Polarized Absorption Spectra of Crystals of Indole and Its Related Compounds

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The polarized absorption spectra of crystals of indole derivatives have been measured in order to confirm the presence of two types of electronic transitions, ^1L_b and ^1L_a, in the long-wavelength absorption region, and in order to determine the direction of their transition moments. The polarization spectra show that the ^1A—^1L_b electronic transition forms a sharp absroption band with vibrational structures lying below a broader absorption band of the ^1A—^1L_a transition. The transition memont direction of the ^1L_b makes an angle of 54° to the long axis of the molecule, while that of the ^1L_a lies at an angle of −38° to the same axis. These results were compared with the results of theoretical calculations of the P-P-P SCF MO-CI method.

The excited states of indole and its related compounds have been studied by many workers by means of absorption and emission spectral measurements in solution.[1-8] The absorption band of indole and tryptophan in the region of 40000—32250 cm^{-1} (250—310 nm) is composed of two different types of electronic transitions.[1,2] One is ^1A—^1L_b, with maxima at 34800 cm^{-1} (287 nm) and 35850 cm^{-1} (279 nm), while the other is the ^1A—^1L_a type forming a broad band with a maximum at 36200 cm^{-1} (276 nm). We have studied the polarized absorption spectra of crystals in order to clarify the vibrational structures of the ^1L_b and ^1L_a electronic levels and to find the direction of their transition moments. This study may be important in connection with the circular dichroism and chromophore interactions of tryptophan and its derivatives.

Experimental

The ultraviolet absorption spectrum of the solution was measured by means of a Carl-Zeiss PMQ-II-type spectrophotometer. The absorption spectra of crystals were obtained by the use of the microspectrophotometer described previously.[9]

Materials. The indole was of a special grade from the Tokyo Chemical Industry Co. and was purified by recrystallization from an ethanol-water mixture and by column chromatography. The crystal used for the spectral measurement was obtained from a methylcyclohexane solution. The 3-indolylacetic acid was purchased from the Nakarai Chemical Co. and was purified by recrystallization. The crystal obtained from chloroform had a form of diamond-shaped plates. The glycyl-L-tryptophan was of a pfs grade purchased from the Sigma Chemical Co. and was used without further purification. The glycyl-L-tryptophan dihydrate was obtained in the form of plate-like crystals from water-methylcellsolve solutions.

Results and Discussion

Absorption Spectra of Crystals. **1)** *Indole*: The crystal structure of indole has not yet been determined. The crystal used for the absorption measurement had an elongated plate-like shape and was thin enough to have an adequate optical density for the spectral measurement. Figure 1 shows the absorption spectrum of the indole crystal produced by a light polarized along the *X*-axis (the vibration direction of the faster ray), which shows maxima at 34250 cm^{-1} (292 nm), 35100 cm^{-1} (285 nm) and 36350—37000 cm^{-1} (275—270 nm), and one produced by a light polarized along the *Z*-axis (the vibration direction of the slower ray), which shows peaks at 34500 cm^{-1} (290 nm), 35350 cm^{-1} (283 nm) and 36650 cm^{-1} (273 nm). These vibrational peaks show a good correspondence to those of the solution spectrum.[1] The 0-0 absorption band of the indole

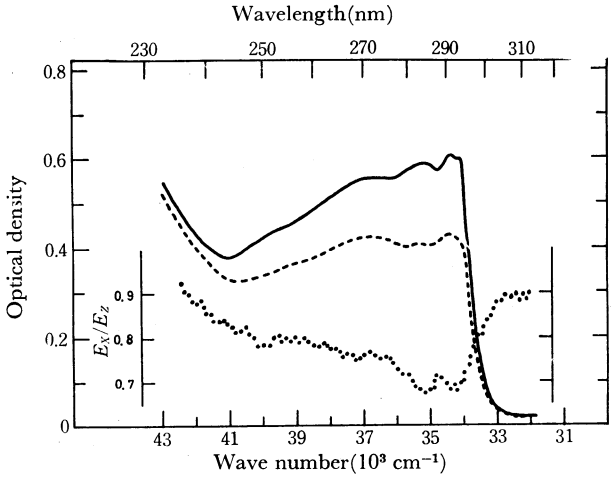

Fig. 1. The polarized absorption spectrum of the single crystal of indole.
The solid and broken lines show the spectrum parallel to the *Z*-axis and the *X*-axis in the crystals respectively. The dotted line indicates the polarization ratio (E_x:E_z). The polarization ratio in the 32400—33700 cm^{-1} region was obtained using a thicker plate which had the optical density of 0.75 at 33400 cm^{-1} for the absorption parallel to the *Z*-axis.

1) S. V. Konev, "Fluorescence and Phosphorescence of Proteins and Nucleic Acids", Plenum Press, New York, N. Y. (1967).
2) G. Weber, *Biochem. J.*, **75**, 335 (1960).
3) H. Zimmermann and H. Joop, *Ber. Bunsenges. Phys. Chem.*, **65**, 61 (1961).
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5) N. Mataga, Y. Torihashi, and K. Ezumi, *Theor. Chim. Acta* (Berl.), **2**, 158 (1964).
6) G. S. Kembrovskii, V. P. Bobrovich, and S. V. Konev, *Zh. Prikl. Spektrosk.*, **5**, 695 (1966).
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crystal is red-shifted nearly 600 cm^{-1} relative to that of indole in *n*-hexane. In order to compare the absorption intensity produced by the light along the *X*-axis with that produced by the light along the *Z*-axis, the polarization ratio ($E_x : E_z$) as a function of the wavenumber was plotted. In the region of 32350 to 33500 cm^{-1} (309 to 298 nm), the polarization ratio has a higher value, 0.9. With an increase in the wavenumber, the polarization ratio is markedly reduced, having its minimum at 34100 cm^{-1} (293 nm). In the 35450—36750 cm^{-1} (283—272 nm) range, there is an increase in the polarization ratio and it has a maximum at 37000 cm^{-1} (270 nm). Thus, the variation in the polarization ratio suggests that the long-wavelength absorption band consists of two different electronic transitions, ^1A—^1L_b and ^1A—^1L_a, and that the former transition forms a sharp absorption band lying below a broader absorption band due to the latter type of transition. The constancy of the polarization ratio in the 32350—33500 cm^{-1} region and in the 37000—40800 cm^{-1} region indicates that the absorptions in these regions are composed of mainly the ^1A—^1L_a transition. The reduction in the polarization ratio in the range from 33500 to 34100 cm^{-1} may be due to the increase in the contribution of the ^1A—^1L_b transition. Therefore, it is clear that the first two vibrational maxima at 34250 and 35100 cm^{-1} for the *X*-axis (34500 and 35350 cm^{-1} for the *Z*-axis) belong to the ^1A—^1L_b transition and that the broad band near 36350—37000 cm^{-1} for the *X*-axis (36650 cm^{-1} for the *Z*-axis) to the ^1A—^1L_a transition. These results are in good correspondence to the results obtained for the absorption polarization spectrum of the fluorescence of tryptophan and indole solutions.[1,2,10]

2) 3-Indolylacetic Acid: According to Karle *et al.*,[11] the crystal belongs to the space group of $P2_1/c$, with four molecules per unit cell. The (100) face develops, and the projection of molecules onto this face is shown in Fig. 2(a). The molecules form a dimeric structure with hydrogen bonds between the acid groups; the NH group in indole is not involved in any hydrogen bonding. The crystalline absorption spectrum was measured with the (100) face by a light polarized along the *b* axis and the *c* axis. Figure 2(b) shows the ^1L_b and ^1L_a absorption bands with six or more vibrational structures. The absorption peaks were clearly seen at 32940, 33550, 34600, 35400, 37250, and 38750 cm^{-1} (303.5, 298, 289, 282.5, 268.5, and 258 nm) in the absorption spectrum by means of the light polarized along the *b* axis, while they are clearly seen at 33000, 33620, 34540, 35340, 36950, and 38050 cm^{-1} (303, 297.5, 289.5, 283, 271, and 263 nm) in that along the *c* axis. The long-wavelength edge of the absorption band in the crystal is displaced by about 1500 cm^{-1} as compared with that in solution. At room temperature, it is difficult to guess accurately the band splitting: therefore, we have examined the peaks with care. The observed band splittings ($\nu_{//c}$—$\nu_{//b}$) are 60, 70, −60, −60, −300, and −700 cm^{-1} for the first, second, third, fourth, fifth, and sixth bands respectively. The experimental error is within 25 cm^{-1}. The band splittings of the higher bands are reversed in sign in comparison with the first two bands; this may suggest that the splitting of the band series is mainly due to the electronic transition of the ^1A—^1L_b type and that the higher bands may be affected by the ^1A—^1L_a electronic transition. The polarization ratio ($E_c : E_b$) against the wavenumber is shown in Fig. 2(b). In the 30600—32000 cm^{-1} (327—312 nm) region, the polarization ratio has a constant value of 0.9, which indicates that the absorption band in this region is due to the ^1A—^1L_a transition. The increase in the polarization ratio in the 32150—33200

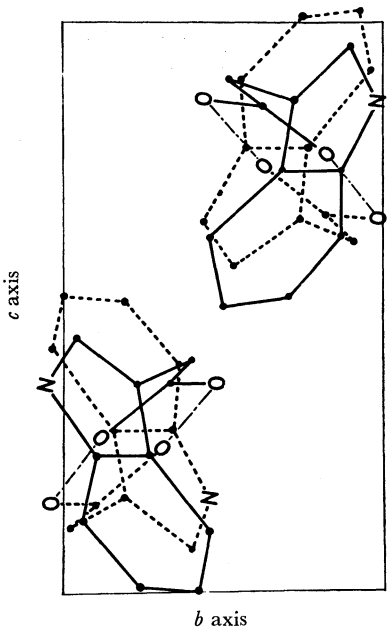

Fig. 2(a). Projection of the four molecules in the unit cell of 3-indolylacetic acid onto the (100) plane.

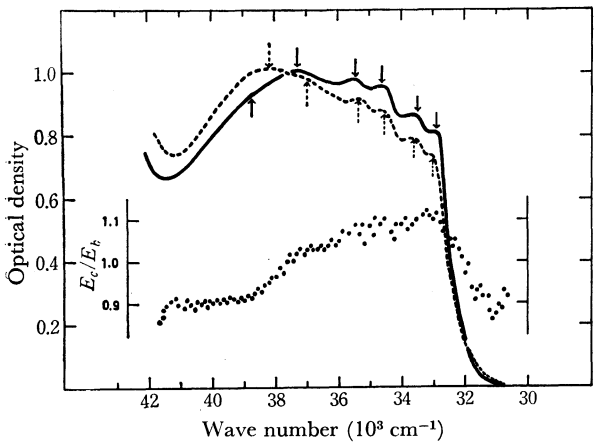

Fig. 2(b). The polarized absorption spectrum in the (100) plane of the single crystal of 3-indolylacetic acid.
The solid and broken lines show the spectrum parallel to the *c* axis and the *b* axis respectively.
The dotted line indicates the polarization ratio (E_c:E_d). The polarization ratio in the 30800—32200 cm^{-1} was obtained using a thicker plate which had the optical density of 0.62 at 32000 cm^{-1} for the absorption parallel to the a axis.

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11) I. L. Karle, K. Britts, and P. Gum, *Acta Crystallogr.*, **17**, 496 (1964).

cm^{-1} (311—301 nm) region reflects the gradual increase in the contribution of the ^1A—^1L_b transition, while the maximum at 32900 cm^{-1} (304 nm) corresponds to the 0–0 band of the ^1L_b transition. The increase in the ^1L_a transition may be responsible for the reduction in the polarization ratio from 34500 to 37700 cm^{-1} (from 290 to 265 nm). Above 37600 cm^{-1} (266 nm) the polarization ratio retains a nearly constant value of 0.9, which shows that the absorption band in this region may be predominantly due to the ^1A—^1L_a transition. The overlapping of the ^1A—^1L_b and ^1A—^1L_a transitions in the long-wavelength absorption band makes it difficult to determine the transition-moment directions of the ^1L_b and ^1L_a states. However, it may be possible to resolve the observed absorption spectrum into two types of electronic transitions, ^1A—^1L_b and ^1A—^1L_a, because the location and the vibrational structures of the ^1A—^1L_b transition have already been determined. The procedure of dividing the long-wavelength absorption band into the ^1L_b and ^1L_a bands was as follows. The absorption band below 32000 cm^{-1} and that above 37500 cm^{-1} may be due to only the ^1A—^1L_a transition; therefore the absorption band due to the ^1A—^1L_a transition was supposedly figured in the 32000—37500 cm^{-1} region to be linked with the absorption curves in the former and the latter regions. Thus, the *b*-axis polarized absorption band due to the ^1L_a transition was obtained. Then, the *c*-axis polarized absorption band originated from the same transition was obtained in the same way; the polarization ratio is 0.9 ($E_c : E_b$). The absorption band belonging to the ^1A—^1L_b transition was determined as the rest of the band after the ^1L_a band was subtracted from the observed spectrum. The thickness of the crystal could not be reliably estimated, and this made it impossible to obtain the oscillator strength. Nevertheless, it is possible to obtain the oscillator strength ratio (f_c^{exp}/f_b^{exp}) by integrating the observed absorption spectra parallel to the *b* and *c* axes. The direction of the transition moment of the ^1L_b and ^1L_a states was determined by the use of the corresponding oscillator strength ratios, namely, $f_c^{exp}(^1L_b)/f_b^{exp}(^1L_b)$ and $f_c^{exp}(^1L_a)/f_b^{exp}(^1L_a)$ respectively. The transition-moment direction of the ^1L_b state makes an angle of $+57$ or $-57°$ to the *b* axis, while that of the ^1L_a state lies at an angle of $+42$ or $-42°$ to the same axis.

3) Glycyl-L-tryptophan Dihydrate: The crystal structure of glycyl-L-tryptophan dihydrate has been determined by Pasternak.[12] The crystal is monoclinic, and the space group is $P2_1$, with two molecules per unit cell. The projection of the molecules in the unit cell onto the (001) plane, which is the developed face, is shown in Fig. 3(a). The optical properties of the crystal had been described in the literature,[12] and the axes were easily determined by a polarization microscope. The crystalline absorption spectra of glycyl-L-tryptophan dihydrate are shown in Fig. 3(b), together with the polarization spectrum. The crystalline absorption spectra obtained by the light polarized parallel to the *a* axis and the *b* axis have maxima at 33100, 34000, 35000, and 36100 cm^{-1} (302, 294, 285.5, and 277 nm), and

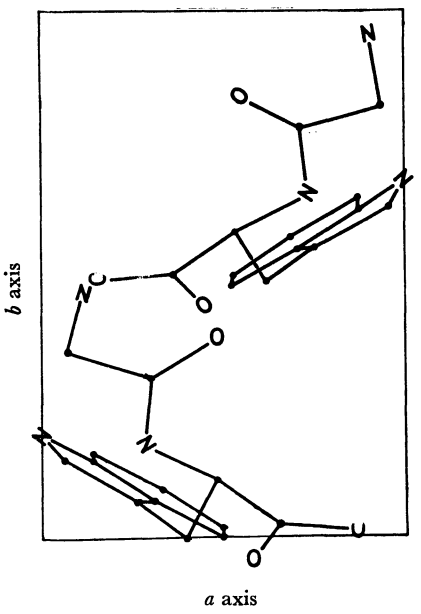

Fig. 3(a). Projection of the two molecules in the unit cell of glycyl-L-tryptophan dihydrate onto the (001) plane.

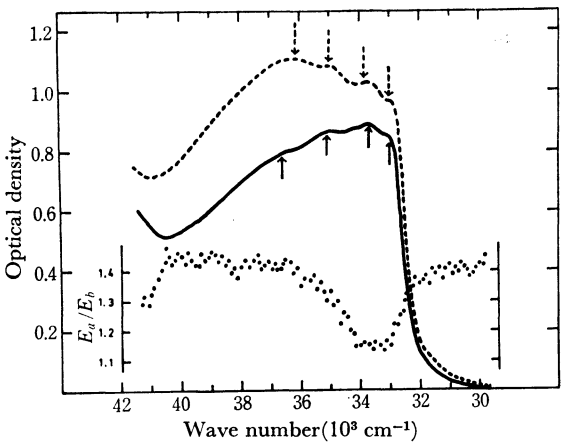

Fig. 3(b). The polarized absorption spectrum in the (001) plane of the single crystal of glycyl-L-tryptopahan dihydrate. The solid and broken lines show the spectrum parallel to the b axis and the a axis respectively. The dotted line indicates the polarization ratio (E_a:E_b). The polarization ratio in the 29800—32300 cm^{-1} was obtained using a thicker plate which had the optical density of 0.78 at 32000 cm^{-1} for the absorption parallel to the *a* axis.

33000, 33800, 35100, and 36750 cm^{-1} (303, 295.5, 285, and 272 nm) respectively. These absorption peaks are in good correspondence with those of the solution spectrum. The band splittings of the first two bands are reversed in sign compared with those of the higher bands, similar to the case of 3-indolylacetic acid. The polarization ratio ($E_a : E_b$) changes with the variation in the wavenumber, as is shown in the case of the indole or 3-indolylacetic acid crystal. In the 30000—31600 cm^{-1} (333—317 nm) region, the polarization ratio is calculated to be nearly 1.4. With an increase in wavenumber, the polarization ratio is sharply reduced; it has its minimum value of 1.15 at 33300 cm^{-1} (300 nm). In the range from 33900 to 36000 cm^{-1} (295 to 278 nm),

12) R. A. Pasternak, *Acta Crystallogr.*, **9**, 341 (1956).

there is an increase in the polarization ratio, and it has its maximum value of 1.4 at 36500 cm^{-1} (274 nm). Thus, the ^1A—^1L_b transition appears at from 31600 to 36350 cm^{-1} (316 to 275 nm), overlapping with the ^1A—^1L_a transition. This overlapping of the ^1L_b and ^1L_a transitions makes it difficult to determine the corresponding transition-moment directions. Therefore, the observed absorption spectrum was resolved into the bands due to the ^1A—^1L_b and the ^1A—^1L_a transitions in the same way as has been described in the case of the 3-indolylacetic acid crystal. With the use of the oscillator strength ratio of the ^1L_b transition ($f_a^{exp}(^1L_b)$: $f_b^{exp}(^1L_b)$) and that of the ^1L_a transition ($f_a^{exp}(^1L_a)$: $f_b^{exp}(^1L_a)$), the corresponding transition-moment directions were estimated. The direction of the transition-moment of the ^1L_b state makes an angle of +41 or −41° to the *a* axis, while that of the ^1L_a state lies at an angle of +30 or −30° to the same axis. When the transition-moment direction obtained from glycyl-L-tryptophan dihydrate was compared with that obtained from 3-indolylacetic acid, it was clear that the direction of the angle of −41 and −30° to the *a* axis in the former crystal coincides with the direction of the angle of −57 and +42° to the *b* axis in the latter crystal for the ^1L_b and ^1L_a states respectively. The experimental error is within 5°.

Determination of the Transition-moment Direction of the ^1L_b and ^1L_a States. According to the polarized absorption spectra of glycyl-L-tryptophan dihydrate and 3-indolylacetic acid crystals, the directions of the transition moments of the ^1L_b and ^1L_a states were determined to be as is shown in Fig. 4. The transition-moment direction of the ^1L_b and ^1L_a states determined from the 3-indolylacetic acid crystal, which is oriented −57 and +42° to the *b* axis, makes angles of +49 and −42° to the long axis of the indole molecule respectively. On the other hand, the orientations of −41 and −30° to the *a* axis, as determined from glycyl-L-tryptophan dihydrate crystal, corresponds to angles of +58 and −35° to the long axis of the indole molecule for the ^1L_b and ^1L_a states respectively. Thus, the orientation of the ^1L_b state makes an angle of +58 or +49° to the long axis of the molecule, while that of the ^1L_a state lies at an angle of −35 or −42° to the same axis. These results

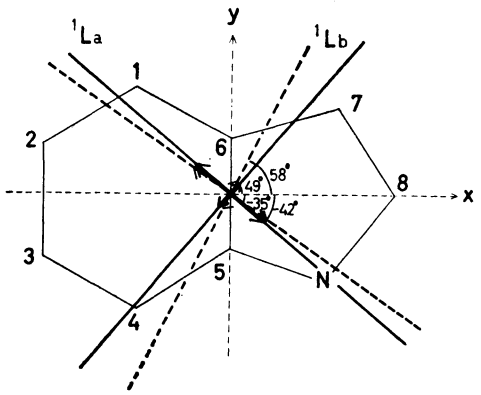

Fig. 4. Transition moments of the ^1L_b and ^1L_a states determined experimentally. The solid line was obtained by the ploarized absorption spectrum of 3-indolylacetic acid and the broken line by that of glycyl-L-tryptophan dihydrate.

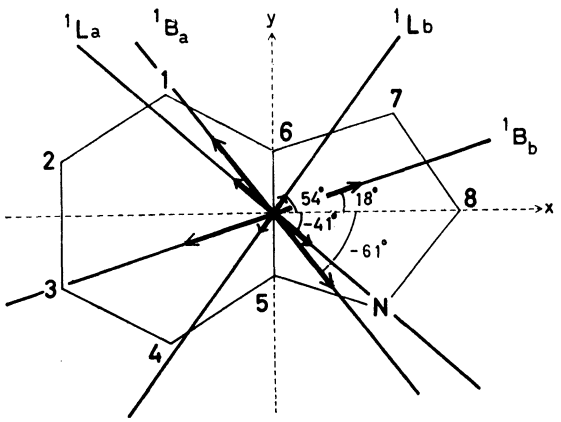

Fig. 5. The theroretical predictions of the transition moments of the four lowest excited singlet states (^1L_b, ^1L_a, ^1B_b, and ^1B_a) of indole.

are in satisfactory agreement with the theoretical orientations of +54 and −41° for the ^1L_b and ^1L_a states respectively, as is shown in Fig. 5. The mutual orientation angle between the ^1L_b and ^1L_a states is about 92° in both molecules, which is in reasonable agreement with the results obtained from the absorption polarization of the indole fluorescence.[1,8] The long-wavelength absorption spectrum of tryptophan in an aqueous solution is shown in Fig. 6, where the proposed resolution into the ^1A—^1L_b and ^1A—^1L_a bands is given. The location and absorption intensity of the ^1A—^1L_b transition were estimated with the help of the results described hitherto. The experimental oscillator strength (f^{exp}) may be obtained from the following equation, $f^{exp}=4.33\times10^{-9}\times\int\varepsilon(\sigma)\,d\sigma$, where $\varepsilon(\sigma)$ is a molar absorption coefficient and where σ means the wavenumber (cm^{-1}). Using the resolved absorption bands shown in Fig. 6, we have found the experimental oscillator strengths to be 0.010 and 0.112 for the ^1A—^1L_b and ^1A—^1L_a transitions. With these experimental oscillator strengths, the magnitude of the transition moments ($|r_{mn}|$) was estimated by means of this equation: $f=1.085\times10^{-5}\times\sigma\times|r_{mn}|^2$. They are 0.15 Å and

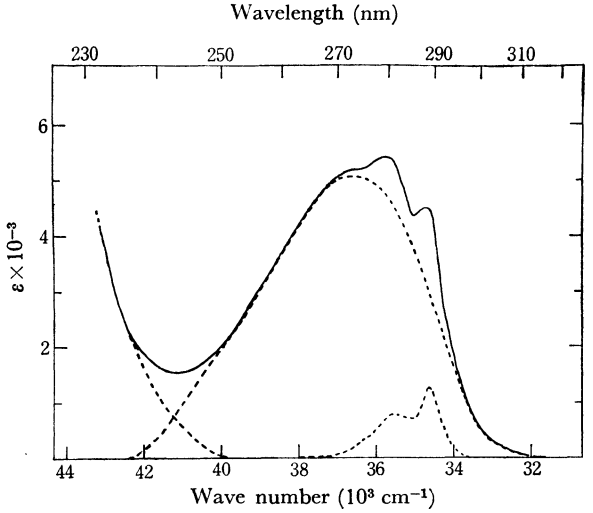

Fig. 6. The absorption spectrum of tryptophan in aqueous solution, showing the proposed resolution into the ^1L_b and ^1L_a absorption bands.

TABLE 1. THEORETICAL PREDICTIONS OF THE SINGLET EXCITED STATES OF INDOLE[a)]

State symbols	State energy[b)]	Transition-moment length		Oscillator strength	Orientation angle[d)]
		r_x[c)]	r_y[c)]		
^1L_b	4.51 (4.4)[e)]	0.161 Å	0.186 Å	0.024 (0.010)	54° (54°)
^1L_a	4.72 (4.6)	0.427 Å	−0.368 Å	0.133 (0.112)	−41° (−38°)
^1B_b	5.82 (5.8)	0.990 Å	0.320 Å	0.553 (0.680)	18°
^1B_a	6.49 (6.2)	0.668 Å	−0.864 Å	0.676	−61°

a) The calculated values were obtained by the P–P–P SCF MO–CI method.
b) The state energies are given in the electron volt (eV) unit.
c) The coordinate designations of x and y correspond to the long and short axis of the indole molecule, as is shown in Fig. 4 or 5.
d) The angle of the transition moment to the long axis (the x axis) of the molecule.
e) The values in the brackets are the experimental results.

0.54 Å for the ^1A—^1L_b and ^1A—^1L_a transitions respectively; these values are a little smaller than the results obtained from the theoretical calculations (0.25 Å and 0.57 Å).

Theoretical Calculation. The P–P–P SCF MO–CI calculations of indole has been done by many workers,[8,13–15] but their results were still not in satisfactory agreement with the experimental results. Momicchioli and Rastelli[13] used only four singly-excited configurations; later Song and Kurtin[8] employed a considerably larger number of configurations (20—25) in their P–P–P SCF MO–CI calculations. Their results on the polarizations are in reasonable agreement with the experimental results, but those on the transition energies and oscillator strengths are not so satisfactory. Recently, Evleth[15] made calculations employing 10 singly-excited and 5 additional doubly-excited configurations. His results on transition energies and oscillator strengths agree well with the observed ones; nevertheless, the transition-moment directions are inconsistent with the experimental results. We have considered all singly-excited configurations in the P–P–P SCF MO–CI calculation of indole and used the geometrical structure of the molecule taken from the data of the Xray analysis of glycyl-L-tryptophan dihydrate[12] or 3-indolylacetic acid crystal.[11] The Coulomb, resonance, and one-center repulsion parameters incorporated in this calculation were the same as have usually been used.[14] The values of the resonance integral at any distance were estimated by the following equation[16]:

$$\beta(r) = \beta^\circ \exp[4.599(1.397 - r)]$$

The results of our calculation for the four lowest singlet-excited states are listed in Table 1, where the state energies, the oscillator strengths, the transition-moment lengths, and the angles of the transition moments to the long axis of the molecule are presented. The calculated spectral features of the ^1A—^1L_b and ^1A—^1L_a transitions are in satisfactory agreement with the observed ones. Although the calculated energies of the ^1L_b and ^1L_a states are a little higher than the experimental ones, the values are in good agreement with the observed spectral characteristic that the ^1L_b and ^1L_a excited states are closely spaced. The experimental polarizations of the ^1A—^1B_b and ^1A—^1B_a transitions are still unknown; however, the calculated transition energies of the ^1B_b and ^1B_a states are reasonably correlated with the observed transition energies.

These calculations were carried out by the use of a computer program written by Dr. Masashi Tanaka of our laboratory, to whom our thanks are due. The calculation was carried out with the HITAC 5050E at the computation center of the University of Tokyo.

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15) E. M. Evleth, *Thoer. Chim. Acta* (Berl.), **16**, 22 (1970).
16) T. Nakajima, "Molecular Orbitals in Chemistry, Physics, and Biology," ed. by P. O. Löwdin and B. Pullman, Academic Press, New York (1964), p. 454.