[CONTRIBUTION FROM THE BIOLOGICAL LABORATORIES, HARVARD UNIVERSITY, CAMBRIDGE 38, MASS.]

N.m.r. of Nucleic Acid Derivatives. V. Deoxyribose Conformation[1]

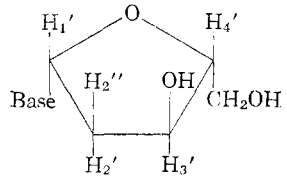
BY CHRISTINE D. JARDETZKY

RECEIVED NOVEMBER 28, 1960

The n.m.r. spectra of the deoxyribonucleic acid derivatives may be analyzed theoretically on the basis of the general XABY type spectrum (where X = H_1', A = H_2', B = H_2'', and Y = H_3') by assuming that the spin–spin coupling constant between A and B is much larger than the difference in their chemical shifts and is large compared to either J_{AX} or J_{BY}. Such an analysis of the spectra of various deoxyribonucleosides and deoxyribonucleotides strongly suggests that the ring oxygen and possibly C_1' may be twisted out of the plane of the five-membered ring.

The 60 megacycle spectra of a number of the biologically active deoxyribonucleosides and -tides were obtained and the spectra were analyzed with the purpose of defining the conformation of the sugar in these compounds in aqueous solution. The method depends on the relationship between the spin coupling constant J_{1-2} for protons H_1 and H_2 and the dihedral angle defined by the H_1–C–C and C–C–H_2 planes in structures of the type H_1–C–C–H_2.[2] Tentative suggestions concerning ribose ring conformation in nucleosides were made previously[3] and an extension of these studies to the ribose nucleotides reveals that either C_2' or C_3' may be twisted out of the plane of the other four ring atoms.[4]

The spectra of the deoxyribose derivatives differ from those of the corresponding ribose compounds



since both H_1' and H_3' are spin coupled to each of the two protons on C_2', H_2' and H_2''. The peaks due to the various protons are easily identified as in Fig. 1 for deoxyuridine.

Spin coupling of the two protons on C_2' with H_1' gives rise to a triplet for the resonance peak due to the latter proton. The spacings between the lines are the same and equal to 6.5 ± 0.5 c.p.s. in all compounds studied. This indicates either that $J_{1'-2'} = J_{1'-2''}$ or that the shift difference between the two protons H_2' and H_2'' is small (of the order of 1 to 3 c.p.s.) and that the coupling constant $J_{2'-2''}$ is much larger than either $J_{1'-2'}$ or $J_{1'-2''}$. Since $J_{2'-2''}$ should be of the order of 13 c.p.s., as demonstrated theoretically[5] and experimentally[6] for the C_{21}-methylene protons in the 21-acetoxy-20-one steroids, the spacing between transitions which have been denoted as 10 and 11 in the ABX case will be small and the spectrum for H_1' or X will appear as a triplet.[7] In this case, $J_{1'-2',2''} = {}^1/_2[J_{1'-2'} + J_{1'-2''}]$. Similarly for H_3' $J^{obsd.}_{3'-2',2''} = {}^1/_2[J_{3'-2'} + J_{3'-2''}]$. The peak spacings and intensities may be explained satisfactorily by theoretical spectra, as seen in Fig. 1 for deoxyuridine. Different possible conformations were considered and the dihedral angles were calculated from equations $J = 8.5\cos^2\phi - 0.28$ for dihedral angles $90° > \phi > 0°$ and $J = 9.5\cos^2\phi - 0.28$ for dihedral angles $180° > \phi > 90°$.[2] Best agreement with the observed coupling constants was obtained with the conformation in which the ring oxygen is twisted out of the plane of the other four ring atoms by about 0.7 Å. as shown in Table I.

TABLE I

	Dihedral angle[a]	O-endo predicted coupling C		Deoxyuridine obsd. coupling C
H_1' – H_2'	135°	4.6	6.0	6.6
H_1' – H_2''	15°	7.3		
H_2' – H_3'	0°	8.2	5.2	5.2
H_2'' – H_3'	120°	2.1		
H_3' – H_4'	135°	4.6		4.0

[a] Estimated from the angles between the projected bonds and the ring plane.

TABLE II

LINE WIDTHS[a] DUE TO THE DEOXYRIBOSE PROTONS IN NUCLEOSIDES AND NUCLEOTIDES

Compound	pH	H_1'	$H_2' + H_2''$	H_3'
Deoxyuridine	5.5	13.2	11.8	14.4
Thymidine	10	14.2	12.4	14.1
Deoxycytidine	5.5	13.5	13.1	14.9
Deoxyguanosine	10	13.6	18.2	12.7
5′-Deoxycytidylic acid	6.5	13.7	13.7	15.6
5′-Thymidylic acid	1	12.6	13.5	11.4
5′-Deoxyadenylic acid	6.5	12.5	11.5	13.2
O-*endo*[b]		12.0	11.2	15.0
C_2'-*endo*[b]		12.2	8.2	4.6
C_4'-*endo*[b]		8.0	10.1	20.7
Planar		10.7	10.7	12.8

[a] In cycles/sec. [b] Predicted from maximally puckered conformations.

In this conformation the large side groups, uracil and CH_2OH, are oriented at a projected angle of about 45° with respect to the ring plane (quasi-equatorial)[4] and the bonds of C_2' and C_3' not involved in ring formation are eclipsed. An eclipsed or nearly eclipsed configuration for these bonds would be characterized by less strain in the deoxyribose as compared with the ribose derivatives since the OH group on C_2' of the latter is substituted by a hydrogen in the former case.

(1) This investigation was supported by a Special Research Fellowship from the Public Health Service, by grants (H-3169) from the Public Health Service and (G-9116) from the National Science Foundation, to Prof. J. T. Edsall.

(2) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959).

(3) C. D. Jardetzky, *J. Am. Chem. Soc.*, **82**, 229 (1960).

(4) C. D. Jardetzky, *ibid.*, submitted for publication.

(5) H. S. Gutowsky, M. Karplus and D. M. Grant, *J. Chem. Phys.*, **31**, 1278 (1959).

(6) J. N. Shoolery and M. T. Rogers, *J. Am. Chem. Soc.*, **80**, 5121 (1958).

(7) J. A. Pople, W. G. Schneider and H. J. Bernstein, "High-resolution Nuclear Magnetic Resonance," McGraw-Hill Book Corp, Inc., New York, N. Y., 1959, p. 134.

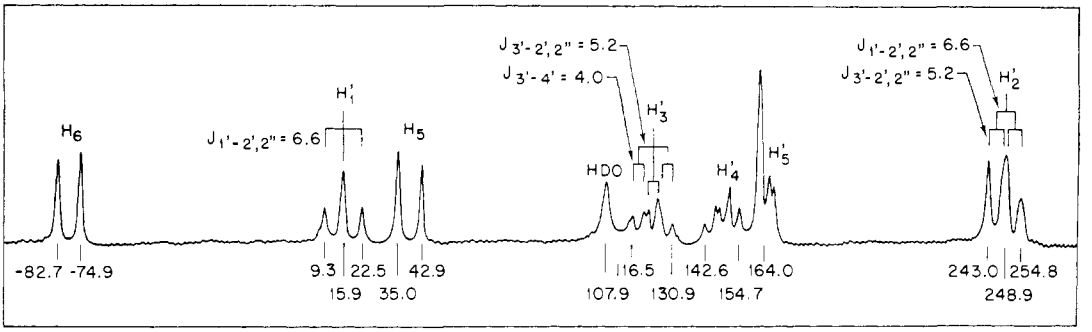


Fig. 1.—Measured and theoretical n.m.r. spectra for deoxyuridine in D_2O at 60 mc. The magnetic field increases from left to right. Primed protons refer to those of the deoxyribose ring, and the unprimed ones to those of uracil. Numbers under the peaks denote the chemical shift in c.p.s. from benzene, the external standard. The peaks due to H_2' and H_2'' (labeled H_2' in the figure) have been analyzed by taking the spacing between the two middle transitions in the AB spectrum (H_2' = A and H_2'' = B) to be less than about 1 c.p.s. This is indeed the case if the difference in chemical shifts of these two protons is of the order of 1 to 3 c.p.s. since J_{AB} = 13 c.p.s. Then spin coupling to H_1' (X) and to H_3' (Y) gives rise to the observed multiplet.

The number of peaks and intensities in the multiplets due to H_1', $H_2' + H_2''$ and H_3' of a number of deoxyribose nucleosides and nucleotides are similar to those of deoxyuridine. With the exception of deoxyguanosine and 5'-thymidylic acid the line widths noted for the other compounds are in good agreement with those predicted on the basis of the O-*endo* conformation, Table II.[8] A more detailed analysis of the multiplet spacings in the spectra of these compounds as well as their temperature dependence will follow.[9]

Acknowledgment.—I am grateful to Prof. J. T. Edsall for his continued interest and support in these studies. I am also indebted to Prof. O. Jardetzky, Department of Pharmacology, Harvard Medical School, for constant encouragement and for the use of the 60 mc. n.m.r. equipment and to Dr. P. Pappas for his expert help in obtaining the spectra.

(8) I am indebted to Dr. R. U. Lemieux for making available to me his manuscript on the configuration and conformation of thymidine prior to publication (*Can. J. Chem.*, in press). The theoretical analysis of the spectra in this case depends on the theory of Richards and Schaefer (*J. Mol. Phys.*, **1**, 331 (1958)) according to which H_2' and H_2'' should have identical chemical shifts. This assumption becomes unnecessary however, if account is taken of the large coupling constant between H_2' and H_2'' (A and B, respectively) and of the small difference between the chemical shifts of these protons. The spectrum may then be treated generally as that of an XABY type ($H_1'H_2'H_2''H_3'$) shown above. Complete analysis of the thymidine spectrum reveals that both O and C_1' are displaced in opposite directions from the plane of the other ring atoms so that the large side groups are more equatorially oriented as compared with those in a completely planar ring (C. D. Jardetzky, *Fed. Proc. Abstracts*, April, 1961). In this case the dihedral angles $H_2'H_3'$ and $H_2''H_3'$ are equal to 5 and 115°, respectively. This conformation is compatible only with an $H_2''H_3'$ dihedral angle of less than 120° and is therefore inconsistent with that suggested by Dr. Lemieux since these angles have been assigned the values of 10 and 130°, respectively. The O-*endo* conformation for deoxyuridine also implies that these angles are equal to 0 and 120°, respectively.

(9) C. D. Jardetzky, Abstracts, Vth International Congress of Biochemistry, Moscow, August 1961.

[CONTRIBUTION FROM THE DEPARTMENT OF MEDICINE AND THE STUDY GROUP ON RHEUMATIC DISEASES, NEW YORK UNIVERSITY SCHOOL OF MEDICINE, NEW YORK, N. Y.]

Interaction in Solution of Lysozyme with Chondroitin Sulfate and its Parent Proteinpolysaccharide[1]

BY MAXWELL SCHUBERT AND EDWARD C. FRANKLIN

RECEIVED DECEMBER 11, 1960

A study was made of the interaction in aqueous solution between the cationic protein lysozyme and the principal parent proteinpolysaccharide of chondroitin sulfate (PP-L) that occurs in bovine nasal cartilage. A series of salt-like compounds appears to exist whose composition is expressed by the equivalence ratio (e.r.) which is equivalent of polyanion per equivalent of lysozyme. These compounds have been divided roughly into three groups by centrifuging: (a) water insoluble, easily sedimented at 700 × *g*, and with e.r. ranging from 1.0 to 2.0; (b) water soluble, giving strongly opalescent solutions, not easily sedimented at 700 × *g* but easily sedimented at 30,000 × *g*, and with e.r. mainly from 1.9 to 5.2; (c) water soluble, giving grossly clear solutions, not easily sedimented at 144,000 × *g*, but detectable in the analytical ultracentrifuge as distinct from PP-L or lysozyme. This centrifugal method of studying lysozyme-polyanion compounds provides a means to determine the proportions of lysozyme and polyanion in those compounds that are sedimentable.

Polyelectrolytes behave as weak electrolytes and a model for this behavior has been developed by Harris and Rice.[2] In mammalian connective tissues polyelectrolytes occur in the form of anionic polysaccharides associated with simple counterions, principally sodium. These polysaccharides are usually bound to proteins by bonds that are not

(1) This investigation was supported by United States Public Health Service Grants A-2594 (C2) and A-28 (C8), and the Arthritis and Rheumatism Foundation.

(2) F. E. Harris and S. A. Rice, *J. Phys. Chem.*, **58**, 725 (1954).