The Synthesis of Potential Anticancer Agents. XXXVII. N-Nitrosoureas. III. 1,5-Bis(2-chloroethyl)-1-nitrosobiuret and Related Derivatives of Biurets, Biureas, and Carboxamides[1]

THOMAS P. JOHNSTON AND PAMELA S. OPLIGER

Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Alabama 35205

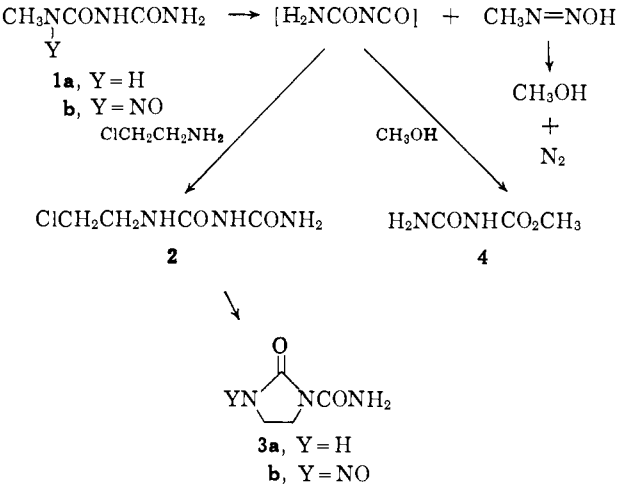
Received December 27, 1966

The search for congeners of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) as anticancer agents has been extended to nitroso derivatives of biurets, biureas, and carboxamides. The aqueous decomposition of N-nitrosobiurets in the presence of 2-chloroethylamine, a method involving *in situ* generation of carbamoyl isocyanates, made possible the preparation of N-(2-chloroethyl)-substituted biurets, from which 5-(2-chloroethyl)-1-methyl-1-nitrosobiuret (**6b**) and 1,5-bis(2-chloroethyl)-1-nitrosobiuret (**7b**) were derived. Alkali cyclizations of N-(2-chloroethyl)biurets produced 2-oxo-1-imidazolinecarboxamides, which could be nitrosated only on the ring nitrogen. Of several new methyl- and 2-chloroethyl-substituted biureas prepared, including 1,6-bis(2-chloroethyl)biurea (**18b**), only 1,3,6-trimethylbiurea (**16a**) yielded a pure mono- or dinitroso derivative. Interception of the nitrosation product of 1-methylbiurea (**13**) with cyclohexylamine resulted in the isolation of 3-cyclohexyl-1-methyl-1-nitrosourea and 1,3-dicyclohexylurea. Unlike N,N'-bis(2-chloroethyl)oxamide (**19**), which resisted nitrosation under favorable conditions, N,N'-bis(2-chloroethyl)hexanediamide (**21a**) and N,N'-bis(2-chloroethyl)-*trans*-1,4-cyclohexanedicarboxamide (**22a**) were converted by nitrosation in acetic anhydride–acetic acid to the crystalline dinitroso derivatives **21b** and **22b**. Some of the nitroso derivatives of biurets, biureas, and carboxamides increased the life span of leukemic mice, but data obtained with a limited number of congeners (**7b**, N-(2-chloroethyl)-N-nitrosocyclohexanecarboxamide (**20b**), **21b**, and **22b**) indicate that substitution by the 2-chloroethyl group does not result in the outstanding activity against L1210 leukemia previously observed with BCNU and related nitrosoureas.

Structural requirements for maximal antileukemic activity of N-nitrosoureas in mice have recently been defined,[2] and one of the most active compounds of this class and the first to be tried clinically is 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU).[3] The synthesis of the structurally analogous 1,5-bis(2-chloroethyl)-1-nitrosobiuret (**7b**) and related compounds was undertaken as a rational extension of the search for active congeners of BCNU. Although nitroso derivatives of methyl-, dimethyl-, and trimethylbiurets were described many years ago,[4] apparently no further precedent for the preparation of **7b** has been reported.

The aqueous decomposition of N-nitrosoureas in the presence of primary and secondary amines has been widely applied as a method for the preparation of variously substituted ureas and undoubtedly involves an effectual, if not actual, intermediacy of isocyanic acid or an isocyanate.[2,5] Application of this method to the decomposition of N-nitrosobiurets offered the possibility of *in situ* generation of carbamoyl isocyanates [RNHCONCO][6] and a new route to substituted biurets adaptable to the introduction of labile substituents such as the 2-chloroethyl group. This speculation was substantiated by the isolation of 1-(2-chloroethyl)-biuret (**2**) from the aqueous decomposition of 1-methyl-1-nitrosobiuret (**1b**) in the presence of 2-chloroethylamine (see Scheme I), which was liberated *in situ* from its hydrochloride with triethylamine. The reaction proceeded under mild conditions with nitrogen evolution; the intermediate isocyanate would be expected to be exceptionally reactive because of electron withdrawal by the carbamoyl group.[8]

SCHEME I



A method based on an undetailed description of the reaction of ethyl allophanate and methylamine by Murray and Dains[9] was found superior to one of Biltz and Jeltsch[4] involving the addition of urea to methyl isocyanate for the preparation of the intermediate 1-methylbiuret (**1a**). A small amount of the product obtained by nitrosation of **1a** in dilute HCl was recrystallized from methanol, and the purified product compared favorably with previously reported **1b**, which was recrystallized from a proportionately large volume of ethyl acetate.[4] An attempted recrystallization of a large amount of **1b** from methanol, however, resulted in vigorous evolution of N_2 at or near the boiling point of the solvent, and the cooled solution

(1) This work was supported by funds from the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health (Contract No. PH-43-64-51), and the C. F. Kettering Foundation. Part XXXVI, ref 2b.

(2) (a) T. P. Johnston, G. S. McCaleb, and J. A. Montgomery, *J. Med. Chem.*, **6**, 669 (1963); (b) T. P. Johnston, G. S. McCaleb, P. S. Opliger, and J. A. Montgomery, *ibid.*, **9**, 892 (1966).

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

(4) H. Biltz and A. Jeltsch, *Ber.*, **56B**, 1914 (1923).

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

(6) Such an intermediate may also be involved in the recently reported synthesis of 1,5-disubstituted biurets from the reaction of 4-substituted allophanyl azides with primary amines in benzene.[7]

(7) H. Stollar, R. J. Ranz, and F. L. Chubb, *Can. J. Chem.*, **44**, 846 (1966).

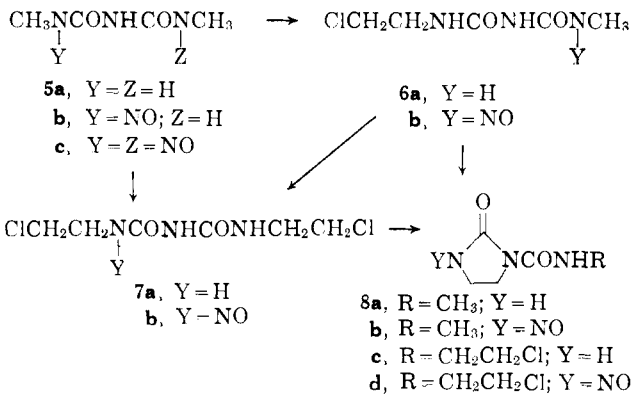
(8) R. G. Arnold, J. A. Nelson, and J. J. Verbanc, *Chem. Rev.*, **57**, 47 (1957).

(9) J. A. Murray and F. B. Dains, *J. Am. Chem. Soc.*, **56**, 144 (1934).

deposited methyl allophanate (**4**) in good yield. An initial breakdown of **1b** into carbamoyl isocyanate and methanediazohydroxide, a mechanism that parallels the one recently proposed[10] for observed decompositions of N-methyl-N-nitrosourea, which presumably include allophanate formation,[11,12] would account for the formation of **2** as well as **4** and the evolution of N_2. Although attempted nitrosations of **2** did not result in the isolation of the desired 1-(2-chloroethyl)-1-nitrosobiuret,[13] the conversion of **1b** to **2** provided a precedent for the eventual synthesis of the title compound **7b**. Ring closure of **2** by the action of KOH in refluxing aqueous ethanol produced chromatographically homogeneous 2-oxo-1-imidazolidinecarboxamide (**3a**), nitrosation of which to give the 3-nitroso derivative **3b** without attack on the carbamoyl group is another example of the easy nitrosation of cyclic ureas.[2a] The structure of **3b** is supported by infrared and pmr spectra. Alkaline ring closure of haloethylureas to 2-imidazolidinones is well known,[14] and the use of pmr spectra in structure determinations of nitrosoureas has recently been described.[2b]

1,5-Bis(2-chloroethyl)biuret (**7a**) was first obtained by the stepwise sequence **5b** → **6a** → **6b** → **7a** (Scheme II), which involved the *in situ* generation of 2-chloroethylcarbamoyl isocyanate from **6b**. A more direct synthesis of **7a** was subsequently achieved by the reac-

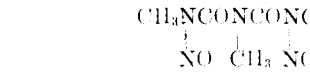
SCHEME II



tion of 1,5-dimethyl-1,5-dinitrosobiuret (**5c**) with 2 molar equiv of 2-chloroethylamine. The results of this reaction may reasonably be explained by sequential generation of two carbamoyl isocyanate intermediates as follows. The over-all yields of **7a** from the common

5c → [$ClH_3N(NO)CONCO$] →
6b → [$ClCH_2CH_2NHCONCO$] → **7a**

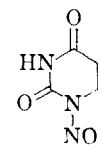
precursor 1,5-dimethylbiuret (**5a**) by the two routes appear to be about the same. Nitrosation of **7a** in undiluted HCOOH with a fivefold excess of nitrite gave the title compound **7b**, which was also the product of a deliberate dinitrosation attempt in which a larger excess of nitrite and a longer reaction time were used. Both 1-(2-chloroethyl)-5-methylbiuret (**6a**) and its mononitroso derivative **6b** also failed to yield an isolable dinitroso derivative. The resistance of **6a** and **6b** to dinitrosation seems particularly surprising when compared with the easy dinitrosation of **5a** and the easy mononitrosation of **7a**. Furthermore, only the dinitroso derivative **9** of 1,3,5-trimethylbiuret (from 1,3-dimethylurea and methyl isocyanate)[14] is known, although attempts to prepare the mononitroso derivative have been made. Ring closure of **6a** and **7a** with alkali



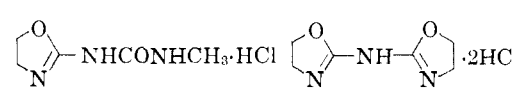
9

as in the preparation of **3a** afforded N-methyl- and N-(2-chloroethyl)-2-oxo-1-imidazolidinecarboxamides (**8a** and **8c**), respectively. The infrared absorption of **3a**, **8a**, and **8c** compared favorably with that recently reported for similarly substituted imidazolidinones.[15] Again, only ring nitrosation to give the 3-nitroso derivatives **8b** and **8d** was observed even under conditions chosen to introduce a second nitroso group.

The easy ring nitrosations of **3a**, **8a**, and **8c** seem less surprising in view of a subsequent observation that hydrouracil, a cyclic acylurea, was also readily nitrosated in dilute HCl. The product, 1-nitrosohydrouracil (**10**), whose structure is cogently supported by pmr spectroscopy, underwent slow and complete hydrolysis of the N–NO bond (denitrosation) when stirred with H_2O. This behavior contrasts the usual reaction of nitrosoureas with H_2O, which evolves N_2 and CO_2.[2b,5]


10

The hygroscopic products resulting from cyclization of the chloroethylbiurets **6a** and **7a** in boiling H_2O without added base are apparently aminooxazoline hydrochlorides: 2-(3-methylureido)-2-oxazoline hydrochloride (**11**) from **6a** and 2,2′-iminobis-2-oxazoline dihydrochloride (**12**) from **7a**. Although these products were not obtained in pure, characterizable form, their identity as oxazoline salts was indicated by their high water solubility and infrared spectral resemblance to the products of similar cyclizations such as that of 1,3-bis(2-chloroethyl)urea.[16]

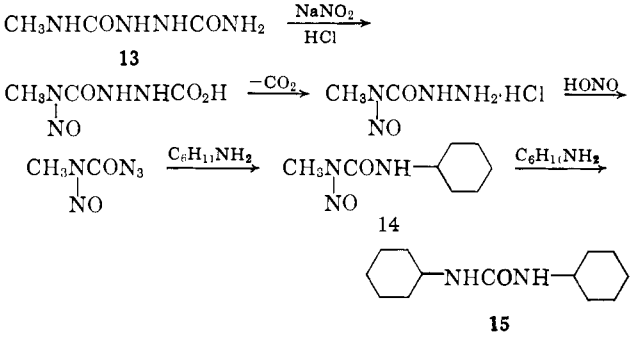

11 **12**

The synthesis of nitrosobiureas related to the nitrosobiurets described above was undertaken as a collateral investigation. One compound of this class, 1,6-dimethyl-1,6-dinitrosobiurea, had already been prepared,[2a] but of several new methyl- and 2-chloroethyl-substituted biureas prepared only one yielded the desired mono- or dinitroso derivative in pure and isolable form. 1-Methylbiurea (**13**) was derived from 4-methylseni-

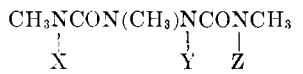
(10) D. L. Muck and W. M. Jones, *J. Am. Chem. Soc.*, **88**, 74 (1966).
(11) E. A. Werner, *J. Chem. Soc.*, **115**, 1093 (1919).
(12) K. Clusius and P. Endtinger, *Helv. Chim. Acta*, **43**, 2063 (1960).
(13) Only unchanged **2** (27%) was isolated from nitrosation in aqueous formic acid, but a yellow product of undetermined structure (but not the desired nitroso derivative of **2**) was isolated in low yield from nitrosation in dilute HCl.
(14) S. Gabriel and R. Stelzer, *Ber.*, **28**, 2929 (1895); H. Najer, R. Giudicelli, J. Menin, and C. Morel, *Bull. Soc. Chim. France*, 323 (1963); H. Nohira, Y. Nishikawa, and T. Mokaiyama, *Bull. Chem. Soc. Japan*, **37**, 797 (1964).
(15) J. N. Tilley and A. A. R. Sayigh, *J. Org. Chem.*, **29**, 3347 (1964).
(16) M.-E. Kreling and A. F. McKay, *Can. J. Chem.*, **37**, 504 (1959).

carbazide by the action of KOCN in dilute HCl. Failure to isolate 1-methyl-1-nitrosobiurea after treatments of **13** with limited amounts of $NaNO_2$ in dilute HCl prompted interception of the nitroso derivative by *in situ* reaction with cyclohexylamine after excess nitrite had been used for the nitrosation. The products isolated in two crops were, according to infrared spectral and thin layer chromatographic comparisons, (1) 3-cyclohexyl-1-methyl-1-nitrosourea (**14**) contaminated with a little 1,3-dicyclohexylurea (**15**), and (2) **15** alone. These products may have resulted from changes involving nitrous acid degradation of the unsubstituted carbamoyl function and leading to methylnitrosocarbamoyl azide, which reacted stepwise with cyclohexylamine as shown in Scheme III. The

SCHEME III

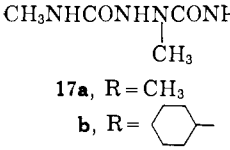


proposed azide displacement is not unlike the recently reported reaction of allophanyl azide with primary amines.[7] Treatment of methylhydrazine with 2 molar equiv of methyl isocyanate gave 1,3,6-trimethylbiurea (**16a**) *via* the unisolated intermediate 2,4-dimethylsemicarbazide. Nitrosation of **16a** in dilute HCl afforded an analytically pure dinitroso derivative, which was indicated by pmr spectroscopy to be an approximately 1:1 mixture of 1,3,6-trimethyl-1,6-dinitrosobiurea (**16b**) and one of the isomers **16c** and **16d** (probably **16c**, since only in cyclic structures are there examples of dinitrosation on both nitrogens of a ureido function). Pure **16b** was isolated in low yield from a nitrosation done in undiluted HCOOH with solid $NaNO_2$, but this result does not exclude the formation of other isomers. The reaction of 2-methylsemicarbazide



16a, X = Y = Z = H
b, X = Z = NO; Y = H
c, X = Y = NO; Z = H
d, X = H; Y = Z = NO

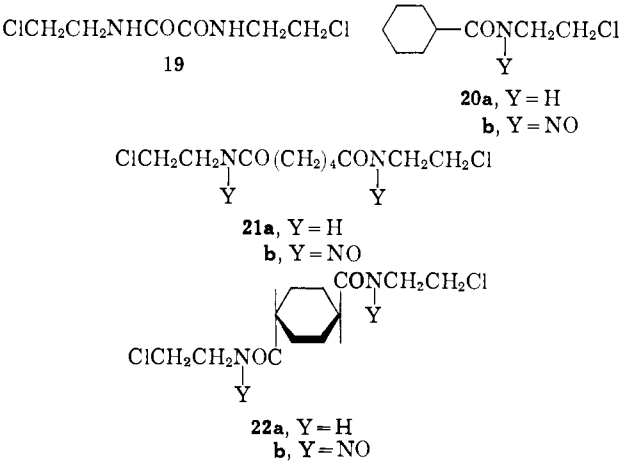
with methyl isocyanate provided 1,4-dimethylbiurea (**17a**), but the nitrosation of **17a** (**17a** + $2NaNO_2$ + 2HCl) gave a mixture of products, one of which was apparently the desired 1-nitroso derivative as evidenced by the identity (mixture melting point and infrared absorption) of one of the products of an *in situ* decomposition with cyclohexylamine and the product (1-cyclohexyl-4-methylbiurea (**17b**)) of the reaction of 2-methylsemicarbazide with cyclohexyl isocyanate. (Another product of the *in situ* decomposition was 1,3-dicyclohexylurea, which indicated some degradation of the type encountered in the nitrosation of **13**.) 1-(2-Chloroethyl)-6-methylbiurea (**18a**) was obtained by the action of 2-chloroethyl isocyanate on 4-methylsemicarbazide, and 1,6-bis(2-chloroethyl)biurea (**18b**), by the action of 2-chloroethyl isocyanate on hydrazine. Nitrosation of **18a** in undiluted HCOOH resulted in the isolation of a low yield of a yellow solid, which decomposed during *in vacuo* drying. Nitrosations of **18b** under various conditions did not yield a pure nitroso- or dinitrosobiurea; ring closure may have been a complicating factor.



17a, R = CH_3
b, R =

18a, R = CH_3
b, R = CH_2CH_2Cl

A successful nitrosation of N,N′-bis(2-chloroethyl)-oxamide (**19**) would have provided an analog of **7b** in which the central imino group had been omitted, but little, if any, nitrosation of **19** was observed when modifications of two methods found by White[17] to be applicable to the nitrosation of a number of amides were employed. The nitrosating systems used consisted of (1) $NaNO_2$, acetic acid, and acetic anhydride, and (2) N_2O_4, sodium acetate, and CCl_4; extended reaction times and large excesses of reagents apparently failed to effect even mononitrosation to an appreciable extent. Effective use of the N_2O_4–sodium acetate–CCl_4 system for the dinitrosation of N,N′-dimethyloxamide has recently been reported.[18] This system was also adapted to the preparation of acid-sensitive N-methyl-N-nitrosoacetamide, after several failures to reproduce the original nitrosation of acetamide in concentrated HCl as described by D'Alelio and Reid.[19] A similar nitrosation of N-(2-chloroethyl)cyclohexanecarboxamide (**20a**) gave the nitroso derivative **20b** as an oil that contained 10% of unchanged **20a** as shown by pmr spectroscopy and thin layer chromatography. N,N′-Bis(2-chloroethyl)hexanediamide (**21a**) and N,N′-bis-(2-chloroethyl)-*trans*-1,4-cyclohexanedicarboxamide (**22a**), unlike **19**, were, respectively, converted by the $NaNO_2$–acetic anhydride–acetic acid system to the crystalline dinitroso derivatives **21b** and **22b**.



Although some of the nitroso compounds described above (biurets, biureas, and carboxamides) significantly increase the life span of leukemic mice (see Table I),

(17) E. H. White, *J. Am. Chem. Soc.*, **77**, 6008 (1955).
(18) H. Reimlinger, *Chem. Ber.*, **94**, 2547 (1961).
(19) G. F. D'Alelio and E. E. Reid, *J. Am. Chem. Soc.*, **59**, 109 (1937).

TABLE I
ACTIVITY OF NITROSOBIURETS, NITROSOBIUREAS, AND NITROSOCARBOXAMIDES AGAINST INTRAPERITONEALLY INOCULATED L1210 LEUKEMIA[a]

Compd	Min effective dose, mg/kg[b]	Opt dose, mg/kg[c]	Therap ratio[d]	Max effectiveness, % ILS at OD[e]
1b	3.8[f]	10[f]	2.5	88
5b	18[f]	37[f]	2	44
5c	9[f]	18–37[f]	2–4	62
9				Inactive[g]
6b	18[g]	187[g]	10	46
7b	4.5[g]	9[g]		47
1,6-Dimethyl-1,6-dinitrosobiurea[2a]	14[f]	20[f]	<2	52
16b		100[g]		25
N-Methyl-N-nitrosoacetamide		25[g]		25
20b[h]				Inactive
N,N'-Dimethyl-N,N'-dinitrosooxamide				Inactive
21b	12.5[g]	100[g]	8	65
22b	1[g]	4[g]	4	46
10		375–500[g]		26

[a] Inoculum: 10^5 cells. The life-span experiments were carried out according to protocols set up by the Cancer Chemotherapy National Service Center, the dose–response plots being interpreted by published procedures [H. E. Skipper and L. H. Schmidt, *Cancer Chemotherapy Rept.*, **17**, 1 (1962)]. [b] MED, the minimum dose that will increase the life span of leukemic mice 40% (ILS_{40}). [c] The dose at which the maximum increase in life span occurs (OD). [d] OD/MED. [e] Average per cent increase in life span of treated mice over control mice [100(T/C − 1)] at the OD. [f] Compound given intraperitoneally from the first day to death. [g] Compound given intraperitoneally on day 1 only. [h] ~90% **20b** and ~10% **20a**.

the data obtained with a limited number of congeners indicate that substitution by the 2-chloroethyl group does not result in the outstanding activity observed with similarly substituted nitrosoureas.[2] The activity of the title compound **7b**, for example, is of the same low order as that of 1,5-dimethyl-1-nitrosobiuret (**5b**) and 5-(2-chloroethyl)-1-methyl-1-nitrosobiuret (**6b**). Comparisons are restricted, however, since the dinitroso derivative corresponding to **7b** and 2-chloroethyl derivatives of biureas were not available because of synthetic difficulties. Complete substitution of NH protons as in **9** resulted in inactivity as previously noted with nitrosoureas.[2] In this regard it should be noted that *N,N'*-bis(2-chloroethyl)-*N,N'*-dinitrosohexanediamide (**21b**), lacking the NH group in a rather drastic departure from the ureido structure, has significant activity.

Experimental Section[20]

1-Methylbiuret (1a).—A mixture of ethyl allophanate (10 g, 0.075 mole) and methylamine [16 g, 0.44 mole (38 ml of 40% aqueous solution)] was allowed to stand in a stoppered flask for 31 days at room temperature with occasional shaking. *In vacuo* evaporation and recrystallization of the residue from ethanol (~45 ml) produced 7.0 g (80%) of **1a** in three crops with essentially identical infrared spectra; mp 171–173° (first crop) (lit. mp 169–172°,[3] mp 175°[9]); infrared absorption (KBr) at 3430–3390 and 3315–3190 (m–s, NH, NH_2), 1720–1680 (s) and 1610 (m) (CO), and 1535 cm^{-1} (CNH).

1-Methyl-1-nitrosobiuret (1b).—A solution of $NaNO_2$ (8.38 g, 121 mmoles) in H_2O (15 ml) was added dropwise to an ice-cold, stirred solution of **1a** (7.75, 66.1 mmoles) in 5 *N* HCl (60 ml) with immediate precipitation of **1b** as a yellow solid (HCl solution of **1a** effected by warming). More H_2O (40 ml) was added just after nitrite addition, and stirring was continued for 1 hr. The product was triturated in cold (5°) water (100 ml) for 30 min, and dried *in vacuo* over P_2O_5; yield 8.84 g (92%); mp 145° dec (lit.[4] mp 139–140° dec); infrared absorption (KBr) at 3430 (m–s, NH), 3300 and 3240 (m–s, NH_2), 1720 (s) and 1695 (m–s) (CO), and 1485 (s, NO).

Anal. Calcd for $C_3H_6N_4O_3$: N, 38.35. Found: N, 38.37.

1-(2-Chloroethyl)biuret (2).—Triethylamine (1.1 ml, 7.7 mmoles) was added to a cold, stirred solution of 2-chloroethylamine hydrochloride (1.00 g, 8.62 mmoles) in H_2O (15 ml); then **1b** (1.12 g, 7.76 mmoles) was added in portions as the mixture was slightly warmed with a warm-water bath. Solution of yellow **1b** was followed by an exothermic reaction, gas evolution, and deposition of a white solid. The mixture was stirred at room temperature for 1 hr, and the solid was collected, washed with water, and dried *in vacuo* over P_2O_5; yield 0.70 g (55%); mp 138–140°; infrared absorption (KBr) at 3435 and 3335 (NH), 1715–1665 (multiplet, strongest at 1695) and 1610 (w–m) (CO), 1540 (m–s), and 1505 (s) cm^{-1} (CNH).

Anal. Calcd for $C_3H_5ClN_3O_2$: C, 29.03; H, 4.87; N, 25.38. Found: C, 29.11; H, 4.79; N, 25.63.

2-Oxo-1-imidazolidinecarboxamide (3a).[21]—A stirred solution of KOH (155 mg, 85%, min, 2.4 mmoles) and **2** (455 mg, 2.75 mmoles) in 70% aqueous ethanol (10 ml) was refluxed for 40 min. The resulting solution (pH ~7) was chilled in ice, and the small amount of solid (10 mg) that precipitated was removed by filtration. The filtrate was evaporated to dryness *in vacuo* at <40°, and the residue was dried *in vacuo* over P_2O_5 and extracted with three 25-ml portions of acetonitrile. Evaporation *in vacuo* of the extracts and drying *in vacuo* over P_2O_5 left crude **3a** (340 mg), which was triturated in $CHCl_3$ (30 ml); the insoluble solid and that which precipitated on dilution of the filtrate with 30–60° petroleum ether (20 ml) were combined, dried, and further triturated in warm (50–60°) ethanol (~10 ml). The dried insoluble portion (70 mg) and the dried solid (80 mg) that precipitated on dilution of the filtrate with ether (5 ml) and petroleum ether (5 ml) were identical with respect to melting point [195° dec (Mel-Temp) with presoftening from 115°], tlc homogeneity (silica gel H and ethyl acetate), and strong infrared absorption (KBr) at 3360, 3270, and 3210 (NH and NH_2), 1740 and 1675 (CO), and 1580, 1385, and 1270 cm^{-1}; total yield 49%. The precipitated sample was analyzed; the infrared spectra of the analytical sample and the originally isolated crude sample were practically identical.

Anal. Calcd for $C_4H_7N_3O_2$: C, 37.20; H, 5.46; N, 32.55. Found: C, 37.04; H, 5.34; N, 32.58.

3-Nitroso-2-oxo-1-imidazolidinecarboxamide (3b).—Sodium nitrite (50 mg, 0.72 mmole) was added to a cold (5–10°), stirred solution of **3a** (90 mg, 0.70 mmole) in 98–100% HCOOH (0.7 ml). After 0.5 hr at 5–10°, the stirred solution was diluted with ice-cold water (5 ml). During the next 0.5 hr, **3b** precipitated as pale yellow flakes, which were washed with a little cold water and dried *in vacuo* over P_2O_5; yield 40 mg (36%); melting point indefinite (dec); infrared absorption (KBr) at 3405 (s) 3300 and 3240 (NH_2), 1775 and 1700 (s, CO), 1595 (m, amide II), 1385 (s), 1345 (s), and 1150 (s) cm^{-1}; nmr peaks (DMSO-d_6) at δ ~7.6 (2 H, $-NH_2$) and 3.72 (4 H multiplet, CH_2CH_2). Extraction of the filtrate with three 5-ml portions of $CHCl_3$ and *in vacuo* evaporation of the dried ($MgSO_4$) extract produced additional **3b** (10 mg), whose infrared spectrum was identical with that of the larger, analyzed sample; the total yield was 45%.

Anal. Calcd for $C_4H_6N_4O_4$: C, 30.38; H, 3.82; N, 35.44. Found: C, 30.19; H, 4.06; N, 35.09.

Methyl Allophanate (4).—A mixture of **1b** (9.66 g, 66.1 mmoles) and anhydrous methanol (550 ml) was heated to boiling; complete solution occurred after vigorous evolution of N_2 and lightening of the yellow color. The filtered, cooled solution deposited **4** in two crops. Recrystallization of the combined crops (5.41 g)

(20) Melting points for which a range is recorded were determined on a Mel-Temp apparatus; those without a range, on a Kofler Heizbank. The infrared spectra were determined in pressed KBr disks (solids) or films (oils) on a Perkin-Elmer spectrophotometer (either Model 221-G or 521). The nmr spectra were obtained on a Varian A-60 spectrometer; chemical shifts (expressed as δ in parts per million downfield from Me_4Si) were measured from the center of complex multiplets unless otherwise indicated.

(21) Preparation by a different method reported by J. Jankiewicz-Wasowska, *Roczniki Chem.*, **34**, 85 (1960), but the abstract [*Chem. Abstr.*, **54**, 16411*f* (1960)] gives no preparative details or physical constants.

from methanol reduced the yield to 4.77 g (61%), but neither the melting point (205–208°, lit.[4] mp 208°) nor the infrared absorption (KBr) at 3430 (NH), 1745 and 1700 (CO), 1260, and 1235 cm^{-1} (major bands), which compared favorably with that of authentic ethyl allophanate at 3405, 1740, 1705, and 1225 cm^{-1}, were altered.

1,5-Dimethylbiuret (5a).—A mixture of methylurea (46.2 g, 0.624 mole) and methyl isocyanate (70 ml, 1.1 moles), divided equally, was heated (oil bath) in two 100-ml stainless steel bombs at 98–101° for 5 hr. The bombs were cooled, opened, and left overnight to allow evaporation of excess isocyanate. The crystalline residues were combined and recrystallized from acetonitrile; yield of **5a** dried *in vacuo* over P_2O_5, 32.3 g (39.5%); mp 166–168° (lit.[4] 162–163°); strong infrared absorption (KBr) at 3355 and 3315 (NH), 1705 and 1680 (doublet, CO), 1545 (CNH), and 1230 cm^{-1}. (A similar run with 0.178 mole of methylurea produced 12.6 g (54%) of **5a** in 2 crops.)

Anal. Calcd for $C_4H_8N_2O_2$: C, 36.63; H, 6.92; N, 32.05. Found: C, 36.42; H, 6.79; N, 31.77.

1,5-Dimethyl-1-nitrosobiuret (5b).—A solution of $NaNO_2$ (4.55 g, 65.9 mmoles) in water (25 ml) was added dropwise during 1 hr to an ice-cold, stirred solution of **5a** (8.50 g, 64.7 mmoles) in 1.1 *N* HCl (82 ml). After 1.5 hr more, the light yellow precipitate (**5b**) that had formed was collected, washed with cold water, and dried *in vacuo* over P_2O_5; yield 8.13 g (78%), mp 108° dec. A pilot experiment produced the analytical sample; mp 111° (lit.[4] mp 108°); infrared absorption (KBr) at 3335 (NH), 1715 (s) and 1700 (s) (doublet, CO), 1545 (CNH), 1510, and 1490 cm^{-1} (doublet, probably NO).

Anal. Calcd for $C_4H_8N_4O_3$: C, 30.00; H, 5.04; N, 34.99. Found: C, 29.76; H, 5.14; N, 34.65.

1,5-Dimethyl-1,5-dinitrosobiuret (5c).—Sodium nitrite (41.4 g, 0.600 mole) was added in portions during 2 hr to a cold (5°), stirred solution of **5a** (13.1 g, 0.100 mole) in 98–100% HCOOH (90 ml). The mixture was stirred 1 hr, diluted with ice-cold H_2O (200 ml), and stirred at 0–5° 1 hr longer. The yellow precipitate, **5c**, was washed with cold H_2O and dried *in vacuo* over P_2O_5; yield 5.74 g (30%), mp 94° dec. The analytical sample (same melting point) was obtained in a pilot experiment; infrared absorption (KBr) at 3350 (NH), 1790 (s, CO), 1525, 1510, and 1480 cm^{-1} (triplet, CNH and NO).

Anal. Calcd for $C_4H_7N_5O_4$: C, 25.40; H, 3.73; N, 37.03. Found: C, 25.32; H, 4.00; N, 36.55.

1-(2-Chloroethyl)-5-methylbiuret (6a).—Triethylamine (10.4 ml, 74.5 mmoles) was added to a vigorously stirred, cold (<10°) solution of 2-chloroethylamine hydrochloride (8.70 g, 75.0 mmoles) in H_2O (100 ml), and then **5b** (11.9 g, 74.3 mmoles) was added all at once. The frothy mixture was stirred at room temperature for 6 hr. The product was washed with cold water and dried *in vacuo* over P_2O_5; yield 6.95 g (52%), mp 134–136°. The analytical sample was obtained similarly from a pilot experiment in 58% yield; strong infrared absorption (KBr) at 3375 and 3310 (NH), 1705 and 1670 (C=O), 1525 (broad, CNH), and 1220 cm^{-1}.

Anal. Calcd for $C_5H_{10}ClN_3O_2$: C, 33.43; H, 5.61; N, 23.40. Found: C, 33.44; H, 5.46; N, 23.44.

5-(2-Chloroethyl)-1-methyl-1-nitrosobiuret (6b).—Solid $NaNO_2$ (26.6 g, 0.386 mole) was added in portions during 1 hr to an ice-cold, stirred suspension of **6a** (20.9 g, 0.116 mole) in 5 *N* HCl (355 ml) and H_2O (75 ml). The frothy mixture was stirred at 0–5° for an additional 0.5 hr; and the light yellow solid product was washed with H_2O and dried *in vacuo* over P_2O_5; yield 23.4 g (97%), mp 108°. The analytical sample, mp 109°, was obtained in a pilot experiment in which the molar ratio of nitrite to biuret was 1.2:1.0; strong infrared absorption (KBr) at 3345 and 3315 (NH), 1730 and 1705 (C=O), 1540 (CNH), and 1480 cm^{-1} (NO); pmr peaks ($CDCl_3$) at δ ~9.2 (1 H, CONHCO), ~8.4 (1 H multiplet, CON*H*CH_2), 3.75 (4 H, A_2B_2X multiplet, NHCH_2CH_2Cl), and 3.20 (3 H singlet, CH_3).

Anal. Calcd for $C_5H_9ClN_4O_3$: C, 28.79; H, 4.37; N, 26.86. Found: C, 29.13; H, 4.59; N, 26.83.

1,5-Bis(2-chloroethyl)biuret (7a). A. From 6b.—Triethylamine (12.0 ml, 85.6 mmoles) was added to a cold (0–10°), stirred, filtered solution of 2-chloroethylamine hydrochloride (10.0 g, 86.2 mmoles) in water (500 ml), and then **6b** (18.0 g, 85.6 mmoles) was added in portions during 20 min. The mixture was stirred 24 hr at room temperature. The product was collected, washed with water, and dried *in vacuo* over P_2O_5. A small crop was obtained by concentration of the filtrate to ~70 ml. Recrystallization of the combined crude products (13.1 g) from $CHCl_3$–petroleum ether gave 10.9 g (56%) of **7a**, mp 142–144°. The analytical sample, mp 145–146°, was obtained in 72% yield from a small pilot run; infrared absorption (KBr) at 3385 and 3335 (m, NH), 1720 (m) and 1670 (s) (C=O), 1540 (s, CNH), and 1245 (m) cm^{-1}. Over-all yield from **5a** was 26%; pmr peaks (DMSO-d_6) at δ ~8.8 (1 H, CONHCO), ~7.6 (2 H multiplet, CON*H*CH_2), and 3.60 (two identical A_2B_2X multiplets, 4 H each, ClCH_2CH_2NH).

Anal. Calcd for $C_6H_{11}Cl_2N_3O_2$: C, 31.60; H, 4.86; N, 18.42. Found: C, 31.63; H, 4.82; N, 18.53.

B. From 5c.—A cold (5–10°), stirred solution of 2-chloroethylamine (880 mg, 7.46 mmoles) in H_2O (10 ml) was treated first with triethylamine (0.98 ml, 7.0 mmoles) and then with **5c** (610 mg, 3.23 mmoles). The mixture was stirred at room temperature for ~23 hr, and the product was collected, washed with water, and dried *in vacuo* over P_2O_5; yield 410 mg (56%), mp 130–135° dec. The infrared spectrum was identical with that of **7a** prepared from **6b**; a thin layer chromatogram (1:2 benzene–ethyl acetate) detected only a trace of impurity. The over-all yield from **5a** was 22%.

1,5-Bis(2-chloroethyl)-1-nitrosobiuret (7b).—Solid $NaNO_2$ (20.0 g, 290 mmoles) was added in portions during 1.5 hr to a cold (5–10°), stirred solution of **7a** (6.62 g, 29.0 mmoles) in 98–100% HCOOH (60 ml). The resulting yellow mixture was stirred for 0.5 hr, diluted with ice-cold H_2O (250 ml), and stirred for an additional 1 hr at 0–5°. The yellow product, which had separated as an oil, solidified during the final stirring period and was collected, washed with cold H_2O, and dried *in vacuo* over P_2O_5 and NaOH pellets; yield 5.45 g (73%), mp 65°. The analytical sample, mp 69°, was obtained in a pilot experiment (67% yield) in which a 5:1 *M* ratio of nitrite to biuret was used; infrared absorption (KBr) at 3370 (m–w, NH), 1720 and 1695 (s) (C=O), 1540 (m–s, CNH), 1470 (s, NO), 1245 (m), and 1070 (m) cm^{-1}; pmr peaks ($CDCl_3$) at δ ~ 9.2 (1 H, CONHCO), ~8.3 (1 H multiplet, CON*H*CH_2), 4.18, 3.52 (strongest signals of 4 H A_2B_2 pseudo-triplets, $ClCH_2CH_2N(NO)$), and 3.73 (4 H A_2B_2X multiplet, ClCH_2CH_2NH).

Anal. Calcd for $C_6H_{10}Cl_2N_4O_3$: C, 28.03; H, 3.92; N, 21.79. Found: C, 28.23; H, 3.99; N, 21.67.

N-Methyl-2-oxo-1-imidazolidinecarboxamide (8a).—The biuret **6a** (795 mg, 4.45 mmoles) was added to a stirred solution of KOH (250 mg, 85% min, 3.79 mmoles) in 70% aqueous ethanol (10 ml). The resulting solution was refluxed for 30 min, then cooled and poured into ice–water (50 ml), but no precipitation occurred. The solution was evaporated to dryness *in vacuo* at ~35°, and the residue was washed with a minimal volume of cold H_2O and dried *in vacuo* over P_2O_5. Recrystallization of the crude product from $CHCl_3$–petroleum ether (~1:1) gave 160 mg (25%) of **8a**: mp 196–198°; infrared absorption (KBr) at 3240 (m, NH), 1725 (s) and 1645 (m–s) (C=O), 1550 (m–s, CNH), and 1265 (m) cm^{-1}. (A run twice as large in which 1.8 equiv of KOH and 1.5-hr reflux were used gave a 23% yield of **8a**.)

Anal. Calcd for $C_5H_9N_3O_2$: C, 41.95; H, 6.34; N, 29.36. Found: C, 41.81; H, 6.21; N, 29.12.

N-Methyl-3-nitroso-2-oxo-1-imidazolidinecarboxamide (8b).—Solid $NaNO_2$ (140 mg, 2.03 mmoles) was added in two portions during 15 min to a cold (5–10°), stirred solution of **8a** (260 mg, 1.81 mmoles) in 98–100% HCO_2H (1.5 ml). The solution was stirred cold for ~20 min, diluted with water (~6 ml), and stirred at 0–5° for ~20 min. The pale yellow precipitate **8b** was washed with water and dried *in vacuo* over P_2O_5; yield 125 mg (40%); mp 150–154° dec; infrared absorption (KBr) at 3340 (m–s, NH), 1765 and 1695 (s, C=O), 1530 (m–s, CNH), 1465 (m), 1395 (s), 1205, 1180, 1165 and 1145 (s, quadruplet) cm^{-1}; pmr peaks ($CDCl_3$) at δ ~7.8 (1 H multiplet, CH_3NHCO), 3.92 (4 H A_2B_2 multiplet, CH_2CH_2), and 2.98 (3 H doublet, J = 4.8 cps, CH_3NH).

Anal. Calcd for $C_5H_8N_4O_5$: C, 34.88; H, 4.68; N, 32.55. Found: C, 34.72; H, 4.70; N, 32.28.

N-(2-Chloroethyl)-2-oxo-1-imidazolidinecarboxamide (8c).—The biuret **7a** (645 mg, 2.83 mmoles) was added to a solution of KOH (190 mg, 85% min, 2.88 mmoles) in 70% aqueous ethanol (8 ml) at room temperature. The magnetically stirred solution was refluxed for 30 min (oil bath, 85–90°), cooled, and filtered. The collected precipitate was washed with ethanol, and the combined filtrate and washings were evaporated to dryness *in vacuo* at 35–40°. The ethanol-extracted precipitate and the residue were triturated in acetonitrile (30 ml). *In vacuo* evaporation of the filtered acetonitrile solution at ~35° left an off-

white crystalline residue, which was washed with water (10 ml) and dried *in vacuo* over P_2O_5 and NaOH; yield of **8c**, 345 mg (64%); mp 155° (Mel-Temp) with softening from 138°; infrared absorption (KBr) at 3320, 3300, and 3240 (m-w, NH), 1720 (s), 1705 (s), 1650 (m–s), 1635 (m) (C=O), 1540 (s, CNH), 1480, and 1270 (m) cm^{-1}; pmr peaks (DMSO-d_6) at δ ~8.4 (1 H multiplet, NH), ~7.5 (1 H multiplet, NH), and 3.64, 3.58, 3.43 (strongest signals of two overlapping A_2B_2X multiplets, 4 H each, $ClCH_2CH_2NH$ and ring CH_2CH_2).

Anal. Calcd for $C_6H_{10}ClN_3O_2$: C, 37.60; H, 5.22; N, 21.93. Found: C, 37.49; H, 5.23; N, 22.11.

N-(2-Chloroethyl)-3-nitroso-2-oxo-1-imidazolidinecarboxamide (8d).—Solid $NaNO_2$ (270 mg, 3.92 mmoles) was added in portions during 30 min to a cold (5–10°), stirred solution of **8c** (140 mg, 0.730 mmole) in 98–100% HCOOH (1.5 ml). The solution was stirred at 5–10° for 1 hr, diluted with cold H_2O (5 ml), and stirred at 0–5° for 1.75 hr. The yellow precipitate (**8d**) was washed with a little H_2O and dried *in vacuo* over P_2O_5; yield 90 mg; mp 102–105° dec; strong infrared absorption (KBr) at 3350 (NH), 1750 and 1700 (C=O), 1545 (CNH), 1390, and 1215, 1180, and 1150 (triplet) cm^{-1}; pmr peaks ($CDCl_3$) at δ ~8.4 (1 H multiplet, ClH_2NHCO), 3.92 (4 H A_2B_2 multiplet, ring CH_2CH_2), and 3.73 (4 H A_2B_2X multiplet, $ClCH_2$-CH_2NH). Additional **8d** (40 mg), whose infrared spectrum was identical with and melting point lower than that of **8d** which precipitated, was recovered by extraction of the filtrate with three 10-ml portions of chloroform and *in vacuo* evaporation at room temperature of the extract. The total yield was 81%. The higher melting sample was analyzed.

Anal. Calcd for $C_6H_9ClN_4O_3$: C, 32.66; H, 4.11; N, 25.40. Found: C, 32.73; H, 4.28; N, 25.15.

1,3,5-Trimethyl-1,5-dinitrosobiuret (9).—Sodium nitrite (15.9 g, 230 mmoles) was added in increments to a cold, stirred solution of 1,3,5-trimethylbiuret[4] [5.02 g, 34.5 mmoles; mp 125–126° after two recrystallizations from benzene and one from ethyl acetate; infrared absorption (KBr) at 3400 (s) and 3280 (m) (NH), 1710 (s) and 1650 (m) (C=O), and 1515 cm^{-1} (s, CNH)] in formic acid (35 ml), cold H_2O being added midway to aid stirring. More cold H_2O (250 ml) was added 30 min after nitrite addition, and stirring was continued for 1.5 hr. The yellow product was washed with cold H_2O (20 ml) and dried *in vacuo* over P_2O_5; yield 2.28 g (33%); mp 102° (lit.[4] mp 102° dec); infrared absorption (KBr) at 1760 (m) and 1710 (s) (C=O), and 1510 cm^{-1} (m–s, NO); pmr peaks ($CDCl_3$) at δ 3.67 (3 H singlet, $CON(CH_3)CO$) and 3.04 (two identical singlets, 3 H each, $CH_3N(NO)$).

1-Nitrosohydrouracil (10).—Sodium nitrite (14.5 g, 210 mmoles) was added in small portions over a period of 3.5 hr to an ice-cold, stirred suspension of hydrouracil[22] (3.94 g, 34.6 mmoles) in 5 *N* HCl (42 ml, 210 mmoles). The cold mixture was stirred 1 hr longer, and the yellow solid, **10**, was washed with cold H_2O (10 ml) and dried *in vacuo* over NaOH and P_2O_5; yield 4.22 g (84%); mp 142° dec (Mel-Temp); infrared absorption (KBr) at 3200 (w–m) and 3095 (m) (NH), 1745 and 1705 (s, CO), 1385 (s), and 1200 (s) cm^{-1}; pmr peaks (DMSO-d_6) at δ ~11.2 (1 H, CONHCO) and 3.85 and 2.68 (strongest signals of 4 H A_2B_2 pseudo-triplets, $COCH_2CH_2N(NO)$).

Anal. Calcd for $C_4H_5N_3O_3$: C, 33.57; H, 3.52; N, 29.37. Found: C, 33.41; H, 3.55; N, 29.47.

Denitrosation of 1-Nitrosohydrouracil (10).—A suspension of **10** (400 mg, 2.79 mmoles) in water (10 ml) was stirred at room temperature for 6 days. The insoluble solid was collected, washed with water (5 ml), and dried *in vacuo* over P_2O_5. The filtrate and washings were combined and evaporated to dryness *in vacuo* at room temperature. Infrared spectra of both the insoluble solid (140 mg, mp 279–281° dec), which was analyzed, and the residue (180 mg, mp ~275–280° dec) were identical with that of authentic hydrouracil, mp 280°; total yield 100%; infrared absorption (KBr) at 1750 (m–s) and 1695 (s) (CO), 1490 (m–s), 1285 (m–s), and 760 (m–s) cm^{-1}.

Anal. Calcd for $C_4H_6N_2O_2$: C, 42.10; H, 5.30; N, 24.53. Found: C, 41.85; H, 5.17; N, 24.49.

1-Methylbiurea (13).—4-Methylsemicarbazide[23] (5.18 g, 58.2 mmoles) was dissolved in 0.5 *N* HCl (116.5 ml) with stirring and heating, and KOCN (4.84 g, 59.6 mmoles) was added in portions to the solution cooled to room temperature. The mixture, now containing a fluffy white precipitate, was stirred for several hours to ensure complete reaction. Recrystallization of the crude product (6.73 g) from H_2O (~375 ml) gave 5.80 g (75%) of **13**: mp 245–246°; infrared absorption (KBr) at 3410 (m), 3315 (m) and 3210 (m) (NH), 1675 (s) and 1610 (m) (CO), and 1570 (m) cm^{-1} (CNH). (A second crop raised the yield to 80%.)

Anal. Calcd for $C_3H_7N_3O_2$: C, 27.27; H, 5.10; N, 42.41. Found: C, 27.61; H, 6.09; N, 42.47.

Nitrosation of 1-Methylbiurea and *in Situ* Decomposition of the Product with Cyclohexylamine.—A solution of $NaNO_2$ (870 mg, 12.6 mmoles) in H_2O (5 ml) was added dropwise to a cold (0–5°), stirred solution of **13** (550 mg, 4.16 mmoles) in 7 *N* HCl (~12.5 ml). After 45 min, enough cyclohexylamine (~10 ml) was added to the solution to bring the pH to ~9 (the temperature of the mixture rose to 20°). The precipitate that had formed was removed by filtration, washed with H_2O, and dried *in vacuo* over P_2O_5; this material (340 mg, mp 75–77° dec) was indicated by infrared spectral and thin layer chromatographic comparisons to be mainly 3-cyclohexyl-1-methyl-1-nitrosourea (**14**) containing a small amount of 1,3-dicyclohexylurea (**15**). The filtrate, stirred for 24 hr at room temperature, deposited additional **15** (70 mg, mp 227° dec; mp of authentic **15**, 230°), which was identified through its infrared spectrum.

3-Cyclohexyl-1-methyl-1-nitrosourea (14). A solution of $NaNO_2$ (884 mg, 12.8 mmoles) in H_2O (20 ml) was added dropwise to a cold (5°), stirred solution of 1-cyclohexyl-3-methylurea[25] (1.00 g, 6.40 mmoles) in HCOOH (10 ml). The mixture was stirred at 0–5° for 30 min and then diluted with cold H_2O (15 ml). Pale yellow **14** that had precipitated was washed with cold H_2O and dried *in vacuo* over P_2O_5; yield 980 mg (83%); mp 77° dec; infrared absorption (KBr) at 3380 (m, NH), 2940 (m–s) and 2860 (m) (CH), 1705 (s, CO), 1530 (s, CNH), 1475 (m) and 1460 (m) (NO), 1170 (m), and 1000 (m) cm^{-1}.

Anal. Calcd for $C_8H_{15}N_3O_2$: C, 51.87; H, 8.16; N, 22.69. Found: C, 51.85; H, 8.16; N, 22.69.

1,3,6-Trimethylbiurea (16a).—A solution of methyl isocyanate (4.95 g, 86.8 mmoles) in dry $CHCl_3$ (20 ml) was added dropwise to an ice-cold, stirred solution of methylhydrazine (2.00 g, 43.4 mmoles) in the same solvent (40 ml). The solution, allowed to warm to room temperature, was stirred overnight. The copious white precipitate (**16a**) that had formed was collected and dried *in vacuo* over P_2O_5; yield 5.58 g (80%), mp 206–207°. The analytical sample, mp 202–204°, was recrystallized from absolute ethanol; infrared absorption (KBr) at 3345 (m), 3180 (w–m), and 3090 (w–m) (NH), 1700 (m–s) and 1665 (s) (CO), and 1560 (s) and 1540 (s) cm^{-1} (CNH).

Anal. Calcd for $C_5H_{12}N_3O_2$: C, 37.49; H, 7.55; N, 34.98. Found: C, 37.59; H, 7.54; N, 34.78.

1,3,6-Trimethyl-1,6-dinitrosobiurea (16b).—Sodium nitrite (1.14 g, 16.5 mmoles) was added in portions to a cold (5–10°), stirred solution of **16a** (645 mg, 4.02 mmoles) in undiluted HCOOH (10 ml). After 15 min, the solution was diluted with ice-cold H_2O (15 ml) and stirred for 30 min more. The yellow precipitate, **16b**, was washed with cold H_2O (5 ml) and dried *in vacuo* over P_2O_5; yield 90 mg (10%); mp ~119° dec; infrared absorption (KBr) at 3300 (m–s, NH), 1735 (s) and 1715 (s, shoulder) (CO), 1490 (s), 1460 (m–s), 1415 (m–s), and 1000 (m–s) cm^{-1}; pmr peaks ($CDCl_3$) at δ ~9.2 (1 H, NH), 3.52 (3 H singlet, $NHN(CH_3)CO$), and 3.16 (two identical singlets, 3 H each, $CH_3N(NO)$).

Anal. Calcd for $C_5H_{10}N_6O_4$: C, 27.52; H, 4.62; N, 38.52. Found: C, 27.44; H, 4.69; N, 38.66.

Nitrosation of 1,3,6-trimethylbiurea (510 mg, 3.18 mmoles) in 2.5 *N* HCl (10 ml) with $NaNO_2$ (850 mg, 12.3 mmoles) at 0–8° gave 450 mg (65%), mp 75° dec, of yellow dinitroso derivative, whose complex pmr spectrum showed it to be a mixture of **16b** (slightly more than half) and one other isomer, probably 1,4-dinitroso-1,3,6-trimethylbiurea (**16c**).

1,4-Dimethylbiurea (17a).—To a filtered solution of 2-methylsemicarbazide[26] (5.62 g, 63.2 mmoles) in $CHCl_3$ (250 ml), prepared by heating and cooled to room temperature, was added methyl isocyanate (3.60 g, 63.2 mmoles). The cloudy mixture was stirred overnight, diluted with petroleum ether (250 ml),

(22) Mann Research Laboratories, New York, N. Y. 10006.

(23) Prepared as previously described[24] except $CHCl_3$ instead of ether was solvent. The crude product (mp 108–110°, yield 95%) that precipitated was used without further purification.

(24) D. J. Backer, *Rec. Trav. Chim.*, **34**, 187 (1915).

(25) Prepared by addition of methyl isocyanate to cyclohexylamine in cold $CHCl_3$ and recrystallized from MeCN: yield 97%, mp 158° (lit.[3] mp 157–158°).

(26) Prepared by method of E. C. Taylor and K. S. Hartke, *J. Am. Chem. Soc.*, **81**, 2456 (1959), but used without recrystallization from $CHCl_3$.

and cooled. Precipitated **17a** (6.04 g) was recrystallized from ethanol and dried *in vacuo* over P_2O_5; yield 4.21 g (46%); mp 190–191°; infrared absorption (KBr) at 3405 (m–s) and 3210 (m) (NH), 1670 and 1655 (s, CO), 1595 (m), 1440 (m–s), and 1395 (m) cm^{-1}.

Anal. Calcd for $C_4H_{10}N_4O_2$: C, 32.87; H, 6.90; N, 38.34. Found: C, 33.13; H, 6.49; N, 38.68.

1-(2-Chloroethyl)-6-methylbiurea (18a).—2-Chloroethyl isocyanate[27] (4.18 g, 39.6 mmoles) was added dropwise to an ice-cold suspension of 4-methylsemicarbazide[24] (3.50 g, 39.4 mmoles) in dry $CHCl_3$ (175 ml). The mixture was stirred at room temperature for ~24 hr. The white solid present was washed ($CHCl_3$) and dried *in vacuo*, then stirred with dilute HCl and redried; yield of **18a**, 6.19 g (81%); mp 238–239°; infrared absorption (KBr) at 3310 (m–s) and 3215 (m) (NH), 1665 (s, CO), 1565 (s, CNH), 1410 (m), and 1325 (m) cm^{-1}.

Anal. Calcd for $C_5H_{11}ClN_4O_2$: C, 30.89; H, 5.70; N, 28.79. Found: C, 31.06; H, 5.58; N, 29.16.

1,6-Bis(2-chloroethyl)biurea (18b).—2-Chloroethyl isocyanate[27] (7.5 g, 71 mmoles) was added dropwise to an ice-cold solution of 95% hydrazine (1.0 ml, 35.5 mmoles) with immediate precipitation of a white solid. The mixture was stirred at room temperature for ~24 hr; then the precipitate was washed with petroleum ether and dried *in vacuo* over P_2O_5; yield of **18b**, 6.9 g (81%); mp 223–225°; infrared absorption (KBr) at 3325 (m–s) and 3215 (m) (NH), 1660 (s, CO), and 1550 (s) cm^{-1} (CNH).

Anal. Calcd for $C_6H_{12}Cl_2N_4O_2$: C, 29.64; H, 4.98; N, 23.05. Found: C, 29.83; H, 5.02; N, 23.20.

N,N'-Bis(2-chloroethyl)oxamide[28] (19).—Ethylenimine (8.50 ml, 0.164 mole) was added dropwise during 3 hr to a stirred, cold (below −30°) solution of oxalyl chloride[29] (7.00 ml, 0.082 mole) in $CHCl_3$ (170 ml). After a short time, the powdery white precipitate was collected and dried *in vacuo* over P_2O_5. Recrystallization of the crude product (11.0 g) from absolute ethanol (1.5 l.) afforded 6.30 g (36%) of **19**: mp 199–201° (lit.[28] mp 200°); infrared absorption (KBr) at 3295 (s, NH), 1660 (s, CO), 1535 (m–s, amide II), 1440 (m–s), and 1245 (m) cm^{-1}.

N-Methyl-N-nitrosoacetamide.[30]—A solution of N_2O_4[31] in CCl_4 (10 ml containing 0.09 mole of N_2O_4) was added to a cold (−15 to −20°), stirred suspension of anhydrous sodium acetate (14.7 g, 0.180 mole) in CCl_4 (100 ml). At about −6°, a solution of N-methylacetamide (4.00 g, 0.055 mole) in the same solvent (15 ml) was added dropwise to the mixture, which was then stirred between −6 and 2° for 1 hr. The solids were removed by filtration and washed (CCl_4); evaporation of the combined filtrate and washings left an oil (4.3 g), which was taken up in ether and filtered. Evaporation of the ether under reduced pressure and in a stream of N_2 left 2.84 g (51%) of the nitrosoamide as an amber oil: $n^{25}D$ 1.4414 (lit.[19] $n^{25}D$ 1.4415); infrared absorption (film) at 1735 (s, CO), 1500 (m–s), 1115 (m–s), and 930 (m–s) cm^{-1}.

N-(2-Chloroethyl)cyclohexanecarboxamide (20a).—A solution of ethylenimine (2.50 g, 58.2 mmoles) in $CHCl_3$ (10 ml) was added dropwise to a cold (−50°), stirred solution of cyclohexanecarbonyl chloride[29] (8.53 g, 58.2 mmoles) in the same solvent (90 ml). The resulting solution was stirred at ~0° for 2 hr. Removal of the solvent *in vacuo* left a crystalline residue (9.9 g), recrystallization of which from ethanol–H_2O gave 6.65 g (60%) of **20a**: mp 94–95°; infrared absorption (KBr) at 3290 (s, NH), 2935 (s) and 2855 (m–s) (CH), 1640 (s, CO), 1540 (s, amide II), 1440 (m), and 1210 (m) cm^{-1}.

Anal. Calcd for $C_9H_{16}ClNO$: C, 56.98; H, 8.50; N, 7.38. Found: C, 56.90; H, 8.41; N, 7.41.

N,N'-Bis(2-chloroethyl)-N,N'-dinitrosohexanediamide (21b).—Solid $NaNO_2$ (10.3 g, 149 mmoles) was added in small portions at 0.5-hr intervals over a period of 8 hr to a cold (0°, ice–NaCl bath), stirred suspension of **21a**[28,32] (8.30 g, 30.8 mmoles) in glacial acetic acid (40 ml) and acetic anhydride (198 ml). The cold mixture was stirred overnight, allowed to warm slightly, and poured into 400 ml of ice and H_2O. The aqueous mixture was extracted with four 75-ml portions of ether, and the combined extracts were washed with 75-ml portions of 5% $NaHCO_3$ solution (until the washings were basic) and then with two 75-ml portions of H_2O. Evaporation of the Na_2SO_4-dried ethereal layer under reduced pressure left **21b** as yellow flakes, which were further dried *in vacuo* over P_2O_5; yield 9.40 g (93%), mp 50–52°. A small pilot run provided the analytical sample: mp 50–52°; infrared absorption (KBr) at 1730 (s, CO), 1505 (s), 1325 (m–s), 1085 (m–s), 985 (s), and 915 (s) cm^{-1}; ultraviolet maximum (EtOH) at 240 mμ (ε 15,400).

Anal. Calcd for $C_{10}H_{16}Cl_2N_4O_4$: C, 36.71; H, 4.93; N, 17.13. Found: C, 36.91; H, 4.71; N, 17.16.

N,N'-Bis(2-chloroethyl)-*trans*-1,4-cyclohexanedicarboxamide (22a).—A solution of ethylenimine (2.05 ml, 39.6 mmoles) in $CHCl_3$ (20 ml) was added dropwise to a cold (~−40°), stirred solution of *trans*-1,4-cyclohexanedicarbonyl chloride[33] (4.14 g, 19.8 mmoles) in the same solvent (35 ml), a white solid precipitating immediately. The mixture was allowed to warm gradually to room temperature and was stirred overnight. The collected product (4.83 g), washed ($CHCl_3$) and dried *in vacuo* over P_2O_5, was recrystallized from absolute ethanol; the yield of **23a**, mp 256–259° dec, was 2.98 g (51%); infrared absorption (KBr) at 3285 (s, NH), 2960 (m, CH), 1635 (s, CO), 1535 (s, amide II), 1440 (m), 1240 (m–s), and 1200 (m–s) cm^{-1}.

Anal. Calcd for $C_{12}H_{20}Cl_2N_2O_2$: C, 48.81; H, 6.83; N, 9.49. Found: C, 48.96; H, 6.70; N, 9.41.

N,N'-Bis(2-chloroethyl)-N,N'-dinitroso-*trans*-1,4-cyclohexanedicarboxamide (22b).—Solid $NaNO_2$ (14.1 g, 205 mmoles) was added in portions at 0.5-hr intervals over a period of 4 hr to a cold (0°), stirred suspension of **22a** (3.53 g, 11.9 mmoles) in glacial acetic acid (35 ml) and acetic anhydride (175 ml). The resulting mixture was stirred at ~0° overnight and then poured into 175 ml of ice and H_2O. The product was deposited as yellow plates, which were washed well with H_2O and dried *in vacuo* over P_2O_5; yield 2.65 g (62%), mp 113–114° dec. The analytical sample (mp 115–116° dec) was recrystallized from ether–hexane; infrared absorption (KBr) at 1730 (s, CO), 1485 (s), 1435 (m), 1085 (m), 995 (m), 935 (s), and 780 (m–s) cm^{-1}.

Anal. Calcd for $C_{12}H_{18}Cl_2N_4O_4$: C, 40.80; H, 5.14; N, 15.86. Found: C, 40.99; H, 5.40; N, 16.00.

Acknowledgments.—The authors are indebted to Mr. G. S. McCaleb for helpful advice and discussion and for the preparation of **14**, to Dr. J. A. Montgomery for encouragement throughout this work, to Mrs. Sarah Jo Clayton for resynthesis of some of the intermediates, to Mrs. Martha Thorpe for determination and interpretation of pmr spectra, and to Dr. W. R. Laster and members of the Cancer Screening Division of Southern Research Institute for L1210 screening data.

(27) W. Siefkin, *Ann. Chem.*, **562**, 75 (1949).

(28) H. Bestian, *ibid.*, **566**, 210 (1950).

(29) Distillation Products Industries, Rochester, N. Y. 14603.

(30) Prepared according to modification of general method of M. Murakami, K. Akagi, and Y. Mori, *Bull. Chem. Soc. Japan*, **35**, 11 (1962), who did not characterize product.

(31) The Matheson Co., East Rutherford, N. J. 07073

(32) J. M. Z. Gladych and E. P. Taylor, *J. Chem. Soc.*, 1481 (1962).

(33) From *trans*-1,4-cyclohexanedicarboxylic acid (Aldrich Chemica Co.) by method of R. Malachowski, J. J. Wasowska, S. Jóźkiewicz, J. Adamiczka, and G. Zimmerman-Pasternak, *Ber.*, **71**, 759 (1938).