The Modes of Decomposition of 1,3-Bis(2-chloroethyl)-1-nitrosourea and Related Compounds[1]

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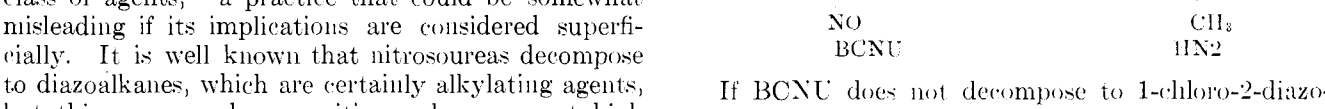
An investigation of the chemistry of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) and other 1,3-disubstituted 1-nitrosoureas has led to a better understanding of the reactions of this class of agents highly active against L1210 leukemia. Aqueous decomposition of 1,3-dimethyl-1-nitrosourea gave 1,3-dimethylurea, methanol, N_2, and CO_2. Reaction of molten BCNU with a limited amount of water at 50° gave the corresponding 2-chloroethanol and 1,3-bis(2-chloroethyl)urea, but reactions in aqueous solution resulted in anomalous decomposition that produced acetaldehyde, HCl, N_2, and derivatives of 2-chloroethyl isocyanate dependent on exact conditions used. No evidence for the formation of 1-chloro-2-diazoethane was noted under any conditions studied. Reaction rates of BCNU decompositions were determined in various aqueous media. Aqueous decomposition of 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea was also anomalous, but 1,3-bis(2-fluoroethyl)-1-nitrosourea decomposed in the "normal" manner, producing 2-fluoroethanol and 1,3-bis(2-fluoroethyl)urea. Half-lives of a number of nitrosoureas in nonaqueous media were also determined, and decompositions of BCNU and 1,3-dimethyl-1-nitrosourea in appropriate hydrocarbon solvents were studied in detail.

1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU) has been found to be highly effective in the treatment of leukemia L1210 in mice implanted either intraperitoneally or intracerebrally.[2] The drug can be administered with equal effectiveness intraperitoneally, intravenously, subcutaneously, or *per os*. Clinical trials, initiated because of the outstanding activity observed in animals, have been promising despite the occurrence of delayed toxicity.[3,4]

The similarity of the biological effects of BCNU and other nitrosoureas to those of the accepted biological *alkylating* agents has led to the inclusion of them in this class of agents,[5,6] a practice that could be somewhat misleading if its implications are considered superficially. It is well known that nitrosoureas decompose to diazoalkanes, which are certainly alkylating agents, but this aqueous decomposition only occurs at high pH[7] that could not possibly be attained *in vivo*. Furthermore, although BCNU contains two 2-chloroethyl groups and thus appears to resemble nitrogen mustard (HN2), several factors would indicate that BCNU does not alkylate through its β carbons: (1) the electron density at the urea nitrogens of BCNU is obviously lower than that of the amino nitrogen of HN2, and, therefore, BCNU almost certainly does not react *via* the cyclic ethylenimmonium ion as does HN2; (2) 1,3-bis(2-chloroethyl)urea is completely devoid of antileukemic activity showing the necessity for the nitroso rather than the chloro groups; (3) 1-methyl-1-nitrosourea, which has no 2-chloroethyl group, shows good antileukemia activity, and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea, which has only one 2-chloroethyl group, is as active, if not more active, than BCNU; and (4) 1-(2-fluoroethyl)-1-nitrosoureas are highly active against L1210 leukemia,[9] although it is well known that 2-fluoroethyl compounds are much less chemically reactive than 2-chloroethyl compounds.[10] In contrast, the less reactive fluoro analogs of certain nitrogen mustards show little, if any, antitumor activity,[11] presumably because of their inability to alkylate readily by displacement of the fluoro group under physiological conditions.



If BCNU does not decompose to 1-chloro-2-diazoethane under physiological conditions and does not alkylate through its β carbon, it either is not an *alkylating* agent in the chemical sense, or it must alkylate by some other mechanism. In order to provide the necessary chemistry for the elucidation of its biologic activity and for the preparation of the drug for clinical administration, a study of the stability and modes of decomposition of BCNU in various solutions was undertaken.

N-Methyl-N-nitrosourea is known to decompose in aqueous solution, on boiling, to give methanol, N_2, and isocyanic acid.[12] It decomposes on heating in inert solvents such as toluene to give a 30% yield of trimethyl isocyanurate, an unspecified amount of methyl isocyanate, and N_2.[13,14] Recent evidence concerning the mechanism of the reaction in toluene[15] indicates, however, that these reactions proceed through a common intermediate, methanediazohydroxide. The

(1) This work was supported by funds from the C. F. Kettering Foundation and the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Contract No. PH-43-64-51; presented in part at the 147th National Meeting of the American Chemical Society, Philadelphia, Pa., April 1964, Abstracts of Papers, p 27M; part XXXVI: T. P. Johnston, G. S. McCaleb, P. S. Opliger, and J. A. Montgomery, *J. Med. Chem.*, **9**, 892 (1966).

(2) F. M. Schabel, Jr., T. P. Johnston, G. S. McCaleb, J. A. Montgomery, W. R. Laster, and H. E. Skipper, *Cancer Res.*, **23**, 725 (1963).

(3) B. A. Nies, L. B. Thomas, and E. J. Freireich, *Cancer*, **18**, 546 (1965).

(4) V. T. DeVita, P. P. Carbone, A. H. Owens, Jr., G. L. Gold, M. J. Krant, and J. Edmonson, *Cancer Res.*, **25**, 1876 (1965).

(5) G. P. Wheeler, *ibid.*, **22**, 651 (1962).

(6) J. A. Montgomery, *Progr. Drug Res.*, **8**, 431 (1965).

(7) It has been suggested that this decomposition proceeds *via* the diazonium ion from which a proton is abstracted to give the diazoalkane.[8]

(8) H. Zollinger, "Azo and Diazo Chemistry," Interscience Publishers, Inc., New York, N. Y., 1961, p 44.

(9) See article cited in ref 1.

(10) P. L. Levins and Z. B. Papanastassiou, *J. Am. Chem. Soc.*, **87**, 826 (1965).

(11) Z. B. Papanastassiou, R. J. Bruni, F. P. Fernandes, and P. L. Levins, *J. Med. Chem.*, **9**, 357 (1966).

(12) J. L. Boivin and P. A. Boivin, *Can. J. Chem.*, **29**, 478 (1951).

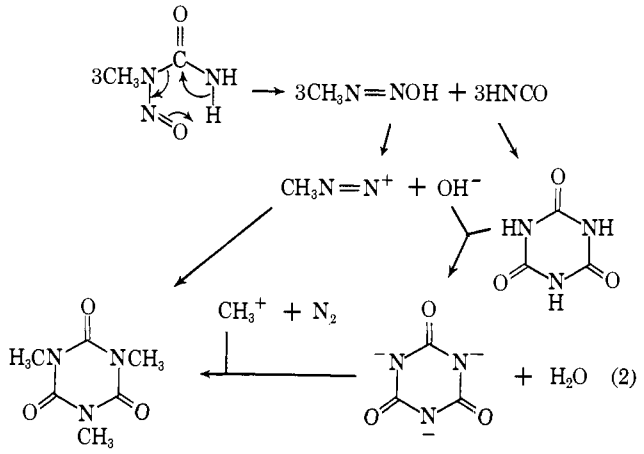
(13) E. A. Werner, *J. Chem. Soc.*, **115**, 1093 (1919); F. Arndt, L. Loewe, and S. Avan, *Ber.*, **73**, 606 (1940); R. Huisgen and H. Reimlinger, *Ann.*, **599**, 183 (1956); K. Clusius and F. Endtinger, *Helv. Chim. Acta*, **43**, 2063 (1960).

(14) Although not mentioned by any of the investigators listed in ref 13, it appears almost certain that another product of this reaction in inert solvents is methanol (*vide infra*).

(15) D. L. Muck and W. M. Jones, *J. Am. Chem. Soc.*, **88**, 74 (1965).

final products are determined by the nature of the solvent.[16] In water, methanol is formed presumably *via* the methyldiazonium ion and the methyl carbonium ion (eq 1), whereas in toluene the isocyanic acid trimerizes to cyanuric acid whose anion is methylated by the methyl carbonium ion (eq 2). Any methyl isocyanate formed results from the methylation of isocyanic acid prior to trimerization.[15]

$$CH_3N(NO)CONH_2 \longrightarrow CH_3N{=}NOH + HNCO$$
$$\downarrow$$
$$CH_3N{=}N^+ + OH^- \longrightarrow$$
$$N_2 + CH_3^+ \xrightarrow{H_2O} CH_3OH + H^+ \quad (1)$$



Results and Discussion

Boivin and Boivin claim to have observed the *transient* formation of methyl isocyanate, indicated by the identification of methylamine and CO_2, from the decomposition of 1,3-dimethyl-1-nitrosourea in boiling water. Nitrogen and methanol were also identified.[12] Previous work in this laboratory[2] has shown that the aqueous decomposition of certain other 1,3-disubstituted 1-nitrosoureas gives a symmetrical urea, an alcohol, N_2, and CO_2. Our results can be explained by assuming that the nitrosourea breaks down initially into a diazohydroxide and an isocyanate.[16] The diazohydroxide releases N_2 and a carbonium ion that reacts with H_2O to give an alcohol. One-half of the isocyanate undergoes hydrodecarboxylation to give an amine which reacts with the remaining one-half of the isocyanate to give a urea (eq 3). Repetition of Boivin

$$2RN(NO)CONHR' \longrightarrow 2RN{=}NOH + OCNR' + OCNR'$$
$$2ROH \longleftarrow 2R^+ + 2N_2 \longleftarrow 2RN{=}N^+ + 2OH^- \quad | \quad HO_2CNHR'$$
$$R'NHCONHR' \longleftarrow R'NH_2 + CO_2 \quad (3)$$

and Boivin's experiment[12] confirmed our earlier work[2]—an 81% yield of dimethylurea was obtained by boiling an aqueous solution of 1,3-dimethyl-1-nitrosourea.

(16) Since the rate of the reaction in water (eq 3) is dependent upon pH,[17] the formation of the diazohydroxide and isocyanic acid may result from attack of hydroxide ions on the nitrosourea, possibly on the nitroso group.[15] In benzene, proton abstraction is probably intramolecular as indicated in eq 4.[15]

(17) E. R. Garrett, S. Goto, and J. F. Stubbins, *J. Pharm. Sci.*, **54**, 119 (1965).

Under one particular set of conditions, heating at 50° with a limited quantity of water, BCNU decomposed in the "normal" manner to give 1,3-bis(2-chloroethyl)-urea, chloroethanol, N_2, and CO_2 (eq 4). Under

$$2ClCH_2CH_2N(NO)CONHCH_2CH_2Cl + H_2O \longrightarrow ClCH_2CH_2NHCONHCH_2CH_2Cl + ClCH_2CH_2OH + 2N_2 + CO_2 \quad (4)$$

all other conditions studied an anomalous decomposition occurred that produced acetaldehyde as one of the various products, this acetaldehyde production being associated with the loss of HCl from BCNU. The other products of the reaction varied depending on the conditions employed. In an aqueous suspension or dilute water solution at room temperature, the other product (besides N_2 and CO_2) was 2-chloroethylamine hydrochloride (eq 5). When

$$ClCH_2CH_2N(NO)CONHCH_2CH_2Cl \longrightarrow ClCH_2CH_2NH_2{\cdot}HCl + CH_3CHO + N_2 + CO_2 \quad (5)$$

the aqueous decomposition was carried out in the presence of 1 molar equiv of triethylamine as acid acceptor, pure 1,3-bis(2-chloroethyl)urea precipitated in 70% yield (eq 6).[9] When triethylamine

$$2BCNU + H_2O + 2Et_3N \longrightarrow 2Et_3N{\cdot}HCl + ClCH_2CH_2NHCONHCH_2CH_2Cl + 2CH_3CHO + 2N_2 + CO_2 \quad (6)$$

was replaced by cyclohexylamine in the reaction, the product isolated in 36% yield was 1-(2-chloroethyl)-3-cyclohexylurea. When 2 molar equiv of cyclohexylamine was used, the yield of urea was increased to 84% (eq 7).[9]

$$BCNU + 2C_6H_{11}NH_2 \longrightarrow C_6H_{11}NH_2{\cdot}HCl + ClCH_2CH_2NHCONHC_6H_{11} + CH_2CHO + N_2 \quad (7)$$

That the dehydrochlorination of 2-chloroethanol is not the source of acetaldehyde and HCl in eq 5–7 is evidenced by the fact that under slightly different conditions 2-chloroethanol was obtained from BCNU (*vide supra*) and by the fact that 1,3-bis(2-chloroethyl)urea is readily formed from 2-chloroethyl isocyanate and H_2O in the presence of 2-chloroethanol since, if 2-chloroethanol released HCl under these conditions, any 2-chloroethylamine resulting from the hydrodecarboxylation of 2-chloroethyl isocyanate would be trapped. Furthermore, 2-chloroethanol yields acetaldehyde directly only under conditions[18] much more vigorous than those employed in this study, and ethylene oxide, obtained from 2-chloroethanol only under basic conditions,[19] is isomerized to acetaldehyde only under more vigorous conditions.[20] If BCNU decomposed in the "normal" manner (eq 3), 2-chloroethanediazohydroxide would be formed (eq 8). It seemed

$$BCNU \longrightarrow ClCH_2CH_2N{=}NOH + OCNCH_2CH_2Cl \quad (8)$$

possible that this diazohydroxide, because of its β chlorine, could decompose abnormally into acetaldehyde, HCl, and N_2. This same intermediate should be derivable from the action of nitrous acid on 2-chloro-

(18) H. Dreyfus, U. S. Patent 2,340,371 (1944); P. V. Zimakov and L. M. Kogan, *Dokl. Akad. Nauk SSSR*, **115**, 297 (1957); L. M. Kogan, *Zh. Prikl. Khim.*, **31**, 1437 (1958).

(19) L. O. Winstrom and J. C. Warner, *J. Am. Chem. Soc.*, **61**, 1205 (1939); J. E. Stevens, C. L. McCabe, and J. C. Warner, *ibid.*, **70**, 2449 (1948); C. L. McCabe and J. C. Warner, *ibid.*, **70**, 4031 (1948).

(20) K. Bauer, U. S. Patent 2,031,200 (1936); H. P. A. Groll and G. Hearne, U. S. Patent 2,106,347 (1938).

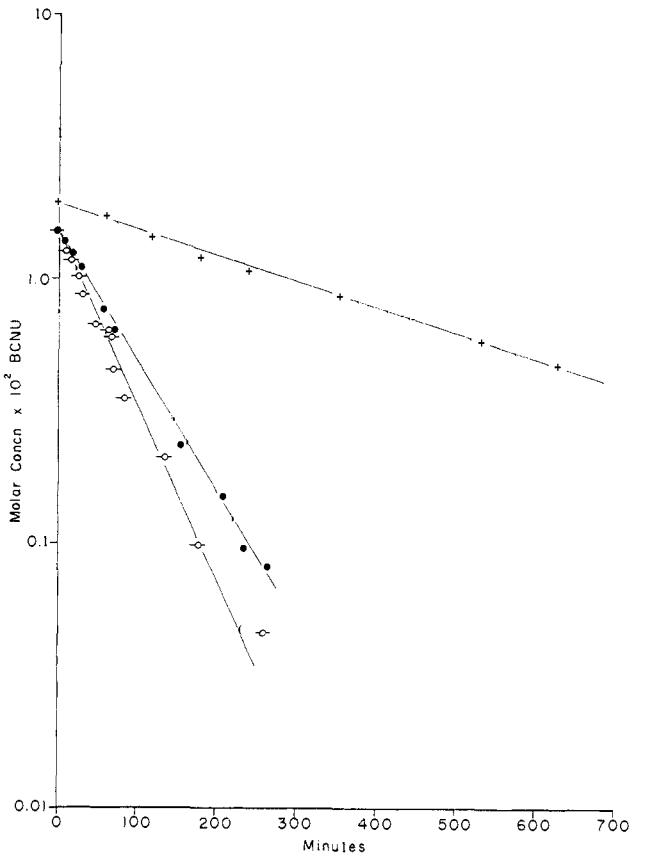
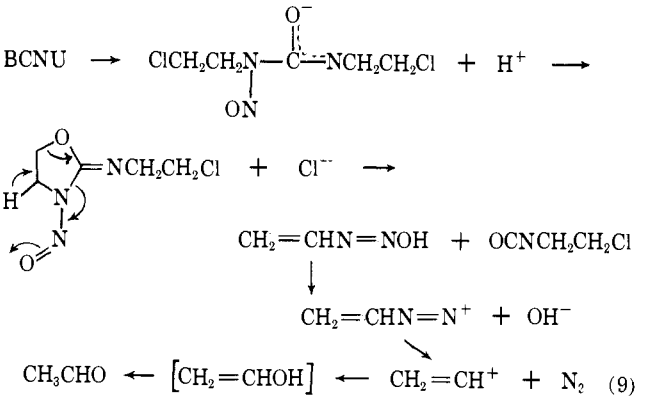


Figure 1.—Apparent first-order plots of the decomposition of BCNU at 37°: +, water; ●, whole blood; -O-, blood serum.

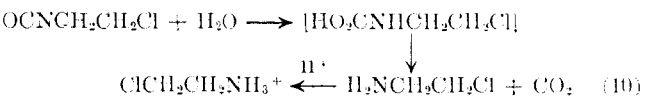
ethylamine,[21] but this latter reaction was studied and found to produce no acetaldehyde. Thus, the elimination of HCl from BCNU must occur prior to, or concomitant with, removal of its NH proton. It seems likely that in aqueous media, the first step in the decomposition of BCNU is loss of the NH proton[22] followed by attack of the anion on the electropositive β carbon to give an unstable oxazolidine intermediate, which then breaks down into ethylenediazohydroxide and 2-chloroethyl isocyanate (eq 9). The ethylenediazohydroxide reacts normally to give vinyl alcohol which rearranges to acetaldehyde. The other products of the reaction result from 2-chloroethyl isocyanate.



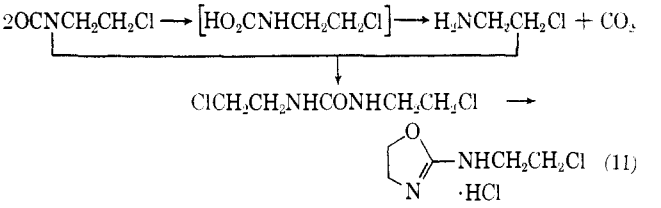
(21) Reference 8, p 68.

(22) The dependence of the rate of this reaction on pH (*vide infra*) supports this mechanism.

Thus in water the isocyanate breaks down to the amine which is trapped by the proton generated initially (eq 10). In the presence of triethylamine, the

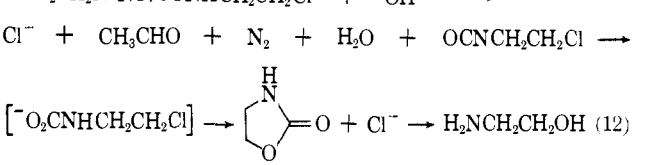


2-chloroethylamine is not trapped and is free to react with undecomposed isocyanate to give 1,3-bis(2-chloroethyl)urea. This same initial result is obtained in buffer solutions, but on long standing 1.5 moles of HCl/mole of BCNU was formed, and the residue was identified as 2-(2-chloroethylamino)-2-oxazoline hydrochloride[23a] (eq 11). In the presence of a primary

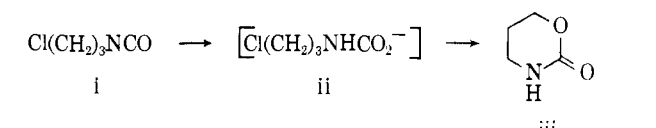


amine, the 2-chloroethyl isocyanate reacts to give an unsymmetrical urea before it can undergo hydrodecarboxylation.

The results described above prompted us to study the decomposition of BCNU at pH 12 (0.1 *N* NaOH). The products resulting from the decomposition at this pH were dependent upon the amount of base used. Thus, if 1 mole of base/mole of BCNU was used, the products were primarily NaCl, acetaldehyde, and 1,3-bis(2-chloroethyl)urea. With 2 moles of base, 2-oxazolidinone was obtained instead of the urea indicating that, with sufficient base, the second reaction that occurred was conversion of the isocyanate to the sodium carbamate, which cyclized to 2-oxazolidinone.[23b] In excess base, no 2-oxazolidinone is obtained, and the product presumably is 2-aminoethanol (eq 12). This reaction sequence was confirmed by treating 2-chloroethyl isocyanate with 1 equiv of base which gave the 2-oxazolidinone, and an excess of base which gave only 2-aminoethanol. Authentic 2-oxazolidinone[24] in turn gave 2-aminoethanol[25] readily on treatment with aqueous base. In view of these results,



(23) (a) M.-E. Kreling and A. F. McKay, *Can. J. Chem.*, **37**, 504 (1959). (b) Evidence for this sequence was provided by the formation of (tetrahydro-2H-1,3-oxazin-2-one(iii) from the action of NaOH on 3-chloropropyl isocyanate (i). Although the alternative route, hydrolysis of the C–Cl bond of 2-chloroethyl isocyanate to give 2-hydroxyethyl isocyanate which could cyclize to 2-oxazolidinone, is not unreasonable, it seems an unlikely path for the 3-chloropropyl isocyanate (i).



(24) Aldrich Chemical Co., Inc., Milwaukee, Wis.

(25) 1-Aryl-2-oxazolidinones are known to decompose in base to the corresponding 2-(arylamino)ethanols [R. Adams and J. B. Segur, *J. Am. Chem. Soc.*, **45**, 785 (1923)].

an attempt was made to prepare 1-chloro-2-diazoethane[26] under optimal conditions for diazoalkane formation, *viz.*, in the conventional manner used for diazomethane.[27] No evidence for the formation of any 1-chloro-2-diazoethane was obtained.

The rates of decomposition of BCNU in various aqueous media were determined by measurement of the nitrogen and CO_2 evolved during the course of the reaction. Although the rates of evolution of the two gases were comparable, the total recovery of nitrogen was usually better (almost quantitative) than that of CO_2 and, therefore, the rate of the reaction was calculated from the measurement of the nitrogen evolution. The decomposition is an apparent first-order reaction at 37 and 50°, as can be seen from Figures 1 and 2.[28] The rate constants and half-lives in the various media are given in Table I. It is obvious from these data that, although the reaction proceeded faster in all buffered media than in H_2O, phosphate buffer has a greater catalytic effect than other buffers. The results with blood serum and whole blood bear out this specific catalysis by the monohydrogen phosphate ion. These results are in agreement with those obtained by Garrett[29] with the antibiotic streptozotocin, presumed to be a nitrosourea. The obvious pH dependence of the reaction is also in agreement with the results Garrett obtained with other nitrosoureas.[17] The rate of reaction in distilled water at 50° was also followed by titration of the chloride ions formed, and the rate found in this way is in good agreement with that determined by gas chromatography of nitrogen evolved.

Table I

Decomposition Rates in Aqueous Media

Compd[a]	Medium	Concn, *M*	$k \times 10^3$, min^{-1}	$t_{1/2}$, min
	A. At 37°			
BCNU	H_2O	0.0191	2.27	305
CCNU	H_2O		1.84	376
BFNU	H_2O	0.0286	2.84	244
BCNU	Acetate buffer, pH 7.2	0.0153	6.73	103
BCNU	Tris buffer, pH 7.2	0.0154	6.96	101
CCNU	Tris buffer, pH 7.2		3.33	208
BCNU	PO_4 buffer, pH 7.2	0.0135	12.2	57
CCNU	PO_4 buffer, pH 7.2		10.8	64
BFNU	PO_4 buffer, pH 7.2	0.0174	12.2	57
BCNU	Blood serum	0.0152	15.3	45.4
BCNU	Whole blood	0.0151	11.2	61.9
BCNU	0.01 *N* NaOH	0.0054	301	2.3
	B. At 50°			
BCNU	0.1 *N* HCl	0.0156	7.33	94.6
BCNU	H_2O	0.0169	5.93	117
BCNU	H_2O		6.13	113[b]
BCNU	PO_4 buffer, pH 7.2	0.00912	37.4	18.5
BCNU	PO_4 buffer, pH 8.5	0.0152	54.7	12.7
BCNU	Blood serum		36.9	18.8
BCNU	Whole blood	0.0127	35.2	19.7

[a] Abbreviations defined in text. [b] Chloride ion titration.

(26) A vague description of the supposed preparation of this compound has appeared [H. Brintzinger and K. Pfannstiel, *Chem. Ber.*, **81**, 378 (1948)].

(27) F. Arndt in "Organic Syntheses," Coll. Vol. II, A. H. Blatt, Ed., John Wiley and Sons, Inc., New York, N. Y., 1943, p 165.

(28) The plot of the decomposition in phosphate buffer at pH 8.5 at 50° does show some departure from linearity, and this decomposition may in fact be more complex. The observed products, however, are the same as those obtained in the other media.

(29) E. R. Garrett, *J. Am. Pharm. Assoc., Sci. Ed.*, **49**, 767 (1960).

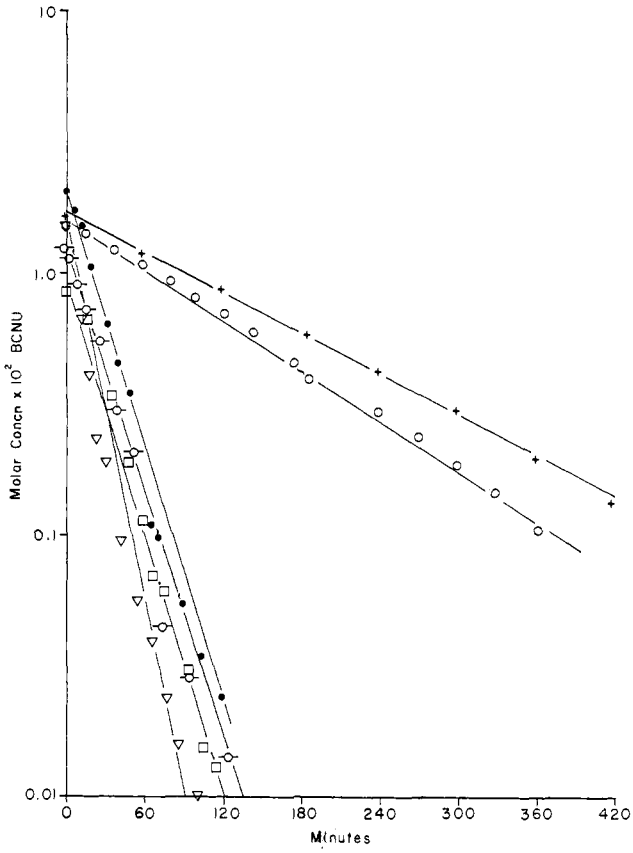


Figure 2.—Apparent first-order plots of the decomposition of BCNU at 50°: +, water; ○, 0.1 *N* HCl; ●, blood serum; -○-, whole blood; □, phosphate buffer (pH 7.2); ▽, phosphate buffer (pH 8.5).

The mode of decomposition of another nitrosourea highly active against leukemia L1210, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU),[9] was also investigated. It decomposed in a manner analogous to BCNU to give acetaldehyde, N_2, CO_2, and cyclohexylamine hydrochloride. The rate of decomposition of this nitrosourea was also studied at 37° in distilled water and in water at pH 7.2 buffered by both phosphate and Tris buffers. The reaction rates were similar to those of BCNU and specific monohydrogen phosphate catalysis was again observed. In Tris buffer the rate was about one-half that of BCNU in this medium.

The anomalous decomposition of BCNU and other 1-(2-chloroethyl)-1-nitrosoureas in aqueous media could be essential to their high order of antileukemic activity. In order to clarify this point, a study of the decomposition of a nitrosourea equally effective against L1210, 1,3-bis(2-fluoroethyl)-1-nitrosourea (BFNU),[9] was initiated, and it was found to decompose in the normal fashion (eq 3); a 95% yield of 2-fluoroethanol was obtained. The decomposition rates of this nitrosourea in water and in phosphate buffer were comparable to those of the (2-chloroethyl)ureas (see Table I). Thus, it would appear that the decomposition of the 1-(2-chloroethyl)-1-nitrosoureas into acetaldehyde and HCl is not essential to their antileukemic activity. It would further appear that the biologic activity of the nitrosoureas may result from their *in situ* decomposition into isocyanates that then react with primary amino groups of some macromolecule thus interfering

with its vital function.[30] Such a mechanism of action would be in keeping with the biochemical studies of Wheeler, *et al.*, using C^{14}-labeled BCNU.[31a] If this postulate is correct, the structure of the isocyanate appears not to be critical to activity. The fact that methyl isocyanate and 2-chloroethyl isocyanate are toxic to mice, but show no selective toxicity for L1210 leukemia cells, does not support or disprove this hypothesis, since the nitrosoureas may be a latent, carrier form of the isocyanate, and this latentiation may be vital for selective action.[31b]

The difference in the chemistry of the 2-chloroethyl- and 2-fluoroethylnitrosoureas could be responsible for the differences observed in their toxicities, since with the 2-chloroethyl compounds the median day of death of mice at the LD_{50} is the seventh to the thirtieth day after administration, whereas with the 2-fluoroethyl compounds only acute deaths (median day of death, second day after administration) were observed. Since the 2-fluoroethyl compounds decompose to 2-fluoroethanol under physiological conditions, this acute toxicity could be due to fluoroacetate.[32-34] At the same time the delayed toxicity of the 2-chloroethyl compounds could result from their anomalous chemical behavior.

Because of the relatively short half-life of the nitrosoureas in aqueous media, the stability of BCNU in nonaqueous media suitable for injection into animals was investigated. Since the decomposition in nonaqueous media at room temperature was slow, the rates were followed by measurement of the change in the height of the ultraviolet absorption maximum at 232 mμ with time. BCNU was found to have a half-life of 9 days in sesame oil, 30 days in propylene glycol, and 74 days in 95% ethanol. Seven other nitrosoureas, highly active against leukemia L1210, were also studied in ethanol in the same way. The half-lives are listed in Table II.

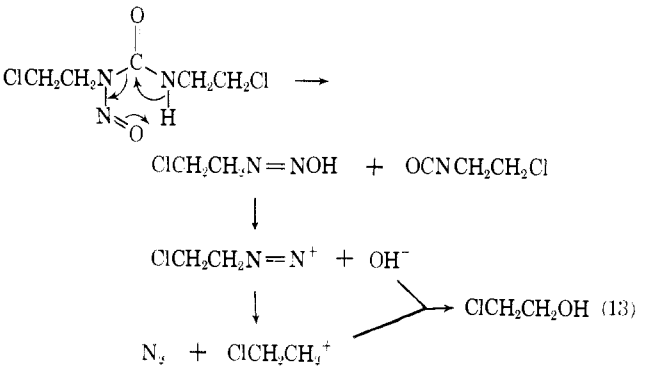
In order to identify the products of the decomposition of BCNU in indifferent solvents, a solution of it in petroleum ether (bp 30-60°) was refluxed for 24 hr. Contrary to the results of Loo, *et al.*,[37] no decomposition was observed under *strictly* anhydrous conditions during this period of time. When a solution of BCNU in 2,2,4-trimethylpentane (bp 98°) was refluxed for 1 hr under anhydrous conditions, it decomposed completely to give N_2, 2-chloroethanol, and 2-chloroethyl isocyanate according to eq 13.[38] Since Loo, *et al.*,[37] observed the formation of 1,3-bis(2-chloroethyl)urea from BCNU in refluxing petroleum ether, a small amount of water was added to the trimethylpentane solution of the 2-chloroethyl isocyanate and 2-chloroethanol. An essentially quantitative yield of 1,3-bis(2-chloroethyl)urea formed overnight.

That decomposition of 1,3-*disubstituted* nitrosoureas normally occurs according to eq 13 was established by observing the decomposition of 1,3-dimethyl-1-nitrosourea in toluene. The only observed products of the reaction were methanol, N_2, and methyl isocyanate.

TABLE II

DECOMPOSITION RATES IN ETHANOL

Compd	Half-life, days
BCNU	74
1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea	10
1,1′-(*trans*-1,2-Cyclohexylene)bis[3-(2-chloroethyl)-3-nitrosourea]	12
3-(*cis*-2-Chlorocyclohexyl)-1-(2-chloroethyl)-1-nitrosourea	9
3-(1-Adamantyl)-1-(2-chloroethyl)-1-nitrosourea	5
3-Cyclohexyl-1-(2-fluoroethyl)-1-nitrosourea	14
3-(*trans*-2-Chlorocyclohexyl)-1-(2-chloroethyl)-1-nitrosourea	6
1-(2-Chloroethyl)-3-(2-norbornyl)-1-nitrosourea	10



Experimental Section

The infrared spectra were determined with a Perkin-Elmer Model 521 spectrophotometer and the ultraviolet spectra with a Perkin-Elmer Model 202 spectrophotometer. Phosphate,[39] acetate,[39] and Tris [tris(hydroxymethyl)aminomethane][40] buffers were prepared by standard procedures. Melting points were determined on a Kofler Heizbank and are corrected.

Decomposition of 1,3-Dimethyl-1-nitrosourea in Water.—A solution of 1,3-dimethyl-1-nitrosourea (2.0 g, 17 mmoles) in 50 ml of H_2O was heated under reflux until gas evolution ceased (3 hr). Removal of the solvent under reduced pressure left a white solid (mp 104°), whose mixture melting point with 1,3-dimethylurea was undepressed; yield 0.06 g (81%).

Reaction of BCNU with a Limited Quantity of Water.—A mixture of BCNU (3.0 g, 14 mmoles) and distilled H_2O (0.80 ml, 4.4 mmoles) was stirred at 50° in a 50-ml flask closed with an adapter fitted with a stopcock and a serum cap. After 96 hr the flask was cooled and the volatiles were removed by connecting the system through a series of cold traps to a vacuum line. The nonvolatile residue was shown by its infrared spectrum to be impure 1,3-bis(2-chloroethyl)urea, from which 0.50 g (20%)

(30) On the other hand, sufficient alkylating activity may be derivable, under physiological conditions, from carbonium ions resulting from the diazohydroxides to explain the biologic activity of this class of compounds. In this regard, however, one should recall the lack of antitumor activity of other monofunctional alkylating agents.

(31) (a) G. P. Wheeler, B. J. Bowdon, and T. C. Herren, *Cancer Chemotherapy Rept.*, **42**, 9 (1964). (b) The efficacy of other latentiated drugs such as cytoxan is well established; see N. J. Harper, *Progr. Drug Res.*, **4**, 221 (1962).

(32) M. R. Chenoweth, *Pharmacol. Rev.*, **1**, 383 (1949).

(33) Personal communication, D. P. Rall.

(34) Initial experiments in this laboratory have shown that the acute toxicity of the 2-fluoroethyl compounds can be reversed in mice by sodium acetate in ethyl alcohol,[35] the best agent for the reversal of fluoroacetate in mice.[32]

(35) F. M. Schabel, Jr., unpublished data.

(36) W. W. Tourtellotte and J. M. Coon, *Federation Proc.*, **8**, 339 (1949).

(37) T. L. Loo, R. L. Dion, R. L. Dixon, and D. P. Rall, *J. Pharm. Sci.*, **55**, 492 (1966).

(38) These results explain our initial observation on the decomposition of BCNU at 50° in the presence of a limited quantity of water. The first step of that reaction occurred in the melt of BCNU without the intervention of water and proceeded according to eq 19. The 2-chloroethyl isocyanate formed in this way, then reacted with the water present to give 1,3-bis(2-chloroethyl)urea. Thus, the over-all reaction *appeared* to be the "normal" nitrosourea reaction.

(39) I. M. Kolthoff, "Acid-Base Indicators," The Macmillan Co., New York, N. Y., 1937, p 239.

(40) R. G. Bates and V. E. Bower, *Anal. Chem.*, **28**, 1322 (1956).

of pure material was obtained. The liquid in the cold traps was a two-phase mixture of H_2O and 2-chloroethanol, identified by comparison of its infrared spectrum with that of an authentic sample. The yield of 2-chloroethanol was 0.48 g (43%).

Decomposition of BCNU in Dilute Aqueous Solution.—The decomposition of an approximately 0.02 *M* solution of BCNU in distilled H_2O was carried out at 37°. Gas chromatography of the volatile products of the reaction established that 1 mole of N_2 and 1 mole of CO_2 were produced per mole of BCNU. A third peak was also observed. This third component, collected and identified by its infrared spectrum as acetaldehyde, was also identified in the reaction medium by its reaction with *p*-phenylphenol and $CuSO_4$ in H_2SO_4.[41]

An aliquot of the spent reaction solution was titrated potentiometrically with 0.1 *N* NaOH. The results of several titrations confirmed that 1 equiv of acid was produced from each mole of BCNU. The apparent pK_a of the acid was 8.6. Three aliquots removed from the original reaction mixture at intervals were evaporated to dryness *in vacuo* at 25–50°, and the residues were examined by infrared spectroscopy, which showed that all three samples were mixtures, the principal component of which was an amine hydrochloride, subsequently identified by comparison of its spectrum with the spectrum of an authentic sample as 2-chloroethylamine hydrochloride. A gas chromatographic examination of an ether extract of the remaining solution showed only traces of 2-chloroethanol.

Titration of another aliquot of the spent solution by the Volhard method indicated that 1 mole of Cl^- was produced per mole of BCNU. A solution of BCNU in H_2O was then prepared and maintained at 50°. Aliquots were removed and titrated potentiometrically at 0, 25, and 50° with $AgNO_3$ in an acetate-buffered medium[42] (three temperatures were used to determine if the Cl^- was a rearrangement product or an initial reaction product). The results of the titrations at all three temperatures were essentially the same; 0.0296 mmole of BCNU produced 0.0293 mmole of Cl^-. The half-life of 113 min, based on rate of formation of Cl^-, was in excellent agreement with that obtained by N_2 evolution measurements.

In a related experiment, 2-chloroethyl isocyanate was allowed to react with water at 25 and 50°, and with 0.1 *N* HCl at 25°. Evaporation of the reaction solutions to dryness *in vacuo* gave residues that were examined by infrared spectroscopy. The infrared spectra of these residues determined in pressed KBr disks indicated the formation of 1,3-bis(2-chloroethyl)urea (C=O, 1620 cm^{-1}) and 2-(2-chloroethylamino)-2-oxazoline hydrochloride (C=N, 1695 cm^{-1}) in the H_2O solutions and 2-chloroethylamine hydrochoride in the HCl solution (*vide supra*).

The Decomposition of BCNU in Water Containing Triethylamine.—BCNU (1.00 g, 4.67 mmoles) was added to a solution of triethylamine (473 mg, 4.67 mmoles) in H_2O (20 ml). The mixture was stirred at 5–10° for 1 hr and at room temperature for 18 hr and was then chilled. The white solid that had formed was collected, washed with cold water (5 ml), and air-dried; yield 301 mg (70%), mp 130–132°. A mixture melting point with authentic 1,3-bis(2-chloroethyl)urea was not depressed.

Reaction of BCNU with Cyclohexylamine.—A cold (0–5°) suspension of BCNU (2.0 g, 9.35 mmoles) in water (15 ml) was treated with cyclohexylamine (0.93 g, 9.35 mmoles), and the resulting mixture was stirred at 5° for 1 hr and then at 10° for 2 hr. The white solid that had formed was collected on a filter and air-dried; its infrared spectrum was identical with that of 1-(2-chloroethyl)-3-cyclohexylurea, with which a mixture melting point was undepressed; yield 0.70 g (36%), mp 130°.

The reaction described above was repeated using 2 equiv of cyclohexylamine. The yield of 1-(2-chloroethyl)-3-cyclohexylurea was 1.63 g (84%).

Decomposition of BCNU in Phosphate Buffer, pH 7.2.—The decomposition of an approximately 0.02 *M* solution of BCNU in phosphate buffer was carried out as described above for the decomposition in water. One mole of N_2 was obtained, but the observed yield of CO_2 was lower, it being held in the buffer. A determination of Cl^- showed that 1.5 moles of Cl^- was produced per mole of BCNU, but 200 hr was required to attain this maximum value, which did not increase even after 500 hr. A comparison of the infrared spectrum of the solid residue from the reaction with that of an authentic specimen[23] showed it to be 2-(2-chloroethylamino)-2-oxazoline hydrochloride.

Reaction of BCNU with 0.1 *N* NaOH. A. One Equivalent.—A suspension of BCNU (500 mg, 2.34 mmoles) in 23.4 ml of 0.1 *N* NaOH was stirred at room temperature for 5 hr, resulting in the formation of a white solid. The mixture was chilled and the solid product was collected, washed with two 3-ml portions of cold water, and dried *in vacuo* overnight; yield 110 mg (51%), mp 130°. A mixture melting point with an authentic sample of 1,3-bis(2-chloroethyl)urea showed no depression.

B. Two Equivalents.—BCNU (500 mg, 2.34 mmoles) was suspended and stirred in 0.1 *N* NaOH (50 ml) in a flask fitted so that evolving gases passed through a cold trap (Dry Ice-acetone) and then a $Ba(OH)_2$ trap. The suspended solid had dissolved after 15 min, and the reaction mixture was stirred at room temperature for 1.5 hr. A trace of acetylene in the gas remaining in the cold trap was detected by infrared spectroscopy. There appeared to be no CO_2 present, but a gas did escape both traps. This gas was undoubtedly N_2, because N_2 was detected quantitatively (1 mole/mole of BCNU by gas chromatography) in another similar decomposition reaction.

A 4.0-ml aliquot from the reaction solution was treated with an excess of 2,4-dinitrophenylhydrazine reagent.[43] The precipitated orange 2,4-dinitrophenylhydrazone of acetaldehyde was collected, washed with two 1-ml portions of cold water, and dried *in vacuo:* yield 7.8 mg (19%), mp ~143°. The infrared spectrum of the product was identical with a reference spectrum.

The remaining reaction solution was concentrated under reduced pressure to give a white residue, whose infrared spectrum was similar to that of 2-oxazolidinone. A gas chromatogram, obtained with an F and M 1720 chromatograph using a 10% Versamid 900 on Ultraport column, of an ethanol extract of the residue was identical with that of authentic 2-oxazolidinone. In another identical decomposition of BCNU, extraction of the concentrated reaction mixture with two 10-ml portions of ethanol gave a semisolid, which was shown by infrared spectroscopy and gas chromatography, on a 10% silicone gum rubber W-98 on 80–100 mesh Diatoport S column using an F and M 5657-A gas chromatograph, to consist chiefly of 2-oxazolidinone; yield 140 mg (68%).

Reaction of 2-Chloroethyl Isocyanate with 0.1 *N* NaOH.—2-Chloroethyl isocyanate (246 mg, 2.34 mmoles) was added to 0.1 *N* NaOH (23.8 ml), and the solution was stirred for 4 hr at room temperature. *In vacuo* concentration left a white residue, which was dried further and examined by infrared spectroscopy. Its spectrum was similar to that of 2-oxazolidinone. Extraction of the residue with 16 ml of hot benzene gave 135 mg (66%) of 2-oxazolidinone, mp 86°, identified by mixture melting point and its infrared spectrum.

Reaction of 2-Oxazolidinone with 0.1 *N* NaOH.—A solution of 2-oxazolidinone (250 mg, 2.87 mmoles) in 0.1 *N* NaOH (28.7 ml) was stirred at room temperature for 30 hr. A gas chromatogram, obtained with an F and M 5657-A chromatograph equipped with a 10% Carbowax 20 M on 70–80 mesh acid-washed DMCS Chromsorb W column, of the reaction solution showed the presence of 2-aminoethanol only.

Decomposition Rates of Nitrosoureas in Aqueous Media by Gas Chromatography of the Evolved Gases.—Gas chromatography was used to follow the rate of evolution of N_2 and CO_2 from solutions of BCNU in H_2O, 0.1 *N* HCl, phosphate buffers (pH 7.2 and 8.4), blood serum, and human whole blood at 37 and 50°. Initially only N_2 was measured, but later it was evident that in some cases CO_2 could also be measured.

A Burrell Kromo-Tog Model K-1 was modified to make the all-glass gas sampling manifold suitable for liquid samples and so that the samples could be maintained at a constant temperature over long periods of time. Solutions of BCNU were prepared by several techniques. When solubility permitted, the sample was weighed into the gas sampling bulb containing the solvent. The bulb was flushed with He and closed. An alternative procedure was to weigh the sample into a volumetric flask, dilute with solvent, mix, and add the desired volume of solution to the flushed, evacuated gas sampling bulb. When the above techniques could not be used because of solubility limitations, the sample was dissolved in a minimum amount of ethanol and injected with a syringe into the flushed gas sampling bulb containing a measured quantity of the desired medium. The latter technique was used to prepare solutions of BCNU in 0.01 *N* NaOH, phosphate

(41) S. B. Barker and W. S. Summerson, *J. Biol. Chem.*, **138**, 535 (1941).
(42) V. J. Shiner, Jr., and M. L. Smith, *Anal. Chem.*, **28**, 1043 (1956).
(43) R. L. Shriner, R. C. Fuson, and D. Y. Curtin, "The Systematic Identification of Organic Compounds," 4th ed, John Wiley and Sons, Inc. New York, N. Y., 1956, p 111.

buffers, serum, and blood samples. A magnetic stirring bar was placed in the gas sampling bulb to aid in mixing and to dislodge bubbles of gas adhering to the walls of the bulb. At time intervals, the gas above the sample was flushed into the chromatographic column with He carrier gas. The amount of N_2 and CO_2 formed was determined by comparing the area under the peaks with areas produced by known quantities of these gases.

Two columns were used for the analysis, Molecular Sieve 5A and silica gel. When it was shown by experiment that oxygen was not a decomposition product, or had not diffused into the system, the remaining analyses were made on a silica gel column maintained at 70°. The results indicated that 1 mole of BCNU gave 1 mole of N_2 and 1 mole of CO_2 in dilute aqueous solution, except in 0.01 *N* NaOH, from which only N_2 was evolved.

The volume of N_2 collected during a given time interval was used as a measure of the amount of BCNU that decomposed during that time interval. Semilog plots of concentration of BCNU *vs.* time were essentially linear. From the slopes of these linear plots, the reaction rate constant and half-life of BCNU in a given solvent were calculated.

In the manner described above, the reaction rate constants for the decomposition of 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea and 1,3-bis(2-fluoroethyl)-1-nitrosourea and their half-lives were determined.

Stability of BCNU in 95% Ethanol, Propylene Glycol, and Sesame Oil.—Stock solutions of BCNU were prepared by weighing samples into dry 20-ml serum bottles, adding solvent, stoppering with serum caps, and shaking to effect solution. Dissolution was instantaneous in 95% ethanol and required less than 15 min in propylene glycol and sesame oil. All stock solutions of BCNU were yellow and remained yellow for several days. The prepared stock solutions were stored at room temperature. As soon as solution was effected, aliquots were removed and diluted with ethanol, and the ultraviolet absorbance was measured at 232 mμ. At timed intervals, aliquots were removed from the original stock solutions, and the absorbance measurements were repeated. Measurements of absorbance were continued for over 1 month with the ethanol and propylene glycol solutions; however, changes in the absorption properties of the solvent itself prevented prolonged study in sesame oil. 2-Chloroethanol, 2-chloroethylamine hydrochloride, 1,3-bis(2-chloroethyl)urea, 2-(2-chloroethylamino)-2-oxazoline hydrochloride, and 2-chloroethyl isocyanate show very little or no absorption in the 232-mμ region; therefore, little or no correction is necessary in the calculations to determine the half-life of BCNU based on absorbance at the absorbance maximum.

The Decomposition of BCNU in 2,2,4-Trimethylpentane.—A solution of BCNU (250 mg, 1.17 mmoles) in 2,2,4-trimethylpentane (20 ml) was refluxed in a flask equipped with a condenser, thermometer, magnetic stirrer, and a He inlet. The condenser outlet was attached to an F and M 1720 gas chromatograph equipped with a Molecular Sieve 5A column, and the evolving gases were analyzed. The only volatile components detected were trimethylpentane, N_2, and O_2. The oxygen detected was found to be that which was dissolved in the trimethylpentane[44,45] and not from the decomposition of BCNU. After 4–5 hr of reflux, the evolution of gases had ceased. The remaining reaction solution, which had become colorless, was analyzed by gas chromatography, and the only volatile components found were 2-chloroethyl isocyanate, 2-chloroethanol, and trimethylpentane. An infrared spectrum of the reaction solution showed NCO absorption as a doublet at 2275 and 2260 cm^{-1}.

Water (5 ml) was added to the reaction solution, and the mixture was stirred for 4 hr. The solvent was removed under reduced pressure, and the residual white semisolid was taken up in hot ethanol (10 ml). Removal of the ethanol under reduced pressure left a white solid which was further dried *in vacuo* and identified as 1,3-bis(2-chloroethyl)urea by infrared spectral comparison and mixture melting point with an authentic sample; yield 65 mg (60%), mp 128°.

The Decomposition of 1,3-Dimethyl-1-nitrosourea in Refluxing Toluene.—A solution of 1,3-dimethyl-1-nitrosourea (500 mg, 4.27 mmoles) in toluene (20 ml) was refluxed for 20 hr with the evolved gases being passed into a cold trap (Dry Ice–acetone). Gas evolution ceased after about 6 hr. The reaction solution and the trace of low-boiling liquid condensed in the trap were combined and analyzed by infrared spectroscopy and gas chromatography. The infrared spectrum showed absorption at 2280 cm^{-1} for NCO and at 3440 cm^{-1} for OH; chromatographic peaks, obtained using an F and M 5657-A gas chromatograph equipped with a 10% silicone gum rubber W-98 on 80–100 mesh Diatoport S column, corresponded to methanol and methyl isocyanate.

A second decomposition was carried out as described above except that the exit gases were led into a solution of cyclohexylamine (423 mg, 4.27 mmoles) in water (7 ml). After 3 hr, the reaction was stopped and the H_2O solution was evaporated to dryness *in vacuo*; yield of 1-cyclohexyl-3-methylurea, 190 mg (28%). One recrystallization gave material that melted at 157° (lit.[12] mp 157–158°).

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(44) R. R. Baldwin and S. G. Daniel, *J. Appl. Chem.* (London), **2**, 161 (1952).

(45) E. S. Thomsen and J. C. Gjaldbaek, *Acta Chem. Scand.*, **17**, 127 (1963).