

## Optical Rotatory Dispersion

### II. The Effect of Circular Dichroism upon Measurements of Optical Rotatory Dispersion by Means of a Spectrophotometer with a Polarimetric attachment. A New Principle for Measuring Circular Dichroism

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The results of recording optical rotatory dispersion by means of a spectrophotometer equipped with a polarimetric attachment of the equal angles type will be influenced by the ellipticity of the light transmitted by samples within their wavelength ranges of circular dichroism. The relation between the angle of rotation,  $\alpha$ , the ellipticity,  $v$ , the equal angles setting,  $\Theta$ , and the apparent optical density recorded,  $D_{\text{app}}$ , is derived; within certain ranges of the variables it may be closely approximated by

$$\alpha = \Theta \tanh 0.576 D_{\text{app}} + \frac{\tanh 0.576 D_{\text{app}}}{\Theta(1 - \tanh^2 0.576 D_{\text{app}})} v^2$$

the validity of which is demonstrated by measurements upon a solution of  $+\text{Coen}_3\text{Br}_3$ . This relation suggests a new principle for the measurement of circular dichroism, possibly of a limited practical applicability.

In previous papers<sup>1,2</sup> the construction and operation of a polarimetric attachment for use with a double beam (Cary 11) spectrophotometer have been described. It was pointed out that within spectral regions where the sample exhibit circular dichroism the measurements of optical rotatory dispersion, ORD, by means of this instrument will be influenced by the ellipticity of the light transmitted by the sample\*\*. Fig. 1 helps visualize this effect\*\*\*.

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\*\* This and the following discussion also apply to measurements made by any principally similar instrument such as the commercially available Keston and Perkin-Elmer attachments and others.<sup>3-9</sup>

\*\*\* Comparison with Fig. 1 of Ref. <sup>2</sup> may prove useful.

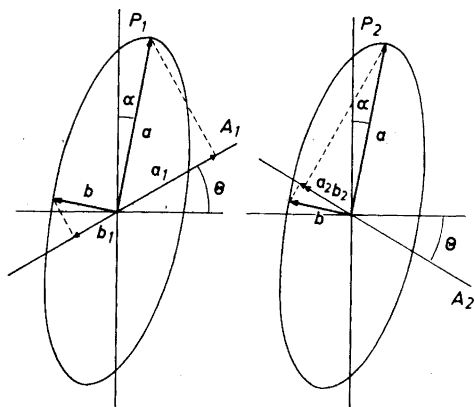


Fig. 1.  $P_1$ ,  $P_2$  and  $A_1$ ,  $A_2$  are the traces in the drawing plane of the planes of polarization of the electric field vector for the polarizers and the analyzers respectively. The analyzers are set at angles  $\pm \Theta$  from the position in which they would cause complete extinction of light polarized by the polarizers. The main axis,  $a$ , of the elliptically polarized light emerging from the samples forms the angle  $\alpha$  with  $P_1$  and  $P_2$ .  $a_1$  and  $b_1$ , respectively  $a_2$  and  $b_2$ , represent the amplitudes of the waves transmitted by  $A_1$ , respectively  $A_2$ , due to the two perpendicular components of the elliptically polarized light whose amplitudes are  $a$  and  $b$ . For clarity the ellipticity as well as the magnitude of  $\Theta$  and  $\alpha$  have been greatly exaggerated.

The light transmitted by the polarizers perpendicular to the drawing plane becomes plane polarized in planes whose traces in the drawing plane are  $P_1$  and  $P_2$ . After transmission by a circularly dichroic sample the light will be elliptically polarized and the main axis of the ellipse may form an angle,  $\alpha$ , with the original plane of polarization. For light of the wavelength,  $\lambda$ , which travels through a distance,  $l$ , in the sample  $\alpha$  is related to the difference between the indices of refraction of the sample for left and right circularly polarized light,  $n_l$  and  $n_d$ , through

$$\alpha = \frac{\pi \cdot l}{\lambda} (n_l - n_d)$$

and corresponds therefore, to the optical rotation of the plane of polarization outside regions of circular dichroism. The ellipticity of the light,  $v$ , defined by

$$\tan v \equiv b/a$$

is related to the difference between the molar decadic extinction coefficients for left and right circularly polarized light (the circular dichroism) through

$$\tan v = \tanh \frac{c \cdot l}{4 \log e} (\epsilon_l - \epsilon_d) \quad (1)$$

where  $c$  designates the concentration of the active substance. For small angles,  $\Theta$ , and for  $\alpha < \Theta$ , the components,  $b$ , of the light perpendicular to the main axis of the ellipse being nearly parallel to the planes of polarization of the analyzers,  $A_1$  and  $A_2$ , will suffer only a slight reduction upon transmission by

the analyzers. The effect of adding the corresponding intensities to those due to the light components,  $a$ , parallel to the main axis will be to make the ratio of the two intensities registered by the spectrophotometer approach unity so that the optical density recorded,  $D_{\text{app}}$ , approaches zero. Thus, if the formula

$$\alpha = \Theta \tanh \frac{1}{4 \log e} D_{\text{app}} \quad (2)$$

(or any of the other relations applicable in the absence of circular dichroism) is applied, the calculated values of  $\alpha$  will be too small.

In former derivations<sup>10,11,1,2</sup> of the relation between  $\alpha$  and  $D_{\text{app}}$  or equivalent relations<sup>4,9</sup> (for exhaustive treatments, see Refs<sup>11,9</sup>), it has been tacitly assumed that  $v = 0$ . Some of the authors who have dealt with this particular principle for ORD measurements have been aware of the fact that eqn. (2) would not be valid for elliptically polarized light<sup>2,7,8,12</sup> but apart from the statement "Actually, this effect was not observed in the optically active absorption band of potassium chromium tartrate, the very compound studied by Cotton . . . (figure 4)" (Ref.<sup>8</sup>, p. 162) no estimates of the possible magnitude of the deviations seem to have been made.

As the present method for measuring ORD becomes more frequently used in recording Cotton effect curves<sup>1,7-9,13</sup>, it becomes increasingly important to control the source of error represented by the influence of the circular dichroism which is as essential a feature of the Cotton effect as is the characteristic shape of the ORD curve. In the following, the effects of ellipticity will be treated quantitatively and experimental evidence for the validity of the treatment will be presented.

#### DERIVATION OF THE RELATION BETWEEN $\alpha$ AND $D_{\text{app}}$

The relation between the apparent optical density recorded by the spectrophotometer,  $D_{\text{app}} = \log (I_1/I_2)$ , and the quantities  $\Theta$ ,  $\alpha$ , and  $v$  is obtained by expressing the ratio of the intensities  $I_1$  and  $I_2$  transmitted by the two polarimetric units in terms of  $\alpha$ ,  $v$  and  $\Theta$ . The elliptically polarized light transmitted by the sample may be resolved into two perpendicular, plane polarized waves of amplitudes  $a$  and  $b$  having the phase difference  $\pi/2$ . The projections of the electric field vectors of these two waves upon the plane of polarization of one of the analyzers, say  $A_1$ , as a function of time may thus be represented by

$$a_1 \cos (\omega t) \text{ and } b_1 \cos \left( \omega t + \frac{\pi}{2} \right)$$

The electric field vector of the plane polarized wave transmitted by the analyzer  $A_1$ , obtained by the addition sketched in Fig. 2, is

$$F_1 = \sqrt{a_1^2 + b_1^2} \cdot \cos (\omega t + \varphi_1)$$

The expression for the electric field vector of the light transmitted by  $A_2$ ,

$$F_2 = \sqrt{a_2^2 + b_2^2} \cdot \cos (\omega t + \varphi_2)$$

is obtained in an analogous way.

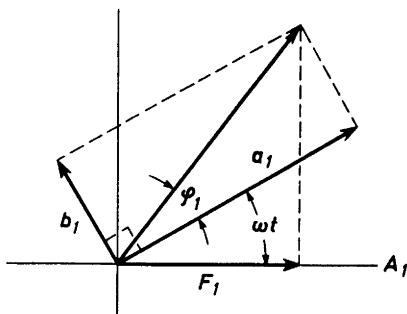


Fig. 2. The electric field vector of the light transmitted by one of the analyzers,  $F_1 = \sqrt{a_1^2 + b_1^2} \cos(\omega t + \varphi_1)$ , is obtained by vectorial addition of the projections,  $a_1 \cos \omega t$  and  $b_1 \cos(\omega t + \frac{\pi}{2})$ , upon the plane of polarization of that analyzer of the two perpendicular, plane polarized waves of amplitudes  $a$  and  $b$ , into which the elliptically polarized light may be resolved.

The intensity of plane polarized light being proportional to the square of the amplitude of the electric vector we obtain

$$\frac{I_1}{I_2} = \frac{a_1^2 + b_1^2}{a_2^2 + b_2^2} = \frac{a^2 \sin^2(\Theta + \alpha) + b^2 \cos^2(\Theta + \alpha)}{a^2 \sin^2(\Theta - \alpha) + b^2 \cos^2(\Theta - \alpha)} \quad (3)$$

For the sake of convenience we restrict the derivation to small ellipticities so that  $v \approx \tan v$ . Since the differential extinction,  $(\epsilon_i - \epsilon_d)$ , causing the ellipticity (1) is always very small in comparison to the (average) extinction,  $(\epsilon_i + \epsilon_d)/2$ , samples causing ellipticities of more than a few degrees will be optically too dense to permit measurements of the type in question. This restriction, therefore, hardly limits the practical applicability of the expression derived.

In order to obtain a reasonable sensitivity in the ORD measurements,  $\Theta$  is kept well below  $10^\circ$  and usually below  $5^\circ$  and, moreover, since we work in the region  $\alpha < \Theta$ , the approximation introduced by replacing the sines and cosines in (3) by the angles resp. unity is good to within  $\pm 1\%$  rel.

Making this approximation and setting  $b \approx va$  we obtain

$$\frac{I_1}{I_2} \approx \frac{(\Theta + \alpha)^2 + v^2}{(\Theta - \alpha)^2 + v^2}$$

(which is the equivalent of eqn. (1) of Ref.<sup>2</sup> for  $v = 0$ ). Setting this equal to  $\exp(D_{\text{app}}/\log e)$  and rearranging, we obtain

$$\alpha^2 - \frac{\exp\left(\frac{D_{\text{app}}}{\log e}\right) + 1}{\exp\left(\frac{D_{\text{app}}}{\log e}\right) - 1} \cdot 2\Theta\alpha + \Theta^2 + v^2 = 0$$

which may be written as

$$\alpha^2 - 2\Theta\alpha \coth(1.151 D_{\text{app}}) + \Theta^2 + v^2 = 0$$

The solutions

$$\alpha = \Theta \coth 1.151 D_{\text{app}} \pm \sqrt{\Theta^2 \coth^2 1.151 D_{\text{app}} - \Theta^2 - v^2}$$

may be written in the form

$$\alpha = \Theta \coth 1.151 D_{\text{app}} + \sqrt{\frac{\Theta^2}{\sinh^2(1.151 D_{\text{app}})} - v^2} \quad (4)$$

and

$$\alpha = \Theta \coth 1.151 D_{\text{app}} - \sqrt{\frac{\Theta^2}{\sinh^2(1.151 D_{\text{app}})} - v^2} \quad (5)$$

We shall be concerned only with (5) which is valid for  $\Theta > \alpha$ . It is easily verified, that for  $v = 0$  (5) may be reduced to the familiar expression (2). ((4) is valid for  $\Theta < \alpha$ ; for  $v = 0$  it may be reduced to

$$\alpha = \Theta \coth 0.576 D_{\text{app}}$$

*cf.* Ref.<sup>11</sup>)

(5) may be written as

$$\alpha = \Theta \coth 1.151 D_{\text{app}} - \frac{\Theta}{\sinh 1.151 D_{\text{app}}} \sqrt{1 - \frac{v^2 \cdot \sinh^2 1.151 D_{\text{app}}}{\Theta^2}}$$

In most, and probably all, practical cases, the last term under the root sign will be small enough compared to unity to warrant the approximation

$$\alpha \approx \Theta \coth 1.151 D_{\text{app}} - \frac{\Theta}{\sinh 1.151 D_{\text{app}}} + \frac{\sinh 1.151 D_{\text{app}}}{2\Theta} v^2$$

Using the proper trigonometric transformations we finally obtain

$$\alpha = \Theta \tanh 0.576 D_{\text{app}} + \frac{\tanh 0.576 D_{\text{app}}}{\Theta(1 - \tanh^2 0.576 D_{\text{app}})} v^2 \quad (6)$$

This is the most convenient form of the relation, partly because the ellipticity appears in a "correction term" to the expression valid outside of wavelength ranges of circular dichroism and partly because only one function of  $D_{\text{app}}$  needs to be read from the recorder paper. (The values of the function  $\tanh 0.576 D_{\text{app}}$  may be read directly by means of a properly divided rule.)

Obviously, for a set of constant values of  $\alpha$  and  $v$  (*i.e.*, a given sample) the relative importance of the correction term will increase with decreasing values of  $\Theta$ , whereas for a constant setting of  $\Theta$  the relative importance of the correction term will decrease with decreasing values of  $\alpha$  (*e.g.*, dilution of the sample) because of the proportionality between  $\alpha$  and  $v$  for any given optically active material.

#### EXPERIMENTAL

In order to test the validity of (6), we have recorded the ORD between 600 and 400  $m\mu$  for an aqueous  $1.640 \times 10^{-2}$  M solution of + Coen<sub>3</sub>Br<sub>3</sub> in 1 cm cells \* using two different

\* When measurements are made within wavelength regions of absorption it is, of course, especially important that the light paths in the two cells be equal. This was ascertained by recording the zero line for  $D_{\text{app}}$  with water as well as with a solution of racemic Coen<sub>3</sub>Br<sub>3</sub> in the cells. The two zero lines thus obtained turned out to be indistinguishable.

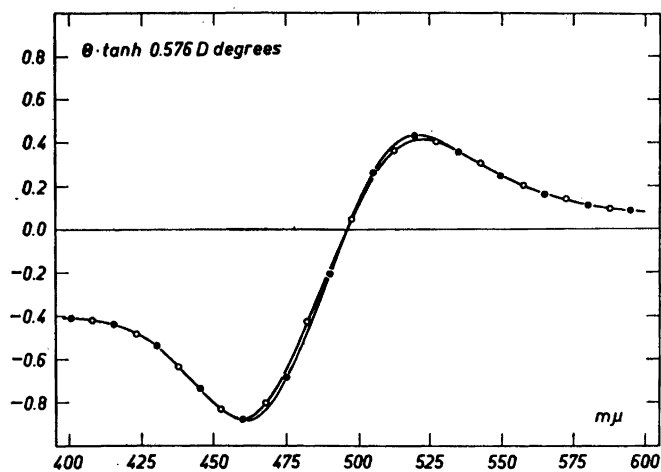


Fig. 3.  $\Theta \tanh 0.576 D_{app}$  as a function of wavelength for a  $1.640 \times 10^{-2}$  M aqueous solution of  $+Coen_3Br_3$ , measured in 1 cm cells at  $\Theta = 2.47^\circ$  and (O—O)  $\Theta = 4.32^\circ$ , (●—●) respectively.

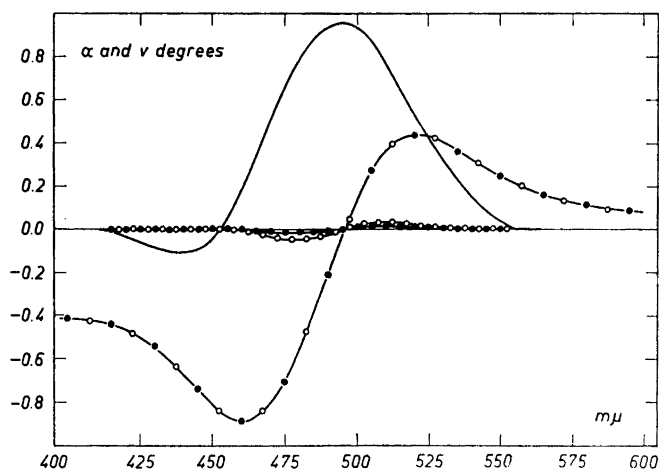


Fig. 4. The continuous curve is the ellipticity,  $v$  (in degrees), of the light transmitted by a 1 cm layer of a  $1.640 \times 10^{-2}$  M aqueous solution of  $+Coen_3Br_3$ , calculated by means of Mathieu's data<sup>14</sup>. —O—O— and —●—●— are the correction terms

$$\frac{\tanh 0.576 D_{app}}{\Theta (1 - \tanh^2 0.576 D_{app})} v^2$$

calculated for  $\Theta = 2.47^\circ$  and  $\Theta = 4.32^\circ$ , respectively. —O—●— is the result,  $\alpha$ , of adding these correction terms to the respective curves of Fig. 3.

settings of the angle  $\Theta$ . Care was taken to record the two curves by identical slit width programs. Fig. 3 shows the results of calculating  $\Theta \tanh 0.576 D_{\text{app}}$  from the two curves. As would be expected from what was said above, the smaller value of  $\Theta$  gives the (numerically) smaller values of  $\Theta \tanh 0.576 D_{\text{app}}$ . Using Mathieu's data for the circular dichroism of  $\text{Coen}_3\text{Br}_3$  in aqueous solution<sup>14</sup>, which are reproduced in Fig. 4, we have calculated the numerical value of the correction term

$$\frac{\tanh 0.576 D_{\text{app}}}{\Theta(1 - \tanh^2 0.576 D_{\text{app}})} v^2$$

as a function of wavelength for the two appropriate values of  $\Theta$  and the results are shown in Fig. 4. The result of adding the two correction curves to the curves of Fig. 3 is also shown in Fig. 4. All of the points calculated (calculation was made at 5  $m\mu$  intervals) fall on a common curve within experimental uncertainty, *ca.*  $\pm 1\%$  relative, which in our opinion proves the validity of the expression (6) derived above.

In this connection it is interesting to note that the deviation between the ORD curves for potassium chromium tartrate observed by Cotton<sup>15,16</sup> and Kirschner *et al.*<sup>7,8</sup>, respectively, (*vide* Fig. 1 in Ref.<sup>7</sup> or Fig. 4 in Ref.<sup>8</sup>) is in perfect qualitative agreement with the deviation expected from (6). In lack of the necessary experimental data concerning  $\Theta$  and  $c$  for the numerous curves obtained by Kirschner *et al.* the correction terms cannot be quantitatively calculated, not even for such compounds, for which the circular dichroism is known, but it appears from the indications of  $\Theta$  given in Ref.<sup>7</sup> that it may not have been quite negligible.

## DISCUSSION

The expression (6) immediately suggests the way in which the interference of the ellipticity with the ORD measurements may be eliminated also in cases where the circular dichroism of the compound studied has not been recorded. If  $D_{\text{app}}$  is recorded as a function of wavelengths for the sample in question at two appropriately different settings of the angle  $\Theta$ , the necessary information is available for calculating both  $\alpha$  and  $v^2$  as functions of wavelength. Besides yielding the correct values of the ORD, this procedure constitutes a new principle for measuring circular dichroism, the magnitude of which may be calculated from  $v^2$  and (1), and the sign of which may, at least in not too complicated cases, be obtained via Natansons rule:<sup>17,18</sup>

*On the long wavelength side of an optically active absorption band its contribution to  $(n_l - n_d)$  has the same sign as  $(\epsilon_l - \epsilon_d)$  within the band.*

Two circumstances, however, will tend to limit the practical applicability of this new principle.

First of all, a little consideration shows that technical sources of error in the apparatus such as stray light, incomplete polarization and depolarization (*e.g.* due to fluorescence or turbidity) will produce an effect similar to that produced by the ellipticity so that the calculated values of this quantity will be extremely sensitive towards such imperfections.

Secondly, it appears from the form of (6) that  $v^2$  will have to be calculated as a relatively small difference between two relatively large quantities. This is certainly true of the case illustrated in Fig. 3 but consideration of other optically active compounds for which absorption spectra as well as ORD and circular dichroism have been recorded indicates that this will generally be so. Relatively favorable conditions seem to prevail within the weak — but strongly optically active — absorption band near 290  $m\mu$  exhibited by the steroids

recently so extensively studied. Work is in progress to determine whether the principle for measurement of circular dichroism suggested above may be applicable to such compounds.

## REFERENCES

1. Woldbye, F. *Optical Rotatory Dispersion of Transition Metal Complexes*, European Research Office, U.S. Army, Frankfurt a. M. 1959.
2. Woldbye, F. *Acta Chem. Scand.* **13** (1959) 2137.
3. Keston, A. and Lospalluto, J. *Federation Proc.* **12** (1953) 229.
4. Carroll, B., Tillem, M. B. and Freeman, E. S. *Anal. Chem.* **30** (1958) 1099.
5. Savitzky, A., Noble, R. H., Slavin, W. and Porro, T. in Djerassi, C. *Optical Rotatory Dispersion*, McGraw Hill Book Company, Inc., New York 1960.
6. Kirschner, S., Sonessa, A. J., Bhatnagar, D. C. and Moy, D. *Abstracts of Papers*, 14 N, Div. of Inorg. Chem., 138th Meeting of the Am. Chem. Soc., New York 1960.
7. Albinak, M. J., Bhatnagar, D. C., Kirschner, S. and Sonessa, A. J. *Can. J. Chem.* **39** (1961) 2360.
8. Albinak, M. J., Bhatnagar, D. C., Kirschner, S. and Sonessa, A. J. in Kirschner (ed.) *Advances in the Chemistry of the Coordination Compounds*. 6ICCC. MacMillan. New York 1961, p. 154.
9. Rouy, A. L. and Carroll, B. *Anal. Chem.* **33** (1961) 594.
10. Gallop, P. M. *Rev. Sci. Instr.* **28** (1957) 209.
11. Poulsen, K. G. *Anal. Chem.* **32** (1960) 410.
12. Heller, W. and Fitts, D. D. in Weissberger, A. (ed.) *Technique of Organic Chemistry*, 3rd ed. Vol. 1 Part 3. Interscience Publishers, New York 1960, p. 2280.
13. Kling, O. and Woldbye, F. *Acta Chem. Scand.* **15** (1961) 704.
14. Mathieu, J. P. *J. Chim. Phys.* **33** (1936) 83.
15. Cotton, A. *Compt. Rend.* **120** (1895) 989, 1044.
16. Cotton, A. *Ann. Chim. et Phys.* **8** (1896) 347.
17. Natanson, L. *Anz. Akad. Wiss., Krakau* **1908** 764; **1909** 25.
18. Natanson, L. *J. Phys. Radium.* (4) **8** (1909) 321.

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