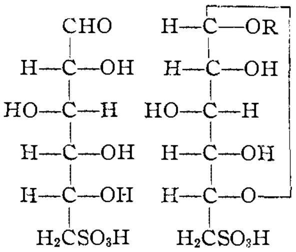
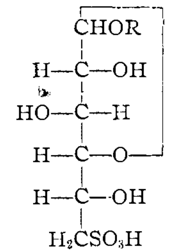


I II

a, R = $-CH_3$
b, R = $-CH_2CH{=}CH_2$
c, R = $-CH_2CHOHCH_2OH$
d, R = $-CH_2CHBrCH_2Br$
e, R = $-CH_2CHBrCH_2OAc$
f, R = $-CH_2CHBrCH_2OH$
g, R = $-CH_2CHOAcCH_2OAc$
h, R = $-CH_2CHOHCH_2OAc$
i, R = $-CH(CH_2Br)_2$



III

a, R = $-CH_2CHBrCH_2Br$(DL)
b, R = $-CH(CH_2Br)_2$
c, R = $-CH_2CHOHCH_2OAc$

Co-recrystallization of Natural and Synthetic Sulfoglycosylglycerols.—A spot of sulfoglycosylglycerol-S^{35} in a radiogram prepared from the natural sulfolipid-S^{35} was cut out and eluted with water to make the standard solution. A portion (25 µl.) of the standard solution was transferred to a 25-ml. bottle for scintillation counting, dried and dissolved in 2 ml. of 95% ethanol. 18 ml. of scintillation liquid (5 g. of PPO and 100 mg. of POPOP in 1 l. of pure toluene) was added and the radioactivity was counted in the Packard Tri-carb scintillation counter (Packard Inst. Co., LaGrange, Ill.).

Co-crystallization was carried out using 75 µl. (32,700 c.p.m.) of the standard solution and 16.7 mg. of the synthetic 6-sulfo-O-α-D-quinovopyranosyl(1→1)-glycerol cyclohexylamine salt. The mixture was dried and recrystallized from methanol-toluene. The specific radioactivity after three recrystallizations was 1,980 c.p.m./mg. After five recrystallizations the specific activity was found to be 1,910 c.p.m./mg., indicating quantitative retention of the S^{35}.

Acknowledgment.—We would like to express our appreciation to Dr. R. O. Mumma for his collaboration in obtaining and interpreting the infrared and nuclear magnetic resonance spectra, to Dr. M. Lepage for optical rotation measurements and to Dr. I. Shibuya and Mr. T. Yagi for their helpful suggestions.

[CONTRIBUTION FROM THE BIOLOGICAL LABORATORIES, HARVARD UNIVERSITY, CAMBRIDGE, MASSACHUSETTS]

Proton Magnetic Resonance of Nucleotides. IV. Ribose Conformation[1,2]

CHRISTINE D. JARDETZKY

RECEIVED MARCH 7, 1961

Analysis of proton magnetic resonance spectra of the cyclic and monocyclic mononucleotide isomers leads to the assignment of specific conformations for the sugar ring. In particular, the spacings in the multiplets due to H_1', H_2' and H_3' agree with a C_2'-*endo* conformation for 2'-AMP in which C_2' is displaced by about 0.5 Å. relative to the ring plane and C_4' is also slightly rotated in the opposite direction. This analysis is valid only if the theory relating coupling constants and dihedral angles in these compounds is quantitatively correct. Increased confidence in defining relative proton orientation from coupling constants in these compounds is based on the excellent agreement between the observed and calculated spin coupling constant, $J_{1'-2'}$, in the case of the cyclic 3':5'-AMP whose sugar conformation (C_3'-*endo* — C_4'-*exo*) is determined solely by steric requirements.

Specific conformations for the D-ribofuranose ring of purine and pyrimidine nucleosides have been previously suggested on the basis of the magnitude of the spin coupling constant between the protons on adjacent carbon atoms of the sugar ring.[3] Conformations for thymidine[4,5] and for other deoxyribonucleosides and nucleotides[6] have also been suggested.

The dependence of the spin coupling constant on the angle formed by the two intersecting planes defined by H_1-C-C and C-C-H_2 in compounds of the type H_1-C-C-H_2 (dihedral angle, ϕ) has been shown theoretically to be described by the function $8.5 \cos^2 \phi - 0.28$ (eq. 1) for angles from 0 to 90° and $9.5 \cos^2 \phi - 0.28$ (eq. 2) for angles from 90 to 180°.[7] Conroy[8] has published a slightly different function for angles from 90 to 180°. The experimentally determined coupling constants from p.m.r. studies on small molecules (substituted ethanes, cyclohexanes, the acetylated aldopyranoses and camphane-2:3-diols) are in good agreement with Karplus' theoretically predicted values.[9,10]

In order to establish the principles governing sugar conformation in the nucleic acid derivatives, a p.m.r. study was made of the various adenylic acid isomers and of the cyclic phosphates of cytidine and uridine. If the magnitude of the coupling constant indeed reflects the relative orientation of the protons on the first and second carbon atoms of D-ribose (and hence the pucker of the 5-membered ring) one would expect that it might vary depending on the position of the phosphate group on the ring.

The spectra and chemical shifts for the protons of the various nucleotides are seen in Fig. 1 The experimentally determined coupling constants be-

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

[2] Abbreviations used: 2'-AMP, 3'-AMP and 5'-AMP = 2'-, 3'-, and 5'-adenosine monophosphate; 2':3'-AMP and 3':5'-AMP = 2':3'-cyclic and 3':5'-cyclic adenosine monophosphate; 2':3'-CMP = 2':3'-cyclic cytidine monophosphate and 2':3'-UMP = 2':3'-cyclic uridine monophosphate.

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

(4) R. U. Lemieux, *Can. J. Chem.*, **39**, 116 (1961).

(5) C. D. Jardetzky, *Fed. Proc. Abstr.*, **20**, 355 (1961).

(6) C. D. Jardetzky, *J. Am. Chem. Soc.*, **83**, 2919 (1961).

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

(8) "Advances in Organic Chemistry," Vol. II, Ed. R. A. Raphael, E. C. Taylor and H. Wynberg, Interscience Publishers, Inc., New York, N. Y., 1960, p. 311.

(9) J. A. Pople, W. G. Schneider and H. J. Bernstein, "High Resolution Nuclear Magnetic Resonance," McGraw-Hill Book Co., Inc., New York, N. Y., 1959.

(10) F. A. L. Anet, *Can. J. Chem.*, **39**, 789 (1961).

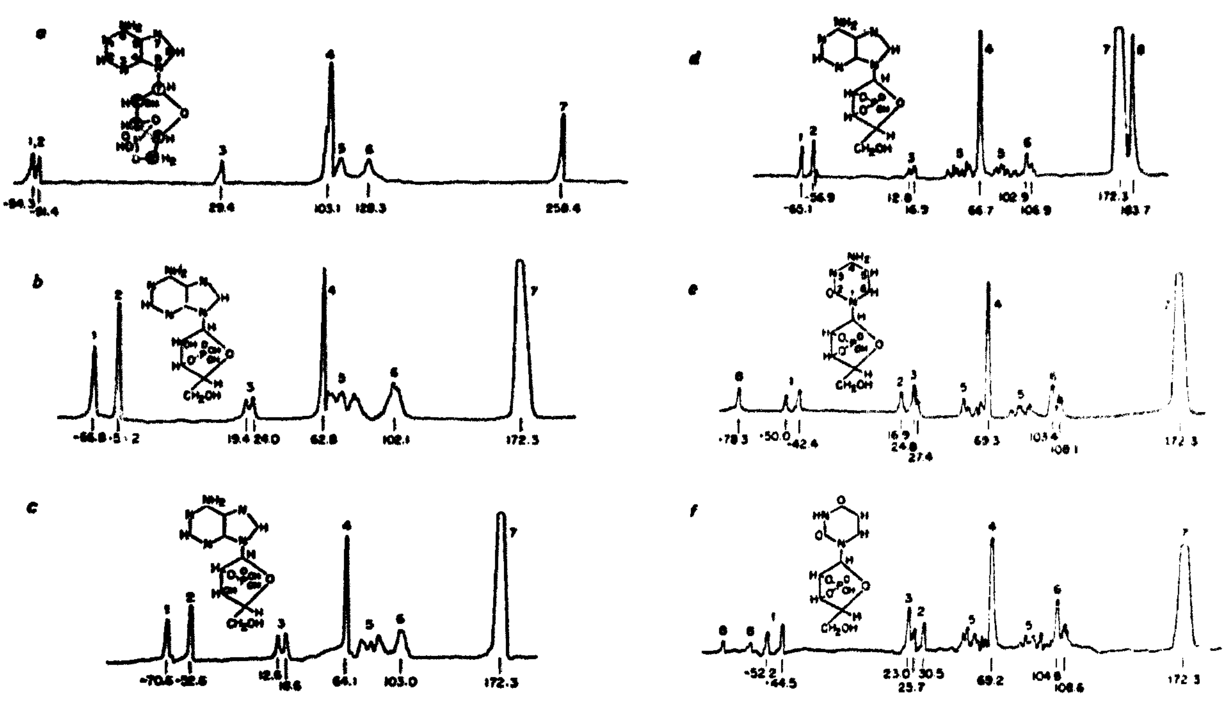


Fig. 1.—(a) The 60 mc. spectrum of 3′:5′-AMP; (b), (c), (d), (e) and (f) the 40 mc. spectra of 3′-AMP, 2′-AMP, 2′:3′-AMP, 2′:3′-CMP and 2′:3′-UMP; respectively. The magnetic field increases from left to right in all cases. The numbers above the peaks indicate the resonances from the protons: 1, H_8; 2, H_2; 3, H_1; 4, HDO; 5, $H'_2 + H_3' + H_4'$; 6, CH_2OD; 7, CH_3 of the internal standard, acetone; 8, unidentified peaks probably arising from impurities. In the pyrimidine nucleoside spectra the doublets 1 and 2 arise from H_6 and H_5, respectively. The numbers below the peaks are the chemical shifts in c.p.s. from the external standard, benzene, which is arbitrarily assigned a chemical shift of zero.

tween the protons of ribose may be found in Table I.

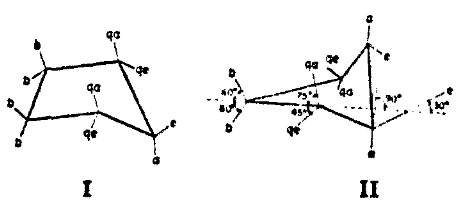
Molecular models were constructed using the wire atomic models referred to in a previous communication.[3] It then became evident that in the case of the 3′:5′-cyclic-AMP the 5-membered sugar ring can assume only one conformation, the C_3'-*endo*-C_4'-*exo* conformation (C_3' pointing on the same side of the plane, defined by atoms C_1', O and C_2', as the C_4'–C_5' sugar bond and C_4' directed on the opposite side).[11] That this conformation is the only possible one is a necessary consequence of the fact that the C_4'–C_5' and the C_3'–O_3' bonds, which belong to the 6-membered ring, are *trans* with respect to each other. The 6-membered ring is formed by atoms C_3',C_4',C_5',O_5', P and O_3' and as noted from Fig. 3 is in the energetically favored chair conformation. (Thus the *trans* protons, H_3' and H_4', lie in the plane defined by H_3'–C_3'–C_4'–$H^{4'}$.) The dihedral angle defined by the intersecting planes H_1'–C_1'–C_2' and

TABLE I

EXPERIMENTALLY MEASURED COUPLING CONSTANTS IN VARIOUS NUCLEOTIDES

Compound[b]	pH	Coupling constants[a] $J_{1'-2'}$	$J_{3'-P}$	$J_{2'-3'}$	$J_{3'-4'}$
2′-AMP	6.2	6.0	6.3	5.2	3.4
3′-AMP	6.0	4.6			
5′-AMP	6.5	4.5			
2′:3′-AMP	6.0	4.1			
3′:5′-AMP	5.3	<1.0			
2′:3′-UMP[c]	6.6	2.7			
2′:3′-CMP[c]	6.0	2.6			

[a] In cycles/sec. [b] The concentration is approximately 0.2 *M* in D_2O. [c] The barium salts.



I II

Fig. 2.—The cyclopentane *envelope*, C_s (Structure I), and *half-chair* C_2, conformations (Structure II). See text for explanation of symbols.

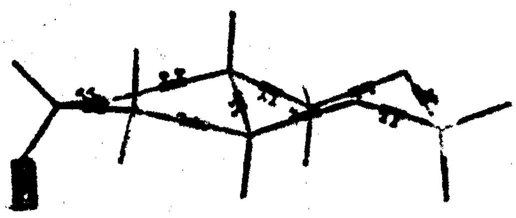


Fig. 3.—The wire model of 3′:5′-AMP. The chair conformation of the cyclic phosphate 6-membered ring on the right, and the *half-chair* conformation of the ribose ring, on the left, are easily discerned. The support for the C_1' bond on the left indicates the position of the adenine base.

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(11) In cyclopentane the form in which one atom is out of the plane of the other four has been referred to as the *cyclopentane envelope*, I, on the basis of the obvious structural similarity, while the form in which two atoms are out of the plane has been referred to as the *cyclopentane half-chair*, II[12] [Fig. 2].

(12) F. V. Brutcher, Jr., T. Roberts, S. J. Barr and N. Pearson, *J. Am. Chem. Soc.*, 81 4915 (1959). These two conformations for cyclopentane have also been referred to according to their symmetry properties as the C_s and the C_2 forms, respectively.[13]

(13) J. E. Kilpatrick, K. S. Pitzer and R. Spitzer, *ibid.*, 69, 2483 (1947).

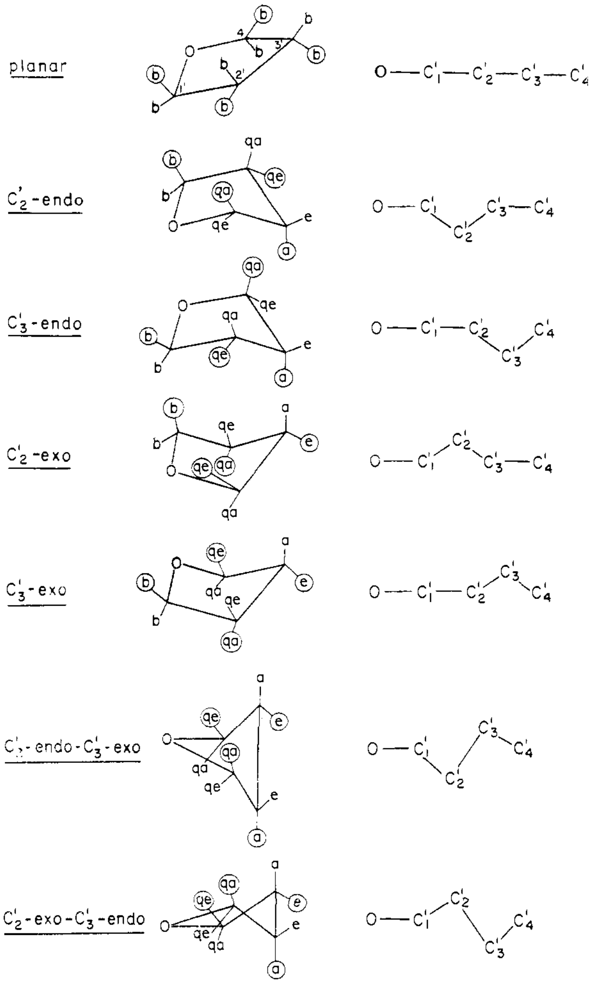

Fig. 4.—Bond orientations in different puckered forms of the ribose ring. The circles represent the protons which give rise to spin–spin coupling multiplets. The diagrams on the right represent the conformation when the ring is viewed side-ways along the $OC_1'C_4'$ plane. The direction of pucker indicated by *endo* and *exo* is defined relative to the direction of the C_4'-C_5' bond.

C_1'–C_2'-H_2' of the sugar ring is 105° and the predicted coupling constant calculated from eq. 2 is 0.36 c.p.s. This is in excellent agreement with the fact that the peak for H_1' is not split (Fig. 1a), as expected in the case of spin coupling of about 0.5 c.p.s. *Thus the situation in the case of 3':5'-AMP is unique insofar that the conformation of the 5-membered sugar ring in solution is determined solely by steric requirements. The agreement with the predicted conformation increases confidence in the use of equations 1 and 2 for determining proton orientations in these compounds.* Similar comparisons based on structures determined by X-ray diffraction, although valuable, are not unique, since the forces which stabilize a specific conformation, and hence the preferred conformation, in the solid may be different from those predominating in the liquid state. In the case of cytidine the measured coupling constant (2.7 c.p.s.) is not in good agreement with that predicted from the conformation of the sugar in the solid state (C_3'-*endo*) which has been determined by X-ray diffraction.[14] Better agreement between observed and predicted coupling constant $J_{1'-2'}$ would be obtained if C_1' were also slightly rotated in a direction opposite to that of C_3' so that the dihedral angle H_1'–H_2' would be equal to 125° instead of 105° which characterizes a simple C_3'-*endo* conformation. Measurement of the coupling constant in 3'-CMP would be of interest since its structure has been recently determined by X-ray diffraction.[15] The ribose of this nucleotide is in the C_2'-*endo* conformation and is different from that found in the corresponding nucleoside, cytidine. Based on the agreement between measured and predicted coupling constant in the case of 3' : 5'-AMP specific conformations may be assigned to the ribose of various nucleosides and nucleotides with a greater degree of confidence.

Different orientations of the bonds in a puckered 5-membered ring are possible. All of these are illustrated in the structures of Fig. 2, where the letters a, e, qa, qe and b stand for axial, equatorial, quasi-axial, quasi-equatorial and bisectional, respectively. The approximate angles formed by projecting each of these bonds on a plane perpendicular to that defined by the three ring atoms as shown in structure II of Fig. 2 are: 30° for equatorial, 45° for quasi-equatorial, 60° for bisectional or planar cyclopentane type of bond, 75° for quasi-axial and 90° for axial bonds. These are angles expected under conditions of maximum puckering; that is under conditions in which either two ring atoms are away from the plane (defined by the other three ring atoms) by about 0.4 Å. or one atom is displaced by about 0.75 Å. relative to the plane defined by the other four ring atoms. Various maximally puckered structures are seen in Fig. 4. It is possible, however, that the extent of puckering may be less than maximal, corresponding to the situation in which the atoms are displaced away from the plane by less than about 0.4 Å. in the *half-chair* conformation and by less than about 0.75 Å. in the *envelope* conformation. In this case the projected angles will have different values depending on the extent of displacement relative to the planar form. The freedom of the 5-membered ring to assume a stable conformation in which it is not puckered maximally may account for the differences between the predicted and the experimentally determined coupling constants, as will be seen in the case of 2'-AMP.

Although on the basis of the spin coupling constant between the first and second protons of the ribose ring one obtains an estimate of the dihedral angle, it is highly desirable to confirm the conclusions based on the data from a single coupling constant by analyzing the rest of the ribose spectrum. Such a first order analysis is possible in the case of the 60 megacycle spectra of 2'-AMP shown in Fig. 5, in which all the peaks due to the different ribose protons can be identified with certainty. For this identification the fact that the H_1'–H_2' coupling constant should be readily recognized in the spectrum due to H_2' was taken into account. However, the multiplet due to H'_2, Fig. 5 arises from coupling with H_1', H_3' as well as with the phosphorus atom. The coupling constants which satisfy the multiplet

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(14) S. Furberg, *Acta Cryst.*, **3**, 325 (1950).

(15) E. Alver and S. Furberg, *Acta Chem. Scand.*, **13**, 910 (1959).

are $J_{1'-2'} = 6.0$, $J_{2'-3'} = 5.2$ and $J_{2'\text{-P}} = 6.3$ c.p.s. giving a total separation between the extreme peaks of the multiplet equal to 17.5 c.p.s. Thus for 2'-AMP the H_1'-H_2' coupling constant of 6.0 c.p.s. is present in the spectrum due to H_2'. It is further noted that the width for the whole H_3' spectrum is 8.7 c.p.s., and its multiplet structure is satisfied with $J_{2'-3'} = 5.2$ and $J_{3'-4'} = 3.4$ c.p.s. If the 5-membered ring in this compound were planar, $J_{1'-2'}$ would be equal to $J_{3'-4'}$ or about 2.1 c.p.s. according to eq. 1. Furthermore $J_{2'-3}$ would be theoretically about 8.2 c.p.s. (eq. 1), since the dihedral angle in this case is equal to zero, Fig. 4. Thus the H_2', and H_3' spectra would be expected to have the same width and to consist of four well resolved component lines. These conditions, however, are not in agreement with the observed spectra; it is therefore justified to conclude that the 5-membered sugar ring in 2'-AMP is puckered.

Approximate dihedral angles for the protons on adjacent carbon atoms in the various conformations of Fig. 4 are seen in Table II.

TABLE II

APPROXIMATE DIHEDRAL ANGLES FOR POSSIBLE COMBINATIONS OF BOND ORIENTATIONS ON ADJACENT CARBON ATOMS IN MAXIMALLY PUCKERED 5-MEMBERED RINGS

Bond orientation	Dihedral angle in degrees
a–a	180
b–b (*trans*)	120
b–b (*cis*)	0
e–e	60
e–a (*trans*)	120
e–a (*cis*)	60
a–qa	165
a–qe	45
b–qa (*trans*)	135
b–qa (*cis*)	15
b–qe (*trans*)	105
b–qe (*cis*)	15
e–qa	45
e–qe	75

Because of the short distance between the two *cis* oxygen atoms on C_2' and C_3', the most probable conformations are those in which one of these two atoms is out of the plane formed by the rest of the ring atoms. The four most probable conformations, the approximate dihedral angles and the calculated coupling constants, are presented in Table III.

TABLE III

THEORETICAL DIHEDRAL ANGLES AND COUPLING CONSTANTS FOR SOME MAXIMALLY PUCKERED 5-MEMBERED RING CONFORMATIONS

	H_1'–H_2'		H_2'–H_3'		H_3'–H_4'	
Ring conformation	Angle	Coupl. const.	Angle	Coupl. const.	Angle	Coupl. const.
C_2'-*endo*	165	8.6	45	4.0	105	0.36
C_2'-*endo*-C_3'-*exo*	165	8.6	60	1.8	75	0.32
C_3'-*endo*	105	0.36	45	4.0	165	8.6
C_3'-*endo*-C_2'-*exo*	75	0.32	60	1.8	165	8.6
Planar	120	2.1	0	8.2	120	2.1

For comparison purposes the angles and coupling constants of the planar ring are also included. It may be seen that the observed coupling constants, Table I, suggest a C_2'-*endo* conformation for 2'-AMP. According to this structure the coupling constant $J_{1'-2'}$ should be greater than $J_{3'-4'}$ as already shown to be the case from the first order analysis of the 60 mc. spectra. The discrepancies between the observed and the calculated coupling constants are probably due to the fact that in the actual structure C_2' is not puckered maximally. A C_2'-*endo* conformation, in which the carbon atom is displaced by about 0.5 Å. (Table IV), is characterized by coupling constants in good agreement with the observed ones. Better agreement between predicted and observed coupling constant ($J_{3'-4'}$) is obtained by assuming that C_4' is also slightly rotated out of the plane so that the H_3'-C_3'-C_4'–H_4' dihedral angle is about 130° (c.c. = 3.5 c.p.s.) instead of 113°. It is conceivable, however, that the actual structure is a time-averaged form of the maximally puckered and the planar conformations. In order to clarify this point a study of the temperature dependence of the spectrum will be undertaken.

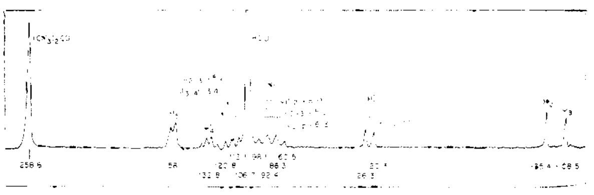

Fig. 5.—First order analysis of the 60 mc. high resolution spectrum of 2'-AMP. The magnetic field is increasing from right to left.

TABLE IV

MEASURED DIHEDRAL ANGLES[a] FOR C_2'-*endo* CONFORMATION AS A FUNCTION OF EXTENT OF PUCKERING

	Angle	Predicted c.c.[b]
C_2' displaced by 0.6 Å.		
H_1'–H_2'	155	7.5
H_2'–H_3'	37	5.1
H_3'–H_4'	110	0.8
C_2' displaced by 0.4 Å.		
H_1'–H_2'	140	5.3
H_2'–H_3'	20	7.2
H_3'–H_4'	115	1.4

[a] A dihedral angle is defined as the angle between the projections of the C–H bonds on a plane perpendicular to the C–C bond for the configurations H–C–C–H. In order to measure this angle the 5-membered ring is placed on a sheet of paper so that the C–C bond is perpendicular to it; the projections of the CH bonds are drawn carefully and the angle is measured with a protractor. [b] Coupling constants calculated from eq. 1 and 2.

Another possible, although improbable, reason for these differences is that the nature of the side groups affects the magnitude of the coupling constant. Other than distortion of the dihedral angles however, side group effects on the coupling constants have not been theoretically considered and experimental evidence for the existence of such effects is still uncertain. Furthermore, the side groups of these compounds are sufficiently similar to make differential effects on the coupling constants improbable. For example, both 3'- and 5'-AMP have $J_{1'-2'}$ equal to 4.5 c.p.s. while in the cyclic form 3':5'-AMP $J_{1'-2'}$ is less than about 1 c.p.s.

Steric considerations also favor a C_2'-*endo* type of pucker for 2'-AMP, since the bulky phosphate and adenine groups are equatorially and quasi-equatorially oriented, respectively, and the CH_2OH group is bisectionally oriented. (This conformation has been previously suggested for the purine nucleosides.)

Based on the observation that the $J_{1'-2'}$ values for 2′:3′-UMP and 2′:3′-CMP are the same within experimental error it is reasonable to suggest that their sugar rings are characterized by the same conformation. In the case of 3′-AMP, 5′-AMP and 2′:3′-AMP the coupling constant of about 4.5 c.p.s. implies a dihedral angle of about 135° between H_1' and H_2'. This could be achieved by having a C_2'-*endo* conformation in which C_2' is displaced by somewhat less than 0.4 Å. However, analysis of the 60 mc. spectra due to the other ribose protons is essential before suggesting a specific puckered form for these compounds.

Experimental

The various nucleotides were dissolved in 0.5 ml. of D_2O and the *p*H was adjusted with NaOD to the desired value. The *p*H was measured with *p*H paper accurate to 0.1 *p*H units. The nucleotide solution was transferred quantitatively to small lyophilizing flasks of 25–35 ml. capacity with an additional 1.5 ml. of D_2O and was lyophilized in a Vis-tis freeze-dry apparatus. The lyophilization was repeated 2 to 3 times and finally the sediment was dissolved in 0.5 ml. of D_2O. An aliquot was pipetted into an n.m.r. tube (5 mm. o.d.). Approximately 0.005 ml. of acetone (internal standard) was then added and the tubes were stoppered with plastic caps and kept at −20°.

High resolution spectra at 40 and 60 megacycles were obtained with standard spectrometers from Varian Associates. The shifts were obtained by superimposing the acetone sideband on the sharp peaks of the spectra while the shifts for broader lines were calculated from at least four spectra by interpolation. However, the spacings for H_1' were obtained by superposition of an *equal* intensity acetone side-band or by interpolation from the spectrum calibrated in the following way; an acetone side band is placed on the low field side near the doublet. The field is slowly swept through the doublet and then the frequency of the audio-oscillator is quickly changed so that the side band appears on the high field side of the doublet. The two side band frequencies are read off from a Hewlett–Packard counter. In all cases the shift of the acetone peak was measured from an external benzene standard introduced in the sample in a small capillary and all shifts were then expressed relative to the benzene peak.

Acknowledgments.—I am indebted to Prof. J. T. Edsall for his interest and support in these studies. I am also grateful to Prof. O. Jardetzky for encouragement and for the use of the n.m.r. equipment at Harvard Medical School, to Dr. P. Pappas for his help in obtaining the spectra of 3′:5′- and 2′-AMP and to the Department of Chemistry. Harvard University, for the use of the 40 mc. n.m.r. equipment.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING, UNIVERSITY OF ILLINOIS, URBANA, ILLINOIS]

Bovine Serum Albumin in Water–Dioxane Mixtures

BY K. E. VAN HOLDE AND S. F. SUN[1]

RECEIVED MAY 26, 1961

In order to investigate the influence of solvent environment upon the configuration of globular proteins, the behavior of bovine serum albumin (BSA) in mixtures of *p*-dioxane and dilute salt solutions has been studied. Experimental methods included sedimentation, optical rotation, viscometry and the measurement of apparent partial specific volume. In the neighborhood of the isoelectric point the configuration of the protein molecule is only slightly affected by dioxane in concentrations up to 35% (vol.). On the other hand, the expansion of the BSA molecule in acid solution is profoundly influenced by the presence of dioxane. The expansion is greater at a given *p*H, and the onset of expansion occurs at a higher *p*H when dioxane is present. The behavior can be semi-quantitatively explained in terms of a simple electrostatic model. The influence of dioxane upon the configuration of BSA appears to result mainly from the effect of the non-polar solvent upon electrostatic interactions within the molecule, rather than from direct interaction of dioxane with the protein.

Introduction

The nature of the forces responsible for maintaining the conformations of globular protein molecules in solution has been the subject of much recent inquiry. It appears that a complex and delicate balance is involved which is sensitive to small changes in the composition of the protein molecule or its environment. Examples of the latter effect are afforded by the recent studies by Tanford and co-workers of the result of the addition of organic solvents to aqueous solutions of ribonuclease[2] and β-lactoglobulin[3] and by Bresler, *et al.*,[4,5] of human and horse serum albumin in dioxane–water mixtures. This paper describes somewhat similar experiments with bovine serum albumin (BSA), in water–1,4-dioxane mixtures. Earlier studies of the intrinsic viscosity and optical rotation of BSA in dioxane–water mixtures have been reported by Foster and Yang,[6] who suggested that the increase in intrinsic viscosity observed upon addition of dioxane was a consequence of the decrease in the dielectric constant of the solution.

Serum albumin provides an interesting material for such study, because of the large number of intrachain disulfide bonds which serve to limit the number and kind of conformations available to the molecule and because of the large body of experimental data which has been accumulated concerning the behavior of this protein.

The choice of a solvent pair was dictated in part by the desirability of minimizing flow interaction in sedimentation. This can be accomplished by using

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(1) Northland College, Ashland, Wisconsin.

(2) R. E. Weber and C. Tanford, *J. Am. Chem. Soc.*, **81**, 3255 (1959).

(3) C. Tanford, P. K. De and V. G. Taggart, *ibid.*, **82**, 6028 (1960).

(4) S. E. Bresler, *Discussions Faraday Soc.*, **25**, 158 (1958).

(5) S. E. Bresler, V. P. Kushner, S. Ya Frenkel, *Biokhimiya* (consultants' Bureau English translation), **24**, 630 (1959).

(6) J. F. Foster and J. T. Yang, "Abstracts 129th Meeting, American Chemical Society," p. 4-C.