A Simple Device for Measurement of Circular Dichroism in the Visible and Ultraviolet Regions

Circular dichroism (CD), i.e. the difference in absorbability for left and right circularly polarized light, is widely used as a tool for determining the structure of organic compounds[1–3].

A commercial recording apparatus for measurement of CD, the Dichrograph[4,5], is available but rather expensive. Therefore a simpler apparatus may be suitable for those who wish to use CD measurements only occasionally. Simple devices for this purpose have been constructed by others[6–9] but in most of the publications only few details are given.

Though the apparatus which we constructed is only another application of a well-known principle, a short description of it may be useful as the construction is simple and fairly precise measurements can be made with it very easily. This device may be attractive for others, especially for those who possess a Zeiss spectrophotometer.

The principle of the apparatus is as follows (Figures 1 and 2): A monochromatic light beam passes an entry slit (A) and a calcite polarizing prism of the Glan type (dimensions 15 · 15 · 16 mm, aperture 9°) so that it becomes plane-polarized.

The polarizer is mounted in such a way that it can be rotated through an angle of 90° by moving a handle (B) in a slot (C). Thus the plane of polarization is caused to make an angle of 45° to the left or the right with the vertical. These angles may be adjusted once and for all by means of the screws (D). The beam passes a diaphragm (E) to eliminate stray light and is totally reflected twice in a Fresnel rhomb (F) of suprasil. This rhomb was made for us by Heraeus Quarzschmelze Hanau; the dimensions are given in Figure 3.

In this rhomb the beam is resolved into two components, vibrating in and perpendicular to the plane of reflection, respectively. The phase difference (δ) between these components is $^1/_4\ \lambda$ or 90° so that they result in a circularly polarized beam (left or right depending on the position of the polarizer). Behind the Fresnel rhomb a

[1] C. DJERASSI, *Optical Rotatory Dispersion* (McGraw-Hill, New York 1960).
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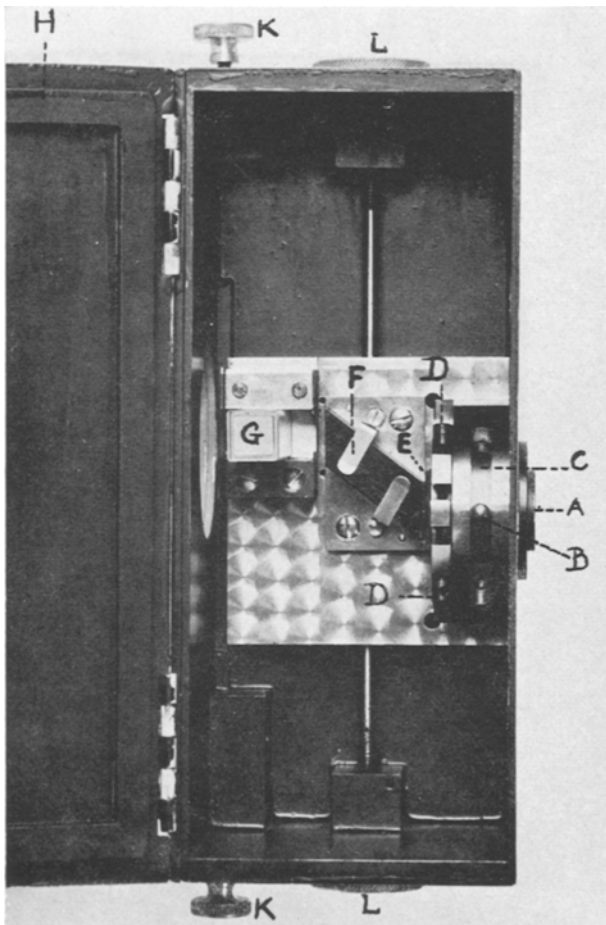

Fig. 1. Top view of circular dichroism attachment.

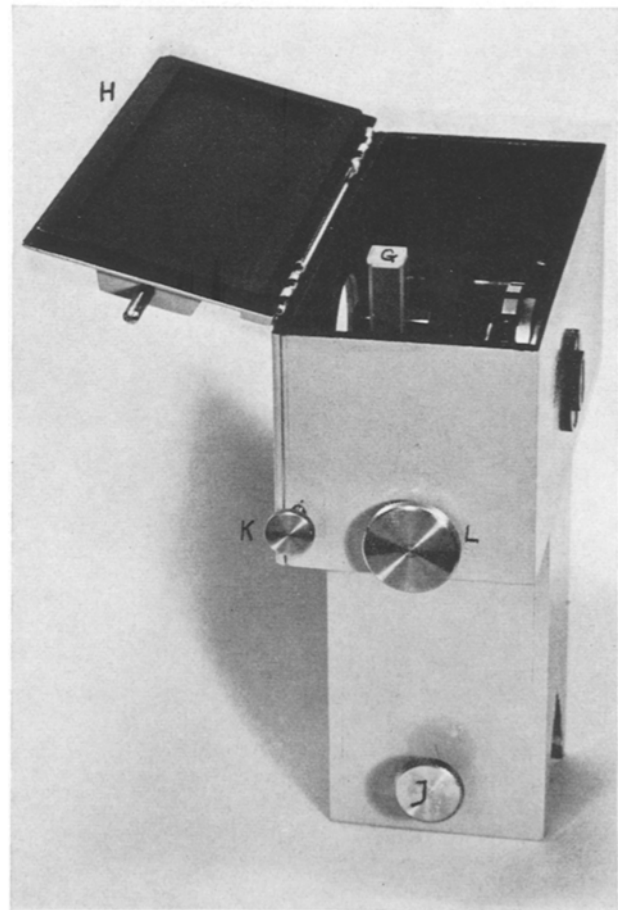

Fig. 2. Overall view of circular dichroism attachment.

suprasil cuvette (G) of 1 or 2 cm light path can be inserted.

It may be necessary to place a diaphragm behind the cuvette in order to remove secondary non-polarized projections produced by the polarizer.

All parts are enclosed in a housing (outer dimensions 86 · 86 · 200 mm) which can be closed light-tight with a lid (H). This housing is screwed onto a stand which can be fixed on the optical bench of the spectrophotometer (by means of the screw (J)) (Figure 2) in place of the normal cuvette-housing of the spectrophotometer. The photocell compartment of the spectrophotometer is fixed behind the housing with the screws (K). The optical parts can be centred in the light beam coming from the monochromator by means of the screws (L).

The photocell compartment must be displaced laterally exactly 19.1 mm from its normal position, corresponding with the lateral displacement of the light beam by the Fresnal rhomb.

The circular dichroism $\Delta\varepsilon$ of a solution at a given wavelength is measured by determining the difference between the extinctions E_L and E_R in left and right circularly polarized light with the polarizer in the 2 extreme positions (e.g. by arbitrarily setting extinction = zero for right circular and reading the extinction for left circular). $\Delta\varepsilon$ is given by the formula:

$$\Delta\varepsilon = \frac{E_L - E_R}{l\,C},$$

wherein: l = path length in cm;
C = concentration in M/l.

The 'molecular ellipticity' $[\theta]$ in degrees per $M/100$ ml/10 cm is:

$$[\theta] = 2303\,\frac{4.500}{\pi}\,\Delta\varepsilon = 3298\,\Delta\varepsilon \text{ (approximately)}.$$

About the construction of the apparatus and the possible errors in the readings, we make the following remarks: In principle, the conversion of plane-polarized light to circularly polarized light could be done by any 'quater-wave plate' but such a plate would only be suitable for one particular wavelength (or a very short range of wavelengths). A device suitable for a wide range of wavelengths without any mechanical correction is given by the Fresnel rhomb[10] as the phase difference (δ) is not caused by delay but by 2 phase jumps, only depending on the angle of reflection (α) and the index of refraction(n) and is governed by the formula:

$$\tan\frac{\delta}{4} = \frac{\cos\alpha\,\sqrt{n^2\sin^2\alpha - 1}}{n\sin^2\alpha}.$$

We chose $\alpha = 53°$, but as the beam coming from the monochromator is not strictly parallel, α actually varied from 50.75°–55.25°.

The index n for quartz varies from 1.50–1.46 in the wavelength region 250–600 nm. With these data, the following values for δ were calculated:

	$\alpha = 50.75°$	$\alpha = 51.5°$	$\alpha = 53°$	$\alpha = 55.25°$
$n = 1.50$	90° 16′	91° 4′[a]	90° 8′	88° 16′
$n = 1.46$	83° 28′		84° 44′[a]	83° 52′

The values, marked with [a], are the maxima of the function.

The influence of variations of δ on the values, measured for $\Delta\varepsilon$, can be calculated approximately with the following formula:

$$\Delta\varepsilon' = \Delta\varepsilon\,\sin\delta,$$

wherein $\Delta\varepsilon'$ = measured value,
$\Delta\varepsilon$ = correct value.

It proves that the error is always $< 0.65\%$ and, therefore, negligible.

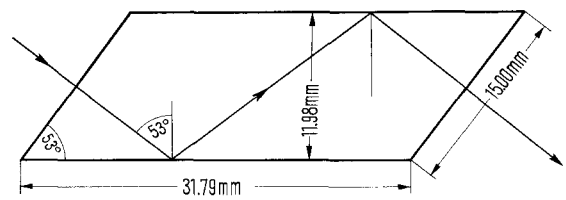

Fig. 3. Dimensions of Fresnel rhomb in circular dichroism attachment.

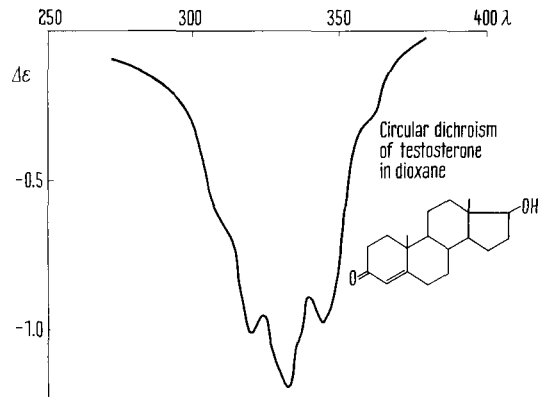

Fig. 4. Circular dichroism spectrum of testosterone (in dioxane).

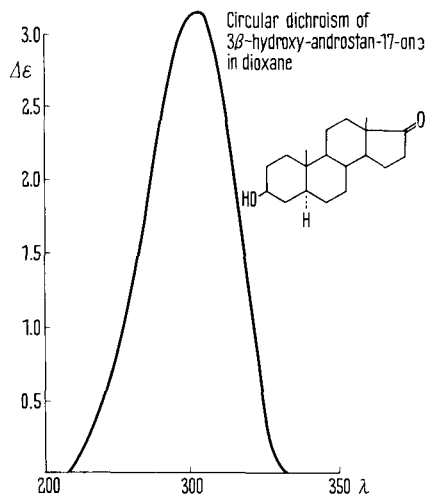

Fig. 5. Circular dichroism spectrum of 3β-hydroxy-androstan-17-one (in dioxane).

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Our instrument has the disadvantage that, without cuvette, the readings of the photometer at the 2 positions of the polarizer are not exactly the same, although they should be theoretically. We do not wholly understand this phenomenon, but partial polarization of the light in the monochromator and inhomogeneity of the polarizer may be two of the causes. Without cuvette we find a small difference ($E_L - E_R$) which varies with wavelength but is independent of slit width and hence of light energy. The values are not altered by placing a cuvette with solvent in the beam. Therefore a blank curve should be taken with a cuvette with solvent, and the values measured for the solution are corrected by subtraction of the blank values. The quality of the cuvettes used for the measurements is very important. Some of our cuvettes appeared to be birefringent (owing to strain) and therefore useless, and in some cases we found high blank values for a particular cuvette which could be influenced by inverting the cuvette. Nevertheless some commercial cuvettes were selected that showed none of these disadvantages.

To demonstrate the utility of the apparatus, the CD curves of testosterone and 3β-hydroxy-(5α)-androstan-17-one obtained with the instrument are given in Figures 4 and 5. These curves agree well with the published values[5]. Probably, the transmission of the apparatus in the UV-region can be improved by using a quartz polarizer instead of a calcite one. (We used the calcite polarizer because it happened to be available[11]).

Zusammenfassung. Es wird ein einfacher Apparat beschrieben, der als Zusatzgerät zum Zeiss-Spektralphotometer PMQ II zur Messung von Zirkulardichroismus zu verwenden ist.

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Immunofluorescent Localization of TAMM-HORSFALL Mucoprotein

TAMM-HORSFALL mucoprotein[1] exists normally in human urine as a highly aggregated, double stranded filamentous molecule[2,3] having a molecular weight of approximately 7 million[1]. It has recently been shown that the large, native form of this protein may be dissociated or disaggregated by various means[4–8], and some evidence has become available to indicate that the basic monomeric subunit, retaining hemagglutination inhibition activity and all the immunologic determinants of the parent molecule, has a molecular weight in the region of 95,000–112,000[9]. The cellular origin of the mucoprotein has been assumed to be rather high in the nephron, since attempts to demonstrate serum precursors have been unsuccessful[10,11], and since cast material from patients with acute renal failure or with the nephrotic syndrome has been found to consist almost entirely of TAMM-HORSFALL mucoprotein[11,12]. An immunofluorescent technique has been used in a study of the localization of this mucoprotein in sections of human kidney.

Native TAMM-HORSFALL mucoprotein was prepared from human urine by precipitation in 0.58 molar NaCl, according to the method described by TAMM and HORSFALL[1]. This procedure has been shown to yield a product which behaves homogeneously in starch gel electrophoresis, ultracentrifugation, gel filtration and immunodiffusion[1,8]. Antiserum was prepared in rabbits by 2 intramuscular injections of purified mucoprotein with complete Freund's adjuvant at 3 week intervals, and the initial harvest was at 6 weeks. Antibody was conjugated with fluorescein isothiocyanate[13,14] and adsorbed with mouse liver powder[15] after dialysis and filtration on G-25 Sephadex.

Kidney sections were obtained at the time of autopsy and were cut at a thickness of 4 or 5 μ and transferred to glass slides. The sections were washed with phosphate-buffered saline, covered with conjugated antiserum and kept for 45–60 min in a moist chamber at room temperature. After 3 subsequent washings with the buffered saline, the slides were mounted in buffered glycerol and examined with a Zeiss UV-microscope.

Bright cytoplasmic fluorescence was found in kidney sections from patients with congenital heart disease, acute lymphocytic leukemia, idiopathic thrombocytopenic purpura and fibrocystic disease of the pancreas. Glomeruli and proximal tubules remained uniformly nonfluorescent in all sections. Cells of the distal tubules and collecting tubules and perhaps the loops of Henle showed specific fluorescence which was blocked by pre-incubation with unconjugated antiserum as well as by mixing antigen with conjugated antiserum (Figure). The binding of antibody was evenly distributed among all cells in a fluor-

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