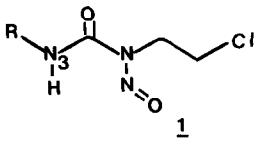
Tetrahedron Letters Vol. 22, pp 401 - 404 0040-4039/81/0129-0401$02.00/0

CONFORMATIONS OF THE ANTITUMOR AGENT 1-(2-CHLOROETHYL)-3-CYCLOHEXYL-1-NITROSOUREA IN SOLUTION STUDIED BY ^{15}N-NMR

J. William Lown* and Shive M. S. Chauhan,
Department of Chemistry
University of Alberta
Edmonton, Alberta, Canada T6G 2G2

ABSTRACT A study of the ^{15}Nnmr spectra of the anticancer agent 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) employing both a natural abundance and selectively ^{15}N enriched CCNU permitted assignment of ^{15}N-resonance and its conformation relevant to its mechanism of action.

The initial abstraction of the NH proton from the clinically useful antitumor agents 2-chloroethyl-1-nitrosoureas (CENUs e.g. 1a-c) leading to the formation of reactive electrophiles which attack cellular macromolecules appears to underlie their biological activity.[1] The selection of a particular pathway of decomposition, of which three have been proposed,[1] depends in part on the conformation of the CENUs in solution and the concomitant control of, for example, their propensity to cyclize and the type of chloroethylated diazotate produced. The unique chemical role of the nitrogens in these agents together with the high sensitivity of ^{15}N nmr to molecular interactions, hydrogen bonding and dynamic changes in solution make ^{15}N nmr especially attractive and informative.[2] We report the ^{15}N nmr study, both natural abundance and selectively ^{15}N enriched of the conformation and solvent interactions of 1-chloroethyl-3-cyclohexyl-1-nitrosourea (CCNU, 1a) as it pertains to its chemical reactivity.



1

a R = C_6H_{11}
b R = $4CH_3C_6H_{10}$
c R = $ClCH_2CH_2$

The proton decoupled natural abundance ^{15}N spectrum of 1M solution of CCNU in $CHCl_3$ containing 0.1M $Cr(AcAc)_3$ in a 20 mm diameter tube was obtained on 86K scans on a Bruker WH-200 spectrometer operating at 20.285 MHz using ammonia as reference and 10-20% $CDCl_3$ as lock signal. The spectrum showed the amide nitrogen resonance at δ 103.1 (see Table) which is within the range of alkyl ureas and alkylamide[3] ^{15}N chemical shifts. CCNU (2a) selectively enriched at N-3 and

TABLE

^{15}N Chemical Shifts of CCNU[a] (1a and 2a)

Solvent (conc.)	δNH	δ N	δN=O
$CHCl_3$ (1M)	103.1	268.4	560.2
$CHCl_3$ (0.01M)	103.6	c	559.9
CF_3CH_2OH (0.01M)	106.5	c	561.9
Dioxane (0.01M)	103.7	c	562.4
Dioxane:H_2O (0.01M)	103.7	c	563.4
DMSO (1M)	107.7	269.5	564.5
DMSO (0.01M)	107.5	c	564.6
DMSO:H_2O (0.01M)	108.0	c	564.5

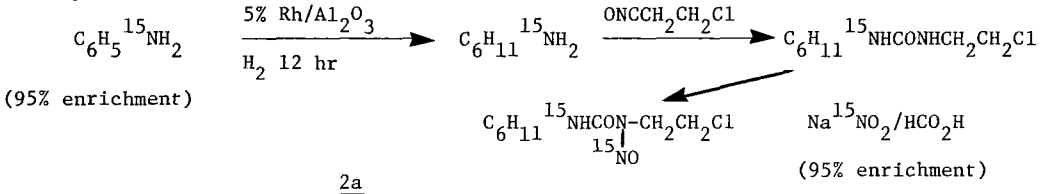
a. Proton decoupled spectra are reported using NH_3 as standard and to (± 0.05 ppm). Approximately 86K scans were required for natural abundance and approximately 1K scans for ^{15}N enriched CCNU.

b. The relaxing agent $Cr(AcAc)_3$ was used in the natural abundance spectrum at 0.1M and with the ^{15}N enriched spectrum at 25-50 mM.

c. Owing to enrichment in N-3 and N=O in 2a the enriched N-1 is not observed for the concentration and the number of scan used.

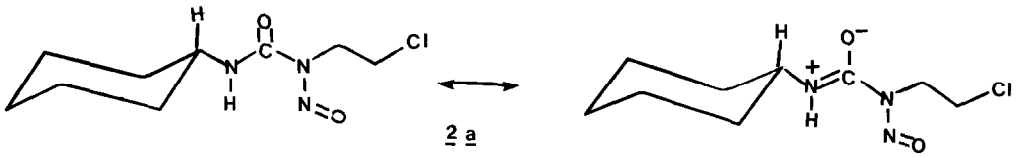
---

in the NO group was prepared as shown employing ^{15}N precursors available from Merck, Sharpe and Dohme.



2a

This compound provided confirmation of the ^{15}N resonance assignments and permitted detection of coupling constants $^1J_{^{15}N-^1H}$ = 90.5 Hz (one bond coupling) and $^2J_{^{15}N-^1H}$ = 1.80 Hz (two bond coupling) in CCl_4. The values of the latter coupling constants showed a solvent dependence and in the more polar solvent dimethyl sulfoxide (DMSO) were $^1J_{^{15}N-^1H}$ = 92.5 Hz and $^2J_{^{15}N-^1H}$ = 0.6 Hz. The change in value of the one bond coupling may indicate an increase in S characte of the N and a consequent increase in double bond character of the amide R-N-C bond [3a] which is in accord with its tendency to cyclize to an oxazolidine.[4]



2 a

Similarly the observed decrease in the two bond coupling upon increasing the solvent polarity may include this factor in addition to the association of the polar DMSO with the nitrogen lone pair.[5] The magnitude of the coupling constant in DMSO for $^{2}J_{^{15}N-^{1}H}$ is close to that of the trans coupling constant average [6] and thus the conformational assignment is in agreement with X-ray diffraction data on MeCCNU(1b).[7]

The chemical shifts of the N-1 and N=O ^{15}N signals appear downfield relative to that of aliphatic nitrosoamines and closer to those of aromatic nitrosamines [8] which may indicate substantial electron delocalization due to the acyl group. There is no detectable $^{3}J_{^{15}N-^{1}H}$ between the N=O nitrogen and the protons alpha to N-1 either in $CHCl_3$ or in polar solvents like aqueous DMSO. This indicates that the chloroethyl group is syn to the N=O group as has been reported in the case of nitrosoamines, sydnones and sydnonimines.[9] In addition upon sequential dilution in $CHCl_3$ (0.1M → 0.01M → 5mM) with the ^{15}N-enriched CCNU there is no change in the chemical shifts of the N=O nitrogen, indicating that there is no hydrogen bonding with the N=O group in nonpolar solvents, in contrast to the anti hydrogen bonded conformation that is conventionally depicted for these compounds.[1] However one observes a downfield shift in the resonance due to the N=O nitrogen upon increasing solvent polarity and basicity from $CHCl_3$ (559.9), dioxane (562.4), dioxane-H_2O (563.4) to DMSO (564.6) which may indicate a rotation about the N-N bond under the influence of base attack. The slow decomposition of nitrosoureas in aqueous solutions [1,4] and in the basic solvent DMSO [10] does not permit a definitive answer to this point at present.

These conclusions on the average conformation of CCNU in solution favor the decomposition pathway involving generation of the 2-chloroethyldiazohydroxide.[1]

Acknowledgements. The authors wish to thank Mr. Glen Bigam of the Department of Chemistry nmr laboratory for assistance in obtaining the ^{15}N nmr spectra. This research was supported by grant 1-R01-CA21488-01 awarded by the National Cancer Institute, DHEW and by a grant from the Alberta Provincial Cancer Hospitals Board.

References and Notes

1. (a) J. A. Montgomery, T. P. Johnston and Y. F. Shealy in Burger's Medicinal Chemistry Fourth Edition Part II. Edited by Manfred E. Wolff, Wiley (1979) 595.
(b) D. J. Reed, H. E. May, R. B. Boose, K. M. Gregory and M. A. Beilstein, Cancer Research 35, 568 (1975).
(c) J. W. Lown, A. V. Joshua and L. W. McLaughlin, J. Med. Chem. 23, 798 (1980).
2. (a) G. C. Levy and R. L. Lichter, Nitrogen-15 Nuclear Magnetic Resonance Spectroscopy. A Wiley Interscience Publication (1979).
(b) M. Witanowski, L. Stefaniak and G. A. Webb, in Annual Reports on NMR Spectroscopy vol. 7, ed. by G. A. Webb, pp. 117-244, Academic Press London (1977).
3. (a) I. Yavari and J. D. Roberts, Org. Mag. Reson. 13, 68 (1980).
(b) B. Coxon, A. J. Fatiadi, A. Cohen, H. S. Hertz and R. Schaffer, Org. Mag. Reson. 13, 187 (1980).
(c) J. Dorie, J. P. Gouesnard, B. Mechin, N. Naulet and G. J. Martin, Org. Mag. Reson. 13, 126 (1980).
4. J. W. Lown and S. M. S. Chauhan, J. Med. Chem., in press.
5. L. Paolillo and E. D. Becker, J. Mag. Reson. 2, 168 (1970).
6. (a) G. Binsch, J. B. Lambert, B. W. Roberts and J. D. Roberts, J. Amer. Chem. Soc. 86, 5564 (1964).
(b) J. A. Sogn, W. A. Gibbon and E. W. Randall, Biochemistry 12, 2100 (1973)
7. H. W. Smith, A. Camerman and N. Camerman, J. Med. Chem. 21, 468 (1978).
8. (a) J. P. Gouesnard and G. J. Martin, Org. Mag. Reson. 12, 263 (1979).
(b) R. Bonnett, R. Holleyhead, B. L. Johnson and E. W. Randall, J. Chem. Soc. Perkin 1 2261 (1975).
9. (a) L. Stefaniak, M. Witanowski, B. Kamienski and G. A. Webb, Org. Mag. Reson. 13, 274 (1980).
(b) L. Stefaniak and M. Witanoski, Bull. Acad. Pol. Sc. Ser. Sci. Chim. 25, 261 (1977).
(c) L. Stefaniak, Tetrahedron 33, 2571 (1977).
10. P. F. Wiley, R. R. Herr, H. K. Jahnke, G. G. Chidester, S. A. Mizasak, L. B. Spaulding and A. D. Argondelis, J. Org. Chem. 44, 9 (1979).

(Received in USA 3 September 1980)