saturated solution for all experiments, its solubility in neutral solution being less than 0.2 mg./ml. Solutions of the latter were prepared at 100°; at room temperature they persisted without crystallization for several days. That no hydrolysis occurred in their preparation was demonstrated by the absence of a ninhydrin reaction.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF TEXAS, AUSTIN, TEX.]

Comparison of Ribose and Deoxyribose Nucleosides by N.m.r. and Deductions Regarding Ribose and Deoxyribose Nucleic Acids. I. Tautomeric Form

BY LILA GATLIN AND JEFF C. DAVIS, JR.

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The 60-megacycle n.m.r. spectra in dimethyl sulfoxide of the eight nucleosides found in ribose and deoxyribose nucleic acids are characterized and compared. Exchangeable proton peaks are identified. It is concluded that cytidine has the amino form but deoxycytidine has the imino form. Biological implications are outlined.

Introduction

The n.m.r. spectra of adenosine, cytidine and uridine have been studied in D_2O at varying pH.[1] Conformation of the ribose ring in these compounds was suggested[2] from the theoretical work of Karplus[3] and the observed H'_1 coupling constants.

It is apparent that considerable study of the nucleic acid derivatives, particularly with regard to ring conformation and tautomeric form, has been made with n.m.r. The importance of such studies is obvious. However, no attempt has been made to formulate a comprehensive picture of the implications of these studies concerning DNA-RNA structure and interaction.

TABLE I

CHEMICAL SHIFTS[a] AND COUPLING CONSTANTS[b] OF THE NUCLEOSIDES IN DIMETHYL SULFOXIDE

Nucleoside	H_1' ±0.01	H_2' ±0.01	H_3' ±0.01	H_4'	H_5'	OH_2' ±0.05	OH_3' ±0.05	OH_5' ±0.05	H_2 ±0.01	H_8 ±0.01	H_5 ±0.01	H_6 ±0.01	NH_2 ±0.05	NH	NH
A	0.55	2.25–2.33	1.83	2.42–2.58	2.75–2.92	*J* 4.5 1.27	0.97	*J* 5.4 1.05	−1.68	−1.87			−0.86		
dA	.09	2.75–3.00	1.92–2.08	2.50–2.67	2.75–3.00		1.12	*J* 5.6 1.16	−1.70	−1.87			− .87		
G	.71	2.25–2.33	2.00	2.53	2.75–3.00	1.25–1.42	*J* 5.7 1.02	1.25–1.33		−1.52			− .06	−4.33–−4.42	
dG	.31	2.75–3.08	2.08–2.17	2.58–2.75	2.75–3.08		1.17–1.25	1.42–1.50		−1.50			− .05	−4.17–−4.50	
C	.70	2.25–2.58	2.25–2.58	2.25–2.58	2.67–2.92						*J* 7.3 0.38	*J* 7.3 −1.7	− .17 − .33		
dC	.39	4.22	2.18	2.60	2.75–2.92						*J* 7.9 0.22	*J* 7.9 −1.83		−2.25–−2.33	−3.42–−3.50
U	.70	2.42–2.67	2.42–2.67	2.42–2.67	2.75–3.08	1.33–1.58	1.08–1.25	1.33–1.58			*J* 8.2 0.82	*J* 8.2 −1.42		−4.92	−4.67
T	.31	4.40	2.17–2.25	2.58–2.83	2.75–3.08		1.31	1.53	4.72			−1.21		−4.72	

[a] All shifts in p.p.m. measured from aromatic toluene peak. The aromatic toluene peak was measured to be 6.86 p.p.m. below the peak of a tetramethylsilane internal standard. [b] *J* in c.p.s. ± 0.5 c.p.s.

A similar, more detailed analysis of the n.m.r. spectra of synthetic α- and β-thymidine in D_2O has been done.[4] Also a conformational study of the n.m.r. spectrum of deoxyuridine in D_2O has been reported with a brief, generalized reference to other deoxyribose nucleosides and nucleotides.[5] Since deoxyuridine does not occur in the nucleic acids, it is not immediately pertinent to our discussion.

Recently the 40-megacycle n.m.r. spectra of adenosine, guanosine, cytidine, uridine and thymidine in d_6-dimethyl sulfoxide were reported; but resolution and spectral analysis were limited.[6] The deoxyribose nucleosides, deoxycytidine, deoxyadenosine and deoxyguanosine, were not studied.

The present work presents the first complete set of 60-megacycle n.m.r. spectra of all eight major ribose and deoxyribose nucleosides found in nucleic acids, in a common solvent (dimethyl sulfoxide), at the same pH, with the exchangeable proton peaks present. Cytidine and deoxycytidine are exceptions in that cytidine is isolated as the hemisulfate and deoxycytidine as the hydrochloride. The slight acidity thus introduced is evidenced only by the lack of exchangeable ring hydroxyl proton peaks. However, since the exchangeable NH_2 and NH protons still appear, it is felt that the comparability of these spectra has not been significantly altered. There are no significant differences in parameters and general spectral character of the nucleosides in dimethyl sulfoxide and D_2O except that the exchangeable protons do not appear in the latter. Hence conclusions are most probably applicable to biological systems.

(1) C. D. Jardetzky and O. Jardetzky, *J. Am. Chem. Soc.*, **82**, 222 (1960).
(2) C. D. Jardetzky, *ibid.*, **82**, 229 (1960).
(3) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959).
(4) R. U. Lemieux, *Can. J. Chem.*, **39**, 116 (1960).
(5) C. D. Jardetzky, *J. Am. Chem. Soc.*, **82**, 2919 (1961).
(6) J. P. Kokko, J. H. Goldstein and Leon Mandell, *ibid.*, **83**, 2909 (1961).

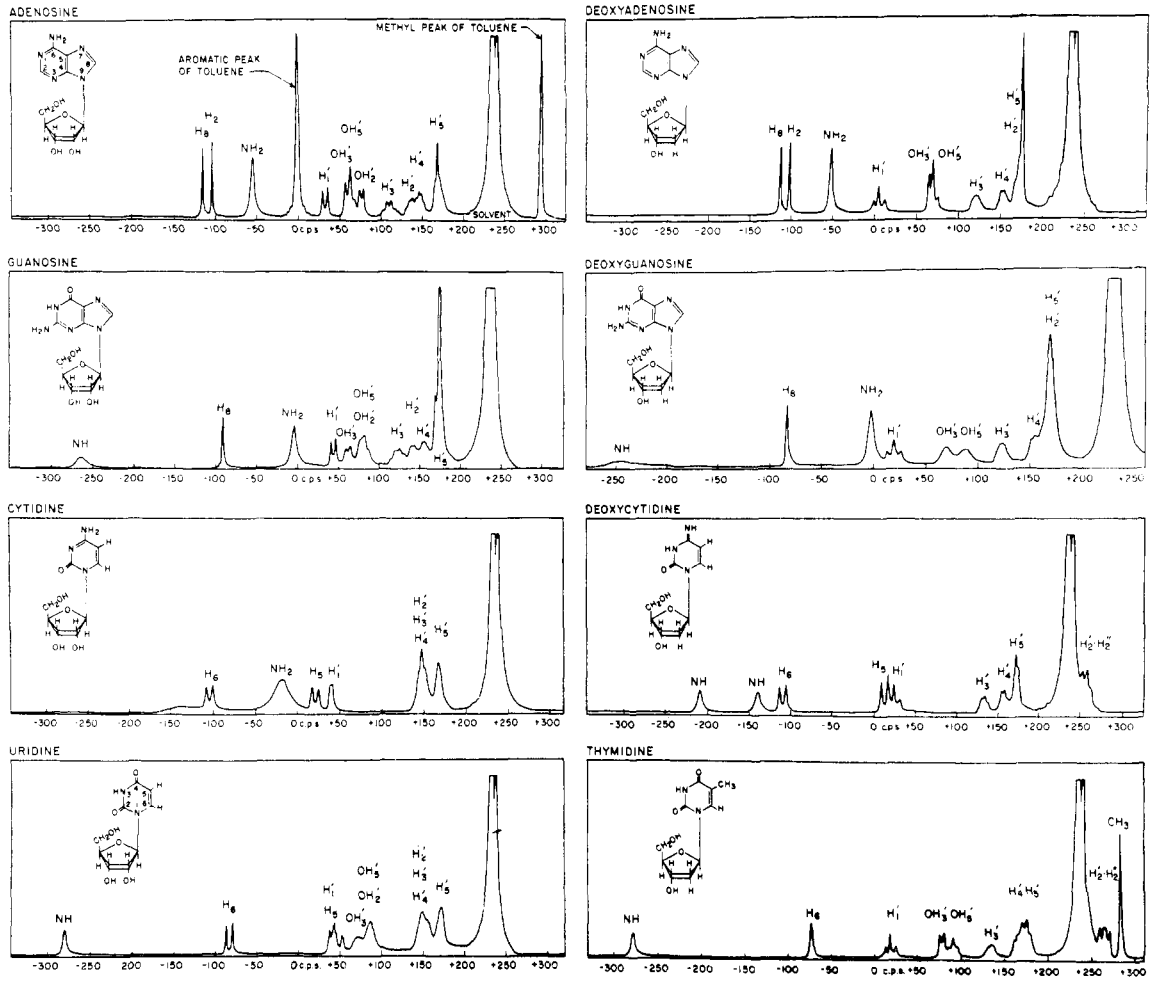


Fig. 1.—60 Mc. n.m.r. spectra of ribose and deoxyribose nucleosides in dimethyl sulfoxide; field increases from left to right; sweep rate 174 c.p.s. min.$^{-1}$, H_1 field 60 db. attenuation below 0.5 watt, frequency response 100 c.p.s. Toluene reference peaks included in the adenosine spectrum. Primed numbers refer always to ribose ring.

Experimental

All spectra were obtained with the Varian DP-60 high resolution spectrometer. Coupling constants and chemical shifts were measured by the method of the Jardetzkys.[1,2] The chemical shift between the aromatic and methyl peaks of toluene at 60 megacycles is 294.9 ± 0.2 c.p.s. All shifts are reported relative to the aromatic peak of toluene as external reference.

Coupling constants were reproducible to within ±0.2 c.p.s. for a given preparation; but the average of several preparations varied ±0.5 c.p.s. All compounds were obtained from the California Corporation for Biochemical Research and met NRC standards.

TABLE II

H_1' COUPLING CONSTANTS IN DIMETHYL SULFOXIDE

Nucleoside	$J_{H1'}$ ± 0.5 c.p.s.	Nucleoside	$J_{H1'}$ ± 0.5 c.p.s.
A	5.9	C	2.5
dA	7.3	dC	7.1
G	5.7	U	4.2[a]
dG	7.0	T	6.9

[a] Overestimated due to overlap with H_5.

All nucleosides were soluble at room temperature (20°) in dimethyl sulfoxide except cytidine which required slight heating. However, heating at 55° for 15 minutes did not alter the spectra of any of the compounds. Standing at room temperature for 2 weeks did not alter the spectra except to decrease peak intensity very slightly. A concentration of 0.8 *M* was used throughout.

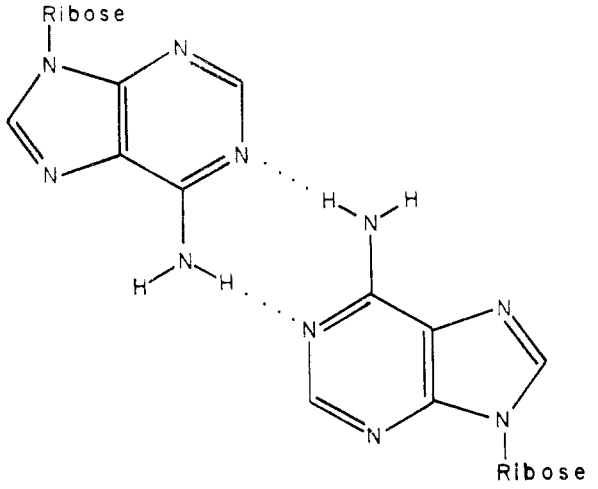
Dimethyl sulfoxide was used as a solvent because it is non-exchanging, easily handled and readily available. Upon comparison of our spectra with those of Kokko, Goldstein and Mandell,[6] it appears that d_6-dimethyl sulfoxide offers no advantages in regard to exchangeability for the nucleosides. It does reduce the large solvent peak which appears in our spectra. Double distillation of the solvent produced no observable changes in spectra.

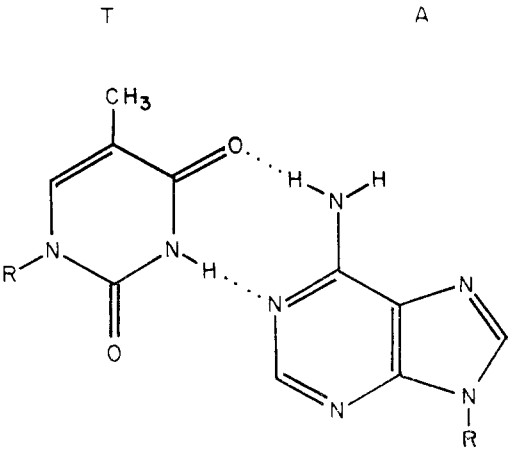
Results and Discussion

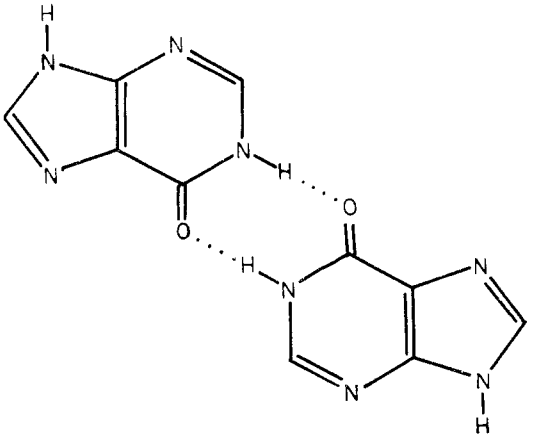
I. Spectral Analysis. Purine and Pyrimidine Protons.—Figure 1 shows the eight nucleoside spectra obtained and Table I lists the measured chemical shifts with pyrimidine and hydroxyl proton coupling constants. When a peak is broadened, a range is indicated. Tables II and III lists the H_1' coupling constants in dimethyl sulfoxide and D_2O, respectively.

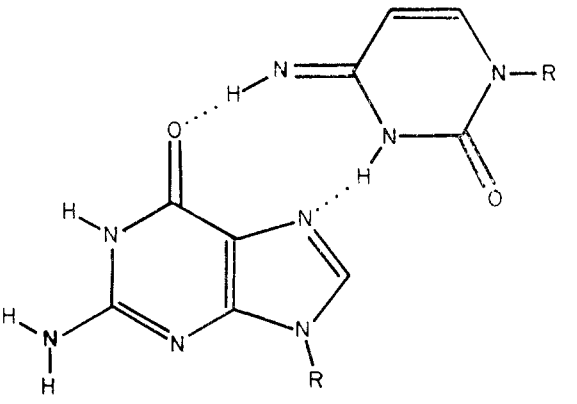
The pyrimidine protons, H_5 and H_6, have been unquestionably identified.[1] The purine protons, H_2 and H_8, in adenosine and deoxyadenosine lie in the H_6 region. The differentiation of H_2 and H_8 is not certain, but we will follow the assignment of Jardetzky.[1] The H_5 and H_6 coupling constants varying from 7.3 to 8.2 c.p.s. are in excellent agreement with those found in D_2O.[1]

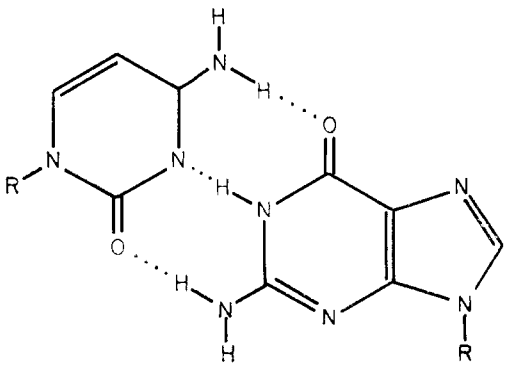
Ribose Ring Protons.—The H_1' proton of the ribose ring has been positively identified[1,4] and

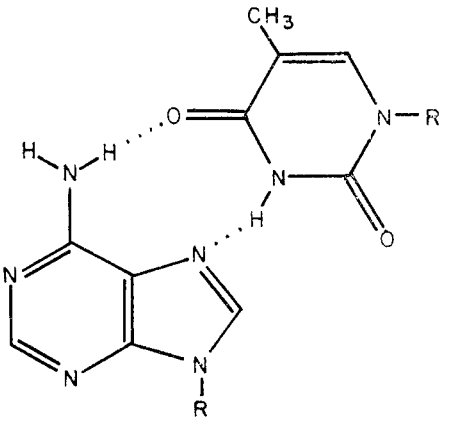












lies in the narrow range of 0.55 to 0.70 p.p.m. for the ribose nucleosides and 0.09 to 0.39 p.p.m. for the deoxyribose nucleosides. This general shift to higher field of the H_1' proton upon going from the deoxyribose to the ribose form can be explained most simply as an electrostatic shielding of H_1' due to substitution of a hydroxyl group for a hydrogen at C_2'. The H_1' peak is consistently a doublet in the ribose nucleosides and a triplet in the deoxyribose nucleosides due to spin–spin interaction with one and two C_2' protons, respectively.

TABLE III

H_1' COUPLING CONSTANTS IN D_2O

Nucleoside	$J_{H_1'} \pm 0.5$ c.p.s.	Nucleoside	$J_{H_1'} \pm 0.5$ c.p.s.
A	5.5[1]	C	3.0[a]
dA	6.6[5]	dC	6.6[5]
G	6.4[1]	U	3.3[a]
dG	6.6[5]	T	7.0[4]

[a] Our own measurements.

In deoxycytidine the H_5 doublet overlaps the first peak of the H_1' triplet. In uridine the H_5 doublet and H_1' doublet overlap. This H_1'–H_5 region of uridine is anomalous. Figure 2 shows the change in spectral character observed in two different preparations; III is preparation I at 52°. Hence II which is similar to III appears to be the

more stable. Uridine is the only nucleoside which shows this behavior. A possible explanation for this variation is that different conformational forms of uridine may be present. Further study in continuing on this point.

Form II has been used for the measurement of chemical shifts and coupling constants, H_1' being considered the first two peaks of lower intensity with $J = 4.2$ c.p.s. and H_5 the last two peaks with $J = 8.2$ c.p.s. ($J_{H_4} = 8.2$ c.p.s. also). The coupling constant of $J = 4.2$ c.p.s. for H_1' would be expected to be somewhat high due to the H_5 overlap. Hence this is in satisfactory agreement with $J = 3.3$ c.p.s. found in D_2O (see Table III).

H_3', H_4' and H_5' lie at 1.83 to 3.08 p.p.m. and always in this order.[1,4,5] While H_4' and H_5' are relatively constant in all nucleosides, H_3' remains at lower field, separated from H_4' in all nucleosides, except cytidine and uridine where increased shielding causes it to be superimposed on H_4'.

The H_2'–H_2'' multiplet appears at high field values in thymidine and deoxycytidine.[4,5] The peak labeled as H_2' in thymidine by Kokko, Goldstein and Mandell[6] is actually H_3', H_4' and H_5'.

H_2' has been placed under H_4' in deoxyadenosine and deoxyguanosine, under H_4' in cytidine and uridine, and between H_3' and H_4' in adenosine and guanosine. These assignments are tentative. Peak areas are consistent with them.

Exchangeable Peaks.—NH_2 and NH peaks have been identified for adenosine, guanosine, cytidine, thymidine and uridine.[6] Our spectra of deoxyadenosine, deoxyguanosine and deoxycytidine also contain such peaks. The NH_2 peaks range from -0.05 to -0.87 p.p.m.; NH peaks range from -2.25 to -4.92 p.p.m. The NH peaks in guanosine and deoxyguanosine appear at higher field than those in adenosine and deoxyadenosine. This could be due to hydrogen bonding structure of type I involving the amino group and N_1, which can be easily drawn for adenosine and deoxyadenosine but not for guanosine and deoxyguanosine. (Such structures in guanosine and deoxyguanosine involve the NH but not the NH_2 group.)

Attention is immediately drawn to deoxycytidine. It displays two peaks definitely of the NH type, each with an area corresponding to one proton. Cytidine, on the other hand, shows the typical NH_2 peak with an area corresponding to two protons. Apparently cytidine has the amino form while deoxycytidine has the imino form, and only deoxycytidine shows this structure. From the pattern of NH and NH_2 peaks the following tautomeric forms are easily deduced. Deoxycytidine, deoxyguanosine and deoxyadenosine have not been reported previously.

Adenosine	= amino	Cytidine	= amino
Deoxyadenosine	= amino	Deoxycytidine	= *imino*
Guanosine	= amino-keto	Uridine	= keto
Deoxyguanosine	= amino-keto	Thymidine	= keto

The two NH peaks of deoxycytidine appear at higher field than those in guanosine, deoxyguanosine, uridine and thymidine. This is, in itself, strong evidence that deoxycytidine has the imino form. Hydrogen bonding structures involving

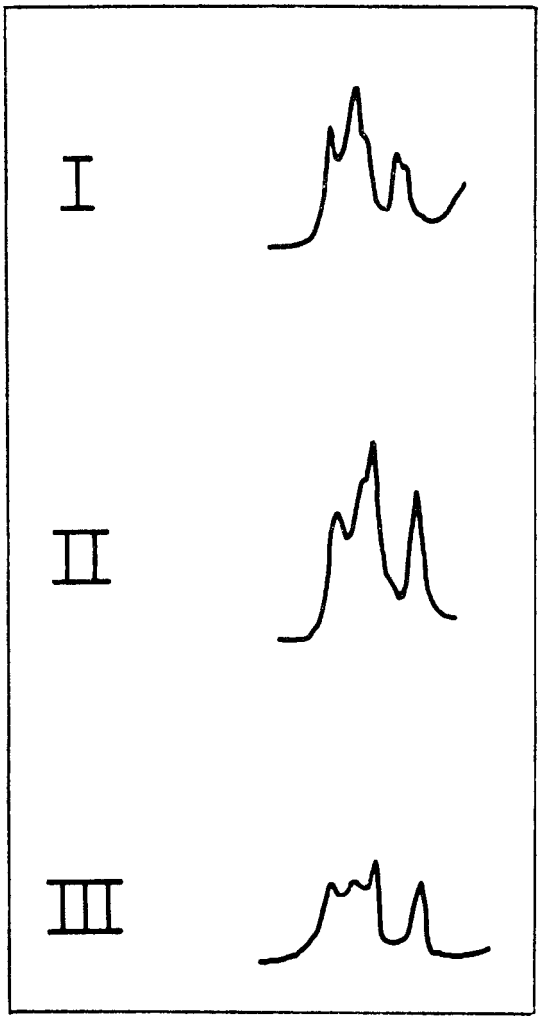


Fig. 2.—Variation in the H_1'–H_5' region of uridine.

N_1H can be drawn for guanosine, deoxyguanosine, uridine and thymidine but not for deoxycytidine in the imino form. Hence the higher field values of the deoxycytidine NH protons confirm that they are not involved in hydrogen bonding to the extent that those in guanosine, deoxyguanosine, uridine and thymidine are.

The peaks appearing in the region from 0.97 to 1.50 p.p.m. are the ribose ring hydroxyl protons. Kokko, Goldstein and Mandell[6] identified these as exchangeable protons by D_2O substitution. Working independently, we found that these peaks disappeared upon heating or addition of acid, confirming that they are exchangeable protons.

In adenosine and deoxyadenosine the OH_5' 1:2:1 triplet is superimposed on another doublet. The doublet at slightly higher field is probably OH_2' since it disappears in deoxyadenosine. In guanosine the peak at 1.25 and 1.33 p.p.m. has an area corresponding to two protons while in deoxyguanosine the area is reduced to one proton. Hence OH_3' must again appear at lower field while OH_2' and OH_5' are superimposed. A similar situation exists for uridine and thymidine. The latter displays a clearly resolved OH_3' doublet and OH_5' 1:2:1 triplet. As mentioned previously, the

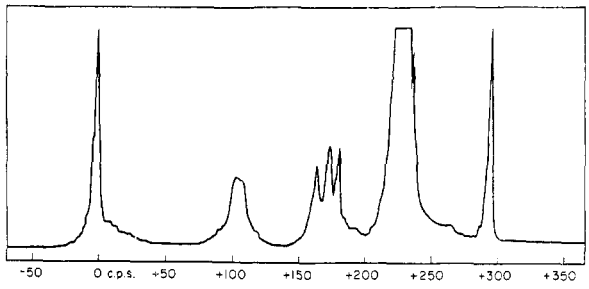


Fig. 3.—Ribose in dimethyl sulfoxide. Toluene reference peaks are included. Conditions are the same as in Fig. 1.

cytidine and deoxycytidine spectra do not show ring hydroxyl protons due to acid exchange.

It is interesting that the nucleosides should display their ring hydroxyl protons at all, particularly with the clear resolution shown in some cases. Ribose itself in dimethyl sulfoxide shows no such clearly resolved spin multiplets. Figure 3 shows ribose in dimethyl sulfoxide. Since, as pointed out previously, the solvent is non-exchanging for the nucleosides, solute–solute exchange appears to be the predominant type. It is known that collapse of spin multiplets can be caused by exchange between protons in identical molecules, but in different spin states.[7] The nucleosides and ribose in dimethyl sulfoxide appear to be excellent examples of this. If exchange occurs upon collision, then the nucleosides with resolved hydroxyl multiplets must be in that intermediate range of correlation time, where collision frequency is low enough to reduce exchange, yet relaxation time is not sufficiently shortened to broaden the line excessively.

Line widths in these systems are undoubtedly influenced strongly by neighboring N^{14} quadrupole fields and quite possibly by preferred intermolecular orientation in solution. In this respect, Jardetzky[1] has pointed out that the spectra of certain purines are concentration dependent. It is stated that "these results cannot be explained on the basis of hydrogen bonding at high concentration, since there are no available protons to bring about such an association."

There *are* protons available for hydrogen bonding, for example the NH and keto groups of hypoxanthine and the NH_2 group of adenine. Structures analogous to those proposed for DNA base pairing[8] can be constructed for hypoxanthine as shown in structure II. Similar structures have already been suggested for adenosine and deoxyadenosine (see structure I).

These shifts cannot be explained as hydrogen bond shifts because they are in the wrong direction. Dilution shifts the peaks to lower field. However, these data are consistent with the idea of a preferred intermolecular orientation in solution for these systems. This could be an additional factor in the relatively unresolved lines for some protons but not for others of the same type. Bound H_2O in the preparations is also a factor in exchange rate and hence line width. H_2O in dimethyl sulfoxide appears at 165 c.p.s. near the

(7) J. T. Arnold, *Phys. Rev.*, **102**, 135 (1956).
(8) J. D. Watson and F. H. C. Crick, *Nature*, **171**, 737 (1953).

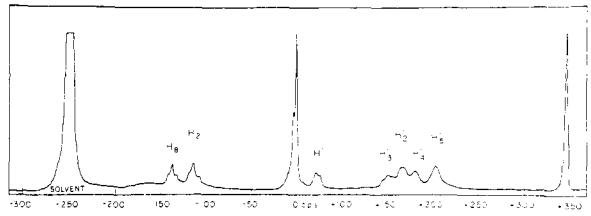


Fig. 4.—Adenosine in trifluoracetic acid; conditions same as in Fig. 1.

H_5' protons. A large amount of H_2O is clearly visible in the guanosine spectrum.

In cytidine a broad, unresolved plateau appears in the H_6 region. This could possibly be due to the hemisulfate proton. The acid proton peak of H_2SO_4 in dimethyl sulfoxide appears at −4.25 p.p.m. The HCl proton peak appears at −0.25 p.p.m. The plateau in cytidine begins at about −2.42 p.p.m. No such peak appears in freshly prepared deoxycytidine samples. However, after standing for one month, a similar plateau appears at about −0.25 p.p.m. This could be due to the HCl proton.

The H_2SO_4 proton may not be completely dissociated as evidenced by its increased shielding. It is interesting to speculate as to where this proton is associated in the cytidine molecule. Possible sites are the nitrogen lone pair electrons. Some evidence in favor of this interpretation is that the H_2 and H_8 peaks of adenosine in trifluoroacetic acid are both 1:2:1 triplets with J = 4.1 c.p.s. as shown in Fig. 4. This could be explained as spin–spin interaction with protons associated at N_1, N_3, N_7 and N_9. It is also possible that the plateau in cytidine is a dissociated H_2SO_4 proton exchanging and associated with the NH_2 peak which is somewhat broadened. That the plateau in cytidine (hemisulfate) is the acid proton is confirmed by the fact that this plateau completely disappears in the spectrum of the free base.

The NH_2 peak in cytidine (hemisulfate) could not be, for example, two superimposed NH peaks due to acid exchange. The NH_2 peak appears in the spectrum of the free base where no acid is present. The spectra of deoxycytidine in 0.1 N NaOH and in 0.1 N H_2SO_4 in dimethyl sulfoxide retain their two NH peaks in the same frequency ranges.

II. Biological Implications of Tautomeric Form.—There is at present a controversy regarding the base pairing scheme in DNA.[9] Watson and Crick[8] originally proposed structures of types III and IV involving hydrogen bonding between cytosine–guanine and adenine–thymine at the N_1 atom of the purine ring system. Pauling and Corey[10] proposed the third hydrogen bond in III. This third hydrogen bond is believed to confer increased stability to the cytosine–guanine base pair.

Recently it has been found[11] that 1-methylthymine and 9-methyladenine co-crystallize in a

(9) H. T. Miles, *Proc. Natl. Acad. Sci.*, **47**, 791 (1961).
(10) L. Pauling and L. R. B. Corey, *Arch. Biochem. Biophys.*, **65**, 179 (1956).
(11) K. Hoogsteen, *Acta Cryst.*, **12**, 822 (1959).

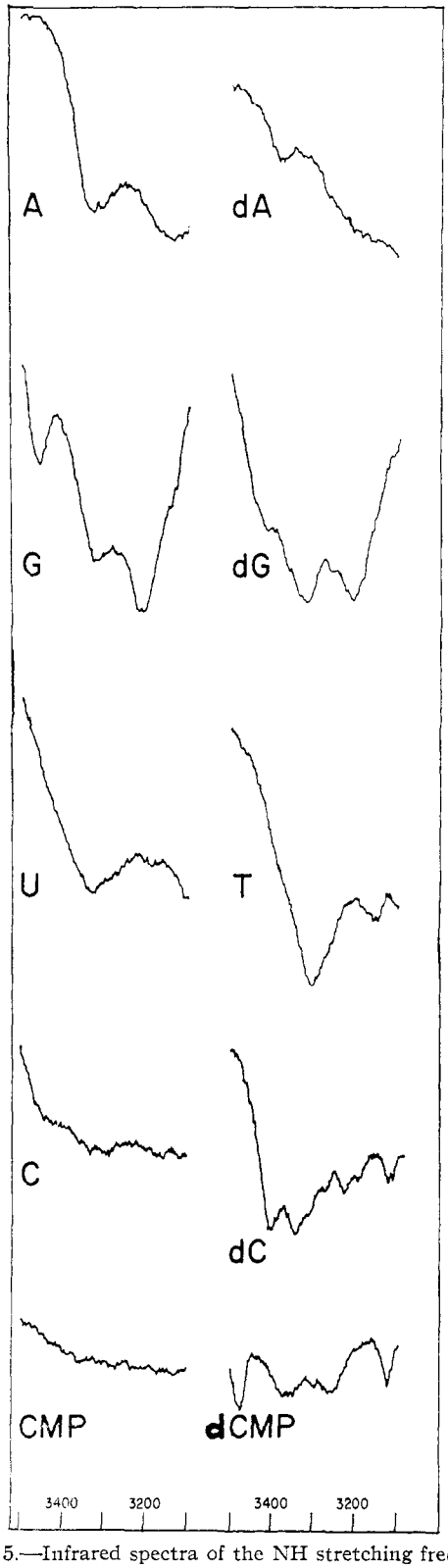


Fig. 5.—Infrared spectra of the NH stretching frequency region in cm.$^{-1}$ of Nujol emulsions of: A, adenosine; dA, deoxyadenosine; G, guanosine; dG, deoxyguanosine; C, cytidine; dC, deoxycytidine; U, uridine; T, thymidine; CMP-5′; and dCMP-5′.

hydrogen bonding arrangement involving N_7 of the purine ring (structure V). Also synthetic (1) poly-adenine + 2 uracils) contains an N_7 bond.[12] Such evidence has aroused a critical re-evaluation of the original base pairing scheme. It has been proposed[13,14] that DNA may involve the base pairs V and VI rather than III and IV.

This proposal automatically requires that deoxycytosine have the imino form since there is no hydrogen at N_7 in deoxyguanine for hydrogen bonding. The major objection to this proposal is that no N_7 structural model has been found which is consistent with the DNA X-ray data.[15] However, the possibility that one will be found in the future has not yet been discarded. A further objection to the N_7 hypothesis[9] is the necessity for another explanation for the additional stability of the cytosine–guanine pair. It is quite possible, however, that the cytosine–guanine hydrogen bonds are intrinsically stronger than the adenine–thymine bonds. N.m.r. studies are now in progress which we hope will produde evidence on this point.

The question remaining is: Are the tautomeric forms we observe *in vitro* also present *in vivo*? The first hydrolysis products of certain nucleases are the 5′-monophosphates. These are insoluble in dimethyl sulfoxide. Figure 5 shows the NH stretching frequency region of the infrared spectra of Nujol emulsions of the eight nucleosides, CMP-5′ and dCMP-5′. As we would expect, cytidine and deoxycytidine show the greatest spectral difference. This is due to their difference in tautomeric form; CMP-5′ and dCMP-5′ show a striking parallel difference. This is evidence that CMP-5′ has the amino form and dCMP-5′ the imino form in the solid state. Hence if DNA is N_1 rather than N_7, deoxycytidine must have been changed from the amino to the imino form by DNAase while cytidine was not changed by RNAase. This is possible, but it does appear unlikely.

The objection that dimethyl sulfoxide spectra are not applicable to biological systems is weak. A comparison of Tables II and III shows that the H_1' coupling constant does not vary greatly between dimethyl sulfoxide and D_2O. In most cases the variation is within experimental error. As pointed out previously, shifts and general spectral character in dimethyl sulfoxide are very similar to that in D_2O. All evidence indicates that dimethyl sulfoxide is a non-exchanging, essentially non-interacting solvent for these systems.

If this were a solvent effect, *i.e.*, if dimethyl sulfoxide had changed deoxycytidine from the amino to the imino form, it would have probably changed cytidine, adenosine, deoxyadenosine, guanosine and deoxyguanosine also. All these clearly display the unmistakable NH_2 peak with a two-proton area. Deoxycytidine does not. It displays two NH peaks, each with a one-proton area, and at higher field then those in uridine, thymidine, guanosine and deoxyguanosine. The infrared evidence suggests that these tautomeric differences

(12) G. Falsenfeld, D. R. Davies and A. Rich, *J. Am. Chem. Soc.*, **79**, 2023 (1957).

(13) L. Pauling, "The Nature of the Chemical Bond," 3rd ed., Cornell University Press, Ithaca, N. Y., 1960, p. 504.

(14) R. Langridge and A. Rich, *Acta Cryst.*, **13**, 1052 (1960).

(15) M. H. F. Wilkins, *J. chim. phys.*, **58**, 891 (1961).

also exist in the solid state and were present in the phosphates.

If future evidence supports the N_7 base pairing scheme for DNA, some interesting deductions follow. If RNA base pairs by hydrogen bonding with DNA in the process of information transfer for subsequent protein synthesis, and if deoxycytosine is in the imino form and cytosine is in the amino form, deoxycytosine–guanine base pairing must be N_7 but deoxyguanine–cytosine must be N_1. Thymine–adenine and deoxyadenine–uracil may theoretically base pair in either form.

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Studies on Polypeptides. XXIII. Synthesis and Biological Activity of a Hexadecapeptide Corresponding to the N-Terminal Sequence of the Corticotropins[1-4]

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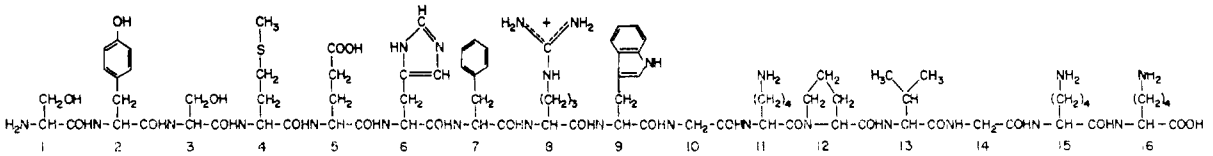
A synthesis is described of the hexadecapeptide seryltyrosylserylmethionylglutamylhistidylphenylalanylarginyltryptophylglycyllysylprolylvalylglycyllysyllysine (14-L) which corresponds to the arrangement of the N-terminal 16 amino acid residues of pig corticotropin. Evidence is presented for the stereochemical homogeneity of this hexadecapeptide. The peptide possessed both *in vitro* melanocyte expanding and adrenocorticotropic activity but the latter activity (<0.1 i.u./mg.) was of the same low order of magnitude as that of a tridecapeptide amide corresponding to the arrangement of the N-terminal 13 amino acid residues in pig corticotropin. It was concluded that the unit of the corticotropin molecule which possesses the full adrenocorticotropic activity of pig corticotropin must be longer than the N-terminal hexadecapeptide but may be shorter than the N-terminal tetracosapeptide.

Definition of the shortest segment of the corticotropin molecule which is endowed with full adrenocorticotropic activity is of considerable significance for the understanding of the physiological function of this hormone. Presumptive evidence[5] has located the adrenocorticotropically active portion of ACTH within the N-terminal 24 amino acid residues, but the smallest fully active sequence remains to be elucidated.

Biological evaluation of homogeneous synthetic peptides of increasing chain-length which correspond to the N-terminal portion of the ACTH molecule appears to provide a rational approach to the solution of this problem.

We have reported[6] that a synthetic tridecapeptide amide which corresponds to the arrangement of the first 13 amino acid residues of the corticotropins possesses low, but reproducible *in vivo* adrenal ascorbic acid depleting and steroidogenic activity in the rat. Thus, the fully active segment must be longer than 13 but may be shorter than 24 amino acid residues.



In the present communication we describe a synthesis of the hexadecapeptide (I) and provide biological data to show that this compound possesses essentially the same low *in vivo* adrenocorticotropic activity as the tridecapeptide amide. We conclude that the smallest fully active segment of ACTH must be longer than 16 but may be shorter than 24 amino acid residues.

The synthesis of (I) is patterned according to the scheme which we developed for the preparation of seryltyrosylserylmethionylglutamylhistidylphenylalanylarginyltryptophylglycyllysylprolylvaline amide.[6]

Two approaches were explored for preparing N^{α}-carbobenzoxy-N^{ϵ}-formyllysylprolylvalylglycyl-

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(2) The peptides and peptide derivatives mentioned in this communication (with exception of glycine) are of the L-configuration. In the interest of space conservation the customary L-designation for individual amino acid residues has been omitted.

(3) See *J. Am. Chem. Soc.*, **84**, 1054 (1962), for paper XXII in this series.

(4) A preliminary account of some of the results presented in this paper was communicated to the "First International Symposium on Polyamino Acids," June 19, 1961, "Polyamino Acids, Polypeptides and Proteins" M. Stahmann, editor, University of Wisconsin Press, 1962, p. 2.

(5) R. G. Shepherd, S. D. Willson, K. S. Howard, P. H. Bell, D. S. Davies, S. B. Davis, E. A. Eigner and N. E. Shakespeare, *J. Am. Chem. Soc.*, **78**, 5067 (1956).

(6) K. Hofmann and H. Yajima, *ibid.*, **83**, 2289 (1961).