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Detection of Extraterrestrial Life

Method II: Optical Rotatory Dispersion

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1.0 INTRODUCTION

The object of this study is to develop polarimetric methods to detect the presence of DNA (deoxyribonucleic acid) or its congeners in soil suspensions, and through these methods determine the existence of life (as known terrestrially) on other planets. The cotton region associated with optically active organic compounds is being used to detect and characterize the compounds above.

An apparatus has been designed and assembled which can measure optical rotations in systems which strongly attenuate incident-polarized, monochromatic light. This instrument was used to measure the optical rotatory dispersion spectra of nucleosides, a polynucleotide, and proteins whose optical density at 260 m\(\mu\) approached 1.0. This work is discussed in the final report on Contract NASr-85, Detection of Extraterrestrial Life, Method II: Optical Rotatory Dispersion. Recent work in Melpar laboratories has reaffirmed these rotatory dispersion spectra. Based upon the analysis of the optical components associated with this apparatus, however, these measurements must be considered qualitative rather than quantitative. The reason for this is discussed in greater detail subsequently in this report.

In addition, an evaluation of the theoretical and instrumental aspects of making rotatory-dispersion measurements in the cotton region has resulted in a procedure for measuring optical rotation.
2.0 BACKGROUND

The measurement of optical rotation in the cotton regions has contributed to several obstacles. These are circular dichroism, which may be associated with unequal absorption of right and left circular-polarized light; absorption of light because of functional groups; and scattering. The Rudolph polarimeter, which measures absolute values of optical rotation, has not been too effective in the ultraviolet region of the spectrum because of absorption and scattering of light which results in a low signal output. Several approaches have been proposed to eliminate the absorption effects. The theoretical aspects of these are adequately covered in a paper by A.L. Rouy and B. Carroll, Anal. Chem. 33, 594 (1961).

The measurement of optical rotations in turbid media results in an enhancement of the scattering effect. It appears from a theoretical development of the Rouy technique in utilizing the ratio of the difference to the sum that optical-rotation measurements may be made on turbid solutions. This aspect was discussed in the first quarterly report on Contract NASr-85. The introduction of the circular dichroic effects into the theoretical development of the ratio of the "difference to the sum" results in the ratio being a complex function of the optical rotation. It remains to be seen, however, whether these dichroic effects are of sufficient magnitude to introduce an error in the measurement of optical rotation.
3.0 EVALUATION OF ROUY-TYPE POLARIMETER

3.1 Experimental Procedures

All rotatory-dispersion measurements were performed on the Rouy-type polarimeter. To eliminate part of the stray white light from the monochromator, a Corning filter, No. 9868, was utilized. The Beckman D. U. spectrophotometer was utilized for all absorption measurements in both the visible and ultraviolet regions of the spectrum.

Adenosine (Grade A), d-tartaric acid (Grade A), and l-tartaric acid (Grade A) were purchased from Calbiochem, Los Angeles, California. L-tyrosine, L-tryptophan, and L-phenylalanine (all of Schwartz O. S. grade) were obtained from Schwartz Biochemicals, Mount Vernon, New York.

The procedure for preparing the potassium tartrate-potassium dichromate complex was as follows: 10 grams of potassium tartrate in 30 ml of distilled water were prepared from d-tartaric acid and reagent-grade potassium hydroxide. A second solution was prepared by dissolving 0.75 grams of potassium dichromate in 30 ml of water. Both solutions were mixed at about 50°C and allowed to stand for several hours. The resulting solution was then allowed to boil for 30 minutes, cooled, and diluted to 100 ml. This solution, which contains the complex between potassium tartrate and potassium dichromate, was permitted to stand for 48 hours before rotatory dispersion measurements were performed on it.

The water-soluble extract of soil was prepared by adding water to 100 grams of soil to make a thick slurry. This slurry was allowed to stand for several days to allow the water-soluble components in the soil to dissolve. The slurry was then
filtered by means of fritted glass filters of course, medium, and fine porosity until a clear solution resulted. Optical-rotation measurements were performed on this solution without further dilution.

The adenosine, d-tartaric acid, l-tartaric acid, L-tryptophan, and L-phenylalanine were prepared with distilled water. The L-tyrosine was prepared with 5 N HCl.

3.2 Results for Rouy-type Polarimeter

A preliminary investigation in the evaluation of the performance of the Rouy-type polarimeter indicated that both beams were of unequal intensity. A description and a theoretical discussion of the Rouy-type polarimeter are given in the proposal submitted by Melpar to NASA on the detection of extra-terrestrial life, Method II, Analysis of Optical Rotation Dispersion Profiles, June, 1961. Upon an investigation of the optical components associated with this instrument, it was found that the differences in intensities between the two beams could be attributed to the following:

(a) If reflecting surfaces are located between a polarizer and analyzer, the reflection of plane-polarized light off these surfaces results in elliptically polarized light being introduced into the plane-polarized beam. To overcome this problem, it was necessary to reposition the polarizing prisms so that their optical axes make equal angles with the optical axes of these reflecting surfaces. On this basis, equal amounts of elliptically polarized light would be present in both beams. By utilizing the ratio of the difference to the sum, this effect of elliptically polarized light will be cancelled.
(b) In an examination of the glan prisms which serve as the polarizers and analyzer, it was observed that they transmit different amounts of light at different wavelengths. Upon closer examination of these prisms, it was found that the prism surfaces which make up the internal plane of the glan prism were not parallel. This resulted in several images being emitted from the prisms. The number of images varied as a function of wavelength. Consideration of the observation above, in the theoretical development of the ratio of the "difference to the sum," indicated that the unequal transmission between the right and left polarizing prisms renders the measured ratio a complex function of the angular rotation.

Despite the findings above, it is interesting that it is possible to observe cotton-effect regions as well as to correlate $\lambda_c$ with the wavelength at which the maximum absorption occurs. The $\lambda_c$ is defined as that wavelength within the cotton region where the angular rotation is equal to zero. From an examination of the rotatory dispersion curves for L-tyrosine, L-phenylalanine, and L-tryptophan (see figure 1), it may be seen that the $\lambda_c$ values are 262 m$\mu$, 270 m$\mu$, and 280 m$\mu$, respectively. The absorption spectra of L-tyrosine, L-phenylalanine, and L-tryptophan indicate maximum absorption at 260 m$\mu$, 275 m$\mu$, and 280 m$\mu$. It is also interesting that the ratio values appear to be unsymmetrical when there is appreciable absorption occurring. The negative measured ratio appears to be much larger in absolute value than the positive measured ratio. This is shown more clearly in figure 2, which represents the rotatory dispersion curves of l-tartaric acid and d-tartaric acid. Both
Figure 1. Rotatory Dispersion Spectra at $5 \times 10^{-5} \text{M/1}$ of Amino Acids: 1) L-Phenylalanine, 2) L-Tyrosine, and 3) L-Tryptophan
Figure 2. Rotatory Dispersion Spectra at 10% of 1) d-Tartaric Acid and 2) l-Tartaric Acid
curves appear symmetrical down to 260 μ. At shorter wavelengths, that is, below 260 μ, both compounds tend toward negative measured ratios. The absorbance, as measured for these two compounds at 255 μ, was about 0.180.

The rotatory-dispersion curve of the complex between potassium dichromate and optically active potassium tartrate in the wavelength range of 500 μ to 630 μ should give a symmetrical cotton curve with \( \lambda_c = 560 \mu \). Figure 3 shows the dispersion curve for this complex. Even though \( \lambda_c \) is equal to 560 μ, the dispersion curve is not symmetrical. The negative measured ratios have much larger absolute values than the positive measured ratios. The absorbance at 560 μ for this solution was found to be approximately 1.80.

Based upon the previous findings, it appeared that quantitative measurements cannot be made at the present time utilizing the Rouy-type polarimeter; anomalies associated with optically active organic compounds, however, can be detected. In figure 4, the dispersion curve for the water-soluble extract of soil is shown. It may readily be seen that there are certain components within this extract that exhibit anomalous dispersion. The absorption spectra for this extract are also shown in figure 4. The absorption spectra appear to give a linear increase in absorbance with wavelength and are not capable of characterizing the components within the extract. A comparison of the dispersion curve of adenosine in figure 5 with the dispersion curve of the extract in figure 4 indicates similarity. Further analysis on the soil extract must be performed, however, to determine whether adenosine-type compounds are responsible for this cotton effect.
Figure 3. Rotatory Dispersion Spectra at 10% of Complex of Potassium Dichromate and Potassium Tartrate
Figure 4. Rotatory and Absorption Spectra of Water Extract of Soil (Rotatory: Solid Line. Absorption: Dotted Line)
Figure 5. Rotatory Dispersion Spectra at $2.9 \times 10^{-5}$ M/l of Adenosine
3.3 Conclusions

At the present time, the Rouy-type polarimeter is capable of detecting anomalous dispersions qualitatively. The reason for this is the difference in transmission between the two polarizing prisms. It is believed that the instrument should yield quantitative results when these prisms are replaced with new ones, which are now on order.

The optical-rotation measurements performed on a water-soluble extract of soil indicate that there are organic compounds present in this extract capable of giving anomalies. A comparison of this dispersion curve with that of adenosine shows that they appear to be similar. Work will be continued, however, on the analysis of the extract by chromatographic means to identify the compounds present in this extract. Once this is achieved, chromatographic separations will be employed to isolate the optically active species. Optical-rotation measurements will then be run on the extract of the remaining supposedly inactive species to determine whether the anomalous dispersion still exists. It is interesting that absorption measurements on this extract apparently yield no characteristic spectra.
4.0 NEW TECHNIQUE FOR MEASURING OPTICAL ROTATORY DISPERSION

4.1 Introduction

It has been stated previously in this report that the introduction of circular dichroic effects into the theoretical aspects of utilizing the ratio of the "difference to the sum" results in the ratio being a complex function of the angular rotation. As a result of this finding, a search for a new technique for measuring optical rotations in the cotton region was initiated. Upon completion of a mathematical analysis of the problems associated with optical-rotation measurements in the cotton region, a new ratio, which may be defined as the ratio of the "difference to a constant," was evolved. This ratio appears to take into account adequately the circular dichroic effect, absorption, and scattering. In addition, not only does it appear possible to obtain angular rotation measurements in both clear and turbid solutions, but also to determine the absorption coefficients associated with circular dichroism.

This new approach can be readily adapted to both single-beam and double-beam instruments. For the present, however, Melpar is planning to utilize this approach in a double-beam instrument. The Cary spectrophotometer, model 15, is now being modified so that dispersion measurements can be performed on this instrument.

4.2 Theoretical Development and Evaluation of the Ratio of the "Difference to a Constant"

It may be readily shown that, in the presence of circular dichroism, absorption, and scattering, the light energy ($E_1$) which passes through an analyzer, with its optical axis set at an angle ($+\theta$) to the optical axis of the polarizer, may be represented by equation (1).
\[ E_1 = \frac{(q+p)^2}{2} \cos^2 (\theta - \alpha) + \frac{(q-p)^2}{2} \sin^2 (\theta - \alpha) + \] (1)

where:
\[ q^2 = 2A^2 e^{-\frac{K_L \ell c}{2}} e^{-\frac{K_R \ell c}{2}} e^{-\frac{K_S \ell c}{2}} \]
\[ p^2 = 2A^2 e^{-\frac{K_L \ell c}{2}} e^{-\frac{K_R \ell c}{2}} e^{-\frac{K_S \ell c}{2}} \]

and
\[ \frac{1}{2} a e^{-\frac{K_L \ell c}{2}} e^{-\frac{K_R \ell c}{2}} e^{-\frac{K_S \ell c}{2}} \]
\[ \frac{1}{2} b e^{-\frac{K_L \ell c}{2}} e^{-\frac{K_R \ell c}{2}} e^{-\frac{K_S \ell c}{2}} \]
\[ Z = \text{amount of unpolarized light passing through the analyzer because scattering results in depolarization of plane-polarized light} \]
\[ \alpha = \text{angular rotation of solution} \]
\[ A = \text{amplitude of plane-polarized beam emitted from the polarizer} \]
\[ K = \text{extinction coefficient associated with the absorption of light} \]
\[ K_R = \text{extinction coefficient associated with the absorption of right, circularly polarized light} \]
\[ K_L = \text{extinction coefficient associated with the absorption of left, circularly polarized light} \]
\[ K = \text{scattering coefficient} \]
\[ \ell = \text{path length} \]
\[ c = \text{concentration of optically active solution} \]
Should another setting be utilized in which the optical axis of the polarizer now makes an angle of \((-\theta)\) with the optical axis of the analyzer, the light energy \(E_2\) which passes through the analyzer may be represented by equation (2).

\[
E_2 = \frac{(q+p)^2}{2} \cos^2(\theta + \alpha) + \frac{(q-p)^2}{2} \sin^2(\theta + \alpha) + \Sigma
\]  

(2)

If a double-beam instrument is being utilized, the constant in the ratio of the "difference to a constant" is defined as that energy as seen by the phototube with only a polarizing prism present. In the absence of an optically active substance, if the intensity of both beams is balanced, the energy \(E\) associated with the constant term may be defined by equation (3).

\[
E = 2A^2 \cos^2 \theta
\]  

(3)

The placing of an optically active sample in this beam modifies equation (3) in the following manner:

\[
E = 2A^2 \cos^2 \theta e^{-Klc} e^{-KRc} e^{-Khc} + 2A^2 \cos^2 \theta e^{-Klc} e^{-KLc} e^{-Khc} e^{-kZlc}
\]  

(4)

Factoring out of the common terms in equation (4) results in equation (5)

\[
E = 2A^2 e^{-Klc} e^{-KZlc} \cos^2 \theta (e^{-KRC} + e^{-KLC})
\]  

(5)

Substitution of equation (1), equation (2), and equation (5) into the ratio of the difference to a constant leads to equation (6)
\[ R = \frac{E_1 - E_2}{E} = \frac{(q+p)^2}{2} \cos^2 (\theta - \alpha) + \frac{(q-p)^2}{2} \sin^2 (\theta + \alpha) + \Sigma}{2A^2 e^{-K_R C} e^{-K_L C} \cos^2 \theta \left( e^{-K_R C} + e^{-K_L C} \right)} \]

Substitution for \( p \) and \( q \) the values which were defined earlier, cancellation of the common terms which appear in both the numerator and denominator, and making use of the trigonometric relationships,

\[ \cos^2 - \sin^2 x = \cos 2x \]

\[ \cos (x + y) = \cos x \cos y + \sin x \sin y \]

results in equation (7)

\[ R = \frac{2e^{-\frac{K_R C}{2}} e^{-\frac{K_L C}{2}}}{e^{-K_R C} + e^{-K_L C}} \left[ \frac{\sin 2 \theta \sin 2 \alpha}{\cos^2 \theta} \right] \]

Dividing both the numerator and denominator of equation (7) by

\[ \frac{K_R C}{2} \quad \frac{K_L C}{2} \]

and taking advantage of the trigonometric relationship

\[ \sin 2x = 2 \cos x \sin x. \]

leads to equation (8)

\[ R = \frac{4 e^{-\frac{2}{2} (K_L - K_R) C}}{(K_L - K_R) C + 1} \tan \theta \sin 2 \alpha \]
If we let \( x = \Delta K = \frac{K_L - K_R}{2} \), equation (8) can be written as

\[
R = 4 \tan \theta \left[ \frac{e^{x}}{e^{2x} + 1} \right] \left[ \sin 2\alpha \right]
\]  

(9)

Both \( \frac{e^{x}}{e^{2x} + 1} \) and \( \sin 2\alpha \) can be expanded around zero

\[
\frac{e^{x}}{e^{2x} + 1} = \frac{1}{2} \left[ 1 - \frac{x^2}{2!} + \frac{5x^4}{4!} \right]
\]

(10)

\[
\sin 2\alpha = 2\alpha = \frac{(2\alpha)^3}{3!} + \frac{(2\alpha)^5}{5!} \cdots
\]

(11)

It can be readily shown that the series represented in equation (10) and equation (11) converge for all values of \( x \) and \( \alpha \). In addition, the series represented by equation (11) converges rapidly when \(-1 < \alpha < 1\). If \( R \) is plotted as a function of \( \alpha \), it should be symmetrical with respect to the \( \alpha \) axis because only odd-power terms exist in the series represented by equation (11). The series represented by equation (10) is independent of the sign of \( x \) because only even powers of \( x \) exist in this series, and it also converges rapidly when \(-1 < x < 1\).

Substitution of equation (10) and equation (11) into equation (9) leads to equation (12).
From equation (12), it can readily be seen that the sign of $R$ is directly related to the angular rotation and not to the dichroic terms because these terms exist only as even powers.

Rearrangement of equation (12) and substitution of $t = \tan \theta$ leads to equation (13)

$$R = 4 \tan \theta \cdot a \left[ 1 - \frac{(2a)^2}{3!} + \frac{(2a)^4}{5!} \right]$$

$$\left[ 1 - \frac{x^2}{2} + \frac{5x^4}{4!} \right]$$

(12)

Substitution of $x = \frac{\Delta Kn}{c}$ into equation (13) leads to equation (14)

$$R = 4t \left[ 1 - \frac{(\Delta Kn)^2}{8} \right] - \left[ 1 - \frac{(\Delta Kn)^2}{8} \right] \left[ \frac{(2a)^3 t}{3!} - \frac{(2a)^5 t}{5!} \right]$$

$$+ \left[ \frac{5(\Delta Kn)^4}{16.4!} \right] \left[ 2at - \frac{(2a)^3 t}{3!} + \frac{(2a)^5 t}{5!} \right]$$

(14)
Both \( \alpha \) and \( AKc \) can be adjusted so that the second term in equation (14) and the terms thereafter can be considered negligible. Thus, equation (14) may be written as

\[
R = 4\alpha \left[ 1 - \frac{(AKc)^2}{8} \right]
\]  

Equation (15) indicates that, in the region where there is no circular dichroism, \( R = 2\alpha \). In the cotton region, however, both the rotation and \( \Delta K \) can be obtained by measuring \( R \) at two different path lengths where one path is some integral number of path lengths of the other. In this way, the angular rotation and the term containing \( \Delta K \) will differ by a fixed whole number. Therefore, two equations can be generated for the two unknowns which can then be readily determined.

In the development of the ratio of the "difference to a constant," the \( \Sigma \) term is eliminated, as may be seen from equation (6), as well as the terms containing \( K_2 \). Thus, scattering does not affect angular-rotation measurements when utilizing the ratio of the "difference to a constant." To calculate the error in nonlinear terms in the absence of circular dichroism, equation (12) may be rewritten:

\[
R = 2 \tan \theta \cdot 2\alpha - 2 \tan \theta \cdot \frac{(2\alpha)^3}{3!}
\]

For \( \alpha = 0.1 \) radians and \( \theta = 45^\circ \), the departure from linearity is less than 0.7\%. 

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5.0 SUMMARY

In the evaluation of the performance of the Rouy-type polarimeter, it was observed that the glan prisms which serve as the polarizers transmitted unequal amounts of light. Consideration of this finding, in the theoretical development of the ratio of the "difference to the sum," indicated that the measured ratio is a complex function of angular rotation. Dispersion measurements on standard solutions, however, have indicated that this instrument may be used qualitatively to determine anomalous dispersions.

Optical-rotation measurements performed on a water-soluble extract of soil have indicated a cotton effect at about 255 m\(\mu\). In addition, ultraviolet absorption measurements on this extract have shown that this approach is not as sensitive as rotatory dispersion for characterizing unknown mixtures.

A new system, the ratio of a difference to a constant, has been developed which should permit measurement of optical rotations in the cotton region for both turbid and homogenous solutions. The theoretical development of this technique indicates that it may be possible to determine the difference in absorption coefficients associated with circular dichroism. The Cary spectrophotometer, model 15, is now being modified for this purpose. It is hoped that by utilizing this spectrophotometer it may be possible to make optical-rotation measurements as far down as 220 m\(\mu\).