Acid-Catalyzed Decomposition of 1-Alkyltriazolines: A Mechanistic Study

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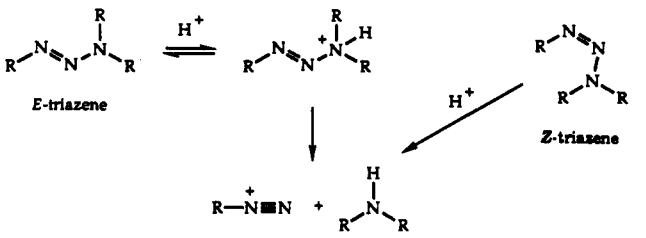
1-Alkyltriazolines are five-membered cyclic triazenes containing the unusual *Z*-configuration for the triazene moiety. The hydrolytic decomposition of these compounds in aqueous or mixed acetonitrile–aqueous buffers leads predominantly to the formation of the corresponding 1-alkylaziridines and lesser amounts of 2-(alkylamino)ethanols, alkylamines, and acetaldehyde. The latter two products presumably result from hydrolysis of a rearrangement product, *N*-ethylidenealkylamine. Neither the nature of the 1-alkyl group nor the pH of the medium greatly influences the product distribution, although decomposition in purely aqueous buffers slightly reduces the aziridine yields. The rate of hydrolysis of 1-alkyltriazolines is about twice as fast as that of the analogous acyclic 1,3,3-trialkyltriazenes and varies in the order *tert*-butyl > isopropyl > ethyl > butyl > methyl > propyl > benzyl. The mechanism of the decomposition is specific acid-catalyzed (A1) involving rapid reversible protonation followed by rate-limiting formation of a 2-(alkylamino)ethyldiazonium ion. The slopes of the log k_{obs} versus pH plots are near −1.0. The solvent deuterium isotope effect, k_{H_2O}/k_{D_2O}, is in all cases <1.0 and ranges from 0.58 for 1-methyltriazoline to 0.86 for 1-benzyltriazoline. The rate of decomposition shows no significant dependence on the concentration of the buffer acid. The proposed mechanism involves rate-limiting formation of a 2-(alkylamino)ethyldiazonium ion, which is then partitioned among several competing product formation pathways. 1-Alkyltriazolines are potent direct-acting mutagens in the alkylation-sensitive TA 1535 strain of *Salmonella typhimurium*. A clear, dose-dependent mutagenicity is observed. At the highest dose level, various 1-alkyltriazolines have activities roughly equivalent to that of the potent methylating agent, 1,3-dimethyltriazene. At low levels of substrate, 1-alkyltriazolines are significantly more active than 1,3-dimethyltriazene, with mutagenicity following the order benzyl > methyl > ethyl.

Introduction

The chemistry of acyclic 1,3 dialkyl- and 1,3,3-trialkyltriazenes has been extensively studied in our laboratories. Efficient methods were developed for their synthesis from alkyl azides,[1,2] and their hydrolytic decomposition in aqueous buffers was shown to be specific acid-catalyzed, A-1.[3,4] In general, the mechanism involves rapid reversible protonation followed by rate-limiting heterolysis of the N(2)–N(3) bond leading to the formation of an alkyl- or dialkylamine, respectively, from N(3) and an alkyl diazonium ion[5] from N(1), Scheme I. Theoretical calculations[6] suggested that neutral triazenes exist preferentially in the *E*-configuration. Calculated proton affinities show

Scheme I



that N(3)-protonation, though slightly less favorable than N(1)-protonation, yields a stable conjugate acid species which is predisposed to heterolysis of the N(2)–N(3) bond. Similar calculations for *Z*-configuration triazenes predict spontaneous scission of the N(2)–N(3) bond upon the approach of a proton. It is thus possible that *E*- and *Z*-triazenes may follow subtly different acid-catalyzed decomposition mechanisms. Of necessity, small- to medium-sized cyclic triazenes constrain the triazene group to a *Z*-configuration, and therefore cyclic triazenes present an opportunity to explore the chemistry of the *Z*-triazene moiety.

Triazolines, 4,5-dihydro-1,2,3-triazoles, are perhaps the best known simple cyclic triazenes. A variety of methods are available for the preparation of triazolines.[7] The most

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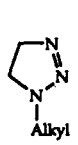
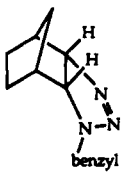
Table I. Structures and Abbreviations of Compounds Investigated





BzHMBT

abbrev	1-alkyl	abbrev	1-alkyl
MT	methyl	iPT	isopropyl
ET	ethyl	tBT	*tert*-butyl
PT	propyl	BzT	benzyl
BT	butyl		

general route to the simplest 1-alkyltriazolines involves the reaction of dimethylsulfoxonium methylide with alkyl azides.[8] The chemistry of triazolines has been dominated by the study of thermolysis reactions.[7] There have, however, been reports of acid-induced decompositions, generally involving bi- and tricyclic 1-aryltriazolines. Several general features of this reaction are perhaps relevant to the chemistry of the simple 1-alkyl analogs. Decomposition appears to involve protonation of N(1)[9] leading to heterolytic cleavage of the N(1)–N(2) bond. The resultant 2-(alkylamino)ethyldiazonium ion then suffers a variety of fates dependent upon the specific structure of the compound and the nature of the reaction medium. The most common pathway is displacement of molecular nitrogen by nucleophiles present in the solvent,[10] possibly involving an aziridinium ion intermediate.[11] Actual isolation of an aziridine product, formed by an intramolecular nucleophilic displacement of nitrogen, occasionally has been observed.[12] Further, an aziridine intermediate has also been postulated as responsible for the formation of a variety of rearranged products.[13] On the other hand, in other cases rearrangement has been reported to occur without the intermediacy of an aziridine.[7] In no instance have simple 1-alkyltriazolines been investigated, and in all cases, detailed mechanistic have not been undertaken.

Recently, we reported on a theoretical investigation of the proton-induced decomposition of monocyclic triazolines. Those results suggested that protonation of N(1) leads spontaneously to heterolysis of the N(1)–N(2) bond followed by collapse to an aziridinium ion with the expulsion of molecular nitrogen.[14] In the present work, we describe the results of an experimental investigation of the products and mechanism of this reaction, the acid-induced decomposition of 1-alkyltriazolines in aqueous buffers. The compounds studied are listed in Table I.

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(9) It should be noted that the numbering of the triazene moiety is different in cyclic and acyclic triazenes. The saturated nitrogen in acyclic triazenes is N(3), while in cyclic triazenes it is designated as N(1).

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Experimental Section

Safety Note. Because acyclic triazenes are potent biological alkylating agents, it is only prudent to assume that triazolines are also potentially toxic and carcinogenic. At all times, efficient hoods and protective clothing should be used in working with these substances. Alkyl azides are treacherously explosive and should be treated with extreme caution. Wherever possible, these compounds should only be handled in solution.

Materials. All chemicals were reagent grade (Aldrich Chemical Co.) and were used as purchased without further purification. The synthesis of the various alkylazides used in the preparation of 1-alkyltriazolines has been reported previously.[2,4,5] The various 1-alkyltriazolines were prepared by the reaction of the corresponding alkylazides with a slight excess of dimethyl sulfoxonium methylide.[9] A crucial modification of the previously reported method, which significantly improves the isolated yields, was the use of an aqueous 1.0 N NaOH solution, instead of water, for hydrolysis of the initial reaction mixture. 1-Ethyl- and 1-benzyltriazoline (ET and BzT, respectively) are known compounds.[9] The preparation of 1-methyltriazoline, MT, has recently been reported.[15] Previously unavailable NMR and UV spectral data for the all of the 1-alkyltriazolines synthesized herein are reported below. For the sake of completeness, the data for 1-methyltriazoline are included. 1-Benzyl-3a,4,5,6,7,7a-hexahydro-4,7-methano-1*H*-benzotriazole, BzHMBT, was prepared by the cycloaddition of benzylazide to norbornene.[16]

A Fisher Accumet Model 825MP digital pH meter and a Fisher (13-620-270) high ionic strength combination electrode (calomel reference) were used in pH measurements. UV spectra were recorded on either a Hewlett-Packard Model 8450A double-beam diode-array processor or a Shimadzu Model UV-2100 spectrophotometer. NMR spectra were obtained on a Varian XL-200 spectrometer.

1-Alkyltriazolines are extremely sensitive to hydrolytic decomposition, and thus combustion analysis is not an appropriate method of establishing purity or identity. The alternative method, a high-resolution mass spectral molecular formula determination, was instead chosen. Exact mass measurements were determined on a VG 70-250 mass spectrometer using a peak matching technique. All samples submitted for exact mass determination were shown to be >97% purity by ^{1}H NMR analysis. (See supplementary material for availability of these spectra.)

1-Methyltriazoline (MT).[15] Obtained in 3.3% yield following purification by distillation, a colorless liquid, bp 55 °C at 8 mm: UV (CH_3CN) λ_{max} 239 nm (log e 3.53), 264 nm (log e 3.45); ^{1}H NMR ($CDCl_3$, Me_4Si) δ 2.95 (2 H, t, J = 10.7 Hz), 3.25 (3 H, s), 4.16 (2 H, t, J = 10.7 Hz); proton-decoupled ^{13}C NMR ($CDCl_3$, Me_4Si) δ 38.00, 48.05, 65.57; exact mass calcd m/z for M^+, $C_3H_7N_3$ 85.06399, found 85.06344 (by EI).

1-Ethyltriazoline (ET). Obtained in 24.0% yield following purification by distillation, a colorless liquid, bp 42–44 °C at 4.4 mm: UV (CH_3CN) λ_{max} 240 nm (log e 3.54), 264 nm (log e 3.48); ^{1}H NMR ($CDCl_3$, Me_4Si) δ 1.29 (3 H, t, J = 7.2 Hz), 2.99 (2 H, t, J = 10.8 Hz), 3.58 (2 H, q, J = 7.2 Hz), 4.13 (2 H, t, J = 10.8 Hz); proton-decoupled ^{13}C NMR ($CDCl_3$, Me_4Si) δ 13.26, 45.35, 45.66, 64.68.

1-Propyltriazoline (PT). Obtained in 25.4% yield following purification by distillation, a colorless liquid, bp 50–52 °C at 2.5 mm: UV (CH_3CN) λ_{max} 240 nm (log e 3.55), 264 nm (log e 3.51); ^{1}H NMR ($CDCl_3$, Me_4Si) δ 1.00 (3 H, t, J = 7.4 Hz), 1.70 (2 H, q of t, J = 7.4, 7.2 Hz), 3.00 (2 H, t, J = 10.8 Hz), 3.49 (2 H, t, J = 7.2 Hz), 4.13 (2 H, t, J = 10.8 Hz); proton-decoupled ^{13}C NMR ($CDCl_3$, Me_4Si) δ 11.40, 21.46, 45.74, 52.82, 64.58; exact mass calcd m/z for M^+, $C_5H_{11}N_3$ 113.0953, found 113.0961 (by EI).

1-Butyltriazoline (BT). Obtained in 27.8% yield following purification by distillation, a colorless liquid, bp 29–30 °C at 0.025 mm: UV (CH_3CN) λ_{max} 239 nm (log e 3.58), 264 nm (log

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e 3.52); ^{1}H NMR ($CDCl_3$, Me_4Si) δ 0.97 (CH_3, t, J = 7.3 Hz), 1.42 (CH_3CH_2–, sextet, J = ca. 7.7 Hz), 1.66 ($CH_2CH_2CH_2$, quintet, J = ca. 7.2 Hz), 2.99 (ring CH_2NN, t, J = 11.0 Hz), 3.53 (exocyclic CH_2CH_2N, t, J = 7.1 Hz), 4.13 (ring CH_2N=N, t, J = 11.0 Hz); proton-decoupled ^{13}C NMR ($CDCl_3$, Me_4Si) δ 13.74 (CH_3), 20.02 (CH_3CH_2–), 30.20 ($CH_2CH_2CH_2$), 45.77 (ring CH_2NN), 50.80 (exocyclic CH_2CH_2N), 64.57 (ring CH_2N=N) [^{1}H and ^{13}C NMR peak assignments determined by HETCOR experiment]; exact mass calcd m/z for M^+, $C_6H_{13}N_3$ 127.1109, found 127.1113 (by EI).

1-Isopropyltriazoline (iPT). Obtained in 24.9% yield following purification by distillation, a colorless liquid, bp 25–27 °C at 0.6 mm: UV (CH_3CN) λ_{max} 241 nm (log e 3.61), 265 nm (log e 3.55); ^{1}H NMR ($CDCl_3$, Me_4Si) δ 1.30 (6 H, t, J = 6.6 Hz), 3.00 (2 H, t, J = 11.0 Hz), 3.93 (1 H, septet, J = 6.6 Hz), 4.09 (2 H, t, J = 11.0 Hz); proton-decoupled ^{13}C NMR ($CDCl_3$, Me_4Si) δ 20.59, 42.30, 51.24, 63.88; exact mass calcd m/z for M^+, $C_5H_{11}N_3$ 113.09553, found 113.0959 (by EI).

1-*tert*-Butyltriazoline (tBT). Obtained in 20.4% yield following purification by distillation, a colorless liquid, bp 25–26 °C at 0.3 mm: UV (CH_3CN) λ_{max} 242 nm (log e 3.63), 266 nm (log e 3.58); ^{1}H NMR ($CDCl_3$, Me_4Si) δ 1.35 (9 H, s), 3.04 (2 H, t, J = 10.9 Hz), 4.07 (2 H, t, J = 10.9 Hz); proton-decoupled ^{13}C NMR ($CDCl_3$, Me_4Si) δ 27.68, 41.33, 55.64 (quaternary C), 63.81; exact mass calcd m/z for M^+, $C_6H_{13}N_3$ 127.1109, found 127.1112 (by EI).

1-Benzyltriazoline (BzT). Obtained in 24.0% yield following purification by distillation, a colorless liquid, bp 55–60 °C at 0.005 mm. UV (CH_3CN) λ_{max} 239 nm (log e 3.69), 263 nm (log e 3.63); ^{1}H NMR ($CDCl_3$, Me_4Si) δ 2.88 (2 H, t, J = 10.9 Hz), 4.14 (2 H, t, J = 10.9 Hz), 4.78 (2 H, s), 7.33 (5 H, m); proton-decoupled ^{13}C NMR ($CDCl_3$, Me_4Si) δ 44.73, 55.0, 65.15, 127.71 (quaternary C), 128.43, 128.60, 136.08 (quaternary C).

1-Methylaziridine. Chlorosulfonic acid (8.5 g, 0.073 mol) was added dropwise at 0 °C to 2-(methylamino)ethanol (5.5 g, 0.073 mol). The resulting dark brown mass was heated at 140–150 °C under vacuum (20 mm) for 2 h. The residue was cooled and dissolved in 10 mL of water, and a solution of potassium hydroxide (20 g, 0.356 mol, in 20 mL of water) was added. The resulting solution was gently warmed to 100 °C and 1-methylaziridine distilled off and collected in a dry ice–acetone trap. The yield was 1.22 g (0.214 mol, 29.3%), bp 24–25 °C: NMR ($CDCl_3$, Me_4Si) δ 1.02 (2 H, "t", J = 2.3 Hz), 1.71 (2 H, "t", J = 2.3 Hz), 2.28 (3 H, s), the two "triplets" each have inverted peak ratios of ~2:1:2;[17] proton-decoupled ^{13}C NMR ($CDCl_3$, Me_4Si) δ 28.29, 48.29; exact mass calcd m/z for M^+, C_3H_7N 57.0578, found 57.0576 (by EI).

Product Studies. The products of the decomposition of MT were determined by carrying out the reactions in 0.05 M buffers of sodium phosphate in D_2O adjusted to the appropriate pH with a D_2O solution of NaOD. Because of the low solubility of BzT in aqueous solutions, the decompositions of this compound were performed in mixed solvents, 25% acetonitrile-d_3 (v/v) in 0.05 M sodium phosphate buffers in D_2O. For comparison purposes, MT decompositions were also run in this mixed solvent. In a typical experiment, buffer (or buffer plus acetonitrile-d_3) was added to a weighed amount of the compound. The sample was mixed thoroughly to ensure a homogeneity, and 0.5 mL was removed and placed in a capped NMR tube. The initial ^{1}H NMR spectrum was recorded and then followed periodically until all of the starting triazoline had been decomposed. Between spectral determinations, the reaction tubes were maintained at 25 °C and protected from light. The initial triazoline concentration in each reaction was 0.050 M. The pH of the remaining reaction mixture was measured at the end of the reaction, as determined by ^{1}H NMR analysis. In each case, the pH had changed by no more than ±0.4 pH units. Assignment of the NMR peaks arising from the various products was made by comparison with authentic samples and by coincidence of peaks upon the addition of authentic materials. Yields were determined by comparative integration of the product peaks. The decomposition of 1-methylaziridine was investigated in a similar manner.

Kinetic Studies. The method employed for the preparation of the buffers used in these kinetic studies has been reported previously.[4] Either $NaClO_4$ or Na_2SO_4 was added as an inert salt to maintain constant ionic strength. Buffers containing large amounts of $NaClO_4$ tended to produce somewhat unstable pH readings, a problem not observed when Na_2SO_4 was used. Several experiments were performed in order to correlate kinetic data obtained with buffers containing $NaClO_4$ versus Na_2SO_4 (data not shown). For a given compound, the rate of decomposition was 1.30 ± 0.05 times faster in a buffer containing Na_2SO_4 as compared to one containing $NaClO_4$. The relative rates for different triazolines, however, were the same irrespective of the inert salt used.

Rates of triazoline decomposition in aqueous solution were followed spectrophotometrically using either a Hewlett-Packard Model 8450A double-beam diode-array processor, a Milton Roy Spectronic 3000 (diode) Array, or a Shimadzu Model UV-2100 spectrophotometer. In the case of the Shimadzu spectrophotometer, the analog output of absorbance versus time was recorded using an Apple IIe computer equipped with an Interactive Microwave Ada-Lab data acquisition board. The reaction solutions were contained in thermostated 1-cm cells, and the temperature was held constant to within ±0.1 °C. The disappearance of each triazoline was followed by monitoring the change in absorbance at its highest wavelength λ_{max} (see Materials section). In a typical kinetic run, the reaction cuvette was charged with 1.341 mL of a 0.1 M lysine buffer (ionic strength = 0.25 M maintained with added $NaClO_4$ or Na_2SO_4), and the reaction was initiated by the addition of 9 μL of a 9.0 × 10^{-3} M solution of the 1-alkyltriazoline in acetonitrile; the initial triazoline concentration was thus 6.0 × 10^{-5} M. The reference cuvette contained 1.341 mL of buffer (the addition of 9 μL of acetonitrile proved unnecessary). A minimum of 100 absorbance vs time readings were obtained over 3.5 half-lives. The first order rate constants were calculated from these data by means of a computer program based on the Guggenheim approximation least-squares method.[18]

Mutagenesis Experiments. The *Salmonella typhimurium* assay was used essentially as previously described,[19] with modifications as subsequently suggested.[20] Dose–response data were obtained over the range of 0.001–10.0 μmol/plate of compound by using the TA 1535 strain, which has in the past proved to be the most sensitive strain to triazene alkylation.[21] The experiments were carried out by adding to each dish an aliquot of 100 μL of an appropriate solution of each compound in dimethyl sulfoxide. The dishes contained 0.2 mL of the tester strain and 20 mL of VBE agar. The plates were incubated at 37 °C for 48 h. The revertant colonies were counted by hand-held tally.

Results

Product Studies. The decomposition reaction of each triazoline was followed periodically by ^{1}H NMR analysis, typically at times of 0, 0.5, 5, 24, 48, and 72 h. Product yield data are recorded in Table II for the earliest time at which all of the triazoline had disappeared. Values are reported as a percentage of the total *N*-methyl- or *N*-benzyl-containing products, respectively.

The identity of each product was determined by the addition of standard compounds. In the early stages of the reactions which produce acetaldehyde (and its hydrate), the protons on the α-carbon atom were clearly visible. These disppear, however, with increasing time due to deuterium exchange, a process which occurs most rapidly at elevated pH. The presence of acetaldehyde and its hydrate could still be detected, with somewhat less

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Table II. Products and Yields[a] from the Hydrolysis of 1-Methyl- and 1-Benzyltriazoline at 25 °C in Aqueous Buffers[b] at Various pH Levels[c]

1-methyltriazoline	pH 5.5 [0.5 h]	pH 7.5 [0.5 h]	pH 11.5 [3 d]
1-methylaziridine	53.4 (49.3)[d]	65.3 (48.7)	64.4 (50.0)
2-(methylamino)ethanol	22.8 (21.7)	18.1 (21.5)	15.9 (26.5)
methylamine	23.8 (29)	16.7 (29.8)	19.7 (23.5)
acetaldehyde[e]	7.8 (26.1)	16.7 (28.7)	8.0
1-benzyltriazoline	**pH 5.5 [0.5 h]**	**pH 7.5 [0.5 h]**	**pH 11.5 [24 h]**
1-benzylaziridine	66.3	66.7	73.6
2-(benzylamino)ethanol	12.4	12.5	11.0
benzylamine	21.3	20.8	15.4
acetaldehyde[e]	9.8	16.7	

[a] Yields were determined by ^{1}H NMR analysis and are based on the % of total N-CH_3 signals present in the spectrum. The total reaction time is shown in brackets. [b] The initial triazoline concentration was 0.05 M in each case. The buffer was 25% (v/v) CD_3CN in D_2O containing 0.05 M sodium phosphate. Values in parentheses are for MT in 0.05 M sodium phosphate in D_2O containing no CD_3CN. [c] Nominal pH reading was actually 0.4 pH units lower than that recorded in the table. [d] Present as the 1-methylaziridinium ion at this pH. [e] Observed yield is progressively lowered with time and increasing pH due to deuterium exchange of the α-protons of acetaldehyde. Reported acetaldehyde yield is the total combined yield of acetaldehyde and its hydrate, present in approximately equal amounts.

Table III. pH Profile for Rate[a] of Decomposition of 1-Alkyltriazolines[b] in 0.1 M Lysine Buffers[c] at 25 °C

	compd		
pH	MT	ET	BzT
10.25			6.42
10.50	7.65	9.44	4.26
10.75	4.48	5.52	2.52
11.00	2.61	3.18	1.51
11.25	1.56	1.80	0.726

[a] k (s^{-1}) × 10^3, average of two runs agreeing within ±3%. [b] Triazoline initial concentration: MT, 4.0×10^{-5} M, ET, 8.1×10^{-5} M, BzT, 6.0×10^{-5} M. [c] 0.25 M ionic strength, maintained with added Na_2SO_4.

accuracy, from the aldehydic and methine proton signals, respectively. The identity of the 1-methylaziridinium ion formed from MT at low pH was confirmed by adjusting the pH of the final reaction solution to 11.0 with NaOD in D_2O. The resulting ^{1}H NMR spectrum was then identical to that of the free base, 1-methylaziridine. The pK_a of 1-methylaziridine is reported at 7.86.[22]

Under the reaction conditions it was observed that 1-methyl- and 1-benzylaziridine slowly decompose with half-lives considerably longer than those of the respective triazolines. This decomposition did not, however, lead to the formation of additional amounts of the other products listed in Table II. This observation was confirmed independently for the decomposition of 1-methylaziridine using the same experimental procedures. No more than trace amounts of 2-(methylamino)ethanol, methylamine, or acetaldehyde were detected. The major 1-methylaziridine decomposition product appeared polymeric in nature, as indicated by several broadened methylene signals in the region δ 2.3–3.0. A similar observation of aziridine polymerization in aqueous media has been reported previously.[23] Likewise, the decomposition of 1-benzylaziridine did not lead to the formation of 2-(benzylamino)-ethanol, benzylamine, or acetaldehyde; the major product again appeared to be polymeric.

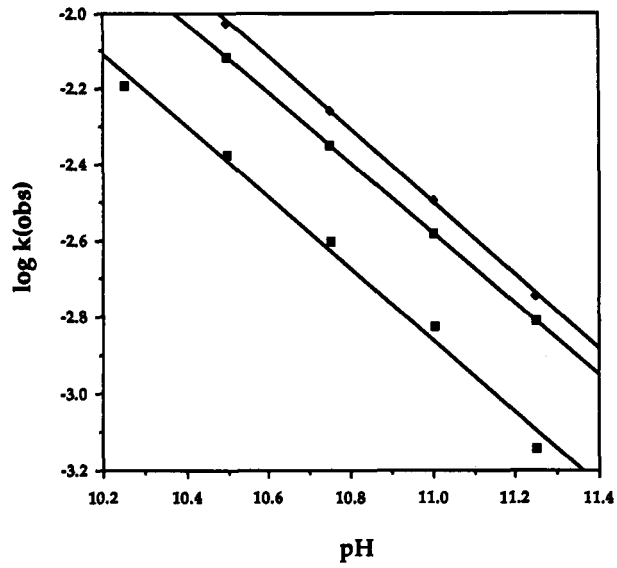

Figure 1. Plots of log k_{obs} vs pH for the decomposition of 1-alkyltriazolines in 0.10 M lysine buffers at 25 °C. Ionic strength held constant at 0.25 M with sodium perchlorate: (a) MT (□), ET (▲), BzT (■).

pH Dependence of the Rate of Triazoline Decomposition. The rate of decomposition of several representative triazolines was determined in buffers over a range of pH levels. The data are presented in Table III. Plots of log $k_{(obs)}$ versus pH for these data are displayed in Figure 1. The slope and intercept for each of the lines in Figure 1 are, respectively, as follows: MT, −0.922, 7.57; Et, −0.959, 8.05; BzT, −0.937, 7.45. The coefficient of determination (r^2) is 0.989 for BzT and >0.995 for the remaining compounds.

Relative Rates of Decomposition of 1-Alkyltriazolines. The rate of decomposition of the complete series of monocyclic 1-alkyltriazolines was determined in pH 10.75, 0.10 M lysine buffer at 25 °C. BzHMBT decomposes very slowly at this pH; consequently, the rate constant for this reaction was determined in pH 9.50, 0.10 M lysine buffer at 25 °C. BzT was included in this latter study, as was an acyclic triazene, 1,3-dimethyltriazene (DMT), for comparison purposes. The rate constants obtained from these studies are included in Table IV.

Solvent Deuterium Isotope Effect. The rate of decomposition of all of the simple 1-alkyltriazolines, BzHMBT, and DMT were measured in D_2O buffers of pD equivalent to the pH (10.75 and 9.50) of the analogous H_2O buffers used in the relative rate studies. In preparing the D_2O buffers, the nominal pH readings were, respectively, 10.35 and 9.10, corrected according to the relationship pD = $pH_{nominal}$ − 0.4.[24] The measured rates of decomposition in D_2O buffers and the calculated values for the solvent deuterium isotope effect, k_{H_2O}/k_{D_2O}, are recorded in Table IV. The solvent isotope effects varied somewhat with triazoline structure, but, in all cases k_{H_2O}/k_{D_2O} was <1.0. The isotope effect (0.40) for DMT, which was included in this study as a control, was comparable to that previously reported, 0.35.[4]

Dependence of the Rate of Triazoline Decomposition of Buffer Concentration. The rates of decom-

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Table IV. Solvent Isotope Effects on the Rate[a] of Decomposition of 1-Alkyltriazolines[b] in Aqueous Buffers[c] at 25 °C

	pH 10.75		
compd	$k_{(H_2O)}$	$k_{(D_2O)}$	$k_{(H_2O)}/k_{(D_2O)}$
MT	1.46	2.54	0.58
ET	1.90	3.06	0.62
PT	1.43	2.02	0.71
BT	1.52	2.20	0.69
iPT	2.42	3.32	0.73
tBT	2.96	3.86	0.77
BzT	0.874	1.01	0.86
	pH 9.50		
compd	$k_{(H_2O)}$	$k_{(D_2O)}$	$k_{(H_2O)}/k_{(D_2O)}$
BzT	14.3	17.6	0.81
BzHMBT	1.44	1.84	0.78
DMT	0.153	0.384	0.40

[a] Rate constants (k_{obs} (s^{-1}) × 10^3) are an average of at least two independent runs varying no more than ±3%. [b] All triazoline initial concentrations, 4.5 × 10^{-5} M. DMT initial concentration 3.0 × 10^{-5} M. [c] 0.1 M Lysine buffers, 0.25 ionic strength (maintained with added Na_2SO_4). Nominal pH reading for D_2O buffers was 0.4 pH units lower than for H_2O buffers.

Table V. Rates[a] of Triazoline[b] Decomposition as a Function of Buffer[c] Concentration at pH 10.5, 25 °C

[lysine], M	ET	BzT	TET
0.020	5.85	2.62	2.55
0.050	5.52	2.45	2.38
0.100	5.60	2.35	2.22
0.200	5.60	2.34	2.30
0.400	5.80	2.45	2.46

[a] k_{obs} × 10^3, an average of at least two independent determinations agreeing within ±3%. [b] ET and BzT initial concentrations were 4.5 × 10^{-5} M. TET initial concentration was 3.0 × 10^{-5} M. [c] 0.25 M ionic strength, maintained with added Na_2SO_4.

position of ET and BzT were measured at pH 10.5 in lysine buffers ranging in concentration from 0.020 to 0.500 M. The ionic strength of the buffers was maintained at 0.25 M by the addition of the appropriate amounts of Na_2SO_4. The data are reported in Table V. For comparison purposes, 1,3,3-triethyltriazene, TET, was included in this study. The slopes (mol^{-1} s^{-1}) of the k_{obs} versus [lysine] lines are: BzT, −2.38 × 10^{-4}; TET, 1.97 × 10^{-5}; and ET, 2.55 × 10^{-4}. The slightly negative slope of BzT may be attributed to minor inaccuracies in the calculation of the amounts of inert salt needed to maintain ionic strength. The essentially zero slope of TET reproduces previous observations for trialkyltriazenes.[4]

Ultraviolet Spectra of Triazolines. All of the simple 1-alkyltriazolines studied have a UV spectrum characterized by two roughly equal intensity bands, λ_{max} ~240 and ~264 nm. This is to be contrasted with the UV spectra of acyclic 1,3-dialkyl[5] and 1,3,3-trialkyltriazenes[3] which evidence only a single band of λ_{max} ~234 or ~245 nm, respectively. The molar absorptivity of the single band in acyclic triazene spectra (log $\epsilon \simeq 3.9$) is roughly twice that (log $\epsilon \simeq 3.6$) of each of the two bands in 1-alkyltriazoline spectra. Interestingly, in the less flexible tricyclic triazoline, BzHMBT, only a single UV band (λ_{max} = 266 nm) of somewhat lower intensity (log ϵ = 3.58) is observed.

Mutagenesis. Dose response assays were performed for three 1-alkyltriazolines, MT, ET, and BzT, and two 1,3-dialkyltriazenes, DMT, and 1,3-diethyltriazene (DET). All five compounds are directly active without the necessity of metabolic activation in TA1535, a base-pair substitution mutant strain of *S. typhimurium*. The data, which represent the average of duplicate runs, are presented in Table VI and displayed in Figure 2. A clear dose dependency can be seen for all five compounds. The overall activity of the three 1-alkyltriazolines are roughly equal and most like that of DMT, which is considerably more active than DET. In a separate experiment, the mutagenicities of MT and 1-methylaziridine were compared. It should be noted that the concentrations in this second study are 10 times those in the first. The results, displayed in Figure 3, indicate very similar dose–response curves for both compounds, with MT being slightly more mutagenic at higher concentrations and exhibiting toxic effects on the bacteria at the highest concentration.

Table VI. Dose–Response Mutagensis Data for 1-Alkyltriazolines in the *S. typhimurium* Strain TA 1535[a]

dose[b]	MT	ET	BzT	DMT	DET
1.0000	3074	2407	3495	2929	631
0.5000	2668	3045	3295	2842	602
0.2500	2146	2117	2995	2573	334
0.1000	1653	1044	2328	1746	123
0.0500	1479	435	1878	638	35
0.0250	1015	240	1682	157	14
0.0100	298	141	1247	24	16
0.0050	122	85	464	15	14
0.0025	81	46	269	10	12
0.0010	41	29	118	9	12

[a] Controls of cells alone and cells treated with 0.10 mL of dimethylsulfoxide were 10 and 13 revertants per plate, respectively. [b] Concentration in μmol/plate, which is equivalent to an initial concentration of 0.049 mM at the highest dosage.

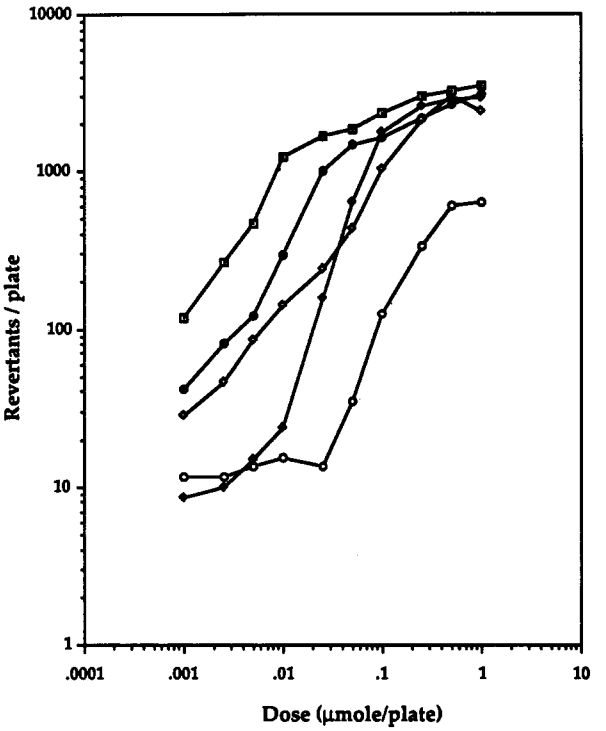


Figure 2. Comparative mutagenic activities of 1-alkyltriazolines toward *S. typhimurium* TA 1535. Representative 1,3-dialkyltriazenes included for comparison: MT (●), ET (△), BzT (□), CMT (▲), DET (○). Background revertants have not been subtracted.

Discussion

1-Aryltriazolines have been reported[10–13] to decompose in the presence of acids with the formation of a variety of nucleophilic substitution, cyclization (aziridine formation),

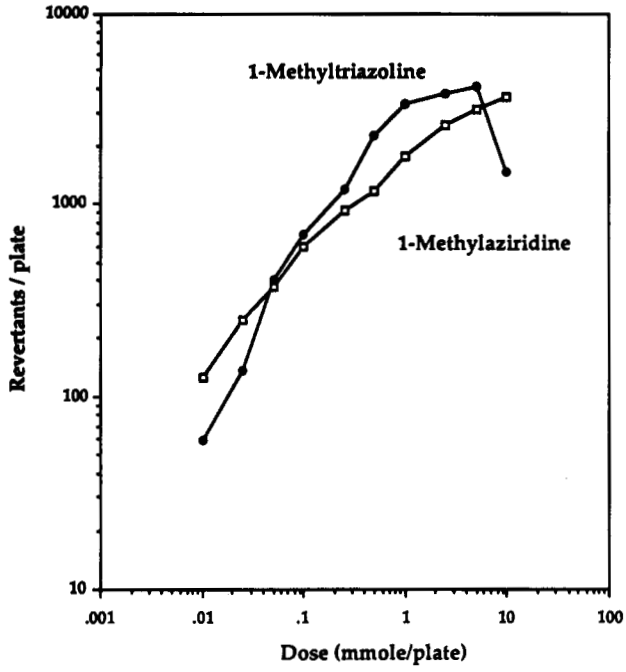


Figure 3. Comparative mutagenic activities of 1-methyltriazoline (●) and 1-methylaziridine (□) toward *S. typhimurium* TA 1535. Background revertants have not been subtracted.

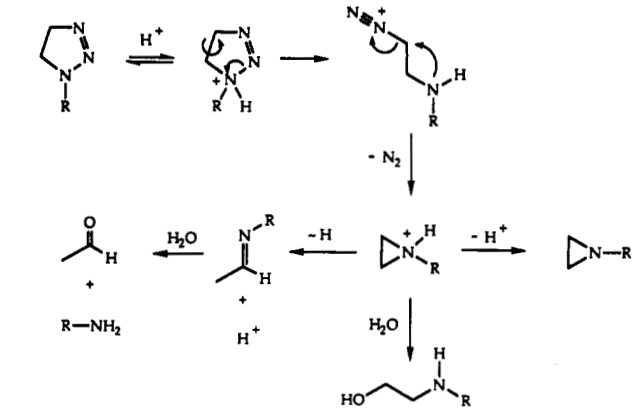
and rearrangement products. The current study demonstrates that the hydrolysis of 1-alkyltriazolines results in a similar array of products.

The data presented in Table II show that the decomposition of MT in 25% acetonitrile–75% aqueous phosphate buffer leads to a mixture of three different products, the relative distribution of which varies little over a wide pH range, 11.5–5.5. The major product, formed in 50–65% yield, is 1-methylaziridine. Lesser and roughly equal amounts (15–20%) of 2-(methylamino)ethanol, methylamine, and acetaldehyde are also formed. The latter two products, methylamine and acetaldehyde, presumably result from the initial formation and subsequent hydrolysis of *N*-ethylidenemethanamine. In pure aqueous phosphate buffer solutions, the same products are formed. The yield of 1-methylaziridine is lower, and those of 2-(methylamino)ethanol, methylamine, and acetaldehyde are correspondingly higher than in the mixed solvent system. This is presumably the result of the increased D_2O concentration combined with higher solvent polarity.

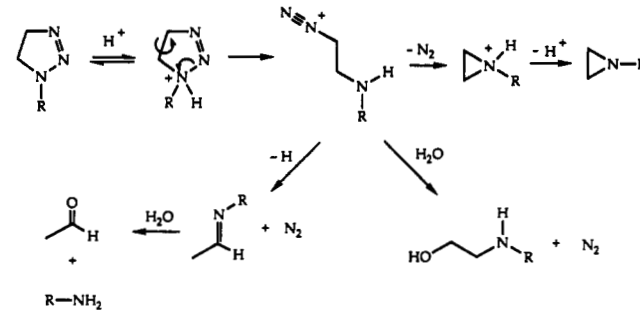
Two possible acid-promoted decomposition sequences can be envisioned to explain the observed products, Schemes II and III. In both schemes, decomposition is initiated by protonation of the triazoline N(1), followed by scission of the N(1)–N(2) bond to generate the 2-(methylamino)ethyldiazonium ion.

The distinguishing feature of Scheme II is the centrality of the 1-methylaziridinium ion, which acts as a common intermediate in the formation of all remaining products. Proton abstraction gives 1-methylaziridine. Simple nucleophilic attack by either hydroxide ion or water, depending upon pH, produces 2-(methylamino)ethanol. Alternatively, ring opening accompanied by a 1,2-hydride shift initially forms *N*-ethylidenemethanamine, which subsequently undergoes further hydrolysis to methylamine and acetaldehyde. This last reaction must be rapid, because at no time are signals attributable to *N*-ethylidenemethanamine observed in the ^1H NMR spectrum. Although the rearrangement of alkyl epoxides to aldehydes

Scheme II



Scheme III



by 1,2-shifts in the presence of acids are known,[25] this process has not been included in reports of the hydrolysis of simple 1-alkylaziridines.[26]

Scheme III differs in that the 2-(methylamino)ethyldiazonium ion serves as the common intermediate from which all product routes diverge. 1-Methylaziridine is formed as in Scheme II. Direct attack by water on the diazonium ion displaces molecular nitrogen and gives, after loss of a proton, 2-(methylamino)ethanol. A concerted 1,2-hydride shift and loss of molecular nitrogen results in an iminium ion which then hydrolyzes to methylamine and acetaldehyde. Rearrangement of 2-hydroxyethyldiazonium ions to aldehydes or ketones by 1,2-shifts are well known.[27] There are, however, no reports of the chemistry of the analogous 2-aminoethyldiazonium ions, other than those related to triazolines.[13]

Under the reaction conditions, 1-methylaziridine (pK_a = 7.86[22]) does not decompose to the remaining observed products, even at pH 5.5, where it is presumably extensively protonated. This observation strongly suggests that Scheme III is the more likely mechanism for the decomposition of MT in aqueous buffers.

The products of the acid-mediated decomposition of BzT in mixed acetonitrile–buffer solutions are in general similar to those observed from MT. 1-Benzyltriazoline gives rise to 1-benzylaziridine, 2-(benzylamino)ethanol, benzylamine, and acetaldehyde. The latter two products again suggest the formation and subsequent hydrolysis of *N*-ethylidenebenzylamine. The aziridine yield is comparable to that observed from MT, while those of the

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rearrangement products, benzylamine and acetaldehyde, are increased at the expense of 2-(benzylamino)ethanol.

The hydrolytic decomposition of triazolines is acid-catalyzed. The data in Table III and Figure 1 show a direct and linear dependence of the rate of decomposition of MT, ET, and BzT on the hydronium ion concentration of the buffer. Consequently, the slopes of the log k_{obs} versus pH plots are all near −1.0.

For simple 1-alkyltriazolines, the rate of decomposition (Table IV) varies in the order *tert*-butyl > isopropyl > ethyl > butyl > methyl > propyl > benzyl. This pattern generally follows the electron-releasing ability of the 1-alkyl group, a property which favors acid-catalyzed decomposition through enhanced N(1) basicity. Minor variations from that order are likely due to steric hinderance of solvation of the positively charged intermediate. 1-Alkyltriazolines decompose about twice as fast as the analogous 1,3,3-trialkyltriazenes; compare the rates for ET and TET in Table V.

It is interesting to note that BzHMBT undergoes hydrolysis about 10 times slower than BzT. In BzHMBT, the triazoline ring is in the endo-configuration. The lower rate compared with that of monocyclic triazolines possibly reflects steric inhibition of the attainment of the transition state required for dissociation. The attendant increase in the energy of activation of a stepwise process would account for the slower overall rate. This observation suggests that for BzHMBT hydrolysis, Scheme II may represent the more likely mechanism.

The proteolytic decomposition of 1-alkyltriazolines proceeds by a specific hydronium ion catalyzed mechanism. For all eight 1-alkyltriazolines, hydrolysis is more rapid in D_2O than in H_2O buffers (Table IV). The calculated solvent deuterium isotope effect, k_{H_2O}/k_{D_2O}, varies from 0.58 for MT to 0.86 (0.81) for BzT. A solvent isotope effect of less than one indicates rapid reversible protonation of the substrate, prior to the rate limiting step. The data in Table V indicate no significant variation in the rate of decomposition of either MT or BzT with buffer concentration. There is no buffer component in the rate-determining step.

Thus, the general pattern by which 1-alkyltriazolines undergo hydrolysis in aqueous buffers involves a specific acid-catalyzed (A1) mechanism, rapid reversible protonation of N(1), followed by rate-limiting heterolysis of the N(1)–N(2) bond. This is the same mechanism previously reported for 1,3,3-trialkyltriazenes[3] and, under most conditions, 1,3-dialkyltriazenes.[4] The key intermediate generated in the hydrolytic decomposition of 1-alkyltriazolines is the 2-(alkylamino)ethyldiazonium ion or, in the case of BzHMBT, possibly the 1-alkylziridinium ion. This common intermediate is then partitioned among several different pathways, with intramolecular cyclization to a 1-alkylaziridine dominating.

Previous experiments have shown that 1,3-dialkyl-[21] and 1,3,3-trialkyltriazenes[28] exhibit a high level of mutagenicity in the TA 1535 strain of *S. typhimurium*. These triazenes presumably act through the generation of an alkyldiazonium ion, which leads to alkylation of the O^6-position of guanine and results in a G → A transitional base-pair mutation. Previous work[21] has shown that both the mutagenic activity in *S. typhimurium* and the level of in vitro calf thymus DNA alkylation by 1,3-dialkyltriazenes follows the order methyl > ethyl > butyl.

1-Alkyltriazolines are potent direct-acting mutagens in the alkylation sensitive TA 1535 strain of *S. typhimurium*. The data in Table VI and Figure 2 show a clear dose-dependent mutagenicity for each triazoline. At the highest dose level, all three triazolines have activities roughly equivalent to that of the methylating agent, DMT. On the other hand, Figure 2 shows that at low levels of substrate, all three 1-alkyltriazolines are significantly more potent mutagens than DMT, the most active of the dialkyltriazenes. At these low concentrations, 1-alkyltriazolines exhibit somewhat different levels of mutagenic potency, following the order BzT > MT > ET. This order parallels the stability of the compounds in aqueous media, Table IV. The strong mutagenic properties of 1-alkyltriazolines suggest that the ultimate mutagenic intermediate is the aziridinium ion rather than the corresponding 2-(alkylamino)ethyldiazonium ion. Substituted alkyldiazonium ions are generally less mutagenic than the methyldiazonium ion[28] viz. the relative mutagenic potencies of DMT and DET. Additional strong support is gained from the observation that 1-methyltriazoline and 1-methylaziridine produce very similar dose-response curves (Figure 3). Thus the mechanism of the proteolytic decomposition of 1-alkyltriazolines provides a rational explanation for the observation that these compounds produce extensive modification of cellular DNA.

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Supplementary Material Available: Supporting ^1H NMR and ^{13}C NMR spectra (13 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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