The Synthesis of Potential Anticancer Agents. XXXVI. N-Nitrosoureas.[1] II. Haloalkyl Derivatives

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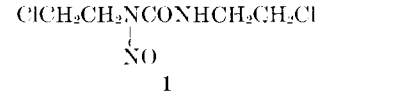
The synthesis, chemical properties, and structure of numerous congeners of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), an experimentally important anticancer agent, have been investigated, and structure–activity relationships have been established with respect to intraperitoneally and intracerebrally inoculated L1210 mouse leukemia. Structural modifications include variation of halogen, alkyl branching, and introduction of variously substituted cycloaliphatic, aromatic, and heterocyclic groups. Decomposition with amines as a method of determining the position of nitrosation in nitroso derivatives of unsymmetrical 1,3-disubstituted ureas has been complemented principally by pmr spectroscopy. The effect of steric factors and aqueous dilution of the nitrosating medium (formic acid) on isomer ratios in the nitrosation of 2-(haloethyl)ureas having certain cyclic substituents has been demonstrated, as well as relative lability of certain nitrosoureas in undiluted formic acid. Screening data indicate that the most active nitrosoureas so far evaluated against both the intraperitoneal and intracerebral disease are 1-[2-(chloro or fluoro)ethyl]-1-nitrosoureas substituted in the 3 position by a 2-(chloro or fluoro)ethyl or cycloaliphatic group. A few exceptions to this generalization were noted.

In previous reports,[2] the somewhat random investigation of congeners of 1-methyl-1-nitrosourea that led to the experimentally and perhaps clinically useful antileukemic agent, 1,3-bis(2-chloroethyl)-1-nitrosourea (**1**, BCNU),[3] was described. The efficiency and



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reproducibility of the activity shown by BCNU against both intraperitoneally and intracerebrally implanted L1210 leukemia in mice have made investigation of the kinetics of leukemic-cell kill possible and have led to a restatement of the criteria associated with curability of experimental leukemia,[4] which is now being extrapolated to the therapy of human neoplasms. These findings and concepts have accelerated interest in structural modifications of BCNU and related N-nitrosoureas that might meliorate the insidious effects of delayed toxicity,[3d,5] but retain intracerebral activity.[2b,6] This continuing search has led to the synthesis of a number of new haloalkylureas (Table I), many of which have been converted to the corresponding nitroso derivatives (Table II); analogs of these compounds having substituents other than halogen will be reported separately. The methods of synthesis are in general based on those previously employed.[2a]

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(2) (a) T. P. Johnston, G. S. McCaleb, and J. A. Montgomery, *ibid.*, **6**, 669 (1963); (b) 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).

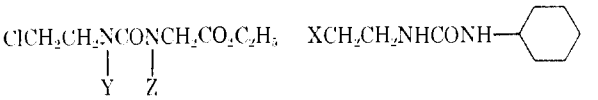
(3) (a) A. Goldin, J. M. Venditti, J. A. R. Mead, and J. P. Glynn, *Cancer Chemotherapy Rept.*, **40**, 57 (1964); (b) B. A. Nies, L. B. Thomas, and E. J. Freireich, *Cancer*, **18**, 546 (1965); (c) J. Hananian, J. F. Holland, and P. Sheehe, *Proc. Am. Assoc. Cancer Res., 56th, Philadelphia, Pa., 1965*, **6**, 26 (1965); (d) 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. E. Skipper, F. M. Schabel, Jr., and W. S. Wilcox, *Cancer Chemotherapy Rept.*, **35**, 1 (1964).

(5) D. P. Ridl, M. Ben, and D. M. McCarthy, *Proc. Am. Assoc. Cancer Res., 5th, Toronto, 1963*, **4**, 55 (1963).

(6) M. A. Chirigos, S. R. Humphreys, and A. Goldin, *Cancer Chemotherapy Rept.*, **49**, 15 (1965).

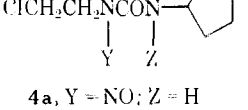
Chemical Properties and Structure.—The preponderance of unsymmetrical 1,3-disubstituted ureas among the ureas synthesized necessitated cautious assignment of structure to the corresponding nitroso derivatives. Rationalization based on relative nucleophilicity of the urea nitrogen atoms can lead to erroneous structural assignments as in the case of the previously reported ethyl 5-(2-chloroethyl)-5-nitrosohydantoate[2a] (**2a**), whose structure became suspect because of a low degree of L1210 activity. Reaction of the nitrosohydantoate with cyclohexylamine in water resulted in the isolation of a 30% yield of 1-(2-chloroethyl)-3-cyclohexylurea (**3a**), which could have formed only from ethyl 5-(2-chloroethyl)-3-nitrosohydantoate (**2b**); further confirmation of structure **2b** was later obtained by pmr spectroscopy.



2a, Y = NO; Z = H
b, Y = H; Z = NO

3a, X = Cl
b, X = Br
c, X = F

Isolation and identification of ureas as decomposition products of nitrosoureas constitute proof of homogeneity only if a single product is isolated in nearly theoretical yield; detection of two products indicates an isomeric mixture of nitrosoureas, but the isolation of a single product corresponding to one isomer in less than theoretical yield does not eliminate the presence of the other isomer. For example, the decomposition of a mixture of 1-(2-chloroethyl)-3-cyclopentyl-N-nitrosoureas with ammonium hydroxide resulted in the isolation of cyclopentylurea in 73% yield, which indicated that at least 73% of the mixture was 1-(2-chloroethyl)-3-cyclopentyl-1-nitrosourea (**4a**). A subsequent



4a, Y = NO; Z = H
b, Y = H; Z = NO

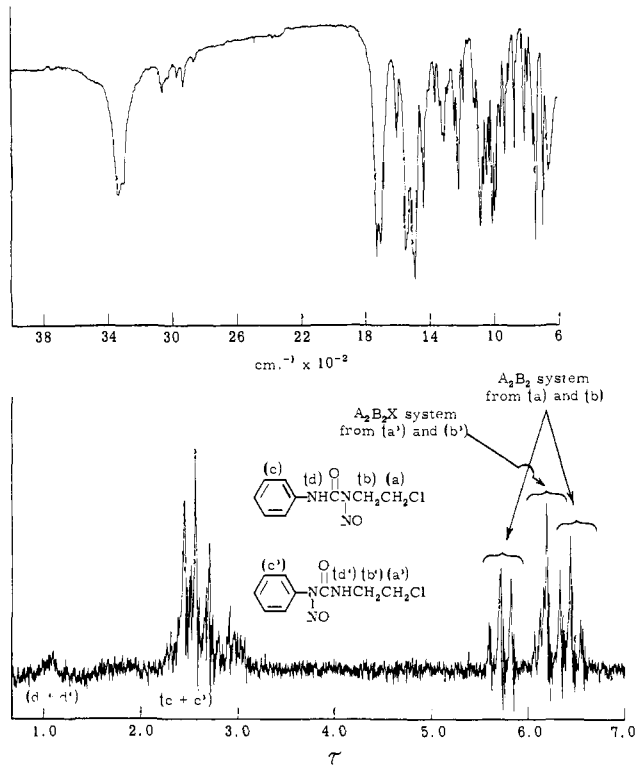


Figure 1.—Infrared (in KBr disk) and pmr [in 10% (w/v) chloroform-*d* solution at 60 Mc/sec] spectra of the product from nitrosation of 1-(2-chloroethyl)-3-phenylurea in aqueous formic acid.

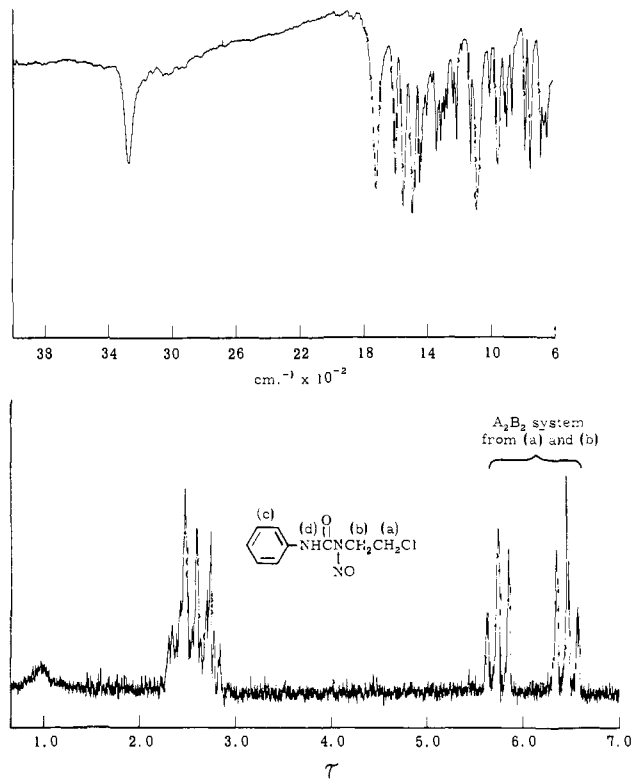
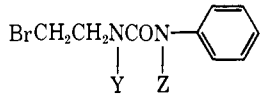


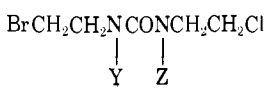
Figure 2.—Infrared (in KBr disk) and pmr [in 10% (w/v) chloroform-*d* solution at 60 Mc/sec] spectra of the product from nitrosation of 1-(2-chloroethyl)-3-phenylurea in undiluted formic acid.

examination of the pmr spectrum revealed that the ratio of **4a** to the isomer **4b** was roughly 3:1.

Clear-cut separation of isomeric pairs of nitrosoureas by thin layer chromatography has been observed only in the case of the 1-(2-bromoethyl)-N-nitroso-3-phenylureas **5a** and **5b**, but several mixtures of a nitrosourea and the corresponding unnitrosated urea, as well as mixtures of products of decomposition of nitrosoureas with amines, have been so separated and identified. Thus, thin layer chromatography showed the isolated product of the reaction of 1-(2-bromoethyl)-3-(2-chloroethyl)-N-nitrosourea with cyclohexylamine in water to be a mixture of 1-(2-bromoethyl)-3-cyclohexylurea (**3b**) and **3a** as would be expected from the isomeric pair **6a** and **6b**; pmr data indicated a composition of approximately equal parts of **6a** and **6b**.

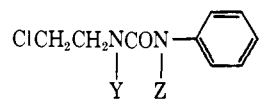


5a, Y = NO; Z = H
b, Y = H; Z = NO



6a, Y = NO; Z = H
b, Y = H; Z = NO

Since nitrosation of a ureido function causes a shift of carbonyl absorption in the infrared to a higher wavenumber,[2a] the completeness of nitrosation can often be established by infrared spectroscopy, and in some instances doublet absorptions (both C=O and NH) are definitely indicative of an isomeric mixture (*cf.* Figures 1 and 2). The most generally useful means of establishing the isomeric purity of a nitrosourea, however, has been pmr spectroscopy. Distinction between RNH and RN(NO) groups was first made in the spectrum of BCNU, the asymmetry of the central portion of which is attributed to NH coupling of the adjacent methylene group since A_2B_2 symmetry is effected (graphic resolution) by deuterium oxide in dimethyl sulfoxide-d_6. Complete deuterium exchange of the NH protons of 1,3-bis(2-chloroethyl)urea, which required a period of about 26 hr, resulted in an A_2B_2 symmetry that could be observed without graphic resolution. This type of coupling has previously been noted in the spectra of certain carboxanides.[7] Spectral asymmetry of the $ClCH_2CH_2NH$ group (A_2B_2X system) and symmetry of the $ClCH_2CH_2N(NO)$ group (A_2B_2 system) are clearly seen in Figures 1 and 2. The composition of the mixture (Figure 1), which was previously reported as pure **7a**,[2a] is estimated by integral ratios to be 60% 1-(2-chloroethyl)-1-nitroso-3-phenylurea (**7a**) and 40% 3-(2-chloroethyl)-1-nitroso-1-phenylurea (**7b**).



7a, Y = NO; Z = H
b, Y = H; Z = NO

The unique F^{19} splitting pattern as seen in the spectra of 2-fluoroethylamines[8] complicates the spectra of 2-fluoroethylnitrosoureas, but the principle of analysis is the same as with simpler spectra. Singlet absorption of the $CH_3N(NO)$ group characterizes all the methylnitrosoureas studied and hence confirms certain tenuous assignments previously based on low yields

(7) L. Skulski, G. C. Palmer, and M. Calvin, *Tetrahedron Letters*, 1773 (1963).

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

TABLE I

(HALOALKYL)UREAS

R	Method[a]	Recrystn solvent[b]	Yield,[c] %	Mp,[d] °C	ν(KBr, cm⁻¹)[e] CO	ν(KBr, cm⁻¹)[e] CNH	Formula	%C Calcd	%C Found	%H Calcd	%H Found	%N Calcd	%N Found
A. (2-Fluoroethyl)ureas, $RNHCONHCH_2CH_2F$, and Bis(2-fluoroethyl)ureas, $FCH_2CH_2NHCONHRNHCONHCH_2CH_2F$													
H	A	Acetonitrile–ether	58–87	83–86	1660	1550	$C_3H_7FN_2O$	33.95	33.88	6.65	6.46	26.40	26.53
Methyl	Ba1	Chloroform–petr ether	81	78–80	1630	1600	$C_4H_9FN_2O$	39.99	39.77	7.55	7.44	23.32	23.11
2-Fluoroethyl	Ca	Ethanol–petr ether	53–87	139–141	1630	1590	$C_5H_{10}F_2N_2O$	39.47	39.74	6.63	6.86	18.42	18.43
2-Chloroethyl	Ba2	Chloroform–petr ether	30–35	95–96	1625	1590	$C_5H_{10}ClFN_2O$	35.62	35.31	5.98	5.76	16.62	16.60
Cyclohexyl	Ba3	Chloroform–petr ether	68	123–125	1630	1590	$C_9H_{17}FN_2O$	57.42	57.50	9.11	9.27	14.88	14.76
trans-4-*t*-Butylcyclohexyl	Ca	Acetonitrile–water	32–40	144	1630	1510	$C_{13}H_{25}FN_2O$	63.90	63.92	10.32	10.41	11.46	11.70
2-Norbornyl	Ca	Acetonitrile–water	41	120–122	1625	1565	$C_{10}H_{17}FN_2O$	59.97	60.15	8.56	8.52	13.99	14.01
1-Adamantyl	Cb	Acetonitrile	63–79	212	1620	1555	$C_{13}H_{21}FN_2O$	64.93	65.06	8.81	8.57	11.65	11.76
5α-Cholestan-3α-yl	Ca	Acetonitrile–ethanol	53–96	242–244	1630	1575	$C_{30}H_{53}FN_2O$	75.58	75.61	11.21	11.08	5.88	5.91
Phenyl	Ba2	Ether–petr ether	50	144–145	1635	1570	$C_9H_{11}FN_2O$	59.33	59.61	6.09	6.05	15.38	15.41
trans-1,4-Cyclohexylene	Ca		86	>280 dec	1625	1565	$C_{12}H_{22}F_2N_4O_2$	49.30	49.43	7.59	7.48	19.17	19.11
p-Phenylene	Ba4		92	270–273 dec	1635	1575	$C_{12}H_{16}F_2N_4O_2$	50.34	50.56	5.63	5.54	19.57	19.45
B. (2,2,2-Trifluoroethyl)ureas, $RNHCONHCH_2CF_3$													
2,2,2-Trifluoroethyl	D		65	157[f]	1645	1595	$C_5H_6F_6N_2O$						
2-Chloroethyl	Ba3	Benzene–cyclohexane	62–85	116	1635	1580	$C_5H_8ClF_3N_2O$	29.35	29.63	3.94	4.06	13.70	13.71
Cyclohexyl	Ba2		41	142–144	1630	1580	$C_9H_{15}F_3N_2O$	48.20	48.49	6.75	6.90	12.50	12.70
C. (2-Chloroethyl)ureas, $RNHCONHCH_2CH_2Cl$, and Bis(2-chloroethyl)ureas, $ClCH_2CH_2NHCONHRNHCONHCH_2CH_2Cl$													
t-Butyl	Bb2		85	107–108	1630	1560	$C_7H_{15}ClN_2O$	47.06	47.25	8.46	8.33	15.68	15.77
Cyanomethyl	Bb5		16	94	1630	1575	$C_5H_8ClN_3O$	37.17	37.23	5.00	4.85	26.00	26.09
2-Bromoethyl	Bb5		77	103–104[g]	1630	1585	$C_5H_{10}BrClN_2O$					12.21	12.14
2-Cyanoethyl	Bb3	Ethanol	67–98	125	1635	1585	$C_6H_{10}ClN_3O$	41.03	41.15	5.74	5.71	23.93	24.03
$H_2NCOCH_2CH_2CH(CO_2H)$	Bb1[h]		41	153–155	1720[i]	1560	$C_8H_{14}ClN_3O_5$	38.18	37.78	5.61	5.40	16.70	16.48
$HO_2CCH(NH_2)(CH_2)_4$	Bb1[j]		40	~200	1620	1575	$C_9H_{18}ClN_3O_3$	42.94	42.64	7.21	7.12	16.70	16.42
$CH_3O_2CCH_2CH_2CH(CO_2CH_3)$	Ba5		58	75	1745[k]	1575	$C_{10}H_{17}ClN_2O_5$	42.78	42.86	6.11	5.91	9.98	10.04
2,6-Dioxo-3-piperidyl	Bb4	Acetonitrile	56–91	180–181	1710[l]	1580	$C_8H_{12}ClN_3O_3$	41.12	41.29	5.18	5.35	17.98	17.90
Cyclopentyl	Bb3	Acetonitrile	68	115	1630	1590	$C_8H_{15}ClN_2O$	50.39	50.35	7.94	7.77	14.70	14.82
1-Methylcyclopentyl	Bb2	Acetonitrile–water	64	92	1635	1575	$C_9H_{17}ClN_2O$	52.81	53.06	8.37	8.39	13.69	13.62
1-Ethoxycarbonylcyclopentyl	Bb2		84	140	1720[m]	1565	$C_{11}H_{19}ClN_2O_3$	50.28	50.40	7.29	7.35	10.66	10.59
Cyclohexyl	Bb3		74	130[n,o]	1635	1595	$C_9H_{17}ClN_2O$						
1-Methylcyclohexyl	Bb2	Cyclohexane	87	100	1630	1560	$C_{10}H_{19}ClN_2O$	54.84	54.93	8.72	8.77	12.81	12.67
2-Methylcyclohexyl	Bb3	Acetonitrile–water	19	70	1620	1565	$C_{10}H_{19}ClN_2O$	54.84	54.61	8.72	8.59	12.81	12.63
3-Methylcyclohexyl	Bb3	Acetonitrile	31	132–134	1620	1585	$C_{10}H_{19}ClN_2O$	54.84	55.08	8.72	8.63	12.81	12.75
4-Methylcyclohexyl	Bb2		59	140	1625	1580	$C_{10}H_{19}ClN_2O$	54.84	54.94	8.72	8.75	12.81	12.90
3,3,5-Trimethylcyclohexyl	Bb2	Hexane	45–73	91	1625	1580	$C_{12}H_{23}ClN_2O$	58.41	58.55	9.40	9.59	11.35	11.34
trans-4-*t*-Butylcyclohexyl	Bb2		85	133–134	1625	1570	$C_{13}H_{25}ClN_2O$	59.87	60.07	9.67	9.44	10.74	10.79
cis-2-Chlorocyclohexyl	Bb2	Benzene–cyclohexane	84–97	130	1620	1560	$C_9H_{16}Cl_2N_2O$	45.20	45.28	6.74	6.54	11.71	11.78
trans-2-Chlorocyclohexyl	Bb2		78	136	1630	1580	$C_9H_{16}Cl_2N_2O$	45.20	45.32	6.74	6.84	11.71	11.74
cis-2-Hydroxycyclohexyl	Bb2		65	135	1655		$C_9H_{17}ClN_2O_2$	48.98	49.10	7.73	7.58	12.69	12.75
1-Ethoxycarbonylcyclohexyl	Bb2	Acetonitrile–water	84	81–83	1735[p]	1565	$C_{12}H_{21}ClN_2O_3$	52.07	52.03	7.65	7.70	10.12	9.96
4-Ethoxycarbonylcyclohexyl	Bb2		81	85–87	1735[o]	1560	$C_{12}H_{21}ClN_2O_3$	52.05	52.15	7.65	7.51	10.12	10.30
1-Phenylcyclohexyl	Bb2		94	130	1635	1560	$C_{15}H_{21}ClN_2O$	63.98	64.32	7.54	7.93	9.98	10.04
2-Norbornyl	Bb2	Benzene	73	126	1630	1575	$C_{10}H_{17}ClN_2O$	55.42	55.59	7.91	7.74	12.93	12.94
2-Bornyl	Bb2	Ethanol–water	30	138	1625	1560	$C_{13}H_{23}ClN_2O$	60.33	60.44	8.96	8.80	10.83	10.87
2-Oxo-3-bornyl	Bb2		43	124–125	1745[q]	1560	$C_{13}H_{21}ClN_2O_2$	57.25	57.04	7.76	7.58	10.27	10.24

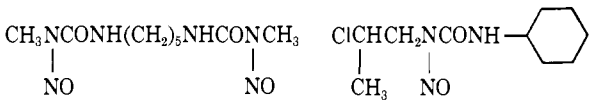
1-Adamantyl	Bb2		91	202	1625	1560	$C_{18}H_{21}ClN_2O$	60.80	60.79	8.25	8.28	10.91	10.87
Cycloheptyl	Bb3		87–97	109	1625		$C_{10}H_{19}ClN_2O$	54.91	54.70	8.76	8.58	12.81	12.71
Cyclooctyl	Bb3		89	83	1625	1575	$C_{11}H_{21}ClN_2O$	56.76	57.02	9.09	9.08	12.04	11.85
Cyclododecyl	Bb3	Ethanol–water	81	138	1620	1565	$C_{15}H_{29}ClN_2O$	62.37	62.13	10.12	9.88	9.70	9.66
5α-Cholestan-3α-yl	Bb2		91	228–230	1625	1560	$C_{30}H_{53}ClN_2O$	73.05	73.01	10.84	10.86	5.68	5.61
2-Indanyl	Bb2		99	129	1620		$C_{12}H_{15}ClN_2O$	60.33	60.29	6.34	6.31	11.73	11.73
p-Fluorophenyl	Ba5	Benzene	65–70	138	1630	1575	$C_9H_{10}ClFN_2O$	49.89	49.73	4.64	4.60	16.37	16.50
o-Chlorophenyl	Ba5		85	145	1630	1565	$C_9H_{10}Cl_2N_2O$	46.37	46.69	4.33	4.44	12.03	11.93
m-Chlorophenyl	Ba5		86	110	1640	1560	$C_9H_{10}Cl_2N_2O$	46.37	46.75	4.33	4.48	12.03	12.21
2,5-Dichlorophenyl	Bb3	Ethanol	24	204–205	1640	1560	$C_9H_9Cl_3N_2O$	40.40	40.66	3.39	3.56	39.76[r]	40.10[r]
4-Amino-3,5-dichlorophenyl	Bb3	Ethanol–water	75	169–171 dec	1620	1575	$C_9H_{10}Cl_2N_3O$	38.25	38.50	3.57	3.58	14.87	15.03
o-Tolyl	Ba3	Ethanol	54	152	1630	1565	$C_{10}H_{13}ClN_2O$	56.61	56.55	6.18	6.19	13.21	13.23
α,α,α-Trifluoro-*p*-tolyl	Bb3	Benzene	68–92	138	1645	1560	$C_{10}H_{10}ClF_3N_2O$	45.05	45.08	3.78	3.98	13.30[r]	13.30[r]
2,6-Xylyl	Bb3	Benzene	67	167[s]	1635	1570	$C_{11}H_{15}ClN_2O$	58.28	58.51	6.67	6.64	12.29	12.33
m-Nitrophenyl	Bb6	Ethanol	50	158	1655	1550	$C_9H_{10}ClN_3O_3$	44.37	44.47	4.14	4.19	17.24	17.31
m-Methoxyphenyl	Bb3	Benzene	73	109	1630	1580	$C_{10}H_{13}ClN_2O_2$	52.53	52.28	5.73	5.84	12.25	12.29
p-Cyanophenyl	Ec1	Ethanol–water	75	150	1675	1550	$C_{10}H_{10}ClN_3O$	53.70	53.87	4.51	4.54	18.79	19.04
o-Carboxyphenyl	Bb2		90	171	1670[t]	1545	$C_{10}H_{11}ClN_2O_3$	49.49	49.60	4.57	4.64	11.55	11.59
m-Carboxyphenyl	Bb3	Ethanol–water	40–75	234	1700[u]	1570	$C_{10}H_{11}ClN_2O_3$	49.49	49.48	4.57	4.62	11.55	11.34
p-Carboxyphenyl	Bb3		36	228–230	1680[u]	1570	$C_{10}H_{11}ClN_2O_3$	49.49	49.48	4.57	4.60	11.55	11.55
p-Ethoxycarbonylphenyl	Bb3	Benzene	67–99	141	1710[u]	1570	$C_{12}H_{15}ClN_2O_3$	53.24	53.26	5.59	5.46	10.35	10.37
p-Acetylphenyl	Bb5	Chloroform–hexane	29	165[v]	1695[w]	1590	$C_{11}H_{13}ClN_2O_2$						
p-(Dimethylcarbamoyl)phenyl	Bb3		42–88	152	1705[t]	1545	$C_{12}H_{16}ClN_3O_2$	53.43	53.72	5.98	6.04	15.58	15.48
p-(Methylthio)phenyl	Bb3	Benzene	69–76	136	1630	1585	$C_{10}H_{13}ClN_2OS$	49.07	49.33	5.35	5.48	11.45	11.38
m-(Fluorosulfonyl)phenyl	Bb3	Ethanol–water	39	104	1655	1560	$C_9H_{10}ClFN_2O_3S$	38.51	38.74	3.59	3.67	9.98	9.84
p-(Fluorosulfonyl)phenyl	Bb3	Benzene	13	141	1655	1555	$C_9H_{10}ClFN_2O_3S$	38.51	38.90	3.59	3.55	9.98	10.02
p-(Carboxymethyl)phenyl	Bb3[x]	Acetonitrile	52	174–176	1700[y]	1570	$C_{11}H_{13}ClN_2O_3$	51.47	51.67	5.10	5.05	10.92	11.04
p-(Carboxymethylthio)phenyl	Bb3[x]	Ethanol–water	64–92	152	1705[y]	1570	$C_{11}H_{13}ClN_2O_3S$	45.76	45.77	4.54	4.40	9.70	9.62
p-(3-Carboxypropyl)phenyl	Bb6	Ethanol	64–83	181	1695[y]	1575	$C_{13}H_{17}ClN_2O_3$	54.83	54.97	6.02	5.97	9.84	9.87
5,6,7,8-Tetrahydro-2-naphthyl	Bb5	Acetonitrile	57	190	1635	1570	$C_{13}H_{17}ClN_2O$	61.78	61.83	6.78	6.76	11.09	11.12
2-Naphthyl	Bb3	Ethanol	78–90	181–183	1625	~1580	$C_{13}H_{13}ClN_2O$	62.77	62.80	5.27	5.22	11.26	11.26
3-Pyridyl	Bb2	Acetonitrile	60–90	155	1665	1560	$C_8H_{10}ClN_3O$	48.13	48.17	5.05	4.85	21.05	21.03
1,2,3,4-Tetrahydro-2,4-dioxo-5-pyrimidinyl	Bb4		88	>260 dec	1745[z]	1580	$C_7H_9ClN_4O_3$	36.15	35.87	3.90	3.92	24.10	24.13
8-Quinolyl	Bb3		75	161	1645	1555	$C_{12}H_{12}ClN_3O$	57.72	57.83	4.85	4.88	16.83	16.66
9-Acridinyl	Bb6	1-Propanol	18	163	1630	1545	$C_{16}H_{14}ClN_3O$	64.10	64.19	4.71	4.71	14.02	13.83
trans-Vinylene[aa]	Ba5		73	195 dec	1635	1590	$C_8H_{14}Cl_2N_4O_2$	35.70	35.86	5.25	5.03	26.34[r]	26.40[r]
trans-1,2-Cyclohexylene	Bb2	Ethanol	86	250	1630	1595	$C_{12}H_{22}Cl_2N_4O_2$	44.31	44.25	6.82	6.94	17.28	17.18
trans-1,4-Cyclohexylene	Bb3		66	265–266 dec	1625	1570	$C_{12}H_{72}Cl_2N_4O_2$	44.31	44.26	6.82	6.90	17.28	16.97
1,8-*p*-Menthylene	Bb3	Acetonitrile–water	13–87	163	1635	1560	$C_{16}H_{30}Cl_2N_4O_2$	50.40	50.34	7.93	7.86	14.70	14.67
o-Phenylene	Bb3	Ethanol	52–93	200	1635	1575	$C_{12}H_{16}Cl_2N_4O_2$	45.15	44.94	5.06	5.06	22.22[r]	22.40[r]
4-Chloro-*o*-phenylene	Bb5	Ethanol–water	38	163–165	1655	1550	$C_{12}H_{15}Cl_3N_4O_2$	40.75	40.94	4.28	4.19	15.84	15.78
4-Methoxy-*m*-phenylene	Bb5	Ethanol–water	19–68	167–169	1630	1560	$C_{13}H_{18}Cl_2N_4O_3$	44.71	44.84	5.20	4.99	16.05	16.20
5-Carboxy-*m*-phenylene	Bb4	Dimethylformamide–water	64	>270 dec	1700[w]	1560	$C_{13}H_{16}Cl_2N_4O_4$	43.00	43.41	4.44	4.73	15.43	15.38
p-Phenylene	Bb4		75	260–262	1635	1595	$C_{12}H_{16}Cl_2N_4O_2$	45.15	45.50	5.06	4.70	17.55	17.75
Tetramethyl-*p*-phenylene	Bb3		56–78	>350	1625	1560	$C_{12}H_{24}Cl_2N_4O_2$	51.20	51.14	6.44	6.41	14.93	14.48
4,4'-Biphenylylene	Bb4	Dimethylformamide–ethanol	42–66	>260	1640	1560	$C_{18}H_{20}Cl_2N_4O_2$	54.69	54.81	5.10	5.24	14.18	14.04
Methylenedi-*p*-phenylene	Ed1		90	130 dec	1640	1575	$C_{19}H_{22}Cl_2N_4O_2$	55.75	56.17	5.42	5.62	13.69	13.81
Oxydi-*p*-phenylene	Bb4	Ethanol	49–87	245 dec	1640	1555	$C_{18}H_{20}Cl_2N_4O_3$	52.56	52.56	4.90	4.96	13.62	13.70

TABLE I (*Continued*)

R	Method[a]	Recrystn solvent[b]	Yield,[c] %	Mp,[d] °C	ν(KBr, cm⁻¹)[e] CO	CNH	Formula	%—Carbon— Calcd	Found	%—Hydrogen— Calcd	Found	%—Nitrogen— Calcd	Found
Dithiodi-*p*-phenylene	Bb3	Dimethylformamide–ethanol	61–99	242	1630	1585	$C_{18}H_{20}Cl_2N_4O_2S_2$	47.06	47.34	4.39	4.47	12.70	12.25
2,6-Pyridinediyl	Bb3 5	Ethanol	30	171–174[dd]	1680[w]	1555	$C_{11}H_{15}Cl_2N_5O_2$	41.26	41.43	4.72	4.68	21.88	22.00
D. (2-Chloropropyl)ureas, RNHCONHCH$_2$CH(CH$_3$)Cl													
2-Chloropropyl	Fa	Benzene–petr ether	~33	~105	1630	1570	$C_7H_{14}Cl_2N_2O$	39.45	39.08	6.63	6.50	13.14	13.15
Cyclohexyl	Bb2	Acetonitrile–water	~31	131	1625	1565	$C_{10}H_{19}ClN_2O$	54.98	54.84	8.76	8.64	12.81	12.76
E. [1-(Chloromethyl)propyl]ureas, RNHCONHCH(CH$_2$CH$_3$)CH$_2$Cl													
1-(Chloromethyl)propyl	Fa		~47	123	1630	1565	$C_9H_{18}Cl_2N_2O$	44.82	45.02	7.52	7.61	11.62	11.68
Cyclohexyl	Bb3	Acetonitrile–water	~15	129	1620	1560	$C_{11}H_{21}ClN_2O$	56.86	56.87	9.09	9.00	12.04	12.18
F. 1,3-Bis(2-chloro-1,1-dimethylethyl)urea, RNHCONHC(CH$_3$)$_2$CH$_2$Cl													
2-Chloro-1,1-dimethylethyl	Fa	Acetonitrile–water	~44	126	1635	1565	$C_9H_{18}Cl_2N_2O$	44.82	44.56	7.52	7.52	11.62	11.51
G. 1-(2-Chloro-2-methylpropyl)-3-cyclohexylurea, RNHCONHCH$_2$C(CH$_3$)$_2$Cl													
Cyclohexyl	Eb2	Acetonitrile–water	67	195	1625	1570	$C_{11}H_{21}ClN_2O$	56.76	56.83	9.09	8.94	12.04	12.02
H. *cis*-(2-Chlorocyclohexyl)ureas, RNHCONHCH(CH$_2$)$_4$CHCl-*cis*													
Methyl	Ba2	Benzene–hexane	75	137	1625	1580	$C_8H_{15}ClN_2O$	50.39	50.44	7.93	8.04	14.70	14.85
cis-(2-Chlorocyclohexyl)	Ce	Acetonitrile	36–77	200	1630	1560	$C_{13}H_{22}Cl_2N_2O$	53.25	53.28	7.57	7.60	9.55	9.63
I. *trans*-(2-Chlorocyclohexyl)ureas, RNHCONHCH(CH$_2$)$_4$CHCl-*trans*													
Methyl	Ba2		92	156	1630	1585	$C_8H_{15}ClN_2O$	50.39	50.54	7.93	7.86	14.70	14.67
trans-(2-Chlorocyclohexyl)	Ce		65	193	1630	1560	$C_{13}H_{22}ClN_2O$	53.25	53.26	7.57	7.68	9.55	9.52
J. (3-Chloropropyl)ureas, RNHCONHCH$_2$CH$_2$CH$_2$Cl and ClCH$_2$CH$_2$CH$_2$NHCONHRNHCONHCH$_2$CH$_2$CH$_2$Cl													
3-Chloropropyl	Fb		99	71–73	1620	1575	$C_7H_{14}Cl_2N_2O$	39.45	39.81	6.63	6.81	13.14	13.05
Cyclohexyl	Bb3		97	105	1630	1580	$C_{10}H_{19}Cl_2N_2O$	54.98	54.90	8.76	8.77	12.81	12.65
Phenyl	Bb3		79	130	1635	1560	$C_{10}H_{13}ClN_2O$	56.61	56.23	6.18	6.22	13.21	13.03
o-Phenylene	Bb6		68	260 dec	1620	1580	$C_{14}H_{20}Cl_2N_4O_2$	48.42	48.38	5.80	5.79	20.43[ff]	20.40[ff]
K. (2-Bromoethyl)ureas, RNHCONHCH$_2$CH$_2$Br													
2-Bromoethyl	Ea2		61	124–125	1625	1590	$C_5H_{10}Br_2N_2O$	21.92	21.91	3.68	3.70	10.23	10.06
Cyclohexyl	Ba5		75	148–150	1625	1585	$C_9H_{17}BrN_2O$	43.38	43.75	6.88	6.69	11.24	11.22
L. (2-Iodoethyl)ureas, RNHCONHCH$_2$CH$_2$I													
2-Iodoethyl	G		73	157–159[ee]	1615	1585	$C_5H_{10}I_2N_2O$					7.61	7.61
Cyclopentyl	G	Ethanol	10	110	1625	1575	$C_8H_{15}IN_2O$	34.06	34.24	5.36	5.51	9.93	9.67
Phenyl	G	Ethanol–water	45–95	154[dd]	1630	1575	$C_9H_{11}IN_2O$						

[a] Examples of procedures are detailed in Experimental Section: hydrochlorides as sources of free amines were neutralized with Et_3N or aqueous NaOH and used *in situ* or extracted with reaction solvent: A, $FCH_2CH_2NH_2$·HCl + KNCO in H_2O; B, (a) $XCH_2CH_2NH_2$, $CF_3CH_2NH_2$, or $ClCH(CH_2)_nCHNH_2$ + RNCO or $RNCO)_2$, or (b) RNH_2 or H_2NRNH_2 + CHBNCO in (1) H_2O, (2) Et_2O, (3) $CHCl_3$, (4) DMF, (5) C_6H_6, or (6) EtOH; C, (a) RNH_2 + $CH_3N(NO)CONHCH_2CH_2X$, (b) $FCH_2CH_2NH_2$ + $RNHCON(NO)CH_2CH_2Cl$, or (c) RNH_2 + $CH_3N(NO)CONHR$ in H_2O; D, $CH_3N(NO)CONHR$ + H_2O; E, (a) [NCON], (b) RNHCON[NCH$_2$], (c) ArNHCON], (d) [NCONH], Ar + (1) concentrated HCl or (2) HCl or HBr in Et_2O; F, RNCO + (a) H_2O [or (b) H_2O in acetone] in the presence of Et_3N; G, $RNHCONHCH_2CH_2Cl$ (R = $ClCH_2CH_2$, C_5H_9, or C_6H_{11}) + NaI in acetone. [b] When no recrystallization solvent is indicated, purification was effected by washing with either an appropriate solvent or with dilute hydrochloric acid. [c] Based on pure compound isolated; a range indicates yields of pure and crude products having favorable melting point or spectral comparison. [d] Melting points without range were observed on a Koller Heizbank; those with range, on a Mel-Temp apparatus. [e] Prominent bands (aromatic CH excluded) in 1500–1750-cm^{-1} range; urea CO and CNH assignments according to N. B. Colthup, L. H. Daly, and S. E. Wiberley, "Introduction to Infrared and Raman Spectroscopy," Academic Press Inc., New York, N. Y., 1964, pp 265, 384–385. [f] Lit.[26] mp 157–158°. [g] Lit.[42] mp 109–111°. [h] L-(+)-2-Chlorobutylamine was neutralized with aqueous NaOH before reaction. [i] Also 1710, 1645, 1625. [j] From L-lysine. [k] Also 1735, 1635. [l] Also 1655 w, 1610. [m] Also 1625. [n] H. Najer, P. Chabrier, and R. Giudicelli [*Bull. Soc. Chim. France*, 352 (1950)] reported mp 130–132°. [o] French Patent 1,313,055 (1962); *Chem. Abstr.*, **59**, 7533 (1963). [p] Also 1630. [q] Also 1730, 1630. [r] Cl. [s] Lit.[27] mp 159–161°. [t] Also 1610. [u] Also 1640. [v] Lit.[43] mp 166°. [w] Also 1645. [x] Amino acid neutralized with Et_3N *in situ* before reaction. [y] Also 1635. [z] Also 1710, 1685, 1640. [aa] *trans*-Vinylene diisocyanate obtained from Aerojet-General Corp., Azusa, Calif. [bb] Resolidified at ~185°, remelted at 214–220° dec. [cc] Lit.[25] mp 156–157° (from EtOH). [dd] L. Birckenbach and M. Linhard, *Ber.*, **64B**, 1076 (1931), reported mp 156° (acetone–ether).

of decomposition products. For example, the pmr spectrum of 1,1′-pentamethylenebis(3-methyl-3-nitrosourea) (**8**) showed no split methyl absorption and therefore no random nitrosation, whereas the previous[2a] assignment was made on the basis of an 18–29% yield of 1,1′-pentamethylenebis(3-ethylurea) isolated after treatment of **8** with ethylamine. The utility of pmr spectroscopy fails in the case of nitrosoureas substituted by alkyl-branched chloroethyl groups because of severe overlapping of signals, but the structure of 1-(2-chloropropyl)-3-cyclohexyl-1-nitrosourea (**9**) was reliably established by a 99% conversion to 1,3-dicyclohexylurea with cyclohexylamine in water.

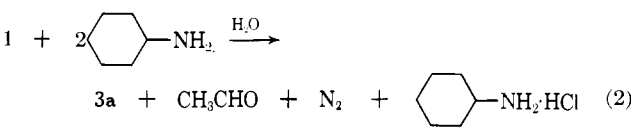


8 **9**

The elimination of hydrochloric acid and its interference with normal urea formation[2a,9] characterizes the aqueous decompositions of chloroethylnitrosoureas and must be taken into account when such reactions are used for structure proofs or synthetic purposes. Studies of the aqueous decomposition of BCNU, which will be reported in detail later, permits the following over-all equation (1) to be written. 2-

(1)

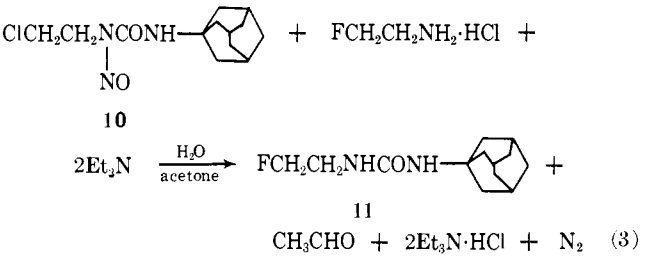
Chloroethyl isocyanate, a primary decomposition product emanating from the unnitrosated side of BCNU, is the source of the 2-chloroethylamine, which is neutralized as it is formed by hydrochloric acid released from the nitrosated side of BCNU and thus cannot add to unhydrolyzed isocyanate. A stronger base will free 2-chloroethylamine from its salt and make it available for urea formation. Thus, when the aqueous decomposition of BCNU was carried out in the presence of 1 molar equiv of triethylamine, 1,3-bis(2-chloroethyl)urea precipitated in 70% yield. When triethylamine was replaced by cyclohexylamine (eq 2), the product isolated in 36% yield was 1-(2-chloroethyl)-3-cyclohexylurea (**3a**); with 2 molar equiv of cyclohexylamine, one as reagent and one as proton acceptor, the yield of **3a** was increased to 84%.



(2)

Triethylamine can be used to conserve an expensive or rare amine. For example, from 2-fluoroethylamine hydrochloride, 3-(1-adamantyl)-1-(2-chloroethyl)-1-nitrosourea (**10**), and an excess of triethylamine in aqueous acetone there was obtained a good yield of 1-(1-adamantyl)-3-(2-fluoroethyl)urea (**11**) (eq 3). Aqueous decomposition of fluoroethylnitrosoureas in the presence of amines is apparently not complicated by release of hydrofluoric acid, since the reaction (eq 4) of 1,3-bis(2-fluoroethyl)-1-nitrosourea (**12**) with 1 molar equiv of cyclohexylamine produced an 85% yield of 1-cyclohexyl-3-(2-fluoroethyl)urea (**3c**), which is consonant with the usual behavior of nitrosoureas.[9,2a]

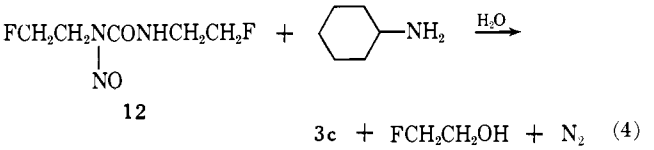
(9) J. L. Boivin and P. L. Boivin, *Can. J. Chem.*, **29**, 478 (1951).



10 **11**

(3)

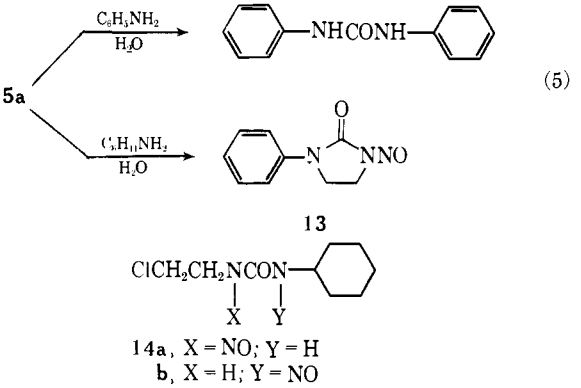
This view was subsequently supported by the identity of **1**,3-bis(2-fluoroethyl)urea and 2-fluoroethanol as products of the decomposition of **12** in dilute, aqueous solution. In apparently saturated aqueous solution, however, **12** was surprisingly stable; after 14 days at room temperature at least 80% was recovered unchanged.



12

(4)

The effect of water on isomer ratio was first observed in the nitrosation of 1-(2-bromoethyl)-3-phenylurea, the nitrosation of which with solid sodium nitrite in 85% formic acid gave an approximately **1**:1 mixture of isomers **5a** and **5b**, whereas in 98–100% formic acid only **5a** was obtained. Decomposition of **5a** with aniline (1 molar equiv) in aqueous dioxane gave the expected carbanilide, but unexpected cyclization occurred with the more basic cyclohexylamine, resulting in the isolation of 1-nitroso-3-phenyl-2-imidazolidinone (**13**) (eq 5). The presence of water had a similar effect on the nitrosations of 1-(2-chloroethyl)-3-phenylurea (Figures 1 and 2) and 1-(2-chloroethyl)-3-cyclohexylurea (**3a**). Nitrosation of **3a** in undiluted formic acid with an equal volume of aqueous nitrite solution (5–6%) gave a mixture of isomers (about 65% **14a** and 35% **14b**). Furthermore, these isomeric mixtures were converted to the pure isomers **5a**, **7a**, and **14a**



13

14a, X = NO; Y = H
b, X = H; Y = NO

(5)

in high yields by solution in cold, undiluted formic acid and, after a while, reprecipitation by addition of water; the reverse process was not effected in aqueous acid. Transfer of the nitroso group in formic acid probably involves the formation of formyl nitrite (**15**), which has apparently been detected spectroscopically in solutions of dry nitrite in 85% formic acid.[10] Such

(10) V. V. Kozlov and B. I. Belov, *J. Gen. Chem. USSR*, **33**, 1898 (1963).

TABLE II

(HALOALKYL)-*N*-NITROSOUREAS

R	Nitrosation[a] Urea, mmoles	Medium Acid	ml	$NaNO_2$, mmoles	Yield, %	Mp,[b] °C	IR, cm⁻¹ CO[c]	CNH[d]	Formula	Carbon, % Calcd	Carbon, % Found	Hydrogen, % Calcd	Hydrogen, % Found	Nitrogen, % Calcd	Nitrogen, % Found
A. Methylbitrosoureas, $RNHCON(NO)CH_3$															
2-Fluoroethyl	84.7	3 *N* HCl	140	378	92	53–55	1710	1530	$C_4H_8FN_3O_2$	32.21	32.51	5.41	5.46	12.74[e]	12.9[e]
cis-2-Chlorocyclohexyl	2.6	HCO_2H	7	7.3	90	60 dec	1725	1530	$C_8H_{14}ClN_3O_2$	43.73	43.88	6.42	6.44	19.13	19.14
trans-2-Chlorocyclohexyl	23.7	HCO_2H	90	65.3	90	121 dec	1700	1555	$C_8H_{14}ClN_3O_2$	43.73	43.80	6.42	6.23	19.13	19.20
B. (2-Fluoroethylbitreso)ureas, $RNHCON(NO)CH_2CH_2F$, and a Bis(2-fluoroethylnitroso)urea, $FCH_2CH_2N(NO)CONHRNHCON(NO)CH_2CH_2F$															
2-Fluoroethyl	30.1	3 *N* HCl	80	120	85	30–34	1725	1530	$C_5H_9F_2N_3O_2$	33.15	32.87	5.01	5.38	23.20	22.95
2-Chloroethyl[f]	3.0	12 *N* HCl	8	22	80	Oil	1720	1530	$C_5H_9ClFN_3O_2$	30.39	30.36	4.59	5.02	21.27	21.21
Cyclohexyl	36.0	HCO_2H	60	153	70	34–37	1720	1530	$C_9H_{16}FN_3O_2$	49.76	50.02	7.42	7.46	19.34	19.35
trans-4-*t*-Butylcyclohexyl	1.2	HCO_2H	10	4.4	74	76 dec	1725	1525	$C_{13}H_{24}FN_3O_2$	57.13	56.86	8.85	8.70	15.38	15.34
2-Norbornyl	1.5	6 *N* HCl	10	7.4	78	63–67	1690	1535	$C_{10}H_{16}FN_3O_2$	52.39	52.41	7.04	7.20	18.33	18.13
1-Adamantyl	9.6	HCO_2H	75	33	83	102 dec	1730	1515	$C_{13}H_{20}FN_3O_2$	57.98	57.86	7.48	7.35	15.66	15.43
5α-Cholestan-3α-yl	0.42	HCO_2H	10	7.3	80–85	99 dec	1735	1520	$C_{30}H_{52}FN_3O_2$	71.25	71.28	10.36	10.34	8.30	8.18
Phenyl	15.6	HCO_2H	30	70.0	85	82–85	1740	1540	$C_9H_{10}FN_3O_2$	51.18	51.18	4.77	5.00	19.90	19.71
trans-1,4-Cyclohexylene	1.0	HCO_2H	15	14.5	44	186 dec	1695	1535	$C_{12}H_{20}F_2N_6O_2$	41.62	41.58	6.03	5.94	23.92	23.87
C. (2,2,2-Trifluoroethyl)ureas, $RNHCON(NO)CH_2CF_3$															
2,2,2-Trifluoroethyl	0.67	HCO_2H	3	2.2	36	54	1730	1556[g]	$C_5H_6F_6N_3O_2$	23.73	23.78	1.99	2.12	16.60	16.58
Cyclohexyl[f]	1.3	HCO_2H	4	4.4	88	54	1720[h]	1540	$C_9H_{14}F_3N_3O_2$	42.85	42.74	5.58	5.69	16.60	16.40
D. (2-Chloroethylnitroso)ureas, $RNHCON(NO)CH_2CH_2Cl$, and Bis(2-chloroethylnitroso)ureas, $ClCH_2CH_2N(NO)CONHRNHCON(NO)CH_2CH_2Cl$															
2-Bromoethyl[f]	27.0	HCO_2H	50	109	75	30–31	1725	1530	$C_5H_9BrClN_3O_2$	23.23	23.16	3.51	3.64	16.25	16.44
2,6-Dioxo-3-piperidyl	19.3	HCO_2H	50	84.0	47	154 dec	1705	1530	$C_8H_{11}ClN_4O_4$	36.58	36.52	4.22	4.42	21.33	21.18
Cyclopentyl[f]	29.5	6 *N* HCl	250	81.2	48	Oil	1720	1525	$C_8H_{14}ClN_3O_2$	43.74	44.18	6.42	6.75	19.13	18.94
1-Methylcyclopentyl	2.4	HCO_2H	5	7.3	60	Oil	1730	1520	$C_9H_{16}ClN_3O_2$	46.25	46.05	6.90	6.84	17.98	17.61
1-Ethoxycarbonylcyclopentyl	17.2	HCO_2H	50	65.3	82	44–45	1735[i]	1510	$C_{11}H_{18}ClN_3O_4$	45.44	45.34	6.25	6.34	14.45	14.19
Cyclohexyl	4.9	HCO_2H	15	14.5	84	90	1710	1545	$C_9H_{16}ClN_3O_2$	46.25	46.40	6.90	6.94	17.98	17.72
Cyclohexyl[k]	46	HCO_2H	95	94[l]	94	70 dec	1705	1535	$C_9H_{16}ClN_3O_2$	46.25	46.34	6.90	7.14	17.98	17.86
1-Methylcyclohexyl	4.6	HCO_2H	12	14.5	57	Oil	1730	1520	$C_{10}H_{18}ClN_3O_2$	48.48	48.50	7.33	7.44	16.97	16.79
3-Methylcyclohexyl	2.3	HCO_2H	10	7.3	81	80 dec	1700	1535	$C_{10}H_{18}ClN_3O_2$	48.48	48.72	7.33	7.29	16.97	16.62
4-Methylcyclohexyl	2.3	HCO_2H	10	7.3	79	64 dec	1700	1535	$C_{10}H_{18}ClN_3O_2$	48.48	48.62	7.33	7.33	16.97	16.90
3,5,5-Trimethylcyclohexyl	2.0	HCO_2H	8	7.3	64	Oil	1725	1520	$C_{12}H_{21}ClN_3O_2$	52.27	52.38	7.68	7.89	15.25	15.28
trans-4-*t*-Butylcyclohexyl	1.9	HCO_2H	10	7.3	90	80 dec	1735	1520	$C_{13}H_{24}ClN_3O_2$	53.88	53.92	8.35	8.26	14.49	14.52
cis-2-Chlorocyclohexyl	2.1	HCO_2H	15	7.3	89	97 dec	1700	1525	$C_9H_{15}Cl_2N_3O_2$	40.31	40.55	5.64	5.68	15.67	15.60
trans-2-Chlorocyclohexyl	13.0	HCO_2H	50	43.5	89	95 dec	1705	1530	$C_9H_{15}Cl_2N_3O_2$	40.31	40.13	5.64	5.73	15.67	15.83
1-Ethoxycarbonylcyclohexyl	1.7	HCO_2H	5	12.6	60	56–65 dec	1730	1520	$C_{12}H_{20}ClN_3O_4$	47.14	47.16	6.59	6.48	13.74	13.56
2-Norbornyl	24.1	HCO_2H	130	75.3	91	42–45	1695	1535	$C_{10}H_{16}ClN_3O_2$	48.88	49.18	6.57	6.85	17.10	17.13
2-Bornyl	1.4	HCO_2H	7	5.8	82	Oil	1725	1520	$C_{13}H_{22}ClN_3O_2$	54.25	54.11	7.71	7.79	14.60	14.76
1-Adamantyl	1.2	HCO_2H	20	4.4	96	74 dec	1725	1535	$C_{13}H_{20}ClN_3O_2$	54.63	54.54	7.05	7.16	14.70	14.65
Cycloheptyl[m]	4.6	HCO_2H	10	14.5	66	Oil	1720	1520	$C_{10}H_{18}ClN_3O_2$	48.48	48.43	7.36	7.18	16.97	16.87
Cyclododecyl	1.7	HCO_2H	25	14.5	97	98 dec	1710	1535	$C_{15}H_{28}ClN_3O_2$	56.67	56.85	8.88	8.94	13.22	13.05
5α-Cholestan-3α-yl	0.61	HCO_2H	15	4.4	79	92 dec	1730	1520	$C_{30}H_{52}ClN_3O_2$	69.00	69.02	10.04	9.92	6.79[n]	6.80[n]
2-Indanyl	2.1	HCO_2H	10	7.3	84	81 dec	1720	1520	$C_{12}H_{14}ClN_3O_2$	53.84	53.65	5.28	5.43	15.70	15.57
Phenyl	35.3	HCO_2H	100	125	66	95 dec	1725	1550	$C_9H_{10}ClN_3O_2$	47.48	47.58	4.43	4.65	18.46	18.55

p-Fluorophenyl	2.3	HCO_2H	10	7.3	47–70	100 dec	1735	1550	$C_9H_9ClFN_3O_3$	44.00	44.10	3.69	3.61	17.10	17.06
o-Chlorophenyl	34.3	HCO_2H	150	116	81	35	1740	1540	$C_9H_9ClN_3O_2$	41.25	41.34	3.46	3.63	16.03	16.27
m-Chlorophenyl	34.3	HCO_2H	150	116	90	121	1730	1545	$C_9H_9ClN_3O_2$	41.25	41.37	3.46	3.54	16.03	16.06
3,5-Dichlorophenyl[o]	1.0	HCO_2H	8	8.6	50	85 dec	1735	1545	$C_9H_8Cl_2N_3O_2$	36.45	36.48	2.72	2.82	14.17	13.66
α,α,α-Trifluoro-*p*-tolyl	18.7	HCO_2H	115	74.0	96	83 dec	1735	1550	$C_{10}H_9ClF_3N_3O_2$	40.69	40.68	3.07	3.37	14.22	14.28
2,6-Xylyl	2.2	HCO_2H	10	7.3	71	83 dec	1730	1510	$C_{11}H_{14}ClN_3O_2$	51.67	51.52	5.52	5.51	16.45	16.16
m-Nitrophenyl	14.0	HCO_2H	100	49.3	97	110 dec	1730	1550	$C_9H_9ClN_4O_4$	39.64	39.50	3.34	3.42	20.55	20.65
m-Methoxyphenyl	2.2	HCO_2H	15	6.0	62	82 dec	1720	1550	$C_{10}H_{12}ClN_3O_3$	46.61	46.59	4.70	4.82	16.31	15.94
p-Cyanophenyl	40.4	HCO_2H	100	100[p]	77–94	131 dec	1735	1535	$C_{10}H_9ClN_4O_2$	47.53	47.77	3.59	3.87	22.18	22.41
o-Carboxyphenyl	2.1	HCO_2H	30	10	93	165 dec	1730[q]	1540	$C_{10}H_{10}ClN_3O_4$	44.27	44.15	3.72	3.65	15.47	15.43
m-Carboxyphenyl	2.1	HCO_2H	60	10	79	170 dec	1740[h]	1555	$C_{10}H_{10}ClN_3O_4$	44.27	44.09	3.72	3.87	15.47	15.24
p-Carboxyphenyl	19.4	HCO_2H	450	94.2	95	180 dec	1730[r]	1540	$C_{10}H_{10}ClN_3O_4$	44.27	44.15	3.72	4.02	15.46	15.44
p-Ethoxycarbonylphenyl	1.8	HCO_2H	15	5.0	94	107 dec	1730[i]	1550	$C_{12}H_{14}ClN_3O_4$	48.09	48.26	4.71	4.86	14.02	14.13
p-Acetylphenyl	2.1	HCO_2H	10	7.3	87	130 dec	1730[s]	1530	$C_{11}H_{12}ClN_3O_3$	48.99	49.00	4.49	4.57	15.58	15.53
p-(Dimethylcarbamoyl)-phenyl	2.2	HCO_2H	10	8.7	65	141 dec	1730[t]	1530	$C_{12}H_{15}ClN_4O_3$	48.25	48.56	5.06	5.17	18.76	18.62
p-(Fluorosulfonyl)phenyl	2.1	HCO_2H	8	8.7	94	135 dec	1735	1540	$C_9H_9ClFN_3O_4S$	34.90	35.06	2.93	3.19	13.57	13.52
p-(Carboxymethyl)phenyl	13.2	HCO_2H	100	69.3	78	125–127 dec	1735[s]	1540	$C_{11}H_{12}ClN_3O_4$	46.24	45.99	4.24	4.16	14.71	14.47
p-(Carboxymethylthio)-phenyl	1.7	HCO_2H	15	7.3	64	104 dec	1730	1540	$C_{11}H_{12}ClN_3O_4S$	41.58	41.55	3.81	3.98	13.23	12.98
p-(3-Carboxypropyl)phenyl	1.7	HCO_2H	20	7.3	80	125 dec	1725[u]	1550	$C_{13}H_{16}ClN_3O_4$	49.76	50.19	5.14	5.42	13.39	13.42
5,6,7,8-Tetrahydro-2-naphthyl	10.3	HCO_2H	130	37.7	81	85 dec	1720	1545	$C_{13}H_{16}ClN_3O_2$	55.42	55.12	5.72	5.70	14.92	14.57
2-Naphthyl	20.0	HCO_2H	250	81.0	91–97	110 dec	1715	1555	$C_{13}H_{12}ClN_3O_2$	56.22	56.11	4.36	4.50	15.13	15.09
1,2,3,4-Tetrahydro-2,4-dioxo-5-pyrimidinyl	30.2	HCO_2H	210	72.5	86	210 dec	1725[q]	1555	$C_7H_8ClN_3O_4$	32.13	32.30	3.08	3.37	13.56[n]	13.50[n]
8-Quinolyl	2.0	HCO_2H	10	7.3	91	138 dec	1720	1540	$C_{12}H_{11}ClN_4O_2$	51.71	51.69	3.98	3.95	20.10	20.27
trans-1,2-Cyclohexylene	3.1	HCO_2H	15	15	93	165 dec	1705	1545	$C_{12}H_{20}Cl_2N_6O_4$	37.61	37.78	5.26	5.26	21.93	21.97
trans-1,4-Cyclohexylene	1.5	HCO_2H	30	7.3	99	170 dec	1730	1520	$C_{12}H_{20}Cl_2N_6O_4$	37.61	37.84	5.26	5.66	21.93	21.51
o-Phenylene	1.6	HCO_2H	12	19	62	88 dec	1715	1530	$C_{12}H_{14}Cl_2N_6O_4$	38.21	38.13	3.73	3.74	18.80	19.00
p-Phenylene	15.7	HCO_2H	300	187	97	185 dec	1725	1560	$C_{12}H_{14}Cl_2N_6O_4$	38.21	37.98	3.73	3.85	22.28	21.91
Tetramethyl-*p*-phenylene	1.3	HCO_2H	25	11.5	75	200 dec	1720	1510	$C_{16}H_{22}Cl_2N_6O_4$	44.35	44.13	5.12	4.93	16.40[n]	16.40[n]
5-Carboxy-*m*-phenylene	1.4	HCO_2H	60	14.5	88	172 dec	1740[u]	1550	$C_{13}H_{14}Cl_2N_6O_6$	37.07	37.12	3.35	3.24	19.95	19.89
4,4'-Biphenylene	1.3	HCO_2H	100	15	85	175 dec	1715	1515	$C_{18}H_{18}Cl_2N_6O_4$	47.69	47.91	4.02	4.04	18.55	18.17
Oxydi-*p*-phenylene	1.2	HCO_2H	35	14.5	87	140 dec	1720	1530	$C_{18}H_{18}Cl_2N_6O_5$	46.06	45.80	3.86	4.05	17.91	17.88
E. (2-Chloropropylnitroso)ureas, $RNHCON(NO)CH_2CH(CH_3)Cl$															
2-Chloropropyl	1.4	HCO_2H	5	4.4	44	Oil	1710	1530	$C_7H_{13}Cl_2N_3O_2$	34.73	34.79	5.41	5.78	17.36	17.22
Cyclohexyl	2.3	HCO_2H	10	7.3	72	77	1705	1570	$C_{10}H_{18}ClN_3O_2$	48.49	48.48	7.33	7.41	16.97	16.97
F. 1,3-Bis[1-(chloromethyl)propyl]-1-nitrosourea, $RNHCON(NO)CH(CH_2CH_3)CH_2Cl$															
1-(Chloromethyl)propyl	1.2	HCO_2H	8	4.4	65	29–31	1720	1520	$C_9H_{17}Cl_2N_3O_2$	40.01	40.43	6.34	6.58	15.55	15.49
G. 1,3-Bis(2-chloro-1,1-dimethylethyl)-1-nitrosourea, $RNHCON(NO)C(CH_3)_2CH_2Cl$															
2-Chloro-1,1-dimethylethyl	1.2	HCO_2H	7	8.7	18	41–43	1710	1535	$C_9H_{17}Cl_2N_3O_2$	40.01	40.04	6.34	6.28	15.55	15.12
H. 1,3-Bis(*trans*-2-chlorocyclohexyl)-1-nitrosourea, $RNHCON(NO)\overline{CH(CH_2)_4CH}Cl$-*trans*															
trans-2-Chlorocyclohexyl	10.2	HCO_2H	90	43.5	93	106 dec	1690	1535	$C_{13}H_{21}Cl_2N_3O_2$	48.45	48.36	6.58	6.55	13.04	12.95

TABLE II (*Continued*)

R	Nitrosation[a] Urea, mmoles	Medium Acid	ml	$NaNO_2$, mmole	Yield, %	Mp,[b] °C	ν_{max}^{KBr}, cm^{-1} CO[c]	CNH[d]	Formula	Carbon, % Calcd	Found	Hydrogen, % Calcd	Found	Nitrogen, % Calcd	Found
I. (3-Chloropropyl)nitrosoureas, RNHCON(NO)$CH_2CH_2CH_2Cl$ and $ClCH_2CH_2CH_2N(NO)CONHRNHCON(NO)CH_2CH_2CH_2Cl$															
Cyclohexyl	4.6	HCO_2H	15	20.0[e]	48	20	1725	1525	$C_{10}H_{18}ClN_3O_2$	48.49	48.34	7.33	7.07	16.97	16.78
Phenyl	4.7	HCO_2H	10	14.1	93	88	1720	1550	$C_{10}H_{12}ClN_3O_2$	49.69	49.68	5.01	5.13	17.35	17.49
p-Phenylene	1.4	HCO_2H	20	14.5	95	165 dec	1725	1565	$C_{14}H_{18}Cl_2N_6O_4$	41.49	41.57	4.48	4.28	17.50	17.70
J. (2-Bromoethyl)nitrosoureas, RNHCON(NO)CH_2CH_2Br															
2-Bromoethyl	23.6	HCO_2H	75	94.2[e]	75	36–38	1715	1530	$C_5H_9Br_2N_3O_2$	19.82	20.19	2.99	2.80	52.75[f]	52.5[f]
Cyclohexyl	24	HCO_2H	50	50	85	75	1705	1530	$C_9H_{16}BrN_3O_2$	38.86	38.91	5.80	5.83	28.73[f]	28.90[f]
Phenyl[g]	26.8	HCO_2H	200	115	86	113 dec	1725	1650	$C_9H_{10}BrN_3O_2$	39.72	39.65	3.70	3.70	15.44	15.46
Phenyl[h]	3.4	HCO_2H	25[i]	15	71	86	1725[m]	1650	$C_9H_{10}BrN_3O_2$	39.72	39.93	3.70	3.87	15.44	15.34
K. 1,3-Bis(2-iodoethyl)-1-nitrosourea, $RNHCON(NO)CH_2CH_2I$															
2-Iodoethyl	16.5	HCO_2H	225	47.3	87	58–60	1715	1530	$C_5H_9I_2N_3O_2$	15.13	15.32	2.29	2.42	10.59	10.53

[a] Solutions or suspensions of ureas in the media indicated (undiluted 98–100% formic acid designated as HCO_2H) were treated cold (0–5°) with $NaNO_2$ (solid unless noted as aqueous solution); after 1–4 hr water was added to formic acid solutions or suspensions, and the precipitates were washed with water and dried *in vacuo*; some typical procedures are described in the Experimental Section. [b] Melting points without range were observed on a Kofler Heizbank; those with range, on a Mel-Temp apparatus. [c] Cf. ref 28 for previously noted effect of nitrosation on urea CO absorption. [d] See Table I, footnote *c*; strong bands in 1400–1500-cm^{-1} region can often be assigned to NO absorption. [e] 6.6 F. [f] About a 1:1 mixture with isomeric nitrosourea. [g] Also 1530 (probably NO). [h] Also 1695. [i] Isomeric nitrosourea content about 25%. [j] Also 1710. [k] Isomeric nitrosourea content about 35%. [l] In 95 ml of H_2O. [m] Isomeric nitrosourea content about 20%. [n] Cl. [o] From 1-(4-amino-3,5-dichlorophenyl)-3-(2-chloroethyl)urea. [p] In 50 ml of H_2O. [q] Also 1665. [r] Also 1690 and 1670. [s] Also 1685. [t] Also 1620. [u] Also 1715. [v] In 10 ml of H_2O. [w] In 25 ml of H_2O. [x] Br. [y] A larger run under about the same conditions gave a 5:1 product/isomer ratio. [z] Diluted with 5 ml of H_2O. [aa] Also 1705, which merged into a band at 1730 in $CHCl_3$.

$$ClCH_2CH_2NHCONR + HCO_2H \longrightarrow \underset{NO}{ClCH_2CH_2NHCONR}$$

$$\left[ClCH_2CH_2NHC(=O)-N(R)-H \;\; O=N-O \;\; O=C-H\right] \longrightarrow ClCH_2CH_2NHCONHR +$$

$$[HCOONO \rightleftharpoons HCO_2^- + NO^+] \longrightarrow$$

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$$ClCH_2CH_2\underset{NO}{NCONHR} + HCO_2H \quad (6)$$

a mechanism may be depicted as shown in eq 6. The isomeric composition may depend on an equilibrium governed by the relative stability of the isomers toward nitroso group abstraction by formic acid, and apparently, with increasing water concentrations, there is a point above which abstraction does not occur. In an aqueous system, formation of isomers may not be reversible, and the isomer ratio may therefore depend primarily on relative rates of formation. It was subsequently demonstrated that intermolecular transfer of nitroso groups can also occur. When a stirred solution of an equimolar mixture of 1-(2-chloroethyl)-3-cyclohexylurea (**3a**) and 1,3-dicyclohexyl-1-nitrosourea (**16**) in formic acid was diluted with water, after 1 hr the product that precipitated consisted of 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (**14a**) in addition to the starting material and undoubtedly 1,3-dicyclohexylurea (**17**). The product composition, estimated from a thin layer chromatogram, was consistent with an equilibrium that favors the more stable **14a**. The equilibrium was also established from the reverse direction, *i.e.*, from **14a** and **17** (eq 7).

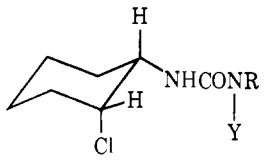
$$\textbf{3a} + \underset{NO}{\text{C}_6\text{H}_{11}\text{NCONH-C}_6\text{H}_{11}} \rightleftharpoons \textbf{14a} + \underset{\textbf{17}}{\text{C}_6\text{H}_{11}\text{NHCONH-C}_6\text{H}_{11}} \quad (7)$$

16

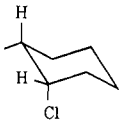
The exclusive formation of **14a** in anhydrous nitrosations of **3a** and the relative instability of the isomer **14b** are attributed to steric hindrance due to the cyclohexyl group. Steric factors are partially counteracted by electron withdrawal in the nitrosation of 1-cyclohexyl-3-(2,2,2-trifluoroethyl)urea under the conditions that afforded pure **14a**, the formation of **18a** being slightly favored over that of **18b**. Steric effects are not as marked in the nitrosations of the cyclopentyl and cycloheptyl analogs of **3a**. The approximately 3:1 ratio of **4a**:**4b** in the isomeric mixture obtained from the nitrosation of 1-(2-chloroethyl)-3-cyclopentylurea in 6 *N* hydrochloric acid was not significantly affected by nitrosation in formic acid under essentially anhydrous conditions, and the isomer content of 1-(2-chloroethyl)-3-cycloheptyl-1-nitrosourea (**19**) obtained under the same conditions was about 20%. A methyl branch enhanced the steric effect in the preparation of 1-(2-chloroethyl)-3-(1-methylcyclo-

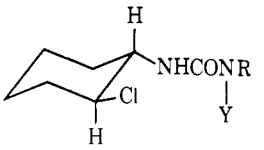
TABLE III

CONFORMATION OF *cis*- AND *trans*-2-CHLOROCYCLOHEXYLUREAS AND -NITROSOUREAS[a]

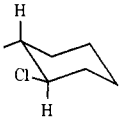
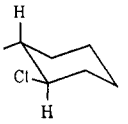


cis

R	Y	ν(CCl), cm⁻¹	ν(NH), cm⁻¹
CH_3	H	690	3340
CH_3	NO	690	3350
CH_2CH_2Cl	H	680	3320
CH_2CH_2Cl	NO	685	3350
	H	690	3340

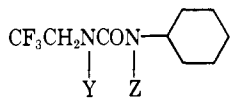


trans

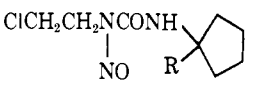
R	Y	ν(CCl), cm⁻¹	ν(NH), cm⁻¹
CH_3	H	735	3320, 3360
CH_3	NO	735	3290
CH_2CH_2Cl	H	735	3320, 3358
CH_2CH_2Cl	NO	735	3340
	H	735	3325
	NO	735	3300

[a] Infrared absorptions determined in pressed KBr disks.

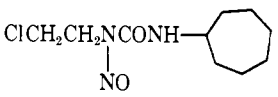
pentyl)-1-nitrosourea (**20a**), and consequently only a trace of the unwanted isomer was detected. No isomeric contamination was detected in ethyl 1-[3-(2-chloroethyl)-3-nitrosoureido]cyclopentanecarboxylate (**20b**), the position of nitrosation in the open-chain hydantoate **2b** having been reversed by steric hindrance. The random nitrosation of 1-cyclohexyl-3-phenylurea (**21**), which was established by identity of **17** and **21** as products of decomposition with cyclohexylamine, further attests steric hindrance by the cyclohexyl group in view of the isomeric purity of **7a** obtained under comparable conditions.



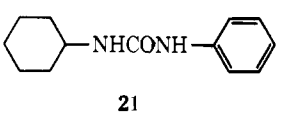
18a, Y = H; Z = NO
b, Y = NO; Z = H



20a, R = CH_3
b, R = $C_2H_5CO_2$



19

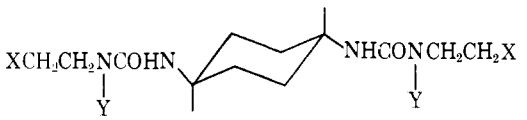


21

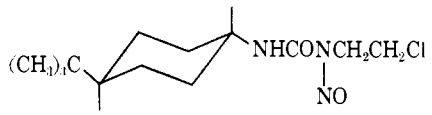
Yoshida has recently determined the conformation of N-(2-chlorocyclohexyl)benzamides[11] and 2-chlorocyclohexylamines[12] on the basis of axial and equatorial C–Cl absorptions in the infrared. Extension of this method to conformational analysis of the ureas and nitrosoureas that have been prepared from *cis*-[13] and *trans*-2-chlorocyclohexylamines[14] (Tables I and II) leads to the conformations shown in Table III, in which axial chlorine atoms are *cis* and equatorial chlorine atoms are *trans*. Therefore, the ureido function is equatorial in every instance, whereas Yoshida found the benzamido group in *cis*-N-(2-chlorocyclohexyl)benzamide to be axial.[11] Of the series of 2-chlorocyclohexyl-substituted ureas listed in Table III only 1,3-bis(*cis*-2-chlorocyclohexyl)urea failed to yield a pure nitrosourea.

The reported four-step conversion of dimethyl *trans*-1,4-cyclohexanedicarboxylate to 1,4-cyclohexanediamine dihydrochloride[15] provided the required intermediate in the preparation of 1,1′-(*trans*-1,4-cyclohexylene)bis[3-(2-chloroethyl)-3-nitrosourea] (**22a**). The *trans* configuration of the diamine dihydrochloride was assigned on the basis of the following comparisons: the melting point of the derived free base agreed with that reported for the *trans*-diamine,[16] and the melting point (350–351° dec) of the analytically pure diacetyl derivative was somewhat higher than that (310–315° dec) of N,N′-(*trans*-1,4-cyclohexylene)bisacetamide and considerably higher than that (208–209°) of the *cis* isomer as recorded by Nielsen.[16] The NH stretching bands at 3290–3350 cm^{-1} shown by the nitrosoureas of Table III seem to be characteristic of equatorial nitrosoureido groups. On this basis the same conformation is indicated for the nitrosoureas derived from *trans*-1,4-cyclohexanediamine and *trans*-4-*t*-butylcyclohexylamine,[17] for example, **22a** (3360 cm^{-1}), the fluoro analog **22b** (3320 cm^{-1}), and 3-(*trans*-4-*t*-butylcyclohexyl)-1-(2-chloroethyl)-1-nitrosourea (**23**) (3360 cm^{-1}). The urea **22c**, from which **22b** was derived, was prepared in two ways (eq 8): (a) protracted treatment of a solution of 2-fluoroethylamine hydrochloride in equal volumes of water, N,N-dimethylformamide (DMF), and triethylamine with 1,1′-(*trans*-

(11) Z. Yoshida, *Tetrahedron Letters*, 2757 (1965).
(12) Z. Yoshida, *ibid.*, 3753 (1965).
(13) G. E. McCasland, R. K. Clark, Jr., and H. E. Carter, *J. Am. Chem. Soc.*, **71**, 637 (1949).
(14) O. E. Paris and P. E. Fanta, *ibid.*, **74**, 3007 (1952); T. Taguchi, M. Kojima, and T. Muro, *ibid.*, **81**, 4322 (1959).
(15) (a) T. Curtius, E. Darmstaedter, H. Pringsheim, and R. Stangassinger, *J. Prakt. Chem.*, **91**, 1 (1915); (b) P. A. S. Smith, *Org. Reactions*, **3**, 386 (1946).
(16) A. T. Nielsen, *J. Org. Chem.*, **27**, 1993 (1962).
(17) D. V. Nightingale, J. D. Kerr, J. A. Gallagher, and M. Maienthal, *ibid.*, **17**, 1017 (1952).

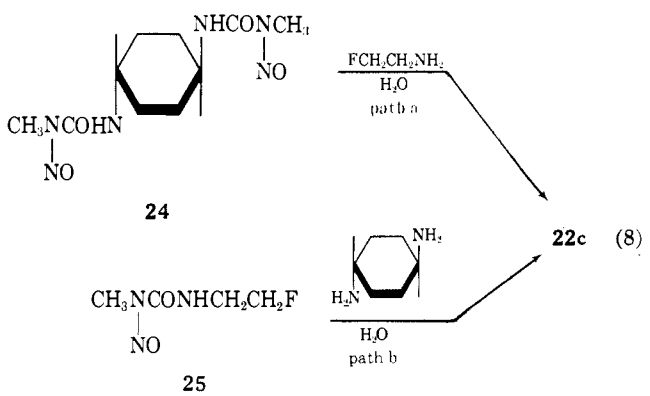


22a, X = Cl; Y = NO
b, X = F; Y = NO
c, X = F; Y = H



23

1,4-cyclohexylene)bis[3-methyl-3-nitrosourea] (**24**), and (b) treatment of a cold solution of *trans*-1,4-cyclohexanediamine dihydrochloride in water first with excess triethylamine and then with 3-(2-fluoroethyl)-1-methyl-1-nitrosourea (**25**). The second route is preferred because of convenience and somewhat better yield.



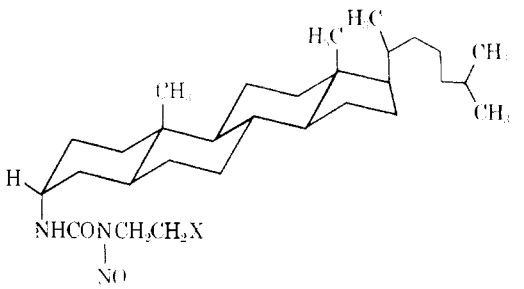
24

25

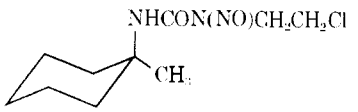
22c (8)

Axial conformation for the nitrosoureido groups of 1-(2-chloroethyl)-3-(5α-cholestan-3α-yl)-1-nitrosourea (**26a**) and the fluoroethyl analog **26b** is deducible from the conformation of 5α-cholestane-3α-amine[18] from which they were derived. The infrared spectra of both **26a** and **26b** show NH stretching bands at 3430 cm^{-1} which is a higher wavenumber than those (3290–3350 cm^{-1}) already assigned to equatorial nitrosoureido groups; this difference is consistent with that previously reported for axial and equatorial benzamido groups.[11] Since 1-(2-chloroethyl)-3-(1-methylcyclohexyl)-1-nitrosourea, which was derived from 1-methylcyclohexylamine,[19] absorbs at 3420 cm^{-1}, it is not unreasonable to assume that the axial conformation for the nitrosoureido group as shown in structure **27** is predominant.

The recent assignment of *trans* configuration to the product of hydrogenation of ethyl *p*-aminobenzoate over platinum oxide in acetic acid[20] prompted an attempt to prepare ethyl *trans*-4-aminocyclohexanecarboxylate by the reported method for conversion to the corresponding chloroethylnitrosourea, but the product obtained was subsequently shown by vapor phase chromatography to be a mixture of nearly equal amounts of *cis* and *trans* isomers. The reported assignment became suspect when the derived urea gave ethyl 4-[3-(2-chloroethyl)-3-nitrosoureido]cyclohexanecarboxylate (**28**) as an oil whose pmr spectrum showed discrepancies unassociated with the position of nitrosation.



26a, X = Cl
b, X = F



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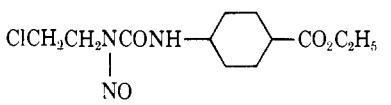
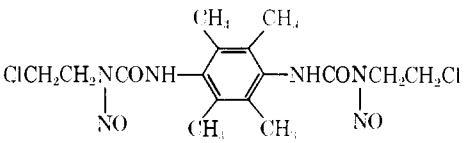


28

During the course of preparation of ureas and nitrosoureas from various phenylenediamines, effects of ring substituents such as those described in the following examples have been noted. Nitrosation was apparently sterically controlled in the preparation of 1,1′-(tetramethyl-*p*-phenylene)bis[3-(2-chloroethyl)-3-nitrosourea] (**29**), as no isomers were detected. The nitrosation of 1,1′-(4-methoxy-*m*-phenylene)bis[3-(2-chloroethyl)urea] (**30**) in formic acid, however, apparently



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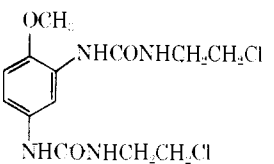


30

produced a mixture of all the possible bis(nitrosoureas), since a thin layer chromatogram showed four components, each different from the starting urea. Treatment of 2,6-dichloro-*p*-phenylenediamine with 2 molar equiv of 2-chloroethyl isocyanate afforded 1-(4-amino-3,5-dichlorophenyl)-3-(2-chloroethyl)urea (**31**) in good yield and catalysis of the reaction by triethylamine in DMF did not force further reaction of compound **31** with isocyanate to give the bisurea. Nitrosation of **31** in formic acid with dry nitrite resulted in concomitant deamination (Scheme I); the yield of pure 1-(2-chloroethyl)-3-(3,5-dichlorophenyl)-1-nitrosourea (**32**) under the favorable conditions of long reaction time and large excess of

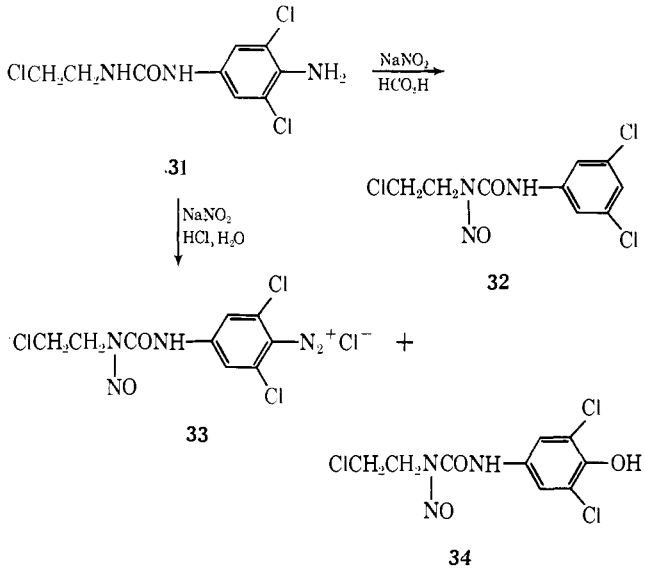
(18) J. L. Pinkus, G. Pinkus, and T. Cohen, *J. Org. Chem.*, **27**, 4350 (1962).

(19) Preparations of 1-methylcyclohexylamine hydrochloride (yield 35%) and 1-methylcyclopentylamine hydrochloride (low yield), modeled after the procedure of V. H. Maddox, E. F. Godefroi, and R. F. Purcell, *J. Med. Chem.*, **8**, 230 (1965), for the preparation of the 1-phenyl analog; see also J. J. Ritter and J. Kalish, *J. Am. Chem. Soc.*, **70**, 4048 (1948); Z. J. Vejdělek, M. Rajšner, and M. Protiva, *Collection Czech. Chem. Commun.*, **25**, 245 (1960); H. J. Barber and E. Lunt, *J. Chem. Soc.*, 1187 (1960).

(20) H. J. Schaeffer and E. Odin, *J. Pharm. Sci.*, **54**, 421 (1965).

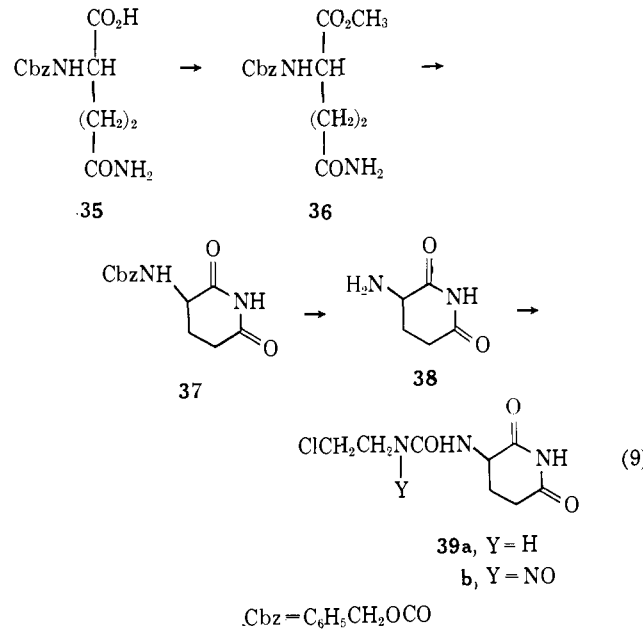
nitrite was about 50%.[21] When the nitrosation of **31** was carried out in dilute hydrochloric acid, the isolated product was a mixture of the diazonium chloride **33** (about 53%) and the phenol **34** (about 47%) as judged from elemental analyses, the infrared spectrum, a thin layer chromatogram, and a positive Bratton-Marshall test.[22]

SCHEME I



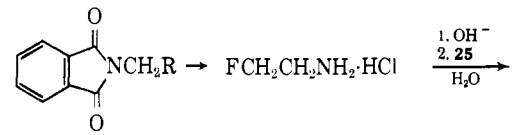
The synthesis of DL-1-(2-chloroethyl)-3-(2,6-dioxo-3-piperidyl)-1-nitrosourea (**39b**) (eq 9) required the preparation of the intermediate DL-2-aminoglutarimide (**38**) by a four-step sequence beginning with DL-glutamine and patterned after published procedures.[23] The reaction of benzyl chloroformate with DL-glutamine at about pH 9 afforded the benzyloxycarbonyl derivative **35** in 77–92% yield, sodium hydroxide being used instead of the specified sodium bicarbonate.[23a] A high yield of the DL-methyl ester **36** was conveniently obtained by treatment of the potassium salt of **35** with iodomethane in DMF, thus avoiding use of diazomethane as in the reported preparation of the L isomer.[23b] Cyclization of **36** with sodium methoxide in benzyl alcohol provided benzyl DL-2,6-dioxo-3-piperidinecarbamate (**37**), which was also the product of a conversion of L-**36** under the same conditions.[23b] Attempted cyclization of DL-**36** in methanol under conditions described for the conversion of L-**36** without racemization[23c] resulted in either incomplete reaction or almost total recovery of starting material. Catalytic hydrogenolysis of **37** over 5% palladium–charcoal was equally effective in acidic and neutral media,[23c] but the resultant DL-2-aminoglutarimide (**38**) was more conveniently converted to the urea (**39a**) as the free base than as the hydrochloride.

The Gabriel synthesis of 2-fluoroethylamine hydrochloride as described by Childs, *et al.*,[24] provided the



(9)

intermediate needed for the preparation of fluoroethyl analogs of the more promising chloroethylnitrosoureas. The fluoroethylation of potassium phthalimide with 2-fluoroethyl *p*-toluenesulfonate in DMF was found to be a convenient method for the preparation of N-(2-fluoroethyl)phthalimide (**40a**), hydrazinolysis of which, according to the published procedure, afforded a theoretical yield of white, crystalline amine hydrochloride in contrast to the "brown glass" reported previously.[24] Its direct conversion to ureas was accomplished in two ways: (1) addition of isocyanates to the free amine, and (2) aqueous decomposition of nitrosoureas in the presence of the free amine. But the aqueous decomposition of **25** in the presence of an amine was sometimes the preferred method and a means of circumventing the preparation of the as yet unknown 2-fluoroethyl isocyanate as illustrated by the preparation of 1,3-bis(2-fluoroethyl)-urea (**41**) (eq 10). The Gabriel synthesis was also applied, apparently for the first time, to the preparation of 2,2,2-trifluoroethylamine hydrochloride. The reaction of potassium phthalimide with 1,1,1-trifluoro-2-iodoethane provided the intermediate N-(2,2,2-trifluoroethyl)phthalimide (**40b**).



40a, R = CH_2F
b, R = CF_3

$FCH_2CH_2NHCONHCH_2CH_2F$ (10)

41

Intermediates for the synthesis of nitrosoureas containing alkyl-branched chloroethyl groups as in **9** and 1,3-bis(2-chloro-1,1-dimethylethyl)-1-nitrosourea (**42a**)

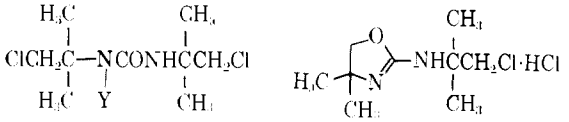
(21) Reduction of diazonium compounds by formates and formic acid has been noted previously (see K. H. Saunders, "The Aromatic Diazo Compounds," Edward Arnold and Co., London, 1949, pp 272, 278).

(22) A. C. Bratton and E. K. Marshall, Jr., *J. Biol. Chem.*, **128**, 537 (1939).

(23) (a) V. Bruckner, J. Kovács, and K. Kovács, *J. Chem. Soc.*, 1512 (1953); (b) E. Sondheimer and R. W. Holley, *J. Am. Chem. Soc.*, **76**, 2467 (1954); (c) E. Sondheimer and R. W. Holley, *ibid.*, **79**, 3767 (1957).

(24) A. F. Childs, L. J. Goldsworthy, G. F. Harding, F. E. King, A. W. Nineham, W. L. Norris, S. G. P. Plant, B. Selton, and A. L. L. Tompsett, *J. Chem. Soc.*, 2174 (1948).

were obtained by the action of phosphorus pentachloride on appropriate ethyl 2-hydroxylalkylcarbamates according to the method of Wenker[25] as applied by Najer, *et al.*,[26] for conversion of a (chloroalkyl)carbamate. Vapor phase chromatography showed that the 2-chloroalkyl isocyanates so obtained were only 74–94% pure even after fast distillation through a short column. Because of acidic impurities, aqueous decompositions of the crude isocyanates to give symmetrical 1,3-disubstituted ureas were carried out in the presence of triethylamine. The urea **42b** was particularly unstable; a dry sample was completely cyclized to 2-(2-chloro-1,1-dimethylethylamino)-4,4-dimethyl-2-oxazoline hydrochloride (**43**) after a few days.



42a, Y = NO
b, Y = H

43

Experimental Section

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 pmr spectra were obtained in chloroform-*d* or dimethyl sulfoxide-d_6 on a Varian A-60 spectrometer with tetramethylsilane as internal reference. Thin layer chromatograms were developed on silica gel H (E. Merck AG, Darmstadt) plates usually with such solvent pairs as 9:1 benzene–chloroform and 9:1 chloroform–methanol; nitrosoureas could often be detected in ultraviolet light after spraying with Ultraphor WT[27] solution; ureas, with Dragendorff solution;[28] and both, by iodine vapor. Nitrosoureas were stored dry and cold to minimize decomposition; 2-fluoroethyl- and 2-chloroethylnitrosoureas that were kept under such conditions for long periods showed no evidence of decomposition.

N-(2-Fluoroethyl)phthalimide (40a).—A mixture of potassium phthalimide (34.0 g, 0.183 mole), 2-fluoroethyl *p*-toluenesulfonate[24] (40.0 g, 0.183 mole), and DMF (300 ml) was stirred at 110° for 2.5 hr. The resulting solution was cooled a little, diluted with water (1.5 l.), and chilled. The white precipitate was washed with water and dried *in vacuo* over P_2O_5. Recrystallization of the crude product (31.8 g) from absolute ethanol (200 ml) afforded 26.9 g (76%) of **40a** as white needles, mp 97–101° (lit.[24] mp 100°).

2-Fluoroethylamine Hydrochloride.[29]—A mixture of **40a** (26.0 g, 0.135 mole), hydrazine hydrate (9.0 g, 0.18 mole), and absolute ethanol (150 ml) was refluxed for 1 hr. The resulting semisolid mixture was acidified to about pH 2 with concentrated HCl (25 ml), stirred, and refluxed for 1 hr more, cooled to about 5°, and filtered to remove phthalhydrazide (21 g, 96%). The filtrate was evaporated to dryness *in vacuo*, and the residue was extracted with water. *In vacuo* evaporation of the filtered, aqueous extract left hygroscopic crystals (18.4 g), which were stirred for 8 hr with redistilled salicylaldehyde (16 ml) and enough ether and benzene to dissolve the yellow solid that formed. The layers were separated and the aqueous phase was evaporated to dryness *in vacuo*. The yield of white, crystalline 2-fluoroethylamine hydrochloride (further dried *in vacuo* over P_2O_5), mp 89–91°, was 13.4 g (100%) (lit. mp 92–93°,[30a] 95°[30b]).

N-(2,2,2-Trifluoroethyl)phthalimide (40b).—2,2,2-Trifluoro-1-iodoethane[31] (27.0 g, 0.13 mole) was added to a stirred suspension of potassium phthalimide (20.0 g, 0.11 mole) in DMF (300 ml). The resulting mixture was heated at 110° for 5 hr, cooled to room temperature, and diluted with water (1.2 l.). The precipitate was washed with water and air dried; yield of crude **40b**, 14.1 g (57%). Purification was effected by extraction into ether and recrystallization from ethanol; yield 9.4 g (38%), mp 130°.

Anal. Calcd for $C_{10}H_6F_3NO_2$: C, 52.41; H, 2.64; N, 6.11. Found: C, 52.74; H, 2.98; N, 6.12.

1,1′-*p*-Phenylenebis[3-(2-fluoroethyl)urea] (Method B). A stirred suspension of 2-fluoroethylamine hydrochloride (2.0 g, 20.3 mmoles) in DMF (15 ml) was treated with triethylamine (2.9 ml, 20.5 mmoles) and then with solid *p*-phenylene diisocyanate[32] (1.6 g, 10.1 mmoles). The resulting suspension was stirred at ambient temperature for about 24 hr, diluted with water (100 ml), and chilled. The insoluble, finely divided, cream-colored bisurea was collected, washed with water, and dried *in vacuo* over P_2O_5; yield 2.63 g (92%).

1-(2-Fluoroethyl)-3-methylurea.—Triethylamine (18.4 ml, 0.13 mole) then methyl isocyanate[32,33] (8.3 ml, 0.13 mole) were added dropwise to a 0–5°, stirred solution of 2-fluoroethylamine hydrochloride (13.1 g, 0.13 mole) in water (70 ml). The resulting solution was stirred at room temperature for 3 hr and extracted with six 50-ml portions of ethyl acetate. *In vacuo* evaporation of the dried ($MgSO_4$) extract left a white solid (7.5 g). *In vacuo* evaporation at 35° of the aqueous filtrate left additional product as a pale yellow solid, which was extracted with three 50-ml portions of ethyl acetate and recovered as described above (6.8 g). Two recrystallizations of the combined samples from $CHCl_3$–petroleum ether (bp 30–60°) (60–75:240–300 ml) afforded 12.7 g (80.5%) of 1-(2-fluoroethyl)-3-methylurea.

3-(2-Fluoroethyl)-1-methyl-1-nitrosourea (35).—Dry sodium nitrite (26.1 g, 0.38 mole) was added in small portions over a period of 1.5 hr to a stirred, 0–5° solution of 1-(2-fluoroethyl)-3-methylurea (10.2 g, 0.085 mole) in 3 *N* HCl (140 ml). The mixture was stirred another 1 hr at 0–5°, and the insoluble light yellow flakes (**35**) were dried *in vacuo* over P_2O_5 and NaOH; yield 8.0 g. The filtrate was extracted with five 25-ml portions of $CHCl_3$; the dried ($MgSO_4$) extract was evaporated *in vacuo* leaving additional **35** (3.6 g); total yield 92%. The infrared spectrum was identical with that of the analytical sample prepared in a pilot experiment.

1,1′-(*trans*-1,4-Cyclohexylene)bis(3-methylurea).—*trans*-1,4-Cyclohexanediamine dihydrochloride[35b] (10.0 g, 0.05 mole) in water (25 ml) was made basic with 50% aqueous NaOH (15 ml) and extracted with three 75-ml portions of ether. The dried (Na_2SO_4), ethereal solution was cooled, stirred, and treated with methyl isocyanate[32,33] (6.8 ml, 0.11 mole). The mixture was stirred at room temperature for 2 hr, then chilled. The bisurea that had formed was washed with ether and dried *in vacuo* over P_2O_5; yield 6.3 g (52%), mp >300°.

Anal. Calcd for $C_{10}H_{20}N_4O_2$: C, 52.61; H, 8.83; N, 24.54. Found: C, 52.62; H, 8.86; N, 24.11.

1,1′-(*trans*-1,4-Cyclohexylene)bis(3-methyl-3-nitrosourea) (24).—Dry $NaNO_2$ (9.7 g, 140 mmoles) was added in small increments to a 5°, stirred solution of 1,1′-(*trans*-1,4-cyclohexylene)bis(3-methylurea) (4.85 g, 21.3 mmoles) in 98–100% formic acid (150 ml). The mixture was stirred for 2 hr at 5°, diluted with water (100 ml), and stirred for 30 min more at 0–5°. The white precipitate was washed with water and dried *in vacuo* over P_2O_5; yield of **24**, 5.1 g (84%); mp >210° dec.

Anal. Calcd for $C_{10}H_{18}N_6O_4$: C, 41.95; H, 6.34; N, 29.35. Found: C, 42.08; H, 6.09; N, 29.36.

1,1′-(*trans*-1,4-Cyclohexylene)bis[3-(2-fluoroethyl)urea] (22c) (Method C). A. From 1,1′-(*trans*-1,4-Cyclohexylene)bis(3-methyl-3-nitrosourea) (24).—A solution of 2-fluoroethylamine hydrochloride (2.23 g, 22.4 mmoles) in water (40 ml), DMF (40 ml), and triethylamine (40 ml) was treated with **24** (3.20 g, 11.2 mmoles) and stirred at room temperature for 2 days. The mixture was concentrated *in vacuo* to a volume of about 35 ml and diluted with water (100 ml). The bisurea **22c** separated as a white solid which was washed with water and dried *in vacuo* over P_2O_5; yield 2.45 g (74%).

(25) H. Wenker, *J. Am. Chem. Soc.*, **58**, 2608 (1936).
(26) H. Najer, P. Chabrier, and R. Giudicelli, *Bull. Soc. Chim. France*, 611 (1959).
(27) BASF Colors and Chemicals, Inc., Charlotte, N. C.
(28) K. Randerath, "Thin-Layer Chromatography," Academic Press Inc., New York, N. Y., 1964, p 129.
(29) By method of ref 24.
(30) (a) V. G. Nemets and G. L. Epshtein, *Izv. Vysshikh Uchebn. Zavedenii Khim. i Khim. Tekhnol.*, **5**, 101 (1962); *Chem. Abstr.*, **58**, 3298 (1962); (b) Z. B. Papanastassiou and R. J. Bruni, *J. Org. Chem.*, **29**, 2870 (1964).
(31) Columbia Organic Chemicals Co., Columbia, S. C.
(32) Distillation Products Industries, Rochester, N. Y.
(33) Olt Chemical Co., Muskegon, Mich.

B. From 3-(2-Fluoroethyl)-1-methyl-1-nitrosourea (25).—Triethylamine (5.6 ml, 40.2 mmoles) and, a few minutes later, **25** (3.0 g, 20.1 mmoles) were added to a cold (ice bath), stirred solution of *trans*-1,4-cyclohexanediamine dihydrochloride[15b] (1.9 g, 10.0 mmoles) in water (75 ml). The mixture was stirred at room temperature for 18 hr, and **22c**, which had precipitated as a white solid, was washed with water and dried *in vacuo* over P_2O_5; yield 2.5 g (86%). The infrared spectrum of **22c** from B was identical with that of the analytically pure **22c** from A.

Mixture (1:1) of 1-(2-Chloroethyl)-3-(2-fluoroethyl)-1-nitrosourea and 3-(2-Chloroethyl)-1-(2-fluoroethyl)-1-nitrosourea.—Sodium nitrite (9.7 g, 140 mmoles) was added in portions over 2.5 hr to a 0–5°, stirred solution of 1-(2-chloroethyl)-3-(2-fluoroethyl)urea (2.8 g, 16.6 mmoles) in concentrated HCl (25 ml). The mixture was diluted with water (20 ml), and stirring was continued for 1.5 hr at 0–5°. Enough water (5 ml) was added to dissolve the NaCl that had precipitated, and the oily mixture was extracted with three 25-ml portions of $CHCl_3$. The dried ($MgSO_4$) $CHCl_3$ extract was evaporated under reduced pressure leaving an orange oil, which was further dried over P_2O_5 and NaOH; yield 2.8 g (86%), $n^{25}D$ 1.4890. Pmr indicated a 1:1 mixture of isomers.

1-(1-Adamantyl)-3-(2-fluoroethyl)urea (11) (Method C).—Triethylamine (4.1 g, 40 mmoles) and then 3-(1-adamantyl)-1-(2-chloroethyl)-1-nitrosourea (**10**) (4.5 g, 16 mmoles) were added to a stirred solution of 2-fluoroethylamine hydrochloride (1.6 g, 16 mmoles) in water (100 ml). After 2 hr the suspension was thinned with acetone (20 ml), stirred for 2 days at room temperature, and concentrated *in vacuo* to 100 ml. The white solid (3.0 g), washed with water and dried *in vacuo* over P_2O_5, was further purified by trituration in ether and recrystallization from acetonitrile; yield of **11**, 2.4 g (63%).

1,3-Bis(2-fluoroethyl)urea (Method C).—A 5–10°, stirred solution of 2-fluoroethylamine hydrochloride (4.1 g, 41.2 mmoles) in water (125 ml) was treated with 50% aqueous NaOH (2.2 ml) then, in portions over a 45-min period, 3-(2-fluoroethyl)-1-methyl-1-nitrosourea (**25**) (6.0 g, 40.3 mmoles); the resulting solution was stirred at room temperature for 18 hr. Removal of water *in vacuo* at 20° or below left a 6.6-g residue which was extracted with ethanol (50 ml). Evaporation of the filtered ethanolic solution left the crude product (3.8 g), which was recrystallized from ethanol–petroleum ether (35:50 ml); yield 2.3 g (38%). The analytical sample was obtained in 53% yield from a pilot experiment in which triethylamine (1 equiv) was used as base.

1,3-Bis(2,2,2-trifluoroethyl)urea (Method D).—A stirred suspension of 3-(2,2,2-trifluoroethyl)-1-methyl-1-nitrosourea[2a] (14.2 g, 76.8 mmoles) in water (150 ml), triethylamine (2 ml), and acetone (25 ml) was warmed slowly, kept at 70–80° for 1 hr as complete solution resulted, then refluxed for 30 min. The cooled, filtered solution deposited the product as a white solid which was washed with water and air-dried; yield 5.6 g (65%).

1-(2-Chloroethyl)-3-cyclohexylurea (3a) from Decomposition of Ethyl 5-(2-Chloroethyl)-3-nitrosohydantoate (2b) with Cyclohexylamine.—A suspension of **2b**[2a] (500 mg, 2.11 mmoles) in water (10 ml) was treated with cyclohexylamine (208 mg, 2.11 mmoles) and stirred overnight at room temperature. The solid present, washed with water and dried *in vacuo*, was identical with authentic 1-(2-chloroethyl)-3-cyclohexylurea with respect to infrared absorption, melting point (130°), and mixture melting point. The yield was 130 mg (30%).

Mixture (about 3:1) of 1-(2-Chloroethyl)-3-cyclopentyl-1-nitrosourea (4a) and 3-(2-Chloroethyl)-1-cyclopentyl-1-nitrosourea (4b).—A 0°, stirred suspension of 1-(2-chloroethyl)-3-cyclopentylurea (5.6 g, 29.5 mmoles) in 6 *N* HCl (250 ml) was treated with $NaNO_2$ (5.6 g, 81 mmoles) in small increments. The reaction mixture was stirred at 0° for 2 hr and extracted with two 75-ml portions of $CHCl_3$. The dried (Na_2SO_4) $CHCl_3$ solution was evaporated *in vacuo* to a yellow oil which was further dried *in vacuo* over P_2O_5 overnight; yield 3.1 g (48%). The approximate 3:1 isomeric ratio was established by pmr.

Cyclopentylurea from Decomposition of a Mixture of 1-(2-Chloroethyl)-3-cyclopentyl-N-nitrosoureas (4a and 4b) with Ammonium Hydroxide.—A solution of the isomeric mixture of **4a** and **4b** described above (100 mg, 0.456 mmole) in 3 *N* NH_4OH (10 ml) was stirred overnight at room temperature, then evaporated *in vacuo*. A carbon-decolorized solution of the residue in water (5 ml) deposited 42.5 mg (73%) of cyclopentylurea as white crystals, mp 200°, alone or in mixture with an authentic sample.

Nitrosation of 1-(2-Chloroethyl)-3-cyclohexylurea (3a). A. In the Presence of Water.—A 5°, stirred solution of **3a** (9.5 g, 46 mmoles) in 98–100% formic acid (95 ml) was treated dropwise with $NaNO_2$ (6.5 g, 94 mmoles) in water (95 ml). The mixture was stirred at 0–5° for 30 min, diluted with cold water (225 ml), and stirred further for 30 min. The light yellow precipitate was washed with water and dried *in vacuo* over P_2O_5; yield of 1-(2-chloroethyl)-3-cyclohexyl-N-nitrosourea (about 65% **14a** and 35% **14b** by pmr in $CDCl_3$), 10 g (94%), mp 70° dec.

B. In the Absence of Added Water.—A 5°, stirred solution of **3a** (1.0 g, 4.9 mmoles) in 98–100% formic acid (15 ml) was treated with dry $NaNO_2$ (1.0 g, 14.5 mmoles) in small increments. After the addition, the mixture was stirred at 5° for 30 min, then slowly diluted with cold water (15 ml), and stirred further at 0–5° for 30 min. The light yellow precipitate was washed with water and dried *in vacuo* over P_2O_5. The yield of 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (**14a**, devoid of **14b** according to pmr in $CDCl_3$), mp 90°, was 0.96 g (84%).

C. By Nitroso Group Transfer in Isomeric Mixture of 14a and 14b.—An isomeric mixture (9.0 g, mp 70° dec) of **14a** and 3-(2-chloroethyl)-1-cyclohexyl-1-nitrosourea (**14b**) as described above under A was dissolved in 98–100% formic acid (150 ml). The solution was stirred at 5° for 2 hr, diluted with cold water (250 ml), and further stirred at 5° for 30 min. The light yellow **14a** was washed with water and dried *in vacuo;* yield 8.3 g (92%), mp 90°. The pmr spectrum in $CDCl_3$ was identical with the product described under B.

1-(*cis*-2-Chlorocyclohexyl)-3-(2-chloroethyl)urea (Method B).—*cis*-2-Chlorocyclohexylamine hydrochloride[13] (3.0 g, 0.18 mole) was treated at 0° with NaOH (0.8 g, 0.02 mole) in water (5 ml). This mixture was extracted with three 50-ml portions of ether. 2-Chloroethyl isocyanate[34] (1.5 ml, 0.18 mole) was added dropwise to the dried (Na_2SO_4), chilled (10°), stirred ether solution. After 2 hr at room temperature, removal of the ether *in vacuo* left the crude product as a white solid (4.1 g), which was recrystallized from benzene (20 ml) by the addition of cyclohexane (100 ml) and dried *in vacuo;* yield 3.6 g (84%).

N^2-[(2-Chloroethyl)carbamoyl]-L-(−)-glutamine (Method B).—To a stirred suspension of L-(+)-glutamine (1.54 g, 10.6 mmoles) in water (20 ml), cooled to 5°, was added 1 *N* NaOH (9.5 ml), and to the resulting solution (~pH 10) 2-chloroethyl isocyanate[34] (0.81 ml, 9.5 mmoles) was added dropwise. The mixture, allowed to warm gradually to room temperature, was stirred overnight, and the pH was adjusted from ~7 to ~2 with 1 *N* HCl (9.5 ml). Evaporation under reduced pressure and below 25° left a solid residue which was washed with ethanol, then with water and dried *in vacuo* over P_2O_5 leaving 1.1 g (41%) of the carbamoylglutamine, $[\alpha]^{22}D$ −10.3 ± 0.2° (*c* 0.995, H_2O).

***p*-[3-(2-Chloroethyl)ureido]phenylacetic Acid (Method B).**—Triethylamine (4.64 ml, 30.0 mmoles) was added to a stirred suspension of *p*-aminophenylacetic acid[35] (4.67 g, 30.3 mmoles) in $CHCl_3$ (150 ml) at about 10°, and then 2-chloroethyl isocyanate[34] (2.6 ml, 30.0 mmoles) was added dropwise. The mixture was stirred at room temperature for 6 hr during which time most of the solid dissolved; the mixture was filtered, and the filtrate was extracted with three 25-ml portions of water. The amber, aqueous solution (pH 8) was made acidic (~pH 1) with concentrated HCl (7.0 ml), stirred, and chilled. The precipitate was washed with water and dried *in vacuo* over P_2O_5. The crude product (6.9 g) was recrystallized from acetonitrile as yellow crystals, yield 4.0 g (52%).

4-{*p*-[3-(2-Chloroethyl)ureido]phenyl}butyric Acid (Method B).—*p*-Aminophenylbutyric acid[36] (5.0 g, 28 mmoles) in ethanol (200 ml) at room temperature was treated with 2-chloroethyl isocyanate[34] (2.45 ml, 28.5 moles), stirred for 2 hr, and chilled. The white precipitate (6.6 g) was recrystallized from ethanol (150 ml) and dried *in vacuo* over P_2O_5; yield 5.1 g (64%).

1-(4-Amino-3,5-dichlorophenyl)-3-(2-chloroethyl)urea (31) (Method B).—2-Chloroethyl isocyanate[34] (4.00 ml, 46.7 mmoles) was added dropwise to a 0–5°, stirred solution of freshly recrystallized 2,6-dichloro-*p*-phenylenediamine[35] (4.14 g, 23.4 mmoles) in $CHCl_3$ (190 ml), and the mixture was stirred overnight at ambient temperature. Recrystallization of the crude, insoluble

(34) W. Siefkin, *Ann. Chem.*, **562**, 75 (1949).
(35) Aldrich Chemical Co., Milwaukee, Wis.
(36) L. R. Moffett, Jr., and H. W. Vaughan, Jr., *J. Org. Chem.*, **25**, 1238 (1960).

solid (6.3 g) from ethanol–water (150:100 ml) afforded 5.0 g (75%) of **31** as a fluffy white solid.

1-(2-Chloroethyl)-3-(3,5-dichlorophenyl)-1-nitrosourea (32). Sodium nitrite (590 mg, 8.55 mmoles) was added in small increments over a period of 2.5 hr to a stirred, 5–10° solution of **31** (280 mg, 0.994 mmole) in 98–100% formic acid (8 ml). The resulting reddish brown solution was stirred cold for 2 hr, poured into cold water (20 ml), further stirred for 3 hr at 0–5°, and extracted with two 15-ml portions of $CHCl_3$. Removal of the solvent under reduced pressure left **32** which was triturated in petroleum ether and dried *in vacuo* over P_2O_5; yield 147 mg (50%).

N^2-[(Benzyloxy)carbonyl]-DL-glutamine Methyl Ester (36).—A stirred mixture of N^2-[(benzyloxy)carbonyl]-DL-glutamine[35b] (**35**) (34.3 g, 0.122 mole) and $KHCO_3$ (12.3 g, 0.122 mole) in water (160 ml) was heated at 75–85° for 1 hr. The resulting solution was evaporated to dryness under reduced pressure with heating. A stirred suspension of the residual salt (further dried *in vacuo* at about 50°) in DMF (180 ml) was treated with iodomethane (23 ml, 0.32 mole), heated at 80–85° for 2 hr, and filtered. The filtrate was diluted with water (3.5 l.) and chilled. The crystalline **36** that formed was washed with water and dried *in vacuo*; yield 33.1 g (92%). The analytical sample was recrystallized from methanol.

Anal. Calcd for $C_{15}H_{18}N_2O_5$: C, 57.13; H, 6.16; N, 9.52. Found: C, 57.19; H, 6.19; N, 9.52.

Benzyl DL-2,6-dioxo-3-piperidinecarbamate (37) was made by the published procedure in which racemization of the L isomer was observed.[35b] The yield of recrystallized DL-**37**, mp 132–134°, from DL-**36** (15.0 g, 51.0 mmoles) (20-min reaction *in vacuo*) was 9.0 g (67%) (lit.[35b] mp 122–123°).

Anal. Calcd for $C_{13}H_{14}N_2O_4$: N, 10.68. Found: N, 10.67.

DL-2-Aminoglutarimide (38).[37]—Hydrogenolysis of **37** was carried out according to a published procedure.[23c] A solution of **37** (6.8 g, 26 mmoles) in methanol (170 ml) was shaken with 5% palladium–charcoal (1.7 g) and hydrogen (initially about 50 psi) for 2 hr with intermittent purging of evolved carbon dioxide. Removal of the catalyst and evaporation of the solvent *in vacuo* left 3.0 g (89%) of crude DL-**38** as white and greenish blue crystals, mp about 140° dec (lit.[23c] mp about 120° dec). Without attempted purification, crude **38** was converted to DL-1-(2-chloroethyl)-3-(2,6-dioxo-3-piperidyl)urea (**39a**, see Table I).

1-(2-Chloroethyl)-3-(1,2,3,4-tetrahydro-2,4-dioxo-5-pyrimidinyl)urea (Method B).—2-Chloroethyl isocyanate[33] (10.0 ml, 0.116 mole) was added slowly to a cold, stirred suspension of 5-aminouracil[38] (7.0 g, 0.055 mole) in DMF (40 ml). The mixture was stirred at 10–20° for 3 hr, diluted with $CHCl_3$ (50 ml), and stirred at room temperature overnight. The mixture was further diluted with $CHCl_3$ (50 ml) and chilled. The urea that formed as a white solid was collected, washed with three 15-ml portions of 2 *N* HCl, water, then ethanol, and dried *in vacuo* over P_2O_5; yield 11.3 g (88%).

1,1'-[*p*-Phenylenebis]3-(2-chloroethyl)urea] (Method B).—2-Chloroethyl isocyanate[33] (6.0 g, 57 mmoles) was added slowly to a 5°, stirred solution of water-recrystallized *p*-phenylenediamine (3.1 g, 28.5 mmoles) in DMF (50 ml). The resulting mixture, stirred at 5–10° for 30 min and at room temperature for 3 hr, was thinned with $CHCl_3$ and stirred for an additional 1 hr and diluted with hexane to complete the precipitation. The crude product was collected, washed with ethanol and then ether, and air dried. It was triturated and washed in ethanol and dried *in vacuo* over P_2O_5; yield 6.8 g (75%).

1,1'-[*p*-Phenylenebis]3-(2-chloroethyl)-3-nitrosourea]. Dry $NaNO_2$ (13 g, 190 mmoles) was added in small portions over a period of 30 min to a 5°, stirred suspension of 1,1'-[*p*-phenylenebis]3-(2-chloroethyl)urea] (5.0 g, 15.7 mmoles) in 98–100% formic acid (300 ml). The mixture was stirred at 5° for 2 hr, and the yellow precipitate that had formed was washed with water and dried *in vacuo* over P_2O_5; yield 5.8 g (97%).

4'-Cyano-1-aziridinecarboxanilide.—*p*-Cyanophenyl isocyanate[32] (10.0 g, 69.4 mmoles) was added dropwise to a 5°, stirred solution of ethylenimine (3.6 ml, 70 mmoles) in $CHCl_3$ (100 ml), and the mixture was stirred at room temperature for 2 hr. The white, solid residue (12.5 g) remaining after the solvent was removed *in vacuo* was taken up in hot benzene (500 ml); the cooled filtrate deposited 4'-cyano-1-aziridinecarboxanilide as white needles which were washed with hexane and dried *in vacuo*; yield 10.0 g (77%), mp 135°.

Anal. Calcd for $C_{10}H_9N_3O$: C, 64.16; H, 4.85; N, 22.45. Found: C, 64.40; H, 4.72; N, 22.45.

1-(2-Chloroethyl)-3-(*p*-cyanophenyl)urea (Method E).—4'-Cyanophenyl-1-aziridinecarboxanilide (10.0 g, 53.5 mmoles) was added in small portions to 0°, stirred concentrated HCl (50 ml), and the resulting mixture was stirred at 5–10° for 1 hr. The white precipitate was washed with cold water and dried *in vacuo*. The crude product (11.2 g) was dissolved in ethanol (50 ml) and the cooled solution was diluted with water (50 ml). 1-(2-Chloroethyl)-3-(*p*-cyanophenyl)urea was deposited as white needles, which were dried *in vacuo* over P_2O_5; yield 9.0 g (75%).

***p*-Amino-N,N-dimethylbenzamide.**—Gaseous dimethylamine was introduced into cold benzene (100 ml) until the weight gain was 7.5 g (0.17 mole). *p*-Aminobenzoyl chloride[39] (12.9 g, 0.083 mole) in benzene (50 ml) was slowly added below 7° with stirring. The mixture was stirred at room temperature for 3 hr, and the solid that had precipitated was removed and washed with two 25-ml portions of benzene. The filtrate combined with the washings was evaporated *in vacuo*, and the orange residue was recrystallized, once from benzene and twice from xylene, giving the benzamide as slightly colored needles (3.7 g), mp 153° (lit.[39] mp 153°). Additional product (1.8 g, mp 154°) was obtained from the original precipitate by extraction into $CHCl_3$ and recrystallization from xylene. The total yield was 41%.

Anal. Calcd for $C_9H_{12}N_2O$: C, 65.82; H, 7.37; N, 17.06. Found: C, 66.01; H, 7.28; N, 16.92.

N,N'-Bis(2-chloroethyl)-1,4-piperazinedicarboxamide.—Piperazine (3.0 g, 35 mmoles) in $CHCl_3$ (100 ml) was treated with 2-chloroethyl isocyanate[31] (7.4 g, 70 mmoles), stirred at room temperature overnight, and chilled. The precipitate was washed with cold $CHCl_3$, air dried, triturated in water, washed with ethanol, and dried *in vacuo* over P_2O_5; yield 8.5 g (93%), mp 220°; ν_{max}^{KBr} in cm^{-1}) 1615 (C=O), 1635 (CNH).

Anal. Calcd for $C_{10}H_{18}Cl_2N_4O_2$: C, 40.41; H, 6.10; N, 18.85. Found: C, 40.59; H, 6.00; N, 18.67.

N,N'-Bis(2-chloroethyl)-N,N'-dinitroso-1,4-piperazinedicarboxamide.—Sodium nitrite (6.0 g, 87 mmoles) was added in small increments to a 5°, stirred solution of N,N-bis(2-chloroethyl)-1,4-piperazinedicarboxamide (6.5 g, 22 mmoles) in 98–100% formic acid (130 ml); the mixture was stirred for 1 hr at 0–5°, resulting in the deposition of a white solid. The mixture was diluted with water and dried *in vacuo* over P_2O_5; yield 6.2 g (80%), mp 114° dec, ν_{max}^{KBr} 1695 cm^{-1} (C=O).

Anal. Calcd for $C_8H_{12}Cl_2N_6O_4$: C, 33.81; H, 4.54; Cl, 19.96; N, 23.66. Found: C, 33.95; H, 4.54; Cl, 20.10; N, 23.22.

1,3-Bis(2-chloro-1,1-dimethylethyl)urea (42b) (Method F).—The reaction of ethyl (2-hydroxy-1,1-dimethylethyl)carbamate[40] (68.3 g, 0.424 mole) with PCl_5 (177 g, 0.85 mole) was carried out according to the procedure of Najer, *et al.*,[26] for similar conversion of ethyl (3-chloropropyl)carbamate. Rapid distillation of the reaction mixture through a short column produced first a large fraction (mostly $POCl_3$) boiling up to 105° at atmospheric pressure, then a crude product (about 20 ml) boiling at 65–70° (about 30 mm). Redistillation of the smaller fraction at atmospheric pressure gave 14 g of crude 2-chloro-1,1-dimethylethyl isocyanate (about 75% pure by vpc) boiling at 145–150°, ν 2260 cm^{-1} (NCO).

A portion of the crude isocyanate (10 ml, estimated 67 mmoles) was added to a cold, stirred solution of triethylamine (20 ml) in water (125 ml), and the mixture was stirred for 1 hr. The aqueous phase was decanted from the semisolid precipitate, which solidified after prolonged triturations in water. The crude vacuum-dried **42b** was recrystallized from acetonitrile–water; yield 3.5 g (estimated 44%).

2-(2-Chloro-1,1-dimethylethylamino)-4,4-dimethyl-2-oxazoline Hydrochloride (43).—The urea **42b**, left standing in a closed vial at room temperature for 3 days, became hygroscopic and water soluble; strong infrared absorption at 1635 (C=N) shifting to 1690 cm^{-1} (C=N). A similar shift from 1620 to 1700 cm^{-1} was observed in the cyclization of 1,3-bis(2-chloroethyl)urea to 2-(2-chloroethylamino)-2-oxazoline hydrochloride[32] in boiling

(35) By method of ref 23c.

(38) Krishell Laboratories, Portland, Ore.

(39) L. McMaster and F. F. Abmann, *J. Am. Chem. Soc.*, **50**, 145 (1928).

(40) H. Wenker, *ibid.*, **60**, 1081 (1938).

(41) A. T. Bloomquist and F. T. Fiedorek, U. S. Patent 2,485,855 (1949).

(42) A. F. McKay and M.-E. Kreling, *Can. J. Chem.*, **37**, 594 (1959).

water. Drying *in vacuo* over P_2O_5 afforded analytically pure **43**, mp 105–107° with softening from 90°.

Anal. Calcd for $C_9H_{17}ClN_2O \cdot HCl$: C, 44.82; H, 7.52; N, 11.62. Found: C, 44.69; H, 7.70; N, 11.63.

Decomposition of 1-(2-Chloropropyl)-3-cyclohexyl-1-nitrosourea (9) with Cyclohexylamine.—A suspension of **9** (200 mg, 0.81 mmole) in cyclohexylamine (161 mg, 1.62 mmoles) in water (10 ml) and acetone (5 ml) was stirred for 20 hr at room temperature. The white precipitate was washed with water and dried *in vacuo* over P_2O_5; identity as 1,3-dicyclohexylurea was established by melting point (228°) and mixture melting point with an authentic sample. The yield was 180 mg (99%).

N-Cyclohexyl-2,2-dimethyl-1-aziridinecarboxamide.—Cyclohexyl isocyanate[43] (3.0 ml, 23.5 mmoles) was added dropwise to a stirred solution of 2,2-dimethylaziridine (2.12 ml, 23.5 mmoles) in petroleum ether (150 ml) with almost immediate precipitation of a white solid. After 30 min the product was collected, washed with petroleum ether, and dried *in vacuo* over P_2O_5; yield 4.06 g (88%), mp 126°. Recrystallization from hexane (125 ml) afforded analytically pure needles (3.8 g) with melting point unchanged.

Anal. Calcd for $C_{11}H_{20}N_2O$: C, 67.30; H, 10.27; N, 14.27. Found: C, 67.50; H, 10.58; N, 14.30.

1-(2-Chloro-2-methylpropyl)-3-cyclohexylurea (Method E).—A 10% solution (8 ml) of dry HCl in ether was added to a stirred solution of N-cyclohexyl-2,2-dimethyl-1-aziridinecarboxamide (300 mg, 1.53 mmoles) in ether (15 ml.). After 30 min, the precipitate was washed with ether and dried *in vacuo* over P_2O_5. The crude product (300 mg) was recrystallized from acetonitrile–water; yield 240 mg (67%). The structural assignment, based on analogy with reported[44] products obtained from 1-(arylsulfonyl)-2,2-dimethylaziridines under similar conditions, was supported by pmr, homogeneity having been indicated by tlc.

1,3-Bis(*trans*-2-chlorocyclohexyl)urea (Method C).—A cold, stirred solution of *trans*-2-chlorocyclohexylamine hydrochloride[14] (2.8 g, 16.4 mmoles) in water (60 ml) was neutralized with 50% NaOH solution and treated with 3-(*trans*-2-chlorocyclohexyl)-1-methyl-1-nitrosourea (3.6 g, 16.4 mmoles). The resulting suspension was diluted with acetone (60 ml) and triethylamine (5 ml), stirred overnight at room temperature, then chilled (0°). The white product that formed was washed with cold water and dried *in vacuo* over P_2O_5; yield 3.1 g (65%).

1-(2-Bromoethyl)-3-cyclohexylurea (Method B).—Aqueous sodium hydroxide (50%) (5.9 g) was slowly mixed with a cold solution of 2-bromoethylamine hydrobromide[32] (15.0 g, 0.07 mole) in water (10 ml). The mixture was stirred at 5° for 10 min, then extracted with four 50-ml portions of benzene. The benzene extract was dried (Na_2SO_4), chilled (0–5°), stirred, and treated dropwise with cyclohexyl isocyanate[43] (9.15 g, 0.07 mole). After 1 hr at 5–10°, the white urea that had formed was collected, washed with petroleum ether (50 ml), and dried *in vacuo* over P_2O_5; yield 13.7 g (75%).

1,3-Bis(2-bromoethyl)urea (Method E).—For 5.5 hr, dry HBr was passed through Drierite and into a 0°, stirred solution of 1,1'-carbonylbisaziridine[45] (4.95 g, 44.0 mmoles) in dry ether (70 ml) protected from moisture. Removal of ether and excess HBr under reduced pressure left the crude urea as a white solid which was triturated 3 min in ice water (25 ml) and dried *in vacuo* over P_2O_5; yield 7.35 g (61%), mp 124–125°. Weak absorption at 1700 cm^{-1} indicated slight contamination by 2-(2-bromoethylamino)-2-oxazoline hydrobromide. The product was stored in a desiccated container in a freezer to minimize further cyclization.

1-Nitroso-3-phenyl-2-imidazolidinone (13) by Reaction of 1-(2-Bromoethyl)-1-nitroso-3-phenylurea (5a) with Cyclohexylamine.—A solution of an isomeric mixture of 1-(2-bromoethyl)-N-nitroso-3-phenylureas (920 mg, 3.38 mmoles; approximately 75% **5a** and 25% **5b**) in *p*-dioxane (15 ml) was added dropwise to a 6°, stirred solution of cyclohexylamine (0.39 ml, 3.4 mmoles) in *p*-dioxane (10 ml) and water (5 ml). The solution was stirred at room temperature for 22 hr, then diluted with water (50 ml), and cooled. The dried orange precipitate (440 mg) was recrystallized from absolute ethanol giving 280 mg (~58% from **5a**) of **13** as tan crystals, mp 186–187° dec, ν^{KBr} 1750 cm^{-1} (C=O).

Anal. Calcd for $C_9H_9N_3O_2$: C, 56.54; H, 4.75; N, 21.98. Found: C, 56.72; H, 4.83; N, 21.67.

The infrared spectrum of **13** described above was identical with that of the light yellow product (mp 184–185° dec) obtained in 96% yield by the nitrosation of 1-phenyl-2-imidazolidinone[46] in aqueous formic acid.

1,3-Bis(2-iodoethyl)urea (Method G).—A solution of 1,3-bis(2-chloroethyl)urea[45] (6.5 g, 35.2 mmoles) and NaI (20.0 g, 133 mmoles) in dry acetone (300 ml), protected from moisture, was refluxed for 24 hr. The precipitate (2.7 g) after 8 hr was removed and, being water soluble, was assumed to be NaCl; acetone (50 ml) was added at this point. The precipitate (6.6 g) after 24 hr was washed with water and dried *in vacuo* over P_2O_5 leaving 6.0 g of the desired urea, mp 158–160° [lit.[47] mp 156–157°]. Dilution of the acetone filtrate with water (400 ml) afforded additional product (3.4 g, mp 155–157° dec); total yield 73%.

Screening Results

Introduction.—The true measure of the effectiveness of a drug against a neoplastic disease is the ability of the drug to kill the neoplastic cells at dosages that are not toxic to the host animal. A quantitative evaluation of drug action can be obtained by using the L1210 leukemia system in mice.[4] In this test 10^5 leukemia cells are injected into the peritoneal cavity of a mouse, and treatment of the mouse with a single injection of a drug may result in an increase in life span or, in some instances, cures[48] of the leukemia. The number of leukemic cells killed can be estimated from the observed increase in life span or from the percentage of cures obtained. The reduction in cell population expressed as a logarithm is a convenient way to compare the efficacy of a series of active structures such as the nitrosoureas. For example, a reduction of an inoculum of 10^5 cells to 10^2 cells (99,900 cells killed) can be called a "3-log kill."[49] In the tables that follow, the LD_{10} in normal animals (when known), optimal doses, percentage of cures, and the "log kill" are given. In addition, the compounds active against the intraperitoneally injected cells were evaluated against 10^4 cells injected intracerebrally to determine their ability to cross the "blood-brain barrier."[2b] Using these standardized tests the greatest log kill that can be determined in the intraperitoneal (ip) test is 6 and in the intracerebral (ic) test is 5. BCNU and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea were evaluated at higher inocula and found to kill 8 logs at their LD_{10}.

In some cases, cures are obtained at doses greater than the LD_{10} (but smaller than the LD_{90}). In other cases when the log kill (5) is great enough to effect some cures (20–50%) but none is obtained, the dose must exceed the LD_{10} and may be close to the LD_{90} for those compounds; toxicity data in normal animals are not available for compounds that effected no cures.

Structure–Activity Relationships.—The 1-(haloalkyl)-1-nitrosoureas investigated in this study can be conveniently divided into six groups for analysis

(43) Carwin Co., North Haven, Conn.

(44) V. I. Markov and S. I. Burmistrov, *Zh. Obshch. Khim.*, **35**, 153 (1965).

(45) H. Bestian, *Ann. Chem.*, **566**, 210 (1950).

(46) A. F. McKay and R. O. Braun, *J. Org. Chem.*, **16**, 1829 (1951).

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

(48) The word cure is employed herein to imply drug-induced, 45-day survival of hosts randomly selected from populations of leukemic mice in which 100% of a significant sample of untreated controls died of the disease.[4]

(49) It should be understood that "log kill" is independent of the size of the inoculum, whereas the number of cells killed is not.[50]

(50) For a detailed discussion of the effect of chemotherapy on the kinetics of leukemic cell behavior and of the concept of "log kill," see ref 4.

TABLE IV

THE EFFECTIVENESS OF 1-(HALOALKYL)-1-NITROSOUREAS AGAINST L1210 LEUKEMIA IMPLANTED BOTH INTRAPERITONEALLY AND INTRACEREBRALLY[a]

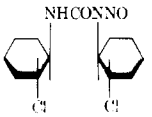
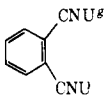
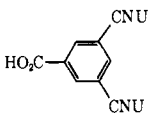
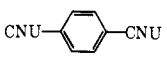
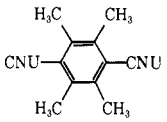
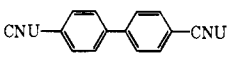
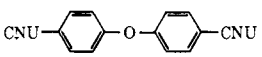
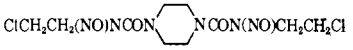
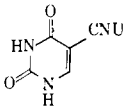
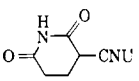
Structure	LD_{50}[b] mg/kg	OOC[c] mg/kg	10^6 cells Log kill[d]	10^6 cells % cures[e]	10^5 cells Log kill	10^5 cells % cures
Group A						
$XCH_2CH_2N(NO)CONHCH_2CH_2Y$						
X = Y = F	7.1	6–7	6	(50–60)	5	(60)
1:1 mixture: X = Cl, Y = F; X = F, Y = Cl	~64	20	6	90–100	5	50–90
X = Y = Cl (BCNU)	30	25–30	6	0–100	5	0–100
1:1 mixture: X = Cl, Y = Br; X = Br, Y = Cl		50–75	6	(100)	0	0
X = Y = Br		200	5	20	0	0
X = Y = I		Inactive			Not tested	
$XCH_2CH_2N(NO)CONHR$						
R = cyclohexyl						
X = F	54	30–45	6	70–90	5	60–100
X = Cl	50	40–50	6	80–100	5	20–100
X = Br	234	300	5	(30)	0	0
R = 2-norbornyl						
X = F	23–56	46–59	6	(90–100)	5	80–90
X = Cl	58	58–62	6	(100)	5	80–90
R = 1-adamantyl						
X = F		187	5	(20)	2	0
X = Cl		62	6	(70)	<2	0
R = phenyl						
X = F		18	4	0	2	0
X = Cl		36	3	0	0	0
X = Br		222	4	0	0	0
Group B						
$ClCH(CH_3)CH_2NHCON(NO)CH_2CH(CH_3)Cl$		1000	5	20	4	0
$c\text{-}C_6H_{11}NHCON(NO)CH_2CH(CH_3)Cl$		750	5	0	2	0
$C_6H_5NHCON(NO)CH_2CH_2CH_2Cl$		200	2	0	Not tested	
$Cl(CH_2)_3N(NO)CONHC_6H_4(NHCON(NO)(CH_2)_3Cl)\text{-}p$		Inactive			Not tested	
$c\text{-}C_6H_{11}NHCON(NO)CH_2CH_2CH_2Cl$		Inactive			Not tested	
$ClCH_2CH(C_2H_5)NHCON(NO)CH(C_2H_5)CH_2Cl$		Inactive			Not tested	
		Inactive			Not tested	
$CF_3CH_2NHCON(NO)CH_2CF_3$		Inactive			Not tested	
$c\text{-}C_6H_{11}NCONCH_2CF_3$ (H | NO (mixture))		Inactive			Not tested	
Group C						
$RC_6H_4NHCON(NO)CH_2CH_2Cl$						
R = 2,6-dimethyl		31	6	(70)	<2	0
$p\text{-}CON(CH_3)_2$		16	6	(70)	Not tested	0
$p\text{-}CH_3O$		40	5	0	<1	0
$m\text{-}CH_3O$		40	5	(20)	Not tested	
p-Cl		75	5	(10)	<1	0
m-Cl		100	5	0	0	0
p-F		25	5	(20)	<1	0
$p\text{-}CF_3$		75	3	0	<1	0
p-CN		150	5	(20)	0	0
$p\text{-}COCH_3$		75	5	(20)	2	0
$p\text{-}CO_2C_2H_5$		9	5	(30)	<1	0
$p\text{-}CO_2H$		30	5	(30)	<1	0
$m\text{-}CO_2H$		12–18	5	(20)	0	0
$p\text{-}CH_2CO_2H$		26	5	(30)	2	0
$p\text{-}SCH_2CO_2H$		18	5	(20)	0	0
o-Cl		50	3	0	0	0
$m\text{-}NO_2$		26	4	0	<1	0
$p\text{-}SO_2F$		125	3	0	Not tested	
H		38	3	0	<1	0
$RNHCON(NO)CH_2CH_2Cl$						
R = 2-naphthyl		300	5	0	<1	0
R = 8-quinolyl		12	5	0	Not tested	

TABLE IV (*Continued*)

Structure	LD_{10},[b] mg/kg	OD,[c] mg/kg	Ip (10^5 cells) Log kill[d]	Ip (10^5 cells) % cures[e]	Ic (10^4 cells) Log kill	Ic (10^4 cells) % cures
		Group D				
RNHCON(NO)CH_2CH_2Cl						
R = H	8.3	5–7.2	5	40	4	20
cyclohexyl	50	40–50	6	20–100	5	80–100
4-methylcyclohexyl	<62	30–45	6	90–100	4	20–50
3-methylcyclohexyl	~143	62	6	70–100	5	50–80
3,3,5-trimethylcyclohexyl		750	6	100	Not tested	
trans-4-*t*-butylcyclohexyl	<1000	375–500	6	20–80	4	20
trans-2-chlorocyclohexyl	69	50–72	6	30–90	4	(50)
cis-2-chlorocyclohexyl	61	46–61	6	80–100	5	30–70
1-ethoxycarbonylcyclohexyl		250	6	70–80	4	20
bornyl		300	3	0	Not tested	
2-norbornyl	58	58–62	6	100	5	80–90
1-adamantyl		62	6	(70)	0	0
cyclopentyl	100	64–93	5	(30)	5	(70)
1-methylcyclopentyl		125–250	6	(100)	5	(90)
2-indanyl		62–125	5	(30)	2	0
cyclododecyl	1190	750–840	5	(30)	<2	0
		Group E				
		10	3	0	Not tested	
		26–40	5	(30)	2	0
	370	600	5	(50)	<2	0
	500	500	5	0–20	<2	0
		1500	5	(20)	Not tested	
		1000	5	(30)	2	0
	255	255	6	100	5	60
	243	150	6	80	4	0
		80–250	6	(100)	4	(50)
		50–100	2	0	Not tested	
		40	6	(70)	0	0
		12–16	≥6	(100)	5	(80)
(5α-Cholestan-3α-yl)-CNU		2500	5	(50)	Not tested	

[a] A detailed description of these screening procedures may be found in ref 4. [b] LD_{10} is defined as the dose required to kill 10% of a test group of normal animals as determined from log dose, probit mortality plots. The fit of the line to the data was obtained with a computer program designed to approximate a least-squares fit by successive approximations. [c] OD is defined as the optimal dose for the therapeutic effect observed, *i.e.*, greatest log kill, largest per cent cures. [d] Defined in text. [e] For the definition of a cure see footnote 48. [f] Parentheses indicate only one determination. [g] CNU = 3-(2-chloroethyl)-3-nitrosoureido, FNU = 3-(2-fluoroethyl)-3-nitrosoureido.

of structure–activity relationships.[51] The compounds listed in group A of Table IV show the effects of varying the halogen at C-2 of an ethyl side chain. There appears to be little difference between the fluoro and chloro compounds. Both types are more active than the bromo compounds, and the only iodo compound studied was inactive. The bromo compounds, although quite active against L1210 implanted intraperitoneally, are all inactive against the intracerebrally implanted cells. A possible explanation for this observation might be that the bromo compounds have a greater tendency to cyclize to inactive structures. When injected intraperitoneally these compounds are able to kill cells injected into the peritoneal cavity, but cyclize during the trip to the brain and before they can cross the blood–brain barrier.

Compounds with variations in the alkyl group attached to the nitrosated nitrogen comprise group B. Although the 2-chloropropyl group gives rise to active compounds, the extremely high optimal doses (1000 and 750 mg/kg) indicate that this group is less effective than the 2-chloroethyl group. Other variations such as transfer of the chlorine atom to C-3 of the propyl group or attachment of an ethyl group to C-1 of the 2-chloroethyl group result in essentially inactive compounds.

The aromatic nitrosoureas of group C show a wide variation in activity, killing 2–6 logs of cells; but most of these compounds kill between 3–5 logs. Against the intracerebrally implanted cells, they are much less effective, killing less than 99% of the cells and effecting no cures. The presence of an aromatic ring seems to interfere with passage across the blood–brain barrier.

On the other hand, the most active nitrosoureas are found among the cycloaliphatic compounds of group D. With one not easily understood exception, the bornyl compound, these compounds all kill 5 or 6 logs of cells and most of them kill 6 logs. Most of the compounds tested against the ic disease killed 4–5 logs. A notable exception is the adamantyl compound which is highly active against the ip disease, but inactive against the ic disease, in spite of the great lipoid solubility imparted by the adamantyl group.

Group E contains the compounds bearing two 1-(2-chloroethyl)-1-nitrosoureido groups. The more effective aromatic compounds are found in this group, and the two bisureido cycloaliphatics are about as good as the best mono compounds. The piperazine derivative, which has no protons on its ureido nitrogens, is inactive.

All the miscellaneous structures in group F are quite active against the ip disease, but the uracil derivative as expected, was inactive against the ic disease. On the other hand, the glutarimide derivative, with rather low lipoid solubility, is highly active against the ic cells.[52] This result and the lack of activity of the adamantyl derivative indicate that our original suggestion about the relationship of lipoid solubility to the ability of a compound to cross the blood–brain barrier is in need of modification. Other factors, such as the rigid geometry[54] of the adamantyl moiety, must now be considered.

Out of this study have come fifteen 1-[2-(chloro or fluoro)ethyl]-1-nitrosoureas, in addition to BCNU, that can effect 90–100% cures of mice injected intraperitoneally with 10^5 L1210 leukemia cells at their LD_{90} or lower doses. Of these, nine[55] are almost as effective against these cells (10^4) implanted intracerebrally. These compounds are listed in Table V. With one exception, the glutarimide derivative, these highly active structures are aliphatic or cycloaliphatic compounds.

Table V

The Most Effective Nitrosoureas against L1210 Leukemia

Compd[a]	% cures of L1210 leukemia ip	ic
$ClCH_2CH_2CNU$ (BCNU)*	0–100[b]	0–100
$ClCH_2CH_2FNU$ and FCH_2CH_2CNU*	90–100	50–90
$BrCH_2CH_2CNU$ and $ClCH_2CH_2BNU$	100	0
1-Methylcyclopentyl-CNU*	100	90
Cyclohexyl-CNU*	80–100	20–100
Cyclohexyl-FNU*	70–90	60–100
3-Methylcyclohexyl-CNU*	70–100	50–80
4-Methylcyclohexyl-CNU	90–100	20–50
3,3,5-Trimethylcyclohexyl-CNU	100	Not tested
cis-2-Chlorocyclohexyl-CNU	30–90	(50)
trans-2-Chlorocyclohexyl-CNU	80–100	30–70
2-Norbornyl-CNU*	100	80–90
2-Norbornyl-FNU*	90–100	80–90
trans-1,4-Cyclohexylenebis(CNU)	(100)	(60)
trans-1,4-Cyclohexylenebis(FNU)	(100)	(50)
2,6-Dioxo-3-piperidyl-CNU*	100	80

[a] CNU = 3-(2-chloroethyl)-3-nitrosoureido, BNU = 3-(2-bromoethyl)-3-nitrosoureido, FNU = 3-(2-fluoroethyl)-3-nitrosoureido. The asterisk indicates the compound is effective in the ip and ic tests. [b] Includes early experience with BCNU. Later data indicate that a high percentage of cures can be consistently obtained with this compound.

The problem now to be faced is that of selecting a limited number of these structures for further study in higher animals and perhaps in man. If suggestive differences in host toxicity are truly significant, they may form the basis for such a selection.

Acknowledgments.—The authors are indebted to Dr. J. R. Dice for gifts of 1-phenylcyclohexylamine, 3,3,5-trimethylcyclohexylamine, ethyl 1-aminocyclopentanecarboxylate, and ethyl 1-aminocyclohexanecarboxylate hydrochlorides, and to Dr. Koert Gerzon for a gift of 1-adamantylamine hydrochloride; to Mr. W. J. Boyle, Jr., for technical assistance during the summer of 1965; to Mrs. Ruby James for vpc studies of the aqueous decomposition of BCNU; to Dr. W. C. Coburn, Jr., for interpretation of pmr and infrared spectra; to Mrs. Martha Thorpe for determination and interpretation of pmr spectra; to Dr. F. M. Schabel, Jr., Dr. W. R. Laster, Miss Valerie Peake, and members of the Cancer Screening Section

(51) In a previous report[2a] the activity of a variety of 1-substituted 1-nitrosoureas was described. Of that group 1,3-bis(2-chloroethyl)-1-nitrosourea showed the highest degree of activity.

(52) Glutamine, but not glutamic acid, is known to penetrate ascites cells as well as the blood–brain barrier.[53]

(53) A. White, P. Handler, E. L. Smith, and D. Stetten, Jr., "Principles of Biochemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1951, p 562.

(54) R. C. Fort, Jr., and P. von R. Schleyer, *Chem. Rev.*, **64**, 277 (1964).

(55) Designated in Table V with an asterisk.

of Southern Research Institute for biological data; to Mrs. Luverne Mattil for assistance in the compilation of the data in Table IV; to Mr. W. E. Fitzgibbon, Jr., and his staff (Mrs. Sarah Jo Clayton, Mr. Francis Chen, Mrs. Lucy Rose, Mrs. Anita Shortnacy) for preparation of some of the intermediates and products in large amounts; and to Dr. W. J. Barrett, Dr. P. D. Sternglanz, and members of the Analytical Chemistry Section of Southern Research Institute who performed the microanalytical and spectral determinations.

The Use of α-Amino Acids in the Synthesis of Derivatives of 2-Aminoethanethiol as Potential Antiradiation Agents[1]

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The utility of α-amino acids as intermediates in multistep syntheses of derivatives of 2-aminoethanethiol as potential antiradiation agents has been further demonstrated; the types of compounds synthesized included S-substituted derivatives of 2-aminoalkanethiols, 2-amino-2-methylalkanethiols, and 1-aminocycloalkanemethanethiols—chiefly inner Bunte salts and phosphorothioates—and cyclic dithiocarbamates, in addition to a number of the aminothiols themselves. A convenient method for the preparation of amino acid esters from 2-alkanones and cycloalkanones was developed by combining a modified Strecker amino acid synthesis with the Fischer amino acid esterification. Applied to 1,4-cyclohexanedione, this method led to the synthesis of a novel bisaziridine, 1,7-diazaspiro[2.2.2.2]decane (**37**), and to a novel synthesis of 1,4-diamino-1,4-cyclohexanedimethanethiol diphosphate (**40**), which involved hydrolysis of the corresponding bis(phosphorothioic acid) **39b** in 1 *M* phosphoric acid. The following products so derived afforded mice good protection against lethal radiation in a standard test: sodium hydrogen S-2-amino-3-methylbutylphosphorothioate (**5d**), S-2-amino-2-methylpropylthiosulfuric acid (**16b**), S-2-amino-2-methylpropylphosphorothioic acid (**16c**), and tetrahydro-1H,3H-thiazolo[4,3-*c*][1,4]thiazine-3-thione (**29**).

The lithium aluminum hydride reduction of α-amino acid esters by Karrer, *et al.*,[2] provided a synthetic route to 2-substituted 2-aminoethanols that is particularly useful if the desired substituent is contained in a readily available amino acid. Vogl and Pöhm demonstrated later that a direct reduction of amino acids could be achieved similarly.[3] Thus, in the present work, 2-amino-1-pentanol (**1**, R = n-C_3H_7) was obtained by the reduction of both ethyl DL-norvalinate and DL-norvaline. Conversion of the resultant 2-aminoalkanols **1** to the corresponding 2-bromoethylamine hydrobromides **3** was accomplished either directly by the action of (1) phosphorus tribromide on the preformed hydrobromide [as with 2-amino-3-phenyl-1-propanol[4] (**1**, R = $C_6H_5CH_2$) from DL-phenylalanine] and (2) refluxing 48% hydrobromic acid[5] [as with L-leucinol (**1**, R = i-C_4H_9) from L-leucine], or indirectly by the hydrobromic acid ring opening of the aziridine derived by the Wenker method[6,7] [as with DL-valinol (**1**, R = i-C_3H_7) from DL-valine *via* 2-isopropylaziridine (**2**)]. These examples, then, typify the amino acid derived intermediates that led to the preparation of a number of S-substituted 2-aminoalkanethiols, chiefly inner Bunte salts and phosphorothioates, which were desired as analogs of known radioprotective compounds.[8] The syntheses outlined in Chart I were based on 2-aminoalkanols derived from common amino acids; a subsequent synthesis based on commercially available 2-amino-2-methylbutyric acid (**7**) is shown in Chart II. Some examples of the utility of amino acids in the synthesis of potential antiradiation compounds have recently been reported.[12]

Commercially available 2-amino-2-methyl-1-propanol (**12**) and later 2,2-dimethylaziridine (**13**, R = CH_3) were used as starting materials for the synthesis of 2-amino-2-methylpropanethiol (**16a**) and several of its S-substituted derivatives by the route outlined in Chart II. The radioprotective activity shown by S-2-amino-2-methylpropylthiosulfuric acid (**16b**) in an initial test inspired the synthesis of a series of S-2-amino-2-methylalkylthiosulfuric acids in which one of the methyl groups of **16b** is replaced by other alkyl groups as in the route **7** → **16e** already mentioned. Development of practical methods for the preparation of the intermediate amino acid esters **10** was requisite since neither these esters nor the corresponding amino acids (except **7**) were readily available. The general procedure that evolved, as applied to 2-alkanones (**8**), combines a modified Strecker amino acid synthesis

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(2) P. Karrer, P. Portmann, and M. Suter, *Helv. Chim. Acta*, **31**, 1617 (1948).

(3) O. Vogl and M. Pöhm, *Monatsh. Chem.*, **83**, 541 (1952).

(4) W. J. Gensler and J. C. Rockett, *J. Am. Chem. Soc.*, **74**, 4451 (1952).

(5) F. Cortese, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p 91.

(6) (a) H. Wenker, *J. Am. Chem. Soc.*, **57**, 2328 (1935); (b) P. E. Fanta, "Heterocyclic Compounds with Three- and Four-membered Rings," Part One, A. Weissberger, Ed., Interscience Publishers, Inc., New York, N. Y., 1964, pp 528–532.

(7) T. L. Cairns, *J. Am. Chem. Soc.*, **63**, 871 (1941).

(8) For example: 2-amino-1-propanethiol,[9] S-2-aminoethylthiosulfuric acid,[10] and S-2-aminoethylphosphorothioic acid.[11]

(9) J. F. Thomson, "Radiation Protection in Mammals," Reinhold Publishing Corp., New York, N. Y., 1962, p 66.

(10) B. Holmberg and B. Sörbo, *Nature*, **183**, 882 (1959).

(11) (a) B. Hansen and B. Sörbo, *Acta Radiol.*, **56**, 141 (1961); (b) S. Åkerfeldt, *Acta Radiol., Therapy, Phys. Biol.*, **1**, 465 (1963); *Chem. Abstr.*, **60**, 13534 (1964).

(12) (a) J. R. Piper and T. P. Johnston, *J. Org. Chem.*, **28**, 981 (1963); (b) D. L. Klayman, J. W. Lown, and T. R. Sweeney, *ibid.*, **30**, 2275 (1965); (c) G. R. Handrick, E. R. Atkinson, F. E. Granchelli, and R. J. Bruni, *J. Med. Chem.*, **8**, 762 (1965); (d) H. Gershon and R. Rodin, *ibid.*, **8**, 864 (1965).