Optical Rotatory Dispersion

I. A Recording Spectropolarimeter Based on the Cary 11MS—50 Spectrophotometer

FLEMMING WOLDBYE

Chemistry Department A, Technical University of Denmark, Copenhagen, Denmark

The increasing interest in measurements of optical rotatory dispersion recently developed within several branches of chemistry [1-6] has accentuated the instrumentation problems of spectropolarimetry. The solution of the structural and theoretical problems formulated in the works referenced will require a detailed knowledge of the rotatory dispersion within the widest possible wavelength range, especially within the ranges of absorption. Much effort is being spent, therefore, in developing recording spectropolarimeters, none of which, however, seem to be commercially available as yet.

In the course of studies of the optical rotatory dispersion of some transition metal complex ions [6] we have developed an accessory for the Cary 11MS—50 spectrophotometer which permits the automatic recording of optical rotatory dispersion in the wavelength range $800 \geq \lambda \geq 228$ mμ with a relative accuracy of *ca.* 1 %.

Although unsatisfactory for high precision quantitative analysis optical rotatory dispersion curves of this accuracy may certainly meet the requirements of the structural and theoretical investigations.

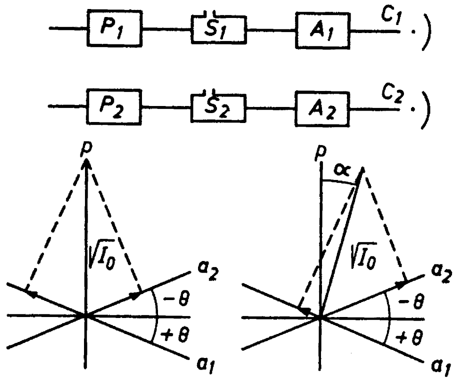

Fig. 1. $P_{1,2}$ polarizers, $S_{1,2}$ identical samples, $A_{1,2}$ analyzers at angles $90^\circ + \Theta$ and $90^\circ - \Theta$, respectively, with the planes of polarization of P_1 and P_2, and C_1 and C_2 sample and standard photomultipliers of the spectrophotometer. p, a_1 and a_2 planes of the electric vector of light polarized by $P_{1 \text{ or } 2}$, A_1 and A_2 viewed from $C_{1 \text{ or } 2}$. The intensities transmitted by the systems are obtained by squaring the lengths of the projections of the electric vector, $\sqrt{I_0}$, upon a_1 and a_2.

The principle by which the rotation, α, of a sample is converted into an apparent optical density which may be recorded by the spectrophotometer is illustrated in Fig. 1 *. If the samples, S, are optically inactive, the two systems, P—S—A, have identical optical densities

$$D_1 = D_2 = \log\frac{I_0}{I} = \log\frac{I_0}{I_0\cos^2(\pm(90^\circ-\Theta))} + D_u = -2\log\sin\Theta + D_u$$

where the first term represents the density caused by the relative setting of polarizer and analyzer while D_u represents the density caused by the initial polarization, the absorption in the sample, reflections *etc.* Thus the density recorded by the spectrophotometer, $D_{app} = D_1 - D_2$, will be equal to zero.

If identical, optically active samples, S, causing a rotation $\alpha < \Theta$, are inserted consideration of Fig. 1 shows that $D_1 = -2 \log \sin(\Theta - \alpha) + D_u$ and $D_2 = -2 \log \sin(\Theta + \alpha) + D_u$ so that

$$D_{app} = D_1 - D_2 = 2 \log \frac{\sin(\Theta + \alpha)}{\sin(\Theta - \alpha)} \quad (1)$$

Provided $\Theta + \alpha < 10^\circ$, $(\alpha < \Theta)$, the approximate solution [6,9] of (1)

---

* This principle has been utilized in polarimetry before, *e.g.* by Keston [7]. Also the accessory [8] marketed by the Perkin-Elmer Corporation, which has been developed simultaneously with, but independently of our accessory, is based on this principle.

$$a \approx \Theta \tanh\left(\frac{1}{4 \log \mathrm{e}} D_{\mathrm{app}}\right) = \Theta \tanh(0.576\, D_{\mathrm{app}}) \qquad (2)$$

is correct to within 0.5 %.

For $D_{\mathrm{app}} < 1$ this relation between a and D_{app} is nearly linear. The curves recorded are, therefore, very similar to plots of a against wavelength. The exact measuring is most conveniently done by means of a rule graduated so as to permit the direct reading of tanh ($0.576\ D_{\mathrm{app}}$).

The necessary attachments to the spectrophotometer consist of two identical units made from brass rod of a square cross section fitting the cell holders of the spectrophotometer equipped with a cylindrical bore accomodating polarizer, polarimeter tube and analyzer. For reasons discussed below the standard light sources of the spectrophotometer have been replaced by a special projector tungsten filament lamp and a 2 kW hydrogen arc for use in the visible and the ultraviolet regions, respectively.

The angles, Θ, are set by means of a special setting apparatus in which adjustments may be made with an accuracy of $\pm$ 0.002°. However, the value of Θ is not measured by means of this instrument but is calculated from measurements of D_{app} for solutions of sucrose of known concentrations by means of (2). The relative accuracy and reproducibility of these determinations of Θ are the same as those obtained in the recording of absorption spectra, *i.e.* $\pm$ 1 % or better.

The details of the construction, operation and performance of the spectropolarimeter together with theoretical considerations regarding the sources of error in the system (ellipticity of the light transmitted by absorbing samples, inequality of the two angles $\pm \Theta$, optical anisotropy of the windows in the sample cells, stray light *etc.*) will be dealt with in a later paper[10], but a few remarks concerning the sensitivity and the accuracy so far obtained should be made.

The main difficulty in measuring optical rotatory dispersion within wavelength ranges of absorption, namely that of getting sufficient light through the sample to be able to measure the rotation, may be expressed quantitatively as

$$A = \frac{D}{|a|} = \frac{100\ \varepsilon}{|[M]|}$$

i.e. the optical density of a sample which will produce a rotation of 1° (ε and $[M]$ being the molar extinction coefficient and rotation, respectively). Being a function of the wavelength this quantity equals zero outside the ranges of absorption but in the vicinity of the peaks (and troughs) of the rotatory dispersion curves which are usually situated within the optically active absorption bands it may often assume values of 7 or 8. Since these parts of the curves are of a particular theoretical interest, a method of measuring rather small rotations with a relatively high accuracy is needed.

Consideration of (2) shows that the sensitivity of the present instrument

$$\frac{D_{\mathrm{app}}}{a} \approx \frac{1}{0.576\ \Theta}$$

(tanh $x \approx x$ for small values of x) may, in principle, be increased to any desired value by decreasing Θ. This is, of course, subject to several limitations, the most important of which is the fact that the optical density of the polarimeter units (polarizer-analyzer) increases very rapidly with decreasing Θ at small values of Θ. We are thus facing the problem of finding the optimum value of Θ.

This problem may be attacked as follows [6]. For a given spectrophotometer there will exist a maximum permissible optical density, $D_{\max}$, which cannot be exceeded by the systems inserted into the light paths without seriously affecting the signal-to-noise ratio or the resolution. $D_{\max}$ will be a function of the wavelength *and* of the resolution demanded. This maximum condition may be written

$$D_{\max} \geq D_{\mathrm{t}} = -2 \log \sin(\Theta - a) + D_{\mathrm{u}}$$

Splitting D_{u} into two terms, the optical density due to the absorption in the sample, $D_{\mathrm{s}} = A \times a$, and a constant term, D_{p}, due to the initial polarization, reflection losses *etc.* we obtain

$$D_{\mathrm{m}} \equiv D_{\max} - D_{\mathrm{p}} \geq -2 \log \sin(\Theta - a) + \\ + A \times a \qquad (3)$$

Approximating (2) by

$$a = \Theta \times 0.576 \times D_{\mathrm{app}} \qquad (2')$$

and $\sin(\Theta - a)$ by $(\Theta - a)$ and eliminating Θ from (2′) and (3) we get

$$\frac{\mathrm{d}D_{\mathrm{app}}}{\mathrm{d}a} = 0 \text{ for } a = \frac{2}{A \ln 10} = \frac{0.87}{A}$$

Inserting this value of a into (3) we may calculate the corresponding minimum value (*i.e.* the desired optimum value) of Θ.

If a rough estimate of A has been obtained from preliminary measurements we may adjust the setting of the prisms according to this result and we may use a combination of sample concentration and tube length such that $a = 0.87/A$. Doing this we ensure the highest pen deflection (D_{app}) which may be produced by any sample of the substance in question subject to our requirements of resolution.

The exchange of the ordinary light sources of the spectrophotometer for more powerful ones serves the purpose of increasing $D_{\max}$. In our present setup this has enabled us to work with values of Θ as small as 1.5°, *i.e.* a rotation of 0.001° corresponds to an apparent optical density of *ca.* 0.001, and we believe that it will prove difficult to increase this sensitivity very much. (The Perkin-Elmer attachment [8] is operated at $\Theta \geq 10°$).

The accuracy of the values of a obtained is comparable, or a little inferior, to that with which absorption spectra are recorded by the spectrophotometer, *i.e.* a is correct to within *ca.* ± 1 %, provided $A < 5$. Substances with appreciably higher values of A must be measured in very dilute solutions, and the correspondingly lower values of D_{app} cannot be evaluated with this relative accuracy.

This work has been sponsored in part by the *European Research Office, U. S. Department of the Army*, Frankfurt a.M.

1. Moffitt, W. *J. Chem. Phys.* **25** (1956) 467, 1189.
2. Djerassi, C. and Klyne, W. *Proc. Chem. Soc.* **1957** 55.
3. Schellman, J. A. *Compt. rend. Lab. Carlsberg Ser. chim.* **30** (1958) 363.
4. Djerassi, C. *Optical Rotatory Dispersion*, McGraw Hill, New York 1960.
5. Hidaka, J., Yamada, S. and Tsuchida, R. *Bull. Chem. Soc. Japan* **31** (1958) 921.
6. Woldbye, F. *Optical Rotatory Dispersion of Transition Metal Complexes*, European Research Office, U S Army, Frankfurt a. M. 1959.
7. Keston, A. and Lospalluto, J. *Federation Proc.* **12** (1953) 229.
8. Savitzky, A., Slavin, W. and Salinger, R. E. *VIth Meeting on Molecular Spectroscopy*, Pergamon Press, London 1960.
9. Gallop, P. M. *Rev.Sci. Instr.* **28** (1957) 209.
10. Woldbye, F. To be published in *Acta Chem. Scand.*

Received November 3, 1959.

Isolation of a New Neutral Amino Acid from *Lactarius helvus*

J. CASIMIR* and ARTTURI I. VIRTANEN

Laboratory of the Foundation for Chemical Research, Biochemical Institute, Helsinki, Finland

During chromatographic investigations on the soluble nitrogen compounds in the mushroom *Lactarius helvus* we found relatively large amounts of an amino acid which could not be identified with any knovn amino acid already found to occur in plant tissues. This new substance corresponded to spot A in Fig. 1. When treated with ninhydrin on paper, it was easily recognized by a strong yellow colour at 80°C. The colour soon turned violet brown. On two-dimensional chromatograms the substance moved just above glutamine when phenol-water-ammonia and butanolacetic acid-water were used as solvents. The R_F-values

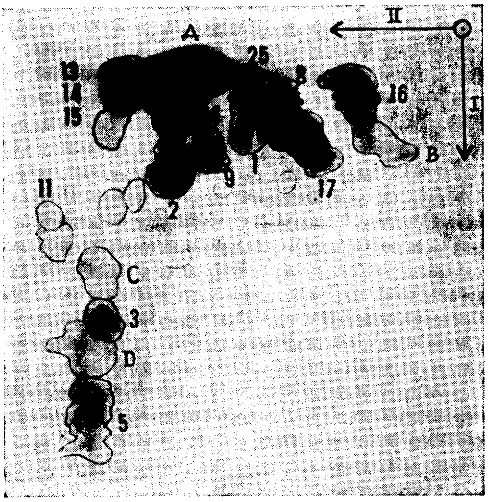

Fig. 1. Two-dimensional paper chromatogram of a 70 % ethanol extract of *Lactarius helvus*. Spots A, C, and D apparently represent unknown neutral amino acids and spot B an acidic one. 1 gly, 2 ala, 3 val, 5 leu, 8 ser, 9 threo, 11 pro, 13–15 basic, 16 asp, 17 glu, 24 glu-NH_2, 25 asp-NH_2. I butanol-acetic acid-water, II phenol-water-NH_3.

---

* Present address: Institut Agronomique de l'Etat, Gembloux, Belgique.