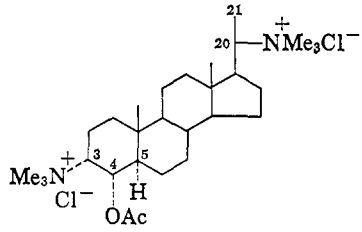
More accumulated data on $J_{H,^{14}N(vic)}$ values for a variety of dihedral angles may be necessary to derive the relationship between them. However, the $J_{H,^{14}N(vic)}$ value of 2.2 Hz observed in ethyltrimethylammonium bromide and some other similar compounds can lead us to infer that the J value for 180° may be about 5–6 Hz[9,10] in view of the $J_{H,^{14}N(vic)}$ value in I at the dihedral angle 60°.[19]

Acknowledgments. We thank Professor T. Kikuchi of Kyoto University and Drs. K. Kitahonoki and Y. Hamashima of this laboratory for providing us with the starting materials used to synthesize the model compounds.

(19) In order to determine a $J_{H,^{14}N(vic)}$ value at the dihedral angle of 180°, we synthesized 4α-acetoxy-3α,20α-di(dimethylamino)-5α-pregnane dimethochloride (IV) (mp 210–213.5° dec) from its free base[20]



IV

by the usual way. However, the pmr signal of $H_{4\beta}$ in IV, which is a quartet with spacings of 5.0, 6.0, and 5.0 Hz at τ 4.67 in D_2O, shows no splittings due to the ^{14}N at C-3α even at 110° but becomes very broad peaks, although the signal of the 21-methyl protons appears as a clear doublet of triplets at τ 8.48 (J = 7.0 and 2.2 Hz) at this temperature. Quadrupolar relaxation of the ^{14}N at C-3α may not be sufficiently slow at this temperature to show splittings by the ^{14}N atom.

(20) M. Tomita, S. Uyeo, and T. Kikuchi, *Chem. Pharm. Bull.* (Tokyo), **15**, 193 (1967).

(21) To whom all inquiries should be addressed.

Yoshihiro Terui, Katsutoshi Aono, Kazuo Tori[21]

Shionogi Research Laboratory, Shionogi and Company, Ltd. Fukushima-ku, Osaka, Japan

Received August 8, 1967

The Optical Rotation of Ribonuclease[1]

Sir:

One of the most characteristic features of the optical rotatory spectrum of the α helix is the trough at 233 mμ. It is an important and interesting fact that in model polypeptide systems this trough diminishes in magnitude without appreciable change in position as the helical content is diminished until at very low helicity it drifts up into the small anomaly of the random polypeptide chain at 239 mμ (Figure 1). Curve C, for example, has a trough at 233 mμ even though it represents only 6–7% helix as estimated by the trough amplitude method.[2] The absorption band responsible for this trough has been located at 222 mμ by studies of circular dichroism[3] and has been assigned to the n,π* transition of the peptide group. This is an unusual position for this transition. Model studies place it at about 228 mμ in solvents which cannot donate hydrogen bonds[4] and at about 212–214 mμ in solvents such as water and methyl alcohol.[5] This is the well-known blue shift of n,π* transitions in highly polar solvents. The constancy of position of the trough of the α helix in a wide variety of solvents and in many proteins suggests that the helix itself provides a relatively constant environment for the n orbitals of the peptide group which is distinct from that of water or nonpolar media. Inspection of space-filling models of helices reveals that the nonbonding n orbitals are nestled in the helix in such a way that direct interaction with solvent is prevented. The hydrogen bonds of the α helix itself are in the nodal planes of the n and π* orbitals and consequently will affect the n,π* energies only as a secondary effect.

The trough of beef pancreatic ribonuclease is at 228 mμ.[6] Since this is a deviation from model helix behavior which may be of deep structural significance, we have performed a number of experiments to demonstrate that this is a general feature of the ORD spectrum of ribonuclease and not dependent on special conditions of solution or the protein preparation. These experiments spanned the following conditions: eight enzyme preparations,[7] pH 2–8, in a variety of salts: KCl, Tris-HCl, and $(NH_4)_2SO_4$ at concentrations of 0.05–3.0 *M*. Measurements were made on a Cary 60 spectropolarimeter. In almost all cases the trough was at λ_t 228–229 mμ. Exceptions were two old preparations, Sigma R6B-053 and Pentex 10669, which had troughs at 230 and 231 mμ, respectively. Evidently long storage or impurities can lead to higher values for λ_t. The mean residue rotation at the trough, averaged over 21 experiments, was −4100° with a standard deviation of 300°. These values are corrected for refractive index using the values for water. Curves from a given preparation were quite reproducible and did not change much with the variation of pH and ionic strength. The standard deviation given above represents essentially the variation among protein preparations.

Circular dichroism was also investigated using a Jasco dichrograph (Figure 2), and the data were analyzed by a curve-fitting procedure derived from the program of Carver, Shechter, and Blout.[8] The data are analyzable in terms of two negative Cotton effects. The upper one at 217 mμ has a molar rotatory strength of −5.6 Debye-magnetons and is responsible for the trough at 228 mμ.[9] The calculated Gaussian band width is 12 mμ, which is a reasonable value for the protein chromophores which may be involved as established in model studies. Consequently, this Cotton effect is evidence either for a single type of transition or the superposition of a number of transitions at the same wavelength. On the other hand, the lower band has an apparent width of only 6 mμ. This indicates that it is a composite of a number of

(1) This research was supported by PHS Grant CA 4216 of the National Cancer Institute.

(2) (a) N. S. Simmons, C. Cohen, A. G. Szent-Gyorgyi, D. B. Wetlaufer, and E. R. Blout, *J. Am. Chem. Soc.*, **83**, 4766 (1961); (b) Y. Tomimatsu, L. Vitello, and W. Gaffield, *Biopolymers*, **4**, 653 (1966).

(3) G. Holzworth and P. Doty, *J. Am. Chem. Soc.*, **87**, 218 (1965).

(4) E. B. Nielsen and J. A. Schellman, *J. Phys. Chem.*, **71**, 3914 (1967).

(5) J. A. Schellman and E. B. Nielsen in "Conformation of Biopolymers," Vol. 1, G. N. Ramachandran, Ed., Academic Press Inc., New York, N. Y., 1967, p 109.

(6) R. E. Cathou, G. G. Hamnes, and P. R. Schimmel, *Biochemistry*, **4**, 2687 (1965).

(7) Pentex, Lots 2626, 10669, 26Z8; Worthington, RNase A, Lot 6504; Mann, Lot F 2789; Sigma, Lots R61B-053, R101B67, R22B70.

(8) J. P. Carver, E. Shechter, and E. R. Blout, *J. Am. Chem. Soc.*, **88**, 2550 (1966).

(9) In calculating the rotatary strength Δε values were corrected for refractive index by means of the formula $\Delta\epsilon' = (3/(3^2 + 2)^2 n\Delta\epsilon$, where Δε is the observed molar dichroism and n is the refractive index of water.

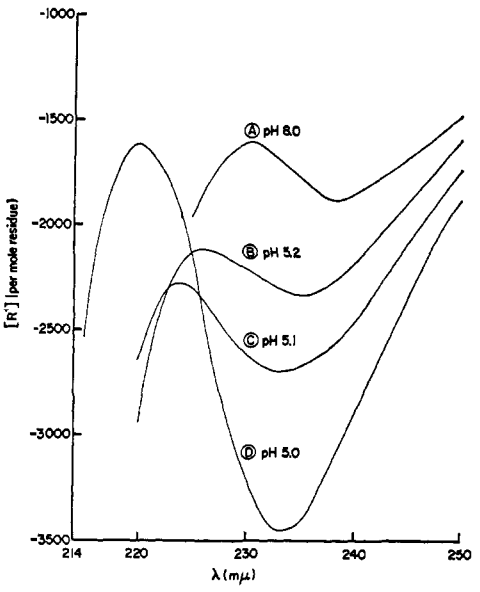


Figure 1. Optical rotatory dispersion of polyglutamic acid as a function of pH (polyglutamic acid: Pilot Chemicals, Lot G-84, molecular weight 68,000, concentration 0.25 mg/ml, in 0.05 *M* NaCl). Residue rotations corrected by the Lorentz factor of $3/(n^2 + 2)$ using the values for the refractive index of water.

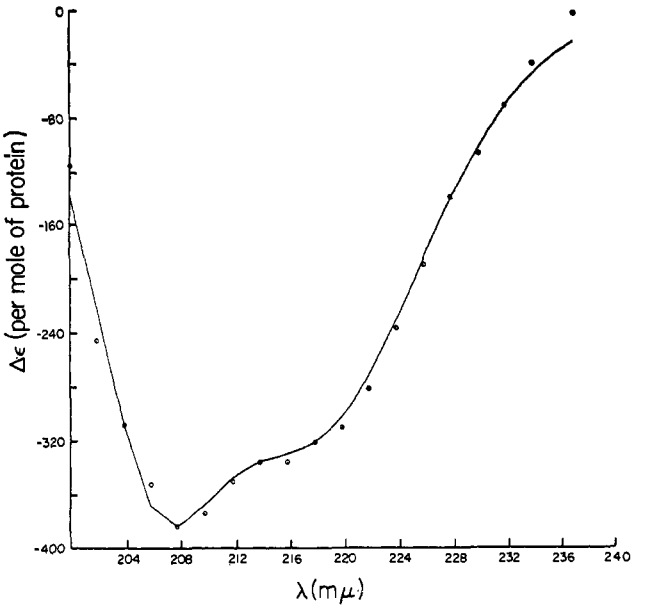


Figure 2. The circular dichroism of ribonuclease (Worthington ribonuclease A, Lot 6504, 3.4 mg/ml in 0.1 *M* KCl, pH 6.4). The smooth curve is derived for two Gaussian dichroic bonds with λ_1 2.7 mμ, $R_1 = -5.6$ Debye-magnetons, $\Delta_1 = 12$ mμ; λ_2 206 mμ, $R_2 = -2.2$ Debye-magnetons, $\Delta_2 = 6$ mμ. These rotatory strengths are for the entire ribonuclease molecule and are corrected for refractive index.

absorption bands and presumably derives its apparent sharpness from positive circular dichroism at lower wavelengths.

The recent X-ray diffraction studies of ribonuclease[10] have revealed that it contains appreciable regions of α helix as well as β structure. A Cotton effect curve centered at 217 mμ with a trough at 228 mμ is in good accord with results obtained with model β structures,[11] and it is reasonable to assume that the observed Cotton effect of ribonuclease is partially comprised of contributions from this structure. β structures display relatively feeble n,π^* Cotton effects, however, and a good fraction of the observed rotation must arise from another structural source. (It requires 100% β structure to account for the observed magnitude of the trough.) The rest of the interpretation is less straightforward, and we are left with the problems of (1) accounting for the extra intensity of the 217-mμ Cotton effect, and (2) explaining the absence of the typical helical Cotton effect at 222 mμ. Constructed curves for a mixture of α, β, and random structures reveal that large quantities of β structure are insufficient to produce a 5-mμ shift in the position of the trough with small quantities of α structure.[12]

Before considering the contribution of the helical regions, we will discuss possible contributions from the side chains. The only side-chain absorption band of ribonuclease in this region is that of tyrosine at 220 mμ. Two lines of evidence cause us to believe that this absorption band makes only a feeble contribution. (1) Titration of ribonuclease to pH 11.3 produces marked changes in the tyrosine Cotton effect of ribonuclease at 278 mμ but has no effect on the Cotton effect at 217 mμ[13] (reconfirmed in this investigation). (2) Extremely large rotatory strengths would be required of the six tyrosine groups to produce a dominant effect on the molar rotation of the entire protein. There is no evidence from model studies that this absorption band is capable of producing such abnormally large Cotton effects.

One could accomplish the resolution of the two problems mentioned above in one stroke by moving the α-helical Cotton effect down 5 mμ in ribonuclease. The magnitude of the now-superimposed Cotton effects of the α and β structures would be in reasonable agreement with the structure as determined by X-ray diffraction. This step cannot be taken casually, however, since it implies a change in the excitation energy of the helical n,π^* transition of about 3 kcal/mole of residues. This in turn requires a considerably different interaction with environment than that of the α helices of polypeptide models and of many proteins which have n,π^* Cotton effects in the normal helical position. It is the purpose of this note to point out that such energy changes might well be expected for short and/or distorted helices in proteins.

Shortness of helices will in general give a blue shift in the n,π^* transition because of exposure of the carbonyl groups at the carboxyl end of the helix to the surroundings. Hydrogen bonding to the exposed groups need not be colinear with the C=O group, and as discussed above this leads to partial participation of the n orbital in the hydrogen bond and to a typical n,π^* blue shift. If we define the length of a helix, N, as the number of peptide residues in a recognizable helical array, then the ratio of end residues to interior residues will be $3/(N - 3)$. For very short helices this leads to a significant fraction of end residues. The same effect

(10) (a) G. Kartha, J. Bello, and D. Harker, *Nature*, **213**, 862 (1967); (b) H. W. Wycoff, K. D. Hardman, N. M. Allewell, T. Inagami, L. N. Johnson, and F. M. Richards, *J. Biol. Chem.*, **242**, 3984 (1967).

(11) R. Townsend, T. Kumosinski, S. Timasheff, G. Fasman, and B. Davidson, *Biochem Biophys. Res. Commun.*, **23**, 163 (1966).

(12) N. Greenfield, B. Davidson, and G. D. Fasman, *Biochemistry*, **6**, 1630 (1967).

(13) A. N. Glazer and N. S. Simmons, *J. Am. Chem. Soc.*, **87**, 3991 (1965).

would be achieved within the interior of helices if the hydrogen bonds were displaced to the side of the C=O group. Distortions of this kind have been observed in certain regions of lysozyme[14] and in myoglobin[15] and have also been predicted theoretically.[14]

In summary the ORD and CD spectra of ribonuclease indicate that its helical contributions are at lower wavelengths than those in polypeptide models and in many proteins. Most of the literature on optical rotation has emphasized the role of conformation on the magnitude and sign of Cotton effects. We are here dealing as well with the effect of conformation and solvation on the energy of the transition which produces the Cotton effect. Hydrogen bonds involving the n orbitals produce such changes, and it is suggested that the n,π^* Cotton effects of helices which are very short or badly distorted will be displaced toward lower wavelengths as in ribonuclease.[15a]

Acknowledgment. We are grateful to J. T. Yang for permitting us to use his instrument for the measurement of circular dichroism.

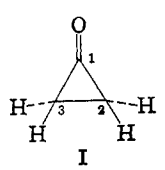
(14) G. Nementhy, D. C. Phillips, S. J. Leach, and H. A. Scheraga, *Nature*, 214, 363 (1967).
(15) J. C. Kendrew, personal communication.
(15a) NOTE ADDED IN PROOF. Since this paper was submitted, we have learned of the complementary work of B. Jirgensons (*J. Am. Chem. Soc.*, 89, 5979 (1967)). Noting that the position of the trough is not that of an α helix, he has used the peak in the ORD spectrum at 199 mμ to estimate helix content.
(16) Public Health Service Predoctoral Fellow.

John A. Schellman, Margaret J. Lowe[16]
Department of Chemistry, University of Oregon
Eugene, Oregon
Received July 5, 1967

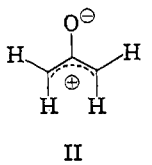
Microwave Spectrum, Structure, and Dipole Moment in Cyclopropanone

Sir:

Various substituted cyclopropanones react with dienes such as furan to give cycloadducts;[1-6] the adducts are formally ascribable to either a 4 + 2 cycloaddition of diene across the C_2–C_3 single bond of the cyclopropanone (I) or a 4 + 3 addition of diene and an acyclic dipolar reactive intermediate (II). Both reaction modes would be, according to orbital symmetry conditions, thermally allowed for *cis* cycloadditions.[7]



I



II

(1) H. G. Richey, Jr., J. M. Richey, and D. C. Clagett, *J. Am. Chem. Soc.*, 86, 3906 (1964), and literature cited in ref 2.
(2) R. C. Cookson, M. J. Nye, and G. Subrahmanyam, *Proc. Chem. Soc.*, 144 (1964).
(3) N. J. Turro and W. B. Hammond, *J. Am. Chem. Soc.*, 87, 3258 (1965).
(4) W. B. Hammond and N. J. Turro, *ibid.*, 88, 2880 (1966).
(5) R. B. Woodward, "Aromaticity," Special Publication No. 21, The Chemical Society, London, 1967, p 241.
(6) R. C. Cookson, M. N. Nye, and G. Subrahmanyam, *J. Chem. Soc., Sect. C*, 473 (1967).
(7) R. Hoffmann and R. B. Woodward, *J. Am. Chem. Soc.*, 87, 2046 (1965).

Extended Hückel calculations[8] have suggested that the ring-closed form I may be unstable relative to the oxyallyl acyclic dipolar structure II. In fact, the approximate calculations give no stability for cyclopropanone with respect to conversion to oxyallyl.[8]

We now report clear-cut spectroscopic evidence for ring-closed cyclopropanone (I). Microwave transitions for the compound thought to be cyclopropanone, prepared from ketene and diazomethane[9] in fluorotrichloromethane, were observed in several microwave absorption cells. The microwave spectrograph has been described before.[10,11] Considerable difficulty was experienced in maintaining the quality of the spectra above Dry Ice temperatures. Known rotational transitions for acetone,[12] ketene,[13] and fluorotrichloromethane[14] were observed. No transitions were observed for cyclobutanone,[15] acrolein,[16] or diazomethane.[17] Rotational transitions of the new molecule were identified by the Stark effect. Only transitions excited by the dipole moment along the axis of least moment of inertia (*a*, principal inertial axis) were observed. The assigned *a*-type transitions, rotational constants, and calculated transitions are listed in Table I. The μ_b- and μ_c-type rotational

Table I. Observed and Calculated Rotational Transitions in Cyclopropanone[a]

Transition	Frequencies	
	Obsd	Calcd
0(0,0)–1(0,1)	13,338.6	13,338.4
1(1,1)–2(1,2)	25,082.1	25,082.1
1(1,0)–2(1,1)	28,271.6	28,271.4
1(0,1)–2(0,2)	26,535.8	26,535.7
2(0,2)–3(0,3)	39,458.0	39,458.1
2(2,1)–3(2,2)	40,014.8	40,015.1
2(1,2)–3(1,3)	37,538.7	37,538.6
3(1,3)–3(1.2)	9,560.4	9,560.0
6(2,5)–6(2,4)	8,623.6	8,623.0
6(1,6)–6(1,5)	32,847.6	32,847.6
7(2,6)–7(2,5)	14,329.8	14,329.5
9(2,8)–9(2,7)	30,697.0	30,697.5

[a] The calculated transitions are for A = 20,153.8 ± 1.0 MHz, B = 7466.50 ± 0.05 MHz, and C = 5871.87 ± 0.05 MHz which were obtained by a least-squares fit of the observed frequencies. Experimental errors are estimated to be 0.1 MHz.

transitions were also predicted, and a careful search was made to find these lines. No μ_b or μ_c transitions were observed, which indicates the molecule has C_{2v} symmetry. The Stark effect was observed with high precision as a function of electric field for the $1_{10} \rightarrow 2_{11}$ and $0_{00} \rightarrow 1_{01}$ transitions. The above Stark effect gives an electric dipole moment in this molecule of μ_a = 2.67 ± 0.10 D which is intermediate between the

(8) R. Hoffmann, unpublished.
(9) N. J. Turro and W. B. Hamond, *J. Am. Chem. Soc.*, 88, 3672 (1966).
(10) W. H Flygare, *J. Chem. Phys.*, 41, 206 (1964).
(11) M. L. Unland, V. W. Weiss, and W. H. Flygare, *ibid.*, 42, 2138 (1965).
(12) R. Peter and H. Dreizler, *Z. Naturforsch.*, 20a, 301 (1965).
(13) H. R. Johnson and M. W. P. Strandberg, *J. Chem. Phys.*, 20, 687 (1952).
(14) M. W. Long, Q. Williams, and T. L. Weatherly, *ibid.*, 33, 508 (1960).
(15) A. Bauder, F. Tank, and H. H. Günthard, *Helv. Chim. Acta*, 46, 1453 (1963).
(16) E. A. Cherniak and C. C. Costain, *J. Chem. Phys.*, 45, 104 (1966).
(17) A. P. Cox, L. F. Thomas, and J. Sheridan, *Nature*, 181, 1000L (1958).