

The Absolute Assignment of the Electronic Transitions of $\alpha,\beta,\gamma,\delta$ -Tetraphenylporphine¹

Sir:

Theoretical treatments of the electronic structure of the porphyrins²⁻⁶ have rationalized the observed spectra in terms of four singlet-singlet electronic transitions occurring at wave lengths longer than about 350 m μ . Platt,⁴ interpreting substituent effects through "spectroscopic moments," and Gouterman,^{5,6a} considering the cyclic polyene² and other models, agree on the relative polarizations of these transitions, but disagree on the absolute assignments. The predictions concerning relative polarizations have been confirmed by polarized fluorescence studies on solutions of $\alpha,\beta,\gamma,\delta$ -tetraphenylporphine (TPP),⁷ but absolute assignments

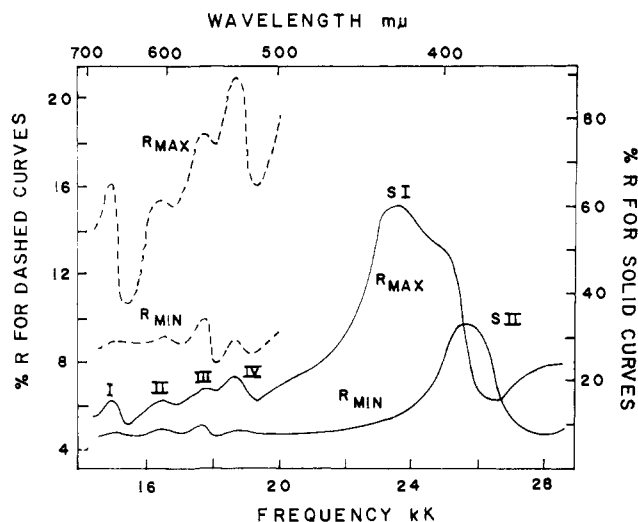


Fig. 1.—The reflection spectra obtained for the (010) face of $\alpha,\beta,\gamma,\delta$ -tetraphenylporphine, the incident light being polarized with its electric vector parallel in one instance to the " R_{\max} " and in the other to the " R_{\min} " principal direction in that face. An expanded plot of the long wave-length spectra is included and the various bands labeled to facilitate reference in the text.

made on a strictly empirical basis have yet to be reported. The recent determination of the TPP crystal structure by Silvers and Tulinsky^{8,9} has raised the possibility of achieving the latter goal through single crystal optical studies. We have thus carried out an investigation in which the techniques of polarized reflection spectroscopy, an approach that is especially suitable for strongly absorbing crystals,¹⁰⁻¹² have been employed.

The TPP used in this work was prepared from a

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highly purified sample of Zn-TPP, and deep purple crystals satisfactory for the optical studies were obtained by recrystallizing several times from very pure dioxane. A microspectrophotometer developed in these laboratories¹³ was used to obtain the polarized reflection spectra for two crystal faces that, in terms of Silvers and Tulinsky's unit cell choice, correspond to the (010) and (001) crystallographic planes. Since the principal directions of these triclinic crystals are not symmetry determined, they had to be found empirically.¹⁴

Figure 1 presents the results for the (010) face. If one recalls that the reflection coefficient rises first to a maximum and then falls to a minimum as one moves through an absorption from the low energy side,¹⁰⁻¹² one may readily correlate the structure found in the R_{\max} curve with the bands in the solution absorption spectrum reported in, for instance, ref. 7a.

Band I, about which the most unambiguous conclusions may be drawn from the present work, appears strongly in the R_{\max} curve of Fig. 1 but is almost undetected in the R_{\min} direction. In relating this observation to the polarization of the corresponding single molecule absorption, TPP has been treated as if it possessed true D_{2h} symmetry. The x symmetry axis was taken as the projection of the line containing the two pyrrole-attached hydrogen atoms on the least-squares plane of the porphine nucleus,⁹ and the y -axis taken as the similar projection of the line containing the other two nitrogens. The projections of a unit vector parallel to the x -axis on the (010) R_{\max} and R_{\min} principal directions are 0.992 and 0.006, respectively, while those of a " y -axis unit vector" are 0.091 and 0.739, respectively. A 100% x -polarized transition would thus exhibit a dichroic ratio [$(R_{\min}$ optical density)/ $(R_{\max}$ optical density)] equal to the appropriate ratio of the squares of the projections, or 0.0004. Since any y -character increases the value of this figure, the near zero observed dichroic ratio noted for the lowest energy TPP band leads one to assign it as being polarized parallel to the "H-H axis," a result which is in accord with the predictions of Gouterman.^{5,6a}

The (001) reflection spectra have permitted experimental verification of the theoretically reasonable assumption of in-plane absorption made in the above argument. Here the R_{\min} direction, which has a large z - (out-of-plane) component and small x - and y -components, possesses a low structureless reflectivity characteristic of a nonabsorbing direction, while the R_{\max} direction, with a small z -component and relatively large x - and y -components, shows all the bands in question.

One may now assign absolutely the remaining TPP bands from the polarized emission data. Since Weigl^{7a} obtained a polarization ratio of 0.30 (instead of the expected 0.50) for the x -polarized band I, Albrecht's procedure¹⁵ for correcting for deviations from ideality has been applied and the results of Table I obtained.¹⁶

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(16) The manner in which Weigl's emission data were obtained^{7a} results in the value of 0.44 for the "randomization factor" probably being an upper limit. A lower value would of course alter the entries in the final column of Table I. It should also be noted that Gouterman and Stryer^{7b} have obtained results for bands I through IV which are similar to Weigl's but more detailed in nature.

TABLE I
PER CENT x INTENSITY IN THE ABSORPTION BANDS
OF $\alpha,\beta,\gamma,\delta$ -TETRAPHENYLPORPHINE^a

Band	x intensity based on uncorrected fluorescence data, %	x intensity based on corrected fluorescence data, % ^b
I	70	100
II	45	55
III	27	22
IV	46	57
SI	54	71
SII	35	37

^a Derived from data of ref. 7a. ^b Corrected as suggested in ref. 15, using a "randomization factor" of 0.44.¹⁶

Although detailed quantitative analysis based on simple inspection of reflection spectra is difficult except in favorable cases, the molecular dichroic ratios listed in Table I seem consistent with the crystal dichroism noted in Fig. 1, and certainly the largely y -character of band III is in accord with the appearance of the R_{\min} curve.

If, accepting the usual interpretation of the porphine spectrum, one takes bands I and II as one electronic transition and bands III and IV as another, and further assumes that bands I and III indicate the intrinsic polarizations, one is now led to assign the first TPP transition x and the second y . Figure 1 and Table I also provide confirmation that the Soret absorption contains two distinct regions of polarization, that of low energy being x and that of high energy y .

The mixed polarizations of bands II, III, and IV indicate that, contrary to the commonly held view, each of these bands is not a single vibronic component, since this latter situation would require unique polarizations. The complex nature of these bands, which has been noted by earlier workers,^{7b,17} is also supported by the additional structure that appears in low temperature solution absorption spectra.¹⁸

This work will be reported in more detail at a later date, as will emission and single crystal absorption work currently being undertaken. It is also intended to extend these investigations to include selected metal porphyrins.

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Introduction of Covalent Cross-Linkages into Lysozyme by Reaction with α,α' -Dibromo- p -xylenesulfonic Acid

Sir:

Extensive studies on the cross-linking of proteins have been carried out. Fibrous proteins¹⁻⁴ and globu-

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lar proteins⁵⁻⁷ have been subjected to cross-linking. In no case were the reagents entirely satisfactory from the point of view of determining intramolecular distances under conditions simulating in part the conditions the proteins are exposed to in their native milieu.

The concept of using a "solubilized" bifunctional reagent of known dimensions was exploited first with various naphtholic and phenolic disulfonyl chlorides⁸⁻¹⁰ and fluorides.¹¹ It was demonstrated that such compounds can react with lysozyme under conditions of homogeneity and that intramolecular bonding had occurred.¹² A tentative assignment of certain intramolecular distances was made.^{10,12}

We wish to report here the synthesis and use of α,α' -dibromo- and α,α' -diiodo- p -xylenesulfonic acid (DBX and DIX). These and their S³⁵-labeled counterparts were synthesized by a variation of the procedures of Karlslake and Huston,¹³ and of Schmid and Karrer.¹⁴

The tritiated material was prepared by exposure of the unlabeled material to tritium by the Wilzbach procedure.¹⁵ DBX (500 γ /ml.) was allowed to react with lysozyme (250 γ /ml. in 0.1 M borate buffer, total volume 1000 ml.) at 37° for 48 hr. at pH 9.1. The treated lysozyme was separated from unreacted DBX on a column of Sephadex G-25 using 0.1 M acetic acid as eluent and was found to emerge as a single peak.

It was homogeneous by ultracentrifugal analysis with essentially the same molecular weight as the native lysozyme. The enzymic activity was not diminished by a significant amount.

Two peaks were apparent by analysis by the Tiseluis technique, neither of which corresponded to lysozyme itself. The relative amount of material in each peak was 70 and 30%. The difference in mobilities between lysozyme and the peak nearest it in mobility was essentially equal to the difference between the mobilities of the two reacted species. This suggests that one and two residues of DBX have been introduced into lysozyme changing the charge on the molecule by two equal increments. Since the peptides A and B were of about equal radioactivity it would appear that all of the lysozyme had reacted with at least one DBX at one of two sites and a smaller fraction had reacted to give the least cationic species (30% of the material) seen in the electrophoretic analysis.

Examination of the tryptic digest of the lysozyme treated with performic acid by peptide mapping revealed some deletions and three additions (peptides A, B, and C) when compared to the lysozyme not exposed to DBX. The new peptides were radioactive when either DBX-S³⁵ or DBX-H³ were used.

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