course of a session showed that k' was constant to a very high degree. Day-to-day variation was larger, about 3%, and linear-regression equations could be used to relate all log k' back to a given day by appropriate choice of standards. For situations in which a very high correlation between log D_{SF} and log k' were obtained, $r > 0.99$, use of standard compounds allowed log D to be calculated each day. Standards were all measured by shake-flask methods (see below) in the identical buffers (without DMOA). For buffer A, log D values are as follows: 2-butanone, 0.28; aniline, 0.91; benzaldehyde, 1.45; acetophenone, 1.65; nitrobenzene, 1.88; anisole, 2.09; benzene, 2.13; benzophenone, 3.10; chlorobenzene, 2.84. Reproducibility in log D was also about 3%. The 20%, v/v, CH_3CN (MCB, Chromatoquality) was prepared by diluting 20 volumes of organic with buffer A to 100 volumes.

Shake-flask partitionings were carried out in 16 × 100 mm culture tubes with an aluminum-lined screw cap. A typical partitioning was conducted in 10 mL of the same isotonic buffer A as used in the HPLC work and varying amounts of buffer-saturated 1-octanol, depending upon the lipophilicity of the sample. Samples were shaken on an automatic shaker for at least 2 h, and the tubes were then centrifuged at 2000 rpm to clarify the two phases. Spectrophotometric determination (Bausch & Lomb Spectronic 200 UV) of concentrations was used for pure and stable compounds; for impure or unstable samples, concentrations in both phases were determined by standard analytical HPLC methods using computer integration or cut-out tracings to determine relative amounts of compound. Final sample concentrations of 10^{-4} to 10^{-5} M in buffer and 10^{-3} to 10^{-4} M in 1-octanol were obtained. Each sample was determined at 3–4 dilutions and extrapolated to infinite dilution if a concentration trend was observed; otherwise, means were taken. Typical precision was 1–2%.

All computer correlations were performed on a commercial APL language system (Proprietary Computer Systems, Van Nuys, CA), using our published regression programs.[9]

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Mechanism of Action of (2-Haloethyl)nitrosoureas on DNA. Isolation and Reactions of Postulated 2-(Alkylimino)-3-nitrosooxazolidine Intermediates in the Decomposition of 1,3-Bis(2-chloroethyl)-, 1-(2-Chloroethyl)-3-cyclohexyl-, and 1-(2-Chloroethyl)-3-(4′-*trans*-methylcyclohexyl)-1-nitrosourea

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Three examples of the postulated but hitherto unisolated 2-(alkylimino)-3-nitrosooxazolidines (**2**) have been prepared containing cyclohexyl, *trans*-4-methylcyclohexyl, and 2-chloroethyl groups at the 2 position, respectively. These compounds correspond to intermediates previously postulated to be formed in the aqueous decomposition of the antitumor agents 1-(2-chloroethyl)-3-cyclohexyl- (CCNU), 1-(2-chloroethyl)-3-(4′-*trans*-methylcyclohexyl)- (MeCCNU), and 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), respectively. Compounds **2** decompose under physiological conditions to give a range of products similar to those formed from the corresponding (2-chloroethyl)nitrosoureas, including the hitherto unrecognized 2-hydroxyethyl *N*-alkylcarbamates (**9**). Compounds **2a** and **2b** are converted with hydrochloric acid into CCNU and MeCCNU, respectively, suggesting that **2a** and **2b** may be reaction intermediates of decomposition. The corresponding 3-alkyl-1-nitroso-1-(2-hydroxyethyl)ureas (**4**) were characterized and, since they also decompose to give the same products as **2**, may arise from the ring opening of **2**. The intermediacy of compounds **4** can explain the formation of hydroxyethylated nucleosides isolated by other workers from the reaction of (2-chloroethyl)nitrosoureas on polynucleotides.

1-3-Bis(2-chloroethyl)-1-nitrosourea (BCNU), 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), 1-(2-chloroethyl)-3-[4′-*trans*-methylcyclohexyl]-1-nitrosourea (MeCCNU), and other (2-haloethyl)nitrosoureas are of clinical value in the treatment of Hodgkin's disease,[1,3] brain tumors[2], lymphomas,[3] and other malignant diseases. BCNU, CCNU, and related (2-chloroethyl)nitrosoureas have been found to give rise to electrophiles upon aqueous decomposition, which may alkylate DNA[4-7] and other macromolecules in the cell. For example, chloroethyl cations, or their equivalent, may alkylate a base in DNA and, following labilization of the C–Cl bond, this can result in an interstrand cross-link.[5-7] Another major decomposition product is the alkyl isocyanate which can result in carbamoylation reactions of amino groups in macromolecules.[8-12] Such reactions may, in part, underlie the cytotoxic action of these agents. Extensive studies on the chemistry of nitrosoureas and their hydroxylated metabolites[13-16] and the method of formation of products from

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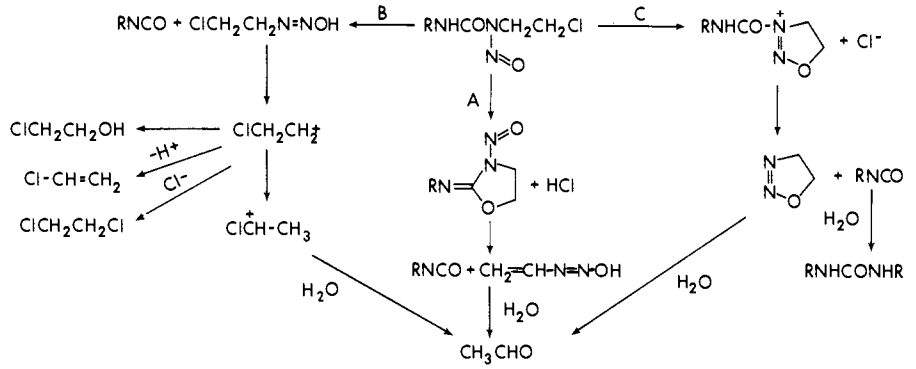
Scheme I



aqueous decomposition under physiological conditions have proven useful in interpreting the mechanism of action of these agents.[4-19]

The (2-chloroethyl)nitrosoureas are rich in their chemistry for molecules of their small size. Three main pathways of decomposition have been proposed and these are outlined briefly in Scheme I. Pathway B[4,6,9] involves formation of a chloroethyl cation or its kinetic equivalent and is considered as the main pathway leading to products including acetaldehyde and 2-chloroethanol. Pathway A involves cyclization to a 2-(alkylimino)-3-nitroso-2-oxazolidine[17] and subsequent cleavage of the latter. Pathway C involves cyclization of the nitrosourea to a postulated 3-acyl-1,2,3-oxadiazolinium salt with subsequent conversion to a oxadiazoline and then to acetaldehyde.[19]

While there is considerable evidence in favor of major pathway B,[4-9,17-19] the possible contribution of minor pathways A and C could not be assessed, since the postulated intermediates have been widely regarded as too unstable to permit isolation. Accordingly, we report the synthesis and characterization of three examples of 2-(alkylimino)-*N*-nitroso-2-oxazolidines. Their properties and reactions under physiological conditions of controlled decomposition have been examined so that the possible contribution of the minor pathway A via these intermediates may be assessed.

Synthesis. Owing to the acid lability of 2-(alkylimino)oxazolines[20,21] in aqueous acidic medium, basic conditions were selected for the nitrosation reactions. Although there have been recent developments in nitrosation methods employing, for example, lithium anions of amines with nitrosyl chloride,[22,23] we have performed the nitrosation of the (cyclohexylamino)oxazoline (**2a**) with butyl nitrite in the presence of an equivalent of sodium methoxide in ether at 0 °C. A mixture of the isomers **2a** and **3a** was formed, and by repeated crystallization from ether/petroleum ether the isomer **2a** could be isolated in a pure form. The composition and the presence of a strong C=N absorption at 1714 cm^{-1}, a weak nitroso absorption at 1470 cm^{-1}, and the absence of an NH frequency in the infrared spectrum of **2a** in $CHCl_3$ confirm the N-nitrosation of **1a**.

The NMR spectrum allowed the differentiation of isomer **2a** and **3a** and provided information on the conformation of **2a**.[24] The downfield shifts of the 4-methylenes at δ 3.95 (J = 7.5 Hz) and the 5-methylenes at δ 4.50 (J = 7.5 Hz) for **2a** compared with the 4-methylenes at δ 3.75 and 5-methylenes at δ 4.20 (J = 7.5 Hz) for **1a**, the disappearance of the exchangeable NH proton at δ 3.45, as well as the downfield shift of the cyclohexylmethine[24,25] at δ 3.65 compared to that of the oxazoline (**1**) at δ 3.40 strongly indicate that the nitrosation of **1a** has taken place at the endocyclic nitrogen and not at the exocyclic one. The ring opening of **2a** with hydrogen chloride to CCNU (see Results), together with its characteristic decompsition pattern, confirms, the structural assignment of **2a**.

The NMR spectrum of the second isomer shows a multiplet at δ 4.75 for cyclohexylmethine, a triplet for 5-methylenes at δ 4.60, and a triplet for 4-methylenes at δ 4.10 as well as the downfield shift[24] of two cyclohexyl protons. These data, together with the production of cyclohexene from decomposition,[25] confirm the structure of this compound as **3a**. Interconversion of **2** and **3** by 1,3 migration of the nitroso groups is conceivable by comparison with other 1,3 migrations,[26] but there is no precedent for this occurring under physiological conditions and we did not obtain evidence for such a rearrangement in these cases.

The utility of ^{13}C NMR in elucidating the configuration and conformation of cyclohexane derivatives,[27] as well as of nitrosoamines and related systems,[28-32] prompted us to examine the ^{13}C NMR of the above nitrosooxazolidines and nitrosoureas to obtain information on the conformation of these compounds. The ^{13}C NMR spectrum of **2a** (Table I) shows upfield shifts of C_4 at δ 41.46 and of C_5 at δ 64.21, as compared to the unnitrosated oxaoline (**1a**) which shows C_4 at δ 52.87 and C_5 at δ 67.51. The ^{13}C NMR assignments of **2a** are in close agreement with those of 3-nitroso-2-ox-

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Table I. Physical and Spectral Data of 2-(Alkylamino)-2-oxazolines (1a–d), 2-(Alkylimino)-3-nitroso-2-oxazolidines (2a–c), 3-Cyclohexyl-1-(2-hydroxyethyl)nitrosourea (4a), and (2-Haloethyl)nitrosoureas (5a–c)

no.	yield, %	mp, °C	IR spectra ($CHCl_3$), cm^{-1}	formula	^1H NMR ($CDCl_3$),[a] δ	^{13}C NMR ($CDCl_3$), δ							
						C_2	C_5	C_4	C_1'	$C_2'C_6'$	C_4'	$C_3'C_5'$	others
1a	80	128–129 (127–128)[b]	3475 (NH), 1670 (C=N)	$C_9H_{16}N_2O$	4.20 (t, 2 H, H_5), 3.75 (t, 2 H, H_4), 3.45 (br m, *N*-Hex), 3.40 (m, 1 H, H_1' ax), 2.00 (H_2' and H_6' eq), 1.50 (m, H_3' and H_5' eq), 1.52 m, 1 H, H_4' eq), 1.00–1.40 (m, 5 H ax)	160.65	67.51	52.87	51.78	33.66	25.63	34.87	
1b	83	155	3475 (NH), 1670 (C=N)	$C_{10}H_{18}N_2O$	4.25 (t, 2 H, H_5), 3.75 (t, 2 H, H_4), 3.45 (br, *N*-Hex), 3.43 (m, 1 H, H_1' ax), 2.05 (m, 2 H, H_2' and H_6' eq), 1.56 (m, 2 H, H_3' and H_5' eq), 1.10–1.50 (m, 5 H ax), 0.93 (d, 3 H, CH_3)	160.60	67.60	53.02	52.15	34.01	32.13	33.75	22.20
1c	72	86 (84–86)[b]	3480 (NH), 1670 (C=N)	$C_5H_9ClN_2O$	4.30 (t, 2 H, H_5), 3.75 (t, 2 H, H_4), 3.65 (m, 4 H, CH_2CH_2Cl)	160.93		52.55	43.87	44.88			
1d	84	heavy liquid	3400–3200 (OH, NH),[c] 1600 (C=N)	$C_5H_{10}N_2O_2$	4.20 (t, 2 H, CH_2), 3.48 (m, 4 H, NCH_2), 3.10 ($HOCH_2$), 5.50 (br m, 2 H, NH, OH ex)[d]	163.39	68.54	50.69	44.65	60.50[e]			
2a	50[f]	109–110	1714 (C=N), 1470 (N=O)	$C_9H_{15}N_3O_2$	4.50 (t, 2 H, H_5), 3.90 (t, 2 H, H_4), 3.65 (m, 1 H, H_1' ax), 1.82 (m, 4 H, H_2', H_3', H_5', and H_5' eq) 1.68 (m, 1 H, H_4' eq), 1.50 (m, 2 H, H_2' and H_6' eq), 1.35 (m, 2 H, H_3', H_5' ax), 0.25 (m, 1 H, H_4' ax)	143.64	64.21	41.46	55.83	33.66	25.67	25.03	
2b	57[f]	95–96[g]	1715 (C=N), 1475 (N=O)	$C_{10}H_{17}N_3O_2$	4.45 (t, 2 H, H_5), 3.95 (t, 2 H, H_4), 3.60 (m, 1 H, H_1' ax), 1.82 (m, 4 H, H_2', H_3', H_5', and H_6' eq), 1.45 (m, 3 H ax), 1.00 (m, 2 H ax), 0.93 (d, 3 H, CH_3)	143.84	64.21	41.46	55.86	33.92	31.94	33.63	22.42
2c	45[f]	68–69[g]	1714 (C=N), 1470 (N=O)	$C_5H_8ClN_2O_2$	4.20 (t, 2 H, H_5), 3.95 (t, 2 H, H_4), 3.80 (m, 4 H, CH_2CH_2Cl)	146.69	64.76	41.70	48.87	44.31			
3a	50[f]	105–106[g]	1714 (C=N), 1470 (N=O)	$C_9H_{15}N_3O_2$	4.75 (m, 1 H, H_1', ax), 4.60 (t, 2 H_5), 4.10 (t, 2 H, H_4), 2.20 (m, 2 H, H_2', H_3', and H_6' eq),[h] 1.10–1.90 (m, 8 H, CH_2)[c]	140.68	68.67	55.84	45.65	33.32	25.55	24.80[h]	
4a	54	50–51[g] (49–50)[i]	3490 (OH), 3370 (NH), 1705 (CO), 1480 (NO)	$C_9H_{17}N_3O_3$	6.85 (br m, 1 H, NH), 4.10 (t, 2 H, H_2), 3.90 (m, 1 H, H_1 ax), 3.65 (q, 2 H, H_2), 2.20 (br m, 1 H, *O*-Hex), 2.05 (m, 2 H, H_2' and H_6' eq), 1.75 (m, 2 H, H_3' and H_5' eq), 1.60 (m, 1 H, H_4' eq), 1.45 (m, 2 H, H_2' and H_6' ax), 1.37 (m, 1 H, H_4' ax), 1.25 (m, 2 H, H_3' and H_5' ax)	153.31	41.88	57.87	50.14	33.05	24.77	25.44[h]	

5a	43	88–89[g] (90)[j]	3375 (NH), 1750 (C=O), 1490 (NO)	$C_9H_{16}ClN_3O_2$	6.90 (br m, 1 H, NH), 4.20 (t, 2 H, H_2), 3.90 (m, 1 H, 2.10 (m, 2 H, H_2' and H_6' eq), 1.80 (m, 2 H, H_3' and H_5' eq), 1.68 (m, 1 H, H_4' eq), 1.45 (m, 2 H, H_2' and H_6' ax), 1.35 (m, 1 H, H_4' ax), 1.28 (m, 2 H, H_2' and H_6' ax)	151.86	40.07	38.89	50.05	33.11	25.45	24.84[h]	
5b	32	68–69[g] (70)[k]	3385 (NH), 1707 (CO), 1485 (NO)	$C_{10}H_{18}ClN_3O_2$	6.85 (br m, 1 H, NH), 4.20 (t, 2 H, H_1), 3.82 (m, 1 H, H_4' ax), 3.50 (t, 2 H, H_2), 2.10 (m, 2 H, H_2' and H_6' eq), 1.80 (m, 2 H, H_3' and H_5' eq) 1.40 (m, 1 H, H_4' ax), 1.26 (m, 2 H, H_2 and H_6 ax), 1.12 (m, 2 H, H_3 and H_5 ax), 0.90 (d, 3 H, CH_3)	151.97	40.02	38.85	50.33	33.96	33.12	33.88	22.06[h]
5c		31–32[g] (30–32)[l]	3395 (NH), 1710 (CO), 1489 (NO)	$C_5H_{10}Cl_2N_3O_2$	7.50 (br m, 1 H, NH), 4.20 (t, 2 H, H_1), 3.80 (m, 4 H, CH_2CH_2Cl), 3.50 (t, 2 H, H_2)	153.26	40.24	38.96	42.55	43.28[m]		

[a] Primes (H_1', H_2', ..., H_6' and C_1', C_2', ..., C_6') signify cyclohexyl protons and carbons. [b] Reference 18. [c] IR were taken neat. [d] Me_2SO-d_6 as solvent. [e] D_2O as solvent. [f] Yields are not critically optimized. [g] Crystallized from ether/petroleum ether. [h] Chemical shifts may be altered since they are taken from a mixture of 2a and 3a. [i] Reference 7. [j] Reference 40. [k] Reference 41. [l] Reference 42. [m] For nitrosoureas (4a and 5a–c) in ^{13}C NMR, $C_2 = C{=}O$ and $C_5 = C_1$ and $C_4{=}C_2$ in ethylene portions.

azolidone (C_4, δ 40.65; C_5, δ 63.53) and confirm the assignment of the nitroso group to position 3. These data suggest that, at least in chloroform solution, **2a** may exist largely in the (*S*)-trans form. This finding is consistent with X-ray diffraction studies of MeCCNU,[33] where it has been shown that the nitroso group is (*S*)-trans to the carbonyl group (and may indicate that the conformation of the nitroso group is maintained during ring opening to CCNU or MeCCNU). The expected product of ring opening of **2a** by attack of water at position 5, 3-cyclohexyl-1-(2-hydroxyethyl)-1-nitrosourea (**4a**), was prepared by nitrosation with sodium nitrite in anhydrous formic acid of 3-cyclohexyl-1-(2-hydroxyethyl)urea (**10a**), which in turn was prepared by the action of 3-cyclohexyl isocyanate with ethanolamine.[7] The structure of **4a** was confirmed by its physical as well as its spectral data presented in Table I.

The isolation of the hitherto unrecognized type of decomposition product from oxazolidines, namely, the 2-hydroxyethyl *N*-alkylcarbamates (**9**) necessitated their preparation as authentic samples for identification purposes. Reaction of the appropriate alkyl isocyanate with ethylene glycol afforded a mixture of the 2-hydroxyethyl *N*-alkylcarbamates (**9**) and the corresponding ethylene glycol bis(*N*-alkylcarbamates) from which samples of pure **9** could be readily separated. Similarly, 3-cyclohexyl-2-oxazolidinone (**12b**) was prepared from 2-chloroethyl *N*-cyclohexylcarbamate (**20a**), which in turn was prepared from 2-chloroethyl chloroformate and cyclohexylamine.

Results

Treatment of the 2-(alkylimino)-3-nitroso-2-oxazolidines **2a** and **2b** with hydrogen chloride in ether afforded the parent nitrosoureas CCNU (**5a**) and MeCCNU (**5b**) in 43 and 32% yields, respectively. The 2-[(2-chloroethyl)imino] analogue **2c** behaves differently under these conditions and gave the denitrosated products **6c** and **7c** quantitatively.

When **2a** was allowed to decompose at 37 °C in a phosphate buffered (0.1 M, pH 7.2) aqueous solution, the volatile product acetaldehyde (**8**, 8–10%) and the involatile products 2-(cyclohexylamine)-2-oxazoline (**1a**, 10–15%), dicyclohexylurea (**11a**, 3–4%), and 2-hydroxyethyl *N*-cyclohexylcarbamate (**9a**, 20–25%) were obtained. In addition to these products, small amounts of cyclohexylamine, 2-oxazolidinone (**12a**), and 3-cyclohexyl-1-(2-hydroxyethyl)urea (**10a**) were also detected.

Decomposition of **2a** at pH 7.2 and 37 °C in the presence of sodium chloride gave vinyl chloride (**13**, ~1%), 1,2-dichloroethane (**14**, ~1%), and 2-chloroethanol (**15**, 2–4%) as volatile products. It was observed that the yields of these products varied slightly according to the concentration of sodium chloride. In addition, the following involatile products were detected by TLC and CIMS: 2-(cyclohexylamino)-2-oxazoline (**1a**), 2-hydroxyethyl *N*-cyclohexylcarbamate (**9a**), 2-oxazolidinone (**12a**), 2-chloroethyl *N*-cyclohexylcarbamate (**16a**), and dicyclohexylurea (**11a**).

Decomposition of the 2-(alkylimino)-3-nitroso-2-oxazolidines **2b** and **2c** at 37 °C in phosphate buffer (pH 7.2) and separately in the presence of sodium chloride gave the corresponding range of products as was obtained for **2a**, and the results are presented in Table II.

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Table II. Mass Spectral Data of 2-(Alkylimino)-3-nitroso-2-oxazolidines (2a–c), 3-Cyclohexyl-1-(2-hydroxyethyl)-1-nitrosourea (4a), and Their Volatile Decomposition Products

compd	reaction conditions	decomp products	yield, %	retention time, min	*m/e* (rel intensity, fragments)
2-(cyclohexylimino)-3-nitroso-2-oxazolidine (2a)					197.1170 (1.27, M^+), 168.1221 (12.73, $M^+ - 29$), 167.1183 (100.00, $M^+ - 30$), 115.0382 (7.10, $C_5H_5N_3O_2^+$), 87.0558 (61.29, $C_3H_7N_2O^+$), 83.0860 (5.94, C_6H_{11}), 81.0704 (17.11, $C_6H_9^+$)
	phosphate buffer[a]	acetaldehyde	8–10	1.04	44 (48.6, M^+), 43 (26.1, $M^+ - 1$), 29 (100.0, ^+CHO)
	sodium chloride[b]	vinyl chloride[c]	>1	1.17	64 (30.7, M^+), ^{37}Cl), 62 (100.0, M^+, ^{35}Cl), 27 (90.6, $CH_2=CH^+$)
		dichloroethane	>1	7.80	102 (1.5, M^+, 2 ^{37}Cl), 100 (11.6, M^+, ^{37}Cl and ^{35}Cl), 98 (18.9, M^+, 2 ^{35}Cl), 64 (31.5, $M^+ - H^{37}Cl$), 62 (100.0, $M - H^{35}Cl$), 27 (90.0, $CH_2=CH^+$)
		chloroethanol	1–4	16.21	82 (1.3, M^+, ^{37}Cl), 80 (4.2, M^+,^{35}Cl), 31 (100.0, $^+CH_2OH$)
mixture of 2a + 3a	phosphate buffer[a]	acetaldehyde	7–8	1.03	44 (66.2, M^+), 43 (24.3, $M^+ - 1$), 29 (100.0$^+$, CHO)
		cyclohexene	10–15	1.84	82 (31.6, M^+), 81 (9.1, $M^+ - 1$), 67 (100.0, $M - 15$), 54 (77.3), 41 (42)
2-[(*trans*-4′methylcyclohexyl)imino]-3-nitroso-2-oxazolidine (2b)					211.1313 (0.42, M^+), 182.1410 (27.98, $M^+ - 29$), 181.1335 (16.81, $M^+ - 30$), 115.0382 (5.15, $C_3H_5N_3O^+$), 97.1015 (3.19, $C_7H_{13}^+$), 95.0857 (6.50, $C_7H_{11}^+$), 87.0553 (100.00, $C_3H_7N_2O^+$)
	phosphate buffer[a]	acetaldehyde	8–10	1.14	44 (74.2, M^+), 43 (34.7, $M^+ - 1$), 29 (100.0, ^+CHO)
	sodium chloride[b]	vinyl chloride[c]	>1	1.21	64 (32.4, M^+), ^{37}Cl), 62 (100.0, M^+, ^{35}Cl), 27 (89.2, $CH_2=CH^+$)
		dichloroethane	>1	7.59	102 (0.5, M^+, 2 ^{37}Cl), 100 (6.5, M^+, ^{37}Cl and ^{35}Cl), 98 (12.2, M^+, 2 ^{35}Cl), 64 (32.2, $M^+ - H^{37}Cl$), 62 (100.0, $M^+ - H^{35}Cl$), 27 (82.3, $CH_2=CH^+$)
		chloroethanol	1–4	16.62	82 (1.1, M^+, ^{37}Cl), 80 (3.7, M^+, ^{35}Cl), 31 (100.0, $^+CH_2OH$)
2-[(2′-chloroethyl)-imino]-3-nitroso-2-oxazolidine (2c)					179.0259 (0.62, M^+, ^{37}Cl), 177.0302 (1.91, M^+, ^{35}Cl), 150.0335 (3.40, $M^+ - 29$, ^{37}Cl), 149.0296 (31.64, $M^+ - 30$, ^{37}Cl), 148.0373 (9.54, $M^+ - 29$, ^{35}Cl), 147.0324 (91.58, $M^+ - 30$, ^{35}Cl), 128.0463 (100.00, $M^+ - CH_2$, ^{35}Cl), 115.0385 (3.21, $C_3H_5N_3O_2$), 86.0483 (13.77, $C_3H_6N_2O^+$)
	phosphate buffer[a]	acetaldehyde	8–12	1.21	44 (56.5, M^+), 43 (30.6, $M^+ - 1$), 29 (100.0, ^+CHO)
	sodium chloride[b]	vinyl chloride[c]	>1	1.15	64 (22.5, M^+, ^{37}Cl), 62 (79.5, M^+, ^{35}Cl), 27 (100.0, $CH_2=CH$)
		dichloroethane	>1	7.44	102 (0.5, M^+, 2 ^{37}Cl), 100 (5.6, M^+, ^{37}Cl and ^{35}Cl), 98 (11.2, M^+, 2 ^{35}Cl), 64 (30.7, $M^+ - H^{37}Cl$), 62 (100.0, $M^+ - H^{35}Cl$), 27 (90.6)
		chloroethanol	2–5	16.31	82,[d] 80 (1.3, M^+, ^{35}Cl), 31 (100.0, $^+CH_2OH$)
3-cyclohexyl-1-(2-hydroxyethyl)-1-nitrosourea (4a)					215.0042 (5.31, M^+), 169.1106 (1.22, $M^+ - 46$), 126.0917 (24.70, $C_7H_{12}NO^+$), 90.0429 (20.00, $C_2H_6N_2O_2^+$), 83.0842 (100.00, $C_6H_{11}^+$)
	phosphate buffer[a]	acetaldehyde	25–35[e]	1.04	44 (60.5, M^+), 43 (32.0, $M^+ - 1$), 29 (100.0, ^+CHO)
	sodium chloride[b]	vinyl chloride[c]	>1[e]		64 (2.3, M^+, ^{37}Cl), 62 (8.8, M^+, ^{35}Cl), 27 (100.0, $CH_2=CH^+$)
		dichloroethane[c]	>1[e]	7.88	102,[d] 100 (5.6, M^+, ^{37}Cl and ^{35}Cl), 98 (13.6, M^+, 2 ^{35}Cl) 64 (35.0, $M - H^{37}Cl$), 62 (100.0, $M^+ - H^{35}Cl$), 27 (94.4, $CH_2=CH^+$)
		chloroethanol	8–10[e]	16.21	82,[d] 80 (0.8, M^+, ^{35}Cl), 31 (100.0, $^+CH_2OH$)

[a] Gaseous phase (1 mL) was injected for GC and GC–MS. [b] Methylene chloride (2 μL) was injected for GC and GC–MS. [c] These were detected by cross scan and mass spectra at their retention time. [d] Isotopic ion could not be detected. [e] Yields are not critically optimized. Acetaldehyde was estimated using propane gas as standard. Chloroethanol was estimated by removing the solvent peak.

Decomposition of 3-cyclohexyl-1-(2-hydroxyethyl)-1-nitrosourea (**4a**) at 37 °C in phosphate buffered (0.1 M, pH 7.2) aqueous solution afforded acetaldehyde (23–25%), dicyclohexylurea (**11a**, 8–10%), 2-hydroxyethyl *N*-cyclohexylcarbamate (**9a**, 10–15%) as well as small quantities of cyclohexylamine, 2-(cyclohexylimino)-2-oxazoline (**1a**), and 3-cyclohexyl-1-(2-hydroxyethyl)urea (**10a**).

When **4a** was decomposed under similar conditions, but in the presence of sodium chloride, vinyl chloride (**13**, ~1%), and 2-chloroethanol (**15**, 1–4%) were detected in addition to the products described above.

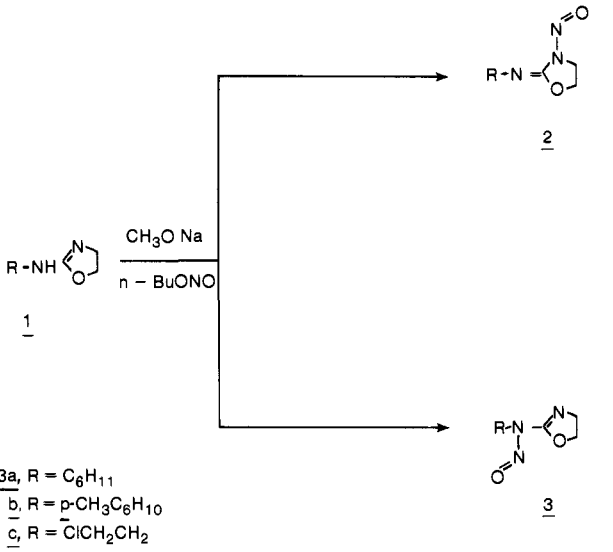
In order to determine if the new 2-(alkylimino)-3-nitrosooxazolidines can, like the parent (2-chloroethyl)-nitrosoureas, give rise to electrophiles which are capable of alkylating DNA, they were allowed to react with PM2 covalently closed circular (CCC) DNA at 37 °C in phosphate buffered (0.1 M, pH 7.2) aqueous solution. The course of the reaction was followed by assaying the change in ethidium fluorescence. The basis of the assay is that alkylation of the DNA is detected by subsequent depurination and alkaline scission of the resulting apurinic site (under the pH 11.8 conditions of the assay), which results in DNA strand scission and subsequent loss of the duplex structure of the DNA required for intercalation of the ethidium.[37,38] The percentage of reduction in fluorescence with time compared with the control is proportional to the extent of DNA alkylation. Compound **2a** gives 75% alkylation after 5 h, **2b** gives 85% alkylation, and **2c** gives 73% alkylation also after 5 h, all comparable with the reactivity of CCNU (**5a**) at 78% and of 3-nitroso-2-oxazolidine at 80%.[7] The ring-opened 3-cyclohexyl-1-(2-hydroxyethyl)-1-nitrosourea[7] (**4a**) by comparison gives more extensive alkylation, i.e., 90% after 4 h.

Discussion

2-(Alkylimino)-*N*-nitroso-2-oxazolidines (**2**), which have been postulated as intermediates in one pathway (A, Scheme I) of decomposition of (2-haloethyl)nitrosoureas, have hitherto been regarded as too unstable to permit isolation;[17] therefore, the contribution of this pathway has been inferred by indirect means. The 2-(alkylimino)-*N*-nitroso-2-oxazolidines (**2a–c**) described herein are stable in the solid form or in aprotic solvents. However, they decompose slowly in protic solvents and readily in buffered aqueous solutions to give a range of products. These products, with the exception of the 2-hydroxyethyl *N*-alkylcarbamate (**9**), have been detected previously by other workers as primary products of decomposition of the parent (2-haloethyl)nitrosoureas.[4,6,17,18,36] Moreover, careful examination by CIMS of the involatile products of decomposition of CCNU, MeCCNU, and BCNU revealed that the respective carbamates (**9**) were also formed directly. It was not possible to establish the formation of **2** from the corresponding nitrosoureas (**5**) under aqueous conditions (owing to the rapid decomposition of both **2** and **5**). Nevertheless, the conversion of **2a** to **5a** and **2b** to **5b** under acid catalysis, together with the similarity of products from **2** and **5**, implicates the compounds **2** as intermediates in one minor pathway of decomposition, as originally suggested by Montgomery and co-workers to account for the formation of acetaldehyde (Scheme III).[17]

In addition to other involatile products, the 2-hydroxyethyl *N*-alkylcarbamates (**9**) have been observed as products of the decomposition of **2**. Two possible modes

Scheme II



of formation of **9** are shown in Scheme IV. The first involves nucleophilic attack by water at position 2 of **2**, resulting in ring opening to form the diazohydroxide (**16**) which could give rise to **9** by loss of nitrogen. Alternatively, the water could attack at position 5 by analogy with observed opening of **2** to **5** (X = Cl) with chloride ion. This would afford the (2-hydroxyethyl)nitrosourea **4**, which could in turn give rise to the intermediate diazotate **17**, which leads to the carbamate **9** as shown in Scheme IV. Preliminary evidence in support of the later pathway is the observed preparation and characterization of the 3-alkyl-1-nitroso-1-(2-hydroxyethyl)urea **4** and the formation from the latter under conditions of controlled decomposition of products identical with those obtained from the oxazolidine **2** (see Schemes III and IV). In addition, the possible mechanistic connection between the (2-haloethyl)nitrosourea **5**, the 3-nitrosooxazolidine **2**, and the (2-hydroxyethyl)nitrosourea **4** is indicated by the detection of small quantities of the 2-hydroxyethyl *N*-cyclohexylcarbamate (**9**) by CIMS in the involatile products of decomposition of CCNU (**5a**). This suggests, but does not prove, that CCNU may in a minor pathway be in equilibrium with **2a**, which in turn forms **4a**. The implied intermediacy of the 2-hydroxyethyl diazotate (**17**) has the merit that it could account for the formation of the hydroxyethylated nucleosides isolated by Ludlum and coworkers from the action of (2-haloethyl)nitrosoureas on polynucleotides.[6,39] These workers identified hydroxyethyl derivatives of cytidine, guanosine, and adenosine, which were shown not to arise from the corresponding haloethyl nucleosides. Tong and Ludlum suggested that the hydroxyethylated derivatives arise from the as yet unidentified 3-acyl-1,2,3-oxadiazolinium postulated intermediate[39] which has been rejected by Weinkam and Lin.[18] The significance of low concentrations of hydroxyethylated compounds is that they have been shown to be especially active in the degradation of DNA[43] and in other physio-

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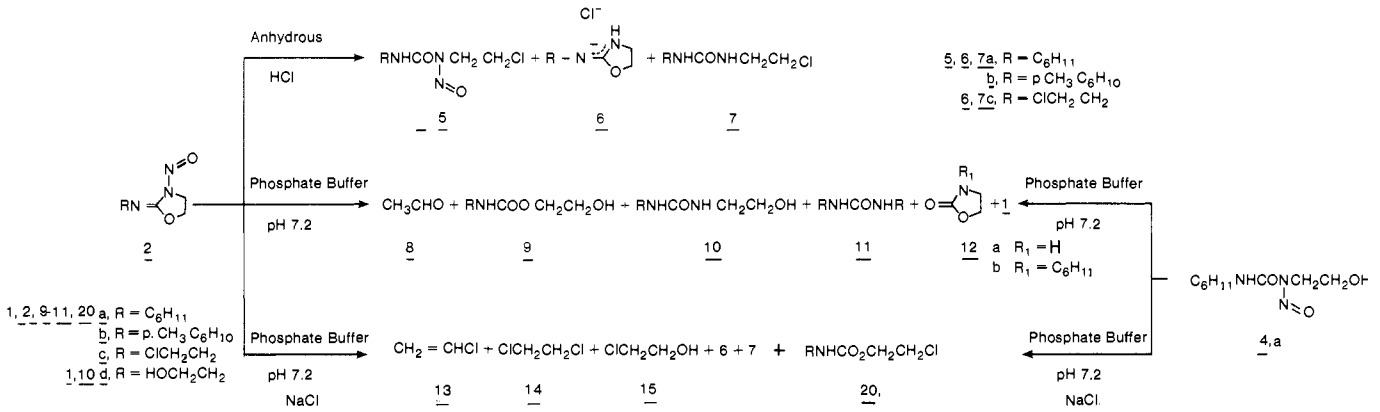
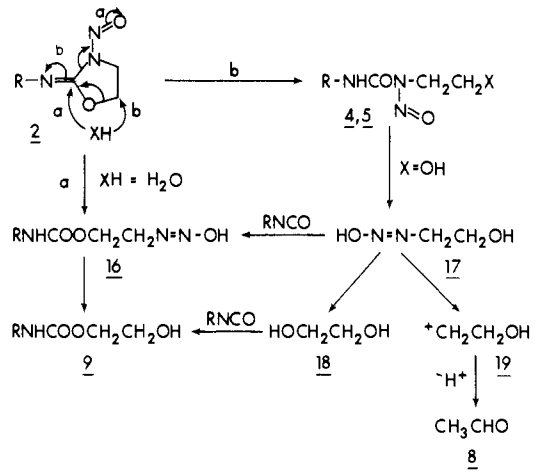
(39) W. P. Tong and D. B. Ludlum, *Biochem. Pharmacol.*, **28**, 1175 (1979).
(40) T. P. Johnston, G. S. McCaleb, P. S. Opliger, and J. A. Montgomery, *J. Med. Chem.*, **9**, 892 (1966).
(41) T. P. Johnston, G. S. McCaleb, P. S. Opliger, W. R. Laster, and J. A. Montgomery, *J. Med. Chem.*, 14, 600 (1971).
(42) T. P. Johnston, G. S. McCaleb, and J. A. Montgomery, *J. Med. Chem.*, **6**, 669 (1963).
(43) J. W. Lown and L. W. McLaughlin, *Biochem. Pharmacol.*, **28**, 1631 (1979).

Scheme III



Scheme IV



logical effects, e.g., mutations.[44-46] For example, compound **4a** shows a high reactivity toward DNA compared to **2a**, **2b**, or **2c** (see Results). Attempts to discriminate between the alternative sources of the 2-hydroxyethylating species by specific deuterium labeling will be reported subsequently.

The detection of vinyl chloride and 2-chloroethanol as products from 2-(cyclohexylimino)-3-nitrosooxazolidine (**2**) in the presence of sodium chloride may suggest that chloride ion is attacking at position 5 of **2** to give rise to CCNU (**5a**) or **4a**. Subsequent decomposition of **5a** by pathway A (Scheme I) could give rise to vinyl chloride and 2-chloroethanol via the 2-chloroethyl cation. If the initial concentration of chloride ion is low then preferential attack by water at position 5 would give rise to **4** and then to the diazotate **17**. The latter could give rise to 2-chloroethanol as shown in Scheme IV by reaction with chloride ion.

In contrast to **2a** and **2b** that interconvert readily with the parent nitrosoureas CCNU and MeCCNU, respectively, under acid catalysis, **2c** did not open to give BCNU but rather underwent quantitative denitrosation to the inert 2-[(2-chloroethyl)amino]-2-oxazoline (**1c**). This difference in behavior may reflect the different preferred sites for protonation on **2c** compared with **2a** or **2b**. The marked difference in behavior of the compounds **2a–c** predict a greater overall contribution of this decomposition pathway for CCNU and MeCCNU than for BCNU (which would then prefer the 2-chloroethyl diazonium ion pathway B).

In conclusion there is substantial evidence from several groups in favor of pathway B as the major source of products including acetaldehyde, 2-chloroethanol, and vinyl chloride from (2-haloethyl)nitrosoureas.[4-9,17-19] However, significant differences in the measured total alkylating abilities of BCNU, CCNU, and MeCCNU[1-3] indicate that other pathways such as A or C may contribute to a small but significant extent depending on the structure of the nitrosourea. For example, a greater propensity of the nitrosourea to cyclize might be expected to favor those pathways, such as A or C.

An examination by specific deuterium labeling of the alternative mechanistic pathways leading to the observed products and an assessment of the contribution of the pathway of decomposition via the 2-(alkylimino)-3-nitroso-2-oxazolidine in the overall scheme of decomposition of (2-haloethyl)nitrosoureas will be reported subsequently.

Experimental Section

Melting points were determined on a Fisher-Jones apparatus and are uncorrected. The IR spectra were recorded on a Nicolet 7199 Fourier transform spectrophotometer, and only the principal, sharply defined peaks are reported. Most of the ^1H NMR spectra were recorded on Perkin-Elmer 90 and Varian HA-100 analytical spectrometers, and only the final nitrosoureas and nitrosooxazolidines were recorded on Bruker WH-200 and WH-400 spectrometers. The ^{13}C NMR spectra were recorded on Varian HA-60 and Bruker HFX-22.6 spectrometers. The spectra were recorded on approximately 5–15% (w/v) solutions, depending upon the spectrometers, in appropriate deuterated solvents with tetramethylsilane as internal standard. Line positions are recorded in parts per million from reference. Electron-impact mass spectra were determined on an Associated Electrical Industries (AEI) MS-9 double-focusing high-resolution mass spectrometer, and chemical-ionization mass spectra were recorded on an AEI MS-12 using isobutane as reagent gas.[34,35] The ionization energy, in general, was 70 eV. The peak measurements were made by comparison with perfluorotributylamine at a resolving power of 15000 kW. Kieselgel DF-5 (Camag, Switzerland), Eastman Kodak, and Waters Associate reverse-phase precoated sheets were used for thin-layer chromatography. In the workup procedures reported for the various syntheses described, solvents were removed with a rotary evaporator under reduced pressure without heating.

GC analyses were performed on a Hewlett-Packard 5840A gas chromatograph equipped with flame-ionization detector. GC–MS analyses were performed on an AEI MS-12 spectrometer using a helium gas flow rate of 22 mL/min. Samples were injected onto a 6-ft 10% Carbowax 20M 80–100 WAW-DMCS 5830 column. The column was heated at 70 °C for acetaldehyde, vinyl chloride, vinyl bromide, and cyclohexene for 5 min and was heated further with a rate of 10 °C/min up to 120 °C for 30 min for 1,2-dichloroethane, chloroethanol, and bromoethanol.

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(46) D. H. Swenson, J. V. Frei, and P. D. Lawley, *J. Natl. Cancer Inst.*, 63, in press (1979).

Materials. Purified acetaldehyde, vinyl chloride, 1,2-dichloroethane, 2-chloroethanol, 2-oxazolidinone, 2-chloroethylamine hydrochloride, cyclohexyl isocyanate and dicyclohexylurea used as authentic samples for GC and GC–MS were obtained from Aldrich. 2-Chloroethyl isocyanate was obtained from Eastman. The following ureas were prepared from knwon procedures:[40-42] bis(2-chloroethyl)urea, mp 127 °C; 1-(2-chloroethyl)-3-cyclohexylurea, mp 130 °C; 1-(2-chloroethyl)-3-(4-methylcyclohexyl)urea, mp 150 °C.

General Method for the Preparation of 2-(Alkylamino)-2-oxazolines (1a–d). A suspension of 1-(2-chloroethyl)-3-alkylurea (20 mmol) was refluxed in water (150 mL) until the solution became almost clear. The reaction mixture was cooled and extracted with ethyl acetate to remove the unreacted starting material, as well as other organic impurities. The aqueous solution was basified with dilute NaOH or NH_4OH solution to pH 10–12 and in the case of **1a** and **1b**, extracted with chloroform. The chloroform layer was dried (Na_2SO_4), the solvent was removed under reduced pressure, and the residue was crystallized from dichloromethane/petroleum ether to afford **1a** and **1b**. In the case of **1c** and **1d**, since they are water soluble, the aqueous solution was concentrated after basification with NaOH, and the residue was triturated with 2-propanol to remove the sodium chloride. Removal of 2-propanol under reduced pressure afforded **1c** and **1d**. The physical and spectral data for these compounds are given in Tables I and II.

General Method for the Preparation of 2-(Alkylimino)-3-nitroso-2-oxazolidines (2a–c). A solution of *n*-butyl nitrite (5.1 g, 50 mmol) was added dropwise to the stirred suspension of sodium methoxide (0.67 g, 11 mmol) and 2-(alkylamino)-2-oxazoline (10 mmol) in dry ether (100 mL) in an ice bath, and the reaction mixture was stirred for an additional 12 h at ambient temperature. The inorganic salt was collected and washed with dry ether, and the filtrate was concentrated under reduced pressure. In some cases, the inorganic salt and excess of butyl nitrite was washed out by cold water cautiously, and the organic layer was dried (Na_2SO_4) and worked up as above. The 2-(alkylimino)-3-nitroso-2-oxazolidines were repeatedly crystallized from ether/petroleum ether, and their physical and spectra data are given in Table I.

Reaction of 2-(Alkylimino)-3-nitroso-2-oxazolidine (2) with Dry Hydrogen Chloride. Formation of (2-Chloroethyl)-nitrosoureas (5a,b). A stream of dry hydrogen chloride was passed into a solution of 2-(alkylimino)-3-nitroso-2-oxazolidine (**2a,b**; 5 mmol) in dry ether (50 mL) at 0–4 °C for 30 min. The solution was washed with ice-cold water to remove the oxazoline hydrochloride formed via denitrosation, as well as excess HCl, the organic layer was dried (Na_2SO_4) and the solvent was removed under reduced pressure. The residue was crystallized from ether/petroleum ether. The products were characterized by their mixture melting point with authentic samples and by comparison with other physical and spectral data as shown in Table I.

Under these conditions, BCNU could not be isolated from the nitrosooxazolidine **2c** and instead was almost quantitatively converted to 2-[(2-chloroethyl)amino]-2-oxazoline hydrochloride (mp 108–109 °C), which on heating slowly solidified and remelted at 126–127 °C which corresponds in properties to the conversion of 2-[(2-chloroethyl)amino]-2-oxazoline (**1c**) hydrochloride to bis(2-chloroethyl)urea (**11c**) by ring opening by Cl^-.[47]

3-Cyclohexyl-1-(2-hydroxyethyl)-1-nitrosourea (4a). Cyclohexyl isocyanate (2.50 g, 20 mmol) was added to a stirred suspension of ethanolamine in ether (75 mL) at ambient temperature. The reaction mixture was stirred for 12 h and crystalline 3-cyclohexyl-1-(2-hydroxyethyl)urea (3.2 g, 85%) was obtained, which was crystallized from acetone/ether: mp 90–91 °C; ^1H NMR ($CDCl_3$) δ 1.00–2.00 (m, 10 H, CH_2), 3.30 (m, 2 H, CH_2OH), 3.45 (m, 1 H, CH), 3.62 (m, 2 H, NCH_2), 4.60 (s, 1 H, OH exchangeable), 5.50 (d, 1 H, NH exchangeable), 5.90 (t, 1 H, NH exchangeable); MS, *m/e*, (relative intensity) 186.1369 (29.66, M^+, calcd for $C_9H_{18}N_2O$, 186.1369), 156.1259 (33.71, calcd for $C_8H_{16}N_2O$, 156.1256), 56.0606 (100.00, calcd for C_3H_6N, 56.0501).

The above urea (1.86 g, 10 mmol) was dissolved in 98% formic acid (25 mL) and cooled to 0 °C. The solid sodium nitrite (2.7 g, 30 mmol) was added portionwise over a period of 1 h, maintaining the temperature below 5 °C. after the mixture was stirred for 30 min, cold water (30 mL) was added dropwise and the mixture was stirred for 1 h. The reaction mixture was extracted with chloroform, the organic layer was removed, washed with cold water, and dried (Na_2SO_4), and the solvent was removed under reduced pressure. The residue was crystallized from ether/petroleum ether to afford (1.35 g (62%) of 3-cyclohexyl-1-(2-hydroxyethyl)nitrosourea (**4a**). The physical and spectral data are given in Table I.

Preparation of 2-Hydroxyethyl *N*-Cyclohexylcarbamate (9) and Ethylene Glycol Bis(*N*-cyclohexylcarbamate). Cyclohexyl isocyanate (6.25 g, 500 mmol) was added dropwise to a stirred suspension of ethylene glycol (3.10 g, 50 mmol) in dry benzene (100 mL) and stirred for 24 h at room temperature. The benzene solution was concentrated to 50 mL under reduced pressure and on cooling a white solid separated, which was washed with benzene to afford 2.25 g (14%) of ethylene glycol bis(*N*-cyclohexylcarbamate) which after two crystallizations with acetone melted at 139–40 °C; ^1H NMR ($CDCl_3$) δ 1.00–2.10 (m, 2 × 10 H, CH_2), 3.50 (m, 2 × 1 H, CH), 4.20 (s, 4 H, CH_2), 4.65 (m, 2 × 1 H, exchangeable); IR ($CHCl_3$) 3465 (NH), 1720 (C=O) cm^{-1}; MS, *m/e* (relative intensity) 312.2048 (7.45, M^+, calcd for $C_{16}H_{28}N_2O_4$, 312.2049), 188.1284 (36.51, calcd for $C_9H_{18}NO_3$, 188.1284), 187.1208 (30.58, calcd for $C_9H_{17}NO_3$, 187.1208), 170.1180 (100.00, calcd for $C_9H_{16}O_2$, 170.1180).

The benzene filtrate was diluted with petroleum ether until the solution became turbid and on cooling it afforded 5.40 g (58%) of **9a**, which on crystallization with petroleum ether melted at 58–59 °C: ^1H NMR ($CDCl_3$) δ 1.10–2.10 (m, 10 H, CH_2), 2.50 (br m, OH, exchangeable), 3.52 (m, 1 H, CH), 3.75 (m, 2 H, CH_2), 4.20 (m, 2 H, CH_2), 4.65 (br m, NH, exchangeable); IR ($CHCl_3$) 3480 (NH, OH), 1720 (C=O) cm^{-1}; MS, *m/e* (relative intensity) 187.1205 (47.37, M^+, calcd for $C_9H_{17}NO_3$, 187.1209), 157.1103 (6.83, calcd for $C_8H_{15}NO_2$, 157.1103) 144.0660 (100.00, calcd for $C_8H_{10}NO_3$, 144.0660).

Preparation of 2-Chloroethyl *N*-Cyclohexylcarbamate (20a). A solution of 2-chloroethyl chloroformate (14.29 g, 100 mmol) in benzene (100 mL) was added dropwise to a stirred solution of cyclohexylamine (19.82 g, 200 mmol) in benzene (100 mL) at ambient temperature. After the solution was stirred for 4 h, the hydrochloride salt was filtered off, the filtrate was concentrated, and the residue was crystallized from ether/petroleum ether to afford 16.50 g (80%) of **20a**: mp 62 °C; NMR ($CDCl_3$) δ 1.00–2.10 (m, 10 H, CH_2), 3.45 (m, 1 H, CH), 3.65 (t, 2 H, CH_2Cl), 4.30 (t, 2 H, OCH_2), 4.80 (br m, 1 H, NH exchangeable); IR ($CHCl_3$) ν_{max} 3480 (NH), 1720 (C=O) cm^{-1}; MS, *m/e* (relative intensity) 207.0847 (5.96; M^+, calcd for $C_9H_{16}{}^{37}ClNO_2$, 207.0840), 205.0872 (22.71, M^+, calcd for $C_9H_{16}{}^{35}ClNO_2$, 205.0869), 164.0295 (32.14, calcd for $C_6H_9{}^{37}ClNO_2$, 164.0292), 162.0222 (100.00, calcd for $C_6H_9ClNO_2$, 162.0321), 126.0134 (12.12, calcd for $C_3H_7{}^{37}ClNO_2$, 126.0136), 124.0167 (35.89, calcd for $C_6H_7{}^{35}ClNO_2$, 124.0122).

Preparation of 3-Cyclohexyl-2-oxazolidinone (12b). Powdered sodium hydroxide (0.44 g, 11 mmol) was added to a solution of **20a** (2.05 g, 10 mmol) in ethanol (80 mL) and refluxed for 5 h. The reaction mixture was cooled, solid sodium chloride was filtered off, and the filtrate was concentrated. The residue was triturated with petroleum/ether to afford 1.30 g (77%) of **12b**: mp 29–30 °C; NMR ($CDCl_3$) δ 1.00–2.00 (m, 10 H, CH_2), 3.55 (t, 2 H, H_4), 3.65 (m, 1 H, CH), 3.55 (t, 2 H, H_5); IR ($CHCl_3$) 1740 (C=O) cm^{-1}; MS, *m/e* (relative intensity) 169.1102 (34.33, M^+, calcd for $C_9H_{15}NO_2$, 169.1103), 126.0552 (100.00, $M^+ - 43$, calcd for $C_6H_8NO_2$, 126.0555), 88.0398 (93.94, calcd for $C_3H_6NO_2$, 88.0398).

General Method for the Decomposition of 2-(Alkylimino)-3-nitroso-2-oxazolidines (2a–c) and 3-Cyclohexyl-1-(2-hydroxyethyl)-1-nitrosourea (4a). (1) In Phosphate Buffer. (a) Identification of Volatile Products. A sample of **2a–c** or **4a** (0.05 mmol) suspended in 0.1M phosphate buffer (0.6 mL), pH 7.2, in 1-mL capacity screw capped Reacti-vials was allowed to decompose for 12 h at 37 °C, after the evacuation of the head space above the liquid. The gaseous sample (1 mL) was injected by pressure-lok syringe onto GC for acetaldehyde and other volatile products, which were confirmed by the retention times by comparison to authentic samples and their masses by GC–MS.

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Table III. Isobutane Chemical-Ionization Mass Spectra of 2-(Alkylimino)-3-nitroso-2-oxazolidines (2a–c), 3-Cyclohexyl-1-(2-hydroxyethyl)-1-nitrosourea (4a), and Their Nonvolatile Products (135–150 °C) in Phosphate Buffer, pH 7.2 at 37 °C

compd	decomp products	yield,[a] %	*m/e* (rel intensity)
2-(cyclohexylimino)-3-nitroso-2-oxazolidine (2a)			199 (5, MH + 1), 198 (16, MH), 169 (100, MH – 29), 168 (21, MH – 30), 151 (MH – 47)
	cyclohexyl-1-(2-hydroxyethyl)-urea[b]	1–2	188 (15, MH + 1), 187 (100, MH), 100 (6, $C_6H_{11}NH_3$)
	2-hydroxyethyl *N*-cyclohexyl-carbamate[b]	20–25	189 (10, MH + 1), 188 (100, MH), 170 (MH – H_2O)
	dicyclohexylurea[c]	3–4	266 (19, MH + 1), 225 (100, MH), 143 (20, MH – C_6H_{11})
	2-oxazolidinone		89 (7, MH + 1), 88 (100, MH)
	cyclohexylamino-2-oxazoline[b]	10–15	170 (13, MH + 1), 169 (100, MH)
	cyclohexylurea		144 (8, MH + 1), 143 (100, MH)
2-[(*trans*-4′-methylcyclohexyl)imino]-3- nitroso-2-oxazolidine (2b)			212 (12, MH + 1), 183 (100, MH – 29)
	4,4′-dimethylcyclohexylurea[c]	3–5	254 (15, MH + 1), 253 (100, MH), 157 (25, MH – $CH_3C_6H_{10}$)
	4′-methylcyclohexyl-2-oxazoline	12–15	184 (15, MH + 1), 183 (100, MH), 114 (9, $CH_3C_6H_{10}NH_3$)
	1-(2-hydroxyethyl)-4′-methyl-cyclohexylurea	1–2	202 (13, MH + 1), 201 (100, MH)
	2-hydroxyethyl *N*-(4′-methyl-cyclohexyl)carbamate	15–25	203 (12, MH + 1), 202 (100, MH), 184 (8, MH – H_2O)
2-[(2′-chloroethyl)imino]-3-nitroso-2-oxazolidine (2c)			181 (3, MH + 1, ^{37}Cl), 180 (30, MH, ^{37}Cl), 179 (8, MH + 1, ^{35}Cl), 178 (100, MH, ^{35}Cl), 151 (9, MH – 29, ^{37}Cl), 149 (35, MH – 29, ^{35}Cl), 113 (10, MH – 65)
	2-[(2′-chloroethyl)amino]-2-oxazoline	15–20	152 (3, MH + 1, ^{37}Cl), 151 (21, MH, ^{37}Cl), 150 (8, MH + 1, ^{35}Cl), 149 (100, MH, ^{35}Cl), 113 (13, MH – HCl)
	3-(2-chloroethyl)-1-(2-hydroxy-ethyl)urea	1–2	170 (3, MH + 1, ^{37}Cl), 169 (36, MH, ^{37}Cl), 168 (8, MH + 1, ^{35}Cl), 167 (100, MH, ^{35}Cl), 131 (10, MH – HCl)
	2-[(2′-hydroxyethyl)amino]-2-oxazoline	1–3	132 (11, MH + 1), 131 (100, MH), 86 (8)
	2-hydroxyethyl *N*-(2-chloro-ethyl)carbamate	8–12	171 (3, MH + 1, ^{37}Cl), 170 (36, MH, ^{37}Cl), 169 (9, MH + 1, ^{35}Cl), 168 (100, MH, ^{35}Cl), 150 (6, MH – H_2O)
	bis(2-chloroethyl)urea		189 (15, MH, 2 ^{37}Cl), 187 (60, MH, ^{37}Cl and ^{35}Cl), 185 (100, MH, 2 ^{37}Cl)
3-cyclohexyl-1-(2-hydroxyethyl)-1-nitrosourea (4a)			217 (13, MH + 1), 216 (100, MH), 198 (10, MH – H_2O), 187 (20, MH – 29), 169 (27, MH – 47)
	ethylene glycol bis(*N*-cyclo-hexylcarbamate)[d]		314 (13, MH + 1), 313 (100, MH), 188 (13, MH – 125), 170 (55, MH – 143)
	3-cyclohexyl-2-oxazolidinone[c]		171 (10, MH + 1), 170 (100, MH)
	cyclohexyl-1-(2-hydroxyethyl)-urea	1–2	188 (13, MH + 1), 187 (100, MH)
	2-hydroxyethyl *N*-cyclohexyl-carbamate	10–15	189 (10, MH + 1), 188 (100, MH)
	dicyclohexylurea[d]	8–10	226 (19, MH + 1), 225 (100, MH)

[a] Yields were not critically optimized. [b] Yields were estimated from the NMR spectra of nonvolatile products soluble in $CDCl_3$. [c] Yields were estimated from the material insoluble both in $CDCl_3$ and H_2O. [d] Presence was detected by only CIMS.

(b) Identification of Nonvolatile Products. A sample of **2a-c** (9.8 mg, 0.05 mmol) in 0.1 M phosphate buffer (10 mL) was allowed to decompose for 12 h at 37 °C. The aqueous solution was lyophilized and the residue was subjected to CIMS using isobutane as reagent gas; the CIMS of their decomposition products are presented in Table III. After CIMS spectra, the residue was extracted with $CDCl_3$ and the extract examined by NMR. This allowed both the identification (by comparison with authentic samples) and quantitation of cyclohexyloxazoline *N*-cyclohexylcarbamate and 2-hydroxyethylcyclohexylurea.

(2) In the Presence of Saturated Sodium Chloride. (a) Identification of Volatile Products. A sample of **2a-c** or **4a** (0.1 mmol) in saturated sodium halide (4 mL) in 0.1 M phosphate buffer was allowed to decompose in 5-mL capacity Reacti-vials as above at 37 °C for 12 h. The 1-mL head space was immediately evacuated by a syringe, and the gaseous contents after 12 h were analyzed by GC-MS. Immediately after the removal of the gaseous contents, 0.5 mL of dichloromethane was injected into the vial and shaken thoroughly, and the dichloromethane solution (2 μL) was injected for GC-MS analysis. The retention time and mass spectra of each compound in different reaction conditions are given in Table II.

(b) Identification of Nonvolatile Products. After the identification of volatile products, the reaction mixture was extracted with hot acetonitrile and the organic phase was concentrated. The products were identified as ureas and oxazolines by TLC on reverse-phase precoated silica plates using a water/acetonitrile mixture (1:9-2:8) and their characteristic mass spectra.

Fluorescence Determination of Alkylation of PM2-CCC-DNA by (2-Haloethyl)nitrosourea Intermediates. All fluorescence measurements were performed on a G. K. Turner and Associates Model 430 spectrofluorometer equipped with a cooling fan to minimize fluctuations in the xenon lamp source. Wavelength calibration was performed as described in the manual for the instrument; 1-cm^2 cuvettes were used. The excitation wavelength was 525 nm and the emission wavelength was 500 nm. The 100X scale of medium sensitivity was generally used, and water was circulated between the cell compartment and a thermally regulated bath at 22 °C. The method has been described in detail elsewhere.[38] The basis of the method is that while PM2-CCC (covalently closed circular) DNA returns to register after heating to 96 °C and cooling, owing to topological constraints, and therefore shows no change in the fluoroscence of intercalated ethidium, alkylated PM2-CCC-DNA shows a decrease in fluorescence under these conditions because of thermally induced cleavage at the site of alkylation. The ratio of the decrease in fluorescence (after heat denaturation and rapid cooling) to that of control is a measure of the extent of alkylation.

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Synthesis and DNA Binding of [3-[2′-(2-Acetamidoethyl)-2,4′-bithiazole-4-carboxamido]propyl]dimethylsulfonium Chloride, a Fragment of Bleomycin A_2

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[3-[2′-(2-Acetamidoethyl)-2,4′-bithiazole-4-carboxamido]propyl]dimethylsulfonium chloride (**1**), the acetyl derivative of the cationic terminal dipeptide of bleomycin A_2, has been synthesized and its binding to DNA and poly(dA-dT) has been studied by proton NMR and fluorescence spectroscopy. The spectral perturbations which occur upon binding of the compound to either nucleic acid indicate that that portion of bleomycin which binds to the nucleic acid can, for the most part, be mimicked by the fragment. The data are discussed in terms of the structure of the drug and the drug-nucleic acid complex.

The antitumor antibiotic bleomycin A_2 (Figure 1) is believed to exert its biological effect(s) by causing the degradation of DNA.[1] The putative mechanism of action involves at least two processes (which may occur stepwise or simultaneously): (i) association of the drug with DNA and (ii) generation of free radicals by the drug, acting in the form of a metal ion complex.[1] It is not known if the radicals are the species responsible for DNA degradation or if the bleomycin molecule also plays a role. Bleomycin A_2 appears to possess two distinct regions, each of which is responsible for one of the functions: a cationic terminus containing an aromatic group for the binding and a metal-ion binding site localized around the pyrimidine moiety necessary for the degradation of the nucleic acid.[1-3] Figure 1 shows the possible ligands involved in the complexation of the metal ions. As a part of ongoing studies on structure-activity relationships in the bleomycin family of drugs, we have synthesized a fragment of bleomycin A_2 which is the acetyl derivative of the cationic terminal "dipeptide" and which contains the presumed DNA binding sites. We have studied the interaction of this derivative with calf thymus DNA and poly(dA-dT) using proton NMR and fluorescence spectroscopy. If the activity of bleomycin A_2 can, in fact, be explained on the basis of a simple bifunctional model in which the two portions of the molecule act more or less independently, the cationic fragment should show all, or nearly all, of the spectral perturbations seen in the binding of the intact drug.

The binding of intact bleomycin A_2 and tripeptide S, the terminal fragment containing threonine (Figure 1), to DNA has been studied using fluorescence[2] and NMR spectroscopy.[2,4] The fluorescence experiments suggested that tripeptide S bound in a manner very similar to the intact

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