1693, 1671 cm^{-1}; NMR ($CDCl_3$) δ 5.95 (d, 1, J = 7.2), 6.25 (d, 1, J = 7.2), 6.85 (s, 1), 7.05–8.13 (m, 8), 10.05 (s, 1); mass spectrum, m/e 288, 133 (base), 129.

Anal. Calcd for $C_{18}H_{12}N_2O_2$: C, 74.98; H, 4.20; N, 9.72. Found: C, 74.88; H, 4.21; N, 9.95.

Phthalideisoquinoline (10) from 7. To a refluxing solution of 0.50 g (1.7 mmol) of **7** in 50 mL of methanol was added 4 mL (0.01 mol) of 10% aqueous NaOH. The solution was refluxed for 45 min, solvent removed in vacuo, and the residue dissolved in 50 mL of water. This solution was poured into a solution of 10 g of ammonium chloride in 50 mL of water. The resulting solution was made strongly acidic with 10% HCl, boiled, cooled, and filtered. The filtrate was made weakly alkaline with 10% NaOH solution, cooled, and scratched to afford 260 mg (58%) of **10** as a white solid. An analytical sample was prepared by several recrystallizations from 95% ethanol: mp 168–169 °C dec (lit.[12] mp 150–152 °C); IR (KBr) 1770 cm^{-1} (lit.[12] IR 1770 cm^{-1}); NMR ($CDCl_3$) δ 7.22 (s, 1), 7.40–8.60 (m, 10); mass spectrum, m/e 261, 232 (base), 133, 128.

Anal. Calcd for $C_{17}H_{11}NO_2$: C, 78.14; H, 4.23; N, 5.36. Found: C, 78.07; H, 4.46; N, 5.24.

1-(*o*-Carbomethoxybenzyl)isoquinoline 2-Oxide. The preparation of 1-(*o*-carbomethoxybenzyl)isoquinoline 2-oxide was carried out by the method of Natsume and Tanabe.[12]

Phthalideisoquinoline (10). An independent synthesis of phthalideisoquinoline (**10**) was effected by the method of Natsume and Tanabe.[12] A solution of 90 mg of 1-(*o*-carbomethoxybenzyl)isoquinoline 2-oxide dissolved in 3 mL of acetic anhydride was refluxed for 3 h and evaporated to dryness under reduced pressure, and the resulting black oil dissolved in 10 mL of methanolic HCl prepared from 0.5 mL of concentrated HCl and 9.5 mL of methanol. The solution was refluxed for 1 h and evaporated in vacuo, and 30 mL of water added. The mixture was filtered and 5 g of ammonium chloride added to the filtrate. The filtrate was made weakly alkaline with 10% sodium hydroxide solution, and 0.1 g of phthalideisoquinoline (**10**) precipitated as an amorphous tan solid; mp 168–169 °C; mp 168–169 °C in admixture with the sample prepared from **7**. IR and NMR spectra of the two samples were identical.

Registry No. 1, 74133-22-5; **3**, 60159-78-6; **4**, 77287-52-6; **5**, 33863-62-6; **7**, 77287-53-7; **8**, 77287-54-8; **10**, 24223-06-1; isoquinoline, 119-65-3; α-chloro-*o*-toluyl chloride, 42908-86-1; 6,7-dimethoxyisoquinoline, 15248-39-2; α-bromo-*o*-toluic acid, 7115-89-1; α-bromo-*o*-toluyl chloride, 7115-90-4; 1-(*o*-carbomethoxybenzyl)isoquinoline 2-oxide, 24223-05-0.

Synthesis and Reactions of Deuterated 2-(Alkylimino)-3-nitrosooxazolidines, 3-Alkyl-1-(2-hydroxyethyl)-1-nitrosoureas, and Related Compounds as Possible Intermediates in the Aqueous Decomposition of 3-Alkyl-1-(2-chloroethyl)-1-nitrosoureas[1a]

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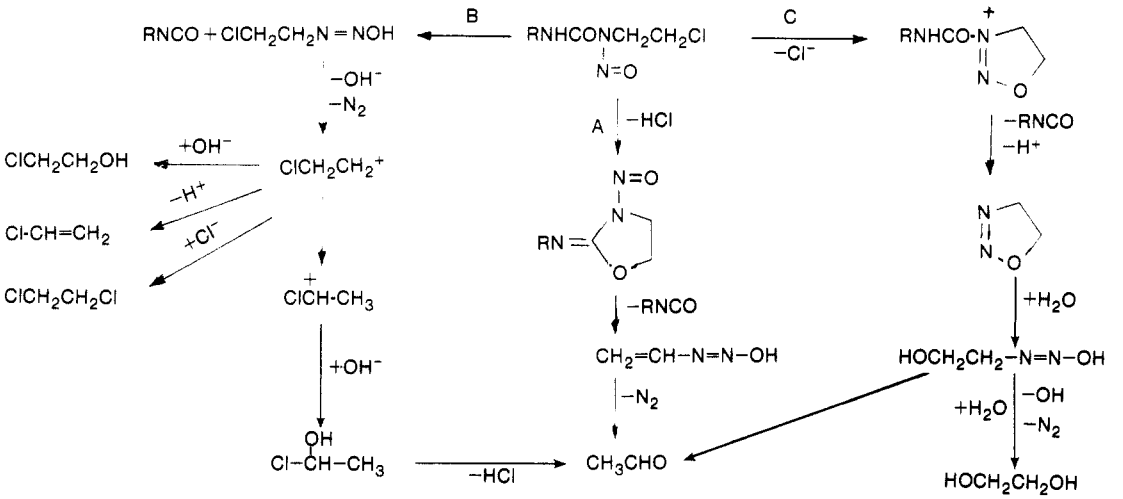
Decomposition of CCNU-α-d_2 (**7**) in pH 7.2 phosphate buffer or of CINO-α-d_2 (**9**) or CHNU-α-d_2 (**8**) with the addition of chloride ion gives rise to the same spectrum of products, including deuterium-free acetaldehyde (**29**), a mixture of the two deuterio-2-chloroethanols, 2-hydroxy-2,2-dideuterioethyl cyclohexyl carbamates, and vinyl chloride containing one deuterium (i.e., opposite the results obtained in the corresponding reaction of BCNU-α-d_4). The products were identified and the number and position of the deuterium labels determined by GC/MS. The results are interpreted in terms of two decomposition pathways for CCNU. The first decomposition pathway operating for CCNU is via an intermediate 2-chloroethanediazohydroxide or the equivalent 2-chloroethyl cation in agreement with the results of other workers. The second pathway may involve reversible conversion of CCNU-α-d_2 (**7**) to CINO-α-d_2 (**9**) and then ring opening of the latter to CHNU-α-d_2 (**8**). Independent decomposition of **8** provides evidence for its conversion to a 1,1-dideuterio-2-hydroxyethanediazohydroxide (**41**) leading to the isolated carbamates **36** and **44**. The intermediacy of species **41** may account for the formation of 2-hydroxyethylated nucleosides observed when (2-chloroethyl)nitrosoureas react with DNA. An alternative ring-opening reaction of **9** leads to a 2-hydroxydiazoethyl cyclohexylcarbamate species (**37**), elimination of which and attack by halide ion may account for the vinyl halide species formed. Further evidence in support of these competing pathways employing additional specifically deuterated intermediates is described and discussed.

(2-Haloethyl)nitrosoureas including 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), and others are of clinical value in the treatment of a range of neoplasms.[1-5] These compounds decompose readily under physiological conditions and have been found to alkylate and cross-link DNA both in vivo and in vitro.[6-9] Studies on the nature

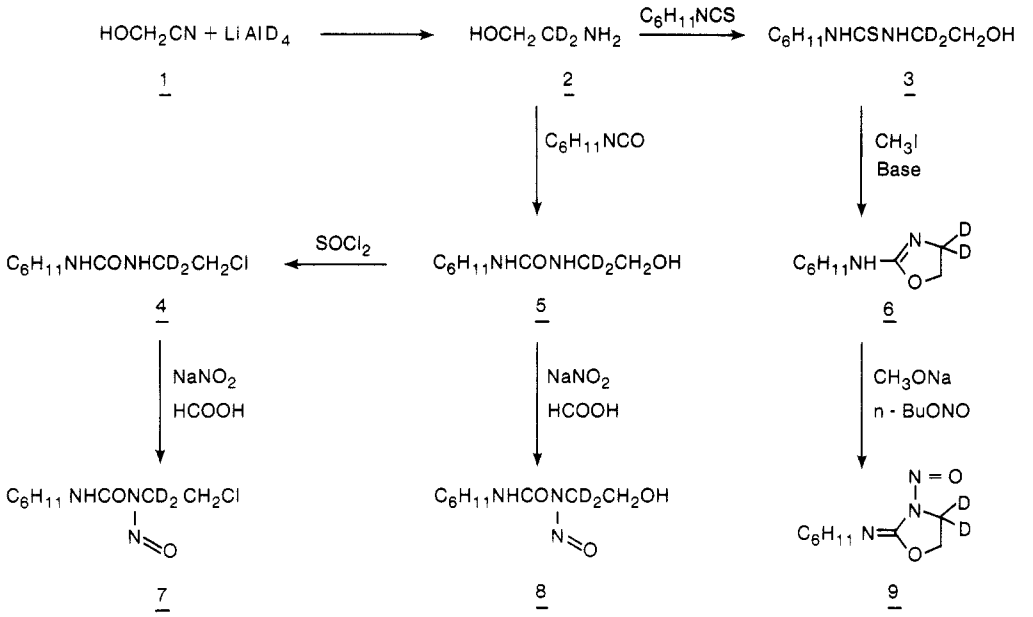
(1) (a) Abbreviations are as follows: CCNU-α-d_2, 3-cyclohexyl-1-nitroso-1-(1,1-dideuterio-2-chloroethyl)urea; CCNU-β-d_2, 3-cyclohexyl-1-nitroso-1-(2,2-dideuterio-2-chloroethyl)urea; CHNU-α-d_2, 3-cyclohexyl-1-nitroso-1-(1,1-dideuterio-2-hydroxyethyl)urea; CHNU-β-d_2, 3-cyclohexyl-1-nitroso-1-(2,2-dideuterio-2-hydroxyethyl)urea; CINO-α-d_2, 2-(cyclohexylimino)-3-nitroso-4,4-dideuteriooxazolidine; CINO-β-d_2, 2-(cyclohexylimino)-3-nitroso-5,5-dideuteriooxazolidine; BCNU-α-d_4, bis(1,1-dideuterio-2-chloroethyl)-*N*-nitrosourea. (b) S. K. Carter, F. A. Schabel, L. E. Broder, and T. P. Johnson, *Adv. Cancer Res.*, **16**, 273 (1972).
(2) G. D. Wheeler ACS *Symp. Ser.*, **No. 30**, 87–119 (1976).
(3) V. A. Levin and C. B. Wilson, *Cancer Treat. Rep.*, **60**, 719 (1976).
(4) J. A. Montgomery, *Cancer Treat. Rep.*, **60**, 651 (1976).
(5) V. DeVita, P. Carbone, A. Owens, G. L. Gold, M. J. Krant, and J. Edmonson, *Cancer Res.*, **25**, 1876 (1965).
(6) K. W. Kohn, *Cancer Res.*, **37**, 1450 (1977).
(7) D. B. Ludlum, B. S. Kramer, J. Wang, and C. Fenselau, *Biochemistry*, **14**, 5480 (1975).
(8) M. Colvin, R. B. Brundrett, W. Cowens, I. Jardine, and D. B. Ludlum, *Biochem. Pharmacol.*, **25**, 695 (1976).
(9) J. W. Lown, L. W. McLaughlin, and Y. M. Chang, *Bioorg. Chem.*, **7**, 97 (1978).

0022-3263/81/1946-2479$01.25/0

Scheme I



Scheme II



and origin of the products have led to the proposal of three distinct pathways of decomposition, the essential features of which are given in Scheme I. They include the following: pathway B, via generation of a chloroethyl cation (or its kinetic equivalent);[7,8] pathway A, via an intermediate 2-(alkylimino)-*N*-nitrosooxazolidine which decomposes further;[10] pathway C, via a postulated but as yet unisolated *N*-acyloxadiazolinium species.[11]

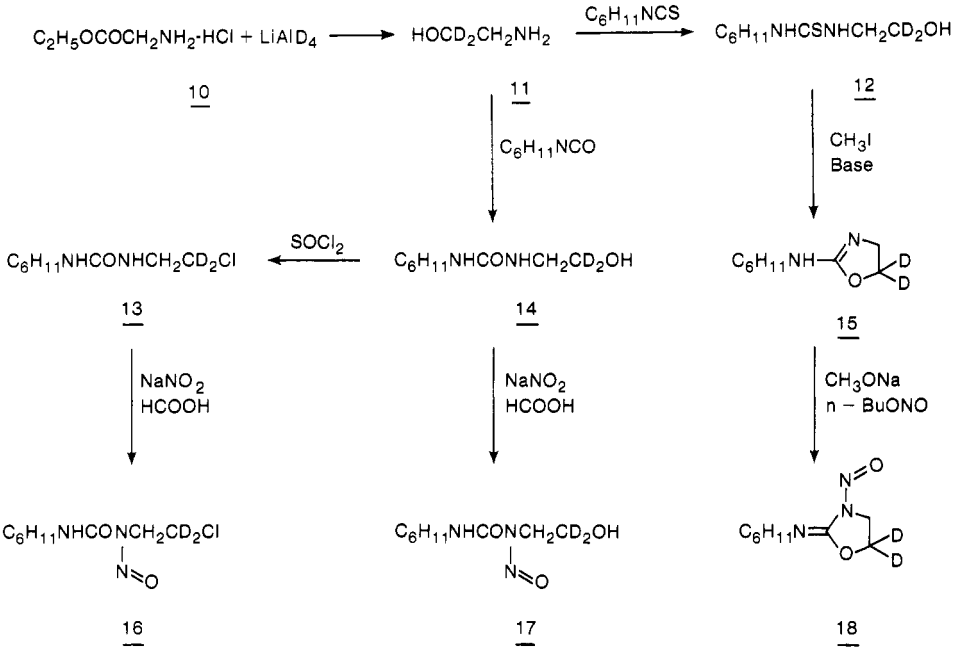
There is compelling evidence that pathway B constitutes one source of chloroethylating species from nitrosoureas.[7,8,17,18,24] However, since the three pathways are competitive, there is still question about the pathway(s) selected for a given agent. For example, those nitrosoureas which are more susceptible to cyclization may be expected to show a relatively greater contribution from pathways A and C.

In the previous paper[12] we reported the characterization of 2-(alkylimino)-3-nitrosooxazolidines and showed that they give rise to acetaldehyde, for which they were proposed as intermediates in pathway A.[10] The 2-(cyclohexylimino)-3-nitrosooxazolidine (CINO) intermediate in the presence of anhydrous HCl gives rise to CCNU and in aqueous buffer at physiological temperature and pH in the presence of chloride ion gives rise to vinyl chloride, 1,2-dichloroethane, and 2-chloroethanol as well as involatile products which are observed from CCNU directly.

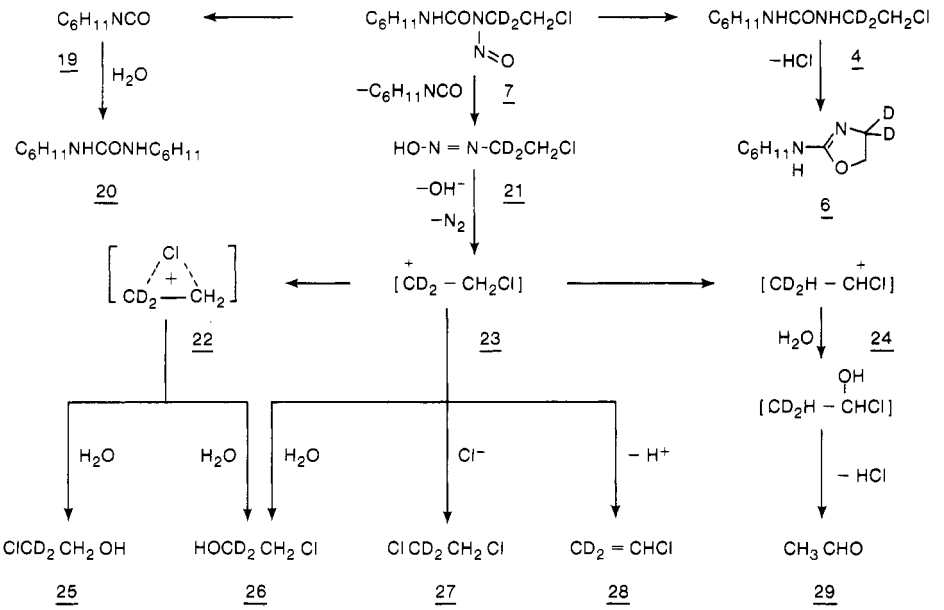
The application of nitrosoureas specifically labeled with ^{13}N,[13] ^{14}C,[14,15] or ^{2}H[16-18] has been useful in the study of nitrosourea metabolism, decomposition, and mode of action. Accordingly, we report the synthesis and the study of the aqueous decomposition of CCNU-α-d_2 (**7**), CCNU-β-d_2 (**16**), CINO-α-d_2 (**9**), CINO-β-d_2 (**18**), CHNU-α-d_2 (**8**), and CHNU-β-d_2 (**17**) in order to determine the mechanistic origin of the various decomposition products and to assess the possible contribution of pathway A to the overall decomposition process for CCNU.

(10) J. A. Montgomery, R. James, G. S. McCaleb, and T. P. Johnston, *J. Med. Chem.*, **10**, 668 (1967).
(11) D. C. Chatterjii, R. F. Green, and S. F. Gallelli, *J. Pharm. Sci.*, **67**, 1527 (1978).
(12) J. W. Lown and S. M. S. Chauhan, *J. Med. Chem.*, **24**, 270 (1981).
(13) W. A. Pettit, R. H. Mortara, G. A. Digenis, and M. F. Reed, *J. Med. Chem.*, **18**, 1029 (1975).
(14) V. T. Oliverio, W. M. Vietzki, M. K. Williams, and R. H. Adamson, *Cancer Res.*, **30**, 1330 (1970).
(15) V. T. Oliverio, *Cancer Treat. Rep.*, **60**, 703 (1976).
(16) P. B. Farmer, A. B. Foster, M. Jarman, M. R. Oddy, and D. J. Reed, *J. Med. Chem.*, **21**, 514 (1978).
(17) M. Colvin, R. B. Brundrett, M. N. Kan, I. Jardine, and C. Fenselau, *Cancer Res.*, **36**, 1121 (1976).
(18) R. B. Brundrett, J. W. Cowens, M. Colvin, and I. Jardine, *J. Med. Chem.*, **19**, 958 (1976).

Scheme III



Scheme IV



Syntheses of specifically deuterated compounds were carried out as follows. CCNU-α-d_2 (**7**), CHNU-α-d_2 (**8**), and CINO-α-d_2 (**9**) were synthesized from 2-amino-2,2-dideuterioethanol (**2**) as shown in Scheme II. Similarly, CCNU-β-d_2 (**16**), CHNU-β-d_2 (**17**), and CINO-β-d_2 (**18**) were synthesized from 2-amino-1,1-dideuterioethanol (**11**) as shown in Scheme III. Examination of the NMR and mass spectra of the intermediates and products confirmed that no scrambling of deuterium had occurred and secured the position of the labels. The 2-hydroxyethylureas **5** and **14**, as well as 2-hydroxycarbamates **36** and **44**, have $M^+ - 30$ (CH_2O) and $M^+ - 32$ (CD_2O) peaks in their mass spectra which are due to β cleavage and are useful for the determination of the position of deuterium in these compounds.[17,18] The deuterated nitrosooxazolidines **9** and **18** display the fragments $M^+ - 30$ (NO) and $M^+ - 82$ (C_6H_{10}). The $M^+ - 30$ fragments suggest that these nitrosooxazolidines (**9** and **18**) have stabilities and reactivities similar to those of nitrosoamines (where the N–NO bond cleaves during mass spectral fragmentation) whereas nitrosoureas fragment during mass spectroscopy by cleavage of N–CO bond.[19,20]

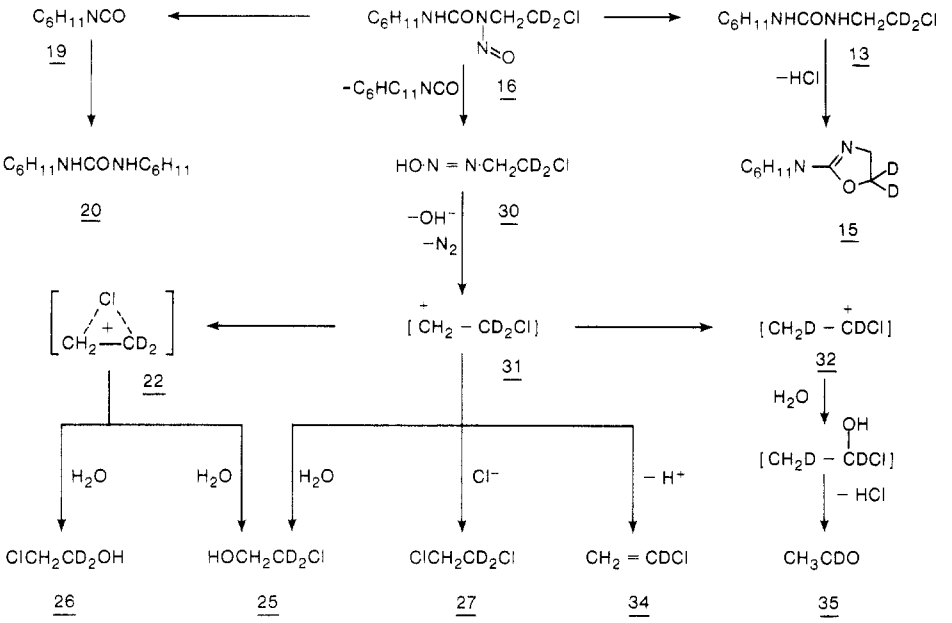
The diazo hydroxide $C_6H_{11}NHCOOCH_2CH_2N{=}NOH$ was prepared in solution by nitrosation of the corresponding amine which was prepared from 2-phthalimido-2-hydroxyethyl cyclohexylcarbamate[21] which was in turn prepared from the 2-phthalimidoethanol[22] and cyclohexyl isocyanate.

Results

The deuterated CCNU's were allowed to decompose at 37 °C in phosphate-buffered (0.1 M, pH 7.2) water in gas-tight reaction vials, and the volatile products were

(19) T. J. Hansen, M. C. Archer, and S. R. Tannenbaum, *Anal. Chem.*, **51**, 1526 (1979).
(20) W. T. Rainey, W. H. Christie, and W. Lijinsky, *Biomed. Mass Spectrom.*, **5**, 335 (1978).
(21) D. R. Sargent, G. G. Clifton, S. R. Brayant, and C. G. Skinner, *Tex. Rep. Biol. Med.*, **33**, 433 (1975).
(22) W. G. Rose, *J. Am. Chem. Soc.*, **69**, 1384 (1947).

Scheme V



analyzed by gas chromatography (GC) and combined gas chromatography–mass spectrometry (GC/MS) as previously described.[12] The nonvolatile products were analyzed and identified by direct mixture analysis chemical-ionization (CI) mass spectrometry on lyophilized reaction mixtures evaporated directly into the ion source.[23,24] Aqueous decomposition of CCNU-α-d_2 (**7**; see Scheme IV) afforded vinyl chloride (~1%) containing one deuterium (**34**), acetaldehyde (~10%) containing no deuterium, 1,2-dichloroethane containing two deuteriums (**27**), and a mixture of 2-chloroethanols **25** and **26** bearing two deuteriums predominantly on the carbon bearing hydroxyl. Additionally, cyclohexylamine, 3-cyclohexyl-1-(1,1-dideuterio-2-chloroethyl)urea (**4**), 2-(cyclohexylamino)-4,4-dideuterio-2-oxazoline (**6**), and 2,2-dideuterio-2-hydroxyethyl cyclohexylcarbamate (**36**) together with some unreacted CCNU-α-d_2 were detected.

Similar aqueous decomposition of the corresponding CINO-α-d_2 (**9**) afforded cyclohexylamine, acetaldehyde, a mixture of the carbamates **36** and **44** (in which **36** predominates), and the oxazoline **6**. Decomposition of **9** in the presence of chloride ion gave, in addition to the products described above, vinyl chloride bearing one deuterium (**34**).

From decomposition of the corresponding CHNU-α-d_2 (**8**) in buffered aqueous media (pH 7, 37 °C) was obtained acetaldehyde, the urea **5**, cyclohexyl isocyanate, and a mixture of the carbamates **36** and **44** in which **36** predominates. Similar decomposition of CHNU-α-d_2 (**8**) in the presence of chloride ion gave, in addition to the products mentioned above, vinyl chloride bearing one deuterium and a mixture of 2-chloroethanols **25** and **26** in a ratio of ca 10:1.

Aqueous decompositon of CCNU-β-d_2 (**16**; see Scheme V) yielded vinyl chloride containing two deuteriums (**28**), acetaldehyde with a deuterium in the formyl group (**35**), 1,1-dideuterio-1,2-dichloroethane (**27**), and a mixture of deuterated 2-chloroethanols **25** and **26** in a ratio of ca. 1:10 in addition to cyclohexylamine, cyclohexyl isocyanate, deuterated (2-chloroethyl)cyclohexylurea **13**, (cyclohexylamino)oxazoline **15**, and the cyclohexylcarbamate **44**, together with some unreacted CCNU-β-d_2 (**16**).

Similar aqueous decomposition of the corresponding CINO-β-d_2 (**18**) afforded acetaldehyde containing a deuterium in the formyl group (**35**), cyclohexylamine, carbamate **44**, and oxazoline **15**. When CINO-β-d_2 (**18**) was allowed to decompose in the presence of chloride ion, dideuterated vinyl chloride **28** was obtained in addition to the products noted above.

Decomposition of CHNU-β-d_2 (**17**) in aqueous buffered solution gave deuterium-labeled acetaldehyde **35**, together with the hydroxyethyl carbamates **35** and **44** (of which **44** predominates), and (in the presence of chloride ion) some doubly labeled vinyl chloride **28**.

The ethylene glycol anticipated as a product of pathway A or C is especially difficult to detect by GC/MS from aqueous solutions.[23-25] Ethylene glycol was, however, identified by GC (employing a Porapak Q column) among the products obtained from both CINO and CHNU and was implicated in the products from CCNU. The products identified together with their calculated yields are given in Table I.

Table I. Product Yields from the Reactions of CCNU, CINO, and CHNU in Phosphate Buffer (pH 7.2-7.4) at 37 °C

component	yield, % CCNU[a]	CINO[b]	CHNU[c]
acetaldehyde	5-10	8-10	25-35
2-chloroethanol	18-25	2-5[e]	8-10[e]
cyclohexylamine	32	*d*	*d*
2-(cyclohexylamino)-2-oxazoline	3-5	10-15	*d*
dicyclohexylurea	1	3-4	8-10
ethylene glycol	*e*	2-5[f]	20-25[f]

[a] Product yields were taken from the results of Weinkam and Lin[24] for reactions for 2 h. [b] Product yields were taken from the results of Lown and Chauhan[12] for reactions for 12 h. [c] Yields are in the presence of added sodium chloride (5 M).[12] [d] Yields were not calculated. [e] Product was not reported in ref 24, and in the present work the yield could not be calculated due to overlap of the peaks of 2-chloroethanol and ethylene glycol on Porapak 2 at 200 °C (isothermal). [f] Yields were calculated in present study after 24 h.

(23) R. J. Weinkam and H. S. Lin, *Anal. Chem.*, **51**, 972 (1979).
(24) R. J. Weinkam and H. S. Lin, *J. Med. Chem.*, **22**, 1193 (1979).
(25) E. Schupp and W. J. Baumann, *J. Lipid Res.*, **14**, 121 (1973).