(0.042 g, 1.75 mmol) in 2 mL of dry tetrahydrofuran. The tosylhydrazone prepared above (0.39 g, 1.2 mmol) was added, and the reaction mixture was stirred for 15 min. The solvent was removed by distillation at reduced pressure, and the reaction flask was connected through a trap to a vacuum line. The system was evacuated to 0.2 mm and the flask was heated; at a bath temperature of 120–130 °C, the pale-yellow sodium salt turned pink, and liquid began to collect in the liquid-nitrogen-cooled trap. The bath temperature was brought to 180–190 °C for 1.5 h to complete the reaction. Purification of the liquid collected in the cold-finger trap by preparative GLC (SE-30, 120 °C) gave 30 mg (19% yield) of tricyclo[4.2.2.0^{1,6}]dec-7-ene: NMR 6.25 (2 H, s), 1.82 (6 H, br s), 1.50 (2 H, m), 1.35 (4 H, m); mass spectrum, *m/e* 134 (M^+), 119, 106, 105, 91 (base peak).

Tricyclo[4.2.2.0^{1,6}]decane (11). Atmospheric hydrogenation of 5 mg of tricyclo[4.2.2.0^{1,6}]dec-7-ene in ethyl acetate with the aid of Adams catalyst gave a colorless low-melting solid. Its NMR spectrum matched the published spectrum of [4.2.2]propellane (tricyclo[4.2.2.0^{1,6}]decane).[27]

3,8-Dimethylenecyclooctene (2). A 5-mg sample of tricyclo[4.2.2.0^{1,6}]dec-7-ene in 0.5 mL of benzene-d_6 was thoroughly degassed, sealed in vacuo, and heated in a salt bath maintained at 221.4 °C. After 10 min, new NMR signals had appeared at δ 6.15 (s), 4.95 (s), 2.4 (br m), and 1.4 (m), with relative intensities 1:2:2:2. After 60 min of thermolysis, more than 90% of the starting material had been converted to this new product. On an SE-30 column at 120 °C, it had a longer retention time than the starting material; mass spectrum, *m/e* 134 (M^+), 119, 106, 91 (base peak).

Kinetics of the Tricyclo[4.2.2.0^{1,6}]dec-7-ene to 3,8-Dimethylenecyclooctene Rearrangement. Open-ended glass capillary tubes were soaked for several hours in dilute HCl and again in an NH_4OH/EDTA solution and then thoroughly washed with distilled water and dried overnight in an oven at 140 °C. One end of each tube was sealed. A benzene solution approximately 0.8 M in tricyclodecene and containing dodecane as internal standard was prepared; 5-μL portions of this solution were placed in each capillary tube with a syringe. These capillary-tube ampules were flushed gently with nitrogen, frozen, sealed, and heated in the salt bath; after pyrolyses the reaction mixtures were analyzed by GLC. The observed first-order rate constants for the thermal conversion of tricyclodecene to 3,8-dimethylenecyclooctene were $(2.52 \pm 0.15) \times 10^{-5}$ s^{-1} at 181.1 °C, $(2.51 \pm 0.24) \times 10^{-4}$ s^{-1} at 211.2 °C, and $(2.31 \pm 0.06) \times 10^{-3}$ s^{-1} at 240.3 °C. The kinetic work at 211.2 °C was done several months after the other two sets of pyrolyses were completed. An Arrhenius plot based on the three rate constants gives the activation parameters $E_a = 35.8 \pm 1.3$ kcal/mol and log $A = 12.6 \pm 0.6$.

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Discrimination between Alternative Pathways of Aqueous Decomposition of Antitumor (2-Chloroethyl)nitrosoureas Using Specific ^{18}O Labeling†

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The synthesis of certain specifically ^2H- and ^{18}O-labeled 1-(2-chloroalkyl)-3-alkyl-1-nitrosoureas is described. When BCNU-β,β'-d_4 (2) was allowed to decompose in phosphate buffer at pH 7.1 and 25 °C in 99% $H_2{}^{18}O$ in the presence of liver alcohol dehydrogenase and NADH, the acetaldehyde initially produced is immediately reduced to ethanol. Gas chromatographic mass spectral analysis of the positions of ^2H and ^{18}O labels in the ethanol permitted a discrimination between competing pathways of decomposition, indicating in this case ca. 21% contribution via a postulated 1,2,3-oxadiazoline intermediate. The observation of $CH_2DCDH^{16}OH$ in addition to $CH_3CDH^{16}OH$ indicates the 1,2,3-oxadiazoline intermediate may decompose partly by a concerted pathway requiring a 5 to 4 deuterium shift. In contrast decomposition of BCNU-α,α'-diMe (3) in 99% $H_2{}^{18}O$ containing phosphate buffer at pH 7.1 and 25 °C in the presence of the alcohol dehydrogenase afforded labeled propanol corresponding to ca. 89% contribution via the 1,2,3-oxadiazoline intermediate. The result was substantiated by the reverse decomposition of BCNU-β,β'-d_4-α,α'-diMe-*N*-^{18}O in $H_2{}^{16}O$, GC–MS analysis of which afforded $CH_3CDHCDH^{18}OH$ and $CH_3CH_2CDH^{18}OH$ corresponding to ca. 88% contribution of the 1,2,3-oxadiazoline pathway. The preference for this pathway in the latter case may be due to the increased stabilization the α-Me group affords the intermediate cation. This would tend to disfavor the two alternative mechanisms which require a hydride shift in the cationic intermediate.

Introduction

The (2-chloroethyl)nitrosoureas (CENUs) such as BCNU, CCNU, and MeCCNU, and chlorozotocin are of clinical value in the treatment of a wide range of neoplasma.[1,2] Pharmacologial evidence indicates that CENUs decompose spontaneously under physiological conditions, giving rise to electrophiles including isocyanate, 2-chloroethyldiazohydroxide, or the 2-chloroethyl cation, and that the latter two species both alkylate and form interstrand cross-links in DNA and between DNA and proteins.[3–10]

† CENU, 1-(2-chloroethyl)-3-alkyl-1-nitrosourea; BCNU, bis(2-chloroethyl)-1-nitrosourea; BCNU-β,β'-d_4, bis(2-chloro-2,2-dideuterioethyl)-1-nitrosourea; BCNU-α,α'-diMe, bis(2-chloro-1-methylethyl)-1-nitrosourea; BCNU-β,β'-d_4-α,α'-diMe, bis(2-chloro-2,2-dideuterio-1-methylethyl)-1-nitrosourea; BCNU-β,β'-d_4-α,α'-diMe-*N*-^{18}O, bis(2-chloro-2,2-dideuterio-1-methylethyl)-1-[^{18}O]-nitrosourea.

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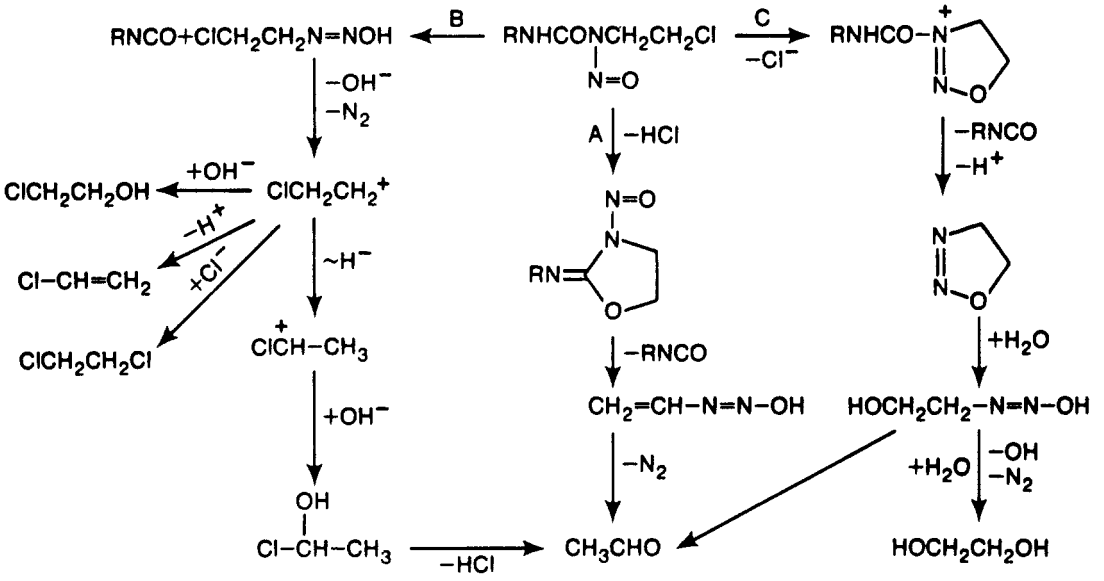
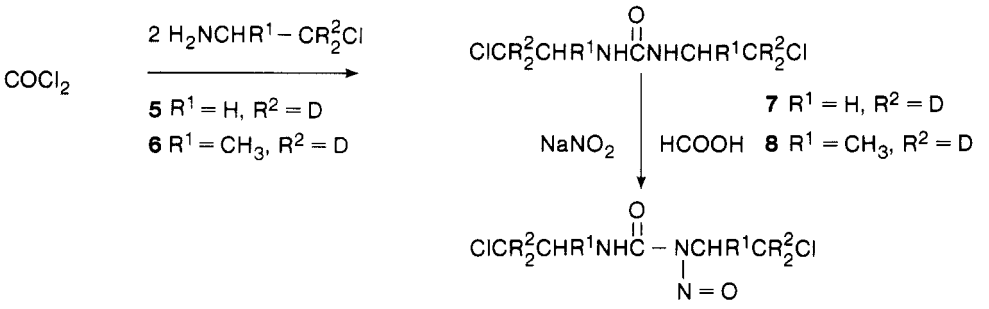
Scheme I



Scheme II



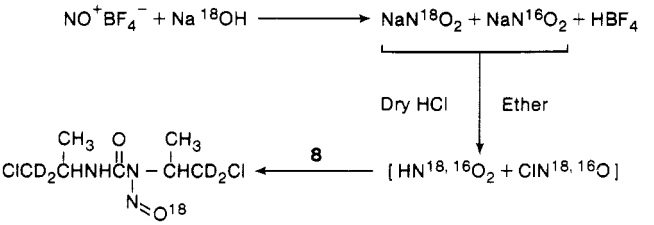
CENUs

1 $R^1 = H$, $R^2 = H$
2 $R^1 = H$, $R^2 = D$
3 $R^1 = CH_3$, $R^2 = H$
4 $R^1 = CH_3$, $R^2 = D$

The events ultimately responsible for the biological activity of CENUs therefore occur before or during the decomposition, i.e., NH proton abstraction by base,[11] and the formation of transient tetrahedral intermediates[12] by hydration of the carbonyl group. These events are governed by the type and nature of the CENU, pH, temperature, solvent polarity, and nature of the buffering medium. In addition, the operation of stereoelectronic control in the decomposition of the CENUs requires that available lone pairs on two heteroatoms in the tetrahedral intermediate be aligned antiperiplanar to the bond to be broken.[12]

The rich chemistry of CENUs in aqueous solution leads to a plethora of products, and three major pathways of decomposition have been suggested[2-10] (Scheme I). We describe experiments with specifically ^{18}O-labeled CENUs in $H_2{}^{16}O$ and the complementary decomposition of ^{16}O-containing CENUs in $H_2{}^{18}O$ as a means of discriminating among the competing pathways and of determining the dependence of the preference for particular pathways on the structure of the CENU.

Scheme III



9

Synthesis of Specifically ^{18}O and ^{2}H Labeled CENUs

The required unlabeled nitrosoureas, BCNU and bis-[2-(chloromethyl)ethyl]-1-nitrosourea (BCNU-α,α'-diMe, **3**), were prepared by literature procedure.[13,14] The deuterated CENUs were prepared by reaction of phosgene with the corresponding specifically deuterium labeled

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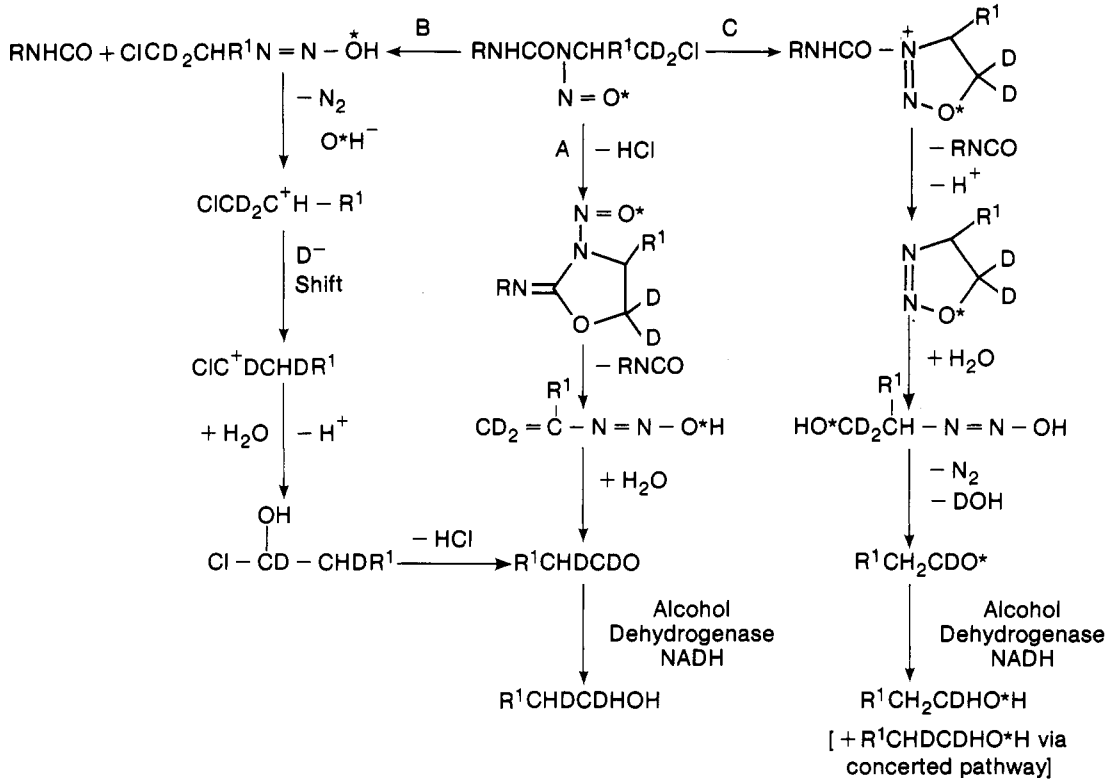
Scheme IV



2-aminochloroethanols or 2-aminochloropropanol as shown in Scheme II. The specifically $N^{18}O$-containing nitrosourea (**9**) was prepared by a modification of the procedure used to make the corresponding nitrosocarbamate,[15] using nitrosonium tetrafluoroborate (Scheme III).

Results and Discussion

The essential steps of the three alternative pathways of aqueous decomposition of CENUs to be considered are shown in Scheme I. The schemes have been deliberately simplified by omitting the critical factors of conformational and stereoelectronic control[12] for the sake of clarity. It may be seen that carbonyl compounds are predicted to be among the common products of all three pathways. There is strong evidence for the formation of acetaldehyde from the aqueous decomposition of BCNU via a 2-chloroethyl cation followed by hydride transfer and subsequent hydrolysis of the newly formed cation.[4,5,9,10] Recently we have shown acetaldehyde is also one of the products in the aqueous decomposition of 2-(alkylimino)-3-nitrosooxazolidine,[9,10] a proposed intermediate in decomposition pathway A.[3] A third possible source of acetaldehyde is via the recently postulated 1,2,3-oxadiazoline intermediate in pathway C.[6,7] We considered that it ought to be possible to distinguish pathways A and B from pathway C by determining the extent of incorporation of ^{18}O label in the acetaldehyde produced. Owing to the possibility of exchange of the ^{18}O label in the carbonyl group, our approach has been to "fix" the carbonyl ^{18}O as soon as it is generated by in situ reduction with equine liver alcohol dehydrogenase.

This approach is based on the following premises. (a) CENU decompositions are not catalyzed or influenced by the enzymes used.[11] (b) ^{18}O to ^{16}O exchange in either the carbonyl or nitroso group is extremely slow in phosphate buffer at physiological pH.[16-18] (c) Acetaldehyde and propionaldehyde are reduced enzymatically to the corresponding alcohols much faster than the ^{18}O exchange with the solvent at pH 7.1 and 25 °C and that in the presence of the coenzyme NADH, the equilibrium acetaldehyde ⇌ ethanol is forced essentially completely in the direction of reduction.[19,20]

A complicating factor in BCNU decomposition, which affords acetaldehyde and thence ethanol after enzyme reduction, is that the equine liver alcohol dehydrogenase coenzyme NADH contains ca. 2% of ethanol as a stabilizer. This difficulty was overcome by the synthesis of specifically labeled BCNU-β-d_4 and with high-resolution mass spectrometry, permitting a discrimination between CH_3CH_2OH, $CH_2DCHDOH$, and CH_3CHDOH products. When BCNU-β,β'-d_4 (**2**) was decomposed at pH 7.1 in phosphate buffer (prepared with 99% $H_2{}^{18}O$) in the presence of liver alcohol dehydrogenase and NADH, the composition of the ethanol-d ($CH_2DCDHOH$ and CH_3CDHOH; determined by GC/MS) was 79 ± 10% containing ^{18}O and 21 ± 10% containing ^{16}O. This result indicates under these conditions that the relative contributions of the competing pathways are (A + B)/C = ca. 79:21 (see Scheme IV).

The observation of $CH_2DCDH^{16}OH$ in addition to $CH_3CDH^{16}OH$ indicates that the 1,2,3-oxadiazoline intermediate in pathway C may decompose partly by a concerted pathway requiring a 5 to 4 deuterium shift. Further evidence in support of this conclusion is provided by the example of BCNU-$\alpha\alpha'$-diMe-$\beta\beta'$-d_4-N-^{18}O below. The reaction mechanisms were further tested by deter-

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Table I. Mass Spectral and Gas Chromatographic Characteristics of BCNU-α,α'-diMe and BCNU-β,β'-d_4-α,α'-diMe and Their Aqueous Decomposition Products

reactant	reaction conditions	decomp products	GC t_R,[a] min	m/e (relative intensity, fragments)[a,b]
BCNU-α,α'-diMe (3)	control			245.0354 (0.12, M^+, $C_7H_{13}{}^{37}Cl_2N_3O_2$, 245.0326), 243.0358 (1.00, M^+, $C_7H_{13}{}^{37}Cl^{35}ClN_3O_2$, 243.0355), 241.0383 (1.63, M^+, $C_7H_{13}{}^{35}Cl_2N_3O_2$, 241.0385) 124.0219 (30.74, $C_3H_7{}^{37}ClN_2O$, 124.0218), 122.0243 (100.00, $C_3H_7{}^{35}ClN_2O$, 122.0247)
	potassium phosphate (0.1 M) buffer, pH 7.2, 25 °C	1-chloroprene	1.17	78 (6.8, M^+, ^{37}Cl), 76 (22.2, M^+, ^{35}Cl), 41 (100.0, $M^+ - {}^{35}Cl$), 39 (76.7, $M^+ - {}^{37}Cl$) $M^+ - {}^{37}Cl$)
		3-chloropropene	1.77	78 (13.5, M^+, ^{37}Cl), 76 (40.6, M^+, ^{35}Cl), 41 (100.0, $M^+ - {}^{35}Cl$)
		propionaldehyde	1.52	58 (19.7, M^+), 29 (100, ^+CHO)
	1,2-	1,2-dichloropropane	10.52	116 (0.2, M^+, $^{37}Cl_2$), 114 (1.5, M^+, ^{37}Cl, ^{35}Cl), 112 (2.8, M^+, $^{35}Cl_2$), 78 (12.5, $M^+ - H^{35}Cl$), 76 (38.8, $M^+ - H^{37}Cl$), 41 (100.0, $M^+ - 2Cl$)
		1-chloro-2-propanol	15.60	81 (2.4, $M^+ - CH_3$), 79 (7.8, $M^+ - CH_3$), 45 (100.0, $M^+ - CH_2Cl$)
BCNU-β,β'-d_4-α,α'-diMe (4)	control			249.0595 (0.88, M^+, $C_7H_9{}^{37}Cl_2D_4N_3O_2$, 249.0579), 247.0616 (5.82, M^+, $C_7H_9{}^{37}Cl^{35}ClD_4N_3O_2$, 247.0616), 245.0644 (9.12, M^+, $C_7H_9{}^{35}Cl{}^{35}Cl_2D_4N_3O_2$, 245.0644), 126.0346 (40.82, $C_3H_5{}^{37}ClD_2N_2O$, 126.0343), 124.033 (100.0, $C_3H_5{}^{35}ClD_2N_2O$, 124.0372)
	potassium phosphate (0.1 M) buffer, pH 7.2, 25 °C	1-chloro-1-deuterio-1-propene	1.45	79 (7.6, M^+, 1 D, ^{37}Cl), 77 (23.2, M^+, ^{35}Cl), 62 (6.8 $M^+ - CH_3$), 42 (100, $M^+ - {}^{35}Cl$)
		1,1-dideuterio-2-propene	1.52	80 (23.1, M^+, 2 D, ^{37}Cl), 78 (59.9, M^+, 2 D, ^{35}Cl), 43 (100, O, $M^+ - {}^{35}Cl$)
		1,1-dideuterio-3-chloropropene	2.13	80 (0.9, M^+, 2 D, ^{37}Cl), 78 (3.2, M^+, 2 D, ^{35}Cl), 43 (100.0, $M^+ - {}^{35}Cl$)
		propionaldehyde-α-d	2.13	59 (31.9, M^+), 43 (19.0, $M^+ - CH_4$), 30 (100.0, CDO)
		1,2-dichloropropane (2D)*	5.58	116 (1.2, M^+, 2 D, ^{37}Cl, ^{35}Cl), 114 (2.6, M^+, 2 D, $^{35}Cl_2$), 80 (9.8, $M^+ - {}^{35}Cl$), 78 (36.1, $M^+ - {}^{37}Cl$), 65 (33.5, $CH_2{=}CD^{37}Cl$), 63 (100.0, $CH_2{=}CD^{35}Cl$)
		1-chloro-1,1-dideuterio-2-propanol	15.72	83 (2.2, $M^+ - CH_3$, 2 D, ^{37}Cl), 81 (7.2, $M^+ - CH_3$, ^{35}Cl), 45 (100.0, $M^+ - CD_2{}^{35}Cl$), 43 (11.9, $M^+ - CD_2{}^{37}Cl$)

[a] Determined on a 6-ft, 10% Carbowax 20M 80–100 mesh WA or DMCS 5830 column with a helium flow rate at 22 mL/min. [b] The mass spectra fragmentation characteristics of BCNU and BCNU-β,β'-d_4 have been reported previously.[4]

mining the effects of substituents adjacent to the incipient cationic center. Therefore a similar experiment with BCNU-α,α'-diMe (3) in 99% $H_2{}^{18}O$ containing phosphate buffer afforded propanol bearing ca. 11% of ^{18}O and ca. 89% of the ^{16}O species. The full spectrum of products of aqueous decomposition of BCNU-α,α'-diMe has been reported previously.[14] The present result shows a marked reversal of mechanistic preference for pathway C at the

Table II. Mass Spectral and Gas Chromatographic Characterization of the Products of Aqueous Decomposition of BCNU and Its Analogues in the Presence of Liver Alcohol Dehydrogenase and NADH

reactant	reaction solvent	product	GC t_R, min	m/e (relative intensity)
	control	ethanol	2.40	46 (14.1, M^+), 45 (42.1, $M^+ - 1$), 31 (100.0, $^+CH_2OH$)
BCNU (1)	H_2O	ethanol	2.41	46 (19.1, M^+), 45 (51.1, $M^+ - 1$), 31 (100.0, $^+CH_2OH$)
BCNU-β,β'-d_4 (2)	H_2O	ethanol-d + ethanol	2.41	47 (6.6, M^+), 46 (18.1, $M^+ - 1$), 32 (100.0, ^+CHDOH)
			2.41	46 (18.1, M^+), 45 (42.5, $M^+ - 1$), 31 (100.0, $^+CH_2OH$)
BCNU-β,β'-d_4 (2)	$H_2{}^{18}O$ (99%)	ethanol-^{18}O	2.45	50 (10.3, M^+, ^{18}O, 2 D), 49 (24.4, M^+, ^{18}O, D), 48 (M^+, ^{18}O)
		ethanol-^{16}O	2.45	47 (48.5, $M^+ - 1$, ^{18}O), 46 (7.6, M^+, ^{16}O), 34 (64.3, $CHD^{18}OH$),[a] 33 (100.0, $^+CH_2{}^{18}OH$), 32 (16.6, CDHOH),[a] 31 (39.6, $^+CH_2OH$)
	H_2O	n-propanol	7.19	60 (6.6, M^+), 59 (10.9, $M^+ - 1$), 31 (100.1, $^+CH_2OH$)
BCNU-α,α'-diMe (3)	H_2O	n-propanol	7.18	60 (4.8, M^+), 59 (8.6, $M^+ - 1$), 31 (100.0, $^+CH_2OH$)
BCNU-α,α'-diMe (3)	$H_2{}^{18}O$ (99%)	n-propanol-^{18}O + n-propanol-^{16}O	7.20	62 (M^+, ^{18}O), 61 (1.8, $M^+ - 1$, ^{18}O), 60 (6.8, M^+, ^{16}O) 59 (12.3, $M^+ - 1$, ^{16}O), 33 (12.6, $^+CH_2{}^{18}OH$), 31 (100.0, $^+CH_2{}^{16}OH$)[a]
BCNU-β,β'-d_4-α,α'-diMe-N-^{18}O (9)	H_2O	n-propanol	7.37	63 (0.6, M^+, ^{18}O), 62 (4.8, $M^+ - 1$, ^{18}O) 61 (7.0, M^+, ^{16}O), 60 (2.1, $M^+ - 1$, ^{16}O) 34 (12.8, $^+CHD^{18}OH$),[a] 33 (3.8, $CH_2{}^{18}OH$), 32 (100.0, $^+CHD^{16}OH$),[a] 31 (7.5, $^+CH_2{}^{16}OH$)

[a] Characteristic mass fragments used to determined the extent of ^{18}O incorporation in the product alcohols.

expense of pathways A and B (see Scheme IV) and was confirmed by performing the experiment in reverse in terms of the ^{18}O labels. BCNU-β-d_4-α,α'-diMe-N-^{18}O (4) specifically labeled with ^{18}O in the nitroso group (15% ^{18}O) was used. Deuterium labels were incorporated in this case also to determine the fate of the 4-methyl-1,2,3-oxadiazoline intermediate. Decomposition of BCNU-$\beta\beta'$-d_4-α,α'diMe-N-^{18}O in $H_2{}^{16}O$ phosphate buffer at pH 7.1 in the presence of the enzyme afforded propanol-d (comprising $CH_3CDHCDH^*OH$ and $CH_3CH_2CDH^*OH$) bearing 88 ± 10% of the ^{18}O-containing propanols and 12 ± 10% of the ^{16}O-containing propanols. This therefore confirms the previous result, within experimental error, and indicates ca. 88% preference for pathway C for the α,α'-diMe compound at least as far as the formation of carbonyl compounds is concerned. Again the detection of $CH_3CDHCDH^{18}OH$ as well as $CH_3CH_2CDH^{18}OH$ indicates that the 4-methyl-1,2,3-oxadiazoline intermediate in pathway C may decompose partly by a concerted pathway requiring a direct 5 to 4 deuterium shift. The preference shown for pathway C may be rationalized by the increased stabilization the α-methyl group affords the intermediate cation[21] generated in pathways A and B, disfavoring the subsequent hydride shift required to generate the propionaldehyde. The fact that 1-chloro-2-propanol accounts for 30% of the volatile products is in accord with this interpretation. Other possible effects of α substitution in causing a change in preferred pathway include those of steric hindrance on the relative rates of decomposition.[22,23]

These findings tend to corroborate the postulate of three competing pathways of aqueous decomposition of CENUs and indicate that it is possible to control the pathway selected by suitable structural modification. The results may therefore be useful in the design, synthesis, and testing of new potential antitumor CENUs.

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Experimental Section

Melting points were determined on a Fisher-Johns apparatus and are uncorrected. The ^1H NMR spectra of the intermediates were recorded on Perkin-Elmer 90 and Varian HA-100 analytical spectrometers and those of the final nitrosoureas were recorded on Bruker WH-200 and WH-400 spectrometers. Mass spectra were determined on an Associated Electrical Industries (AEI-MS-9) double-focusing high-resolution mass spectrometer with ionization energy at 70 eV. Peak measurements were made by comparison with perfluorotributylamine at a resolving power of 15 000.

GC analyses were performed on a Hewlett-Packard 5840A gas chromatograph equipped with a flame-ionization detector. GC/MS analyses were performed on an AEI-MS12 spectrometer. Samples were injected onto a 6-ft, 10% Carbowax 20M 80–100 WAW-DMCS 5830 column with helium flow rate of 22 mL/min. The column was heated at 70 °C to detect acetaldehyde, vinyl chloride, acetone, propionaldehyde, and chloropropenes for 5 min and was heated further at a rate of 5 °C/min up to 120 °C for 30 min to detect 1,2-dichloroethane, 1,2-dichloropropane, 2-chloroethanol, and 2-chloropropanol. Ethanol and propanol were detected by chromatography on a Porapak T (80–100 mesh) column under isothermal conditions at 175 °C with a helium flow rate of 35 mL/min.

Materials. 2-Chloroethylamine hydrochloride and 2-chloroethanol were obtained from Aldrich Chemical Co. DL-Alanine ester hydrochloride, lactonitrile, 2-chloro-1-propanol, and 1-chloro-2-propanol were obtained from Eastman Kodak Co. Phosgene (12.5% in benzene) was obtained from Matheson, Coleman and Bell. $H_2{}^{18}O$ (99% enriched) was obtained from Merck Sharp and Dohme of Canada Ltd.

Alcohol dehydrogenase (alcohol:NAD^+ oxidoreductase; EC 1.1.1.1) from equine liver substantially ethanol free, activity 1–2 units/mg of protein, and β-nicotinamide adenine dinucleotide, reduced form (β-NADH) from yeast, grade III disodium salt, anhydrous, mol wt 709.4, were obtained from Sigma. Bis(2-chloroethyl)-1-nitrosourea [1; mp 31–32 °C (lit.[12] mp 30–32 °C)] and bis(2-chloro-1-methylethyl)-1-nitrosourea [3; mp 31 °C (lit.[14] mp 30–31 °C)] were prepared following literature procedures.[12-14] The additional specifically ^2H and ^{18}O labeled nitrosoureas required were prepared as described below.

2-Chloro-2,2-dideuterioethylamine hydrochloride (5) was prepared from the 2-aminoethanol, which was in turn prepared by standard literature procedures.[4,10,12] 1-Chloro-1,1-dideuterio-2-propylamine (6) was obtained from 1,1-dideuterio-2-aminopropanol, which in turn was prepared from the reduction of alanine ethyl ester hydrochloride with lithium aluminum deuteride.

Bis(2-chloro-2,2-dideuterio-1-methylethyl)-1-nitrosourea (4). A solution of phosgene (8.3 mL, 12.5% in benzene) was added dropwise to 1-chloro-1,1-dideuterio-2-propylamine [6; prepared from its hydrochloride (2.50 g, 20 mmol) and sodium hydroxide (1.0 g, 25 mmol) in water (20 mL)] at 5–6 °C, and then the solution was diluted with ether (3 × 150 mL) and triethylamine (2.2 g, 20 mmol) in ether (150 mL) added and the solution cooled to 0 °C. The reaction mixture was stirred mechanically for 6 h, and then water (20 mL) was added dropwise and the resulting solid urea was collected by filtration, washed with water, and finally crystallized from dichloromethane and petroleum (1:10) to afford 1.10 g (50% yield) of 8: mp 108 °C (lit.[14] mp for undeuterated material 117–118 °C); ^{1}H NMR ($CDCl_3$) δ 1.25 (d, 6 H, 2 CH_3), 4.20 (q, 2 H, 2 CH), 5.24 (d, 2 H, 2 NH exchangeable); mass spectrum, *m/e* (relative intensity) 218.0715 (0.53, M^+, $C_7H_{10}{}^{37}Cl^{35}ClD_4N_2O$, 218.0704), 216.0742 (0.94, M^+, $C_7H_{10}{}^{35}Cl_2D_4N_2O$, 216.0734), 167.0735 (32.30, $C_6H_{10}{}^{37}ClD_2N_2O$, 167.0734), 165.0761 (100.00, $C_6H_{10}{}^{35}ClD_2N_2O$, 165.0763).

Sodium nitrite (0.69 g, 10 mmol) was added in portions to a stirred solution of the above urea (8; 0.49 g, 2 mmol) in concentrated HCl (10 ml) at 0–5 °C during a period of 30 min and stirring was continued for an additional hour. Water (10 ml) was added slowly, the reaction mixture was extracted with ether, and the extract was dried (Na_2SO_4) and concentrated in vacuo. The residue was crystallized from ether/petroleum ether to afford 210 mg (62%) of nitrosourea 4: mp 66–67 °C (mp of undeuterated material 65 °C); ^{1}H NMR ($CDCl_3$) δ 1.35 (d, 3 H, CH_3), 1.45 (d, 3 H, CH_3), 4.40 (q, 1 H, CH), 5.15 (q, 1 H, CH), 7.05 (br m, 1 H, NH exchangeable).

BCNU-β-d_4 (2) was prepared by a similar procedure as described above and its physical and spectral data matched those of the literature values.[4,12]

Bis(2-chloro-2,2-dideuterio-1-methylethyl)-1-[^{18}O]-nitrosourea (9). A solution of hydrogen chloride in ether was added dropwise to a solution of $NaN^{18}O_2$ in $Na^{18}OH$ [prepared from sodium (0.46 mol) and $H_2{}^{18}O$ (99%, 3 mL), then by addition of nitrosonium tetrafluoroborate (1.16 g, 10 mmol) at 0 °C] at 0–4 °C until the pH of the solution reached 1–2. The resulting mixture of $HN^{18}O_2$ and $ClN^{18}O$) was distilled in ether (20 mL) cooled to −20 °C and then sodium acetate (0.82 g, 10 mmol) was added to this solution and the mixture stirred. The above urea (8) (545 mg, 2.5 mmol) was added in portions to the above stirred solution of [^{18}O]nitrosyl chloride and the mixture stirred for a further 2 h. The ether layer was removed and the aqueous layer was extracted with ether (2 × 10 mL). The combined ether extract was washed successively with saturated sodium chloride and then 2% sodium bicarbonate solutions. The organic layer was dried (anhydrous $CaCl_2$) and the solvent was removed in vacuo to afford 200 mg (40%) of the ^{18}O-labeled nitrosourea 9: mp 65 °C; mass spectrum, *m/e* (relative intensity) 249.0607 (0.69, $C_7H_9{}^{35}Cl^{37}ClD_4N_3{}^{16}O^{18}O$, 249.0563, $C_7H_9{}^{37}Cl_2D_4N_3O_2$ peak ratio of 15:85, 249.0577), 247.0622 (2.71, $C_7H_9{}^{37}Cl^{35}ClD_4N_3O_2$, 247.0607), 245.0634 (3.37, $C_7H_9{}^{35}Cl_2N_3O_2$, 245.0636), 128.0385 (3.88, $C_3H_5{}^{37}ClD_2N_2{}^{18}O$, 128.0385), 126.0370 (36.30, $C_3H_5{}^{37}ClD_2N_2{}^{16}O$, 126.0343, $C_3H_5{}^{35}ClD_2N_2{}^{18}O$, 126.0325), 124.0370 (80.23, $C_3H_5{}^{35}ClD_2N_2{}^{16}O$, 124.0373).

General Method for the Analysis of the Products of Aqueous Decomposition of CENUs in Phosphate Buffer. A sample of each compound (0.05 mmol) was suspended in potassium phosphate buffer (0.1 M, 0.6 mL) at pH 7.1 in a 1-mL-capacity screw-capped Reacti-vial. The head space was evacuated and the solution allowed to decompose at 37 °C. At intervals a gaseous sample was removed by a pressure-lock syringe and injected into the GC for the detection of acetaldehyde, vinyl chloride, propionaldehyde, acetone, or a mixture of chloropropenes.

Dichloromethane (0.2 mL) was injected into the reaction vials and the mixture was shaken thoroughly. An aliquot of the dichloromethane solution (2 μL) was injected for GC and GC/MS analysis. Each compound was confirmed by its retention time by comparison with the corresponding undeuterated authentic sample. The retention times and the corresponding mass spectral data for the products are given in Table I.

Decomposition of CENUs in Aqueous Phosphate Buffer in the Presence of Equine Liver Alcohol Dehydrogenase and NADH at pH 7.1. A sample of the CENU (0.05 mmol) was suspended in potassium phosphate buffer (0.1 M, 20 mL) at pH 7.1, containing equine liver alcohol dehyrogenase (8 mg, 16 units of activity) and NADH, (8 mg, 0.01 mmol) in a 5-μL-capacity Reacti-vial and the solution was allowed to decompose for 12 h at 25 °C. An aliquot of the aqueous solution (1 μL) was injected for GC/MS on a Porapak T column at 175 °C (isothermal). The retention times and the corresponding mass spectra data for the alcohols are given in Table II.

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