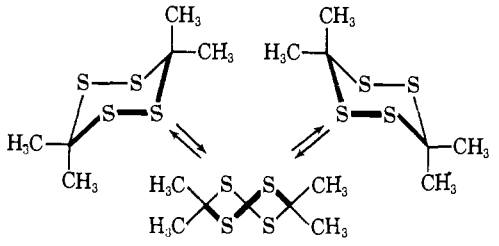
Examination of the nmr spectrum[4] of duplodithioacetone (15% by weight in $Cl_2C{=}CCl_2$) at 35° reveals a set of two broad resonances of unequal intensities at δ 2.00 and 1.66 ppm. Upon lowering the temperature, these resonances sharpened in the usual manner (Figure 1) into a set of three peaks consisting of two resonances of *equal area* at δ 1.53 and 2.03 ppm and a larger sharp singlet at δ 1.68 ppm. When the sample temperature was raised, the spectrum showed kinetic broadening characteristic of an intermediate exchange rate on the nmr time scale (Figure 1), and at 80°, the two broad resonances observed at 35° had coalesced into a singlet at δ 1.73 ppm. Upon again lowering the temperature, an identical sequence of spectral transitions was observed.

The above results would seem to be best rationalized by the presence of a thermodynamically stable twist-boat form of duplodithioacetone in equilibrium with the two chair conformers (eq 1).[5] At lower temperatures

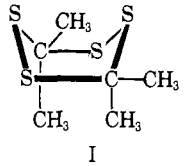


(1)

(−30°), the two peaks of equal area at δ 1.53 and 2.03 ppm (Figure 1) are assigned to the axial and equatorial methyl resonances of the chair conformers of duplodithioacetone (eq 1) and the sharp singlet at δ 1.68 ppm to the symmetrical twist-boat conformation (eq 1). The ratio of boat to chair conformers is 2.2:1.0, respectively, at 0° ($\Delta F° = -0.8$ kcal/mole).

The free energy of activation ($\Delta F^{\ddagger}$) for the chair to boat process is estimated to be 16 ± 1 kcal/mole at 50°.

Entropy considerations, the long carbon–sulfur and sulfur–sulfur bonds, and the complete lack of any Pitzer strain due to carbon–hydrogen bonds in the flexible twist-boat form apparently render it more stable than the rigid chair form of duplodithioacetone. Indeed, X-ray crystallographic studies[6] indicate that duplodithioacetone exists in a boat conformation with a sulfur atom at each prow position (I).



I

In acetone diperoxide, it is apparent that the shorter carbon–oxygen and oxygen–oxygen bond lengths play an important role in causing the chair form to be the most stable conformer.[7] It would be expected that

(4) The nmr spectra were recorded on a Varian Associates A-60 nmr spectrometer using a V-6040 temperature controller. Temperature was measured using a methanol sample.

(5) The data as presented do not rule out unequivocally some kind of dissociative process for rendering all the methyl groups equivalent. However, the very sharp lines in the nmr spectrum observed at high temperatures (>80°) would seem to rule out the intervention of radicals in such a process. Experiments are in progress to test such a hypothesis.

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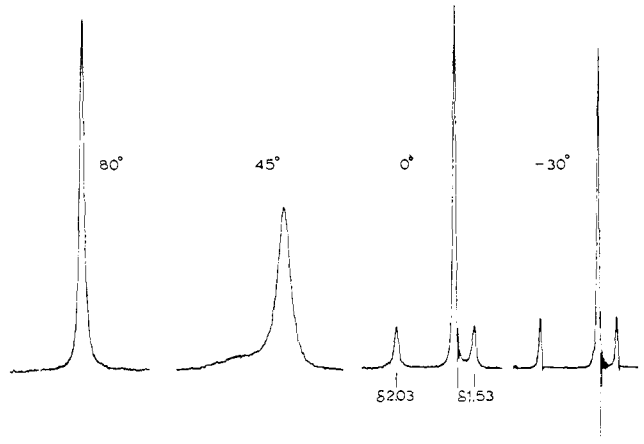

Figure 1. The nmr spectrum (60 Mc) of duplodithioacetone at various temperatures.

lone-pair repulsions would be larger in acetone diperoxide than in duplodithioacetone. Indeed, a consideration of models indicates that the true boat form of acetone diperoxide is incapable of existence due to serious nonbonded compressions between methyl groups. However, the twist-boat conformer would appear to be relatively strain-free, although lone-pair repulsions are still probably serious.

The barrier to conformational isomerism in duplodithioacetone is comparable to that in acetone diperoxide ($\Delta F^{\ddagger}$ = 15.4 kcal/mole at 30°)[7] and substantially higher than in 1,1,4,4-tetramethylcyclohexane ($\Delta F^{\ddagger}$ = 11.4–11.6 kcal/mole at −65°).[8] The barriers to chair–chair equilibration in 3,3,6,6-tetramethyl-1,2-dithiane ($\Delta F^{\ddagger}$ = 13.8 kcal/mole at −2°) and 3,3,6,6-tetramethyl-1,2-dioxane ($\Delta F^{\ddagger}$ = 14.6 kcal/mole at 12°) are of intermediate value.[9]

Duplodithioacetone was prepared according to the method of Magnusson.[10]

Our effort toward the conformational analysis of other multisulfur ring systems, as well as the measurement of the conformational enthalpy and entropy of chair ⇄ boat equilibria, is continuing.

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C. Hackett Bushweller
Oxidation and Catalyst Research Section
Mobil Chemical Company, Edison, New Jersey 08817
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The Far-Ultraviolet Cotton Effects and Conformation of Ribonuclease and Pepsin[1]

Sir:

The conformation of ribonuclease has been disclosed recently by high-resolution X-ray diffraction.[2,3] This

(1) This study was supported in part by Grant CA-01785 from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service, and by Grant G-051 from the Robert A. Welch Foundation, Houston, Texas.

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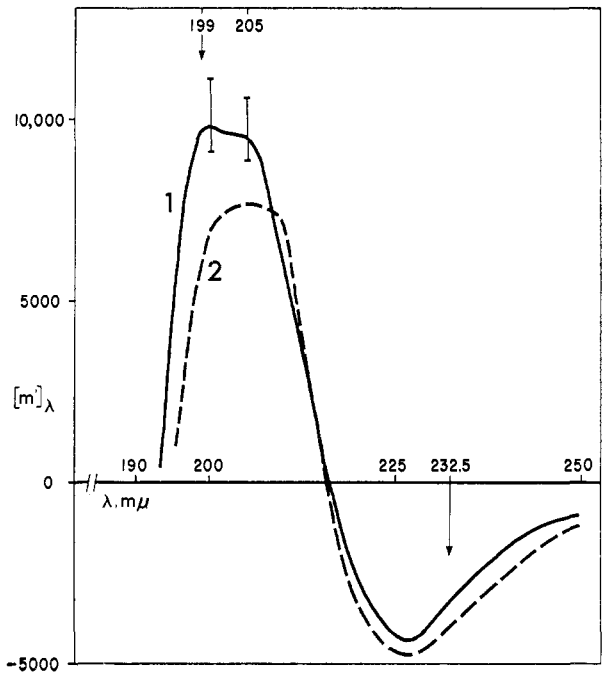

Figure 1. The far-ultraviolet rotatory dispersion of ribonuclease (curve 1) and pepsin (curve 2). The optical activity is plotted as corrected mean residual specific rotation: $[m']_\lambda = [\alpha]_\lambda \bar{M}/100[3/(n^2+2)]$, where $[\alpha]_\lambda$ is the specific rotation, $\bar{M}$ the mean residual weight of the constituent amino acids, and n the refractive index of the solvent. Curve 1 shows average values from eight recorded curves, while curve 2 represents average values from three runs. The bars on curve 1 indicate maximal variation in the eight runs of several separate solutions of the enzyme. In five of the eight runs the variation was smaller than indicated by the bars.

presents an opportunity to compare the optical rotatory dispersion (ORD) data with the results of the X-ray study to obtain a clearer idea of how to interpret the far-ultraviolet Cotton effects in terms of conformation. The X-ray diffraction shows very little of α helix in both ribonuclease[2] and ribonuclease-S,[3] and some of the helices are short and imperfect. Parts of the polypeptide chain are strongly extended, resembling in some segments the β form, and some sections of the chain are disordered.

The ORD of ribonuclease has been studied by several authors, chiefly by the Moffit–Yang method, using data obtained from visible and near-ultraviolet spectra.[4,5] According to these data, ribonuclease has about 15 to 20% of the α helix. Studies in the ultraviolet at 220 to 300 mμ have revealed several weak Cotton effects,[6-8] *e.g.*, a trough at 227–230 mμ.[7]

The present communication deals with the Cotton effects of ribonuclease and pepsin in the 190–250-mμ spectral zone. Circular dichroism studies have shown that the rotational strength of the 190-mμ π–π^* peptide bond transition is about four times greater than the 222-mμ transition,[9] and this favors the $[m']_{199}$ values for the estimation of the α-helix content. A positive extremum characteristic of the extended β structure has been found recently at 204–207 mμ.[10-12]

A Rudolph Model RSP-3-4 spectropolarimeter with a double-prism monochromator specially designed for the far-ultraviolet was used, as described previously.[13,14] The optical path through the solutions was 0.05–0.50 cm, the slit width 0.05–0.2 cm; the enzyme concentration was 0.012 to 0.031%. Chromatographically pure ribonuclease-A preparations from Worthington (sample RAF) or Sigma (sample XII-A) were used. Pepsin was a twice-crystallized product from Worthington (sample PM 6HF). The enzymes were dissolved in water.

The results are shown in Figure 1. Curve 1 represents ribonuclease and curve 2 shows the far-ultraviolet ORD of pepsin. For ribonuclease, the trough at 227 to 230 mμ and the peaks at 198–200 and 204–206 mμ are characteristic. The position of the trough is characteristic of structures having the β conformation, although it may not be unique for it. The peaks indicate the presence of both the α helix and a conformation causing the same effect as the antiparallel β form. Since a perfect hydrogen-bonded β structure has not yet been revealed in the crystals of ribonuclease by X-ray diffraction, one has to assume that imperfectly oriented chain segments produce the same ORD effect as the β form of silk[11] or polylysine.[10,12] According to Wyckoff, *et al.*,[3] hydrogen bonding should be possible at least between some parts of the imperfectly oriented segments. The α-helix content of ribonuclease, as estimated from the magnitude of the positive extremum at 198–200 mμ[13,14] (Figure 1), is approximately 9800/75,000 = 0.13 or 13%, if it is assumed that the optical activity contribution of the other conformations is negligible. This amount of the α helix is in fair agreement with the X-ray data.[2,3] (Since curve 1 has no trough at 233 mμ, an estimate of the α-helix content from this part of the curve would be questionable.) Making these estimates, it is assumed that the conformation of ribonuclease in solution is the same as in wet crystals; this may be justified on the basis of the extensive studies of others on myoglobin.[15-18]

Comparison of the X-ray data and the findings of ORD indicate that further refinements in the methods are needed in order to describe the conformation of ribonuclease in detail. A precise quantitative estimate of the α-helix and β-form content from the Cotton-effect data is difficult, because of the following reasons: (1) partial overlap of the ORD effects produced by the various conformations, (2) the presence of imperfect α-helical and β structures, and (3) the amino acid side-chain effects.[19-22] In spite of these uncertainties, the reported observations show the usefulness of the ORD

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method for the study of proteins with a low α-helix content, such as ribonuclease.

If the same considerations are applied to pepsin, curve 2 indicates that the conformations of ribonuclease and pepsin are similar, and that pepsin probably has even less of the α helix than ribonuclease. It will be intriguing to see whether future X-ray diffraction work on pepsin crystals will support this prediction.

B. Jirgensons
The University of Texas
M. D. Anderson Hospital and Tumor Institute
Department of Biochemistry, Houston, Texas 77025
Received April 27, 1967

On the Relation of Nematic to Cholesteric Mesophases

Sir:

Friedel[1] suggested in 1922 that the nematic and cholesteric mesophases are closely related. He showed that two cholesteric compounds of opposite optical rotational power could produce a nematic liquid when mixed in the correct proportions. Friedel concluded,[2] "The cholesteric compounds are but a special form of the nematic compounds. When the rotatory power and the structural properties connected with it disappear..., a nematic material is obtained...." The purpose of this note is to report a number of our recent observations which are related to Friedel's suggestion.

It is believed that in the nematic liquid crystalline phase, intermolecular forces align the molecules with their long axes parallel in large regions, called swarms or domains. A modest magnetic field couples to the magnetic anisotropy of the domains and aligns them to give a macroscopically oriented sample. Molecules dissolved in such solvents give proton nmr spectra of many sharp lines.[3] The spectra are theoretically well understood.[3,4] It is thought that in the cholesteric phase the molecular axes are parallel within planes, while the direction of alignment changes smoothly in a direction perpendicular to the planes. The helical structure thus created is right-handed for some compounds and left-handed for others. No sharp nmr lines can be observed for molecules dissolved in the cholesteric phase. We have employed the proton nmr spectrum of dissolved benzene to differentiate a nematic phase from a cholesteric one.

Our first observations developed from an attempt to find a nematic phase consisting of optically active molecules. We hoped that the built-in screw sense of such a solvent would cause *d*- and *l*-active solute molecules to show different nmr spectra.[5] For this purpose we obtained *p,p'*-diactive amyloxyazoxybenzene. The pure compound did not form a mesophase, but a 1:1 mixture with *p,p'*-di-*n*-hexyloxyazoxybenzene did. Benzene dissolved in the mesophase of this mixture did not give the sharp spectrum characteristic of solution in a nematic medium. The same solvent mixture made from the racemic amyl derivative did produce the expected spectrum. We concluded that the liquid crystal containing the optically active component was in the cholesteric phase. We understand these observations in terms of a simple analogy, which assumes that optically active molecules behave like screws. When right-handed screws are stacked in an orderly manner, layer upon layer, the threads tend to interlock so that the screw axes gradually change direction from one layer to the next and a "cholesteric" structure results. On other hand, an equal mixture of right- and left-handed screws stacks so that their axes remain on the average parallel over long distances, and forms a "nematic" phase.

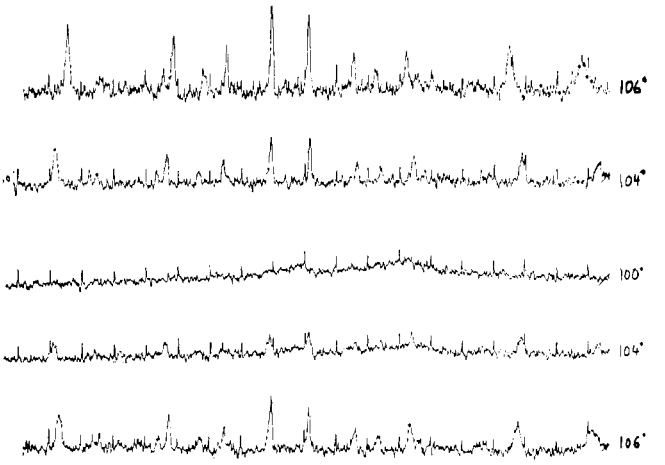

Figure 1. Nmr spectra at 60 MHz of a mixture of 0.013 g of optically active amyloxyazoxybenzene, 0.574 g of hexyloxyazoxybenzene, and 0.03 g of benzene. The spectra were obtained in succession from top to bottom, at the temperatures indicated at right. The small sharp peaks, most distinct in the center spectrum, are frequency markers, spaced 106 Hz apart. Total width of the spectra is about 2000 Hz.

We have observed a phase transition from cholesteric to nematic caused by an applied magnetic field. A solvent mesophase of 2.4 mole % of the active amyloxyazoxybenzene in the hexyloxyazoxybenzene is weakly cholesteric. Above 104° the typical nematic phase nmr spectrum is observed for dissolved benzene. With decreasing temperature the sharp lines suddenly disappear, and at 100° only some broad lines, typical for the cholesteric phase, are observed. These reversible changes are shown in Figure 1. The transition can also be observed as a large increase in turbidity as the liquid becomes cholesteric. Preliminary observations of this effect in fields up to 100 kgauss show that the transition field is roughly proportional to the concentration of the active amyloxyazoxybenzene. These experiments were done at constant temperature, the variation in the magnetic field strength inducing the phase transition in a reversible manner. We conclude that a magnetic field can produce a change of phase from cholesteric to nematic. The interaction of the field with the diamagnetic anisotropy of the nematic phase overcomes the weak intermolecular forces which favor the helical cholesteric structure.

We have extended our nmr studies to solvents which are mixtures of derivatives of cholesterol. Benzene in a 1.9:1 by weight mixture of cholesteryl chloride (*l* rotatory at long wavelengths) and cholesteryl myristate (*d* rotatory at long wavelengths) at 40° give a nematic

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