# 210. Magnetic Circular Dichroism Studies XXIII [1]
# Magnetic Circular Dichroism Spectra of Indole Alkaloids

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(25. VI. 72)

*Summary.* The magnetic circular dichroism spectra of a number of indole alkaloids show two B-terms of opposite sign in the 250–330 nm wavelength region associated with the $^1L_b$ and $^1L_a$ electronic transitions, the long wavelength, $^1L_b$, band being of positive sign, whereas both bands strongly overlap in absorption. Various substituents in different positions of the indole ring cause a red shift of both bands and a broadening of the long wavelength B-term. The sign pattern, however, remains unchanged in all examples thus far investigated. Dihydroindole and oxindole, on the other hand, exhibit MCD. bands with the opposite sign sequence as compared to the indole chromophore. This observation allows identification of the indole chromophore in alkaloids from the sign pattern of the MCD. bands.

**1. Introduction.** – In our recent studies of the magnetic circular dichroism (MCD.) spectra of proteins [2] [3] [4] we observed that the indole chromophore of tryptophan shows a very characteristic and comparatively intense MCD. band pattern in the 250–300 nm wavelength region that could be used for the unambigious determination of this amino acid in intact proteins. Because of the widespread occurrence of this chromophore in a variety of natural products and the importance of spectroscopic methods for the identification and structural determination of these compounds, it was of interest to extend our studies to a series of indole alkaloids and related systems in order to evaluate the potential applications of this technique in organic and biochemical problems.

**2. Results and Discussions.** – Absorption spectroscopy has been a very useful method for identifying the chromophoric group in indole alkaloids, their spectra being characterized by two bands at 225 and 280 nm. The longer wavelength band, which shows some fine structure but otherwise has the appearance of a single electronic band, has been considered as being composed of two unresolved overlapping absorption bands. Evidence for this conclusion came primarily from fluorescence [5] [6] and fluorescence polarization spectra [7] [8]. More recently, *Strickland et al.* [9] have used the differences in wavelength shift which both bands undergo on changing the solvent polarity to identify individual vibrational components. These bands have been assumed to have the same origin as the low energy transitions of benzene, i.e., $^1L_b$, $^1L_a$ and $^1B_a$ (in the order of decreasing wavelength). Consequently, the benzene nomenclature has been adopted for the indole chromophore [10].

Because of the low symmetry of the indole $\pi$-electron system only B-terms are expected to be observed in the MCD. spectrum. These result from the coupling of different energy levels by the magnetic field [11] [12] [13]. The general quantum mechanical expression for the B-term of an allowed electronic transition, $\mathbf{a} \rightarrow \mathbf{j}$ is given by equation (1) [14] [15] where $f(\nu, \nu_0, \Delta)$ is a band shape function (usually *Gaussian*

or *Lorentzian*), $\vec{\mu}$ and $\vec{m}$ are the magnetic and electric dipole moment operators and $E_{ka}$ und $E_{kj}$ are the energy differences between the states **k** and **a**, and **k** and **j**, respectively. The MCD. associated with a single absorption band is therefore deter-

$$[\theta(\mathbf{a}\rightarrow\mathbf{j})]_M = -\frac{4\pi NH}{\hbar c} f(\nu,\nu_0\Delta)\ \mathrm{Im}\left\{\sum_{k\neq a}\frac{\langle \mathbf{k}|\vec{\mu}|\mathbf{a}\rangle\cdot\langle \mathbf{a}|\vec{m}|\mathbf{j}\rangle\times\langle \mathbf{j}|\vec{m}|\mathbf{k}\rangle}{E_{ka}} + \sum_{k\neq j}\frac{\langle \mathbf{j}|\vec{\mu}|\mathbf{k}\rangle\cdot\langle \mathbf{a}|\vec{m}|\mathbf{j}\rangle\times\langle \mathbf{k}|\vec{m}|\mathbf{a}\rangle}{E_{kj}}\right\} \quad (1)$$

mined by the sum of the contributions resulting from the coupling with all energy levels of the molecule, a situation too complex to allow even a qualitative interpretation of the spectral data. There are, however, situations in which equation (1) can be made sufficiently tractable so that qualitative correlations can be made. Several aspects of the MCD. of compounds of low symmetry have recently been discussed by *Caldwell, Thorne & Eyring* [13]. We have also had occasion to apply equation (1) in simplified form to particular compounds, e.g., to adenine [14]. Within in the context of the present work, we note that the widespread occurrence of the purine and indole ring systems in nature causes the detailed knowledge of the electronic structures and the optical properties of these chromophoric moieties to be of general interest. In particular, there is interest in the low energy electronic transitions of two chromophores which share the geometrical features of structural similarity, here both are 6,5 fused ring systems but which differ in the number of heteroatoms. In the case of adenine we were able to establish a working simplification of equation (1) to a form which contained only the second part of this equation. That is, we found some support for the proposition that the dominant effect of the magnetic field on the optical properties of adenine in the UV. could be ascribed mostly to intermixing of the $^1L_b$ and $^1L_a$ states with each other rather than with higher energy states of the appropriate symmetry. The conditions are explicitly given in equation (1): If the separation between the excited states **k** and **j** is much less than the separation between excited state **k** and the ground state **a** (i.e., $E_{ka} \gg E_{kj}$) then the second summation in equation (1) dominates the MCD. in the vicinity of the transition $\mathbf{a}\rightarrow\mathbf{j}$, and (2): If the separation between the two lowest excited states **j** and **k** is small compared to the separation of **j** or **k** with another excited state **s** ($E_{jk} \ll E_{sk}$) then a single term will dominate the MCD. of states **k** and **j**. Equation (1) can now be rewritten as:

$$[\theta(\mathbf{a}\rightarrow\mathbf{j})]_M = -\frac{4\pi NH}{\hbar c} f(\nu,\nu_0,\Delta)\ \mathrm{Im}\frac{\langle \mathbf{j}|\vec{\mu}|\mathbf{k}\rangle\cdot\langle \mathbf{a}|\vec{m}|\mathbf{j}\rangle\times\langle \mathbf{k}|\vec{m}|\mathbf{a}\rangle}{E_{kj}} \quad (2)$$

For this very hypothetical situation, the appearance of two B-terms of opposite sign and equal magnitude corresponding in wavelength position with the absorption bands is predicted. The sign sequence (+− or −+) is determined by the relative orientation of the two electric and the magnetic dipole transition moment vectors because of the form of the scalar triple product.

The MCD. spectrum of yohimbine (Fig. 1) is very similar to the spectra obtained from a number of tryptophan derivatives [2] [3] indicating that the additional alkyl

substituent in the 2 position of the indole ring has very little influence, as would be expected. The long wavelength positive B-term at 290 nm and the vibrational component at 283 nm correlate with the 0 – 0 and 0 + 730 transitions of the $^1L_b$ band which have been identified in the absorption spectrum [9] and the broad negative B-term with a maximum at about 265 nm corresponds to the $^1L_a$ band.

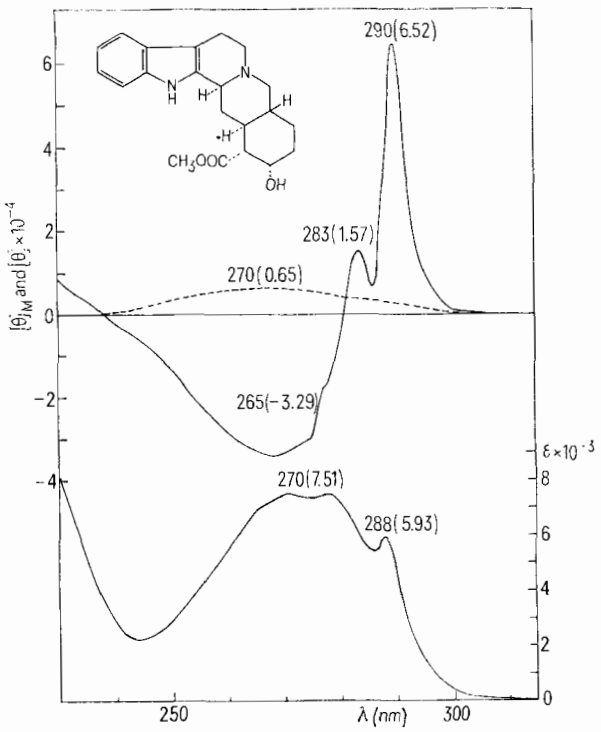


Fig. 1. *MCD.* (——), *CD.* (----) *and absorption* (lower curve) *spectra of yohimbine hydrochloride in methanol*

It is quite apparent from the MCD. spectrum of yohimbine hydrochloride, which is given in Fig. 1, that the indole chromophore does not accurately mimic the adenine system even though the qualitative interpretation is the same. For the latter compound, integration of the positive and negative MCD. associated with the lowest energy transitions shows them to be nearly equal (Fig. 5) [14]. It can be seen in Fig. 1 that the area of the negative MCD. band is greater than that of the positive band (approximately 2-to-1). This disparity in the integrated intensities of the MCD. associated with the $^1L_b$ and $^1L_a$ transitions supports the view that conditions appropriate to complex mixing of several excited states exist for the indole chromophore. This experimental result is surprising in view of the known [8] [16] near orthogonality of the three transition moments and the small energy difference between the $^1L_b$ and $^1L_a$ states (0.12 eV).

All perturbations that influence the direction or magnitude of the electric and magnetic dipole moments will lead to changes in the MCD. spectrum. In the absence of distortions induced in the indole $\pi$-electron system, changes in the stereochemistry or structure of rings D and E of the alkaloid skeleton are expected to have only little influence on the MCD. spectrum. The comparison between the spectra of yohimbine (Fig. 1) and akuammidine (Fig. 2) clearly demonstrates that despite the differences

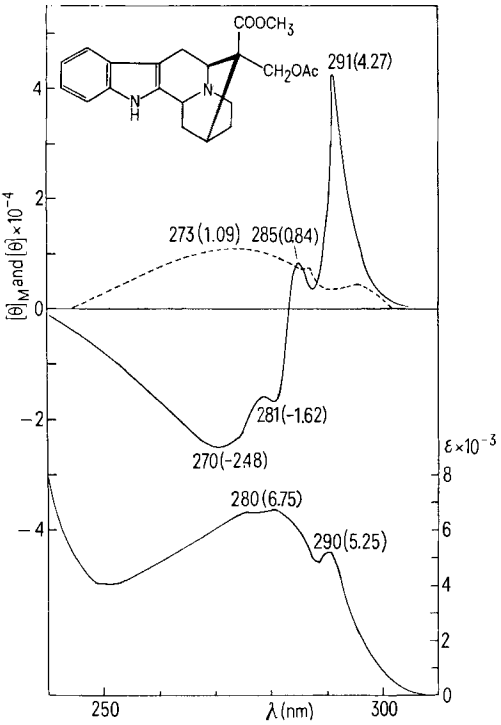


Fig. 2. *MCD.* (——), *CD.* (----) *and absorption* (lower curve) *spectra of acetyl akuammidine in methanol*

within the extrachromophoric part of the molecule the basic band pattern remains the same. Substituents that are able to interact directly with the $\pi$-electron system of the indole chromophore should cause more significant changes. The N-methyl substituent in N-methyl quebrachamine (Fig. 3) causes a broadening of the positive B-term with concommittant loss of vibrational fine structure. The influence of the N-acetyl group in diacetyl-yohimbine (Fig. 4) results in an increase in the energy separation between the $^1L_b$ and $^1L_a$ bands and is accompanied by the diminution of the corresponding B-terms. A vinyl group in the 2-position of the indole ring as in uleine (Fig. 5) again causes a band broadening of the positive B-term together with a red shift of both bands. Methoxy- or hydroxy substituents in the 5- or 6-position as in reserpine (Fig. 6) and serotonin (Fig. 7) cause broadening of the bands and an increase in the energy separation of both the $^1L_b$ and $^1L_a$ bands which are now clearly

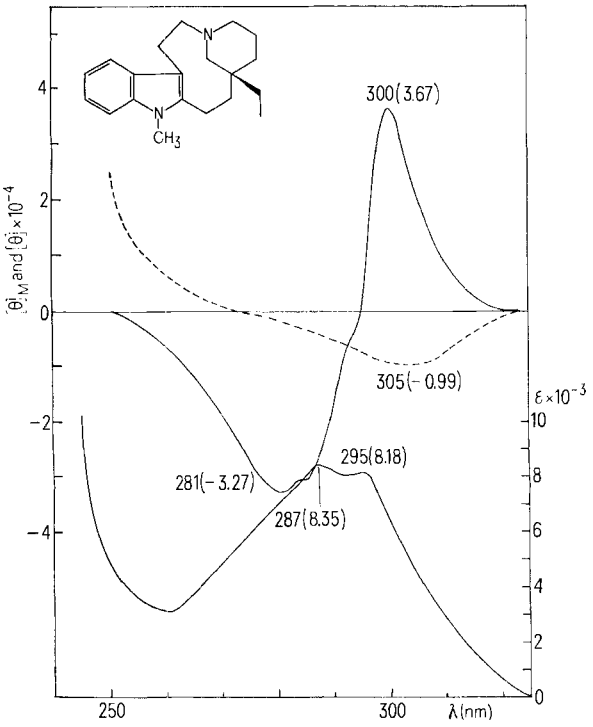


Fig. 3. *MCD.* (——), *CD.* (----) *and absorption* (lower curve) *spectra of* (–)-*N-methyl-quebrachamine in methanol*

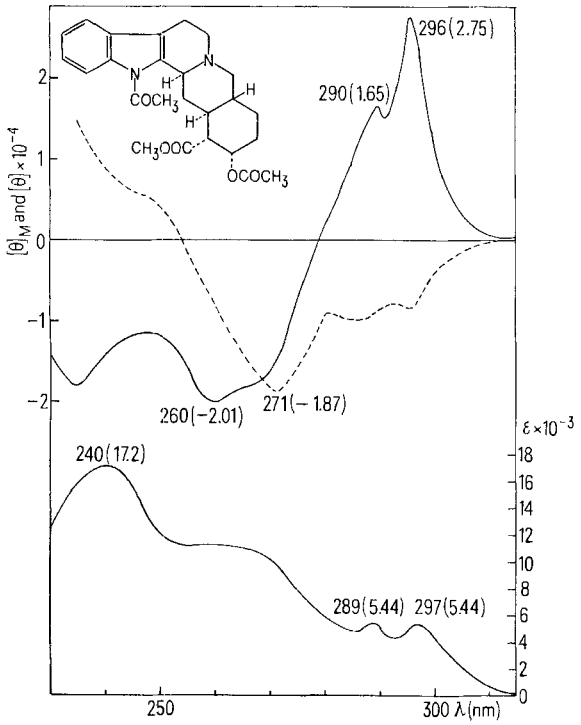


Fig. 4. *MCD.* (——), *CD.* (----) *and absorption* (lower curve) *spectra of diacetyl yohimbine hydrochloride in methanol*

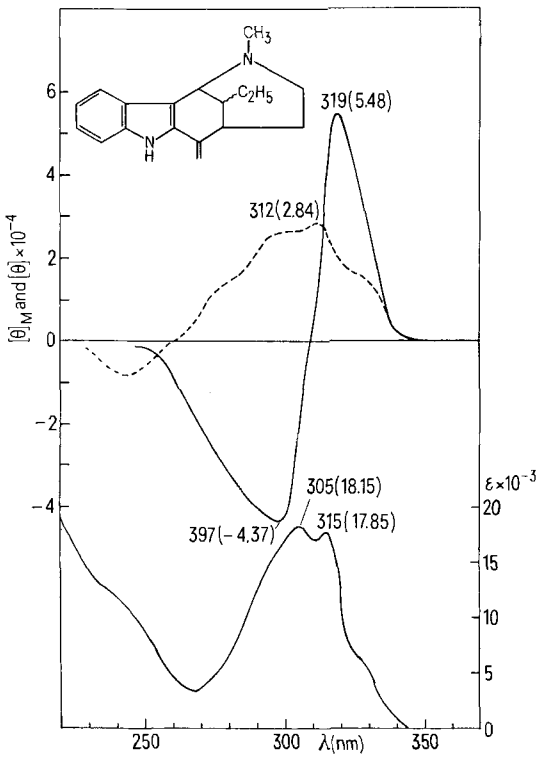


Fig. 5. *MCD.* (——), *CD.* (----) *and absorption* (lower curve) *spectra of uleine in methanol*

resolved in the absorption spectrum. In all of the examples discussed, however, the sign sequence of the B-terms remains unchanged by substitution.

In contrast to the effect of perturbing groups, hydrogenation of the 2,3 double-bond causes sign inversion of the MCD. B-terms associated with the long wavelength transitions. The absorption spectrum of indoline (Fig. 8) shows the $^1L_b$ and $^1L_a$ bands at 290 and 238 nm, respectively, to be particularly well separated. The B-term corresponding to the longest wavelength transition is found at 292 nm and is now of negative sign whereas the $^1L_a$ band shows a posisitive B-term at 240 nm. A very similar curve is observed for oxindole (Fig. 9).

**3. Application to the Recognition of Indole Alkaloids.** – The factors that determine sign and magnitude of the MCD. bands are very different from those operative for natural CD. The ORD./CD. spectra of yohimbine type alkaloids have been reported recently [17] [18] and from the observation that they show only one *Cotton* effect in the 250–300 nm wavelength region it was concluded that there is but a single electronic transition. In view of the results from the MCD. spectra which indicate the presence of two transitions, it must be assumed that the situation is more complex since in principle both transitions can contribute to the CD. spectrum with either the same or opposite sign, the latter possibility having in fact been observed in some ibogamine type alkaloids [19]. The resolution of both overlapping *Cotton* effects can be very difficult but appears to be necessary for the establishment of sector rules that

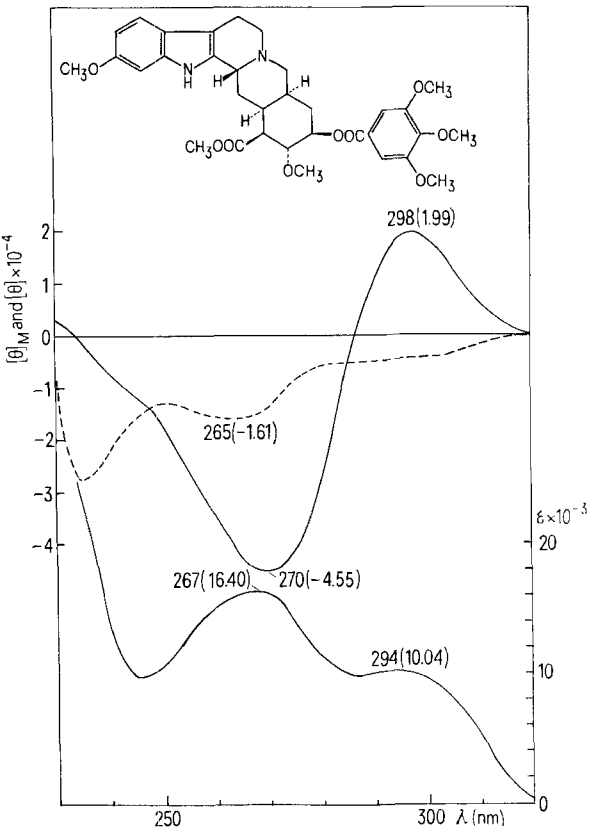


Fig. 6. *MCD.* (——), *CD.* (----) *and absorption* (lower curve) *spectra of reserpine in methanol*

relate the sign of the *Cotton* effect with the spatial distribution of the perturbing groups.

The inversion of the MCD. sign pattern between indole alkaloids on the one hand and those alkaloids having the indoline or oxindole chromophore on the other can certainly be of analytical value in those cases where, on the basis of the absorption spectrum alone, no definite distinction can be made. For example, the absorption spectrum of brucine (Fig. 10) shows two bands at 300 and 264 nm and is therefore with respect to wavelength position and intensity very similar to the one of reserpine (Fig. 6). The MCD. spectrum, however, showing a $-+$ sign sequence for brucine and $+-$ for reserpine, clearly indicates the presence of an indole chromophore only for the latter compounds. Cylindrocarpine, another example shown in Fig. 11, has an intense absorption band at 285 nm which could be interpreted as resulting from an indole ring. The MCD. spectrum again is unambiguous at longer wavelengths showing only the negative band at 280 nm suggestive of a dihydro indole structure although at shorter wavelengths a more complex MCD. curve is found than has been here-to-fore encountered.

In conclusion, these results show that, in view of the ease with which MCD. measurements are made, whenever the natural CD. of these alkaloids are required,

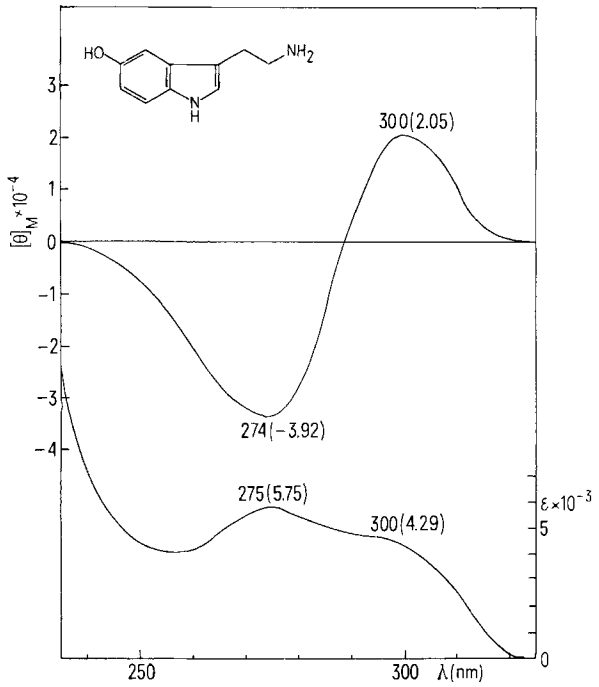


Fig. 7. *MCD.* (upper curve) *and absorption* (lower curve) *spectra of serotonin creatinine sulfate hydrate in methanol*

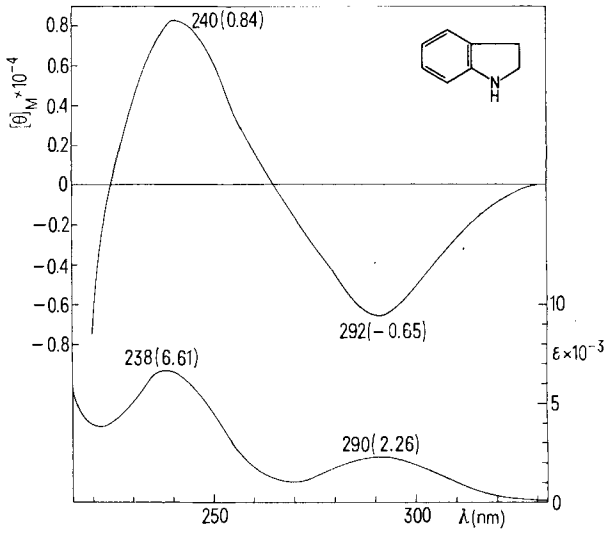


Fig. 8. *MCD.* (upper curve) *and absorption* (lower curve) *spectra of indoline in methanol*

it may often be worthwhile repeating the measurement in the presence of a strong magnetic field.

We are indebted to Professor *M. M. Janot* (Laboratoire de Pharmacie Galénique, Faculté de Pharmacie, Université de Paris, France) for a sample of diacetyl yohimbine, and to Professor *J. D. Barchas* (Department of Psychiatry, Stanford University, Stanford, California, USA) for

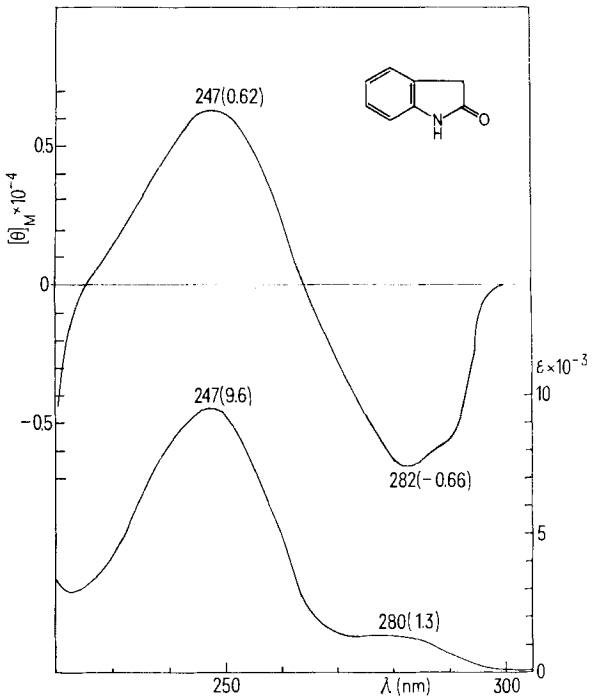


Fig. 9. *MCD.* (upper curve) *and absorption* (lower curve) *spectra of oxindole in methanol*

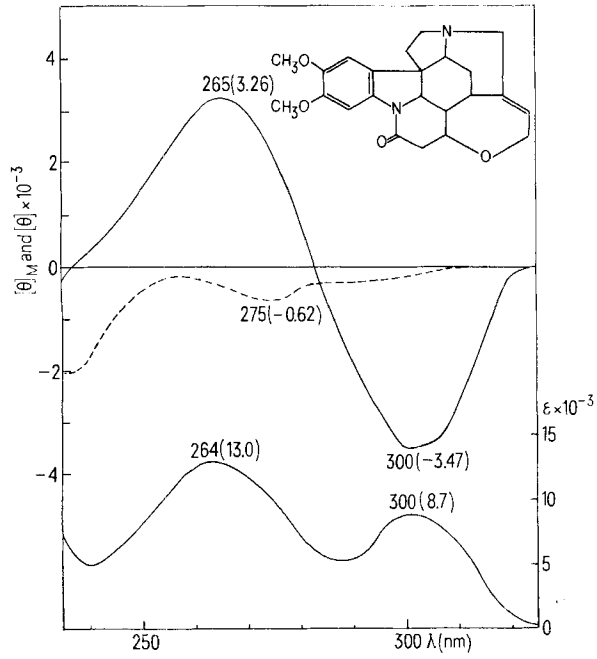


Fig. 10. *MCD.* (——), *CD.* (----) *and absorption* (lower curve) *spectra of brucine sulfate in methanol*

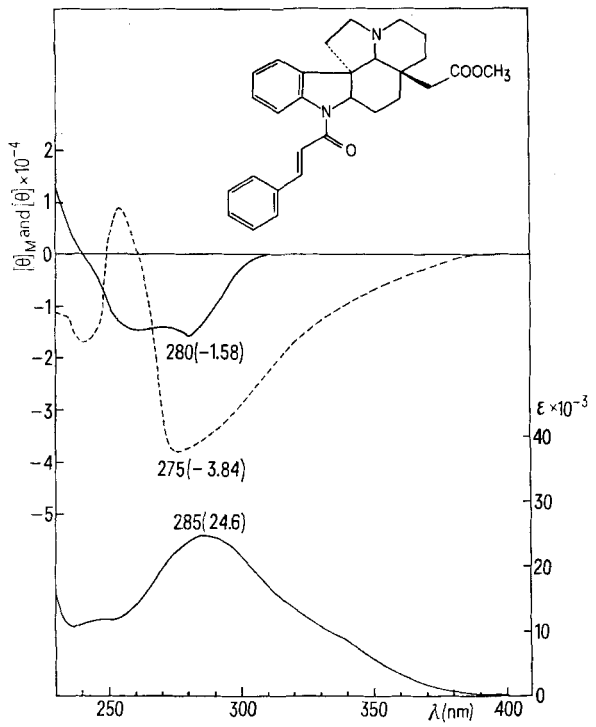


Fig. 11. *MCD.* (——), *CD.* (----) *and absorption* (lower curve) *spectra of cylindrocarpine in methanol*

a sample of serotonin. Financial support was provided by the *National Institutes of Health* (Grant No. AM-12758) and the *Stanford University Center* for Materials Research. The excellent assistance of Mrs. *Ruth Records* is appreciated.

**Experimental Part.** – A Japan Spectroscopic Co. Spectropolarimeter (*Durrum*-JASCO Model ORD-UV-5) modified to allow CD. measurements and to except a superconducting magnet built by *Lockheed Palo Alto Research Laboratories* (model OSCM 103) (20) was used for MCD. and CD. measurements. CD. spectra were measured on the same solution used for MCD. measurement but in the absence of the magnetic field. $[\theta]$ values are expressed in deg $cm^2$ $dmol^{-1}$ and for reasons of better comparison the MCD, $[\theta]_M$ values are given in the same units for a magnetic field of 49.5 kGauss. Absorption spectra were taken on a *Cary* 14 Spectrophotometer. Spectro grade methanol (*J. T. Baker Co.*, Phillipsburg, New York) was used as solvent.

The compounds used in this study were obtained from the following sources: Yohimbine hydrochloride, dihydroindole and oxindole (*Aldrich* Chem. Co., Milwaukee, Wis.); brucine sulfate and reserpine (*Matheson Coleman Bell*, Norwood, Ohio); acetyl akuammidine, (–)-N-methyl-quebrachamine, uleine and cylindrocarpine (private sample collection). Diacetyl yohimbine hydrochloride was a gift from Professor *M. M. Janot*. Serotonin creatinine sulfate hydrate was supplied by Professor *J. D. Barchas*. The purity of the samples was checked by thin layer chromatography and comparison of the absorption spectra with the literature values [21]. Indoline was distilled and oxindole recrystallized from benzene.

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## 211. Exchange Equilibrium of Oxygen Isotopes between $BrO_3^-$, $ClO_3^-$, $IO_3^-$ and Water

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*Summary.* The liquid phase fractionation factors $\alpha_H = (^{18}O/^{16}O)_{H_2O}/(^{18}O/^{16}O)_{XO_3^-}$ and $\alpha_D = (^{18}O/^{16}O)_{D_2O}/(^{18}O/^{16}O)_{XO_3^-}$ (X = Cl, Br, I) were calculated quantum mechanically between 0 and 100°. Experimental values were obtained in the case of $BrO_3^-$ at 60° showing good agreement with the calculated results.

In connection with the investigation of the exchange kinetics of oxygen between halogenate ions and water [1] it was of interest to determine the equilibrium fractionation factors

$$\alpha_H = (^{18}O/^{16}O)_{H_2O} / (^{18}O/^{16}O)_{XO_3^-} \quad (1a)$$

$$\alpha_D = (^{18}O/^{16}O)_{D_2O} / (^{18}O/^{16}O)_{XO_3^-} \quad (1b)$$

of the respective exchange reactions in the liquid phase

$$X^{16}O_2{}^{18}O^- + H_2{}^{16}O \rightleftharpoons X^{16}O_3^- + H_2{}^{18}O \quad (2a)$$

$$X^{16}O_2{}^{18}O^- + D_2{}^{16}O \rightleftharpoons X^{16}O_3^- + D_2{}^{18}O. \quad (2b)$$

[1]) Institut für anorganische, analytische und physikalische Chemie der Universität Bern.