Magnetic Circular Dichroism Studies of Indole, DL-Tryptophan, and Serotonin[1]

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Abstract: The magnetic circular dichroism (MCD) spectrum of serotonin between 350 and 240 nm is presented and compared to known MCD spectra of indole and DL-tryptophan. Measurements made using simple optical-electronic equipment and model calculations using deorthogonalized CNDO/S wave functions both confirm a red shift of the first (+)290 nm peak and an increase in intensity of the second (−)270 nm bands of the indole system upon 5-hydroxy substitution.

The recent use of magnetic circular dichroism (MCD) spectroscopy by Djerassi et al.[3] and by Holmquist and Vallee[4] to determine the tryptophan content of proteins has great long-term significance for the increased use of MCD in applied chemistry. The observation by Djerassi et al. that tryptophan is the only naturally occurring amino acid with a positive MCD peak at 290 nm is easily extended to the observation that tryptophan is the only such amino acid also containing an indole moiety. Since many psychopharmacological compounds (serotonin, reserpine, lysergic acid diethylamide, etc.) as well as over 800 plant hormones[5] contain the indole moiety, a great many potential applications of MCD spectroscopy to substituted indoles await research.

Our previous work in applying molecular orbital techniques to the study of natural optical activity[6,7] together with the recently developed formalism of Buckingham and Stephens[8,9] for interpreting MCD spectra led us to begin a program of investigation into the mechanism of the magnetically induced optical activity of indole and possible effects chemical substitution may have on its MCD spectrum. In this first study we consider a simple nonvibronic model of the MCD spectra of indole and the effect of hydroxy substitution in the 5 position. Using a low-resolution MCD spectrometer constructed in this laboratory we have measured the MCD spectrum of serotonin in the near ultraviolet range to test our model and we have repeated the measurement of the MCD spectra of DL-tryptophan and indole for calibration and assessment of our instrument's performance.

Experimental Apparatus

The spectra were measured using a single-beam MCD spectrometer constructed in this laboratory. The design follows that of Johnson[10] very closely except that a Beckman DU monochrometer was used and a large permanent magnet was placed in a 22 × 20 × 12 in. aluminum sample compartment. In a gap suitable for a 1 mm quartz cell the field was measured to be 10.0 ± 0.3 kG using a Hall-effect gaussmeter. The Beckman DU scanning attachment was coupled to the slit in order to monotonically open the slit toward the high dispersion end of the nonlinear band-pass DU monochrometer so that roughly the same light intensity was maintained and to obtain a more nearly constant band pass of 3 ± 2 nm half-width. A single quartz focusing lens is followed by a 15 mm square cross-section uv-quartz Rochon polarizer which produces two beams of linearly polarized light, one being blocked off, and a Morvue Photoelastic Modulator[11] (Tigard Oregon) is used to convert the other beam to right and left circularly polarized light alternately at 50.26 kHz. After passing through the 1 mm quartz cell placed between the magnet poles the light was depolarized by a 25 mm diameter "Depol" prism[12] from Karl Feuer Optical Associates (Fairview, N.J.) to reduce possible polarization effects in the EMI9783B side-window photomultiplier tube (PMT). The PMT was mounted 15 in. away from the magnet and shielded with mu-metal. A Power Designs, Inc. 2K20 high-voltage power supply was modified to be controlled in a feed-back loop from the DC output of the photomultiplier as described by Johnson.[10] Although the best signal-to-noise ratio was obtained when the PMT was operated at 500 V, the operating range of the feed-back loop was from 300 to 1400 V. The PMT output was amplified by a Fairchild 709 operational amplifier for a gain of 100 and sent to an Ithaco 391 lock-in voltmeter. The feed-back mechanism maintained the dc output of the PMT at about 4.8 V after the gain of 100 and across a 499 Ω load resistor via a comparison circuit to within a maximum fluctuation of less than 1 mV. Slow scans of about 30 min were used for the 350 to 200 nm range with a lock-in time constant of 40 sec and a sensitivity of from 1 to 10 mV to measure the ac signal superimposed on the PMT dc output by the different absorption of left and right circularly polarized light alternating at 50.26 kHz. A special crystal filter with a Q of about 300 and a half-band-width of only 100 Hz centered at 50.26 kHz was inserted in the circuit between the preamplifier and the lock-in voltmeter and the oscillator signal driving the photoelastic modulator was used for lock-in synchronization. All MCD spectra reported here were taken between 23 and 26°C in an air-conditioned room as temperature drift in electronic components would not allow operation with the sharply tuned crystal filter above or below these temperatures. In our estimation the main limiting factors in our instrument were large band pass and high stray light of about 0.1% in the DU monochrometer and the low source intensity of the DU deuterium lamp.

The circular dichroism values were obtained by removing the magnet from the optical path and measuring the spectra of D(+)-10-camphorsulfonic acid (Eastman) in water. This standard had been first recrystallized twice from benzene, vacuum dried over P_2O_5 at 60°C for seven days, and then sublimed under vacuum. A discrepancy exists between Detar[13] and Cassim and Yang[14] as to the true $\Delta\epsilon$ value of this compound. After comparing the two treatments we chose to use Detar's value of $\Delta\epsilon = 2.49$ at 290 nm and $[\Theta]_M = 8176$ based on what appears to be a better fit to the Kronig-Kramers transform between ORD and CD curves obtained by Detar. We also checked Detar's transform data using a different program (MOSCOW, Q.C.P.E. No. 107, University of Indiana) and obtained close agreement with his reported ORD values using his gaussian-fitted CD representation. While other workers in the past[3b,4] can trace their standards to the value of Cassim and Yang,[14] the self consistency within their data is no guarantee of an absolute standard and we prefer Detar's value because of the better agreement between measured ORD and ORD data obtained from his transformed CD data. We plan to investigate this matter further and those who prefer the Cassim and Yang standardization merely have to multiply our results by (2.20/2.49) for $\Delta\epsilon$ values. Indole (Aldrich 99%) and DL-tryptophan (Eastman) were both recrystallized from 95% ethanol–5% water. Indole was then sublimed twice under vacuum. Serotonin solutions were prepared from oxalate-stabilized fluorescence standards obtained from Regis Chemical Co. The final solution concentrations used are shown on the spectra. With the magnet back in place, the lock-in voltmeter output (ac signal rectified and amplified) was displayed on a Sargent MR recorder and compared to the average signal from five spectra of D(+)-camphorsulfonic acid. Our "instrument constant" was de-

Table I. CNDO/2-D CI MCD Parameters for H_2O

Transition	λ (exptl),[a] nm	λ (calcd), nm	$f(\nabla)$[b]	(A/D)	(B/D)[c]
1	166	131	0.19478	0.0	$+1.66 \times 10^{-4}$
2		124	0.0	0.0	0.0
3	133	98	0.43194	0.0	$+2.74 \times 10^{-4}$
4	123	96	0.32222	0.0	-2.34×10^{-4}

[a] References 31 and 32. [b] Oscillator strength via dipole velocity. [c] Bohr magnetons per l./cm.

Table II. Origin Dependence of the Lowest Energy *B* Terms of Indole Using a 20 × 20 CI

Origin λ, nm	C-5 (B/D)[a]	C.O.M. (B/D)[a]	N-1 (B/D)[a]
290	-3.24×10^{-3}	-2.94×10^{-3}	-2.63×10^{-3}
261	$+2.14 \times 10^{-5}$	$+2.30 \times 10^{-5}$	$+2.85 \times 10^{-5}$
223	$+2.26 \times 10^{-5}$	$+2.28 \times 10^{-5}$	$+2.41 \times 10^{-5}$
205	$+6.49 \times 10^{-4}$	$+7.66 \times 10^{-4}$	$+9.07 \times 10^{-4}$
202	-7.09×10^{-4}	-7.17×10^{-4}	-7.24×10^{-4}
196	$+2.29 \times 10^{-4}$	$+1.04 \times 10^{-4}$	$+1.09 \times 10^{-5}$
192	$+6.20 \times 10^{-6}$	$+2.40 \times 10^{-4}$	-2.58×10^{-5}
180	-1.07×10^{-3}	$+6.42 \times 10^{-4}$	-3.74×10^{-4}

[a] Bohr magnetons per l./cm.

termined assuming $[\Theta]_M = 8176$ at 290 nm and the magnetic circular dichroism values were estimated by dividing the CD values by our field of 10,000 G. It is of interest to note that by using Detar's standardization and assuming 10,000 G for our field our $[\Theta]_M$ values for DL-tryptophan are actually closer to what Djerassi et al. reported than if we use the Cassim and Yang standardization. Supportive uv spectra, not shown, were measured on a Perkin-Elmer 202 and a Beckman Acta VI spectrophotometer as well as single-beam absorbance measurements obtained with a Heath 703 spectrophotometer. All MCD spectra shown are direct tracings of individual scans.

Computational Model

Most of our computational procedure has been described in great detail before[15] but several modifications have been made. In this study we have used the CNDO/S parameters of Jaffe et al.[16] for the SCF-MO procedure and the single-excitations configuration interaction, but we still use the inverse Löwdin[17] transformation to deorthogonalize the molecular orbitals; we call this procedure CNDO/S-D. We have now recoded the previous Algol program (ANGMOMT) in Fortran suitable for double-precision arithmetic on an IBM 370/145 system. In doing so our previous inclusion of spin-orbit perturbation and singlet-triplet transitions was neglected as nonessential for interpretation of strong singlet transitions in closed-shell molecules. Two improvements in the procedure were made during recoding. First, the translation of origin in the angular momentum matrix elements is now carried out in the local diatomic framework before transformation to the molecular coordinate framework (eq 15, 16, and 17 in ref 16). Second, the Buckingham-Stephens[8,9] equations were coded to permit evaluation of (A/D) and (B/D) parameters characteristic of singlet electronic transitions.

$$A(a \rightarrow j) = \left(\frac{1}{2}\right) \sum_{\sigma} \sum_{\lambda} \operatorname{Im}[\langle a|\mathbf{m}|j_\sigma\rangle \times \langle j_\lambda|\mathbf{m}|a\rangle \cdot \langle j_\sigma|\boldsymbol{\mu}|j_\lambda\rangle] \tag{1}$$

$$B(a \rightarrow j) = \sum_{\lambda} \sum_{k \neq j} \operatorname{Im}\left[\langle a|\mathbf{m}|j_\lambda\rangle \times \langle k|\mathbf{m}|a\rangle \cdot \frac{\langle j_\lambda|\boldsymbol{\mu}|k\rangle}{(E_k - E_j)} + \langle a|\mathbf{m}|j_\lambda\rangle \times \langle j_\lambda|\mathbf{m}|k\rangle \cdot \frac{\langle k|\boldsymbol{\mu}|a\rangle}{(E_k - E_a)}\right] \tag{2}$$

$$D(a \rightarrow j) = \Sigma|\langle a|\mathbf{m}|j_\lambda\rangle|^2 \tag{3}$$

Here $\langle a|\mathbf{m}|j_\lambda\rangle$ is the electric transition moment and $\langle a|\boldsymbol{\mu}|j_\lambda\rangle$ is the magnetic transition moment for the $(a \rightarrow j_\lambda)$ transition. Using our low-resolution spectrometer and semiempirical CNDO/S-D wave functions we adopted the simpler rigid-shift model of Stephens[18] and Stephens, Mowery, and Schatz.[19] In this model Boltzmann distributions within the vibrational manifold of a given electronic state are ignored and temperature-dependent assymmetry of the shape of the B-type MCD curves is assumed to be zero. In reality this type of assymmetry affects the values of both (B/D) and (A/D) and the method of moments[18] should be used to analyze high-resolution MCD spectra. Although in our case the solution spectra showed considerable vibronic structure for indole even at 25°C and using our low-resolution optical system, our signal-to-noise ratio averaged only about 5 to 1 and this seemed insufficient to justify any attempt at moment fitting our spectra. While recent work by Rosenfield and Moscowitz[20] has shown that semiempirical wave functions can be used successfully to interpret weak vibrationally induced transitions in benzene, the major features of the substituted indole spectra are so large that we believe a single fixed-geometry model is adequate for understanding the MCD spectra within the rigid-shift model. With these assumptions we adopt the expression of Stephens, Mowery, and Schatz[19] in our eq 4–6 where k is the absorption coefficient and $\Delta k = k_L - k_R$ is the circular dichroism in the magnetic field H, where α is the polarizability, n is the refractive index, and N is the number of molecules in the sample.

$$k = (2\pi^2\alpha^2N/3\hbar n)D(a \rightarrow j)f^0(\omega) \tag{4}$$

$$\Delta k = (4\pi^2\alpha^2N/3\hbar n) \times \left\{\frac{A(a \rightarrow j)}{\hbar}f'(\omega) - B(a \rightarrow j)f^0(\omega)\right\}H \tag{5}$$

$$\int f^0(\omega)\mathrm{d}\omega = 1; \quad f'(\omega) = \partial f^0/\partial\omega; \quad \int f'(\omega)\mathrm{d}\omega = 0; \quad \int \omega f'(\omega)\mathrm{d}\omega = -1 \tag{6}$$

Here A, B, and D are understood to be constructed from zero-field ($H = 0$) state functions and $f^0(\omega)$ is the normalized zero-field absorption band shape.

As one gains experience in computer programming, the ease with which subtle errors can be made becomes quite evident, hence two checks were made on the computational scheme. First the atomic orbital basis Z-angular momentum matrix elements for twisted (45°) glyoxal were checked against the published values of Hug and Wagniere.[21] Second, the complete treatment was used to compute the MCD parameters for the water molecule, one of the primary MCD standards. In Table I we show the results using deorthogonalized CNDO/2 orbitals and a complete singles-only CI (9 × 9) using standard Pople[22] parameters which give a better spectral fit than CNDO/S parameters in this case and allowed us to check against previous results[23] using the Algol program. We note that in eq 5 circular dichroism has an opposite sign from the B term and also (B/D) since D is always positive; a positive B term means negative circular dichroism. By convention[24] water is taken to have *negative* circular dichroism in the near uv so that while the wavelengths predicted by the CNDO/2-D CI calculations are poor in this case, it is clear that the two lowest transitions predict the sign of the near-uv edge of the MCD correctly. (Only the four lowest states pertaining to this convention are shown.) While this is a weak constraint it is encouraging that qualitative agreement was obtained.

At one time there was great concern over the practicality of calculating MCD B terms within a mathematically incomplete set of states and it was believed that parameteri-

Table III. (20 × 20) CI Indole (Idealized Geometry) CNDO/S-D Results

Transition	λ, nm	f (ZDO/∇)	$(B/D)^a$	(A/D)	MX^b	MY^b	MZ^b	Type (C_s)
1	290	0.00542			−0.118	−0.025	0.0	$\pi \to \pi^*$
		0.00051	-2.94×10^{-3}	0.0	−0.011	−0.004	0.0	A″ → A″
2	261	0.43339			−0.995	+0.230	0.0	$\pi \to \pi^*$
		0.20904	$+2.30 \times 10^{-5}$	0.0	−0.233	+0.024	0.0	A″ → A″
3	223	0.42742			0.579	0.737	0.0	$\pi \to \pi^*$
		0.21474	$+2.28 \times 10^{-5}$	0.0	0.182	0.180	0.0	A″ → A″
4	205	0.37751			−0.828	−0.163	0.0	$\pi \to \pi^*$
		0.20928	$+7.66 \times 10^{-4}$	0.0	−0.260	−0.048	0.0	A″ → A″
5	202	0.52839			−0.646	+0.754	0.0	$\pi \to \pi^*$
		0.22747	-7.17×10^{-4}	0.0	−0.178	+0.213	0.0	A″ → A″
6	196	0.02480			0.0	0.0	0.212	$\sigma \to \pi^*$
		0.00927	$+1.04 \times 10^{-4}$	0.0	0.0	0.0	−0.057	A′ → A″
7	192	0.30748			−0.732	0.093	0.0	$\pi \to \pi^*$
		0.17230	$+2.40 \times 10^{-6}$	0.0	−0.246	0.027	0.0	A″ → A″
8	180	0.10728			0.0	0.0	−0.422	$\sigma \to \pi^*$
		0.01230	$+6.42 \times 10^{-4}$	0.0	0.0	0.0	−0.068	A′ → A″

[a] Bohr magnetons per l./cm. [b] Debyes (f, MX, MY, and MZ upper values ZDO, lower CNDO/S-D).

Table IV. (40 × 40) CI 5-Hydroxyindole (Idealized Geometry) CNDO/S-D Results

Transition	λ, nm	f(ZDO/Δ)	$(B/D)^a$	(A/D)	MX^b	MY^b	MZ^b	Type (C_s)
1	301	0.04096			−0.280	−0.188	0.0	$\pi \to \pi^*$
		0.01262	-4.82×10^{-3}	0.0	−0.044	−0.031	0.0	A″ → A″
2	262	0.34669			+0.888	−0.223	0.0	$\pi \to \pi^*$
		0.16124	$+1.11 \times 10^{-4}$	0.0	+0.204	−0.021	0.0	A″ → A″
3	228	0.38226			+0.581	+0.682	0.0	$\pi \to \pi^*$
		0.20238	-6.78×10^{-5}	0.0	+0.186	+0.162	0.0	A″ → A″
4	215	0.66498			−1.128	+0.212	0.0	$\pi \to \pi^*$
		0.36252	$+1.47 \times 10^{-4}$	0.0	−0.334	+0.060	0.0	A″ → A″
5	205	0.48832			+0.551	−0.786	0.0	$\pi \to \pi^*$
		0.22105	-3.24×10^{-4}	0.0	+0.154	−0.224	0.0	A″ → A″
6	202	0.03784			0.0	0.0	−0.265	$\sigma \to \pi^*$
		0.01638	$+1.17 \times 10^{-4}$	0.0	0.0	0.0	+0.074	A′ → A″
7	198	0.15174			−0.520	−0.080	0.0	$\pi \to \pi^*$
		0.10920	$+1.69 \times 10^{-4}$	0.0	−0.192	−0.026	0.0	A″ → A″
8	191	0.00327			0.0	0.0	−0.076	$\sigma \to \pi^*$
		0.00171	$+2.20 \times 10^{-5}$	0.0	0.0	0.0	+0.027	A′ → A″

[a] Bohr magnetons per l./cm. [b] Debyes (f, MX, MY, and MZ upper values ZDO and lower CNDO/S-D).

zation and approximation would render such calculations useless.[25] We were inclined to be more optimistic based on earlier unpublished work[23] and recently Michl[26,27] has investigated the problems of CI truncation and origin dependence[28] using a method limited to π electrons. Michl has shown that qualitatively correct results are entirely feasible provided one uses a sufficiently large CI and locates the origin somewhere within the molecular framework. In Table II we present only the (B/D) ratios obtained for indole using a small 20 × 20 CI for three different choices of origin. In the lower states of most interest we note about a 10% uncertainty associated with origin displacement within the molecule. This seems entirely acceptable to us in a semiempirical framework, but in the future we urge the adoption of a *convention of computing the MCD B terms relative to the center of mass* of the molecule just as dipole moments of molecular ions are computed at their center of mass. Although we did not vary the CI size, it seems that about four or five times as many excited configurations are needed as the number of excited states of interest. At the outside, the first three excited states are adequately represented here with a 20 × 20 CI (albeit culled from a larger set; see below).

While we believe it is sensible to report (B/D) ratios as calculated with the molecular center of mass as the origin, there is no denying the usefulness of Michl's[27] technique of actually carrying out several calculations relative to different origins. Since it is usual that one would choose an origin somewhere within the molecule when one specified the positions of the atoms, the practical range of ambiguity is that associated with the change in (B/D) values from one "edge" of the molecule to another. Clearly the choice of an origin external to the molecular volume aggravates the incompleteness of an LCAO basis set localized around the atoms in the molecule. A center-of-mass origin should make near optimum use of the degree of completeness in the orbital basis while the change in (B/D) across the molecular volume is a direct measure of the incompleteness of the orbital basis in the region of primary interest. Since the N-electron basis degree of completeness limits the completeness in the hierarchy of orbitals and configurations, the center-of-mass origin should help at several levels.

In Table III we present the computed results for indole using the CNDO/S-D parameter treatment. The geometry was idealized to be composed of a hexagon and pentagon of 1.397 Å sides each and C–H and N–H bond lengths of 1.08 Å each. In view of our adoption of a rigid shift model and the experimental observation of vibrational splitting in the MCD spectra, we chose to consider our frozen nuclear framework as merely one sensible average structure accessible within some $(3N - 6)$ 42 vibrational manifolds at 25°C. For the 5-hydroxy substitution a phenolic C–O bond of 1.36 Å[29] was used with a C–O–H angle of 105° and an arbitrarily symmetrical O–H bond length of 1.36 Å, which is probably somewhat long but not as critical as the C–O distance. In order to keep the problem small, we first ran a 50 CI CNDO/S calculation for indole and selected the 20 configurations most important to the uv-vis bands. The smaller 20 CI CNDO/S results were nearly identical for the first

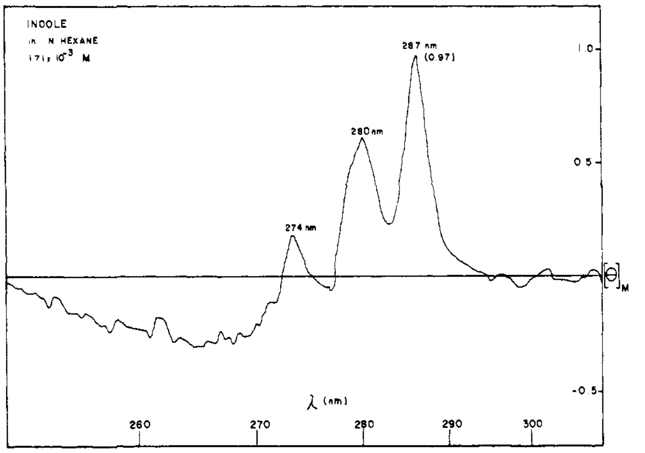

Figure 1. MCD spectrum of indole in *n*-hexane. Vertical scale in (deg cm^2)/(dmol G).

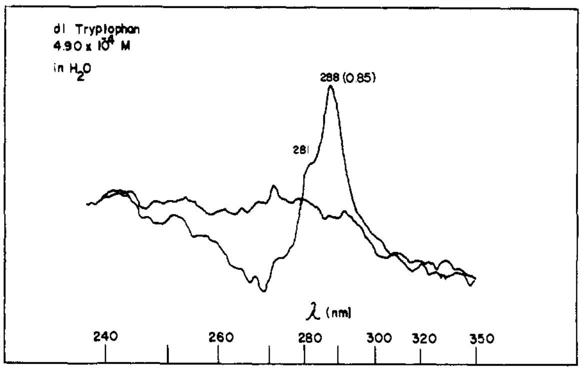

Figure 2. MCD spectrum of DL-tryptophan in water. Vertical scale in (deg cm^2)/(dmol G).

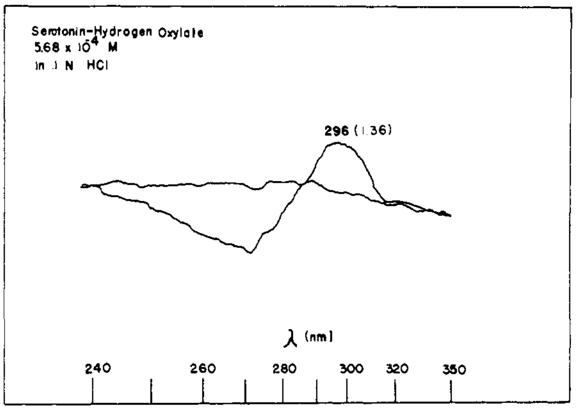

Figure 3. MCD spectrum of serotonin stabilized by hydrogen oxalate in 0.1 *N* HCl. Vertical scale in (deg cm^2)/(dmol G).

five bands with the larger CI treatment. Warnick and Michl[26] have recently shown the importance of MCD *B* terms in organic molecules using the π electron Pariser-Parr-Pople (PPP) method, and Miles and Eyring have already treated indole using this model.[27] In very large aromatic systems the π electron states alone may be sufficient, but our method has an advantage in including all valence electrons. Our results indicate that the main perturbing mechanism for the large indole positive MCD peak at about 290 nm comes from magnetic mixing of two σ to π^* transitions (No. 6 and 8) which would have been neglected in a PPP treatment while Miles and Eyring[27] obtained only a small 290 nm peak for indole using only π electrons without this mechanism. The wavelength predictions for the first two bands are in excellent agreement with experiment as shown in Figure 1. The third band maxima is uncertain from our experimental data but the calculated wavelength at 223 nm is quite reasonable. Note that while the lower values under each entry are computed from deorthogonalized orbitals and the upper values from the CNDO/S orbitals using the ZDO neglect of cross terms, there are few sign discrepancies giving us confidence in the computed (B/D) signs. On our spectra only the peaks are given values because baseline drift limits the certainty of the zero position and hence the vertical scale. Our spectra are mainly intended to show qualitative features and are only calibrated vertically near 290 nm by direct comparison with D(+)-camphorsulfonic acid. Our spectra are not as good as those obtained by others[3,4,27] but we believe it is essential to show the three spectra in Figures 1, 2, and 3 together in order to be able to assess the red shift we have found relative to the same limitations in instrumentation. Here we calculate that the 290 positive peak will be over 100 times the negative second band, an exaggeration but qualitatively correct. The third band is predicted to have negative MCD which agrees with our measurements, but which are not shown due to a very poor signal-to-noise ratio. In DL-tryptophan the third band is shown by Djerassi et al.[3a] to be negative in support of this sign assignment; our DL-tryptophan spectra agrees with theirs and is shown in Figure 2. Our DL-tryptophan

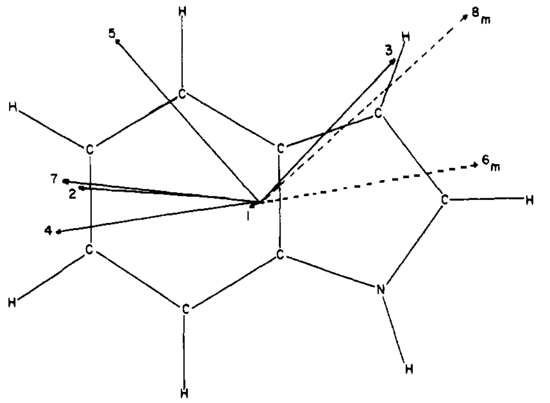

Figure 4. Computed transition moments for indole. Solid vectors (1 Å/D) are electric (ZDO) transition moments in the molecular plane. Dashed vectors (1 Å/μ_B) are magnetic (CNDO/S-D) transition moments in the molecular plane. The corresponding electronic moments for No. 6 and 8 are perpendicular to the plane.

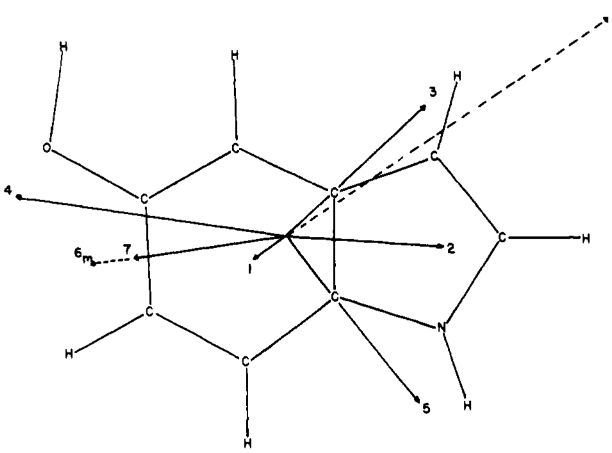

Figure 5. Computed transition moments for 5-hydroxyindole. Same notation as Figure 4.

spectra is not artistically smoothed and is given to assess our spectra of serotonin discussed below and shown in Figure 3.

The uv spectrum of Holmquist and Vallee[4] shows that the large MCD peak at 290 nm is a very weak uv transition and our oscillator strength (CNDO/S-D) of 0.0005 bears this out. Figure 4 shows the electric transition moments drawn with respect to the center of mass of the molecule and to a scale of 1 Å/D. The dashed vectors are magnetic transitions within the plane of the molecule and the solid vectors are electric transitions in the molecular plane. Magnetic transitions 6 and 8 are very large so that even when the electric part of no. 1 is small the resulting product in eq 2 is still very large. Our model does not need to invoke a vibronic mechanism for the large 290 nm peak per se, we assume that whatever vibronic structure is present in the electric part of the weak uv band it is coupled by the external magnetic field to the magnetic transitions 6 and 8 (mainly).

In Table IV we present the computed MCD parameters for 5-hydroxyindole. To keep the problem small we assumed that the saturated ethylamine group of serotonin (5-hydroxytryptamine) would have a negligible chromophoric contribution to the uv–vis spectra above 200 nm. This time 40 low-lying configurations were used in the CNDO/S CI treatment. Several qualitatively correct predictions are confirmed by our spectra in Figure 3. First the 290 nm band is predicted to shift to the red some 10 nm upon 5-hydroxy substitution. It is difficult to assess the effect of the presence of hydrogen oxalate on the serotonin spectra but the spectra of 5-hydroxyindole unbuffered in water (not shown) is very similar to Figure 3; the 296 nm peak is less broad but still red shifted with respect to indole. Note that the second MCD band (negative) increases in intensity relative to the longest wavelength (301 nm) band and the wavelength agreement is excellent. The third MCD band is predicted to be positive but close to a larger negative MCD band at 215 nm. Our experimental data support the sign of the third band but the signal-to-noise ratio is poor and baseline drift does not allow us to unambiguously assign the signs of the third and fourth bands. However, the red shift of the first band and the change in ratio of intensities of the first two bands to more nearly equal is consistent between the experimental spectra and the CNDO/S-D computations.

In Figure 5 we show the electric and magnetic transition moments for 5-hydroxyindole. Again the first transition is very weak electrically and two low σ to π^* transitions have very large magnetic transition moments. Note the change in direction of the magnetic transitions compared to indole. This change indicates the mechanism by which 5-hydroxy substitution effects mainly the second MCD (negative) band at about 262 nm. The red shift of the first 290 nm band of indole can be qualitatively explained as due to pushing up the energy of the occupied carbon orbitals by the addition of the more electronegative oxygen atom to the system via the requirement of orbital orthogonality.[30]

Conclusions

It seems that deorthogonalized CNDO/S all-valence-electron computations can serve as a useful interpretative model for the lowest energy features of MCD spectra. Experimentally our instrumentation is adequate to measure the MCD spectra of low-symmetry organic molecules at least down to 240 nm and, under favorable signal-to-noise conditions, to estimate MCD bands below 240 nm. The model calculations interpret the large 290 nm peak (+) of indoles as due mainly to coupling between low-lying π to π^* excited states and higher energy σ to π^* excitations occurring around 200 nm via the magnetic field. While the π electron only (PPP) methods of Warnick and Michl and Miles and Eyring[27] do a good job for π–π^* excitations, our results indicate a need to include σ to π^* excitations as well. We interpret 5-hydroxy substitution as causing an increase in intensity of the second (−)270 nm band by changing the direction of one of the σ to π^* *magnetic* transition moments, and by raising the occupied energy levels upon substitution of a more electronegative oxygen atom to the heterocyclic ring system, a red shift of the 290 nm (+) first band is obtained. We urge the adoption of a convention of computing MCD B terms at the molecular center of mass, although repeated computation at several origins is a useful check on the validity of computed values. We have used this model to interpret the new MCD spectrum of serotonin obtained with our instrumentation.

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References and Notes

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