A SELF-CONSISTENT-FIELD STUDY OF TRYPTOPHAN

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ABSTRACT The aromatic amino acid, tryptophan, was studied with the semi-empirical self-consistent-field method. Ground-state bond lengths and π-election charge densities as well as the energies and transition moment directions for the first three absorption transitions were calculated. Published experimental work indicates that the indole part of tryptophan may enter into excited state interactions with neighboring solvent molecules; since this interaction will lead to emissive properties different from those of absorption, the present work includes a calculation of the transition moment direction for light *emission* by tryptophan.

INTRODUCTION

Tryptophan is a rather unique amino acid because it has the most intense UV absorption and the lowest absorption frequency among these compounds. These two features make a tryptophan residue a likely sink for energy transferred by the "resonance-transfer" process, the latter being of considerable importance in determining the nature of radiation inactivation of proteins (cf. Förster, 1960 and McRae and Kasha, 1964). A reasonable description of energy transfer, however, requires knowledge of: (*a*) ground-state properties (since these will determine the inter-residue separations and interactions in a normal peptide prior to light absorption), (*b*) of transitions other than the lowest (since molecules in their higher energy states may interact with solvent or other solute molecules) (Walker et al., 1966, 1967), and (*c*) of absolute transition moment directions and magnitudes (since resonance energy transfer can be described as the result of a multipole-multipole interaction) (McRae and Kasha, 1964; Craig and Thirunamachandran, 1964).

Direct experimental investigations of *a*, *b*, and *c* are difficult; thus, data are lacking. Preiss and Setlow (1956) and Yeargers and Augenstein (1968) have reported on vacuum UV studies of amino acids and proteins while Weber (1960), Chernitskii et al. (1963, 1965), Zimmerman and Joop (1961), and Lynn and Fasman (1968) have investigated the fluorescence polarization of indole and tryptophan. Walker et al. (1966, 1967) have investigated excited state interactions of indole with solvents.

I report here the results of a semiempirical self-consistent-field (SCF) molecular-orbital investigation of these properties of tryptophan, almost equally applicable to the similar compound indole, in the Pariser-Parr-Pople approximation using configuration interaction of all singly excited states. The general method has been described in great detail by others (e.g., Parr, 1963).

METHOD AND PARAMETERS

The semiempirical SCF method as normally applied starts with a reasonably accurate quantum-mechanical description of an arbitrary system, including electron-electron interactions; certain practical simplifications are then applied to make the problem solvable for complicated molecules; finally, a refining technique is used to offset possible inaccuracies introduced by the previous approximations. The simplifications are of two kinds and constitute the Pariser-Parr-Pople modification: first, a molecular wave function is assumed to be a weighted sum of atomic wave functions and second, the complicated integrals inherent in the SCF method are not evaluated by explicit mathematical means but, rather, their values are obtained whenever possible by measurement of the physical quantity which the integrals represent. (This is the "semiempirical" aspect.) The accuracy of the final results frequently can be improved by refining the molecular wave functions through configuration interaction: that is, the final wave functions are considered to be weighted sums of the SCF wave functions. From these final wave functions various electronic properties can be numerically evaluated by a computer—in the present case, a Univac 1108 (Univac Div., Sperry Rand Corp., New York).

The semiempirical SCF method, with configuration interaction, has been used for about a decade and has given highly satisfactory results for a large number of physical problems. Parr (1963) has written a very readable discussion of the important points of the technique.

In the present work, the various integrals were evaluated as follows: (*a*) The ionization potential used for carbon was 11.16 ev, as calculated semiempirically (Dewar and Gleicher, 1966), and that for nitrogen was 24.75 ev—as optimalized along with β_{CN} (see below) to give the best fit for the lowest absorption maximum in a group of ten compounds containing pyrrole rings. The quantity I_N was not computed by the same means as I_C because the hybridization of the indole nitrogen is in question (Clementi et al., 1967). An inductive effect was assumed in order to allow for the methyl group; accordingly, I_C was reduced by 10% at the ring carbon attached to the methyl group in keeping with the data of Allinger and Tai (1965). (In the inductive model the delocalized π-electron cloud from the indole ring is assumed not to extend to the methyl carbon but, rather, the latter is assumed to exert an electrostatic effect on the nearest ring carbon atom.) (*b*) Resonance integrals were optimalized using the absorption spectra (as described in *a*) and were $\beta_{CC} = 2.32$ ev and $\beta_{CN} = 1.85$ ev. (*c*) In separate computer runs, resonance integrals were reevaluated at each iteration according to $\beta_{ij} = \beta_{ij}^{0} \exp [\alpha_{ij} \cdot (P_{ij} - 0.666)]$; where $\alpha_{CC} = \alpha_{CN} = 0.30$, and P_{ij} is the bond order. The exponential dependence of β_{ij} on bond order is quite general and when expanded in series gives an approximately linear dependence as suggested by Nishimoto (1967). Subsequently, bond lengths were calculated using the linear relationships between bond lengths and bond orders given by Dewar and Gleicher (1966). (*d*) Electronic repulsion integrals were evaluated as follows: the two-center terms were calculated from the Mataga-Nishimoto formula (Nishimoto and Mataga, 1957); the one-center repulsion integrals were the theoretical values given by Orloff and Sinanoglu (1965): $\gamma_{NN} = 16.47$ ev and $\gamma_{CC} = 10.69$ ev.

RESULTS

Comparison with Experiment

The predicted π-electron charge densities and bond lengths for the ground state appear in Fig. 1, along with the π-electron dipole moment direction. Fig. 2 shows the absorption spectrum of tryptophan: the vertical lines mark the frequency predicted by the SCF method to be that for maximum absorption and their heights are proportional to the calculated oscillator strengths. Fig. 3 shows the predicted directions for the transition moments corresponding to the absorption bands shown in Fig. 2.[1]

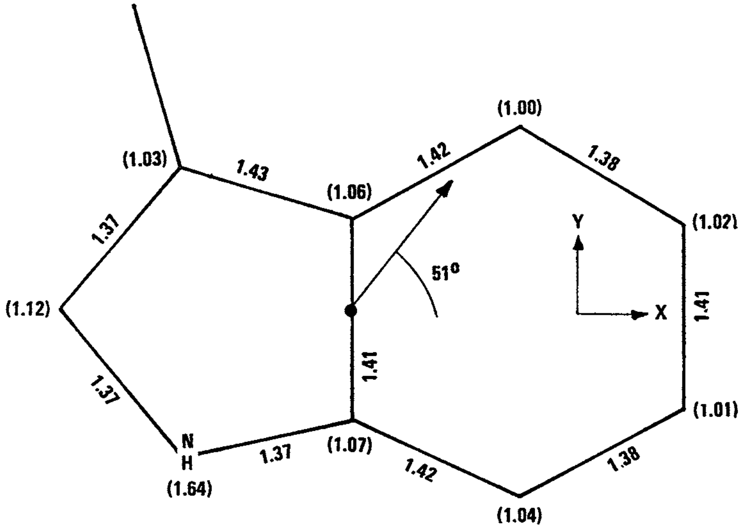

FIGURE 1 The predicted bond lengths in angstroms, π-electron charge densities in units of e, and the π-electron dipole moment (magnitude-2.3 D) for the ground-state of tryptophan.

Bond lengths at the pyrrole end of the molecule agree with crystallographic measurements of pyrrole (Bak et al., 1956). Thus, while the crystallography of indole or tryptophan is not available, the bond lengths in Fig. 1 should suffice for many purposes, e.g., estimating the size of tryptophan or indole to determine interresidue-interaction strengths in polypeptides and to determine if the dipole approximation is valid in a given case.

The three predicted transitions are consistent with the observed absorption spectrum in both intensity and wavelength.

It has been pointed out that the use of fluorescence polarization spectra to resolve

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[1] Henceforth, these same states will be referred to as *SG*, *S*1, *S*2, and *S*3, respectively, instead of using the Platt notation, because of nomenclatural difficulties in determining their orders in emission *and* absorption (see below).

two or more electronic transitions with a single, more or less continuous, absorption envelope can be compromised by several factors, e.g., vibrational interactions and low solvent viscosity (Weber, 1960). The SCF results are, however, in clear agreement with the published fluorescence polarization results mentioned above in which it was concluded that the longest wavelength envelope contains two separate electronic transitions. Weber (1960), in order to explain the large positive fluorescence polarization of tryptophan and indole at 310 mμ, and Mataga et al. (1964), in order to explain the emission red shift of indole in polar solvents, have proposed that fluorescence from indole structures apparently originates from *S*2, which has

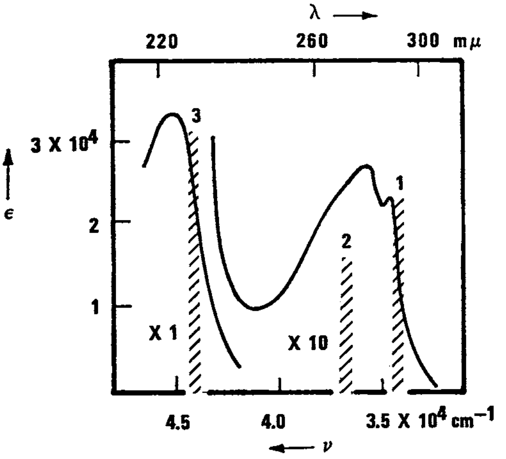

FIGURE 2 Absorption spectrum of tryptophan in water. The energies of Franck-Condon maxima predicted by the SCF method are marked by the vertical lines, the heights of which are proportional to the predicted oscillator strengths. (Changes of pH do not strongly affect the absorption spectrum of tryptophan, Beaven and Holliday, 1952).

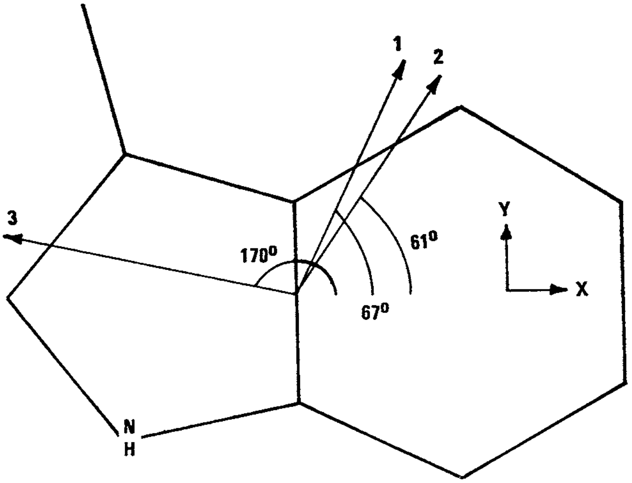

FIGURE 3 The predicted transition moment directions for the first three transitions of tryptophan.

become the lowest excited state in emission. That is, following absorption ($SG \rightarrow S2$), solvent interaction with indole stabilizes $S2$ through dipolar interactions sufficiently to lower its energy below that of $S1$. There are at least two lines of evidence contrary to this proposal, however. First, dipolar stabilization of $S2$ would have to occur before the molecule could radiationlessly relax to $S1$—the latter process taking $\sim 10^{-13}$ sec (the period of a thermal vibration). However, this is *at least* 100 times faster than water molecules can dielectrically readjust to a changed electrical field (e.g., Smyth, 1955 and Hasted, 1961). Other common solvents readjust even more slowly. Secondly, Walker et al. (1966, 1967) have indicated convincingly by means of a kinetic argument that indole forms excited state complexes—called *exciplexes*—with strongly interacting solvents, thus, explaining the emission redshift mentioned above without invoking solvent-solute dipolar interactions. Bobrovich et al. (1966) have given similar results.

Exciplex polarization

It is possible to calculate a probable transition moment direction for the emitting exciplex since the angle θ between absorbing and emitting dipoles is related to the magnitude of the polarization by the following equation (Weber, 1966):

$$P = \frac{3\cos^2\theta - 1}{\cos^2\theta + 3}.$$

Because θ enters in the square of the cosine and because polarization experiments do not distinguish the sign of a vector, the above equation gives four possible directions for a given value of P. These constitute two pairs, each pair comprised of two vectors 180° apart—thus, only two directions are independent. Use of the above equation with Weber's polarization data (Weber, 1960) and the SCF predictions of Fig. 3 indicate that the indole, or tryptophan, exciplex transition moment is located either at 105°–115° or at 10°–40° from the x axis of an undistorted indole molecule (Fig. 3). Both of these assignments lead to the following polarizations: $P_{230} \sim 0.0$, $P_{270} \sim 0.10$–0.20, and $P_{290} \sim 0.10$–0.20. The experimental measurements for tryptophan in propylene glycol at -70°C are $P_{230} = 0.0$, $P_{270} = 0.15$, and $P_{290} = 0.09$.

A large positive polarization of tryptophan in the weakly absorbed region around 310 mμ has been reported as being due to an altered molecular species (Chernitskii et al., 1963). Thus, this species must have a transition moment direction nearly parallel to that for the exciplex or, in fact, may be due to a very small concentration of a ground-state complex resembling the exciplex.

Further Work

This work is being continued in an investigation of excited state-charge densities in order to help determine the likelihood of a charge-transfer interaction of tryptophan and indole with solvents, resulting in exciplex formation. The relative locations of higher singlet and triplet excited states should also shed light on the effects of high-energy-radiation absorption in tryptophan. Knowledge of transition moment strengths and directions will hopefully lead to estimates of the probability of resonance-transfer of energy between tryptophan residues in proteins.

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