

ethoxy-2,5-hexadione and 6.2 g. of *o*-toluidine was heated on an oil-bath at 130–150° for two and one-half hours. The reaction mixture was then refluxed for an hour with an excess of alcoholic potash and poured into cold dilute hydrochloric acid. The crude acid was purified by crystallizing from 95% alcohol; m. p. 184.5–185.5° (corr.).

Anal. Calcd. neut. equiv.: 229. Found: 227.2. Calcd. for $C_{14}H_{16}O_2N$: C, 73.32; H, 6.60. Found: C, 73.36; H, 6.68.

N-(2-Methyl-6-methoxyphenyl)-2,5-dimethyl-3-carboxypyrrole (VI).—The procedure followed was the same as that used in the preparation of N-(*o*-tolyl)-2,5-dimethyl-3-carboxypyrrole. 2-Methyl-6-methoxyaniline was prepared by the method of Gibson.⁵ The product was purified by crystallization from 95% alcohol and melted at 198–199° (corr.). The yield was 26% of the theoretical.

Anal. Calcd. neut. equiv.: 259. Found: 256.7. Calcd. for $C_{18}H_{17}O_3N$: C, 69.46; H, 6.61. Found: C, 69.58; H, 6.71.

Summary

1. Unlike the N-(2-carboxyphenyl)-2,5-dimethyl-3-carboxypyrrole, N-(3-carboxyphenyl)-2,5-dimethyl-3-carboxypyrrole and the N-(4-carboxyphenyl)-2,5-dimethylcarboxypyrrole could not be resolved.
2. It was also impossible to resolve N-(2-carboxyphenyl)-2,5-dimethyl-3,4-dicarboxypyrrole.
3. A discussion of the optical isomerism in phenyl pyrroles is given.

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[CONTRIBUTION FROM THE CONVERSE MEMORIAL LABORATORY OF HARVARD UNIVERSITY]

STUDIES IN THE CHLOROPHYLL SERIES.

VII. EVIDENCE AS TO STRUCTURE FROM MEASUREMENTS OF ABSORPTION SPECTRA

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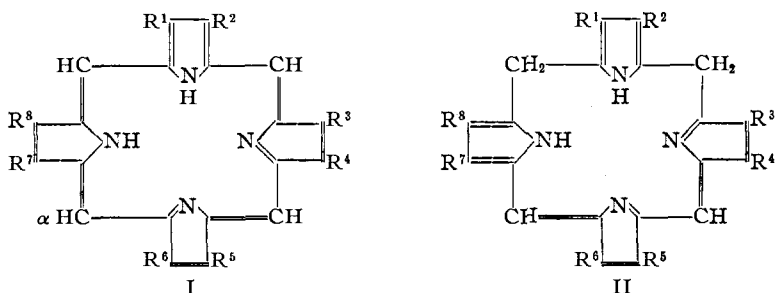
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The synthesis of a variety of porphyrins by Fischer has shown the structure of this class of compounds. However, the relation between the porphyrins and chlorophyll *a* and *b* has not yet been definitely established. Willstätter expressed as his final opinion the probability that a very drastic chemical change was involved in the transition from chlorophyll and its near relatives (the phaeophorbides, chlorins and rhodins) to the porphyrins. Much milder methods of forming porphyrins from chlorophyll derivatives are now known and indicate a close connection between the two classes. The results of the investigation of chlorophyll in this Laboratory have led us to write structural formulas for chlorophyll derivatives which contain a dihydro porphyrin ring or its equivalent.¹ In this paper we shall present a certain number of physical facts which we believe strongly support our formulation.

⁵ Gibson, *J. Chem. Soc.*, 123, 1273 (1923).

¹ Paper V, *THIS JOURNAL*, 53, 2382 (1931).

The general formulas for the porphyrins (I) and chlorins (II) are represented below; in the natural compounds the R's are usually methyl, or ethyl, propionic acid or carboxyl groups. The first, which is the Küster formula, may be regarded as clearly established by Fischer's synthetic work, the position of the double bonds being somewhat arbitrary. The other formula is that suggested in this Laboratory.



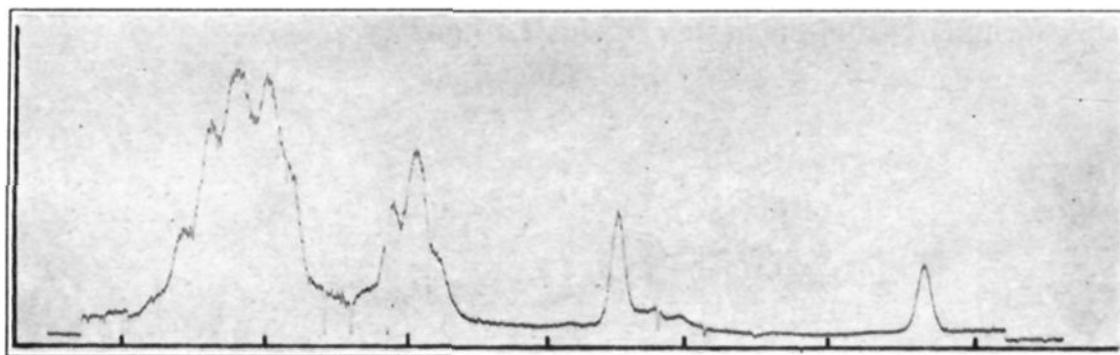
An examination of these formulas makes it evident that they are related to each other as are benzene and dihydrobenzene (cyclohexadiene). In the porphyrin ring, as in benzene, we have a complete conjugation of linkages and a rigid planar ring. (This can be shown easily by constructing models.) As was shown in a joint publication with Professor Crawford,² the absorption spectra of porphyrins like that of benzene at low temperatures is composed of relatively sharp and narrow bands. On the other hand, the absorption bands of most colored substances (including the chlorophyll derivatives) do not consist of narrow bands even at low temperatures.

We have continued this work and examined the absorption spectra at liquid air temperatures of seven porphyrins, fourteen greenish and reddish compounds from chlorophyll, and nine representatives of other colored compounds. The unique behavior of the porphyrins is very striking.³ A microphotometric curve corresponding to a typical low temperature spectrum of a porphyrin (etioporphyrin I) is shown in Fig. 1. Our method of procedure was identical with that described in the previous paper, the spectra being photographed with a Zeiss grating "Spectroscope for Chemists." Similar plates were obtained with the following porphyrins as their methyl esters: pyrrporphyrin, rhodoporphyrin, phylloporphyrin, deutero-porphyrin, mesoporphyrin, protoporphyrin.

² *Proc. Nat. Acad. Sci.*, 16, 552 (1930).

³ The porphyrins are listed in Table I. The chlorophyll compounds are: chlorophyll *a*, chlorophyll *b*, methyl phaeophorbide *a*, methyl phaeophorbide *b*, chlorin *e*, chlorin *e* trimethyl ester, pyrochlorin *e* methyl ester, chlorin *a* trimethyl ester, chlorin *f* methyl ester, mesochlorin, phyllochlorin *e*, phaeopurpurin 7 methyl ester, phaeopurpurin 18 methyl ester, rhodin *g*. The other colored compounds are: indothymol, quinizarine, crystal violet, 1-amino-6-chloroanthraquinone, pinacyanole, erthyrosin bluish, orthochrome T bromide, carotin and orange II.

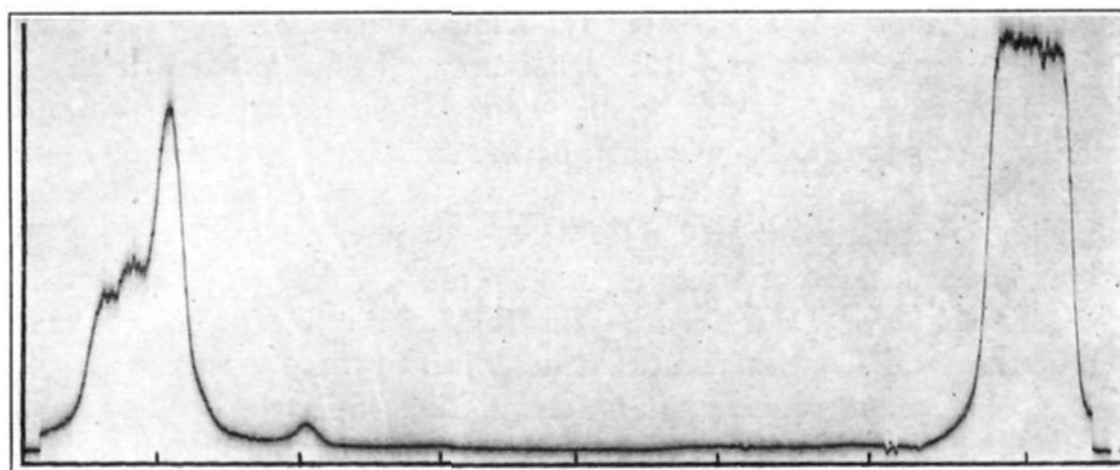
Of all the other substances examined, the one which showed the most similarity to a porphyrin was pyrochlorin *e* (to which we have assigned a structure corresponding to the substitution of a methylene group for two hydrogens on one of the $-\text{CH}_2-$ bridges of formula II). This chlorin



4750Å. 5000Å. 5250Å. 5500Å. 5750Å. 6000Å. 6250Å.

Fig. 1.—Microphotometric curve of photograph of absorption spectrum of etioporphyrin in an alcohol-ether solution (12.5 mg. per liter) at liquid air temperatures, through 18-mm. test-tube.

shows only very slightly the phenomenon characteristic of the porphyrins, namely, having a series of narrow sharp bands in the absorption spectrum at liquid air temperatures. A comparison of Fig. 1 and Figs. 2 and 2a brings out the contrast clearly. In the extreme blue we have a group of



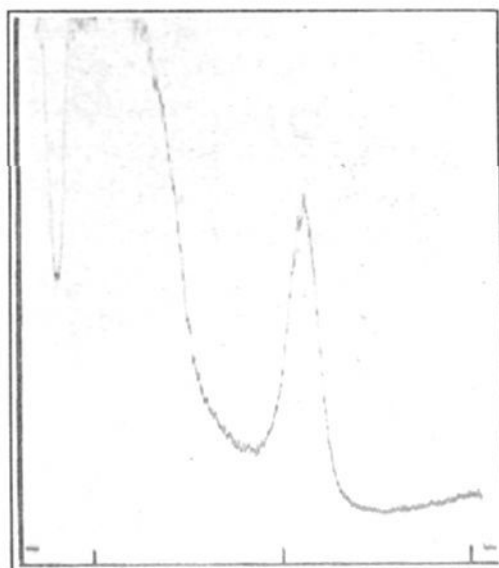
5000Å. 5250Å. 5500Å. 5750Å. 6000Å. 6250Å. 6500Å.

Fig. 2.—Microphotometric curve of absorption spectrum of pyrochlorin *e* methyl ester in an alcohol-ether solution 12.5 mg./liter at liquid air temperatures, through 18-mm. test-tube.

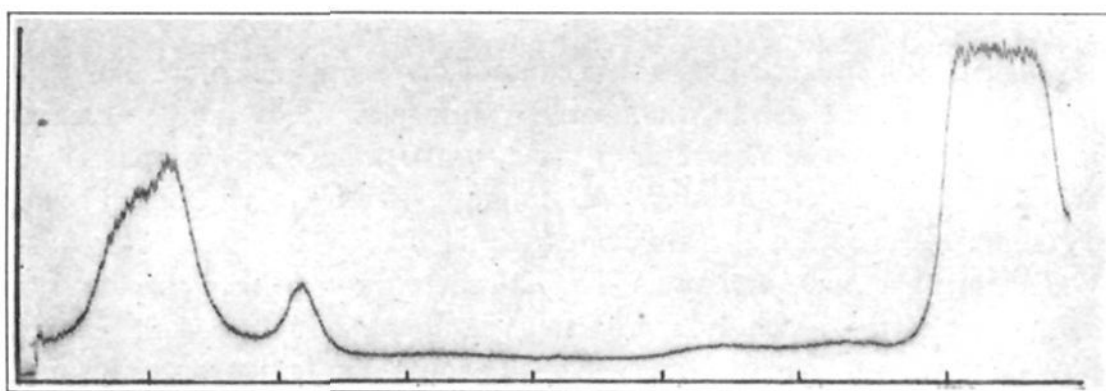
five narrow bands in the porphyrin as compared with three less distinct in pyrochlorin *e*, and in the next group three bands compared with one; in addition the extreme red band is very wide in the pyrochlorin *e* as in all chlorins. The spectrum of pyrochlorin *e* is reproduced here to show the

most pronounced case of narrow bands we have found (in the visible) outside of the field of the porphyrins. A more typical chlorin spectrum is that of chlorin *e* (Fig. 3). The transition from the porphyrins to the chlorins in spectral type is thus clear and definite.

The situation in regard to the absorption spectrum of benzene as compared to non-aromatic unsaturated compounds is completely analogous to our findings with the porphyrins. As is well known, the benzene spectrum (of course in the ultraviolet) consists of a series of relatively narrow bands. These bands are most striking in solvents of low dielectric constant and at very low temperatures.^{4,5} No similar phenomenon has been reported with non-aromatic hydrocarbons even if they are as highly unsaturated as butadiene. Moreover, Kistiakowsky and Arnold⁶ have recently shown that even at liquid air temperatures the ultraviolet spectrum of cyclohexadiene (1,3)



5000 Å. 5250 Å. 5500 Å.
Fig. 2a.—As Fig. 2, conc. 25 mg./liter, green band only.



5000Å. 5250Å. 5500Å. 5750Å. 6000Å. 6250Å. 6500Å.
Fig. 3.—Microphotometric curve of chlorin *e* trimethyl ester in alcohol-ether solution (12.5 mg./liter) at liquid air temperatures, through 18-mm. test-tube.

⁴ Pringsheim and Kronenberger, *Z. Physik*, **40** 75 (1926).

⁵ In this investigation we have found similar effects. A solution of a porphyrin in non-polar hexane (dielectric constant about 2.5) shows sharper-edged absorption bands than an alcoholic solution (dielectric constant about 30) at room temperature. Upon cooling there is further sharpening of the band edges. Thus by cooling alcohol-ether mixtures to liquid air temperatures both effects are operating to produce the sharpest absorption spectra because these super-cooled solutions have a low dielectric constant (of about 3).

⁶ Kistiakowsky and Arnold, to be published shortly.

does not show a series of sharp narrow bands but is more like that of pyrochlorin *e*. Cyclohexene is similar to the open-chain conjugated compound dimethylbutadiene, which shows continuous absorption even at liquid air temperatures. It thus seems that in regard to ultraviolet absorption at low temperatures the non-aromatic cyclic conjugated unsaturated compound occupies an intermediate place between aromatic compounds and open-chain unsaturated compounds. The chlorins occupy exactly this place in regard to the visible absorption at low temperatures. This seems to us strong evidence in support of our formula.

Measurements of the porphyrin spectra at low temperatures have brought out certain interesting regularities. The narrow red band characteristic of the porphyrins shifts to the blue about 50 Å. on cooling from room temperature to liquid air temperature, but remains a single band.

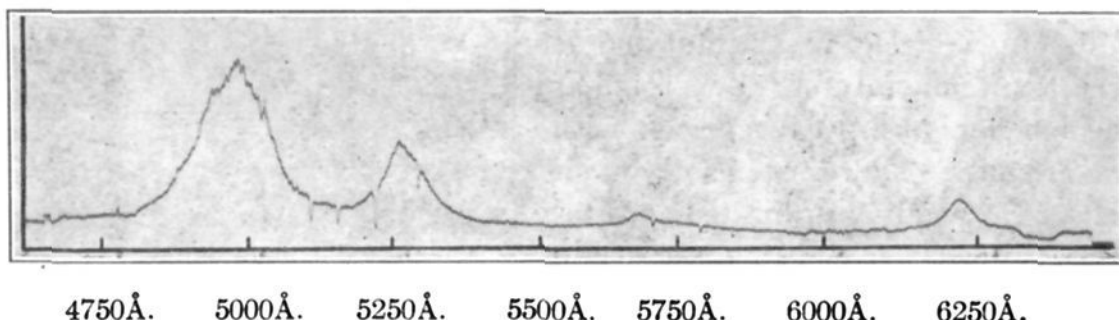


Fig. 4. —Microphotometric curve of etioporphyrin I in alcohol-ether solution (12.5 mg./liter) at room temperature, through 18-mm. test-tube.

The other bands are split into smaller bands or, as appears more likely, the edges of constituent bands which are so diffuse at room temperature are sharpened and the true character of the absorption appears (compare Fig. 4 with Fig. 1). Thus the band in the orange-yellow band shows two bands, the green three, and the blue six component bands. This sharpening of the visible absorption bands upon cooling has been found to occur with all the porphyrins studied. In addition, it may be noted that the red and orange-yellow bands always shift to the blue with decrease in temperature for more than the green and blue bands. It is interesting that Hartridge found a sharpening and shift of the oxyhemoglobin band at liquid air temperatures.⁷

For purposes of microphotometric recording the plates obtained with solutions containing 12.5 mg. per liter were chosen. In not all cases could the band center be found accurately from the curves although a visual examination of the plates showed the band clearly. (Phylloporphyrin and rhodoporphyrin esters presented the most difficulty, the latter because of its extreme insolubility.) In Table I are given the positions (in wave numbers) of the band centers and the frequency difference between ad-

⁷ Hartridge, *Proc. Phys. Soc.*, 54, 128 (1920-21).

TABLE I
RESULTS OF MEASUREMENTS

	Pyrroporphyrin methyl ester Band center, cm. ⁻¹	Diff.	Etioporphyrin ¹ Band center, cm. ⁻¹	Diff.	Protoporphyrin dimethyl ester Band center, cm. ⁻¹	Diff.	Denteroporphyrin dimethyl ester Band center, cm. ⁻¹	Diff.	Mesoporphyrin dimethyl ester Band center, cm. ⁻¹	Diff.	Phylloporphyrin methyl ester Band center, cm. ⁻¹	Diff.	Rhodoporphyrin dimethyl ester Band center, cm. ⁻¹	Diff.
I	16236		16207		15978		16245		16202		16136		15868	
II a	17562	213	17553	203	17266	203	17569	227	---				17289	219
b	17775		17756		17469		17796		17756		17553 (center) ^c		17508	
III a	18837	166	18858	132	---		18892	131	---					
b	19003	132	18990	132	18653	166	19023	155	18986	149	18819 (center) ^c		18351 (center) ^c	
c	19135		19122		18819		19178		19135					
IV a	---		19824	157	19554	128	---		---		---			
b	19967	238	19981	219	19682	230	20013	251	19996	212	19809	210		
c	20205	183	20200	220	19912	200	20264	181	20208	207	20019		19708 (center) ^a	
d	20388	183	20420	181	20112	220	20445	202	20415		---			
e	20571	213	20601		20332		20647		---		---			
f	20784		---		---		---		---		---			

The structure of these compounds is as follows (Formula I)

Etioporphyrin I^a R¹R³R⁵R⁷, --CH₃; R²R⁴R⁶R⁸, --C₂H₅

Pyrroporphyrin ester R¹R³R⁵R⁸, --CH₃; R²R⁴, --C₂H₅; R⁶, --H; R⁷, --CH₂CH₂COOCH₃

Protoporphyrin ester R¹R³R⁵R⁸, --CH₃; R²R⁴, --CH=CH₂; R⁶R⁷, --CH₂CH₂COOCH₃

Denteroporphyrin ester R¹R³R⁵R⁸, --CH₃; R²R⁴, --H; R⁶R⁷, --CH₂CH₂COOCH₃

Mesoporphyrin ester R¹R³R⁵R⁸, --CH₃; R²R⁴, --C₂H₅; R⁶R⁷, --CH₂CH₂COOCH₃

Phylloporphyrin ester R¹R³R⁵R⁸, --CH₃; R²R⁴, --C₂H₅; R⁶, --H; R⁷, --CH₂CH₂COOCH₃; α, --CH₃

Rhodoporphyrin ester R¹R³R⁵R⁸, --CH₃; R²R⁴, --C₂H₅; R⁶, --COOCH₃; R⁷, --CH₂CH₂COOCH₃

^a Kindly furnished by Mr. A. H. Corwin, who synthesized it according to the method of Hans Fischer. ^b Indicates band whose center cannot be found accurately from the microphotometric curve. ^c At the concentration chosen for comparison, only the absorption center of these bands shows on the microphotometric curve. Visual observation of other photographs shows the pattern to be the same as the other porphyrins.

acent bands in the same group. It will be noted that there are differences in the absolute positions of corresponding bands in different porphyrins. This is also true at room temperature (a group of narrow bands being usually taken as one band) and leads to the well-known methods of spectroscopic identification of the porphyrins. In order to compare the pattern of absorption bands in a variety of porphyrins, it is convenient to eliminate the variations in absolute position of corresponding bands. This we have done in Fig. 5 by arbitrarily placing the red band in each porphyrin at the

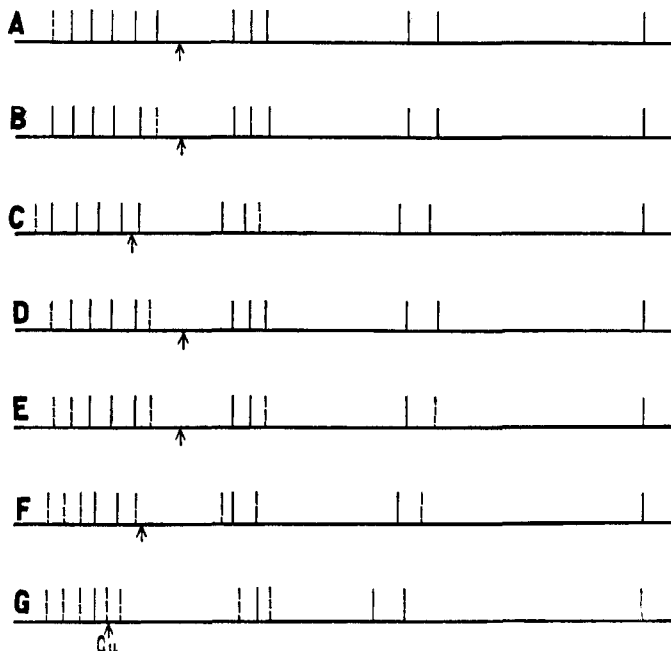


Fig. 5.—The absorption pattern of the porphyrins at liquid air temperature constructed from microphotometric curves; the dotted lines are approximate locations of band centers from visual observation of the plates; Cu \uparrow indicates position of 5106 Å line. A, Etio-I-; B, Pyrro-; C, Proto-; D, Deutero-; E, Meso-; F, Phyllo-; G, Rhodo porphyrin; all carboxyl groups methylated. For structure and numerical values of absorption centers see Table I.

same position. (The data for constructing Fig. 5 were taken from the microphotometric curves mentioned above.) The shift along the wavelength scale caused by changes in substituent groups is shown in Fig. 5 by the position of the copper line at 5106 Å. (marked Cu) which was recorded on all plates.

An inspection of Table I and Fig. 5 shows that with changes in substituents there may occur three different kinds of spectral change. The first is a shift in the absolute position of the red band, the second involves the

relative positions of the other three groups of bands to each other and to the red band and the third is the change in interval within a group. As would be expected, etioporphyrin (R's all methyl and ethyl) and mesoporphyrin (R's all methyl, ethyl and $-\text{CH}_2\text{CH}_2\text{COOCH}_3$) are nearly identical in all respects; the carbomethoxy group in a β -position of an ethyl group would be expected to exert very little influence. The substitution of an alkyl or propionic ester group by hydrogen produces a slight change in relative position of groups and in the interval within a group. This is clearly seen by comparing mesoporphyrin (practically identical with etioporphyrin) with pyrroporphyrin (one $-\text{CH}_2\text{CH}_2\text{COOCH}_3$ replaced by hydrogen), and with deuteroporphyrin (two ethyl groups by hydrogen); pyrroporphyrin lies between meso and deuteroporphyrins. In contrast to these small changes the introduction of an unsaturated group such as vinyl (proto) or carbomethoxy (rhodo) has a marked effect on absolute position, relative position and interval. However, the general pattern appears to be identical as judged from the results with protoporphyrin. The introduction of a methyl group on a bridge carbon atom (phylloporphyrin compared with pyrroporphyrin) produces a big change, as would be expected. Unfortunately not sufficiently good plates could be obtained to be sure of the exact pattern but it is visually the same as the others.⁸

We are greatly indebted to Professor H. H. Plaskett of the Harvard Astronomical Observatory for the use of the microphotometer.

Summary

1. The visible absorption spectra at liquid air temperatures of a number of colored substances have been photographed. The porphyrins show a unique pattern of groups of narrow bands comparable to the pattern of the ultraviolet absorption spectra of aromatic hydrocarbons. The chlorophyll derivatives have spectra with wider bands and occupy a position intermediate between the usual colored substance and the porphyrins just as the spectrum of cyclohexadiene is intermediate between those of benzene and butadiene.

2. The change of spectral patterns of the porphyrins with change of substituents has been discussed.

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⁸ This similarity in pattern seems to us to be additional evidence for the structure of phylloporphyrin as a methyl pyrroporphyrin (as we have assumed in this Laboratory) instead of another type of ring structure with an ethylene link. Fischer and Helberger's synthesis [*Ann.*, **480**, 235 (1930)] established that one or the other of these alternatives must be correct.