

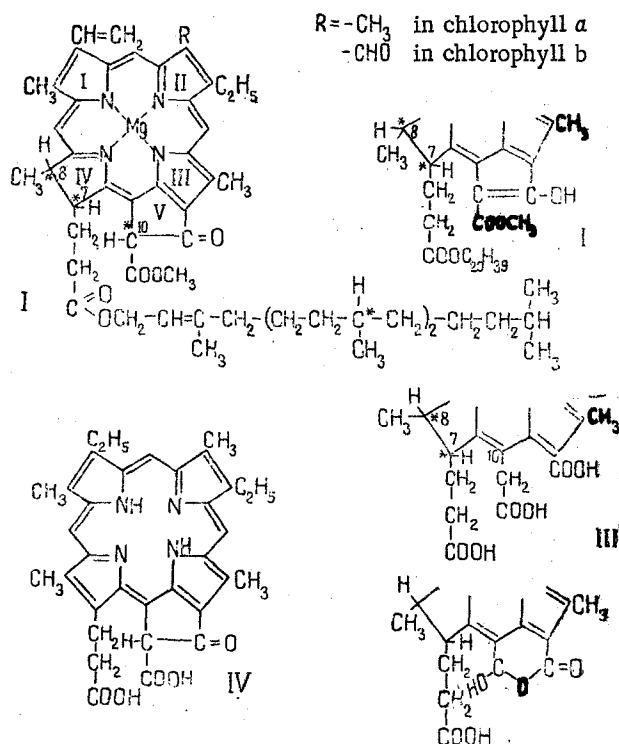
# OPTICAL ACTIVITY OF CHLOROPHYLL AND THE KETO-ENOL TAUTOMERISM OF THE CARBOCYCLIC RING

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According to the structural formula, chlorophylls *a* and *b* (I) possess three asymmetric carbon atoms in positions 7, 8, and 10, and two in the phytol residue. The mobility of the hydrogen atom attached at C<sub>10</sub> [1] and the possibility of keto-enol tautomerism of the carbocyclic ring (II) must lead to the racemization of the asymmetric C<sub>10</sub> atom. The optical activity of chlorophyll will therefore be determined only by atoms 7 and 8 in the macrocyclic ring and the asymmetric atoms of the phytol residue. In this case, the molecular rotation of chlorophyll should be similar to that of chlorophyllin (III) (since the optical activity of phytol is very small [7]) and other derivatives of the chlorin type. The testing of this hypothesis was the main object of the present investigation. In addition, it was necessary to elucidate the dependence of the optical activity on the concentration of the pigments and the nature of the solvents. The sparse data on the optical activity of chlorophyll and its derivatives indicate the existence of anomalous rotatory dispersion. This fact, together with the question of the keto-enol equilibrium is of great interest for the study of the photochemical properties of the chlorophylls and of sensitizers of photosynthesis.

Stoll and Wiedemann [2], who were the first to detect the optical activity of acetone solutions of chlorophylls *a* and *b* and their derivatives at a wavelength  $\lambda$  of 720 m $\mu$ , considered that the C<sub>10</sub> of the carbocyclic ring is also an optically active center.



Fischer and Stern [3], on the other hand, considered that the optical activity was associated only with C<sub>7</sub> and C<sub>8</sub> in view of the fact that pheophorbide (IV) did not possess optical activity and all the derivatives of the chlorin type did possess it. Woodward [4], on measuring the optical activity of "chlorin 5" (V) at a wavelength  $\lambda$  of 546 m $\mu$  found that it was +1810° for the synthetic product and +1823° for the natural product, while Fischer and Stern [3] found specific activities of the opposite sign both for chlorins and for chlorophylls at  $\lambda$  720 m $\mu$ : -141° for chlorin *e* and -337° for chlorin *e*<sub>4</sub> dimethyl ester. This discrepancy indicated either an anomalous dispersion of the rotation of the pigments or its dependence on the concentration, since the measurements were carried out with solutions of different concentrations.

While our investigation was in progress, a paper by Ke and Miller was published [5] in which the existence of an anomalous rotatory dispersion in chlorophylls *a* and *b* was reported. However, no reference was made to quantitative data on the specific rotation or its dependence on the concentration and other factors.

We had first to determine whether a deuterioexchange at C<sub>10</sub> had any effect on the optical activity of chlorophylls *a* and *b*. The replacement of ordinary water by D<sub>2</sub>O led to no change in  $\alpha_c$  of either chlorophyll in 1–2 hr; it is known that the replacement of H in the carboxyl ring by D is complete in 30 min [6]. The absence of an influence of deuterioexchange at C<sub>10</sub> on the optical activity could be due either to intramolecular racemization at C<sub>10</sub> in consequence of keto-enol equilibrium or to an ionization mechanism of the S<sub>N</sub>1 type for the replacement of the hydrogen atom under the condition that in this case the replacement of H by D does not appreciably change the specific rotation.

Table 1

Experiment no.	Substance	Concentration, mole/l	$[M]_{546}^{22}$	$[\alpha]_{546}^{22}$
1	Chlorophyll <i>a</i>	$1 \cdot 10^{-4}$	$3650 \pm 150$	$406 \pm 15$
	Chlorophyllin <i>a</i>	$9 \cdot 10^{-5}$	$3400 \pm 140$	$550 \pm 20$
	Pheophytin <i>a</i>	$9 \cdot 10^{-5}$	$5700 \pm 240$	$640 \pm 30$
2	Chlorophyll <i>b</i>	$5 \cdot 10^{-5}$	$2960 \pm 210$	$326 \pm 25$
	Pheophytin <i>b</i>	$5 \cdot 10^{-5}$	$5300 \pm 160$	$585 \pm 20$
	Chlorophyll <i>a</i>	$9 \cdot 10^{-5}$	$3050 \pm 120$	$344 \pm 15$
3	Pheophytin <i>a</i>	$9 \cdot 10^{-5}$	$5600 \pm 100$	$635 \pm 10$
	Chlorophyllin <i>a</i>	$8 \cdot 10^{-5}$	$2900 \pm 90$	$470 \pm 15$
	Chlorophyll <i>a</i>	$1 \cdot 10^{-4}$	$3650 \pm 100$	$406 \pm 10$
4	Chlorophyllin <i>a</i>	$9 \cdot 10^{-5}$	$3450 \pm 100$	$560 \pm 10$
	"	$5 \cdot 10^{-5}$	$3080 \pm 200$	$500 \pm 20$
	"	$2 \cdot 10^{-5}$	$2700 \pm 200$	$430 \pm 20$

The fact that the observed constancy of  $\alpha_c$  is due to the first-mentioned factor is shown by the molecular rotations of chlorophylls *a* and *b* and chlorophyllin *a* (Table 1). The molecular rotation of chlorophyll and chlorophyllin are very similar, i. e. the absence from the chlorophyllin molecule of a phytol residue, which possesses slight dextrorotatory power  $[\alpha]_D^{20} + 0.76^\circ$  [7], and the asymmetric C<sub>10</sub> atom has no appreciable effect on the optical activity of the pigments. The keto-enol equilibrium in the carbocyclic ring can explain not only the loss of optical activity at C<sub>10</sub> but also the rapid replacement of hydrogen by deuterium in the same position [6, 8]. It is characteristic that the chlorophylls and their pheophytins show a substantial difference in their molecular (and specific) rotations – the replacement of a magnesium atom by two hydrogen atoms in the molecule of pheophytin changes the electronic state of the C<sub>7</sub>\* – C<sub>8</sub>\* bond. Thus, although the C<sub>7</sub>\* – C<sub>8</sub>\* bond does not participate in the system of conjugation of the pigments that is mainly responsible for their spectral and chemical properties, as can be seen from the results obtained, its state depends on the state of the conjugated system of double bonds.

It must also be noted that  $\alpha$  depends on the concentration, and this fact must be taken into account in comparing the optical activities of the chlorophylls and their derivatives (Table 2).

Table 2 gives part of the results of determinations of  $\alpha$  with data on the relative accuracy of the measurements. Since the monochromaticity of the measuring beam in the polarimeter which we used (see Experimental) was achieved by means of interference filters, the figures given are integral magnitudes for a half-width of the beam of  $546 \pm 10$  m $\mu$ . The use of other filters transmitting at 578, 589, and 436 m $\mu$  enabled us to establish the relationship between the specific rotation and the concentration (Fig. 1) and the anomalous rotatory dispersion for the wavelengths given (Fig. 2). It was not possible to measure solutions with concentrations  $> 1 \cdot 10^{-4}$  mole/l because of the high absorption capacity of the pigments.

The measurement of samples of the chlorophylls obtained from various starting materials showed a difference in  $[\alpha]_{546}^{22}$  for a concentration of  $1 \cdot 10^{-4}$  mole/l in the range  $344\text{--}416 \pm 15$  for chlorophyll *a* and  $320\text{--}340 \pm 20$  for chlorophyll *b* (regardless of the time of storage of the samples); the cause of this is obscure.

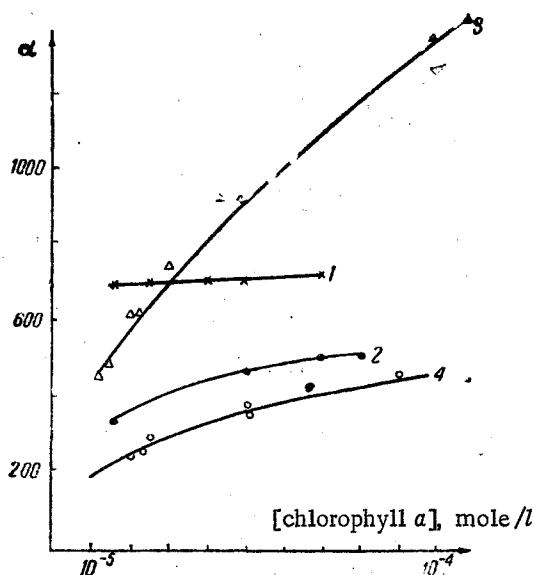
The use of various organic solvents – acetone, ethyl alcohol, ether – had no effect on the rotatory power of the pigments.

#### Experimental

**Polarimetric measurements.** These measurements were carried out on a French photometric polarimeter (Quick-Polarimetre Roussel Jonan) with a sensitivity of  $\pm 0.0005^\circ$  in a cell with an optical length of 2 cm. Continuous measurements for 5–7 min did not lead to any change in the angle of rotation, which we feared because of the photochemical

sensitivity of the pigments and the heating of the solutions. The deuterated water  $D_2O$  was added to the cell after the solution had previously been brought to room temperature ( $22^\circ C$ ).

Preparation of the chlorophylls and their derivatives. The starting material for the preparation of chlorophylls *a* and *b* was a finely ground powder of dried nettles and cells of the alga *Scened. obl.* The pigments were isolated and separated by a previously published method [9].



$\alpha$  as a function of the concentration for various wavelengths. 1)  $\lambda$  589 2)  $\lambda$  578; 3)  $\lambda$  436; 4) 546 m $\mu$ .

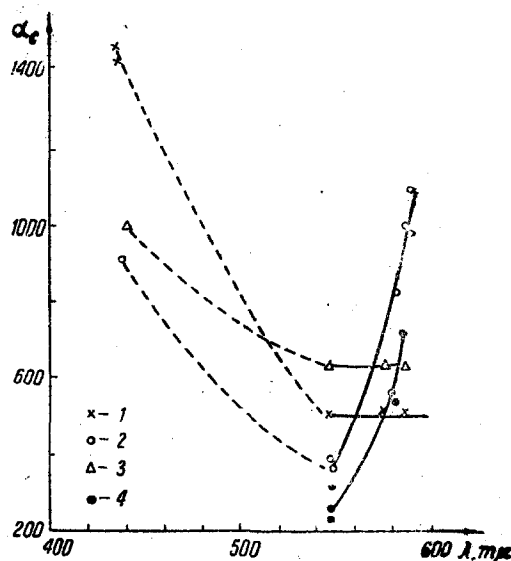


Fig. 2. Specific rotations of the pigments as functions of the wavelengths. 1) Chlorophyllin *a*; 2) chlorophyll *a*; 3) pheophytin *a*; 4) chlorophyll *b*.

K-Chlorophyllin *a* was obtained by saponifying chromatographically pure chlorophyll *a* with 7% methanolic alkali as described by Oster et al. [10]. The only modification was that the methanolic solution containing the chlorophyllin and the excess of caustic potash was chromatographed directly on paper in the system pyridine–phosphate buffer, pH 7 (4 : 5) system. The alkali remained at the starting point. The K-chlorophyllin was eluted from the paper with methanol, and the solution was filtered and its optical activity measured. The pheophytins *a* and *b* were obtained by the action of 2N hydrochloric acid on alcoholic solutions of chlorophylls *a* and *b*.

### Summary

1. The optical activity of the chlorophylls is determined only by the asymmetric carbon atoms in positions 7 and 8 and the two asymmetric atoms of the phytyl residue. Because of keto-enol equilibrium, the asymmetric  $C_{10}$  atom in the carbocyclic ring poses no optical activity.

2. The optical activity of solutions of the chlorophylls has an anomalous rotatory dispersion and increases with an increase in the concentration of the solutions.

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