Specificity of DNA Alkylation by 1-(2-Chloroethyl)-3-alkyl-3-acyltriazenes Depends on the Structure of the Acyl Group: Kinetic and Product Studies

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The reactions of calf thymus DNA with ten 1-(2-chloroethyl)-3-alkyl-3-acyltriazenes of varying acyl side chain structure were studied alone, or in the presence of porcine liver esterase in pH 7.0 phosphate buffer. In several of the key triazenes, the acyl substituent contained a free carboxylic acid group. With esterase present in the reaction mixture, the resultant levels of DNA alkylation could be correlated with the kinetic rates of decomposition of the triazenes. Under these conditions, the predominant pathway of decomposition involved deacylation of the parent triazene and eventual production of an alkanediazonium ion. This intermediate subsequently alkylated DNA−guanine to give 7-alkylguanine as the principal reaction product. In the absence of esterase, the order of DNA alkylation for all of the acyltriazenes did not correlate with their respective rates of decomposition, leading to the conclusion that the triazenes did not decompose by the expected mode of uncatalyzed N(2)−N(3) heterolyic cleavage. The major DNA alkylation product from the *N*(3)-methyltriazenes was 7-methylguanine, instead of the expected 7-(chloroethyl)- and 7-(hydroxyethyl)guanine products, which suggested that the acyl group was being hydrolyzed. However, acyltriazenes with an *N*(3)-benzyl group rather than a methyl in this position produced very little 7-benzylguanine product, contrary to prediction. An alternative mechanism involving internally assisted hydrolysis of the side chain ester is proposed to explain these results. NMR product analysis and computational studies were carried out to lend support to the postulated mechanism.

Introduction

The chemistry and biochemistry of 1-(2-chloroethyl)-3-acyl-3-methyltriazenes have been explored in our laboratory for several years. These compounds have been shown to be alkylating agents for DNA (*1*), reacting in a base sequence-selective manner (*2*). Most of the alkylation was due to (chloroethyl)- and (hydroxyethyl)guanine diazonium ions, which were formed at neutral or acidic pH following N(2)−N(3) heterolysis of the parent triazene. In the presence of base or a hydrolytic enzyme such as esterase, deacylation was the predominant pathway and resulted in the production of highly labile dialkyltriazenes. These compounds, in turn, could competitively methylate, chloroethylate, or hydroxyethylate DNA−guanine (Figure 1).

In order to be effective as antitumor agents, drugs must have reasonable stability under physiological conditions. It has been shown previously (*3*) that the stability in buffer of the extremely unstable 1,3-dialkyltriazenes could be dramatically enhanced by the addition of an acyl group at the 3-position. The resultant compounds have half-lives of 2−1000 min, depending on the structure of the acyl group. Several of the acyltriazenes have subsequently been shown to possess significant chemotherapeutic properties (*4*).

Recently, acyltriazenes were used as cytotoxic "bullets" to target gastrin receptor (GR)[1] expressing tumor cells. GR is expressed in various human cancers, such as colon adenocarcinoma (*5*, *6*). Since the crucial feature for the recognition of gastrointestinal peptides by this receptor is the carboxyl terminal sequence Trp-Met-Asp-Phe amide (*7*, *8*), the drugs were designed to contain an acyl linker between the peptide and the triazene which resulted in a carrier-linked conjugate of reasonable stability. One of the resulting triazenes, *N*-[3-benzyl-3-(carboxypropanoyl)-1-(2-chloroethyl)triazene]-*β*-Ala-Trp-Met-Asp-Phe amide (CBS-5), effectively competed with gastrin in an assay using either guinea pig stomach fundus or the rat acinar tumor cell line AR42J as the

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[1] Abbreviations: GR, gastrin receptor; CMA, 1-(2-chloroethyl)-3-methyl-3-acetyltriazene; CMC, 1-(2-chloroethyl)-3-methyl-3-carbethoxytriazene; CBSA, 1-(2-chloroethyl)-3-benzyl-3-(3-carboxypropanoyl)-triazene; CBC, 1-(2-chloroethyl)-3-benzyl-3-carbethoxytriazene; CEC, 1-(2-chloroethyl)-3-ethyl-3-carbethoxytriazene; CMSA, 1-(2-chloroethyl)-3-methyl-3-(3-carboxypropanoyl) triazene; CMGA, 1-(2-chloroethyl)-3-methyl-3-(4-carboxybutanoyl)triazene; CBGA, 1-(2-chloroethyl)-3-benzyl-3-(4-carboxybutanoyl)triazene; DMAP, 4-(dimethylamino)-pyridine; CMS, 1-(2-chloroethyl)-3-methyl-3-(3-carbomethoxypropanoyl)-triazene; CBS, 1-(2-chloroethyl)-3-benzyl-3-(3-carbomethoxypropanoyl)-triazene; CMG, 1-(2-chloroethyl)-3-methyl-3-(4-carbomethoxybutanoyl)-triazene; CBG, 1-(2-chloroethyl)-3-benzyl-3-(4-carbomethoxybutanoyl)-triazene; CMAD, 1-(2-chloroethyl)-3-methyl-3-(5-carbomethoxypentanoyl)triazene; CMT, 1-(2-chloroethyl)-3-methyltriazene; CBT, CMT, 1-(2-chloroethyl)-3-benzyltriazene; RHF, restricted Hartree−Fock; SCRF, self-consistent reaction field; 7-MeG, 7-methylguanine; 7-EtG, 7-ethylguanine; 7-BzG, 7-benzylguanine; 7-HOEtG, 7-(hydroxyethyl)guanine; 7-ClEtG, 7-(chloroethyl)guanine; O^6-MeG, O^6-methylguanine; O^6-HOEtG, O^6-(hydroxyethyl)guanine.

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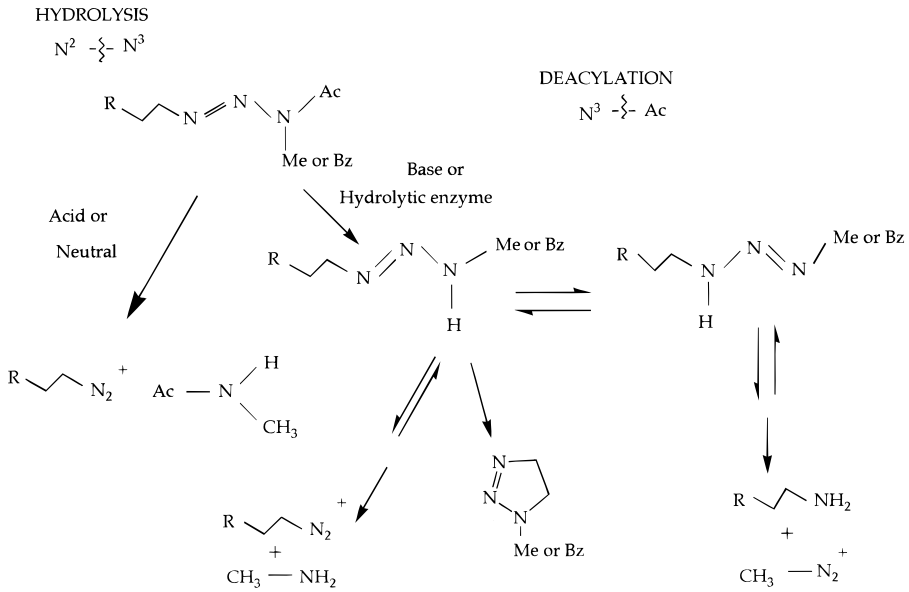
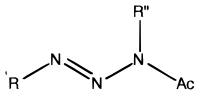


Figure 1. Proteolytic decomposition scheme for 1,3-dialkyl-3-acyltriazenes.



R′	R″	Acyl	Acyltriazene
ClEt	CH_3	$COCH_3$	CMA
ClEt	CH_3	CO_2Et	CMC
ClEt	CH_3CH_2	CO_2Et	CEC
ClEt	Bz	CO_2Et	CBC
ClEt	CH_3	$CO(CH_2)_2COOCH_3$	CMS
ClEt	CH_3	$CO(CH_2)_2COOH$	CMSA
ClEt	Bz	$CO(CH_2)_2COOCH_3$	CBS
ClEt	CH_3	$CO(CH_2)_3COOCH_3$	CMG
ClEt	CH_3	$CO(CH_2)_4COOCH_3$	CMAD
ClEt	Bz	$CO(CH_2)_3COOCH_3$	CBG

Figure 2. Structures and abbreviations of 1-(2-chloroethyl)-3-alkyl-3-acyltriazenes.

source of the receptor (*9*). In addition, this triazene was cytotoxic to AR42J cells, but was not toxic to A549 human lung cancer cells. The latter cell line does not express the gastrin receptor.

In the present study, we examined DNA alkylation by the parent triazene CBS and related compounds (Figure 2), where the acyl side chains are derivatives of dicarboxycyclic (succinic, glutaric, and adipic) acids. Several other acyltriazenes were also studied. Our goals were twofold: to evaluate how different acyl side chains modulate the resultant DNA alkylation levels and to determine the mechanism of the alkylation through kinetic and product studies. Such information would be extremely valuable in the development of new, more potentially active alkylating drugs.

Materials and Methods

Chemicals. Calf thymus DNA was obtained from Sigma Chemical Co. (St. Louis, MO). Standard alkylated bases were either purchased (7-methylguanine, Sigma) or synthesized in our laboratory (*11*) except for 7-(2-chloroethyl)guanine, which was a generous gift of Dr. David Ludlum, University of Massachusetts Medical School, Worcester, MA. Acyltriazenes which had been prepared previously were synthesized, purified, and characterized according to the procedures of Smith et al. (CMA, CMC) (*4*, *10*) or Schmidt et al. (CBSA) (*9*) and were >99% pure (NMR and UV/vis spectroscopy). The other acyltriazenes were prepared according to the following procedures. **Note: Extreme caution must be taken in working with low molecular weight alkyl azides, as these compounds are treacherously explosive!**

Synthetic Procedures. 1-(2-Chloroethyl)-3-benzyl-3-carbethoxytriazene (CBC). Chloroethyl azide (570 mg, 5.4 mmol) was dissolved in 25 mL of dry THF and cooled to −30 °C under nitrogen. Benzylmagnesium chloride (2.7 mL of a 2 M solution in THF) was added dropwise. The reaction mixture was allowed to warm to 0 °C over a 45 min time period. Ethyl chloroformate (516 μL, 5.4 mmol) in 5 mL of THF was added at −20 °C. The reaction mixture was allowed to warm to room temperature and was stirred for 1 h. Diethyl ether (30 mL) was added, and the reaction mixture was washed with 10% (w/w) $NaHCO_3$ solution (2 × 15 mL), H_2O, and brine. The organic phase was then dried over Na_2SO_4. The solvent was removed, and the desired oil was purified by column chomatography on SiO_2 (eluent: pentane/diethyl ether = 2/1). Yield: 600 mg (41%). ^{1}H-NMR ($CDCl_3$/200 MHz): δ 7.24 (arom, s), 5.11 (CH_2-arom, s), 4.41 (CH_2, q, 7.1 Hz), 4.08 ($ClCH_2$, AA′), 3.79 (CH_2N═, BB′), 1.37 (CH_3, t, 7.1 Hz). Exact mass calcd for $C_{12}H_{16}ClN_3O_2$ (M^+) = 269.093105, found 269.0909.

1-(2-Chloroethyl)-3-ethyl-3-carbethoxytriazene (CEC). This compound was prepared by a procedure analogous to the preceding using 30 mmol of chloroethyl azide and 30 mmol of ethyl chloroformate. Yield: 2.0 g (32%). ^{1}H-NMR ($CDCl_3$/200 MHz): δ 4.38 (NCH_2, q, 7.11 Hz), 4.09 ($ClCH_2$, AA′, ~6.5 Hz); 3.96 (OCH_2, q, 7.0 Hz), 3.90 (CH_2N═, BB′, ~6.5 Hz); 1.38 (NCH_2CH_3, t, 7.11 Hz), 1.07 (OCH_2CH_3, t, 7.0 Hz). Exact mass calcd for $C_7H_{14}ClN_3O_2$ (M^+) = 207.077455, found 207.0790.

General Procedure for the Synthesis of CMSA, CMGA, and CBGA. Chloroethyl azide (2.58 mL, 30 mmol) was dissolved in 80 mL of dry THF and cooled to −50 °C under nitrogen. Methylmagnesium chloride (10.5 mL of a 3 M solution in THF) or benzylmagnesium chloride (15 mL of a 2 M solution in THF) was added dropwise. The reaction was allowed to warm to room temperature over a 1 h time period. The reaction mixture was recooled to −30 °C, and a solution of succinic anhydride (3.0 g, 30 mmol) or glutaric anhydride (3.42 g, 30 mmol) in THF (20 mL) was added dropwise. The cooling bath was removed, and the reaction mixture was stirred for 1.5 h (CMSA) or 3 h (CMGA

and CBGA) at room temperature. Water (20 mL) was added, and the reaction mixture was acidified to pH 3−4 with 1 N HCl and extracted with ethyl ether (2 × 100 mL). The organic phase was washed with brine and dried over Na_2SO_4. After evaporation of the solvent, a crude, oily product was obtained. The products were either recrystallized from ethyl acetate (CMSA) or chomatographed (CMGA, CBGA) on SiO_2 (eluent: hexane/diethyl ether = 2:1). Attempts to prepare 1-(2-chloroethyl)-3-methyl-3-(5-carboxypentanoyl)triazene (CMADA) by this procedure were unsuccessful.

1-(2-Chloroethyl)-3-methyl-3-(3-carboxypropanoyl)triazene (CMSA). Yield: 2.3 g (34%), mp 75−83 °C dec. ^{1}H-NMR ($CDCl_3$/200 MHz): δ 4.127 ($ClCH_2$, AA′), 3.90 (CH_2N═, BB′), 3.246 (CH_3, s), 3.147 (CH_2, t), 2.779 (CH_2, t). ^{13}C-NMR ($CDCl_3$/200 MHz): δ 178.41, 173.81, 62.75, 41.72, 28.90, 28.62, 27.33. Exact mass calcd for $C_7H_{13}ClN_3O_3$ $(M + H)^+$ = 222.064544, found 222.0646.

1-(2-Chloroethyl)-3-methyl-3-(4-carboxybutanoyl)triazene (CMGA). Yield: 4.52 g (64%). ^{1}H-NMR ($CDCl_3$/200 MHz): δ 4.118 ($ClCH_2$, AA′, ∼6.0 Hz), 3.90 (CH_2N═, BB′, ∼6.0 Hz), 3.235 (CH_3, s), 2.91 (CH_2, t, 7.3 Hz), 2.49 (CH_2, t, 7.3 Hz), 2.05 (CH_2, qi, 7.3 Hz). Exact mass calcd for $C_8H_{15}ClN_3O_3$ $(M + H)^+$ = 236.080194, found 236.0798.

1-(2-Chloroethyl)-3-benzyl-3-(4-carboxybutanoyl)triazene (CBGA). Yield: 5.7 g (61%). ^{1}H-NMR ($CDCl_3$/200 MHz): δ 7.23 (Ph, s), 5.13 ($PhCH_2$, s), 4.06 ($ClCH_2$, AA′, ∼6.0 Hz), 3.81 (CH_2N═, BB′, ∼6.0 Hz), 2.94 (CH_2, t, 7.3 Hz), 2.49 (CH_2, t, 7.3 Hz), 2.07 (CH_2, qi, 7.3 Hz). Exact mass calcd for $C_{14}H_{19}ClN_3O_3$ $(M + H)^+$ = 312.111494, found 312.1112.

General Esterification Procedure for CMSA, CBSA, CMGA, and CBGA. Carboxylic acid (10 mmol), methanol (0.5 mL), and a catalytic amount of 4-(dimethylamino)pyridine (DMAP) were dissolved in 20 mL of CH_2Cl_2. A solution of dicyclohexylcarbodiimide (2.06 g, 10 mmol) in methylene chloride (15 mL) was added at 0 °C. The reaction mixture was stirred for 5 h at room temperature. The precipitated dicyclohexylurea was filtered off, and the filtrate was concentrated to give a colorless oil. This oil was purified by column chomatography on SiO_2 (eluent: hexane/diethyl ether = 3/1).

1-(2-Chloroethyl)-3-methyl-3-(3-carbomethoxypropanoyl)triazene (CMS). Yield: 34%. ^{1}H-NMR ($CDCl_3$/200 MHz): δ 4.12 ($ClCH_2$, AA′, ∼6.5 Hz), 3.89 (CH_2N═, BB′, ∼6.5 Hz), 3.71 (OCH_3, s), 3.23 (NCH_3, s), 3.14 (CH_2, t, 6.75 Hz), 2.72 (CH_2, t, 6.75 Hz). Exact mass calcd for $C_8H_{14}ClN_3O_3$ (M^+) = 235.072369, found 235.0712.

1-(2-Chloroethyl)-3-benzyl-3-(3-carbomethoxypropanoyl)-triazene (CBS). Yield: 74%. ^{1}H-NMR ($CDCl_3$/200 MHz): δ 7.23 (Ph, s), 5.14 ($PhCH_2$, s), 4.07 ($ClCH_2$, AA′, ∼6.0 Hz), 3.81 (CH_2N═, BB′, ∼6.0 MHz), 3.71 (CH_3O, s), 3.18 (CH_2, t, 6.8 Hz), 2.76 (CH_2, t, 6.8 Hz). Exact mass calcd for $C_{14}H_{18}ClN_3O_3$ (M^+) = 311.103669, found 311.1066.

1-(2-Chloroethyl)-3-methyl-3-(4-carbomethoxybutanoyl)triazene (CMG). Yield: 87%. ^{1}H-NMR ($CDCl_3$/200 MHz): δ 4.12 ($ClCH_2$, AA′, ∼6.0 Hz), 3.895 (CH_2N═, BB′, ∼6.0 Hz), 3.68 (CH_3O, s), 3.23 (CH_3, s), 2.88 (CH_2, t, 7.3 Hz), 2.44 (CH_2, t, 7.3 Hz), 2.04 (CH_2, t, 7.3 Hz). Exact mass calcd for $C_9H_{17}ClN_3O_3$ $(M + H)$ = 250.0958, found 250.0928.

1-(2-Chloroethyl)-3-benzyl-3-(4-carbomethoxybutanoyl)triazene (CBG). Yield: 87%. ^{1}H-NMR ($CDCl_3$/200 MHz): δ 7.23 (Ph, s), 4.06 ($ClCH_2$, AA′, ∼6.0 Hz), 3.81 (CH_2N═, BB′, ∼6.0 Hz), 3.67 (CH_3O, s), 2.91 (CH_2, t, 7.3 Hz), 2.44 (CH_2, t, 7.3 Hz), 2.06 (CH_2, qi, 7.3 Hz). Exact mass calcd for $C_{15}H_{24}ClN_4O_3$ $(M + NH_4)^+$ = 343.153694, found 343.1571.

1-(2-Chloroethyl)-3-methyl-3-(5-carbomethoxypentanoyl)-triazene (CMAD). Adipic acid monomethyl ester (2.96 mL, 20 mmol) and hydroxysuccinimide (2.416 g, 21 mmol) were dissolved in 30 mL of dry THF. Dicyclohexylcarbodiimide (4.326 g, 21 mmol) was added, and the reaction mixture was stirred overnight at room temperature. After filtration of the precipitated dicyclohexylurea, the filtrate was concentrated, taken up in 10 mL of dry THF, and refiltered. This solution of the crude hydroxysuccinimide ester of adipic acid monomethyl ester was used in the next reaction step.

Chloroethyl azide (2.1 g, 20 mmol) was dissolved in 30 mL of dry THF and cooled to 0 °C under nitrogen. Methylmagnesium chloride (7 mL of a 3 N solution in THF) was added dropwise. The reaction was allowed to warm to 0 °C over a 45 min time period. After recooling to −30 °C, the above-described THF solution of the activated adipic acid monomethyl ester was added dropwise. The cooling bath was removed and the reaction mixture was stirred at room temperature for 1 h. Diethyl ether (60 mL) and saturated $NaHCO_3$ solution (10 mL) were added. The reaction mixture was filtered, and the filtrate was washed with (10%) $NaHCO_3$ solution (2 × 20 mL) and saturated brine. The organic phase was dried over Na_2SO_4. After evaporation of the solvent, a colorless oil was obtained. The product was purified by column chromatography on silica gel (eluent: pentane/diethyl ether = 2/1 v/v). Yield: 750 mg (15%). ^{1}H-NMR ($CDCl_3$/200 MHz): δ 4.11 ($ClCH_2$, AA′, ∼6.5 Hz), 3.89 (CH_2N═, BB′, ∼6.5 Hz), 3.67 (NCH_3, s), 3.23 (OCH_3,s), 2.83 ($(O)CCH_2$), 2.36 ($(O)CCH_2$), 1.72 ($(CH_2)_4$, m). Exact mass calcd for $C_{10}H_{18}ClN_3O_3$ (M^+) = 263.103669, found 263.1048.

Reactions of Triazenes with Calf Thymus DNA. A 10 μL aliquot of a 0.5 M dimethyl sulfoxide solution of a particular triazene was added to 990 μL of a solution of calf thymus DNA (1 mg/mL) in 0.02 M sodium phosphate/0.01 M sodium chloride buffer (pH 7.0). The reactions (5 mM final triazene concentration) were incubated at 37 °C either alone (48 h) or in the presence of porcine liver esterase (2 h) (EC 3.1.1.1) (10 μL/1 mL reaction solution, 5.35 mg/mL). In addition, CMS was also incubated for 48 h in the presence of esterase. The DNA was precipitated with 3 mL of absolute ethanol and centrifuged for 15 min at 5000*g*. The DNA pellet was washed with 2% sodium acetate in ethanol and then allowed to sit in this solution for 15 min. This solution was decanted and was replaced by anhydrous ether for 5 min. The ether was decanted off, and the DNA was dried in a vacuum desiccator overnight and then stored at −20 °C until hydrolysis.

Analysis of DNA Adducts. Hydrolysis and HPLC of the DNA adducts (7-MeG, 7-EtG, 7-HOEtG, 7-ClEtG, O^6-MeG, and O^6-HOEtG) were carried out as previously described (*11*). The presence of expected 7-BzG from the 7-benzyl triazenes, identified by coelution with a standard sample, was also estimated. All results are reported as millimoles of alkylated base per mole of guanine on at least duplicate experiments.

Kinetic Studies. Rates of triazene decomposition in aqueous solution at 70 °C were carried out on a Milton Roy MR3000 UV/visible spectrophotometer. The disappearance of each triazene (final concentration 3.0×10^{-5} M) was followed by monitoring the change in absorbance at its respective λ_{max}, ca. 245 nm. The experimental procedure for a typical kinetic run in 0.1 M phosphate buffer, pH 7.0 (ionic strength = 0.45 M maintained with added NaCl), and the calculation of the first-order rate constants have been previously detailed (*12*). A minimum of 100 absorbance vs time readings were obtained over at least 3.5 half-lives. The values reported are the average of two separate determinations which agree within ±2%.

Product Studies. The products of the decomposition of the various triazenes were determined by ^{1}H-NMR following reaction at 37 and 70 °C in 0.05 M sodium phosphate/0.25 M Na_2SO_4 in D_2O, adjusted to pH 7.5 with a D_2O solution of NaOD. Buffer was added to a weighed amount of triazene, sealed in vials, and incubated for at least four half-lives, as determined by kinetic measurements. At the end of the reaction time, an aliquot of the reaction solution was removed and analyzed by ^{1}H NMR. Assignment of the NMR peaks arising from the various products was made by comparison and spiking with authentic samples. Yields were determined by comparative integration of the product peaks versus an internal standard, fumaric acid.

The potential conversion of acyltriazenes esters to their respective free acid forms was checked by HPLC following incubation at 37 °C. Reactions with CMC, CBS, CMS, CBG, CMG, or CMAD in buffer alone, or in the presence of calf thymus DNA in buffer (1 mg/mL), both in the presence and in the absence of esterase, were carried out for times up to 48 h. The

Table 1. Rates[a] of Decomposition of 1,3-Dialkyl-3-acyltriazenes[b] in pH 7.0 Phosphate Buffer[c] at 70 °C

triazene	k_{obs} (s^{-1})	triazene	k_{obs} (s^{-1})
CMA	4.46E−05[d]	CBSA	9.84E−05
CMC	2.39E−04	CMG	5.64E−05
CBC	6.38E−04	CMGA	5.11E−05
CEC	3.03E−04	CBG	1.26E−04
CMS	6.42E−05	CBGA	1.06E−04
CMSA	6.73E−05	CMAD	4.56E−05
CBS	1.62E−04		

[a] The rate constants are an average of at least two independent runs varying no more than ±3%. [b] The triazene initial concentration was 3.0×10^{-5} M. [c] The buffer also contained 0.01 M sodium chloride. [d] Data taken from ref 12.

various triazenes were separated on a Waters HPLC equipped with a 5-μm ODS reverse-phase Beckman column at a flow rate of 1 mL/min using an isocratic solvent mixture of 60/40 methanol/water. The retention times for the various triazenes were as follows: CMC, 8 min; CMS, 9 min, and its free acid CMSA, 4.5 min; CBS, 22 min, and its free acid, 5 min; CMG, 8 min, and its free acid, 5 min; CBG, 27 min, and its free acid, 14 min; CMAD, 16 min, and its free acid, 5.0 min. Aliquots were removed from the nonesterase containing reaction mixtures at 0 and 48 h and were injected immediately onto the column for product identification. Coinjection of the 48 h samples with their respective free acid forms was used to verify conversion of the starting triazene to product. The reactions which included esterase were monitored for times up to 1 h in order to follow the disappearance of the starting material.

Computational Studies. Calculations were performed using the *ab initio* quantum mechanics program Gaussian 92 (*13*), adapted to run on the Cray YMP-2 computer at FCRDC. Optimized geometries of all structures were determined from restricted Hartree−Fock (RHF) calculations using the standard 6-31G* basis set. Although the Berny optimization algorithm was used in the exploration of the hypersurface to find energy minima, the analytic force field was calculated for all stationary points to verify that they were minima. An estimate of the effect of solvent was calculated by the self-consistent reaction field (SCRF) method, which is included in the Gaussian 92 package, using a dielectric constant of 78.3 for water at 25 °C.

Results

Rate of Triazene Decomposition. The rate of decomposition of all 13 acyltriazenes (see Figure 2) was determined at pH 7.0 in sodium phosphate buffer. The observed rate constants, which were cleanly first-order, are shown in Table 1.

Alkylation Studies with Calf Thymus DNA. Ten of these acytriazenes were reacted with calf thymus DNA in pH 7.0 phosphate buffer (48 h), or in the presence of esterase (2 or 48 h reactions) in the same buffer. Depending on the structure of the triazene, analysis by HPLC for 7-methyl, 7-ethyl-, 7-(hydroxyethyl)-, and/or 7-(chloroethyl)guanine adducts was carried out, as well as for the O^6-methyl- and O^6-(hydroxyethyl)guanines.

The data for the alkylation of DNA−guanine by the various acyltriazenes without esterase present in the reaction mixture are tabulated in Table 2. The best alkylating agent, based on total 7-alkyl product detected in the 48 h time of reaction, was CMSA, followed in order by CMS > CMC > CMG > CBC = CEC > CMAD > CMA > CBS > CBG. The levels of total 7-alkylguanine products and the rates of decomposition of the respective triazenes revealed no obvious correlation. In fact, the benzyltriazenes, which decomposed with the fastest rate, gave the lowest total levels of alkylation. As expected, mainly 7-ClEtG and 7-HOEtG products were detected

Table 2. Alkylation of DNA−Guanine by Acyltriazenes

	mmol/mol of guanine				
triazene	7-MeG	7-HOEtG	7-ClEtG	O^6-MeG	O^6-HOEtG
CMA	0.35	0.14	0.72	0.11	nd[a]
CMC	0.33	0.65	2.60	0.40	0.02
CBC	nd[b]	1.12	1.03	nd	0.01
CEC	nd[c]	0.60	1.56	nd	nd
CMS	3.46	0.31	0.70	1.77	0.01
CMSA	6.05	0.15	0.47	2.72	nd
CBS	~0.02[d]	0.50	0.34	nd	0.01
CMG	1.28	0.42	1.05	0.49	nd
CBG	nd	0.25	nd	nd	nd
CMAD	0.89	0.17	0.63	1.05	nd

[a] nd = adduct not detected following reaction at 37 °C for 48 h. [b] No 7-BzG detected. [c] No 7-EtG detected. [d] Adduct is presumed 7-BzG.

Table 3. Alkylation of DNA−Guanine by Acyltriazenes in the Presence of Esterase

	mmol/mol of guanine				
triazene	7-MeG	7-HOEtG	7-ClEtG	O^6-MeG	O^6-HOEtG
CMA	0.48	nd[a]	nd	0.23	nd
CMC	9.76	nd	nd	4.40	nd
CBC	~0.03[b]	nd	nd	nd	nd
CEC	nd[c]	nd	nd	nd	nd
CMS	0.24	nd	nd	0.13	nd
CMSA	1.25	0.33	nd	0.35	nd
CBS	~7[b]	nd	nd	nd	nd
CMG	0.41	nd	nd	0.17	nd
CBG	nd	nd	nd	nd	nd
CMAD	0.51	nd	nd	0.17	nd

[a] nd = adduct not detected following reaction at 37 °C for 2 h. [b] Adduct presumed to be 7-benzylguanine. [c] nd = no 7-ethylguanine detected.

from all the triazenes except for CMS and CMSA, where large quantities of 7-MeG were observed. CMG and CMAD also gave significant amounts of 7-MeG. Further, the rates of decomposition of CMS, CMG, and CMAD correlated with the amount of 7-MeG which was detected from these triazenes; i.e., CMS produced more 7-MeG than CMG, which in turn yielded more than CMAD.

When esterase was added to the reaction mixture, the major guanine adduct detected (Table 3) from all of the acyltriazenes where R″ = CH_3 was 7-MeG. In addition, in the presence of esterase, CMSA was the only triazene to hydroxyethylate guanine in the 7-position. Compared with the levels of production of 7-MeG in the simple hydrolytic reactions, the addition of esterase led to an increase with CMC (30-fold), no change with CMA or CMAD, and a decrease with CMS and CMSA (14- and 5-fold, respectively). The reaction of CMS with guanine in the presence of esterase for 48 h instead of 2 h resulted in a 7-MeG level of 2588 μmol/mol of guanine (an 11-fold increase). In the case of the *N*(3)-benzyltriazenes (when R″ = Bz), very small amounts of 7-BzG were observed.

The alkylation product ratios are shown in Table 4. The 7-HOEt/7-ClEtG ratios for all of the *N*(3)-methyl or *N*(3)-ethyl compounds are in the range of 0.2−0.4, while those for the two *N*(3)-benzyltriazenes were 1.1 and 1.5. The 7-HOEtG/7-MeG ratios vary greatly depending on the structure of the triazene; from 0.09−0.03 for CMS and CMSA, to 0.2 and 0.4 for CMAD and CMA, and finally to 2.0 for CMC. The O^6/7-MeG ratios were the same (0.3−0.5) for all the triazenes with or without esterase present in the reaction mixture with the exception of CMC and CMAD, where the ratio changed from ~0.4 to 1.2 in the absence of esterase.

HPLC Product Studies. The conversion in buffer of CMS, CBS, CMG, CBG, CMAD, and CMC to their

Table 4. DNA Alkylation Ratios from Acyltriazenes

triazene	$O^{6/7}$-MeG	7-HOEt/MeG	7-HOEt/ClEtG	$O^{6/7}$-MeG (esterase)
CMA	0.32	0.41	0.2	0.47
CMC	1.21	1.95	0.25	0.45
CBC			1.09	
CEC			0.38	
CMS	0.51	0.09	0.44	0.52
CMSA	0.45	0.025	0.32	0.28
CBS			1.50	
CMG	0.38	0.32	0.34	0.43
CBG				
CMAD	1.2	0.19	0.27	0.34

respective free acid forms in the presence and absence of calf thymus DNA and/or esterase was investigated by HPLC. The levels of starting triazene ester and the free acid product were monitored at times of 0 and 48 h. It was determined that CMS, CMG, and CMAD were all at least partially converted during the time course of the reaction to their respective free acid forms, in buffer alone and/or in the presence of DNA or esterase (data not shown). The amount of free acid detected from CMS was greater than that from CMG or CMAD, in line with the amounts of 7-MeG produced from the respective triazenes. CBS and CBG evidenced the formation of only a small amount of their respective free acids. The CMC reactions (buffer, esterase, or calf thymus DNA) only showed starting material in the buffer or DNA reactions; no new peak was discerned in the 48 h DNA or buffer reactions.

NMR Product Studies. The production of methanol from the hydrolysis of the various acyltriazenes in buffer was determined by ^{1}H NMR. Following a 90 h incubation at 70 °C in sealed ampules, methanol was not detected as a product from CMC, while methanol (maximal yield = 1.3%) was produced from the decomosition of CMA. The other products of hydrolysis of CMC and CMA at neutral pH have been reported previously (*12*).

Decomposition of CMSA, CMGA, CBSA, and CBGA at 37 and 70 °C (Table 5) showed the production of methanol, methylamine, benzylamine, benzyl alcohol, and either succinic or glutaric acid, or their respective monoamides.

Modeling Studies. The energies of the optimized structures of CMS and its decomposition products (Figure 3) calculated in the gas phase and in the presence of a generic dielectric medium for the various possible decomposition pathways of this triazene are shown in Table 6. The results of the theoretical calculations utilizing Hartree−Fock theory at the RHF/6-31G* level allowed the determination of enthalpy of reaction (ΔH) values for selected reactions, which were calculated by subtracting the sum of the energy(ies) of the starting material(s) from the sum of the energy(ies) of the product(s).

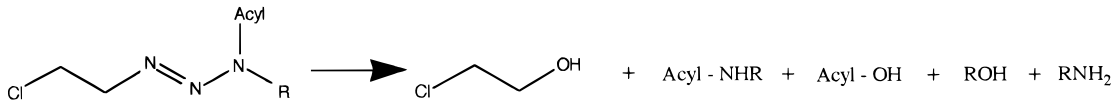
The conversion of CMS to CMSA in the presence of esterase, for example, is highly exothermic, as can be determined from the analysis of the energies of the optimized structures in Table 6. The neutral, free acid form of CMSA was found to be more stable than its anionic form by 325 kcal in the presence of a dielectric, indicative of the more reactive nature of the anion form. The results from the enthalpy calculations revealed that, from neutral CMSA, pathway B to 1-(2-chloroethyl)-3-methyltriazene (CMT) and succinic acid (Figure 3) was an exothermic process by 8.5 kcal/mol in the presence of a dielectric (6.7 kcal/mol gas phase), while pathway A to the diazonium ion and an alkylaminoacetyl-amidyl anion was endothermic by 150 kcal/mol (163 kcal/mol gas phase).

The CMT or 1-(2-chloroethyl)-3-benzyltriazene (CBT) formed via pathway B from CMA or CBS, respectively, can exist as two tautomers. Calculations on these dialkyltriazenes revealed that the N(3)-H tautomers of both CMT or CBT were more stable by 3.0 or 3.5 kcal/mol, respectively, than the corresponding N(1)-H tautomers. Enthalpy calculations on the further decomposition of the methyl- or benzyltriazenes via pathways C and D showed that both were exothermic processes, with pathway D to the alkanediazonium ion and chloroethylamine favored over pathway C by 1.4 kcal/mol for the methyl analogue and by 45 kcal/mol for the benzyltriazene (SCRF values). The cyclization pathway E was endothermic in the presence of a dielectric for both the monomethyl- and monobenzyltriazenes, with $\Delta H = +79.6$ kcal/mol for CMT and $\Delta H = +80.2$ kcal/mol for CBT for conversion to their respective triazoline products.

Discussion

Several 1-(2-chloroethyl)-3-acyltriazenes have been shown to be biological alkylating agents with chemotherapeutic potential (*1*). It is believed that the cytotoxic properties of these triazenes are closely linked with their ability to modify DNA and that many of these compounds may require metabolic activation in order to be active, chemotherapeutically effective agents. We had previously postulated an activation mechanism for CMC in the presence of esterase (*1*), whereby the triazene became a DNA alkylating agent following deacylation to form CMT. We wished to determine whether CMS and related

Table 5. Triazene Hydrolysis Product Yields[a] at 70 °C As Determined by NMR Analysis



acyltriazene	2-chloroethanol	N(3)-derived amide	carboxylic acid	R-OH	$R-NH_2$
CMA	40.8	93.6	5.0	0.32	1.6
CMS	76.4	105.0	11.0	10.4	6.0
CMSA	21.6	30.8	28.0	10.4	10.4
CMGA	44.0	106.0	<3.0	0.5	2.0
CBSA	43.6	85.6	18.4	2.7	4.3
CBGA	56.0	126.0	<3.0	3.0	<3.0

[a] Product yields were estimated by comparison of the integration of the NMR signal of the products with the integrations of lines due to the internal standard, fumaric acid. The product signals for most of the compounds accounted for >90% of the expected yields; however, the amount of the expected primary products of CMSA was only 60% of the total. The remaining 40% probably underwent secondary reactions, which were not identified.

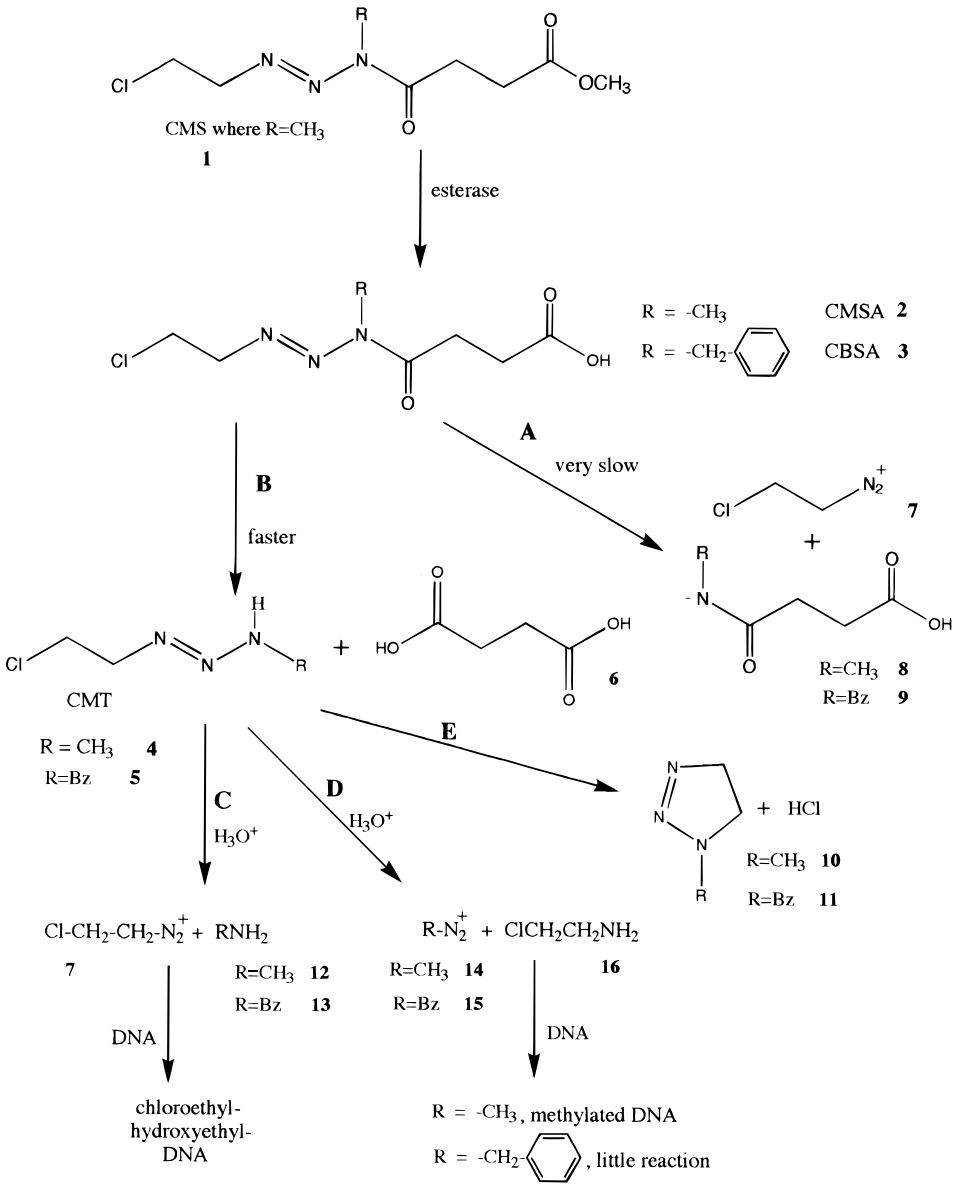


Figure 3. Possible decomposition pathways of CMS in the presence of esterase.

Table 6. Total Energies Calculated for the Products of Decomposition of CMS or CBS

compd no.	gas phase[a]	solvent[b]
1	−1158.4758	−1158.4776
2	−1119.4495	−1119.4512
3	nd[c]	nd
4	−741.0177	−741.0205
5	−970.5656	−970.5676
6	−378.4212	−378.4270
7	−646.1469	−646.1576
8	−473.6562	−473.0542
9	−702.5914	−702.5926
10	−280.9516	−282.0790
11	−510.4964	−510.4985
12	−95.2098	−95.2108
13	−324.7589	−324.7592
14	−148.2161	−148.2181
15	−377.8329	−377.8355
16	−593.1506	−593.1525

[a] Energies (Hartrees) were calculated using the Gaussian 92 program at the HF/6-31G* level of theory (1 Hartree = 627.5 kcal). [b] Energies were calculated using the SCRF option of Gaussian 92 using a dielectric of 78.30 for water. [c] Energy not determined.

compounds were also activated to form alkylating agents through this same intermediate. To this end, we measured the calf thymus DNA alkylation levels of nine additional acyltriazenes both in the presence and in the absence of esterase. In addition, the products and rates of decomposition of these compounds, plus three triazene analogues bearing a free acid moiety (CMGA, CBSA, and CBGA), were also determined.

The rates of decomposition of the new acyltriazenes, along with those of CMC and CMA, which had been measured previously, are listed in Table 1. From these data, it can be seen that the order of decomposition of the compounds is CBC > CEC > CMC > CBS > CBG > CBGA > CBSA > CMSA > CMS > CMG > CMGA > CMAD > CMA. It is clear from these data that the *N*(3)-acyl group, rather than the *N*(3)-alkyl group, is the key predictor of the decomposition rate. For example, the carbethoxytriazenes (CBC, CEC, or CMC) decomposed faster than all other acyltriazenes. Secondly, within the series of triazenes bearing an esterified *N*(3)-acyl group derived from dicarboxylic acids of varying chain lengths, the rate decreased with increasing chain length (CMS > CMG > CMAD), as one would predict. The simple acetyltriazene, CMA, had the slowest rate of decomposition. For a given acyl group, however, the *N*(3)-benzyl

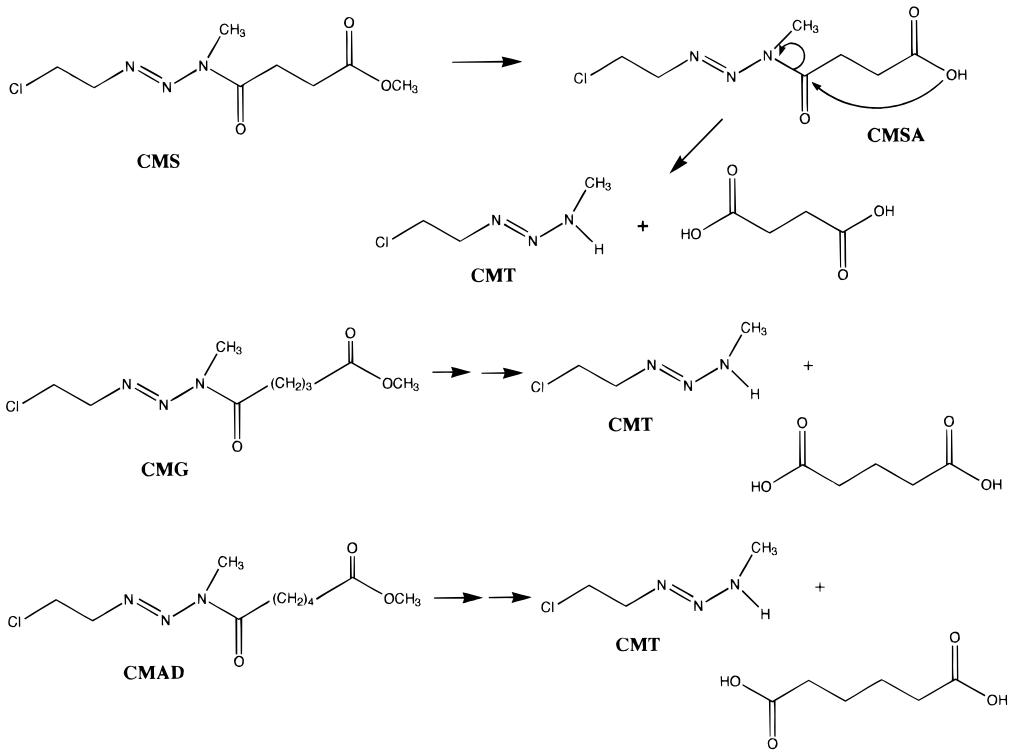


Figure 4. Scheme of internally assisted deacylation in the conversion of CMS, CMG, and CMAD to products.

derivative decomposes more rapidly than does the *N*(3)-methyl, consistent with the more electronegative character of the benzyl group (*14*). This pattern with respect to the structure of the *N*(3)-alkyl group is in agreement with the rates for *N*(3)-carbethoxy- and acetyltriazenes determined previously (*12*). At that time, we postulated an explanation for this trend at neutral pH: electron withdrawal from N(3), which correspondingly enhances the uncatalyzed rate of hydrolysis of the triazene through stabilization of the developing negative charge on N(3) in the transition state (Figure 1).

For those acyltriazenes bearing a free carboxylic acid group, the observed decrease in the rate of hydrolytic decomposition with increasing chain length (CMS > CMG > CMAD) is consistent with enhancement of N(2)−N(3) heterolysis by electron withdrawl (Table 1). In keeping with this trend, the analogous acyltriazene which bears no electron withdrawing carboxyl group, CMA, is even slower to hydrolyze. Further supporting this notion is the fact that the rates for CMS and CMSA (and likewise for the other ester/free acid pairs) are very similar. The electron withdrawing ability of a carbethoxy and a carboxyl group, which terminate the acyl groups of CMS and CMSA, respectively, would be expected to be roughly equivalent.

The DNA alkylation data (Table 2), however, suggest that two of the studied triazenes, CMS and CMSA, do not produce alkylating species in the same manner as do the other acyltriazenes. These two succinoyl triazenes give much higher levels of DNA methylation than do triazenes derived from other dicarboxylic acids, e.g., CMAD and CMG. A possible explanation for this unusual behavior is depicted in Figure 4. Methylation of DNA by 1-(2-chloroethyl)-3-methyl-3-acyltriazenes can arise only though the intermediacy of CMT. This highly labile dialkyltriazene, formed by initial deacylation of the starting material, has previously been shown to give rise to products derived primarily from the methanediazonium ion. Thus, our DNA alkylation results suggest that an alternative decomposition pathway, deacylation, becomes more favored in the cases of CMSA and CMS. As shown in Figure 4, nucleophilic attack by the carboxyl or ester group could internally assist the deacylation of CMS or CMSA. Because a carboxylate anion would be expected to be a significantly better nucleophile than an ester group, it is consistent that CMSA gives higher 7-MeG yields than does CMS. Further, the yields of methanol- and acyl group-derived carboxylic acid (both indicative of the deacylation pathway) from simple hydrolytic decomposition in aqueous buffer (Table 5) increase across the series CMA ≈ CMGA ≪ CMS < CMSA. Correspondingly, the amounts of N(2)−N(3) heterolysis products (chloroethanol and the N(3) amide) decrease across this same series (Table 5). Further, it is clear that acyltriazenes derived from longer chain dicarboxylic acids (e.g., glutamic or adipic acid) do not give similar evidence of enhanced deacylation. This would be expected given the general preference for formation of five-membered, as opposed to larger, cyclic intermediates.

The rate data obtained from the NMR studies for the hydrolysis of the various triazenes in aqueous buffers (Table 1) are also consistent with the above postulation of two competing decomposition mechanisms. Within the mechanistic scheme pictured in Figure 1, the overall rate of decomposition would be given by $k_{obs} = k_{het} + k_{deacyl}$, where k_{het} is the rate constant for N(2)−N(3) heterolysis and k_{deacyl} is that for deacylation. The yields of alcohol + amine and N(3)-derived amide can be taken, respectively, as measures of the extent to which decomposition occurs by deacylation versus heterolysis. Given this assumption, it is therefore possible to derive a second relationship between k_{het} and k_{deacyl}:

$$([ROH] + [RNH_2])/[\text{N(3)-amide}] = k_{het}/k_{deacyl}$$

Simultaneous solution of these two equations, using the

Table 7. Estimated Rate Constants for Acyltriazene Hydrolysis at 70 °C by Competing Deacylation and N(2)−N(3) Heterolysis Pathways

	rate const ($\times 10^5$ s^{-1})			rate const ($\times 10^5$ s^{-1})	
acyltriazene	k_{deacyl}	k_{het}	acyltriazene	k_{deacyl}	k_{het}
CMA	0.09	4.4	CMGA	0.12	5.0
CMS	0.87	5.6	CBSA	0.75	9.1
CMSA	2.7	4.0	CBGA	<0.49	10.0

experimental data in Tables 1 and 5, thus allows estimation of the values of k_{het} and k_{deacyl} for each of the compounds tested (see Table 7).

From these results, it can be seen that the rate of deacylation, k_{deacyl}, for CMSA is enhanced by a factor of 30 over that of CMA. While a portion of this enhancement is possibly due to electronic effects, the fact that k_{deacyl} for CMGA is only slightly greater (1.3) than that of CMA suggests that this is not the sole determinative factor. A more plausible explanation for the unusually high deacylation rate of CMSA, compared with that of CMA and CMGA, is its exclusive ability to form a cyclic intermediate, which greatly facilitates loss of the *N*(3)-acyl group (Figure 5). It is well established that five-membered cyclic intermediates, as would be seen with CMSA, are considerably more favorable than those involving a six-membered ring (as might occur with CMGA) (*15*). Kluger and Lam have observed (*16*, *17*) that internally assisted deacylation in monoamides of maleanilinic acid produced a rate increase of 1−3 orders of magnitude over that for simple amides or those derived from longer chain dicarboxylic acids. The fact that a similar difference is not observed in the benzyl series (k_{deacyl} for CBSA is only slightly greater than that for CBGA) is likely the result of the electron withdrawing nature of the benzyl group and steric hindrance. The considerably larger benzyl group would significantly hinder the formation of the more crowded tetrahedral intermediate, irrespective of ring size.

The rather substantial deacylation rate enhancement (a factor of 9.7) for the ester CMS as compared with CMA is somewhat surprising. Given the above, this is too large to be explained by an electronic effect. Instead, we believe that the apparent k_{deacyl} for CMS actually is the result of partial hydrolysis of the ester to the free acid during the course of the reaction. Evidence for this adventitious hydrolysis has already been presented (see above).

With regard to the rates of N(2)−N(3) heterolysis, k_{het}, it can be seen that all of the *N*(3)-methyltriazenes have essentially the same rate. Varying the structure of the acyl group has little impact on the heterolysis rate. Replacement of methyl by benzyl, however, does cause a 2-fold increase in k_{het}. This effect is reasonable given the enhanced electron withdrawing nature of the benzyl group which, as previously noted, would stabilize the resultant amide anion and favor N(2)−N(3) heterolysis.

In the presence of esterase, the alkylation profile is as one would expect based on the postulated mechanism of activation (Figure 1), with the amount of 7-MeG observed roughly paralleling the order of decomposition for the triazenes. The amounts of this adduct produced by CMS and CMSA were both greatly reduced over those seen in the absence of esterase, suggesting that the enzyme may compete with DNA in reaction with the putative alkylating agent, the methanediazonium ion. In the esterase-mediated decomposition of CMS, the protein, de facto, would be in close proximity to and favorably disposed to react with alkylating agents generated by the product of its catalytic process, CMSA. This idea is further bolstered by the data obtained from the CMS reaction with esterase and DNA for 48 h. In this experiment, the level of 7-MeG observed (2.6 mmol/mol of guanine) was lower than that seen in the non-esterase reaction (3.5 mmol/mol of guanine). It is unclear why esterase causes a marked decline in methylation by CMSA, in that CMSA is not a substrate for esterase. 7-MeG levels from CMG and CMAD were also reduced in the presence of esterase, but less dramatically than those from CMS and CMSA. However, methylation of the esterase does not necessarily imply that inhibition results from this alkylation. In the case of CMC, esterase dramatically increased the level of 7-MeG observed. This is so because esterase efficiently removes the CMC acyl group, and thus the yield of 7-MeG is from essentially pure 1-(2-chloroethyl)-3-methyltriazene. That compound is not formed at all during the solvolysis of CMC. Presumably, however, substantial methylation of the esterase also occurs in the case of CMC.

Clearly the succinoyl, adipoyl, and acetyl triazenes yield less of the highly active methanediazonium ion precusor, CMT. CMA, bearing an *N*(3)-acetyl group, is not a substrate for esterase and thus methylates DNA only to a small extent both in the presence and absence of the enzyme. The benzylated triazenes CBS and CBC, on the whole, showed only very low levels of alkylation, as compared with the corresponding methyl compounds. This is to be expected, given the low efficiency of DNA benzylation, as compared with methylation (*14*).

The differing 7-HOET/7-MeG ratios for the various triazenes are reflective of the differences in mechanism of activation for the compounds, as mentioned above. CMC, with a ratio of 2.0, decomposes predominately by N(2)−N(3) heterolysis, resulting only in production of the chloroethanediazonium ion, and thus does not methylate the DNA. However, in the cases of CMS and CMSA this ratio is low, ca. 0.1, consistent with a shift in mechanism to auto-catalyzed N(3)-cleavage leading to CMT production. CMG and CMAD give an intermediate 7-HOEtG/7-MeG ratio (0.3), reflecting their ability to undergo, at best, an inefficient deacylation reaction.

Interestingly, the ratio of the alkylation products derived from the *N*(1)-alkyl group (7-HOEtG/7-ClEtG, Table 4) varies considerably with the nature of the *N*(3)-alkyl group. Triazenes with an *N*(3)-methyl or -ethyl group favor chloroethylation (7-HOEtG/7-ClEtG ca. 0.3), while those bearing an *N*(3)-benzyl group favor hydroxyethylation (ca. 1.3). The highest yields (Table 5) of the simple N(2)−N(3) cleavage products (an amide and chloroethanol) are obtained from *N*(3)-benzyltriazenes (CBSA and CBGA), an observation which is in keeping with the ability of a benzyl group to stabilize the amide anion initially produced by this heterolysis pathway. The obligatory intermediate, the chloroethanediazonium ion, thus leads to the production of 7-ClEtG and 7-HOEtG (via the intermediate formation of oxadiazoline (*18*, *19*)). The alternative pathway, deacylation, would give CBT, a compound which should also give substantial amounts of products attributable to the chloroethanediazonium ion. Parallel decomposition pathways can also be postulated for the *N*(3)-methyl- and -ethyltriazenes and differ from the benzyl case only in that deacylation produces CMT. It has been shown previously (*1*) that CMT decomposes mainly to the methanediazonium ion

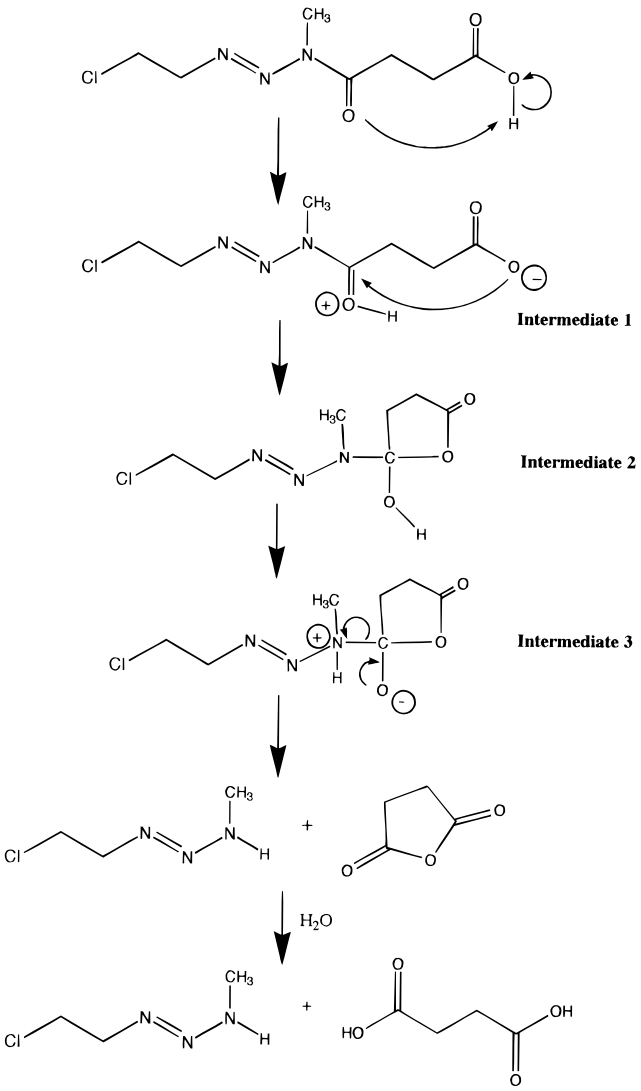


Figure 5. Scheme depicting the cyclic intermediates in the conversion of CMSA to products.

with lesser amounts of chloroethanediazonium ion. The dramatic shift in the 7-HOEtG/7-ClEtG ratio toward 7-HOEtG suggests that 1-(2-chloroethyl)-3-alkyltriazenes do not decompose directly to the chloroethanediazonium ion, but instead give rise to substantial quantities of oxadiazoline (and ultimately, hydroxyethylation products with DNA). Although the exact nature of this process is unknown, it may involve the attack of water at the N(2) position of the dialkyltriazene during decomposition. This mechanism would lead to the direct formation of 2-chloroethanediazotic acid and subsequently, though intramolecular displacement of chloride ion, to oxadiazoline. An alternative explanation would invoke a similar attack by water on N(2) of the 3-acyltriazene during N(2)−N(3) heterolysis. Though plausible, it is hard to see why the presence of a benzyl moiety, in contrast to a methyl or ethyl group, would shift the mechanism in favor of attack by water at N(2).

Ab initio quantum mechanical calculations allowed determination of the stability of the various postulated intermediates in the decomposition of these acyltriazenes. In addition, they supplied information concerning the most favorable pathways of decomposition of the triazenes, based on the calculation of enthalpies of reaction (ΔH). Decomposition of CMSA via pathway B (internally assisted deacylation, see Figure 3) leading to the dialkyltriazene CMT and succinic acid ($\Delta H = -8.5$ kcal/mol) was shown to be favored by 159 kcal/mol over the heterolysis pathway to ionic products (pathway A, $\Delta H = +150.2$ kcal/mol), as one would predict. The route to formation of CMT in pathway B is probably composed of several steps (Figure 5). CMSA is first converted to its linear zwitterionic form (intermediate 1), which can then cyclize to intermediate 2, followed finally by conversion to the cyclic zwitterionic intermediate 3. N−C scission followed by a proton transfer would then lead to CMT and succinic anhydride. Under the conditions of reaction, succinic anhydride would undergo subsequent hydrolysis to succinic acid.

Pathways C and D leading from CMT to chloroethane- and methanediazonium ion products, respectively, are both exothermic ($\Delta H = -38$ or -44 kcal, respectively), with the formation of methanediazonium ion predictably favored over that of chloroethanediazonium ion by 6 kcal/mol. These data account for the much larger amounts of methylated, as compared with chloro- or hydroxyethylated, guanine products that were detected from the triazenes in the absence of esterase.

In conclusion, these data have shown that the structural composition of the triazene *N*(3)-acyl group can radically affect the DNA adduct spectrum in a non-enzyme-catalyzed alkylation. Thus, this study provides useful information to permit modulation of the alkylation of specific bases in DNA. The ability to control the pattern of DNA alkylation though structural modification could be very important in the design of better alkylating agents for use in cancer chemotherapy.

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