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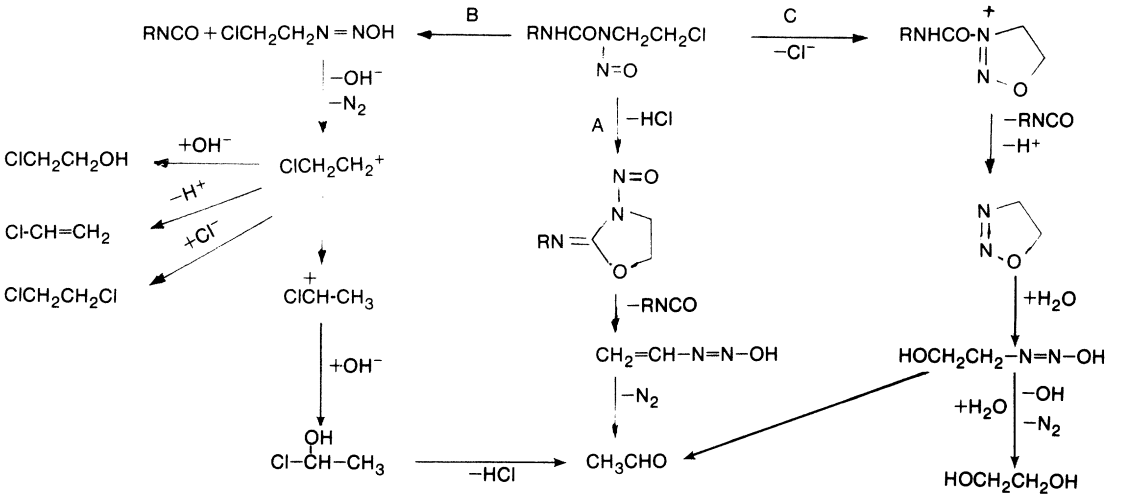
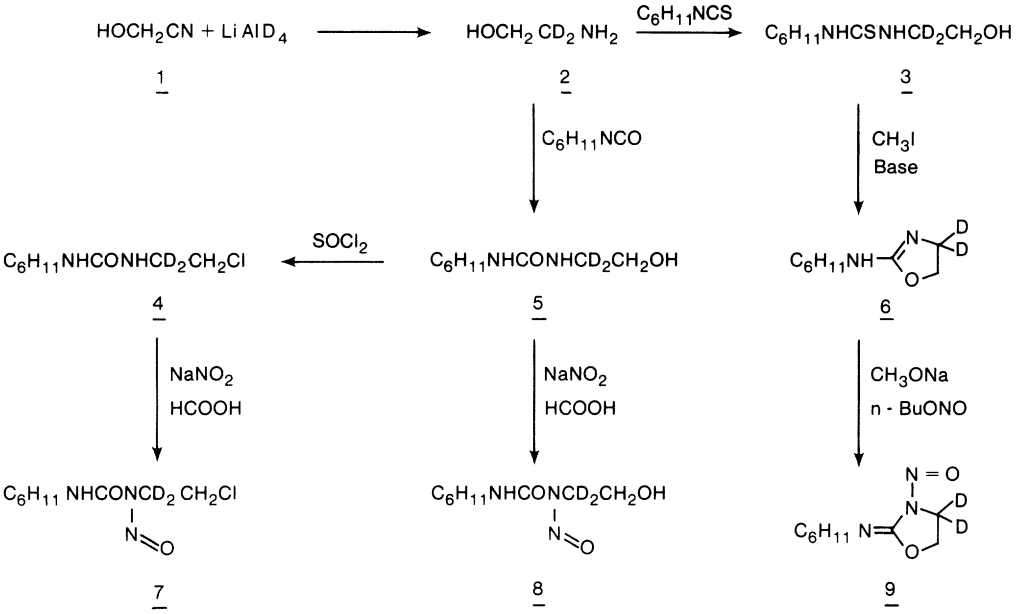
0022-3263/81/1946-2479$01.25/0

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.

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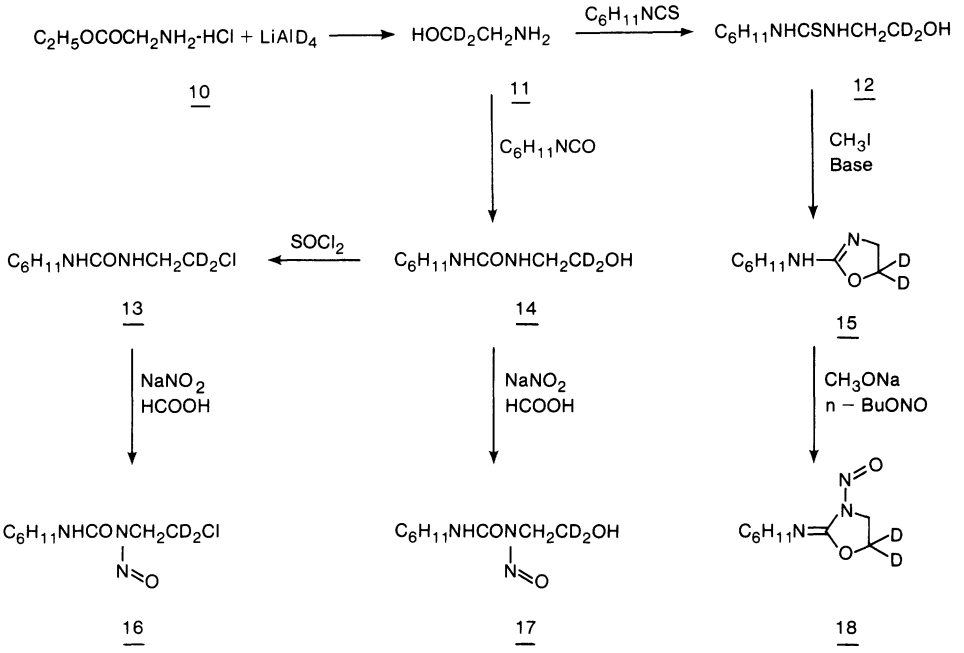
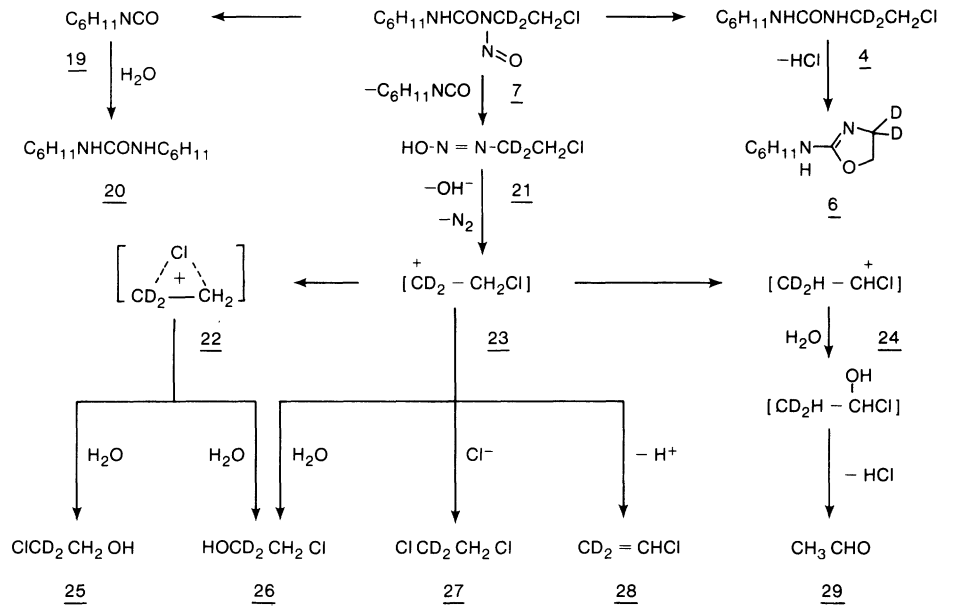
Scheme III



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

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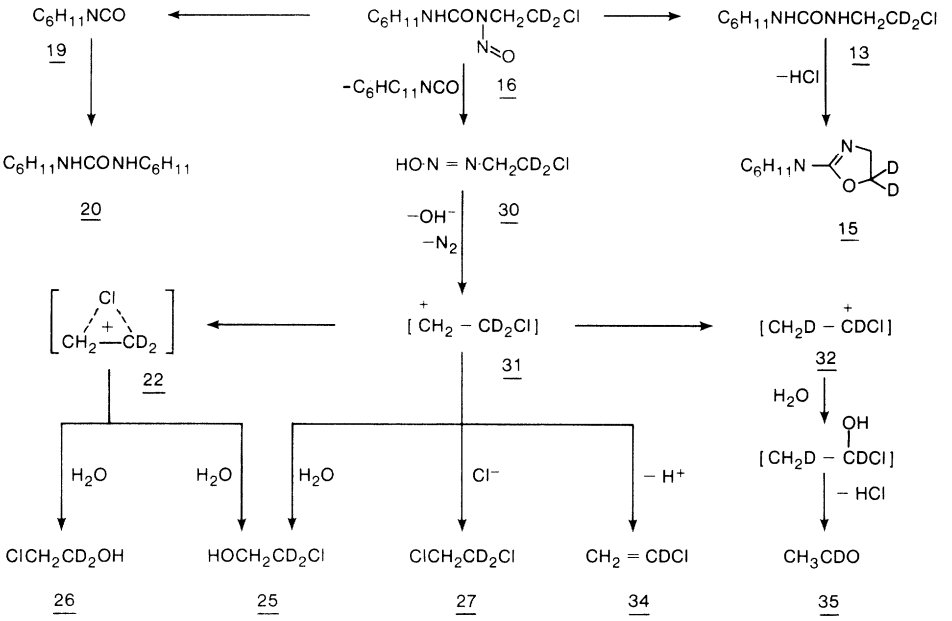
Scheme V



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[c]	8-10[c]
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.

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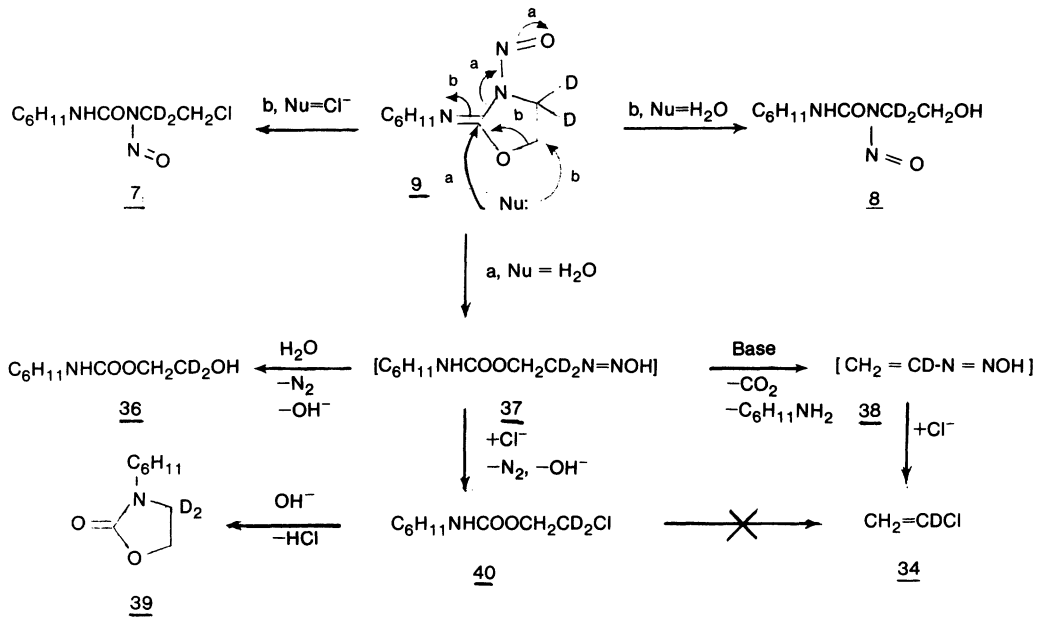
Scheme VI



Discussion

The first step in the aqueous decomposition of CCNU and related (2-haloethyl)nitrosoureas is the abstraction of the NH proton and subsequent cleavage to form the reactive intermediate 2-chloroethanediazohydroxide and cyclohexyl isocyanate (pathway B, Scheme I).[2,7,8] The 2-chloroethanediazohydroxide (or the kinetically equivalent 2-chloroethyl cation) is held to account for the subsequent formation of the observed products 2-chloroethanol, 1,2-dichloroethane, vinyl chloride, and acetaldehyde.[2,7,8] The cyclohexyl isocyanate gives rise to cyclohexylamine and dicyclohexylurea.[2,10] The formation of 2-(cyclohexylamino)oxazoline from CCNU can best be explained by cyclization to CINO followed by denitrosation of the latter.

The position and number of deuterium labels in the products 2-chloroethanol, 1,2-dichloroethane, and acetaldehyde are consistent with pathway B for the aqueous decomposition of CCNU-α-d_2 and CCNU-β-d_2.

Formation of Vinyl Chloride. However, in contrast to similar studies on BCNU-α-d_4,[18] this pathway B does *not* account for the labeling in the minor product vinyl chloride (Scheme IV). An alternative mechanism is required. One possible pathway consistent with the facts involves the formation of 2-(hydroxydiazo)-2,2-dideuterioethyl cyclohexylcarbamate (**37**) followed by elimination to give the singly deuterium labeled diazohydroxide **38**. The formation of the intermediate diazohydroxide **37** from **9** can be explained through nucleophilic attack of water at position 2 of CINO (**9**) and ring opening by path a (Scheme VI). This has been further substantiated by isolation of carbamate **36** from **9** and by the preparation of an authentic sample of **36** by the aqueous diazotization of 2-amino-2,2-dideuterioethyl cyclohexylcarbamate which plausibly proceeds through an intermediate similar to **37**.

There are precedents for the suggested ring opening of **9** by attack of nucleophiles at position 2 in the reported reaction of aromatic amine bases with 3-nitroso-2-oxazolidones to give 2-(arylamino)ethyl arylcarbamates[26] and in the decomposition of 2-methyl-2-oxazoline in aqueous solution to give *N*-(2-acetoxyethyl)-*N'*-(2-hydroxyethyl)-acetamidate,[27] a reaction which involves initial hydroxide attack at position 2 of the oxazoline and subsequent ring opening.

The longer lifetime of primary diazohydroxide compared with secondary[28] diazohydroxide as well as the equilibration of primary diazohydroxide with the corresponding diazoalkanes increases the probability that elimination from **37** may give rise to **38**. This requires the loss of a carbamate group and, while the carbamate group is not normally regarded as having good leaving properties, several instances are known in which elimination of carbamates leads to olefinic compounds.[28-31] A carbamate group moiety acts as a leaving group, for example, during the alkylation of DNA which appears to underlie the antibiotic action of mitomycin C.[32] Nucleophilic attack by chloride ion (**38**) would give rise to the observed vinyl chloride bearing one deuterium (**34**, Scheme VI). Additional precedents or analogous decompositions of *N*-nitrosooxazolidinones to ethenediazohydroxides and the trapping of vinyl derivatives have been reported.[33-35]

A similar explanation can account for formation of vinyl chloride bearing two deuteriums (**38**) observed to be formed from either CCNU-β-d_2 (**16**), CINO-β-d_2 (**18**), or CHNU-β-d_2 (**17**) in the presence of sodium chloride (Scheme VII).

Formation of Acetaldehyde. CCNU-α-d_2 (**7**) gives rise to acetaldehyde (**29**) via the chloroethyl cation **23** through hydride transfer to the intermediate cation **24** and thence by hydrolysis and elimination of hydrogen chloride[18] (Scheme IV). It should be emphasized that the extent of generation of the 2-chloroethyl cation compared to 2-chloroethanediazohydroxide from the nitrosourea has not been settled.[18] It seems likely that only a relatively small

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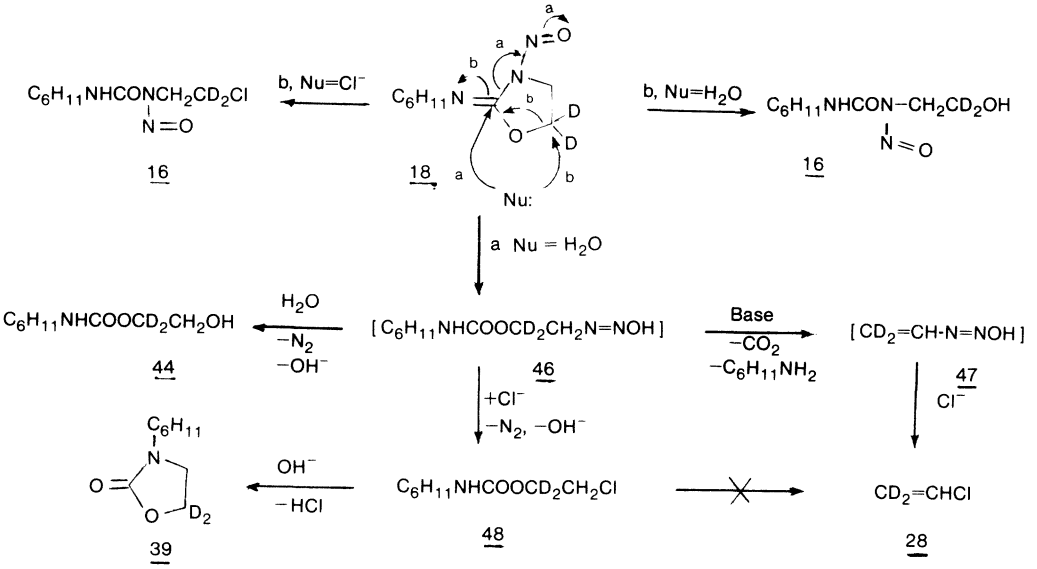
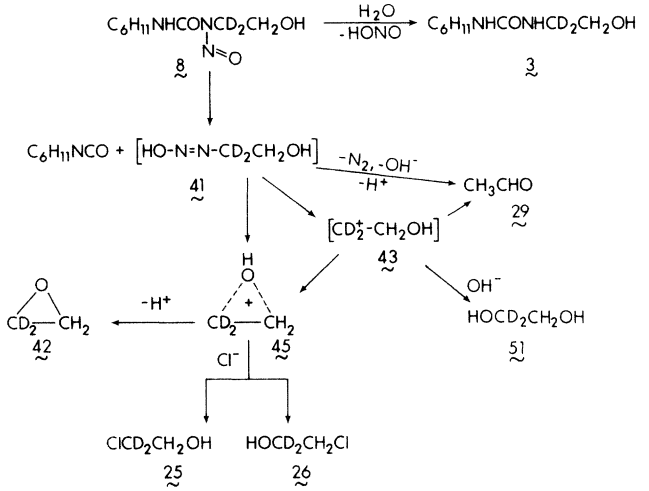
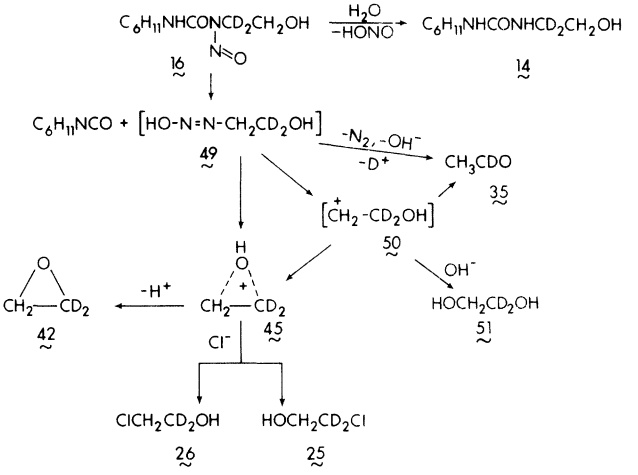
Scheme VII



Scheme VIII



Scheme IX



amount of 2-chloroethyl cation **23** is formed, a proportion of which is trapped in forming the chloronium ion **22** and the major product 2-chloroethanol, as well as 1,2-dichloroethane.[7,8] It follows that the origon of the bulk of acetaldehyde formed from nitrosourea is in question. In our preceding paper we demonstrated that 2-(alkylimino)-3-nitrosooxazolidines give rise to acetaldehyde,[12] and the corresponding CHNU gives rise to relatively more CH_3CHO. Thus one possible additional source of acetaldehyde is that CCNU-α-d_2 (**7**) may cyclize to CINO-α-d_2 (**9**) which opens to CHNU-α-d_2 (**8**) which finally leads to acetaldehyde (Scheme VIII). The suggested Scheme VIII for the formation of CH_3CHO from CHNU-α-d_2 via the 2-hydroxyethanediazohydroxide or equivalent 2-hydroxyethyl cation receives support from the identification of oxirane-2,2-d_2 (**42**) among the decomposition products which plausibly arises from the stabilized[37,38] oxiranium ion intermediate. The fact that the oxirane **42** was not detected among the products of decomposition of either CCNU-α-d_2 or CINO-α-d_2 is probably a question of the level of detection. In the latter case, **42**, representing a minor product of a minor product, presumably escapes detection. The reason oxirane is not detected in the conventional acid diazotization of, e.g., ethanolamine is probably because the strongly acidic conditions employed favor its conversion to ethylene glycol.

It could be argued that α attack by water on the ethenediazohydroxide **38** would predict the formation of acetaldehyde bearing deuterium in the formyl group. Although a critical study on the properties of ethenediazohydroxides is needed, there are literature precedents[39-42] that would lead one to expect α attack by halide ion but β displacement (leading to CH_3CHO) in the case of water. Another possible source of acetaldehyde production through pathway C (Scheme I) is via the recently postulated[11] but as yet unidentified 3-acyl-1,2,3-oxadiazolinium intermediate.

Explanations similar to those offered above may be given to account for the formation of CH_3CDO (**35**) from CCNU-β-d_2 (**16**), CINO-β-d_2 (**17**), and CHNU-α-d_2 (**18**) (Scheme IX).

Formation of 2-Chloroethanol. The formation of the major isomeric 2-chloroethanol **26** from CCNU-α-d_2 (**7**) can

(37) H. Lischka and J. H. Kohler, *Chem. Phys. Lett.*, **63**, 326 (1979).
(38) R. H. Staley, R. R. Corderman, M. S. Foster, and J. L. Beauchamp, *J. Am. Chem. Soc.*, **96**, 1260 (1974).
(39) W. Kirmse, *Top. Curr. Chem.* **80**, 125 (1979).
(40) W. Kirmse and T. Engbert, *Angew. Chem., Int. Ed., Engl.*, **18**, 228 (1979).
(41) K. Bott, *Angew. Chem., Int. Ed. Engl.*, **18**, 259 (1979).
(42) J. E. Backvall, B. Akermark and S. O. Ljunggren, *J. Chem. Soc., Chem. Commun.*, 204 (1977).

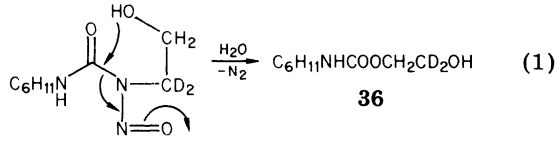
be explained via S_N2 attack of water on the 2-chloroethanediazohydroxide (21) or via the chloroethyl cation 23, whereas the minor isomeric 2-chloroethanol 25 may arise from the chloronium ion intermediate[18] 22 (Scheme IV). In the case of CINO-α-d_2 (9) the major 2-chloroethanol 26 and 1,2-dichloroethane-d_2 formed in the presence of sodium chloride can be explained by the attack of chloride ion at position 5 via path b to give CCNU-α-d_2 (7, Scheme VI) followed by decomposition according to Scheme IV.

A similar interpretation accounts for the production of the major isomeric deuterated 2-chloroethanol (25) and the minor isomer 26 from CCNU-β-d_2 (16) in phosphate buffer and from CINO-β-d_2 (18) in the presence of sodium chloride (Schemes V and VII).

By contrast, the formation of the major isomer 2-chloroethanol 25 from CHNU-α-d_2 (8) can arise either by S_N2 attack of chloride ion on the 2-hydroxyethanediazohydroxide 41 or by S_N1 reaction with the 2-hydroxyethyl cation 43. Formation of the minor isomer (26) in this instance can be explained via the transient conjugate acid of the epoxide[43] (45, Scheme VIII). In accord with this interpretation, CHNU-α-d_2 does not give rise to 1,2-dichloroethane.

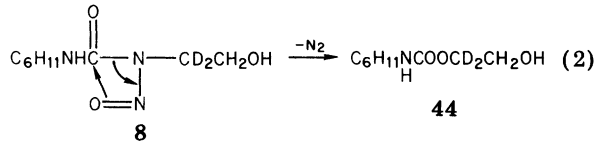
A similar pathway via the intermediate epoxide species 45 may account for the formation of the major labeled 2-chloroethanol 25 and the minor isomer 26 from CHNU-β-d_2 (16) in the presence of sodium chloride (Scheme IX), although 26 may also arise directly from 49.

Formation of Ethyl Cyclohexylcarbamates. The presence of the minor and previously undetected product 2-hydroxyethyl cyclohexylcarbamate 36 from the aqueous decomposition of CCNU-α-d_2 (7) or CINO-α-d_2 (9) was confirmed by ^1H NMR and mass spectra. The formation of the major carbamate 36 can be explained via the intramolecular attack of the OH group on the carbonyl group (eq 1). A similar intramolecular attack of a thiol group



8 36 (1)

on an amide carbonyl leading to rearrangement has recently been reported.[44] The minor carbamate isomer 44 may plausibly arise from the intramolecular reaction shown in eq 2, for which precedents exist.[45]



8 44 (2)

A similar explanation accounts for the reverse formation of major carbamate 44 and the minor carbamate 36 from the aqueous decomposition of CHNU-β-d_2 (16). Ring opening of CINO-α-d_2 and nitrogen loss explain the formation of the hydroxyethyl carbamate 44 from the aqueous decomposition of CINO-β-d_2 (18, Scheme VII). In contrast to CINO-α-d_2 (9) which gives rise to only one deuterated carbamate (36), the comparable decomposition of CHNU-α-d_2 (8) gives rise to a mixture of the differentially isotope labeled carbamates 36 and 44. In view of the recent recognition of the carcinogenicity and mutagenicity of certain carbamates,[46] their detection among the products of nitrosourea decomposition may have significance with respect to toxic effects in the clinical administration of these agents.

Formation of Ethylene Glycol. Recently Brundrett[47] has shown that ethylene glycol is formed in 2% yield when BCNU is allowed to decompose in phosphate buffer at pH 7.4 and 37 °C for 4 days. In the present work dideuterioethylene glycol (51) is probably formed from CCNU-α-d_2 (8) and CCNU-β-d_2 (16), but owing to the overlap in the GC peak position of ethylene glycol and 2-chloroethanol, positive identification and quantitation of 51 was difficult under the conditions used. However, ethylene glycol is definitely identified as a product from CINO-α-d_2 (9) or CHNU-α-d_2 (8) after decomposition in phosphate buffer pH 7.2 and 37 °C for 24 h and GC analysis of the products on a Poropak Q column under isothermal conditions at 200 °C.[48]

The formation of 51 can be explained by the attack of water at position 5 of CINO-α-d_2 which gives rise to the (2-hydroxyethyl)nitrosourea (8, Scheme VI). The formation of 2-hydroxyethanediazohydroxide 41 via path B of the nitrosourea decomposition and subsequent attack of water on 41 either by an S_N2 or S_N1 mechanism through the hydroxyethyl cation 43 can also lead to 51 (Scheme VIII). A similar mechanism for the formation of ethylene glycol from a (2-hydroxyethyl)nitrosourea in pH 7.2 phosphate buffer has been proposed.[47] Another possible source of ethylene glycol may be the opening of the epoxide 42 or its conjugate acid (45) by water.[49,51] The relatively low yield of 51 obtained from CINO may be due to a competing attack of water at position 2 of the ring rather than at position 5.

Conclusions

The present results show that CCNU reacts in buffered solution at physiological temperature and pH to yield a variety of products that are consistent with two pathways of decomposition together with minor denitrosation. Reaction may occur in one pathway through cyclization to the hitherto uncharacterized 2-(cyclohexylimino)-3-nitrosooxazolidine which reacts further by attack of nucleophiles at positions 2 and 5 to lead to the various volatile and nonvolatile products. The second and major pathway which has been recognized and examined by other workers[7,8,17,23,24] is through formation of 2-chloroethanediazohydroxide and isocyanate intermediates leading ultimately to volatile and nonvolatile products. The deuterium labeling results from CCNU for vinyl chloride are opposite those of BCNU;[18] in addition, the formation of ethylene glycol from CINO indicates a proportionally greater contribution of the cyclization for CCNU[12] compared with the case for BCNU.

The above results should assist in our understanding of the mechanisms of decomposition and action of 2-(chlo-

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(44) J. W. Lown, A. V. Joshua, and L. W. McLaughlin, *J. Med. Chem.*, **23**, 798 (1980).
(45) J. March in "Advanced Organic Chemistry, Reactions, Mechanisms and Structure", McGraw-Hill, New York, 1977, p 956.
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(47) R. B. Brundrett, *J. Med. Chem.*, **23**, 1245 (1980).
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(50) J. G. Pritchard and F. A. Long, *J. Am. Chem. Soc.*, **78**, 2667 (1956), and references therein.
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roethyl)nitrosoureas although further studies are clearly warranted particularly to substantiate or reject the alternative pathway C (Scheme I).

Experimental Section

Melting points were determined on a Fisher-Johns apparatus and are uncorrected. The IR spectra were recorded on a Nicolet 7199 FT spectrophotometer, and only the principal, sharply defined peaks are reported. The NMR spectra of the intermediates were recorded on Perkin-Elmer 90 and Varian HA-100 analytical spectrometers, and only the spectra of the final nitrosourea and nitrosooxazolidines were recorded on Bruker WH-200 and WH-400 spectrometers. The spectra were measured on approximately 5–15% (w/v) solutions, depending upon the spectrometers, in appropriate deuterated solvents with tetramethylsilane as an internal standard. Line positions are recorded in parts per million from the reference. Mass spectra were determined on an Associated Electrical Industries MS-9, double-focussing, high-resolution mass spectrometer. The ionization energy, in general, was 70 eV. Peak measurements were made by comparison with perfluorotributylamine at a resolving power of 15 000. Kieselgel DF-5 (Camag, Switzerland) and Eastman Kodak precoated silica gel sheets were used for thin-layer chromatography. In the workup procedures reported for the various syntheses described, the solvents were removed with a rotary evaporator under reduced pressure without heating.

GC analyses were performed on a Hewlett-Packard 5840A gas chromatograph equipped with flame-ionization detector. GC/MS analyses were performed on an AEI MS12 spectrometer using a helium gas flow rate of 22 mL/min. Samples were injected onto a 6 ft, 10% Carbowax 20M 80–100 WAW-DMCS 5830 column. The column was heated at 70 °C for acetaldehyde and vinyl chloride for 5 min and was heated further with a rate of 5 °C/min up to 120 °C for 30 min for 1,2-dichloroethane and chloroethanol. Ethylene glycol was chromatographed on Porapak Q (80–100 mesh) at 200 °C (isothermal) with a hydrogen flow rate of 30 mL/min.

2-Amino-2,2-dideuterioethanol (2). Compound **2** was prepared by following the procedure of Brundrett et al.,[18] i.e., lithium aluminum deuteride reduction of glycolonitrile (**1**); bp 40–41 °C (~0.15 mm) [lit.[18] bp 85–90 °C (~10 mm)].

3-Cyclohexyl-1-(1,1-dideuterio-2-hydroxyethyl)thiourea (3). A solution of cyclohexyl isothiocyanate (3.98 g, 35 mmol) in ether (50 mL) was added dropwise to a stirred suspension of **2** (2.10 g, 35 mmol) in ether (150 mL) cooled in an ice bath. After the addition was completed, the reaction mixture was stirred for 12 h at room temperature. The precipitate formed was filtered off and crystallized from ethanol to afford white crystals (6.30 g, 90%) of **3**: mp 122 °C (melting point for undeuterated compound 120–121 °C); NMR (Me_2SO-d_6) δ 0.90–2.10 (m, 10 H, CH_2), 3.50 (m, 2 H, H_2), 3.95 (m, 1 H, $H_{1'ax}$), 4.70 (s, 1 H, OH exchangeable), 7.30 (br m, 2 H, 2 NH, exchangeable); mass spectrum, m/e (relative intensity) 204.1256 (100, M^+; calcd for $C_9H_{16}D_2N_2OS$, 204.1265).

3-Cyclohexyl-1-(1,1-dideuterio-2-hydroxyethyl)urea (5). A solution of cyclohexyl isocyanate (3.50 g, 35 mmol) in ether (50 mL) was added slowly to a stirred and cooled suspension of **2** (2.10 g, 35 mmol) in ether (100 mL), and the reaction mixture was stirred at room temperature for 12 h. The solid which separated was filtered off and crystallized from a mixture of acetone/ether to afford white crystals (5.30 g, 88%) of **5**: mp 93 °C (lit.[12] mp (undeuterated) 90–91 °C); NMR ($CDCl_3$) δ 0.90–1.95 (m, 10 H, CH_2), 3.45 (m, 1 H, $H_{1'ax}$), 3.60 (s, 2 H, H_2), 4.40 (s, 1 H, OH exchangeable), 5.35 (d, 1 H, NH exchangeable, J = 7.00 Hz), 5.65 (s, 1 H, NH, exchangeable); mass spectrum, m/e (relative intensity; calcd value) 188.1491 (33.37, M^+; calcd for $C_9H_{16}D_2N_2O_2$, 188.1494), 158.1397 (31.86, $M^+ - CH_2O$; 158.1388), 56.015 (100.00; C_3H_6N, 56.0501).

3-Cyclohexyl-1-(1,1-dideuterio-2-chloroethyl)urea (4). To a stirred sample of **5** (1.88 g, 10 mmol) cooled in an ice bath was added thionyl chloride (10 mL) dropwise during 20 min, and the reaction mixture was refluxed for 1 h. The excess of thionyl chloride was removed under reduced pressure, and the residue was chromatographed on silica gel with ethyl acetate and crystallized from ethyl acetate to afford 0.84 g (41%) of **4**: mp 126–127 °C (lit.[24] mp (undeuterated) 120–121 °C); NMR (Me_2SO-d_6) δ 1.00–2.20 (m, 10 H, CH_2), 3.60 (m, 1 H, $H_{1'ax}$), 3.75 (s, 1 H, H_2), 6.65 (br m, 2 H, 2 NH, exchangeable); mass spectrum, m/e (relative intensity; calcd value) 208.1129 (1.85, M^+, ^{37}Cl; 208.1125), 206.1161 (6.90, M^+, ^{35}Cl; 206.1156), 56.0501 (100.00, C_3H_6N; 56.0501).

2-(Cyclohexylimino)-4,4-dideuterio-2-oxazoline (6). Methyl iodide (3.53 g, 25 mmol) was added dropwise to a stirred solution of **3** (4.08 g, 20 mmol) in ethanol (150 mL) at room temperature. After being stirred for 45 min at room temperature, the reaction mixture was refluxed for an additional 8 h. The solvent was removed under reduced pressure, the residue was dissolved in water (300 mL), and the solution was extracted with chloroform to remove the organic impurities. The aqueous layer was basified with dilute NaOH solution to pH 10–11 and extracted with chloroform, and the organic layer was dried (Na_2SO_4). Evaporation of the solvent under reduced pressure afforded 2.60 g (76%) of **6**: mp 128–129 °C (lit.[24] mp (undeuterated) 127–128 °C); NMR ($CDCl_3$) δ 1.00–2.20 (m, 10 H, CH_2), 3.50 (m, 1 H, $H_{1'ax}$), 3.80 (s, 1 H, NH exchangeable), 4.25 (s, 2 H, H_5); mass spectrum, m/e (relative intensity; calcd value) 170.1381 (32.50; M^+, $C_9H_{14}D_2N_2O$, 170.1389), 89.0679 (100.00; $C_3H_5D_2N_2O$, 89.0684).

3-Cyclohexyl-1-(1,1-dideuterio-2-chloroethyl)-1-nitrosourea (7). Anhydrous sodium nitrite (0.69 g, 10 mmol) was added in portions to a stirred solution of **4** (0.41 g, 2 mmol) at 0–5 °C in anhydrous formic acid (10 mL) during a period of 1 h. After the mixture was stirred for 30 min, water (10 mL) was added, and the mixture was stirred for an additional 1 h. The separated solid was collected and crystallized from ether/petroleum ether to afford 350 mg (74%) of **7**: mp 85–86 °C (lit.[53] mp (undeuterated) 88–89 °C); NMR ($CDCl_3$) δ 1.28 (m, 2 H, H_3', $H_{5'ax}$), 1.35 (m, 1 H, $H_{4'ax}$), 1.48 (m, 2 H, H_2', $H_{6'ax}$), 1.68 (m, 1 H, $H_{4'eq}$), 3.50 (s, 2 H, H_2), 3.90 (m, 1 H, $H_{1'ax}$), 6.80 (br m, 1 H, NH); CI mass spectrum (isobutane, 125 °C), m/e (relative intensity) 238 (32), 236 (100), 126 (20), 133 (19), 111 (57).

3-Cyclohexyl-1-(1,1-dideuterio-2-hydroxyethyl)-1-nitrosourea (8). A portion of **5** (950 mg, 5 mmol) was dissolved in 97% formic acid (15 mL) at 0–5 °C, and sodium nitrite (1.05 g, 15 mmol) was added slowly over a period of 1 h, maintaining the temperature between 0 and 5 °C. After the mixture was stirred for 30 min, cold water (15 mL) was added cautiously. The reaction mixture was extracted with chloroform, the extract was washed with cold water and dried (Na_2SO_4), and the solvent was removed under reduced pressure. The residue was recrystallized from ether/petroleum ether, affording 100 mg (65%) of **8**: mp 48–49 °C (lit.[9] mp (undeuterated) 49–50 °C); NMR ($CDCl_3$) δ 1.10–1.50 (m, 5 H, axial protons), 1.55–1.85 (m, 3 H, H_3', H_4', $H_{5'eq}$), 2.05 (m, 2 H, H_2', $H_{5'eq}$), 2.10 (br s, 1 H, OH, exchangeable), 3.65 (s, 2 H, H_2), 3.90 (m, 1 H, $H_{1'ax}$), 6.80 (m, 1 H, NH, exchangeable); CI mass spectrum (isobutane, 135 °C), m/e (relative intensity) 218 (100), 189 (19), 169 (13), 93 (18).

2-(Cyclohexylimino)-4,4-dideuterio-3-nitrosooxazolidine (9). To a stirred suspension of **6** (1.70 g, 10 mmol) and sodium methoxide (0.67 g, 11 mmol) in ether (150 mL) was added *n*-butyl nitrite (5.1 g, 50 mmol) dropwise at 0 °C, and the reaction mixture was stirred for 18 h at 10–15 °C. The inorganic salts were filtered off, and the filtrate was concentrated under reduced pressure. The residue was crystallized with ether/petroleum ether to afford 0.95 g (46%) of **9**: mp 108–109 °C (lit.[12] mp (undeuterated) 108–110 °C); NMR ($CDCl_3$) δ 1.25 (m, 1 H, $H_{4'ax}$), 1.30 (m, 2 H, H_3', $H_{5'ax}$), 1.50 (m, 2 H, H_2', $H_{6'ax}$), 1.65 (d, 1 H, $H_{4'eq}$), 1.85 (m, 4 H, H_2', H_3', H_5', $H_{6'eq}$), 3.65 (m, 1 H, $H_{1'ax}$), 4.50 (s, 2 H, H_5); CI mass spectrum (isobutane, 135 °C), m/e (relative intensity) 200 (33), 171 (100).

2-Amino-1,1-dideuterioethanol (11). Compound **11** was prepared by following the procedure of Brundrett et al.[18] from lithium aluminum deuteride reduction of glycine ethyl ester hydrochloride (**10**); bp 35–38 °C (~0.15 mm) [lit.[18] bp 83–88 °C (~10 mm)].

3-Cyclohexyl-1-(2,2-dideuterio-2-hydroxyethyl)thiourea (12). This compound was prepared (by following the procedure described for the thiourea **3**) from cyclohexyl isothiocyanate (2.82 g, 20 mmol) in ether (20 mL) and **11** (1.26 g, 20 mmol) in ether (100 mL), and crystallization from ethanol afforded 3.50 g (86%) of **12**: mp 124 °C (mp (undeuterated) 120–121 °C); NMR ($CDCl_3$) δ 1.00–1.20 (m, 10 H, CH_2), 2.25 (br m, 1 H, OH, exchangeable), 3.60 (d, 2 H, H_1), 3.85 (m, 1 H, $H_{1'ax}$), 6.20 (br m, 2 H, 2 NH,

exchangeable); mass spectrum, m/e (relative intensity) 204.1271 (100.00, M^+; calcd for $C_9H_{16}D_2N_2OS$, 204.1266).

3-Cyclohexyl-1-(2,2-dideuterio-2-hydroxyethyl)urea (14). Compound **14** (3.2 g, 85%) was prepared from **11** (1.26 g, 20 mmol) and **19** (2.50 g, 20 mmol) by following the method for the synthesis of **5**: mp 91–92 °C; NMR ($CDCl_3$) δ 1.00–2.00 (m, 10 H, CH_2), 3.25 (d, 2 H, H_1, J = 5.5 Hz), 3.40 (m, 1 H, $H_1'_{ax}$), 4.35 (s, 1 H, OH, exchangeable), 5.25 (d, 1 H, NH, exchangeable, J = 7.00 Hz), 5.60 (m, 1 H, NH, exchangeable); mass spectrum, m/e (relative intensity; calcd value) 188.1549 (22.18, M^+; $C_9H_{16}D_2N_2O$, 188.1494), 156.1270 (25.50, $M^+ - CD_2O$; $C_8H_{16}N_2O$, 156.1263), 56.0507 (100.00; C_3H_6N, 56.0501).

3-Cyclohexyl-1-(2,2-dideuterio-2-chloroethyl)urea (13). Compound **13** (0.95 g, 46%) was prepared from **14** (1.88 g, 10 mmol) by following the method for the synthesis of **4**: mp 126 °C; NMR (Me_2SO-d_6) δ 1.00–2.10 (m, 10 H, CH_2), 3.60 (m, 1 H, $H_1'_{ax}$), 3.75 (br s, 2 H, H_1), 6.45 (br m, 2 H, 2 NH, exchangeable); mass spectrum, m/e (relative intensity; calcd value) 208.1143 (2.43, M^+, ^{+7}Cl; 208.1125), 206.1156 (9.39, M^+, ^{35}Cl; 206.1156), 56.0509 (100.00; C_3H_6N, 56.0501).

3-Cyclohexyl-1-(2,2-dideuterio-2-chloroethyl)-1-nitrosourea (16). Compound **16** (380 mg, 81%) was prepared from **13** (410 mg, 2 mmol) by following the method for the synthesis of **7**: mp 87–88 °C; NMR ($CDCl_3$) δ 1.25 (m, 2 H, H_3', $H_5'_{ax}$), 1.32 (m, 1 H, $H_4'_{ax}$), 1.45 (m, 2 H, H_2', $H_6'_{ax}$), 1.65 (m, $H_4'_{eq}$), 1.78 (m, 2 H, H_3', $H_5'_{eq}$), 2.08 (m, 2 H, H_2', $H_6'_{eq}$), 3.90 (m, 1 H, $H_1'_{ax}$), 4.80 (s, 2 H, H_1), 6.80 (br m, 1 H, NH); CI mass spectrum (isobutane, 135 °C), m/e (relative intensity) 238 (27), 236 (100), 126 (15), 113 (16), 111 (47).

3-Cyclohexyl-1-(2,2-dideuterio-2-hydroxyethyl)-1-nitrosourea (17). Compound **17** (1.35 g, 62%) was prepared from **14** (1.88 g, 10 mmol) by following the method for the synthesis of **8**: mp 50–51 °C; NMR ($CDCl_3$) δ 1.10–1.50 (m, 5 H, axial protons), 1.55–1.85 (m, 3 H, H_3', H_4', $H_5'_{eq}$), 2.08 (m, 2 H, H_2', $H_6'_{eq}$), 2.12 (m, 1 H, OH exchangeable), 3.90 (m, 1 H, $H_1'_{ax}$), 4.08 (s, 2 H, H_1), 6.85 (m, 1 H, NH); CI mass spectrum (isobutane, 135 °C), m/e (relative intensity) 218 (100), 189 (17), 169 (27), 93 (34).

2-(Cyclohexylamino)-5,5-dideuterio-2-oxazoline (15). Compound **15** (1.40 g, 80%) was prepared from **12** (2.04 g, 10 mmol) by following the method for the synthesis of **6**: mp 130 °C; NMR ($CDCl_3$) δ 1.00–2.20 (m, 10 H, CH_2), 3.40 (m, 1 H, $H_1'_{ax}$), 3.85 (s, 2 H, H-4), 3.95 (s, 1 H, NH exchangeable); mass spectrum, m/e (relative intensity; calcd value) 170.1387 (21.12, M^+; $C_9H_{14}D_2N_2O$, 170.1389), 89.0683 (100; $C_3H_5D_2O$, 89.0684).

2-(Cyclohexylimino)-5,5-dideuterio-3-nitrosooxazolidine (18). Compound **18** (0.75 g, 47%) was prepared from **15** (1.36 g, 8 mmol) by following the method for the synthesis of **9**: mp 106–108 °C; NMR ($CDCl_3$) δ 1.25 (m, 1 H, $H_4'_{ax}$), 1.34 (m, 2 H, H_3', $H_5'_{ax}$), 1.50 (m, 2 H, H_2', $H_6'_{ax}$), 1.55 (m, 1 H, $H_4'_{eq}$), 1.85 (m, 4 H, H_2', H_3', H_5', $H_6'_{ax}$), 3.65 (m, 1 H, $H_1'_{ax}$), 3.95 (s, 2 H, H_4); CI mass spectrum (isobutane, 135 °C), m/e (relative intensity) 200 (35), 171 (100).

General Method for the Decomposition of CCNU-α-d_2 (7), CINO-α-d_2 (9), CHNU-α-d_2 (8), CCNU-β-d_2 (16), CINO-β-d_2 (18), and CHNU-β-d_2 (17). (1) In Phosphate Buffer. (a) Identification of Volatile Products. A sample of each compound (0.05 mmol) suspended in 0.1 M phosphate buffer (0.6 mL, pH 7.2) in a 1-mL capacity, screw-capped Reacti-vial was allowed to decompose for 1 week at 37 °C after the evacuation of the head space above the liquid. The gaseous sample (1 mL) was injected with a Pressure-lok syringe into the gas chromatograph for detection of acetaldehyde and vinyl chloride. Immediately after the removal of the gaseous contents, dichloromethane (0.2 mL) was injected into the vials, the mixture shaken thoroughly, and the dichloromethane solution (2 μL) then injected for GC/MS analysis. Each compound was confirmed by its retention time by comparison with the corresponding authentic undeuterated compound and by its mass spectrum. The retention times and the corresponding mass spectra of the volatile products are given in Table II.

(b) Identification of Nonvolatile Products. A sample of each compound (0.05 mmol) in 0.1 M phosphate buffer pH 7.0 (10 mL) was allowed to decompose for 12 h at 37 °C. The aqueous solution was lyophilized, and the residue was subjected to CI mass spectroscopy using isobutane as the reagent gas. The identification of each compound was established by comparison with its undeuterated analogue.

(2) In the Presence of 5 M Sodium Chloride. (a) Identification of Volatile Products. A sample of each compound (0.1 mmol) in 5 M sodium chloride (4 mL) in 0.1 M phosphate buffer was allowed to decompose in 5-mL capacity Reacti-vials as above at 37 °C for 1 week. After the end of the reaction time, dichloromethane (0.5 mL) was injected into the vials, the mixture was shaken thoroughly, and the dichloromethane solution (2 μL) was then injected for GC/MS analysis. The retention time and mass spectral data of each compound under different reaction conditions are given in Table II.

(b) Identification of Nonvolatile Products. After the identification of volatile products, the reaction mixture was concentrated to dryness and triturated with hot acetonitrite (3 × 10 mL), and the acetonitrile solution was concentrated. The products were identified by TLC on reverse-phase, precoated, silica plates by using a water–acetonitrile mixture (1:9 to 2:8) as well as by their characteristic mass spectra.

Isolation of 2,2-Dideuterio-2-hydroxyethyl Cyclohexylcarbamate (36) from the Decomposition of CINO-α-d_2 (9) in 0.1 M Phosphate Buffer. A sample of **9** (19.9 mg, 0.1 mmol) in 0.1 M phosphate buffer (10 mL) was allowed to decompose for 12 h at 37 °C. The solvent was removed under reduced pressure, the residue was triturated with chloroform (2 × 5 mL), and 10 mL of ether was added to the mixture which was filtered. The filtrate was concentrated, and the remaining **36** (crude) melted at 55–57 °C: NMR ($CDCl_3$) δ 1.00–2.10 (m, 10 H, CH_2), 3.00 (br s, 1 H, OH, exchangeable), 3.45 (m, 1 H, $H_1'_{ax}$), 4.20 (s, 2 H, OCH_2), 4.80 (m, 1 H, NH exchangeable); IR ($CHCl_3$) ν_{max} 3480 (NH, OH) 1705 (C=O) cm^{-1}; mass spectrum, m/e (relative intensity; calcd value) 189.1323 (30.69, M^+; $C_9H_{15}D_2NO_3$, 189.1323), 157.1094 (5.01, $M^+ - CD_2O$; $C_8H_5NO_2$, 157.1103), 146.0780 (100.00, $M^+ - 43$; $C_6H_8D_2NO_3$, 146.0780).

Isolation of 1,1-Dideuterio-2-hydroxyethyl Cyclohexylcarbamate (44) from the Decomposition of CINO-β-d_2 (18) in 0.1 M Phosphate Buffer. Compound **44** was isolated from the decomposition of **18** according to the procedure described for **36**: mp 54–55 °C; NMR ($CDCl_3$) δ 1.10–2.10 (m, 10 H, CH_2), 2.80 (t, 1 H, OH, exchangeable), 3.45 (m, 1 H, $H_1'_{ax}$), 3.80 (br s, 2 H, CH_2OH_2OH), 4.70 (br m, 1 H, NH, exchangeable); IR ($CHCl_3$) ν_{max} 3485 (NH, OH), 1710 (C=O) cm^{-1}; mass spectrum, m/e (relative intensity; calcd value) 189.1333 (37.48, M^+; $C_9H_{15}D_2NO_3$, 189.1334), 159.1228 (4.58, $M^+ - CH_2O$; $C_8H_{13}D_2NO_2$, 159.1228), 146.0781 (100.00, $M^+ - 43$; $C_6H_8D_2NO_3$, 146.0780).

2,2-Dideuterio-2-hydroxyethyl Cyclohexylcarbamate (36) from Acid Diazotization of 2-Amino-2,2-dideuterioethyl Cyclohexylcarbamate. A mixture of **2** (1.89 g, 30 mmol) and phthalic anhydride (4.44 g, 30 mmol) was refluxed for 10 min at 150 °C. The reaction mixture was allowed to cool to 90 °C and poured into water (200 mL). The crystals so obtained were collected, dried, and crystallized from chloroform to afford 2,2-dideuterio-2-phthalimidoethanol: 3.90 g (67%); mp 125–126 °C (lit.[22] mp (for protium compound) 126–127 °C); NMR ($CDCl_3$) δ 2.80 (br m, 1 H, OH, exchangeable), 3.85 (s, 2 H, CH_2), 7.55–8.90 (m, 4 H, aromatic); mass spectrum, m/e (relative intensity; calcd value) 193.0709 (5.04, M^+; $C_{10}H_7D_2NO_3$, 193.0708), 163.0584 (46.21, M - CH_2O; $C_9H_5D_2NO_3$, 163.0602), 162.0525 (100.00; $C_9H_4D_2NO_2$, 162.0525).

A mixture of the above phthalimidoethanol (1.93, 10 mmol) and cyclohexyl isocyanate (1.25 g, 10 mmol) in toluene (35 mL) was refluxed for 10 h and cooled, and petroleum ether (30 mL) was added to the reaction mixture. The crystals so obtained were collected to yield 2,2-dideuterio-2-phthalimidoethyl cyclohexylcarbamate: 2.50 g (78%); mp 154–155 °C (lit.[21] mp (for protium compound) 157–158 °C); NMR ($CDCl_3$) δ 1.00–2.00 (m, 10 H, CH_2), 3.40 (m, 1 H, CH), 4.30 (s, 2 H, CH_2), 4.62 (br m, 1 H, NH, exchangeable), 7.80–8.00 (m, 4 H, aromatic); mass spectrum, m/e (relative intensity; calcd value) 318.1547 (5.73, M^+; $C_{17}H_{18}D_2N_2O_4$, 318.1547), 194.0785 (30.82; $C_{10}H_8D_2NO_3$, 194.0787), 162.0521 (100.00; $C_9H_4ND_2O_2$, 162.0524).

A mixture of the above carbamate (2.00 g, 6.3 mmol) and hydrazine hydrate (1.00 g, 20 mmol) in ethanol (30 mL) was refluxed for 3 h. The ethanol was removed under reduced pressure, and the residue was treated with 1 N HCl (20 mL). The resulting suspension was stirred at room temperature for 3 h, and

Table II. Mass Spectral Data on CCNU-α-d_2 (7), 2-(Cyclohexylimino)-3-nitroso-4,4-dideuterio-2-oxazolidine (9), 3-Cyclohexyl-1-(1,1-dideuterio-2-hydroxyethyl)nitrosourea (8), CCNU-β-d_2 (16), 3-(Cyclohexylimino)-3-nitroso-5,5-dideuterio-2-oxazolidine (18), 3-Cyclohexyl-1-(2,2-dideuterio-2-hydroxyethyl)nitrosourea (17), and Their Decomposition Products

substrate	reaction conditions	decomposition products	retention time, min	*m/e* (relative intensity, fragments)
CCNU-α-d_2 (7)				237.1040 (1.62, M^+, 2 D, ^{37}Cl), 235.1061 (5.22, M^+, 2 D, ^{35}Cl), 126.0917 (47.24, $C_6H_{11}NHCO$), 112.0181 (1.38, $^{37}ClCH_2CD_2N_2OH$), 110.0210 (4.88, $^{35}ClCH_2CD_2N_2OH$), 83.0856 (100, C_6H_{11}), 75.0527 (1.62, $CH_2CD_2N_2OH$), 67.0101 (0.93, $^{37}ClCH_2CD_2$), 65.0137 (3.61, $^{35}ClCH_2CD_2$)
	phosphate buffer	vinyl chloride (34)	1.24	65 (23.5, M^+, 1 D, ^{37}Cl), 63 (79.6, M^+, 1 D, ^{35}Cl), 28 (100, $M^+ - Cl$),
		acetaldehyde[a] (29)	1.30	44 (38.2, M^+, no D), 43 (17.9, CH_3CO), 29 (100, CHO)
		1,2-dichloroethane (27)	8.57	104 (1.6, M^+, 2 D, 2 ^{37}Cl), 102 (13.3, M^+, 2 D, ^{37}Cl and ^{35}Cl), 100 (20.5, M^+, 2 D, 2 ^{35}Cl), 66 31.2, $M^+ - H^{37}Cl$), 64 (100.0, $M^+ - H^{35}Cl$), 65 (44.1, $M^+ - D^{37}Cl$), 63 (61.9, $M^+ - D^{35}Cl$), 29 (90.0, $C_2HD_2^+$)
		chloroethanol (26)	16.53	84 (0.9, M^+, 2 D, ^{37}Cl), 82 (1.1, M^+, 2 D, ^{35}Cl), 33 (100.0, $^+CD_2OH$), 31 (9.7, $^+CH_2OH$)
CINO-α-d_2 (9)				199.1301 (0.43, M^+, 2 D), 182.1267 (0.44, $M^+ - 17$), 170.1356 (20.95, M − 29), 169.1309 (61.86, $M^+ - NO$), 127.0840 (10.78, C_6H_{11}-PNCN), 117.0507 (5.08, $C_3H_3D_2N_3O_2$), 89.0683 (100, $C_3H_5D_2N_2O$), 88.0609 (8.23, $C_3H_4D_2N_2O$), 83.0856 (4.83, $C_6H_{11}^+$), 81.0696 (12.83, C_6H_9), 74.0453 (1.00, $C_2H_2D_2N_2O$).
	phosphate buffer	acetaldehyde[a] (29)	1.30	44 (68.7, M^+, no D), 43 (38.5, CH_3CO), 29 (100, CHO)
	sodium chloride	vinyl chloride (34)	1.45	65 (31.5, M^+, 1 D, ^{37}Cl), 63 (100.0, M^+, 1 D, ^{35}Cl), 28 (96.5, $CH_2{=}CD^+$)
		1,2-dichloroethane (27)	8.90	104 (1.8, M^+, 2 D, 2 ^{37}Cl), 102 (9.8, M^+, 2 D, ^{37}Cl and ^{35}Cl), 100 (18.5, M^+, 2 D, 2 ^{35}Cl), 66 (33.5, $M^+ - H^{37}Cl$), 64 (100, $M^+ - H^{35}Cl$), 65 (39.4, $M^+ - D^{37}Cl$), 63 (43.8, $M^+ - D^{35}Cl$), 29 (89.5, $C_2HD_2^+$)
		chloroethanol (25 and 26)	18.17	84 (1.2, M^+, 2 D, ^{37}Cl), 82 (4.8, M^+, 2 D, ^{35}Cl), 33 (100, $^+CD_2OH$), 31 (9.7, $^+CH_2OH$)
CHNU-α-d_2				217.1395 (0.44, M^+, 2 D), 126.0920 (17.92, $C_6H_{11}NHCO$), 92.0555 (33.38, $HOCH_2CD_2N_2OH$), 83.0862 (100.00, C_6H_{11}), 75.0528 (2.29, $CH_2CD_2N_2OH$)
	phosphate buffer	acetaldehyde[a] (29)	1.30	44 (60.9, M^+, no D), 43 (32.2, CH_3CO), 29 (100, CHO)
		oxirane-2-d_2 (42)	1.26	46 (52.2, M^+, 2 D), 44 (20.1, M − D), 30 (100, ODO)
	sodium chloride	vinyl chloride (34)	1.45	65 (31.5, M^+, 1 D, ^{37}Cl), 63 (100, M^+, 1 D, ^{35}Cl), 28 (96.5, $CH{=}CD^+$)
		chloroethanol (25 and 26)	17.49	84 (0.5, M^+, 2 D, ^{37}Cl), 82 (1.5, M^+, 2 D, ^{35}Cl), 33 (12.9, CD_2OH), 31 (100, CH_2OH)
CCNU-β-d_2 (16)				237.1031 (2.14, M^+, 2 D, ^{37}Cl), 235.1060 (6.52, M^+, 2 D, ^{35}Cl), 126.0917 (47.24, $C_6H_{11}NHCO$), 112.0185 (1.54, $^{37}ClCD_2CH_2N_2OH$), 110,0213 (5.05, $^{35}ClCD_2CH_2N_2OH$), 83.0857 (100, C_6H_{11}), 75.0528 (1.36, $CD_2CH_2N_2OH$), 67.0106 (0.82, $^{37}ClCD_2CH_2$), 65.0139 (2.95, $^{35}ClCD_2CH_2$)
	phosphate buffer	vinyl chloride (28)	1.30	66 (21.0, M^+, 2 D, ^{37}Cl), 64 (67.8, M^+, 2 D, ^{35}Cl), 29 (100, $M^+ - Cl$)
		acetaldehyde[a] (35)	1.30	45 (46.2, M^+, 1 D), 43 (13.2, CH_3CO), 30 (100, CDO)
		1,2-dichloroethane (27)	8.50	104 (2.1, M^+, 2 D, 2 ^{37}Cl), 102 (12.6, M^+, 2 D, ^{37}Cl and ^{35}Cl), 100 (21.3, M^+, 2 D, 2 ^{35}Cl), 66 (32.9, $M^+ - H^{37}Cl$), 64 (100.0, $M^+ - H^{35}Cl$), 65 (38.9, M − $D^{37}Cl$), 63 (29.3, $M^+ - D^{35}Cl$), 29 (87.6, C_2HD_2)
		chloroethanol (25 and 26)	16.11	84 (3.1, M^+, 2 D, ^{37}Cl), 82 (2.2, M^+, 2 D, ^{35}Cl), 33 (21.4, CD_2OH), 31 (100, CH_2OH)

Table II (*Continued*)

substrate	reaction conditions	decomposition products	retention time, min	*m/e* (relative intensity, fragments)
CINO-β-d_2 (18)				199.1290 (0.81, M^+, 2 D), 182.1266 (0.87, M^+ – O), 170.1370 (14.90, M – 29), 169.1310 (100, M^+ – NO), 127.0843 (14.04, C_6H_{11}-NCN), 117.0507 (8.02, $C_3H_3D_2N_3O_2$), 89.0683 (80.81, $C_3H_5D_2N_2O$), 88.0613 (3.83, $C_3H_4D_2N_2O$), 83.0857 (6.89, C_6H_{11}), 81.0702 (21.34, C_6H_9), 74.0450 (1.00, $C_2H_2D_2N_2O$)
	phosphate buffer	acetaldehyde (35)	1.24	45 (49.5, M^+, 1 D), 43 (18.3, CH_3CO), 30 (100, CDO)
	sodium chloride	vinyl chloride (28)	1.35	66 (25.5, M^+, 2 D, ^{37}Cl), 64 (68.5, M^+, 2 D, ^{35}Cl), 29 (100, M^+ – Cl)
		1,2-dichloroethane (27)	9.20	104 (1.5, M^+, 2 D, 2 ^{37}Cl), 102 (11.6, M^+, 2 D, ^{37}Cl, ^{35}Cl), 100 (18.9, M^+, 2 D, ^{35}Cl), 66 (31.5, $M^+ - H^{37}Cl$), 64 (100, $M^+ - H^{35}Cl$), 65 (36.6, $M - D^{37}Cl$), 63 (42.9, $M - D^{35}Cl$), 29 (90.6, C_2HD_2)
		chloroethanol (25 and 26)	18.57	84 (2.4, M^+, 2 D, ^{37}Cl), 82 (4.5, M^+, 2 D, ^{35}Cl), 33 (10.5, $^+CD_2OH$), 31 (100, $^+CH_2OH$)
CHNU-β-d_2 (17)				217.1395 (2.14, M^+, 2 D), 126.0920 (21.62, $C_6H_{11}NHCO$), 92.0555 (40.10 $HOCD_2CH_2N_2$-OH), 83.0860 (100.00, C_6H_{11}), 75.0528 (0.60, $CD_2CH_2N_2OH$)
	phosphate buffer	acetaldehyde (35)	1.34	45 (44.1, M^+, 1 D), 43 (16.3, CH_3CO), 30 (100, CDO)
		oxirane-2-d_2 (42)	1.26	46 (52.2, M^+, 2 D), 44 (20.1, M – D), 30 (100, CDO)
	sodium chloride	vinyl chloride (28)	1.43	66 (24.8, M^+, 2 D, ^{37}Cl), 64 (78.9, M^+, 2 D, ^{35}Cl), 29 (100, $CD_2{=}CH^+$)
		chloroethanol (25 and 26)	18.05	84 (0.4, M^+, 2 D, ^{37}Cl), 82 (1.6, M^+, 2 D, ^{35}Cl), 33 (100, $^+CD_2OH$), 31 (10.9, $^+CH_2OH$)

[a] Detected in the gaseous phase of the sample.

the solid hydrazide was filtered off. The filtrate was evaporated to dryness under reduced pressure and triturated with hot ethanol, and insoluble hydrazine hydrochloride was filtered off. The warm filtrate was mixed with an equal volume of ether and cooled in order to precipitate 2-amino-2,2-dideuterioethyl cyclohexylcarbamate hydrochloride. Recrystallization was done from an ethanol/ether mixture, and the product was dried at room temperature: mp 160–161 °C (lit.[21] mp (undeuterated) 162–163 °C); NMR (Me_2SO-d_6) δ 1.00–2.00 (m, 10 H, CH_2), 3.25 (m, 1 H, CH), 4.15 (s, 2 H, CH_2), 7.10 (d, 1 H, NH, exchangeable), 3.86 (br m, 2 H, NH, exchangeable); mass spectrum, *m/e* (relative intensity; calcd value) 189.1577 (1.0, M^+; $C_9H_{17}D_2N_2O_2$, 189.1572), 157.1100 (9.15; $C_8H_{15}NO_2$, 157.1103), 156.1025 (9.65; $C_8H_{14}NO_2$, 156.1024), 83.0859 (100.00; C_6H_{11}, 83.0860), 55.0562 (88.50; C_4H_7, 55.0547).

A dilute solution of formic acid (50%, 10 mL) was added to a solution of the above 2-amino-2,2-dideuterioethyl cyclohexylcarbamate hydrochloride (540 mg, 2.5 mmol) at 0 °C and sodium nitrite (350 mg, 5 mmol) in water (20 mL), and the reaction mixture was stirred for 4 h. The mixture was extracted with chloroform, the extract was dried (Na_2SO_4) and evaporated, and residue was crystallized from ether/petroleum ether to afford 2,2-dideuterio-2-hydroxyethyl cyclohexylcarbamate: 120 mg (26%); mp 57–58 °C (lit.[12] mp (for protium compound) 58–59 °C). The other physical as well as spectral data are in agreement with those of the compound obtained from the decomposition of CINO-α-d_2 (9).

2-Chloro-2,2-dideuterioethyl Cyclohexylcarbamate (40). A solution of sodium nitrite (350 mg, 5 mmol) in water (10 mL) was added dropwise to a stirred suspension of 2-amino-2,2-dideuterioethyl cyclohexylcarbamate hydrochloride (540 mg, 2.5 mmol) in hydrochloric acid (10 mL) at 0–5 °C. The reaction mixture was stirred for 3 h, diluted with water (20 mL), and extracted with chloroform (2 × 15 mL). The organic layer was dried and concentrated to afford 250 mg (49%) of **40**: mp 58–60 °C (lit.[12] mp (undeuterated) 60–61 °C); NMR ($CDCl_3$) δ 0.95–2.10 (m, 10 H, CH_2), 3.50 (m, 1 H, CH), 4.30 (s, 2 H, OCH_2), 4.70 (br m, 1 H, NH, exchangeable); mass spectrum, *m/e* (relative intensity; calcd value) 209.0973 (3.03, M^+; $C_9H_{14}D_2$–$^{37}ClNO_2$, 209.0966), 207.0992 (10.07; $C_9H_{14}D_2$–$^{35}ClNO_2$, 207.0995), 166.0418 (32.52; $C_6H_7D_2$–$^{37}ClNO_2$, 166.0418), 164.0444 (100.00; $C_6H_7D_2$–$^{35}ClNO_2$, 164.0448).

3-Cyclohexyl-5,5-dideuterio-2-oxazolone (39). Powdered sodium hydroxide (44 mg, 1.1 mmol) was added to a solution of **40** (205 mg, 1 mmol) in ethanol (10 mL), and the mixture was refluxed for 5 h. The reaction mixture was cooled, solid sodium chloride was collected by filtration, and the filtrate was concentrated. The residue was triturated with petroleum ether to afford 120 mg (70%) of **39**: mp 28–30 °C (lit.[12] mp (for protium compound) 29–30 °C); NMR ($CDCl_3$) δ 1.00–2.10 (m, 10 H, CH_2), 3.50 (s, 2 H, NCH_2), 3.55 (m, 1 H, CH); mass spectrum, *m/e* (relative intensity; calcd value) 171.1228 (28.42, M^+; $C_9H_{13}D_2NO_2$, 171.1229), 128.0678 (100.00; $C_6H_6D_2NO_2$, 128.0681), 90.0523 (85.69; $C_3H_4D_2NO_2$, 90.0524).

Caution: All nitrosoureas should be handled with extreme care owing to their potential mutagenicity.

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Registry No. **1**, 107-16-4; **2**, 59099-82-0; **3**, 77081-25-5; **4**, 77081-26-6; **5**, 77081-27-7; **6**, 77081-28-8; **7**, 77081-29-9; **8**, 77081-30-2; **9**, 77081-31-3; **10**, 623-33-6; **11**, 59099-87-5; **12**, 77081-32-4; **13**, 77081-33-5; **14**, 77081-34-6; **15**, 77081-35-7; **16**, 77081-36-8; **17**, 77081-37-9; **18**, 77081-38-0; **19**, 3173-53-3; **25**, 77081-39-1; **26**, 77081-40-4; **27**, 61596-07-4; **28**, 6745-37-5; **29**, 75-07-0; **34**, 4984-12-7; **35**, 4122-13-8; **36**, 77081-41-5; **39**, 77081-42-6; **40**, 77081-43-7; **42**, 57178-82-2; **44**, 77081-44-8; cyclohexyl isothiocyanate, 1122-82-3; phthalic anhydride, 85-44-9; 2,2-dideuterio-2-phthalimidoethanol, 77081-45-9; 2,2-dideuterio-2-phthalimidoethyl cyclohexylcarbamate, 77081-46-0; 2-amino-2,2-dideuterioethyl cyclohexylcarbamate, 77081-47-1; ethylene glycol, 107-21-1; CINO, 76310-06-0; CHNU, 66929-45-1.