# DECOMPOSITION OF BCNU (1,3-BIS(2-CHLOROETHYL)-1-NITROSOUREA) IN AQUEOUS SOLUTION

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Received July 26,1974

SUMMARY. BCNU, labelled with $^{14}C$ in the chloroethyl groups, decomposes in neutral aqueous solution to release half of its radioactivity as volatile products. These have been identified by a combination of gas chromatography and mass spectrometry as vinyl chloride, acetaldehyde, dichloroethane, and chloroethanol. This set of products is consistent with the existence of chloroethylcarbonium ions as reactive intermediates which would produce the previously described substituted nucleotides.

BCNU (1,3-Bis(2-chloroethyl)-1-nitrosourea) and related compounds are useful agents for the treatment of lymphomas and other malignant diseases. Although they have a structural relationship to the typical alkylating agents, their pharmacological properties are different and their mechanism of action is not understood.

BCNU does, however, possess some alkylating activity, and we have found that it adds a two-carbon unit to certain nucleotides. Thus, we have identified 3-hydroxyethyl-CMP and 3,$N^4$-ethano-CMP in the acid hydrolysate of poly C treated with BCNU (1). This has led us to investigate the source of the two-carbon fragment. In this paper, we describe the spectrum of volatile products obtained when BCNU decomposes in water.

MATERIALS AND METHODS. Crystalline BCNU (NSC-409962) and BCNU labelled with $^{14}C$ in the chloroethyl groups (specific activity, 10 μCi/μmole) were obtained from Dr. Robert Engle, Drug Research and Development Branch, National Cancer Institute, Division of Cancer Treatment. All other reagents were obtained from standard sources.

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To determine the relative amount of volatile decomposition products from BCNU, a dilute solution of the $^{14}C$ labelled BCNU was incubated in pH 7 buffer under the conditions shown in Fig. 1. Aliquots were withdrawn at varying times, spotted on filter papers, air dried, and counted in a liquid scintillation counter.

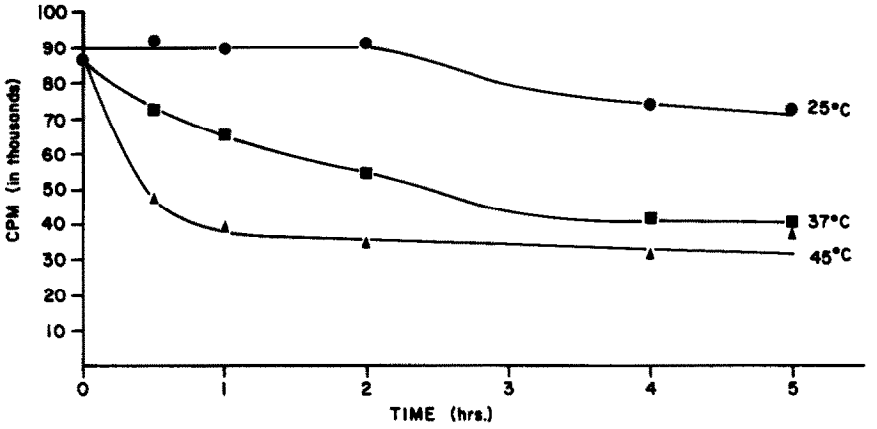


Figure 1. Decomposition of BCNU. 30 µg of $^{14}C$ labelled BCNU, 3.5 µCi/µmole, dissolved in 25 µl of ethanol was added to 0.475 ml of 0.05 M Na cacodylate buffer, pH 7, containing 0.1 M NaCl. Aliquots (50 µl) were withdrawn at the indicated time points and analysed as described in the text.

The volatile materials formed from the decomposition of BCNU were trapped as follows: One hundred milligrams of BCNU suspended in 5-10 mls. of pH 7.4 phosphate buffer were allowed to decompose at 37°C. A slow stream of nitrogen was bubbled through the incubation mixture and then into a small amount of ether maintained at -70°C in a dry ice-isopropanol bath.

In other experiments, the BCNU suspended in the aqueous buffer was allowed to decompose completely, as measured by a colorimetric technique (2), in a gas tight vial fitted with a Teflon-lined rubber septum. After complete decomposition of the BCNU, ether was injected into the vial, the phases were mixed thoroughly, and both the ethereal and aqueous phases were analysed by gas chromatography (GC).

Deamination of 2-chloroethylamine was carried out in a two phase system

of 0.5 M hydrochloric acid and ether by the addition of solid sodium nitrite. After the vigorous evolution of nitrogen had ceased, both the aqueous and ethereal phases were analysed by GC.

GC was carried out in a Varian 2700 instrument fitted with a flame ionization detector. A ten foot Chromosorb 101 column (with no liquid stationary phase) was used isothermally at 150°. Mass spectral analyses were carried out on a DuPont 21/491 GC/MS equipped with an all glass single stage jet separator using the GC conditions cited above.

RESULTS AND DISCUSSION. As shown in the experiment illustrated in Fig. 1, the aqueous decomposition of BCNU produces volatile products. After two hours of incubation, almost 50% of the radioactivity was present in materials which evaporated from the paper.

Direct injection of BCNU onto the Chromosorb 101 column resulted in thermal decomposition and gave a number of peaks on the chromatogram. Therefore, an apparatus was set up, as described above, to trap the volatile materials produced during the decomposition of the BCNU. Aliquots of the ether trap solution were then injected onto the Chromosorb 101 column (Fig. 2). By comparison of GC retention times to those of authentic compounds and by combined gas chromatography-mass spectrometry, we identified vinyl chloride, acetaldehyde, and 1,2-dichloroethane (Table 1).

In another type of experiment, BCNU was allowed to decompose completely in a gas tight vial. The reaction mixture was then extracted with ether injected directly into the vial, and both the ethereal and aqueous phases were analysed. In addition to the volatile products described above, a new peak (Peak 6 in Fig. 2) was present in both phases. By GC retention time and mass spectrum, this peak was identified as chloroethanol. The relatively high boiling point of chloroethanol (129°) would be consistent with its failure to be carried into the ether trap in the first type of experiment, and with its long retention time on the gas chromatographic column. In these experiments, the sum of the four volatile products is at least 0.5 moles for each mole of BCNU decomposed.

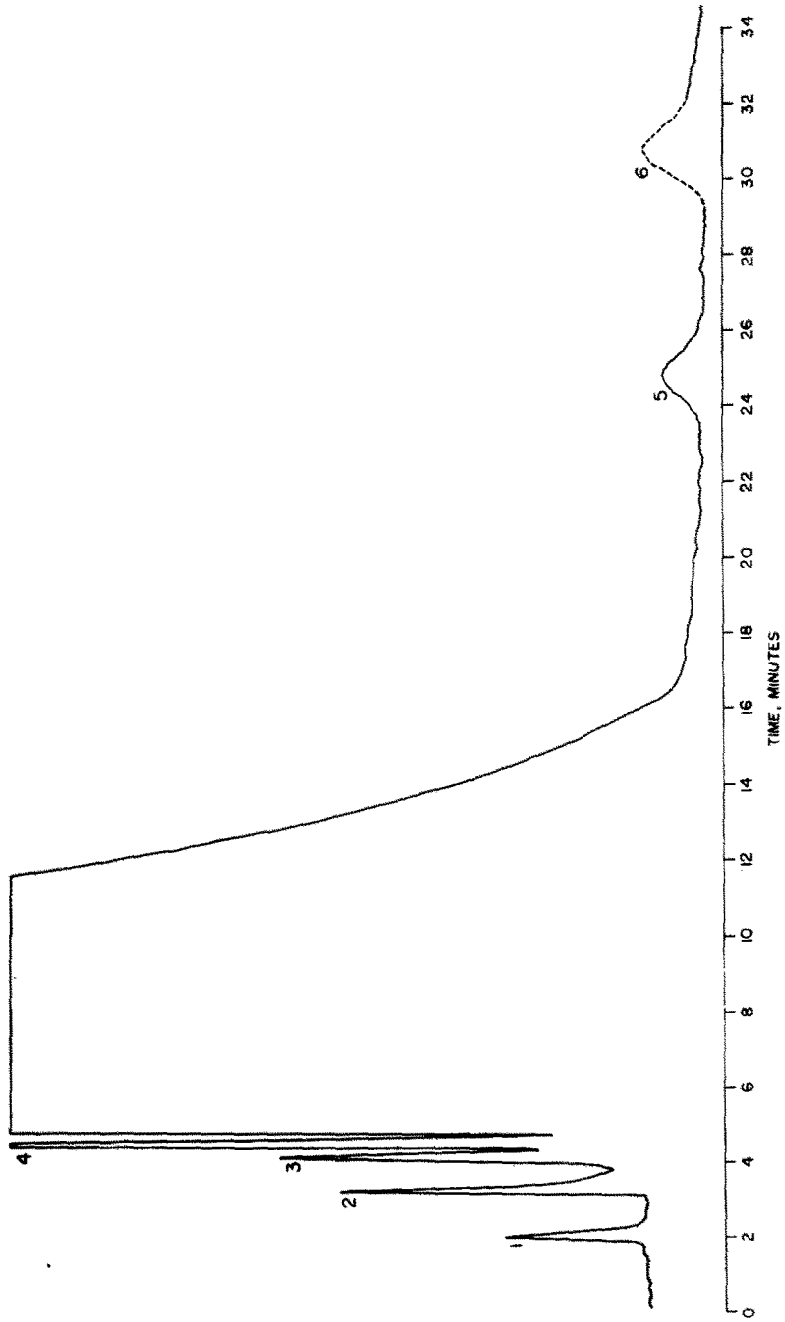


Figure 2. Gas chromatography of products on Chromosorb 101. Peaks 3, 4, 5, and 6 are vinyl chloride, acetaldehyde, dichloroethane, and chloroethanol, respectively. The very broad peak is the ether solvent, peak 2 is $H_2O$, and peak 1 has not been identified. Peak 6 was not present in the ether trap solution (see text).

TABLE 1

Mass Spectral Data on Peaks in Figure 1

| Peak | Identification | m/e [Relative Intensity (%)] |
|---|---|---|
| 3 | Vinyl Chloride | 62(100, 1Cl, $M^+$); 27(44, M-35) |
| 4 | Acetaldehyde | 44(85, $M^+$); 43(55, M-1); 29(100, M-15) |
| 5 | Dichloroethane | 98(11, 2Cl, $M^+$); 62(82, 1Cl, M-36); 49(43, 1Cl, M-48); 27(100, M-71) |
| 6 | Chloroethanol | 80(3, 1Cl, $M^+$); 31(100, M-49) |

In a previous study, Montgomery, *et al* (3) reported acetaldehyde as the principle volatile product of the aqueous decomposition of BCNU, found only traces of chloroethanol, and did not mention vinyl chloride or dichloroethane. When these workers treated chloroethylamine with nitrous acid, no acetaldehyde was detected. These results were cited as evidence that chloroethane diazohydroxide was not produced by the aqueous decomposition of BCNU.

We reacted chloroethylamine with nitrous acid in aqueous solution and analysed the reaction mixture on the Chromosorb 101 column. Using this technique, acetaldehyde, vinyl chloride, dichloroethane, and chloroethanol were produced in the same relative amounts as from the aqueous decomposition of BCNU, as shown in Table 2. Since treatment of a primary amine with nitrous acid is a classical means of generating carbonium ions (4), we believe that the above products are being produced from a chloroethylcarbonium intermediate. The higher yield of dichloroethane from chloroethylamine is probably due to the high concentration of chloride ion in this reaction mixture.

We have interpreted the results of these experiments to indicate that a chloroethylcarbonium ion (or chloroethyldiazonium precursor) is generated by the aqueous decomposition of BCNU at physiologic pH and temperature. The production of this reactive moiety may well account for the alkylating activity

TABLE 2

Volatile Products Produced from the Decomposition of BCNU and Chloroethylamine*

| | BCNU | Chloroethylamine |
|---|---|---|
| Acetaldehyde | 31% | 18% |
| Dichloroethane | 2% | 13% |
| Chloroethanol | 63% | 66% |
| Vinyl Chloride | 4% | 3% |
| | 100% | 100% |

*For experimental details, see Materials and Methods. Values expressed as percent of total identified volatiles.

of BCNU. The reaction of this alkylating species with nucleic acids may play a significant role in the biologic effects of BCNU.

ACKNOWLEDGMENTS. We thank Drs. Catherine Fenselau and Ian Jardine of The Johns Hopkins University Mass Spectrometry Center for their assistance with the mass spectrometry. This work was supported by grants from the National Cancer Institute (CA 15696, CA 16299, CA 06973) and the Maryland Division of the American Cancer Society.

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