The measurement of adenyl cyclase activity of guinea pig lung was carried out as previously described.[32] The materials used were beef heart PDE (Boehringer Mannheim, 15153, EPAY, control no. 7205306), Russel's viper venom (Sigma Chemical Co.), cyclic [8-^{3}H]AMP (specific activity 21 Ci/mmol, Schwarz-Mann), [α-^{32}P]ATP [adenosine 5'-triphosphate, tetrakis(triethylammonium salt), specific activity 33.54 Ci/mmol, New England Nuclear], Dowex 1-X-2 (~400 mesh, chloride form), Dowex 50 W-X-8 (100–200 mesh, hydrogen form, Bio-Rad Laboratories), and theophyllinemonoethylamine (K and K laboratories).

Acknowledgment. The authors express their appreciation to Dr. G. Schilling and his associates for microanalytical and spectral data. The skillful assistance of Miss M. Mihelic and Mr. J. Csakvary in chemical synthesis and of Ms. M. Wong and Ms. M. T. Silvestre in the biochemical studies is gratefully acknowledged. We also thank Dr. E. Greselin and Dr. D. Hicks for their cooperation in the bioavailability studies and in the synthesis of ^{14}C-labeled esters, respectively.

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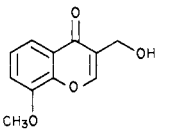

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Reactions of 1,3-Bis(2-chloroethyl)-1-nitrosourea and 1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea in Aqueous Solution

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Products formed from the reaction of two chloroethylnitrosoureas in neutral aqueous solution have been identified and quantified. Mixture components recovered after a 1-h incubation period accounted for 75–85% of the starting nitrosourea. Approximately 65–85% of the reaction products were formed by an initial cleavage of the nitrosourea to the proposed intermediates 2-chloroethyl azohydroxide and an isocyanate and by subsequent hydrolytic reactions. A minor pathway, 5–10% of products, involves denitrosation of the nitrosourea with oxazoline formation. Stable isotope labeling and mass spectrometry have been used to determine the reaction sequence and product origins. Reaction product identification has been made using high-performance LC isolation and comparison with synthetic material.

1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU) and other chloroethylnitrosoureas are used in the treatment of brain tumors,[1,2] lymphomas,[3] and other malignant diseases. These agents are not biologically active in their parent form but are converted to active alkylating intermediates by chemical reactions in aqueous media.[4] The active alkylating agent is thought to be 2-chloroethyl azohydroxide or diazonium ion.[5,6] BCNU and related chlo-

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roethylnitrosoureas have been found to alkylate[7] and cross-link DNA,[8] presumably through 2-chloroethylation of an amino function to generate $RNHCH_2CH_2Cl$, which may undergo further alkylation reactions. Cross-linking of DNA may be one mode of BCNU cytotoxic activity, but because BCNU has a rapid onset of activity[9] and is active against nondividing cells,[10] other mechanisms are probably operative.

The reactions of BCNU in aqueous solution at physiological temperature and pH may be related to the fraction of parent drug undergoing activation and degradation processes in vivo. For this reason, the decomposition reactions of chloroethylnitrosoureas have been investigated. BCNU, the most widely studied representitive of this class, is relatively stable at pH 4–5, where its half-life exceeds 500 min, but degrades rapidly under basic conditions with a half-time of 5 min at pH 8.0.[11,12] BCNU disappearance at pH 7.4 is first order with a half-life of 50 min.[11] Conflicting reports have appeared on the dependence of BCNU kinetics on buffer,[11-13] salt effects,[11] and specific hydroxide ion catalysis,[12,13] although decomposition appears to occur by a mechanism that involves general base catalysis.[13]

Colvin and co-workers[14] have analyzed the volatile reaction products generated from the 1-(2-chloroethyl) moiety of BCNU (5×10^{-2} M) at pH 7.4 and 37 °C in phosphate buffer. 2-Chloroethanol (~32%) and acetaldehyde (~16%) were identified as major products. Vinyl chloride (~2%) and 1,2-dichloroethane (~1%) were observed as minor products. Approximately 50% of the radioactivity was present as nonvolatile material and was not identified. Montgomery and co-workers[15,16] have confirmed these results; they isolated 2-chloroethanol (40%) and acetaldehyde (20%) after 2 h at 50 °C. 2-Chloroethylamine and 1,3-bis(2-chloroethyl)urea were also identified as products. 1,3-Bis(2-chloroethyl)urea (BCU) and 2-[(2-chloroethyl)amino]-2-oxazoline (CAO) were also identified as products from BCNU after reaction for 15 h.[16]

1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), 6×10^{-4} M, in pH 7.4 phosphate buffer reacts in the same way. 2-Chloroethanol (18–25%), acetaldehyde (5–10%), and cyclohexylamine (32%) are formed after 3 h at 37 °C.[17] CCNU has a half-life of 48 min under these conditions.[17] The products are similar to those observed for other alkylnitrosoureas.[18] The yields of these products from 2-chloroethylnitrosourea reactions are lower than those reported from other alkylnitrosoureas under the same reaction conditions.[18] Only a fraction (<45%) of BCNU and CCNU reaction products have been identified, and unidentified nonvolatile products are known to be formed.[14,17] This report describes additional chloroethylnitrosourea reactions at physiological pH and temperature that were determined through the analysis of nonvolatile reaction products.

Results

Direct mixture analysis chemical-ionization mass spectrometry (CIMS) techniques have been used to identify components of complex chemical and metabolic reaction mixtures.[19-23] This approach has been applied to the reaction of BCNU in aqueous buffer.[19] Repetitive CI scans were made of lyophilized reaction mixtures and extracts as these materials were being evaporated into the ion source. The limited protonated molecular ion fragmentation that occurs under CI conditions permits the preliminary identification of mixture components that are introduced simultaneously. Figure 1 shows an isobutane CI mass spectrum in which the protonated molecular ion

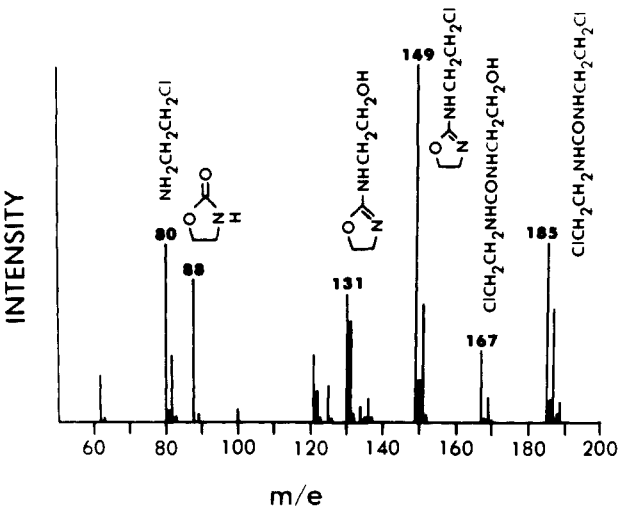


Figure 1. Isobutane CIMS of nonvolatile products formed during the reaction of BCNU in pH 7.4 phosphate buffer at 37 °C. Unreacted BCNU was extracted with cyclohexane prior to lyophilization.

Table I. Products from the Reaction of BCNU (9.5×10^{-4} M) in pH 7.4 Phosphate Buffer at 37 °C after 2 h

component	% recovered
2-chloroethanol	25[a]
acetaldehyde	13[a,b]
vinyl chloride	3[a,b]
2-chloroethylamine	11[c]
2-oxazolidone	26[c]
BCU	8[d]
HCU	4[d]
BHU	2
CAO	12
HAO	2[e]
BCNU unreacted	19
total recovered:	73-84

[a] Percent of theoretical yield, assuming 1 mol of BCNU gives 1 mol of product by way of 2-chloroethyl azohydroxide. [b] Calculated from 2-chloroethanol/acetaldehyde or vinyl chloride ratios of ref 14 and 15. [c] Assumes 1 mol of BCNU gives 1 mol of product by way of 2-chloroethyl isocyanate. [d] Assumes 1 mol of BCNU gives 0.5 mol of product by way of 2-chloroethyl isocyanate. [e] Value obtained from a different experiment under similar conditions.

(MH^+) of six nonvolatile BCNU reaction products are apparent. Tentative identification of these compounds may be made on the basis of ion masses and chlorine isotope ratios evident in the spectrum. The structures of these reactions products were confirmed by independent synthesis and comparison of product thin-layer chromatography (TLC) R_f values, gas chromatography (GC) or high performance liquid chromatography (LC) retention times, and the mass spectra of product isolated using TLC, GC, or high performance LC with synthetic compounds.

The direct mixture analysis CIMS technique may also be used for the quantitative analysis of these ureas, oxazolines, 2-oxazolidone, and 2-chloroethylamine.[19] This procedure involved summation of the total ion current generated from each of the reaction products and a known amount of standard compound. The relative ionization efficiency of each compound was determined using the respective synthetic compound. 2-Chloroethanol was analyzed using GC.

Table I shows the reaction mixture components measured after 2 h at 37 °C in a pH 7.4 phosphate buffer. 2-Chloroethanol, acetaldehyde, and vinyl chloride are

Scheme I

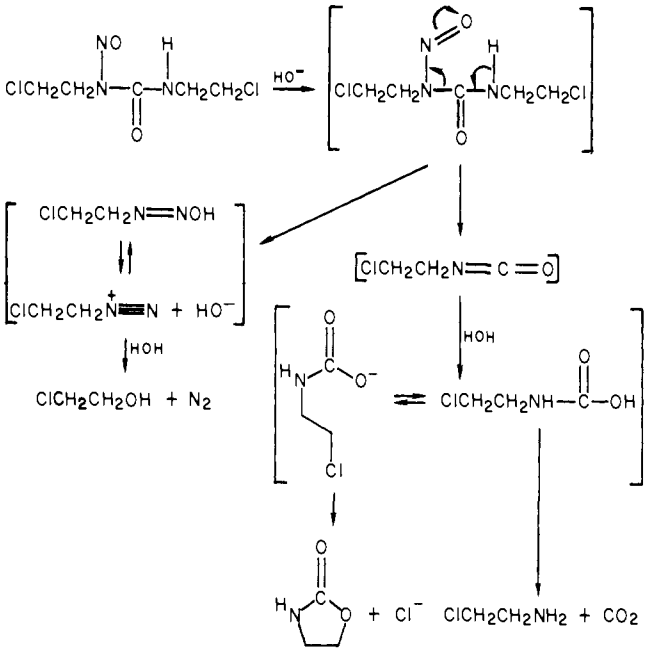


Table II. BCNU/BCNU-d_8 Isotope Ratios from the Reaction of 3×10^{-3} M Total BCNU in pH 7.4 Phosphate Buffer at 37 °C

compd	H/^{2}H	SD
BCNU starting mixture	1.51	
BCNU recovered	1.51	0.06
2-oxazolidone	1.62	0.10
2-chloroethylamine	1.41	0.10

compd	d_0	d_4	d_8
BCU			
recombination (calcd)	1.0	1.3	0.42[a]
denitrosation (calcd)	1.0	0.0	0.66
obsd	1.0	1.2	0.42
CAO			
from BCNU (calcd)	1.0	0.0	0.66[a]
obsd: 10 min	1.0	0.37	0.56
20 min	1.0	0.69	0.43
30 min	1.0	0.73	0.37
80 min	1.0	0.89	0.35
HAO			
obsd	1.0	1.2	0.46

[a] Calculated isotope ratios assuming that there is no deuterium isotope effect on the reaction.

derived from 2-chloroethyl azohydroxide.[14] The measured amount of 2-chloroethanol (31% of reacted BCNU) is in good agreement with the amount found by Colvin et al. under identical conditions.[14] Amounts of acetaldehyde and vinyl chloride were not measured but were calculated using published ratios for the relative amounts of 2-chloroethanol and these compounds.[14,15] 2-Chloroethylamine, 2-oxazolidone, BCU, and subsequent hydrolysis products are derived from 2-chloroethyl isocyanate, as shown in Scheme I.

Aqueous decomposition reactions were conducted using mixtures of BCNU and BCNU-d_8 labeled on the ethylene positions. The relative amounts of deuterium present in the products are indicative of the reaction pathway leading to their formation. Isotope ratios were determined from repeated CIMS scans of reaction mixture aliquots following extraction of unreacted BCNU. Table II shows the H/^{2}H ratios of starting BCNU/BCNU-d_8 and products formed from incubation of a 3×10^{-3} M BCNU solution. The

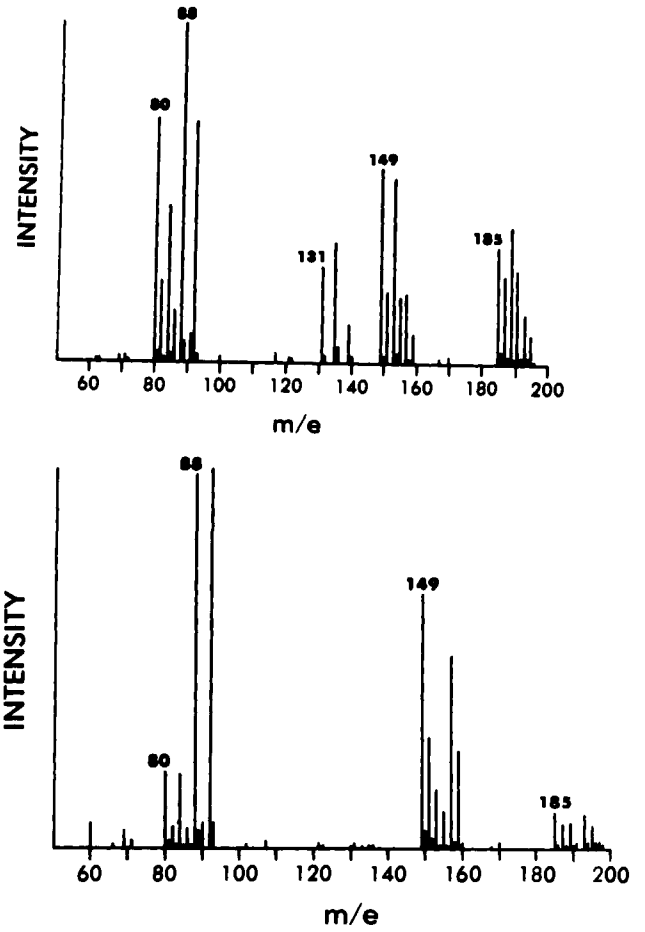


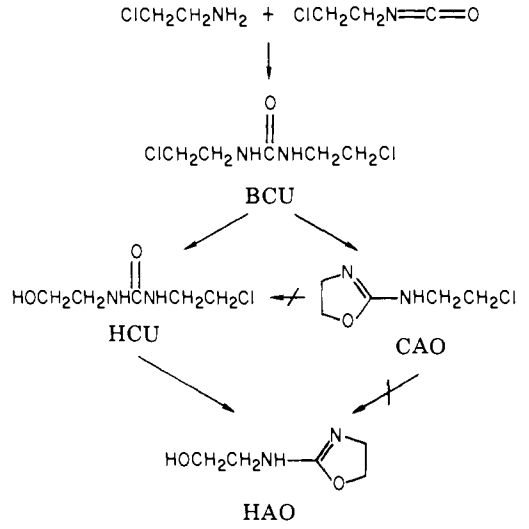
Figure 2. Mass spectra of products from the reaction of (top) a 3×10^{-3} M 1.5:1 mixture of BCNU/BCNU-d_8 and from (bottom) a 4×10^{-4} M 1:1 mixture of BCNU/BCNU-d_8, showing 2-chloroethanol, m/e 80; 2-oxazolidone, 88; HAO, 131; CAO, 149; and BCU, 185.

isotope ratios of unreacted BCNU and products 2-oxazolidone, 2-chloroethylamine, BCU, and HAO do not change during the course of the reaction. The isotope ratio of CAO, however, is both concentration and time dependent.

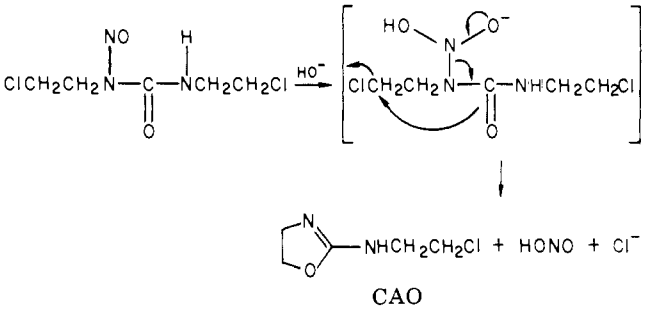
Figure 2 shows an isobutane CI spectrum of a 3×10^{-3} M 1.51:1 and a 4×10^{-4} M 1:1 BCNU/BCNU-d_8 reaction mixture. The CAO-d_4 ion, m/e 153–155, has a higher relative intensity in the more concentrated mixture and is virtually absent in the more dilute solution. This observation suggests that CAO may be formed by two pathways, directly from BCNU and from BCU cyclization. The amount of CAO formed from BCU cyclization and the amount of BCU formed at low initial BCNU concentrations are negligible. BCU is formed by a bimolecular process (Scheme II). At initial BCNU concentrations below 10^{-3} M, the products 2-chloroethylamine and 2-chloroethyl isocyanate are also dilute, and their combination reaction rate cannot compete with the concentration independent rate of isocyanate hydrolysis.

CCNU decomposition reactions were also investigated at 37 °C in pH 7.4 phosphate buffer. The nonvolatile reaction products cyclohexylamine, 1,3-dicyclohexylurea (DCU), 1-(2-chloroethyl)-3-cyclohexylurea (CCU), and 2-(cyclohexylamino)-2-oxazoline were identified from CI spectra of lyophilized reaction mixtures. The structures of DCU, CCU, and 2-(cyclohexylamino)-2-oxazoline were confirmed by TLC isolation and mass spectral analysis; R_f values and spectra were identical with those of synthetic samples. The ureas were quantitated using high-per-

Scheme II



Scheme III



formance LC, and the amount of oxazoline was estimated from mass spectral analysis. Table IV combines these results with those of Reed et al.[17] to show the distribution of CCNU reaction products.

Discussion

BCNU reacts in aqueous solution through two competing pathways, either by cleavage to the proposed 2-chloroethyl azohydroxide and 2-chloroethyl isocyanate intermediates (Scheme I) or by denitrosation and cyclization to CAO (Scheme III). The first step in Scheme I apparently involves general-base-catalyzed cleavage of the urea N–H bond to initiate formation of the active intermediate.[7,13,16] 2-Chloroethyl azohydroxide, which is in equilibrium with and kinetically indistinguishable from 2-chloroethyldiazonium ion, reacts with water predominantly by an S_N2 mechanism at neutral pH to give 2-chloroethanol. Lesser amounts of acetaldehyde and vinyl chloride are formed by way of 2-chloroethyldiazonium ion dissociation to carbocation intermediates as described by Colvin et al.[5,6] 2-Chloroethyl isocyanate reacts by addition of water to give a carbamic acid. This unstable intermediate may decarboxylate to 2-chloroethylamine and carbon dioxide or may ionize to the *N*-(2-chloroethyl)-carbamate anion. Cyclization by displacement of chloride ion gives 2-oxazolidone. This reaction sequence is supported by the isotope abundance data of Table II. 2-Oxazolidone formed from a BCNU/BCNU-d_8 mixture is depleted in deuterium, while 2-chloroethylamine is deuterium enriched. This suggests that 2-oxazolidone and 2-chloroethylamine are formed from a common intermediate, *N*-(2-chloroethyl)carbamic acid. The slower decarboxylation reaction occurs from a carbamic acid pool enriched in deuterium to the extent that the α and β secondary deuterium isotope effects prevent the more rapid 2-oxazolidone cyclization reaction. If deuterium does not alter the decarboxylation rate, the cumulative effect on 2-oxazolidone formation from the carbamic acid is $k_{H/D} = 1.16$ (Scheme I).

Table III. Dependence of the Origin of 2-[(2-Chloroethyl)amino]-2-oxazoline (CAO) on Initial BCNU Concentrations

concn,[a] M	BCNU		CAO			% CAO from BCNU
	d_0	d_8	d_0	d_4	d_8	
3×10^{-3}	1.0	0.66	1.0	0.73	0.37	44
8×10^{-4}	1.0	0.84	1.0	0.22	0.78	87
4×10^{-4}	1.0	0.98	1.0	0.22	0.77	89

[a] Reaction of BCNU and BCNU-d_8 at 37 °C for 30 min in pH 7.4 phosphate buffer.

Table IV. Products from the Reaction of CCNU (1×10^{-3} M) in pH 7.4 Phosphate Buffer at 37 °C after 2 h

component	% recovered
2-chloroethanol	18–25[a]
acetaldehyde	5–10[a]
cyclohexylamine	32[a]
DCU	1
CCU	3–5
2-(cyclohexylamino)-2-oxazoline	3–5
CCNU unreacted	12–17

[a] Calculated from the results of Reed et al. (ref 17).

The cleavage reaction is the major BCNU pathway under these conditions. All of the major products of this reaction, 2-chloroethanol, acetaldehyde, 2-oxazolidone, and 2-chloroethylamine, are toxic substances.[24] 2-Oxazolidone displays delayed toxicity.[24]

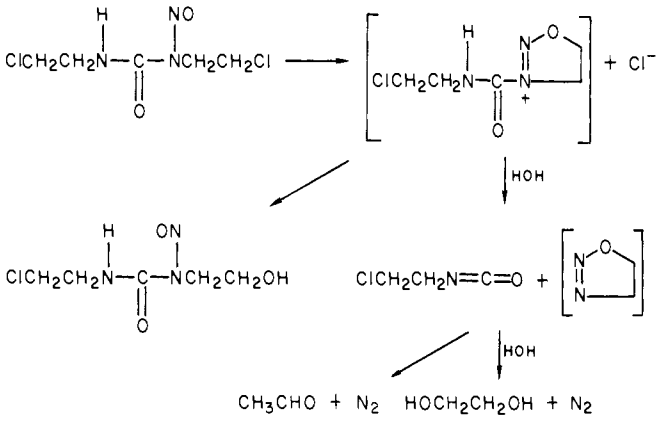
2-Chloroethyl isocyanate can also react with 2-chloroethylamine to give BCU (Scheme II). Reaction of a BCNU/BCNU-d_8 mixture will give BCU with the same isotope abundance as starting BCNU if it is formed by denitrosation or as a BCU-d_0, -d_4, -d_8 mixture if formed from a combination of 2-chloroethyl isocyanate and 2-chloroethylamine. The BCU isotope abundances are equal to the amounts calculated for combination, indicating that this is the only route of formation (Table II).

Urea formation is in competition with the isocyanate–water reaction of Scheme II, such that the amount of urea formed is related in a complex manner to the initial concentrations of BCNU. The yield of BCU is less than 10% if the initial BCNU concentration is below 10^{-3} M at physiological pH and temperature. BCU undergoes cycloelimination of chloride ion to give CAO.[25] The half-time of this reaction is approximately 1 h. Solvolysis of BCU gives HCU by a competitive reaction. HCU will also cyclize to give HAO.

This scheme is also supported by the isotope abundance data of Table II. HAO has the same isotopic composition as BCU, indicating that it is formed from this compound rather than by hydrolysis of CAO. The secondary reactions of BCU are slow relative to the rate of BCNU decomposition. These reaction products are important only when high concentrations ($>10^{-3}$ M) of BCNU are reacted for several hours. The toxicity of CAO is not known, but other substituted 2-aminooxazolines are highly toxic.[24]

CAO may be formed in relatively high yield (20% of recovered products) from reaction of 10^{-3} M BCNU (Table I). Reactions of more dilute BCNU/BCNU-d_8 mixtures (Table III) gave CAO/CAO-d_8 with little CAO-d_4, indicating that CAO may be formed directly from BCNU. CAO is also formed by cyclization of BCU as discussed above. The amount of CAO formed directly from BCNU

Scheme IV

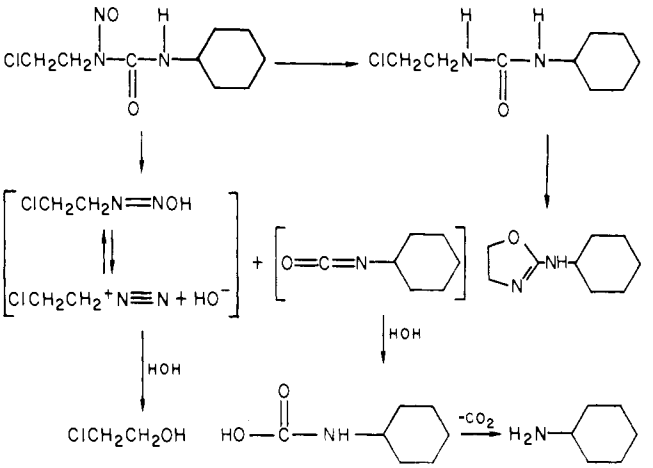


is 3–6% in 2 h at pH 7.4, 37 °C. Scheme III shows a possible mechanism for this reaction. Nitrite ion, another product of this reaction, was found to be present in 1.5% yield after 2 h when analyzed using the Bratton–Marshal method.[26] All other BCNU reaction products are derived from the initial formation of azohydroxide and isocyanate intermediates.

Recent reports indicate that the *N*-nitroso group of dialkylnitrosoamines may act as a nucleophile and displace β substituents in a cyclization reaction.[27] An analogous mechanism has been proposed for 2-chloroethylnitrosourea degradation to 1,2,3-oxadiazolidine in order to explain the pH dependence of acetaldehyde formation during these reactions[13] (Scheme IV). Substituted 1,2,3-oxadiazolidines are proposed intermediates in the reaction of aldehydes with diazomethane to give aldehyde, ketone, and epoxide products.[28] 1,2,3-Oxadiazolidine, by analogy, would give acetaldehyde and ethylene oxide, which reacts rapidly in aqueous solution in the presence of trace acid to give ethylene glycol. Ethylene glycol and 1-(2-hydroxyethyl)-2-(2-chloroethyl)nitrosourea could not be detected as products of BCNU decomposition using mass spectrometric and GC–MS methods with sensitivities capable of detecting 1% yields. Stable isotope labeling experiments by Brundrett et al.[5] are consistent with the formation of acetaldehyde by the dissociation of 2-chloroethyl azohydroxide to chloroethylcarbocation. This dissociation would be favored in acid solution and increase the amount of acetaldehyde formed, which is consistent with the kinetic measurements of Chatterji et al.[13] and do not require the existence of 1,2,3-oxadiazolidine[13] or vinylcarbonium ions.[15] The electron-withdrawing effect of the *N*-acyl group may decrease the nucleophilicity of the nitroso group and raise the energy of the positively charged intermediate formed from chloride ion displacement. These forces and the poor chloride leaving group may combine to inhibit this reaction pathway.

CCNU reactions in aqueous buffer occur primarily through the initial cleavage to chloroethyl azohydroxide and cyclohexylamine intermediates (Scheme V). These intermediates decompose to 2-chloroethanol, acetaldehyde, and cyclohexylamine as previously described.[17] 2-Oxazolidone cannot be formed from CCNU. Cyclohexylamine can react with cyclohexyl isocyanate to give DCU if the initial CCNU concentration is above 10^{-3} M. A surprisingly high amount of CCU (11–25% recovered products) and its cyclization product, 2-(cyclohexylamino)-2-oxazoline, was formed during this reaction. This is not analogous to BCNU reactions, as no BCU is formed directly from BCNU. Less than 0.8% nitrite is generated during the CCNU reaction, so that simple denitrosation does not appear to be the mechanism of formation.

Scheme V



Experimental Section

BCNU-d_8 labeled in the methylene positions was obtained from the Drug Development Branch, Division of Cancer Treatment, National Cancer Institute, through a contract with SRI International, Menlo Park, CA 94025. Proton magnetic resonance (^{1}H NMR) spectra were obtained on Varian A-60 and XL-100 spectrometers. Mass spectra were performed using a Finnigan 3200 mass spectrometer operated in the chemical-ionization mode with isobutane reagent gas. Selected ion monitoring utilized a Data General 830 computer and programs to control the mass spectrometer and analyze data.[29] Gas chromatography was performed on a Varian 2100 chromatograph with flame-ionization detection. High-performance liquid chromatography utilized an Altex pump with a Waters C_{18}-μBondapak column. Column effluent was monitored at 205 nm using an Hitachi 100-10 spectrophotometer. ^{1}H NMR data are reported as parts per million downfield from Me_4Si = 0. CIMS data are reported as the nominal mass m/e. Relative intensely values include ^{13}C and ^{37}Cl isotope peaks.

Reaction Conditions. BCNU (CCNU) in 0.2 mL of ethanol was dissolved in 10 mL of 0.07 M, pH 7.4, phosphate buffer to give 1.0×10^{-2}–1.0×10^{-4} M solutions. Reactions were carried out at 37 °C in septum capped tubes. At the end of the reaction period, a 2.0-mL aliquot was removed and analyzed for 2-chloroethanol using GC. The remaining reaction mixture was extracted with 2 × 8 mL of ether containing a known amount of BCNU-d_8. The ether solution was analyzed for unreacted BCNU using the direct-insertion CIMS method previously described.[29] A known amount of CCU was added to the aqueous solution, and the mixture was lyophilized. The residue was analyzed for nonvolatile products. 2-Chloroethylamine and CAO, which are protonated at pH 7.4, and other nonvolatile products were analyzed using direct mixture analysis techniques that employ chemical-ionization mass spectrometric ion summation methods[19,20] and high-performance LC.

2-Chloroethanol was analyzed by GC on a 2% KOH–2% Carbowax (100:120) Gas-chrom Q column at 70 °C. An aliquot of the above reaction mixture was mixed with a known amount of cyclohexanol or an internal standard and extracted with ether. The ether solutions may be injected onto the column. Unreacted BCNU does not interfere with this assay at the column temperature used.

Ureas were analyzed with high-performance LC on a C_{18}-μBondapak column with 40% acetonitrile in water as solvent. The ureas, CCU, and 1,3-dicyclohexylurea were analyzed using BCU as a standard.

1,3-Bis(2-chloroethyl)urea (BCU). 2-Chloroethyl isocyanate (1.97 g, 18.7 mmol) was added to a filtered solution of 2-chloroethylamine hydrochloride (2.12 g, 18.2 mmol) and potassium hydroxide (1.24 g) in 5 mL of 2-propanol. The solution was stirred for 2 h at room temperature and the solvent was evaporated under reduced pressure. The solid residue was washed with water and crystallized from methanol–ether (1:1, v/v) to give 3.4 g (18.3 mmol) of BCU: mp 126.5–127.5 °C, lit.[30] 126.5–128 °C; ^{1}H NMR (Me_2SO-d_6) 3.3 (CH_2N), 3.5 (CH_2Cl), 6.1–6.6 ppm (NH); CIMS

(isobutane, 150 °C) m/e (relative intensity) 185 (100), 149 (6).

1-(2-Chloroethyl)-3-cyclohexylurea (CCU) was prepared from cyclohexyl isocyanate and 2-chloroethylamine hydrochloride using the above procedure: mp 120–121 °C, lit.[31] 130–132 °C; ^{1}H NMR (Me_2SO-d_6) 3.3 (CH_2N), 3.6 (CH_2Cl), 6.6 (NH) (NCH), 0.9–1.9 ppm (C_6H_{10}); CIMS (isobutane, 150 °C) m/e (relative intensity) 205 (100), 169 (6).

1,3-Bis(2-hydroxyethyl)urea (BHU) was prepared from 2-aminoethanol and 2-oxazolidone according to Brundrett et al.:[5] mp 86–88 °C, lit.[32] 86 °C; ^{1}H NMR (Me_2SO-d_6) 3.1 (CH_2O), 3.4 (CH_2N), 4.7 (OH), 6.1 ppm (NH); CIMS (isobutane, 150 °C) m/e (relative intensity) 149 (100), 131 (2), 80 (3), 62 (15).

1-(2-Chloroethyl)-3-(2-hydroxyethyl)urea (CHU) was prepared from 2-aminoethanol and 2-chloroethyl isocyanate using the above procedure: mp 83–85 °C; ^{1}H NMR (Me_2SO-d_6) 3.1 (CH_2O), 3.3–3.5 (CH_2N), 3.5 (CH_2Cl), 6.3 ppm (NH); CIMS (isobutane, 150 °C) m/e (relative intensity) 167 (100), 149 (1), 131 (8), 80 (3), 62 (15).

2-[(2-Chloroethyl)amino]-2-oxazoline (CAO) was prepared in 50% yield from BCU following the method of Kreling and McKay:[25] mp 103–104 and 118–120 °C, lit.[25] 103–104 and 121–123 °C; ^{1}H NMR (Me_2SO-d_6) 4.85 (CH_2N), 39 (CH_2O), 3.6 ($NHCH_2CH_2Cl$), 6.6 ppm (NH); CIMS (isobutane, 150 °C) m/e (relative intensity) 149 (100), 113 (6), 86 (2). The CIMS spectrum of the hydrochloride salt was the same as observed for BCU, suggesting that ring opening occurred. The free base was prepared by stirring CAO·HCl with AG1-10X (hydroxide form) ion-exchange resin in methanol for 5 h. The resin was filtered and washed with methanol, and the combined methanol fractions were evaporated to give the free base in 18% yield: mp 82–84 °C; ^{1}H NMR ($CDCl_3$) 4.85 (CH_2N), 3.9 (CH_2O), 3.6 ($NHCH_2CH_2Cl$), 6.5 (NH).

2-(Cyclohexylamino)-2-oxazoline was prepared from CCU (0.48 g, 21 mmol) by refluxing in 5 mL of water for 90 min. The hot reaction mixture was filtered and the pH adjusted to 9.0 using 2.5% NH_4OH. This solution was extracted with ethyl acetate and evaporated to give 0.18 g of product: yield 38%; mp 127–128 °C, lit.[31] 131 °C.

Conclusion

These studies show that BCNU reacts in aqueous solutions at physiological temperature and pH to yield a variety of products. The majority of these products follow from the initial cleavage of BCNU to yield reactive alkylating and carbamoylating agents. These intermediates react in biological systems to initiate antitumor and toxic effects. It is significant to note, however, that virtually all of the stable reaction products are known to be toxic or are structurally related to toxic substances. These products will contribute to the toxic manifestations of chloroethylnitrosoureas to the extent that they are formed in vivo. The ease with which formation of the aminooxazoline ring may be reversed by chloride ion[25] suggests that this product may covalently bind to more nucleophilic functions present in biological systems.

Reactions of BCNU in aqueous buffer occur predominantly through cleavage to azohydroxide–diazonium ion and isocyanate intermediates. A minor competitive reaction leads to formation of 2-(2-chloroethyl)-2-oxazoline. Competitive pathways that occur from a common intermediate are subject to changes from conditions that may produce only minor alterations in the activation energy of the reactions. It is known that the stability of BCNU in serum ($t_{1/2}$ = 11–17 min) is less than that observed in aqueous solution ($t_{1/2}$ = 51 min in Ringer's solution).[33] The fact that the half-time for the elimination constant from patient plasma (21.5 min) is similar to the half-time for in vitro disappearance of BCNU from patient serum (15.6 min)[33] implies that the serum reaction has a major influence on the fate of BCNU in vivo. The chemical reactions that occur under these conditions are not necessarily the same as those observed in aqueous solutions. Serum-catalyzed reactions of BCNU are currently under investigation.

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