# Measurement of Optical Activity: New Approaches

Spectropolarimeters of new types, which give promise of wider application, are being developed.

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During the past decade, interest in the theory and measurement of optical activity has increased rapidly, particularly among biological scientists. This sudden growth has not been accompanied by sufficient consideration of the relative merits of new, commercially available instruments (1).

Thus, the principal purpose of this article is to consider the concepts underlying modern instrumental approaches to the measurement of optical activity. But first, in order to point out the need for more reliable and versatile instrumentation and to view in better perspective the current surge of instrumental development in response to that need, we present a survey of some of the more interesting recent applications of polarimetry. These are applications in the explication of chemical and biological problems, especially of problems in the field of biological macromolecules.

### **Applications of Polarimetry**

While the exact mechanism of optical rotation remains unknown, abundant correlation between rotation and chemical structure has been found. The extensive study by Djerassi on steroids is an example of this approach (2).

The description of a polymeric molecule is essentially incomplete in the absence of data on the spatial relationships among the groups comprising the molecule. Seven years have passed since Moffitt and Yang pointed out that measurements of optical rotatory dispersion provide a means of distinguishing between random-coil and regular helical arrangements of polypeptides in solution (3). In the interim many such studies have been made. The concept of the existence of polymers in a helical or partially helical state in solution, in contrast with the solid state, has been firmly established. These ideas have also proved useful in explaining the optical activity of stereo regular vinyl polymers in dilute hydrocarbon solutions (4). An excellent account of these investigations is given elsewhere (5), together with a presentation of the application of rotatory dispersion to a variety of problems in the field of organic chemistry.

All these studies were based on analysis of the dispersion curves obtained at wavelengths remote from absorption bands. Recently, measurements on polypeptides and proteins have been extended to the region of shorter wavelengths, where dispersion anomalies have been found (6). The spectra are strongly dependent on conformation, and they disappear when the polypeptide assumes a random-coil arrangement.

Of the two anomalies in the far-ultraviolet which have been investigated, one is apparently associated with the peptide bond  $n > \pi^*$  transition around 220 m $\mu$  and the other is associated with the intense  $\pi > \pi^*$  transition of this chromophore near 185 m $\mu$ . The magnitude of the trough in the anomaly at 233 m $\mu$  was used to calculate the helix content (in percentage) of paramyosin; the results agreed well with data obtained through use of the  $b_0$  term of the Moffitt equation (7). Later studies showed that, in the random-coil form,

the absorption due to the peptide bond resulted in a symmetrical, weak Cotton effect about an inflection point at 197  $m\mu$  (8). This weak, negative Cotton effect was completely swamped by an intense positive Cotton effect associated with the helical form of the polypeptide, with inflection around 190 m $\mu$ . Certainly these studies illustrate the advantages of using synthetic polypeptides in attacking the problems of protein structure. Furthermore, it is apparent that, as instrumentation develops which permits penetration into spectral regions where rotatory anomalies occur, more sophisticated chemical approaches to these problems will be feasible.

The very high optical rotatory power of a liquid crystalline phase of synthetic polypeptides has been recently described by Robinson (9). The liquid crystalline phase formed by the polypeptides was the nematic phase, in which the long axes of the molecules are parallel, and in which the molecules are free to move principally in the direction of these axes. These mesophases are produced when the concentration of a solution of long-chain, stiff molecules is such that the total hydrodynamic excluded volume of the solute exceeds the volume available to the solute in the solution. At that point, separation into two phases occurs-one, highly concentrated and birefringent; the other, dilute and isotropic. In the case of synthetic polypeptides, the birefringent phase has a high optical rotatory power. These structures give rise to what is sometimes called "form" optical activity, since the optical rotatory power is not derived from the selective absorption of circularly polarized light by active centers but arises from selective reflections within the twisted structure.

Further, there is no relationship between the "form" optical activity and the sense of the  $\alpha$ -helix of the molecules of the crystal. This is shown by the fact that the sense of the "form" optical activity reverses in changing from one solvent to another in which the sense of the  $\alpha$ -helix is known to be the same.

The probable existence of a threedimensional structure formed in the liquid crystal as a result of regular contacts along the chains was demonstrated by the fact that the periodic appearance of these solutions under crossed Polaroid filters showed elastic recovery after the application of small

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shearing forces. There was also evidence of a small yield value for plastic flow which irreversibly affected the structure.

Robinson also found that "form" optical activity could be observed in concentrated DNA solutions-a finding which indicates that the twisted structures found in synthetic systems have their counterparts in biological systems. Since changes in these twisted structures arise not only by changes in temperature and concentration but also by the addition of selected solutes, this approach may provide an excellent method for studying the reactions of small molecules with large molecules which result in chemically active complexes, and in changes of phase. Such studies may clarify the role of twistedlong-chain molecules in biological systems in general.

A new and promising method for establishing the sense of the polypeptide helix was recently described (10). It involved the reaction between a synthetic polypeptide in helical form and a non-optically-active dye; in such a reaction the entire complex becomes optically active. The dye provided a chromophore in the visible portion of the spectrum and a Cotton effect was observed in its absorption band, the magnitude and direction depending on the helical sense of the polypeptide chain. At neutral values of pH the polypeptides were principally in the form of polyions and therefore of random coils. In this case, the dye-polypeptide complex showed the same dispersion as the random-coil polyion alone. Although the absolute sense of the helix could not be determined, it was clearly shown that dye-polypeptide complexes of poly-L-glutamic acid and poly-D-glutamic acid exhibited Cotton effects which were approximately mirror images of each other. The induced Cotton effects could have been the result of simple chemical combination between the nonchromophoric, optically active helix and the non-optically active dye, or they could have arisen from the polypeptide's having imposed a helical sense on microaggregates of dye.

Since a naturally occurring protein with a chromophore in the visible region of the spectrum was thus available for investigation, some structural properties of sickle-cell hemoglobin have recently been elucidated (11). The oxygenated form of sickle-cell hemoglobin showed complex behavior within

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its absorption band around 560 m $\mu$ . with two peaks and two troughs. The deoxygenated form exhibited only one peak and one trough within the same spectral region. Of greatest interest was the finding that the magnitude of the Cotton effect exhibited by the sickle-cell hemoglobin was strongly dependent on temperature, whereas the normal hemoglobin showed only a slight response to change of temperature in the same spectral region. Previous investigations, limited to measurement of optical activity at a single wavelength, had failed to provide satisfactory differentiation between normal and sickle-cell hemoglobin (12).

Glass (13) has recently measured high doses of radiation (doses greater than about  $2.5 \times 10^5$  rad/hr for a total dose of up to  $1 \times 10^{8}$  rad) by means of changes in the optical activity of a sugar solution upon exposure to a cobalt-60 radiation source. He surveyed a variety of optically active materials and found that mono- and disaccharides, particularly D(+) maltose, was useful. He showed that, upon irradiation, the initial, high value of rotation of D(+) maltose solution decreased, and that there was a linear relationship between the change in optical activity and the radiation dose. The problems with this technique lie in the fact that the stoichiometry of the reaction is not well known. The change in optical activity was probably the result of the action of the freeradical dissociation products of water. The components of the solution after irradiation, therefore, were probably products of the hydrolysis of maltose and products of subsequent oxidation of these moieties. Because these products were also optically active, the absolute dosage could not be estimated. It may be possible to solve these problems in the future by choosing a material which loses optical activity as a result of irradiation, and by working in the ultraviolet region of the spectrum, where the values for optical rotation are much higher than they are in the visible region.

There has been interest in recent years in applying methods of inducing optical activity through the application of magnetic fields to problems of analytical chemistry. The earliest approach to the problem of magnetooptical rotation of mixtures was that of Verdet (14). He based his rule on the assumption that each component of a mixture behaves as if it alone occupied

Fig. 1. Schematic diagram of a photoelectric polarimeter based on the symmetricalangle principle. L, Light source; O, collimating lens; F, filter;  $D_1$ , diaphragm;  $P_1$ and  $P_2$ , polarizing prisms;  $D_2$ , diaphragm; C, cell containing solution;  $D_3$  diaphragm; An, analyzer; Ph, photomultiplier; M, meter.

the total volume of the mixture. This rule is reasonably accurate in the case of "ideal" mixtures. "Ideal," here, is taken to mean that no change in volume occurs on mixing. However, in cases where marked changes in volume occur on mixing, the Verdet rule fails. In such cases, it has been shown, the use of apparent molar volumes for the solute and of the molar volume of the pure liquid for the solvent overcomes this difficulty and makes it possible to use magneto-optical rotatory measurements in the quantitative analysis of solutions (15).

Induced magnetic rotation has also proved useful in characterizing spectroscopic transitions which overlap. Recent work indicates that singlet states do not contribute to magnetic-rotation spectra (16). Furthermore, multiplet states appear in both magnetic-rotation



Fig. 2. Vector diagram indicating the effect of placing a sample with optical activity  $\alpha$  between the polarizers and the analyzer.

and simple absorption spectra. Therefore, making an assignment of multiplet or singlet state to spectroscopic transitions becomes a simple matter of comparing magnetic-rotation spectra with absorption spectra. Those bands which appear in both spectra may be assigned to the triplet excited state; those which appear only in absorption spectra, to the singlet state. This approach should prove extremely useful in the study of certain aspects of energy transfer in biological systems—for example, photosynthesis.

An exhaustive review of the applications of optical activity measurements in chemical analysis would serve no purpose here. Our intent is to sketch broadly and to indicate the promise of the technique.

In the next section we discuss the present status and the underlying principles of a group of instruments, some of which may be expected to appear on the commercial scene over the next few years.

### Instrumentation

The trend in polarimetry, as in most other fields, has been toward automation. Simple mechanization of the conventional visual polarimeter is not quite possible because the instrument requires observation of a balance point by the observer. Replacing the eye with modern light transducers and accompanying electronic gear has opened up new fields of research. A question that is now being asked is: What is the ultimate sensitivity of the polarimeter? It appears feasible to measure and to automatically record rotations of 10<sup>-3</sup> degree and less. This advance in instrumentation may herald new interest in "forced" optical activity-the capacity of a substance to rotate the plane of incident radiation when subjected to applied electrical and magnetic fields (17) or to a screw-shear pressure.

General principles. Two basic components of a polarimeter are the polarizer and the analyzer, which are usually made of calcite or quartz. In some instruments, polaroids are used. The law of Malus may be considered the fundamental relation governing the energy of the light signal that passes the analyzer and is intercepted by the light transducer of the polarimeter. According to this law, the energy of the linearly polarized light beam transmitted by the analyzer varies as the square of the cosine of the angle between the optic axes of the polarizer and the analyzer. The equation is written

$$E = E_o \cos^2 \theta \tag{1}$$

where  $E_o$  is the energy of the light beam as it leaves the polarizer; E is the energy of the linearly polarized light beam as it leaves the analyzer; and  $\theta$  is the angle between the optic axis of the polarizer and the optic angle of the analyzer.

Let us suppose that an optically active sample having an absorption coefficient K and an optical rotation  $\alpha$ is introduced between the polarizer and the analyzer. Then

$$E = E_{\alpha} e^{-\kappa \iota c} \cos^2(\theta + \alpha).$$
 (2)

Here, l and C are the path length and the concentration, respectively, of the solution. We refer frequently to the term  $e^{-\kappa \iota \sigma}$  as the absorption factor. Introducing the effect of possible unattenuated light that passes the analyzer, or  $\phi$ , we may finally write Malus' law as

$$E = E_{\alpha} e^{-\kappa \iota c} \cos^2(\theta + \alpha) + \phi.$$
 (3)

The form of the function,  $\phi$ , depends on the nature of the unattenuated light which is caused by scattering and circular dichroism (1).

In many cases the contribution of  $\phi$  is negligible in comparison to that of the cos<sup>2</sup> term. In those cases, Eq. 2 may be adequate for analyzing the energy relations of photoelectric polarimeters. When absorption and unattenuated radiation are both lacking, we have the widely used expression

$$E = E_o \cos^2(\theta + \alpha). \tag{4}$$

### General Methods

A number of investigators have reported the successful use of simple accessories for rapidly converting colorimeters and spectrophotometers into photoelectric polarimeters. Accessories are marketed by Keston, Perkin-Elmer, Weston, and others. They have been applied to the Beckman (18), Perkin-Elmer (19), and Cary (20) spectrophotometers. Since the modification of the instruments indicates in a simple way the general optical methods around which the recent automatic spectropolarimeters are designed, let us discuss these methods at this point.

Practically all the currently used methods are based on the symmetrical angle principle (see Fig. 1). The polarizer can make an angle of  $+\theta$  or  $-\theta$  with the analyzer, and thus the phototransducer has two measured outputs. The various methods differ from one another in the way the two outputs are combined to give the angle of rotation. The various combinations have been made, in part, in an effort to achieve two desirable features—elimination of the absorption factor in Eq. 2 and linearity between the combined signal and the optical rotation of the sample.

Keston method. The respective energies  $E_1$  and  $E_2$  for positions  $+\theta$  and  $-\theta$  of the optic axis of the polarizer can be combined in many ways to eliminate the absorption factor of the sample. For the ratio  $E_2/E_1$ , according to Eq. 2, one may write

$$R_{K} = \frac{E_{2}}{E_{1}} = \frac{\cos^{2}\left(-\theta + \alpha\right)}{\cos^{2}\left(-\theta + \alpha\right)}.$$
 (5)

Such a ratio could also eliminate losses from reflection. A detailed description of this method and some results obtained with the Perkin-Elmer spectrophotometer have been given by Savitsky and his co-workers (5, 19). The  $R_{\kappa}$  ratio is directly determinable with a double-beam instrument.

For our purposes we consider the functional relation between  $R_{\kappa}$  and the optical rotation. This may be written as a series (21):

$$R_{\kappa} = 1 + 4\alpha \tan \theta + 8\alpha^2 \tan^2 \theta + \dots$$
(6)

The series contains all powers of  $(\alpha$  $\tan \theta$ ) and thus indicates poor linearity between  $R_{\kappa}$  and  $\alpha$  or  $\theta$ . The aberrant factor of second order with respect to the factor of first order is  $2(\alpha \tan \theta)$ , and it is excessive for an analog system. For example, an angular rotation of 0.1 radian at  $\theta = 45^{\circ}$  yields a departure of more than 20 percent from a linear scale. Another undesirable feature of the Keston ratio is indicated by the lack of symmetry for the series with respect to the origin of the  $R_{\kappa}$ and  $\alpha$  coordinates. Because terms of even power do not change sign with changes in sign of  $\alpha$ , the value of  $R_{\pi}$ varies in magnitude when  $\alpha$  changes sign, while remaining constant in absolute value. As a result, tables or graphical computers must be used to convert  $R_{\kappa}$  to  $\alpha$ .

Rouy method. The two unfavorable aspects of the Keston method are avoided by the Rouy (22) method. Unfortunately, existing instruments cannot be readily converted to yield the Rouy ratio directly. However, the individual signals  $E_1$  and  $E_2$ , corresponding to fixed angles  $+\theta$  or  $-\theta$ , may be observed individually, and therefore the Rouy method may be employed if colorimeters and spectrophotometers are available for use in connection with the polarizer.

The Rouy method is based on the ratio  $(E_2 - E_1)/(E_2 + E_1)$ , or the "difference-to-sum" ratio:

$$R_{d} = \frac{(E_{2} - E_{1})}{(E_{2} + E_{1})} \tag{7}$$

Note that as in the Keston method the absorption factor is removed. Substitution of Eq. 2 in Eq. 7 and expansion of terms gives the equation

$$R_{a} = 2\alpha \tan \theta - 2\left(1 - \frac{1}{3\tan^{2}\theta}\right) \times \alpha^{3}\tan^{3}\theta + \dots \qquad (8)$$

The series now contains only odd powers of  $\alpha$  and  $\theta$ ; thus the relationship between  $R_a$  and  $\alpha$  is symmetrical with respect to the  $\alpha$  axis. Departure of  $R_a$ from linearity at  $\theta = 45^{\circ}$  and at  $\alpha =$ 0.1 radian is less than 7 parts per thousand. It is interesting to note that when  $\theta$ , the angle between the optic axis of the polarizer and the optic axis of the analyzer, is set at 30 degrees, the difference-to-sum ratio is linear with  $\alpha$  to within about 1 part per million.

Null-point and small-angles method. The "null-point" method may be considered an absolute method. It is the one upon which the conventional visual polarimeter is based. In the case of photoelectric polarimetry, the signals  $E_1$  and  $E_2$  are balanced to correspond with angles  $+\theta$  and  $-\theta$ . Upon the introduction of the optically active sample, this balance is upset, as illustrated in Fig. 2. Now, let us suppose that a rotation is imposed upon the analyzer until the balance of signals is restored. The imposed rotation will be exactly equal to the optical rotation of the sample. Usually, in this null-point method,  $\theta$  is set at about 85 degrees or more, so that in the experimental procedure small angular oscillation of  $\pm \varepsilon$  (the complement of  $\theta$ ) are made about the axis of the polarizer when it is in the orthogonal position with respect to the analyzer. The angle  $\varepsilon$  is sometimes referred to as the "halfshade" angle or the angle of scan.

Upon the introduction of a sample with an optical activity equal to  $\alpha$ , the response of the phototransducer depends upon the general energy equation

$$E_{2} - E_{1} = E_{0} e^{-K IC} \times [\cos^{2} (\theta - \alpha + \varepsilon) - \cos^{2} (\theta - \alpha - \varepsilon)]$$
(9)

according to Eqs. 2 or 3. The value for  $E_2 - E_1$  will become 0 when

$$\theta - \alpha = \pi/2$$

Thus, by rotating the analyzer  $\alpha$  degrees (or the polarizer  $-\alpha$  degrees), the mean position of the plane of polarization as the beam leaves the sam-



Fig. 3. Schematic diagram of the Rudolph spectropolarimeter. The polarizer is rotated through an angle  $(-\alpha)$  which is equal and opposite to the angle of rotation of the sample. The angle of scan,  $\pm \varepsilon$ , is induced by a mechanical oscillator. *L*, Light source; *M*, monochromator;  $P_{\varepsilon}$ , initial position of polarizer axis; *P*, final position of polarizer axis; *S*, sample;  $\varepsilon$ , angle of scan.

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ple will be orthogonal with respect to the optical axis of the analyzer.

Equation 9 may be rewritten as

$$E_2 - E_1 = E_0 e^{-K l C} \sin 2 \varepsilon \sin 2 \alpha \quad (10)$$

Frequently Eq. 10 is replaced by the expression

$$E_2 - E_1 = k\alpha \tag{11}$$

where k represents a constant obtained by calibrating the instrument with a known substance (23). Although Eq. 11 obviates the necessity of imposing a precise rotation to achieve a null point, the restrictions of Eq. 11 are not always adhered to. It should be noted that, for Eq. 11 to be valid, not only must the activity be small enough so that the sine of the angle may be replaced by the angle itself but also the rotation  $\alpha$  must be less than  $\varepsilon$ . Furthermore, Eq. 11 should not be used for measurements in the absorption band, since the constant k conceals the absorption coefficient as well as the concentration. This may be seen by comparing Eqs. 10 and 11.

### **Automatic Recording Polarimeters**

Several automatic recording polarimeters have been reported. With the exception of the Rudolph instrument, they are of very recent development. As a result, few assessments of their performance have been made. Herewe try to describe the broad aspects of these instruments and their possible relative merits.

The instruments may be placed broadly in two classes—(i) instruments in which the null-point method is used, and (ii) instruments in which a ratio method is used. At present all commercially available instruments fall into the first class. They differ among themselves in the manner in which the null point is achieved. Some very recent instruments which are not yet available commercially are based on the Rouy ratio method (24). Let us consider the null-point instruments first.

Rudolph spectropolarimeter. Most of the measurements of optical rotatory dispersion that have been reported during the past several years have been obtained with the instruments developed by Rudolph (5). It may be said that the reawakened interest in this field has been due in large part to the availability of, first, Rudolph's manual spectropolarimeter and, later, his automatic recording spectropolarimeter (25).



Fig. 4. Schematic diagram of the Cary spectropolarimeter. The angle of scan,  $\pm_{\varepsilon}$ , is induced by a Faraday cell,  $M_1$ . L, Light source; M, monochromator;  $M_1$ , Faraday-cell modulator; PM, phase-sensitive motor.

The latter instrument works on the null-point principle, the null point being ascertained by means of an imposed mechanical oscillation  $(\pm \epsilon)$  of the analyzer, whose mean angular position is orthogonal with respect to the plane of polarization of the entering light beam (see Fig. 3). At this point, angular changes of  $+\varepsilon$  and  $-\varepsilon$  produce the same current in the phototransducer system. The optical rotation,  $\alpha$ , produced by the introduction of a sample between the polarizer and the analyzer is measured by the angular rotation  $(-\alpha)$  of the polarizer which is required to restore the balance of the signal output.

The analyzer prism is mechanically

oscillated to produce a 20-cycle-persecond modulation of the light beam striking the photomultiplier tube. A portion of its signal is separated, by means of a chopper, into two signals, corresponding to the right and left oscillations of the analyzer. The difference between these two signals is fed into a null-point-seeking servo system, which drives the polarizer to a position which compensates for the rotation of the sample. The recording of the optical activity is obtained by means of a linkage between the angular position of the polarizer and an X-Y recording system.

An estimate of the restriction that the optical arrangement imposes upon



Fig. 5. Schematic diagram of the Bendix-Ericsson polarimeter. The angle of scan,  $\pm_{\varepsilon}$ , is induced by Faraday cell  $M_1$ , and the compensatory angle  $(-\alpha)$ , by Faraday cell  $M_2$ . L, Light source; F, filter for monochromation;  $M_1$  and  $M_2$ , modulators.

the signal strength, which is related to the sensitivity of the polarimeter, may be obtained through consideration of the maximum transmission through the polarizer-analyzer system in the absence of any absorption by the sample. An estimate of the transmission may be written as

$$\frac{E}{E_{\rm o}} = \sin^2 \varepsilon \tag{12}$$

where  $E_0$  is the energy of the polarized light beam entering the analyzer and E is the energy of the polarized light beam emerging from the analyzer. Equation 12 yields a maximum transmission of 0.85 percent for  $\varepsilon = 5^{\circ}$  and 0.034 percent for  $\varepsilon = 1^{\circ}$ . The small value for the transmission is characteristic of instruments where the balance points are achieved at orthogonal positioning of the polarizer and analyzer. It imposes a limitation on the signal strength and results in reduced precision with highly absorbing samples.

Cary spectropolarimeter. The Cary spectropolarimeter is similar in principle to the Rudolph instrument except that in the Cary instrument the mechanical oscillation of the analyzer is replaced by an oscillation brought on by a magneto-optical effect. To achieve this, a Faraday cell is placed ahead of the analyzer (see Fig. 4). The Faraday cell consists of a silica cylinder surrounded by a coil. An alternating current (60 cy/sec) passes through the coil, thus cyclically displacing the plane of polarization of the beam. A motor energized by the amplified current from the photomultiplier moves the polarizer by means of a mechanical linkage.

Although the use of the Faraday cell may be considered an advantage over the mechanical system for producing oscillations of the analyzer, its use may introduce several possible drawbacks in work requiring the greatest accuracy. This is due to the fact that the Verdet constant, and hence the magnitude of the oscillatory angle  $\varepsilon$ , is sensitive to both temperature and wavelength. Also, the magneto-optical effect results in a small value for  $\varepsilon$ , and thus limits the range of the polarimeter. Increasing the value of  $\varepsilon$  enhances the problem of temperature control for the Faraday cell. The Verdet constant decreases with wavelength, producing a decrease in the sensitivity of the instrument as the spectrum is scanned. The oscillatory angle  $\varepsilon$  has values of from  $\pm$  2 degrees to about  $\pm$  ½ degree; when the corresponding transmission

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factor is fixed at 0.14 and 0.009 percent, respectively.

Bendix-Ericsson polarimeter. The Bendix-Ericsson polarimeter, an automatic recording instrument in which filtered radiation is used, appears to be particularly well suited for measuring systems in flow and process control. In the Bendix-Ericsson instrument the Faraday effect is utilized for both modulation of and compensation for rotation of the sample. Thus, the instrument has no moving parts.

As may be seen in Fig. 5, the beam of filtered polarized light traverses a Faraday cell,  $M_1$ , which is fed by an alternating current. The oscillating beam passes through the optically active sample, introducing the rotation  $\alpha$ . After passing through a second Faraday cell, the light beam reaches the analyzer, whose axis is orthogonal with respect to the axis of the polarizer. The purpose of the second Faraday cell is to introduce a rotation of  $-\alpha$  to compensate for the optical activity of the sample, so as to achieve a null point. The compensating angle is known through the intensity and sign of the current feeding the energizing coil of the Faraday cell  $M_2$ . The rotation in the cell is directly proportional to the current producing it. Unlike the Rudolph or Cary instruments, the Bendix-Ericsson polarimeter requires calibration.

Here again the "half-shade" angle  $\pm \varepsilon$  is of the order of 2 degrees and sometimes as small as  $\frac{1}{2}$  degree. Hence, the maximum transmission for the polarizer-analyzer system is about the same as for the Cary—from 0.14 to 0.009 percent.

Bendix-Gillham-King spectropolarimeter. An ingenious design for a spectropolarimeter was reported by Gillham and King (26). The instrument is being developed and will probably be available commercially in the near future (27). In principle, this instrument is similar to the Bendix-Ericsson instrument, with the difference that scanning over the visible and ultraviolet spectrum is provided. The instrument will probably enable the investigator to probe further into the ultraviolet range because of the use of quartz instead of calcite polarizing prisms.

As indicated in Figs. 6 and 7, the function of monochromator and polarimeter are combined by using two crystalline quartz prisms, to produce the necessary decomposition of white light into its spectral components and



Fig. 6. Schematic diagram of the Gillham-King spectropolarimeter. Quartz prisms  $P_1$  and  $P_2$  serve as polarizer and analyzer, respectively, and also monochromatize the incident radiation.  $E_1$  and  $E_2$ , entrance and exit slits;  $C_1$ ,  $C_2$ ,  $C_3$ , and  $C_4$ , concave mirrors;  $X_1$  and  $X_2$ , plane mirrors; M, modulator; F, Faraday cell; S, sample.

also to serve as polarizer and analyzer, placed orthogonally with respect to each other. The prisms  $(P_1 \text{ and } P_2)$ are fixed in position, and wavelength scanning is achieved by means of two plane mirrors,  $X_1$  and  $X_2$  rotating in unison about a common axis. The concave mirrors— $C_1$  and  $C_2$  in the first half of the system and  $C_3$  and  $C_4$  in the second half-are placed off-axis in the arrangement; this produces some compensation of the extra-axial aberrations. The modulator  $(M_1)$ , the sample (S), and the Faraday cell compensator (F)are all mounted close to the intermediate slit (I). Two types of modulator have been used. In one type the axis of the plane of polarized light is shifted back and forth by the angle  $\pm \epsilon$ around the orthogonal position by means of left- and right-handed quartz plates carried by a rotating disk. More recently, a Faraday-cell modulator has been used, as in the Bendix-Ericsson polarimeter.

The Gillham-King instrument appears to have the advantage of extreme simplicity of design and construction in comparison with other recently developed spectropolarimeters. It is not an absolute instrument, however, since the compensating Faraday cell requires calibration. The transmission factor for the instrument should be the same as that for the Bendix-Ericsson.

Rouy spectropolarimeter. The Rouy spectropolarimeter represents a departure from the null-point, orthogonal instruments. It is designed to obtain rotary dispersion spectra by the method of difference-to-sum ratio. An instrument based on this method may be designed in any one of a number of different ways; the automatic Rouy spectropolarimeter offers a novel approach. The preliminary results appear promising as the instrument nears completion.

The instrument (28) offers a possible solution to the problem of determining the optical activity of highly absorbing and light-scattering systems because its transmission factor is generally greater by a few orders of magnitude than that for the null-point instruments. A schematic diagram of the Rouy instrument is shown in Fig. 8. A beam from a light source enters a prism-grating monochromator. At the exit slit of the monochromator an optical system collimates the light onto the optical axis of the instrument. Two polarizers are used, making angles of  $\pm \theta$  with the optical axis of the analyzer. The value of  $\theta$  may be varied over a wide range; usually it is kept at 45 degrees. The rotating mirror R carries an odd number of trapezoidal teeth and is driven at constant velocity by a synchronous motor. The plenums and voids (all of equal angular length) of the rotating mirror form a simple beamswitching device.

The traces of the two beams, indicated by the dashed lines in Fig. 8, are intercepted or not intercepted by the rotating mirror, depending upon its angular position. When a void occurs at the entrance of the switching system, the beam follows a path through the polarizer  $P_2$  and is reflected on the back surface of the rotating mirror. It then enters the sample and analyzer before reaching the light transducer. When a plenum of the revolving mirror occurs at the entrance of the switching system, the light follows the path that passes through polarizer  $P_1$ . It is then reflected by the mirror  $M_1$ , and from then on it follows the optical axis of the instrument. The two paths are contained in the same plane, and their space geometry through sample, analyzer, and phototransducer window remains invariant.

A wave shape characteristic of the signal is represented in Fig. 9. The wave passes through a low dip, a, which represents the dark current of the phototransducer, then rises to a plateau b, which indicates the energy  $E_2$ . The second dip, d, also represents the dark current, and the second plateau ef, yields the energy  $E_1$ . The distance aa' represents a single period.



Fig. 7. (Top) Bendix-Gillham and King spectropolarimeter. (Bottom) Top view of the optical train of the Bendix-Gillham and King spectropolarimeter.



Fig. 8. Schematic diagram of the Rouy spectropolarimeter, showing the rotating beamswitching mirror, R.  $M_1$  and  $M_2$ , Plane mirrors;  $P_1$  and  $P_2$ , polarizing prisms; R, rotating mirror with trapezoidal teeth; D, diaphragm; C, sample holder.

The ratio of the difference  $(E_2 - E_1)$  to the sum  $(E_2 + E_1)$  of the peak energies is linear with respect to the alternating-current to direct-current signal components. The method lends itself readily to digital and analog readouts. All readings of the peak energy values  $E_1$  and  $E_2$  may be averaged through integration over one or more seconds, depending upon the precision desired. The transmission of the instrument depends upon the setting for  $\theta$ . The transmission, which is equal to  $\cos^2 \theta$  (or  $\sin^2 \varepsilon$ ), reaches a value of 50 percent at 45 degrees.

The usefulness of the Rouy approach is strongly dependent on the mechanical and optical characteristics of the component parts of the instrument. The two polarizing prisms must have identical optical and mechanical axes. The transmission of the prisms must be uniform in all orientations around the optical-mechanical axis, and their transmission factors must be identical. Differences in reflectivity from different portions of the chopping mirror, as well as ellipticity introduced by reflections, reduce the accuracy of the instrument. These problems are being studied and probably can be solved (27).

It should be added that Rouy has achieved a new approach for designing polarizing prisms (27). This should appreciably extend the transmission of calcite prisms in the ultraviolet range. Preliminary measurements show as high as a fourfold increase in transmission over the Glan prism at 220  $m\mu$ . Moreover, the lateral displacement of the light beam is practically eliminated. The latter property is essential for precise measurement of optical activity through turbid media.

## Light Scattering and Circular Dichroism in Polarimetry

Unattenuated radiation passing through the analyzer may arise from scattering and circular dichroism. Let us consider briefly the determination of the optical rotation of a system that scatters a large amount of radiation. Under such conditions the orthogonal or null-type instrument gives only qualitative measurements. This is attested by the experience of chemists for the past hundred years. Clarification of turbid solutions has always been a preliminary step in polarimetry-a step which, at times, has caused some anxiety lest the clarified sample might

not quite represent the sample of original interest.

The reason for the need for clarification becomes immediately apparent when one considers Malus's law in its general form (Eq. 3). The  $\phi$  term, which is independent of the optical activity, is a noise component, and the ratio of signal to noise may be written as

$$\rho = \frac{A_o^2 e^{-K1C} \cos^2(\theta + \alpha)}{\phi} \qquad (13)$$

As the effective angle  $(\theta + \alpha)$  is made to approach 90 degrees—the balance point for orthogonal polarimeters— $\phi$ remains essentially constant, so that  $\rho$ approaches zero and the signal becomes swamped. It may be seen from Eq. 13 that highly absorbing samples will have a similar effect. The nonorthogonal polarimeter offers a possible solution to the problem of precisely measuring the rotation of highly scattering systems (29).

A similar situation exists for the case of circular dichroism. In this case the nonorthogonal instrument may be used to evaluate the quantity and kind of unattenuated radiation, whereas this information is inaccessible to the orthogonal instrument. Let us examine the origin of circular dichroism.

Plane-polarized light may be considered to be made up of right-handed and left-handed circularly polarized light. In the vicinity of an optically active absorption band the optical rotation increases to a maximum, then decreases abruptly to zero near the center of the absorption band, then reverses in sign, as indicated in Fig. 10. This is referred to as the Cotton effect and arises from differences in the absorption of right-handed and lefthanded circularly polarized light. The unequal absorption causes the planepolarized light to become elliptically polarized, and the effect, or ellipticity, is expressed in terms of the ratio of the minor to the major axis of the ellipse.

The ellipticity, when plotted against wavelength, is Gaussian in shape (see Fig. 10), with its maximum at the center of the corresponding optical rotatory dispersion curve. These curves are usually much narrower than optical absorption bands. As for the rotation, it decreases rather gradually from the center of the band. Thus, ellipticity associated with a particular electronic transition may be more readily isolated when many transitions are involved, in a complex compound, than

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Fig. 9. Wave shape of the signal for the Rouy spectropolarimeter.  $aa^1$ , One period of rotation of the beam-switching mirror.

when there are only a few transitions. Moreover, it is possible to deduce the optical rotation of substances from data on ellipticity. This was pointed out by Kuhn in 1931 and more recently by Moscowitz (5). The application of this procedure to coordination compounds has been reported in a recent paper by Brushmiller and his co-workers (30).

Woldbye (31) has shown how one may obtain the ellipticity from polarimetric measurements obtained by the Keston method. In the case of the Rouy method, the procedure is very simple. One may bring the optical axis of the analyzer parallel to one of the polarizers, which originally were at  $\theta = \pm 45^{\circ}$ . Under these conditions the difference-to-sum ratio  $R_4$ , is related to the ellipticity  $\psi$  as follows:

$$\Gamma \mathrm{an}^2 \psi = \frac{(1-R_d)}{(1+R_d)}$$

# Range and Sensitivity of Spectropolarimeters

One may well inquire about the useful range and the corresponding sensitivity of the various spectropolarimeters. The "half-shade" angle or angle of scan,  $\varepsilon$ , (the complement to the angle  $\theta$ ), sets the upper limit for the range of measurement of the optical



Fig. 10. Plot of the idealized (positive) Cotton effect. Optical rotation,  $\alpha$ , and ellipticity,  $\psi$ , are plotted against wavelength,  $\lambda$ , at an isolated, optically active absorption band.

activity. For orthogonal polarimeters, the effective range may vary from about 6 degrees to as little as 0.02 degree, the limits being set by practical considerations.

In the absence of scattering or circular dichroism, analog systems have a maximum sensitivity of 0.1 percent of the range of the instrument. Decreasing the range to increase the sensitivity may not achieve the expected result, since the overall light transmission of the instrument is decreased. For example, at an angle of scan of 0.02 degree, the transmission of the polarimeter falls below 0.000012 percent. Under these conditions a sensitivity of 0.0002 percent could be achieved only outside the absorption band. The possible advantages of the ratio methods, in regard to range and sensitivity, become immediately apparent. In the case of the instrument based on the difference-to-sum ratio, where the angle of scan may be 45 or even 60 degrees, the sensitivity appears to be limited mainly by the resolving power of the digital readout system, the time of integration, and the amplification of the alternating-current signal component. Sensitivities of the order of 10<sup>-5</sup> degree seem approachable. Whereas only samples of optical density up to 2 may be measured with orthogonal instruments, with instruments based on the ratio method, samples of optical density up to more than twice this value may be used. Thus, it appears that in the not-too-distant future we should be able to make polarimetric measurements on a much larger number of substances than we can at present.

### Conclusion

Most of the useful information to be obtained in determinations of optical activity will be measured in spectral regions that correspond to optical absorption bands. This information cannot be satisfactorily extracted at present, owing to the large attenuation of light attributable to dichroic absorption, to Rayleigh scattering in clear solutions, and to Mie scattering in turbid solutions. Present instruments either cannot permit enough light energy to penetrate solutions which are simultaneously absorbing and optically active or do not have the proper signal-tonoise ratio to yield accurate measurements of optical activity when there is extensive attenuation of depolarization,

or both, of the incident light beam. When these problems have been solved, the polarimeter will be useful in studying many absorbing and scattering systems-perhaps the living cell itself.

#### **References** and Notes

- A. L. Rouy, B. Carroll, T. J. Quigley, Anal. Chem. 35, 627 (1963).
   C. Djerassi, Science 134, 649 (1961).
   W. Moffitt and J. T. Yang, Proc. Natl. Acad. Sci. U.S. 42, 506 (1956).
- S. A. Montel and S. I. Lang, Froc. Natl. Acad. Sci. U.S. 42, 596 (1956).
   Pino and Lorenzi, J. Am. Chem. Soc. 82, 4745 (1960).
- 4/45 (1960).
  5. C. Djerassi, Optical Rotatory Dispersion (McGraw-Hill, New York, 1960).
  6. N. S. Simmons, C. Cohen, A. G. Szent-Gyorgyi, D. B. Wetlaufer, E. R. Blout, J. Am. Chem. Soc. 83, 4766 (1961).
- W. Moflitt, J. Chem. Phys. 25, 467 (1956).
   E. R. Blout, I. Schmier, N. S. Simmons, J. Am. Chem. Soc. 84, 3193 (1962).
   C. Robinson, Tetrahedron 13, 219 (1961).
   E. R. Blout and L. Stryer, Proc. Natl. Acad. Sci. U.S. 45, 1591 (1959).
   M. Murayama, Nature 194, 933 (1962).
   E. Havinga, Proc. Natl. Acad. Sci. U.S. 39, 59 (1953).
   A. L. Glass, Nuclearize 26, 55 (1975). 7. W. Moflitt, J. Chem. Phys. 25, 467 (1956).

- 59 (1953).
  A. L. Glass, Nucleonics 20, 66 (1962).
  Verdet, Am. Chim. Phys. 52, 129 (1858).
  R. Fleming and L. Saunders, J. Chem. Soc. 1955, 4147 (1955). 15.
- 16. W. H. Eberhardt and H. Renner, J. Mol. Spectr. 6, 483 (1961).
- 17. H. F. Hameka, J. Chem. Phys. 36, 2540 (1962).
- (1962).
  18. A. Keston and J. Laspalluto, Federation Proc. 12, 229 (1953).
  19. A. Savitsky, W. Slavin, R. E. Salinger, paper presented at the Conference on Analytical Chemistry and Applied Spectroscopy, Pitts-burgh (1959).

- 20. F. Woldbye, Acta Chem. Scand. 13, 2137 (1959).
- (1959).
  21. A. L. Rouy and B. Carroll, Anal. Chem. 33, 594 (1961).
  22. B. Carroll, H. B. Tillem, E. S. Freeman, *ibid.* 30, 1099 (1958).
  23. S. Kirchner, Advances in the Chemistry of Coordination Compounds (Macmillan, New York 1961) p. 145
- Coordination Compounds (Macmilian, New York, 1961), p. 145.
  24. A. L. Rouy, U.S. patents Nos. 2,986,066; 2,993,404; 3,001,439 (1961); patent application No. SN 654,929 (1957).
  25. H. Rudolph, J. Opt. Soc. Am. 45, 50 (1955).
  26. E. J. Gillham and R. J. King, J. Sci. Instr. 38, No. 1 (1961).
  27. Private communication

- 38, No. 1 (1961).
  27. Private communication.
  28. The instrument will probably be available from Weston-Schlumberger, Newark, N.J.
  29. B. Carroll, Methods in Carbohydrate Chemistry (Academic Press, New York, in press).
  30. Brushmiller, E. L. Amma, B. E. Douglas, J. Am. Chem. Soc. 84, 3227 (1962).
  31. F. Woldbye, Acta Chem. Scand., in press.

# News and Comment

### **High-Energy Physics: Major Fight Brewing as Midwestern Legislators** Take Stand on MURA Accelerator

A high-energy scientific-political row is shaping up in Washington as the deadline approaches for the administration to say yes or no to a proposed \$150-million, 12.5-Bev proton accelerator near Madison, Wisconsin.

For a decade, a high energy accelerator has been the principal raison d'être for the 15-institution Midwestern Universities Research Association (MURA). With the construction time estimated at 7 years, and accelerator technology moving fast, the time is getting close to build it or forget it. If it's the latter, the project's supporters argue emotionally, the administration will, in effect, be endorsing second-class status for the Midwest as a scientific and technical center. It can be argued that the Midwest, with its long-standing industrial emphasis on mass consumer products, has no one to blame but itself if the East and West Coasts have run away with basic research and advanced technology. But the midwesterners, with considerable justification, contend that they are the victims of a closed system-that federal money goes east and west because federal money helped establish a level of quality, east and west, that inevitably attracts more federal money. Their agitation over this cycle has been mounting for some time, and now, with the MURA proposal-which calls for one of the costliest single pieces of federally-financed research hardware ever built-as a rallying point, the midwesterners are lobbying with a ferocity and skill that would command the respect of any aerospace manufacturer.

Their efforts have enlisted the active support of at least 50 of the region's congressmen and senators, and these, in turn, have directed their energies toward the Atomic Energy Commission, the White House Office of Science and Technology, and the Bureau of the Budget. Interestingly, the MURA issue has created (or, more probably, simply revealed) some splendid rifts among leaders of the scientific community, rifts sufficiently wide and deep to provide permanent burial for any still-quivering remnants of the notion that the elder statesmen of science approach their duties with dispassion, disinterest, and precision.

There is a lot of power, prestige, and money riding on the MURA decision, and the attendant scientific politiking and maneuvering are themselves in the Bev range. Not surprisingly, a great deal of this originates with MURA's Chicago neighbor, the AEC's Argonne National Laboratory, which that its 12.5-Bev Zero contends Gradient Synchrotron, now nearing completion, will do very nicely for the Midwest. MURA rejects this claim as self-serving nonsense and charges that not only will Argonne be overwhelmed by requests for its machine but that the MURA accelerator would provide intensities far beyond Argonne's reach. Argonne, however, has enlisted the support of many of the AEC's other national laboratories, which have their own ideas of what could be done with \$150 million.

As a result of all this agitating, it is generally agreed that John F. Kennedy himself, who, for all his talents, wouldn't know an accelerator from an automated olive stuffer, will issue the final verdict. (This will have to be in hand before the end of the year for inclusion in the fiscal 1965 budget, which goes to Congress in January.) For despite the most pious assertion that technical considerations alone will dictate the outcome, no one concerned with the issue can get away from the fact that the politics of MURA cannot be extracted from the technology of MURA; that whatever the technical merits of the MURA proposal, it has become the symbol of Midwest bitterness over the distribution of the nation's research budget, and that the midwesterners in Congress are not only promoting MURA but threatening grim reprisals against other accelerator proposals if the MURA machine doesn't come off the drawing boards.

These threats, significantly, are not at