THE ABSORPTION SPECTRA OF CHLOROPHYLL AND RELATED COMPOUNDS[1]

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The absorption spectra of chlorophyll and homologous compounds are described qualitatively. Included are brief discussions of the contribution of polar species to the ground state, the influence of the spatial orientation of the substituent on the spectra, chelate and acid *vs.* free-base spectra, fluorescence and phosphorescence, and the initial contemporary analyses of the band structure.

Absorption spectra have been the most prominent of the physical properties used for analysis of the so-called "fine" structure of porphyrins,[2] including chlorophyll, and involve such questions as hydrogen isomerism in the porphyrins, nitrogen equivalence of the pyrrole rings, and the arrangement of the double bonds in a typical porphyrin such as chlorophyll. An appreciation of the absorption spectra of the chlorophylls is obtained by comparing them with homologous compounds and with derivatives. This review does not attempt to list all the diverse phenomena and data in this field; rather, a selection has been made of certain absorption spectra studies which appear to be of most significance.

Physical characteristics, such as high melting points and sharpness of absorption bands, and organochemical characteristics, such as substitution rather than addition reactions, unusual stability of the metal chelates, etc., have long ago attested to the "aromatic" character of the porphyrin nucleus. Although the Kekulé type of resonance, as exemplified in benzene and homologs, is assumed by most workers in this field,[3] later studies (2, 14) pointed out the contribution

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[1] Contribution No. 91 from the Institute for Atomic Research and Department of Botany, Iowa State College, Ames, Iowa.

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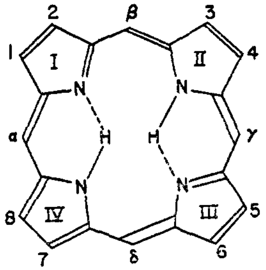
[2] The term "porphyrins" is used to denote all tetrapyrrolic compounds in which the rings are linked by methine carbons in a closed conjugated system. Porphyrins are then subdivided into porphines and chlorines; in the former *none* of the β- or β'-carbons within the rings are reduced, while in the latter, two adjacent carbons (β,β') are thus reduced. Phorbines are a type of chlorine in which an isocyclic ring (carbons 9 and 10) joining C_γ and C_6 is present. Phorbides are chlorines (or phorbines) in which the carbon substituent on C_γ is in a state of oxidation higher than methyl.

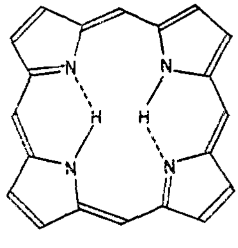
The names "porphin," "chlorin," and "phorbin" appear in much of the literature dealing with these compounds: however, in 1946 *Chemical Abstracts* adopted the "ine" ending, which is therefore used throughout this paper.

[3] A notable exception was H. Fischer and his school. For their arguments, which rely primarily on spectral data, see below. In a variety of cases, of which porphyrins are only a prominent example, the question arises as to the mobility of hydrogen in the hydrogen bond. If mesomeric forms occur in which the movement is over a large distance, we term them tautomers. The two tautomers are distinct compounds, often of sufficient differences of energy so that they can be separated physically. If we can *write* different forms in which there is no movement of the hydrogen, we denote these as structures which contribute to the resonance. If the distance over which the hydrogens can move is so small that separation of the tautomers is improbable because of small energy differences, then we can conceive of a

of dipolar and tetrapolar species to the total structure. A pair of Kekulé resonance structures is depicted by skeletal formulas I and II, while examples of dipolar and tetrapolar species are given in III, IV, and V.



I



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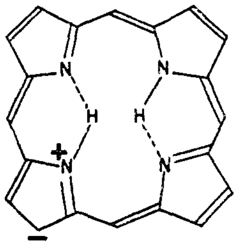

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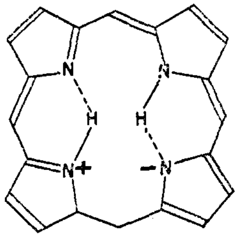

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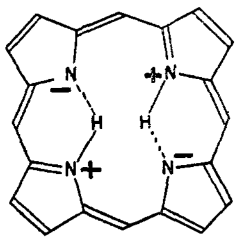

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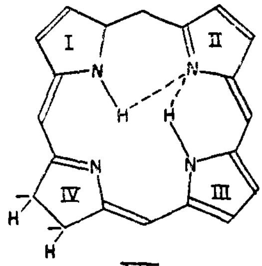

VI

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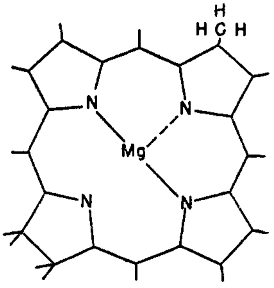
difficulty in distinction between a tautomer and a resonance form. [Although claim has actually been made of the preparation of porphine tautomers (P. Rothemund: J. Am. Chem. Soc. **58**, 625 (1936); **61**, 2912 (1939)), this was shown to be incorrect (S. Aronoff and M. Calvin: J. Org. Chem. **8**, 205 (1943); M. Calvin, R. H. Ball, and S. Aronoff: J. Am. Chem. Soc. **65**, 2259 (1943))]. A true distinction between tautomerism and resonance in this instance is made by Erdman and Corwin (10), although their evidence is not conclusive (see text and accompanying footnote). It was therein concluded that "a hydrogen atom in a porphyrin ring is bonded to a single nitrogen atom at any instant *even though it may be within the field of force of the adjacent nitrogen at the time*. . . . This does not mean that stable opposite and adjacent isomers necessarily exist but simply that the hydrogens remain fixed to specific individual nitrogens for periods of time exceeding the period of existence of an excited form." (Italics, S. A.) In the text no distinction is drawn between such tautomers with extremely low energy requirements for interconversion and the identically depicted resonance-contributing forms.

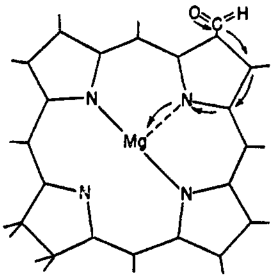
The change in the spectral type on substitution in the porphine ring was associated by Haurowitz *et al.* (14) with the predominance of different ionic species. The substitution of a relatively electropositive alkyl group for hydrogen tends to cause the contribution of species such as III (above) to be more prominent. This probably affords the beginning of an explanation of the effect on the absorption spectra of "adjacent" and "opposite" substitution (see below).

The structure of a chlorine (VI), although bearing some analogies to the above, is not completely analogous, for the nitrogens are no longer equivalent, i.e., the probability of the nitrogen of ring IV forming a homopolar bond with the internal hydrogens is small. Although the homopolar type of resonance occurs, the dipolar type is strongly diminished, the contribution of the nitrogen of ring IV being reduced to a minimum. This relationship may be of importance, e.g., in explaining the difference in the rates of hydrolysis of magnesium from chlorophylls a and b (23, 24), where it has been found that pheophytin a is formed about eight times as rapidly as pheophytin b. The magnesium of chlorines is undoubtedly not held as strongly as that of porphines; according to the above reasoning it would have only a tripole instead of quadripole coördination.



VII

Schematic for chlorophyll a[4]



VIII

Schematic for chlorophyll b[4]

Consequently the effect of an electronegative chromophore, as present in the 2-formyl group of chlorophyll b, in direct conjugation with a pyrrole nitrogen, which would tend to make the magnesium–nitrogen bond stronger, would in turn decrease the rate of hydrolysis. A similar problem, though more difficult and obscure, is presented in the different rates of the phase test undergone by the two chlorophylls.

The porphines, generally forming disalts, exhibit characteristic changes in spectrum from the four-banded to a two-banded spectrum. The general structures must be as given in formula IX and/or formula X.

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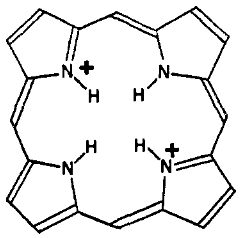
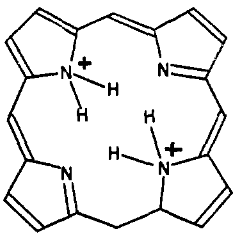
[4] The formulas as indicated are not intended to denote homopolar bonding. Although exchange experiments with radioactive Mg^{+2} and *purified* chlorophylls were negative (S. Ruben, A. W. Frenkel, and M. D. Kamen: J. Phys. Chem. **46**, 710–14 (1942)), they may well be interpreted as in the corresponding negative experiments of radio-Fe^{+3} and ferrihemin (S. Ruben, M. D. Kamen, M. B. Allen, and P. Nahinsky: J. Am. Chem. Soc. **64**, 2297 (1942)) —a lack of exchange because of spatial hindrance. The ionic nature of the magnesium bonding is not, however, proved.



IX



X

The typical free base porphyrin generally displays four main bands in the visible (with occasional secondaries) and a primary band in the near ultraviolet. The bands in the visible are usually numbered I, II, III, IV, beginning with the longest wave lengths. Among the twenty-four possible types of spectra (that is, with varying degrees of prominence of bands) only three occur naturally in the porphines, while a fourth is found in the chlorines (see figure 1). The etio type is characteristic not only of the etioporphines, but of the majority of porphine spectra. We may speculate that deviations from this type of spectra occur only when substitutions in the pyrrole rings cause the density distribution of π-electrons to lie more on one half of the ring than the other (e.g., compare the diethyldimethylporphines in figure 2).[5] This statement will be discussed in somewhat greater detail below. The etio-type spectrum thus occurs in such divergently substituted materials as the monosubstituted etioporphines, the tetramethyl-, tetraethyl-, and octamethyl-porphines, and tetramethylporphine-tetrapropionic acid. It is therefore a distinct surprise to note that the spectrum of porphine itself (figure 3) is of the phyllo type, which normally results either from a substitution within the ring itself, i.e., on a methine carbon as in phylloporphine or by "adjacent" rather than the "opposite" substitutions. The meth-

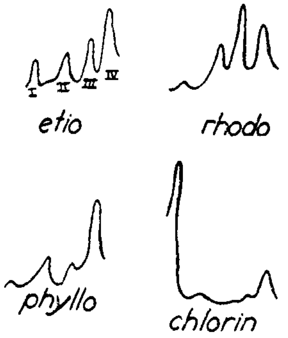


FIG. 1. Types of porphyrin spectra (from Fischer and Orth (11))

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[5] An exception to this generalization appears to exist in the spectra of the dibromodeuteroetioporphines II (2,6-dibromo) and III (2,3-dibromo) (35). In this instance the 2,6-dibromo compound appears to result in a rhodo-type spectrum, while the 2,3-dibromoporphine results in an etio-type spectrum. These data appear, however, to be at variance with the qualitative descriptions of the spectra of the same compounds (11).

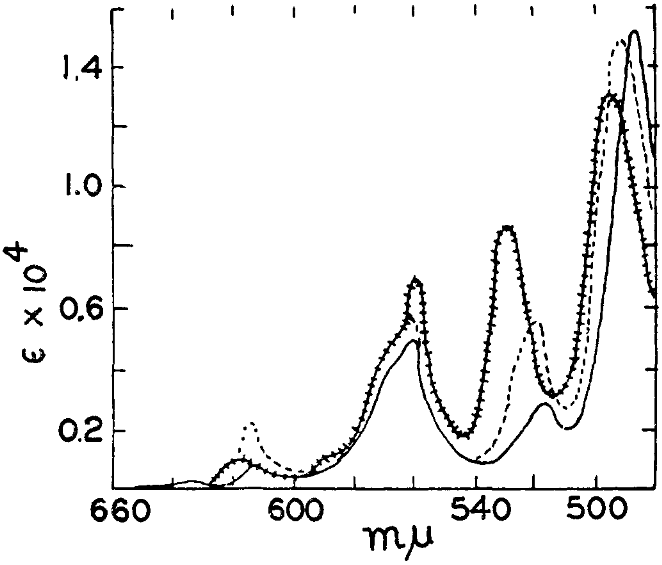


FIG. 2. The similarity of absorption spectra of porphine isomers (from Stern, Wenderlein, and Molvig (40)).——, porphine; - - -, 1,4-diethyl-2,3-dimethylporphine in dioxane; +++, 2,6-diethyl-1,5-dimethylporphine in dioxane.

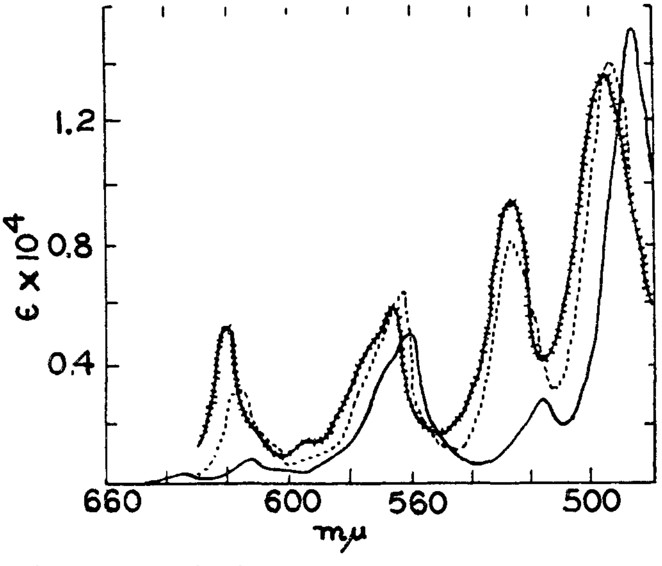


FIG. 3. The abnormality of the absorption spectrum of porphine (from Stern, Wenderlein, and Molvig (40)). ——, porphine; +++, etioporphine I; - - -, deuteroetioporphine II (3-free).

ods of synthesis of porphine in this instance are by no means unequivocal, and the question thus arises as to whether "porphine" may not be a mixture of materials, as was shown to be the case in a similar type of synthesis (1), resulting

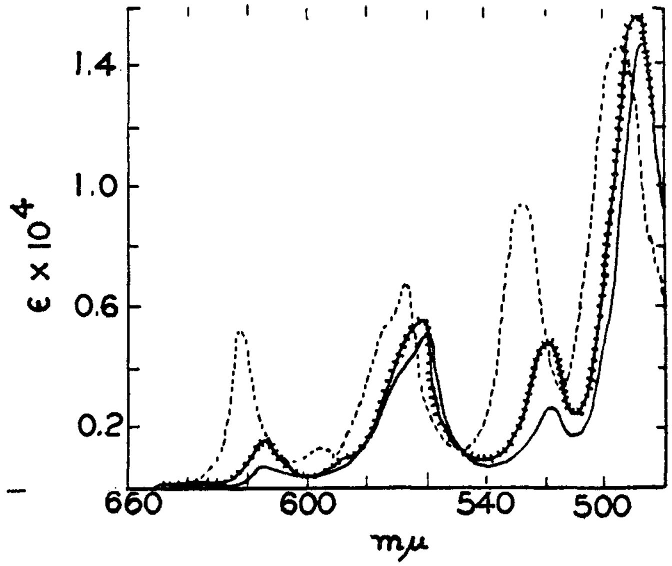


FIG. 4. The phyllo-type spectrum, resulting from "adjacent" substitutions (from Stern, Wenderlein, and Molvig (40)). ——, porphine; ###, methyl ester of porphine-1,4-dipropionic acid; - - -, tetramethyl ester of coproporphine II.

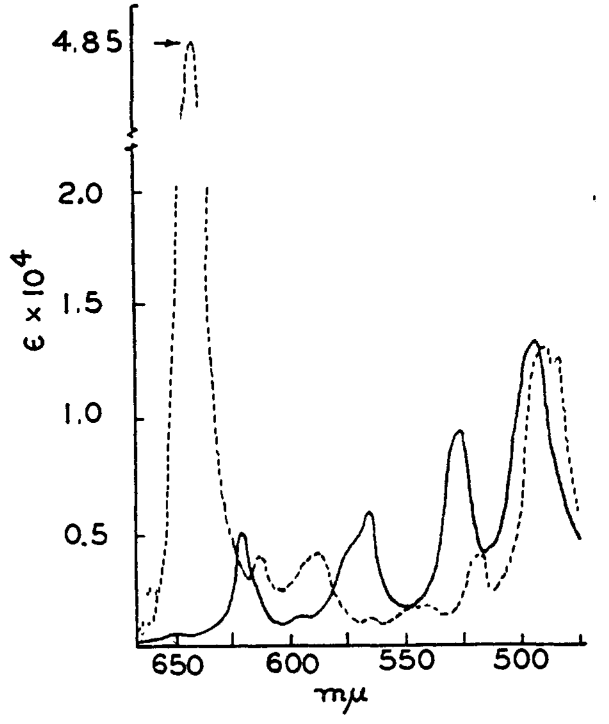


FIG. 5. Absorption spectra of homologous chlorines and porphines (from Pruckner (28)). ——, etioporphine I; - - -, etiochlorine I.

not only in the methine-substituted porphine, α,β,γ,δ-tetraphenylporphine, but its chlorine and at least four other substances also showing porphyrin spectra. The rhodo-type spectrum, like the phyllo, results from "adjacent" substitutions. Thus, e.g., in rhodoporphine (XV) itself, or as in figure 4 in the dimethyl ester of porphine-1,4-dipropionic acid, a rhodo-type spectrum results, while in the tetramethyl ester of the symmetrical porphinetetrapropionic acid, coproporphine II, an etio type results. A typical chlorine spectrum, in contrast to the corresponding porphine spectrum, is shown in figure 5.

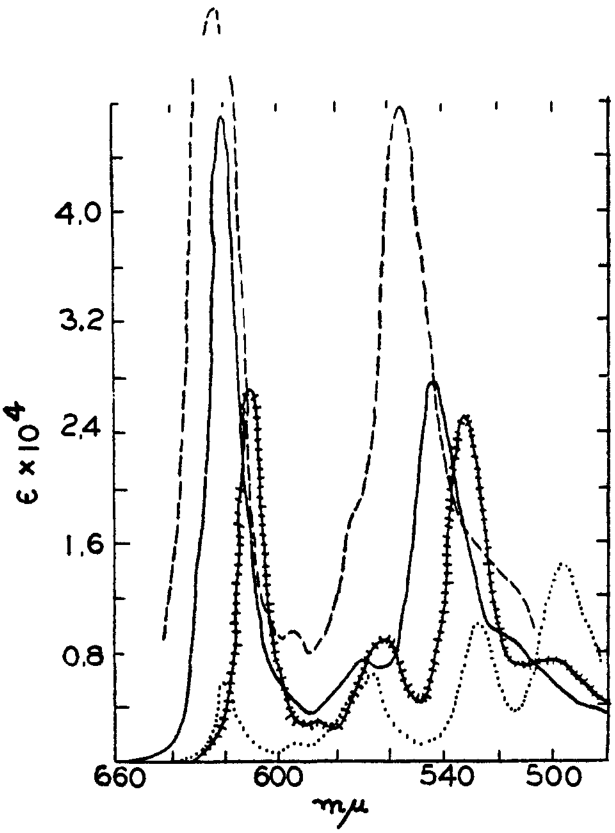


FIG. 6. Absorption spectra of imidoporphines (from Stern and Pruckner (37)). ——, β,δ-diimidoetioporphine II; ǂǂǂ, monoimidoetioporphine II; . . ., etioporphine II; – – –, tetraimidoetioporphine.

That a difference in spectra should occur when substitutions are "adjacent" or "opposite" has been used by Fischer (*loc. cit.*) as an argument against the equivalence of the nitrogens in porphines and for the presence of both pyrrole and pyrrolin rings. This argument could be used with equal facility in another closed conjugated system such as benzene, where, e.g., *m*-dinitrobenzene would be expected, with this reasoning, to have a spectrum identical with that of *o*-dinitrobenzene. A difference in symmetry relationships, however, can result in considerably different spectra, and a similar situation should be found in the porphines.

The substitution of nitrogen for a methine carbon (as in the phthalocyanines) yields a polybanded spectrum (figure 6) considerably different from the porphine type. The intense predominance of two of the four bands, occurring even in the monoimidoporphines, is more apparent in the diimidoporphines and most apparent in the tetraimidoporphines. These differences are emphasized by the increasing absorption coefficients, which are proportional to the number of nitrogens substituted. For example, for the mono-, di-, and tetra-imido compounds, the molar extinction coefficients of the primary red band are, respectively, 2.8 × 10⁴, 4.8 × 10⁴, and (by extrapolation) 9.5 × 10⁴. (A triimidoporphine has not yet been synthesized.)

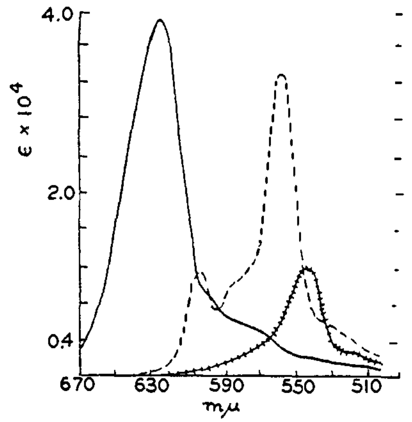


FIG. 7

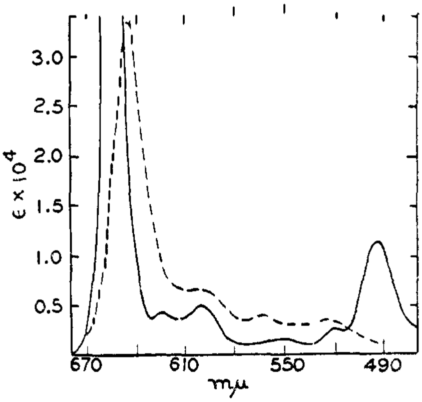


FIG. 8

FIG. 7. Acid spectra of porphines (from Stern and Pruckner (37)). ——, tetramethyl ester of coproporphine II in 3 *N* hydrochloric acid; - - -, tetramethyl ester of β,δ-diimidocoproporphine II in 6 *N* hydrochloric acid; +++, porphine in 6 *N* hydrochloric acid.

FIG. 8. Acid spectra of chlorines (from Stern and Molvig (36)). ——, dimethyl ester of mesorhodochlorine in dioxane; - - -, dimethyl ester of mesorhodochlorine in aqueous hydrochloric acid.

Inasmuch as the porphines are bases in which the basic atoms participate strongly in the excited state, their spectra might be expected to vary with the acidity. Typical acid spectra are shown in figures 7 and 8. Since porphines are generally dibasic (8), it might be expected that spectroscopic investigation of the course of titration of a porphine would reveal the existence of two acid spectra (2). Such is apparently not the case, intermediate spectra appearing to be capable of calculation from the spectra of the free base and the disalt. It is concluded that if the monosalt exists, it does so only over a very short range. Of unique interest is the similarity of the spectra of divalent metal chelates and disalt spectra (10), which also have full equivalence structurally (see figure 9).

One of the more spectacular achievements in porphyrin chemistry was the synthesis of *N*-alkylporphyrins (10, 21). The synthesis of these compounds was performed in an attempt to settle the question of whether a hydrogen isomerism (i.e., a tautomerism) existed, or whether the hydrogens were essentially static and only resonance occurred. It was reasoned that if an *N*-substituted porphine could be synthesized, this substituted nitrogen would not be able to partake in resonance, and the spectrum should be significantly different from that of the normal porphine, e.g., the etio-type spectrum. It might, for example, be similar to that of a chlorine, although in one case the path of the π-electrons would include nitrogen and in the other the β-carbon atoms. It should, at any event, destroy the equivalence of the nitrogens. As shown in figure 10, the absorption spectrum of the free base of *N*-methyletioporphine was very similar to that of the normal bases, though shifted somewhat to the red. The conclusion was therefore reached that tautomerism, rather than resonance, exists in normal porphines, with a very low energy barrier between, e.g., tautomers XI and XII. More specifically, the life of the tautomers is greater than the life of the excited (singlet) state (10).[6]

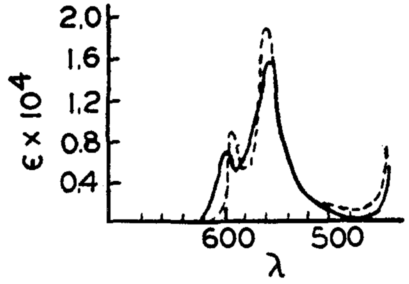


FIG. 9

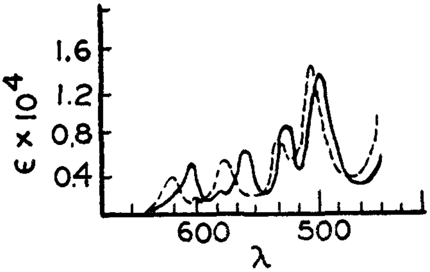
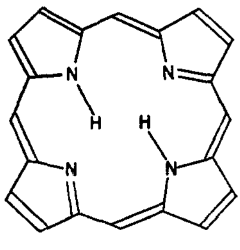


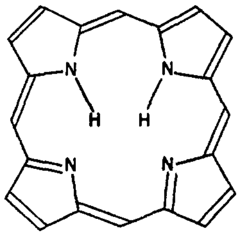
FIG. 10

FIG. 9. Equivalence of salt and monovalent metal spectra of porphines (from Erdman and Corwin (10)). ——, etioporphine *II* dihydrochloride; - - -, disodium salt of etioporphine II.

FIG. 10. Similarity of normal and *N*-methylporphines (from Erdman and Corwin (10)). ——, etioporphine II; - - -, *N*-methyletioporphine II.



XI



XII

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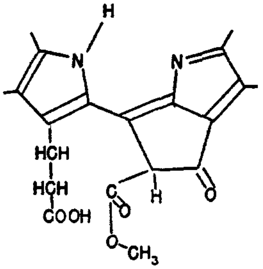
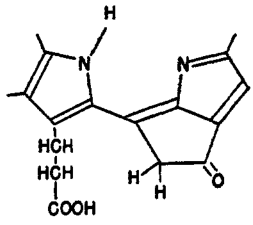
[6] It should be pointed out that even in *N*-methylporphines the Kekulé resonance remains, as does the contribution of the ionic forms. Only tautomerism is partially restricted. Furthermore, since the nitrogens are equivalent, *the remaining hydrogen is still mobile* and tautomerism is still possible. True restriction could occur only if the nitrogens were not equivalent, as in chlorines, or if a di(*N*-methyl)porphine rather than an *N*-methyl, were considered. To some extent this is realized in comparing the metal chelates of the chlorines (see figure 15), which are essentially single-banded, with the corresponding chelates of porphines (figures 12, 13, 14), which are doubly banded.

Numerous instances have been observed in which structural relationships would be expected by analogy to be observed in spectra, but which do not exist. Thus, the dimethyl ester of pheoporphine a_5 should exhibit a spectrum very



Pheoporphine a_5



Phylloerythrine

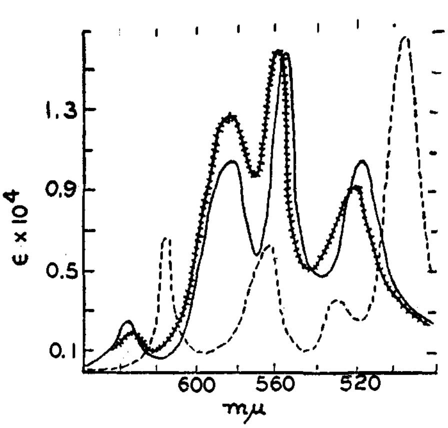


FIG. 11

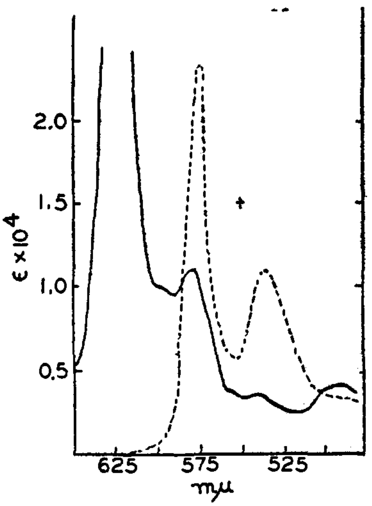


FIG. 12

FIG. 11. Influence of C_{10} substituents on absorption spectra (from Stern (34)). - - -, dimethyl ester of pheoporphine a_5; ——, monomethyl ester of phylloerythrine; ++++, monomethyl ester of desoxophylloerythrine.

FIG. 12. Absorption spectra of metal chelates of corresponding porphines and chlorines (from Pruckner (28)). ——, copper salt of dimethyl ester of mesorhodochlorine; - - -, copper salt of dimethyl ester of rhodoporphine.

similar to that of phylloerythrine, but it does not do so (figure 11). On the contrary, that of desoxophylloerythrine, which might be expected to differ exceedingly from phylloerythrine (because of enolization and other influences), does not. Profound differences, however, do occur in strong acid (2), as would be expected in oxonium-ion formation.

Outstanding among the properties of the porphyrins is the ability to chelate with metals. All classes of metals form porphyrin complexes, which, however, vary considerably in the strength of chelation (and undoubtedly bond type).

Thus, the alkali and the alkaline earth metals are readily removed from porphyrins by acid, substances like zinc are less so, transition metals like iron only with difficulty, while the copper chelates are stable even in concentrated sulfuric acid. The spectra of the synthetic copper and zinc tetraphenylporphines and chlorines (7) indicate similar wave lengths (actually increasing with increasing weight of metal) but with significant differences in numerical value of the absorption coefficient (26). Metal chelates of the chlorins appear to possess a more complex spectrum than the corresponding porphine (see figure 12). Although spectra of copper chelates of porphines are generally two-banded, as compared to their singly banded chlorines (see figures 12, 13, 14), this type of generalization is of little value, not only because it is not true for other classes of porphyrins, e.g., the methine-substituted porphines and for the imidoporphines (figure 15), but also because closer examination of the copper chlorine spectra often reveals within the complexity a second band with as great an absorption coefficient as the weaker of the two in the corresponding copper porphine.

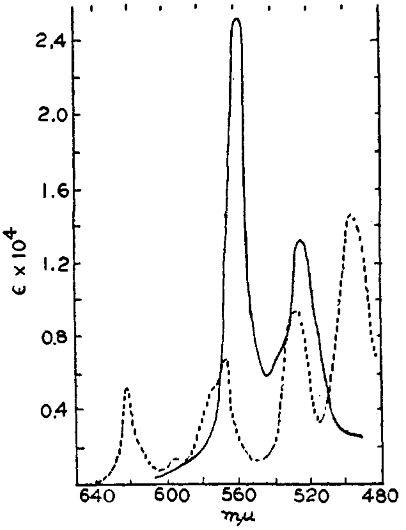


FIG. 13

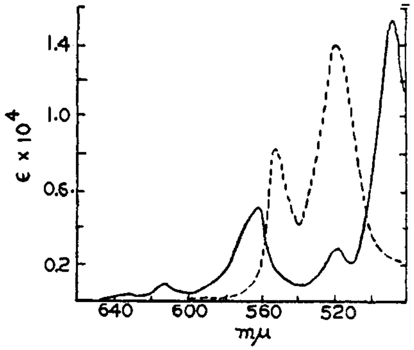


FIG. 14

FIG. 13. Absorption spectra of a typical porphine and its copper chelate (from Stern, Wenderlein, and Molvig (40)). ——, copper salt of tetramethyl ester of coproporphine II; - - -, tetramethyl ester of coproporphine II.

FIG. 14. Absorption spectra of porphine and its copper chelate (from Stern, Wenderlein, and Molvig (40)). ——, porphine; - - -, copper salt of porphine.

Magnesium chelates of closely related porphines and chlorines show a relationship (figure 16) similar to the above.

The spectra for chlorophylls a and b, which were long a subject of controversy, now appear to be a matter of general agreement (43) (see figure 17). Most conspicuous is the extraordinarily high absorption coefficient for chlorophyll a in the red, being *ca.* 9.1 × 10^4 at its maximum, whereas most chlorines or phorbines, or their metal chelates, do not exceed two-thirds this value. Ultraviolet absorption spectra in various solvents are also available (13, 30). Some (4, 22) have believed that the position of the absorption maximum of chlorophyll a was not always a function of the index of refraction of the solvent (Kundt's rule). Mackinney (22) gave examples of additional failures for chlorophyll b in acetone ($N_D = 1.35886$) and dichloroethane ($N_D = 1.45026$) which have identical positions (and the importance of apparent deviations in visual instruments caused by skewness was emphasized). Egle (9), however, disputed Mackinney's findings as the result of investigations at different concentrations of chlorophylls a and b in different solvents (including the above) and attributed Mackinney's results to pheophytin formation. It is of interest to note that the data of Zscheile and Comar (43) on absorption spectra are not in agreement with Egle's, nor does the work (43) on the fluorescence of the chlorophylls (figure 18) show any relation between the wave length of the primary fluorescence and the refractive index of the solvent. While the earlier work of Stern *et al.* included spectra of a variety of examples of chlorophyll derivatives, a more recent specific study has been made by Stern and Pruckner (38). Additional spectra on some derivatives of bacteriochlorophyll have been given by Pruckner alone (27), as well as the dihydroxy derivatives of the chlorines (28), in which the hydrogen atoms in the 7- and 8-positions have been replaced by hydroxyl. In general, the bands

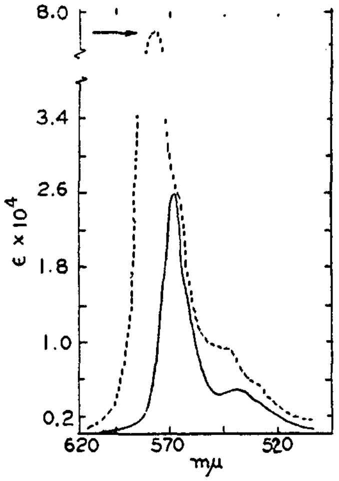


FIG. 15

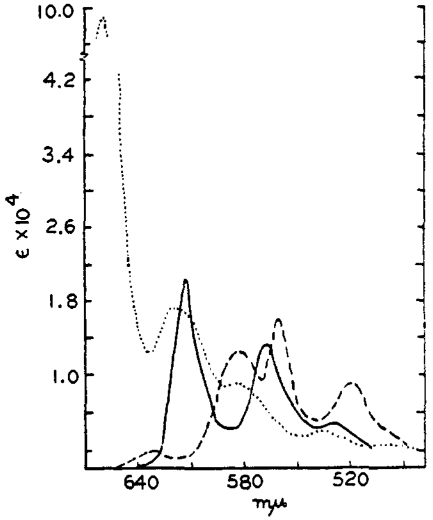


FIG. 16

FIG. 15. Absorption spectra of copper chelates of imidoporphines (from Pruckner and Stern (30)). ——, copper salt of monoimidoetioporphine; - - -, copper salt of tetramethyl ester of β,δ-diimidocoproporphine II.

FIG. 16. Absorption spectra of chelates of corresponding porphines and phorbines (from Stern and Wenderlein (39)). ——, magnesium salt of dimethyl ester of pheoporphine a_5; - - -, dimethyl ester of pheoporphine a_5; . . ., methylchlorophyllide a (magnesium salt of dimethyl ester of pheophorbine a).

of the dihydroxy compounds are very similar in form and magnitude to those of the chlorines, but are shifted somewhat to the red. A further paper (29) is primarily concerned with the relation of structure to spectra. The results, e.g., of Calvin, Ball, and Aronoff's (5) interpretation of Rothemund's tetraphenylporphine

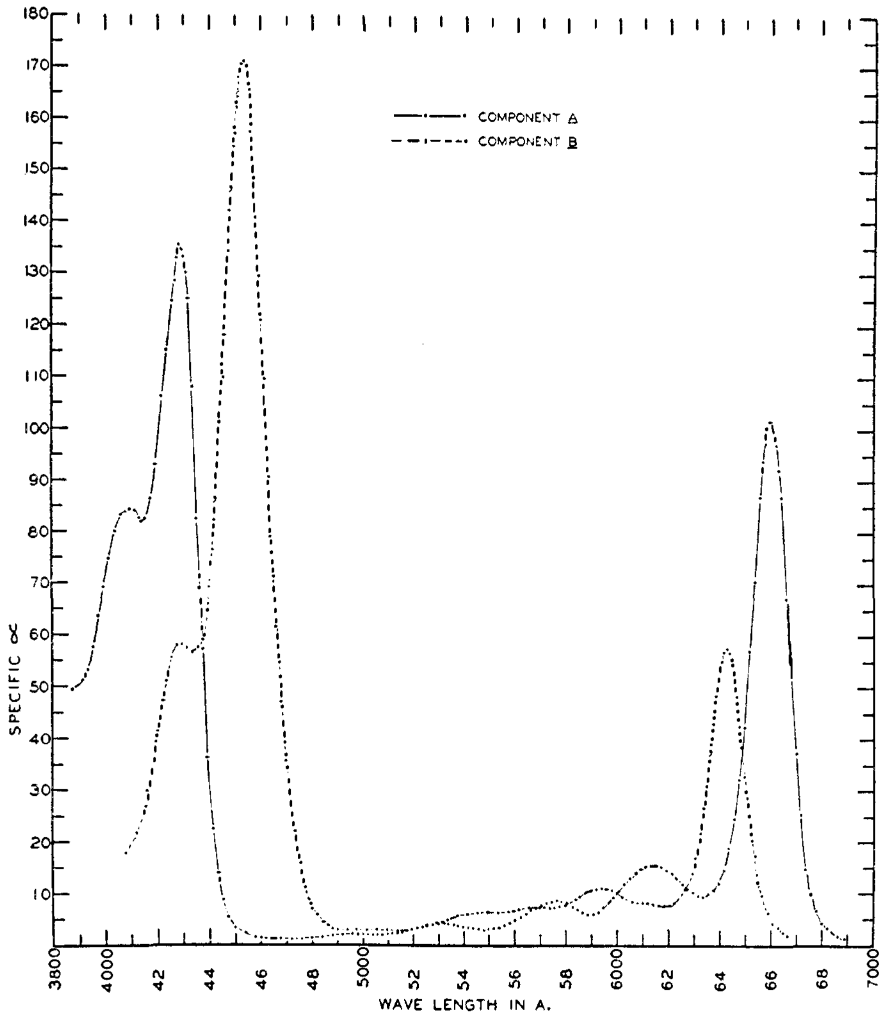


FIG. 17. Absorption spectra of chlorophylls a and b in ether (from Zscheile and Comar (43))

"isomers" as being due to the porphine and corresponding chlorine, are here also suggested. Rothemund remarks, in addition, that by comparison with the di-imidoporphines, the chlorines are more "symmetrical" than the porphines, and that for this reason their general absorption is shifted toward the red. This point of view presumably stems from the "interference" in the resonance of the inner ring by coupling with the β-carbons in the pyrrole rings of porphines, a "disturbance" which occurs to a lesser extent in the chlorines (*viz.* XIII and XIV). Nevertheless, a more generally accepted qualitative explanation of the shift of the primary absorption band in conjugated compounds is associated with an increased resonance or length of conjugation (see Lewis and Calvin (19)). Although the primary bands in the near ultraviolet of the chlorines and porphines are not significantly different in height or position, one interesting example is again found in tetraphenylchlorine, whose absorption coefficient in the ultraviolet is only half that of the corresponding porphine[7]. It is, however, still unexplained as to why the addition of hydrogens to the β-positions in the pyrroles, a phenomenon which decreases the permissible resonance, should result in the formation of prominent bands in the red or infrared, aside from the improbability that the excited state is actually a dissociation (proton ejection), which would be easier with chlorines than with the nuclear hydrogens of porphines.

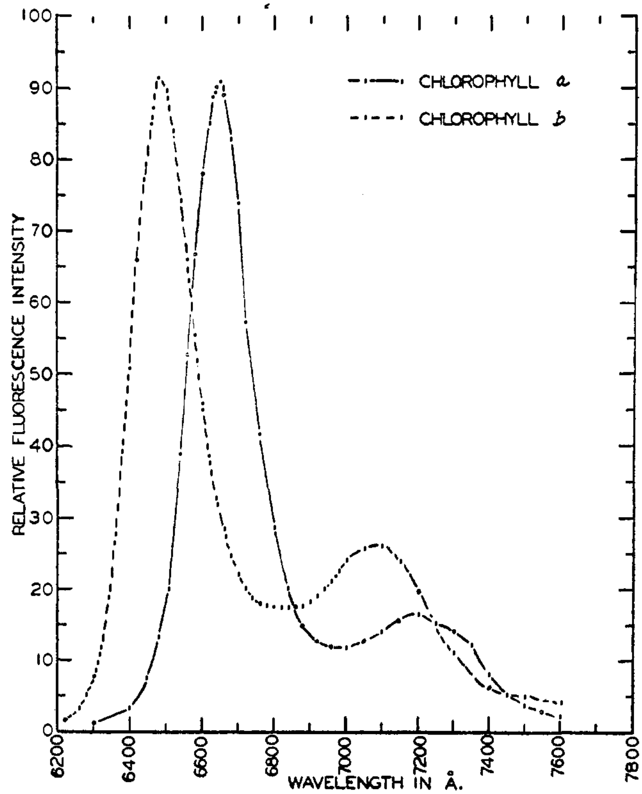
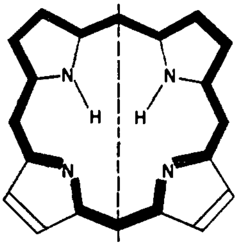


FIG. 18. Fluorescence spectra of chlorophylls a and b (from Zscheile and Harris (44))

Three analyses of the spectra of porphyrins have appeared. An earlier and more elaborate scheme (15) gives a scheme of fundamentals for deuteroporphine (figure 19). Vibration frequencies of 1525 and 1145 cm.$^{-1}$, ascribed to the —C═C—

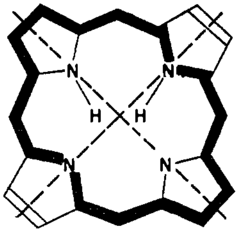
[7] G. Dorough: Thesis, University of California, 1946.

conjugation, are made the basis of the term system. Vibrational states within the first electronic level are indicated, and the second electronic level, at 23,000 cm.$^{-1}$, is only inferred. The first main band in the red is ascribed to a vibrational band in the lowest level of the first electronic state, and subsequent bands to succeeding higher vibrational levels. The main band in the near ultraviolet presumably is another electronic transition. An almost identical scheme, of a more general character, has been suggested by Rabinowitch (31), who proposes, in addition, that the large red band in chlorines be ascribed to a new electronic transition of lower energy (figure 20a), with still a fourth, of even lower energy, for the tetrahydroporphines (bacteriochlorines) (figure 20b).



XIIIa

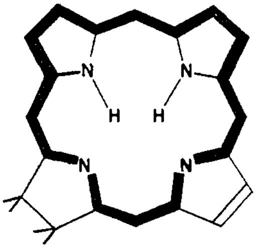
(symmetrical)



XIIIb

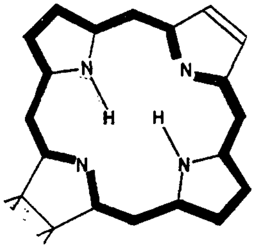
(symmetrical)

Some paths of π-electrons in porphines



XIVa

(unsymmetrical)



XIVb

(unsymmetrical)

Some paths of π-electrons in chlorines

Very recently Simpson (32) has given a simplified LCAO calculation of the "center of gravity" of the bands of a porphine. In Simpson's model porphine is depicted as oscillating between the two tautomers XI and XII. Contributions of other forms are not considered but the system is "calibrated" by reference to the "center of gravity" of benzene. On the basis of a 24-electron system, calculations would indicate a center of gravity for the bands in the near infrared. Such absorption does not occur. On the basis of an 18-electron system in which the pair of double bonds not involved in the conjugation is neglected, and considering the conjugated system to be a circle, a center of gravity of 615 mμ for the absorption bands is found. The ability to obtain a more correct result by neglect of the two nonconjugated double bonds is indeed interpreted as an indication of a partial lack of complete aromaticity. The argument is adduced that the reduction of a porphine to a chlorine does not change the position of the bands, and, indeed, by virtue of its asymmetry, diminishes degeneracy and results in an increased absorption in the band of lowest frequency. This reasoning does not, however, explain why the addition of two more hydrogens as in bacteriochlorine, giving increased symmetry, should result in a new band, further in the infrared, also of very great intensity. This disappearance of bands in this acid spectrum is explained as a tendency of tautomerism to favor the tautomer in which the charges are at the greatest distance. A "free-electron gas"

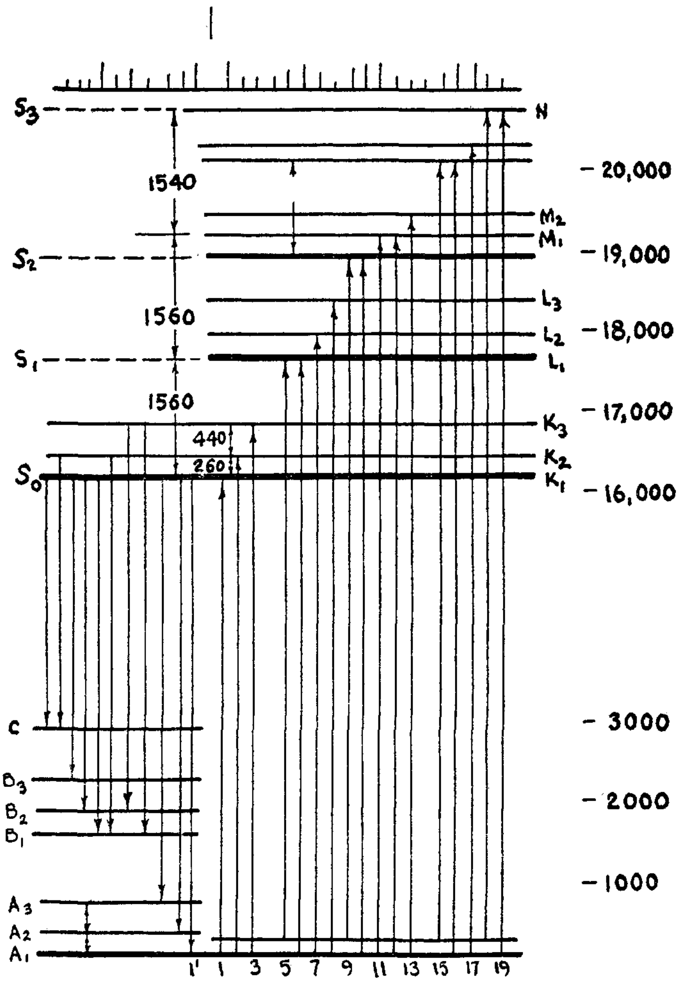


FIG. 19. Term system for deuteroporphine (from Hellström (15))

approach, in calculation similar to Simpson's, has been suggested by H. Kuhn (17), but results of calculations and arguments are not yet available. These approaches show great promise in interpretation of porphyrin spectra.

Relatively little attention has been given to the infrared spectra of the porphines, primarily because of the difficulties encountered with the interpretation of such complex molecules. Two works on infrared spectra have appeared. Vestling and Downing (42) used this technique in an attempt to determine the degree of N—H—N bonding in porphines. As is well known, the infrared OH band of phenols disappears in chelates as of salicylaldehyde. It was of interest that these infrared studies showed a band not only in the dipyrrylmethenes, but also in the porphines at 3μ, where one would expect such a band from —NH. This

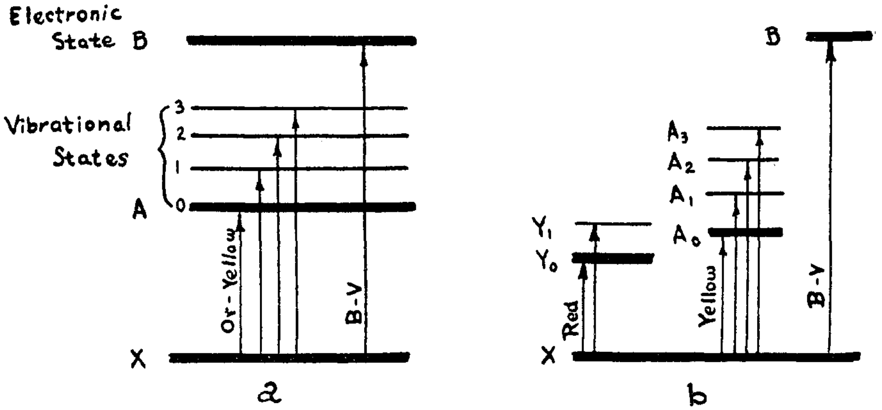


FIG. 20. Term system of (a) a porphine and (b) a chlorine (from Rabinowitch (31))

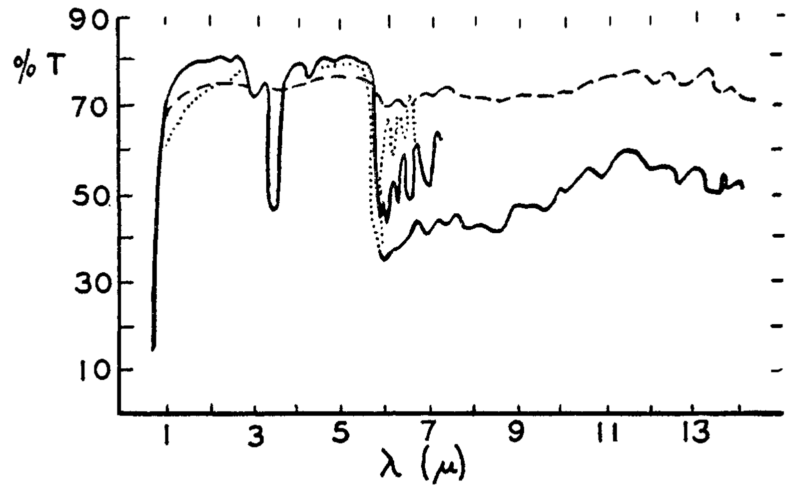


FIG. 21. Infrared spectra of chlorophyll and derivatives (from Stair and Coblentz (33)). ——, chlorophyll; . . ., pheophytin; - - -, ethyl chlorophyllide.

investigation did not rule out the possibility of N—H—N bonding, but neither did it substantiate it. A broader examination of the spectra was given earlier by Stair and Coblentz (33), in which spectra were presented for chlorophyll, pheophytin, copper pheophytin, ethyl chlorophyllide, phytol, "carotene," and xanthophyll in the region from 1 to 15 μ (figures 21 and 22). Two difficulties appeared prominent in this work. First, there was the complete disappearance of structure in ethyl chlorophyllide, including the band near 3 μ. Since this band is characteristic of compounds with —NH, or —OH, and apparently of the metal porphines (at least that of copper), there seems to be little reason to expect its disappearance. Indeed, on a logical deduction, from the pronounced appearance of this band in phytol (which is, of course, still present in pheophytin and its copper complex but not in ethyl chlorophyllide) one might have presumed that this absorption band in chlorophyll was due solely to the presence of phytol. Another singularity was the presence of this same band in the hydrocarbon carotene. Although the material used was designated as crystalline, the known difficulty of separating sterols from carotenoids may account for this, in part.

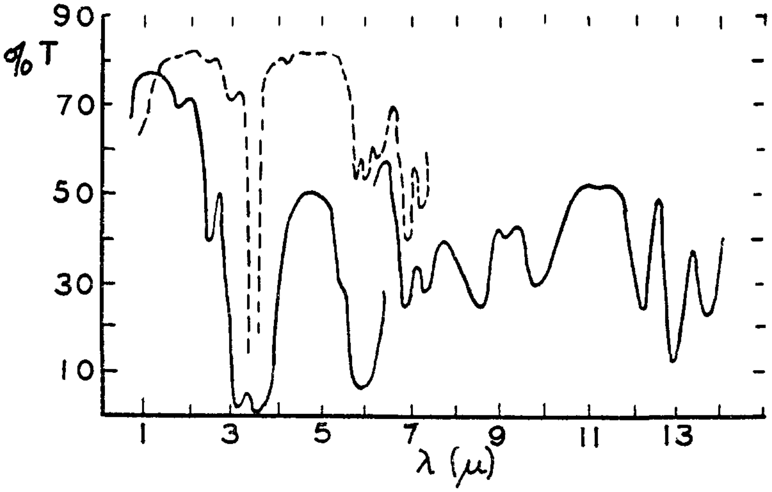


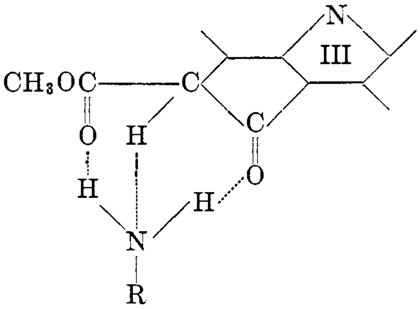
FIG. 22. Infrared absorption spectra of phytol and carotene mixture (from Stair and Coblentz (33)). ——, phytol; - - -, carotene (plus sterols ?).

FLUORESCENCE

The fluorescence spectra of chlorophylls a and b have been measured (44). Chlorophyll a in diethyl ether has a primary maximum at 665 mμ and a secondary at 720 mμ. Chlorophyll b has a similar structure, though shifted to the blue, with the corresponding maxima at 649 and 709 mμ. Variations, measured for the a component, are found in a variety of different solvents, the lowest being that for diethyl ether (664.5 mμ) and the highest for 2-ethyl-1-hexanol (676.5 mμ). The fluorescence is also temperature-dependent, the primary maximum being shifted to the red and increased in magnitude with decreasing temperature

(669.0 at −62°C. to 666.7 at 28°C.). No anti-Stokes fluorescence was found. The spectrum, as was already well known for the overall emission, was found to be independent of the wave length of the exciting source.

Livingston, Watson, and McArdle (20) have shown the necessity for the presence of polar solvent (0.01 per cent or more of the total solution) in order that chlorophyll should fluoresce in its normal yield. Fluorescence is almost nonexistent in the pure dry hydrocarbon. They explain their results by suggesting that the chlorophyll and the polar solvent form an addition compound (possibly *via* hydrogen bonding), the last being a requirement for fluorescence. Only those compounds which can form hydrogen bonds (with few exceptions, an outstanding one being ethyl ether) permit the fluorescence. The fluorescent entity is depicted as:



It would be interesting to know whether these same requirements for fluorescence hold for porphines and chlorines without the isocyclic ring and electronegative substituent such as oxygen. Furthermore, unless the yield of the phosphorescence (or other long-lived state) is measured, we do not know whether or not such an entity is required for activation to the excited state. In other words, the possibility exists (until experimentation shows otherwise) that the function of these substances may be to diminish the yield of the long-lived state and that fluorescence may not be an indication of the ability of chlorophyll to react photochemically.

Hagene (12) has discussed briefly some of the visible changes of necrobiosis in chloroplasts. Two optical changes have been followed: the cessation of fluorescence and the displacement of the maximum. If chloroplasts are killed, e.g., by being placed in water at 50°C., the fluorescence is gone after only 3 min., while the shift in the red band of chlorophyll (681 to 673–671 mμ) does not occur until 160 min. later. This obviously indicates that subtle changes, not visible in the absorption spectrum, occur rapidly, inactivating the assimilatory system.

Porphyrin–gelatin phosphors have been reported by both Bandow and Klaus (3) and Klaus (16). Unfortunately, the only portion of work reported by the first authors is concerned with the fluorescence, rather than the phosphorescence, properties of porphyrins in the solid state, although the existence of phosphorescence is mentioned. Vannotti indeed makes the highly interesting statement (41) that a phosphorescence with a duration of 0.01–1.0 sec. is present, quoting from

Klaus (whose thesis has been unavailable to the writer). Of particular and inexplicable interest is the statement by Vannotti that only wave lengths of the region 580–640 mμ can excite phosphorescence. The assumption has been made that no difficulty is found in the transition from the second electronic state (the large band in the ultraviolet) to the first. This is substantiated by the fact that all porphines have the same characteristic fluorescence spectra regardless of the wave length of the absorbed light. A phosphorescence of chlorophyll has long been sought as an aid in explaining the kinetics of photosynthesis. This has recently been found by Calvin and Dorough (6, 7) who, following the theory of Lewis and Kasha (18), identify the phosphorescence with a forbidden transition from the triplet to the singlet state. The lifetime of chlorophyll phosphorescence was then estimated at 0.2 sec. (6). In more recent work with purified pigments (7) it was found that the reported phosphorescence occurs only with chlorophyll b, with a lifetime of 0.03 sec. The existence of a long-lived state for chlorophyll a is thus still open to question. Furthermore, the diminution in the lifetime of the phosphorescence upon the purification of chlorophyll suggests that in the natural state, where the high concentrations of chlorophyll (*ca.* 0.1 *M*) permit energetic coupling of adjacent molecules, the above value may not be quantitative for chlorophyll b or even qualitative for chlorophyll a.

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