Chemistry of Nitrosoureas. Intermediacy of 4,5-Dihydro-1,2,3-oxadiazole in 1,3-Bis(2-chloroethyl)-1-nitrosourea Decomposition

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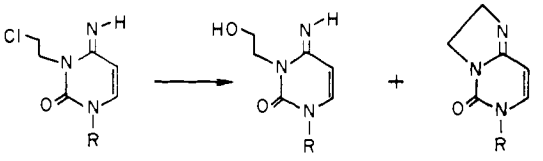
A new product, ethylene glycol, was identified from BCNU [1,3-bis(2-chloroethyl)-1-nitrosourea] decomposition. The variation of ethylene glycol yield with pH indicates that there are two competing mechanisms of decomposition. At pH 7.4 the decomposition is predominantly through 2-chloroethyldiazohydroxide, and chloroethanol and acetaldehyde are the major products. At pH 5, the decomposition is predominantly through 4,5-dihydro-1,2,3-oxadiazole and 2-hydroxyethyldiazohydroxide, and ethylene glycol and acetaldehyde are the major products. Deuterium labeling shows that at both pH's the acetaldehyde arises through a mechanism involving a hydride shift. At pH 5 in the presence of bromide, 2-bromoethanol is a major product and deuterium labeling shows that the hydroxyl is predominantly on the carbon which bore the chlorine in BCNU.

The 2-haloethylnitrosoureas are a class of highly active antitumor agents, and some of them [e.g., BCNU [1,3-bis(2-chloroethyl)-1-nitrosourea] and CCNU [1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea]] are currently in clinical use. It is believed that the cytotoxicity of these compounds is due to their decomposition to a diazotate which alkylates DNA with 2-haloethyl groups.[1-3] The attached 2-haloethyl groups are alkylating and can then cross-link the DNA.[4] The chemistry of the haloethylnitrosoureas is, however, complex, and there are a number of questions which have not yet been definitely answered.

Kramer et al. have shown that BCNU reacts with poly(cytidylic acid) to form both ethano and 2-hydroxyethyl derivatives and proposed that these arose from a common 2-chloroethyl intermediate (Scheme I).[5] However, it was later found that, although BFNU [1,3-bis(2-fluoroethyl)-1-nitrosourea] reacts with cytidine to form both the hydroxyethyl and ethano derivatives as well as the 2-fluoroethyl intermediate, the 2-fluoroethyl intermediate formed only the ethano derivative on further incubation.[6] To explain the 2-hydroxyethyl product, Tong et al. have proposed 4,5-dihydro-1,2,3-oxadiazole as an intermediate in the decomposition of the haloethylnitrosoureas.[7] This intermediate has also been proposed by other workers.[8,9] In this note, we present more experimental evidence supporting the oxadiazole intermediate.

Scheme I



Experimental Section

BCNU was provided by Dr. Harry Wood of the Drug Development Branch of the National Cancer Institute. DMCNU [1-(2-chloroethyl)-3,3-dimethyl-1-nitrosourea],[10] BCNU-α-d_4 [1,3-bis(2-chloro-1,1-dideuterioethyl)-1-nitrosourea], and BCNU-β-d_4 [1,3-bis(2-chloro-2,2-dideuterioethyl)-1-nitrosourea][11] were described previously. Gas chromatography was performed on a Varian 2400 instrument with flame-ionization detectors. High-performance LC was carried out on a Waters Associates 244 W/D using a R401 differential refractometer detector. Electron-impact mass spectrometry was performed on a DuPont 491 instrument equipped with an INCOS data system, using probe insertion of the sample.

1,3-Bis(2-hydroxyethyl)-1-nitrosourea (BHNU). 1,3-Bis(2-hydroxyethyl)urea (600 mg) was nitrosated at room temperature in CH_2Cl_2 (30 mL) with NaOAc (650 mg) and N_2O_4 (200 mL of gas). After stirring for 1 h, the mixture was neutralized with aqueous $NaHCO_3$ and extracted three times with CH_2Cl_2. Drying, evaporation, and chromatography (CH_2Cl_2, SiO_2) gave a yellow oil: MS, m/e 178 ($M^+ + 1$), 117 ($HOCH_2CH_2NHCO$).

Product Determinations. Nitrosourea (0.05 M) was incubated at 37 °C in sodium phosphate buffer (0.1 M, pH 5.0 or 7.4) for 4 days in a sealed vial. Where indicated, 2 M potassium bromide was included in the buffer.

Acetaldehyde was quantitated by gas chromatography using a 10 ft Chromosorb 101 column at 120 °C. Chloroethanol, ethylene glycol, and bromoethanol were quantitated by high-performance LC using a Waters C_{18} μ-Bondapak column with water as eluant. Bromoethanol and ethylene glycol were confirmed by GC at 70 °C using a 6 ft, 0.4% Carbowax 1500 on Carbopack A column.

Deuterium Distribution in Acetaldehyde. A slow stream of nitrogen was bubbled through a mixture of BCNU-β-d_4 (55 mg) and 0.1 M sodium phosphate buffer (pH 7.4 or 5, 5 mL) incubated at 37 °C. The nitrogen stream was then passed through a saturated solution of 2,4-dinitrophenylhydrazine in 2 N HCl (10 mL). After 12 h, the precipitated hydrazone was collected, washed with distilled water, dried, and analyzed by MS and NMR. The proton NMR spectra were recorded on a JEOL FX-100 instrument and the deuterium NMR spectra on a home-built 270-MHz spectrometer.

Deuterium Distribution in Bromoethanol. BCNU-α-d_4 or BCNU-β-d_4 (0.1 M) was incubated at 37 °C for 4 days in sodium phosphate buffer (0.2 M, in D_2O pH 5.0) containing potassium bromide (2 M). The 300-MHz proton FT NMR spectra of the two reaction mixtures were recorded on a Bruker WM-300 spectrometer. Peaks were identified by comparison of chemical shifts relative to internal TSP to authentic material in the same buffer. The expected β-deuterium isotope effects on chemical shift were seen.

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Table I. Product Yields from the Decomposition of BCNU, BHNU, and DMCNU

nitrosourea	conditions: pH	conditions: 2 M KBr	products[a]: acetaldehyde	products[a]: ethylene glycol	products[a]: chloroethanol	products[a]: bromoethanol
BCNU	7.4	-	19	2	51	
			21	2	53	
BCNU	5.0	-	58	22	6	
			59	25	6	
BHNU	7.4	-	36	25		
			31	22		
DMCNU	7.4	-	33	13	5	
			30	14	6	
BCNU	7.4	+	18	b	54	4
			19		63	4
BCNU	5.0	+	57	b	5	30
			51		5	33

[a] Mole percent of nitrosourea reacted; values from two independent runs are given. [b] Interference from KBr peak prevented determination of ethylene glycol.

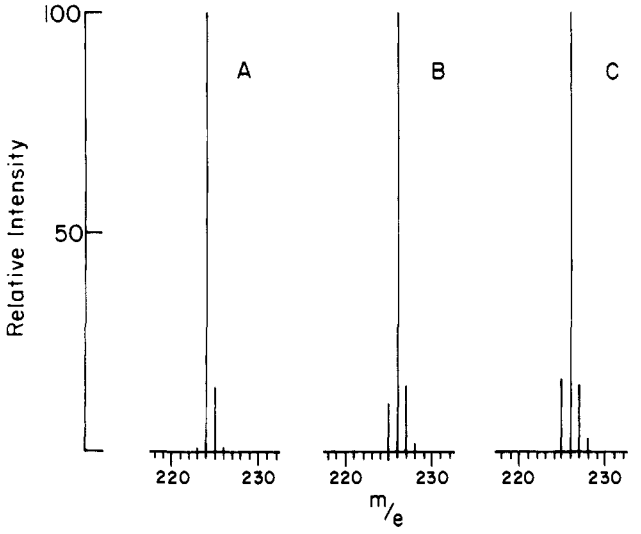


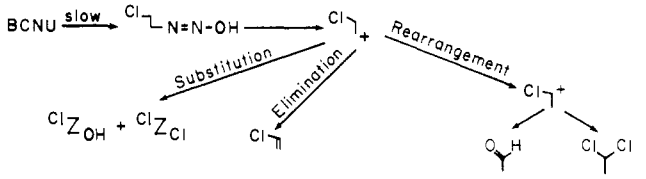
Figure 1. Mass spectra of acetaldehyde dinitrophenylhydrazones: A, acetaldehyde from BCNU; B, acetaldehyde from BCNU-β-d_4 decomposed at pH 7.4; C, acetaldehyde from BCNU-β-d_4 decomposed at pH 5.0.

Results

It was previously shown that BCNU-β-d_4 decomposed at pH 7.4 to acetaldehyde which contained one deuterium.[11] This was done under conditions in which the α-hydrogens (or deuteriums) of the acetaldehyde exchanged with the solvent (H_2O). This experiment has been repeated with the acetaldehyde being removed as it is formed by a stream of nitrogen and trapped as the 2,4-dinitrophenylhydrazone. Mass spectrometry of the acetaldehyde DNP from BCNU-β-d_4 decomposed at both pH 5 and 7.4 shows approximately 90% contained 2 deuteriums (Figure 1). The proton NMR of the hydrazones generated at both pH's shows no signal at δ 7.64 (methine proton) and a triplet (J = 2.5 Hz) at δ 2.16 (methyl group). The deviation of the δ 2.16 signal from a two-hydrogen, equal-intensity triplet (expected for a CH_2D group) indicates a small (~10%) contribution from CH_3. The deuterium NMR shows the methyl signal to be about 10% smaller than the methine signal. Thus, 90% of the acetaldehyde generated at both pH's contains two deuteriums, one in the aldehyde group and one in the methyl group.

BCNU, BHNU, and DMCNU were decomposed in buffers, and the amount of acetaldehyde, ethylene glycol, and chloroethanol was quantitated. The results are shown in Table I. BCNU at pH 7.4 gives acetaldehyde and chloroethanol as the major products and only a few percent ethylene glycol. At pH 5, little chloroethanol is formed, and the major products are acetaldehyde and ethylene glycol. Acetaldehyde and ethylene glycol are also the major products produced from both BHNU and DMCNU at pH 7.4.

Scheme II



Inclusion of 2 M potassium bromide in the pH 7.4 buffer has little effect on the decomposition products (Table I). Bromoethanol is only a minor product at this pH. However, when 2 M potassium bromide is included in the pH 5.0 buffer, bromoethanol is a major product. Proton NMR of the BCNU-β-d_4 reaction mixture in D_2O shows the bromoethanol signals to be singlets at δ 3.57 and 3.91. The ratio of the area of the δ 3.57 singlet (corresponding to protons in the CH_2Br group of 2-bromo-1,1-dideuterioethanol) to the δ 3.91 singlet (corresponding to protons in the CH_2OD group of 2-bromo-2,2-dideuterioethanol) is 2 to 1. The ratio of the bromoethanol peaks from BCNU-α-d_4 is 1 to 2.

Discussion

The fact that BCNU-β-d_4 at both pH 7.4 and 5 gives acetaldehyde with 2 deuteriums, one on the aldehydic carbon and one on the methyl carbon, is direct evidence that this product arises via a hydride shift. These data are consistent with the mechanism we previously proposed,[10,11] which is summarized in Scheme II. Although the eliminations, substitutions, and rearrangements probably occur concerted with the loss of nitrogen from the diazotate,[12] the reactions are shown as going through a free carbonium ion, as this is easier to visualize. At least some of the acetaldehyde at pH 7.4 comes via this mechanism, since the rearranged 1-chloroethyl carbonium ion can be trapped by chloride to give 1,1-dichloroethane.[10] However, a mechanism involving 4,5-dihydro-1,2,3-oxadiazole can be drawn which also gives acetaldehyde by a rearrangement (path D, Scheme III). The mechanisms proposed by Chatterji[8] to yield acetaldehyde from the oxadiazole do not give the right deuterium distribution.

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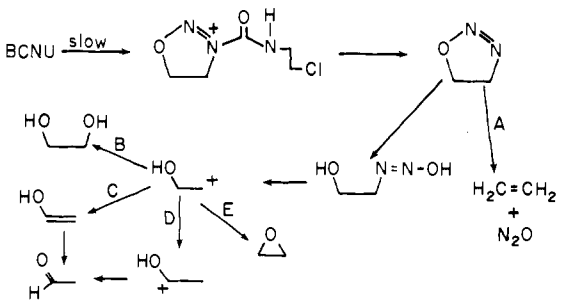
Scheme III



4,5-Dihydro-1,2,3-oxadiazole is, at present, an unknown compound. However, the 3-methyl derivative (formed by cyclization of β-tosylethylmethylnitrosoamine)[13] and the 2-oxo derivative [formed by cyclization of *N*-(2-bromoethyl)-*N*-nitrotosylamide][14] are known and support the proposed cyclization of the 2-chloroethylnitrosourea to give, after loss of alkyl isocyanate, the oxadiazole. It seems unlikely the cyclization could compete with loss of nitrogen once the diazotate is formed. Scheme III shows some of the reactions one might postulate for 4,5-dihydro-1,2,3-oxadiazole. The 3-methyl and 2-oxo derivatives have a positive charge on the nitrogens which should stabilize the N-O bond to heterolytic cleavage and, thus, these compounds are probably not good models for the chemistry of the parent compound. Path A involves an electrocyclic reaction to form ethylene and nitrous oxide, which is analogous to the decomposition of the isomeric *N*-nitrosoaziridine to ethylene.[15] Reed has reported that ethylene is a minor product of CCNU decomposition.[2] The other paths involve an initial hydrolysis of the N-O bond to form 2-hydroxyethyldiazohydroxide, which then undergoes reaction analogous to those proposed for 2-chloroethyldiazohydroxide. Substitution by hydroxide (path B) to yield ethylene glycol is analogous to the formation of chloroethanol. Elimination (path C) to form the enol of acetaldehyde is analogous to the formation of vinyl chloride. Hydride shift (path D) ultimately yields acetaldehyde from both diazotates. Cyclization (path E) to ethylene oxide is analogous to the formation of the chloronium ion[11] in BCNU decomposition. Ethylene oxide should react with water (or other nucleophiles) to give ethylene glycol (or other hydroxyethylated compounds).

2-Hydroxyethyldiazohydroxide should also be an intermediate in the decomposition of BHNU. BHNU gives acetaldehyde and ethylene glycol as major products on decomposition at pH 7.4 (Table I). This fact supports the prediction that both acetaldehyde and ethylene glycol will be products of BCNU decomposition if the oxadiazole is an intermediate.

The decomposition of BCNU at pH 7.4 gives only a 2% yield of ethylene glycol (control experiments show that chloroethanol does not hydrolyze to ethylene glycol at pH 7.4 or 5.0). This indicates that oxadiazole formation is only a minor (~5%) side reaction and, therefore, that the acetaldehyde comes primarily via the rearranged 1-chloroethyl carbonium ion at this pH. At pH 5, however, ethylene glycol is a major product (25%). This indicates that most of the reaction is going through the oxadiazole intermediate and, therefore, that the acetaldehyde comes primarily via the rearranged 1-hydroxyethyl carbonium ion (protonated acetaldehyde) at this pH.

This shift in decomposition mechanisms is probably due to the fact that the rate-limiting reaction to form 2-chloroethyldiazohydroxide (Scheme II) is base catalyzed and at lower pH's becomes slower than the cyclization (Scheme III). DMCNU at pH 7.4 also gives acetaldehyde and ethylene glycol. In this case, the lack of an N3 hydrogen blocks decomposition to 2-chloroethyldiazohydroxide. The difference in the acetaldehyde to ethylene glycol ratio between BCNU at pH 5 and DMCNU (2.5 to 1) and BHNU (1.4 to 1) is possibly due to differences in the conformation of the 2-hydroxyethyl diazotates generated from 4,5-dihydro-1,2,3-oxadiazole hydrolysis (diazo and hydroxyl groups initially cis) and nitrosourea decomposition (diazo and hydroxyl groups initially trans).

Further evidence supporting the intermediacy of the oxadiazole at pH 5.0 comes from the decomposition of BCNU in the presence of a trapping agent, bromide. When BCNU is decomposed at pH 7.4 in the presence of bromide, 2-bromoethanol is only a minor product, a result which agrees with the previous work of others.[9] Under these conditions, 1-bromo-2-chloroethane should also be a product,[9] but it was not looked for in this experiment. At pH 5.0 in the presence of bromide, however, 2-bromoethanol becomes a major product. That BCNU-β-d_4 gives 2-bromo-1,1-dideuterioethanol and 2-bromo-2,2-dideuterioethanol in a 2 to 1 ratio indicates that two-thirds of the bromoethanol arises through the opening of ethylene oxide by HBr to give approximately equal amounts of the two isomers and one-third arises through the reaction of bromide with the 2-hydroxy-2,2-dideuterioethyl diazotate (or carbonium ion). That BCNU-β-d_4 gives the reverse ratio shows that the product ratio is not due to a deuterium isotope effect on the opening of ethylene oxide.

The production of ethylene glycol as well as the production of 2-bromoethanol in the presence of bromide during BCNU decomposition at pH 5.0 support the idea that the oxadiazole can yield alkylating species (2-hydroxyethyl diazotate, 2-hydroxyethyl carbonium ion, and/or ethylene oxide) capable of delivering 2-hydroxyethyl groups. This intermediate can thus explain the 2-hydroxyethyl nucleotides found by Tong and Ludlum.[6,7] They carried out the BCNU-nucleotide reactions at pH 6.0 which probably increased production of the hydroxyethyl derivatives over what would occur at pH 7.4. Thus, 4,5-dihydro-1,2,3-oxadiazole plays only a minor role in the decomposition of BCNU at pH 7.4 and probably has no antitumor effect, since DMCNU, which decomposes via this mechanism at pH 7.4, is not cytotoxic to cells.

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