TABLE V
EFFECT OF SULFONIC ACIDS ON HERPES SIMPLEX VIRUS INJECTED INTRACEREBRALLY INTO MICE (FOR DETAILS SEE EXPERIMENTAL SECTION)

| Substance | Injected amount, μg | % survivors of virus and substance | % survivors of virus |
|---|---|---|---|
| Vital new red | 10 | 30 | 13 |
| | 20 | 90 | 67 |
| | 30 | 67 | 42 |
| Evan's blue | 10 | 40 | 13 |
| | 20 | 100 | 67 |
| | 30 | 92 | 42 |
| Suramin | 10 | 55 | 10 |
| | 20 | 100[a] | 67 |
| Chromatropic acid | 50 | 40 | 10 |
| | 50 | 100 | 67 |
| Aminomethanesulfonic acid | 50 | 50 | 10 |
| | 50 | 80 | 67 |
| | 10 | 0 | 13 |

[a] 40% of the animals died of toxic symptoms during the first few days. All the remaining animals survived.

thalene ring does not seem to be as important as with herpes simplex virus.

**Effect against Rhino Virus 33342** *in Vitro*.—A rather striking difference is seen between the protective effect of a number of substances in the two *in vitro* test systems used. For instance, several of the dyes give excellent protection in the lung cell system, whereas they are completely inactive or almost inactive in the amnion cell system.

Several of the compds being protective in both test systems (**11, 13, 14, 20, 22, 24**) are closely related to chromotropic acid.

It is noteworthy that the one-carbon compd, $H_2N$-$CH_2SO_3H$, is active in both test systems. The distance between the $H_2N$ and $SO_3H$ groups in this type of compd is crucial (*cf.* **47, 48, 49**).

**Acknowledgment.**—The authors are indebted to Mrs. S. Geijer, G. Fuchs, E. Holubars, and M. Brundin for skillful technical assistance.

# Synthesis of Potential Anticancer Agents. 38. *N*-Nitrosoureas. 4.[1] Further Synthesis and Evaluation of Haloethyl Derivatives[2]

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Additional congeners of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) were synthesized with special emphasis on alicyclic and heteroalicyclic analogs of 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), which further exemplified steric control of nitrosation. Steric control of nitrosation by noncyclic tertiary branching was also demonstrated. Attempted modifications of the nitrosoureido function were successful only in the case of 1-(2-chloroethyl)-1-nitroso-3-(*p*-tolylsulfonyl)urea (**51**), isolation of characterizable nitroso derivatives of (methylsulfonyl)-, thio-, and alkoxyureas and a nitronitrosourea being thwarted by instability. Activities of the new 2-chloroethyl- and 2-fluoroethylnitrosoureas against both intraperitoneally (ip) and intracerebrally (ic) inoculated murine leukemia L1210 were compared, in terms of the chemotherapeutic indices $ED_{50}/LD_{10}$ and $ED_{99}/LD_{10}$, with BCNU, CCNU, and the isomeric mixture **6** derived by nitrosation of 1-(2-chloroethyl)-3-(2-fluoroethyl)-urea. The most effective compound against these two forms of leukemia L1210 was found to be the isomeric mixture **6** with 1-(2-fluoroethyl)-1-nitroso-3-(tetrahydro-2*H*-thiopyran-4-yl)urea *S,S*-dioxide (**25**), 1-(2-fluoroethyl)-1-nitroso-3-(tetrahydro-2*H*-thiopyran-4-yl)urea (**23**), and 3-(4-acetoxycyclohexyl)-1-(2-chloroethyl)-1-nitrosourea (**47**) being almost as active. High activity against the ip disease and slight activity against the ic disease were shown by **51**, which is another example of structural limitation to crossing the blood–brain barrier.

The synthesis of numerous congeners of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), a clinically promising antineoplastic agent,[3] led to a definition of structural requirements for exceptional activity against murine leukemia L1210, implanted both intraperitoneally and intracerebrally.[4] Such activity was limited, for the most part, to 1-(2-haloethyl)-1-nitrosoureas substituted in the 3 position by a 2-haloethyl or an alicyclic group, the halogen atom being either Cl or F; for example, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU),[4] which is also undergoing clinical trials, was particularly effective against both forms of leukemia L1210.

**Chemistry.**—Further synthesis in this area made available the additional haloethylnitrosoureas (from haloethylureas of Tables I and II) of Tables III and IV for comparative evaluation against experimental animal tumor systems. Nitrosations were carried out in undiluted $HCO_2H$ with $NaNO_2$, a system known to minimize random nitrosation of chloroethylureas substituted at the 3 position by cyclic groups.[4] Such steric control was apparently operative also in the nitrosation of 1,1′-(2-chlorotrimethylene)bis(3-cyclohexylurea) (**1**), since decomposition of the product with cyclohexylamine gave 1,3-dicyclohexylurea, the product expected from structure **2**.[5] The conversion of 1-(2-chloroethyl)-3-(α,α-dimethylphenethyl)urea (**3**)

(1) Part 3: T. P. Johnston and P. S. Opliger, *J. Med. Chem.*, **10**, 657 (1967).

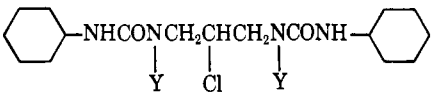
(2) This work was supported by funds from the C. F. Kettering Foundation, the Southern Research Institute, and Chemotherapy, National Cancer Institute, National Institutes of Health, Contract No. PH43-64-51.

(3) (a) S. K. Carter and J. W. Newman, *Cancer Chemother. Rep. (Part 3)*, **1**, 115 (1968); (b) H. E. Lessner, *Cancer*, **22**, 451 (1968); (c) V. B. Rege and R. E. Lenhard, Jr., Fifth Annual Scientific Meeting of the American Society of Clinical Oncology, Inc., San Francisco, Calif., March 1969, Abstract 36; (d) C. B. Wilson, E. B. Boldrey, and K. J. Enot, *Cancer Chemother. Rep. (Part 1)*, **54**, 273 (1970).

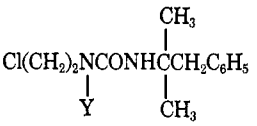
(4) T. P. Johnston, G. S. McCaleb, P. S. Opliger, and J. A. Montgomery, *J. Med. Chem.*, **9**, 892 (1966).

(5) The structure of **2** could not be definitely decided by pmr spectroscopy[4] because of overlapping of signals, but the NH protons appeared to be split by single (1-cyclohexyl) protons and not by two ($CH_2$) protons.

to the nitrosourea **4**, whose structure was verified by pmr spectroscopy, exemplifies yet another type of steric control of nitrosation, the first example by adjacent noncyclic tertiary branching. Factors other than steric hindrance, however, were apparently encountered in attempted resynthesis of the 1:1 mixture of isomers[4] obtained by nitrosation of 1-(2-chloroethyl)-3-(2-fluoroethyl)urea (**5**) in concd HCl; repetitions of the original, confirmed experiment have given **6a**:**6b** ratios of ~2:5 twice and ~1:1 once. Equilibration in cold $HCO_2H$ changed the 2:5 ratio to 1:6, but since the recovery was low, this change could not be definitely attributed to nitroso group migration.[4] The results of subsequent small-scale nitrosations have indicated that a relatively slow, constant-rate addition of an aq soln of $NaNO_2$ will consistently give the 1:1 mixture.



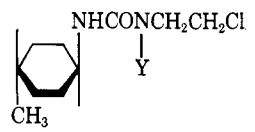
**1**, Y = H
**2**, Y = NO



**3**, Y = H
**4**, Y = NO

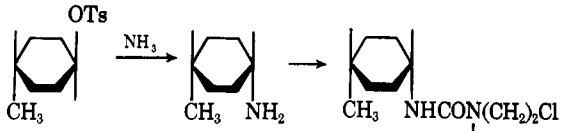
**5**, Y = Z = H
**6a**, Y = NO; Z = H
**b**, Y = H; Z = NO

Several ring-substituted analogs of CCNU were described previously,[4] but special interest in the 4-methyl derivative[6] prompted the synthesis of other 4-alkylcyclohexyl derivatives. First, however, the assumption that the original sample of the 4-methyl derivative (prepared from commercial 4-methylcyclohexylamine) was predominantly trans was verified by the preparation of cis and trans forms of the amine and their conversion to the nitrosoureas **8** and **12**. 4-Methylcyclohexylamine derived by the Na–EtOH reduction[7] of 4-methylcyclohexanone oxime was converted to a three-times recrystallized hydrochloride whose melting point agreed with that reported for the trans form prepared from *p*-acetotoluidide.[8] Pure **8** derived from the trans amine melted 6° higher than the original sample, whose pmr spectrum had shown no contamination due to random nitrosation. In subsequent preparations the urea derived from commercial amine was recrystallized until its melting point agreed with that of the pure trans isomer **7**. The preparation of **12** (Scheme I) involved an inversive ammonolysis of *trans*-4-methylcyclohexyl tosylate (**9**); the melting point of the isolated cis amine **10**·HCl agreed with that reported for an authentic sample prepared from *p*-acetotoluidide.[8] The derived **12** was an analytically pure oil in which a trace of the urea **11** was detected by tlc.



**7**, Y = H
**8**, Y = NO

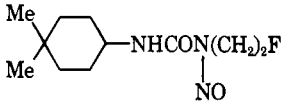
SCHEME I



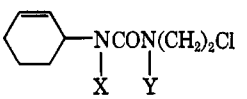
**9** **10**

**11**, Y = H
**12**, Y = NO

Preparations of the 4-ethyl- and 4-isopropylcyclohexyl derivatives of Tables III and IV involving Raney Ni reductions of the corresponding cyclohexanone oximes probably resulted, without design, in a predominance of the trans isomers, since recrystallization of the intermediate ureas entailed considerable loss with sharpening of melting points. This assumption seems to be supported by data in Table V showing conformations based on empirical observations of differences in ir absorptions between equatorial and axial nitrosoureido groups.[9] 4,4-Dimethylcyclohexanone[10] was similarly converted to 1-(2-fluoroethyl)-3-(4,4-dimethylcyclohexyl)-1-nitrosourea (**13**), and 2-cyclohexen-1-ylamine[11] was converted to 1-(2-chloroethyl)-3-(2-cyclohexen-1-yl)-1-nitrosourea (**14a**), an unsaturated analog of CCNU, the latter containing a small amount of the isomeric nitrosourea **14b**. The chloroethylnitrosourea corresponding to **13** and the fluoroethylnitrosourea corresponding to **14a** were both oils, which, as was often the case with oily nitrosoureas, could not be obtained pure. A decomposition of 3-(2-cyclohexen-1-yl)-1-methyl-1-nitrosourea catalyzed by $Et_3N$ gave 1,3-di-2-cyclohexen-1-ylurea (**15**), an alicyclic analog of 1,3-diallylurea, which itself has shown some activity against leukemia L1210.[12]



**13**



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

**15**

(6) J. A. Montgomery, *Annu. Rep. Med. Chem.*, **1969**, 144 (1970).
(7) *Cf.* D. V. Nightingale, J. D. Kerr, J. A. Gallagher, and M. Maienthal, *J. Org. Chem.*, **17**, 1017 (1952); D. H. R. Barton and R. C. Cookson, *Quart. Rev. Chem. Soc.*, **10**, 44 (1956); C. W. Shoppee, D. E. Evans, H. C. Richards, and G. H. R. Summers, *J. Chem. Soc.*, 1649 (1956); D. Y. Curtin, R. D. Stolow, and W. Maza, *J. Amer. Chem. Soc.*, **81**, 3330 (1959).
(8) M. Tichy, J. Jonas, and J. Sicher, *Collect. Czech. Chem. Commun.*, **24**, 3434 (1959).
(9) *Cf.* similar observations on equatorial nitrosoureido groups in ref 4.
(10) F. G. Bordwell and K. M. Wellman, *J. Org. Chem.*, **28**, 1347 (1963).
(11) L. Goodman, S. Winstein, and R. Bochan, *J. Amer. Chem. Soc.*, **80**, 4312 (1958).
(12) An observation made in the Cancer Chemotherapy National Service Center screening program. Also see British Patent 1,117,387, 1968; *Chem. Abstr.*, **69**, 54290 (1968).

TABLE I
(2-CHLOROETHYL)UREAS
RNHCONHCH$_2$CH$_2$Cl

| R | Method[a] | Reaction solvent | Recrystn solvent[b] | Yield, % | Mp,[c] °C | ν KBr, cm$^{-1}$ [d] C=O | ν KBr, cm$^{-1}$ [d] CHN | Formula[e] |
|---|---|---|---|---|---|---|---|---|
| 2-Hydroxy-1,1-dimethylethyl[f] | A | $Et_2O$[g] | | 87 | 85 | 1645 | 1560 | $C_7H_{15}ClN_2O_2$ |
| $\alpha,\alpha$-Dimethylphenethyl | A | $CHCl_3$ | $CCl_4$ | 82 | ~118 | 1630 | 1565 | $C_{13}H_{19}ClN_2O$ |
| 2-Cyclohexen-1-yl | A | Hexane | | 95 | 115 | 1620 | 1575 | $C_9H_{15}ClN_2O$ |
| *trans*-4-Methylcyclohexyl | B | $Et_2O$ | | 80 | 150 | 1620 | 1580 | $C_{10}H_{19}ClN_2O$ |
| *cis*-4-Methylcyclohexyl | C | $CHCl_3$ | MeCN–$H_2O$ | 79 | 112 | 1620 | 1560 | $C_{10}H_{19}ClN_2O$ |
| 4-Ethylcyclohexyl | C | $CHCl_3$ | MeCN | 39 | 140 | 1620 | 1575 | $C_{11}H_{21}ClN_2O$ |
| 4,4-Dimethylcyclohexyl | C | $CHCl_3$ | MeCN | 64 | ~118 | 1625 | 1570 | $C_{11}H_{21}ClN_2O$ |
| 4-Isopropylcyclohexyl | C | $CHCl_3$ | MeCN | 50 | 110 | 1625 | 1580 | $C_{12}H_{23}ClN_2O$ |
| 3-*tert*-Butylcyclohexyl[h] | C | $CHCl_3$ | MeCN | 38 | ~145 | 1620 | 1570 | $C_{13}H_{25}ClN_2O$ |
| 4-Hydroxycyclohexyl | A | $H_2O$–EtOH | | 36 | 137 | 1655, 1645 | 1555 | $C_9H_{17}ClN_2O_2$ |
| 4-Acetoxycyclohexyl | C | $CHCl_3$ | $C_6H_6$ | 62 | 157 | 1720,[i] 1625 | 1575 | $C_{11}H_9ClN_2O_3$ |
| *trans*-2-Phenylcyclopropyl | D | $CHCl_3$ | | 60 | 105 | 1630 | 1585 | $C_{12}H_{15}ClN_2O$ |
| 1-(*p*-Fluorophenyl)cyclohexyl | A | $Et_2O$[g] | | 93 | 136 | 1630 | 1565 | $C_{15}H_{20}ClFN_2O$ |
| *trans*-4-(Ethoxycarbonyl)cyclohexyl | C | $CHCl_3$ | | 65 | 135 | 1720,[i] 1625 | 1575 | $C_{12}H_{21}ClN_2O_3$ |
| *cis*-3-(Ethoxycarbonyl)cyclohexyl | B | $CHCl_3$ | | 64 | 103 | 1725,[i] 1625 | 1575 | $C_{12}H_{21}ClN_2O_3$ |
| *cis*-4-(Ethoxycarbonyl)cyclohexyl | C | $CHCl_3$ | | 82 | 99 | 1730,[i] 1725,[i] 1625 | 1555 | $C_{12}H_{21}ClN_2O_3$ |
| 1-(Ethoxycarbonyl)-2-methylcyclohexyl | A | $Et_2O$ | MeCN–$H_2O$ | 87 | ~125 | 1730,[i] 1635 | 1560 | $C_{13}H_{23}ClN_2O_3$ |
| 1-(Ethoxycarbonyl)-3-methylcyclohexyl | A | $Et_2O$ | MeCN–$H_2O$ | 80 | 128 | 1735,[i] 1715,[i] 1655, 1625 | 1560 | $C_{13}H_{23}ClN_2O_3$ |
| Tricyclo[2.2.1.0$^{2,6}$]hept-3-yl[j] | A | $Et_2O$ | $C_6H_6$ | 87 | 135 | 1635 | 1575 | $C_{10}H_{15}ClN_2O$ |
| 2-Adamantyl | A | $C_6H_6$[g] | MeCN | 55 | 203 | 1615 | 1560 | $C_{13}H_{21}ClN_2O$ |
| 3,5,7-Trimethyl-1-adamantyl | B | $Et_2O$ | Cyclohexane | 84 | 160 | 1625 | 1560 | $C_{16}H_{27}ClN_2O$ |
| 1,3,4,6-Tetra-*O*-acetyl-2-deoxy-D-gluco-pyranos-2-yl | B | $CHCl_3$ | EtOH | 81 | 148–149 | 1750,[i] 1740,[i] 1630 | 1570 | $C_{17}H_{25}ClN_2O_{10}$ |
| Tetrahydro-2*H*-pyran-4-yl | B | $Et_2O$ | | 73 | 144 | 1625 | 1580 | $C_8H_{15}ClN_2O_2$ |
| Tetrahydro-2,6-dimethyl-2*H*-pyran-4-yl | C | $CHCl_3$ | MeCN | 50 | 186 | 1630 | 1590 | $C_{10}H_{19}ClN_2O_2$ |
| Tetrahydro-3-thienyl (*S,S*-dioxide) | C | $CHCl_3$ | EtOH | 70 | 150 | 1620 | 1570[k] | $C_7H_{13}ClN_2O_3S$ |
| Tetrahydro-2*H*-thiopyran-4-yl | C | $CHCl_3$ | $C_6H_6$ | 65 | 134–137 | 1625 | 1575 | $C_8H_{15}ClN_2OS$ |
| Tetrahydro-2*H*-thiopyran-4-yl (*S,S*-dioxide) | C | $CHCl_3$ | $Me_2CO$–hexane | 54 | 148 | 1635 | 1570[l] | $C_8H_{15}ClN_2O_3S$ |
| Thiochroman-4-yl | C | $CHCl_3$ | MeCN–$H_2O$ | 85 | 133 | 1615 | 1560 | $C_{12}H_{15}ClN_2OS$ |
| *m*-Dithian-5-yl | A | MeCN | MeCN | 93 | 180 | 1620 | 1570 | $C_7H_{13}ClN_2OS$ |
| *m*-Dithian-5-yl (*S,S,S',S'*-tetraoxide) | *m* | | | 76 | 242–244 dec | 1640 | 1560 | $C_7H_{13}ClN_2O_5S_2$ |
| Mesityl | A | $C_6H_6$ | MeCN | 61 | 165 | 1635 | 1570 | $C_{12}H_{17}ClN_2O$ |
| 3,4,5-Trimethoxyphenyl | A | $CHCl_3$ | MeCN | 48 | 160 | 1640 | 1560 | $C_{12}H_{17}ClN_2O_4$ |
| 3-Carboxy-2,6-dimethylphenyl | C[n] | $CHCl_3$ | | 62 | 260 dec | 1690,[o] 1630 | 1560 | $C_{12}H_{15}ClN_2O_3$ |
| *p*-(Piperidinocarbonyl)phenyl[p] | C | $CHCl_3$ | MeCN–$H_2O$ | 22 | ~120 | 1700 | 1535 | $C_{15}H_{20}ClN_3O_2$ |
| *p*-(Dimethylsulfamoyl)phenyl[q] | A[r] | THF | $C_6H_6$ | 12 | 125 | 1670 | 1540 | $C_{11}H_{16}ClN_3O_3S$ |
| 5-Nitro-2-thiazolyl | A[s] | THF | $MeC_6H_5$ | 57 | 172[t] | 1670 | 1545[u] | $C_6H_7ClN_4O_3S$ |
| Methylsulfonyl | *m* | | EtOH | 20 | 180 | 1645 | 1550 | $C_4H_9ClN_2O_3S$ |
| *p*-Tolylsulfonyl | E[m] | $C_6H_6$ | $C_6H_6$ | 84 | 153 | 1670 | 1545 | $C_{10}H_{13}ClN_2O_3S$ |
| | | $ClCH_2CH_2NHCONHRNHCONHCH_2CH_2Cl$ | | | | | | |
| 3-Aza-3-nitropentamethylene | D[v] | $CHCl_3$ | (1) MeCN, (2) EtOH | 35 | 140 | 1620 | 1560 | $C_{10}H_{20}Cl_2N_6O_4$ |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 5,5-Dimethyl-1,3-cyclohexylene | B | $Et_2O$ | MeCN | 24 | 165 | 1625 | 1560 | $C_{14}H_{26}Cl_2N_4O_2$ |
| Methylenedi-1,4-cyclohexylene[w] | A | $CHCl_3$ | EtOH | 27 | 240 | 1625 | 1560 | $C_{19}H_{34}Cl_2N_4O_2$ |

[a] A, $RNH_2$ + $Cl(CH_2)_2NCO$, product pptg from reaction medium in most cases (otherwise solvent evapd *in vacuo*); B, $RNH_2$ [by extraction, after basification of $RNH_2 \cdot HCl$ with aq NaOH (or NaOAc in case of R = 1,3,4,6-tetra-*O*-acetyl-), with reaction solvent] + $Cl(CH_2)_2NCO$; C, $RNH_2 \cdot HCl$ + $Et_3N$ + $Cl(CH_2)_2NCO$ (solvent evapd *in vacuo*, residue washed with hexane or $Et_2O$ and then $H_2O$); D, RNCO + $Cl(CH_2)_2NH_2 \cdot HCl$ + $Et_3N$; E, RNCO + $Cl(CH_2)_2NH_2$. [b] If no recrystn solvent indicated, product was appropriately washed and dried *in vacuo*. [c] Determined with a Kofler Heizbank (no range) or Mel-Temp apparatus (range). [d] Prominent bands (aromatic CH excluded) in 1500–1750 $cm^{-1}$ range; urea C=O (Amide I) and CNH (Amide II) assignments according to N. B. Colthup, L. H. Daly, and S. E. Wilberly, "Introduction to Infrared and Raman Spectroscopy," Academic Press, Inc., New York, N. Y., 1964, pp 265, 384–385. [e] All compds analyzed for C, H, N (see ref 29). [f] Cyclized in storage to oily 2-(2-hydroxy-1,1-dimethylethylamino)-2-oxazoline·HCl, ir (KBr) 1700 (oxazoline C=N) $cm^{-1}$. *Anal.* ($C_7H_{14}N_2O_2 \cdot HCl$) C, H, N. [g] Pptd with hexane. [h] From 3-*tert*-butylcyclohexylamine·HCl, mp 235–240° [lit. mp 267–268° (*cis*), 234–235° (*trans*); J. Sicher, M. Tichy, F. Sipos, M. Svoboda, and J. Jonas, *Collect. Czech. Chem. Commun.*, **29**, 1561 (1964)] prepd by Raney Ni hydrogenation of the oxime in EtOH. [i] Ester C=O. [j] From tricyclo[2.2.1.$0^{2,6}$]heptan-3-amine prepd according to G. Mueller and R. Merten, *Chem. Ber.*, **98**, 1097 (1965). [k] Also 1300 and 1110 $cm^{-1}$($SO_2$). [l] Also 1285 and 1125 $cm^{-1}$ ($SO_2$). [m] See Experimental Section. [n] Residue triturated in 3 *N* HCl and washed with $H_2O$. [o] Carboxyl C=O. [p] From 1-(*p*-aminobenzoyl)piperidine, mp 160° [lit. mp 162°; H. Wenker, *J. Amer. Chem. Soc.*, **60**, 1081 (1938)], prepd from *p*-aminobenzoyl chloride in 44% yield (recrystd from xylene) (*cf.* prepn of *p*-amino-*N*,*N*-dimethylbenzamide[4]). [q] From $N^1$,$N^1$-dimethylsulfanilamide prepd according to J. Walker, *J. Chem. Soc.*, 686 (1940). [r] Heated in pressure vessel at 90° for 20 hr; solvent removed *in vacuo*. [s] Heated in pressure vessel according to M. Wilhelm, F.-H. Marquardt, K. Meier, and P. Schmidt, *Helv. Chim. Acta*, **49**, 2443 (1966); product pptd from concd (0.4) reaction mixture with warm hexane and recrystd from THF with hexane (1:14), then from toluene. [t] Lit. 135–140° (see ref in footnote *s*). [u] Also 1515 and 1345 $cm^{-1}$ ($NO_2$). [v] Oily product triturated in $Et_2O$. [w] From 5,5-dimethyl-1,3-cyclohexanediamine·2HCl [mp >350°. *Anal.* ($C_8H_8N_2 \cdot 2HCl$) C, H, N; *cf.* free base, K. Hosino, *Nippon Kagaku Zasshi*, **62**, 599 (1941); *Chem. Abstr.*, **37**, 4698 (1943)] prepd by Raney Ni hydrogenation in EtOH of the dioxime, mp 200° [lit. mp 171–173° (Hosino), 176° [D. Vorlaender and J. Erig, *Justus Liebigs Ann. Chem.*, **294**, 302 (1897)]]. *Anal.* ($C_8H_{14}N_2O_2$) C, H, N.

## TABLE II

### (2-FLUOROETHYL)UREAS

### $RNHCONHCH_2CH_2F$

| R | Method[a] | Reaction solvent | Recrystn solvent[b] | Yield, % | Mp,[c] °C | KBr, $cm^{-1}$ [d] C=O | KBr, $cm^{-1}$ [d] CNH | Formula[e] |
|---|---|---|---|---|---|---|---|---|
| 2,2,2-Trifluoroethyl | *f* | $H_2O$ | $C_6H_6$ | 38 | 138 | 1635 | 1590 | $C_5H_8F_4N_2O$ |
| 2-Cyclohexen-1-yl | Aab | $H_2O$–EtOH | | 57 | 124 | 1625 | 1580 | $C_9H_{15}FN_2O$ |
| 4-Methylcyclohexyl | Ac | $H_2O$–$Me_2CO$ | MeCN | 33 | 177 | 1630 | 1585 | $C_{10}H_{19}FN_2O$ |
| 4-Ethylcyclohexyl | B | $H_2O$–EtOH | MeCN | 69 | 115 | 1625 | 1560 | $C_{11}H_{21}FN_2O$ |
| 4,4-Dimethylcyclohexyl | Bbc | $H_2O$–EtOH | MeCN–$H_2O$ | 59 | 92 | 1620 | 1570 | $C_{11}H_{21}FN_2O$ |
| 4-Isopropylcyclohexyl | Bbc | $H_2O$–EtOH | MeCN–$H_2O$[g] | 58 | 159 | 1620 | 1580 | $C_{12}H_{23}FN_2O$ |
| 4-Acetoxycyclohexyl | B | $H_2O$ | $C_6H_6$–hexane | 43 | 178 | 1720,[h] 1625 | 1580 | $C_{11}H_{19}FN_2O_3$ |
| 2-Bornyl | Ca | $H_2O$–$Me_2CO$ | MeCN | 32 | 155 | 1620 | 1560 | $C_{13}H_{23}FN_2O$ |
| 2-Adamantyl | Cab | $H_2O$–EtOH | MeCN | 71 | 210 | 1620 | 1565 | $C_{13}H_{21}FN_2O$ |
| 3,5,7-Trimethyl-1-adamantyl | Bab | $H_2O$–EtOH | | 44 | 202 | 1625 | 1560 | $C_{16}H_{27}FN_2O$ |
| Cyclododecyl | Aab | $H_2O$–EtOH | MeCN | 74 | 166 | 1630 | 1565 | $C_{15}H_{29}FN_2O$ |
| 1,3,4,6-Tetra-*O*-acetyl-2-deoxy-D-glucopyranos-2-yl | *f* | $MeC_6H_5$ | EtOH | 8 | 150 | 1750,[h] 1630 | 1570 | $C_{17}H_{25}FN_2O_{10}$ |
| Tetrahydro-2*H*-thiopyran-4-yl | Bb | $H_2O$ | $C_6H_6$–hexane | 57 | 170 | 1620 | 1580 | $C_8H_{15}FN_2OS$ |
| Tetrahydro-2*H*-thiopyran-4-yl (*S*,*S*-dioxide) | Bd | $H_2O$ | EtOH | 80 | 190 | 1620 | 1585 | $C_8H_{15}FN_2O_3S$ |
| Thiochroman-4-yl | B | $H_2O$–$Me_2CO$ | EtOH–$H_2O$ | 79 | 177 | 1620 | 1575 | $C_{12}H_{15}FN_2OS$ |
| Thiochroman-4-yl (*S*,*S*-dioxide) | *f* | | *n*-PrOH | 36 | 190 | 1630 | 1575 | $C_{12}H_{15}FN_2O_3S$ |
| *m*-Dithian-5-yl | C[f] | $H_2O$–EtOH | | 73 | 210 | 1625 | 1580 | $C_7H_{13}FN_2OS_2$ |
| *m*-Dithian-5-yl (*S*,*S*,*S*′,*S*′-tetraoxide) | *f* | | | 85 | 256–258 dec | 1640 | 1560 | $C_7H_{13}FN_2O_5S_2$ |
| *p*-Tolylsulfonyl | *f* | | $C_6H_6$ | 86 | 151 | 1680 | 1540 | $C_{10}H_{13}FN_2O_3S$ |
| Nitro | *f* | | $C_6H_6$ | 42 | 120 | 1680 | 1535 | $C_3H_6FN_3O_3$ |

[a] A, $RNH_2$ + MFNU at room temp followed by (a) warming at, *e.g.*, 50–60° (mixt chilled before isolation of product or after (b) diln with $H_2O$ or (c) concn *in vacuo* to remove more volatile solvent); B, $RNH_2 \cdot HCl$ + $Et_3N$ or (a) NaOH + MFNU at room temp followed by (b) warming, (c) diln with $H_2O$, and/or (d) evapn of solvents *in vacuo*, and chilling when needed; C, $RNH_2$ + MFNU + $Et_3N$ (catalyst) followed by (a) concn *in vacuo* to remove more volatile solvent and (b) diln with $H_2O$ and chilling. [b–e] See *b–e*, Table I. [f] See Experimental Section. [g] Recrysted product triturated in hot hexane. [h] Ester C=O.

TABLE III

*N*-(2-CHLOROETHYL)-*N*-NITROSOUREAS

RNHCON(NO)$CH_2CH_2Cl$

| R | Nitrosation[a] Urea, mmoles | $HCO_2H$, ml | $NaNO_2$, mmoles | Yield, % | Mp,[b] °C | ν KBr, $cm^{-1}$ C=O[c] | CNH[d] | NN=O[e] | Formula[f] |
|---|---|---|---|---|---|---|---|---|---|
| α,α-Dimethylphenethyl | 29.4 | 75 | 109 | 90 | ~64 | 1705 | 1530 | 1480 | $C_{13}H_{18}ClN_3O_2$ |
| 2-Cyclohexen-1-yl[g] | 21.8 | 50 | 26.5 | 75 | 52 | 1700 | 1520 | 1480 | $C_9H_{14}ClN_3O_2$ |
| *trans*-4-Methylcyclohexyl | 13.8 | 50 | 43.5 | 80 | 70 | 1700 | 1535, 1530 | 1490 | $C_{10}N_{18}ClN_3O_2$ |
| *cis*-4-Methylcyclohexyl[h] | 9.14 | 20 | 29 | 84 | *i* | 1720 | 1520 | 1485 | $C_{10}H_{18}ClN_3O_2$ |
| 4-Ethylcyclohexyl[i] | 6.87 | 20 | 23 | 83 | 70 dec | 1705 | 1525 | 1485 | $C_{11}H_{20}ClN_3O_2$ |
| 3-*tert*-Butylcyclohexyl[k] | 7.68 | 25 | 29 | 61 | 55 | 1700 | 1540 | 1485 | $C_{13}H_{24}ClN_3O_2$ |
| 4-Acetoxycyclohexyl | 12.3 | 50 | 47 | 80 | 125 dec | 1730,[l] 1710 | 1530 | 1480 | $C_{11}H_{18}ClN_3O_4$ |
| *trans*-4-(Ethoxycarbonyl)cyclohexyl | 18.5 | 75 | 74 | 80 | 61 | 1730,[l] 1715 | 1520 | 1480 | $C_{12}H_{20}ClN_3O_4$ |
| *cis*-3-(Ethoxycarbonyl)cyclohexyl[m] | 17.9 | 100 | 72.5 | 67 | 73 | 1705[n] | 1520 | 1485 | $C_{12}H_{20}ClN_3O_4$ |
| 2-Adamantyl | 7.40 | 20 | 27.5 | 83 | 55 | 1710 | 1520 | 1470 | $C_{13}H_{20}ClN_3O_2$ |
| 3,5,7-Trimethyl-1-adamantyl | 8.10 | 70 | 35 | 86 | 70 dec | 1735 | 1520 | 1485 | $C_{16}H_{22}ClN_3O_2$ |
| 1,3,4,6-Tetra-*O*-acetyl-2-deoxy-D-glucopyranos-2-yl | 9.75 | 25 | 64 | 70 | 146 dec | 1745,[l] 1730,[l] 1710 | 1525 | 1495 (m) 1480 (m) | $C_{17}H_{24}ClN_3O_{11}$ |
| Tetrahydro-3-thienyl (*S,S*-dioxide)[o] | 20.4 | 70 | 71 | 80 | 115 dec | 1720 | 1525 | 1480[p] | $C_7H_{12}ClN_3O_4S$ |
| Tetrahydro-2*H*-thiopyran-4-yl | 19.0 | 40 | 91 | 82 | 92 | 1690 | 1540 | 1480 | $C_8H_{14}ClN_3O_2S$ |
| Tetrahydro-2*H*-thiopyran-4-yl (*S,S*-dioxide) | 20.2 | 75 | 74 | 63 | 150 | 1700 | 1530 | 1480[q] | $C_8H_{14}ClN_3O_4S$ |
| Thiochroman-4-yl | 2.49 | 10 | 7.3 | 86 | 88 dec | 1690 | 1520 | 1490 | $C_{12}H_{14}ClN_3O_2S$ |
| *m*-Dithian-5-yl | 17.0 | 50 | 59 | 94 | 110 | 1705 | 1520 | 1485 | $C_7H_{12}ClN_3O_2S_2$ |
| *m*-Dithian-5-yl (*S,S,S',S'*-tetraoxide) | 10.6 | 200 | 87 | 88 | 210 dec | 1710 | 1530 | 1490[r] | $C_7H_{12}ClN_3O_6S_2$ |
| Mesityl | 16.2 | 60 | 56.5 | 74 | 76 | 1730 | 1500 | 1480 | $C_{12}H_{16}ClN_3O_2$ |
| 4-Carboxy-3,5-dimethylphenyl | 9.63 | 75 | 38[s] | 87 | 157 dec | 1730, 1685[t] | 1520 | 1490 | $C_{12}H_{14}ClN_3O_4$ |
| 5-Nitro-2-thiazolyl | 10.0 | 25 | 36 | 86 | 151 dec | 1710 | 1535 | 1480 | $C_6H_6ClN_5O_4S$ |
| *p*-Tolylsulfonyl | 1.81 | 20 | 7.3 | 90 | 120 dec | 1730 | 1530 | 1425[u] | $C_{10}H_{12}ClN_3O_4S$ |
| $ClCH_2CH_2N(NO)CONHRNHCON(NO)CH_2CH_2Cl$ | | | | | | | | | |
| Methylene-1,4-cyclohexylene | 1.19 | 15 | 14.5 | 96 | 140 | 1700 | 1525 | 1490 | $C_{19}H_{32}Cl_2N_6O_4$ |

[a] Soln or suspension of the urea in 98–100% $HCO_2H$ was treated at 0–5° with $NaNO_2$; after 1–2 hr $H_2O$ was added and ppt was washed with $H_2O$ and dried *in vacuo* ($P_2O_5$); position of nitrosation was checked by pmr.[4] [b] Determined with a Kofler Heizbank. [c] *Cf.* previously noted effect of nitrosation on urea C=O absorption.[4,23] [d] See *d*, Table I. [e] Usually strong bands in 1495–1470-$cm^{-1}$ region. [f] See *e*, Table I. [g] Contains ~5% of isomeric nitrosourea (pmr). [h] Contains trace of unnitrosated urea (tlc, ir). [i] Yellow oil; ir (film). [j] Contains 10–20% of isomeric nitrosourea (pmr). [k] Pptd as oil; solid from EtOH–$H_2O$. [l] Ester C=O. [m] Repptd from EtOH with $H_2O$. [n] Shoulders at 1720, 1710 $cm^{-1}$ (ester C=O). [o] Contains ~25% of isomeric nitrosourea (pmr). [p] Also 1310, 1120 $cm^{-1}$ ($SO_2$). [q] Also1320, 1290, 1280, 1125 $cm^{-1}$ ($SO_2$). [r] Also 1320, 1300, 1140 $cm^{-1}$ ($SO_2$). [s] Nitrosation mixt stirred 3 hr. [t] Carboxyl C=O. [u] Also 1360, 1160 $cm^{-1}$ ($SO_2N$).

TABLE IV

*N*-(2-FLUOROETHYL)-*N*-NITROSOUREAS

RNHCON(NO)$CH_2CH_2F$

| R | Nitrosation[a] Urea, mmoles | $HCO_2H$, ml | $NaNO_2$, mmoles | Yield, % | Mp,[b] °C | ν KBr, $cm^{-1}$ C=O[c] | CNH[d] | NN=O[e] | Formula[f] |
|---|---|---|---|---|---|---|---|---|---|
| 4-Methylcyclohexyl | 2.47 | 8 | 7.3 | 82 | 77 dec | 1720, 1700 | 1525 | 1480 | $C_{10}H_{18}FN_3O_2$ |
| 4-Ethylcyclohexyl | 8.80 | 30 | 27.5 | 69 | 65 | 1720, 1695 | 1525 | 1480 | $C_{11}H_{20}FN_3O_2$ |
| 4-Isopropylcyclohexyl | 5.65 | 15 | 19 | 89 | 104 | 1720, 1700 | 1525 | 1480 | $C_{12}H_{22}FN_3O_2$ |
| 4,4-Dimethylcyclohexyl | 28.5 | 40 | 90 | 74 | 67 | 1720, 1695 | 1535 | 1485 | $C_{11}H_{20}FN_3O_2$ |
| 4-Acetoxycyclohexyl | 8.50 | 25 | 30.5 | 79 | 133 | 1730, 1710 | 1520 | 1465 | $C_{11}H_{18}FN_3O_4$ |
| 2-Adamantyl | 4.16 | 10 | 14.5 | 85 | ~55 | 1720 | 1510 | 1480, 1460 | $C_{13}H_{20}FN_3O_2$ |
| 3,5,7-Trimethyl-1-adamantyl | 12.4 | 110 | 51 | 91 | 103 | 1725, 1695 (1720)[g] | 1530 (1515)[g] | 1485 (1475)[g] | $C_{16}H_{28}FN_3O_2$ |
| Cyclododecyl | 17.2 | 75 | 67 | 92 | 110 dec | 1700 | 1520 | 1460, 1465 | $C_{15}H_{28}FN_3O_2$ |
| Tetrahydro-2*H*-thiopyran-4-yl | 16.0 | 50 | 47.8 | 86 | 113 dec | 1700 | 1520 | 1480 | $C_8H_{14}FN_3O_2S$ |
| Tetrahydro-2*H*-thiopyran-4-yl (*S,S*-dioxide) | 17.4 | 40 | 60 | 67 | 159 dec | 1725 | 1525 | 1480, 1465[h] | $C_8H_{14}FN_3O_4S$ |
| Thiochroman-4-yl | 13.7 | 110 | 51 | 80 | 91 dec | 1710, 1685 | 1525 | 1490 | $C_{12}H_{14}FN_3O_2S$ |
| *m*-Dithian-5-yl | 17.8 | 80 | 58 | 75 | 95 | 1705 | 1515 | 1480 | $C_7H_{12}FN_3O_2S_2$ |
| *m*-Dithian-5-yl (*S,S,S',S'*-tetraoxide) | 7.3 | 150 | 61 | 82 | 220 dec | 1710 | 1530 | 1490[i] | $C_7H_{12}FN_3O_6S_2$ |
| *p*-Tolylsulfonyl[j] | 1.92 | 15 | 7.3 | 38 | ~80 dec | 1735 | 1530 | 1425[k] | $C_{10}H_{12}FN_3O_4S$ |

[a] See *a*, Table III. [b,c] See *b* and *c*, Table III. [d] See *d*, Table I. [e] See *e*, Table III. [f] See *e*, Table I. [g] In $CHCl_3$. [h] Also 1275, 1140, and 1120 $cm^{-1}$ ($SO_2$). [i] Also 1320, 1305, 1155, and 1140 $cm^{-1}$ ($SO_2$). [j] Pilot run not successfully scaled-up. [k] Also 1360 and 1160 $cm^{-1}$ ($SO_2NH$).

The original sample of ethyl 4-[3-(2-chloroethyl)-3-nitrosoureido]cyclohexanecarboxylate was an impure oil derived inadvertently from a cis–trans amine mixture.[4] Esterification after separation of the isomers obtained by catalytic hydrogenation of *p*-aminobenzoic acid,[13] however, enabled the preparation of the pure trans nitrosourea **16**, but several attempts to obtain the oily cis nitrosourea pure were unsuccessful.




**16**

In the attempted synthesis of heteroalicyclic analogs of CCNU from the tetrahydro-4*H*-pyran-4-ones **17** and **18**, nitrosation of the chloroethylureas **19** and **20** produced unstable, impure oils. The methylnitrosourea **21**, a secondary goal, was obtained pure. Tetrahydro-2*H*-thiopyran-4-amine[14] was, however, a more productive precursor than its oxygen counterparts, leading to the haloethylnitrosoureas **22–25**.




**17**, R = H
**18**, R = $CH_3$




**19**, R = H
**20**, R = $CH_3$




**21**

Oxidation at the tetrahydro-4*H*-thiopyran-4-one stage and Raney Ni catalyzed reduction of the derived oxime[14] provided intermediates for the sequence leading to the *S,S*-dioxides **24** and **25**. The corresponding 5-membered ring exerted only moderate steric control of the nitrosation of the chloroethylurea **26**, and consequently the isolated product was a mixture of the nitrosoureas **27a** and **27b** (~3:1). *m*-Dithian-5-amine[15] (**29**) derived from *m*-dithian-5-one[16] (**28**) was also a




**22**, X = Cl
**23**, X = F




**24**, X = Cl
**25**, X = F




**26**, Y = Z = H
**27a**, Y = H; Z = NO
**b**, Y = NO; Z = H

(13) G. Wendt, *Ber.*, **75**, 425 (1942).

(14) C. Barkenbus and J. A. Wuellner, *J. Amer. Chem. Soc.*, **77**, 3866 (1955).

(15) E. G. Howard, Jr., and R. V. Lindsay, Jr., *ibid.*, **82**, 158 (1960); E. G. Howard, Jr., U. S. Patent 2,790,811, 1957; *Chem. Abstr.*, **52**, 457*d* (1958).

(16) A. Luettringhaus and H. Prinzbach, *Justus Liebigs Ann. Chem.* **624**, 79 (1959).

TABLE V

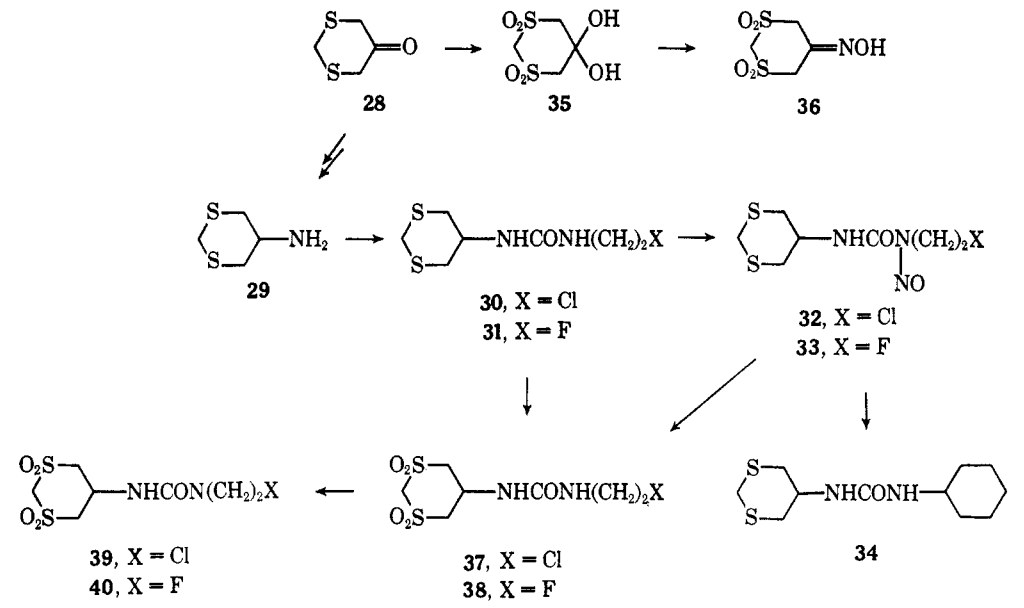
INFRARED NH ABSORPTION OF CIS AND TRANS ISOMERS OF ALICYCLIC-SUBSTITUTED NITROSOUREAS

RNHCONR′
|
NO

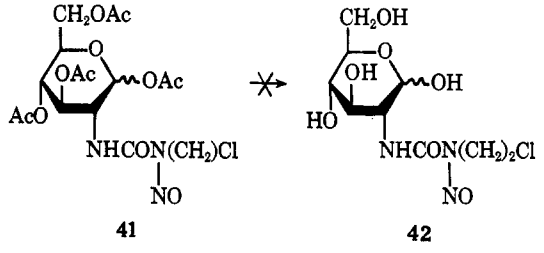
| R | R′ | Configuration | ν KBr (NH) | Conformation of NHCON(NO)R′[a] |
|---|---|---|---|---|
| 2-Chlorocyclohexyl | Me | Cis | 3350 | e |
| 2-Chlorocyclohexyl | Me | Trans | 3290 | e |
| 2-Chlorocyclohexyl | $CH_2CH_2Cl$ | Cis | 3350 | e |
| 2-Chlorocyclohexyl | $CH_2CH_2Cl$ | Trans | 3340 | e |
| 2-Chlorocyclohexyl | 2-Chlorocyclohexyl | Cis,cis | 3340 | e |
| 2-Chlorocyclohexyl | 2-Chlorocyclohexyl | Trans,trans | 3325 | e |
| 3-Methylcyclohexyl | $CH_2CH_2F$ | Cis | 3345 | e |
| 4-Methylcyclohexyl | $CH_2CH_2Cl$ | Cis | 3425, 3350 (sh) | a ⇌ e |
| 4-Methylcyclohexyl | $CH_2CH_2Cl$ | Trans | 3335 | e |
| 4,4-Dimethylcyclohexyl | $CH_2CH_2Cl$ | | 3325 | e |
| 4-Ethylcyclohexyl | $CH_2CH_2Cl$ | Trans | 3335, 3410 (sh) | a ⇌ e |
| 4-Ethylcyclohexyl | $CH_2CH_2F$ | Trans | 3310 | e |
| 4-*tert*-Butylcyclohexyl | $CH_2CH_2Cl$ | Trans | 3360 | e |
| 1,2-Cyclohexylene | $CH_2CH_2Cl$ | Trans | 3310 | e |
| 1,4-Cyclohexylene | $CH_2CH_2Cl$ | Trans | 3360 | e |
| 1,4-Cyclohexylene | $CH_2CH_2F$ | Trans | 3320 | e |
| 4-(Ethoxycarbonyl)cyclohexyl | $CH_2CH_2Cl$ | Trans | 3365 | e |
| 5α-Cholestan-3α-yl | $CH_2CH_2Cl$ | | 3430 | a |
| 5α-Cholestan-3α-yl | $CH_2CH_2F$ | | 3430 | a |

[a] a = axial; e = equatorial.

SCHEME II




convenient precursor of the haloethylnitrosoureas **32** and **33** (Scheme II); but suitable conditions were not




found for continuation of the sequence **28**→**35**[15]→**36**, which was initially proposed as an approach to the tetraoxides **39** and **40**. Oxidation of **30** and **31** by $H_2O_2$ in AcOH proved an effective alternative and provided the respective haloethylureas **37** and **38**. When **32** and **33** were similarly treated, denitrosation as well as oxidation occurred and, in the case of **33**, a high yield of **38** was produced.[17] The conversion of **32** to 1-cyclohexyl-3-*m*-dithian-5-ylurea (**34**) in high yield with cyclohexylamine supported the assigned structure in conjunction with the pmr spectrum, which by itself was not conclusive.

The first of 3 reported syntheses of streptozotocin[18-20]—a natural nitrosourea, broad-spectrum antibiotic, and experimental anticancer agent—prompted a similar effort to prepare the chloroethyl analog **42**, but attempted deacetylations of the chloroethylnitro-

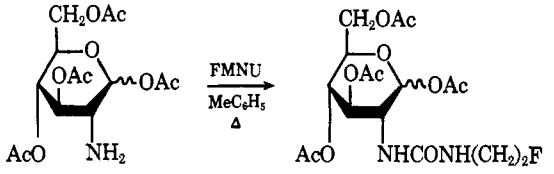
(17) Denitrosation did not occur in AcOH alone.

(18) R. R. Herr, H. K. Jahnke, and A. D. Argoudelis, *J. Amer. Chem. Soc.*, **89**, 4808 (1967).

(19) E. Hardegger, A. Meier, and A. Stoos, *Helv. Chim. Acta*, **52**, 2555 (1969).

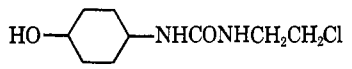
(20) E. J. Hessler and H. K. Jahnke, *J. Org. Chem.*, **35**, 245 (1970).

sourea **41** by ammonolysis in MeOH resulted in excessive decomposition. Similar results were observed in a recently described, independent attempt to duplicate the original synthesis of streptozotocin.[19] The conventional treatment of an amine·HCl with $Et_3N$ and 3-(2-fluoroethyl)-1-methyl-1-nitrosourea (FMNU) in aq soln[4] was unsatisfactory for preparation of the fluoroethylurea **44**, but pure **44** was eventually obtained in low yield by refluxing a toluene soln of FMNU and the free base **43**. This *in situ* generation of 2-fluoroethyl isocyanate parallels the previously reported thermal decompositions of 1,3-dimethyl-1-nitrosourea and BCNU in anhydrous solvents.[21] Since the nitro-



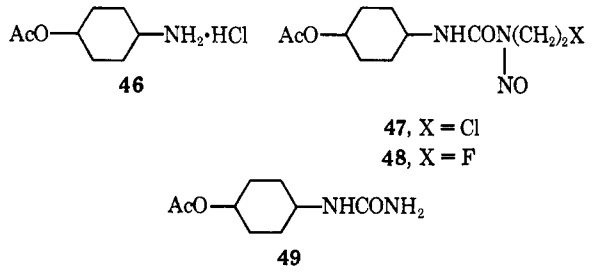

**43** **44**

sations of 1-(2-chloroethyl)-3-(4-hydroxycyclohexyl)-urea (**45**) and similar hydroxyalkyl-substituted ureas




**45**

have proved, with few exceptions, conspicuously unsuccessful in formic acid (a medium chosen for favorable direction of the position of nitrosation), deblocking of the acetylated nitrosoureas **47** and **48** derived from 4-aminocyclohexyl acetate·HCl (**46**) was attempted as in the streptozotocin synthesis. In each case the acetoxy function remained intact, and good yields of (4-acetoxycyclohexyl)urea (**49**) resulted by virtue of a typical nitrosourea decomposition.



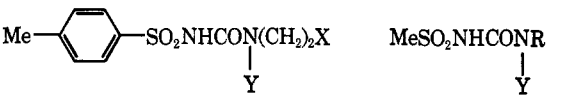

**46**

**47**, X = Cl
**48**, X = F

**49**

Several severe modifications of the nitrosoureido function were attempted in addition to those already described,[22] the ultimate goal being the preparation of sulfonylureas, thioureas, alkoxyureas, and nitroureas substituted by haloethyl and nitroso groups.

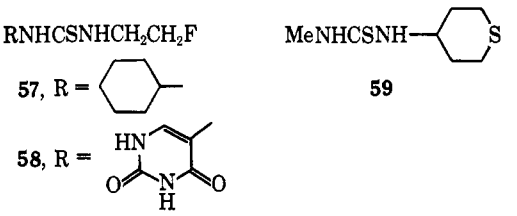
The 1-(2-haloethyl)-1-nitroso-3-(*p*-tolylsulfonyl)-ureas **51** and **53** were prepared from *p*-tolylsulfonyl isocyanate *via* the respective ureas **50** and **52**, but a 13-fold scale-up of the pilot preparation of **53** gave a product that decomposed. The yellow nitroso derivative (presumably **55**) of 1-methyl-3-(methylsulfonyl)urea (**54**), which was intended for the *in situ* generation of $MeSO_2NCO$, was unstable, decomposing spontaneously shortly after isolation and drying. The use of **55** was circumvented, however, by a direct preparation of 1-(2-chloroethyl)-3-(methylsulfonyl)urea (**56**), but the isolation of a nitroso derivative of **56** was also thwarted by instability.




**50**, X = Cl; Y = H
**51**, X = Cl; Y = NO
**52**, X = F; Y = H
**53**, X = F; Y = NO

**54**, R = Me; Y = H
**55**, R = Me; Y = NO
**56**, R = $(CH_2)_2Cl$; X = H

The nitrosation of 1-cyclohexyl-3-(2-fluoroethyl)-2-thiourea (**57**), which was prepared instead of the corresponding chloroethylthiourea to minimize the possibility of thiazoline ring closure, gave promise of a thio analog related to CCNU; but the pmr spectrum of the isolated product indicated considerable decomposition. The search for a haloethyl-substituted nitrosothiourea having suitable stability for characterization and comparison with the corresponding haloethylnitrosourea[4] was extended to the nitrosation of the uracil **58**: mild nitrosation in dil $H_2SO_4$ was apparently incomplete, whereas a product could not be isolated after nitrosation in $HCO_2H$ under forcing conditions, *i.e.*, long reaction time with excess reagent. The elemental analysis of the product isolated after nitrosation of the methylthiourea **59** was satisfactory, but extrinsic absorption in the ir spectrum indicated both random nitrosation and the presence of a decomposition product. Thus, 1,3-dimethyl-1-nitroso-2-thiourea[23] remains the sole example of a successfully characterized nitrosothiourea.




**57**, R =

**58**, R =

**59**

No attempt was made to characterize 1,3-diethyl-1-nitroso-2-thiourea (**60**), however; its immediate conversion to 1-ethyl-3-(2-norbornyl)-2-thiourea (**61**) in high yield established an analogy with the reactions of 1,3-disubstituted nitrosoureas with primary and secondary amines.[4,23,24]


**60** **61**

Although products of the nitrosation of 1-(2-chloroethoxy)-3-phenylurea (**62**) and several other alkoxyureas were so unstable that none could be characterized, the position of nitrosation, at least in part, was deduced by identification of decomposition products. The nitrosation of **62** in $HCO_2H$ produced a low yield of carbanilide as the only characterizable product as did the nitrosation of 1-methoxy-3-phenylurea (**64**)—results that indicate the intermediacy of the nitrosoureas **63** and **65** and phenyl isocyanate formed from them.

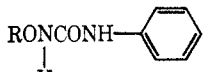
(21) J. A. Montgomery, R. James, G. S., McCaleb, and T. P. Johnston, *J. Med. Chem.*, **10**, 668 (1967).

(22) *Cf.* the prepn of nitrosobiurets, -biureas, and -carboxamides (ref 1).

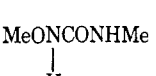
(23) T. P. Johnston, G. S. McCaleb, and J. A. Montgomery, *J. Med. Chem.*, **6**, 669 (1963).

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

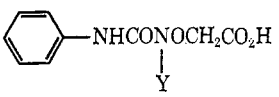
The structure of the yellow nitroso derivative of 1-methoxy-3-methylurea (**66**), which could be isolated and kept briefly, was indicated to be **67** by immediate conversion to 1-(*p*-chlorobenzyl)-3-methylurea by treatment with *p*-chlorobenzylamine. Carbanilide was similarly produced from (3-phenylureido)oxyacetic acid (**68**) *via* aniline treatment of the crude unstable nitroso derivative **69**. A good yield of 1-methoxy-3-(1,2,3,4-tetrahydro-2,4-dioxo-5-pyrimidinyl)urea (**70**)



**62**, R = $Cl(CH_2)_2$; Y = H
**63**, R = $Cl(CH_2)_2$; Y = NO
**64**, R = Me; Y = H
**65**, R = Me; Y = NO

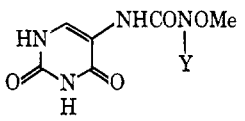


**66**, Y = H
**67**, Y = NO

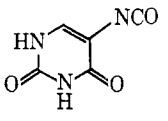


**68**, Y = H
**69**, Y = NO

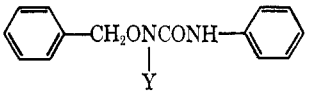
was obtained by allowing the corresponding methylnitrosourea[23] to decompose in hot $H_2O$ in the presence of methoxyamine. The chief component of the crude product isolated after nitrosation of **70** in $HCO_2H$ was apparently the isocyanate **72** [ir (KBr) 2230 $cm^{-1}$ (NCO)], which indicated the intermediacy of the methoxynitrosourea **71**. Decomposition of the yellow nitroso derivative of 1-benzyloxy-3-phenylurea (**73**) with $MeNH_2$ produced 1-methyl-3-phenylurea, which would be expected from the nitrosourea **74**; moist **74** could be preserved for several days in a freezer, but it decomposed spontaneously within 2 hr when stored in dry air at room temp.



**70**, Y = H
**71**, Y = NO



**72**

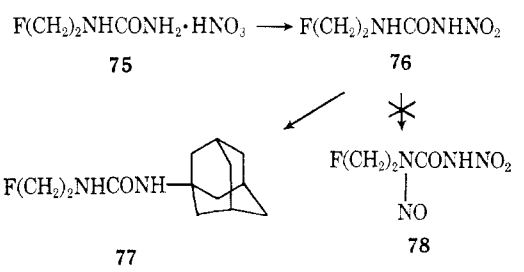


**73**, Y = H
**74**, Y = NO

These varied examples of the decomposition of alkoxynitrosoureas lead to the conclusion that 3-substituted alkoxyureas nitrosate readily on the alkoxy side of the ureido function to give highly unstable nitroso derivatives. The low yields of ureas isolated as decomposition products would suggest random nitrosation; but no products were isolated that would indicate the existence of isomers. Since methoxyamine ($pk_b$ 9.40)[25] is a much weaker base than $MeNH_2$ ($pk_b$ 3.38),[26] the relative nucleophilicity of the 1 and 3 positions of alkoxyureas is not predictable on the basis of apparent relative basicity.

The preparation of 1-(2-fluoroethyl)-3-nitro-1-nitrosourea (**78**) was attempted by the sequence shown in Scheme III. The treatment of (2-fluoroethyl)urea

SCHEME III



nitrate (**75**) with $H_2SO_4$ gave 1-(2-fluoroethyl)-3-nitrourea[27] (**76**), whose pmr spectrum indicated the absence of the 1-nitro isomer. The assigned structure was also supported by the conversion of **76** to the known 1-(1-adamantyl)-3-(2-fluoroethyl)urea[4] (**77**), although in low yield. Several attempts to nitrosate **76** in various media (50% aq $HNO_3$, AcOH, and 6 *N* HCl) failed to give isolable **78**; some unchanged **76** was isolated from nitrosations attempted in the aq media.

**Screening Results.**—The details of the evaluation of nitrosoureas for their effectiveness against murine leukemia L1210 have been discussed.[4] Quantitative comparisons based on the reduction in cell population expressed as a logarithm are convenient, but they do not take into account relative toxicities and do not differentiate between a number of highly active structures.[4] Because of these limitations, comparisons now being made are based on therapeutic indices obtained in two ways. The $ED_{50}/LD_{10}$ is the quotient of the dose required to obtain 50% 45-day survivors of the tested animals divided by the dose that kills 10% of a test group of normal animals, both values being determined from log-dose, probit-survival plots. The second index ($ED_{99}/LD_{10}$) is the quotient of the dose required to kill two logs (99%) of leukemic cells, as determined by increase in lifespan, divided by the $LD_{10}$. There is a reasonable, but far from perfect, correlation between these two indices indicating that the dose-response curve is not a straight line for all the compounds evaluated.

The therapeutic indices against the disease caused by both ip and intracerebrally (ic) implanted leukemia L1210 cells are given in Table VI, but the compds are arranged in order of decreasing activity based on $ED_{50}/LD_{10}$ against the ip disease. Included in this table are 3 compds previously reported, BCNU, CCNU, and the isomeric mixture **6**. The activity values given here are based on cumulative data obtained both before and after the last report,[4] and these values provide a point of reference for the activity of the new compds reported. The correlation between ip and ic activity is reasonably good, although there are notable exceptions

(25) T. C. Bissot, R. W. Parry, and D. H. Campbell, *J. Amer. Chem. Soc.*, **79**, 796 (1957).

(26) H. K. Hall, *ibid.*, **79**, 5441 (1957); A. H. Beckett and J. V. Greenhill, *J. Med. Pharm. Chem.*, **4**, 423 (1961).

(27) *Cf.* the reported prep of 1-methyl-1-nitrourea [mp 156–158°, T. L. Davis and N. D. Constan, *J. Amer. Chem. Soc.*, **58**, 1800 (1936)] and 1-methyl-3-nitrourea [mp 105–106°, O. Degner and H. von Pechmann, *Ber.*, **30**, 646 (1897)]. Our attempt to duplicate the reported prepn of the 1-nitro isomer apparently gave an analytically pure mixt of isomers, whose mp was only slighly higher than that reported for the 3-nitro isomer but whose pmr spectrum indicated roughly an 80% content of the 1-nitro isomer.

TABLE VI

EFFECTIVENESS OF 1-(2-HALOETHYL)-1-NITROSOUREAS AGAINST L1210 LEUKEMIA IMPLANTED BOTH INTRAPERITONEALLY AND INTRACEREBRALLY

| No. | RNHCON(NO)$CH_2CH_2$X R | X | Cytotoxicity index, m$M$[a] | $LD_{10}$, mmoles/kg[b] | Ip ($10^6$ cells) Survival, $ED_{50}/LD_{10}$[c] | Ip ($10^6$ cells) Cell kill, $ED_{99}/LD_{10}$[d] | Ic ($10^4$ cells) Survival, $ED_{50}/LD_{10}$[c] | Ic ($10^4$ cells) Cell kill, $ED_{99}/LD_{10}$[d] |
|---|---|---|---|---|---|---|---|---|
| 39 | *m*-Dithian-5-yl (*S,S,S',S'*-tetraoxide) | Cl | 0.03 | 0.13 | 0.20 | 0.07 | Ns,[e] | 0.88 |
| 6(a,b) | 2-Chloroethyl, 2-fluoroethyl | F, Cl[f] | 0.04[g] | 0.24 | 0.22 | 0.06 | 0.53 | 0.13 |
| 51 | *p*-Tolylsulfonyl | Cl | 0.07 | 1.1 | 0.22 | 0.05 | Ns | >0.44 |
| 32 | *m*-Dithian-5-yl | Cl | 0.10 | 0.082 | 0.29 | 0.17 | 1 | 0.45 |
| 33 | *m*-Dithian-5-yl | F | 0.40 | 0.16 | 0.30 | 0.09 | >1 | 0.27 |
| 25 | Tetrahydro-2*H*-thiopyran-4-yl (*S,S*-dioxide) | F | 0.40 | 0.12 | 0.31 | 0.09 | 0.58 | 0.19 |
| | 4-Ethylcyclohexyl | Cl[h] | 0.10 | 0.38 | 0.31 | 0.13 | 1.0 | 0.22 |
| | 2-Adamantyl | Cl | 0.04 | 0.85 | 0.31 | 0.15 | >1.0 | 0.27 |
| 23 | Tetrahydro-2*H*-thiopyran-4-yl | F | 0.03 | 0.18 | 0.37 | 0.10 | 0.66 | <0.59 |
| 27 | Tetrahydro-3-thienyl (*S,S*-dioxide) | Cl[i] | 0.04 | 0.09 | 0.38 | <0.17 | Ns[i] | 0.39 |
| 16 | *trans*-4-(Ethoxycarbonyl)cyclohexyl | Cl | 0.09 | 0.14 | 0.39 | 0.17 | >1 | 0.46 |
| 47 | 4-Acetoxycyclohexyl | Cl | 0.06 | 0.15 | 0.40 | ~0.17 | 0.55 | ~0.37 |
| | Thiochroman-4-yl | Cl | 0.10 | 0.21 | 0.46 | 0.14 | 0.76 | ~0.28 |
| 24 | Tetrahydro-2*H*-thiopyran-4-yl (*S,S*-dioxide) | Cl | 0.15 | 0.06 | 0.48 | 0.18 | 0.75 | <0.30 |
| 8 | *trans*-4-Methylcyclohexyl | Cl[k] | 0.12[g] | 0.15 | 0.50 | 0.14 | >1 | 0.47 |
| | 2-Chloroethyl | Cl | 0.01[g] | 0.19 | 0.50 | 0.17 | >1 | 0.22 |
| 48 | 4-Acetoxycyclohexyl | F | 0.05 | 0.13 | 0.51 | 0.28 | 0.86 | <0.44 |
| | Mesityl | Cl | 0.12 | 0.44 | 0.52 | 0.04 | Ns | 0.33 |
| 22 | Tetrahydro-2*H*-thiopyran-4-yl | Cl | 0.12 | 0.06 | 0.53 | 0.16 | 0.86 | 0.31 |
| | *cis*-3-(Ethoxycarbonyl)cyclohexyl | Cl | 0.13 | 0.16 | 0.55 | 0.22 | Inactive | |
| 40 | *m*-Dithian-5-yl (*S,S,S',S'*-tetraoxide) | F | 0.12 | 0.21 | 0.58 | 0.08 | Inactive | |
| | Cyclohexyl | Cl | 0.04[g] | 0.17 | 0.65 | 0.19 | 0.53 | 0.15 |
| | 4-Methylcyclohexyl | F | 0.17 | 0.16 | 0.67 | 0.40 | >1 | 0.76 |
| 4 | $\alpha,\alpha$-Dimethylphenethyl | Cl | 0.11 | 3.5 | 0.75 | 0.07 | Ns | 1 |
| | 4-Isopropylcyclohexyl | F | 0.15 | 0.41 | 0.76 | 0.21 | Not tested | |
| | 3,5,7-Trimethyl-1-adamantyl | Cl | 0.04 | 2.9 | 0.79 | 0.03 | Ns | 0.26 |
| | Thiochroman-4-yl | F | 0.04 | 0.13 | 0.80 | 0.39 | Not tested | |
| | 3-*tert*-Butylcyclohexyl | Cl | 0.07 | 0.72 | 0.9 | 0.14 | Not tested | |
| 13 | 4,4-Dimethylcyclohexyl | F | 0.11 | 0.19 | 0.94 | 0.41 | Not tested | |
| 41 | 1,3,4,6-Tetra-*O*-acetyl-2-deoxy-D-glucopyranos-2-yl | Cl | 0.03 | 0.05 | >1 | <0.27 | Inactive | |
| | 4-Ethylcyclohexyl | F | 0.19 | 0.39 | >1 | 0.46 | Not tested | |
| | 2-Adamantyl | F | 0.07 | 0.24 | >1 | 0.62 | Not tested | |
| 14 | 2-Cyclohexen-1-yl | Cl[l] | 0.09 | 0.12 | >1 | 0.53 | Not tested | |
| | 4-Carboxy-3,5-dimethylphenyl | Cl | >0.33 | 0.31 | Ns | 0.85 | Not tested | |
| | 3,5,7-Trimethyl-1-adamantyl | F | 0.07 | 0.84 | Ns | 0.42 | Ns | 0.50 |
| | Cyclododecyl | Cl | 0.04 | 0.43 | Ns | 0.72 | Not tested | |

[a] The concn necessary to inhibit the growth of HEp-2 cells (except where noted) in culture to 50% of control growth measured by protein assay as detd from semilog plots of concn *vs.* the ratio of the growth of treated cells to the growth of control cells. [b] $LD_{10}$ is defined as the dose required to kill 10% of a test group of normal mice as detd from log-dose, probit-survival plots. [c] $ED_{50}$ is defined as the dose required to produce 50% 45-day survivors in a group of treated mice as detd from log-dose probit-survival plots. [d] $ED_{99}$ is defined as the dose required to kill two logs (99%) of leukemic cells as detd from arithmetic plots of log cell kill based on increase in life span *vs.* dose. [e] No survivors. [f] 1:1 (and 2:5) mixture of **6a** and **6b**. [g] KB cells. [h] Isomer content ~5%. [i] Isomer content ~25%. [j] $10^5$ Cells. [k] Limited testing indicated the cis isomer to be less toxic and less active than the trans on an equimolar basis. [l] Isomer content 10–20%.

probably due to variations in the ability of various structural types to cross the blood–brain barrier.[28] In every case, however, the ic activity of a particular compound is less than its ip activity. The most effective compd considering both ip and ic activity is the 1:1 mixture of **6a** and **6b**; the activity of the 2:5 mixture seems indistinguishable from that of the 1:1 mixture. Almost as active are **25**, **23**, and **47**, indicating a lack of structural specificity. This lack of structural specificity is further exemplified by the tosylurea **51**, which is highly active against the ip disease, but only slightly active against the ic form. The last seven compds in Table VI either produced no survivors at any dose tested ($\geq LD_{10}$) or failed to produce 50% survivors at the $LD_{10}$, indicating their lack of specificity for leukemic cells. Two compds reported herein, the CCNU analog **2** and the benzyloxynitrosourea **73** (neither of which contains a 2-haloethyl group), were completely inactive; the methylnitrosourea **21** was moderately active, but effected no cures. The cytotoxicity of these nitrosoureas for either HEp-2 or KB cells in culture is also given in Table VI to emphasize again[23] the lack of correlation between the cytotoxicity of this type of agent and its antileukemic activity, or for that matter between cytotoxicity and whole animal toxicity. Such a lack of correlation could be due to differences in metabolism or distribution of the various compds in the whole animal.

(28) 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).

## Experimental Section[29]

***N,N'*-(2-Hydroxytrimethylene)diphthalimide.**—A stirred mixt of potassium phthalimide (20.0 g, 108 mmoles), DMF (200 ml), and 1,3-dichloro-2-propanol (6.85 g, 54.0 mmoles) was gradually heated to 100°, kept there for 8 hr, chilled, and diluted with $H_2O$ (200 ml). The $H_2O$-washed and vacuum-dried ppt (19 g) was recrystd from MeCN (400 ml) by addition of $H_2O$ (400 ml): yield 15.0 g (80%); mp 204° (lit.[30] mp 204°).

***N,N'*-(2-Chlorotrimethylene)diphthalimide** was prepared by the action of $PCl_5$ on *N,N'*-(2-hydroxytrimethylene)diphthalimide (13.0 g) according to Gabriel.[31] Recrystn of the crude product (11 g) from MeCN (220 ml) gave 8.35 g (61%): mp 214°, 213–214° (lit.[31] 208–209°). *Anal.* ($C_{19}H_{13}ClN_2O_4$) C, H, N.

**2-Chloro-1,3-propanediamine Dihydrochloride.**—A suspension of *N,N'*-(2-chlorotrimethylene)diphthalimide (7.25 g, 19.8 mmoles) in concd HCl (400 ml) and AcOH (300 ml) was refluxed for 48 hr (soln occurring after 4 hr) and then concd under reduced pressure to ~100 ml. The pptd phthalic acid was removed and evapn contd to dryness. The residue was dissolved in $H_2O$ and the soln was clarified by filtration. The filtrate was again evapd and the residue (3.47 g, mp 210–214°) was triturated in EtOH (20 ml) and dried *in vacuo* ($P_2O_5$): yield 2.70 g (76%); mp 217–219° dec (lit.[31] mp 216°).

**1,1'-(2-Chlorotrimethylene)bis(3-cyclohexylurea) (1).**—Cyclohexyl isocyanate (3.70 ml, 29.0 mmoles) was slowly added to a stirred mixt of 2-chloro-1,3-propanediamine dihydrochloride (2.63 g, 14.5 mmoles), $CHCl_3$ (250 ml), and $Et_3N$ (6.25 ml). Stirring was contd at room temp for 3 hr as the suspension became thicker. Volatile material was removed under reduced pressure, and the residue was stirred in $H_2O$ (80 ml) for 30 min, collected, washed with $H_2O$, and dried *in vacuo* ($P_2O_5$): yield 4.20 g (81%); mp 210°, 211–212° dec; ir (KBr) 1625 (C=O), 1570 (CNH) $cm^{-1}$. *Anal.* ($C_{17}H_{31}ClN_4O_2$) C, H, N.

**1,1'-(2-Chlorotrimethylene)bis(3-cyclohexyl-1-nitrosourea) (2).**—$NaNO_2$ (7.4 g, 0.11 mole) was added in small portions to a cold (0–5°), stirred soln of **1** (3.70 g, 10.3 mmoles), and stirring was contd at 0–5° for 2 hr. The light yellow ppt was collected, washed with cold $H_2O$, and dried *in vacuo* ($P_2O_5$): wt 2.55 g; mp 128° dec; ir (KBr) 1705 (C=O), 1540 (CNH) $cm^{-1}$. A 0.35-g second crop (same mp, ir) increased yield to 67%. *Anal.* ($C_{17}H_{29}ClN_6O_4$) C, H, N.

**Reaction of 2 with Cyclohexylamine.**—Cyclohexylamine (10.7 mg, 0.108 mmole) was added to a suspension of **2** (15.0 mg, 0.036 mmole) in $H_2O$ (2 ml) and $Me_2CO$ (0.5 ml). The mixt was stirred at ambient temp overnight, then heated at 80–90° for 30 min, and cooled. The ppt was collected, washed with $H_2O$, dried *in vacuo* ($P_2O_5$), and recrystd from EtOH to give 1,3-dicyclohexylurea as colorless plates: mp 227–230° (lit.[32] mp 229–230°); yield 11 mg (68%). Identity was also confirmed by tlc and mmp.

**1,1'-(2-Hydroxytrimethylene)bis(3-methylurea)**, which pptd when MeNCO (7.6 ml, 120 mmoles) was added to 1,3-diamino-2-propanol (5.0 g, 56 mmoles) in $CHCl_3$ (100 ml), was purified by trituration in warm MeCN (50 ml): yield 9.3 g (82%); mp 172–174°; ir (KBr) 1620 (C=O), 1585 (CNH) $cm^{-1}$. *Anal.* ($C_7H_{16}N_4O_3$) C, H, N. The action of $SOCl_2$ on this urea in an attempted chlorodehydroxylation gave an uncharacterizable oil.[33]

**Nitrosation of 1-(2-Chloroethyl)-3-(2-fluoroethyl)urea (5).—[Caution: Slow-developing and slow-healing erythema (and tanning) can result from exposure of skin to 6, either neat or in $CHCl_3$ soln.]** A soln of $NaNO_2$ (3.0 g, 43.5 mmoles) in $H_2O$ (10 ml) was added dropwise (at as nearly uniform rate as could be achieved with a constant-addition funnel in an open system) over a period of 60 min to a cold (5°), stirred soln of **5** (1.0 g, 5.96 mmoles) in concd HCl (15 ml). After the addn, the reaction soln was stirred at 0–5° for 15 min and then extd with $CHCl_3$ (2 × 20 ml). The exts were combined, dried ($MgSO_4$), and evapd under reduced pressure to a yellow oil, which was further dried (3–4 hr) *in vacuo* ($P_2O_5$): av yield 0.95 g (81%); isomer ratio (**6a**:**6b**) as determined by pmr[4] ~1:1. A reaction temp of 12–14° (90 min) and addn times of 27, 90, and 150 min also gave a ~1:1 ratio, but an addition time of 17 min gave varied ratios, *e.g.*, ~2:3 and ~1:2.

Nitrosations of **5** by portionwise addition of solid $NaNO_2$ gave, unpredictably, **6a**:**6b** ratios of ~1:1 (av yield of 3 runs 77%) and ~2:5 (av yield of 2 runs 73%). The ~2:5 mixts were isolated as oils, which crystd (mp 38–40°). *Anal.* ($C_5H_9ClFN_3O_2$) C, H, N. A soln of the ~2:5 mixt (500 mg) in 98–100% $HCO_2H$ (5 ml) was stirred at 0–5° for 1 hr, dild with cold $H_2O$ (20 ml), and extd with $CHCl_3$ (2 × 15 ml); evapn of the dried ext left 150 mg (30%) of a mixt of **6a** and **6b**: mp 47–48°; isomer ratio ~1:6 (pmr); ir (KBr) 1725 (C=O), 1525 (CNH), 1485 (NN=O) $cm^{-1}$.

**4-Methylcyclohexanone Oxime.**—A stirred mixt of 4-methylcyclohexanone (20.0 g, 0.179 mole), $NH_2OH \cdot HCl$ (8.70 g, 0.224 mole), $H_2O$ (75 ml), and EtOH (25 ml) was treated dropwise with a soln of $Na_2CO_3$ (11.9 g, 0.112 mole) in $H_2O$ (50 ml) at a rate that kept the reaction temp at ~40°. Stirring at 40–45° was contd for 2 hr; most of the EtOH was then removed at 60° under reduced pressure. The remaining mixt was chilled, and the oily layer was sepd, dissolved in $Et_2O$ (300 ml), dried ($MgSO_4$), and concd to a thick oil, which solidified after drying *in vacuo* ($P_2O_5$): yield 11.1 g (49%); mp 36–37° (lit.[34] mp 36°).

***trans*-4-Methylcyclohexylamine Hydrochloride.**—Na (20 g, 0.87 g-atom) was added in small pieces to a stirred soln of 4-methylcyclohexanone oxime (11.0 g, 86.5 mmoles) in EtOH (150 ml) at a rate that maintained refluxing. The mixt was refluxed for 1 hr, cooled, cautiously treated with $H_2O$ (200 ml), concd to ~200 ml under reduced pressure, and extd with $Et_2O$ (3 × 100 ml). The dried ($MgSO_4$) $Et_2O$ soln was treated with ethereal dry HCl until no further pptn occurred. The ppt was dried *in vacuo* ($P_2O_5$): wt 9.50 g; mp ~250°. Three recrystns from MeCN progressively raised the mp to 260° (lit.[8] mp 260.5–261.5°), yield 3.50 g (27%). *Anal.* ($C_7H_{15}N \cdot HCl$) C, H, N. The once-recrystd amine·HCl, mp 253–256°, was converted (Schotten-Baumann, in 2:1 1 *N* NaOH–MeCN followed by dilu with $H_2O$) to an unrecrystd *N*-benzoyl derivative, mp 179° [lit.[8] mp 180–180.5° (trans)], in 99% yield.

***trans*-4-Methylcyclohexyl *p*-Toluenesulfonate.**—A soln of *trans*-4-methylcyclohexanol[35] (6.84 g, 60.0 mmoles) in pyridine (40 ml) was added to a cold (0°) soln of *p*-TsCl (20 g, 0.10 mole) in pyridine (40 ml). The reaction flask was sealed and allowed to stand overnight at ambient temp. The soln was poured into ice-cold 10% HCl (~140 ml) and extd with $Et_2O$ (2 × 200 ml). The ext, washed successively with dil HCl, $H_2O$, dil $NaHCO_3$ soln, and again with $H_2O$, was dried ($Na_2SO_4$) and concd to a solid (16.2 g, mp ~65°), which was recrystd from hexane (50 ml) and dried *in vacuo*: yield 11.2 g (70%); mp 69–70°, 69° (lit.[36] 70–71°).

***cis*-4-Methylcyclohexylamine Hydrochloride.**—A mixt of *trans*-4-methylcyclohexyl *p*-toluenesulfonate (4.00 g, 14.9 mmoles) and liquid $NH_3$ (30 ml) was heated in a Parr pressure vessel at 95–100° for 40 hr. Evapn of $NH_3$ in a stream of $N_2$ left a residue, which was dissolved in $H_2O$ (20 ml), made alkaline with 50% NaOH (5 ml), and extd with $Et_2O$ (50 ml) and then $CHCl_3$ (50 ml). The exts were combined, dried (Drierite), and treated with excess 3 *N* ethanolic dry HCl. Solvent evapn under reduced pressure left a white solid (850 mg, mp ~225°), which was twice recrystd from MeCN: yield 550 mg (25%); mp 230–231°, 234° (lit.[8] mp 233–234°). *Anal.* ($C_7H_{15}N \cdot HCl$) C, H, N. The *N*-Bz derivative, mp 130° (lit.[8] mp 130–130.5°), was prepd in the manner described above for the trans compd and recrystd from MeCN by dilu with $H_2O$; yield 91%.

**4-Ethylcyclohexylamine Hydrochloride.**—A soln of $Na_2CO_3$ (4.75 g, 44.8 mmoles) in $H_2O$ (20 ml) was added dropwise to a stirred soln of 4-ethylcyclohexanone (9.00 g, 71.5 mmoles) and $NH_2OH \cdot HCl$ (6.22 g, 89.5 mmoles) in $H_2O$ (50 ml) and EtOH (25 ml). The mixt was refluxed for 3 hr and then concd under reduced pressure to ~50 ml. The oxime sepd as a clear oil, which was washed with $H_2O$ and dried by distn of added $C_6H_6$;

(29) Melting points recorded without a range were determined with a Kofler Heizbank; those with a range, with a Mel-Temp apparatus. Ir spectra were determined in KBr disks (solids) or films (oils) with a Perkin-Elmer spectrophotometer (Model 521 or Model 621). Pmr spectra were determined in $CDCl_3$ or DMSO-$d_6$ (TMS as internal ref) with a Varian A-60A spectrometer (no satisfactory solvent was found for the tosylnitrosourea **51**, which appeared to react with $CF_3CO_2H$). Analytical results indicated by element symbols were within ±0.4% of the theoretical values. Microanalyses were performed for the most part by Galbraith Laboratories, Knoxville, Tenn. Nitrosoureas were stored cold and dry to minimize decomposition.

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crude yield 7.5 g (74%). A soln of the oxime in EtOH (100 ml) was hydrogenated over Raney Ni at ~3.5 kg/cm² for 4 hr. Treatment of the filtered soln with satd ethanolic dry HCl and evapn under reduced pressure left the amine·HCl, which was triturated in $Et_2O$ (50 ml) and dried *in vacuo* ($P_2O_5$): yield 7.57 g (65% overall); mp 195–196° (lit.[37] mp 234–247°). *Anal.* ($C_8H_{17}N \cdot HCl$) C, H, N.

The conversion of 4-isopropylcyclohexanone[38] (10.0 g, 71.5 mmoles) to **4-isopropylcyclohexylamine hydrochloride** was like the prepn of 4-ethylcyclohexylamine·HCl described above with the following exceptions. A $CHCl_3$ extn supplemented the yield of the oily oxime (total 7.7 g), and the amine·HCl was recrystd from PhMe (100 ml) by addn of hexane (100 ml): yield 6.25 g (49% overall); mp 195–200°. *Anal.* ($C_9H_{19}N \cdot HCl$) C, H, N.

**4,4-Dimethylcyclohexanone Oxime.**—A soln of $Na_2CO_3$ (13.8 g, 130 mmoles) in $H_2O$ (30 ml) was added dropwise to a stirred soln of 4,4-dimethylcyclohexanone[10] (12.6 g, 100 mmoles) and $NH_2OH \cdot HCl$ (9.03 g, 130 mmoles) in EtOH (50 ml) and $H_2O$ (60 ml). The mixt was refluxed for 2 hr and chilled (0°). The ppt was washed with cold $H_2O$, dried *in vacuo* ($P_2O_5$), and recrystd by diln of a filtered EtOH (100 ml) soln with $H_2O$ (150 ml): yield 10.5 g (75%); mp 83°. *Anal.* ($C_8H_{15}NO$) C, H, N.

**4,4-Dimethylcyclohexylamine Hydrochloride.**—A soln of the oxime (9.50 g, 67.4 mmoles) in EtOH (100 ml) was hydrogenated over Raney Ni at ~3.5 kg/cm² for 5 hr. The filtered soln was treated with excess ethereal dry HCl. Removal of solvents under reduced pressure left the amine·HCl which was triturated in $Et_2O$ and dried *in vacuo* ($P_2O_5$): yield 8.3 g (75%); mp 320–335° dec (indefinite). *Anal.* ($C_8H_{17}N \cdot HCl$) C, H, N.

**1-(2-Cyclohexen-1-yl)-3-methylurea.**—Treatment of a cold (5°), stirred soln of 2-cyclohexen-1-ylamine[11] (3.00 g, 31.0 mmoles) in hexane (90 ml) with MeNCO (1.96 ml, 31.0 mmoles) resulted in the pptn of a white solid. After being stirred at room temp for 2 hr, the mixt was again cooled; the ppt was collected, washed with cold hexane (20 ml), and dried *in vacuo*: yield 4.50 g (94%); mp 127°. *Anal.* ($C_8H_{14}N_2O$) C, H, N.

**3-(2-Cyclohexen-1-yl)-1-methyl-1-nitrosourea.**—$NaNO_2$ (1.44 g, 20.9 mmoles) was added in small portions to a cold (0–5°), stirred soln of 1-(2-cyclohexen-1-yl)-3-methylurea (3.00 g, 19.5 mmoles) in 98–100% $HCO_2H$ (30 ml). The mixt was stirred at 0–5° for 1 hr, dild with cold $H_2O$ (180 ml), and stirred at 0–5° for 1 hr longer. The yellow oil that solidified after scratching with a glass rod was collected, washed with cold $H_2O$, and dried *in vacuo* ($P_2O_5$): yield 2.75 g (77%); mp 38°; ir (KBr) 1720 (C=O), 1520 (CNH) $cm^{-1}$. *Anal.* ($C_8H_{13}N_3O_2$) C, H, N.

**1,3-Di-2-cyclohexen-1-ylurea (15).**—$Et_3N$ (2 ml) was added to a stirred soln of 3-(2-cyclohexen-1-yl)-1-methyl-1-nitrosourea (2.75 g, 15.0 mmoles) in $H_2O$ (50 ml) and EtOH (20 ml). The mixt was stirred at room temp for 3 hr, then boiled for 15 min, and cooled. The ppt was recrystd from EtOH (20 ml) by dilution with $H_2O$ (75 ml): yield 1.4 g (85%); mp 247°; ir (KBr) 1615 (C=O), 1560 (CNH) $cm^{-1}$. *Anal.* ($C_{13}H_{20}N_2O$) C, H, N.

**Ethyl *trans*-4-Aminocyclohexanecarboxylate Hydrochloride.**—Dry HCl was bubbled into a stirred suspension of *trans*-4-aminocyclohexanecarboxylic acid[13] (2.75 g, 19.3 mmoles) in ethanolic dry HCl soln (100 ml) until the solid dissolved completely (~15 min). The soln was refluxed for 4 hr, dild with $C_6H_6$ (20 ml), and distd until the distn temp reached 82°. The white solid remaining after removal of the solvent under reduced pressure was washed with $Et_2O$ and further dried *in vacuo* ($P_2O_5$): yield 3.70 g (93%); mp 168°; ir (KBr) 1730 $cm^{-1}$ (C=O). *Anal.* ($C_9H_{17}NO_2 \cdot HCl$) C, H.

Similar esterifications of *cis*-3-aminocyclohexanecarboxylic acid[39] and *cis*-4-aminocyclohexanecarboxylic acid[13] produced the corresponding esters: **ethyl *cis*-3-aminocyclohexanecarboxylate·HCl,** yield 82%, mp 161°, ir (KBr) 1720 $cm^{-1}$ (C=O) [*Anal.* ($C_9H_{17}NO_2 \cdot HCl$) C, H, N]; and **ethyl *cis*-4-aminocyclohexanecarboxylate·HCl** (three times recrystd from MeCN), yield 56%, mp 190° (lit.[40] mp 193–194°), ir (KBr) 1725 $cm^{-1}$ (C=O).

**Tetrahydro-2*H*-pyran-4-amine Hydrochloride.**—This precursor of **19** and **21** was prepared in 4 steps beginning with the hard-to-control Cu-catalyzed thermal decarboxylation of $H_2O$-recrystd commercial chelidonic acid to give a low yield of 4*H*-pyran-4-one, which was converted to tetrahydro-4*H*-pyran-4-one (**17**), bp 55° (9 mm), by Raney Ni hydrogenation.[41] A soln of the oxime[42,43] (9.55 g, 83.0 mmoles) in EtOH (200 ml) was hydrogenated over Raney Ni at ~3.5 kg/cm² for 4 hr. The catalyst was removed and the filtrate was treated with excess ethereal dry HCl soln. The pptd amine·HCl[44] was collected and dried *in vacuo* ($P_2O_5$): yield 9.6 g (84%); mp 230°. *Anal.* ($C_5H_{11}NO \cdot HCl$) C, H, N. A similar prepn of the free amine has been described.[45]

**1-Methyl-3-(tetrahydro-2*H*-pyran-4-yl)urea.**—A cold, stirred soln of tetrahydro-2*H*-pyran-4-amine·HCl (5.00 g, 36.4 mmoles) in $H_2O$ (15 ml) was made alk with 50% NaOH (5 ml) and extd with $Et_2O$ (3 × 80 ml). The dried ($Na_2SO_4$) extract was treated with MeNCO (2.35 ml, 37.0 mmoles), and the mixture was stirred at 0–5° for 2 hr. The ppt was washed with $Et_2O$ and dried *in vacuo* ($P_2O_5$): yield 4.80 g (84%); mp 200°; ir (KBr) 1620 (C=O), 1580 (CNH) $cm^{-1}$. *Anal.* ($C_7H_{14}N_2O_2$) C, H, N.

**1-Methyl-1-nitroso-3-(tetrahydro-2*H*-pyran-4-yl)urea (21).**—$NaNO_2$ (3.25 g, 47.1 mmoles) was added in small portions to a cold (0–5°), stirred soln of 1-methyl-3-(tetrahydro-2*H*-pyran-4-yl)urea (3.25 g, 20.5 mmoles) in 6 *N* HCl (60 ml). After 1 hr the mixt was dild with cold $H_2O$ (120 ml), stirred 30 min longer at 0–5°, and extd with $CHCl_3$ (2 × 180 ml). Evapn of the dried ($MgSO_4$) $CHCl_3$ soln under reduced pressure left **21** as a light yellow solid, which was further dried *in vacuo* ($P_2O_5$): yield 3.1 g (81%); mp 70° dec; ir (KBr) 1720 (sh), 1695 (C=O), 1525 (CNH) $cm^{-1}$; pmr ($CDCl_3$) indicated no $CH_3NH$. *Anal.* ($C_7H_{13}N_3O_3$) C, H, N.

**Tetrahydro-2,6-dimethyl-4*H*-pyran-4-one (18).**—A soln of 2,6-dimethyl-4*H*-pyran-4-one[46] (30.0 g, 242 mmoles) in EtOH (250 ml) was hydrogenated over Raney Ni at ~3.5 kg/cm² for 24 hr. After removal of the catalyst, evapn of the solvent under reduced pressure left an oil, which was distd at atm pressure: yield of crude **tetrahydro-2,6-dimethyl-2*H*-pyran-4-ol,** 31.4 g (80%); bp 182–185° [lit. bp 190°,[42] 96–98° (20 mm)[47]]. A cold (15°) soln of $Na_2Cr_2O_7 \cdot 2H_2O$ (13.8 g, 46.3 mmoles) in AcOH (21 ml) was added all at once to a cold (15°) stirred soln of the tetrahydrodimethylpyranol (15.0 g, 115 mmoles) in AcOH (15 ml). The exothermic reaction temp was kept at 55–60° by intermittent cooling, and, after 30 min and until the color of the soln became green, this temp was maintained by warming. The mixt was dild with $H_2O$ (300 ml) and steam distd; NaCl (60 g) was added to the aq dist (~300 ml) and the resulting suspension extd with $Et_2O$ (3 × 100 ml). The dried ($Na_2SO_4$) $Et_2O$ soln was evapd to an oil (8.7 g), which was distd at atm pressure: yield of **18**, 5.53 g (38%, 30% overall); bp 170–173° [lit. bp 59–62° (14 mm),[48] 52° (8 mm)[49]]; $n^{25}D$ 1.4400 (lit. $n^{14}D$ 1.447,[48] $n^{25}D$ 1.440[49]): ir (film) 1725 $cm^{-1}$ (C=O). *Anal.* ($C_7H_{12}O_2$) C, H. The oxime, mp 85° (from hexane) [lit. mp 82–83°,[42] 92–93° [47]], was prepd for use in the following experiment. *Anal.* ($C_7H_{13}NO_2$) C, H, N.

**Tetrahydro-2,6-dimethyl-2*H*-pyran-4-amine Hydrochloride.**—A soln of tetrahydro-2,6-dimethyl-4*H*-pyran-4-one oxime (4.65 g, 32.2 mmoles) in EtOH (200 ml) was hydrogenated over Raney Ni at ~3.5 kg/cm² for 4 hr. The catalyst was removed by filtration, and the filtrate was treated with excess ethanolic dry HCl soln. Evapn of the solvent under reduced pressure left a semisolid, which was triturated in $Et_2O$. The product was dried *in vacuo* ($P_2O_5$): yield 5.0 g (94%); mp ~205°. A sample was twice recrystd from MeCN for analysis, mp 210°. *Anal.* ($C_7H_{15}NO \cdot HCl$) C, H, N.

**Tetrahydro-3-thiophenamine 1,1-Dioxide Hydrochloride.**[50]—A soln of 2,5-dihydrothiophene dioxide (50.0 g, 424 mmoles) in 29% $NH_4OH$ (180 ml) was heated in a Parr pressure vessel at ~86° for 7 hr. The reaction soln was evapd to a yellow oil, which was filtered, dild with EtOH (150 ml), and treated with

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concd HCl (100 ml). Addition of $Et_2O$ (100 ml) to the resultant mixt pptd the cryst hydrochloride, which was collected, washed with $Et_2O$, and dried *in vacuo* ($P_2O_5$): yield 51.7 g (71%); mp 220°. The analytical sample, mp 220°, was obtd from an earlier run in which the oily amine was not filtered before conversion to the hydrochloride and was ultimately recrystd from MeOH–$Et_2O$.[51] *Anal.* ($C_4H_9NO_2S \cdot HCl$) C, H, N.

**1-Methyl-3-(tetrahydro-3-thienyl)urea *S,S*-Dioxide.**—A stirred suspension of tetrahydro-3-thiophenamine 1,1-dioxide·HCl (1.0 g, 6.3 mmoles) in MeOH (30 ml) was treated with a soln of $Ba(OH)_2 \cdot 8H_2O$ (1.0 g, 3.2 mmoles) in MeOH (50 ml), and, after 1 hr, the solvent was evapd under reduced pressure, and the residue was extd with EtOH (2 × 20 ml). Evapn of the EtOH soln under reduced pressure left an oil, a soln of which in EtOAc (25 ml) was treated with MeNCO (0.40 ml, 6.3 mmoles). The ppt was recrystd from EtOAc (30 ml) and dried *in vacuo* ($P_2O_5$): yield 700 mg (58%); mp 136°; ir (KBr) 1630 (C=O), 1560 (CNH) $cm^{-1}$. *Anal.* ($C_6H_{12}N_2O_3S$) C, H, N. A characterizable nitroso derivative of this urea was not obtained.

**1-(1-Adamantyl)-3-(tetrahydro-3-thienyl)urea *S,S*-Dioxide.**—A stirred soln of 1-adamantanamine·HCl[35] (188 mg, 1.00 mmole) in $H_2O$ (10 ml) and $Me_2CO$ (10 ml) was treated with $Et_3N$ (1 ml) and then with the isomeric nitrosourea mixt **27** (241 mg, 1.00 mmole). After 1 hr, the stirred mixt was heated at 60° for 30 min, then concd to ~10 ml, and cooled. The ppt, washed with $H_2O$ and 1 *N* HCl, was recrystd from MeCN (3 ml) by diln with $H_2O$ (20 ml): yield 80 mg (26%); mp 250°; ir (KBr) 1625 (C=O), 1550 (CNH) $cm^{-1}$. *Anal.* ($C_{15}H_{24}N_2O_3S$) C, H, N.

**1-(*m*-Dithian-5-yl)-3-(2-fluoroethyl)urea (31).**—1-(2-Fluoroethyl)-3-methyl-1-nitrosourea[4] (2.90 g, 19.5 mmoles) and then $Et_3N$ (0.5 ml) were added to a stirred soln of **29**[15] (2.64 g, 19.6 mmoles) in $H_2O$ (60 ml) and EtOH (60 ml). The mixt was stirred at room temp for 2 hr and cooled. The ppt was washed with cold $H_2O$ and dried *in vacuo* ($P_2O_5$), yield 3.20 g (see Table II).

***m*-Dithian-5-one 1,1,3,3-Tetraoxide Oxime (36).**—NaOAc (5.58 g, 68.0 mmoles) was added gradually to a stirred mixt of *m*-dithiane-5,5-diol 1,1,3,3-tetraoxide[15] (**35**) (8.00 g, 37.0 mmoles) $NH_2OH \cdot HCl$ (4.70 g, 67.6 mmoles), and $H_2O$ (240 ml), which was then refluxed for 3 hr and cooled. The ppt was collected, washed with cold $H_2O$, and dried *in vacuo* ($P_2O_5$): yield 7.10 g (90%); mp 249–250° dec. For analysis, a small sample from a previous run was recrystd from $H_2O$: recovery 68%; mp 248–249° dec. *Anal.* ($C_4H_7NO_5S_2$) C, H, N.

**1-(2-Chloroethyl)-3-*m*-dithian-5-ylurea *S,S,S',S'*-Tetraoxide (37).**—A cold, stirred suspension of **30** (5.00 g, 20.8 mmoles) in AcOH (150 ml) was treated with 30% $H_2O_2$ (100 ml). After being stirred at 5° for ~2 hr and then at room temp for 4 days, the mixt was dild with $H_2O$ (550 ml). The pptd **37** was collected, washed with cold $H_2O$, and dried *in vacuo* ($P_2O_5$): yield 4.80 g; ir (KBr) 1330, 1315, and 1145 $cm^{-1}$ ($SO_2$). (See Table I).

**1-(2-Fluoroethyl)-3-*m*-dithian-5-ylurea *S,S,S',S'*-Tetraoxide (38). A. From 31.**—A cold, stirred suspension of **31** (3.10 g, 13.8 mmoles) in AcOH (100 ml) was treated with 30% $H_2O_2$ (60 ml); the resulting soln was stirred at room temp for 2 days, during which time some ppt formed. The suspension was dild with $Et_2O$ (300 ml), and the ppt was collected, washed with $Et_2O$, and dried *in vacuo* ($P_2O_5$): yield 3.40 g; ir (KBr) 1330, 1315, 1300, and 1145 $cm^{-1}$ ($SO_2$). (See Table II).

**B. From 33.**—A stirred soln of **33** (100 mg, 0.395 mmole) in AcOH (5 ml) was treated with 30% $H_2O_2$ (2 ml) and then allowed to stand at room temp for 4 days, during which time a ppt formed. The mixt was dild with cold $H_2O$ (15 ml) and the ppt collected, washed with $H_2O$, and dried *in vacuo* ($P_2O_5$): yield 105 mg (92%); ir (KBr) identical with that of the analytical sample derived from **31**.

**1-Cyclohexyl-3-*m*-dithian-5-ylurea (34).**—To a stirred soln of cyclohexylamine (155 mg, 1.56 mmoles) in $H_2O$ (15 ml) and EtOH (5 ml) was added **32** (210 mg, 0.777 mmoles); the mixt was stirred at room temp for 2 hr and at 60–70° for 1 hr and was then chilled. The white ppt was washed with cold $H_2O$ and dried *in vacuo* ($P_2O_5$): yield 178 mg (88%); mp 239°; ir (KBr) 1615 (C=O), 1565 (CNH) $cm^{-1}$. *Anal.* ($C_{11}H_{20}N_2OS_2$) C, H, N.

**2-Deoxy-2-[3-(2-fluoroethyl)ureido]-D-glucopyranose 1,3,4,6-Tetraacetate (44).**—A soln of 2-amino-2-deoxy-D-glucopyranose 1,3,4,6-tetraacetate·HCl[52] (1.00 g, 2.61 mmoles) in $H_2O$ (20 ml) was treated with NaOAc (427 mg, 5.22 mmoles) and the resulting suspension was extd with $CHCl_3$ (2 × 50 ml). The ext was dried ($MgSO_4$) and concd under reduced pressure. A soln of the residual free amine (800 mg, 2.31 mmoles) and 3-(2-fluoroethyl)-1-methyl-1-nitrosourea[4] (373 mg, 2.52 mmoles) in toluene (25 ml) was refluxed for 3 hr, then cooled, and dild with hexane (10 ml). The dried ppt (705 mg, mp ~140°) was three-times recrystd from EtOH (5 ml) and then dried *in vacuo* ($P_2O_5$), yield 80 mg (8% from the free amine, 7% overall). (See Table II.)

**1-(2-Fluoroethyl)-3-(thiochroman-4-yl)urea *S,S*-Dioxide.**—A stirred soln of 1-(2-fluoroethyl)-3-(thiochroman-4-yl)urea (254 mg, 1.00 mmole) in AcOH (8 ml) was treated at 5° with 30% $H_2O_2$ (2 ml), then stirred at 5° for 3 hr, and left standing at room temp overnight. The soln was heated at 50° for 30 min, concd to ~10 ml under reduced pressure, and chilled. The vacuum-dried ppt (152 mg, mp 190°) was recrystd from *n*-PrOH (3 ml): yield 102 mg; mp 190°; ir (KBr) 1305, 1295, 1285 and 1145, 1130 $cm^{-1}$ ($SO_2$). (See Table II.)

**4-Aminocyclohexyl Acetate Hydrochloride (46).**—A soln of 4-aminocyclohexanol·HCl (11.6 g, 76.5 mmoles) in AcCl (100 ml) and AcOH (60 ml) was refluxed for 3 hr. Evapn under reduced pressure left a pink solid, which was triturated in $Et_2O$ (125 ml) and then in boiling MeCN (500 ml). This last mixt was chilled, and the product was collected and dried *in vacuo* ($P_2O_5$): yield 4.85 g (33%); mp 207°. The analytical sample (450 mg) was obtained by recrystn of the crude product (1.15 g) of a previous run from MeCN (150 ml): mp 226–230°; ir (KBr) 1730 $cm^{-1}$ (C=O). *Anal.* ($C_8H_{15}NO_2 \cdot HCl$) C, H, N.

**(4-Acetoxycyclohexyl)urea (49).**—A cold (−10°) soln of **47** (500 mg, 1.71 mmoles) in $NH_3$-satd MeOH was allowed to stand at −8° in a sealed flask for 2 hr. (Complete disappearance of **47** with formation of a single product was indicated by tlc.) Removal of $NH_3$ at −10° in a stream of $N_2$ and evapn of MeOH at <0° under reduced pressure left a white solid, which was triturated in cold $H_2O$ (10 ml) and dried *in vacuo* ($P_2O_5$): yield 295 mg (86%); mp 240°; ir (KBr) 1720 (ester C=O), 1645 (urea C=O), 1550 (CNH) $cm^{-1}$. *Anal.* ($C_9H_{16}N_2O_3$) C, H, N. A similar treatment of **48** for 5 hr also gave **49** in 83% yield (mp ~245°, but ir identical with analytical sample).

**1-Methyl-3-(methylsulfonyl)urea (54).**[53]—MeNCO (1.35 ml, 21 mmoles) was added to a stirred soln of $MeSO_2NH_2$ (2.0 g, 21 mmoles) in $Et_3N$ (8 ml) and DMF (8 ml). The flask was sealed and stirring was contd for 20 hr. The mixt was dild with $H_2O$ (50 ml) and extd with $Et_2O$ (2 × 20 ml). The aq layer was acidified with 1 *N* HCl and evapd under reduced pressure to a white solid, which was triturated in $H_2O$ (25 ml), dried *in vacuo* ($P_2O_5$), and recrystd from EtOH (50 ml): yield 550 mg (17%); mp 170°, 168–170°; ir (KBr 1690 and 1660 (C=O), 1545 (CNH), 1330 and 1150 ($SO_2N$) $cm^{-1}$. *Anal.* ($C_3H_8N_2O_3S$) C, H, N.

**1-(2-Chloroethyl)-3-(methylsulfonyl)urea (56).**—A soln of $MeSO_2NH_2$ (1.00 g, 10.5 mmoles) and 2-chloroethyl isocyanate[54] (1.27 g, 12.0 mmoles) in DMF (6 ml) was heated in a Parr pressure vessel at 70° for 17 hr. Evapn of the solvent under reduced pressure with the aid of two additions of toluene (10-ml portions) left a semisolid, which was dissolved in EtOH (20 ml) and filtered. The chilled filtrate deposited needles, which were dried *in vacuo* ($P_2O_5$): yield 430 mg; ir (KBr) 1330 and 1115 $cm^{-1}$ ($SO_2N$). (See Table I.)

**1-(2-Chloroethyl)-3-(*p*-tolylsulfonyl)urea (50).**—2-Chloroethylamine·HCl (5.00 g, 43.1 mmoles) was neutralized with a cold soln of NaOH (1.74 g, 43.1 mmoles) in $H_2O$ (5 ml) and extd with $C_6H_6$ (4 × 50 ml). The dried ($MgSO_4$) extract was treated with *p*-TsNCO[55] (8.50 g, 43.1 mmoles). After being stirred at room temp for 2 hr, the mixt was chilled and the ppt was recrystd from $C_6H_6$ (450 ml) and dried *in vacuo* ($P_2O_5$): yield 9.95 g; ir (KBr) 1350 and 1160 $cm^{-1}$ ($SO_2N$). (See Table I.) **1-(2-Fluoroethyl)-3-(*p*-tolylsulfonyl)urea (52)** was similarly prepd from 2-fluoroethylamine·HCl[56] (2.50 g, 25.1 mmoles) and recrystd from $C_6H_6$: yield 6.4 g; ir (KBr) 1330 and 1155 $cm^{-1}$ ($SO_2N$). (See Table II.)

**1-(2-Fluoroethyl)-3-(2,2,2-trifluoroethyl)urea.**—1-Methyl-1-nitroso-3-(2,2,2-trifluoroethyl)urea[23] (mp 34–35° dec) (8.40 g,

(51) The simplified work-up described above was devised by Dr. R. D. Elliott.

(52) M. Bergmann and L. Zervas, *Ber.*, **64B**, 975 (1931).

(53) Procedure adapted from prepn of some 3-substituted 1-(phenylsulfonyl)ureas [G. F. Holland, *J. Org. Chem.*, **26**, 1662 (1961)].

(54) W. Siefkin, *Justus Liebigs Ann. Chem.*, **562**, 75 (1949); now available from Eastman Kodak Co.

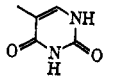
(55) Purchased from Eastman Kodak Co., Rochester, N. Y.

(56) Z. B. Papanastassiou and R. J. Bruni, *J. Org. Chem.*, **29**, 2870 (1964).

TABLE VII

ALKOXYUREAS

RONHCONHR′

| Compd No. | R | R′ | Method[a] | Reaction solvent | Recrystn solvent[b] | Yield, % | Mp,[c] °C | ν KBr, cm⁻¹[d] C=O | ν KBr, cm⁻¹[d] CNH | Formula[e] |
|---|---|---|---|---|---|---|---|---|---|---|
| 62 | $ClCH_2CH_2$ | $C_6H_5$ | A[f] | $CHCl_3$ | EtOH | 70 | 78.5–80 | 1680 | 1545 | $C_9H_{11}ClN_2O_2$ |
| | Me | H[g] | B | $H_2O$[h] | $C_6H_6$ | 72 | 84[i] | 1660 | 1600 | $C_2H_6N_2O_2$[j] |
| 66 | Me | Me | A | $CHCl_3$ | $C_6H_6$ | 63 | 84–86 | 1665 | 1545 | $C_3H_8N_2O_2$ |
| 64 | Me | $C_6H_5$ | A | $CHCl_3$ | $C_6H_6$ | 60 | 113–114[k] | 1655 | 1535 | $C_8H_{10}N_2O_2$[j] |
| 68 | $HO_2CCH_2$ | $C_6H_5$ | C[f] | $H_2O$ | | 89 | 183–184 | 1695,[l] 1625 | 1565 | $C_9H_{10}N_2O_4$ |
| 70 | Me |  | D[f] | $H_2O$ | | 67 | >260 | *m* | 1545 | $C_6H_8N_4O_4$ |
| 73 | $C_6H_5CH_2$ | $C_6H_5$ | A | $C_6H_5$ | | 95 | 106[n] | 1660 | 1535 | $C_{13}H_{14}N_2O_2$[j] |

[a] A, $RONH_2$ (by extn, after basification of $RONH_2 \cdot HCl$ with aq NaOH, with reaction solvent )+ R′NCO; solvent evapd *in vacuo* and residue recrystd; B, $RONH_2 \cdot HCl$ + KNCO; C, $RONH_2 \cdot HCl$ + R′NCO + aq NaOH; D, $RONH_2$ (from $RONH_2 \cdot HCl$ + aq NaOH) + R′NHCON(NO)Me. [b–e] See *b–e*, Table I. [f] See Experimental Section. [g] Nitrosation of this compd in 5 *N* HCl resulted in virtually complete decompn into volatile products. [h] Solvent evapd *in vacuo;* filtered EtOH extract of residue evapd *in vacuo* and recrystd. [i] Lit. mp 82–83° [W. Traube, H. Ohlendorff, and H. Zender, *Ber.*, **53B**, 1477 (1920)], 84.5° [L. W. Jones and R. T. Major, *J. Amer. Chem. Soc.*, **49**, 1537 (1927)]. [j] Not analyzed. [k] Lit. mp 115° (Jones and Major, footnote *h*). [l] Carboxy C=O. [m] 1710, 1670, 1650, and 1630 $cm^{-1}$ (C=O). [n] Lit. mp 106° (from $C_6H_6$); L. Voltmer, *Ber.*, **24**, 378 (1891).

45.4 mmoles) was added to a stirred soln of 2-fluoroethylamine·HCl[56] (4.57 g, 46.0 mmoles) in $H_2O$ (100 ml) and $Et_3N$ (4.0 ml). The mixt was stirred at room temp for 4 hr and then at 60° for 30 min and cooled. The ppt was dried and recrystd twice from $C_6H_6$ (100 ml), yield 3.2 g. (See Table II.)

**1-Cyclohexyl-3-(2-fluoroethyl)-2-thiourea (57).**—A cold, stirred suspension of 2-fluoroethylamine·HCl[56] (2.16 g, 21.7 mmoles) in $CHCl_3$ (50 ml) was treated with 50% NaOH (3 ml) and stirred 30 min longer. The $CHCl_3$ layer was sepd and the aq residue extd with $C_6H_6$. The combined exts were dried ($Na_2SO_4$) and treated with cyclohexyl isothiocyanate[35] (3.06 g, 21.7 mmoles) with stirring, which was continued for 3 hr. Removal of the solvent under reduced pressure left an oil, which solidified when triturated in cold $H_2O$ (20 ml). The crude product (1.75 g) was recrystd by dissolving in $CCl_4$ (9 ml) and dilg with hexane (30 ml): yield 1.65 g (37%); mp 62°. *Anal.* ($C_9H_{17}FN_2S$) C, H, N.

**1,2,3,4-Tetrahydro-2,4-dioxo-5-pyrimidinyl Isothiocyanate.**—5-Aminouracil[57] (5.92 g, 46.8 mmoles) was added to a stirred mixt of $CSCl_2$ (6.00 g, 52.0 mmoles) in $H_2O$ (200 ml), and stirring was contd until the red color of $CSCl_2$ disappeared (~3 hr). The cooled mixt was filtered, and the collected yellow solid was washed with 1.2 *N* HCl (3 × 20 ml) and then $H_2O$, air-dried, and triturated in $Et_2O$ (35 ml). The vacuum-dried crude product was repptd from a filtered DMF (60 ml) soln by addn of $H_2O$ (160 ml). After the mixt had been stirred at 0–5° for 1 hr, the light yellow ppt was collected and dried *in vacuo* ($P_2O_5$): yield 6.1 g (77%); mp >300° dec. A pilot run in which the repptn step was omitted produced the analytical sample: uv max in nm (ε × $10^{-3}$) 260 (sh), 294 (14.7) at pH 1; 260 (sh), 295 (14.1) at pH 7; 267 (12.8), 314 (12.7) at pH 13. *Anal.* ($C_5H_3N_3O_2S$) C, H, N.

**1-(2-Fluoroethyl)-3-(1,2,3,4-tetrahydro-2,4-dioxo-5-pyrimidinyl)-2-thiourea (58).**—$Et_3N$ (3.5 ml, 25 mmoles) was added to a stirred suspension of 1,2,3,4-tetrahydro-2,4-dioxo-5-pyrimidinyl isothiocyanate (3.05 g, 20.7 mmoles) and 2-fluoroethylamine·HCl[56] (2.25 g, 22.8 mmoles) in DMF (35 ml). The mixt was heated in a Parr pressure vessel at 60° for 2 hr and then left at room temp overnight. The soln was filtered, and the filtrate was dild with cold $H_2O$ (125 ml). The grayish ppt was washed with $H_2O$ and dissolved in warm DMF (175 ml). The soln was treated with Norit, filtered, dild with $H_2O$ (150 ml), and cooled. The white ppt was washed with $H_2O$ and dried *in vacuo* ($P_2O_5$): yield 3.40 g (71%); mp ~245° dec. A pilot run provided the analytical sample. *Anal.* ($C_7H_9FN_4O_2S$) C, H, N.

**1-Methyl-3-(tetrahydro-2*H*-thiopyran-4-yl)-2-thiourea (59).**—$Et_3N$ (2.8 ml, 20 mmoles) and then MeNCS (1.25 g, 17.1 mmoles) were added to a stirred suspension of tetrahydro-2*H*-thiopyran-4-amine·HCl[14] (2.62 g, 17.1 mmoles) in $CHCl_3$ (50 ml); stirring was contd overnight. Evapn under reduced pressure left a white solid, which, after being triturated in $H_2O$ and dried *in vacuo* ($P_2O_5$), was twice recrystd from EtOH (40 ml): yield 2.0 g (61%); mp 180°. *Anal.* ($C_7H_{14}N_2S_2$) C, H, N.

**1-Ethyl-3-(2-norbornyl)-2-thiourea (61).**—Cold 3.6 *N* $H_2SO_4$ (25.0 ml) was added to a cold (0°), rapidly stirred soln of 1,3-diethyl-2-thiourea (11.5 g, 87 mmoles) and $NaNO_2$ (6.20 g, 90 mmoles) in $H_2O$ (100 ml), and stirring was contd for 30 min at 0–5°. Washed with cold $H_2O$ and dried briefly *in vacuo* ($P_2O_5$), the yellow ppt (6.0 g, mp ~40°) was added to a soln of 2-norbornanamine·HCl (5.0 g, 34 mmoles) in $H_2O$ (100 ml), which had been basified with $Et_3N$ (5.0 ml, 42.5 mmoles). Reaction was evidenced by evoln of gases. The mixt was stirred at room temp for 4 hr, refluxed for 20 min, and cooled. The air-dried ppt (6.6 g, mp 146°) was recrystd from $C_6H_6$ (100 ml): yield 5.3 g (33% overall); mp 149°. *Anal.* ($C_{10}H_{18}N_2S$) C, H, N.

**1-(2-Chloroethoxy)-3-phenylurea (62).**—A soln of 2-chloroethoxyamine·HCl[58] (2.00 g, 15.2 mmoles) in $H_2O$ (7 ml) was added to a cold, stirred mixt of 1 *N* NaOH (15.2 ml) and $CHCl_3$ (80 ml). The layers were sepd, and the aq layer was extd with $CHCl_3$ (3 × 20 ml). The $CHCl_3$ layers were combined, dried ($Na_2SO_4$), and treated (cold and stirred) with $C_6H_5NCO$ (1.81 g, 15.2 mmoles). After being stirred overnight, the soln was evapd under reduced pressure and the residue (3.05 g) recrystd from EtOH; yield 2.27 g. (See Table VII.)

**Nitrosation of 62.**—$NaNO_2$ (425 mg, 6.17 mmoles) was added in portions to a cold (~5°), stirred soln of **62** (1.00 g, 4.67 mmoles) in 98–100% $HCO_2H$ (4 ml). A yellow ppt, which turned brown, was formed, and, after a few min, the mixt was dild with $H_2O$, stirred for 45 min in the cold, and filtered. A soln of the isolated solid in EtOH, decolorized with Norit, and dild with $H_2O$, deposited 50 mg (10%) of carbanilide (ir, mmp).

**Nitrosation of 66.**—A soln of $NaNO_2$ (535 mg, 5.12 mmoles) in $H_2O$ (2 ml) was added dropwise to a cold (~3°), stirred soln of **66** (526 mg, 5.05 mmoles) in 1.5 *N* HCl (4 ml). The yellow ppt was collected immediately and dissolved in cold $H_2O$ (5 ml); the soln was cooled and treated with *p*-chlorobenzylamine (730 mg, 5.1 mmoles). The stirred mixt foamed and deposited 1-(*p*-chlorobenzyl)-3-methylurea in 2 crops during 45 min. The combined crops were recrystd from $H_2O$ (50 ml) and dried *in vacuo* ($P_2O_5$): yield 248 mg (25%); mp 160–161° (lit.[23] mp 160–161°). *Anal.* ($C_9H_{11}N_2OCl$) C, H, N.

**[(3-Phenylureido)oxy]acetic Acid (68).**—$C_6H_5NCO$ (1.95 ml, 18.0 mmoles) was added to a cold (5°), stirred soln of (aminooxy)-acetic acid hemihydrochloride[55] (2.19 g, 10.0 mmoles) in $H_2O$ (10 ml), which had been neutralized with 2 *N* NaOH (15.0 ml, 30.0 mmoles). The mixt was stirred overnight at ambient temp and filtered to remove carbanilide (mp, ir). The filtrate was acidified with 3 *N* HCl (8.0 ml), and the ppt was washed with $H_2O$ and dried *in vacuo* ($P_2O_5$), yield 3.35 g. (See Table VII.)

**Nitrosation of 68.**—$NaNO_2$ (390 mg, 5.65 mmoles) was added in portions to a cold (5–10°), stirred suspension of **68** (500 mg, 2.38 mmoles) in 98–100% $HCO_2H$ (8 ml). The resulting yellow soln was stirred for ~20 min, dild with cold $H_2O$ (15 ml), and stirred at 0–5° for an addl 15 min. The yellow ppt was washed with a little cold $H_2O$ and added to a cold (~5°), stirred soln of

(57) Purchased from Krishell Laboratories, Portland, Ore.

(58) E. L. Schumann, L. A. Paquette, R. V. Heinzelman, D. P. Wallace, J. P. DaVanzo, and M. E. Greig, *J. Med. Pharm. Chem.*, **5**, 464 (1962).

aniline (0.25 ml, 2.75 mmoles) in $H_2O$ (2.5 ml); stirring was contd at room temp for ~5 hr. The ppt, washed with $H_2O$ and dried *in vacuo* ($P_2O_5$), was identified (mp, tlc, ir) as carbanilide, yield 230 mg (49%).

**Nitrosation of 73.**—When $NaNO_2$ (255 mg, 3.70 mmoles) was added in portions to a cold (~8°), stirred soln of **73** (615 mg, 2.54 mmoles) in 98–100% $HCO_2H$ (4 ml), a yellow ppt formed; the mixt was thinned with cold $H_2O$ (10 ml) and stirred an addl 20 min. The ppt was collected on a fritted-glass filter, and, while still wet, half of it was immediately stirred in cold $H_2O$ (10 ml) and treated with 40% aq $MeNH_2$ (0.5 ml). Immediate dissoln resulted followed by gradual pptn of a white solid, which, after 2–3 hr at room temp, was collected, dried *in vacuo* ($P_2O_5$), and identified as 1-methyl-3-phenylurea by ir comparison with a conventionally prepared sample: wt 135 mg (~71%); mp 140–141° dec. One recrystn from $H_2O$ gave 45 mg (~24%), mp 147–149° (lit.[23] mp 151°). The other half of the nitrosated product decompd within 2 hr when stored over $P_2O_5$ in a desiccator at atm pressure.

**1-Methoxy-3-(1,2,3,4-tetrahydro-2,4-dioxo-5-pyrimidinyl)urea (70).**—A soln of $MeONH_2$, prepd by dissolving $MeONH_2 \cdot HCl$[55] (1.00 g, 12.0 mmoles) in 1 *N* NaOH (12 ml), was added to a stirred suspension of 1-methyl-1-nitroso-3-(1,2,3,4-tetrahydro-2,4-dioxo-5-pryimidinyl)urea[23] (2.55 g, 12.0 mmoles) in $H_2O$ (100 ml). The mixt was warmed gradually, then refluxed for 1 hr, cooled to 50°, and filtered to remove insol matter. The filtrate was evapd to dryness *in vacuo*, and the residue was stirred with 1 *N* HCl (18 ml). The white product was washed with $H_2O$ and dried *in vacuo* ($P_2O_5$) at 100° for 4 hr: yield 1.60 g (67%); $\lambda_{max}$ in nm ($\epsilon \times 10^{-3}$) 267 (7.07) at pH 1, 267 (6.85) at pH 7, and 287 (6.25) at pH 13. (See Table VII.)

**(2-Fluoroethyl)urea Nitrate (75).**—Concd $HNO_3$ (4.57 ml) was added dropwise to a stirred paste consisting of (2-fluoroethyl)urea[4] (5.2 g, 49 mmoles) and $H_2O$ (3.0 ml), and the resulting soln was chilled in an ice–salt bath. The crystals that formed were collected, dried *in vacuo* ($P_2O_5$), and recrystd from $C_6H_6$ (100 ml): yield 4.85 g (59%); mp 68–70°; ir (KBr) 1375 (s) and 825 (w) $cm^{-1}$ ($NO_3^-$). *Anal.* ($C_3H_7FN_2O \cdot HNO_3$) C, H, N.

**1-(2-Fluoroethyl)-3-nitrourea (76).**—The nitrate **75** (3.50 g, 20.7 mmoles) was added in small portions to cold (−15 to −20°), stirred, concd $H_2SO_4$ (7.0 ml). After being stirred for 1 hr at −15°, the mixt was poured over ice-$H_2O$ slush (35 ml), and stirring was contd at 0° for 1 hr. The cryst ppt was collected, washed with cold $H_2O$ (3.5 ml), dried *in vacuo* ($P_2O_5$), and recrystd from $C_6H_6$ (~50 ml): yield 1.30 g (42%); mp 120°. A pilot run afforded the analytical sample: ir (KBr) 1600 and 1270 $cm^{-1}$ ($NO_2$); pmr ($CDCl_3$) $\delta$ ~8 (**NH**) and ~11.5 (**NH**) ppm. (See Table IV).

**1-(1-Adamantyl)-3-(2-fluoroethyl)urea (77) (from 76).**—The nitrourea **76** (36 mg, 0.24 mmole) was added to a soln prepd by adding $Et_3N$ (3 drops) and then $Me_2CO$ (3 ml) to a soln of 1-adamantanamine·HCl[35] (45 mg, 0.24 mmole) in $H_2O$ (3 ml). The mixt was heated at 70° for 1 hr, and the $Me_2CO$ was evapd under reduced pressure. The pptd **77** was washed with $H_2O$ and dried *in vacuo* ($P_2O_5$): yield 10 mg (17.5%); mp 212° (lit.[4] mp 212°); ir (KBr) 1610 (C=O), 1550 (CNH) $cm^{-1}$. The concd filtrate gave a negligible second crop.

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# Selectivity of Action of Alkylating Agents and Drug Resistance. 4. Synthesis of Tritium-Labeled Chlorambucil and a Study of Its Cellular Uptake by Drug-Sensitive and Drug-Resistant Strains of the Yoshida Ascites Sarcoma *in Vitro*[1]

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The synthesis of $^3H$-labeled chlorambucil is described and its uptake and utilization by drug-sensitive and drug-resistant strains of a Yoshida ascites sarcoma have been studied *in vitro*. Drug uptake is markedly influenced by the cell concentration and drug concentrations used. By selecting conditions similar to those achieved following *in vivo* drug treatment, the resistant cells have been shown, *in vitro*, to take up 50% less drug than the sensitive cells. This twofold difference in gross uptake of drug was also reflected in the absolute amounts of drug bound to protein. Chlorambucil appears to associate with an alcohol-soluble fraction of the Yoshida ascites cell, before extensive protein binding occurs. The fraction involved may be lipoprotein. It is unlikely that this represents a general reaction mechanism for all alkylating agents, since busulphan has been shown to combine directly with the intracellular protein of the cells.

A large number of neoplasms, both in man and experimental animals, appear to acquire resistance to treatment with alkylating agents following repeated exposure to these drugs: various mechanisms have been proposed to account for this. Several authors have detected an impaired transport of the drug by resistant cells,[2-4] though Wheeler and Alexander found that both drug-sensitive and drug-resistant plasmacytomas were equally effective in taking up cyclophosphamide,[5] while Novikova has demonstrated an enhanced uptake of phenylalanine mustard into several drug-resistant

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