, does not mean that it could not become a mutagen in one of the other variants of the Ames assay (e.g., the liquid preincubation assay). The fact that the three active triazenes express their mutagenicity in the base pair substitution strain TA1535 is a good indication that the DNA of the bacteria is being damaged by alkylation. In the case of DMA and DMP, addition of rat liver homogenates (S9) to the incubation mixtures failed to increase or to decrease the mutagenic response. In the case of DMC, however, the mutagenic response was enhanced by the addition of S9. A similar enhancement could be produced by adding porcine liver esterase to the incubation mix. In separate experiments (data not shown) we were able to establish that DMC was a substrate for the esterase (K_M = 148 μM, estimated from a Lineweaver–Burke plot). We suspect that the rat liver S9 has an

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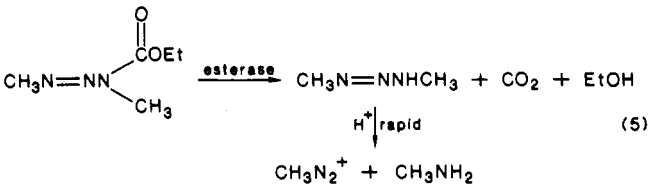
enhancing effect on the mutagenicity of DMC because it also contains nonspecific esterases. The esterase enhancement appears to be due to the liberation of 1,3-dimethyltriazene from DMC according eq 5.



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Registry No. DMT, 3585-32-8; DMA, 87031-64-9; DMC, 88211-12-5; DMP, 103711-39-3; DMM, 103711-40-6; diethyl *N*-methylphosphoramidate, 6326-73-4; ethyl *N*-methylcarbamate, 105-40-8.

Electrochemically Induced Aromatic Substitution. The 2-Nitropropane Anion, a Powerful Nucleophile in $S_{RN}1$ Aromatic Substitution

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In contrast with previous reports, the 2-nitropropane anion reacts readily with aryl radicals in the context of aromatic $S_{RN}1$ processes. This is shown in the examples of 4-bromobenzophenone and iodobenzene under electrochemical stimulation. The substitution products do not, however, result from the simple addition of the 2-nitropropyl anion on the aryl radical. The ensuing anion radical is indeed unstable, cleaving off a nitrite ion and thus leading to the cumene derivative as the main substitution product. Comparison of reactivity with diethyl phosphite and thiophenoxide ions shows that the 2-nitropropane anion is a quite powerful nucleophile in aromatic $S_{RN}1$ reactions.

$S_{RN}1$ aromatic substitution[2a] is a well-documented reaction.[2-4] To proceed, it requires the injection of electrons which can be provided photochemically,[2] or electrochemically[3] or by alkali metals dissolved in liquid ammonia[2] or by other redox reagents.[3b,c,f,h,4] As suspected from the effect of radical scavengers on the reaction[2b,g] and demonstrated on electrochemical grounds,[3] the reaction mechanism involves as starting step the transfer of an electron to the substrate, ArX, followed by the cleavage of the resulting anion radical $ArX^{\bullet-}$. The latter cleaves off the nucleofugal group X^- (commonly a halide ion) giving rise to a σ-aryl radical which is the actual electrophile. This combines with the nucleophile, leading to the anion radical $ArNu^{\bullet-}$ of the substitution product. Removal of an electron from $ArNu^{\bullet-}$ yields the final substitution product ArNu. Reaction 4 represents the removal of the $ArNu^{\bullet-}$ odd electron by the same reactant, electrode, or redox reagent that served to inject an electron into ArX. Reaction 5 shows the possibility of a chain process when $ArNu^{\bullet-}$ transfers its odd electron to the substrate itself.

$$ArX + e^- \rightleftarrows ArX^{\bullet-} \quad (1)$$

$$ArX^{\bullet-} \rightarrow Ar^{\bullet} + X^- \quad (2)$$

$$Ar^{\bullet} + Nu^- \rightarrow ArNu^{\bullet-} \quad (3)$$

$$ArNu^{\bullet-} - e^- \rightarrow ArNu \quad (4)$$

$$ArNu^{\bullet-} + ArX \rightarrow ArNu + ArX^{\bullet-} \quad (5)$$

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