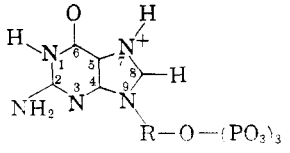
guanosine triphosphate (GTP) (Table XII) where the titration of the group with pK_A equal to 3.30 causes a shift to lower field by about 0.90 and 0.20 p.p.m. of the C_8 and the C_1' protons, respectively. In this instance, the conclusion that the proton is probably attached to N_7 of the imidazole portion of the ring as shown below rather than to the $-NH_2$ group seems warranted. This conclusion



is further supported by the X-ray crystallographic data for the guanine cation which has four hydrogens on N_1, N_9, N_{10} and C_8 and two hydrogen bonds between N_3 and N_{10} and between O_6 and N_7.[23,24]

A comparison of the spectra in Figs. 4a and 4c, as compared with those in the Figs. 4b and 4d, respectively, reveals that the peaks due to the C_2 protons of ATP and to the C_8 proton of GTP are not only shifted to lower fields but are also broadened at *p*H's near the pK_A's of the titratable groups. This broadening may be accounted for by an exchange reaction of the type as depicted for ATP.

$$ATP^*—NH_2 + ATP—NH_3^+ \rightleftharpoons ATP—NH_2 + ATP^*—NH_3^+$$

Contrary to the marked effects due to protonation of the amino groups, essentially no effects in the chemical shifts were observed for the protonation of the $(CON)^-$ group as seen in the case of inosine (Table VIII), inosinic acid (Table XIII) and guanosine triphosphate (Table XII).

As may be seen from Table XIV the coupling constant for the C_1' proton in the purine nucleotides ranges between 4 and 5 c.p.s. Inferences concerning the conformation of the ribose ring from the magnitude of this coupling constant are discussed in a subsequent paper.[26]

Acknowledgment.—The authors are very grateful to Dr. John T. Edsall for a critical perusal of the manuscript and helpful suggestions.

(26) C. D. Jardetzky, THIS JOURNAL, **82**, 229 (1960).

CAMBRIDGE, MASS.

[CONTRIBUTION FROM THE RETINA FOUNDATION, PAPER 81, AND THE CHEMISTRY DEPARTMENT OF HARVARD UNIVERSITY]

Proton Magnetic Resonance Studies on Purines, Pyrimidines, Ribose Nucleosides and Nucleotides. III. Ribose Conformation[1]

BY CHRISTINE D. JARDETZKY[1a]

RECEIVED JULY 13, 1959

Specific conformations for D-ribose in nucleosides and nucleotides have been proposed on the basis of their proton magnetic resonance spectra. In the purine nucleosides C_2' is considered to be out of the plane defined either by C_1', O and C_4' or by C_1', O, C_3' and C_4' and is pointing on the same side as the C_4'–C_5' bond, while in the pyrimidine nucleosides, C_3' is out of the plane defined either by C_1', O and C_4' or by C_1', C_2', O and C_4'. Available information from X-ray diffraction and specific rotation studies further supports the existence of these structures.

Introduction

On the basis of proton magnetic resonance studies (p.m.r.), it was previously concluded that the five-membered ring of D-ribose in nucleosides assumes different puckered conformations depending on whether the base is a purine or a pyrimidine.[2]

Although the stereoconfiguration of five-membered rings has not been established, it has long been suspected that they are not planar structures. In the case of cyclopentane, Kilpatrick, Pitzer and Spitzer[3] have calculated a potential energy for the planar configuration which is higher by about four kcal./mole as compared with that corresponding to the two types of puckered structures which were considered possible. In the puckered conformations, either one atom is out of the plane of the other four, or two atoms are twisted with respect to one another and are out of the plane of the other three ring atoms. Thermodynamic and other measurements on cyclopentane disclose that the puckering of the ring is not of a definite type, but that the angle of maximum puckering rotates around the ring.[3] More recently information on the conformation of five-membered rings from X-ray diffraction studies has been summarized by Spencer.[4]

The purpose of this communication is to present specific three-dimensional structures for the furanose ring of D-ribose in nucleosides and nucleotides inferred from the p.m.r. measurements, and to point out that these structures are also in agreement with the available information from X-ray diffraction studies and with other physico-chemical properties.

Experimental

The spectra were taken with the Varian 40 mc. high resolution n.m.r. spectrometer at a field of about 9,400 gauss. The distance between the aromatic and methyl peaks of toluene, 196.6 c.p.s. was used to calculate the frequency difference between any two peaks in the spectrum. The results obtained in this manner agreed within 0.5 c.p.s. with those obtained by the side band modulation technique.[5]

(1) This investigation was supported by a research grant (B-904) from the National Institute of Neurological Diseases and Blindness, Public Health Service.

(1a) Biological Laboratories, Harvard University, Cambridge, Massachusetts.

(2) (a) C. D. Jardetzky and O. Jardetzky, THIS JOURNAL, **81**, 222 (1959). (b) C. D. Jardetzky, presented at the Symposium on Physical Methods in Biochemistry, Federated Society Meetings, Atlantic City, April, 1959.

(3) (a) J. E. Kilpatrick, K. S. Pitzer and R. Spitzer, THIS JOURNAL, **69**, 2483 (1947). (b) K. S. Pitzer and W. E. Donath, *ibid.*, **81**, 3213 (1959).

(4) M. Spencer, *Acta Cryst.*, **12**, 59 (1959).

(5) J. T. Arnold and M. G. Packard, *J. Chem. Phys.*, **19**, 1608 (1951).

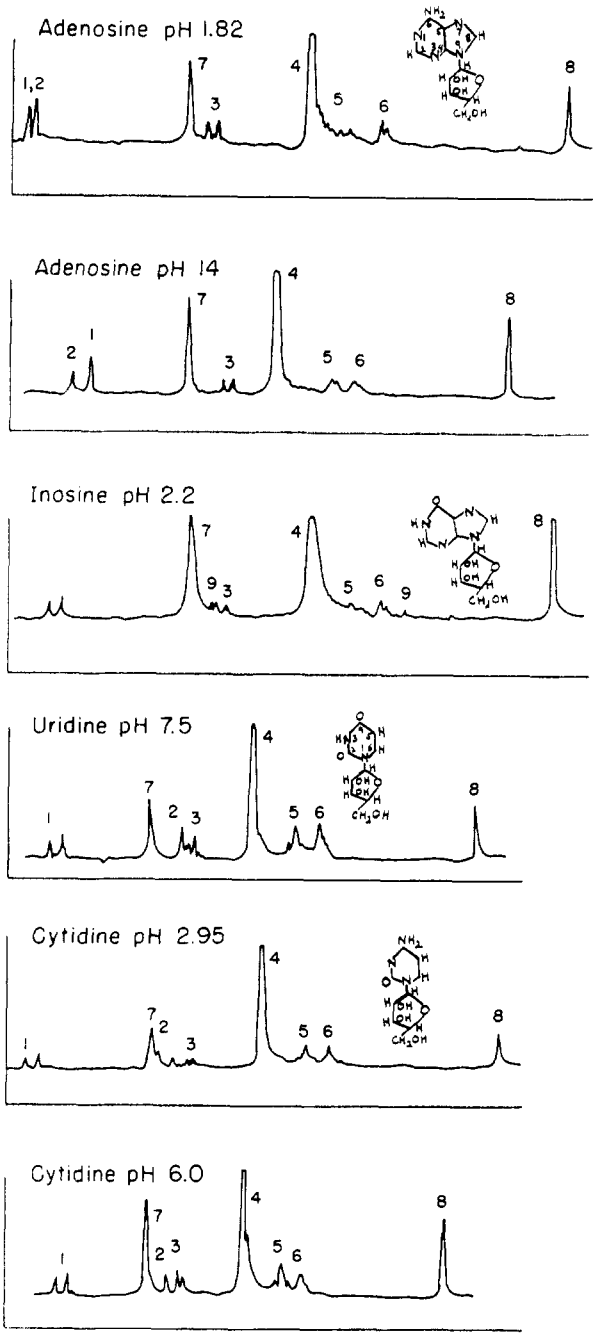


Fig. 1.—Peak identification: peaks 1 and 2 are due to H_6 and H_5 of the heterocyclic rings, respectively, in uridine and cytidine. 3, H_1'; 4, H_2O; 5, $H_2' + H_3' + H_4'$; 6, $2H_5'$; 7, toluene phenyl-Hs; 7, Tol CH_3; 9, side band. Peaks 1 and 2 in adenosine and inosine correspond to H_2 and H_8 of the bases, but not necessarily, respectively. The primed protons refer to those of ribose.

The compounds were dissolved in 3 to 5 ml. of D_2O to give a final concentration of about 2×10^{-1} *M* and the *p*H was measured with a Beckman Model G *p*H meter. Aliquots of about 0.3 ml. were pipetted into the n.m.r. tubes (5 mm. o.d. and about 6 in. long) after adjusting the *p*H to the desired value by adding either concentrated HCl or anhydrous Na_2O_2. In the presence of D_2O, the latter is rapidly converted to NaOD and molecular oxygen. The *p*H of the solution may be increased in this manner and in many instances has been adjusted back to the original value with hydrochloric acid. The only change noted in the spectrum of the back titrated solution was the enlargement of the water peak due to the protons of the added acid.

Results and Discussion

Evidence for Different Ribose Conformations in Nucleosides and Nucleotides.—A close examination of the ribose spectra of purine and pyrimidine nucleosides and nucleotides reveals these differences.

(a) The constant for the nuclear spin–spin coupling[6] between the *trans* protons H_1' and H_2' of ribose is of the order of 2 to 3 c.p.s. in cytidine and uridine, while in adenosine, guanosine, inosine and xanthosine, it varies from about 5.0 to 7.0 c.p.s. and in 5′-adenylic acid (AMP) and in adenosine and guanosine triphosphates (ATP and GTP respectively) it varies from 4 to 5 c.p.s.[2]

(b) The rest of the ribose spectrum, which is made up of peaks due to H_2', H_3', H_4' and the two protons on C_5', occurs at a field higher than that of the water peak. This portion of the ribose spectrum of the pyrimidine nucleosides looks strikingly different from that of the purine nucleosides (Fig. 1). In the former case, two major peaks with an intensity ratio of about 1 to 2 are observed. Each of these peaks is seen to be split further into a few narrower ones. The larger of the two peaks which occurs at a lower field is probably due to H_2', H_3' and H_4', while the smaller one at the higher field is due to the two methylenic protons on C_5'. In agreement with this assignment of peaks is that given by Dr. W. E. Cohn for the ribose spectrum of pseudouridine.[7]

In the purine nucleosides, in contrast to the pyrimidine nucleosides, the peak due to H_2', H_3' and H_4' is spread over a larger frequency range and is made up of a number of well-resolved peaks. Furthermore, the line due to the methylene protons is broader and is characterized by a somewhat different line-splitting than that observed in the pyrimidine derivatives.

In connection with the appearance of the ribose spectrum, it is noted that at *p*H about 14, the center of gravity of the large broad peak moves to higher field, while the position of the peak due to the protons on C_5' remains unchanged. This shift may be explained by the ionization of either the C_2' or the C_3' hydroxyl groups which would bring about the shielding of the proton attached to the same carbon atom.

Specific Ribose Conformations.—Specific ribose conformations are proposed on the basis of the quantitative information available on the H_1'–H_2' coupling constant. The magnitude of the spin coupling constant between protons on adjacent carbon atoms depends primarily on three factors: (a) on the electron density of the carbon–carbon

(6) The nuclear spin–spin coupling between protons on adjacent carbon atoms results from an indirect interaction of the spin vector of one proton with that of the other *via* the electrons of the proton–carbon and carbon–carbon bonds. Consequently, the line due to one of the protons is a doublet because of the two possible spin states of the neighboring proton which are $+1/2$ and $-1/2$, and the distance in cycles/sec. between the peaks of the doublet is a direct measure of the energy of interaction.

(7) (a) W. E. Cohn, presented at the Symposium on Physical Methods in Biochemistry, Federated Society Meetings, Atlantic City, April, 1959. (b) W. E. Cohn, *Biochim. Biophys. Acta*, **32**, 569 (1959).

bond, (b) on configuration and (c) on conformation.

Since the D-ribofuranose ring of all naturally occurring nucleosides and nucleotides is a saturated ring in which all the bonds are single, one must consider only differences in conformation to account for the observed differences in the coupling constants.

Analysis of the proton magnetic resonance spectra of the substituted ethanes and ethylenes has shown that the coupling constant between *trans* protons is larger than that between *gauche* or *cis* protons.[8] Furthermore, Lemieux and coworkers[9] in a thorough study of a large number of acetylated aldopyranoses have demonstrated that the coupling constant between axial protons on adjacent carbon atoms is two to three times greater than that between proton pairs in any other conformation. The coupling constant between two axial protons was found to vary from 5 to 8 c.p.s.

In a very interesting theoretical study, Karplus has calculated the coupling constant due to the contact electron–spin interaction between protons on the adjacent carbons of ethane as a function of the dihedral angle[10] (Fig. 2). His treatment is based on the assumptions that the carbon hybrid orbitals are tetrahedral (sp^3), that the carbon–carbon bond is 1.543 Å. and that the electron–orbital and electron–dipole dipole interactions do not contribute significantly to the magnitude of the coupling constant. It is also pointed out by Karplus that the experimental results on the acetylated aldopyranoses are in good qualitative agreement with the theoretical predictions.

A model of ribose was constructed using wire atoms,[11] 1Å. = 5 cm., and the dihedral angle between H_1' and H_2' was measured in various conformations of the furanose ring. The possibility of having a completely planar ring was excluded on the basis that: (1) the eclipsed configuration of the bonds on adjacent carbon atoms is known to be less stable than the staggered arrangement, and (2) the O_2'–O_3' distance of 2.6 Å. is too small, since the van der Waals radius for oxygen is 1.4 Å. On the other hand, the strain due to the close contact between the two *cis*-hydroxyl groups may be relieved and the eclipsed configuration of bonds on adjacent carbon atoms can be avoided by having either C_2' or C_3' out of the plane of the other four. There are four such possible structures which are characterized by different H_1'–H_2' dihedral angles. Table I depicts the measured angles, the orientation of H_1' and H_2' with respect to the plane of the four ring atoms and the predicted coupling constant.

It is noted that for the structures where C_2' or C_3' are out of the plane of the other four atoms and are directed toward the same side of the D-ribose ring as the β-C_1'–N and the C_4'–C_5' bonds, the predicted coupling constants are 6.9 and 1.7 c.p.s. There is then a close agreement between the predicted and the measured coupling constants for the purine and pyrimidine nucleosides, respectively. However, on the basis of the predicted coupling constants for the reverse pucker (5.4 and 0.05 c.p.s.), it is not possible to exclude these structures. For the purpose of visualizing the pucker of ribose, the following structures are presented (Fig. 3).

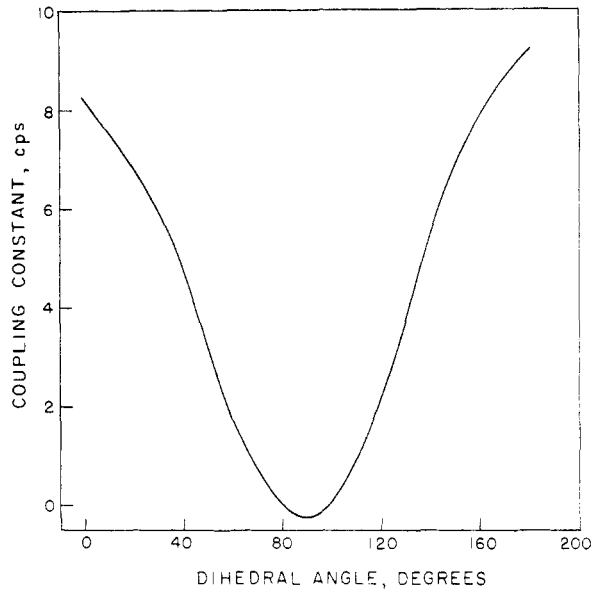


Fig. 2.—Plot of Karplus' calculated contact coupling constant *vs.* the dihedral angle defined by two protons on the adjacent carbon atoms of ethane.[10]

TABLE I

Struc.	Atom out of plane[b]	Dihedral angle H_1'–H_2'[a]	Orientation H_1'	Orientation H_2'	Pred. coupling const.[c]
A	C_2'-*endo*	150°	Axial	Axial	6.9
A′	C_3'-*exo*	140°	Axial	Axial	5.4
B	C_3'-*endo*	115°	Equatorial	Equatorial	1.7
B′	C_2'-*exo*	105°	Equatorial	Equatorial	0.05

[a] ± 5°. [b] "*Endo*" means that the atom is located on the same side of the plane defined by the ring atoms C_1', O and C_4' as the C_4'–C_5' bond, while "*exo*" means that it is found on the opposite side. [c] Extrapolated values from Fig. 2.

Structures in which both C_2' and C_3' point in opposite directions (and are out of the plane of the other three ring atoms) are also quite reasonable. Indeed such structures could be considered to be intermediates in going from configuration A to A′ or from B to B′. This is clearly demonstrated on the wire model of ribose whose structure with the C_2'-*endo* conformation can be changed to that with the C_3'-*exo* conformation without altering the O_2'–O_3' distance and without going through the planar configuration. This is accomplished by slightly twisting the bonds between C_1' and O and between C_4' and O, and applies also to the C_2'-*exo*–C_3'-*endo* pair, but not to the C_2'-*endo*–C_3'-*endo* pair. The intermediate structures A–A′ and B–B′ should be characterized by H_1'–H_2' coupling constants ranging between the limiting values de-

(8) A. D. Cohen, N. Sheppard and J. J. Turner, *Proc. Chem. Soc.*, 118 (1958).

(9) R. V. Lemieux, R. K. Kullnig, H. J. Bernstein and W. G. Schneider, THIS JOURNAL, **80**, 6098 (1958).

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

(11) I am very grateful to Dr. A. Rich for making available the wire model of ribose.

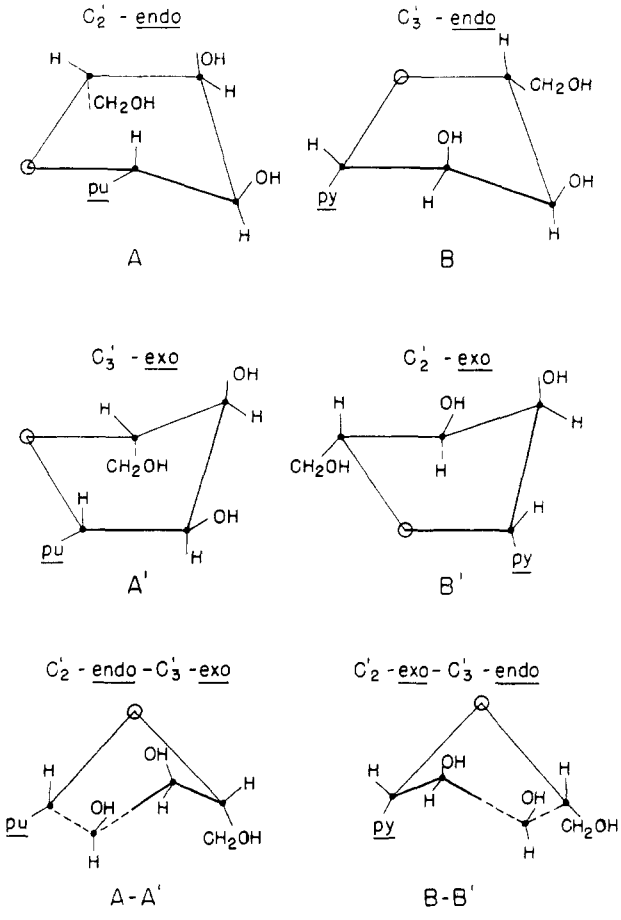


Fig. 3.—Possible conformation of D-ribose in nucleosides and nucleotides: pu, purine; py, pyrimidine, *endo-* and *exo-* indicate the direction of the pucker with respect to the direction of the C_4'–C_5' bond and the plane defined by the ring atoms C_1', O and C_4'.

fined by the structures A and A′ and B, B′, respectively.[11a]

It should be pointed out at this stage that the coupling constant range measured experimentally for the pyrimidine nucleosides is somewhat overestimated because of partial overlap of the C_5 proton spectrum on that due to H_1'. The C_5 substituted nucleosides such as 5-methyl -uridine or -cytidine will permit a better estimation of the coupling constant.

Other conformations in which C_1', O or C_4' are out of the plane of the other four ring atoms are considered to be unlikely, since either the O_2'–O_3' distance would be too short, or some bonds on adjacent carbon atoms would be found in the eclipsed configuration. Furthermore, the conformations in which O and C_4' are out of the plane can be discarded at least for the purine nucleosides because the H_1'–H_2' coupling constant implies an axial–axial orientation of these protons.

Further evidence for the existence of the proposed structures is provided from the crystal structure determinations on compounds with five-membered rings. The B conformation of the furanose ring is found in sucrose sodium bromide dihydrate,[12] cytidine,[13] 5′-bromo-5′-deoxythymidine,[14] calcium thymidylate,[15] and for the five-membered ring of muscarine iodide.[16] However, in the case of 5,6-dimethylbenzimidazole 1-α-D-ribofuranoside (3′-phosphate), the nucleotide attached to the porphyrin nucleus of vitamin B_{12}[17,17a] the second carbon atom is out of the plane of the other four ring atoms.

That the conformation of D-ribose and D-2-deoxyribose in purine nucleosides and nucleotides is different from that in the corresponding pyrimidine derivatives is also suggested by specific rotation measurements. While all the purine derivatives are characterized by a negative specific rotation, which varies from about −20° to −60°, the pyrimidine derivatives show a positive specific rotation which varies from about 0° to +50° in aqueous solution.[18] In this connection it is interesting that D-ribofuranose tetra-O-acetate exists in the solid in an unstable form (m.p. 56–58° and $[\alpha]^{15}D$ +20° in $CHCl_3$) which spontaneously changes to a more stable form (m.p. 82–85° and $[\alpha]^{20}D$ −12° in $CHCl_3$).[19]

It is reasonable to expect that intramolecular oscillations between structures A and A′ or B and B′ require less energy than that required to change structure A to B or A′ to B′ and *vice versa*. From Fig. 3 it becomes clear that the intermediate structure A–A′ of the purine derivatives is a mirror image of B–B′, a possible structure for the pyrimidine derivatives only. The three-dimensional arrangement of the carbon–carbon bonds in the ribose ring may possibly account for the *levo-* and *dextro-*rotatory properties of the purine and pyrimidine nucleosides and nucleotides.[20]

It should be pointed out, furthermore, that the distance between C_5' and the nitrogen atom of the

(11a) While this paper was in press a study of the infrared spectra of α-halocamphors, -2-indanones and -cyclopentanones was published by Brutcher, *et al.* [F. V. Brutcher, Jr., T. Roberts, S. J. Barr and N. Pearson, THIS JOURNAL, **81**, 4915 (1959)] in which they conclude that α-halocyclopentanones assume a stable conformation characterized by having the two β-ring atoms twisted with respect to each other and pointing in opposite directions with respect to the plane formed by the carbonyl group and the two α-carbon atoms. Their conclusions are based on the observed shift to higher frequency of the band due to the carbonyl group in going from axial to equatorial orientation of the α-halogen atom, as well as on the agreement between observed and calculated dipole moments.

(12) C. A. Beevers and W. Cochran, *Proc. Roy. Soc.* (*London*), **A190**, 257 (1947).

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

(14) M. Huber, *ibid.*, **10**, 129 (1957).

(15) P. Horn, V. Luzzati and K. N. Trueblood, *Nature*, **183**, 880 (1959).

(16) F. Jellinek, *Acta Cryst.*, **10**, 277 (1957).

(17) D. Hodgkin, *et al.*, *Proc. Roy. Soc.* (*London*), **A242**, 228 (1957).

(17a) The equation describing the mean plane through a set of points was solved using the space parameters of the ring atoms of ribose in the crystal of vitamin B_{12}. The distances of the ring atoms from the mean plane were calculated and found to have the values: C_1' − 0.156 Å., C_2' + 0.359 Å., C_3' − 0.262 Å., C_4' + 0.045 Å. and O + 0.014 Å. It is clear then that C_2' is further out of the plane than the other ring atoms and is pointing in the same direction as the α-glycosyl bond and in opposite direction from the C_4'—C_5' bond since the atoms N and C_5' are found at about +0.950 Å. and −0.925 A., respectively with respect to the mean plane. I am greatly indebted to Dr. David M. Blow for the set of equations which made these calculations possible.

(18) E. Chargaff and J. N. Davidson, "The Nucleic Acids," Vol. I, Academic Press, Inc., New York, N. Y., 1955, p. 156–178.

(19) Ref. 18, p. 44.

(20) W. Kauzmann, "Quantum Chemistry," Academic Press, Inc., New York, N. Y., 1957, pp. 630–635.

glycosyl bond is considerably smaller in the C_2'-*exo* and C_3'-*exo* conformation than in any other conformation which has been considered acceptable. Thus, intramolecular conversions from one conformation to another of the ribose ring will cause changes in the diameter of polynucleotides and nucleic acids which may or may not be followed by changes in specific rotation. A systematic survey of the stereo configuration of five-membered rings and their derivatives by p.m.r. and optical rotation studies is planned, as well as studies on the implications of the furanose conformation on nucleic acid structure and on the specificity of enzymes which attack these compounds.

Acknowledgment.—I am very grateful to Dr. J. T. Edsall for helpful criticism of the manuscript.

CAMBRIDGE, MASS.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, CORNELL UNIVERSITY MEDICAL COLLEGE, THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF FLORIDA SCHOOL OF MEDICINE, AND THE BUREAU OF MEDICAL RESEARCH, EQUITABLE LIFE ASSURANCE SOCIETY OF THE UNITED STATES]

Coördination Complexes and Catalytic Properties of Proteins and Related Substances. IV. Reactions of Glycine-containing Dipeptides with Cupric Ions and with *p*-Nitrophenyl Acetate[1]

BY WALTER L. KOLTUN, MELVIN FRIED[2,3] AND FRANK R. N. GURD[2,4]

RECEIVED JUNE 10, 1959

Equilibria between Cu(II) ions and glycylglycine (GG), sarcosylglycine (GS), L-prolylglycine (PG), glycyl-L-valine (GV), L-valylglycine (VG), glycylsarcosine (GS) and glycyl-L-proline (GP) have been measured by potentiometric titration. All the peptides except the last two form complexes with Cu(II) in which the peptide hydrogen atom is displaced. The potentiometric results are correlated with spectral measurements. Analysis of the equilibria above *p*H 7 in Cu(II)–dipeptide mixtures is coupled with the demonstration that a certain basic complex catalyzes the hydrolysis of *p*-nitrophenyl acetate (NPA). The formation of a catalytically-inert olate complex is also explored. The rate of acetylation of the various dipeptides by NPA is correlated with their basicity. Lastly, a kinetic method is used to measure the formation of a mixed complex of Cu(II) with both glycylglycine and imidazole.

Introduction

The preceding paper of this series[5] dealt with the effect of zinc and cupric ions on the reaction of glycylglycine (GG) with *p*-nitrophenyl acetate (NPA). By a combination of kinetic and equilibrium measurements similar to previous studies[6,7] on systems containing imidazoles, it was possible to determine unambiguously the association constant for the formation of the complex $CuGG_2^-$, according to equation 3, and to confirm the picture of successive equilibria described by the following sequence of reactions[8]

$$Cu^{++} + GG^- \rightleftarrows CuGG^+ \quad k_1^+ = \frac{(CuGG^+)}{(Cu^{++})(GG^-)} \quad (1)$$

$$CuGG^+ \rightleftarrows CuGG + H^+ \quad k_{I,0} = \frac{(H^+)\,(CuGG)}{(CuGG^+)} \quad (2)$$

$$CuGG + GG^- \rightleftarrows CuGG_2^- \quad k_{II,-} = \frac{(CuGG_2^-)}{(CuGG)(GG^-)} \quad (3)$$

In reaction 2 a hydrogen ion is displaced from the peptide linkage. The participation of the peptide linkage as well as the α-amino group in the formation of metal complexes means that a knowledge of the reactivity of only the first residue in a peptide sequence is insufficient to permit the prediction of the behavior of the N-terminus of a peptide or protein. For this reason the present study was undertaken to compare the reactivities of several dipeptides with Cu(II) ions and with NPA.

The dipeptides studied primarily are glycylglycine (GG), sarcosylglycine (SG), L-prolylglycine (PG), glycyl-L-valine (GV) and L-valylglycine (VG), all of which follow the reaction sequence 1–3 with Cu(II) ions. The evidence of Datta and Rabin[9] that glycylsarcosine (GS) and glycyl-L-proline (GP) follow equations 1 and 4 has been confirmed.

$$CuGS^+ + GS^- \rightleftarrows CuGS_2 \quad k_{II,0} = \frac{(CuGS_2)}{(CuGS^+)(GS^-)} \quad (4)$$

The values for the separate formation constants for these various peptides are compared, and with their help the properties of the absorption spectra of the individual complexes are estimated.

Equilibria in equimolar mixtures of Cu(II) ion and dipeptide at higher *p*H values have been explored and the processes described in equations 5, 6 and 7 are shown to be possible sequels to that in equation 2.

$$CuGG \rightleftarrows CuGGOH^- + H^+ \quad k_{I,-} = \frac{(H^+)(CuGGOH^-)}{(CuGG)} \quad (5)$$

$$CuGGOH^- + CuGG \rightleftarrows (CuGG)_2OH^- \quad k_{2I,-} = \frac{((CuGG)_2OH^-)}{(CuGGOH^-)(CuGG)} \quad (6)$$

(1) This investigation was supported by research grant No. H-2739 from the National Heart Institute, U. S. Public Health Service.

(2) A preliminary report presented at the 132nd National Meeting, American Chemical Society, New York, N. Y., Sept. 8–13, 1957, described work begun in the Department of Biochemistry, Washington University School of Medicine, St. Louis, Missouri.

(3) Senior Postdoctoral Fellow of the United States Public Health Service.

(4) John Simon Guggenheim Memorial Fellow and Helen Hay Whitney Fellow, Washington University, St. Louis, 1954–1955.

(5) W. L. Koltun and F. R. N. Gurd, THIS JOURNAL, **81**, 301 (1959).

(6) W. L. Koltun, R. N. Dexter, R. E. Clark and F. R. N. Gurd, *ibid.*, **80**, 4188 (1958).

(7) W. L. Koltun, R. E. Clark, R. N. Dexter, P. G. Katsoyannis and F. R. N. Gurd, *ibid*, **81**, 295 (1959).

(8) The nomenclature of the association constants is defined in ref. 5; see especially footnote 17.

(9) S. P. Datta and B. R. Rabin, *Trans. Faraday Soc.*, **52**, 1117 (1956).