Tetrahedron Letters No. 11, pp 813 - 816, 1976. Pergamon Press. Printed in Great Britain.

HIGH RESOLUTION ^1H AND ^{13}C NMR INVESTIGATIONS OF CYCLOPHOSPHAMIDE CONFORMATIONAL MOBILITY

William Egan*

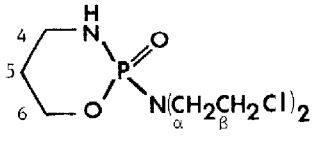
Reproduction Research Branch, National Institute of Child Health and Human Development
National Institutes of Health, Bethesda, MD 20014

Gerald Zon

Maloney Chemical Laboratory, The Catholic University of America
Washington, D C. 20064

(Received in USA 28 October 1975; received in UK for publication 5 February 1976)

In connection with determining the enzymatic stereospecificity for in vivo activation (C-4 hydroxylation) of the anticancer drug cyclophosphamide (1),[1] it became necessary to evaluate the dynamic conformational properties of the six-membered heterocyclic skeleton We now report the results of high resolution ^1H and ^{13}C nmr studies with 1 which are consistent with a relatively low barrier-height for ring reversal The present findings are in



1

accord with studies[2] of related 1,3,2-oxazaphosphorinane derivatives, but contrast several reports[3] suggesting conformational rigidity in systems akin to 1

Freshly prepared samples of 1 (chromatographed on silica gel, $CHCl_3$ CH_3OH = 9 1) were analyzed at various temperatures by high resolution ^{13}C (68 MHz) and ^1H (220 MHz) nmr spectroscopy As indicated in the Table, only one set of ^{13}C resonances were evident for either D_2O, CD_2Cl_2, or $CDCl_3$ solutions of 1 (ca 0 5 M) at ambient probe temperature (40°)[4] Cooling the latter solution to -60° caused minor shifts in the resonance positions and loss of fine splitting arising from ^{31}P-^{13}C spin-spin coupling, however, no additional signals were detected Changing the solvent to acetone-d_6 also yielded one set of ^{13}C resonances at -60°

Detailed investigation of the temperature dependence of the ^1H nmr spectra of 1 in various solvents was hampered by complex spin-spin coupling patterns and sample decomposition at elevated temperatures[5] Nevertheless, several significant conclusions regarding the con-

Table. ^{13}C NMR Parameters for Cyclophosphamide[a]

Solvent	Temp.	C-4	C-5	C-6	C-α	C-β
D_2O	40°	42.90 (3.7)	27.16 (5 6)	71 44 (5 6)	44 24	49 60 (3 7)
CD_2Cl_2	40°	41 68 (2 9)	26 28 (6 1)	68.28 (6 7)	42.70	49.20 (4.2)
$CDCl_3$	40°	41.44 (3 1)	25 85 (6 1)	67 76 (7 7)	42.32	48 92 (4.6)
$CDCl_3$	-60°	41 24	25.44	68 13	41 95	47 29
$(CD_3)_2CO$	-60°	42 40	27 00	69.13	43 00	49 02

[a] Chemical shifts are in ppm downfield from internal TMS or external TSP (for D_2O). Values in parentheses are $|J_{CP}|$ in Hz, proton decoupled spectra obtained at 68 MHz. Assignments for C-α and C-β are tentative

formational dynamics of 1 derive from these 'H studies The relative signal area of each resonance multiplet allowed its assignment to either an individual hydrogen atom or group of atoms, in no instance were non-integral ratios of signal groupings observed Additionally, when a given multiplet pattern was clearly resolved, the number of lines did not exceed that expected on the basis of a simple first-order coupling analysis. These observations provide telling evidence for the existence in solution[6] of either a single "frozen" diastereomer, or a rapidly interconverting set of diastereomers, and are in accord with our more direct ^{13}C results. The 'H nmr spectrum of 1 was, however, temperature dependent, in that the appearance of several resonance multiplets, upon sample cooling, underwent simplification without significant resonance broadening. This was particularly evident for the α-CH_2 protons (see Figure) While such behavior is not to be expected for the freezing out of a conformational isomer, it is explicable in terms of perturbing a distribution of conformers which are rapidly interconverting on the nmr time-scale[7]

A low barrier-height to ring reversal in 1 would, by extension, be applicable to related systems and thus accommodate high resolution 'H and ^{13}C data for diversely substituted cyclophosphamide derivatives, which we have found without exception to give only one set of 'H and ^{13}C resonances.[1,8] Special substituent effects could, of course, seriously restrict conformational mobility, however, the nature of such effects are not obvious to us in cases related to 1 where rigidity has been claimed[3]

Cyclophosphamide thus presents itself to the activating enzyme system as a "floppy" substrate, interpretation of data obtained in an effort to probe intimate details of the substrate-enzyme interaction must take this into account. Further insights into the conforma-

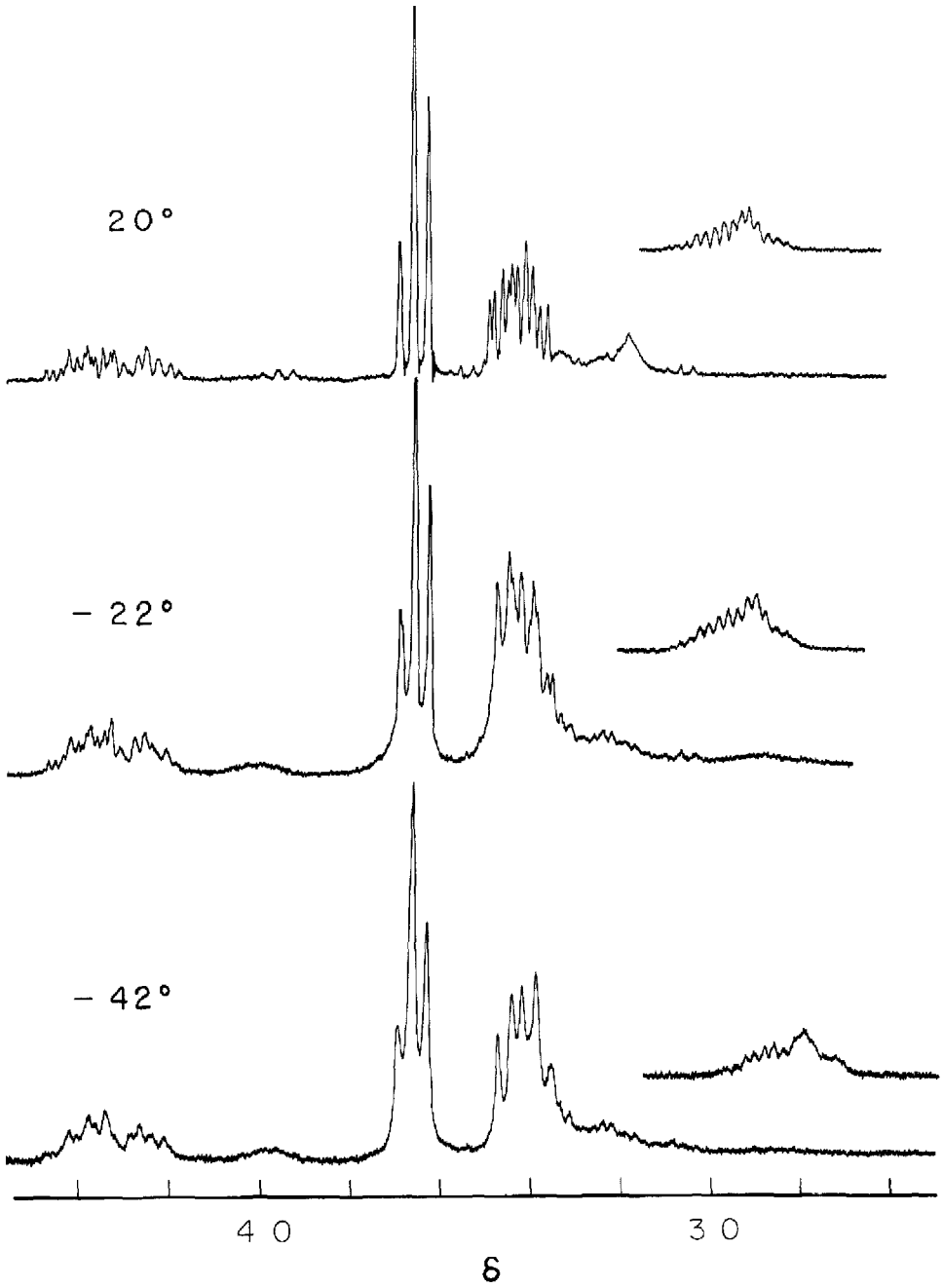


Figure. 220 MHz ^{1}H NMR spectra of 1 in CD_2Cl_2 (TMS) at various temperatures Proton assignments δ 4.5-4 2, C-6, 3.65 (t), C-β, 3 5 - 3 3, C-α, 3.3 - 3 1, C-4, 1 85 - 1.95 (offset), C-5, N-H variable

tional behavior of 1 are being pursued by means of molecular orbital calculations[9]

Acknowledgments This investigation was supported in part by NIH grant number CA-17421 awarded to G Z by the National Cancer Institute, PHS/DHEW We thank Ms Maria L. Thomas for manuscript preparation.

REFERENCES AND FOOTNOTES

1 G Zon, Tetrahedron Lett , 3139 (1975)

2 R. Arshinova, R Kraemer, J. P Majoral, and J Navech, Org. Magn Resonance, 7, 309 (1975), J Durrieu, R Kraemer, and J Navech, ibid , 5, 407 (1973), J Durrieu, R Kraemer, and J. Navech, ibid., 4, 709 (1972).

3 R. S Edmundson and E. W Mitchell, J Chem Soc. (C), 3033 (1968), W. S. Wadsworth, Jr , and Y. G. Tsay, J. Org Chem , 39, 984 (1974), M Mikołajczyk, J Krzywański, and B. Ziemnicka, Tetrahedron Lett , 1607 (1975) An earlier report by W. Wadsworth, Jr., and H Horton, J Amer Chem Soc , 92, 3785 (1970) has been retracted W. S. Wadsworth, Jr., S Larsen, and H L Horten, J Org Chem , 38, 256 (1973).

4 The ^{13}C nmr chemical shifts for 1 in $CDCl_3$, which were recorded at 25 16 MHz, have been reported R F Struck, M C Thorpe, W C Coburn, Jr , and W. R Laster, Jr , J Amer Chem Soc., 96, 313 (1974)

5. For 1 in DMSO-d_6 at 100°, $\tau_{1/2}$ ~15 min, in D_2O at 85°, $\tau_{1/2}$ ~30 min. The hydrolysis of 1 has been studied by Friedman and co-workers [cf J Heterocyclic Chem , 10, 55 (1973)] Our reinvestigation of this work will be the subject of a future publication

6 A single chair-like structure for the monohydrate of 1 obtains in the solid state S. García-Blanco and A Perales, Acta Cryst., B28, 2647 (1972), J C. Clardy, J A Mosbo, and J. G Verkade, J Chem. Soc , Chem. Commun., 1163 (1972)

7 Spectral changes associated with temperature dependent conformational equilibria have been discussed for 1,3,2-dioxyphosphorinane derivatives. R. S Edmundson, Tetrahedron Lett., 1905 (1969), R S Edmundson and E. W. Mitchell, J. Chem Soc (C), 752 (1970), D. W White, G. K. McEwen, R. D Bertrand, and J G. Verkade, J. Chem. Soc. (B), 1454 (1971) For a recent theoretical analysis of conformational preference in these systems, see R F. Hudson and J G Verkade, Tetrahedron Lett., 3231 (1975).

8 G Zon and W Egan, unpublished work

9 A. Rauk, private communication.