Synthesis of Specifically ^{15}N- and ^{13}C-Labeled Antitumor (2-Haloethyl)nitrosoureas. The Study of Their Conformations in Solution by Nitrogen-15 and Carbon-13 Nuclear Magnetic Resonance and Evidence for Stereoelectronic Control in Their Aqueous Decomposition†

J. William Lown* and Shive M. S. Chauhan

Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2

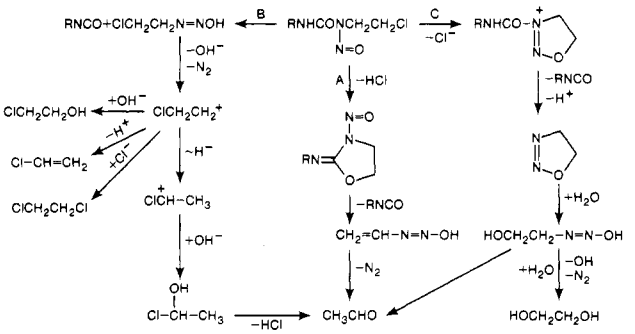
Received June 17, 1981

The synthesis of certain specifically ^{15}N, ^{13}C, and ^{2}H isotope labeled 1-(2-chloroethyl)-3-alkyl-1-nitrosoureas (CENUs) is described. Spectroscopic examination of CENUs and their isotope-labeled counterparts by ^{1}H, ^{15}N, and ^{13}C NMR and infrared spectra indicates that they adopt preferred conformations in nonpolar aprotic solvents in which the NO group is aligned toward the 2-chloroethyl group. The result is in accord with the conformation of MeCCNU in the crystalline state derived from X-ray diffraction. The chemical shifts and coupling constants in the CENUs change with both solvent polarity and basicity. In aqueous phosphate buffer there is evidence for the formation of a tetrahedral intermediate, the conformation of which alters according to the reaction conditions and ultimately controls the formation of the aqueous decomposition products of CENUs. This is revealed most clearly by ^{13}C NMR of carbonyl-^{13}C- and nitroso-^{15}N-labeled BCNU and CCNU where two distinct ^{15}N-coupled ^{13}C doublets with different chemical shifts are observed. The rate of conformational change is comparable with the rate of decomposition of CENUs (via the second conformer) and may therefore represent the critical initial step of the latter process in vivo. The intermediacy of the postulated tetrahedral intermediates for CENUs is supported by observed ^{18}O exchange into the carbonyl group in ^{18}O-enriched water. Consideration of the conformations of the intermediates and of the alignment of the heteroatom lone pairs provides a satisfactory interpretation of the reactions of CENUs in aqueous solution as well as their pH dependence in terms of strict stereoelectronic control and accounts for the formation of the observed products. The latter analysis requires that available lone pairs on the two heteroatoms in a particular tetrahedral intermediate be aligned antiperiplanar to the bond which is cleaved.

The (2-chloroethyl)nitrosoureas (CENUs) including BCNU, CCNU, MeCCNU, and chlorozotocin are of clinical use in the treatment of a wide range of neoplasms.[1-4] In contrast to other anticancer agents such as mitomycin C, streptonigrin, bleomycin, and the anthracyclines that require bioactivation prior to reaction with their cell targets,[5-11] the evidence on CENUs suggests that they react in the cell without activation[12,13] although they are subject to oxidative metabolism in the alkyl group.[1,2] They decompose spontaneously under physiological conditions, giving rise to electrophiles including the (2-chloroethyl)diazo hydroxide or the 2-chloroethyl cation[12-27] which both alkylate and form interstrand cross-links[18,19] in DNA and proteins. Another decomposition product is the alkyl isocyanate[28,29] which can result in carbamoylation of amino groups in biological macromolecules. Extensive studies on the mechanism of the action of these agents have led to the proposal of at least three pathways of decomposition of CENUs, the essential features of which are given in Scheme I. Pathway B proceeds via generation of a (2-chloroethyl)diazo hydroxide and 2-chloroethyl cation, pathway A proceeds via an intermediate 2-(alkylimino)-3-nitrosooxazolidine[14,23-24] which we have recently characterized and which decomposes further (although not by the pathway shown), and pathway C proceeds via a postulated but as yet uncharacterized *N*-acyloxadiazolinium species.[21,22]

There is considerable evidence in favor of pathway B as the major source of electrophiles from CENUs.[12-27] However there are indications that pathways A and C may contribute to a greater or lesser extent depending on the

Scheme I



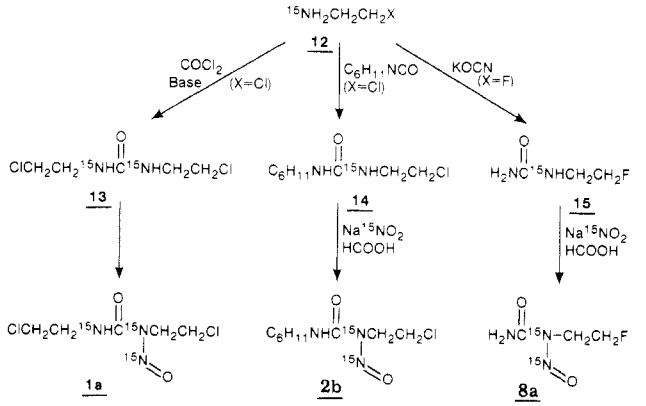
propensity of the individual CENUs to cyclize.[23,24] Since pathway A can lead to toxic and mutagenic carbamates,[23]

† CENU, 1-(2-chloroethyl)-3-alkyl-1-nitrosourea; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea; BCNU-α-d_4, 1,3-bis(1,1-dideuterio-2-chloroethyl)-1-nitrosourea; CCNU, 3-cyclohexyl-1-(2-chloroethyl)-1-nitrosourea; CCNU-α-d_2, 3-cyclohexyl-1-(1,1-dideuterio-1-chloroethyl)-1-nitrosourea; MeCCNU, 3-(*trans*-4-methylcyclohexyl)-1-(2-chloroethyl)-1-nitrosourea; CFNU, 3-cyclohexyl-1-(2-fluoroethyl)-1-nitrosourea; CHNU, 3-cyclohexyl-1-(2-hydroxyethyl)-1-nitrosourea.

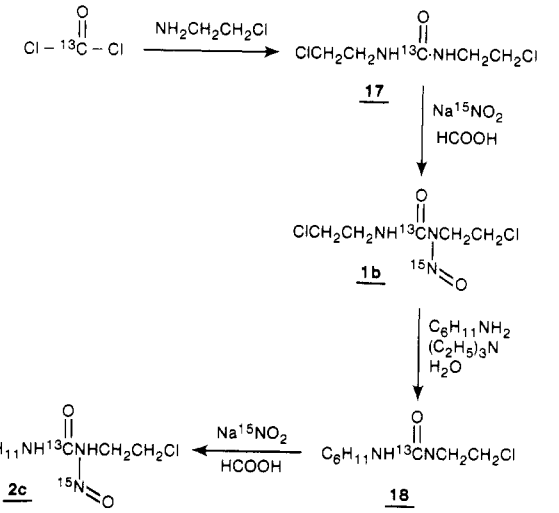
(1) Wheeler, G. D. *ACS Symp. Ser.* **1976**, *No. 30*, 87-119.
(2) Proceedings of the 7th New Drug Nitrosoureas in *Cancer Treat. Rep.* **1976**, *60*, 651-811.
(3) Montgomery, J. A. *J. Med. Chem.* **1980**, *23*, 1063.
(4) Hansch, C.; Leo, A.; Schmidt, C.; Jow, P. C. C.; Montgomery, J. A. *J. Med. Chem.* **1980**, *23*, 1095.
(5) Frank, R. W. *Fortschr. Chem. Org. Naturst.* **1979**, *38*, 1.
(6) Moore, H. W. *Science* **1977**, *197*, 527.
(7) Rao, G. M.; Lown, J. W.; Plambeck, J. A. *J. Electrochem. Soc.* **1977**, *124*, 195, 199.
(8) Lown, J. W.; Chen, H. H. *Can. J. Chem.* **1981**, *59*, 390.
(9) Cone, R.; Hasan, S. K.; Lown, J. W.; Morgan, A. R. *Can. J. Biochem.* **1976**, *54*, 219.
(10) Umezawa, H. "Medicinal Chemistry"; Cassady, J. M., Douros, J. D., Eds.; Academic Press: New York, 1980; Vol. 16, p 148.
(11) Bachur, N. R.; Gee, M. V.; Gordon, S. L. *Proc. Am. Assoc. Cancer Res.* **1978**, *19*, 75.
(12) Thomas, C. B.; Osieka, R.; Kohn, K. W. *Cancer Res.* **1978**, *38*, 2448.
(13) Brundrett, R. B.; Colvin, M.; White, E. H.; McKee, J.; Eartman, P. E.; Brown, D. L. *Cancer Res.* **1979**, *39*, 1328.
(14) Montgomery, J. A.; James, R.; McCaleb, G. S.; Johnston, T. P. *J. Med. Chem.* **1967**, *10*, 668.
(15) Colvin, M.; Cowens, J. W.; Brundrett, R. B.; Kramer, B. S.; Ludlum, D. B.; *Biochem. Biophys. Res. Commun.* **1974**, *60*, 515.
(16) Montgomery, J. A.; James, R.; McCaleb, G. S.; Kirk, M. C.; Johnston, J. P. *J. Med. Chem.* **1975**, *18*, 568.

0022-3263/81/1946-5309$01.25/0

Scheme II



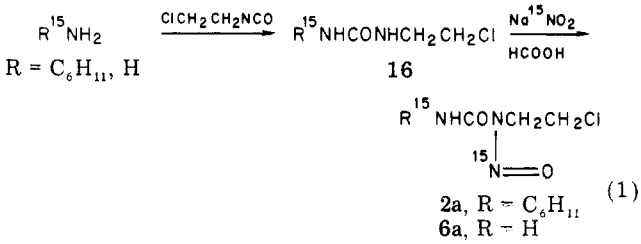
Scheme III



the factors controlling the tendency of CENUs to cyclize are of direct concern. The events ultimately responsible for the biological activities of CENUs may occur before or during the decompositions, e.g., proton abstraction by base from N_3H, formation of a tetrahedral intermediate at the C=O group, or a change in the conformation of the CENU. In order to investigate these phenomena and to detect and characterize intermediates, and thereby to understand the mechanism of action of CENUs under physiological conditions, we synthesized certain specifically ^{15}N- and ^{13}C-labeled CENUs. The properties and reactions of these labeled compounds were then investigated by ^{15}N and ^{13}C NMR spectroscopy among other techniques. On the basis of these results attempts were then made to rationalize the formation of the observed products of decomposition of CENUs in aqueous solution in terms of stereoelectronic control in the several tetrahedral intermediates involved.

Synthesis of Specifically Labeled (2-Haloethyl)nitrosoureas

The unlabeled CENUs were prepared by conventional methods by nitrosation with sodium nitrite in anhydrous formic acid or aqueous HCl of the corresponding ureas, which were in turn prepared either by reaction of 2-chloroethyl isocyanate and the appropriate amine or of alkyl isocyanate and 2-chloroethylamine hydrochloride in the presence of nonnucleophilic base in nonpolar solvents.[30-35] Other special water-soluble nitrosoureas, i.e., *N'*-(2-hydroxyethyl)-*N*-nitroso-*N*-(2-chloroethyl)urea[36,37] and chlorozotocin[33] as well as 2-chloroethyl carbamate,[38] were prepared by following literature procedures. The required specifically ^{15}N-labeled 2-chloroethylamine hydrochloride was prepared from the (95%) ^{15}N-enriched precursors available commercially and converted to ureas[38] as presented in Schemes II and III. Additional specifically ^{15}N-labeled CENUs were prepared from the appropriate labeled amine as in eq 1. The ureas were examined by



(1)

^{15}N NMR ^1H NMR, and mass spectrometry to confirm the position and extent of substitution. The majority of the subsequent N-nitrosations of the ureas were performed with solid $Na^{15}NO_2$ in 97% formic acid which permits regiospecific nitrosation at the less hindered position,[31] e.g., CCNU.

The specifically ^{13}C carbonyl group labeled BCNU was prepared by reaction of 2-chloroethylamine with labeled phosgene (90% ^{13}C enrichment) followed by nitrosation with $Na^{15}NO_2$ (95% enrichment) by the usual procedure (Scheme III). The specifically ^{13}C carbonyl group labeled CCNU was prepared by reaction of cyclohexylamine and ^{13}C-labeled BCNU in the presence of triethylamine and water to afford CCU which on nitrosation with $Na^{15}NO_2$ in formic acid afforded the ^{13}C carbonyl group enriched CCNU. The extents of the incorporation of ^{13}C and ^{15}N were confirmed by ^{13}C NMR, ^{15}N NMR, and mass spectra.

Results and Discussion

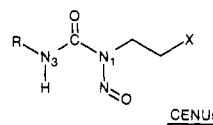
^1H NMR Spectra. The positions of the proton resonances of the methylene groups in the 2-chloroethyl side chains of CENUs have been confirmed by specific deuteration and are especially useful in confirming the position

(17) Brundrett, R. B.; Cowens, J. W.; Colvin, M.; Jardine, I. *J. Med. Chem.* **1976**, *19*, 958.
(18) Kohn, K. W. *Cancer Res.* **1977**, *37*, 1450.
(19) Lown, J. W.; McLaughlin, L. M.; Chang, Y. M. *Bioorg. Chem.* **1978**, *7*, 97.
(20) Weinkam, R. J.; Lin, H. S. *J. Med. Chem.* **1979**, *22*, 1193.
(21) Chatterjii, D. C.; Green, R. F.; Gallelli, S. F. *J. Pharm. Sci.* **1978**, *67*, 1527.
(22) Brundrett, R. B. *J. Med. Chem.* **1980**, *23*, 1245.
(23) Lown, J. W.; Chauhan, S. M. S. *J. Med. Chem.* **1981**, *24*, 270.
(24) Lown, J. W.; Chauhan, S. M. S. *J. Org. Chem.* **1981**, *46*, 2479.
(25) Tong, W. P.; Ludlum, D. B. *Biochem. Pharmacol.* **1979**, *28*, 1175.
(26) Gombar, C. T.; Tong, W. P.; Ludlum, D. B. *Biochem. Pharmacol.* **1980**, *29*, 2639.
(27) Reed, D. J.; May, H. E.; Boose, R. B.; Gregory, K. M.; Beistein, M. A. *Cancer Res.* **1975**, *35*, 568.
(28) Babson, J. R.; Reed, D. J.; Sinkey, M. A. *Biochemistry* **1977**, *16*, 1584.
(29) Kann, H. E.; Schott, M. A.; Petkas, A. *Cancer Res.* **1980**, *40*, 50.
(30) Johnston, T. P.; McCaleb, G. S.; Montgomery, J. A. *J. Med. Chem.* **1963**, *6*, 669.
(31) Johnston, T. P.; McCaleb, G. S.; Opliger, P. S.; Montgomery, J. A. *J. Med. Chem.* **1966**, *9*, 892.
(32) Johnston, T. P.; McCaleb, G. S.; Opliger, P. S.; Laster, W. R.; Montgomery, J. A. *J. Med. Chem.* **1971**, *14*, 600.
(33) Johnston, T. P.; McCaleb, G. S.; Montgomery, J. A. *J. Med. Chem.* **1975**, *18*, 104.
(34) Hyde, K. A.; Acton, E.; Skinner, W. A.; Goodman, L.; Greenberg, J.; Baker, B. R. *J. Med. Chem.* **1962**, *5*, 1.
(35) Colvin, M.; Brundrett, R. B.; Cowens, W.; Jardine, I.; Ludlum, D. B. *Biochem. Pharm.* **1976**, *25*, 695.
(36) Eisenbrand, G.; Fiebig, H. H.; Zeller, W. J. *Z. Krebsforsch* **1976**, *86*, 279.
(37) Gladkovskii, G. A.; Skorokhodov, S. S. *J. Org. Chem. USSR (Engl. Transl.)* **1967**, *3*, 22.
(38) Volford, J.; Banfi, D. *J. Labelled Compd.* **1975**, *11*, 417.

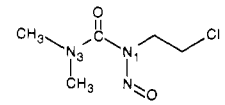
of selective nitrosation.[17,24] The CH_2 groups adjacent to N_1—N=O change relatively little in position upon increasing the solvent polarity ($CDCl_3$ to Me_2SO and pyridine). In contrast, the CH_2 group adjacent to Cl suffers a progressive downfield shift (δ 3.40–3.65). The NH_2 protons of monoalkylnitrosoureas appear in the ranges δ 5.60–5.80 and 6.90–7.10, while the NH proton of dialkylnitrosoureas appears from δ 6.90 to 7.32 in nonpolar solvents. The low-field NH resonance and the relatively large chemical shift separation in monoalkylnitrosoureas have been attributed to intramolecular hydrogen bonding with the N=O group.[39,40] However, recent X-ray crystallographic analysis of the CENU (**3a**) revealed no evidence for intramolecular hydrogen bonding with the N=O group, whose N=O bond is shown aligned toward the 2-chloroethyl group.[41] Although the analogy with solution conditions must be drawn cautiously, independent infrared evidence from the spectra of CENU in 1×10^{-3}–1×10^{-4} M solutions in $CHCl_3$ or CCl_4 reveal NH frequencies between 3420 and 3434 cm^{-1} which are within the range of free NH groups.[42] The NH chemical shifts move progressively to lower fields, and the separation between the two NH signals becomes smaller upon increasing the solvent polarity. For example, in pyridine solution the NH_2 protons of **6** are well separated at −30 °C whereas they coincide at +30 °C, and the spectrum shows evidence by the appearance of additional peaks of a slow decomposition at this temperature. This result indicates that this particular nitrosourea in a polar solvent changes from one conformation to another which then initiates decomposition. In addition, the NH proton in CENUs exchanges with D_2O faster than the rate of decomposition which indicates that the NH proton is not strongly hydrogen bonded.[42] The problems attending this NH proton exchange and line broadening due to ^{14}N quadrupole effects tend to make ^1H spectra less informative in the aqueous phosphate media of interest to CENU chemistry. Accordingly, our interest in CENU behavior in aqueous solution stimulated us to examine the ^{13}C and ^{15}N spectra of CENUs in different solvents and reaction conditions.

^{15}N NMR Spectra. The unique chemical role of the nitrogens in CENUs[43] together with the high sensitivity of ^{15}N NMR to molecular interactions,[44–46] inter- and intramolecular hydrogen bonding,[47–50] dynamic changes such as keto–enol equilibria,[51,52] N atom hybridization,[53] and orientation of the N lone pair[54] either expressed as chemical shift or coupling constant changes (i.e., ^{15}N–^1H; ^{15}N–^{15}N, or ^{15}N–^{13}C) make ^{15}N NMR especially informative in the study of the conformational properties of CENUs both before and during their decomposition.

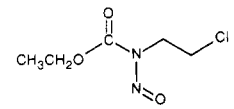
Chemical Shifts. ^{15}N chemical shift values in the amide groups of CENUs depend on the nature and extent of substitution, e.g., for the primary amides **6** and **8** they appear at 77.5 ppm (see Table I), and for BCNU (**1**) and BFNU (**9**) they appear at ~84.3 ppm while the ^{15}N signals



CENUs



10



11

1	X=Cl,	R=$ClCH_2CH_2$	[BCNU]
2	X=Cl,	R=C_6H_{11}	[CCNU]
3	X=Cl,	R=trans 4 $CH_3C_6H_{10}$	[MeCCNU]
4	X=Cl,	R=glucosamino	[Chlorozotocin]
5	X=Cl,	R=$HOCH_2CH_2$	[CHNU]
6	X=Cl,	R=H	[CNU]
7	X=F,	R=C_6H_{11}	[CFNU]
8	X=F,	R=H	[FNU]
9	X=F,	R=FCH_2CH_2	[BFNU]

in substituted cyclohexanes, i.e., **2**, **3**, and **7**, appear at ~103.1 ppm. The amide ^{15}N resonances shift downfield upon increasing the solvent polarity, reaching a maximum in the case of Me_2SO-d_6 and pyridine. The ^{15}N chemical shifts of amides change regularly with the π bond order of the C–N bond and with the π charge density on nitrogen in addition to solvent effects.[55–57] The chemical shift of the N-1 nitrogen is also dependent on substitution but is fairly constant in the range δ 268.4–269.3 in the CENUs (**1–6**) and δ 266.3–268.9 for the corresponding fluoro compounds **7–9** and shows an approximately 1–2-ppm downfield shift upon changing the solvent from $CDCl_3$ to Me_2SO-d_6. A similar solvent-dependent ^{15}N resonance in *N*-acetylproline has been attributed to changes in the orientation of the carbonyl group as a result of rotation about the N–C bond.[58]

The ^{15}N chemical shift of the N=O nitrogen appears downfield relative to aliphatic nitrosamines and closer to those of aromatic nitrosamines which may indicate extensive electron delocalization within the acyl group.[59,60] A downfield shift of the N=O nitrogen also occurs upon increasing the solvent polarity and basicity which may be either due to rotation about the N–N bond (for which there is independent evidence; q.v.) or due to solvent interaction with the nitrogen lone pair. Within the group of CENUs in $CDCl_3$ solution there is observed an upfield shift from **6** (564.5 ppm), to **1** (561.1 ppm) and **2** (560.1 ppm) which is plausibly attributed to the different extents of cross-conjugation in to the acyl carbonyl.[60]

Nitrogen–Proton Couplings. The one-bond coupling $^1J_{^{15}N-^1H}$ values of nitrosoureas generally fall within the range of trigonal nitrogen coupling constants[61–63] (Table III). In the case of BCNU (**1**) and CNU there is a decrease in coupling constant from this range to 93.5–92.6 and 91.5–90.0 Hz, depending on the solvent (Table II). A small positive difference in such couplings on changing solvent

(39) Lien, E. J.; Chou, J. T. Guadauskas, G. A. *Spectrosc. Lett.* **1972**, *5*, 293.
(40) Snyder, J. K.; Stock, L. M. *J. Org. Chem.* **1980**, *45*, 886.
(41) Smith, H. W.; Camerman, A.; Camerman, N. *J. Med. Chem.* **1978**, *21*, 468.
(42) Orchinnikov, Y. A.; Ivanov, V. T. *Tetrahedron* **1975**, *31*, 2177.
(43) Snyder, J. K.; Stock, L. M. *J. Org. Chem.* **1980**, *45*, 1990.
(44) Levy, G. C.; Lichter, R. L. "Nitrogen-15 Nuclear Magnetic Resonance Spectroscopy"; Wiley-Interscience: New York, 1979.
(45) Witanowski, M.; Stefaniak, L.; Webb, G. A. In *Ann. Rep. NMR Spectrosc.* **1977**, *7*, 117.
(46) Alei, M., Jr.; Morgan, L. O.; Wageman, W. E.; Whaley, T. W. *J. Am. Chem. Soc.* **1980**, *102*, 2881.
(47) Poulter, C. D.; Livingston, C. L. *Tetrahedron Lett.* **1979**, 755.
(48) Hawkes, G. E.; Randall, E. W.; Hull, W. N. *J. Chem. Soc., Perkin Trans. 2* **1977**, 1268.
(49) Dyllick-Brenzinger, C.; Sullivan, G. R.; Pang, P. P.; Roberts, J. D. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 5580.
(50) Axenrod, T.; Wieder, M. J. *J. Am. Chem. Soc.* **1971**, *93*, 3541.
(51) Dudek, G. O.; Dudek, E. P. *J. Am. Chem. Soc.* **1964**, *86*, 4283.
(52) Dudek, G. O.; Dudek, E. P. *J. Am. Chem. Soc.* **1966**, *88*, 2407.
(53) Schulman, J. M.; Venanzi, T. *J. Am. Chem. Soc.* **1976**, *98*, 4701, 6739.
(54) Lichter, R. L.; Roberts, J. D. *J. Am. Chem. Soc.* **1971**, *93*, 5218.
(55) Yavari, I.; Roberts, J. D. *Org Magn. Reson.* **1980**, *13*, 68.
(56) Done, J.; Gouesnard, J. P.; Mechin, B.; Naulet, N.; Martin, G. J. *Org. Magn. Reson.* **1980**, *13*, 126.
(57) Irving, C. S.; Lapidot, A. *J. Chem. Soc., Chem. Commun.* **1976**, 43.
(58) Hawkes, G. E.; Randall, E. W.; Bradley, C. H. *Nature (London)* **1975**, *257*, 767.
(59) Bonnett, R.; Holleyhead, R.; Johnson, B. L.; Randall, E. W. *J. Chem. Soc., Perkin Trans. 1* **1975**, 2261.
(60) Gouesnard, J. P.; Martin, G. J. *Org. Magn. Reson.* **1979**, *12*, 263.
(61) Binsch, G.; Lambert, J. B.; Roberts, B. W.; Roberts, J. D. *J. Am. Chem. Soc.* **1964**, *86*, 5564.
(62) Sogn, J. A.; Gibbon, Randall, E. W. *Biochemistry* **1973**, *12*, 2100.
(63) Kricheldorf, H. R. *Org. Magn. Reson.* **1980**, *13*, 52.

Table I. ^{15}N Chemical Shift Data for 1-(2-Chloroethyl)-3-alkyl-1-nitrosoureas (1-6 and 10), 1-(2-Fluoroethyl)-3-alkyl-1-nitrosoureas (7-9), and Ethyl (2-Chloroethyl)nitrosocarbamate (11)

compd	R group	solvent	^{15}N chemical shifts,[a,b] ppm		
			N_3	N_1	N=O
1	2-chloroethyl	$CHCl_3$ (1.0 M)	84.3	268.1	562.1
1a		$CHCl_3$ (0.01 M)	84.4	268.1	562.1
1a		dioxane (0.01 M)	84.9	268.4	563.6
1a		CF_3CH_2OH (0.01 M)	88.0	267.2	563.6
1		Me_2SO (1.0 M)	90.0	269.5	565.6
1a		Me_2SO (0.01 M)	90.0	269.5	565.5
1a		pyridine (0.01 M)	89.0	269.6	565.2
1a		dioxane + phosphate buffer (pH 5.0, 0.01 M)	89.1	268.8	565.1
1a		dioxane + phosphate buffer (pH 7.2, 0.01 M)	89.1	268.9	565.2
1a		dioxane + phosphate buffer (pH 9.2, 0.01 M)	89.1	268.8	565.3
2	cyclohexyl	$CHCl_3$ (1.0 M)	103.1	268.4	560.2
2a		$CHCl_3$ (0.01 M)	103.6	*c*	559.9
2a		CF_3CH_2OH (0.01 M)	106.5	*c*	561.9
2a		Me_2SO (0.01 M)	107.5	*c*	564.6
2		Me_2SO (1.0 M)	107.7	269.5	564.3
2b	cyclohexyl	$CHCl_3$ (0.01 M)	*c*	268.5	559.9
2b		dioxane + phosphate buffer (pH 5.0, 0.01 M)	*c*	269.2	564.1
2b		dioxane + phosphate buffer (pH 7.2, 0.01 M)	*c*	269.3	564.2
3	*p*-methylcyclohexyl	dioxane + phosphate buffer (1 h 7.2, 1.0 M)	103.7	268.2	560.2
4	*O*-glucopyranosyl	Me_2SO (1.0 M)	93.5,[d] 95.0	270.0, 269.4	564.9, 564.3
5	2-hydroxyethyl	$CHCl_3$ (1.0 M)	86.1	268.3	562.6
6	H	$CHCl_3$ (1.0 M)	77.5	268.3	564.5
6a		dioxane (0.01 M)	76.5	*c*	566.0
6a		CF_3CH_2OH (0.01 M)	77.3	*c*	566.9
6a		acetonitrile (0.01 M)	79.1	*c*	565.4
6a		Me_2SO (0.01 M)	84.7	*c*	568.3
6a		pyridine (0.01 M)	83.3	*c*	567.9
7	cyclohexyl	$CHCl_3$ (1.0 M)	103.9	266.3	560.6
8	H	$CHCl_3$ (0.01 M)	77.5	266.5	565.2
8a		$CHCl_3$ (0.01 M)	*c*	266.5	565.2
8a		$CHCl_3$ (0.01 M)	*c*	266.5	565.2
8a	H	Me_2SO (0.01 M)	*c*	268.9	567.8
8a		pyridine (0.01 M)	*c*	268.7	567.6
9	2-fluoroethyl	$CHCl_3$ (1.0 M)	84.3	266.1	563.1
10	dimethyl	$CHCl_3$ (1.0 M)	87.5	267.5	569.3
11		$CHCl_3$ (1.0 M)		266.4	582.7

[a] Proton-decoupled spectra were reported by using external ammonia as a standard and recorded by using dimethylformamide as external reference. Approximately 84-86K scans were required for natural-abundance compounds and approximately 1-4K scans for ^{15}N-enriched compounds. For simplicity splitting patterns arising from enriched compounds are not given. Individual couplings are given in Table III. [b] The relaxing agent $Cr(AcAc)_3$ was used in the natural-abundance spectra at 0.1 M and with the ^{15}N enriched spectra at 10-50 mM in organic solvents. In aqueous solution no relaxing agent was used. [c] Owing to enrichment for other nitrogens, this unenriched nitrogen was not observed for the concentration and the number of scans used. [d] The two sets of peaks were in a ratio of ca. 5:1 due to an anomeric mixture.

Table II

	J_{N-H}, Hz		
	Me_2SO	$CDCl_3$	ΔJ_{N-H}, Hz
CCNU	92.5	90.6	1.9
BCNU	92.6	93.5	-0.9
CNU	90.1	91.5	-1.5

from Me_2SO to $CDCl_3$ has been attributed to strong hydrogen bonding in ortho-substituted benzamides,[50] but in the case of BCNU and CNU only small negative differences were observed, in contrast to that of CCNU. The change in coupling constant may be due the difference in orientation of the lone pair or the N–H hydrogen in CCNU compared with that CNU and BCNU in the less polar solvents whereas the nitrosoureas adopt similar conformations in Me_2SO solution.

The average magnitude of the $^1J_{^{15}N-^1H}$ coupling constant for 2 and 3 in Me_2SO is close to that of the trans coupling constant in the case of peptide bonds.[62]

Simiarly, the $^2J_{^{15}N-^1H}$ two-bond coupling values for CCNU and MeCCNU in $CDCl_3$ is 1.60 Hz which changes to 0.6 Hz in Me_2SO. The observed decrease in the two-bond coupling upon increasing the solvent polarity is also attributed to rotation about the N_3–C_1' bond in addition to the association of the polar Me_2SO with the nitrogen lone pair.[64]

There is no detectable three-bond coupling, $^3J_{^{15}N-^1H}$, between the N=O group nitrogen and the proton α to N-1 either in $CDCl_3$ or in Me_2SO-d_6. This may indicate that the chloroethyl group is syn to the N=O group as has been reported in the case of nitrosamines, sydnones, and sydnonimines.[64-66] However, this may also be due to the noncoplanarity of the N=O and chloroethyl groups as is

(64) Danoff, A.; Franzen-Sieveking, M.; Lichter, R. L.; Fanso-Free, S. N. Y. *Org. Magn. Reson.* **1979**, *12*, 83.
(65) Stefaniak, L.; Witanowski, M.; Kamienski, B.; Webb, G. A. *Org. Magn. Reson.* **1980**, *13*, 274 and references therein.
(66) Stefaniak, L.; Witanoski, M. *Bull. Acad. Pol. Sci., Ser. Sci. Chim.* **1977**, *25*, 261.

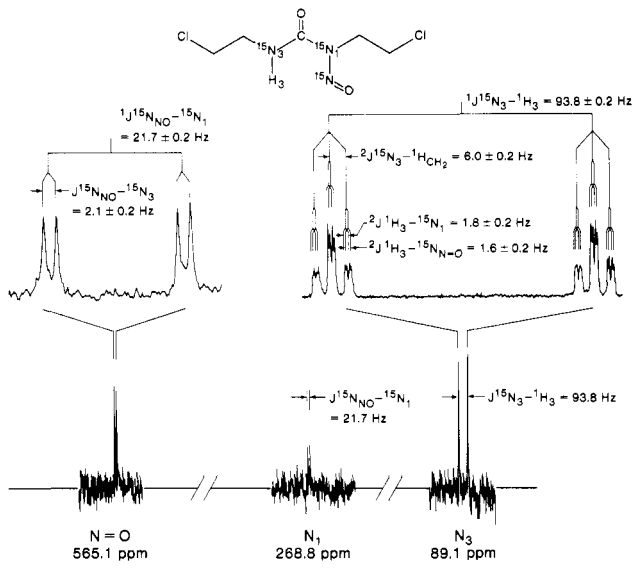


Figure 1. Proton-coupled ^{15}N NMR spectrum (Bruker WH200 instrument operating at 20.285 MHz) of ^{15}N specifically labeled 0.01 M BCNU (**1a**) in 50:50 dioxane–potassium phosphate buffer (pH 7.2) at 30 °C by employing 5K scans with a pulse width of 60 μs and a repetition rate of 0.67 s showing all three ^{15}N signals. (a) The proton-decoupled ^{15}N=O signal is expanded to show additional ^{15}N fine splitting by employing 100 scans with a pulse width of 100 μs and a repetition rate of 8.2 s. (b) The expanded ^1H spectrum at 200 MHz of the NH proton in $CHCl_3$ reveals additional ^{15}N and ^1H couplings. (These small couplings were not well resolved in the dioxane aqueous buffer medium owing to exchange processes.)

indicated (at least in the case of MeCCNU) for the solid state by X-ray diffraction.

^{15}N–^{15}N Coupling Constants. The value of the one-bond coupling constant $^1J_{^{15}N-^{15}N}$ depends upon hybridization, the Fermi contact term, and lone-pair delocalization.[67-70] The ^{15}N–^{15}N coupling constants for nitrosoureas **1**, **2**, and **8** are between 21.0 and 22.5 Hz (Figure 1) which may be due to the greater electron delocalization in the acyl group when compared with the reported values for diphenylamine[70] ($^1J_{^{15}N-^{15}N}$ = 22.0 Hz).

The $^1J_{^{15}N-^{15}N}$ coupling constant is normally greater for the cis isomer than the trans isomer. The $^1J_{^{15}N-^{15}N}$ for the nitrosoureas **1**, **2**, and **8** increase slightly as a result of increasing solvent polarity and pH. The two-bond coupling constants $^2J_{^{15}N-^{15}N}$ for the nitrosoureas have values in the range 2.0–3.0 Hz as compared with those reported for ureas in the range of 4.5–5.0 Hz.[71] The three-bond coupling, $^3J_{^{15}N-^{15}N}$, between the N=O group nitrogen and N_3 for BCNU in dioxane–phosphate buffer is 2.1 Hz (Figure 2, supplementary material), whereas for CNU in Me_2SO-d_6 the value is 1.6 Hz.

^{13}C NMR Spectra. The potential of ^{13}C NMR in the study of dynamic processes including conformational analysis,[72-75] e.g., its successful application in the differentiation of the possible orientations of the N=O group in nitrosamines by the use of both chemical shifts[74] and coupling constants,[54] prompted us to examine the ^{13}C NMR spectra of (2-haloethyl)nitrosoureas. In addition, the sensitivity of ^{13}C chemical shifts toward hybridization changes dictated that ^{13}C NMR was an ideal method for studying the possibility of hydration of the amidic carbon during the aqueous decomposition of clinically used CENUs.

Chemical Shifts. The carbonyl carbons in nitrosoureas resonate at 151.32–155.40 ppm in chloroform solution and shift slightly to lower field in more polar solvents (Table III). The greatest upfield shifts in $CDCl_3$ are observed for CCNU and MeCCNU which is in keeping with the increasing double bond character of the amide bonds in these compounds (Table III). For a particular nitrosourea the carbonyl resonance is broad and of relatively low intensity which only at low temperature sharpens and increases in signal height without changing position. This seems to indicate a large value for the conformational equilibrium constant, and the nitrosoureas exist predominantly in one preferred conformation over a wide temperature range. The chemical shifts for the ethylene portion of **2a** can be assigned as δ 40.67 (C_1) and 38.89 (C_2) since the positions of these carbons are confirmed by examination of the specifically deuterium-labeled compounds BCNU-α-d_4, BCNU-β-d_4, CCNU-α-d_2, and CCNU-β-d_2. The deuterium-labeled carbon does not appear, owing to partial signal loss by the nuclear Overhauser effect, signal broadening due to coupling to the deuterium, and comparatively long T_1 values. The chemical shifts of the adjacent carbons appear 0.1 ppm per deuterium to higher field than for their protium counterparts. In the case of BCNU (**1**) the nitroso groups bearing side-chain carbons resonate at higher field δ 40.24 (C_1) and 38.96 (C_2) than the carbons for the chloroethyl side chain that bear no N=O group, i.e., δ 42.55 (C_1') and 43.23 (C_2') in $CDCl_3$ solution which are in accord with the chloroethyl group being aligned syn to the N=O group. A close analogy exists for this assignment in the case of the corresponding nitrosamines. The shielding influence of the NO group extends to the C_1 carbon which is significantly shielded compared with C_2'. Further support for this preferred conformational assignment is obtained from the two-bond ^{15}N–^{13}C coupling constants obtained with specifically labeled ^{15}N compounds (q.v.).

The C_1 carbon in (2-fluoroethyl)nitrosoureas (FENUs, **7**) is unambiguously assigned since it appears as a doublet at 39.00 ppm coupled to fluorine ($^2J_{^{13}C-^{19}F}$ = 22.5 Hz), and C_2 appears at 79.42 ppm ($^1J_{^{13}C-^{19}F}$ = 173.2 Hz).[75] Similar carbon–fluorine couplings are observed in the case of other FNUs (**8** and **9**) and change little with a change of solvent (Table III).

^{15}N–^{13}C Coupling Constants. The one bond couplings $^1J_{^{15}N_3-^{13}C=O}$ in the case of CCU and BCU are 20.0 and 19.9 Hz, respectively, which are in accord with those found for other ureas.[71,76,77] A number of factors including hybridization, solvent properties, and the Fermi contact term are known to contribute to ^{13}C coupling constants. However, when compared in the same solvent (Me_2SO), the $^1J_{^{15}N_3-^{13}C}$ values for CENUs are somewhat higher, 24.5, 22.5, and 21.5 Hz (Table IV), indicative of a decrease of lone-pair delocalization on the N_3 nitrogen atom compared with that for

(67) Lichter, R. L.; Srinivasan, P. R.; Smith, W. A. B.; Dieter, R. K.; Denny, C. T.; Schulman, J. M. *J. Chem. Soc., Chem. Commun.* **1977**, 366.
(68) Balusu, R. S.; Autera, J. R.; Axenrod, T. *J. Chem. Soc., Chem. Commun.* **1973**, 602.
(69) Axenrod, T.; Mangiaracina, P.; Pregosin, P. S. *Helv. Chim. Acta* **1976**, *59*, 1655.
(70) Schultheiss, H.; Fluck, E. *Z. Naturforsch.* **1977**, *32*, 257.
(71) Coxon, B.; Fatiadi, A. J.; Cohen, A.; Hertz, H. S.; Schaffer, R. *Org. Magn. Reson.* **1980**, *13*, 187.
(72) Binsch, G.; Kessler, H. *Angew. Chem., Int. Ed. Engl.* **1980**, *79*, 411.
(73) Wehrli, F. W.; Wirthlin, T. "Interpretation of Carbon-13 NMR Spectra"; Heyden: London, 1976.
(74) Pregosin, P. S.; Randall, E. W. *J. Chem. Soc., Chem. Commun.* **1971**, 399.
(75) Stothers, J. B. "Carbon-13 NMR Spectroscopy"; Academic Press: New York, 1972; p 332.
(76) Kricheldorf, H. R. *Org. Magn. Reson.* **1980**, *14*, 455 and references therein.
(77) London, R. E.; Walker, T. E.; Whaley, T. W.; Matwiyoff, N. A. *Org. Magn. Reson.* **1977**, *9*, 598.

Table III. ^{13}C Chemical Shift Data for 1-(2-Chloroethyl)-3-alkyl-1-nitrosoureas (1-6 and 10), 1-(2-Fluoroethyl)-3-alkyl-1-nitrosoureas (7-9), and Ethyl (2-Chloroethyl)nitrosocarbamate (11)

			chemical shifts,[a] ppm							
compd	R group	solvent	C=O	C_1	C_2	C_1'	C_2' (C_6')	C_3' (C_5')	C_4'	others
1	2-chloroethyl	$CDCl_3$	153.00	40.24	38.96	42.55	43.28			
1		$CDCl_3$	153.14	*c*	38.65	*c*	43.15			
1		$CDCl_3$	153.13	40.04	*c*	42.37	*c*			
1		Me_2SO-d_6	153.00	40.17	39.73	42.12	42.65			
1		pyridine	154.16	40.65	39.97	43.14	43.51			
1a		$CDCl_3$	153.21	40.17	38.87	42.50	43.36			
1a		Me_2SO-d_6	153.10	40.42	39.77	42.17	42.67			
2	cyclohexyl	$CDCl_3$	151.86	40.07	38.89	50.05	33.11	24.80	25.80	
2		$CDCl_3$	152.05	*c*	38.60	50.05	33.50	24.97	25.52	
2		$CDCl_3$	152.02	40.01	*c*	50.05	33.37	24.97	25.52	
2		pyridine	151.78	40.61	40.04	50.76	33.12	25.43	25.69	
2a		$CDCl_3$	151.87	40.05	38.90	50.00	33.13	24.76	25.44	
2a		Me_2SO-d_6	151.78	40.37	39.81	49.95	32.01	24.77	24.96	
2b		$CDCl_3$	151.32	40.01	38.87	50.04	33.09	24.77	25.43	
3	*p*-methylcyclohexyl	$CDCl_3$	151.91	40.02	38.85	50.33	33.96	33.88	33.12	22.06
3		pyridine	152.95	40.64	40.04	50.82	34.14	33.03	32.07	22.22
4	*O*-glycopyranosyl[e]	Me_2SO-d_6	152.45	40.23	39.77	56.24	70.32, 90.24	70.30	73.22	60.97
4		pyridine	153.83	40.60	39.77	57.64	72.98, 92.37	72.98	73.95	63.03
5	2-hydroxyethyl	$CDCl_3$	153.60	40.20	38.90	40.10	61.60			
5		D_2O	155.50	41.20	40.20	43.00	60.66			
6	H	$CDCl_3$	154.59	39.89	38.89					
6	H	pyridine	155.14	40.23	40.04					
6a	H	$CDCl_3$	154.21	39.83	38.85					
6a	H	Me_2SO-d_6	153.50	40.36	39.77					
7	cyclohexyl	$CDCl_3$	151.97	39.00 ($^2J_{C-F}$ = 22.5)	79.42 ($^1J_{C-F}$ = 173.2)	49.95	33.12	24.96	25.49	
8	H	pyridine[d]	155.38	39.32 ($^2J_{C-F}$ = 21.9)	80.61 ($^1J_{C-F}$ = 169.7)					
8	H	$CDCl_3$ + CD_3OD	154.95	38.97 ($^2J_{C-F}$ = 22.2)	78.60 ($^1J_{C-F}$ = 170.9)					
8a	H	$CDCl_3$ + acetone	154.43	38.89 ($^2J_{C-F}$ = 22.9)	79.55 ($^1J_{C-F}$ = 170.9)					
8a	H	Me_2SO-d_6	153.8	39.20 ($^2J_{C-F}$ = 21.3)	80.00 ($^1J_{C-F}$ = 167.5)					
9	2-fluoroethyl	$CDCl_3$	153.17	39.28 ($^2J_{C-F}$ = 22.5)	79.55 ($^1J_{C-F}$ = 172.7)	40.62[b] ($^2J_{C-F}$ = 20.5)	82.29 ($^1J_{C-F}$ = 168.8)			
9		Me_2SO-d_6	153.17	38.52 ($^2J_{C-F}$ = 21.9)	79.94 ($^1J_{C-F}$ = 167.4)	40.86 ($^2J_{C-F}$ = 20.9)	81.64 ($^1J_{C-F}$ = 166.6)			
9		pyridine[d]	154.39	39.76 ($^2J_{C-F}$ = 22.9)	80.35 ($^1J_{C-F}$ = 170.0)	40.60 ($^2J_{C-F}$ = 21.6)	82.73 ($^1J_{C-F}$ = 166.4)			
10	dimethyl	$CDCl_3$	155.55	39.98	38.95	42.55				
10		pyridine	155.56	40.97	40.97	42.85				
11		$CDCl_3$	153.74	41.45	38.91	64.83	14.25			
11		pyridine[d]	153.81	41.80	40.05	64.90	14.17			

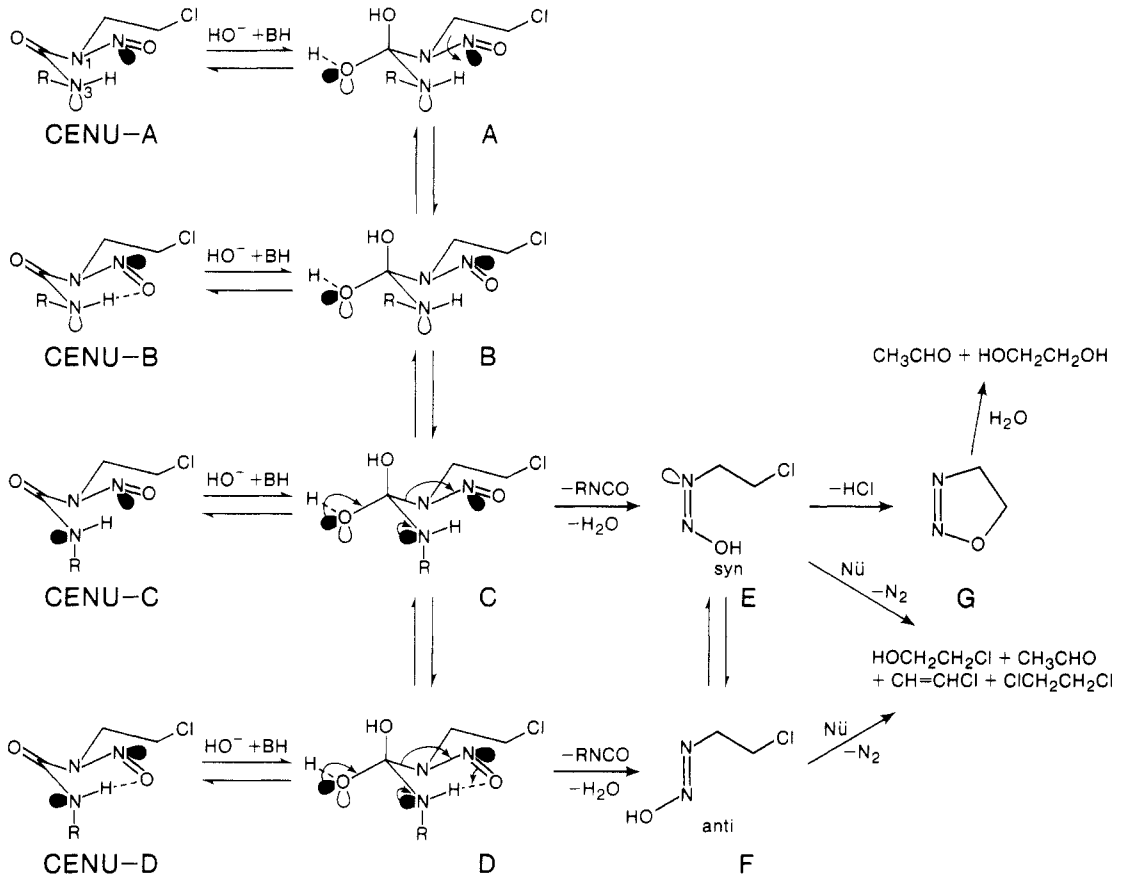
[a] ^{13}C proton-decoupled spectra were recorded by using Me_4Si as an internal reference. The carbons are numbered sequentially from the ureido function. The J values are in hertz. [b] Normally 40-86K scans were used for natural-abundance carbonyl carbon. A greater number of scans are required for the ^{15}N-labeled nitrogen compounds because of relaxation effects. [c] This carbon peak was not detectable when hydrogen is substituted with deuterium, owing to concomitant partial loss by NOE deuterium signal splitting and the comparatively longer relaxation time T_1. [d] Spectra were recorded at 0 °C because at room temperature the compound decomposes during the number of scans used. [e] The ^{13}C spectrum of chlorozotocin in phosphate buffer and triethylammonium bicarbonate buffer (pH 7.4) at 37 °C has been reported (Hammer, C. F.; Loranger, R. A.; Schein, P. S. *J. Org. Chem.* **1981**, *46*, 1521). This showed only decomposition products which we have also observed in phosphate buffer (pH 7.2) at 31 °C. For chlorozotocin the carbonyl peaks and the peak for the carbon adjacent to the nitroso group are broadened owing to the presence of an anomeric mixture.

Table IV. ^{15}N Coupling Constants for 1-(2-Chloroethyl)-3-alkyl-1-nitrosoureas 1a, 2a,b, and 6a and 1-(2-Fluoroethyl)-3-alkyl-1-nitrosourea (8a) in Hertz

compd	solvent	proton coupling constants[a]		carbon ^{13}C coupling constants[b]						nitrogen coupling constant,[c] $^1J_{^{15}N-^{15}N}$
		$^1J_{^{15}N-H}$	$^2J_{^{15}N-H}$	$^1J_{^{13}C(O)-^{15}N_3}$[e]	$^1J_{^{13}C_1(O)-N_1}$[e]	$^2J_{^{13}C(O)-^{15}N}$[e]	$^1J_{^{13}C-^{15}N_1}$	$^2J_{^{13}C-^{15}N=O}$	$^1J_{^{13}C-^{15}N_3}$	
1a	$CDCl_3$	93.5	1.5	22.5	17.2	4.5	7.5	1.4	12.0	21.0 ± 1.5
1a	CF_3CH_2OH	94.0	1.6							22.5 ± 0.2
1a	Me_2SO-d_6	92.6	0.6	21.5	17.6	5.0	7.5	1.2	11.0	22.5 ± 1.5
1a	pyridine	92.3	0.6							21.9 ± 0.2
1a	dioxane + phosphate buffer (pH 5.0)	93.8[d]								21.7 ± 0.2
1a	dioxane + phosphate buffer (pH 7.0)	93.8[d]								21.7 ± 0.2
2a	$CDCl_3$	90.6	1.8	24.0		4.5			10.5	
2a	CF_3CH_2OH	92.0	1.5							
2a	Me_2SO-d_6	92.5	0.6	23.8		4.5			11.6	
2b	$CDCl_3$				16.2	3.7	7.3			22.2 ± 1.5
2b	Me_2SO-d_6				16.2	4.5	8.5			
2b	dioxane + phosphate buffer (pH 5.0)					4.5				22.3 ± 1.5
2b	dioxane + phosphate buffer (pH 7.2)					4.5				21.7 ± 0.2
6a	$CDCl_3$	91.5		24.2		4.5	4.5			
6a	CF_3CH_2OH	73.5								
6a	Me_2SO-d_6	90.0		24.5		5.0				
8a	$CDCl_3$				18.3	5.1	8.0			22.3 ± 1.5
8a	Me_2SO-d_6				16.2	5.1	7.3			22.3 ± 1.5
8a	pyridine-d_5									22.3 ± 1.5

[a] Nitrogen–proton coupling constants were obtained from proton spectra or proton-coupled ^{15}N spectra. Proton coupling with nitroso nitrogen and the proton α to nitroso group are less than 1.0 Hz. [b] Nitrogen carbon coupling constants were obtained by proton-decoupled ^{13}C spectra and are reported ±1.5 Hz. [c] Nitrogen–nitrogen coupling constants were obtained by proton-decoupled ^{15}N spectra. Two-bond $^2J_{^{15}N-^{15}N}$ coupling with N_1 and N_2 are solvent dependent within the range (1.8–3.0) ± 0.2 Hz. The three-bond couplings observed are within the range of (1.6–2.0) ± 0.2 Hz and are also solvent dependent. [d] The observed one-bond $^1J_{^{15}N-^1H}$ coupling constant in aqueous solution indicates that in the case of BCNU under these conditions proton exchange with the solvent is slow on the ^{15}N NMR time scale. [e] Carbonyl carbon appeared only after at least 50–85K scans due to longer relaxation times, T_1's, and the splitting with neighboring ^{15}N nitrogens.

Scheme IV



the corresponding ureas. The latter exhibit a smaller lone-pair delocalization than for normal amide bonds.[53,71] In contrast, the $^{1}J_{^{15}N_1-^{13}C}$ values are smaller at 17.6 and 16.2 Hz, which may signify an increase of s character of the N_1 lone-pair orbital, making a positive contribution to the Fermi contact term.[53] A similar explanation may be advanced for the difference in one-bond coupling with the α-carbon to the N_3 and N_1 nitrogen atoms in **1a** (Table IV).

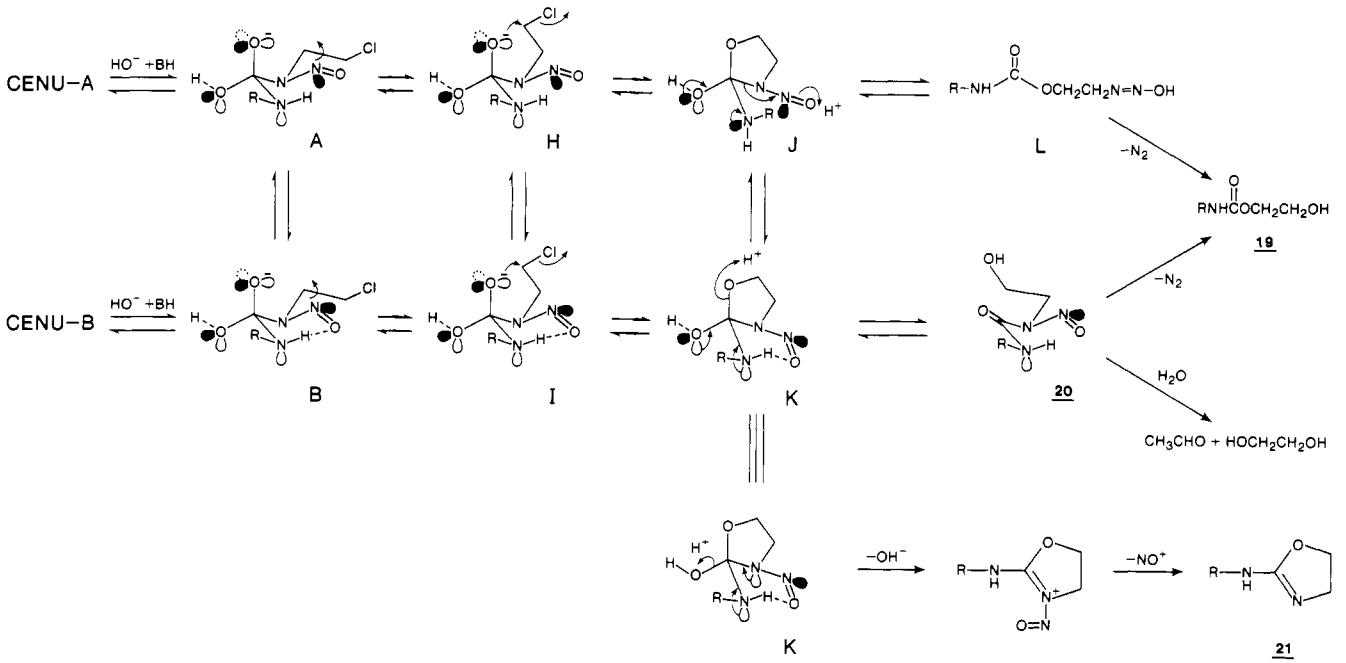
The relatively large $^{2}J_{^{15}N-^{13}C}$ between the N=O group and the C=O group of 5.0–3.66 Hz (Table IV) is in accord with a W conformation of this moiety. The very small ~1.2–1.4-Hz value of $^{2}J_{^{15}N=O-^{13}C}$ with C_1 is also in agreement with models for a syn arrangement of the N=O and chloroethyl groups, i.e., corresponding to an orientation of the lone pair close to C_1 and leading to a larger numerical value for the coupling.[44] If the nitroso group prefers to be anti to the carbonyl group (owing to an extended W conjugation) in aprotic solvents, this would account for the single conformation observed. Further, the $^{2}J_{^{15}N-^{13}C}$ for the CENUs are different and solvent dependent (in contrast to the $^{1}J_{^{15}N-^{13}C}$ values) as would be anticipated if a rotation occurred about the N–N bond as a result of interaction of the solvent with the nitrogen lone pair.[64] This conclusion is also in accord with reesults from the $^{3}J_{^{15}N-^{1}H}$ coupling values.

Since the CENUs adopt a single conformation in aprotic solvents in which the nitroso group is syn to the chloroethyl group and the nitroso nitrogen lone pair is anti to it, one may consider the possibility of a weak hydrogen bond between the N_3H proton and the nitrogen lone pair of the nitroso group. Evidence has been obtained for such intramolecular hydrogen bonding[78,79] in which the minimum distance between the bonded atoms should be 2.60 Å and the maximum distance at which any bonding is possible should be 3.2 Å. Under these circumstances Schiener and Kolb have shown that such a hydrogen bond results in substantial bending of the bond and stretching of the nitrogen lone pair.[78] The bonding distance between the hydrogen of the N_3H and the nitroso group nitrogen is estimated to be 2.28 Å from the X-ray diffraction data on MeCCNU[41] and so falls within possible bonding distance. A weak intramolecular hydrogen bond of this type may be one of the reasons for the CENUs to adopt a single conformation in nonpolar solvents. The $^{2}J_{^{15}N-^{13}C}$ values for CENUs are solvent dependent as is to be expected if facile rotation about single bonds, as well as inversion of configuration at the N_3 nitrogen, and preferential solvent interaction with the N=O nitrogen lone pair readily break a weak intramolecular hydrogen bond.[80,81]

Formation of Tetrahedral Intermediates in Aqueous Decomposition of CENUs. Specifically ^{13}C carbonyl group enriched BCNU and CCNU bearing additional N—^{15}N=O labels were synthesized in order to overcome the longer relaxation time, T_1, of the C=O group, to permit the examination of rapid conformational changes, and possibly to detect tetrahedral intermediates anticipated from the hydration of the carbonyl group. The behavior of these compounds in different solvents including aqueous buffer was examined by ^{13}C NMR. Incorporation of an ^{15}N label affords an opportunity via the magnitude of the $^{2}J_{^{13}C-^{15}NO}$ coupling of assessing conformational changes. Compound **1b** (CENU-A in Scheme IV) shows a sharp

(78) Schreiner, S.; Kolb, V. M. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 5607.
(79) Chiu, Y. J.; Lipscomb, W. N. *J. Am. Chem. Soc.* **1975**, *97*, 2525.
(80) Butter, R. N.; Lambe, T. M.; Tobin, I. C.; Scott, F. L. *J. Chem. Soc., Perkin Trans. 1* **1973**, 1357.
(81) Obvsson, Z.; Jonnson, P.-G. In "The Hydrogen Bond"; Schuster, P., Zundel, G., Sandorfy, C., Eds.; North-Holland Publishing Co.: Amsterdam, 1976; Part II, p 393 and references therein.

Scheme V



doublet at 155.56 ppm with $^2J_{^{13}C-^{15}N=O}$ = 4.5 ± 0.1 Hz. Virtually the same spectrum was obtained in a mixture of aqueous buffer at pH 7.2 and deuterated methanol. Hydration of the C=O group to form a long-lived, intermediate, *stable* species with concomitant change of hybridization from sp^2 to sp^3 would have resulted in a marked upfield chemical shift. No such peak appeared when the solution was kept at ambient temperature in the pH range 9.2–5.0; however, a new doublet appeared (δ 156.28, $^2J_{^{13}C-^{15}NO}$ = 4.9 ± 0.1 Hz) which was attributed to a carbonyl group and was in accord with slow formation of rotamer CENU-C (Scheme IV). When the solution was maintained at 31 °C, the second peak increased in intensity at the expense of the first doublet. During the slow conformational change from A to B, C, or D additional smaller peaks accumulated in the carbonyl region of the ^{13}C NMR spectrum which could be assigned to decomposition products including the previously identified carbamates as well as BCU and the oxazoline **21**[23,24] (see Scheme V).

Similar treatment of the specifically ^{13}C enriched CCNU **2c** afforded evidence for the appearance of a new carbonyl species appearing at 155.42 ppm with a somewhat larger $^2J_{^{15}N-^{13}C}$ (5.1 Hz) than the peak at 154.36 ppm with $^2J_{^{15}N-^{13}C}$ = 4.5 Hz.

It was conceivable that these new doublets could be the result of the deuterium exchange at the NH group to ND. However, such isotope shifts have been observed to be small (0.1–0.4 ppm)[82,83] and upfield, whereas the observed shift in the present case is larger (~1.0 ppm) and downfield.

Incubation of compound 11 with $H_2{}^{18}O$ (22.2% ^{18}O enriched) in dioxane–water at pH 7 and examination of the extracted material by mass spectrometry gave evidence of a small but definite extent of ^{18}O incorporation specifically in the carbonyl group (recognized by the appropriate fragment) and none in the nitroso group. The most common fragmentation is the rupture of the bond between the carbonyl group and the *N*-nitroso moiety.[84] This bond breakage which also corresponds to the primary event in solution affords two fragments, $ClCH_2CH_2NHC{\equiv}{}^{18}O$ and $ClCH_2CH_2N{=}NOH$, and was used in the above exchange studies.

In this context it may be mentioned that there are some parallels between the behavior of CENUs in solution and under electron impact. Mass spectral examination of the specifically prepared N_3D deuterium-exchanged species gave the corresponding nitroso group containing the fragment $ClCH_2CH_2N{=}NOD$ which supports the conclusion obtained from the ^{13}C NMR and ^{15}N NMR that upon activation a change to the amide *syn*-nitroso conformation occurs followed by intramolecular hydrogen bonding and proton abstraction (in this case deuteron abstraction). Precisely the same rearrangement and selective deuteron abstraction occurs under the low-temperature conditions of chemical ionization.

When the ^{13}C carbonyl labeled BCNU **1b** was examined in $H_2{}^{18}O$ although (as noted above), there was mass spectral evidence of oxygen exchange at the carbonyl group, and there was no detectable shift in the carbonyl ^{13}C NMR resonance.[85] Therefore, the new doublets that appear in protic solvents for BCNU and CCNU cannot be attributed to isotopic shifts but instead are more plausibly explained by the appearance of the new rotamer CENU-C (Scheme IV). When the reaction of BCNU **1b** or CCNU **1c** was examined in dioxane–phosphate buffer at pH 7.2, the appearance of the new ^{13}C doublets was slower than in the corresponding reaction in methanol–phosphate. These new species do not appear in nonpolar aprotic solvents, e.g., $CHCl_3$, dioxane, or pyridine, in a wide range of temperatures.

Therefore, the new ^{13}C doublets that appear only in phosphate buffer can be explained via the formation of tetrahedral intermediates (A–D, Scheme IV), followed by rearrangement to give new isomeric CENUs. Rotation about the N–N bond in, e.g., A (Scheme IV), will be easier than rotation in the corresponding CENU-A owing to

(82) Feeney, J.; Partington, A.; Roberts, G. C. K. *J. Magn. Resn.* **1974**, *13*, 268.
(83) Coppola, G. M.; Domon, R.; Kahle, A. D.; Shapiro, M. J. *J. Org. Chem.* **1981**, *46*, 1221.
(84) Rainey, W. T.; Christie, W. H.; Lijinsky, W. *Biomed. Mass Spectrom.* **1978**, *5*, 335.
(85) Vederas, J. C.; Nakashima, T. T. *J. Chem. Soc., Chem. Commun* **1980**, 183.

delocalization in the latter resulting in substantial N–N double bond character. Similarly, the N_2 lone pair is also more delocalized in the CENU than in the tetrahedral intermediate, favoring nitrogen inversion in the latter case. The evidence for the formation of tetrahedral intermediates in the aqueous decomposition of CENUs rests on the following criteria. (i) The yields of the major decomposition products, e.g., 2-chloroethanol and acetaldehyde, are dependent on the pH of the reaction medium.[14-16,21,22] (ii) The rate of decomposition of CENUs is dependent on the nature and concentration of the buffer used. The hydrolysis rates of CENUs at pH 7.0 are about 25% greater in phosphate buffer than in tris(hydroxymethyl)-aminomethane buffer.[21] (iii) Monoalkylnitrosoureas decompose more readily than the corresponding dialkylnitrosoureas[1,2] which may be due to steric hindrance to the nucleophilic attack of hydroxyl on the carbonyl group. (iv) There is ^{18}O exchange at the carbonyl group exclusively in CENUs which is most plausibly explained by a tetrahedral intermediate and may involve stereoelectronic control.[86,87]

General Schemes IV and V have been formulated on this basis which account for the characteristic behavior of CENUs in aqueous media and for the observed products of decomposition. Application of the principles of conformational analysis and of the stereochemical interrelationships of reactants and products led to the realization of the operation of stereoelectronic control in these processes.

Implications of Stereoelectronic Control in the pH-Dependent Aqueous Decomposition of (2-Haloethyl)nitrosoureas. The above results, providing evidence for strict base and solvent control of the conformation of CENUs and for the existence of tetrahedral intermediates, provide an opportunity for analysis by stereoelectronic control of the reactions as has been carried out, e.g., in the analogous tetrahedral intermediates in the hydrolysis of esters and amides.[86,87] The general CENUs adopt the initial conformation 1 depicted in Scheme IV. In aqueous solutions it will first form the tetrahedral intermediate[86] A which will exist as A^+, $A^{\pm}$, or A^-, depending on the pH of the reaction medium. Deslongchamps and others[86,87] have delineated the stereoelectronic factors controlling the cleavage of a tetrahedral intermediate in the following way: specific cleavage of a carbon–oxygen or carbon–nitrogen bond occurs when two heteroatoms (oxygen or nitrogen) of the tetrahedral intermediate each have an orbital oriented antiperiplanar to the departing *O*-alkyl or *N*-alkyl leaving group. The preferred rotamer conformation depicted in A (which places the largest group anti) cannot break down via stereoelectronic control, owing to the inappropriate orientation of the N_3 lone pair. The stereoelectronic theory implies that when a tetrahedral intermediate cannot break down with stereoelectronic control, the energy barrier for its cleavage becomes larger than that for rotation to give other conformers. Consequently, it seems reasonable that conformer A may undergo either rotation about the N—N=O bond to give conformer B or rotation about the N_3—C bond or configurational inversion[88-90] at the N_3 nitrogen to give intermediate C. (It is difficult to differentiate between rotation about the N_3—C bond or inversion at present.)

Reversible dehydration of B affords CENU-B, the more stable conformer that CENUs adopt in protic solvents and the appearance of which was revealed by the specific ^{13}C labeling. Since conformation CENU-B is not detected in aprotic solvents, one may conclude that the conversion of CENU-A to CENU-B (or CENU-C or CENU-D) probably does not occur directly.

In the tetrahedral intermediate C the lone-pair orbitals on both heteroatoms may be aligned antiperiplanar to the C–N_1 bond, thus favoring cleavage of the latter to give the *syn*-(2-chloroethyl)diazohydroxide (E) and the alkyl isocyanate. The disposition of the nitrogen lone pair on the former species (i.e., anti to the leaving group) favors decomposition to give the observed products. The corresponding inversion of configuration at N_3 gives the tetrahedral intermediate D directly from B. In species D the lone-pair orbitals are also approximately aligned antiperiplanar (as in C), affording in this case the *anti*-(2-chloroethyl)diazohydroxide (F) together with isocyanate. Because of the intramolecular hydrogen bonding that obtains in D, the resultant conjugative withdrawal of electronic charge from the C–N_1 bond may tend to weaken it and promote the cleavage of intermediate D compared with C.

Moss has estimated that the interconversion of syn to anti isomers of arene diazotates requires $\Delta G \approx 20$ kcal mol^{-1} at 300 K.[91] Therefore, the corresponding ΔG should be greater for alkanediazoates in which the effects of resonance are absent. While it is recognized that the interconversion of *syn*- and *anti*-alkanediazotates is extremely difficult;[91,92] however, there are no direct thermodynamic data available concerning the possible rates of interconversion of the corresponding alkyldiazo hydroxides.[93,94]

The *anti*-(2-chloroethyl)diazohydroxide (F) may also decompose directly to give the observed products.[23,24] It may be noted that the *syn*-(2-chloroethyl)diazohydroxide (E) is correctly aligned to afford the 1,2,3-oxadiazoline species G by intramolecular displacement of chloride. This process may be expected to be especially favored under somewhat higher pH conditions where the diazotate may be formed. Species G has been suggested as an intermediate and may plausibly be expected to give rise to acetaldehyde and ethylene glycol.[22]

A second sequence of chemical changes can also take place (see Scheme V). Conformational changes in A and B may bring the chlorine of the side-chain into proper alignment (conformers H and I, respectively) to permit intramolecular nucleophilic displacement by the oxygen of the species A and B to form new tetrahedral intermediates J and K, respectively. In the tetrahedral intermediates of the type A to I the deprotonation or reprotonation of the oxygen is assumed to be faster than other chemical events.[87] The suggestion of the intermediacy of such oxazolidines receives support from the isolation, characterization, and examination of the aqueous decomposition products of 2-(alkylimino)-3-nitrosooxazolidines corresponding to BCNU, CCNU, and MeCCNU.[23,24] Stereoelectronic control may also plausibly operate in the subsequent decomposition of species J and K. In the case of J the two heteroatom lone-pair orbitals are correctly aligned to promote cleavage of the C_2–N_3 bond of the oxazolidine to afford the alkyl carbamate diazohydroxide

(86) Bizzozero, S. A.; Dulter, H. *Bioorg. Chem.* **1981**, *10*, 46.
(87) Deslongchamps, P. *Tetrahedron* **1975**, *31*, 2463.
(88) Lambert, J. B. *Top. Stereochem.* **1971**, *6*, 19.
(89) Jackson, W. G.; Sargeson, G. In "Rearrangements in Ground and Excited States"; de Mayo, P. D., Ed.; Academic Press: New York, 1980; Part 2, p 273.
(90) Saavedra, J. E. *J. Org. Chem.* **1979**, *44*, 860.
(91) Moss, R. A. *Acc. Chem. Res.* **1974**, *7*, 421.
(92) Moss, R. A.; Powell, A. J. *J. Am. Chem. Soc.* **1976**, *98*, 283.
(93) Sterba, V. In "The Chemistry of Diazonium and Diazo Groups"; Patai, S., Ed., Wiley: New York, 1978,; Part I, p 71.
(94) Hegarty, A. F. In "The Chemistry of Diazonium and Diazo Groups"; Patai, S., Ed.; Wiley: New York, 1978; p 511.

L. Elimination of nitrogen from the latter accounts for the carbamates **19** which are isolated among the decomposition products of both the CENUs and the corresponding 2-(alkylimino)-3-nitrosooxazolidines.[23,24]

In contrast, the antiperiplanar alignment of heteroatom orbitals in K (which like B may be stabilized by intramolecular hydrogen bonding) predicts the preferential breakage of the 1,2-oxazolidine bond to give the known compound **20**. Compounds **20** have already been demonstrated to give rise to acetaldehyde and ethylene glycol as well as carbamates **19**.[23,24] Finally, it may be noted that the alignment of the heteroatom lone pairs in the tetrahedral intermediate K (invoking in this case the lone pair of the 3-oxazolidine nitrogen) predicts a competing pathway of decomposition leading to elimination of nitrous acid to afford the 2-(alkylamino)oxazoline **21** which is observed as one of the decomposition products of CENUs.

The above discussion and Schemes IV and V which attempt to interpret much of the known chemistry of CENUs in aqueous media in terms of strict stereoelectronic control also predicts a strong pH dependence. For example acidic conditions leading to the protonation of the lone pairs of the N_3 nitrogen in, e.g., tetrahedral intermediates C and D, or of the nucleophilic oxygens in H and I will disfavor decomposition which is in accord with the observed stability of CENUs in acidic media, although denitrosation of the nitrosooxazolidine corresponding to K (Scheme V) has been observed.[24] Conversely, high pH conditions should favor decomposition which is also in accord with the facts.

The series of equilibria outlined in Schemes IV and V meet the criteria for application of the Curtin–Hammett principle;[95] i.e., a nonstoichiometric mixture of reaction products which are not readily interconvertible is formed from a single reactant which can exist in conformational equilibria. This principle cautions us against assuming that the *anti*-diazohydroxide should necessarily predominate as a product merely because the hydrogen-bonded conformer of the nitrosourea tends to form in polar solvents. The ratio of the rates of formation of the *syn*- and *anti*-diazoates is determined solely by the difference in standard potentials of the transition states involved in their formation. The product distribution, i.e., the *syn*- and *anti*-diazohydroxides and the corresponding electrophiles generated therefrom, may well be kinetically controlled. Our present knowledge of the equilibria existing in solution which precede the biological lesions may be summarized in Schemes IV and V. Efforts directed toward determining the actual configuration of the diazohydroxide generated from CENUs employing specific ^{15}N labeling will be reported subsequently.

Conclusions

Spectral evidence, especially that derived from ^{13}C NMR, ^{15}N NMR, and infrared spectra, supports the view that CENUs adopt a preferred conformation in nonpolar aprotic solvents in which the N=O group is aligned toward the CH_2CH_2Cl group which is in accord with the conformation adopted in the crystalline state. This conformation is also indicated for CCNU by the average magnitude of the $^2J_{^{15}N-^{13}C}$ coupling which is close to the expected trans value and by the negligible $^3J_{^{15}N-^1H}$ coupling to the adjacent CH_2 group by reference to model compounds. This conformation places the N_3H proton within recognized bonding distance to the N=O nitrogen lone pair which may afford additional stabilization for this conformer.

In aqueous solution there is evidence from ^1H NMR, ^{13}C NMR, and ^{15}N NMR for a change in conformation via a tetrahedral intermediate to the second conformation in which there is intramolecular hydrogen bonding. This is revealed most clearly by the ^{13}C NMR spectrum of the specifically ^{13}C and ^{15}N enriched BCNU where two distinct ^{15}N-coupled ^{13}C doublets with different coupling constants are identified. The rate of this conformational change is comparable with the rate of decomposition and may well be the critical initial step in the latter process. The base-catalyzed abstraction of the N_3H proton may well be a concomitant process. This ^{13}C NMR experiment also permitted the identification of individual decomposition products including the (2-alkylamino)oxazoline, the urea, and carbamates. While the ^{13}C NMR did not provide direct evidence for a stable tetrahedral intermediate as a result of carbonyl group hydration, the observation of ^{18}O exchange at this moiety in BCNU confirmed its transient existence. There is similar evidence for the intermediacy of a short-lived tetrahedral intermediate involved in the conformational inversion of CCNU in aqueous solutions. Considerations of the conformational and stereoelectronic factors of the intermediates provided a satisfactory interpretation of the reactions in terms of strict stereoelectronic control. Thus the various reactions of CENUs in aqueous solution and their pH dependence may be explained provided the criterion is met that available lone pairs on the two heteroatoms in the tetrahedral intermediates be aligned antiperiplanar to the bond to be broken. This series of events which precede the generation of electrophiles in solution which attack biological macromolecules may well be ultimately responsible for their in vivo activity.

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 ^1H NMR spectra of the intermediates were recorded on Perkin-Elmer 90 and Varian HA-100 analytical spectrometers, and final nitrosoureas were recorded on Bruker WH-200 and WH-400 spectrometers. The spectra were measured on approximate 5–10% (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. Most of the ^{13}C spectra were recorded on Varian HA-60 and Bruker HEX-90 spectrometers, and specially ^{15}N-labeled ^{13}C spectra were recorded on a Bruker WH-200 spectrometer.

The ^{15}N spectra were recorded on a Bruker WH-200 spectrometer opening at 20.283 MHz. The spectra were obtained by using dimethyl formamide as an external reference, and chemical shift values are reported relative to 10–20% ammonia at 0.0 ppm with the respective deuterated solvent as a lock signal.[44] Most of the proton-decoupled natural-abundance ^{15}N spectra were taken by using 1 M solutions containing 0.05–0.1 M $Cr(AcAc)_3$ in a 20-mm-diameter tube after 85–86K scans. Specifically labeled compounds spectra were recorded with 0.01 M solutions, with (0.01–0.1 M) or without $Cr(AcAc)_3$, and in approximately 1–4K scans.

Materials. (2-Chloroethyl)amine hydrochloride and cyclohexyl isocyanate were obtained from Aldrich Chemical Co. 2-Chloroethyl isocyanate was obtained from Trans World Chemicals. Ammonium chloride ^{15}N (95–99%), sodium nitrite ^{15}N (95–99%), potassium phthalimide ^{15}N (95–99%), phosgene-^{13}C (90% as a 1.1 M solution in benzene), and $H_2{}^{18}O$ (22% enriched) were obtained from Merck Sharp and Dohme.

General Methods for the Preparation of Ureas and Nitrosoureas. A solution of the appropriate alkyl isocyanates (100 mmol) in ether (100 mL) was added dropwise to a stirred and cooled suspension or solution of the respective amines (100 mmol)

(95) Hammett, L. P. In "Physical Organic Chemistry", 2nd ed.; McGraw-Hill: New York, 1970, p 119.

in ether (200 mL) at ambient temperature and the mixture stirred at room temperature for 6–12 h. In most cases the solid which separated was collected and recrystallized from dichloromethane/ether or ethanol/ether mixture.

To a stirred solution of the above ureas (10 mmol) in anhydrous formic acid (20–30 mL) at 0–5 °C was added anhydrous sodium nitrite (30–50 mmol) in portions during 1–2 h. After the mixture was stirred for 30 min, water (40–50 mL) was added, and the reaction mixture was stirred for an additional 1 h. The separated solid was collected and crystallized from ether/petroleum ether. In the event that no solid separated, the reaction mixture was extracted with ether, the extract was washed with sodium bicarbonate and water and dried (Na_2SO_4), the solvent was removed, and the residue was crystallized from ether/petroleum ether. The following CENUs were prepared in this way: N_1N_3-bis(2-chloroethyl)-N_1-nitrosourea (**1**), mp 31–32 °C (lit.[30] mp 30–32 °C); N_1-(2-chloroethyl)-N_3-cyclohexyl-N_1-nitrosourea (**2**), mp 88–89 °C (lit.[31] mp 90 °C); N_1-(2-chloroethyl)-N_3-(4-methylcyclohexyl)-N_1-nitrosourea (**3**), mp 68–69 °C (lit.[32] mp 70 °C); N_1-(2-chloroethyl)-N_3-glucosamino-N_1-nitrosourea (**4**), 139–140 °C (lit.[33] mp 140–141 °C); N_1-(2-chloroethyl)-N_1-nitrosourea (**6**), mp 78 °C (lit.[34] mp 75–78 °C); N_3-(cyclohexyl)-N_1-(2-fluoroethyl)-N_1-nitrosourea (**7**), mp 35 °C (lit.[31] mp 34–37 °C); N_1-(2-fluoroethyl)-N_1-nitrosourea (**8**), mp 54–55 °C (lit.[31] mp 53–55 °C); N_1N_3-bis(2-fluoroethyl)-N_1-nitrosourea (**9**), mp 34–35 °C (lit.[31] mp 30–34 °C); N_3N_3-dimethyl-N_1-(2-chloroethyl)-N_1-nitrosourea (**10**), as heavy oil.[35]

N_1-(2-Hydroxyethyl)-N_3-(2-chloroethyl)-N_3-nitrosourea [**5**, mp 56 °C (lit.[36] mp 56–58 °C)] was prepared by a modified procedure. Ethyl *N*-(2-chloroethyl)-*N*-nitrosocarbamate (**11**) was prepared from ethyl (2-chloroethyl)carbamate by nitrosation with N_2O_3 in the presence of anhydrous sodium acetate.[37]

(2-Bromoethyl)phthalimide-^{15}N was prepared by following the literature procedure starting from potassium phthalimide-^{15}N and 1,2-dibromoethane.[38]

(2-Fluoroethyl)phthalimide-^{15}N was prepared by following the literature procedure[31] for the unlabeled material and starting from potassium phthalimide-^{15}N and 2-fluoroethyl *p*-toluenesulfonate.

^{15}N-Labeled 2-Chloroethylamine Hydrochloride 12. (2-Bromoethyl)phthalimide-^{15}N (6.9 g, 27 mmol) was treated with NaOH (100 mL, 30%) and refluxed for 2 h. Liquid 2-aminoethanol-^{15}N was distilled from the reaction mixture into an excess of dilute hydrochloric acid solution, and the water was removed under reduced pressure. The residue was crystallized from methanol/ether to afford 1.8 g (69%) of 2-aminoethanol-^{15}N hydrochloride: mp 70 °C (lit.[38] mp 68–70 °C); ^{1}H NMR (Me_2SO-d_6) 2.80 (t, 2 H, C**H**$_2$OH) 3.61 (m, 2 H, NCH_2), 5.15 (t, 1 H, OH exchangeable), 7.85 (br m, 2 H, NH_3 exchangeable).

Thionyl chloride (11.9, 100 mmol) was added dropwise to a cooled and stirred solution of the above 2-aminoethanol-^{15}N hydrochloride (1.3 g, 13 mmol) in chloroform (20 mL), stirred at room temperature for 3 h, and refluxed for 3 h at 60 °C. The thionyl chloride and chloroform were removed under reduced pressure, and the residue was crystallized from ethanol/ether to afford 1.0 g (67%) of 2-chloroethylamine-^{15}N hydrochloride **12**: mp 148 °C (143–146 °C for the N^{14} authentic sample; NMR (Me_2SO-d_6), 3.18 (t, 2 H, CH_2, $^2J_{H-H}$ = 6 Hz), 3.90 (td, 2 H, CH_2, $^2J_{H-H}$ = 6 Hz, $^2J_{^{15}N-H}$ = 3.2 Hz), 8.50 (br m, 3 H, $^+NH_3$ exchangeable); mass spectrum, *m/e* (relative intensity; calcd value) 82.0130 (30.10; $C_2H_6{}^{15}N^{37}Cl$, 82.0129), 80.0160, (100.000; $C_2H_6{}^{15}N^{35}Cl$, 80.0160).

1,3-Bis(2-chloroethyl)-1-nitrosourea-$^{15}N_1$,$^{15}N_3$,^{15}N=*O* (1a). Phosgene solution (4 mL, 12.5% in benzene) was added dropwise to 2-chloroethylamine-^{15}N [prepared from **12** (1.18 g, 10 mmol) and NaOH (0.4 g, 10 mmol) in 10 mL of water and extracted with ether (3 × 50 mL) in ether (150 mL), and the mixture was cooled at 0 °C and stirred mechanically for 6 h. Water was added dropwise, and the urea was collected by filtration, washed with water, and finally crystallized from ethanol to afford 0.75 g (80%) of the pure urea **13**: mp 127 °C; ^{1}H NMR (Me_2SO-d_6) 3.30 (t, 4 H, 2 CH_2Cl), 3.55 (td, 2CH_2, $^2J_{H-H}$ = 5.0 Hz, $^2J_{^{15}N-H}$ = 1.8 Hz), 6.50 (dt, 2 NH, $^1J_{^{15}N-H}$ = 90.0 Hz, $^2J_{H-H}$ = 5.0 Hz); ^{13}C NMR (Me_2SO-d_6) 41.90 (d, 2C_1, $^1J_{^{15}N-^{13}C_1}$ = 12.5 Hz), 44.38 (2C_2), 157.55 (t, $^1J_{^{15}N-^{13}C=O}$ = 20.0 Hz); mass spectrum, *m/e* (relative intensity; calcd value) 190.0051 (5.15; $C_5H_{10}{}^{15}N_2{}^{37}Cl_2O$, 190.0051), 188.0080 (32.57; $C_5H_{10}{}^{35}Cl^{37}Cl^{15}N_2O$, 188.0070), 186.0104 (52.22; $C_5H_{10}{}^{35}Cl_2{}^{15}N_2O$ 186.0093), 139.0234 (31.90; $C_4H_8{}^{37}Cl^{15}N_2O$, 139.0236), 137.0262 (100.00; $C_4H_8{}^{35}Cl^{15}N_2O$, 137.0266).

Solid $Na^{15}NO_2$ (700, 10 mmol) was added in portions to a stirred solution of 1,3-bis(2-chloroethyl)urea **13** (700 mg, 3.7 mmol) in formic acid (98%, 5 mL) at 0 °C, the reaction mixture was stirred for 2 h, additional cold water (10 mL) was added to the mixture, and the separated solid was collected and crystallized from ether/petroleum ether to afford 450 mg (56%) of nitrosourea **1a**: mp 25–26 °C; ^{1}H NMR ($CDCl_3$) 3.50 (td, 2 H, CH_2Cl, $^2J_{^{15}N-H}$ = 2.0 Hz), 3.76 (td, 2 H, CH_2Cl), 3.76 (m, 2 H, CH_2Cl), 3.88 (m, 2 H, $NHCH_2$), 4.20 (t, 2 H, NCH_2), 7.30 (dtd, NH exchangeable, $^1J_{^{15}N-^1H}$ = 93.5 Hz, $^2J_{^{15}N-^1H}$ = 1.6 Hz); mass spectrum, *m/e* (relative intensity; calcd value) 217.9946 (4.10; $C_5H_9{}^{15}N_3O_2{}^{35}Cl^{37}Cl$, 217.9953), 215.9979 (6.15; $C_5H_9{}^{15}NO_2{}^{35}Cl_2$, 215.9981), 108.9945 (19.87; $C_2H_5{}^{15}N_2O^{35}Cl$, 108.9953), 64.9983 (31.86; $C_2H_4{}^{37}Cl$, 64.9972), 63.0017 (100.0; $C_2H_4{}^{35}Cl$, 63.001).

3-Cyclohexyl-1-(2-chloroethyl)-1-nitrosourea-$^{15}N_1$,^{15}N=*O* (2b). Cyclohexyl isocyanate (480 mg, 4 mmol) was added dropwise to a suspension of 2-chloroethylamine-^{15}N hydrochloride (440 mg, 4 mmol), triethylamine (400 mg, 4 mmol) in ether (50 mL) at 0 °C was added, and the reaction mixture was stirred for 6 h. Ice-cold water (10 mL) was added and the mixture stirred for another 30 min. The crystalline urea was filtered and washed with water to afford 610 mg (74%) of urea **12**: mp 123–125 °C; ^{1}H NMR (Me_2SO-d_6) 1.00–1.90 (m, 10 H, CH_2), 3.32 (t, 2 H, CH_2Cl), 3.55 (td, 2 H, CH_2, $^2J_{^{15}N-H}$ = 2.5 Hz, $^2J_{H-H}$ = 5.0 Hz) 6.81 (dt, ^{15}NH, $^1J_{^{15}N-H}$ = 90.0 Hz, $^1J_{H-H}$ = 8.0 Hz), 6.86 (d, NH, $^1J_{H-H}$ = 8.0 Hz); mass spectrum, *m/e* (relative intensity; calcd value) 205.0997 (33.38; $C_9H_{17}{}^{14}N^{15}N^{37}ClO$, 205.0999), 124.0297 (100.00; $C_3H_8{}^{35}Cl^{14}N^{15}NO$, 124.0295). $Na^{15}NO_2$ (350 mg, 5 mmol) was added in portions to the above urea (408 mg, 2 mmol) in formic acid (10 mL) at 0 °C and stirred for 2 h. After the usual workup the nitrosourea (320 mg, 68%) was obtained: mp 85–80 °C; NMR (CCl_4) 1.20–2.20 (m, 10 H, CH_2), 3.45 (t, 3 H, CH_2Cl), 3.85 (m, 1 H, $H_{1(ax)}$', 4.15 (t, 3 H, CH_2), 6.82 (ddd, $^1J_{^{15}N-H}$ = 90.5 Hz, $^2J_{^{15}N-H}$ = 1.8 Hz, $^2J_{H-H}$ = 8.5 Hz); mass spectrum, *m/e* (relative intensity; calcd value) 237.0846 (1.21; $C_9H_{16}{}^{14}N^{15}N_2{}^{37}ClO_2$, 237.0842), 235,0868 (3.03; $C_9H_{16}{}^{35}Cl^{14}N^{15}N_2O$, 235.0872), 83.0860 (100.00; C_6H_{11}, 83.0860).

1-(2-Chloroethyl)-3-nitrosourea-$^{15}N_3$,^{15}N=*O* (6a). $^{15}NH_3$ (generated from $^{15}NH_4Cl$ and 40% sodium hydroxide, 50 mL) was passed through a solution of 2-chloroethyl isocyanate (1.78 g, 15 mmol) at −5 °C. The reaction mixture was allowed to warm to room temperature, and ether was removed under reduced pressure. The residue was triturated with petroleum ether, and the solid was collected to afford 1.3 g (70%) of urea **16b**: NMR ($CDCl_3$) 3.55 (m, 4 H, CH_2), 4.50 (d, 2 H, $^1J_{^{15}N-H}$ = 87.2 Hz), 5.20 (br m, 1 H, NH); mass spectrum, *m/e* (relative intensity; calcd value) 125.0187 (8.86; $C_3H_7{}^{14}N^{15}N^{37}ClO$, 125.0187), 123.0215 (27.25; $C_3H_7{}^{14}N^{15}N^{35}ClO$), 74.0364 (100; $C_2H_5{}^{14}N^{15}NO$, 74.0372).

Sodium nitrite-^{15}N (1.0, 14 mmol) was added in portions to the above urea (1.23 g, 10 mmol) in concentrated HCl (10 mL) at 0 °C, and after the usual workup the nitrosourea (450 mg, 29%) was obtained: mp 78–79 °C; ^{1}H NMR (CCl_4) 3.40 (t, 2 H, CH_2Cl), 4.10 (t, 2 H, NCH_2), 6.06 (d, 1 H, NH, J = 91.5 Hz), 7.05 (d, 1 H, NH, J = 91.5 Hz); ^{1}H NMR (Me_2SO-d_6) 3.60 (t, 2 H, CH_2Cl), 4.06 (t, 2 H, NCH_2), 7.85 (d, 1 H, NH, $^1J_{^{15}N-H}$ = 90.0 Hz), 8.25 (d, 1 H, NH, $^1J_{^{15}N-H}$ = 90.0 Hz); mass spectrum, *m/e* (relative intensity) 153.0095 (3.32; $C_3H_6{}^{35}Cl^{14}N^{15}N_2O_2$, 153.0089), 111.0033 (31.98; $C_2H_5{}^{37}Cl^{14}N^{15}NO$, 111.0032), 109.0062 (100.00; $C_2H_5{}^{35}Cl^{14}N^{15}NO$, 109.0061).

1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea-3'-^{15}N (2a). Cyclohexylamine-^{15}N (1 g, 10 mmol), obtained by reduction of aniline-^{15}N (1.5 g) with 5% rhodium on alumina (5.0 g) in a Paar apparatus for 20 h, was added dropwise to a solution of chloroethyl isocyanate (1.05 g, 10 mmol) at 0 °C and the mixture stirred for an additional 6 h. A solid separated and was collected and washed with ether to give 1.80 g (80%) of urea **16a**: mp 125–125 °C; ^{1}H NMR (Me_2SO-d_6) 0.90–1.90 (m, 10 H, CH_2), 3.30 (m, 1 H, CH + CH_2), 3.60 (t, 2 H, CH_2), 5.94 (dd, 1 H, NH, $^1J_{^{15}N-H}$ = 88.0 Hz), 6.00 (t, NH, $^1J_{H-H}$ = 6.00 Hz); mass spectrum, *m/e* (relative intensity; calcd value) 207.0966 (6.79; $C_9H_{17}{}^{37}Cl^{14}N^{15}NO$, 207.0970), 207.0990 (21.93; $C_9H_{17}{}^{35}Cl^{14}N^{15}NO$, 205.0980), 57.0496 (100.00; $C_3H_6{}^{15}N$, 57.0471); ^{13}C NMR (Me_2SO-d_6) 24.42 (C_3', C_5'), 25.29 (C_4'), 33.19 (C_2', C_6'), 41.35 (C_1'), 44.53 (C_9'), 47.50 (d, $^1J_{^{15}N-^{13}C}$ = 10.8), 157.10 (d, $^1J_{^{15}N-^{13}C}$ = 20.0 Hz). The above urea (300 mg,

1.5 mmol) was nitrosated with $Na^{15}NO_2$ (300 mg, 4.3 mmol) in formic acid (10 mL) at 0 °C. After the usual workup, the nitrosourea (200 mg, 60%) was obtained: mp 86–87 °C; ^1H NMR ($CDCl_3$) 1.20–2.20 (m, 10 H, CH_2), 3.55 (td, CH_2Cl, $^2J_{^{15}N-H}$ = 1.6 Hz, NH exchangeable); mass spectrum, *m/e* (relative intensity; calcd value) 237.0845 (0.59; $C_9H_{16}{}^{37}Cl^{14}N^{15}N_2O_2$, 237.0842), 235.0870, (1.83; $C_9H_{16}{}^{35}Cl^{14}N^{15}N_2O_2$, 235.0870), 111.0034 (1.24; $C_2H_5{}^{37}Cl^{14}N^{15}NO$, 111.031), 109.0062 (3.82; $C_2H_5{}^{35}Cl^{14}N^{15}NO$, 109.0062), 83.0853 (100.00; C_6H_{11}, 83.0845).

1-(Fluoroethyl)-3-cyclohexyl-1-nitrosourea-*1*,*N*$_1$-$^{15}N_2$ (8a). Potassium cyanate (1.32 g, 15 mmol) was added to a solution of 2-fluoroethylamine-^{15}N hydrochloride [1.35 g, 15 mmol; which in turn was prepared by the procedure of Montgomery et al.[14] for the unlabeled compound from (2-fluoroethyl)phthalimide] in water (10 mL), and the solution was stirred for 6 h. The solid which precipitated (1.2 g, 67%) was collected after the reaction mixture was cooled.

To the above fluoroethylurea-*1*-^{15}N (1.20 g, 10 mmol) in formic acid (10 mL) was added solid sodium nitrite-^{15}N (1.5 g, 21 mmol) in portions at 0 °C, and the mixture was stirred for 1 h. The reaction mixture was stirred for an additional 30 min after slow addition of water (15 mL). The solid which precipitated was collected and crystallized (350 mg, 26%) from ether and petroleum ether, giving purified **8a**: mp 80 °C (for unlabeled, 81–83 °C); NMR ($CDCl_3$) 4.20 (dt, 2 H, CH_2CH_2F, $^2J_{H-F}$ = 24.0 Hz), 4.38 (dt, 2 H, CH_2CH_2F, $^1J_{H-F}$ = 48.8 Hz), 5.70 (br m, 1 H, NH, exchangeable), 5.90 (br m, 1 H, NH exchangeable); mass spectrum, *m/e* (relative intensity; calcd value) 137.0384 (3.61, M^+, $C_3H_6NO_2{}^{15}N_2F$), 137.0385), 94.0327 (100.00; $C_2H_5FO^{15}N_2$, 94.0327).

1,3-Bis(2-chloroethyl)-1-nitrosourea-2-^{13}C=*O*,^{15}N=*O* (1b). This compound was prepared by starting from $^{13}COCl_2$ and employing the procedures described for **13** or **1a** to afford 36% of 1,3-bis(2-chloroethyl)urea (**17**) which was subsequently nitrosated with $NaNO_2$ in HCOOH acid to give **1b** in 50% yield: mp 30 °C; mass spectrum, *m/e* (relative intensity; calcd value) 217.0004 (3.24 M^+; $C_4{}^{13}C_1H_9{}^{35}Cl^{37}ClN_2{}^{15}N_1O_2$, 217.0046), 215.0073 (3.79, M^+; $C_4{}^{13}C_1H_9{}^{35}Cl^{35}ClN_2{}^{15}N_1O_2$, 215.0075).

3-Cyclohexyl-1-(2-chloroethyl)-1-nitrosourea-2-^{13}C=*O*,^{15}N=*O* (2c). To a solution of cyclohexylamine (100 mg, 1 mmol) and triethylamine (1 mL excess) in water (20 mL) was added 1,3-bis(2-chloroethyl)-1-nitrosourea (**1b**; 100 mg, 0.46 mmol), and the reaction mixture was stirred for 4 h at ambient temperature. The solid which separated was collected to afford the urea **18**, 80 mg (86%). The above urea (80 mg) was nitrosated with $Na^{15}NO_2$ (150 mg, 2.17 mg) and after the usual workup afforded the nitrosourea **2c**: 50 mg (62%); pale yellow solid; mp 86 °C; mass spectrum, *m/e* (relative intensity; calcd value 237.0910 (1.87; $C_8{}^{13}C_1H_{16}{}^{37}Cl^{35}ClN_2{}^{15}N_1O_2$, 237.0905), 235.0935 (5.52, M^+; $C_8{}^{13}C_1H_{16}{}^{35}Cl_2N_2{}^{15}N_1O_2$, 235.0935).

^{18}O Exchange of the Carbonyl Oxygen of BCNU (1). A solution of 0.05 mmol of **1** in a mixture of 0.1 mL of acetonitrile and 0.9 mL of $H_2{}^{18}O$ (22% enrichment) with potassium phosphate buffer (pH 7.2) was sealed in a Reacti-vial for 12 h at 25 °C. The reaction mixture was extracted with ether (3 × 10 mL), the extract dried (Na_2SO_4), and the solvent removed. The residue was analyzed by mass spectrometry. The carbonyl oxygen containing fragment (*m/e* 56.0154, relative intensity 8.8, calcd for $C_2H_2NO^+$ *m/e* 56.0138) was compared with $C_2H_2N^{18}O$ (*m/e* 58.0) and found to be ca. 1% enriched in ^{18}O.

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

Acknowledgment. This investigation was supported by Grant 1-R01-CA21488-01 awarded by the National Cancer Institute, DHEW, and by a grant from the Provincial Cancer Hospitals Board. S.M.S.C. acknowledges the award of an Alberta Heritage Foundation for Medical Research Post-Doctoral Fellowship. We thank Dr. Tom Nakashima and Mr. Glen Bigam and their associates for extensive NMR measurements.

Registry No. **1**, 154-93-8; **1** (C=^{18}O), 79664-68-9; **1a**, 79664-69-0; **1b**, 79664-70-3; **2**, 13010-47-4; **2a**, 79664-71-4; **2b**, 79664-72-5; **2c**, 79664-73-6; **2d**, 79664-74-7; **3**, 33073-59-5; **4**, 54749-90-5; **5**, 60784-46-5; **6**, 2365-30-2; **7**, 13908-93-5; **7a**, 79664-75-8; **8**, 69112-98-7; **9**, 13908-91-3; **10**, 59960-30-4; **11**, 6296-45-3; **12**·HCl (X = Cl), 79664-76-9; **12**·HCl (X = F), 79664-77-0; **13**, 79664-78-1; **14**, 79664-79-2; **15**, 79664-80-5; **16** (R = H), 79664-81-6; **16** (R = C_6H_{11}), 79664-82-7; **17**, 79664-83-8; **18**, 79664-84-9; 2-chloroethylamine, 689-98-5; 2-fluoroethylamine, 406-34-8; (2-chloroethyl) isocyanate, 1943-83-5; cyclohexyl isocyanate, 3173-53-3; *trans*-4-methylcyclohexyl isocyanate, 32175-00-1; 2-deoxy-D-glucos-2-yl isocyanate, 79664-85-0; isocyanic acid, 75-13-8; (2-hydroxyethyl) isocyanate, 4747-84-6; (2-fluoroethyl) isocyanate, 505-12-4; ethyl (2-chloroethyl)carbamate, 6329-26-6; (2-bromoethyl)phthalimide-^{15}N, 58551-02-3; (2-fluoroethyl)phthalimide-^{15}N, 79680-95-8; aminoethanol-^{15}N·HCl, 58265-67-1; cyclohexylamine-^{15}N, 78441-12-0; aniline-^{15}N, 7022-92-6.

Supplementary Material Available: Figures 2–5 containing NMR spectra data for **1a**, **1b**, **2a**, and **6** (six pages). Ordering information is given on any current masthead page.

Benzo- and Indoloquinolizines. 21.[1] Allylic Strain Competition in 4b,5,6,7,8,8a,10,11,16,16b-Decahydrodibenz[*f*,*h*]indolo[2,3-*a*]quinolizine Isomers. Detection of Boat Conformers by Carbon-13 Nuclear Magnetic Resonance

D. Tourwé,* E. De Cock, and G. Van Binst

Vrije Universiteit Brussel, Laboratorium voor Organische Chemie, Pleinlaan 2, B-1050 Brussel, Belgium

Received August 1, 1980

The concept of allylic strain between the benzene or the indole ring and the benzylic carbon–carbon bond is used to explain the conformational equilibria in the 4b,5,6,7,8,8a,10,11,16,16b-decahydrodibenz[*f*,*h*]indolo[2,3-*a*]quinolizine isomers, in 5,6,8,9-tetrahydro-14b*H*-benz[*h*]indolo[2,3-*a*]quinolizine, and in their indole *N*-methyl analogues. The conformational changes were monitored by ^{13}C chemical shifts. Boat conformers with bowsprit–flagpole interactions between γ-positions show upfield shifts, whereas these are not observed for δ-interacting groups.

Comparison of the ^{13}C NMR spectra of the *rel*-(4bβ,8aα,16bβ)-**1a** and the *rel*-(4bα,8aβ,16bβ)-**2a** isomers has indicated the cis-cisoid-trans conformation[2] **4a** for the latter (Chart I).[4] The *trans*-quinolizidine conformation **5a** was excluded because of the very similar chemical shifts

(1) Part 20: F. Vlaeminck, E. De Cock, D. Tourwé, and G. Van Binst, *Heterocycles*, **15**, 1213 (1981).

(2) For a discussion of the conformational equilibria in these compounds, see ref 3.

(3) G. Van Binst and D. Tourwé, *Org. Magn. Reson.*, 6, 590 (1974).

0022-3263/81/1946-5321$01.25/0