Decomposition of Pyridinyltriazenes in Aqueous Buffer: A Kinetic and Mechanistic Investigation

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Received March 1, 1994®

1-(X-pyridinyl)-3-butyltriazenes (X-PBT), where X = 2, 3, or 4, were prepared as prototypes of new chemotherapeutic agents. The acid-catalyzed decomposition of the 1-(X-pyridinyl)-3-butyltriazenes leads to the formation of the corresponding aminopyridines and butyl alcohols. pH–rate profiles, determined over a pH range of 3.5–12.00, show sigmoidal curves with slopes asymptotically approaching 0 at the extremes. The transitions have slopes where rate is inversely proportional to pH, indicating regions of acid catalysis. The solvent kinetic isotope effect, k_{H_2O}/k_{D_2O}, for each reaction is <1.0. The determination of the kinetics of the decomposition in amino buffers, ranging in pK_a from 8.95 to 10.4 and concentrations from 0.03 to 0.15 M, indicates negligible variation in the rate constant. These data strongly support the conclusion that the decomposition is specific acid catalyzed (A1) for each X-PBT isomer. This implies that the reactions involve fast, reversible protonation followed by the rate-determining heterolysis of the protonated species to the *n*-butyldiazonium ion and X-aminopyridine. Near neutral pH, the half-lives of the 2- and 4-isomers are ~100-fold shorter than that of the 3-isomer. This difference can be explained by protonation of the pyridinyl N, which leads to direct dissociation only for the 2- and 4-isomers. Experimental pK_a's were obtained for each isomer: 2-PBT, 5.19 ± 0.19; 3-PBT, 4.89 ± 0.12; 4-PBT, 7.77 ± 0.16. These values are lower than, but follow the same order as, the pK_a values for the analogous isomers of aminopyridine.

Introduction

Triazenes are open chain compounds of the general structure $RN_1{=}N_2N_3R'R''$, where R and R′ are either alkyl or aryl groups and R″ is hydrogen, alkyl, aryl, or acyl. Since the first report of the preparation of a triazene,[1] 1,3-diphenyltriazene, a large amount of information on aryltriazenes has been obtained.[2–4] The most widely studied triazenes have been the diaryl (ArN=NNHAr), arylalkyl (ArN=NNHR), and aryldialkyl (ArN=NNR′R″) types. Of these, the latter two have attracted significant attention because of their mutagenic and carcinogenic properties.[5]

The decomposition of the arylalkyltriazenes has been extensively studied. The first reports of the acid-induced decomposition[6,7] showed that arylamines and alkyl alcohols are the products of the decomposition. Subsequent studies have revealed the involvement of alkyl diazonium ions.[8,9] A mechanism involving protonation of the triazene followed by the release of an alkyl cation was initially proposed.[10] More recently, evidence supporting[11] three different decomposition mechanisms, buffer catalyzed, general acid catalyzed (A-S_E2), and uncatalyzed unimolecular N–N heterolysis, has been reported.[12] In contrast, the hydrolysis of 1,3-dialkyltriazenes has been shown to involve either specific acid (A-1) or specific acid–general base (A-2) catalysis depending on the type of buffer.[13,14] 1,3,3-Trialkyltriazenes have been shown to decompose exclusively by a specific acid-catalyzed (A-1)[15] pathway.

There has been much interest in the structure of arylalkyltriazenes, since acid-induced decompositions yield products resulting from only one (1-alkyl-3-aryl) of the two possible tautomeric forms of the triazene. Surprisingly, studies have shown[10,16–18] that the 3-alkyl-1-aryl form is the dominant tautomer in most cases,

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® Abstract published in *Advance ACS Abstracts,* September 15, 1994.

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0022-3263/94/1959-5942$04.50/0

although the equilibrium distribution does show some sensitivity to substituents on the aryl moiety. The equilibrium appears unaffected by the nature of the alkyl group or the solvent.

$$ArN{=}NNHR \rightleftharpoons ArNHN{=}NR$$

Only a few reports have dealt with pyridinyl as the aryl group. Several of these were concerned with the carcinogenic activity of specific pyridinyl- and phenyldialkyltriazenes.[5,19] Others[20-23] discussed the synthesis of different pyridinylalkyl- and dialkyltriazenes, but had little to say about either the products or mechanism of their hydrolytic decomposition. One report,[23] however, described the synthesis, cytotoxic effects, and stability of several derivatives of 3-pyridinylmethyltriazene.

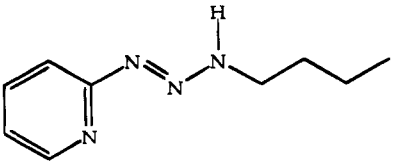
Recent work (B. Pruski, R. H. Smith, Jr., and C. J. Michejda, to be published) on dipyridinyl bistriazenes[24] revealed complex decomposition kinetics for three novel compounds, with the rates being highly dependent on the position of attachment of the triazene moiety to the pyridine ring. The presence of an additional basic site, the pyridyl nitrogen, was assumed to be the complicating factor. In the present work, we describe the results of an experimental investigation of simple 1-(X-pyridinyl)-3-butyltriazenes and the mechanism of their decompositions. Also, we compare these results with those of other 1-phenyl-3-alkyl- and 1,3-dialkyltriazenes. The specific compounds investigated are shown in Figure 1.

Experimental Section

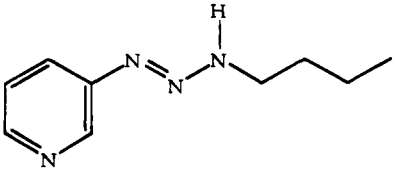
Safety Note. Arylalkyltriazenes are known to be mutagenic and carcinogenic;[5] thus, precautions such as wearing the appropriate protective clothing (rubber gloves, lab jacket, and safety glasses) need to be followed when working with these substances. All reaction mixtures and concentrated samples should be handled in efficient fume hoods. Alkyl azides are treacherously explosive and should be treated with extreme caution. Wherever possible, these compounds should only be handled in solution. Where unavoidable, distillations of alkyl azides should be carried out under vacuum at low temperature and behind a shield in a chemical fume hood.

Materials. All chemicals were reagent grade (Aldrich Chemical Co., Milwaukee, WI) and were used as purchased without further purification. Buffers for kinetic measurements and product analyses were prepared as previously described[15] using water distilled from $KMnO_4$. The synthesis of butyl azide, used in the preparation of 1-(X-pyridinyl)-3-butyltriazenes, has been reported previously.[25] A pure sample of 1,3-dimethyltriazene (DMT) was available from other studies.[13]

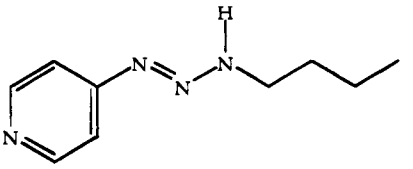
A Fisher Accumet Model 750 MP digital pH meter and a Fisher (13-620-270) high ionic strength combination electrode (calomel reference) were used in pH measurements. Ultraviolet spectra and kinetic measurements were recorded using a combination of three different systems. For reactions lasting longer than 30 s, a Hewlett-Packard Model 8450A double-beam UV/visible diode-array processor was used. Reactions lasting longer than 4 s were followed with a Hi-Tech Scientific rapid kinetics stopped-flow accessory connected to a Milton Roy



1-(2-pyridinyl)-3-butyltriazene (2-PBT)



1-(3-pyridinyl)-3-butyltriazene (3-PBT)



1-(4-pyridinyl)-3-butyltriazene (4-PBT)

Figure 1. Structures of the isomers of the 1-(X-pyridinyl)-3-butyltriazenes.

Model MR3000 spectrophotometer. For reactions faster than 4 s, a Hi-Tech Scientific multi-mixing stopped-flow spectrophotometer, Model SF-51MX, was used in a manner described previously.[26] A computational curve fitting program, Table Curve (Jandel Scientific, Corte Madera, CA), was used for the analysis of stopped-flow data and pK_a determination. Melting points were obtained from an Electrothermal capillary melting point apparatus. NMR spectra were obtained on a Varian XL-200 spectrometer. High-resolution mass spectral molecular formula determinations were obtained on a VG 70-250 mass spectrometer using a peak matching technique. All samples submitted for mass determination were shown to be >96% pure by ^1H NMR analysis. (See the supplementary material for availability of these spectra.)

1-(2-Pyridinyl)-3-butyltriazene (2-PBT). The synthesis was carried out according to a procedure developed in our laboratory.[27] To a 5 mL (12.5 mmol) solution of 2.5 M butyllithium in 10 mL of hexane, at ~−78 °C under nitrogen, was added dropwise 1.2 mL (12.5 mmol) of 2-bromopyridine in 8.8 mL of hexane. The reaction mixture was stirred for an additional hour, followed by the slow addition of 1.38 mL (12.5 mmol) of 1-azidobutane in 8.62 mL of hexane. The reaction temperature was held at −78 °C until addition was complete and then allowed to slowly warm to room temperature (~2.5 h). The reaction mixture was then cooled to ~−20 °C, and a 10% sodium hydroxide solution was added until the solution cleared and bubbling stopped. The organic layer was separated, dried over anhydrous sodium sulfate for several minutes, filtered through anhydrous sodium sulfate, and then concentrated on a rotary evaporator. The residue was dissolved in a small amount of petroleum ether/anhydrous ethyl ether (15:5), placed under nitrogen, and stored in a −20 °C freezer until crystals formed. A final recrystallization from anhydrous ethyl ether at 4 °C afforded 1.21 g (6.81 mmol, 54.5%): mp 53−54 °C; UV (CH_3CN) λ_{max} 277 nm (ϵ = 18 600); ^1H NMR (CD_2Cl_2, Me_4Si) δ 0.96 (3H, t, J = 7.2 Hz), 1.42 (2H, sextet, J = 7.2 Hz), 1.71 (2H, bd quintet), 3.72 (2H, t, J = 7.1 Hz), 6.89 (1H, ddd, J = 0.9, 5.0, 7.2 Hz), 7.36 (1H, dt, J = 0.9, 8.4 Hz), 7.63 (1H, ddd, J = 1.8, 7.2, 8.4 Hz), 8.27 (1H, dq, J = 0.9, 5.0 Hz), 10.73 (1H, bd singlet); proton-decoupled ^{13}C NMR

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(CD_2Cl_2, Me_4Si) δ 14.07, 20.90, 31.29, 61.30, 108.15, 117.25, 138.55, 148.02, 156.14; MS m/z 178 (M^+, 2), 150 (2), 121 (20.5), 107 (12.3), 94 (100), 93 (8.2), 79 (7.8), 78 (28), 67 (49.2), 66 (17.2), 57 (95.7), 52 (10.4), 51 (16); exact mass calcd for M^+, $C_9H_{14}N_4$ 178.1218, found 178.1215 (by EI). Anal. Calcd for $C_9H_{14}N_4$: C, 60.67; H, 7.86; N, 31.46. Found: C, 60.79; H, 7.87; N, 31.40.

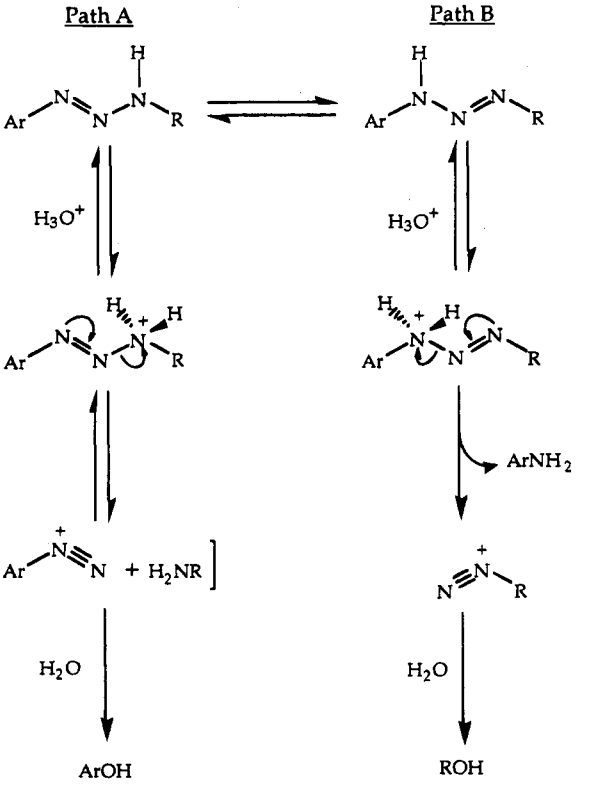
1-(3-Pyridinyl)-3-butyltriazene (3-PBT). This synthesis followed the procedure and scale of 2-PBT above, using 3-bromopyridine as the starting material. Recrystallization from anhydrous ethyl ether at 4 °C afforded 0.996 g (5.59 mmol, 44.8%): mp 47–48 °C; UV (CH_3CN) λ_{max} 274 nm (ϵ = 13 800); ^1H NMR (CD_2Cl_2, Me_4Si) δ 0.96 (3H, t, J = 7.2 Hz), 1.43 (2H, sextet, J = 7.2 Hz), 1.68 (2H, bd quintet), 3.67 (2H, t, J = 7.1 Hz), 7.25 (1H, ddd, J = 0.7, 4.6, 8.2 Hz), 7.66 (1H, d, J = 8.2 Hz), 8.34 (1H, d, J = 4.4 Hz), 8.58 (1H, bd singlet); proton-decoupled ^{13}C NMR (CD_2Cl_2, Me_4Si) δ 13.92, 20.65, bd 29.42, bd 31.83, bd 44.06, 124.00, bd 126.52, bd 144.20, bd 146.98; MS m/z 178 (M^+, 5.5), 150 (2), 121 (3), 107 (13.4), 106 (10.1), 94 (25), 93 (4), 78 (100), 67 (8.4), 57 (18.5), 51 (22.6); exact mass for M^+, $C_9H_{14}N_4$ 178.1218, found 178.1215 (by EI). Anal. Calcd for $C_9H_{14}N_4$: C, 60.67; H, 7.86; N, 31.46. Found: C, 60.77; H, 7.89; N, 31.35.

1-(4-Pyridinyl)-3-butyltriazene (4-PBT). The starting 4-bromopyridine, purchased as the hydrochloride salt, was neutralized by washing a diethyl ether solution with 10% sodium hydroxide. The ethereal solution was then concentrated on a rotary evaporator. The reaction then followed the procedure described above for 2-PBT. The product (>96% purity) was obtained by recrystallization from a solution of hexane:ether (15:1) and cooled for several hours, affording 0.601 g (3.38 mmol, 27%): mp 63–64 °C; UV (CH_3CN) λ_{max} 283 nm (ϵ = 12 300), shoulder λ 274 nm (ϵ = 12 000); two tautomers (A-tautomer) ^1H NMR (CD_2Cl_2, Me_4Si) δ 0.95 (3H, t, J = 7.2 Hz), 1.41 (2H, sextet, J = 7.2 Hz), 1.70 (2H, bd quintet), 3.73 (2H, t , J = 7.1 Hz), 7.06 (2H, d, J = 6.2 Hz), 8.38 (2H, d, J = 6.2 Hz), 9.91 (1H, bd singlet); (B-tautomer) δ 0.95 (3H, t, J = 7.2 Hz), 1.41 (2H, sextet, J = 7.2 Hz), 1.70 (2H, bd quintet), 3.73 (2H, t , J = 7.1 Hz), 7.29 (2H, d, J = 3.7 Hz), 8.50 (2H, d, J = 3.8 Hz), 9.21 (1H, bd singlet); proton-decoupled ^{13}C NMR (CD_2Cl_2, Me_4Si) δ 13.96, 20.74, 28.98, 31.19, 44.28, bd 60.56, bd 109.06, 109.85, 115.74, 127.53, 150.54, 150.68; MS m/z 178 (M^+, 5.2), 150 (1.7), 121 (6.6), 107 (10.1), 106 (12.8), 94 (55.7), 79 (7.5), 78 (85.8), 67 (14.5), 57 (100), 56 (8), 51 (28.4); exact mass for M^+, $C_9H_{14}N_4$ 178.1218, found 178.1214 (by EI). Anal. Calcd for $C_9H_{14}N_4$: C, 60.67; H, 7.86; N, 31.46. Found: C, 60.79; H, 7.81; N, 31.30.

1-Phenyl-3-butyltriazene (PhBT). To a solution of 1.25 g (12.6 mmol) of 1-azidobutane in 20 mL of dry ethyl ether was added slowly 8.78 mL of a 1.8 M solution of phenyllithium (15.8 mmol) in 20 mL of dry ethyl ether at −78 °C under nitrogen. The solution was stirred for ~1 h at −78 °C, and then stirring was continued while the solution warmed to room temperature (~2.5 h). The product was isolated according to the procedure described above to yield 0.689 g of a crude oil, which was chromatographed through silica gel [pretreated with (95:5) pentane/isopropylamine] and eluted with the same to give 0.632 g (3.57 mmol, 28.3%) of a pale yellow oil: UV (CH_3CN) λ_{max} 271 nm (ϵ = 10 500); two tautomers (A-tautomer) ^1H NMR ($CDCl_3$, Me_4Si) δ 0.96 (3H, t, J = 7.2 Hz), 1.42 (2H, sextet, J = 7.14 Hz), 1.67 (2H, bd quintet), 3.66 (2H, t, J = 7.12), 7.30–7.48 (5H, multiplet); (B-tautomer) δ 0.96 (3H, t, J = 7.2 Hz), 1.42 (2H, sextet, J = 7.14 Hz), 1.67 (2H, bd quintet), 3.96 (2H, t, J = 6.5 Hz), 7.56–7.62 (5H, multiplet); MS m/z 177 (M^+, 3.8), 163 (1.6), 155 (11.4), 154 (100), 153 (20.8), 152 (16.4), 120 (5.4), 107 (13.9), 106 (30.9), 105 (9.5), 94 (24), 93 (48.6), 92 (6.9), 86 (8.8), 84 (13.9), 78 (6.3), 77 (46.7), 76 (11.4), 57 (8.8); exact mass for M^+, $C_{10}H_{15}N_3$ 177.1266, found 177.1266 (by EI).

Product Studies. The products of decomposition of the isomers of 1-(X-pyridinyl)-3-butyltriazene were determined from reaction mixtures in which the initial triazene concentration was 5×10^{-2} M. Initial studies were performed in a 0.05 M phosphate buffer (pH 7.5), subsequent product studies used 0.1 M lysine buffers (μ = 0.25 M maintained with Na_2SO_4) in D_2O, adjusted to the appropriate pH with a D_2O solution of

Scheme 1



NaOD. Due to insolubility of X-PBT's in aqueous solutions, the decompositions were performed in mixed solvents, 30% acetonitrile-d_3 (v/v) in 0.05 M sodium phosphate or 0.1 M lysine buffers. The triazene was dissolved in 0.3 mL of acetonitrile-d_3. The triazene solution was then added to a 0.7 mL solution of the aqueous buffer containing DMSO and allowed to stand sealed, maintained at 25 °C, and protected from light, for a minimum of 72 h in phosphate buffer and between 48 h to 18 days (depending on the pH) in lysine buffers, before analysis. DMSO (2.8 mg) was used as an internal standard. The decomposition solution was analyzed by ^1H NMR. The pH of each final reaction mixture was measured and found to vary no more than ±0.45 pH units from the starting pH. Assignments for spectra of the decomposition solutions were made by comparison with NMR spectra of authentic materials. Since there are two possible pathways by which 1-aryl-3-alkyltriazenes may decompose (Scheme 1), depending on the tautomeric distribution,[5,11,28,29] the anticipated decomposition products were pyridylamines, pyridyl alcohols, 1-butylamine, 1-butanol, and 2-butanol.

Solutions of authentic samples of each of the anticipated products were prepared in the appropriate buffer. To assure that all the possible compounds of each decomposition reaction could be detected, these solutions were then combined sequentially and the resultant spectra were compared with those of the individual compounds. The peaks for each of the individual compounds, except 1-butylamine in lysine buffer, were found to be distinctive, thereby allowing a direct determination of the products of decomposition of the different isomers (2-, 3-, and 4-PBT).

Kinetic Studies. The method employed for the preparation of the buffers used in these kinetic studies has been reported previously.[15] Sodium sulfate was added as an inert salt to

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maintain constant ionic strength. Previous work[30] demonstrated that 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 buffer used in our earlier work.

Rates of triazene decomposition in aqueous solution were followed spectrophotometrically on either a Hewlett-Packard (HP) Model 8450A double-beam diode-array spectrophotometer, a Hi-Tech Scientific rapid kinetics stopped-flow accessory (SFA) connected to a Milton Roy Spectronic Model MR3000 (diode array), or a Hi-Tech Scientific multi-mixing stopped-flow spectrophotometer, Model SF-51MX. In the case of the SF-51MX spectrophotometer, the mV readings from the output were converted in Microsoft Excel to readable files for the PC/AT based least-squares program.

In the steady state kinetics experiments the reaction solutions were contained in thermostated 1-cm cells and the temperature was held constant at 25 ± 0.1°C. The disappearance of each triazene was followed by monitoring the maximal absorbance (λ_{max}) change (see the Materials section). In a typical kinetic run, carried out on the HP 8450A spectrometer, the reaction cuvette was charged with 1.341 mL of a 0.1 M lysine buffer (ionic strength = 0.25 M maintained with added Na_2SO_4), and the reaction was initiated by the addition of 9 μL of a 4.5×10^{-3} M solution of the 1-aryl-3-alkyltriazene in acetonitrile; the initial triazene concentration was thus 3.0×10^{-5} M. The reference cuvette contained 1.341 mL of buffer (previous work has shown the addition of 9 μL of acetonitrile to be unnecessary[29]). For kinetics carried out on the SFA-MR3000 spectrometer, the reaction cuvette was charged with 400 μL of 0.1 M lysine buffer and the reaction was initiated by the addition of 7 μL of a 1.5×10^{-3} M solution of X-PBT in acetonitrile, giving an initial triazene concentration of 2.6×10^{-5} M. The fast reactions studied by the SF-51MX instrument were obtained from a mix ratio of 60:1, 0.1 M lysine buffer to 1.5×10^{-3} M solution of X-PBT, to give the initial triazene concentration of 2.5×10^{-5} M. In cases where the SFA-MR3000 or SF-51MX instruments were used, the reaction cuvette was also the reference cuvette and so, before each reaction, a reference reading was taken of the cuvette containing only 0.1 M lysine buffer. Typically a minimum of 100 absorbance vs time points were measured over 3.5 half-lives.[15] In some instances, where reaction rates were too fast to obtain 100 points within 3.5 half-lives, no less than 16 absorbance vs time points were used to obtain k_{obs}. Each triazene sample at each pH was run at least twice, with a deviation between runs of <3%. The first-order rate constants were calculated from these data by means of a computer program based on the Guggenheim approximation least-squares method.[31,32]

Results

Product Studies. The products of the decomposition of 2-PBT, 3-PBT, and 4-PBT were determined by ^1H NMR analysis. The study using phosphate buffer was performed at near neutral pH; the studies using lysine buffers were performed at pD 3.5, 6.75, and 10.0 for 2-PBT and 3-PBT and pD 6.75, 7.75, and 10.0 for 4-PBT in D_2O buffers containing 30% acetonitrile-d_3. DMSO was used as an internal standard to determine yields quantitatively. Product yield data are recorded in Table 1. For each of the isomers, whether in phosphate or lysine buffers, the products that formed were exclusively those resulting from the 3-aryl tautomeric form, Ar-NHN=NR (Scheme 1, path B), yielding only the appropriate aminopyridines, 1- and 2-butanol, and a small amount of butenes. The reaction spectra, when compared with authentic samples, showed no trace of the corresponding hydroxypyridines. Spectra of authentic samples were obtained in the same solvent mixture as were the reaction samples.

A small triplet peak found in the reaction spectra was originally thought to come from butylamine; however, the addition of authentic material showed this not to be the case. The triplet peak obtained from spiking with authentic butylamine was shifted downfield relative to the unknown. Most likely this triplet is due to trace amounts of butenes.[33] Butenes have previously been shown[15,33] to be formed in small amounts in hydrolysis reactions which generate the butyldiazonium ion.

pH Dependence of the Rate of 1-(X-Pyridinyl)-3-butyltriazene Decomposition. The rate of decomposition of 2-PBT and 3-PBT was determined in lysine buffers over a range of pH values (3.50−12.00), while 4-PBT was studied in buffers ranging in pH from 6.09 to 12.00. All observed kinetics were distinctly first-order, and the rate

Table 1. Products and Yields from the Hydrolysis of 1-(X-Pyridinyl)-3-butyltriazenes[a] in Aqueous Phosphate Buffer[b] and Aqueous Lysine Buffer[c] at 25 °C

products	yields[d] (%) 2-PBT	3-PBT	4-PBT
Phosphate Buffer			
X-aminopyridines[e]	96.3	98.7	97.4
X-hydroxypyridines[e]			
1-butanol	53.7	56.1	54.2
2-butanol	29.0	32.1	25.9
1-aminobutane			
butenes[f]	~13.6	~10.5	~17.3

products	pH	yields[d] (%) 2-PBT	3-PBT	4-PBT
Lysine Buffer				
	10.00	97.4	97.6	98.1
X-aminopyridines[e]	7.75	NA[g]	NA[g]	96.4
	6.75	97.2	97.9	97.6
	3.50	98.0	96.3	NA[g]
	10.00			
X-hydroxypyridines[e]	7.75	NA[g]	NA[g]	
	6.75			
	3.50			NA[g]
	10.00	54.2	56.4	55.6
1-butanol	7.75	NA[g]	NA[g]	56.4
	6.75	52.5	55.4	54.6
	3.50	55.1	56.3	NA[g]
	10.00	28.7	31.6	25.7
2-butanol	7.75	NA[g]	NA[g]	23.4
	6.75	30.4	31.9	25.6
	3.50	28.4	31.6	NA[g]
	10.00			
1-aminobutane	7.75	NA[g]	NA[g]	
	6.75			
	3.50			NA[g]
	10.00	~14.5	~9.6	~16.8
butenes[f]	7.75	NA[g]	NA[g]	~16.6
	6.75	~14.3	~10.6	~17.4
	3.50	~14.5	~8.4	NA[g]

[a] Triazene concentration 5.6×10^{-2} M. [b] 0.05 M, pH 7.50 phosphate buffer (μ = 0.25 M maintained with Na_2SO_4)/CD_3CN (70:30), containing DMSO as internal standard. [c] 0.1 M, lysine buffer (μ = 0.25 M maintained with Na_2SO_4)/CD_3CN (70:30), containing DMSO as internal standard. [d] Obtained by comparing the integrations of product signals vs those of DMSO. [e] X = 2-, 3-, or 4-, as the attachment site on the pyridine ring. [f] Values determined by taking the difference between the X-aminopyridines (their values account for total product formed, the butanols and butenes arise from the other part of the original triazene) vs butanols from corresponding triazene. [g] Not applicable, compound was not determined at the particular pH.

(30) Smith, R. H., Jr.; Wladkowski, B. D.; Taylor, J. E.; Thompson, E. J.; Pruski, B.; Klose, J. R.; Andrews, A. W.; Michejda, C. J. *J. Org. Chem.* **1993**, *58*, 2097.
(31) Guggenheim, E. A. *Phil. Mag.* **1926**, *2*, 538.
(32) King, E. L. *J. Am. Chem. Soc.* **1952**, *74*, 563.
(33) Sieh, D. H.; Michejda, C. J. *J. Am. Chem. Soc.* **1981**, *103*(2), 442.

Table 2. pH Profile for Rates of Decomposition of 1-(X-Pyridinyl)-3-butyltriazenes[a] (2-, 3-, and 4-PBT) and 1-Phenyl-3-butyltriazene[a] in 0.1 M Lysine Buffers

	$k_{obsd} \times 10^3$,[c] s^{-1}			
pH[b]	2-PBT	3-PBT	4-PBT	PhBT
3.50	3845.4 (±61.6)[d]	94.51 (±2.46)[d]		
4.00	4006.5 (±102.0)[d]	84.72 (±0.71)[d]		
4.50	3705.8 (±24.9)	70.44 (±5.75)[e]		
4.75		59.10 (±0.21)		
5.00	2215.9 (±84.7)[d]	41.67 (±3.16)[e]		
5.25		30.25 (±1.74)[e]		
5.50	977.3 (±10.7)[d]	22.64 (±0.05)		
5.75		13.53 (±0.40)[f]		
6.09	724.0 (±15.8)[d]	8.88 (±0.45)[e]		
6.28		6.12 (±0.20)[f]		
6.50		4.16 (±0.18)[f]	248.7 (±3.18)[d]	
6.72	227.8 (±40.4)[e]	2.30 (±0.02)		
6.94	115.9 (±1.6)	1.48 (±0.03)[d]	270.5 (±89.9)[e]	
7.21		0.83 (±0.007)	225.4 (±50.3)[e]	
7.36	65.8 (±0.35)[d]	0.58 (±0.003)		9.74 (±0.06)
7.69	30.9 (±0.33)[d]	0.30 (±0.006)	124.2 (±0.24)	4.40 (±0.10)
7.94	20.0 (±2.59)[e]	0.20 (±0.000)	84.59 (±5.52)[e]	2.63 (±0.01)
8.17	11.4 (±0.43)[e]	0.14 (±0.003)	50.22 (±0.53)[d]	1.52 (±0.002)
8.44	6.36 (±0.06)		31.85 (±1.87)[e]	0.86 (±0.004)
8.67	3.81 (±0.54)		17.68 (±0.13)	
8.95	2.31 (±0.02)	0.071 (±0.000)	9.79 (±0.04)	
9.19	1.53 (±0.04)		5.83 (±0.04)	
9.43	1.09 (±0.01)		3.50 (±0.02)	
9.68	0.84 (±0.004)		2.28 (±0.01)	
9.91	0.68 (±0.000)	0.055 (±0.001)	1.51 (±0.006)	
10.17	0.59 (±0.003)		1.05 (±0.003)	
11.00	0.49 (±0.001)	0.052 (±0.000)	0.57 (±0.000)	
12.00	0.46 (±0.002)		0.44 (±0.000)	

[a] Concentration of substrate was 3×10^{-5} M. [b] Actual pH, measured at 25 °C. [c] Average of two independent runs (<3% variability). [d] Average of three independent runs. [e] Data from two separate instruments (at least three independent runs from each), all data within 1.25 standard deviations. [f] Data from two separate instruments (at least three independent runs from each), <3% variability.

constants are given in Table 2. Plots of log $k_{(obs)}$ vs pH for these data are displayed in Figure 2. Each isomer clearly produced a sigmoidal curve. These curves contained an intermediate region in which the rate was inversely proportional to pH, and regions at either extreme of the pH range in which the slope approached 0; that is, the rate was pH independent. A computational program (Table Curve, refer to the Materials section) designed for curve fitting was used to determine the actual curve type. Best fits were obtained from the sigmoidal equation $y = a + b/(1 + \exp(-(x - c)/d))$, where a = lower plateau, b = height of transition, c = the inflection point, and d = the slope. Table Curve uses F statistic calculations (F statistics = mean square regression (MSR)/mean square error (MSE)), where the higher the F value the better the data fit to the curve. In each case, F values of ~1000 (meaning relatively good fit with some points lying outside of the 95% confidence level) to 5000 (all values lying within the 95% confidence level) were obtained with coefficients of determination (r^2) equal to 0.997 or better. The p$K_{a'}$ for each isomer in 0.1 M lysine buffer was obtained using the above formula, where the p$K_{a'}$ is the inflection point for the k vs pH curve. Alternatively, the p$K_{a'}$ can be obtained from the pH at which the second derivative of the k vs pH curve equals 0. Finally, a third method was employed to give the p$K_{a'}$ values from the point at which tangents to the plateau and descending limb intersect[34] for a plot of log k vs pH. Upon averaging the values obtained by the three methods for each compound, the p$K_{a'}$ values are 5.19 ± 0.19 for 2-PBT, 4.89 ± 0.12 for 3-PBT, and 7.77 ± 0.16 for 4-PBT.

(34) Bruice, T. C.; Benkovic, S. J. *Bioorganic Mechanisms*; W. A. Benjamin, Inc.: New York, 1966; Vol. 1, Chapter 1.

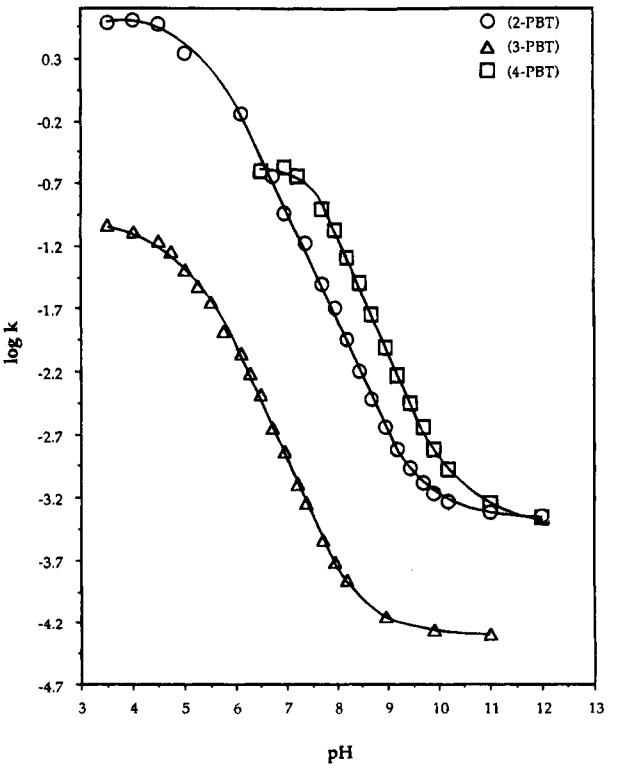


Figure 2. Plots of the logarithms of the observed pseudo-first-order rate vs pH for the decomposition of 2-PBT, 3-PBT, and 4-PBT in lysine buffers. Values computed from Table Curve for each curve; pK_a', k_1, k_2: 2-PBT, 5.10, 2.69×10^{-2}, and 4.21; 3-PBT, 4.91, 7.49×10^{-4}, and 9.71×10^{-2}; 4-PBT, 7.64, 2.64×10^{-3}, and 2.99×10^{-1}.

Relative Rates of Decomposition of 1-Aryltriazenes. The rate of decomposition of 1-phenyl-3-butyltria-

Table 3. Relative Rates of Decomposition in pH 7.69 Lysine Buffer[a] for the X-PBT's[b] and for Representative Arylalkyl-[b] and Dialkyltriazenes[b]

	$k_{obsd} \times 10^3$,[c] s^{-1}		$k_{obsd} \times 10^3$,[c] s^{-1}
DMT	11.0 (±0.42)	4-PBT	120.0 (±0.24)
2-PBT	31.0 (±0.33)	PhBT	4.0 (±0.1)
3-PBT	0.3 (±0.006)		

Table 4. Solvent Isotope Effects on the Rates of Decomposition

substrate	buffer pH[a]	$k_{obsd}^{H_2O} \times 10^3$,[b] s^{-1}	$k_{obsd}^{D_2O} \times 10^3$,[b] s^{-1}	k_{H_2O}/k_{D_2O}
1-(2-pyridinyl)-3-butyltriazene	7.5	50.0 ± 0.03	120.0 ± 2.3	0.42
	8.5	5.5 ± 0.07	16.0 ± 0.02	0.34
1-(3-pyridinyl)-3-butyltriazene	7.5	0.44 ± 0.01[c]	0.62 ± 0.01	0.71
1-(4-pyridinyl)-3-butyltriazene	8.5	26.0 ± 0.09	78.0 ± 0.06	0.33
1-phenyl-3-butyltriazene	7.5	7.1 ± 0.04	10.0 ± 0.08	0.71
1,3-dimethyltriazene[d]	8.5	1.3 ± 0.00	3.2 ± 0.00	0.41

[a] Buffer system used was lysine with concentration in each case held at 0.1 M and ionic strength of 0.25 M held constant with Na_2SO_4. [b] Obtained averages and standard deviations from two independent determinations. [c] Average obtained from three independent determinations. [d] Triazene was used to confirm the reliability of the data. From previously reported data,[13] it was shown to be specific acid catalyzed with k_{H_2O}/k_{D_2O} of 0.35–0.39.

zene (PhBT) was determined over a pH range of 7.36–8.44 in 0.10 M lysine buffer. Throughout this range, the reaction was cleanly first-order. The observed rate constants are listed in Table 2. The rate was found to be inversely proportional to the pH. The rates, when compared with those calculated from the corresponding linear regions of the 1-(X-pyridinyl)-3-butyltriazene, showed PhBT rates fell between the rates for 2-PBT and 3-PBT. The rate of decomposition of a dialkyltriazene, 1,3-dimethyltriazene (DMT), was determined in pH 7.69, 0.10 M lysine buffer for comparison purposes. These data are included in Table 3.

Solvent Deuterium Isotope Effect. The rates of decomposition of 2-PBT, 3-PBT, 4-PBT, PhBT, and DMT were determined by carrying out parallel reactions in aqueous and deuterium oxide buffers. The reactions were carried out in buffers of the same pH (pD), ionic strength, buffer, and buffer concentration. In preparing the D_2O buffers, the nominal pH readings were corrected according to the relationship $pD = pH_{nominal} - 0.4$.[35] The measured rates of decomposition in each pH and pD buffer, and the calculated values for the solvent deuterium isotope effect, k_{H_2O}/k_{D_2O}, are recorded in Table 4. These ratios varied somewhat, but in all cases k_{H_2O}/k_{D_2O} was <1.0. DMT was included as a control and produced a value of 0.40, comparable to the previously reported value of 0.35.[13]

Dependence of the Rates of 1-(X-Pyridinyl)-3-butyltriazene Decomposition on Buffer Concentration for a Range of Buffers. The rates of decomposition of 2-, 3-, and 4-PBT were measured at pH 8.00 in lysine, glycine, sarcosine, and (for 3- and 4-PBT) 3-(*N*-cyclohexylamino)propane sulfonic acid (CAPS) buffers. All buffers were prepared in concentrations ranging from 0.030 to 0.150 M, with ionic strength maintained at 0.50 M by using appropriate amounts of Na_2SO_4. The data, shown in Table 5, for each buffer series indicated no significant variation in rate with buffer concentration,

(35) Glasoe, P. K.; Long, F. A. *J. Phys. Chem.* **1960**, *64*, 188.

Table 5. Rates of Triazene Decomposition as a Function of Buffer Concentration[a]

buffer	[buffer], M	$k_{obsd} \times 10^3$,[b] s^{-1} 2-PBT	3-PBT	4-PBT
lysine	0.03	20.55 (±0.14)	0.206 (±0.002)	73.87 (±0.43)
	0.06	20.64 (±0.79)	0.199 (±0.001)	73.55 (±0.57)
	0.09	20.50 (±0.53)	0.205 (±0.009)	73.66 (±0.38)
	0.12	20.62 (±0.43)	0.193 (±0.001)	73.76 (±0.22)[c]
	0.15	20.53 (±0.51)	0.199 (±0.004)	73.63 (±0.68)
glycine	0.03	20.74 (±0.18)	0.271 (±0.005)	85.96 (±0.75)
	0.06	20.59 (±0.38)	0.235 (±0.001)	87.81 (±1.61)
	0.09	20.69 (±0.39)	0.257 (±0.004)	88.02 (±0.78)
	0.12	20.63 (±0.10)	0.239 (±0.006)	85.72 (±0.55)
	0.15	20.67 (±0.12)	0.262 (±0.000)	86.68 (±2.97)
sarcosine	0.03	20.86 (±0.23)	0.310 (±0.008)	84.61 (±0.20)
	0.06	20.78 (±0.17)	0.303 (±0.006)	85.64 (±2.40)
	0.09	20.92 (±0.26)	0.326 (±0.004)	85.87 (±0.35)
	0.12	20.69 (±0.30)	0.312 (±0.010)	84.38 (±0.56)
	0.15	20.84 (±0.22)	0.298 (±0.006)	84.51 (±0.12)
CAPS	0.03		0.343 (±0.012)	89.28 (±0.15)
	0.06		0.313 (±0.002)	89.25 (±0.82)
	0.09		0.328 (±0.002)	88.10 (±2.13)
	0.12		0.326 (±0.007)	89.55 (±1.16)
	0.15		0.321 (±0.005)	89.01 (±0.92)

[a] All rate determinations were carried out at 25 ± 0.1 °C, at a substrate concentration of 3.0×10^{-5} M, at pH 8.00 and ionic strength of 0.50 M (Na_2SO_4). [b] The rate constants are an average of two independent runs. [c] Value is an average of three independent runs.

as evidenced by least-squares lines with slopes ~0 (slightly negative, but not considered significant) and correlation coefficients <0.4. These results suggest that the rate of decomposition is not dependent upon buffer concentration. In order for the data to show statistically significant correlation of rate with buffer concentration, given that only five buffer concentrations were used, would have required a coefficient of 0.878 or higher.[36]

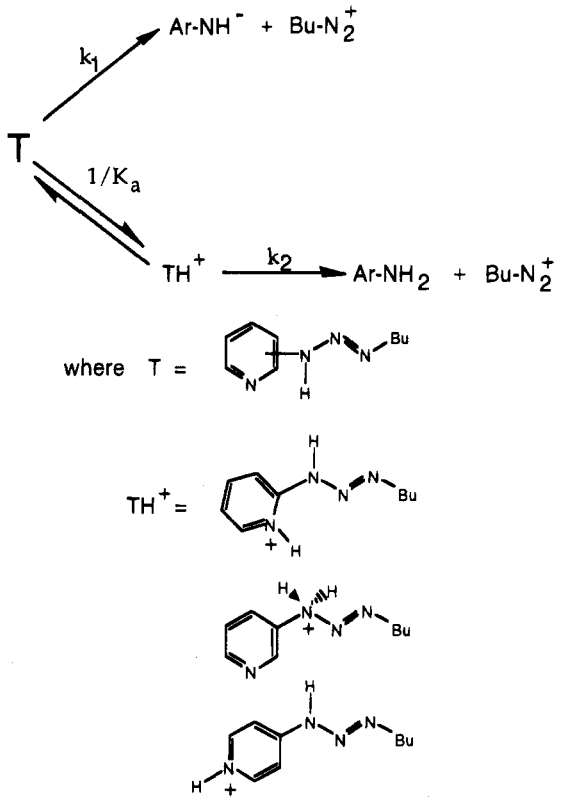
Rates of Decomposition as a Function of Buffer pK_a. The rates of decomposition of the 1-(X-pyridinyl)-3-butyltriazenes were measured at pH 8.0 in the four amino buffers used in the preceding buffer concentration study. The four buffers ranged in pK_a from 8.95 to 10.4. The rate constants for this study were obtained from the buffer concentration study (taken at 0.12 M, Table 5). There was essentially no variation in rate with changing buffer pK_a. The slight positive slopes detected for the 3- and 4-PBT are not considered to be significant. These data are consistent with and add support to the notion that the reaction for each isomer in amino buffers is specific acid-catalyzed.

Discussion

1-Aryl-3-alkyltriazenes[6,7,10,16–18] typically decompose to yield only the corresponding arylamine and alkyl alcohols. The sole exception is a study which reported, in addition to the normal products, significant yields of alkylamines, phenols, and diaryltriazenes.[29] It is perhaps relevant that the authors also reported the presence of both the 1-aryl-3-alkyl and 3-aryl-1-alkyl tautomeric forms of the starting material. In the present study, the initial work was performed in a phosphate buffer at near physiological pH (in order to mimic biological conditions and for future studies); we have observed that 1-(X-pyridinyl)-3-butyltriazenes decomposed exclusively to

(36) Rohlf, F. J.; Sokal, R. R. *Statistical Tables*, 2nd ed.; W. H. Freeman and Company: San Francisco, CA, 1956; pp 166–168.

Scheme 2. Generalized Scheme for Decomposition of N3-Pyridyl Tautomer of X-PBT



aminopyridines and alkyl alcohols, despite the presence of only a small amount of the 3-(X-pyridinyl)-1-butyl tautomer in the NMR spectra. It was satisfying when studies carried out in 0.1 M lysine buffers (the kinetic buffers from which all other data were obtained) at three separate pH's, for each of the X-PBT's, yielded the same products.

The relationship between the structure of the starting material and that of the products bears some comment, particularly in light of the generally accepted mechanism of proteolytic decomposition (Scheme 1). Surprisingly, the observed products arise from the minor tautomer and are the result of the formation of the less stable alkyl (as compared with aryl) diazonium ion. A possible explanation for this observation lies in the potential reversibility of the alternative aryl diazonium ion–alkylamine pathway (Scheme 1, path A). In short, the equilibrium dissociation/reassociation of the aryldiazonium ion and alkylamine can be contrasted with the irreversible formation of the arylamine and the alkyldiazonium ion (Scheme 1, path B). Thus, eventually all the reactants are depleted by path B. Rapid reaction of the resultant alkyldiazonium ion with water then gives alkyl alcohols and, to a lesser extent, the corresponding olefins by elimination. At the present time we have no evidence to either support or refute this hypothesis but are continuing to investigate this point.

The pH–rate profiles for isomers of 1-(X-pyridinyl)-3-butyltriazene show a clear acid-catalyzed domain with pH independent regions at both higher and lower pH. Throughout, the observed well-behaved kinetics predict first order behavior and are consistent with the mechanism shown in Scheme 2, which leads to the rate equation[34]

$$k_{obs} = k_{H^+}[H^+] + k_0 \quad (1)$$

where $k_{H^+} = k_2/(K_a + [H^+])$ and $k_0 = k_1K_a/(K_a + [H^+])$.[37] At relatively low pH values, this equation reduces to

$$\log k_{obs} = \log k_2 \quad (2)$$

thus generating the upper, pH independent plateau. At higher pH, the equation instead takes the form

$$\log k_{obs} = \log k_1 \quad (3)$$

producing the lower plateau, also pH independent. Through the intermediate pH region, where $[H^+] \sim K_a$, one can make the approximation that $(K_a + [H^+]) \sim 2K_a$ and hence

$$k_{obs} = (k_2/2K_a)[H^+] + (k_1/2) \quad (4)$$

Thus, in this region, k_{obs} follows a linear, inverse dependence upon pH.

The rates of decomposition at a given pH in various amine buffers are independent of buffer concentration and pK_a of the buffer conjugate acid. The solvent isotope effect, k_{H_2O}/k_{D_2O}, is < 1.0 for all compounds studied; that is, the decomposition is faster in D_2O than in H_2O. Taken together, these results indicate that the hydrolysis reaction follows a specific acid-catalyzed (A-1) mechanism. The triazene undergoes rapid reversible protonation followed by rate-limiting unimolecular fragmentation.

Previous reports by Sinnott and co-workers[11] have proposed a general acid-catalyzed mechanism for the decomposition of arylalkyltriazenes. In contrast, the data from the present study clearly indicate a specific acid-catalyzed mechanism. To resolve this discrepancy, we investigated the decomposition of phenylbutyltriazene, PhBT. In our hands the evidence points to a specific acid-catalyzed mechanism for PhBT. The solvent isotope effect is < 1.0, and there is no dependence of rate upon either buffer concentration or pK_a of the buffer conjugate acid. One explanation for the difference in observed results may lie in the aqueous systems employed in the two studies. Sinnott and co-workers used a TRIS buffer containing 1.0 M KCl, in contrast to the present study which used lysine buffers of significantly lower total ionic strength (0.25 M).

The solvent isotope effects for 2-PBT and 4-PBT are substantially lower (ca. 0.35) than those for 3-PBT and PhBT (0.70). This difference could be interpreted as indicating an earlier transition state for the heterolysis step of 2-PBT and 4-PBT, as compared with that for the other two compounds. Similar conclusions have been drawn from the isotope effects reported for the acid-catalyzed hydrolysis of acetals, ketals, and ortho esters bearing electron-withdrawing substituents.[38]

(37) From the mechanism in Scheme 2 and the assumption that $[T]_{tot} = [T] + [TH^+]$, then

$$-d[T]_{tot}/dt = k_{obs}[T]_{tot} = k_2[TH^+] + k_1[T]$$

Substituting appropriately from the K_a equation for TH^+ and T ionizations, this becomes

$$k_{obs}[T]_{tot} = k_2([T]_{tot}[H^+])/(K_a + [H^+]) + k_1([T]_{tot}K_a)/(K_a + [H^+])$$

Further simplification gives

$$k_{obs} = (k_2/(K_a + [H^+]))[H^+] + k_1K_a/(K_a + [H^+])$$

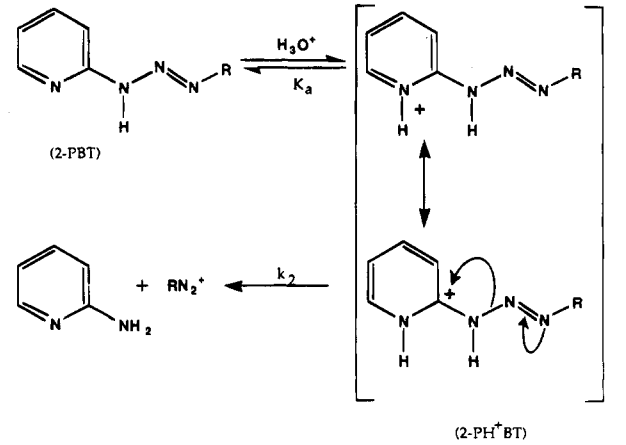
(38) Cordes, E. H.; Bull, H. G. *Chem. Rev.* **1974**, *74*, 581.

The sigmoidal shape of the pH–rate profiles (Figure 2) led us to postulate that these data could be used to obtain $pK_{a'}$ values for each of the triazenes. The only other published determination of a triazene pK_a, that for N_3H_3,[39] involved a rather specialized method which is not generally applicable. Several different methods (see Results) are available for calculating $pK_{a'}$ values from pH–rate data. Taking an average of the result obtained from each of these methods, we have calculated the following $pK_{a'}$ values for pyridinylbutyltriazenes: 2-PBT, 5.19 ± 0.19; 3-PBT, 4.89 ± 0.12; and 4-PBT, 7.77 ± 0.16. Computer fitting the data with the rate equation (eq 4) adds support to the above mentioned $pK_{a'}$ values, in that it yields values which lie within the error range. The $pK_{a'}$ values obtained from the kinetic eqution fit are 2-PBT, 5.10, 3-PBT, 4.91, and 4-PBT, 7.64, which is within the standard error of the methods of calculation. Caution must be exercised in assuming the site of protonation to which these $pK_{a'}$ values pertain, especially in the case of 3-PBT. Only protonation at sites leading to decomposition will be reflected in our $pK_{a'}$ values. That site may not be the same for all of the pyridinyltriazenes studied herein. Nonetheless, we note that these values follow the same order as that of the corresponding aminopyridines [2-aminopyridine, 6.71; 3-aminopyridine, 6.03; and 4-aminopyridine, 9.11].[40] *Ab initio* gas phase proton affinities calculated at the 6-31G* basis set level and then solvent corrected at the gas phase minimum (SCRF[41] and AM1-SM2[42]) for the three isomeric aminopyridines, reveal preferential protonation on the pyridinyl nitrogen. The gas phase difference between exocyclic and endocyclic nitrogens was >20 kcal/mol in the gas phase. This was decreased to ≥10 kcal/mol using the SCRF method and ≥11 kcal/mol using the AM1-SM2 method (data to be published separately). As a group, the pyridinylbutyltriazenes' $pK_{a'}$'s are lower than those of the analogous aminopyridines, presumably a reflection of the electron-withdrawing nature of the triazene moiety. It should also be stressed that $pK_{a'}$ values are only an approximation of the thermodynamic pK_a values. In this case, however, it is expected that $pK_{a'}$ values are close to their accurate thermodynamic counterparts.

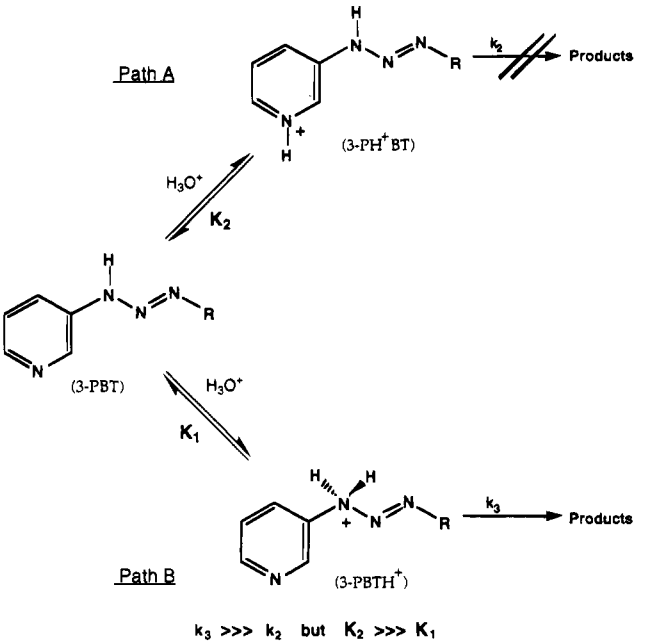
Implicit in the foregoing analysis is the fact that the rate of pyridinyltriazene decomposition correlates directly with basicity, that is, 4-pyridinyl > 2-pyridinyl > 3-pyridinyl. The fact that this correlation is far from linear (viz. a plot of log k versus $pK_{a'}$ for the three isomers) strongly suggests that triazene basicity is not the sole determining factor in the rate of decomposition.

Another important factor lies in the ability of the most probable protonated species to undergo heterolysis of the N(2)–N(3) bond. For simplicity we will consider only the 3-(X-pyridinyl) tautomers in that they alone can give rise to the observed products via protonation and subsequent heterolysis. For each of the three isomeric X-pyridinyltriazenes, the thermodynamically most favored site for protonation is the pyridinyl nitrogen. The data to support this contention are derived from *ab initio* computational results (manuscript in preparation). Briefly,

Scheme 3. Mechanism of Decomposition of 2-PBT in Aqueous Buffer



Scheme 4. Mechanism of Decomposition of 3-PBT in Aqueous Buffer



that study reveals that pyridinyl protonation of the 1-(X-pyridinyl)-3-methyltriazene, like the aminopyridines, is favored over triazene N(3) or N(1) protonation by at least 12 kcal/mol (gas phase).

Mechanistically, simple triazenes (e.g., 1,3-dimethyltriazene[13]) undergo decomposition via protonation of N(3) of the triazene moiety. It can be seen (Scheme 3) that, for the 2- and by analogy the 4-pyridinyltriazenes, the pyridinyl nitrogen is conjugated to N(3) of the triazene moiety. Hence, pyridinyl protonation can lead directly to scission of the N(2)–N(3) bond. For both of these isomers, this destabilizing effect of the protonated pyridinyl ring on the triazene moiety enhances the rate of decomposition as compared with that of PhBT (Table 2). In the case of the 3-pyridinyltriazene, however, due to lack of conjugation of the pyridinyl N with the triazene moiety, protonation of the pyridinyl ring (Scheme 4, path A) does little to favor N(2)–N(3) cleavage. Here protonation actually retards decomposition as evidenced by the fact that the 3-PBT decomposes at a rate slower than that of PhBT (Table 2). We surmise that the most likely route for the decomposition of the 3-PBT (Scheme 4, path B) and PhBT is via N(3) protonation of the neutral

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triazene and that the pyridinyl ring disfavors this route through inductive withdrawal of electron density from the triazene moiety.

In summary, we find that pyridinylalkyltriazenes decompose in aqueous buffers by a specific acid-catalyzed mechanism[43] to give exclusively aminopyridines and alkyl alcohols. By comparison with the analogous phenylalkyltriazene, PhBT, a 2- or 4-pyridinyl substituent exerts a rate-enhancing effect, while a 3-pyridinyl group slows the rate of decomposition. In addition, this study provides the first general method for determining the $pK_{a'}$ of acid-labile triazenes. Pyridinylalkyltriazenes have $pK_{a'}$'s in the range of 5–8 depending upon the site of attachment to the pyridine ring and the site of protonation.

The results of this study suggest that a pyridinyl substituent, appropriately attached, can be used to either increase or decrease the stability of arylalkyltriazenes. Thus, pyridinyltriazenes may prove useful as a means of modulating arylalkyltriazene reactivity in the development of a new generation of these important cancer chemotherapeutic drugs.[44]

Acknowledgment. Research sponsored in part by the National Cancer Institute, DHHS, under Contract No. NO1-CO-74101 with ABL and Contract No. NO1-CO-74102 with PRI, and (R.H.S., in part) by a grant from the National Science Foundation (CHE-8910890 and CHE-9215925). The contents of this publication do not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government. We are grateful to John Roman for providing mass spectrometry data and John Klose for providing NMR spectra. We are also grateful to Raymond W. Nims for his assisstance in analyzing much of the kinetic data. Further, the authors acknowledge the work of Nathan A. Smith and Preetam Shah in providing proton affinity data for aminopyridines and pyridinyltriazenes.

Supplementary Material Available: Copies of supporting ^1H NMR and ^{13}C NMR spectra (7 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.

(43) One reviewer correctly pointed out that the decomposition should be characterized as specific acid catalyzed (A-1) and spontaneous. Further, the spontaneous reaction could be as shown in Scheme 2 or perhaps water catalyzed.

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