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 C4 and the C1' protons, respectively. In this instance, the conclusion that the proton is probably attached to N7 of the imidazole portion of the ring as shown below rather than to the \(-\text{NH}_2\) group seems warranted. This conclusion is further supported by the X-ray crystallographic data for the guanine cation which has four hydrogens on N7, N8, N9 and C4 and two hydrogen bonds between N7 and N10 and between O6 and N7.24,25

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 \( \text{C}_1' \) protons of ATP and to the \( \text{C}_2' \) proton of GTP are not only shifted to lower fields but are also broadened at \( \rho \)'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.

\[
\text{ATP}^+ - \text{NH}_4 + \text{ATP} - \text{NH}_4^+ \rightleftharpoons \text{ATP} - \text{NH}_4 + \text{ATP}^+ - \text{NH}_4^+
\]

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 \( (\text{CON})^- \) 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 \( \text{C}_1' \) 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, 299 (1960).

CAMBRIDGE, MASS.
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 between the trans protons H1' and H2' 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.

(b) The rest of the ribose spectrum, which is made up of peaks due to H2', H3', H4' and the two protons on C4', 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 H2', H3' and H4', while the smaller one at the higher field is due to the two methylenic protons on C4'. In agreement with this assignment of peaks is that given by Dr. W. E. Cohn for the ribose spectrum of pseudouridine.

In the purine nucleosides, in contrast to the pyrimidine nucleosides, the peak due to H2', H3' and H4' 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 pH 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 C4' remains unchanged. This shift may be explained by the ionization of either the C4' or the C4' 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 H2'-H3' coupling constant. The magnitude of this spin coupling constant between protons on adjacent carbon atoms depends primarily on three factors:

(a) on the electron density of the carbon-carbon bonds

(b) 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.
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. Furthermore, Lemieux and co-workers 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 1.545 \(\text{Å} \), 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,\(^{11}\) 1 Å = 5 cm., and the dihedral angle between \(\text{H}_2'\) and \(\text{H}_3'\) was measured in various conformations around 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 \(\text{O}_5'-\text{O}_2'\) 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 \(\text{C}_2'\) or \(\text{C}_4'\) out of the plane of the other four.

There are four such possible structures which are characterized by different \(\text{H}_2'\)-\(\text{H}_3'\) dihedral angles. Table I depicts the measured angles, the orientation of \(\text{H}_2'\) and \(\text{H}_3'\) with respect to the plane of the four ring atoms and the predicted coupling constant.

It is noted that for the structures where \(\text{C}_2'\) or \(\text{C}_4'\) are out of the plane of the other four atoms and directed toward the same side of the ribose ring as the \(\beta-\text{C}_1'\)-N and the \(\beta-\text{C}_4'\)-C bond, 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 puckering of ribose, the following structures are presented (Fig. 3).

Structures in which both \(\text{C}_4'\) and \(\text{C}_4'\) 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 \(\text{C}_4'\)-endo conformation can be changed to that with the \(\text{C}_4'\)-exo conformation without altering the \(\text{O}_5'-\text{O}_2'\) distance and without going through the planar configuration. This is accomplished by slightly twisting the bonds between \(\text{C}_1'\) and O and between \(\text{C}_4'\) and O, and applies also to the \(\text{C}_4'\)-exo-\(\text{C}_4'\)-endo pair, but not to the \(\text{C}_4'\)-endo-\(\text{C}_4'\)-endo pair. The intermediate structures A-A' and B-B' should be characterized by \(\text{H}_2'\)-\(\text{H}_3'\) coupling constants ranging between the limiting values de-

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(11) I am very grateful to Dr. A. Rich for making available the wire model of ribose.
formations in which O and C' are out of the plane can be discarded at least for the purine nucleosides because the H' - H' 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 configuration of the furanose ring is found in sucrose sodium bromide dihydrate,\(^{17}\) 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 \(1\)-\(a\)-d-ribouronoside (3'-phosphate), the nucleotide attached to the porphyrin nucleus of vitamin B\(_{12}\),\(^{17,18}\) 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 \(-25^\circ\) to \(-60^\circ\), the pyrimidine derivatives show a positive specific rotation which varies from about \(+5^\circ\) to \(+35^\circ\) in aqueous solution.\(^{14}\) In this connection it is interesting that d-ribofuranose tetra-O-acetate exists in the solid in an unstable form (m.p. 56-59\(^\circ\) and [\(\alpha\)]\(^{20}\) +20\(^\circ\) in CHCl\(_3\)) which spontaneously changes to a more stable form (m.p. 82-85\(^\circ\) and [\(\alpha\)]\(^{20}\) -12\(^\circ\) 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 levorotatory properties of the purine and pyrimidine nucleosides and nucleotides.\(^{20}\)

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

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Fig. 3.—Possible conformation of d-ribose in nucleosides and nucleotides: pu, purine; py, pyrimidine, endo and exo indicate the direction of the puckering with respect to the direction of the C' - C' bond and the plane defined by the ring atoms C', O and C'.

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Coördination Complexes and Catalytic Properties of Proteins and Related Substances.

IV. Reactions of Glycine-containing Dipeptides with Cupric Ions and with p-Nitrophenyl Acetate

BY WALTER L. KOLTUN, MELVIN FRIED2-3 AND FRANK R. N. GURD2,4

RECEIVED JUNE 10, 1959

Equilibria between Cu(II) ions and glycylglycine (GG), sarcosylglycine (SG), L-prolylglycine (PG), glycyl-L-valine (GV), and dipeptides 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 pH 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 Cu(II) and with 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 series5 dealt with 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 glycyl-L-proline (GP) follow equations 1 and 4 have been confirmed.

In reaction 2 a hydrogen ion is displaced from the peptide linkage. The participation of the peptide linkage as well as the a-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.

Equilibria in equimolar mixtures of Cu(II) ions and dipeptide at higher pH 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.

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.

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\[
\text{Cu}^{2+} + \text{GG} \rightarrow \text{CuGG}^+ \quad k_{1}\text{Cu} \quad \text{(1)}
\]

\[
\text{CuGG}^+ \rightarrow \text{CuGG} + \text{H}^+ \quad k_{1,0} = \frac{(\text{CuGG})}{(\text{CuGG}^+)} \quad \text{(2)}
\]

\[
\text{CuGG} + \text{GG} \rightarrow \text{CuGGG}^+ \quad k_{1,1} = \frac{(\text{CuGGG}^+)}{(\text{CuGG})(\text{GG})} \quad \text{(3)}
\]

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\text{CuGG} \rightarrow \text{CuGGG}^+ + \text{H}^+ \quad k_{1,1} = \frac{(\text{CuGGG}^+)}{(\text{CuGG})} \quad \text{(5)}
\]

\[
\text{CuGGG}^+ + \text{CuGG} \rightarrow (\text{CuGG})_2\text{OH}^- \quad k_{1,1} = \frac{(\text{CuGG})_2\text{OH}^-}{(\text{CuGGG}^+)(\text{CuGG})} \quad \text{(6)}
\]

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.

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