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The Optical Rotatory Dispersion of Ribosomes and Their Constituents*

P. McPhie and W. B. Gratzer

ABSTRACT: Optical rotatory dispersion measurements have been performed on solutions of ribosomes from yeast, *Escherichia coli*, and rabbit reticulocytes. The ultraviolet Cotton effect of ribonucleic acid (RNA) is observed in the same position as in the free molecule, and an additional feature is present in the 233-mμ region, which arises from the presence of α-helical structure in the ribosomal protein. All the ribosomes obey a single-term Drude law toward longer wavelengths. Dissociation of yeast ribosomes, and their disorganization by high concentrations of chelating agent, are found to have essentially no effect on their optical rotatory dispersion. By subtracting the contribution of the RNA, the optical rotatory dispersion curve of the protein *in situ* is obtained, and values of $[m']_{233}$ and the Moffitt constant, b_0, are reported. The helix content of the ribosomal protein *in situ* is some 60% of the maximal value attained by the extracted protein in 2-chloroethanol. The extracted proteins in aqueous solution contain no detectable proportion of α-helix. The nature of the specificity of the protein–RNA complex in the ribosome is discussed. The optical rotatory dispersion curves of the three species of ribosome are quantitatively similar, except insofar as they reflect the differences in RNA:protein ratio. Analytical applications are suggested.

The ribosome is a compact structure, made up of ribonucleic acid (RNA) and a seemingly wide range of basic proteins (*e.g.* Waller, 1964; Leboy *et al.*, 1964). It is highly specific in nature, and has eluded attempts at reconstitution from the dissociated nucleic acid and protein components. The native form is stabilized by poorly understood factors, involving among other things magnesium ions and probably diamines (Petermann, 1964). The RNA has been shown, at least in the case of *Escherichia coli* ribosomes (Schlessinger, 1960), to have the same degree of helical structure in the ribosome as in the free state. The conformation of the proteins has not however been hitherto examined. As a first step toward the investigation of the morphology of the ribosome, and the nature and specificity of the relationship between the protein and RNA, we have examined the optical rotatory dispersion under varying conditions of three types of ribosomes of widely different RNA–protein ratio. We have related the results to the characteristics of the RNA and proteins alone. These findings are described below.

Experimental Section

The greater number of our experiments were performed on ribosomes from yeast, *Saccharomyces fragilis*. The ribosomes were prepared in the manner previously described (McPhie *et al.*, 1966). The RNA was extracted with phenol (Tissières *et al.*, 1959), and three times precipitated with ethanol. All experiments with the intact ribosomes were performed in a buffer of 1 mM Tris, 2 mM magnesium chloride, 0.1 M potassium chloride, pH 7.2. All preparations were screened by sedimentation in the analytical ultracentrifuge (Spinco Model E). Ribosome concentrations were measured spectrophotometrically, taking the specific absorptivity as $E^{1\%}_{1cm} = 113$ (Yin, 1960). The molar residue absorptivity of the RNA was determined by phosphorus analysis by the procedure of Jones *et al.* (1951); it was found to be $\epsilon(P) = 7400$. For a mean residue weight of 317 (from the base composition given by Maeda, 1960), neglecting counterions, this leads to $E^{1\%}_{1cm} = 233$ for the RNA.

The proteins were prepared by extraction with acetic acid (Waller, 1961), with guanidinium hydrochloride (Cox and Arnstein, 1961), and with lithium chloride (Curry and Hersh, 1962). The first of these methods was found to give the best yield of protein and the most stable preparations, and was routinely used. The acetic acid extract was dialyzed at 4° into dilute acetic acid, pH 4, adjusted to pH 4.5 with alkali, and passed through a column of Sephadex G-75 to remove nucleotides, which

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otherwise always remained bound to the protein. The resulting solution was free of nucleotide material, as judged by spectrophotometric analysis. For storage, the solution was once more dialyzed against dilute acetic acid and lyophilized. At least 80% of the protein was extracted in this way.

The amino acid composition of the protein was determined with a Spinco amino acid analyzer; the mean residue weight and nitrogen factor from these data were used. Protein concentrations were determined by nitrogen analysis using the procedure of Jacobs (1962). The reproducibility of determinations was better than 1%.

Ribosomes from *E. coli* (ribonuclease-free strain, MRE 600) were prepared by Mr. Z. Kosinski, using the method of Tissières *et al.* (1959). The preparations were purified with the aid of a DEAE-cellulose column, as described in Clark and Marcker (1965). Rabbit reticulocyte ribosomes, prepared as described by Arnstein *et al.* (1964), were a gift of Dr. H. J. Gould. They were used without further treatment.

All reagents were analytical (Analar) grade, except 2-chloroethanol; the latter was used from newly opened bottles, and samples of poor ultraviolet transparency were rejected.

Optical rotatory dispersions were measured from 220 to 590 mμ with a Bellingham and Stanley polarmatic recording spectropolarimeter, using cells of 1-cm path, in a thermostated cell housing. The instrument was calibrated with standard sucrose solutions. For measurements in the region of absorption, the absorbance was never allowed to exceed 1.5, and the linearity of measured rotations with concentration was in all cases checked. Slit widths in regions of high absorption were up to 1 mm. The independence of readings of slit width could be demonstrated.

Results

Intact Yeast Ribosomes. Most of the present studies were performed on yeast ribosomes. After the procedures of washing and purification on a DEAE-cellulose column, these ribosomes are stable for considerable periods at room temperature. The optical rotatory dispersion of such ribosomes is highly reproducible, and is depicted in Figure 1. The prominent Cotton effect is centered at about 266 mμ, the position characteristic of RNA (Samejima and Yang, 1964), but the existence of an additional feature due to the presence of α-helical protein is evident in the negative limb.

Effect of Subunit Formation. The yeast ribosomes have also been examined in a solution containing 2 mM EDTA. Under these conditions they are seen by sedimentation in the analytical ultracentrifuge to be dissociated into 60 and 40S subunits. No change in any part of the optical rotatory dispersion curve was observed. In 10 mM EDTA a new, more slowly sedimenting, and apparently expanded particle is formed (Weller and Horowitz, 1964), in which the specificity of the ribosome structure is seemingly lost. Again however no change in optical rotatory dispersion is observed. At

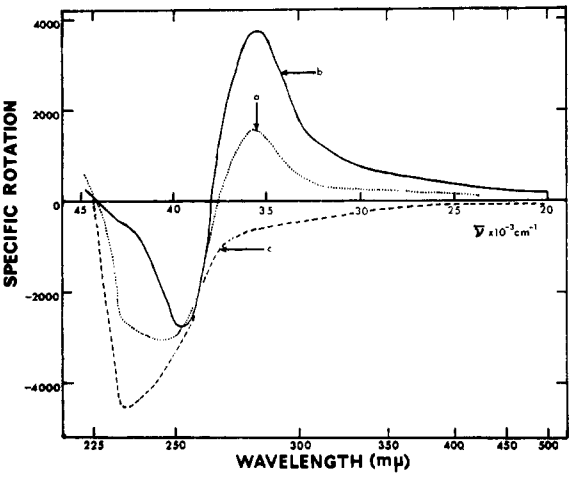

FIGURE 1: Optical rotary dispersion of yeast ribosomes, ribosomal RNA, and ribosomal protein: (a) optical rotatory dispersion of yeast ribosomes; (b) optical rotatory dispersion of yeast ribosomal RNA; (c) calculated optical rotatory dispersion of yeast ribosomal protein in the ribosome. The solvent is 1 mM Tris, 2 mM magnesium chloride, 0.1 M potassium chloride, pH 7.2.

pH 9.5 (carbonate buffer) dissociation also takes place, and in this case it is irreversible (Chao and Schachman, 1956). Here $[\alpha]_{233}$ falls to $-1700°$, and the α-helical structure is seen to be drastically diminished.

Optical Rotatory Dispersion of Ribosomal Protein in the Ribosome. Figure 1 shows in addition the optical rotatory dispersion of the extracted RNA. The rotations are expressed in terms of specific rotation, $[\alpha] = 100\alpha/cd$ where α is the measured rotation, c the concentration in g/100 ml, and d the path length in decimeters. The composition of the yeast ribosome is known to be 43% RNA, 57% protein (Chao and Schachman, 1956), and we may, therefore, break down the specific rotation of the ribosome into its constituent parts by weight, according to the relation

$$[\alpha]_{\text{rib}} = 0.43[\alpha]_{\text{RNA}} + 0.57[\alpha]_{\text{protein}}$$

In order to calculate $[\alpha]_{\text{RNA}}$ we use for the specific absorptivity our value, based on phosphorus determination (see above). It is now possible to deduce the specific rotation of the protein, and to convert this to the mean molar residue rotation

$$[m']_{\text{protein}} = \frac{3}{n^2 + 2}\frac{\bar{M}_{\text{res}}}{100}[\alpha]_{\text{protein}}$$

where n is the refractive index of the medium (dispersion of refractive index being neglected), and $\bar{M}_{\text{res}}$ the mean residue weight, here 108 (from the amino acid composition of the protein, as given in Table I). The protein contribution to the optical rotatory dispersion of the

TABLE I: Amino Acid Composition of Yeast Ribosomal Protein.

Amino Acid	Residues/100 Residues
Asp	9.8
Thr	5.4
Ser	6.3
Glu	10.8
Pro	4.8
Gly	8.1
Ala	10.1
Half-Cys	0.2
Val	7.6
Met	1.7
Ileu	5.2
Leu	8.4
Tyr	2.8
Phe	3.3
Lys	8.7
His	1.7
Arg	5.4
Amide N	15.3
Total	100.3
Total nitrogen (%)	14.0
Mean residue wt	108

ribosome is shown in Figure 1 on a specific rotation, and in Figure 2 on a mean residue rotation basis. It is seen to show the minimum of a negative Cotton effect at 233 mμ, the position characteristic of the α-helical polypeptide chain, with $[m']_{233} = -3900°$.

Away from the absorption band, both the ribosomes and the extracted RNA follow a single-term Drude law, with λ_c 260 and 258 mμ (see Table II), respectively. Subtracting as before to give the contribution of the protein, the latter is found also to give a linear Drude plot, with λ_c 200 mμ. When the optical rotatory dispersion contribution of the protein is plotted in terms of the Moffitt equation (Moffitt and Yang, 1956), taking λ_0 212 mμ in

$$[m'] = a_0 \frac{\lambda_0^2}{\lambda^2 - \lambda_0^2} + b_0 \left(\frac{\lambda_0^2}{\lambda^2 - \lambda_0^2} \right)^2$$

there emerges a value for a_0 of −190, and b_0 of −200. On the usual scale ($b_0 \simeq -630$ for a fully α helical, and 0 for a random chain) we obtain an α-helix content of some 32%. It is not justified to draw any conclusions from the value of a_0, since we are unable to describe the microscopic environment of the protein on the ribosome.

The Extracted Protein. The optical rotatory dispersion of the extracted protein was measured under a number of solvent conditions. The protein is soluble in aqueous buffer systems at pH 4.5 at quite low ionic strengths (we have used 0.05 M acetate buffer), and at pH 7 only at rather high ionic strengths (in our experiments 1 M Tris, pH 7.2). It is also soluble in strongly alkaline solution (0.1 M sodium hydroxide). The optical rotatory characteristics of the protein under these circumstances are summarized in Figures 2 and 3 and Table III. It is clear that in the isolated state it is devoid of α helix in aqueous solution except in small degree in alkali, where the basic groups are uncharged. [The protein is also soluble in 8 M urea at neutrality, and in this solvent b_0 is slightly positive, in consequence probably of the formation of β structures (Imahori, 1960)]. When dissociation of the protein from the RNA is brought about by 1 M Tris at neutral pH (Spitnik-Elson, 1962), there is likewise no α-helical structure.

The Moffitt plot of the protein in 2-chloroethanol solution is also shown, and the b_0 here is −330 (*ca.* 55% α helix on the usual scale).

TABLE II: Optical Rotatory Dispersion Parameters of Ribosomes and Ribosomal RNA.

Species	Solvent	Cotton Effect (mμ)	$[\alpha]_{max}$ (deg) (280–282 mμ)	$[\alpha]_{min}$ (deg) (241–253 mμ)	$[\alpha]_{233}$ (deg)	λ_c (mμ)
Yeast RNA	1 mM Tris, 2 mM $MgCl_2$, 0.1 M KCl, pH 7.2	263	+3700	−2800	−400	258
Yeast ribosomes	1 mM Tris, 2 mM $MgCl_2$, 0.1 M KCl, pH 7.2	266	+1550	−3050	−2700	260
E. coli ribosomes	1 mM Tris, 5 mM $MgCl_2$, pH 7.2	266	+2400	−2600	−1400	252
Rabbit reticulocyte ribosomes	0.25 M sucrose, 1 mM $MgCl_2$, 0.025 M KCl, 5 mM Tris pH 7.6	268	+1550	−2750	−2400	266
Rabbit reticulocyte ribosomes	4 M GuHCl	268	+1600	−2700	−900	...

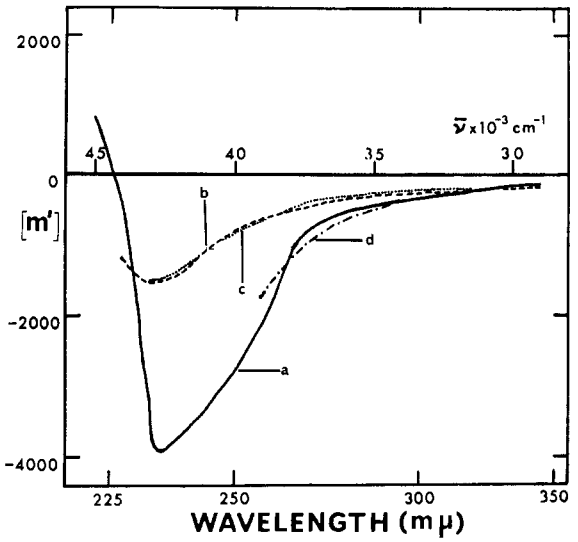

FIGURE 2: Optical rotatory dispersion of yeast ribosomal protein, expressed as mean molar residue rotations: (a) protein on ribosome, calculated from data of Figure 1; (b) extracted protein in 0.05 M sodium acetate pH 4.5; (c) extracted protein in 1 M Tris, pH 7; (d) extracted protein in 2-chloroethanol.

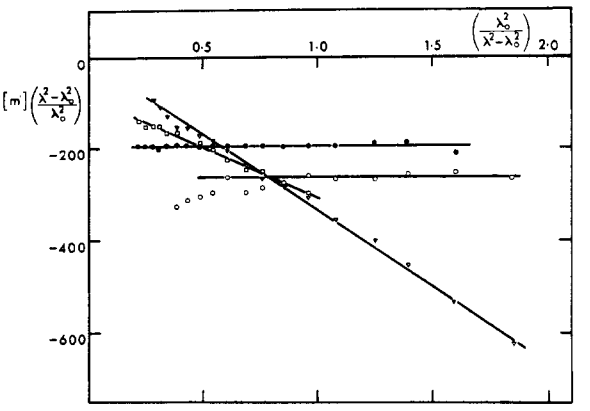

FIGURE 3: Moffitt plots of yeast ribosomal proteins, in ribosome (□); extracted in acetate buffer pH 4.5 (○); in 1 M Tris, pH 7 (●); in 2-chloroethanol (▽).

TABLE III: Optical Rotatory Dispersion Parameters of Yeast Ribosomal Protein.

Sample	$[m']_{233}$ (deg)	a_0 (deg)	b_0 (deg)	λ_c (mμ)
Protein in ribosome	−3900	−190	−200	200
Extracted protein in 0.05 M Na-OAc, pH 4.5	−1500	−290	0	227
Extracted protein in 1 M Tris, pH 7	−1500	−200	0	220
Extracted protein in 0.1 M NaOH	−2500	−325	−75	...
Extracted protein in 2-chloroethanol	...	0	−330	Nonlinear

Comparative Studies on Different Ribosomes. We turn now to the comparison between the yeast ribosomes, and those of two widely different cells, *E. coli* and rabbit reticulocytes. Specific rotations were calculated as before from the known compositions of *E. coli* (Tissières *et al.*, 1959), and reticulocyte (Dribble and Dintzis, 1960) ribosomes, taking $E_{1\,cm}^{1\%} = 157$ and 135, respectively.

The optical rotatory dispersion curves are superficially similar to one another, and to that of yeast (Figure 4). The atypically low protein content of *E. coli* ribosomes makes the 233 mμ feature appear much less prominent however. The salient features of the optical rotatory dispersions of the three types of ribosome are summarized in Table II.

In Figure 4 is shown the effect of 4 M guanidinium hydrochloride on the optical rotatory dispersion of reticulocyte ribosomes. In this solvent, the protein and RNA are fully dissociated from one another, but remain in solution (Cox and Arnstein, 1961). It is clear that the RNA is essentially unaffected (Figure 4 and Table II), but the disappearance of the 233-mμ feature is graphically demonstrated.

Discussion

It was demonstrated by Schlessinger (1960) that the hypochromicity of ribosomal RNA, at least in *E. coli*, is identical in the intact ribosome and in the free state. Our data on yeast ribosomes indicate a similar conclusion. If then it is permissible to assume that the conformation, and therefore the optical rotatory dispersion, of the RNA in the ribosome is as that of the free molecule in solution, the interpretation of the data, which we have presented, is much simplified. We have proceeded in this manner. A comparison of the results for the three species of ribosome, in which the protein contribution to the optical rotatory dispersion clearly reflects the proportion of protein present, provides additional justification.

Figures 2–4 show clearly the presence by the accepted criteria of α-helical structure in the ribosomal protein. In Figure 4 (inset) the disappearance of rotation at 233 mμ when the protein is transferred from the ribosomal environment into aqueous guanidinium hydrochloride is most vividly shown. The origin of the minor irregularities in the difference curve is not clear: they may arise from refractive index effects, but it should be noted that the curve represents a rather small difference between two large quantities, and one is here very close to the limits of error of the polarimetric technique. The immeasurably small α-helix content of

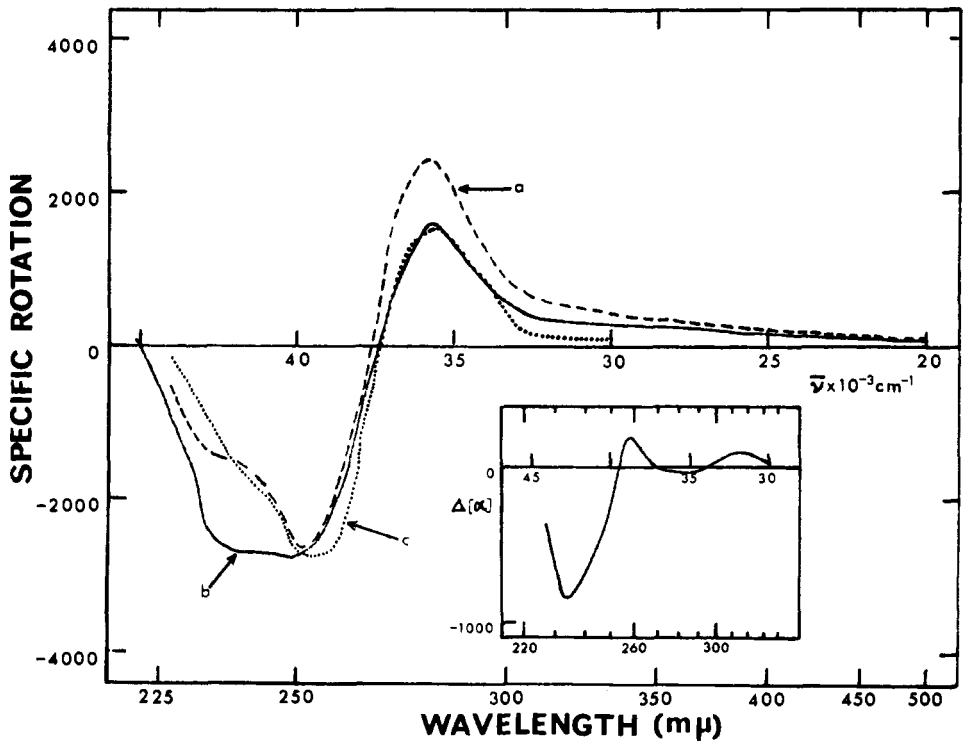

FIGURE 4: Optical rotatory dispersion curves of two different types of ribosome: (a) *E. coli* ribosomes in 1 mM Tris, 5 mM magnesium chloride, pH 7.2; (b) rabbit reticulocyte ribosomes in 0.025 M potassium chloride, 1 mM magnesium chloride, 5 mM Tris, 0.25 M sucrose, pH 7. (Optical rotations have been corrected for the sucrose rotation); (c) rabbit reticulocyte ribosomes, made 4 M in guanidinium hydrochloride. Inset: difference in specific rotation between (b) and (c).

the isolated protein is to be expected from comparison with, for instance, the protamines (Yang and Doty, 1957), which are also small, highly basic, proteins, of not very dissimilar composition; indeed synthetic polypeptides, rich in lysine residues, also possess little or no helix at neutral pH (Friedman *et al.*, 1962). The relatively low value of b_0 in 2-chloroethanol is less expected. In this type of solvent it is supposed (see, for instance, Urnes and Doty, 1961) that proteins are able to assume their highest possible degree of α-helical conformation. The ribosomal proteins do not have a notably high proline content, and are very low in cystine, and it must, therefore, seem that it is their sequences which are for the most part unfavorable (see, *e.g.*, Schellman and Schellman, 1964) for the formation of α-helices.

It is interesting to note therefore that the protein in its native environment possesses a large proportion (over 60%) of its maximum possible content of α helix. This is very remarkable for such highly charged chains. A specific mechanism whereby the helical conformation is promoted must therefore be involved. We have found (Gratzer and McPhie, 1966) that whereas poly-L-lysine at neutral pH can acquire a substantial degree of α-helical structure when it is complexed with polyacrylic or polyphosphoric acid, no such effect occurs with RNA, or even with polyuridylic acid. Neither is any measurable amount of α helix induced into ribosomal proteins when they are complexed with polyacrylic acid. The very low degree of helix induced in 0.1 M alkali and the disruption of the structure in the ribosome subunits by minor adjustment of charge balance at pH 9.5 indicate also that the elimination of positive charges is insufficient to produce anything approaching the *in situ* structure of the protein. We conclude that the manner in which the proteins are arranged around the RNA chain in the ribosome is of a precise specificity, which determines their conformation. Whether the over-all α-helix content of the protein in the ribosome reflects a wide spread between ones of low or zero helix content, and other highly helical ones, or whether a general similarity obtains between the different proteins, cannot at this stage be determined.

It is concluded that the ribosomal proteins may be regarded as native only when they are in their proper environment. In the absence of cystine or sulfhydryl groups, small proteins will normally assume their most stable (native) structure in solution, even after the most drastic treatment; it does not seem profitable in the present context to invoke the concept of denaturation by the extraction procedures *per se*. In any case, it has been noted that α-helical structure is annihilated when the protein is dissociated from the RNA by high ionic strength alone at neutral pH.

It is worth noting that the specificity of the protein-

RNA complex is not disturbed when the ribosomes are dissociated into their subunits, nor yet when magnesium ions are wholly removed, and a new and highly expanded particle is formed. This suggests that the tertiary packing is not essential to the conformational stability.

The results invite comparison with work on the histones, which in their native state are complexed with fully helical DNA. The histones are substantially richer in basic residues than the ribosomal proteins (see, *e.g.*, Jirgensons and Hnilica, 1965, for amino acid compositions). Their optical rotatory dispersion shows that they differ markedly in this respect also: both Bradbury *et al.* (1965) and Jirgensons and Hnilica (1965) have found that nearly all fractions appear to contain appreciable proportions of α helix in dilute salt solutions. Several lines of evidence (see, *e.g.*, Bradbury and Crane-Robinson, 1964) suggest a rather high degree of α helix in native nucleohistone.

The extent of similarity between the three types of ribosome which have been examined supports earlier evidence of the far-reaching basic similarity between such species (see for instance Petermann, 1964). The three species span almost the whole known range of RNA–protein ratios, and may, therefore, be regarded as reasonably representative. Taking account of this range, it appears that the protein portions are rather similar in α-helix content. Tinoco and Maestre (1965) observed large differences in absolute rotation, though not in position and amplitude of Cotton effects, in the optical rotatory dispersion curves of deoxyribonucleic acid (DNA) bacteriophages. A linear relation was reported between specific rotation and DNA content. This type of effect is absent in the ribosomes. Presumably differences in density of packing do not exist in significant degree.

Lastly we point out the possibilities which our results offer of gathering taxonomic information on ribosomes, and in particular of determining the approximate RNA–protein ratio with little difficulty and a minimum of material. Optical rotatory dispersion curves in the Cotton effect region may be obtained in a modern recording instrument on samples of no more than *ca.* 0.2 mg.

Acknowledgments

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