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A COMPARATIVE STUDY OF PHEOPHYTIN a AND OF
PHEOPHYTIN b MONOLAYERS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

by

Walter Earl Ditmars Jr., B.Sc., M.Sc.

* * * * *

The Ohio State University
1967

Approved by

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CONTENTS

ACKNOWLEDGMENTS.	ii
VITA	iv
TABLES	viii
ILLUSTRATIONS.	xi
ABBREVIATIONS AND SYMBOLS.	xii
	Page
INTRODUCTION	1
The Chlorophylls in Vivo	1
Statement of Problem	4
LITERATURE SURVEY.	7
EXPERIMENTAL	17
Reagents, General Procedures, and General Facilities.	17
Preparation of the Chlorophylls and their Derivatives	22
Spectroscopic Measurements	30
Oscillator Strengths	31
Constituent Analysis	32
Monolayer Apparatus	39
Monolayer Procedures	45
Experimental Accuracy - Spectroscopic Data.	52
Experimental Accuracy - Monolayer Data.	67
RESULTS AND DISCUSSION	76
Monolayer Stability.	76
Surfaces Pressures and Surface Potentials.	83
Monolayer Model.	96

CONTENTS (Contd.)

	Page
SUMMARY.	122
APPENDIX A. CHEMICAL AND PHOTOCHEMICAL STABILITY TESTS OF THE CHLOROPHYLLS AND SEVERAL DERIVATIVES IN VARIOUS SOLVENTS.	127
INTRODUCTION	128
EXPERIMENTAL	129
RESULTS AND DISCUSSION	135
Chemical Stability	135
Photochemical Stability.	145
SUMMARY	174
APPENDIX B. VISIBLE ABSORPTION SPECTRA OF THE CHLOR- OPHYLLS AND SEVERAL DERIVATIVES IN VARIOUS SOLVENTS	180
INTRODUCTION	181
RESULTS AND DISCUSSION	186
Table 27	187
Table 28	198
Tables 29, 30, and 31.	207
Tables 32, 33, 34, and 35.	241
Table 36	261
SUMMARY	271
BIBLIOGRAPHY	277

TABLES

Table	Page
1. Comparison of the Spectral Parameters of this Research With Those of the Chlorophyll Derivatives of Holt.	28
2. Optical Density Values and Wavelengths for the $0.99 \times 10^{-5}M$ Solutions, and Values of the Denominator-Determinants Used for Component Correlation	35
3. Comparison of the Visible Absorption Parameters of the Chlorophylls in Ethyl Ether.	54
4. Visible Absorption Parameters of the Pheophytins	60
5. Estimated Uncertainties in Band Peak Positions and Band Cut-Offs	65
6. Estimated Errors in Oscillator and Dipole Strengths Arising from Uncertainties in Choice of Band Cut-Offs	66
7. Procedural Errors in the Area Per Molecule	70
8. Percentage Variations in σ calculated from Observed Variations in ΔV	74
9. Stability Tests on Chlorophyll <u>a</u> Monolayers.	77
10. Stability Tests on Pheophytin Monolayers at Air-Water Interfaces.	80
11. Surface Pressure and Surface Potential Values for Pheophytin <u>a</u> and <u>b</u> at pH = 4.0 and pH = 3.0.	84
12. Average Compressibilities of ph <u>a</u> , ph <u>b</u> , chl <u>a</u> , and chl <u>b</u> Monolayers	89
13. Effect of pH on $(\mu/D) \cos \theta$ for ph <u>a</u>	91

TABLES (Contd.)

Table	Page
14. Variation of Monolayer Molecule Characteristics With Angle of Tilt (θ).	109
15. Areas Per Molecule and Free Areas Per Monolayer Unit at the Start of Transition	115
16. Initial Energy Absorption of Solutions in the Photochemical Experiments	131
17. Description of the Systems in Chemical Stability Tests	136
18. Chemical Stability of Chlorophyll <u>a</u> in Benzene Solutions	139
19. Chemical Stability of the Chlorophylls and the Pheophytins in Various Solvents	140
20. Systems Used in Low Light Intensity Photobleaching Tests	146
21. Photobleaching Behavior at Low Light Intensities, chl <u>a</u> , chl <u>b</u> , al chl <u>a</u> , al ph <u>a</u> , and ph <u>a</u> . . .	149
22. Photobleaching Behavior at Low Light Intensities, chl <u>a</u>	152
23. Systems Used in High Light Intensity Photo- bleaching Tests	156
24. Photobleaching Behavior at High Light Intensities. Benzene Solutions	161
25. Photobleaching Behavior at High Light Intensities. Solvents Different than Benzene	162
26. Apparent Quantum Yield in the Initial Period of Photodecomposition	167
27. Band Peak Wavelengths and Wave Numbers of the Chlorophylls and Several Derivatives in Various Solvents.	188

TABLES (Contd.)

Table		Page
28.	The Change of Band Peak Wave Numbers With Change of Solvent in Reference to Ethyl Ether.	199
29.	Oscillator Strengths of the Chlorophylls and Several Derivatives in Various Solvents	208
30.	Dipole Strengths of the Chlorophylls and Several Derivatives in Various Solvents	220
31.	Changes in Oscillator and Dipole Strengths With Change of Solvent in Reference to Ethyl Ether	229
32.	Spectroscopic Parameters of the Chlorophylls and Several Derivatives in Various Solvents	242
33.	Ratios of Peak Absorbances, Oscillator Strengths, and Dipole Strengths	244
34.	The Change of Band Peak Wave Numbers With Change of Substituent in the Same Solvent.	248
35.	Changes in Oscillator and Dipole Strengths With Change of Substituent in Ethyl Ether.	251
36.	Distances Between Band Peaks	262

ILLUSTRATIONS

Figure	Page
1. Chlorophyll <u>a</u> and Chlorophyll <u>b</u>	3
2. Surface Pressure and Surface Moment Versus Molecular Area for Pheophytin <u>a</u>	85
3. Surface Pressure and Surface Moment Versus Molecular Area for Pheophytin <u>b</u>	86
4. Molecular Model of Chlorophyll <u>b</u>	97
5. Close Packed Arrangement in a Pheophytin Monolayer	103
6. Variations in ΔV With σ ph <u>a</u> -pH = 3.0	117

ABBREVIATIONS AND SYMBOLS

chl a = chlorophyll a

chl b = chlorophyll b

ph a = pheophytin a

ph b = pheophytin b

al chl a = allomerized chlorophyll a

al ph a = allomerized pheophytin a

π = surface pressure

σ = molecular area

ΔV = surface potential

mv = millivolts

A = angstrom unit

m μ = millimicron

dyn = dyne, or dynes

cc = cubic centimeter

ppm = parts per million

f = oscillator strength

D = dipole strength, dielectric constant

d = optical density

λ = wavelength

$\bar{\nu}$ = wave number in cm⁻¹

$\epsilon_{\bar{\nu}}$ = molar extinction coefficient at $\bar{\nu}$, in liters/mole cm

cm = centimeter

ABBREVIATIONS AND SYMBOLS (Contd.)

mg = milligram

gm = gram

mm = millimeter

μ = overall electrical moment of the molecule

θ = angle of tilt of the molecular dipole with respect to a
normal to the interface

INTRODUCTION

The Chlorophylls in Vivo

There is good evidence, principally from light and electron microscopy studies, that the photoconversion apparatus (the chloroplast) of most photosynthesizing organisms has a highly ordered lamellar structure [1], [2]. The layers appear to be comprised of paired membranes joined at their ends to form closed discs. These discs are spaced regularly apart in a matrix, and are in ordered stacks. Differences in reaction with various chemical reagents have shown that the discs probably have a higher concentration of lipids, while the matrix is higher in proteins. Light-microscopic and fluorescence-microscopic evidence indicate that the chlorophyll is concentrated in the stacks of discs and absent in the matrix [2], [3]. Exposure of the discs to silver nitrate solution in light results in silver deposits on the discs as revealed by electron microscopy. Since chlorophyll can reduce silver nitrate photochemically, the silver coating of the discs can be taken as further evidence that the chlorophyll is bound in the discs. Calculations, using the dimensions and number of discs, and the amount of chlorophyll present in the chloroplast, suggest that the chlorophyll could be present in the form of non-crystalline monolayers at the

interfaces of the discs and the matrix. Considerations of this type led to the generally assumed model of monomolecular chlorophyll lying between relatively thick layers alternately high in concentration of lipids and aqueous proteins, or at essentially an oil-water interface. As a consequence of their heterophilic nature the chlorophyll molecules will probably be oriented with the phytol group in the oil phase. Such orientation will result in electrical polarization of the interface. The direction and degree of polarization will depend on the normal (to the plane of the interface) component of the dipole moment of chlorophyll, and the constituents contiguous to the chlorophylls at the phase boundaries. In the former instance, a change in substituent pattern on the chlorin ring may be expected to alter the effective interfacial moment. As shown in Figure 1 change of the $-\text{CH}_3$ group of chl a to the $-\text{CHO}$ group of chl b should produce a different resultant molecular moment, and perhaps a significantly different contiguous molecular environment.

Definition of the actual composition of the chlorophyll-containing interfacial regions in vivo remains a matter of continuing research interest. It is generally thought, however, that intimately associated with chlorophyll at the interface there are at least three other primary constituents: lipoproteins, lipides, and carotenoid pigments. These constituents play several roles in the primary conversion process. From a general functional view the most important roles would be: (a) stabilization

Chl *a* Chl *b*

Figure 1

and protection of the chlorophyll monolayer, (b) provision of phase boundaries that can allow for efficient transfer and conversion of the light energy absorbed by the chlorophyll, and (c) provision for separated (by the chlorophyll monolayer) phases that can allow for efficient utilization of the converted energy (i.e., a chemical oxidant, and a chemical reductant).

Statement of Problem

As a step toward an understanding of the complex interfacial systems in vivo, information on the properties of oriented monolayers of the chlorophylls and their derivatives at simple air-water interfaces may be expected to be of value. The properties of interest include chemical and photochemical stability, surface pressure (π) vs. molecular area (σ) behavior, surface potential (ΔV) vs. σ behavior, and spectral properties. The property of stability is of first concern, since only measurements on films of intact compounds can be regarded as significant.

It has been rather generally known that the chlorophylls and their derivatives are quite labile organic molecules. In particular, gross photodecomposition of chlorophyll in solution, resulting in destruction of the chlorin ring, has often been noted. Until only recently, there appeared to be less general awareness of the ease of intact ring derivative formation, chemically and photochemically, both in solutions and in monolayers.

In 1961 G. Colmano [4] showed that the chlorophylls are chemically unstable at ordinary air-distilled water interfaces, and in benzene solutions prepared under normal laboratory conditions. In monolayers significant decomposition occurs within the first five minutes after spreading, under conditions of dim, diffuse lighting. Some retardation of decomposition is obtained if the pH of the aqueous phase is maintained at about 8.0.

W. D. Bellamy, G. L. Gains, Jr., and A. G. Tweet in 1963 [5] confirmed Colmano's findings, and showed further that decomposition of the monolayer is also lessened in nearly total darkness. Results of the exploratory phases of this work were in agreement with these findings, and will be discussed subsequently. We find that, although pheophytin formation (where the central Mg atom is replaced by two hydrogen atoms) is inhibited, a process that is identified as essentially allomerization (oxidation at the isocyclic ring between the γ and 6 positions of the chlorin ring) continues to occur. The rate is inhibited considerably when the chl a monolayer is under an atmosphere of nitrogen containing about 40 ppm of O_2 .

We find that, at air-water interfaces, the pheophytins undergo a process identified as allomerization unless the pH of the aqueous phase is maintained at less than about 5.0. The monolayers are then stable, in the absence of light, or in dim, diffuse green light (i.e., at wavelengths absorbed only slightly by the molecules), for periods of six hours or more. In view of these findings, a quantitative, comparative study of the $\pi - \sigma$

and $\Delta V - \sigma$ behavior of pH a and pH b monolayers at air-water interfaces was undertaken.

Knowledge of the absolute values of σ will depend on knowledge of the absolute purity of the samples. While there has been increasing agreement between different researches in recent years, absolute purity remains a problem of continued research interest. Study of conditions required for obtaining and maintaining highly purified samples constituted an important phase of this work.

LITERATURE SURVEY

A number of studies of chlorophyll and pheophytin monolayers have been reported over the past 30 years. These will be reviewed briefly in historical order.

W. Sjoerdsma, in 1936 [6], studied the $\pi - \sigma$ behavior of 3 to 1 mixtures of chl a: chl b spread at air-0.1N H_2SO_4 , air-0.01N H_2SO_4 , air-water, and CO_2 -water interfaces. He reports that the $\pi - \sigma$ behavior at the first three interfaces were identical within experimental error, while in the fourth system the force-area curve was shifted to about 7 per cent larger molecular areas. Limiting molecular areas (σ extrapolated to $\pi = 0$) of about $124A^2$, and collapse areas of about $80A^2$, at $\pi \sim 26$ dyn/cm, were observed. No information on film homogeneity, sample purity, or film stability was given. It seems certain that on the acid substrates the chlorophylls were converted to the pheophytins.

A. Hughes, in 1936 [7], summarizes the results of $\pi - \sigma$ and $\Delta V - \sigma$ studies of chl a and chl b monolayers at air-pH = 7.0 - 7.5 buffer interfaces. Limiting areas of $132A^2$ for chl a, and $137A^2$ for chl b were found. Film collapse was observed between $80 - 90A^2$. Over the range of area studied the surface potential of chl a increased from 300 mv to 375 mv on compression, while that for chl b increased from 250 to 300 mv. If the pH of the

aqueous substrate was made less than 6.0, a sharp increase in ΔV was observed, and attributed to the formation of pheophytin. Alkali was reported to have little effect on the properties of the films. No information on sample purity or film homogeneity was given.

I. Langmuir and V. J. Schaefer, in 1937 [8], studied chlorophyll monolayers at air-water interfaces where the pH of the subphase was varied from 3.0 to 10.0. Surface viscosities as a function of surface pressure and pH are reported. Multilayers of up to 600 layers in thickness were prepared and the conditions leading to fluorescence investigated. No information on sample purity, film stability, or film homogeneity was given.

In 1937, A. E. Alexander [9] made a comparative study on the $\pi - \sigma$ and $\pi - \Delta V$ behavior of a variety of porphyrin derivatives, including chl a and chl b, at air-pH = 7.3 buffer interfaces. By use of the dark-field ultramicroscope method the chlorophyll films were found to be homogeneous at large areas. Limiting areas of $135A^2$ and $138A^2$ for chl a and chl b respectively were found. The areas, at a collapse pressure of about 28 dyn/cm, were $80A^2$ for chl a and $76A^2$ for chl b. Over the range of areas studied the surface potential of chl a increased from 300 to 385 mv on compression, while that of chl b rose from 250 to 300 mv. At pH's less than 6.0 the surface potentials rose sharply; the effect was attributed to the formation of pheophytin. It was suggested without elaboration that the $\pi - \sigma$ behavior of

the pheophytins was quite similar to that of the chlorophylls. No information on sample purity was given, and no indication was given of problems of chemical or photochemical instability.

E. A. Hanson, in 1939 [10], studied ethyl chlorophyllide and chlorophyll monolayers at air-water interfaces. Limiting areas of $70 \text{ \AA}^2$ for the chlorophyllide (at $\text{pH} = 5.4$), and of $106 \text{ \AA}^2$ for chlorophyll (at $\text{pH} = 4.1$) were found. Thus, the phytol tail increases the area occupied by about $36 \text{ \AA}^2$. Hanson reported that chlorophyll is not converted into pheophytin at $\text{pH} = 4.1$ which appears to be in variance with the work of Hughes [7] and Alexander [9], and is not corroborated by the results of this research. Hanson further reports that the surface requirement and compressibility increase with increasing pH , effects which are ascribed to increasing degree of hydration. No indication was made of problems of stability, or film homogeneity.

In several papers published in the years 1954 through 1957, E. E. Jacobs and co-workers report studies on the visible absorption spectra of ethyl chlorophyllide and chlorophyll monolayers [11], [12], [13]. Monolayers were prepared by spreading a solution containing a known amount of pigment onto a given area of air-water interface. No $\pi - \sigma$ or $\Delta V - \sigma$ data were reported, and no information on film stability or homogeneity was given. The work showed that the main red absorption band is shifted significantly to the red for both chlorophyll and

chlorophyllide monolayers, in comparison to its position in dilute solution spectra. Such red shifts are observed for chlorophyll *in vivo*. The band shifts were greater for the chlorophyllide and closely resembled shifts observed for the pigment microcrystals.

In 1959, H. J. Trurnit and G. Colmano studied chl a and chl b monolayers at air-water and oil-water interfaces [14]. They reported $\pi - \sigma$ data at air-distilled water interfaces and absorption spectra at both air-distilled water and oil-distilled water interfaces. Considerable attention was given to sample preparation, and spectroscopic criteria for sample purity are presented. Limiting areas for chl a ranged from 103 to 130 Å², depending on the chlorophyll sample used and the spreading solvent. Use of acetone as a spreading solvent, for example, appeared to result in a loss of chlorophyll to the aqueous phase and therefore gave a smaller apparent area per molecule. For chl b the range was from 111 to 140 Å². Collapse pressures for chl a ranged from 26 to 29 dyn/cm and the areas at collapse from 54 to 73 Å²; for chl b the ranges were from 30 to 42 dyn/cm, and from 51 to 54 Å². Detailed consideration was given to spectral changes of chlorophyll films in comparison to spectra in dilute solutions. The most marked changes found were: (a) a shifting of all bands to longer wavelengths, (b) the shrinkage of the blue absorption band of chl a to the extent that it almost disappeared within the blue satellite band, and (c) the shrinkage of the blue satellite band of chl b to the extent of almost disappearing in the blue band. By redissolving the films in solvent after experiment, and

recording the solution spectra, the changes observed for the films were deduced to be due primarily to changes in the physical state of the molecules. In a later report [4] (referred to above), where film stability was discussed, it is stated that measurements were generally completed within about 15 minutes after spreading of the films. In that time period degradation to the extent of perhaps only 10 per cent had occurred, which lends some support to the conclusion regarding the spectral changes observed in the films.

In the period from 1963 through 1965 W. D. Bellamy, G. L. Gains, Jr., and A. G. Tweet reported a number of studies on monolayers of chl a and ph a at gas-water interfaces, [5], [15], [16], [17], [18], [19]. Measurements, using highly sophisticated instrumentation, were carried out under controlled, inert atmospheres, filtered lighting (or total darkness), and with the pH of the aqueous subphase maintained at 8.0. The chl a samples were apparently of high purity, as indicated by the spectral parameters of dilute solutions. The pheophytin a, however, appeared to be of lesser quality (comparisons with this work are made in the discussion of sample purity). Numerous tests were made to assess the chemical and photochemical stability of the monolayers. It was generally concluded that, in the absence of light, the monolayers remained stable for times sufficient for meaningful experiments on their chemical and physical properties. Main band absorbance, for both ph a and chl a films, was reported

to decrease only a few per cent in 2 hours in the absence of light. Degradation of chl a was also determined in terms of change of the following absorption band peak ratios: Blue/Blue satellite (B/BS), Blue/Red (B/R), and Red/475m μ (R/min.), where the denominator in the last ratio refers to the optical density of the absolute minimum in the pure absorption spectrum. In typical experiments, for monolayers spread for two hours in darkness, decreases of 7 per cent in B/BS and 45 per cent in R/min. were observed, with B/R apparently remaining unchanged. Although it is difficult to decide on the actual amount of degradation from peak ratio changes, we estimate, from similar type experiments, that the changes reported represent from 5 to 15 per cent decomposition. This estimate would appear to be in line with the general conclusion of the authors.

In the first report of Bellamy et al [5], $\pi - \sigma$, $\Delta V - \sigma$, and absorption spectral data for chl a and ph a monolayers are given. No information on film homogeneity was reported. Experimental accuracy in σ was indicated to vary from ± 8 per cent to ± 3 per cent, depending largely on sample purity. The limiting areas were, for chl a, 125A², and for ph a, 109A². Chl a monolayers collapsed at 22-23 dyn/cm, at a molecular area of about 77A², while ph a monolayers collapsed at about 11.0 dyn/cm and 90A². For chl a, ΔV varied from 230 to 310 mv over the range 120 - 85A²; for ph a, the increase was from 545 to 590 mv in the range 105 to 90A². The absorption spectra of the monolayers are

compared with earlier work [11], [12], [13], [14]. Similar changes, in comparison to the spectra of dilute solutions, were found. The absorption spectra of both ph a and chl a monolayers are reported to be essentially independent of molecular area over most of the stable mechanical range of pressures.

On the basis of the experimental results and considerations of molecular dimensions, a monolayer model was proposed. It was suggested that the monolayer comprises a liquid film conforming to Langmuir's theory [20] of liquid expanded monolayers.[†] The molecules are anchored to the water surface by the ester linkages, a configuration suggested earlier by Hughes [7] and Alexander [9]. The chlorin heads and the phytol tails, in a folded configuration rising above the water surface, are interspersed in a randomly oriented array. The planes of the chlorin heads are tilted at an angle of 35° , or less, with respect to a normal to the interface. On the basis of the relatively small change of surface moment with molecular area, it was concluded that there is no major change in the configuration of the polar groups with respect to the water surface as the films are compressed.

Following the initial report, Bellamy, Gains, and Tweet reported studies on: a) fluorescence of chl a monolayers [15],

[†]The theory was originally proposed for straight chain hydrocarbons with hydrophilic polar head groups. Monolayers of these substances were visualized as essentially duplex films with the hydrocarbon tails tending to form a coherent liquid phase above the water surface, and the polar heads tending to form a gaseous film, within the water surface, and occupy all the available interfacial area.

b) angular dependence of fluorescence from chl a in monolayers [16], c) interaction between chl a and fatty alcohol molecules in mixed monomolecular films [17], d) fluorescence quenching and energy transfer in monomolecular films containing chlorophyll [18], and e) interaction between chl a and vitamin K, in monomolecular films [19]. A brief summary of the results follows.

Fluorescence in pure chl a monolayers is strongly concentration quenched; relative fluorescence yield increases nearly 1000-fold when the pigment molecules are effectively separated by a two-dimensional diluent. The intensity of fluorescence emission was found to be significantly dependent on the angle of detection with respect to the plane of the interface. On the basis of the proposed monolayer model, the results were interpreted to show that the transition moment of the red fluorescence, lying in the plane of the chlorin ring, makes an angle of 20° or less with the plane of the water surface. Quenching of the fluorescence of chl a in monolayers by copper pheophytin a, a non-fluorescent chlorophyll derivative, was measured as a function of quencher concentration. Energy transfer is detected through the quenching. Since the nature of the description of energy transfer depends on the distances between chromophores, both undiluted and highly diluted chl a monolayers were studied. The results were discussed theoretically with appropriate modification of available theories.

Chl a and oleyl alcohol form nearly ideal two-dimensional solutions up to chlorophyll mole fraction of at least 0.2.

Chlorophyll is essentially insoluble in stearyl alcohol monolayers. Chl a-oleyl alcohol monolayers gave collapse pressures that depended on the composition, while chl a-stearyl alcohol monolayers collapsed at the collapse pressure of chl a at all compositions. Oleyl, but not stearyl, alcohol produces significant wave length shifts to the blue in both the absorption and emission spectra of chl a monolayers. Oleyl alcohol, as a diluent, increases the fluorescence yield of chl a but stearyl alcohol does not. The wavelength shifts with dilution are evidence that chlorophyll-chlorophyll interactions, as well as chlorophyll-water interactions [5], are responsible for the red shift observed in the spectra of chl a monolayers.

Vitamin K₁ (a quinone) quenches the fluorescence of chl a both in diluted (by oleyl alcohol) and undiluted mixed monolayers. The quenching is interpreted as arising from collisional interaction, possibly requiring a preferred orientation between pigment molecule and quencher. Vitamin K₁ and chl a form non-ideal two-dimensional solutions; departures from ideality are small.

M. Rosoff and C. Aron, in 1965, report a study [21] of the conversion of chl a to ph a in monolayers at air-pH = 4.0 buffer interfaces. They indicate that the properties of ph a monolayers at pH = 8.0 are the same as at pH = 4.0 which is in variance with the results of this work. The $\pi - \sigma$ data at pH = 8.0 of Rosoff and Aron do not agree well with that reported by Bellamy et al [5]. At given values of π , the σ values reported by former authors are about 7 per cent greater for chl a, and

about 5 per cent smaller for ph a. Because of the lower compressibility found by Rosoff and Aron, they consider the chl a and ph a monolayers to be of the liquid condensed type rather than liquid expanded as assumed by Bellamy et al [5]. These discrepancies may relate to variations in sample purity or problems in obtaining completely spread monolayers. Rosoff and Aron give no information on these points, nor do they give information on the stability of ph a films at pH = 8.0.

No systematic study of ph b appears to have been reported.

EXPERIMENTAL

Reagents, General Procedures, and General Facilities

All solvents and reagents used were C.P. analytical grade as received from the manufacturer. In the chlorophyll preparation work, monolayer studies, and in some of the spectroscopic determinations the solvents were used without further treatment. For work under controlled atmospheres (in sealed glove enclosures) all solvents, except the ethyl ether, were degassed by evaporation and agitation under vacuum. The benzene was twice recrystallized to about one half its original volume, and dried by storage (for more than 3 days) over calcium hydride powder. It was filtered under a pure nitrogen atmosphere ($O_2 < 30$ ppm, $CO_2 < 3$ ppm, H_2O vapor < 1 ppm), with predesiccated materials and apparatus, into a special pyrex vacuum evaporation vessel, and subsequently degassed. Where it was desired to obtain exceptionally high purity benzene, the following technique was necessary. The already purified benzene was put into a stoppered flask, under pure nitrogen atmosphere, which contained a mixture of CaH_2 and high grade (Woelm) chromatography alumina. The calcium hydride removed adsorbed water from the alumina, as evidenced by an initial, vigorous evolution of gas. Nonaqueous polar molecules in the benzene were then adsorbed on the

activated alumina. With the doubly recrystallized and degassed cyclohexane it was sufficient to pass the cyclohexane through an alumina column and then store over CaH_2 . Attempting to dry a solution of chlorophyll (10^{-5}M) in benzene directly with CaH_2 resulted primarily in loss of the chlorophyll by adsorption on the CaH_2 . Ethyl ether solutions, prepared and used for quantitative measurements, were handled in closed polyethylene bags. The atmosphere within was saturated with ether vapor. This procedure reduced error due to solvent evaporation to less than 1 per cent.

In the exploratory work on the chemical stability of chl a monolayers several additives to the monolayers were examined. The α -L-lecithin was the high purity grade obtained from the Fluka Chemical Manufacturing Co., Buche, Switzerland. The phytol was the C grade product obtained from the California Corporation for Biochemical Research, Los Angeles. The β -carotene was obtained from the Eastman Kodak Co. These chemicals were used without further purification except for removal of adsorbed or dissolved gases (O_2 and CO_2) and water before being put into solution. All spreading solutions were made with highly purified benzene, except for those containing the α -L-lecithin. These latter solutions were made from dry, CP ethyl ether with 10 vol. per cent absolute alcohol which was needed to dissolve the lecithin crystals. The ethyl alcohol was thoroughly degassed (to remove O_2 and CO_2) before use.

The buffer solutions were made with distilled water and reagent grade chemicals. The solutions were made more concentrated than used and subsequently diluted volumetrically. For work under controlled atmospheres degassing (to remove dissolved O_2) was done by allowing the solutions to equilibrate (in the monolayer trough) with the controlled atmosphere for at least 15 hours before spreading the monolayer. The surfaces of all aqueous solutions were cleaned by suction at the surfaces through a capillary prior to transfer to the monolayer trough. Double recrystallization of the Baker's reagent grade phthalate salt, used to prepare the 0.025M buffer solutions, had no detectable influence on the experimental accuracy of the monolayer runs (see below). Within experimental error, increase or decrease of buffer concentration by a factor of four had no effect on monolayer properties. Use of a citrate, instead of a phthalate, buffer did not affect the results.

Double distilled demineralized water (specific resistance 1.1×10^6 ohm cm) was used in chlorophyll preparation. Water used in monolayer work was single distilled (specific resistance 8.0×10^5 ohm cm). For work under controlled atmospheres the water was degassed (to remove O_2 and CO_2) by bubbling (through a fritted glass disk, which also provided for stirring of the water) with high purity nitrogen for at least 18 hours before use. The apparatus consisted essentially of a twelve liter round bottom pyrex flask with suitable glass inlets, stopcocks, ground glass joints, etc., to permit filling, emptying, and

degassing without the water contacting anything other than clear glass surfaces, and short sections of cleaned, heavy wall (5/32 in.) Tygon tubing. The high purity nitrogen ($O_2 \sim 30$ ppm, $CO_2 \sim 3$ ppm as received in the tank) was passed through a fritted glass bubble trap with acid chromous chloride solution [22] (to remove O_2), an 8N NaOH solution (to remove CO_2 and acid spray) and finally through a trap filled with glass-wool (to remove spray) before being passed into the distilled water.

Experiments under controlled atmosphere were conducted in conventional sealed glove boxes with gasketed airlocks and suitable outlets for the nitrogen lines, recirculation purification system, distilled water, system for testing oxygen content, vacuum-trap system for removal of water and cleaning the gas-water interface before spreading the monolayer, etc. The atmosphere box used for preparation of special spectroscopic solutions had a recirculation purification system. The nitrogen in the box, kept at a slight positive pressure with respect to atmospheric, was continuously pumped through (a) an activated charcoal column, (b) condensation traps (filled with fine copper screen to increase surface area) refrigerated to liquid nitrogen temperature, and (c) a tube filled with 200-mesh copper gauze squares heated to about $400^\circ C$ [23], in that order. The oxygen content, during experimental work, averaged ≤ 20 ppm. When experimental results showed that control of polar molecule concentrations was insufficient for working with very high purity solutions, specially designed (to provide maximum surface

exposure and good circulation) containers with CP KOH pellets and (separately) with anhydrous magnesium perchlorate were installed within the box. The amount of condensable materials collecting in the liquid nitrogen traps per unit time was considerably reduced.

Deliberate introduction of gaseous impurities (e.g. wet and dry CO_2 , wet and dry O_2) into the chlorophyll solutions was done within the atmosphere box. The gases were carefully purified and, where intended, saturated with pure water vapor using conventional techniques. The addition of water vapor alone was done by bubbling pure nitrogen saturated with water vapor from degassed distilled water.

The system for testing oxygen content consisted essentially of a trap containing a piece of dry, yellow phosphorus with freshly cut surfaces. At 30 ppm of oxygen a fairly heavy evolution of phosphorous pentoxide smoke is observable in a slow moving dry nitrogen gas stream. At less than 10 ppm the smoke is no longer observable [24].

Sample weighings were made with a Sartorius microbalance to at least ± 0.005 mg. In addition to usual care to achieve great cleanliness, the apparatus and glassware used in controlled atmosphere work were thoroughly desiccated and flushed with pure nitrogen before use. Operations with the chlorophylls or their derivatives were carried out in low intensity diffuse green light. Commercially available transparent green plexiglass in 1/4 inch thicknesses, or greater was used for light filter. This

particular plastic transmits light almost entirely in the region of the green minimum of the chlorophyll-a absorption spectrum, and reduced the problem of photobleaching of samples greatly. Dilute solutions of chlorophyll-a when viewed in this green light, appear as colorless as water, and show no observable fluorescence.

The crystalline chlorophyll samples were predesiccated and degassed with a liquid nitrogen trap under high vacuum for 2 to 6 days at 25°C before use. The preparations of allomerized chlorophyll-a were carried out under controlled atmosphere conditions.

Monolayer experiments were carried in a constant temperature laboratory maintained at 20°C $\pm$ 1°. The laboratory air was maintained at less than 50 per cent relative humidity, and was continuously circulated through dust-removing filters and an activated charcoal air purifier to remove surface active vapors.

Preparation of the Chlorophylls and their Derivatives

Crystalline samples of chlorophyll a and chlorophyll b were prepared by a method similar to that used by E. E. Jacobs, A. E. Vatter, and A. S. Holt [11].[†] Variations will be described.

All operations were carried out at 4°C and very low light intensities. Humidity level was considerably below saturation.

[†]This work confirms the results of Jacobs et al [11] on the stability of the crystalline material. After 36 months of storage in the dark at 4°C there was no trace of decomposition, as determined by visible spectra measurements.

The work was carried out so that a minimum of time elapsed during transfer of the chlorophylls from the fresh spinach leaves to the mixed crystalline mat in the first phase of the process, and during the chromatographic separation through to the final crystallization of the pure products.

The spinach was carefully trimmed of all stems, and only the freshest, greenest leaves used. Prior to blending with acetone, the selected leaves were thoroughly washed with cold water to remove dirt and juices from the cut portions. Following blending, all insoluble material was filtered out through a Hy-Flo Supercell (cleaned diatomaceous earth) pad. The first effluent (about 10 per cent of the total liquid volume) contained no chlorophyll and, hence, was discarded. The insoluble material and filter pad were washed with fresh acetone in order to obtain all the chlorophyll.

The chlorophyll pigments were transferred to a petroleum ether (boiling range 30° - 60°) solution, having about 1/10 the volume of the initial acetone solution, in the following manner. Portions of the initial acetone solution (twice the volume of the petroleum ether) were shaken in a separatory funnel. Then twice as much distilled water as acetone was slowly added and gently shaken. In this way very little chlorophyll was lost in colloidal form to the aqueous phase. The chlorophyll is solubilized in the petroleum ether phase by the acetone in that phase. Consequently, the use of too large a ratio of distilled water to acetone in transfer step can result in the formation

of pigment crystals (particularly toward the end of the transfer process) and greater amounts of colloidal chlorophyll dispersed in the aqueous phase. In the operation of washing the petroleum ether solution of chlorophyll with 80 vol. per cent aqueous methanol solution, it is important to have sufficient volume of petroleum ether so that all the chlorophyll remains in solution (due to the presence of methanol in the petroleum ether phase). If this is not done, removal of the precipitated carotenols by filtration cannot be carried out. In most of the washing and transfer procedures, where two phases are present, mixing in the separatory funnel should be done gently. Otherwise, very stable, fine emulsions can be formed that can cause much loss of time in obtaining the final product.

Confectioner's 10X sugar with 3 per cent cornstarch was used for chromatography. It was pretreated as follows to remove moisture and other volatile materials: (a) heated at 90°C in an oven in a large, open crystallizing dish for about 10 hours, (b) evacuated for 1 1/2 to 2 hours, while still hot after removal from the oven, through a liquid nitrogen trap with a two stage mechanical pump. It was sifted with a triple stage flour sifter, directly before use in the column, into anhydrous reagent grade petroleum ether (30° - 60°C). The column was packed from slurries of the sugar in petroleum ether. The packed sugar was thoroughly washed with petroleum ether. These treatments provided for uniform and channel-free packing, and excellent adsorption and elution characteristics for the pigments. Pressure (with high

purity nitrogen) was used, rather than suction, since it provided a more uniform flow. The first, and last 1/10 of the chlorophyll-a solution was discarded. The chlorophyll-b was removed from the column by elution with acetone; the first and last 1/10's were discarded.

During chromatography the only colored bands observed were the chl a and chl b bands. Ultraviolet illumination of the column did not reveal any fluorescence from colorless bands. No odors were ever detected from the sugar, or from the final crystals (see below).

Crystallization of the chlorophylls was done by careful vacuum removal of solvent on a distilled water surface. In some batches, a small amount of amorphous residue (< 5 per cent of the material) was left on the distilled water surface, indicating that impurities, which may have been picked up from the sugar, were separated out during crystallization. The final crystalline aggregates were vacuum dried through a liquid nitrogen trap for about 1 week. The chlorophyll-a crystal aggregates were dark purplish blue in color with dull metallic lustre. The chlorophyll-b crystal aggregates were dark rich green in color with high metallic lustre. While there were variations from one batch of spinach to another, about one gram of chlorophyll-a, and one-half gram of chlorophyll-b was obtained from 20 lbs. of whole spinach plants.

The pheophytins were prepared as follows: to 100 cc of $10^{-5}M$ acetone solutions of the chlorophylls in a 500 ml

separatory funnel was added 5 cc of degassed 10 per cent (by vol.) HCl solution. After a minute with shaking, about 90 cc of benzene or ethyl ether were added. Then about 200 ml of distilled water was added slowly (to inhibit fine emulsion formation upon phase separation) to transfer the pheophytin to the benzene (or ether) phase. The benzene (or ether) solution was subsequently washed with eight 150 ml portions of distilled water to remove all acid and acetone. This was done with care so that little or no pigment was lost (in the form of fine droplets) in the water phases. Finally, the solution was diluted to 100 ml in a volumetric flask.

Three methods were tried for the preparation of allomerized chlorophyll-a. While only one was considered to give satisfactory results, it is of interest to compare all three. A 100 ml of 10^{-5} M solution of chlorophyll-a in degassed CP methanol was the starting solution in all cases. In all instances the following procedures were followed. The chlorophyll solution was placed in a 500 ml volumetric flask provided with a tight-sealing, standard taper, Teflon-sleeved stopper. Oxygen gas, which had been passed through a long column of Ascarite then through several large surface area traps cooled to dry ice-alcohol bath temperature, was bubbled through the methanol solution for 30 minutes with agitation. The flask was sealed with an oxygen atmosphere within and allowed to stand in the dark, at room temperature for more than 18 hours. About 80 ml of benzene (or ether) was then added to the solution in a separatory funnel. Then a procedure identical with that for pheophytin was followed

to attain the final benzene (or ether) solution. In the first instance nothing was added to the methanol solution. The absorption spectrum was almost identical in every detail with that of pure chlorophyll in a wet benzene. Constituent analysis (see below) of the spectral data showed 98.2 per cent chlorophyll-a, 0.2 per cent allomerized chlorophyll-a, and 1.6 per cent unknown.

In the second instance about 50 mg lanthanum chloride per 100 cc methanol solution was used. In this instance a definite chemical change occurred. However, there were significant intensity increases at 535 $m\mu$ and 505 $m\mu$ in the absorption spectrum which showed that the oxidation reaction was complicated by simultaneous loss of magnesium. This would indicate that the dissolution of lanthanum chloride in the methanol resulted in the formation of sufficient hydrogen ion for replacement of the magnesium in the chlorophyll. Constituent analysis showed 6.9 per cent chlorophyll-a, 66.6 per cent allomerized chlorophyll-a, 24.4 per cent pheophytin-a, and 2.1 per cent unknown.

In the last instance magnesium was dissolved in the methanol (before making up the chlorophyll solution) to give a solution of magnesium methoxide (about 10 mg Mg/100 ml methanol). The spectrum of the product showed no gross complications and closely resembled that of fraction number two reported by A. S. Holt [25]. The method of preparation was similar to that used by Holt to obtain pure fraction 2.

In Table 1 are compared the spectroscopic parameters of

Table 1

Comparison of the Spectral Parameters of This Research
With Those of the Chlorophyll Derivatives of
Holt, 25°C, Solvent is Ethyl Ether

Compound	λ_R^c	λ_B	λ_{BS}	B/R ^d	B/BS ^d	B/505 ^d	R/min ^{d,e}	$R_{1/2}$ m μ	$B_{1/2}$ m μ
Chlorophyll-a ^a	660.0	428.1	408.0	1.29	1.57	64	109	17.0	38.0
Pheophytin-a ^a	667.0	408.5	393.8	2.03	1.23	10.5	18.1	16.6	51.8
Allomerized ^a Chlorophyll-a	653.0	417.0	392.0	2.00	2.00	58.7	49.9	21.6	36.6
Allomerized ^a Pheophytin-a	670.3	399.8		2.77		18.0	19.2	20.5	40.7
Fraction 1 ^b	670	420		2.71		43.3	17.3	(46)	(55)
Fraction 2 ^b	652	417	(400)	1.88		(50)	67	(22)	(35)
Fraction 3 ^b	660	429	411	1.4	1.47	59	65	(20)	(37)

^aThis Research. ^bFrom the data of Holt, Table 1, Figure 6. ^cWavelengths are in m μ .

^dRatios of optical densities. ^eThe minimum occurs in the neighborhood of 470 m μ .

Holt [25] and this research. Data on chl a, ph a, and al ph a are included to illustrate the differences from Holt's fractions. The numbers in parentheses were estimated from Figure 6 of Holt. In the absence of molar extinction coefficient data, it is not possible, by comparison, to decide on absolute pigment purity, which will depend not only on the presence of pigments absorbing in the visible, but on the presence of colorless impurities. This matter is discussed subsequently.

From a comparison of the parameters available, it seems likely that the al chl a of this work was probably less pure than the fraction 2 of Holt. This is evidenced by the smaller R/min ratio and the larger $B_{1/2}$ value (width of the main blue band at 1/2 peak optical density) of this research. Since the spectrum of the al chl a of this work showed a small "tail" on the long wave length side of the main red band, extending over the region occupied by the main red band of Holt's fraction 1, it seems reasonable to suppose that a small amount of this compound was present in our sample. This view is also supported by the fact that the B/R ratio of this work is about 6 per cent larger than that of Holt's fraction 2. If it is assumed that the red molar absorption coefficients of fractions 1 and 2 are about equal, then we estimate, from the magnitude of the absorbance of the red "tail" of our spectrum, that our sample may have had from 5 to 10 per cent impurity. This will introduce some uncertainty in the constituent analysis method, discussed subsequently.

The allomerized pheophytin-a (here, we mean allomerized chlorophyll-a where the Mg atom has been replaced by two hydrogens, as in pheophytin-a) was prepared in the same manner as pheophytin-a, only starting with a solution of allomerized chlorophyll-a.

Spectroscopic Measurements

Visible absorption spectra measurements were made with a Cary 14 recording spectrophotometer run on automatic slit control over a scanning range of 750 $m\mu$ to 350 $m\mu$ at 5A/sec. For a typical chlorophyll spectrum manual variation of the slit widths to about ± 10 per cent of the automatic setting produced only negligible differences in optical densities at a given wavelength. The accuracy of the instrument was usually ± 0.002 unit of optical density but occasionally was as low as ± 0.006 . These errors were generally small compared to those from other sources (to be discussed subsequently). The accuracy in wavelength was generally better than $\pm 0.5 m\mu$. Corrections for scattering and fluorescence were estimated to be negligible.

The Beckman pyrex absorption cells were modified by sealing No. 9 standard taper outer joints on the tops. The cells could then be stoppered with ground glass, polyethylene, or Teflon-sleeved stoppers. The rate of leakage[†] of impurity

[†]Leakage of polar molecules through a ground glass stopper was sufficiently rapid that within a period of 10 to 15 minutes in ordinary laboratory air the chlorophyll spectrum of a dry solution could be observed to be changing in a manner characteristic for the addition of polar molecules.

molecules (H_2O , O_2 , CO_2 , etc.,) through the stoppered joint was in the order: ground glass >> polyethylene >> Teflon sleeve. The use of Teflon-sleeved stoppers was absolutely essential when highly purified solutions were being measured. In fact, as a supplementary precaution, the cells were often kept in a vacuum desiccator (in darkness) filled with pure nitrogen until just before scanning.

All cells were calibrated with respect to a control cell and individual cell corrections over the scanning range determined. Procedural errors were estimated to have introduced an uncertainty of ± 3 per cent or less in concentrations of the pigments. Crystalline samples were re-desiccated at 25°C under vacuum through a liquid nitrogen trap at least two days before weighing. Weighing was done at 20°C , where the relative humidity was 50 per cent or less. Spectra measurements were made at 25°C and concentrations corrected for temperature change.

Oscillator Strengths

The oscillator strengths were determined [26], [27] with

$$f = \frac{2303mc^2}{\pi Ne^2} \int \epsilon_{\bar{\nu}} d\bar{\nu} = 4.318 \times 10^{-9} \int \epsilon_{\bar{\nu}} d\bar{\nu}, \quad 1)$$

$$D = \frac{2303}{N} \frac{3hc}{8\pi^3 e^2} \int \epsilon_{\bar{\nu}} d\bar{\nu} / \bar{\nu} = 3.980 \times 10^{-20} \int \epsilon_{\bar{\nu}} d\bar{\nu} / \bar{\nu}, \quad 2)$$

where $m = 9.1072 \times 10^{-28}$ gm, $c = 2.99776 \times 10^{10}$ cm/sec,
 $N = 6.0254 \times 10^{23}$ mole $^{-1}$, $e = -4.8023 \times 10^{-10}$ e.s.u.,
 $h = 6.6238 \times 10^{-27}$ erg sec, $\bar{\nu}$ = wave number in cm $^{-1}$, and
 $\epsilon_{\bar{\nu}}$ = molar extinction coefficient at $\bar{\nu}$.

An Ott type 30 compensating polar planimeter was used for measurement of areas under the curves. Errors in measurement were negligible compared to those from other sources to be discussed subsequently.

Constituent Analysis

In early phases of this work, observations on the variations of the absorption spectra of dilute solutions of chl a indicated that mixtures of several intact ring derivatives can be rather easily formed under normal laboratory conditions (results are summarized in Appendix A). Similar observations were made, for example, by F.P. Zscheile and co-workers [28], [29], whose classical work on chlorophyll purity established the values of the optical density ratios B/R, R/505 (for chl a), R/520 (for chl b), and R/min as criteria for sample purity. Variations of these ratios, and increases of main band half-widths have been used as an indication of degradative reactions (for example references [4] and [5]).

No work appears to have been reported establishing what mixtures of products are usually formed under normal laboratory conditions leading to degradation. Under usual conditions,

oxygen, water vapor, and carbon dioxide are present to interact. Water and carbon dioxide can interact to cause pheophytinization (for example, reference [2] p. 452), while the cyclopentanone ring is readily attacked by oxygen (for example, reference [2] pp. 400, 462, 1773-1774). This suggested that mixtures of intact ring derivatives, under normal laboratory conditions, would arise primarily from processes of pheophytinization and allomerization.

Although Holt [25] showed that allomerization can result in at least three products, he found that fractions 1 and 3 can both be converted into fraction 2. Under circumstances leading to formation of several derivatives, fraction 2 is the pre-dominant product (70 - 80 per cent). When water is present to give hydroxyl ions, fraction 2 is formed in preference to fraction 1. This evidence suggests that allomerization, occurring under most laboratory conditions, will lead primarily to products having the expanded isocyclic ring structure of Holt's fraction 2. Allomerization under laboratory conditions will give products having a hydroxyl group on the C_{10} atom rather than a methoxy group as obtained in methanol solutions. This difference in substitution does not give rise to significant differences in the visible absorption spectrum [25].

It was assumed, in the absence of extensive light activation and where water, carbon dioxide, and oxygen are present, that chl a could give mixtures of al chl a, ph a, and al ph a. Similarly al chl a could degrade to al ph a, and ph a to al ph a. In dilute solutions (total concentration the order of $10^5 M$),

where the solvent is wet benzene or ethyl ether, mixtures of derivatives are likely to be non-interacting. The absorption spectra at given wavelengths were assumed, therefore, to be additive. Consequently, the optical density of a mixture is given by

$$d = \log I_0/I = (\epsilon_1 C_1 + \epsilon_2 C_2 + \dots)l, \quad 3)$$

where ϵ_i = the molar extinction coefficient for the i^{th} absorbing component, C_i = its concentration, and l = the path length. If there are n components, then n simultaneous equations for the densities at n different wave lengths are needed to solve for the C_n 's. Solutions can be obtained by the method of determinants.

To minimize the effect of small errors, wavelengths in the regions of the maxima of the main red and blue bands were chosen. The optical densities of the four components (i.e. chl a, al chl a, al ph a and ph a) at a concentration of $0.99 \times 10^{-5}M$, and the values of the denominator determinants are given in Table 2. It is desirable to calculate the unknown concentrations from at least two different sets of simultaneous equations to reduce the possibility of spurious correlations. The solvents used were reagent grade, saturated with water, and having dissolved oxygen and carbon dioxide in amounts probably $\geq 10^{-5}M$.

From application of the method to a large number of solutions containing various amounts of the a derivatives, an uncertainty of ± 5 per cent or less was found when the primary components were chl a, ph a, and al ph a. If large percentages

Table 2

Optical Density (d) Values and Wavelengths for the $0.99 \times 10^{-5} \text{M}$
Solutions, and Values of the Denominator Determinants
Used for Component Correlation

A. For Benzene (H ₂ O, O ₂ , CO ₂) Solutions					
Compound Wave- length m μ	Chl. <u>a</u>	Allom. Chl. <u>a</u>	Allom. Pheo. <u>a</u>	Pheo. <u>a</u>	
		d			
667.5	0.723	0.226	0.282	0.501	= Determinant A = -96.8686×10^{-3}
405.0	0.569	0.652	0.989	0.902	
427.5	0.840	0.646	0.283	0.465	
415.0	0.633	0.907	0.720	1.088	
Compound Wave- length m μ	Chl. <u>a</u>	Allom. Chl. <u>a</u>	Allom. Pheo. <u>a</u>	Pheo. <u>a</u>	
		d			
405.0	0.569	0.652	0.989	0.902	= Determinant B = $+40.1329 \times 10^{-3}$
662.5	0.696	0.343	0.182	0.329	
427.5	0.840	0.646	0.283	0.465	
415.0	0.633	0.907	0.720	1.088	

Table 2 (Contd.)

B. For Ethyl Ether (H ₂ O, O ₂ , CO ₂) Solutions					
Wave-length mμ	Compound	Chl. <u>a</u>	Allom. Chl. <u>a</u> d	Allom. Pheo. <u>a</u>	Pheo. <u>a</u>
420		0.796	0.955	0.347	0.609
665		0.659	0.221	0.300	0.505
415		0.690	0.983	0.521	0.882
405		0.688	0.725	0.902	1.022
= Determinant C = +46.11707 × 10 ⁻³					
Wave-length mμ	Compound	Chl. <u>a</u>	Allom. Chl. <u>a</u> d	Allom. Pheo. <u>a</u>	Pheo. <u>a</u>
405		0.688	0.725	0.902	1.022
415		0.690	0.983	0.521	0.882
667.5		0.477	0.121	0.344	0.525
420		0.796	0.955	0.347	0.609
= Determinant D = +39.917798 × 10 ⁻³					

of al chl a were present uncertainties as large as ± 10 per cent were sometimes found. In many instances study of the whole spectrum provided for resolution of the uncertainties. If extensive photobleaching had occurred, as evidenced by rise in the minimum of the spectrum, it was not possible to obtain good correlations. Products arising from rupture of the chlorin ring are formed.

The accuracy of this method of analysis depends on the purity of the four original pigments used to obtain the characteristic optical densities, and the absence of different intact ring derivatives in the solution to be analyzed. The purities of the chl a and ph a components were high, and are discussed subsequently. As stated previously, the allomerized compounds probably had a few per cent of a second constituent present as impurity. This could account for some of the observed uncertainties. It seems more likely, however, that small amounts of intact ring derivatives, different than the four components used for analysis, were being encountered. The surprisingly good correlations found are evidence that most of the oxidized intact ring products had the expanded isocyclic ring structure.

The percentage decomposition of ph b in the monolayer stability tests was roughly estimated by determining the change in the ratio (B/BS) of the peak optical densities of the blue and blue satellite bands. The basis for this was as follows.

Partial allomerization of ph a causes a significant increase in absorption in the region of the blue satellite peak of

ph a, and a corresponding decrease in the blue peak. A decrease in the ratio B/BS is observed. This ratio for ph b was also observed to decrease significantly under the same monolayer or solution conditions that led to allomerization of ph a. Other changes in the visible absorption spectrum were similar to those occurring for ph a on partial allomerization. The wavelength of the main red band shifted to the red and the main blue band shifted to the blue. The half widths of the main red and blue bands increased, which is good evidence for the presence of a mixture of different intact ring chlorin derivatives. Under conditions providing for monolayer stability the spectral parameters showed little or no change. These observations were taken as indirect evidence that allomerization of ph b in monolayers or solutions occurred, when it occurred for ph a. Consequently, the change of B/BS was used as a crude estimation of degree of allomerization of ph b. It must be clearly emphasized that the evidence for allomerization of ph b is quite indirect. It seems certain, however, that different intact ring products were formed.

Under identical conditions of testing, the B/BS ratio of ph b was found to change in fairly constant proportion to the percentage of al ph a formed from ph a. By assuming that the rate of decomposition of ph a and ph b were roughly equivalent, the percentage change of ph b could be calculated from the change in B/BS.

The percentage recovery of ph b was estimated from knowledge of the amount spread, and comparison of the peak optical densities of the red, blue, and blue satellite bands with those from a solution of known concentration of pure pigment. Since peak optical densities vary with change of substitution on the chlorin ring, this procedure can give, at best, only rough qualitative values.

Monolayer Apparatus

The Wilhelmy plate method [30], [31], with use of a Christian Becker chainomatic analytical balance (accuracy, ± 0.2 mg), was used to measure surface pressure. The balance was modified by putting a hole through the floor beneath one of the arms to allow for attachment of the plate. This was done with suitably formed glass rods, and a specially constructed clamp with Teflon jaws. Stops were placed under the pans to prevent accidental vertical motion. In use, vertical motion was the order of 0.06 mm.

Change of surface tension, which is related directly to the amount of water drawn up on the sides of the plate, was determined directly from the change of weight on the balance arm. A 20 foot long optical arm was used for zeroing. A circular, front surface galvanometer mirror was mounted on the beam balance above the fulcrum. The light source was a zirconium arc point source which was focused on the mirror with an achromatic

condensing lens to give a sharp, undistorted image on the distant scale.

The Langmuir trough was heavily coated with Fisher Scientific Company's white purified paraffin (m. p. $65^{\circ} - 70^{\circ}\text{C}$). Of a number of paraffins evaluated, this material was the only one that did not give off significant amounts of surface active impurities in the experimental periods. The cleaned trough, with the buffer solutions used, gave off, in a period of eight hours under the conditions of experimental testing, impurities that could have introduced the following maximum positive errors in π : 0.05 dyn/cm in the range $210\text{A}^2 \geq \sigma \geq 155\text{A}^2$, 0.10 dyn/cm in the range $150\text{A}^2 \geq \sigma \geq 130\text{A}^2$, and 0.20 dyn/cm in the range $125\text{A}^2 \geq \sigma \geq 100\text{A}^2$. Since the testing periods were usually five hours, or less, smaller errors than indicated were probably introduced.

The trough edges and the barriers were machined from Teflon which, with suitable weights on the barriers, provided good gasket-like seals at the barrier-edge intersections. With the pheophytin monolayers, however, at high surface pressures, spreading of the water (which was maintained at a height of about 6 mm above edge level) onto the Teflon-edge surfaces occurred. This could be prevented by applying a very thin film of paraffin onto the Teflon from a petroleum ether solution. The movable barrier was motor driven, and could be run at different speeds.

Both the vibrating disk [32], [33], and the ionizing air electrode [33], [34] methods were used to measure surface potential. Both methods gave the same results within experimental accuracy. The latter method was used almost exclusively because of the greater ease, flexibility, and range of surface scanning.

The vibrating disk apparatus was constructed with the use of a 25-watt high fidelity, floating magnetic core, loud speaker driver, which was suitably mounted and run by a Jackson Model No. 652 audio frequency oscillator. The polished, circular gold plated electrode was attached to the driver mouth through a two-inch long, 1/8-inch diam. bakelite rod which was bolted to the center of a two mil thick spring steel circular diaphragm.

Signal transformation (i.e. alternating current to alternating voltage) was made with the use of a 30-megohm, low-noise resistor, manufactured by the Victoreen Instrument Co. The resistor consisted essentially of a deposited carbon element sealed in a glass envelope which contained an inert gas. The potential balancing and measuring systems were conventional. A specially constructed coaxial cable (45:1 ratio insulation diam. to conductor diam., 7 mm as average separation distance from conductor to shielding braid), having a very low capacity grounding loss of signal, was used to bring the signal into the amplifier.

A General Radio type 1231-B amplifier and null detector, in conjunction with a G.R. type 1231-P5 wave filter, was used to

amplify the signal. The signal was detected with a Dumont type 208-B high-gain oscilloscope. The total gain was about 50,000.

The ionizing air electrodes were made by electrodepositing polonium 210, and then a very thin (0.05 - 0.15 mil) layer of gold onto circular platinum disks with platinum lead wires. The gold plating insured against the occurrence of flaking off of the polonium. The direct current signal was detected by amplification with a Cary Model 31 Vibrating Reed Electrometer. The instrument was used, as recommended by the manufacturer, with a shielded, Victoreen 10,000 meg-ohm resistor installed between the input and feedback terminals. This allows for continuous measurement or detection of small currents. The output signal was led into a Sargent Model MR recorder, so that the surface potential variations could be recorded continuously, or balanced to a null point with the external potential balancing system. Since sensitivity of detection was limited only by the height of the electrode from the water surface, its determination provided for direct reading of the surface potential changes on the recorder chart. The electrode was provided with a mounting stand that allowed for complete scanning of the water surface. As a consequence, it was a simple matter to determine surface heterogeneity of the monolayer, patches of surface impurities during the initial cleaning processes, and the presence, or absence, of leaking (i.e., of the compressed monolayer) past the movable and fixed barrier bars. A saturated calomel electrode, a clean silver wire, or a clean platinum wire

could be used interchangeably as opposing electrode without affecting the values of ΔV .

Use of a polonium electrode, in contrast to the vibrating disc electrode, requires care in preventing the α -particles, and the high energy ions, from causing significant decomposition of the monolayer. Thus, in exploratory tests with the apparatus, it was very soon discovered that reduction of the surface potential[†] (from 100 to 150 mv) rapidly occurred if the electrode was allowed to remain in position over a particular area, or if it was positioned too close to the surface during scanning motion. It seemed likely that primarily decomposition reactions occurred. Consequently, the polonium electrode was positioned at about 1 cm from the water surface during measurements, and positioned entirely off the surface during equilibration periods and measurement of surface pressure. At the rather great distance of the electrode from the surface, the magnitude of the measured potential was not affected if proper control of humidity was maintained. The signal rise time was increased slightly, however, because of the smaller current flow across the air gap.

Measurements were carried out within a heavy plywood enclosure, having suitable ports, windows, and adjustable legs for leveling. The inside was painted black and then coated with paraffin wax. The outside was covered with layers of

[†]Similar changes have been observed for protein monolayers [35].

s3-3 Netic foil, and where additional shielding was needed Co-Netic AA foil was used. These ferromagnetic foils are manufactured by the Perfection Mica Company in Chicago Illinois. They are characterized by a high efficiency in screening out both magnetic and electrostatic fields. Because of their thinness (0.004 to 0.005 inch) and dead softness, they can be easily applied in most shielding problems.

During experiments the relative humidity within the enclosure was held to about 100 per cent with the aid of large sheets of chromatography paper dipped in beakers of water. After a monolayer had been spread, the cabinet was effectively closed off from the laboratory during the measurement period.

A Gilmont precision micropipetburet with micrometer drive was used in spreading the monolayers. It was a direct reading instrument with an accuracy of ± 0.001 cc for most liquids. In use with ethyl ether solutions, however, the accuracy was probably ± 0.003 cc. The instrument was used with a luer fitting and a number 27 hypodermic needle. The ether solutions contacted only glass, the Teflon gasket, and the stainless steel needle. To prevent accumulation of pigment on the needle tip, due to solution creep while delivering solution to the water surface,

the tip was touched to the water surface immediately after delivery.

Monolayer Procedures

Both benzene and ethyl ether were tried as spreading solvents. The latter was adopted since homogeneously spread films could be obtained more easily from it. The ether was C.P. reagent grade and used from freshly opened containers without further purification. It was tested for surface residue by spreading various amounts of pure solvent onto a clean water surface and determining changes of surface pressure and surface potential upon compression. To get measurable changes over the whole range of trough area about three times as much solvent as used in spreading pheophytin had to be used. Then the amount of change that would have occurred, using one third the amount of solvent was calculated. The results showed that maximum positive errors in π of: 0.05 dyn/cm in the range $210\text{Å}^2 \geq \sigma \geq 155\text{Å}^2$, 0.10 dyn/cm in the range $150\text{Å}^2 \geq \sigma \geq 130\text{Å}^2$, and 0.20 dyn/cm in the range $125\text{Å}^2 \geq \sigma \geq 100\text{Å}^2$ could have been introduced.

The need for rather unusual care in spreading the pheophytin monolayers was first evidenced by large variations in ΔV (as great as ± 150 mv) over the surface after spreading. This heterogeneity did not diminish in stand periods of five hours, nor did it diminish significantly on compression of the film. Incompletely spread films showed smaller values of π and ΔV at

given values of σ , in comparison with homogeneous monolayers.

The following procedure was found to give satisfactory results. A $10^{-4}M$ solution of the pigment was added dropwise in increments of about 0.005 cc every 6 to 10 seconds.[†] The drops were distributed evenly over the surface. The amount of pigment added was such that the initial total surface area was from 40 to 80 cm² greater than that corresponding to the start of measurable changes of surface pressure. The initial area per molecule was from 230 to 245 Å². This procedure appeared to provide a suitable avoidance of undesirable piston oil effects of the spreading solvent, while allowing for a uniform molecular distribution with the absence of three dimensional aggregation.

A well spread monolayer showed variations in ΔV of ± 20 mv or less at $\pi < 1.0$ dyn/cm, and ± 2 mv at higher compression. These variations were reproducible on decompression and recompression, provided that the collapse pressure had not been greatly exceeded. If the film had been collapsed, lower surface potentials and greater variations in ΔV were observed at all areas. Variation of electrode area from about 0.8 cm² to 0.07 cm² did not reveal significant differences in magnitude of potential variation in homogeneous monolayers. Scanning with the smaller electrode, however, gave a greater number of fluctuations,

[†]It was observed that the slight rippling of the water surface, due to spreading from the droplet, had ceased after these time periods.

but primarily at molecular areas for $\pi \leq 1.0$ dyn/cm. From these observations it cannot be concluded that the ultimate in monolayer homogeneity was achieved. However, the areas found at collapse and experimental reproducibility indicate that the films must have been at least 95 per cent mono-molecular. This is discussed subsequently.

Use of a spreading peg[†] (wire, slide, etc.), which provides for smooth, even flow of the spreading solution onto the water surface, was extensively investigated in this work. While providing for some improvement toward lessened heterogeneity, it proved to be a minor factor. As observed at $\pi \sim 0.10$ dyn/cm, homogeneously spread films were more easy to obtain at low pH's than at high pH's.

The procedures for testing the stability of various monolayers at air-water, or nitrogen (≤ 100 ppm O_2 , ≤ 10 ppm CO_2)-water interfaces were as follows. The monolayers were spread at areas per molecule ($\geq 240 \text{ \AA}^2$) corresponding to pressures less than about 0.10 dyn/cm. Some monolayers were subsequently compressed. After the test period, a portion of the monolayer was compressed onto a large clean glass plate wetted with the same aqueous substrate used during the test. The pigment on the plate was then collected (with the use of solvent in a hypodermic syringe) into a known volume of reagent grade

[†]This technique was developed by H.J. Trurnit and co-workers in several of their surface chemistry researches. For example, see reference [14].

solvent (i.e. benzene, or ethyl ether), and analyzed spectroscopically as described above. All of the apparatus (glassware, and nickel tongs) used in transferring the monolayer were thoroughly clean and wetted with the test aqueous substrate. It was unlikely, therefore, that the pigment contacted any surface other than the test aqueous substrate.

On the basis of the areas of the plate and the final aqueous substrate area in the trough, it was estimated that a maximum of 80 per cent $\pm$ 10 per cent of total pigment could be recovered. In practice, an average of about 57 per cent was recovered with variations from 49 to 90 per cent. Excessive mechanical losses of pigment probably occurred during removal of the plate from the trough and subsequent transfer to solution, since the whole operation had to be carried out quite rapidly so that drying of the glassware did not occur. When the latter occurred, spurious observations of chemical instability were sometimes made. The reason for this is not known, although interaction with the glass or dried (or concentrated), salt may have been an important factor.

Microscope cover glasses, 0.15 mm thick and 128 mm working perimeter, were used as Wilhelmy plates. Thin tantalum and platinum sheets were tried, but were found not to preserve a zero contact angle.

At air-distilled water interfaces, in exploratory phases of the work, little problem was encountered in obtaining and maintaining zero contact angle on the glass slides, even on

decompression of the monolayers. The conventional cleaning treatment, using hot chromic acid solution, followed by rinsing and storing in distilled water was found suitable. Spreading of the monolayer could be done with the slide in position in the water surface. Considerable difficulties, however, were encountered in obtaining and maintaining zero contact angle with the pheophytins on low pH buffer substrates.

It was necessary to etch[†] the glass slides slightly in hot (~80°C) solution of Haemosol (Meineche and Co., Inc.) and tri-sodium phosphate. Following etching, the slides were rinsed thoroughly in hot, then cold distilled water, and stored under distilled water, the pH of which had been adjusted with HCl near to that of the experimental substrate. This procedure usually provided for an initial zero contact angle when the slide was in position during spreading of the monolayer.

Lack of reproducibility of results and a fortunate observation of a color change in the slide after a run quickly led to the observation that the pigments were adsorbing strongly on the wet glass. The adsorption occurred primarily at low (< 1.0 dyn/cm) surface pressures, and was uneven if the slide was present during spreading. It was necessary, therefore, to insert the slide after the monolayer had been spread. By

[†]Etching, or roughening a surface is known to decrease contact angle of surface active solutions on solids [36].

observation of surface potential changes (after insertion), and spectroscopic analysis[†] of the amount of adsorbed pigment it was established that 75 per cent or more of the adsorption occurred at $\pi \sim 0.10$ dyn/cm. Nevertheless variable additional adsorption occurred upon compression and accounts for part of the reported experimental uncertainty (see below).

Correction of surface concentration for every run was made for the adsorption on the glass. Since adsorption occurred so strongly, it was assumed that the area of the glass plate was not accessible to the monolayer in calculation of area per molecule.

The procedures of etching the slide and installing it after spreading always insured a zero contact angle initially and during compression. Almost invariably, however, the contact angle increased from zero (see below) on decompression. Consequently, only surface pressure values obtained on compression were considered reliable.

Glass slides that had been thoroughly cleaned gave no water breaks when wetted (i.e. water spread evenly over the whole surface). They showed menisci, when inserted vertically into a clean water surface, that rose sharply and curved in parallel to the plane of the slides. Different clean slides of the same dimensions, or the same glass slide recleaned and used on different occasions required the same counterbalancing weight,

[†]The spectra did not reveal any signs of chemical degradation for the pigments which had been adsorbed.

within a few milligrams, when inserted into a clean water surface under identical experimental conditions. Neither the counterbalancing weight, nor the appearance of the menisci changed during a stand period of 18 hours in a clean water surface. When a milligram of weight was added, or subtracted from the counterbalancing arm, the slides were vertically displaced out of, or into the water surface by a small distance (determined by the deflection of the light beam of the scale). For different clean slides of the same dimensions, in clean water surfaces, the displacements were identical, viz. 12 mm $\pm$ 1. The preceding observations were considered to be good evidence that zero contact angle had been obtained.

In monolayer runs with the pheophytins at low pH substrates, compression of the monolayers did not give rise to changes in behavior of the slides. On decompression, however, the vertical displacements, described above, were usually drastically reduced (e.g. 3 to 4 mm), and the line of contact of the water to the plate was often observed to be uneven, giving a crinkled appearance. These phenomena clearly indicated an increase of contact angle from zero, and hysteresis of wetting. Calculated surface pressures, at various areas on decompression, were erratic; values were obtained that were both larger and smaller than those obtained on compression.

The change in slide behavior was considered to arise primarily from additional slight interaction of the pheophytins with the wet glass at the water contact line. In most of the

runs, it was observed that the surface potentials on decompression were consistently lower by a few millivolts (2 to 10 mv) at given areas, which showed that the surface concentration of pigment had been reduced. Since the film heterogeneity had not increased, as evidenced by potential scanning over the surface, it did not appear that any film collapse had occurred. When no slide was present, repeated compression and decompression did not result in a change of surface concentration, as evidenced by surface potential values. If the film area had been reduced below one corresponding to about $90 \text{ \AA}^2$ at $\text{pH} = 4.0$, and about $100 \text{ \AA}^2$ at $\text{pH} = 3.0$, surface potential values decreased with time and an increase in surface heterogeneity occurred, which indicated film collapse.

Barrier motion was $\leq 0.3 \text{ mm/sec}$, corresponding to about $9 \text{ \AA}^2$ /molecule/minute or less. After an area change, corresponding to about 4 per cent of the total area per molecule, the film was allowed to equilibrate from 5 to 10 minutes. More rapid and continuous barrier motions often resulted in increased surface heterogeneity and transient hysteresis effects in both pressure and potential.

Experimental Accuracy - Spectroscopic Data

The primary uncertainty in the measured spectral parameters lies in the uncertainty of the absolute purity of the pigments. Accuracy of the monolayer data also depends on pigment purity, but other factors are involved and will be discussed subsequently.

Preparations of different batches of chlorophyll crystals were made until the visible spectral parameters from batch to batch were reproducible to within about ± 3 per cent. This amount of variation was the maximum found in weighing and volumetric procedures. Use of a magnesium sulfate column in this laboratory [37], for chromatographic purification and separation for completely different preparations of the chlorophylls, gave crystals the spectral parameters of which agreed to within better than ± 1 per cent with those from the method described in this work. A comparison is given in Table 3 of the visible absorption spectral parameters of the chlorophylls of this research and those of the following authors:

- a) H.H. Strain et al [38], b) W.D. Bellamy et al [5],
- c) F.P. Zscheile et al [28], d) J.H.C. Smith et al [39],
- e) H.J. Trurnit et al [14], f) H.J. Perkins et al [40],
- g) D.G. Harris et al [29], and h) A.S. Holt et al [41].

Apart from the problems of allomerization, pheophytin formation, removal of water from the chlorophyll crystals, and photodecomposition, separation of the xanthophylls and carotenols (which absorb in the blue region), and colorless impurities from the chlorophylls by chromatography have currently been emphasized as problems.[†] In terms of variation of the visible absorption parameters given in Table 2, the following

[†]For example, references [38], [40], and [42].

Table 3

Comparison of the Visible Absorption Parameters
of the Chlorophylls in Ethyl Ether

Reference ^a	$\epsilon_{\text{Red}} \times 10^{-4}$ liters mole cm	λ_R m μ	λ_B m μ	λ_{BS} m μ	B/R	B/BS	R/min ^b	R/505 for a R/520 for b	R _{1/2} m μ	B _{1/2} m μ
Chlorophyll <u>a</u> (M.W. 893.5)										
This	8.63 $\pm$	660.0	428.1	408.0	1.290	1.573	109	46	17.2	38.2
Research	0.20 ^c	± 0.1	± 0.3	± 0.3	± 0.007	± 0.018	± 13	± 4	± 0.2	± 0.3
(38)	8.63	660.5	428.1		1.295					
(5)	8.40	661	427		1.280	1.59	98	46	[15] ^d	
(28)	9.10	660.0	429	410	1.32	1.58	114	52.4	18.5	38
(39)	9.00	662	430	410	1.30	1.54				
(14)	[6.61] ^d	660.9	427.7	409.0	1.32	1.57	63		17.3	38.9
(40)		660.0	428		1.29					
(29)	(8.70) ^e	660.0	429.0	410.0	1.33			53		
(41)	8.55 ± 0.18	660.0	427.8 ± 0.3	408	1.33	1.57	105	53.9 ± 1	18	39

Table 3 (Contd.)

Reference ^a	$\epsilon_{\text{Red}} \times 10^{-4}$ liters mole cm	λ_R m μ	λ_B m μ	λ_{BS} m μ	B/R	B/BS	R/min ^b	R/505 for <u>a</u> R/520 for <u>b</u>	R _{1/2} m μ	B _{1/2} m μ
Chlorophyll <u>b</u> (M.W. 907.5)										
This Research	5.09 $\pm$ 0.10	641.8	452.3	427.5	2.81 $\pm$ 0.01	2.71	22.9	18.0	16.1	21.2
(38)	5.69	642.0	452.5		2.84					
(28)	5.15	642.5	453	430	3.00	2.92	21	18.9	17.0	22
(39)	5.61	644	455	430	2.81	2.78				
(29)	(4.97) ^e	642.5	453	428.5	2.98					
(14)	5.21	642.4	452.7	428.4	3.00	2.72	23.9		16.3	22.2

^aSee reference numbers in the text.

^bFor a the minimum occurs at about 468 m μ ; for b at about 497.5 m μ .

^cThe same per cent deviations were found for all other major bands.

^dThis value may have been reported erroneously.

^eValues estimated from the data given.

major changes (with reference to pure chlorophyll) occur in the contamination problems stated above.

Allomerization.[†]--For chl a, the peak wavelengths of the main red (λ_R) and blue (λ_B) bands are shifted significantly to the blue. The red molar extinction coefficient (ϵ_R) decreases about 40 per cent, with the result that the ratio of the peak optical densities of the blue to the red band (B/R) increases from 1.29 to 2.00. Similar changes may occur for chl b.

Pheophytinization.--For both chl a and chl b, λ_R shifts significantly to the red, while λ_B shifts significantly to the blue. For chl a, ϵ_R decreases by about 40 per cent and B/R increases from 1.29 to 2.03; in chl b ϵ_R decreases by about 38 per cent and B/R increases from 2.81 to 4.89. The appearance of the green bands at about 505 $m\mu$ (for chl a) and at about 520 $m\mu$ (for chl b) results in a decrease of R/505 from 46 to 4.52, and a decrease of R/520 from 18.0 to 2.97. Correspondingly large decreases in the R/min ratios occur. The blue half width $B_{1/2}$ of chl a increases from 38.2 to 51.8 $m\mu$; for chl b there is a decrease from 21.2 to 16.2 $m\mu$. If chl a and chl b samples had varying amounts of the allomerized products and/or the pheophytins, both the red and blue half widths would be increased, and the ratio B/BS would be smaller.

Water of hydration.--It is possible that from 1/2 to 2 molecules of water could be present in imperfectly desiccated

[†]See discussion on constituent analysis.

crystals [41], [43]. This would have the effect primarily of lowering the molar extinction coefficients if the dry molecular weights were used for calculation.

Photodecomposition.--In general, light activation of the chlorophylls in dilute solutions, when oxygen is present, can result in decomposition where products are formed derived from rupture of the chlorin ring. Decreases of $R/\min$, $R/505$, and $R/520$ occur, there is a lowering of ϵ_R , and both blue and red half widths increase. If the decomposition products interacted specifically with the chlorophylls, shifts in the band peak wavelengths might also be expected to occur.

Xanthophylls and carotenols.--As impurities absorbing in the blue and green regions, their presence would increase B/R and $B_{1/2}$. Both ϵ_R and $R/\min$ would be reduced.

Colorless impurities.--In general, if the impurities are noninteracting, a decrease of ϵ_R without change of B/R would be expected. If they interacted specifically with the chlorophylls in dilute solution, changes in other parameters could occur, shifts in band peak wavelengths and increase of half widths would perhaps be most predominant.

The value of the red molar absorption coefficient provides the best criterion for absolute purity. Knowledge of the other parameters discussed is important in helping to decide which type of impurities might be present.

There is considerable variation in the reported values of ϵ_R . Differences in technique in determining total

chlorophyll, and procedural errors could give rise to both positive and negative deviations in ϵ_R . In this work, large positive deviations were observed unless special precautions were taken to prevent evaporation of the ether. It seems reasonable to calculate average values of ϵ_R as a basis for estimating absolute pigment purity. Using the values in Table 3 (except that for chl a reported by Trurnit et al [14]) and the values obtained independently in this laboratory [37] which were nearly the same as in this research, the average for chl a is 8.70×10^4 liters/mole cm, and for chl b, 5.25×10^4 liters/mole cm. The corresponding averaged deviations are $\pm 0.17 \times 10^4$ and $\pm 0.21 \times 10^4$ liters/mole cm. On this basis, the chl a of this work is estimated to have had an impurity of about 1 per cent, and chl b about 3 per cent. These impurity levels are within the range of reproducibility of the spectral parameters of different chlorophyll preparations of this work. It seems reasonable to conclude that the calculated average values of ϵ_R may be very close to those for the 100 per cent pure chlorophylls.

The recent careful work of Perkins and Roberts [40] showed that the limiting value of B/R, for extremely pure chl a, is probably 1.29. This value was also found in this research, and in other work in this laboratory [37].

The research of Zscheile and co-workers [28], [29] showed that the values of the R/505 and R/520 ratios are very sensitive for detection of traces of pheophytins as impurities. However,

so also are the half widths and the values of $R/\min$. Since our values of these latter parameters are equal to, or better than those reported by Zscheile and Comar [28], it seems likely that our slightly lower values of $R/505$ and $R/520$ signifies the presence of only very small amounts of pheophytins.

As shown in Table 3, there is considerable variation in B/R for chl b. The other work in this laboratory [37] gave a value of 2.83, in close agreement with our 2.81. Since several factors can account for an increase in B/R , it is not possible to decide what the causes of the discrepancies are. The lower values are more characteristic of chlorophyll preparations done in recent work with improved methods, and probably can be considered to be more reliable.

In this work, the $R/\min$ values were high and the half width values were low. This is regarded as evidence that the main impurities present were colorless and inert toward the chlorophylls. Further evidence on the last point is that both chl a and chl b gave the typical dry spectra (see Appendix B) that occurs in inert solvents free from polar impurities.

In Table 4 are compared the visible absorption parameters of the pheophytins of this work and those reported by the following authors: a) F.P. Zscheile et al, [28], b) J.H.C. Smith et al, [39], c) A.S. Holt et al, [41], and d) W.D. Bellamy, et al, [5].

The pheophytins were prepared from solutions of the pure chlorophylls by volumetric procedures (see above). Consequently,

Table 4

Visible Absorption Parameters of the Pheophytins

Reference	Red Molar Absorption Coefficient $\epsilon_R \times 10^{-4}$	λ_R max m μ	λ_B max m μ	B/R	R/min	R/505 ^a	$\frac{R}{1/2}$ m μ	$\frac{B}{1/2}$ m μ
Pheophytin <u>a</u> (M.W. 871), ethyl ether								
This Research	5.30 $\pm$ 0.16	667.0	408.5	2.03	18.1	4.52	16.6	51.8
(28)	5.14	665.0	410	2.14	18	4.38	17.0	
(39)	5.55	667	408.5	2.07		4.37		
(41)	5.10	666	408.5	2.10	$\sim$ 23	4.44	16.5	
Pheophytin <u>a</u> , benzene								
This Research	5.42	670.2	414.3	2.03	19.2	4.97	18.4	47.9
(5)	4.88	669	414	2.06	16.3	4.65	19.0	50
Pheophytin <u>b</u> (M.W. 885.2), ethyl ether								
This Research	3.18 $\pm$ 0.10	654.3	433.2	4.89	7.33	2.97	16.0	16.2
(28)	3.27	653	433	5.32	8.0	2.92	16.0	22.0
(39)	3.73	655	434	5.15		2.96		
(41)	3.30	654.8	432	5.26	$\sim$ 10	3.0	17.0	18.0

^aThe ratio is that of the optical densities of the peaks of the main red band and the second green band. The latter for pheophytin b occurs at 522.1 m μ .

some of the same contamination problems are to be considered for the pheophytins. These include the possible presence of:

- a) allomerized impurities, b) xanthophylls and carotenols,
- c) products from photodecomposition, and d) colorless impurities.

In the preceding discussion on the purity of the chlorophylls, evidence was presented that indicated that the chl a of this work may have had 1 per cent non-interacting, colorless impurity, while the chl b may have had 3 per cent. The same type and percentage impurities would be expected in the pheophytins. The volumetric procedure used in their preparation could have introduced a maximum concentration error of ± 3 per cent. This will be reflected in the values of ϵ_R .

In terms of the spectral parameters shown in Table 4, the presence of xanthophylls and carotenols, and the occurrence of photodecomposition will have the same effects as discussed above. Allomerization of ph a produces the following changes (see Table 1). The main red band peak is shifted to the red, while the main blue band is shifted to the blue. ϵ_R decreases about 30 per cent and B/R increases from 2.03 to 2.77. The R/min values remain about the same, while the R/505 value increases from 4.52 to 6.5. The $R_{1/2}$ value increases from 16.6 to 20.5, while the $B_{1/2}$ decreases from 51.8 to 40.7. Similar type changes appear to occur in the allomerization of ph b. Contamination of the pheophytins by small amounts of the allomerized pheophytins will increase both the blue and red half-widths.

To estimate absolute purity of the pheophytins of this work, the same procedure is used as for the chlorophylls. From the ethyl ether data, the average value of ϵ_R for ph a is 5.27×10^4 liters/mole cm, while for ph b it is 3.37×10^4 liters/mole cm. The corresponding average deviations are $\pm 0.15 \times 10^4$ and $\pm 0.18 \times 10^4$ liters/mole cm. Using the average values of ϵ_R , the indicated impurity in the ph a of this work is 0 per cent, while in ph b it is 5.6 per cent. Taking into account our estimated ± 3 per cent concentration uncertainty, these impurity levels are within the range of expected impurity content derived from the pure parent chlorophylls. This suggests that the average values of ϵ_R for the pheophytins may also be very close to those for the 100 per cent pure compounds.

Perkins and Roberts [40] report a value of 2.08 for B/R for ph a in ethyl ether. This is the same as the average of the values shown in Table 4. Lower values of B/R would be indicative of greater freedom from xanthophyll and carotenol impurities. This should also be accompanied by smaller values of $B_{1/2}$. Since there are no values for comparison, the significance of the variations is not clear. For ph b our value of B/R is significantly lower than the average, viz., 4.89 versus 5.15. The lower value is accompanied by a significantly smaller value of $B_{1/2}$. These facts indicate a higher purity in terms of freedom from xanthophylls and carotenol impurities.

There is good agreement for the R/min values for the ethyl ether data. The values shown for Holt and Jacobs [41] were

estimated from small scale molar extinction curves and may be too large. The values of $R/505$ and $R/522$ for ethyl ether also agree well.

Comparison of the data for benzene shows considerable differences between ϵ_R , $R/\min$, and $R/505$ values. The higher values in this work, particularly of ϵ_R which is close to that for ethyl ether, are indicative of higher purity. Since the values of B/R and the half widths are close, the sample of Bellamy et al, [5] would appear to have contained colorless impurities.

The data available shows that the half width values of this work are low, and that the $R/\min$ and $R/505$ values are high. This is evidence for absence of allomerized products, and of other pigments absorbing in the visible. It is concluded therefore that the impurities that may have been present in the samples of this work were colorless, and derived from the parent chlorophyll samples.

In the preceding discussions it was estimated that the chlorophylls were from 97 to 99 per cent pure, and the pheophytins from 94 to 99 per cent pure. It was concluded that the impurities present were probably colorless and non-interacting. The presence of these impurities will cause uncertainties in values of intensity, but not in values of band peak positions and band cut-offs (i.e. positions of minima or inflections between bands chosen in order to evaluate values of f and D).

Errors in the positional values were considered to depend primarily on the choosing of the values so that band overlap would be correctly compensated for. They are given in Table 5. The letters refer to peak designations, and are far red (FR), red (R), blue (B), blue satellite (BS), ultraviolet (UV), and the small protuberance appearing on the red side of the main blue band (B_1) in, for example, the dry, nonpolar solvents. The percentage errors are in terms of the total values of energy in cm^{-1} . The choosing of positional values was based primarily on empirical observations of band shape symmetries, and change of these symmetries with change of solvent and substitution. Almost all peaks and cut-offs were chosen on plots of d (or $d/\bar{\nu}$) versus $\bar{\nu}$. In the higher energy regions, selection from d versus λ curves leads to considerable inaccuracy.

Uncertainties in the integrated f and D values depend on both the presence of impurities in the original samples, and on choosing cut-offs between bands so that the band overlap areas are correctly compensated for. Estimated errors arising from the latter source are given in Table 6. The additional letter designations are orange (O), yellow (Y), and green (G). The subscript "T" refers to the total value for the particular band, and was included since in some solvents band splitting was

Table 5

Estimated Uncertainties in Band Peak Positions and Band Cut-Offs

Quantity	Probable Error Range $\pm$	Maximum Error Range $\pm$
FR Peaks	0.2%	0.5%
BS Peaks	0.2%	0.5%
UV Peaks	0.3%	0.7%
(Error in other peak positions is less than 0.2%, except for B_1 which could be relatively high)		
FR Cut-off	0.3%	0.7%
FR---R Cut-off	0.3%	0.7%
B---BS Cut-off	0.2%	0.7%
BS---UV Cut-off	0.2%	0.8%
UV---UV ₂ Cut-off	0.2%	0.7%
(Error in other cut-off positions is less than 0.2% except for B_1 which could be relatively high)		

Table 6

Estimated Errors in Oscillator and Dipole
Strengths Arising From Uncertainties
in Choice of Band Cut-Offs

Quantity ^a	Probable Error Range $\pm$	Maximum Error Range $\pm$
$f(B_T)$, or $f(B + BS)$	2%	5%
$f(BS_T)$	4%	10%
$f(O_T)$	< 1%	3%
$f(Y)$	1%	4%
$f(G_T)$	< 1%	3%
$f(R_T)$	< 1%	2%
$f(UV_T)$ or $f(BS + UV_1)$	3%	8%
$f(R_T + O_T + Y_T)$, or $f(R_T + O_T + Y_T + G_T)$	< 1%	2%

^aError estimates apply both to f and D values.

observed. The per cent uncertainties are in reference to the total calculated values.

Experimental Accuracy - Monolayer Data

The presence of a few per cent of non-chlorin derivative impurities could result in either a negative or positive error in the absolute area per molecule of the pheophytins. This would depend on the surface requirement per molecule of the impurity. Two extreme cases would be: a) the impurity was twice as great as that of the pheophytin. In the former case, the calculated total number of pheophytin molecules would be too large; then the calculated area per molecule would be too small by a per cent corresponding to the per cent impurity present. In the latter case, the calculated area per molecule would be too large by a per cent corresponding to the per cent impurity present. From this view, the uncertainties in σ , due to the presence of colorless impurities, are estimated to be ± 1 per cent for ph a, and ± 3 per cent for ph b. The impurity percentages are taken to be those derived from the parent chlorophylls.

Spectroscopic analysis of the pigment solutions used for spreading the monolayers showed that they each contained about 5 per cent intact ring chlorin derivative. Use of the determinantal method showed the impurity in ph a to be al ph a. The direction of change of the spectral parameters for the ph b solution indicated that the impurity was al ph b (see above).

Calculation of the change in B/BS ratio was made to estimate percentage impurity.

In a test on the stability of an α monolayer, it was observed that the surface potential had the same sign as that of α and was of the same magnitude.[†] This was considered evidence that the molecular orientation of the chlorin heads at the interface was quite similar. The molecular surface requirements of the two compounds would not be expected to be greatly different. It seems probable therefore that the presence of 5 per cent allomerized pheophytin in pheophytin monolayers would cause less than ± 5 per cent uncertainty in σ . A reasonable estimate of maximum error is ± 5 per cent.

The uncertainties in σ arising from the presence of impurities, represent constant limits in the experimental accuracy. The variations in the data points that were observed in different runs are a measure of the procedural errors. These were determined in the manner described below.

The data for each run were smoothed graphically. For each system, points were first taken from the curves of all runs,^{††} and averaged at given values of σ . Comparison was made of the deviations of each run from the averages of ΔV and π over the

[†]The heterogeneity of monolayer was such that a definitive estimate of the average value of the surface potential could not be made.

^{††}Runs were not used that did not have low values in the variation of ΔV over the surface at given values of σ (i.e. ± 30 mv or less at $\pi < 1.0$, and ± 4 mv or less at higher compressions).

whole range of σ . Runs were rejected whose deviations in ΔV and π were not consistent in sign and magnitude, or showed significantly different per cent deviations at different values of σ . The types of internal inconsistencies observed were considered indicative of either accidental contamination with fairly large amounts of polar surface active impurities during the run, or adsorption of unusually large amounts of pigment on the glass slide during compression. In the first instance, deviations in π were more positive than in ΔV , and usually increased with decreasing σ . In the second instance the deviations markedly decreased with decreasing σ . This type of behavior was less frequent than the first type. Average values and deviations were recalculated, and comparison again made of deviations. The new set of average values differed only a few per cent from the first set, deviations were considerably smaller, and nearly all internal discrepancies were eliminated.

The deviations were as follows: ± 0.04 dyn/cm for the range $0.10 \text{ dyn/cm} \leq \pi \leq 0.50 \text{ dyn/cm}$, ± 0.20 dyn/cm for the range $0.50 \text{ dyn/cm} \leq \pi \leq 2.00 \text{ dyn/cm}$, and ± 0.40 dyn/cm for $\pi \geq 2.0 \text{ dyn/cm}$. These deviations are averaged over the range of π shown, and represent average values for all systems. The corresponding average per cent deviations in σ for all systems are shown in Table 7. The average over all ranges and systems is ± 3.1 per cent.

The major sources of error were considered to be:

- 1) differences in adsorption of small amounts of pigment on the

Table 7
Procedural Errors in the Area Per Molecule

System	Range of π dyn/cm	Average Percent Deviation of σ in the Range
ph <u>a</u> , pH = 3.0	0.15 to 0.50	± 4.7
ph <u>a</u> , pH = 3.0	0.50 to 2.25	± 3.5
ph <u>a</u> , pH = 3.0	2.25 to 11.04	± 1.2
ph <u>a</u> , pH = 4.0	0.10 to 0.41	± 5.0
ph <u>a</u> , pH = 4.0	0.41 to 1.60	± 4.0
ph <u>a</u> , pH = 4.0	1.60 to 12.85	± 0.9
ph <u>b</u> , pH = 3.0	0.20 to 0.60	± 2.3
ph <u>b</u> , pH = 3.0	0.60 to 2.63	± 3.6
ph <u>b</u> , pH = 3.0	2.63 to 8.03	± 1.7
ph <u>b</u> , pH = 4.0	0.10 to 0.50	± 4.2
ph <u>b</u> , pH = 4.0	0.50 to 1.84	± 4.5
ph <u>b</u> , pH = 4.0	1.84 to 8.25	± 1.6

glass slide during monolayer compression, 2) uncertainties in the amount of pigment spread, and 3) introduction of varying trace amounts of contaminants during a run. The first source of error can explain in part the tendency for decrease of magnitude of error at smaller values of σ .

The amount of pigment adsorbed was determined after each run. All values of σ were calculated on the basis that all adsorption on the glass occurred before compression. Where additional adsorption had occurred on compression, the calculated values of σ would be too large at low pressures, but would be correct at high pressures. Comparison of the small decreases in ΔV at given values of σ , observed on decompression in most runs, showed that variations of about ± 1.8 per cent occurred. This is a good indication of the variation in percentage adsorption on the glass.

Uncertainties in the amount of pigment spread would be reflected in variations of σ at all values of π . It seems reasonable to suppose that the variations of σ at high values of π reflect error from this source. These values range from 0.9 per cent to 1.7 per cent, and are consistent with the magnitude of the errors that could have arisen from the estimated inaccuracy of the spreading micropipetburet, and from the estimated error in the volumetric procedures in preparing the pheophytins.

F.C. Goodrich [44], in work with mixed monolayers, points out that the presence of small amounts of surface contaminants,

accidentally introduced onto the substrate surface, can expand the low pressure regions without having appreciable effect on the high pressure regions. This may be related to the phenomena of small amounts of non-polar oils dissolved in monolayers (reference [31], P. 156). The areas are more expanded at low pressures than at high pressures, since compression of the film reduces the solubility of the oil and it is squeezed out. It seems possible then that variable trace amounts of non-polar impurities in pheophytin monolayers could give rise to errors in σ in the low pressure regions which would not be reflected in the high pressure region. The magnitude of the errors would be from ± 1.0 to ± 2.0 per cent. The runs which were rejected on the basis of surface contamination showed positive deviations in π at high pressures. The impurities in these cases were evidently not squeezed out of the film on compression.

If significant variations in incompleteness of spreading had occurred, it seems likely that this would have been reflected in the errors in σ at all molecular areas. Errors in monomolecular pigment concentration, however, could be related to other more probable sources. The degree of incomplete spreading, therefore, must have been nearly the same in every run. Residual variation in ΔV over the surface (from ± 2 mv to ± 4 mv) at small areas per molecule was observed in every run. These variations, in part, were probably due to the presence of trace amounts of impurities. They are also evidence that some of the pigment was consistently present in small three dimensional

aggregates in every run. Film collapse occurred at molecular areas from 10 to 15 per cent larger than the smallest possible area per molecule calculated from molecular dimensions (see below). This is evidence that the percentage of pigment in the form of three dimensional aggregates must have been small. A reasonable maximum estimate is 5 per cent.

The average deviations in ΔV for all systems were: ± 9 mv in the range $0.10 \text{ dyn/cm} \leq \pi \leq 0.50 \text{ dyn/cm}$, ± 9 mv in the range $0.50 \text{ dyn/cm} \leq \pi \leq 2.00 \text{ dyn/cm}$, and ± 7 mv for $\pi \geq 2.00 \text{ dyn/cm}$. The corresponding percentage variations in σ are shown in Table 8. These values agree well with the deviations in σ calculated from observed variations in π . Unlike the latter values they do not show a tendency to decrease with decrease of σ . This indicates that surface potentials remained sensitive to the presence of impurities which could have been squeezed out of the film on compression, as suggested above.

The surface potential (in mv) can be related [45], [46] to the vertical component of the overall electrical moment, $\mu \cos \theta$, of the monolayer molecule and its associated counterion by

$$\Delta V = 4 \pi 300 \times 10^3 n \mu / D \cos \theta, \quad 4)$$

where n = the number of monolayer molecules per cm^2 , θ = the angle of tilt of the dipole with respect to a normal to the interface, and D = the interfacial dielectric constant which includes the effects of re-orientation and re-distribution of

Table 8

Percentage Variations in σ Calculated from Observed Deviations in ΔV

System	Range of π dyn/cm	Average Percent Variation of σ in the Range
ph a, pH = 3.0	$0.15 \leq \pi \leq 0.50$	± 2.4
ph a, pH = 3.0	$0.50 \leq \pi \leq 2.25$	± 2.6
ph a, pH = 3.0	$2.25 \leq \pi \leq 11.04$	± 3.0
ph a, pH = 4.0	$0.10 \leq \pi \leq 0.41$	± 2.3
ph a, pH = 4.0	$0.41 \leq \pi \leq 1.60$	± 2.1
ph a, pH = 4.0	$1.60 \leq \pi \leq 12.85$	± 2.3
ph b, pH = 3.0	$0.20 \leq \pi \leq 0.60$	± 3.2
ph b, pH = 3.0	$0.60 \leq \pi \leq 2.63$	± 3.5
ph b, pH = 3.0	$2.63 \leq \pi \leq 8.03$	± 3.3
ph b, pH = 4.0	$0.10 \leq \pi \leq 0.50$	± 3.3
ph b, pH = 4.0	$0.50 \leq \pi \leq 1.84$	± 3.0
ph b, pH = 4.0	$1.84 \leq \pi \leq 8.25$	± 2.8

water molecules. In general μ and D can not be determined independently. With this formula the value of μ/D is given in Debye units (1×10^{-18} e.s.u. cm). The overall average deviation in μ/D was determined to be 0.03 Debye unit.

RESULTS AND DISCUSSION

Monolayer Stability

In Table 9 are summarized representative results of exploratory stability tests on chl a monolayers at air-water and nitrogen (30-50 ppm O₂, 3-10 ppm CO₂)-water interfaces. The percentage variations, shown with per cent products formed, represent the uncertainties in total percent chlorophyll calculated. Their magnitudes are in the range of the order estimated for the accuracy of the analytical method. The differences from run to run, however, indicate that products other than al chl a, ph a, and al ph a were formed. The surprisingly good correlations found, however, signify that the percentages of such products were small.

The tests showed that increase of pH greatly reduced the total rate of decomposition. Pheophytin formation was progressively inhibited as pH was increased, but apparently never entirely eliminated. With increase of pH, the amount of allomerization that occurred, relative to the amount of pheophytinization, increased. Increase of pH to 8.0 and above inhibited the rate of allomerization.

Decrease of oxygen concentration in the gas phase decreased the rate of allomerization. This factor had significantly less effect than increase of pH. This suggests that

Table 9
Stability Tests on Chlorophyll a Monolayers

Run Number	pH	Time, Hours	Per Cent Products Formed	Per Cent Decomposition Per Hour
Air-Water Interface				
98 ^a	6.2	0.42	1.9% al chl <u>a</u> , 28.6% ph <u>a</u> 3.2% al ph <u>a</u> , $\pm$ 1.5%	84%
338 ^a	9.1	4.5	11.3% al chl <u>a</u> , $\pm$ 1.5%	2.5%
Nitrogen-Water Interface				
187 ^a	5.3	1.0	0.9% al chl <u>a</u> , 79.7% ph <u>a</u> 1.0% al ph <u>a</u> , $\pm$ 0.5%	81.6%
182 ^a	7.0	1.0	1.1% al chl <u>a</u> , 9.3% ph <u>a</u> , $\pm$ 2.7%	13.1%
183 ^a	7.0	18.0	24.8% al chl <u>a</u> , 42.9% ph <u>a</u> 13.0% al ph <u>a</u> , $\pm$ 4.3%	4.7%
202 ^b	7.4	18.0	33.2% al chl <u>a</u> , 7.1% ph <u>a</u> 22.1% al ph <u>a</u> , $\pm$ 3.8%	3.7%
207 ^a	8.1	15.0	12.4% al chl <u>a</u> , 7.5% ph <u>a</u> 3.0% al ph <u>a</u> , $\pm$ 0.0%	1.5%

Table 9 (Contd.)

Run Number	pH	Time, Hours	Per Cent Products Formed	Per Cent Decomposition Per Hour
197 ^a	8.9	18.0	12.6% al chl <u>a</u> , 3.3% al ph <u>a</u> , $\pm 1.0\%$	0.9%
192 ^{a,c}	7.0	1.0	12.0% al chl <u>a</u> , $\pm 1.5\%$ traces of ph <u>a</u> and al ph <u>a</u>	13.1%
200 ^{a,d}	7.1	1.0	1.4% al chl <u>a</u> , 4.1% ph <u>a</u> 2.0% al ph <u>a</u> , $\pm 3.0\%$	10.6%
203 ^{b,d}	7.1	21.0	31.4% al chl <u>a</u> , 23.6% ph <u>a</u> 15.9% al ph <u>a</u> , $\pm 2.3\%$	3.5%
205A ^{b,e}	7.4	20.0	17.9% al chl <u>a</u> , 44.4% ph <u>a</u> 11.0% al ph <u>a</u> , $\pm 4.0\%$	3.9%

^aSurface pressure ≤ 0.10 dyn/cm.

^bSurface pressure ~ 5 dyn/cm.

^c50% trans β carotene in the monolayer.

^d50% L- α -lecithin in the monolayer.

^e50% phytol in the monolayer.

polarization of the chlorin heads, through specific interactions with the aqueous substrate, may be an important factor in inhibiting oxidation.

The presence of β carotene, lecithin, or phytol in the monolayer, and compression of the monolayer had little effect on the total rate of decomposition. The presence of β carotene and lecithin appeared to promote the occurrence of allomerization over pheophytinization. Phytol appeared to have the reverse effect.

In Table 10 are summarized the results of stability tests on pheophytin monolayers. The percentage variations shown for ph a and al ph a represent the uncertainties in the total per cent calculated for these compounds. They signify the inherent uncertainties in the method of analysis and the possible presence of unknown chlorin derivatives.

Decrease of pH to below 5.0 gave a marked decrease in rate of allomerization for both ph a and ph b monolayers. Monolayers of al ph a did not appear to undergo any changes. The decrease in rate for ph a, in the range $5.0 \leq \text{pH} \leq 9.1$ is approximately linear, the slope being -3.7 per cent/hour/pH unit. The line intersects zero per cent decomposition at $\text{pH} = 4.8$. At $\text{pH} < 5.0$, the rates of decomposition level out to low values that are independent of pH. This suggests that the decomposition did not occur when the monolayers were in darkness.

Tests showed that when pheophytin monolayers were exposed to direct green lighting much greater changes occurred than when

Table 10

Stability Tests on Pheophytin Monolayers
at Air-Water Interfaces

pH	Time, Hours	Per Cent Product Formed	Per cent Decomposition Per Hour
Pheophytin <u>a</u>			
9.1	4 1/2	74.6% al ph <u>a</u> , $\pm$ 0.0%	16.6%
8.4	6 1/4	70.4% al ph <u>a</u> , $\pm$ 2.6%	11.3%
7.4	6 1/4	65.4% al ph <u>a</u> , $\pm$ 1.2%	10.5%
5.6	6 1/4	17.0% al ph <u>a</u> , $\pm$ 4.0%	2.7%
5.0	6 1/4	4.6% al ph <u>a</u> , $\pm$ 3.7%	0.5%
4.0	7 1/4	1.8% al ph <u>a</u> , $\pm$ 1.1%	0.2%
3.0	6 1/4	6.4% al ph <u>a</u> , $\pm$ 1.0%	1.0%
2.0	6 1/4	0.0% , $\pm$ 0.3%	0.0%
Pheophytin <u>b</u>			
8.4	6 1/4	71.0% al ph <u>b</u>	11.4%
6.2	6 1/4	55.0% al ph <u>b</u>	8.8%
5.6	6 1/4	20.0% al ph <u>b</u>	3.2%
5.0	6 1/4	9.0% al ph <u>b</u>	1.4%
4.5	6 1/4	4.0% al ph <u>b</u>	0.6%
4.0	5 3/4	5.0% al ph <u>b</u>	0.9%
3.0	6 1/4	4.0% al ph <u>b</u>	0.6%
2.0	6 1/4	4.0% al ph <u>b</u>	0.6%
Allomerized Pheophytin <u>a</u>			
6.2	2 1/4	0.0% , $\pm$ 2.4%	0.0%

the lighting was of very low intensity and diffuse,[†] as used in spreading. During collection of the monolayers, it was necessary to use direct, low intensity green lighting for short periods (5-7 minutes). The procedures, lighting, and time periods were very uniform from run to run. It seems quite likely, then, that photoactivated decomposition can account for the constant small percentages of decomposition products found at pH's less than 5.0. Since direct illumination was not used during measurement of surface pressure and surface potential, it is probable that the films remained intact during the test periods (~5 hours).

The stabilization of the pheophytins against allomerization is interpreted as arising from salt formation with the entry of one or more protons into the center of the chlorin ring. Protons, in the center of the chlorin ring, can act as strong electron acceptors. Such action, transmitted to the cyclopentanone ring would decrease its electron density. This would increase the potential barrier in the first step of the allomerization process. If there existed uninterrupted conjugation between the constituent in the center of the ring, the π electron system of the chlorin head, and the cyclopentanone

[†]W.D. Bellamy et al [5] measured the changes in optical absorption of ph a monolayers in situ that had been strongly illuminated. They found not only a general decrease in optical densities, but also a rather large increase in B/R ratio. We have found that partial allomerization was accompanied by an increase in B/R. It seems possible that photoactivation can cause some allomerization.

ring, the increase of potential barrier could be quite large.

In support of this suggested interpretation, it is interesting to note that the rate of allomerization of chl a at air-pH = 9.1 buffer interfaces is about 6.5-fold slower than the rate for ph a under identical conditions. The magnesium atom can be visualized as decreasing electron density in the cyclopentanone ring in the same manner as the protons in pheophytin. It seems certain that the pheophytins at pH = 9.0 are present as the free bases. There may be minor differences in interaction with the aqueous substrate between the chlorophylls and the pheophytins. Such differences would probably be small in comparison to the perturbing effect of the presence of the magnesium atom.

The strong perturbing effect of acid on the visible absorption spectra of some of the chlorins has been studied [47] and attributed to salt formation in the manner suggested above. A. Neuberger and J. Scott [48] present evidence that the closely related porphyrin derivatives can exist in mono- and di-protonated forms. The results of studies on the effect of acid on the visible absorption spectra of the pheophytins in this work are given in Appendix B. Salt formation is a reversible process, as evidenced by the restoration of the free base spectrum when the acid is washed out of the solvent with water.

Addition of protons to the center of the chlorin ring of ph a tends to make its spectrum resemble that of the more centrally symmetric chl a. The main red and blue peaks are shifted toward wavelengths characteristic of chlorophyll, and

the green bands at about 505 $m\mu$ and 535 $m\mu$, characteristic of pheophytin, are greatly diminished in intensity. Similar type changes occur with al ph a.

Surface Pressures and Surface Potentials

The surface pressure and surface potential data are summarized in Table 11 and Figures 2 and 3. The monolayers were mechanically stable at all values of σ shown. If the films were compressed to areas per molecule smaller than those shown, surface pressures and surface potentials decreased with time. This was considered evidence of film collapse.

A study was made of the change of surface potential on repeated compression and decompression down to and below the area corresponding to mechanical stability. If the area per molecule had not been reduced below about 5 per cent of that of the mechanical stability limit, the surface potentials were unchanged on decompression. This indicated that the changes occurring in the film on collapse were reversible on decompression. If the area had been reduced to below about 15 per cent of that of the mechanical stability limit, changes in the film occurred that were not reversible in a period of an hour, on decompression. The heterogeneity was increased as evidenced by increase of variation of surface potential over the surface. This indicated that, where extensive film collapse had occurred, three dimensional aggregates of the monolayer molecules were

Table 11

Surface Pressure (π) and Surface Potential (ΔV) Values for
Pheophytin a and b at pH = 4.0 and pH = 3.0

Area Per Molecule (σ) $\text{\AA}^2$	Pheophytin <u>a</u>				Pheophytin <u>b</u>			
	pH = 3.0		pH = 4.0		pH = 3.0		pH = 4.0	
	π dyn/cm	ΔV millivolts	π dyn/cm	ΔV millivolts	π dyn/cm	ΔV millivolts	π dyn/cm	ΔV millivolts
100			12.85	597			8.25	434
105			10.46	586	8.03	423	6.35	422
110	11.04	561	8.25	573	6.92	415	4.90	410
115	9.50	553	6.24	561	5.83	407	3.65	399
120	8.00	544	4.35	548	4.90	398	2.62	387
125	6.56	536	2.80	535	4.02	390	1.84	377
130	5.30	527	1.60	521	3.30	381	1.27	364
135	4.10	517	1.04	508	2.63	372	0.98	354
140	3.05	508	0.68	492	2.10	364	0.75	342
145	2.25	497	0.50	480	1.64	355	0.62	333
150	1.50	486	0.41	463	1.29	347	0.50	322
155	1.05	476	0.36	450	1.01	339	0.40	313
160	0.80	464	0.31	436	0.83	330	0.35	303
165	0.63	454	0.27	424	0.70	322	0.30	295
170	0.50	442	0.24	412	0.60	314	0.29	286
175	0.42	431	0.21	401	0.50	306	0.25	279
180	0.37	420	0.18	387	0.45	298	0.23	271
185	0.30	409	0.16	377	0.35	290	0.18	263
190	0.27	398	0.14	367	0.30	283	0.15	257
195	0.23	388	0.12	358	0.25	275	0.12	249
200	0.20	379	0.10	349	0.20	269	0.10	244
205	0.17	369						
210	0.15	361

Figure 2

Surface Pressure and Surface Moment Versus
Molecular Area for Pheophytin a

Figure 3

Surface Pressure and Surface Moment Versus
Molecular Area for Pheophytin b

formed that had a high potential energy barrier against resspreading.

The surface potentials are positive in sign. The most reasonable orientation to account for this is one in which the esters groups are anchored in the water phase, and where the phytol tails and the major portion of the chlorin heads are elevated into the air phase.[†] In such a configuration the ester groups, which are the most hydrophilic parts of the molecule, can interact strongly with the water surface and provide for spreading (for example, reference [45], p. 93). The ester groups, anchored in the water surface, would make a positive contribution to the surface moment. This might range from 0.5 to 0.2 Debye unit depending on orientation (for example, reference [36], p. 72). With the ester groups in the water surface, the chlorin head will be oriented with the cyclopentanone ring toward the water surface. This group probably represents the negative end of the overall dipole moment of the head. The total dipole moment of the whole molecule will be a sum of the component parts. In the configuration suggested, the moments of the head and the ester groups will both be positive toward the air phase, and will add to give an overall moment greater than that of an ester group, as observed.

For given values of π above 1.0 dyn/cm, decrease of pH from 4.0 to 3.0 results in an average increase in σ of

[†]This was considered probable also by Hughes [7], Alexander [9], and W.D. Bellamy et al [5].

11 per cent for ph a, and 8 per cent for ph b. The expansion below 1.0 dyn/cm averages about 13 per cent. Comparison with the data given by W.D. Bellamy et al [5], shows that the values of σ for ph a average about 20 per cent larger at pH = 4.0 than at pH = 8.0, at given values of π above 1.0 dyn/cm.

Table 12 shows the average compressibilities (C_s) for ph a, ph b, chl a, and chl b monolayers at several pH's and ranges of π . Comparison is made with data from the work of W.D. Bellamy et al [5], A.E. Alexander [9], and H.J. Trurnit and G. Colmano [14]. C_s decreases with increase of π ; the effect is more pronounced for the pheophytins at low pH's than for the other systems. For the pheophytins, decrease of pH results in an increase of C_s in a given range of π . This does not appear to be the case for the chlorophylls. At pH = 8.0, the C_s values for chl a are greater than those of ph a in both pressure ranges; in the two lower ranges, they are close to those of ph a at pH = 4.0. In a given pressure range and at a given pH, the C_s values of the b compounds are consistently larger than those of the a compounds.

In the range $2.0 \text{ dyn/cm} \leq \pi \leq 8.0 \text{ dyn/cm}$, the σ values of ph a average 9 per cent larger than those of ph b at pH = 3.0. In the same π range at pH = 4.0, the σ values of ph a average 7 per cent larger than those of ph b. From the data of Alexander [9], the σ values of chl a average 7 per cent larger than those of chl b in the range $3.0 \text{ dyn/cm} \leq \pi \leq 20.0 \text{ dyn/cm}$. The work of Trurnit and Colmano [14] shows that the values of

Table 12

$$\text{Average Compressibilities, } C_s = \frac{1}{1/2(\sigma_1 + \sigma_2)} \frac{\sigma_1 - \sigma_2}{\pi_2 - \pi_1}, \text{ cm/dyn}$$

of ph a, ph b, chl a, and chl b Monolayers

Reference	Compound	pH	Range of π dyn/dm	C_s , cm/dyn, $\times 10^2$
This Research	ph <u>a</u>	3.0	1.0 - 3.0	5.1
This Research	ph <u>a</u>	3.0	3.0 - 10.0	3.1
This Research	ph <u>a</u>	4.0	1.0 - 3.0	4.6
This Research	ph <u>a</u>	4.0	3.0 - 10.0	2.2
This Research	ph <u>b</u>	3.0	1.0 - 3.0	8.0
This Research	ph <u>b</u>	3.0	3.0 - 8.0	4.5
This Research	ph <u>b</u>	4.0	1.0 - 3.0	6.5
This Research	ph <u>b</u>	4.0	3.0 - 8.0	3.2
(5)	ph <u>a</u>	8.0	1.0 - 3.0	2.7
(5)	ph <u>a</u>	8.0	3.0 - 10.0	1.4
(5)	chl <u>a</u>	8.0	1.0 - 3.0	3.8
(5)	chl <u>a</u>	8.0	3.0 - 10.0	2.0
(5)	chl <u>a</u>	8.0	10.0 - 20.0	1.7
(9)	chl <u>a</u>	7.3	1.0 - 3.0	2.2
(9)	chl <u>a</u>	7.3	3.0 - 10.0	1.9
(9)	chl <u>a</u>	7.3	10.0 - 20.0	1.8
(9)	chl <u>b</u>	7.3	1.0 - 3.0	3.5
(9)	chl <u>b</u>	7.3	3.0 - 10.0	2.7
(9)	chl <u>b</u>	7.3	10.0 - 20.0	2.1

Table 12 (Contd.)

Reference	Compound	pH	Range of π dyn/cm	C_E , cm/dyn, $\times 10^2$
(14)	chl <u>a</u>	distilled H ₂ O	1.0 - 3.0	3.5
(14)	chl <u>a</u>	distilled H ₂ O	3.0 - 10.0	2.1
(14)	chl <u>a</u>	distilled H ₂ O	10.0 - 20.0	1.9
(14)	chl <u>b</u>	distilled H ₂ O	1.0 - 3.0	4.1
(14)	chl <u>b</u>	distilled H ₂ O	3.0 - 10.0	2.6
(14)	chl <u>b</u>	distilled H ₂ O	10.0 - 20.0	2.4

σ for chl a average 9 per cent greater than those for chl b, in the same pressure range.

Plots of $\mu/D \cos \theta$ versus π are linear, for π greater than about 1.0 dyn/cm up to the collapse pressures, for the pheophytins at low pH's. The slopes, with units of Debye/dyn/cm, are -0.030, -0.017, -0.032, and -0.020 for ph b-pH = 3.0, ph b-pH = 4.0, ph a-pH = 3.0, and ph a-pH = 4.0, respectively. In the same ranges of π , the value of $\mu/D \cos \theta$ averages 0.08 Debye greater at pH = 3.0 than at pH = 4.0 for ph b, and 0.09 Debye larger for ph a. For $\pi < 1.0$ dyn/cm, the average difference is about 0.14 Debye for both ph a and ph b. Comparison with the data of W.D. Bellamy et al [5] shows the following for ph a.

Table 13
Effect of pH on $\mu/D \cos \theta$ for ph a

pH	π , dyn/cm	$\mu/D \cos \theta$, Debye units
8.0	1.0	1.52
8.0	10.0	1.41
4.0	1.0	1.82
4.0	10.0	1.64

The surface moments average 18 per cent greater at pH = 4.0 than at pH = 8.0.

The expansion of the films, the increase of compressibilities, and the increase of the values of the surface moment with decrease of pH are evidence for salt formation, with entry

of one or two protons into the center of the chlorin ring. The amount of expansion of the pheophytin monolayers was relatively small in comparison to that observed for fully ionized straight chain amines (for example, reference [36], p. 226). This suggests that the degree of ionic dissociation of the pheophytins was probably quite small. Close association of the charged heads and their counterions would also account for the relatively small increase of $\mu/D \cos \theta$.

J.T. Davies developed an equation for estimation of the pressure arising from coulombic repulsion for films of symmetrical long chain ions (reference [36], p. 231, [49]). The development is based on the theory of the diffuse electrical double layer of J. Gouy. For the equation to be useful, the counterions must be distributed diffusely beneath the charged monolayer, and the dipole moment associated with head group must not undergo orientation changes with change of σ . To evaluate the possibility for using the equation, one assumes a constant value for $\mu/D \cos \theta$, and calculates ψ_0 from (reference [36], p. 78)

$$\Delta V_{\text{meas.}} - 4 \pi 300 \times 10^3 \sigma^{-1} \times 10^{16} \mu/D \cos \theta = \psi_0, \quad 5)$$

where θ and D are as previously described. It is assumed now that μ relates only to the dipole moment of the head group. The term ψ_0 (in mv) gives the contribution of the ionic double layer to the surface potential. If the surface dipoles do not reorient, and if there is no specific association and change of

specific association of the counterions with the monolayer on compression, ψ_0 should increase in a characteristic fashion with decrease of σ . Exploratory calculations for the pheophytins at low pH with equation 5) showed a decrease in calculated ψ_0 with decrease of σ . This was considered further indication that the degree of ionic dissociation was small.

If compression changed the angle of tilt (θ) of the chlorin heads with respect to a normal to the interface, a decrease of θ would be expected. This would have the effect of increasing the contribution of the dipole moment of the head, to the total molecular moment. If there were no ion effects, plots of $\mu/D \cos \theta$ versus σ would show an increase of $\mu/D \cos \theta$ as σ decreased. This was not observed. A reasonable explanation is that, on compression, the angle of tilt remained essentially constant, and an increase in the relative vertical displacement (vertical staggering, see below) between monolayer molecules occurred, accompanied by a slight increase of ionic association (decrease of separation between charged heads and counterions).[†]

According to Davies' theory for simple ionized monolayers, the surface pressures and the surface potentials, at given values of σ , should increase with decrease of salt concentration in the bulk aqueous phase. Decrease of salt concentration increases the mean thickness of the ionic double layer, which results in an increase in ψ_0 and intermolecular coulombic repulsions.

[†]The value of D is assumed to be independent of compression, see below.

Experiments with pheophytin monolayers were made to test the effect of change of buffer salt concentration. Concentrations were made from 2 to 4-fold smaller than normally used (0.025 M). Within experimental accuracy, no differences in π - σ and ΔV - σ behavior were found. This was considered further evidence that separation between charged heads and counterions was small.

Measurements were made with both ph a and ph b monolayers at pH = 4.5. Areas per molecule and surface moments, at given values of π , were smaller at pH = 4.5 than at pH = 4.0 by about 1 1/2 per cent on the average. This is proportionately much less change than was observed in decreasing the pH from 4.0 to 3.0. Two different effects must have been involved. The results of the monolayer stability tests showed that decomposition either ceased, or leveled out to a nearly constant low value, at a pH between 4.0 and 5.0. This can be interpreted to mean that all of the monolayer molecules must have become mono-protonated in that pH range. Since salt formation in a monolayer can be regarded as similar to a neutralization process in solution, it is reasonable to expect that, near the point of 100 per cent conversion, the percentage change in composition per unit change in pH would be small. The relatively small changes in π - σ and $\mu/D \cos \theta$ - σ behavior between pH = 4.0 and 4.5 can be attributed to the completion of mono-protonation of the monolayer. The differences observed, when the pH was decreased from 4.0 to 3.0, can then be viewed as arising from some degree of di-protonation at the lower pH.

Comparison of the values of $\mu/D \cos \theta$ for ph a and ph b shows that significant differences exist over the range of σ . At pH = 4.0, in the range $200 \text{ \AA}^2 \geq \sigma \geq 140 \text{ \AA}^2$, $\Delta\mu/D \cos \theta$ is constant and equal to 0.56 Debye. In the range $140 \text{ \AA}^2 \geq \sigma \geq 100 \text{ \AA}^2$, $\Delta\mu/D \cos \theta$ decreases from 0.56 to 0.43 Debye. At pH = 3.0, a constant difference of 0.58 Debye, in the range $200 \text{ \AA}^2 \geq \sigma \geq 160 \text{ \AA}^2$, is found, while $\Delta\mu/D \cos \theta$ decreases from 0.58 to 0.42 Debye in the range $160 \text{ \AA}^2 \geq \sigma \geq 110 \text{ \AA}^2$.

The variations in $\Delta\mu/D \cos \theta$ are small compared to the differences themselves. This suggests that the lower values of $\mu/D \cos \theta$ for ph b result largely from the presence of the carbonyl group at the 3-position on the chlorin ring. At this position its group moment will, on the average, be negative toward the air phase, and therefore decreases the total moment of the molecule.

The decreases in $\Delta\mu/D \cos \theta$ are very similar at both pH's, and reflect the fact that the $\mu/D \cos \theta$ values of ph b show smaller decrease with decrease of σ than those of ph a. This smaller decrease for the ph b monolayers indicates that compression, above about 1.0 dyn/cm, to the collapse configuration results in a smaller increase in relative vertical staggering, and thus a smaller decrease in $\mu/D \cos \theta$ due to increase of ionic association as suggested above. If the collapse configuration for both ph a and ph b monolayers involves about the same degree of relative vertical staggering among molecules, which is reasonable to suppose, then below 1.0 dyn/cm the ph b monolayers

would on the average have a greater degree of relative vertical displacement among molecules than the ph a monolayers.

From the surface potential data of Alexander [9], an average value for $\Delta\mu/D \cos \theta$ of 0.25 Debye, between chl a and chl b, was calculated. This was for the range $128\text{\AA}^2 \geq \sigma \geq 90\text{\AA}^2$, and was nearly constant. The absolute values of $\mu/D \cos \theta$, in this area range, decreased from 1.08 to 0.94 Debye for chl a, and from 0.85 to 0.68 Debye for chl b. The corresponding pressure coefficients of $\mu/D \cos \theta$ were -0.007 Debye/dyn/cm for chl a and -0.011 Debye/dyn/cm for chl b.

The values of $\Delta\mu/D \cos \theta$ between ph a and ph b are larger than that between chl a and chl b. This could be due to relative differences in the angles of tilt of the chlorin heads, and differences in degree of ionic association between the pheophytins.

Monolayer Model

In Figure 4 is shown a molecular model of chl b based on known atomic radii and bond angles. The scale used was about 0.83Å /inch. The diameter of the central covalent magnesium atom was taken to be 2.8Å.. The chlorin ring positions are shown clockwise in the figure. The carbonyl group, at the three position, is at the top left, and the ethyl group, at the four position, is at the top right. If the chlorin head were rotated clockwise in the figure so that the vinyl group was at 11 o'clock and the carbonyl group at 1 o'clock, this would represent the probable orientation of the head at the air-water interface.

Figure 4
Molecular Model of Chlorophyll b

The area of the chlorin plane without substituents corresponds to about $108\text{\AA}^2$, as determined from the present model. This assumes the plane to be square; the measured dimension of a side corresponds to about 10.4. The area calculated this way agrees very well with estimates ($100\text{--}110\text{\AA}^2$) given by Rabinowitch (reference [2], p. 448).

The substituents on the chlorin ring extend the area of the plane considerably, and in a fairly uniform manner. Calculation of the dimensions of length in the substituted chlorin plane along various axes passing through the center gave the following: axis between the midpoints of the 3,4 and 7,8 positions-- $13.7\text{\AA}$; [†] (b) axis between the midpoints of the 1,2 and 5,6 positions-- $13.3\text{\AA}$; (c) axis through the carbon atoms at the β and δ methine bridges-- $12.4\text{\AA}$, and (d) axis through the carbon atoms at the α and γ methine bridges-- $12.8\text{\AA}$. With the ester groups oriented in the aqueous phase, as suggested above, the axis passing through the β and δ methine bridges would be nearly parallel to the plane of the interface. If change of angle of tilt (θ) of the chlorin plane, with respect to a normal to the interface, involved only a simple folding movement of one plane with respect to the other, the β - δ axis would remain nearly parallel to the interface at all values of θ . In this case, the greatest extension of length of the chlorin plane in the

[†]The extension in length of the chlorin head due to the $-\text{CH}_2-\text{CH}_2-\overset{\text{O}}{\underset{\parallel}{\text{C}}}-$ group at the 7 position is approximately equal to that due to an ethyl group.

interface would be close to $12.4\text{\AA}$, for θ less than about 68° . Since the exact orientation of the chlorin head, with ester groups immersed, is not known it seems reasonable to select the average of the lengths, $13.1\text{\AA}$, for purposes of calculation. Using this average value and assuming the plane to be square, the total area of the plane is $172\text{\AA}^2$.

The thickness of the chlorin plane will depend on the projections of the substituents from the plane of the head. With the present model this was determined to be $4.5\text{\AA}$. As an approximation, the chlorin head can be represented by a rigid, rectangular parallelepiped with the dimensions $13.1\text{\AA} \times 13.1\text{\AA} \times 4.5\text{\AA}$.

Estimates of chlorin head size, from results of x-ray measurements, gave $15.48\text{\AA} \times 15.62\text{\AA} \times 3.9\text{\AA}$. (Reference [2], pp. 448-449). Although these dimensions do not agree well with those calculated above, the differences in calculated area of the chlorin plane projected on the interface are not more than $2\text{\AA}^2$, for $\theta \leq 55^\circ$. M. Calvin (reference [1], p. 172) gives lengths in the substituted chlorin plane ranging from 12 to $15\text{\AA}$, but does not estimate the area of the plane.

When fully extended, the length of the phytol chain is about $20\text{\AA}$. In the folded configuration with the chlorin head in the monolayer it can extend about $5\text{\AA}$ above the head. The diameter of the extended phytol chain is about $5.8\text{\AA}$, as determined from the present model. This value allows for motion

of the methyl groups around the axis of the chain, arising from rotational motions within the chain. The corresponding excluded area is $26\text{\AA}^2$. This represents the least projected area, or surface requirement for the phytol group in pheophytin monolayers. It is the area that would be expected for the most closely packed state in the monolayer.

Hanson suggested that the phytol group had a surface requirement of about $36\text{\AA}^2$ in chlorophyll monolayers at zero compression (reference [10] and reference [2], p. 449). This was based on a comparison of the limiting areas of ethyl chlorophyllide monolayers at $\text{pH} = 5.4$, and chlorophyll monolayers at $\text{pH} = 4.1$. At these pH 's the monolayers were probably not stable. Larger values for the apparent phytol surface requirement could have been calculated by comparison with chlorophyll at high pH 's, since the limiting areas were greater. To assign a value for the surface requirement of phytol, it was necessary to assume that the heads of the chlorophyllide and chlorophyll molecules had the same angle of tilt with respect to a normal to the interface. If the angle of tilt of chlorophyll had been greater, the calculated surface requirement for phytol would have been smaller than $36\text{\AA}^2$, or vice versa. Unfortunately no surface potential data appears to have been taken, so that the assumption could not be more clearly evaluated. It does not seem possible to decide from Hanson's work whether or not use of $26\text{\AA}^2$, in calculation of minimum areas per molecule, will give rise to significant errors. It is likely, however, that any

uncertainties will be in the direction of estimating too small an area per molecule.

It is possible to describe certain features of the monolayers in terms of molecular dimensions and the surface moment characteristics of the molecules. In the considerations that follow, it is assumed that the chlorin head is approximated by a rigid, rectangular parallelepiped, and the phytol tail by a long flexible cylinder.

With the ester groups oriented in the water phase, one of the lower edges of the chlorin head will be negative with respect to the other, due to the presence of the cyclopentanone ring. The counterion, associated with the head, will tend to be located as far as possible from the ester groups and the cyclopentanone ring to minimize repulsion. A probable arrangement is one in which the plane of the chlorin head is tilted[†] at some angle between 0° and 60° from the normal, and the counterion located slightly beneath, and slightly to one side of the positively charged head center. Close packing of monomolecular units in such an orientation would require that the chlorin heads lie approximately in rows of parallel planes, alternate rows being displaced both horizontally and vertically to minimize

[†]Tilting of the head would allow for closer approach of the charged head center and the counterion. It would also increase the area excluded by each pheophytin molecule (see below) which would allow more space for, and greater separation between counterions. The phthalate anion is quite bulky with an average excluded area of about $24\text{\AA}^2$.

repulsion.[†] The direction of the electrical moments in alternate rows would be essentially congruous in such an orientation. Each phytol tail would have four nearest neighbor chlorin heads, and each chlorin head would have four nearest neighbor tails.

A scale, schematic view of an idealized arrangement in the plane of the interface is given in Figure 5. The rectangles represent cross-sectional projections of the heads. The angle of tilt of the head is taken to be 42° from the normal, so that the effective thickness of the head represents $6A$ instead of $4.5A$. The closed circles represent projections of the tails, and the dashed circles drawn inside the head area represent the counterions. The crosses on the long side of the heads represent the ester groups, while the crosses on the short side of the heads represent the cyclopentanone rings. These are not drawn to scale. The area excluded by the ester groups and the cyclopentanone ring is about $36A^2$. This is close to one half the area excluded by the entire head, which is $79A^2$. As drawn in Figure 5, the average area available to each molecule is about $130A^2$.

The suggested arrangement represents a highly ordered array of both the heads and the tails. It is visualized that an ordering of this type prevails throughout the film down to surface pressures where it is proposed that a transition in order takes place (see below). It is not to be inferred, however,

[†]This type of vertical staggering has been suggested also for symmetrical long chain ions (reference [36], p. 80).

Scale 2mm = 1A

Figure 5

Close Packed Arrangement in a Pheophytin
Monolayer (Scale 2 mm = 1A)

that at high pressures all the chlorine heads of the film, in their average positions, have their planes parallel. Thermal motion of the molecules is likely to result in differences of orientation of large regions of molecules in the film without significantly increasing the average repulsion pressures.

The cohesive pressure within the monolayer will be due primarily to dispersion interactions between the hydrocarbon parts of the molecules. These interactions will increase continuously with compression, roughly in proportion to the minus sixth powers of the various intermolecular distances. Where local ordering prevails, head-tail and head-head interactions will be large and tail-tail interactions small. Dispersion interactions are approximately additive in all directions. For the heads the interactions will be greater along axes parallel to the head planes, since the electronic oscillations along the π -bond paths in the planes of the heads will give rise to larger oscillating dipoles than will arise from motions normal to the head plane. Head-head interactions, therefore, will be favored by parallel orientations of the planes of the heads.

The net coulombic interactions in the film are repulsive and will give rise to a positive pressure. The predominant interactions are probably interionic, so that repulsions will vary approximately as the reciprocal of interchange distances. Vertical displacement into the water surface, of the molecules in alternate rows, will reduce repulsion not only by increasing distances between charges, but also by allowing for the

interposition of more water molecules between charges. This will increase the effective dielectric constant and reduce the repulsive interaction energies. Partial submersion of molecules into the water surface will be opposed primarily by the work of hydrophobic immersion of the hydrocarbon parts of the molecules.[†]

Because of the highly complicated cooperative interactions occurring in pheophytin monolayers, a definitive calculated estimate of depths of immersion can not be made. Estimates have been made, however, for simple long chain ions (reference [36], p. 80). A typical depth might be around 3A. This would entail the immersion of 2 or 3 -CH₂- groups. It seems reasonable to suppose that 3 to 5A is of the right order for the depth of immersion of the ester groups for the pheophytins.

The kinetic motions of the tails will make the main contribution to the thermal pressure of the films. Since the phytol groups extend only about 5A above the planes of the heads, it can be seen from Figure 5 that the motion of the tails will be quite restricted in a close packed array. The restriction will be greater, the greater the angle of tilt of the heads, at a given area per molecule. Assume that the film is expanded

[†]Slight decreases in cohesive interactions between heads, due to increased relative depths of submersion, will be offset by decreases in intermolecular distances. Head-tail interactions, between nearest neighbors, will probably not be decreased by increase of relative immersion. The reason is that the part of the tails, which rise some 5A above the heads, will be brought into the film on immersion.

from $130A^2$ per molecule to $150A^2$, and the same molecular arrangement and angle of tilt of the head persists as depicted in Figure 5. Then, with the molecules centered in their T-shaped cells, the increase in average accessible free area,[†] per head or tail, is from about $13A^2$ to about $23A^2$. The proportionate increase of free area to excluded area is clearly greater for the tails. Because of their flexibility the tails will be able to undertake a greater degree of thermal motion than the rigid heads.

In expansion from the most close packed state to $150A^2$, the average separation between adjacent molecules increases only by about $1.5A$. Intermolecular coulombic repulsions will still be significant, so that local ordering of the heads would be expected to persist. Motions of a molecular unit as a whole, then, would be confined largely to translatory oscillations about the center of its cell.

For the smallest excluded dimensions given in Figure 5, and with the molecules centered in their cells, the smallest area per molecule at which free rotation (about an axis normal to the interface) could occur is about $240A^2$. Since configurational fluctuations will occur wherein one, or two nearest neighbors of a particular molecule will not be centered in their cells, free rotation could occur at smaller areas per molecule

[†]Here, this is assumed to be the area available for the head, or tail to move about in, with all nearest neighbors remaining motionless.

than $240\text{\AA}^2$. Where free rotations set in, the π - σ behavior would reveal discontinuities in C_s which are characteristic of order-disorder transitions (reference [31], p. 109). Such discontinuities were observed, but at areas per molecule where free rotation of whole molecular units would be improbable (see below). For ph a at pH = 3.0, for example, the slope of C_s vs σ started to increase discontinuously at about $150\text{\AA}^2$. For this system, assume that the angle of tilt is 42° , then the average free area per molecule is $45\text{\AA}^2$. Free rotation of a whole molecular unit could just occur if all its nearest neighbors simultaneously moved away from their cell centers to positions where they were contiguous with their nearest neighbors. A fluctuation of this type, however, seems highly improbable.

With the head and tail folded together, and the plane of the head perpendicular to the water surface, the smallest excluded area per molecule is about $90\text{\AA}^2$.[†] This is the smallest area that should be observed, if the entire film is monomolecular, and if the ester groups are oriented in the water surface as suggested. Calculation of the excluded area, using head dimensions estimated from x-ray data and Hanson's suggested surface requirement for phytol, gives about $96\text{\AA}^2$.

If the chlorin heads were inverted into the water surface, an area per molecule of $75\text{\AA}^2$ or less might be observed. It is

[†]In the most close packed molecular array, there would be interstitial spaces unoccupied by the monolayer molecules. This corresponds to about $5\text{\AA}^2$ per molecule, and is included in the value of $90\text{\AA}^2$.

probable that the sign of the surface potential would be reversed. To attain this configuration considerable work would be required for hydrophobic immersion of the hydrocarbon parts of the head.

In the collapse region, where it appeared that some sort of reversible process was occurring, it is conceivable that a configurational fluctuation could result in a vectorial reversal of some of the chlorin heads, without their complete immersion. Such a reversal would result in a reduction in average occupied area by about 16 per cent. The phytol tail would be elevated further into the air phase. Attractive coulombic interactions between the ester groups and the cyclopentanone ring with neighboring head centers could occur. These would tend to compensate for the removal of the ester groups from the water surface. The surface potential would decrease with decrease of σ , and the slope of $\mu/D \cos \theta$ versus σ would increase sharply, which was observed. Apparent reversibility was observed at calculated areas per molecule down to about 5 per cent less than the area determined at the limit of mechanical stability. This corresponds to an inversion of about 16 per cent of the chlorin heads, if the suggestion for the process is correct.

With head and tail folded together and largely elevated above the water surface, an increase of angle of tilt will increase the smallest excluded area and reduce the overall electric moment of the monolayer. In Table 14 are illustrated the changes for several values of θ . A value of 1.50 Debye

is taken for μ/D , and it is assumed that D remains constant.

Table 14
Variation of Monolayer Molecule Characteristics
with Angle of Tilt (θ)

θ , Degrees	Smallest Excluded Area, ^a $\text{\AA}^2$	$\mu/D \cos \theta$, Debyes
0	90	1.50
20	94	1.41
31	100	1.29
37	105	1.20
42	110	1.11
60	149	0.75

^aThis includes $5\text{\AA}^2$ to allow for interstitial areas between molecules in the closest packed state.

The area per molecule, where film collapse began, increased with decrease of pH for both pH a and pH b. This can be related to an increase in the average angle of tilt of the chlorin heads as shown in Table 14.[†] Increase of tilt would not only reduce the vertical component of the dipole of the head, but it would also allow for closer association between charged heads and counterions. This would produce a rather significant decrease in the overall electric moment, which would be consistent with the relatively small increase in $\mu/D \cos \theta$.

[†]It is possible that the larger areas at pH = 3.0 could have resulted from a partial penetration of counterions into the monolayer. However, pheophytin monolayers spread on unbuffered HCl solutions at pH = 3.0 and 4.0 showed the same behavior, within experimental accuracy, as on buffered substrates. No differences were observed between citrate and phthalate buffers.

It was suggested above that di-protonation occurred at $\text{pH} = 3.0$. From the viewpoint of the present monolayer model, the degree of di-protonation must have been rather small. If it had been large, the average angle of tilt of the heads would have been great, and much larger increases in the areas at collapse would have been observed. Alternatively, a large degree of di-protonation could have been accompanied by a relatively great depth of submersion of alternate rows of heads, e.g. 7 or 8 Å. Close association of immersed head centers and their counterions would have greatly diminished the overall electric moment. However, a rather large work of hydrophobic immersion would have been required. Further, a large increase of extent of relative vertical displacement, in contrast to increase of average angle of tilt, would have brought the film closer to the collapse configuration. In this case, it seems likely that greater decreases in collapse pressures should have been observed. If di-protonation had been accompanied by penetration of counterions into the monolayer, compression would have decreased the extent of penetration (reference [36], p. 295). The value of $\mu/D \cos \theta$, then, would probably have increased rather than decreased.

If D changed with compression, it would be due primarily to re-orientation of specifically associated water molecules. Adam (reference [45], p. 38) points out that reorientation and redistribution of specifically associated water molecules is often independent of compression. Consideration of the relative

sizes of water molecules and the pheophytins shows that this is probably true for the pheophytin monolayers. The largest excluded area of a water molecule can be taken as the cross sectional area of the widest cylinder of revolution around one of the axes of rotation. This is approximately $3.0\text{\AA}^2$. The area excluded by the ester groups and the cyclopentanone ring is about $36\text{\AA}^2$, and will remain constant at all areas per molecule. Clearly, twelve primary waters of hydration could be associated with these groups and not be re-oriented on compression. Using the longest dimension of the water molecule, it is estimated that the greatest thickness, which two hydrated protons could impart to the center of the chlorin head, is about $4.3\text{\AA}$. This is slightly less than the value of $4.5\text{\AA}$ estimated for the side chain substituents. It seems unlikely, therefore, that one or two primary waters of hydration at the center of the ring would undergo appreciable rearrangement on compression.

Decrease of separation between head centers and counterions, suggested to explain the decrease of $\mu/D \cos \theta$ on compression, could involve some re-orientation of water molecules. From the following order of magnitude considerations, this effect would appear to be small. In the calculations, no attempt is made to account for the polarization of the chlorin heads, the counterions, or the water molecules. The interaction between a head and its counterion is assumed to be independent of the neighboring heads and counterions. The dielectric constants used, were estimated for the reorientation of water

molecules in electric fields of one sign originating from one source (reference [36], p. 142). In the monolayer region between the charged heads and counterions, there could exist cooperative field effects that would reduce the orienting effect of a single charge center. Thus, the dielectric constants used in the calculations may be considerably smaller than those actually occurring in the film.[†]

Assume that the separation between the charge centers of the heads and the counterions is 8Å, and that a layer of water is effectively interposed. The field strength, at half the distance of separation, due to a mono-valent ion would be [50]

$$\frac{4.8 \times 10^{-10}}{16 \times 10^{-16}} = 3 \times 10^5 \text{ esu cm}^{-1},$$

At this field strength the water molecules would be highly oriented, and would have an estimated dielectric constant of about 7. Using this value and a typical change of $\mu/D \cos \theta$ (e.g. 0.30 Debye), a decrease in ionic separation of

$$\frac{0.30 \times 10^{-18} \times 7}{4.8 \times 10^{-10}} = 0.44 \times 10^{-8} \text{ cm}$$

is calculated. Using one half the new distance of separation, 7.56Å, a field strength of $3.4 \times 10^5 \text{ esu cm}^{-1}$ is found, giving an estimated dielectric constant of about 6.5. The changes in

[†]Davies and Rideal use a value as large as 80 in calculation of the electric moments between heads and counterions in completely ionized films (reference [36], p. 78).

relative distance and dielectric constant are both small. On this bases, then, decrease in distance between head and counterion would not be accompanied by significant rearrangements of water molecules. Using the value of 7 for the dielectric constant and 8A for separation, the electric moment for the ion pair is

$$\frac{4.8 \times 10^{-10} \times 8 \times 10^{-8}}{7} = 5.5 \text{ Debyes}$$

This is somewhat too large and reflects the approximations used in the calculations, and the likelihood that, on the average, separation of charges was considerably smaller than assumed. In this latter case, the rearrangements of water molecules would be of even less significance than indicated.

The areas per molecule, where collapse begins to occur, are similar for the ph a and ph b systems. Collapse pressures for the ph b systems, however, are significantly smaller. At surfaces pressures greater than about 1.0 dyn/cm, the compressibilities of the b systems are greater than those of the a systems, resulting in smaller areas per molecule at given values of π . These phenomena can be related to the presence of the 3-position carbonyl group on the ph b head units. As compression on the film is increased the molecules approach a close packed ordered array with increasing degree of relative vertical displacement between alternate rows of molecules. In the vertical staggering arrangement, intermolecular configurations

are possible where there are ion-dipole attractions between neighboring positively charged head centers and 3-position carbonyl groups. An arrangement might be one in which the heads of the molecules, which are more deeply immersed in the aqueous phase, are rotated slightly around an axis perpendicular to the plane of the head so that the carbonyl groups are brought closer to the plane of the water surface. Increase of degree of relative vertical staggering by compression can increase ion-dipole attractions. These interactions, not present in the ph a monolayers, will contribute to the cohesive pressure and tend to promote relative vertical displacement. Consequently, less compressional work is required to reduce film area and reach the collapse configuration.

As shown in Figures 2 and 3, there are distinct related changes in π - σ and $\mu/D \cos \theta$ - σ behavior in the neighborhood of 1.0 dyn/cm. Above about 1.0 dyn/cm, the compressibilities were characteristic of cohering films, $\mu/D \cos \theta$ decreased linearly with increase of π , and surface potentials were uniform over the surface. In Table 15 are given the areas per molecule where plots of C_s versus σ start to show discontinuities with increase of σ , and where plots of $\mu/D \cos \theta$ versus π cease to be linear with decrease of π . The values of $\sigma_{f/2}$, which represent the approximate average free area accessible to a head or tail, are one half the difference between the average of the transition areas and the smallest excluded areas, σ_0 . These latter values are calculated using the angles of tilt shown in Table 14;

Table 15

Areas Per Molecule and Free Areas Per Monolayer
Unit at the Start of Transition

System	C_s , cm/dyn	σ_{cs} , ^a A ²	π_{μ} , ^b dyn/cm	σ_{μ} , ^c A ²	σ_o , ^d A ²	$\sigma_{f/2}$, ^e A ²
ph <u>a</u> - pH = 3.0	0.055	148	1.5	150	105	22.0
ph <u>a</u> - pH = 4.0	0.055	133	1.2	134	95	19.2
ph <u>b</u> - pH = 3.0	0.09	146	1.5	145	100	22.7
ph <u>b</u> - pH = 4.0	0.08	133	1.1	134	95	19.2

^aArea per molecule where C_s vs σ starts to show discontinuity with increase of σ .

^{b,c}Surface pressure and area per molecule where $\mu/D \cos \theta$ vs π is no longer linear with decrease of π .

^dSmallest excluded area per molecule.

^eAverage free area per head or tail.

the $5A^2$ for interstitial area is included in the free area. The average value of $\sigma_{f/2}$ for all four systems is $20.8A^2$ with average deviations of $\pm 1.6A^2$.

At pressures less than the values shown in Table 15, reproducible small fluctuations in surface potentials over the surface were observed,[†] and the surface pressures tended to level out to constant low values in a manner similar to that observed for fluid type surface films of high polymers [51]. These phenomena indicate that the observed transitions involved a change in the state of ordering in the films.^{††} The close agreement in the average free area, per head or tail, suggests that the transitions for the different systems arose from the same cause. The magnitude of the free area per unit is almost as large as the smallest excluded area of the phytol tail. It seems reasonable to suggest that the changes in film behavior were due primarily to significant increases in the kinetic motions of some of the phytol tails.^{†††}

[†]These corresponded to an average difference in surface moment of about 0.12 Debye in different regions of the surface, or about ± 0.06 Debye from the average values shown in Figures 2 and 3. Variations in ΔV for a typical run are shown in Figure 6.

^{††}It is possible that impurities could have produced part of the pressure in the regions of large molecular area. However, the consistency and reproducibility of the behavior for well spread films rules out the presence of impurities as the primary explanation.

^{†††}D.J. Crisp [51] suggested, similarly, that the expanded low pressure regions for high polymers arose from the onset of kinetic motions of the hydrocarbon chains. The transition in

Figure 6

Variations in ΔV with σ
 $\text{ph } \underline{a} - \text{pH} = 3.0$

The thawing out of thermal motion with decrease of π would produce regions in the film of different states of order. The occurrence of measurable fluctuations in surface moment over the surface suggests that the regions coexisting in equilibrium were macroscopic in size. The regions where the motions of the tails were largely frozen out[†] would preserve the type of ordering suggested in Figure 5. Compression of the films at high pressures, then, would involve relatively large increases in vertical staggering, and relatively small decreases in average intermolecular distances to allow for closer packing.

In the regions of relatively greater disorder, each molecular unit would have a significantly larger average free area than in the ordered regions to allow for the greater degree of kinetic agitation. For example, assume that the average area per molecule in the regions of order increase relatively little on expansion of the film, and that 50 per cent of the molecules

the intermediate liquid region for straight chain monolayers has been ascribed to the gradual liberations of thermal motions (reference [33], p. 134, [52]). Extremely high compressibilities are found in these transitions, and often heterogeneity in surface potential [20].

[†]In discussion of the condensing effect of cholesterol on straight chain fatty acids in mixed monolayers, Adam (reference [45], p. 70) suggests that the rigid heads of the cholesterol molecules, which are not undergoing oscillations, exert a mechanical interference with the oscillatory motions of the long chains. A similar interference can be visualized for the chlorin heads on the phytol tails. At small inter-unit distances, dispersion interactions between heads and tails will be large and tend to make the tails lie as close as possible to, and extended along one of the nearest neighbor heads.

are disordered at an overall average molecular area of $175\text{\AA}^2$. For the $\text{ph a} - \text{pH} = 3.0$ system, the average free area per molecule in the disordered regions would be about $50\text{\AA}^2$ greater than in the ordered regions. With greater free areas per molecule, the probability of rotation of entire molecules about an axis normal to the interface would be increased. Intermolecular configurations would then occur where numbers of phytol chains were interacting. Because of the concerted kinetic motions among the chains, average distances between chain units would remain relatively small. Their dispersive interactions would therefore make a significant contribution to the cohesive pressure.

To account for the variations in surface moment it is suggested that in the disordered regions of the film, where the molecules on the average have a larger accessible free area than in the ordered regions, there is a decrease in the extent of relative vertical staggering between molecules accompanied by a slight increase in the separation between charged heads and their counterions. This would produce a small increase in overall surface moment in comparison to that in the ordered regions and could thus account for the fluctuations in surface potential observed. With a larger accessible free area per molecule, lateral coulombic repulsions would be reduced. This reduction in repulsion would lead to a decrease in relative vertical displacement of the molecules since displacement of some of the molecules into the water surface requires a positive

work of hydrophobic immersion of the hydrocarbon parts of the molecule.

If the commonly observed free area, at which transitions in the behavior of surface moment and compressibility became apparent, corresponds to a sort of critical area per molecule, where the balance of negative cohesive pressure and positive thermal and repulsive pressures is such as just to damp out the random wagging motions of the phytol tails, then a decrease in critical area with increase of temperature might be expected, along with an increase in the measured surface pressure at the start of transition. The transition that is suggested to explain the phenomena can not be considered to be a regular first order transition, primarily because a constant surface pressure was never attained, and the transition from one regime of behavior to the other is not at all sharp.

At equilibrium, with coexisting ordered and disordered regions,

$$\pi_{kin}^{\alpha} + \pi_{coh}^{\alpha} + \pi_{rep}^{\alpha} = \pi_{kin}^{\beta} + \pi_{coh}^{\beta} + \pi_{rep}^{\beta} = \pi_{meas} \quad 6)$$

where α = region of disorder, β = region of order,

$$\pi_{kin}^{\alpha} = \pi_{kin}^{mol} + \pi_{kin}^{tails}, \quad \pi_{coh}^{\beta} = \pi_{coh}^{heads-heads} + \pi_{coh}^{heads-tails},$$

and

$$\pi_{coh}^{\alpha} = \pi_{coh}^{heads-heads} + \pi_{coh}^{heads-tails} + \pi_{coh}^{tails-tails}$$

The following inequalities exist:

$$\pi_{\text{rep}}^{\beta} > \pi_{\text{rep}}^{\alpha}, \pi_{\text{coh}}^{\beta} > \pi_{\text{coh}}^{\alpha}, \pi_{\text{kin}}^{\beta, \text{mol}} > \pi_{\text{kin}}^{\alpha, \text{mol}},$$

$$\pi_{\text{kin}}^{\alpha, \text{tails}} \gg \pi_{\text{kin}}^{\beta, \text{tails}}$$

If the transition was regular first order, then for a given temperature with $\pi_{\text{meas}} = \text{constant}$, the average free areas per molecule in both the ordered and disordered regions would remain essentially constant with change in film area, and each of the terms in equation 6) would remain constant. Since π_{meas} was not constant, particularly in the first part of the transition region with decreasing compression, it seems likely that secondary configurational changes occurred in both the ordered and disordered regions. In the disordered phase such a secondary change might be the gradual freeing of the rotational motions of the molecular units as a whole, while in the ordered phase it might be the liberation of rocking motions of the chlorin heads. The presence of secondary configurational changes might be further revealed in an alteration of shape of the transition region with change of temperature, particularly in the initial part. Thus an increase of temperature might be expected to broaden the transition region.

SUMMARY

A study has been made of the stability of chl a, ph a, ph b, and al ph a monolayers at air-water interfaces, and chl a monolayers at nitrogen-water interfaces. To avoid photodecomposition, the monolayers must be kept in darkness, or under very low intensity, diffuse green light. Ph a and ph b monolayers are stable at air-water interfaces if the pH of the aqueous substrate is 4.5 or less. Al ph a monolayers are stable at air-unbuffered water interfaces. At pH's greater than 4.5, ph a and ph b monolayers undergo a chemical change that appears to be essentially allomerization. The rate of change increases approximately linearly with pH above 5.0, with a slope of 3.7 per cent/hour/pH unit. Chl a monolayers at air-water interfaces rapidly undergo both pheophytinization and allomerization at pH = 6. At pH = 9 pheophytinization is inhibited, but allomerization of chl a occurs at a rate of about 2.5 per cent/hour. Under a nitrogen atmosphere (30-50 ppm O₂) and at pH = 9.0, allomerization of chl a is reduced to about 0.9 per cent/hour.

Since it was possible to obtain chemical stability of ph a and ph b monolayers at low pH, a quantitative study of the surface pressure (π) and surface potential (ΔV) as a function

of area per molecule (σ) was made at 20°C , on $\text{pH} = 4.0$ and $\text{pH} = 3.0$ substrates. At $\text{pH} = 4.0$, as σ was varied from 200 to $100\text{\AA}^2$, π changed from 0.10 to 12.85 dyn/cm and ΔV increased from 349 to 597 mv. for ph a, while for ph b the corresponding changes were 0.10 to 8.25 dyn/cm and 244 to 434 mv. For ph a at $\text{pH} = 3.0$ and for σ in the range 210-110 $\text{\AA}^2$, π varied from 0.15 to 11.04 dyn/cm and ΔV increased from 361 to 561 mv. For ph b at $\text{pH} = 3.0$, as σ was varied from 200 to 105 $\text{\AA}^2$, π changed from 0.20 to 8.03 dyn/cm and ΔV increased from 269 to 423 mv. If the films were compressed to values of σ smaller than those recorded above, surface pressures and surface potentials decreased with time. This was considered as evidence of film collapse.

The positive sign of the surface potentials is indicative of a molecular orientation in which the ester groups are anchored in the water surface, and the major portion of the chlorin head and the phytol tail are elevated into the air phase. The smaller values of the surface potentials for ph b are visualized as arising primarily from the presence of the $-\text{CHO}$ group at the 3-position on the chlorin ring. At this position its group moment will on the average be negative toward the air phase, and therefore decrease the total moment of the molecule.

For given values of π above 1.0 dyn/cm, decrease of pH from 4.0 to 3.0 results in an average increase of σ of about $9\frac{1}{2}$ per cent. Comparison with the results of studies of ph a on $\text{pH} = 8$ substrates shows that σ averages about 20 per cent

larger at $\text{pH} = 4.0$ than at $\text{pH} = 3$, for given values of π above 1.0 dyn/cm . This expansion at the low pH's suggests that salt formation occurs with the entry of one or two protons into the center of the chlorin ring. Protonation would increase the repulsion between molecules and thus result in a larger area per molecule at a given value of the film compression. Salt formation would be expected to affect the electronic distribution in the molecule and therefore alter its susceptibility to allomerization. Thus protonation of the pheophytins can be visualized as resulting in a decrease of electron density in the isocyclic ring, thereby increasing the potential barrier in the first step of an oxidation process.

For both $\text{ph } \underline{a}$ and $\text{ph } \underline{b}$ monolayers at $\text{pH} = 4.0$ and 3.0 , the effective surface moments, $(\mu/D) \cos \theta$, of the molecules decrease linearly with increase of π above about 1.0 dyn/cm . At smaller surface pressures, the surface moment, averaged over the film area, is essentially constant with small reproducible fluctuations appearing over the surface of the monolayer. The change in behavior of surface moment is accompanied quite closely by a change in the character of the average compressibility. At compressions less than 1.0 dyn/cm , the compressibility is characteristic of a gaseous film, while at greater pressures it is characteristic of a liquid expanded film. It is suggested that these phenomena signify an order-disorder transition occurring in the region of surface pressures near 1.0 dyn/cm .

A simple monolayer model is discussed which is based on the dimensions of the molecules and the characteristics of their packing together at different states of compression. A reasonable interpretation can be given for most of the observed characteristics of the monolayers. The essential features of the model are as follows. At surface pressures above about 1.0 dyn/cm, the molecules are packed in a highly ordered array of local extent. To account for the decrease of $(\mu/D) \cos \theta$ with increase of π , it is suggested that molecules in alternate rows undergo an increase in relative vertical displacement with respect to the plane of the interface. This relative motion would be expected to lessen intermolecular repulsion and decrease the distance of separation of some of the monolayer molecules and their counterions. At surface pressures less than about 1.0 dyn/cm it is suggested that regions of order and disorder coexist throughout the film. The disordered regions are characterized by a relatively greater degree of thermal motion of the phytol tails and a smaller degree of relative vertical staggering among the molecules, as compared to the ordered regions. These features could account for the greater compressibility and the fluctuations in surface moment over the surface in the low pressure regime.

A spectroscopic method is presented for the analysis of intact chlorin ring decomposition products of chl a in dilute benzene or ethyl ether solutions. It is applicable to

noninteracting mixtures where the components are chl a, al chl a, ph a, and al ph a, or any other mixtures of these components. From application of the method to a large number of solution samples from chemical, photochemical, and monolayer stability tests, it is found that allomerization and/or pheophytinization are the predominant degradation reactions forming intact chlorin ring derivatives under many usual laboratory conditions.

APPENDIX A

CHEMICAL AND PHOTOCHEMICAL STABILITY TESTS ON
THE CHLOROPHYLLS AND SEVERAL DERIVATIVES
IN VARIOUS SOLVENTS

INTRODUCTION

During the work on preparation of the chlorophylls and their derivatives, it became evident that decomposition of the materials in solution could occur quite readily under normal laboratory conditions. Since it was necessary to obtain pure compounds and to store them under conditions in which they would remain intact, chemical and photochemical stability studies were undertaken. The objectives were to obtain a qualitative evaluation of the various types of solvents used in this work and to intercompare the behavior of the different compounds.

EXPERIMENTAL

In all tests decomposition was evaluated by measuring the changes in the visible absorption spectra. Chemical stability was determined simply by storing the different solutions ($1 \times 10^{-5}\text{M}$, or $1 \times 10^{-4}\text{M}$) in stoppered flasks for various time periods in the dark. Illumination at low light intensities was done with either a 15-watt "Daylight" fluorescent lamp, or a 100-watt tungsten lamp. Solution samples, in stoppered pyrex absorption cells (volume, 4.0 cc) were exposed directly to the light, or through various filters. Condensing lenses were not used. In some experiments, where apparatus was not available to measure light intensity, comparisons were made with different samples placed at equal distances from the light source. Illumination at high light intensities was done with a 1000-watt tungsten projection lamp using suitable condensing and focusing lenses. The light was passed through an eight inch column of water in a flat ended pyrex cylinder and then through one or more Corning heat resistant glass filters. The Corning filters used were as follows:

- a) 5-60, transmitting in the blue region;
- b) 4-64, transmitting in the green region;
- c) 2-59 and 2-64, both transmitting in the red, and

d) 1-69, 1-57, and 1-59, transmitting in the visible and absorbing in the infrared and ultraviolet.

In Table 16 are shown the initial rates of absorption of energy of some of the solutions used in the photochemical experiments. The designations high, and low under the heading "Light Source" refer to the 1000-watt tungsten lamp and the 15-watt fluorescent lamp, respectively. Energy and quanta absorbed were calculated from light intensities measured with an Eppley thermopile. Calculations were made as follows: per cent transmissions of the various filters were applied at wavelength intervals to relative energies of the source which resulted in a relative energy incident to the sample vs wavelength. The absorption spectra data for chlorophyll and related derivatives were changed from d vs λ to per cent absorbed vs λ . The relative incident energies and the absorption curves were combined for each compound. The areas under the resulting curve were measured with a planimeter. Using the measured energy values the relative energy values were changed to absolute energies. The relative energy vs λ emission of the "Daylight" fluorescent source was taken from "Electrical Illumination," J.O. Kraehenbuel, Wiley (1951). The relative energy vs λ emission of the 1000-watt tungsten source was calculated from data in the RCA Tube Handbook and "Measurements of Radiant Energy," W.E. Forsythe, McGraw-Hill (1937). By comparison of measured energies in various regions of the spectrum and relative energies from above references the

Table 16

Initial Energy Absorption of Solutions
in the Photochemical Experiments

System	Conc. $\underline{M}(\times 10^5)$	Spectral Range ($m\mu$)	Filters Light Source	$I_{\text{abs/sec}}$ (ergs/sec) $\times 10^{-5}$	Quanta _{abs/sec} $\times 10^{-16}$
ph <u>a</u> - ether	1.00	750 - 350	1 - 56	3.3	9.1
		750 - 625	High	0.9	3.0
		625 - 575		0.5	1.4
		575 - 475		1.1	2.8
		475 - 350		0.9	1.8
ph <u>b</u> - ether	1.00	750 - 350	1 - 56	4.0	10.4
		750 - 625	High	0.7	2.2
		625 - 575		0.4	1.3
		575 - 475		1.3	3.5
		475 - 350		1.6	3.4
al ph <u>a</u> - ether	1.00	750 - 350	1 - 56	2.5	6.9
		750 - 625	High	0.8	2.6
		625 - 575		0.2	0.7
		575 - 475		0.8	2.1
		475 - 350		0.7	1.5
chl <u>a</u> - ether	1.15	750 - 350	1 - 56	4.4	12.3
		750 - 625	High	1.4	4.5
		625 - 575		1.0	3.0
		575 - 500		0.8	2.1
		500 - 350		1.3	2.7

Table 16 (Contd.)

System	Conc. $\underline{M}(\times 10^5)$	Spectral Range ($m\mu$)	Filters Light Source	$I_{\text{abs/sec}}$ (ergs/sec) $\times 10^{-5}$	Quanta _{abs/sec} $\times 10^{-16}$
chl <u>b</u> - ether	0.94	750 - 350	1 - 56	4.7	12.5
		750 - 625	High	0.9	2.7
		625 - 575		0.8	1.9
		575 - 500		0.8	1.9
		500 - 350		2.2	6.0
chl <u>a</u> - benzene	1.00	750 - 640	2 - 64 + 1 - 56 High	0.5	1.5
chl <u>a</u> - benzene	1.00	750 - 610	2 - 59 + 1 - 56 High	1.0	3.3
chl <u>a</u> - benzene	1.00	525 - 375	5 - 60 + 1 - 56 High	0.7	1.6
chl <u>a</u> - pyridine	1.15	750 - 350	1 - 56	4.8	13.5
		750 - 625	High	1.6	5.4
		625 - 575		0.9	2.9
		575 - 500		0.5	1.4
		500 - 350		1.8	3.9
chl <u>b</u> - pyridine	0.94	750 - 350	1 - 56	5.6	14.9
		750 - 625	High	1.0	3.2
		625 - 575		0.8	2.3
		575 - 500		0.8	2.2
		500 - 350		3.1	7.2

Table 16 (Contd.)

System	Conc. $\underline{M}(\times 10^5)$	Spectral Range ($m\mu$)	Filters Light Source	$I_{\text{abs/sec}}$ (ergs/sec) $\times 10^{-5}$	Quanta _{abs/sec} $\times 10^{-16}$
al chl <u>a</u> - pyridine	0.57	750 - 350	1 - 56	2.5	6.8
		750 - 625	High	0.8	2.6
		625 - 575		0.4	1.3
		575 - 500		0.2	0.6
		500 - 350		1.0	2.2
al ph <u>a</u> - pyridine	0.55	750 - 350	1 - 56	2.1	5.6
		750 - 625	High	0.5	1.6
		625 - 575		0.2	0.7
		575 - 500		0.7	1.9
		500 - 350		0.7	1.4
chl <u>a</u> - wet CCl_4	1.00	750 - 350	Water	0.016	0.041
		750 - 625	Low	0.003	0.011
		625 - 575		0.002	0.006
		575 - 500		0.001	0.003
		500 - 350		0.010	0.022
chl <u>a</u> - dry CCl_4	1.00	750 - 350	Water	0.020	0.052
		750 - 625	Low	0.004	0.013
		625 - 575		0.002	0.007
		575 - 500		0.001	0.004
		500 - 350		0.013	0.028
chl <u>a</u> - benzene	1.00	750 - 610	2 - 59 + 1 - 69 Low	0.002	0.006

approximate temperature of the tungsten source was determined to be 3070°K.

The energy absorbed in each indicated region was converted to quanta absorbed by using an average wavelength absorbed in each region. The total number of quanta absorbed was obtained by adding the number of quanta in each region.

Errors could include source temperature, thermopile measurements, area of exposure measurements, reflection, transmission, plotting, and area measurement. Possible total error in values could be as high as ± 45 per cent; however, the probable error in intercomparison between runs should be ± 15 per cent or perhaps less.

All values calculated are for the indicated solutions in 1 cm absorption cells containing 4 cc of solution.

The only difference between the low intensity wet and dry calculations for chl a in CCl_4 is that the dry series was run slightly closer to the source.

Details of each experimental system are given in the tables describing the systems.

RESULTS AND DISCUSSION

Chemical Stability

In Table 17 are given descriptions of some representative systems studied in the chemical stability tests. If no decomposition occurred it is stated in this table. Tables 18 and 19 give the results where decomposition was encountered. To facilitate tabulation the following abbreviations for the solvents are used throughout: Ac = acetone, py = pyridine, bz-D = dry benzene, E = ethyl ether, EHCl = ethyl ether saturated with dry HCl gas, EHClH₂O = 2 parts ethyl ether + 3 parts ethyl ether saturated with concentrated aqueous HCl solution, bz-H₂O = wet benzene (i.e. [H₂O] > 10⁻⁵M), dry cyclohexane = cyclo-D, dry carbon tetrachloride = CCl₄-D, wet carbon tetrachloride = CCl₄-H₂O, and 1 1/2 per cent py-pet. = 1 1/2 per cent (by volume) of pyridine in petroleum ether.

With reference to the dry benzene solutions, the absorption of polar molecules, such as water, has the effect of decreasing B/R,[†] and increasing both B/505 and R/min as shown for the control runs in Table 17. In the long storage period of 7 months, leakage of water through the plastic stopper seals

[†]The pick up of water or other polar molecules in dry benzene solutions also has the effect of decreasing R_{1/2}B_{1/2}.

Table 17
Description of the Systems in Chemical Stability Tests

System, Run No.	Storage Time	Remarks ^d
chl <u>a</u> , bz - D 235A	7 Months ^a	Solution was prepared under N ₂ atmosphere.
chl <u>a</u> , bz - D 232C	7 Months ^a	Solution was prepared under N ₂ atmosphere, and then saturated with dry O ₂ gas.
chl <u>a</u> , bz - D 235B	7 Months ^a	Solution was prepared under N ₂ atmosphere, and then saturated with dry CO ₂ gas.
chl <u>a</u> , bz - H ₂ O 234C	7 Months	Solution was prepared under N ₂ atmosphere, and then saturated with H ₂ O and O ₂ .
chl <u>a</u> , bz - D 232D	12 Hours	Under N ₂ atmosphere, the chl <u>a</u> was evaporated onto a pyrex dish, allowed to stand for 12 hours, then redissolved in dry benzene.
chl <u>a</u> , bz - D 230C	4 Hours	Under N ₂ atmosphere, a stream of CO ₂ saturated with H ₂ O was blown over dried amorphous chl <u>a</u> before redissolving in dry benzene.
chl <u>a</u> , bz - D 234A	4 Hours	Under N ₂ atmosphere, a stream of O ₂ saturated with H ₂ O was blown over dried amorphous chl <u>a</u> before redissolving in dry benzene.

Table 17 (Contd.)

System, Run No.	Storage Time	Remarks ^d
chl <u>a</u> , Ac ^b	2 Months	No decomposition.
chl <u>a</u> , 1 1/2% py-pet., ^b 8	18 Months	
chl <u>a</u> , py ^b 287	11 Days	No decomposition.
al chl <u>a</u> , py ^b	2 Days	No decomposition.
ph <u>a</u> , py ^b 290	11 Days	
ph <u>a</u> , E ^b	129 Days	No decomposition.
ph <u>a</u> , EHCl ^b	7 Days	No decomposition.
ph <u>a</u> , EHCl H ₂ O ^b	9 Days	No decomposition.
al ph <u>a</u> , EHCl ^b	7 Days	No decomposition.
al ph <u>a</u> , py ^b	11 Days	No decomposition.
al ph <u>a</u> , E ^b	125 Days	No decomposition.
chl <u>b</u> , Ac ^b 273A	2 Years	

Table 17 (Contd.)

System, Run No.	Storage Time	Remarks ^d
chl <u>b</u> , E ^{b,c}	132 Days	No decomposition
chl <u>b</u> , py ^b 288	9 Days	
chl <u>b</u> , bz-H ₂ O ^b 284A		This solution was obtained from 288 by washing out the pyridine with distilled H ₂ O
ph <u>b</u> , E ^b	132 Days	No decomposition.
ph <u>b</u> , EHCl ^b	6 Days	No decomposition.
ph <u>b</u> , EHCl H ₂ O ^b	8 Days	No decomposition.
ph <u>b</u> , py ^b 286	11 Days	

^aThe solution picked up polar molecules (H₂O) during the storage period.

^bThe solution was prepared in normal laboratory atmospheres.

^cChl a, in ether, stored for a similar period, also showed no decomposition.

^dThe amount of oxygen present in the N₂ atmosphere varied from about 40 ppm to 100 ppm; the amount of CO₂ was estimated to be about 10 ppm.

Table 18

Chemical Stability of Chlorophyll a
in Benzene Solutions

System, or Run No.	B/R	B/505	R/min.	%chl <u>a</u>	%al chl <u>a</u>	%ph <u>a</u>	%PBP ^b	%Unknown ^c
Control, ^a bz-D	1.336	52.1	66	100.0				
Control, ^a bz-H ₂ O	1.261	55.7	107	100.0				
235A	1.27	60	89	95.2	1.6		1.1	2.1
232C	1.27	40	58	92.2	3.0		2.8	2.0
235B	1.26	55	87	94.6	2.5		1.1	1.8
234C	1.28	17	17	73.3	13.2		10.5 ^d	3.0
232D	1.35	15	12	56	14	5	20 ^d	5
230C	1.48	14	29	39	6	40	2	13
234A	1.28	12	12	49.5	17.2	9.4	13.7 ^d	10.2

^aThis refers to solutions of pure chl a.

^bThe abbreviation means photobleached products not absorbing, or only slightly absorbing in the regions of the main red and blue peaks. See text.

^cThis represents the uncertainties found in the determinantal correlations, and is indicative of the presence of unknown intact ring chlorin compounds.

^dSee text.

Table 19

Chemical Stability of the Chlorophylls and
the Pheophytins in Various Solvents

System, or Run No.	λ_R m μ	λ_B m μ	B/R	B/BS	B/505	R/min.	$R_{1/2}$ m μ	$B_{1/2}$ m μ
Control, ^a 1 1/2% py-pet.	663.7	434.5	1.169	1.327	54.8	94		
8, chl <u>a</u>	663.0	431.6	1.237	1.486	42	47		
Control, py	669.3	414.6	2.21			13.4	20.4	44.9
290, ph <u>a</u>	671.0	406.0	2.7			10.0	21.6	37.4
Control, Ac	645.5	456.6	2.85	2.52	47.6 ^b	17.4	20.1	25.9
273A, chl <u>b</u>	645.6	457.3	2.91	2.46	43.4	16.0	20.7	26.3
Control, py	655.2	474.0	3.10	2.57		11.6		25.4
288, chl <u>b</u>	641.4	461.0	5.8	2.4		4.5		35.4
Control, bz-H ₂ O	646.2	457.9	2.67	2.81		20.4		21.8
284A, chl <u>b</u> ²	633.6	446.8	4.9	2.2		5.1		35.2
Control, py	656.7	438.0	4.90	2.21		5.04	20.6	25.2
286, ph <u>b</u>	656.2	433.8	5.3	2.5		4.1	22.0	22.2
al ph <u>a</u> , py	671.7	405.8	3.02			8.3	24.4	39.6

^aThe control refers to a solution of the intact compound.

^bThe ratio for the b compound is B/520.

of the flasks, or desorption of water from the glass surfaces could have accounted for the partial increases in R/min for tests 235A and 235B, and for the decreases in B/R for 235A, 232C, and 235B. The former ratio usually decreases when irreversible photobleaching has occurred, while the occurrence of allomerization and/or pheophytinization will tend to increase B/R. If these processes occurred in dry benzene solutions, along with pick up of polar molecules, it is possible that the ratios would not be much changed. Consequently, detection of decomposition from change of ratios alone would be ambiguous.

As will be shown subsequently, the rate of irreversible photobleaching of chlorophyll in benzene solution containing dissolved oxygen is very much greater than the rates in polar solvents.[†] Consequently, brief exposure of benzene solutions to dim diffuse light resulted in detectable amounts of irreversible photobleaching as indicated in Table 18. Photodecomposition results primarily in products that are derived from oxidative break up of the chlorin ring. The per cent of photobleached products was estimated from the decrease of total amount of chlorin derivatives at the end of the test period. In the instances of runs 234C, 232D, and 234A, it seems likely that the high percentages of nonchlorin derivative products

[†]It can be seen, in Table 17, that chl a solutions in Ac, E, and py showed no decomposition. These solutions had dissolved oxygen in concentrations of the order of $10^{-3}M$, or greater. The amount of exposure to light was comparable to the benzene solutions.

may have resulted from dark oxidation reactions as well as photoactivation processes.

Comparison of tests 235A,[†] 232C, and 235B[†] in Table 18 shows that, in the absence of water, a large excess of oxygen did not significantly increase the degree of decomposition. Dry CO₂ has no apparent effect. This result is consistent with past work (for example, reference [2], pp. 451-453). The presence of dissolved oxygen and water together, as shown in 234C, resulted in a significant increase in amounts of al-
lomerized chlorophyll and products formed from the break up of the chlorin ring.

Tests 232D, 230C, and 234A show that when chlorophyll is in the dry amorphous state it is very rapidly decomposed. Pheophytin formation, but not break up of the chlorin ring, is greatly enhanced by CO₂ and water. These results are consistent with the findings of Zscheile and Comar [28].

A 1:1 mixture of chl a and β carotene in benzene was prepared under N₂ atmosphere and stored in the dark for 18 months. Neither the chlorophyll nor the β carotene showed any decomposition. Under the same conditions of preparation and storage chl a alone decomposed to the extent of 5 per cent in seven

[†]The benzene for the solutions in these runs was degassed under vacuum before use. During preparation of the solutions under controlled atmospheres, however, small amounts ($\leq 1.0 \times 10^{-6}$ M) of oxygen were absorbed in the benzene.

months, and β carotene alone underwent 80 per cent decomposition in 18 months.[†]

In pyridine solutions ph a, chl b, and ph b showed decomposition in relatively short time periods, while under the same conditions chl a, al chl a, and al ph a were stable. In a long storage period, however, chl a underwent a change in the 1 1/2 per cent py-pet. solution. In all instances the changes in the spectral parameters that were found were similar to those that occur in allomerization.

Allomerization of chl b gives a principal product, presumably with the expanded isocyclic ring structure, whose main red band peak is at 631 m μ and the main blue peak at 442 m μ with petroleum ether as solvent (reference [2], p. 1775). This corresponds closely to the observed wavelengths of the product of run 284A in benzene shown in Table 19. The parameters of run 290 were in close agreement with those of al ph a in pyridine as shown in the table. However, there were differences greater than would be expected from probable experimental error. The

[†]It seemed probable that the decomposition was oxidation. The source of oxygen was that chemisorbed on the β carotene crystals. Although the crystals were put under high vacuum, with a liquid nitrogen trap in the line, for 15 hours before preparing the solution under controlled atmosphere, the vacuum desiccator was not heated above room temperature. Consequently, a considerable amount of oxygen was probably still contained in the crystals.

product of run 290 was transferred to a wet benzene solution,[†] the spectrum measured, and the spectral parameters compared with those of al ph a in wet benzene. Again there was close agreement, but differences were found that were greater than could be accounted for by experimental error. An analysis, using the determinantal method, showed that unknown chlorin derivatives had been formed, but indicated that the major product was al ph a. These findings suggest that the main reaction in pyridine solutions is oxidative alteration of the cyclopentanone ring with the formation of two or more different products.

The benzene solution of 284A was treated with HCl solution to remove the magnesium atom from the chlorin ring. The acid was washed out with water. The spectrum of the resulting solution was compared to that of the product of 286 which had been transferred to a wet benzene solution. The spectral parameters were nearly identical. This showed that the same reactions for both chl b and ph b had occurred in pyridine.

Chl b in acetone proved to be quite stable. In a period

[†]The pyridine was removed by repeated washings with distilled water. Additional treatment of the resulting benzene solution with HCl, and then removal of the acid by washing with water, did not cause any change in the spectrum of the solution. This was good indication that washing with water had removed all the pyridine.

of two years it appeared that only a small amount of ph b was formed.

Photochemical Stability

In Table 20 are given the descriptions of representative systems studied in low light intensity photobleaching tests. Where no decomposition occurred it is stated in this Table. The runs to be intercompared are spaced together; these had the same conditions of illumination. The concentrations of all solutions were about $1.0 \times 10^{-5} M$.

The results of the low intensity photobleaching tests are given in Tables 21 and 22. Both the presence of water and β carotene inhibited decomposition of chl a significantly. The result with β carotene is consistent with the findings of H. Claes [53], and H. Claes and T. Nakayama [54]. β carotene was not observed to undergo any change on photoactivation, either alone or in solution with chl a.

Brief exposure of benzene solutions to normal atmospheres before testing, or use of glass stoppers that allowed exposure during testing, resulted in significant increases in the amount of decomposition for chl a and chl b. The increase of decomposition was due to the absorption of oxygen in solution. In CCl_4 solutions, comparing P_{23} and P_{24} , it appeared that the inhibiting effect of water outweighed the effect of increasing the oxygen content of the CCl_4 solution (P_{23}).

Under identical conditions of testing al ph a and ph a

Table 20

Systems Used in Low Light Intensity
Photobleaching Tests

System Run No.	Expose Time, Hours	Light Source ^a Filters	Remarks ^c
chl <u>a</u> , bz-D ^b 224A	2.5	F1-3	Teflon stopper.
chl <u>a</u> , bz-H ₂ O ^b 224B	2.5	F1-3	Teflon stopper.
chl <u>a</u> , bz-H ₂ O 231B	1	W-6 1-56	Glass stopper. Solution prepared under normal laboratory conditions.
chl <u>a</u> , bz-H ₂ O 231A	1	W-6 1-69 + 4-64	Glass stopper. Solution prepared under normal laboratory conditions.
chl <u>a</u> , cyclo-D ^b 220A	5	W-12	Teflon stopper.
chl <u>a</u> , cyclo-D ^b 220B	2	W-12	Teflon stopper. Solution was exposed briefly to laboratory air before the test.
chl <u>b</u> , bz-H ₂ O ^b 240A	1.17	W-6 1-57	Teflon stopper.

Table 20 (Contd.)

System Run No.	Expose Time, Hours	Light Source ^a Filters	Remarks ^c
chl b, bz-H ₂ O ^b 240B	1.17	W-6 1-57	Teflon stopper. Solution was exposed briefly to laboratory air before the test.
al chl <u>a</u> , bz-H ₂ O ^b 227B	1.25	F1-3	Glass stopper.
al ph <u>a</u> , bz-H ₂ O ^b 236C	45.5	F1-3	Glass stopper.
ph <u>a</u> , bz-H ₂ O ^b 239 ⁻	48.5	F1-3	Glass stopper.
chl <u>a</u> , bz-D ^{b,d} P25 ⁻	21.6	F1-24 2-59 + 1-69	Teflon stopper.
chl <u>a</u> + β C, bz-D ^b P26, ⁻ See Remarks	21.6	F1-24 2-59 + 1-69	Teflon stopper. This solution was a 1:1 mixture chl <u>a</u> and β -carotene in dry benzene.
β -carotene, bz, C.P. P27, See Remarks	12.9	F1-24	Glass stopper. The solution was prepared in normal laboratory air with C.P. benzene. No decomposition occurred.

Table 20 (Contd.)

System Run No.	Expose Time, Hours	Light Source ^a Filters	Remarks ^c
chl <u>a</u> , CCl ₄ -H ₂ O ^{b,d} P23, See Remarks	3	F1-24 Water	Teflon stopper. Distilled water was added to the solution in laboratory air before the test.
chl <u>a</u> , CCl ₄ -D ^{b,d} P24	3	F1-24 Water	Teflon stopper.

^aThe abbreviations used are F1 = 15 watt fluorescent "Daylight" lamp, and W = 100 watt tungsten lamp. The number after the hyphen designates the distance in inches of the sample from the source.

^bThe solution was prepared under N₂ atmosphere, and contained traces of O₂.

^cThe stopper designation refers to the type used in the absorption cell. Use of glass stoppers allowed leakage of O₂, CO₂, and H₂O into the cell during testing, while the teflon stopper did not.

^dThe initial energy absorbed is shown in Table 15, and the apparent quantum yields in Table 25.

Table 21

Photobleaching Behavior at Low Light Intensities,
chl a, chl b, al chl a, al ph a, and ph a

System Run No.	% Decrease of d per hour			B/R ^c	B/505 ^c	R/min. ^{c,d}
	R	O	B			
Control, ^{a,e} bz-D				1.42	64.7	78
chl <u>a</u> , bz-D 224A	16.9	10.9	15.6	1.50	6.6	4.4
Control, bz-H ₂ O ^e				1.278	83.2	156
chl <u>a</u> , bz-H ₂ O 224B	9.5	0.0	9.0	1.30	15.8	14.0
Control, bz-H ₂ O				1.26	55.7	107
chl <u>a</u> , bz-H ₂ O 231B	64.7	34.4	56.6	1.57	4.7	2.1
chl <u>a</u> , bz-H ₂ O 231A	28.8	14.1	25.1	1.34	13.5	8.1
Control, cyclo-D				1.49	42.8	54
chl <u>a</u> , cyclo-D 220A	3.9	3.4	4.0	1.48	14.4	10.0

Table 21 (Contd.)

System Run No.	% Decrease of d per hour			B/R ^c	B/505 ^c	R/min. ^{c,d}
	R	O	B			
chl a, cyclo-D 220B ⁻⁻⁻	30.6	8.1	26.8	1.55	3.1	2.2
Control, bz-H ₂ O ^e				2.67		20.4
chl b, bz-H ₂ O 240A ⁻⁻⁻	48.5	— ^f	54.5	2.33		2.0
chl b, bz-H ₂ O 240B ⁻⁻⁻	74.6 ^g					
Control, bz-H ₂ O				2.13		44.8
al chl <u>a</u> , bz-H ₂ O 227B	61.0	46.0	53.0	3.0		2.5
Control, bz-H ₂ O				2.87		16
al ph <u>a</u> , bz-H ₂ O 236C	0.24	0.14	0.18	2.94		11.1
Control, bz-H ₂ O				2.03		19.2

Table 21 (Contd.)

System Run No.	% Decrease of d per hour			B/R ^c	B/505 ^c	R/min. ^{c,d}
	R	O	B			
ph <u>a</u> , bz-H ₂ O	0.28	0.21	0.26	2.06		16.0

^aThis refers to solutions of the pure, intact compounds.

^bThis gives the percentage decrease of the peak optical densities for the main red, orange, and blue bands respectively.

^cThe values of the ratios are those at the end of the entire test period.

^dThe minimum d value was determined at the wavelength where the minimum of the intact material occurs.

^eThese particular control solutions were more nearly free of dissolved O₂ and CO₂ than any others prepared.

^fA product was formed that had a band maximum at 545 mμ; this obscured the intensity change in the orange band.

^gOnly the red band could be identified with reasonable certainty.

Table 22

Photobleaching Behavior at Low Light Intensities, chl a

System Run No.	% Decrease chl <u>a</u> per hour	% Total Chlorin Derivatives Formed ^a	% Other Products ^e
chl <u>a</u> , bz-D P25	0.83 ^b 0.75 ^c	42 ± 7 ^d	58
chl <u>a</u> + βC, bz-D P26	0.31 ^c		
chl <u>a</u> , CCl ₄ -H ₂ O P23	15.0 ^b	17 ± 9	83
chl <u>a</u> , CCl ₄ -D P24	28.6 ^b	28 ± 13	72

^aThis represents the per cent of al chl a, and al ph a formed from the total amount of chl a that decomposed.

^bThis was determined from analysis by determinants.

^cThis value was obtained from the decrease of the main red band.

^dThe variations in total per cent known derivatives indicate that unknown products absorbing in the visible were present.

^eThis refers to decomposition products weakly absorbing, or not absorbing in the visible.

showed about a 230-fold lesser rate of photodecomposition than al chl a. Comparing chl a of run 224B (where only trace amounts of O₂ were present) to al ph a of run 236C, or to ph a of run 239 (where continuous exposure to oxygen during testing could occur) showed that the latter two compounds were essentially resistant to decomposition under conditions that would have led to complete irreversible photobleaching of chl a.

As shown in Table 21 photodecomposition was accompanied by a significant decrease in the ratio R/min for all compounds. This resulted both from decreases in the optical densities of the red bands and from increases in the optical densities of the minima. Photodecomposition of chl a in benzene and in cyclohexane that had been exposed to normal atmospheres, and of chl b in wet benzene resulted in significantly larger decreases of the main red and blue bands than in the orange bands. This phenomenon and the increases in the region of the minima clearly indicate that products absorbing in the visible were formed. In the case of chl a, as shown in Table 21, some of the products were identified as intact chlorin ring compounds. However, such compounds usually derived from the chlorophylls do not have their main bands in the orange, yellow, and the blue-green regions. On the other hand, phycobilin type derivatives can absorb strongly in these regions, usually with one main band (reference [2], pp. 666-667, p. 1789). This type of compound could result from the oxidative rupture of one of the methine

bridges of the chlorin ring. A conjugated open chain of substituted pyrrole nuclei linked by $=CH-$ bridges would be formed. The presence of small amounts of such compounds could account for the relatively smaller absorption changes in the region between the main red and blue bands.

In some instances it was found that photobleaching resulted in a product (or products) that gave weak absorption at 700 $m\mu$ and longer wavelengths. While this might have arisen from a phycobilin type compound, it is more likely that it was due to a chlorin derivative. A.S. Holt [25] found that one of the intact ring chlorin products (i.e. fraction 1), formed from chl a under certain conditions of allovermerization, had a main red band whose absorption extended to wavelengths longer than 720 $m\mu$. The isocyclic ring, which had been oxidized, was no longer intact. The formation of products of this type could account for the observed absorption in the far red region.

The major percentage of products formed in photobleaching did not absorb in the visible. These must have been derived from extensive breakup of the chlorin ring with formation of small molecules having a relatively small degree of conjugation. The process of oxidative breakup probably begins with rupture at one methine bridge,[†] resulting in the formation of a phycobilin type compound. It is reported that usually these

[†]On the basis of the recent work of R.B. Woodward and V. Skaric [55], the bridge most likely to be attacked would be the 8 methine bridge.

compounds are quite easily decomposed by light and oxygen (for example, reference [2], p. 522). Where they are formed photochemically from the chlorins they would probably, in most instances, undergo extensive decomposition themselves, with the formation of products not absorbing in the visible. It is for this reason that only small amounts of phycobilin type derivatives[†] were usually manifested.

In Table 23 are given the descriptions of representative systems studied in the high light intensity photoactivation tests. The concentrations of all solutions were about $1 \times 10^{-5} M$. All solutions were prepared under identical conditions of exposure to laboratory atmosphere, and low intensity green light. The solubilities of oxygen, carbon dioxide, and water, in all of the solvents used, are such that their concentrations were probably greater than that of the chlorophyll (or derivative) in each case. As a consequence of the standardization in procedures, intercomparison of the various tests will have significance in regards to the effect of solvent and substituent changes on kinetic behavior. The uncertainties in per cent change of band peak optical densities were estimated from the experimental accuracy in measurement of d in various regions of the spectrum. This depended on the inherent accuracy of the instrument and on the variations in the amounts of

[†]The chlorin derivatives, in contrast to the phycobilins, have multiple band absorption throughout the range $\sim 700 m\mu$ to $\sim 350 m\mu$.

Table 23

Systems Used in High Light Intensity Photobleaching Tests

System, Run No. ^a	Remarks ^b
chl <u>a</u> , bz, P1 chl <u>a</u> , bz sat'd with CO ₂ , P2 chl <u>a</u> , bz sat'd with CO ₂ , P3	The compositions of the solutions were obtained by the determinantal method. The average uncertainty in the percentage of known compounds is $\pm 4\%$.
chl <u>a</u> , E, P4	Average uncertainty in change of d is $\pm 0.8\%$. The primary photochemical change was probably destruction of the chlorin ring. Only small amounts of products were formed that absorbed in the visible.
chl <u>a</u> , py, P5 ^c	Average uncertainty in change of d is $\pm 0.8\%$. Destruction of the chlorin ring occurred. As evidenced by the unequal decreases in the bands, products absorbing in the visible were formed.
al chl <u>a</u> , py, P6 ^c	Average uncertainty in change of d is $\pm 1.9\%$. Destruction of the chlorin ring occurred. The unequal decreases in the peaks showed that products absorbing in the visible were formed.
ph <u>a</u> , E	No photodecomposition occurred in an illumination period of 65 minutes.

Table 23 (Contd.)

System Run No. ^a	Remarks ^b
ph <u>a</u> , py, P8 ^c	Average uncertainty in change of d is $\pm 2.4\%$. Extensive dark reaction, almost to completion, had occurred before the photoactivation tests. Destruction of the chlorin ring was the primary reaction. Some of the products formed absorbed in the visible.
ph <u>a</u> , EHCl, P9	Average uncertainty in change of d is $\pm 1.8\%$. Changes in the initial red band, the final blue band, the initial blue band, and initial blue satellite band are reported, respectively. Photodecomposition resulted primarily in a product absorbing strongly at 433.9 m μ . This derivative was quite stable against photodecomposition.
ph <u>a</u> , EHCl H ₂ O, P10	Average uncertainty in change of d is $\pm 2.4\%$. Same remarks as for P9. The main product absorbed strongly at 435 m μ .
al ph <u>a</u> , E, P11	Average uncertainty in change of d is $\pm 1.3\%$.
al ph <u>a</u> , py, P12 ^c	Average uncertainty in change of d is $\pm 2.9\%$. Destruction of the chlorin ring occurred. The unequal decreases in the peaks showed that products absorbing in the visible were formed.

Table 23 (Contd.)

System Run No. ^a	Remarks ^b
al ph <u>a</u> , EHCl, P13	Average uncertainty in change of d is $\pm 2.5\%$. A dark reaction of significant rate occurred during the test period. This reaction occurred only after photoactivation, and appeared to involve primarily decomposition of the photoformed product absorbing in the visible.
chl <u>b</u> , E, P21	Average uncertainty in change of d is $\pm 1.2\%$. A dark reaction occurred during the test period and only after photoactivation. It appeared to involve primarily the decomposition of the photoformed product absorbing in the visible.
chl <u>b</u> , py, P15 ^c	Average uncertainty in change of d is $\pm 1.2\%$. Destruction of the chlorin ring occurred. Products absorbing in the visible were formed; one of these may have been an intact chlorin ring derivative.
ph <u>b</u> , E, P22	Average uncertainty in change of d is $\pm 0.7\%$. A very slow dark reaction occurred after photoactivation, but not before.
ph <u>b</u> , py, P17 ^c	Average uncertainty in change of d is $\pm 2.7\%$. An extensive dark reaction nearly to completion, had occurred prior to photoactivation. As evidenced by the unequal decreases in the bands products absorbing in the visible were formed. A dark reaction occurred during testing.

Table 23 (Contd.)

System Run No. ^a	Remarks ^b
ph <u>b</u> , EHCl, P18	Average uncertainty in change of d is $\pm 3.0\%$. Changes in the initial red band, the final orange band, the initial blue band, and the final blue band are reported, respectively. A rather rapid dark reaction, initiated by photoactivation, occurred during the test period. In the first 30 minutes of photoactivation, the primary process was formation of a product absorbing strongly at 442.5 m μ . Subsequently the process was primarily photodecomposition of this new product.
ph <u>b</u> , EHCl H ₂ O, P19	Average uncertainty in change of d is $\pm 3.0\%$. Same remarks as for P18. The main product from photobleaching absorbed at about 445.0 m μ .

^aTeflon stoppers were used in the reaction cells unless noted otherwise. In all high light intensity runs, a Corning 1-56 filter was used. In runs P1 and P3 a Corning 2-64 filter was used in addition. In run P2, a Corning 5-60 filter was used in addition.

^bNo significant dark reactions occurred in the test periods unless noted otherwise.

^cA glass stopper was used in the reaction cell.

products formed that absorbed in the visible. Estimates of the latter were made from observations on the variations in change of absorbance at the far red side of the main red band (where the chlorophyll or derivative did not originally absorb), or in the region of the absolute minimum of the initial spectrum. Uncertainties were greatest for intensity changes in the bands near which photoformed products absorbed strongly. These were usually for changes in the orange, yellow, and green bands. The average uncertainties reported are about one half as great as the largest uncertainty, and twice as great as the smallest uncertainty.

In Table 24 are given the results of chl a in benzene solutions, while in Table 25 are given the results of the chlorophylls and several of their derivatives in solutions other than benzene. The rates of photodecomposition, which in Table 25 are given by per cent decreases of the peak optical densities of the main red bands, are averages for all the periods of photoactivation for each run. To allow for the decrease of pigment due to the photodecomposition of preceding periods, the rates, for periods following the first, were calculated with photoactivation times decreased in proportion to the amount of the pigment present at the beginning of the particular period. With this procedure, the rates for different periods showed deviations ranging from ± 9 per cent to ± 32 per cent of the average values tabulated, for those tests in which there were no significant concurrent dark reactions. The average of the

Table 24

Photobleaching Behavior at High Light
Intensities. Benzene Solutions

System Run No.	Total Time Illuminated, min.	% Present After Illumination			Other ^a Products	Average % Decrease chl <u>a</u> Per Minute
		chl <u>a</u>	al chl <u>a</u>	al ph <u>a</u>		
chl <u>a</u> , bz P1	5	62.0	11.0	4.8	22.2	10.6
	10	41.3	15.9	9.5	33.3	
	20	25.4	19.0	11.0	44.6	
	30	15.9	20.6	12.8	50.7	
chl <u>a</u> , bz P2	5	57.5	10.6	7.6	24.3	11.1
	10	38.0	21.2	10.6	30.2	
	20	18.2	19.7	18.2	43.9	
chl <u>a</u> , bz P3	5	66.0	11.4	10.2	12.4	8.0
	15	34.1	10.2	11.4	44.3	
	25	22.7	10.2	14.8	52.3	

^aThis refers to products not absorbing, or only weakly absorbing, in the visible that result from photochemical destruction of the chlorin ring.

Table 25

Photobleaching Behavior at High Light Intensities.
Solvents Different than Benzene

System ^a Run No.	Total Time Illuminated Min.	% Decrease d_R	Peak Optical Densities ^b d_0	d_B	d_{BS}	$d()$	Average % Decrease in d_R Per Min.
chl <u>a</u> , E	5	8.2	7.2	8.7	7.5		1.6
P4	10	16.7	12.5	17.2	15.9		
	15	21.5	16.4	21.7	20.3		
P4'	15	21.2	17.1	21.9	19.6		
Chl <u>a</u> , py	5	8.8	5.7	9.1	4.5		2.9
P5	10	25.6	15.0	24.6	14.4		
	15	37.4	25.4	35.6	23.0		
al chl <u>a</u> , py	5	8.7	1.5	8.1	4.7		1.3
P6	10	13.6	7.7	12.2	9.1		
	20	23.1	13.8	21.3	17.2		
	30	31.1	20.0	28.0	20.4		
ph <u>a</u> , py						(G ₁)	
P8	5	1.7	2.4	1.4	-1.2		0.27
	15	4.6	6.0	3.1	2.3		
	35	7.5	9.6	5.3	8.1		
	65	15.1	18.1	9.1	10.5		
P8'	65	18.8	26.5	10.4	17.4		

Table 25 (Contd.)

System ^a Run No.	Total Time Illuminated Min.	% Decrease d_R	Peak Optical Densities ^b d_0	d_B	d_{BS}	$d_{()}$	Average % Decrease in d_R Per Min.
ph <u>a</u> , EHC1 P9		(663.9)	(433.9)	(423.5)	(397.5)		1.5
	5	5.7	2.0	2.9	2.0		
	15	16.8	-1.0	6.4	6.4		
	35	35.8	-15.6	13.8	12.7		
	65	69.2	-30.5	30.8	31.7		
P9 :	65	71.4	-29.5	30.8	32.2		
	95	86.0	-29.1	38.5	43.9		
ph <u>a</u> , EHC1 H ₂ O P10		(666.0)	(435.0)	(415.0)	(395.0)		
	5	7.9	-12.9	5.3	4.9		2.8
	15	40.0	-81.9	21.7	23.1		
	25	59.3	-102	30.9	34.7		
al ph <u>a</u> , E P11	65	3.1		1.5			0.048
al ph <u>a</u> , py P12					(G ₁)	(G ₂)	
	5	1.7	0	2.1	0	6.1	0.25
	15	4.0	0	3.8	0	10.2	
	35	7.4	0	6.5	0	14.3	
al ph <u>a</u> , EHC1 P13	5	11.0	0	8.3			1.2
	10	14.5	3.1	11.4			
P13'	10	18.6	25.0	14.4			
	30	28.3	21.9	22.4			

Table 25 (Contd.)

System ^a Run No.	Total Time Illuminated Min.	% Decrease Peak Optical Densities ^b				d()	Average % Decrease in d _R Per Min.
		d _R	d _O	d _B	d _{BS}		
chl <u>b</u> , E	5	5.0	0	6.1	6.1		1.1
P21	10	11.0	0	11.7	11.3		
	15	16.0	-1.1	17.5	16.3		
P21'	15	16.2	8.9	17.1	15.9		
	26	26.6	15.6	27.2	25.4		
chl <u>b</u> , py						(G ₁)	
P15	5	4.7	0	11.1	4.3	0	1.9
	10	15.7	3.8	27.4	9.1	0	
	15	25.3	8.7	39.2	14.2	0	
ph <u>b</u> , E	50	0.7		0.8			0.014
P22							
ph <u>b</u> , py						(G)	
P17	5	0	0	1.3	0.6	-3.6	0.08
	15	0.9	1.2	2.7	3.3	-2.5	
	35	2.9	1.2	3.8	4.5	-2.4	
	65	7.2	2.0	5.8	4.5	1.2	
ph <u>b</u> , EHC1		(652.5)	(582.5)	(432.5)	(442.5)	(412.5)	
P18	5	11.7	-9.4	5.5	-4.7	0	3.2
	10	29.9	-30.1	12.6	-12.1	9.4	
	15	48.7	-49.0	20.7	-20.0	13.8	
	20	68.0	-60.1	31.0	-23.7	21.8	
	30	82.0	2.4	41.7	6.1	31.7	

Table 25 (Contd.)

System ^a Run No.	Total Time Illuminated Min.	% Decrease d_R	Peak d_O	Optical d_B	Densities ^b d_{BS}	$d()$	Average % Decrease in d_R Per Min.
P18'	30	83.8	9.4	43.9	12.5	34.0	
	40	83.8	16.5	49.3	23.5	40.5	
	50	86.3	34.1	53.6	32.5	44.2	
ph <u>b</u> , EHC1 H ₂ O		(653.9)	(598.0)	(432.7)	(445.0)	(412.0)	
P19	5	2.7	0	0.7	0.7	0.8	0.82
	15	4.6	0	1.6	0	1.2	
	35	19.8	3.5	6.9	-6.0	4.1	
P19'	35	27.0	10.5	14.0	0	9.8	
	65	61.2	21.0	33.8	6.5	23.3	

^aA prime superscript in the run designation signifies that, after a period of about 10 hours in the dark at 20°C, the spectrum of the solution was again measured.

^bA minus sign for the recorded % change means an increase of the band peak optical density. Numbers or symbols given in parentheses refer to band position wavelength or band at which the change in optical density was determined.

deviations for these runs was ± 21 per cent. The deviations for tests, where rather rapid dark reactions were occurring (P13, P17, P18, and P19) were significantly greater. The average was ± 50 per cent.

In Table 26 are given the apparent quantum yields in the initial periods of photobleaching. The absolute uncertainty in the values may be as great as ± 50 per cent. The relative uncertainty for intercomparison of different tests is estimated to be ± 15 per cent or less. The quantum yields of the chlorophylls reported in Table 26 appear to be from 1.25- to 38-fold greater than those reported in the literature (for example, reference [2], pp. 497, 1494). The major discrepancy is for the non-polar CCl_4 solutions. In CCl_4 the presence of polar impurities such as water results in significant decreases in quantum yield. There is agreement with past work that quantum yields in polar solvents are significantly less than in nonpolar solvents.

Comparison of the tests in Table 24 shows the following points of interest. Within experimental error, the rate of photodecomposition of chl a was the same for excitation in the blue (P_2) and in the red (P_1 and P_3) absorption regions. As shown in Table 26 the initial quantum yields were identical. A large excess of CO_2 in benzene appeared to have no effect on the photochemical changes. Destruction of the chlorin ring was the main photochemical reaction. Significant amounts of al chl a were also formed. It appeared that this compound

Table 26

Apparent Quantum Yield in the Initial Period of Photoconversion

System, Run No. ^a	Average Number of Quanta Absorbed Per Second in the First 5 Minute Photoactiva- tion Period $\times 10^{-16}$	Total No. Molecules Converted
		Average Total No. Quanta Absorbed $\times 10^3$
chl <u>a</u> , CCl ₄ -H ₂ O, P23		3.2
chl <u>a</u> , CCl ₄ -D, P24		9.6
chl <u>a</u> , bz-D, P25		2.0
chl <u>a</u> , β Car., bz-D, P26		0.91
chl <u>a</u> , bz, P1	1.0	1.9
chl <u>a</u> , bz., Sat'd CO ₂ , P2	1.1	2.0
chl <u>a</u> , bz., Sat'd CO ₂ , P3	1.2	2.0
chl <u>a</u> , E, P4	12.1	0.062
chl <u>a</u> , py, P5	13.2	0.062
al chl <u>a</u> , py, P6	6.6	0.063
ph <u>a</u> , E	9.6	0.000
ph <u>a</u> , py, P8	8.0	0.017
ph <u>a</u> , EHC1, P9	8.9	0.052
ph <u>a</u> , EHC1 H ₂ O, P10	4.1	0.16
al ph <u>a</u> , E, P11	4.7	0.000
al ph <u>a</u> , py, P12	5.7	0.013

Table 26 (Contd.)

System, Run No. ^a	Average Number of Quanta Absorbed Per Second in the First 5 Minute Photoactiva- tion Period $\times 10^{-16}$	Total No. Molecules Converted
		Average Total No. Quanta Absorbed $\times 10^3$
al ph <u>a</u> , EHC1, P13	4.6	0.11
chl <u>b</u> , E, P21	12.5	0.030
chl <u>b</u> , py, P15	14.7	0.026
ph <u>b</u> , E, P22	9.8	0.000
ph <u>b</u> , py, P17	9.7	0.011
ph <u>b</u> , EHC1, P18	9.4	0.10
ph <u>b</u> , EHC1 H ₂ O, P19	9.4	0.023

^aThe first four runs tabulated, namely P23, P24, P25, and P26 were low light intensity tests. The apparent quantum yields are for time periods of 180 minutes for the first two runs, and 190 minutes for the latter two runs.

underwent a partial decomposition to al ph a. No ph a appeared to be formed in the testing periods.

Intercomparison of the results in Tables 24 and 25 shows the following relationships between the rates. For comparison between runs the rates are changed by factors that make the initial number of quanta absorbed for each system the same. The adjusted average rates are given in parentheses after the abbreviated designation of the system:

$$\begin{aligned} \text{Chl } \underline{a}, \text{ bz}(10.6) &>> \text{chl } \underline{a}, \text{ py}(0.22) \approx \text{al chl } \underline{a}, \\ \text{py}(0.20) &> \text{chl } \underline{a}, \text{ E}(0.13) \end{aligned}$$

The more polar solvents clearly provided for greater photostability of chl a. This is consistent with the results on the relative chemical stability of chl a in polar solvents. In the chl a, E system there appeared to be considerably less amounts of products formed that absorbed in the visible. For chl a, py and al chl a, py the blue satellite bands decreased significantly less than the blue and the red bands, and the orange bands decreased significantly less than the red bands. The former type of behavior, which has also been observed in past work (for example, reference [2], pp. 497-98), might be expected where allomerization and/or pheophytinization had occurred photochemically. For both chl a and al chl a the red half widths increased with photobleaching, which is good evidence that mixtures of chlorin derivatives were being formed. The predominant direction of increase was toward the red; this is what

would be expected for the formation of al ph a. There is evidence from past work that pheophytinization can be promoted by photoactivation (for example, reference [2], p. 493). The relatively small decreases of the orange bands could have been due to formation of phycobilin type compounds absorbing in that region. This behavior is similar to that observed for the low light intensity tests.

Salt formation of ph a greatly increased the rate of photodecomposition, making it comparable to that of chl a in ether, ph a, EHCl(2.0) $\approx$ chl a, E(1.6). The ph a salt gave a photodecomposition product that absorbed strongly, with apparently only one main band, in the blue-green region. As suggested for chl a and chl b this may have been a phycobilin type compound. While ph a in pyridine photobleached more rapidly than in ether, the rate was considerably less than that of chl a or al chl a in pyridine, chl a, py(2.9) $\approx$ al chl a, py(2.6) > ph a, py(0.44). Except in the final photobleaching period, all bands of ph a in pyridien decreased the same amount within experimental error. If a phycobilin type derivatives was formed, it underwent rapid decomposition to give products that did not absorb in the visible.

Salt formation of al ph a resulted in an increased rate of photobleaching like ph a, however less amounts of product absorbing in the visible were formed. The product absorbed primarily in the orange and yellow regions. If it was a phycobilin type derivative, it was clearly different than the one formed from ph a. The decomposition rates of al ph a and ph a in both EHCl and pyridine were comparable, al ph a, EHCl(1.2) $\approx$ ph a, EHCl(0.77), al ph a, py(0.25) $\approx$ ph a, py(0.19). Photodecomposition of al ph a in pyridine, in contrast to ph a, resulted in formation of small amounts of a product absorbing in the orange and yellow regions. This product may have been similar to the one formed from al ph a in the EHCl solvent.

Chl a, py(2.7) > chl a, E(1.6) $\approx$ chl b,

py(1.56) > chl b, E(1.07)

Photodecomposition of chl b in ether gave rise to significant amounts of a product absorbing in the orange region, in contrast to chl a in this solvent. The product may have been a phycobilin type compound similar to that formed from chl b in benzene solutions. If a similar product was formed from chl a in ether, it was clearly less stable than that from chl b. It is to be noted that the product formed from chl b underwent fairly rapid decomposition in the dark. In pyridine, products absorbing in the visible were formed from both chl a and chl b. Chl b in py showed behavior like chl a. The blue satellite band peak decreased less than the blue band peak, the orange band decreased

less than the red band, and the increase of the red band half width was predominantly toward the red. This behavior, similar to that for chl a, indicated that allomerized pheophytin b and a phycobilin type of derivative might have been formed photochemically.

Salt formation of ph b, similar to ph a and al ph a, resulted in an increased rate of photodecomposition that was comparable to chl b, chl b, E(0.83) $\approx$ ph b, EHClH₂O(0.82). The ph b salt, like the ph a salt, gave rise to a product with one main band absorbing strongly in the blue green region. The effect was greatest in the EHCl solvent. The product formed from the ph b salt was apparently much less stable photochemically and chemically than the one formed from the ph a salt. All the pheophytin salts had roughly the same rates, ph b, EHCl(1.6) > al ph a, EHCl(1.2) > ph a, EHCl(0.77). The increase in rate of photodecomposition of ph b in pyridine over ether was relatively less than the increase for ph a, ph a, py(0.27) > ph b, py(0.066) > ph b, E(0.011) > ph a, E(0.00). The rate of ph b relative to chl b in pyridine was significantly smaller than for the corresponding a compounds, chl b, py(1.25) > ph b, py(0.03). Photodecomposition of ph b, in pyridine, like al ph a

gave rise to a product absorbing in the orange, yellow, and green regions that, similarly, may have been a phycobilin type derivative.

SUMMARY

The results, which were reproducible and found to be internally consistent, are in general agreement with past work (for example, reference [2], pp. 400, 495). They have shown that, for maintaining intact pigments, it is desirable to exclude dissolved oxygen[†] and to use dim, diffuse green light (that is not strongly absorbed) when preparing and working with solutions of the chlorophylls and their intact ring derivatives. This is particularly true for the magnesium containing derivatives in non-polar solvents, and for solutions of the pheophytin salts. Allowing the pigments to become dry, in the amorphous form, results in rapid degradation even under essentially inert atmospheres.

Polar solvents provide for greater photochemical stability against oxidative processes for the chlorophylls than inert solvents such as benzene, carbon tetrachloride, and cyclohexane. With the exception of basic organic solvents, polar solvents also provide for greater chemical stability. It seems likely that the protective action arises from specific solvating

[†]There is evidence in past work (for example, reference [2], pp. 486, 501) that, when there is no dissolved oxygen, and if ultraviolet and violet light are excluded, chlorophyll does not undergo photodecomposition in acetone and methanol solutions.

interactions. Such interactions could be effective either through displacement of specifically associated oxygen, or steric hinderance of the interaction of oxygen, or through electronic inductive effects that increased, the potential barrier in the first step in an oxidative attack. In the latter case specific interactions at the magnesium atom and the cyclopentanone ring would be expected to be of greatest importance. In the case of the inert solvents, such as benzene or CCl_4 , specific interactions at these sites would be unlikely, since their interactions would be limited largely to dispersion interactions, and in addition, for benzene, weak π -electron charge transfer interactions.

In either inert or pclar solvents the apparent quantum yield of irreversible photobleaching is quite small. Several types of mechanisms have been put forward to explain this (for example, reference [2], pp. 499, 501, 1493). In the first type of mechanism, it is postulated that electronic excitation of chlorophyll leads essentially to the formation of a long-lived activated state of the chlorophyll. The chlorophyll in this state reacts with molecular oxygen in an almost completely reversible manner, yielding only small amounts of irreversibly oxidized chlorophyll. In the second instance, it is suggested essentially that excitation of the chlorophyll results primarily in a photosensitized oxidation of solvent molecules. In view of the facts that rather extensive photodecomposition occurred when relatively small amounts of dissolved oxygen was present,

and that the rate of irreversible photobleaching reaches a maximum at relatively small concentrations ($\sim 1 \times 10^{-5} \text{M}$) of dissolved oxygen (reference [2], p. 1493), mechanisms of the first type seem more plausible.

The use of pyridine as a solvent (containing traces of water and dissolved oxygen) resulted in rather rapid chemical reaction of chl b, ph a, and ph b. Chl a, in the presence of pyridine, reacted much more slowly. The reactions in every case appeared to involve primarily oxidative alteration of the cyclopentanone ring. Al chl a and al ph a were stable in pyridine. These results are in agreement with past work on the effect of basic organic solvents (for example, reference [2], pp. 1780-1781, 1799-1801, 1804). It has been suggested that the primary reaction, with primary or secondary amines, is cleavage of the cyclopentanone ring between the 9 and 10 carbon atoms with a hydrogen atom being added in the 10 position and the amide residue in the 9 position (reference [2], pp. 1780-1781).

Comparing the change of photochemical stabilities in going from ether to pyridine, the pheophytins showed greater increases (~ 5 - fold) in rates of decomposition than the chlorophylls (~ 1.5 - fold). The absolute rates of the pheophytins, however, were significantly smaller than those of the chlorophylls and al chl a. Past work has shown that pyridine forms solvates with the chlorophylls, the allomerized chlorophylls, and the pheophytins (reference [2], p. 1803). The fact that the

solvating interactions had greater perturbing effects on the pheophytins indicates that the mode of solvation was different for the pheophytins than for the compounds with the central magnesium atom. It has been suggested for the metallo-porphyrin derivatives that the pyridine, acting as an electron donor, interacts with the vacant orbitals of the central metal atom [56]. Such an interaction would not be possible for the pheophytins.

The pheophytins in all solvents were much more photochemically stable than the chlorophylls and al chl a. When converted to the salt form, with addition of protons to the center of the ring, their photochemical stability decreased to that of the chlorophylls. Salt formation, however, did not appear to decrease chemical stability in contrast to complexation with pyridine. The marked differences in behavior of the magnesium-containing compounds and the pheophytin salts on the one hand, and the free pheophytin bases on the other hand are clearly related to the differences in perturbing effects of the substituents of the center of the chlorin ring. The differences in the perturbing effects of pyridine on ph a and ph b compared to that of salt formation, suggests that the former effect was relatively greater on the ground electronic state, while the latter effect was relatively greater on the excited state.

Alteration of the substituent pattern of the isocyclic ring has significantly less effect on photochemical behavior. Thus, chl a and al chl a decomposed at about the same rates in

pyridine, and the rates of al ph a and ph a were comparable in that solvent. The corresponding salts underwent about the same rate of photodecomposition in ether.

Three types of decomposition products were encountered. The first type were intact chlorin ring compounds that absorbed strongly in the red and blue regions, characteristically of the chlorins. These compounds appeared to be mostly allomerized, or allomerized pheophytin derivatives. The second type of product absorbed strongly, with apparently one main band, in the orange, yellow, or blue-green regions where the usual derivatives of the chlorophylls have bands of relatively low intensity. It was suggested that these products may have been phycobilin type compounds, which can have fairly extensive conjugated double bond systems and are known to absorb in the visible. Compounds of this type, which are themselves chemically and photochemically unstable, could be formed by the oxidative rupture of a bond at one of the methine bridges of the intact chlorin ring. The third type of product did not absorb at all, or at least only very weakly, in the visible. These products must have had relatively short conjugated double bond systems. They would be formed by extensive oxidative break up of the chlorin ring, probably starting from a phycobilin type derivative.

The chemical stability of the ph a and ph b salts in ethyl ether in contrast to the fairly rapid dark decomposition in pyridine bears some parallel to the stability properties of

the pheophytin monolayers. On low pH substrates both ph a and ph b monolayers displayed significantly greater chemical stability than on high pH substrates. At high pH's or in pyridine solutions, fairly rapid decomposition occurred that appeared to involve primarily oxidative alteration of the cyclopentanone ring.

APPENDIX B

VISIBLE ABSORPTION SPECTRA OF THE CHLOROPHYLLS AND SEVERAL DERIVATIVES IN VARIOUS SOLVENTS

INTRODUCTION

Some of the results of the spectroscopic measurements have been discussed above in connection with characterization of the compounds and evaluation of their absolute purity. Since it was found that the samples of this work were of high purity, it was of interest to determine their spectra in a number of common solvents. The data obtained are useful for characterization of the compounds in different solvents, and provide information of value for the eventual detailed interpretation of the spectra. While the data are limited in scope, they give insight into the problems that appear to exist in the present theoretical interpretation, and reveal interesting variations in solvent effects for the different compounds.

It has been suggested (reference [2], p. 646) that determination of the integrated oscillator (f) and dipole (D) strengths would be of value in characterizing band intensity changes occurring with variation of solvent. The integrated intensities are also of value in characterizing substituent effects particularly for the low intensity bands where band half-widths can not be determined.[†] Consequently these values were

[†]As a rough approximation f values can be calculated from band half-widths and molar extinction coefficients [26].

obtained for a number of the systems studied. It does not appear that these quantities have been determined before for most of the systems studied in this work.

In recent years J.R. Platt [57] and later M. Gouterman [58], [59], [60] have put forward interpretations of the porphyrin spectra which have had good qualitative success with the more simply substituted compounds. The chlorophylls and their intact ring derivatives (which are actually chlorins[†]) represent very complexly substituted porphyrins and continue to present difficulties in the detailed interpretation of their spectra. Problems may reside not only in the possibility that the substituent effects are not independent of each other, but also in the possibility that transitions polarized along different axes in the chlorin ring are not independent of each other [58].

The essential features of the interpretations are as follows. Because the porphyrin ring is planar, with the conjugated π -electron system extended in two dimensions, electronic transitions can occur along two different, mutually perpendicular axes in the plane of the ring. Where substitution patterns are identical along each axis, the transitions are degenerate. Differences in substituent pattern remove the degeneracy. The absorption bands extending from the red through the green region

[†]Reduction in one of the pyrrole rings (i.e. hydrogenation of the 7-8 double bond) is treated as a substituent perturbation [58].

of the spectrum are assigned to two nearly degenerate electronic transitions, along the two different axes in the molecule, that are weak in intensity because of the large change in electronic angular momentum on transition. The occurrence of more than two bands is ascribed to the fact that vibrational sub-bands can be associated with each transition. Four distinct bands are usually found in the spectra of the porphyrins with simple substituent patterns. Change of substituent pattern will result in variations in band intensities, and changes in the relative positions of the bands. Some specific predictions are mentioned subsequently and compared with experimental findings. Because of the near degeneracy of the transitions and the presence of vibrational sub-bands, it is suggested that rather complicated interdigitation of the bands can occur with some loss of individual band identity. Such overlapping might be expected for the low intensity bands in the orange through green region of the spectrum.

The bands in the blue and violet region of the spectrum are assigned to two nearly degenerate electronic transitions, along the two different axes, that have high intensity because of the small change in electronic angular momentum on transition. No specific assignments have been made for the bands in the ultraviolet. These bands do not show as clearly defined

structure as those in the visible.[†] The assigned electronic transitions are considered to be singlet-singlet π - π transitions; n - π transitions are too weak in intensity and charge transfer transitions have been considered to be too high in energy to lie in the visible and near ultraviolet regions.

Study of the effect of change of solvent on the positions and intensities of the various bands might be expected to provide some insight as to which bands are related in direction of polarization. For example, related vibrational bands belonging to the same transition should experience nearly the same perturbation in a given solvent, so that variations in position and intensity should be very similar with change of solvent. If, for a given solvent, specific effects were predominant along a given axis, then it is possible that, with change of solvent, the transitions in the upper and lower energy systems polarized along that axis would both show larger (or smaller) variations in relation to those transitions polarized along a different axis. Thus, the orders of sizes of band changes, with change of solvent, might reveal relationships in regards to direction of polarization. If solvent effects were not specific, or if transitions polarized along different axes were not independent,^{††} the effect of change of solvent might not be significantly

[†]It has been suggested that the blurring of structure can arise from the great speed ($\sim 0.3 \times 10^{-13}$ sec.) of the radiationless transitions between higher excited states, which can produce an uncertainty or diffuseness in the region of absorption between bands (reference [57], p. 88).

^{††}It has been suggested [58] that some substituents can cause interaction between differently polarized transitions and thus render them dependent.

different for bands of different polarization. If bands belonging to different transitions overlapped extensively, broadness and lack of symmetry in the mutual regions of absorption would be expected, and determination of peak positions would be uncertain. A change of solvent might reveal such a complication by differential variations of band positions in the overlap region.

RESULTS AND DISCUSSION

The results of the measurements are given in Tables 27, 29, 30, 32, 33, and 36. Determinations were made on $1.0 \times 10^{-5}M$ solutions at $25^{\circ}C$. The systems studied were as follows:

- 1) chl a-ethyl ether (E), 2) chl a-acetone (Ac),
- 3) chl a-methanol (MeOH), 4) chl a-1 1/2 volume per cent pyridine in low boiling (30° - $60^{\circ}C$) petroleum ether (1 1/2 percent py-pet),
- 5) chl a-pyridine (py), 6) chl a-dry, pure benzene (bz-D),
- 7) chl a-pure benzene with $\geq 10^{-5}M$ water (bz- H_2O), 8) chl a-dry, pure carbon tetrachloride (CCl_4 -D), 9) chl a-pure carbon tetrachloride with $\geq 10^{-5}M$ water (CCl_4 - H_2O), 10) chl a-dry, pure cyclohexane (cyclo-D), 11) chl a-pure cyclohexane with about $10^{-5}M$ water (cyclo- H_2O), 12) chl a-pure, dry cyclohexane with from $10^{-6}M$ to $10^{-5}M$ benzene (cyclo-D + bz),[†] 13) chl a-pure cyclohexane with about $10^{-5}M$ water and from $10^{-6}M$ to $10^{-5}M$ benzene (cyclo- H_2O + bz),[†] 14) chl b-ethyl ether, 15) chl b-acetone, 16) chl b-pyridine, 17) chl b-dry, pure benzene,
- 18) chl b-pure benzene with $\geq 10^{-5}M$ H_2O , 19) al chl a-ethyl ether, 20) al chl a-pyridine, 21) al chl a-dry pure benzene,

[†]Increase of the concentration of the benzene in these cyclohexane solutions up to 0.2 volume per cent effected no further change in the spectra.

22) al chl a-pure benzene with $\geq 10^{-5}M$ water, 23) ph a-ethyl ether, 24) ph a-pyridine, 25) ph a-benzene with $\geq 10^{-5}M$ water,[†] 26) ph a-ethyl ether saturated with dry HCl gas (EHCl), 27) al ph a-ethyl ether, 28) al ph a-pyridine, 29) al ph a-pure benzene with $\geq 10^{-5}M$ water, 30) al ph a-ethyl ether saturated with dry HCl gas, 31) ph b-ethyl ether, 32) ph b-pyridine, and 33) ph b-ethyl ether saturated with dry HCl gas. Inter-comparisons of corresponding bands of the different systems are given in Tables 28, 31, 34, and 35.

Table 27

The estimated probable errors in determination of the band peak positions given in Table 27 are as follows:

1) far red (FR)--- $\pm 30 \text{ cm}^{-1}$, 2) red (R)--- $\pm 15 \text{ cm}^{-1}$, 3) orange (O_1 and O)--- $\pm 16 \text{ cm}^{-1}$, 4) yellow (Y)--- $\pm 18 \text{ cm}^{-1}$, 5) first green (G_1)--- $\pm 19 \text{ cm}^{-1}$, 6) second green (G_2)--- $\pm 20 \text{ cm}^{-1}$, 7) third green (G_3)--- $\pm 21 \text{ cm}^{-1}$, 8) blue (B)--- $\pm 24 \text{ cm}^{-1}$, 9) blue satellite (BS)--- $\pm 51 \text{ cm}^{-1}$, and 10) the ultraviolet bands (UV_1 , UV, UV_3 , and UV_4)--- $\pm 34 \text{ cm}^{-1}$. In general determination of band peak positions was unambiguous, even where band overlap created asymmetry in band shapes. Although changes of band-overlap were encountered, it seems unlikely that spurious

[†]Pheophytin a was also studied in dry, pure benzene. The visible absorption spectrum was essentially identical to that in wet benzene, in contrast to the changes occurring with the magnesium containing compounds. This observation is consistent with the findings in past work (e.g. reference [2], pp. 648, 772).

Table 27
Band Peak Wavelengths and Wave Numbers of the Chlorophylls
and Several Derivatives in Various Solvents

System Band, λ m μ cm ⁻¹	chl <u>a</u> , E	chl <u>a</u> , AC	chl <u>a</u> , MeOH	chl <u>a</u> , 1 1/2% py-pet	chl <u>a</u> , py
R	660.0 15152	662.1 15103	665.3 15031	663.7 15068	670.3 14925
O	613.9 16289	616.2 16228	617.0 16207	615.3 16252	618.6 16194
Y	575.8 17367	580.0 17241	581.7 17190	580.0 17241	583.0 17153
G ₁	531.9 18800	534.2 18720	538.8 18559	535.0 18692	542.5 18525
G ₂	500.0 20000	500.0 20000		504.0 19841	509.5 19660
B	427.8 23378	430.6 23222	432.5 23121	434.5 23015	442.9 22585
BS	407.7 24529	409.9 24399	410.1 24383	413.2 24200	420.7 23720
UV	378.5 26397	382.3 26158	383.6 26042	383.5 26050	394.0 25350
				O ₁ , 630.1 15870	O ₁ , 638.7 15625
					UV ₃ , 374.5 26640
					UV ₄ , 342.2 29200

Table 27 (Contd.)

Band, $m\mu$ cm^{-1}	System				
	chl <u>a</u> , bz-D	chl <u>a</u> , bz-H ₂ O	chl <u>a</u> , CCl ₄ -D	chl <u>a</u> , CCl ₄ -H ₂ O	chl <u>a</u> , cyclo-D
R	666.4 15006	664.7 15044	665.9 15008	664.8 15038	663.8 15065
O	625.0 16000	618.5 16168	622.5 16036	618.6 16166	623.7 16033
Y	584.2 17117	579.3 17262	581.4 17212	579.0 17241	581.8 17188
G ₁	535.4 18678	533.7 18738	535.9 18822	535.9 18822	533.9 18730
G ₂	502.0 19900	502.0 19900	497.0 20121	497.0 20121	502.5 19870
B	432.5 23121	432.0 23148	433.3 23073	432.2 23132	430.5 23229
BS	413.5 24181	408.5 24478	412.5 24242	410.5 24372	411.9 24280
UV	380.5 26240	377.6 26460	377.5 26490	377.5 26490	380.0 26312
	FR, 682.8 14646		FR, 685.0 14599		FR, 676.8 14775
	B ₁ , 449.9 22225				B ₁ , 450.7 22187
					BS ₂ 405.0 24630

Table 27 (Contd.)

Band, μ cm ⁻¹	System				
	chl <u>a</u> , cyclo-H ₂ O	chl <u>a</u> , cyclo-D+bz ^b	chl <u>a</u> , cyclo-H ₂ O+bz ^b	chl <u>b</u> , E	chl <u>b</u> , AC
R	661.3 15122	665.2 15033	660.8 15133	641.8 15586	645.5 15492
O	617.5 16194	623.7 16033	615.6 16244	593.5 16850	596.1 16776
Y	577.7 17310	582.5 17167	576.8 17337	566.3 17650	567.5 17621
G ₁	533.9 18730	535.0 18692	532.1 18792	544.5 18450	545.0 18305
G ₂	502.5 19870	500.0 20000	500.0 20000	509.8 19608	513.0 19500
B	428.3 23348	430.6 23223	428.2 23354	452.3 22110	456.6 21901
BS	410.7 24350	411.8 24286	409.0 24450	427.5 23400	430.4 23205
UV	380.1 26300	379.3 26340	380.0 26316	377.8 26450	379.0 26375
	FR, 686.7 14562	FR, 678.2 14746	FR, 681.5 14674	O ₁ , 610.5 16350	O ₁ , 613.5 16300
	B ₁ , 448.4 22300	B ₁ , 451.8 22136	B ₁ , 450.0 22222	UV ₁ , 401.2 24865	UV ₁ , 404.0 24750
	BS ₂ , 403.7 24771	BS ₂ , 404.0 24752	BS ₂ , 401.7 24845	UV ₃ , 357.0 28000	UV ₃ , 361.5 27650

Table 27 (Contd.)

Band, μ cm ⁻¹	System				
	chl <u>b</u> , py	chl <u>b</u> , bz-D	chl <u>b</u> , bz-H ₂ O	al chl <u>a</u> , E	al chl <u>a</u> , py
R	655.2 15265	646.3 15473	646.2 15475	653.0 15315	661.1 15126
O	603.3 16565	601.1 16636	597.5 16736	608.3 16425	622.0 16077
Y		570.0 17544	568.6 17588	566.6 17675	585.0 17065
G ₁	557.1 17930	548.9 18218	546.8 18288	523.1 19100	538.0 18587
G ₂	515.0 19420	503.8 19850	503.8 19850	488.9 20440	505.0 19802
B	474.0 21195	459.5 21763	457.9 21839	417.0 23985	430.0 23256
BS	445.4 22450	431.2 23192	430.1 23250	387.5 25800	404.5 24691
UV	389.8 25625	383.5 26062	382.5 26160	363.5 27485	370.0 27000
	UV ₁ , 417.0 23975	FR, 665.5 15025	O ₁ , 614.6 16271	UV ₃ , 353.5 28290	
	UV ₃ , 372.3 26825	O ₁ , 615.4 16250	UV ₁ , 405.5 24700		
	UV ₄ , 347.0 28800	B ₁ , 485.0 20600	UV ₃ , 363.5 27499		
		UV ₃ , 367.5 27211			

Table 27 (Contd.)

System Band, μ cm^{-1}	al chl <u>a</u> , bz-D	al chl <u>a</u> , bz-H ₂ O	ph <u>a</u> , E	ph <u>a</u> , py	ph <u>a</u> , bz-H ₂ O
R	659.1 15172	655.5 15256	667.0 14995	669.3 14940	670.2 14921
O	615.7 16241	611.3 16359	609.1 16415	611.4 16355	612.5 16327
Y	571.0 17513	569.8 17550	560.0 17850	560.8 17750	561.2 17819
G ₁	525.0 19048	524.8 19055	533.6 18742	544.5 18385	536.5 18625
G ₂	491.2 20360	491.1 20362	507.0 19700	503.0 19625	507.5 19704
B	422.0 23697	419.5 23838	408.5 24457	414.6 24110	414.3 24137
BS			389.5 25650	395.3 25300	394.5 25316
UV	363.5 27500	363.5 27500	368.5 27100	372.3 26810	371.9 26882
			O ₁ , 635.0 15740	G' ₁ , 538.5 18585	G ₃ , 473.7 21110
			G' ₂ , 496.0 20165	G' ₂ , 501.0 19935	
			G ₃ , 465.5 21450	G ₃ , 473.0 21150	

Table 27 (Contd.)

Band, μ cm ⁻¹	System				
	ph <u>a</u> , EHCl	al ph <u>a</u> , E	al ph <u>a</u> , py	al ph <u>a</u> , bz-H ₂ O	al ph <u>a</u> , EHCl
R	663.8 15065	670.3 14920	671.7 14888	673.2 14854	663.0 15094
O	615.0 16260	613.1 16300	615.0 16284	617.3 16199	615.0 16260
Y	565.8 17674	563.9 17750	565.0 17699	563.9 17735	576.1 17316
G ₁	535.0 18692	529.2 18905	533.1 18758	532.0 18797	535.2 18650
G ₂	500.0 20000	498.6 20030	503.4 19841	501.9 19924	501.2 19952
B	423.6 23607	399.8 25015	405.8 24643	405.3 24673	420.9 23759
BS	391.0 25575				392.2 25478
UV	365.0 27397	368.0 27190		366.5 27248	367.2 27211
		G ₃ , 467.0 21400	G ₃ , 470.0 21249	G ₃ , 471.1 21227	
		UV ₃ , 349.0 28610	UV ₃ , 360.0 27778	UV ₃ , 350.0 28577	

Table 27 (Contd.)

System Band, μ -1 cm	ph $\underline{b}$, E	ph $\underline{b}$, py	ph $\underline{b}$, EHC1
R	654.3 15289	656.7 15235	652.7 15307
O	598.8 16690	601.0 16635	596.5 16750
Y	558.0 17910	560.0 17825	555.5 18002
G ₁	531.5 18790	535.5 18650	532.5 18786
G ₂	521.1 19190	525.0 19050	522.1 19205
B	433.2 23080	438.0 22835	433.5 23068
BS	411.3 24335	413.8 24200	410.1 24390
UV	368.2 27125	369.5 27050	368.1 27137
	G ₃ , 487.5 20525	UV ₁ , 392.0 25500	B ₁ , 446.5 22375
	B ₁ , 448.0 22335		UV ₁ , 389.5 25641
	UV ₁ , 388.0 25775		

^aAdditional bands, present in some systems, are tabulated after the UV band, and designated by the letter preceding the wavelength value.

^bVery small amounts (1.0×10^{-6} to 1.0×10^{-5} M) of pure, dry benzene were added to the cyclohexane solutions.

identification of band positions were made. Thus it did not appear that the problem of complex interdigitation of bands was encountered where band identity was lost.

Most of the systems studied showed at least five distinct bands (R, O, Y, G_1 , G_2) in the red through the green region of the spectrum, and at least three bands (B, BS, UV) in the blue through the near ultraviolet region. Variations occurred as a result of change of solvent and change of substituent pattern.

For chl a the following major variations in the structure of the spectrum were observed with change of solvent. In methanol all bands were broadened and the peak position of the G_2 band became uncertain. Pyridine caused a splitting of the O band and gave increased structure in the near ultraviolet region. In dry, nonpolar solvents such as CCl_4 , benzene, and cyclohexane the R band was split, and in the latter two solvents a small protuberance (B_1) was observed on the long wavelength side of the B band. All bands were broadened with some loss of distinctness of structure throughout the spectrum.[†] It

[†]There is evidence that dimeric aggregation of the chlorophylls can occur in dry, pure nonpolar solvents [61]. Dimeric aggregation can be expected to produce different effects on bands polarized along different axes, and consequently can be viewed as a special type of solvent effect. If the dimer absorbed as a unit, structure in the dry spectra could occur that could not be explained in terms of band overlap of the monomeric unit. The general broadening of all bands accompanied by splitting of the R band, which occurs only in the dry nonpolar solvents, suggests the possibility that there may be a superposition of the spectrum of the dimer on the perturbed spectrum of the monomer.

Also see--A.F.H. Anderson and M. Calvin, Arch. Biochem. Biophys., 107, 251 (1964).

was still possible to determine peak positions clearly. In cyclohexane the BS band was also split. Addition of water to cyclohexane, in contrast to CCl_4 and benzene, did not entirely remove band splitting. The presence of a small amount of benzene in the wet cyclohexane solution did not diminish the residual band splitting. Addition of a small amount of benzene to the dry cyclohexane solution did not change the splitting of the bands, but produced red shifts in the R, Y, and G_1 bands.

In the solvents where band splitting of chl a did not occur, and with the exception of methanol, the O, Y, and G bands were quite symmetrical with well defined peaks. In methanol the O, Y, and G bands lost symmetry with a resulting lack of definition of minima between bands. In pyridine, in addition to the O band splitting, there was loss of symmetry in the Y and G bands. The blurring effects of pyridine and methanol appeared to arise primarily from thermal broadening of existing bands, due to the motions of loosely attached solvent molecules (e.g. reference [57], p. 88).

Chl b, in contrast to chl a, showed splitting of the O band in all solvents studied except in pyridine, and was characterized by more band structure in the UV region. In pyridine the G_1 band appeared to shift to the extent of completely overlapping the Y band. The large red shift effect of pyridine caused the appearance of an additional band in the region of the UV scanned. The effect of dry nonpolar solvents on chl b was very similar to that on chl a. In acetone, ether,

and wet benzene, where splitting of the O band was observed, the Y and G bands showed a lack of symmetry. In pyridine the O and G bands were broadened but more symmetrical, while the R band lost shape symmetry on the short wavelength side.

No band splitting was observed for al chl a in the solvents studied. In dry benzene most bands showed a shift to the red as compared to wet benzene, but there was no splitting of the R band and no smearing out of the spectrum as occurred for chl a and chl b. In dry and wet benzene a BS band could not be distinguished. In ether and pyridine the BS band was manifested by a slight protuberance near the short wavelength side of the main blue band. In wet benzene and ether the O, Y, and G bands of al chl a, like those of chl a, were quite symmetrical in shape with well defined peaks. In pyridine, however, the symmetry of these bands was reduced.

In ethyl ether, a small additional O band could be distinguished for ph a near the R band. This did not resemble the O band splitting observed for chl a and chl b. Ph a, in comparison to the chlorophylls, had an additional band in the green region, which, however, could not be distinguished in the EHCl solvent. In ether the second green band showed splitting, while both G_1 and G_2 were split in pyridine. The O and Y bands of ph a had well defined peak positions but did not show much symmetry in shape. In benzene, where splitting of the G_2 band could not be defined, there was distinct asymmetry of shape suggesting the close overlapping of two bands.

Al ph a, like ph a, had a third band in the green region. No band splitting was observed in any of the solvents. A BS band could be distinguished only in the EHCl solvent in which the third green band could not be discerned. In pyridine and EHCl only one UV band could be distinguished, while two bands were observed in ether and wet benzene. In benzene, ether, and pyridine, the O and Y bands of al ph a were less symmetrical than those of ph a; the G bands, however, showed more symmetry with no evidence of splitting.

Ph b showed two UV bands, and a third green band which could be distinguished only in ether. The G_1 and G_2 bands lay much closer together than in ph a and al ph a. In ether and EHCl, but not in pyridine, there was a protuberance on the long wavelength side of the B band. No band splitting was observed. The O band of ph b was quite symmetrical, whereas the Y band, lying rather close to the G_1 , was not.

Chl a, chl b, and al chl a, but not ph a, in the dry nonpolar solvents showed a marked (~10-fold) decrease in fluorescence intensity as observed visually. This is consistent with earlier observations in dry nonpolar solvents (reference [2], pp. 766-772). Ph a and al ph a, but not ph b, in EHCl showed significant decreases in fluorescence intensity.

Table 28

Table 28 shows the change of band peak wave number, and the percentage change with respect to the energy of the

Table 28

The Change of Band Peak Wave Numbers with Change
of Solvent in Reference to Ethyl Ether

Solvent and Compound Band, ^a $\delta \text{ cm}^{-1}$ % Change	AC, chl <u>a</u>	MeOH chl <u>a</u>	1 1/2% py-pet, chl <u>a</u>	py, chl <u>a</u>	bz-D, chl <u>a</u>	bz-H ₂ O, chl <u>a</u>	CCl ₄ -D, chl <u>a</u>	CCl ₄ -H ₂ O, chl <u>a</u>	cyclo-D, chl <u>a</u>
R	49 0.32	121 0.80	84 0.55	227 1.50	146 0.96	108 0.71	144 0.95	144 0.75	87 0.57
O	61 0.37	82 0.50	37 0.23	95 0.58	289 1.78	121 0.74	253 1.56	123 0.76	256 1.57
Y	126 0.73	177 1.02	126 0.73	214 1.23	250 1.44	205 1.18	155 0.89	126 0.73	179 1.03
G ₁	80 0.43	241 1.28	108 0.56	275 1.46	122 0.65	62 0.33	+ 22 + 0.12	+ 22 + 0.12	70 0.37
G ₂	100 0.50		259 1.29	440 2.19	200 1.0	200 1.0	+ 121 + 0.61	+ 121 + 0.61	200 1.00
B	156 0.67	257 1.10	363 1.55	793 3.40	257 1.10	230 0.99	305 1.31	246 1.05	149 0.64
BS	130 0.53	146 0.60	329 1.34	809 3.30	348 1.42	0 0	287 1.17	157 0.64	249 1.02
UV	239 0.91	355 1.35	347 1.32	1047 3.96	157 0.59	0 0	0 0	0 0	0 0

Table 28 (Contd.)

Band, δcm^{-1} % Change	Solvent and Compound		cyclo- H ₂ O, chl <u>a</u>	cyclo- D + bz chl <u>a</u>	cyclo- H ₂ O + bz chl <u>a</u>	AC, chl <u>b</u>	py, chl <u>b</u>	bz-D, chl <u>b</u>	bz-H ₂ O, chl <u>b</u>	py, al chl <u>a</u>	bz-D, al chl <u>a</u>
R	30	119	19	94	321	113	111	189	143		
	0.20	0.79	0.13	0.60	2.06	0.73	0.71	1.23	0.93		
O	95	256	45	74	285	214	114	348	184		
	0.58	1.57	0.28	0.44	1.69	1.27	0.68	2.11	1.12		
Y	57	200	30	29		106	62	610	162		
	0.33	1.15	0.17	0.16		0.60	0.35	3.46	0.92		
G ₁	70	108	0	145	520	232	162	513	52		
	0.37	0.57	0	0.79	2.82	1.26	0.88	2.68	0.27		
G ₂	200	100	100	108	188	+ 242	+ 242	638	80		
	1.00	0.50	0.50	0.55	0.96	+ 1.23	+ 1.23	3.11	0.39		
B	30	155	0	209	915	347	271	729	288		
	0.13	0.66	0	0.95	4.14	1.57	1.23	3.04	1.20		
BS	179	243	79	195	950	258	200	1109			
	0.73	0.99	0.32	0.83	4.06	1.10	0.85	4.30			
UV	0	0	0	0	825	388	290	485	0		
	0	0	0	0	3.12	1.47	1.10	1.76	0		
				0 ₁	UV ₁	0 ₁	0 ₁				
				50	890	100	79				
				0.31	3.58	0.61	0.48				
				UV ₁	UV ₃	UV ₃	UV ₁				
				115	1175	789	165				
				0.46	4.2	2.82	0.66				
				UV ₃			UV ₃				
				350			501				
				1.25			1.79				

Table 28 (Contd.)

Band, δcm^{-1} % Change	Solvent and Compound		bz-H ₂ O, al chl <u>a</u>	py, ph <u>a</u>	bz-H ₂ O, ph <u>a</u>	EHCl, ph <u>a</u>	py, al ph <u>a</u>	bz-H ₂ O, al ph <u>a</u>	EHCl, al ph <u>a</u>	py, ph <u>b</u>	EHCl, ph <u>b</u>
R	59	55	74	+ 70	32	66	+ 174	54	+ 18		
	0.39	0.37	0.49	+ 0.47	0.21	0.44	+ 1.17	0.35	+ 0.12		
O	66	60	88	155	0	101	40	55	+ 60		
	0.40	0.37	0.54	0.94	0	0.62	0.25	0.33	+ 0.36		
Y	125	100	31	176	51	0	434	85	+ 92		
	0.71	0.55	0.17	0.99	0.29	0	2.45	0.48	+ 0.51		
G ₁	45	357	117	50	147	108	255	140	0		
	0.24	1.91	0.62	0.27	0.78	0.57	1.35	0.75	0		
G ₂	78	75	0	+ 300	189	106	78	140	0		
	0.38	0.38	0	+ 1.52	0.95	0.53	0.39	0.73	0		
B	147	347	320	850	372	342	1256	245	0		
	0.61	1.42	1.31	3.47	1.49	1.37	5.01	1.06	0		
BS		350	334	75				135	0		
		1.36	1.30	0.29				0.56	0		
UV	0	290	218	+ 297		0	0	0	0		
	0	1.07	0.81	+ 1.09		0	0	0	0		
		G ₁ 2	G ₃		G ₃	G ₃		UV ₁	B ₁		
		230	340		151	173		275	+ 40		
		1.14	1.58		0.71	0.81		1.07	+ 0.18		
		G ₃			UV ₃	UV ₃		UV ₁			
		300			832	0		134			
		1.40			2.90	0		0.52			

^aChanges less than the estimated probable error (see text) are omitted. Changes tabu-
without sign are negative, that is shifts to the red; changes with plus signs are positive.

transition, with change of solvent in reference to ethyl ether. Shifts are to the red unless accompanied by a plus sign which signifies a blue shift. Ethyl ether was chosen as reference solvent since it is one of the most commonly used solvents in the study of the chlorophylls and their derivatives. The main red and blue band peaks are at shorter wave lengths than in other solvents, and the red band half-widths ($R_{1/2}$) and most of the blue band half-widths ($B_{1/2}$) are smaller (see Table 32). These phenomena indicate that the solvent perturbations in ethyl ether are somewhat less than in the other solvents.

In general, it can be seen that for a given compound the sizes of the individual band shifts change markedly with different solvents. There is no general tendency for any particular band, or group of bands, to show predominantly the largest percentage change, although the largest absolute band shifts occur primarily in the upper energy system. For a given solvent change, bands at corresponding positions in the spectrum shift differently for each compound. In pyridine, for example, the bands of the magnesium containing compounds in general show greater changes than the bands of the corresponding pheophytins. Not only is there a variation in the magnitude of a particular shift, but also the order of the sizes of the shifts changes from system to system. These patterns of variation clearly indicate the occurrence of specific solvent interactions that differ for each system. In general the observed changes support the view that there are bands representing transitions of

different polarization in both the upper and lower energy systems.

For the chl a, chl b, and al chl a systems there is no consistent change of the same per cent, within probable error (≤ 0.1 per cent for the R through the B bands, ≤ 0.2 per cent for the BS band, and ≤ 0.3 per cent for the UV bands), in any of the neighboring bands, nor in next neighbor bands. Consistently similar changes for two or more bands would be good evidence that those bands were related vibrational sub bands belonging to the same transition.

In comparing the orders of sizes of band shifts in the upper and lower energy systems no consistent relationship between upper and lower bands is apparent. As example for chl a, in many of the instances when the B band shows a larger (or smaller) shift than the BS band, the R band shows a larger (or smaller) shift than the O band. However, this relationship between upper and lower bands is not consistent for all solvent changes. There remains some uncertainty, therefore, in drawing a conclusion from these data about which bands may be polarized along the same axis. The difficulty may lie in the possibility that a substituent has effect on transitions polarized along different axes. Interaction of a solvent molecule at such a substituent could therefore have significant effects along two axes. Since the electron distributions in the different chlorin orbitals are not expected to be the same [58], the effects produced by a solvent molecule would in general not be identical along each

axis, and could vary in relative size depending on the specific nature of the interaction.

In contrast to chl a, the shifts in the R and O bands and in the B and BS bands for ph a in benzene and pyridine are consistent in size. This raises the possibility that the R and O bands, and the B and BS bands might be related vibrational bands belonging to the same transition. However, the relative variations in intensities, discussed subsequently, do not support this possibility. Moreover the relationship for the R and the O bands is not preserved for al ph a in benzene and pyridine.

In the EHCl solvent the R and the O bands of ph a shift in opposite directions, there is a comparatively large red shift of the B band, and the G_1 and G_2 bands shift in opposite directions. The net result is that the positions of the band peaks tend to be close to those of the corresponding bands of chl a in ether. These changes are consistent with the suggestion that salt formation occurs with entry of protons into the center of the chlorin ring. Thus, in EHCl the effect is essentially a change in substituent pattern to one resembling that of chl a. There is a similar, though less close correspondence between the band positions of al ph a in EHCl and those of al chl a in ether. Only the R and O bands of ph b in EHCl shift toward the positions of the corresponding bands of chl b in ether. The overall changes in the spectrum for ph b in EHCl are small compared to those for ph a and al ph a. This suggests that the

extent of salt formation was less for ph b, in the particular solvent used.

For chl a in polar solvents the larger percentage shifts are predominantly in the high energy system. In the more inert solvents, either dry or with traces of water, the low energy bands tend to show the larger shifts, and the UV band consistently shows little or no change. In the dry solvents, the O band consistently shows the largest percentage changes. Pyridine produced the greatest band shifts, while cyclohexane with traces of benzene and water gave the least.

The upper energy bands of chl b consistently show the larger shifts, the greatest being in the UV₃ band. The O band in dry benzene undergoes a significantly larger shift than the R band, which resembles the behavior for chl a. The Y band of chl b, however, shows a smaller shift than that of chl a. Pyridine produced the largest changes and acetone the least.

The order of size of band shifts of al chl a in wet benzene closely resembles that of chl a in the same solvent. The actual size of the shifts for al chl a, however, are about one-half those for chl a. There is less correspondence in orders of size for al chl a and chl a in dry benzene and pyridine. In pyridine the Y band of al chl a shows a large shift while the UV band shift is relatively small. The reverse is true for chl a in pyridine. If the bands of the two compounds, at corresponding points throughout the spectrum, represent transitions with the same polarization in the chlorin ring, then it would appear that

the differences in effect for a given solvent change must be directly related to the alteration of the isocyclic ring. Thus, alteration of the isocyclic ring may result in change of specific interactions with it, or change in the interactions with another substituent that is not independent of the isocyclic ring. In the latter case the substituent might be the magnesium atom.

In comparing the effects of a given solvent change for $\text{ph } \underline{\text{a}}$ vs $\text{al ph } \underline{\text{a}}$, differences in the sizes of particular band shifts and in the order of sizes are also observed. In pyridine the shifts of the B bands are very similar, but shifts in the lower energy bands and the UV bands differ considerably. In wet benzene, band shifts are more nearly alike, large differences occurring only in the G_2 , G_3 , and UV band shifts. In EHCl , significant differences exist in all band shifts. The R, Y, G_1 , and B bands of $\text{al ph } \underline{\text{a}}$ show larger shifts than in $\text{ph } \underline{\text{a}}$, while the shifts in the O and G_2 bands of $\text{ph } \underline{\text{a}}$ are larger. These results also reflect the influence of the differences in the isocyclic rings of $\text{ph } \underline{\text{a}}$ and $\text{al ph } \underline{\text{a}}$ on solvent effects. The differences in the effects between pyridine and benzene on the one hand, and EHCl on the other suggest that not only are direct interactions with the isocyclic rings important, but also that the structure of the isocyclic ring can have significant influence on the substituents in the center of the chlorin ring, and thus on the solvent interactions occurring there.

For the pheophytins the B bands consistently show large

shifts with solvent change, except for ph b in EHCl where all spectral changes were relatively small. The R, O, and Y bands undergo relatively smaller shifts, except for the Y band of al ph a in EHCl. The G bands show rather wide variations in sizes of shifts depending on the solvent and the compound. For a given solvent change, the effects on the bands of the pheophytins are generally smaller than those for the magnesium containing compounds. This suggest that specific solvent interactions at the magnesium atom can occur with relatively strong perturbing effect.

Tables 29, 30, and 31

In Table 29 are given the oscillator strengths (f) and band widths[†] for the individual bands of a number of the systems studied. The dipole strengths (D) and their square-roots are shown in Table 30. In Table 31 are given the changes in f and D with change of solvent with respect to ethyl ether. Changes less than probable error (see Table 6) are neglected.

For chl a and al chl a the strengths of the transitions in the lower energy band decrease consistently in the order R, O, Y, G_1 , and G_2 . For chl b the order of strengths is changed only in that G_1 precedes Y. Where O band splitting occurs, the strengths are about equal for chl a, but not for chl b where the

[†]The numbers tabulated represent the distances between band cut offs which were in many instances well defined minima between bands. Actual band widths, which are larger than distances between cut offs, are obscured by band overlap.

Table 29

Oscillator Strengths (f) of the Chlorophylls and Several
Derivatives in Various Solvents

Band, a, b (cm ⁻¹) f	System						
	Width	chl <u>a</u> , E	chl <u>a</u> , AC	chl <u>a</u> , MeOH	chl <u>a</u> , 1 1/2% py-pet	chl <u>a</u> , py	chl <u>a</u> , bz-D
R		1627 0.170	1613 0.167	1987 0.173	1595 0.159	1475 0.170	836 0.116
O		1165 0.049	1161 0.055	1070 0.062	981 0.036	795 0.038	1192 0.063
Y		1320 0.027	1333 0.031	1400 0.032	1154 0.018	1405 0.022	1555 0.035
G ₁		1313 0.015	1393 0.015	2583 ^c 0.018	1284 0.013	1185 0.019	2509 ^c 0.016
G ₁		1741 0.009	1529 0.008		1483 0.008	1575 0.009	
B		2771 0.515	2823 0.463	2837 0.363	2830 0.523	2525 0.516	1670 0.416
BS		1590 0.410	1627 0.413	1550 0.369	1700 0.399	1435 0.336	1755 0.387

Table 29 (Contd.)

Band, a,b (cm ⁻¹) f	System					
	Width	chl a, E	chl a, AC	chl a, MeOH	chl a, 1 1/2% py-pet	chl a, py chl a, bz-D
UV		2861 0.444	2957 0.476	3351 0.560	3046 0.403 0 ₁ ,468 0.023	2996 0.452 0 ₁ ,525 FR,1151 0.035 0.063 UV+UV ₃ ,2740 B ₁ ,1108 0.391 0.024 UV ₄ ,2653 0.301
First Cut off (cm ⁻¹)		14183	14135	13793	14035	13990 13699
Last Cut off (cm ⁻¹)		28571	28571	28571	28571	30303 28571
Total f		1.640	1.628	1.589	1.572	1.840 1.570 (1.635) ^d

Table 29 (Contd.)

Band, Width (cm ⁻¹) f	System				
	chl a, bz-H ₂ O	chl a, cyclo-D	chl a, cyclo-H ₂ O	chl a, cyclo-D ⁺ + bz	chl a, cyclo-H ₂ O + bz
R	1508 0.168	887 0.111	1133 0.146	819 0.114	1133 0.171
O	1158 0.048	1236 0.059	1189 0.045	1262 0.063	1165 0.051
Y	1348 0.028	1331 0.028	1328 0.022	1290 0.030	1290 0.028
G ₁	3002 ^c 0.022	1201 0.012	1201 0.012	1540 0.012	1540 0.014
G ₂		1426 0.008	1563 0.009	1186 0.008	1243 0.012
B	2764 0.506	1695 0.401	1659 0.419	1832 0.407	1659 0.505
BS	1461 0.347	1570 0.328	1402 0.337	1689 0.345	1710 0.377

Table 29 (Contd.)

Band, Width (cm^{-1}) f	System				
	chl a, bz-H ₂ O	chl a, cyclo-D	chl a, cyclo-H ₂ O	chl a, cyclo-D + bz	chl a, cyclo-H ₂ O + bz
UV	3146 0.437	3021 0.450	3090 0.453	2931 0.434	2765 0.503
		FR,1162 0.057	FR,824 0.013	FR,1244 0.069	FR,836 0.015
		B ₁ ,1343 0.033	B ₁ ,1341 0.015	B ₁ ,1129 0.034	B ₁ ,1384 0.018
First Cut off (cm^{-1})	14184	13699	13841	13650	13841
Last Cut off (cm^{-1})	28571	28571	28571	28571	28571
Total f	1.560	1.488	1.481	1.567	1.699

Table 29 (Contd.)

Band, Width (cm ⁻¹) f	System			
	chl <u>b</u> , E	chl <u>b</u> , Ac	chl <u>b</u> , py	chl <u>b</u> , bz-D
R	1845 0.107	1699 0.104	1795 0.113	968 0.090
O	985 0.033	O ₁ +O, 1252 0.043	O ₁ +O, 1385 0.049	897 0.035
Y	610 0.016	627 0.015	Y+G ₁ , 1615 0.032	648 0.016
G ₁	2365 ^c 0.035	2082 ^c 0.035		1275 0.026
G ₂			625 0.009	448 0.008
B	2800 0.683	2777 0.673	2500 0.662	2420 0.663
BS	1350 0.213	BS+UV ₁ , 2439 0.279	1425 0.206	BS+UV ₁ , 2183 0.238
UV	1550 0.105	1694 0.120	1400 0.094	1833 0.142

Table 29 (Contd.)

Band, Width (cm^{-1}) f	System			
	chl <u>b</u> , E	chl <u>b</u> , Ac	chl <u>b</u> , py	chl <u>b</u> , bz-D
	O ₁ ,255	UV ₃ ,1240	UV ₁ ,1000	FR,1342
	0.008	0.116	0.058	0.033
	UV ₁ ,900		UV ₃ ,1400	O ₁ ,334
	0.056		0.128	0.015
	UV ₃ ,1571		UV ₄ ,2775	B ₁ ,705
	0.142		0.308	0.037
				UV ₃ ,1032
				0.108
First Cut off (cm^{-1})	14340	14440	14280	13793
Last Cut off (cm^{-1})	23571	28250	30200	27878
Total f	1.395	1.385	1.658 (1.473) ^d	1.412

Table 29 (Contd.)

System Band Width, (cm ⁻¹) f	chl <u>b</u> , bz-H ₂ O	al chl <u>a</u> , E	al chl <u>a</u> , bz-H ₂ O	ph <u>a</u> , E
R	1812 0.113	1995 0.136	2300 0.144	1250 0.103
O	940 0.033	1200 0.036	1167 0.035	1300 0.027
Y	684 0.017	1220 0.018	1249 0.020	1055 0.011
G ₁	1433 0.026	1540 0.017	2748 ^c 0.024	905 0.028
G ₂	750 0.009	1305 0.008		775 0.029
B	2605 0.684	4055 0.766	B+BS, 5152 0.959	3050 0.700
BS	BS+UV ₁ , 2570 0.246	1365 0.244		1075 0.358
UV	1333 0.088	1235 0.155	UV+UV ₃ , 2255 0.252	2271 0.390
	O ₁ , 320 0.011	UV ₃ , 1120 0.108		O ₁ , 375 0.007

Table 29 (Contd.)

Band Width, (cm ⁻¹) f	System			
	chl <u>b</u> , bz-H ₂ O	al chl <u>a</u> , E	al chl <u>a</u> , bz-H ₂ O	ph <u>a</u> , E
	UV ₃ ,1450 0.129			G' ₂ ,780 0.023
				G ₃ ,425 0.024
First Cut off (cm ⁻¹)	14286	13990	13700	14300
Last Cut off (cm ⁻¹)	28183	29025	28571	28571
Total f	1.357	1.488 (1.450) ^d	1.434	1.700

Table 29 (Contd.)

System Band Width, (cm ⁻¹) f	ph <u>a</u> , py	ph <u>a</u> , bz-H ₂ O	al ph <u>a</u> , E	al ph <u>a</u> , bz-H ₂ O
R	1800 0.113	1637 0.115	1800 0.090	2043 0.103
O	1385 0.031	1357 0.026	1340 0.016	1221 0.016
Y	815 0.012	923 0.009	1100 0.006	1156 0.010
G ₁	475 0.009	990 0.024	1070 0.018	988 0.018
G ₂	760 0.028	1594 0.050	1762 0.040	1512 0.037
B	3125 0.709	2978 0.652	4490 0.941	4750 1.035
BS	1300 0.420	1129 0.373		
UV	2321 0.410	2597 0.484	1365 0.267	1482 0.230
	G ₁ , 590 0.020	G ₃ , 1193 0.018	G ₃ , 925 0.010	G ₃ , 990 0.012

Table 29 (Contd.)

System Band Width, (cm^{-1}) f	ph <u>a</u> , py	ph <u>a</u> , bz-H ₂ O	al ph <u>a</u> , E	al ph <u>a</u> , bz-H ₂ O
	G'2,775		UV3,1545	UV3,1353
	0.023		0.180	0.172
	G3,1225			
	0.022			
First Cut off, (cm^{-1})	14000	14184	14000	13830
Last Cut off, (cm^{-1})	28571	28571	29400	29325
Total f	1.797	1.752	1.568 (1.490) ^d	1.634

Table 29 (Contd.)

System Band, Width, (cm ⁻¹) f	ph <u>b</u> , E	ph <u>b</u> , py
R	1525 0.063	2075 0.067
O	1225 0.023	1120 0.021
Y	970 0.019	1045 0.018
G ₁	710 0.025	550 0.018
G ₂	1090 0.038	G ₂ +G ₃ , 2200 0.070
B	1640 0.670	3000 0.874
BS	1375 0.276	BS+UV ₁ , 1950 0.236
UV	2221 0.223	2670 0.236
	G ₃ , 1125 0.022	
	B ₁ , 1185 0.086	

Table 29 (Contd.)

System Band, Width, (cm ⁻¹) f	ph <u>b</u> , E	ph <u>b</u> , py
	UV ₁ ,975	
	0.103	
First Cut off (cm ⁻¹)	14530	13950
Last Cut off (cm ⁻¹)	28571	28571
Total f	1.547	1.593

^aThe band width is the distance between minima, or band cut offs.

^bAdditional bands, present in some systems, are tabulated after the UV band, and designated by the letter preceding the band width value.

^cTotal green band.

^dTotal f with the last cut off at 28571.

Table 30

Dipole Strengths (D) of the Chlorophylls and Several
Derivatives in Various Solvents

Band, ^a D ^{1/2} , A	System					
	chl <u>a</u> , E	chl <u>a</u> , AC	chl <u>a</u> , MeOH	chl <u>a</u> , 1 1/2% py-pet	chl <u>a</u> , py	chl <u>a</u> , bz-D
R	1.022	1.015	1.144	0.968	1.047	0.714
	1.010	1.007	1.070	0.984	1.023	0.845
O	0.263	0.308	0.352	0.200	0.215	0.359
	0.513	0.555	0.593	0.447	0.464	0.599
Y	0.136	0.169	0.155	0.098	0.134	0.187
	0.369	0.411	0.394	0.313	0.366	0.432
G ₁	0.070	0.078	0.086 ^b	0.064	0.076	0.078 ^b
	0.265	0.279	0.293	0.253	0.276	0.279
G ₂	0.033	0.038		0.038	0.037	
	0.182	0.195		0.195	0.192	
B	2.015	1.839	1.505	2.085	2.100	1.650
	1.419	1.356	1.227	1.444	1.449	1.285
BS	1.520	1.539	1.249	1.462	1.106	1.450
	1.233	1.240	1.119	1.209	1.052	1.204
UV	1.520	1.693	2.075	1.377		1.550
	1.233	1.301	1.441	1.173		1.245

Table 30 (Contd.)

Band, ^a D,A ² D ^{1/2} , A	System					
	chl <u>a</u> , E	chl <u>a</u> , AC	chl <u>a</u> , MeOH	chl <u>a</u> , 1 1/2% py-pet	chl <u>a</u> , py	chl <u>a</u> , bz-D
				O ₁ ,0.132 0.363	O ₁ ,0.206 0.454	FR,0.398 0.631
					UV+UV ₃ ,1.511 1.229	B ₁ ,0.100 0.316
					UV ₄ ,0.925 0.962	
Total D, A ²	6.580	6.680	6.566	6.425	7.357 (6.750) ^c	6.486
Total D ^{1/2} , A	6.244	6.344	6.137	6.381	7.467	6.836

Table 30 (Contd.)

Band, D, A ² D ^{1/2} , A	System				
	chl a, bz-H ₂ O	chl a, cyclo-D	chl a, cyclo-H ₂ O	chl b, E	chl b, Ac
R	1.025	0.676	0.890	0.653	0.621
	1.012	0.822	0.943	0.808	0.788
O	0.268	0.329	0.255	0.185	0 ₁ +0 ₂ , 0.238
	0.518	0.574	0.504	0.430	0.488
Y	0.148	0.147	0.118	0.081	0.080
	0.385	0.383	0.344	0.285	0.283
G ₁	0.083 ^b	0.059	0.059	0.171 ^b	0.171 ^b
	0.288	0.243	0.243	0.414	0.414
G ₂		0.038	0.043		
		0.195	0.207		
B	2.019	1.600	1.666	3.005	2.821
	1.421	1.265	1.290	1.733	1.679
BS	1.290	1.225	1.269	0.846	BS+UV ₁ , 1.080
	1.136	1.107	1.126	0.920	1.039
UV	1.483	1.543	1.557	0.367	0.420
	1.218	1.242	1.247	0.606	0.648
		FR, 0.355	FR, 0.074	0 ₁ , 0.043	UV ₃ , 0.387
		0.596	0.272	0.207	0.622

Table 30 (Contd.)

Band, D, A ² D ^{1/2} , A	System chl a, bz-H ₂ O	chl a, cyclo-D	chl a, cyclo-H ₂ O	chl b, E	chl b, Ac
		B ₁ , 0.131 0.362	B ₁ , 0.066 0.257	UV ₁ , 0.268 0.518	
				UV ₃ , 0.358 0.598	
Total D, A ²	6.316	6.106	5.997	5.973	5.821
Total D ^{1/2} , A	5.778	6.739	6.433	6.519	5.961

Table 30 (Contd.)

Band, D, A^2 $D^{1/2}, A$	System			
	chl <u>b</u> , bz-D	chl b, bz-H ₂ O	al chl <u>e</u> , E	al chl <u>a</u> , bz-H ₂ O
R	0.534 0.731	0.675 0.822	0.825 0.908	0.876 0.936
O	0.193 0.439	0.179 0.423	0.200 0.447	0.196 0.443
Y	0.086 0.293	0.092 0.303	0.095 0.308	0.105 0.324
G ₁	0.128 0.358	0.108 0.329	0.081 0.285	0.113 ^b 0.336
G ₂	0.036 0.190	0.046 0.214	0.034 0.184	
B	2.842 1.686	2.704 1.645	2.913 1.707	B+BS, 3.638 1.907
BS	BS+UV ₁ , 0.793 0.891	BS+UV ₁ , 1.126 1.061	0.866 0.931	
UV	0.504 0.710	0.306 0.553	0.523 0.723	UV+UV ₃ , 0.852 0.923
	FR, 0.208 0.456	O ₁ , 0.063 0.251	UV ₃ , 0.350 0.592	

Table 30 (Contd.)

Band, D, A^2 $D^{1/2}, A$	System			
	chl <u>b</u> , bz-D	chl b, bz-H ₂ O	al chl <u>a</u> , E	al chl <u>a</u> , bz-H ₂ O
	O ₁ , 0.087 0.295	UV ₃ , 0.432 0.657		
	B ₁ , 0.143 0.378			
	UV ₃ , 0.364 0.603			
Total D, A^2	5.918	5.730	5.888 (5.762) ^c	5.783
Total $D^{1/2}, A$	7.030	6.258	6.285	4.869

Table 30 (Contd.)

Band, D, A ² D ^{1/2} , A	System			
	ph <u>a</u> , E	ph <u>a</u> , py	ph <u>a</u> , bz-H ₂ O	al ph <u>a</u> , E
R	0.629 0.793	0.698 0.835	0.707 0.840	0.561 0.749
O	0.151 0.389	0.175 0.418	0.152 0.390	0.108 0.329
Y	0.057 0.239	0.054 0.232	0.045 0.212	0.034 0.184
G ₁	0.117 0.342	0.054 0.232	0.120 0.346	0.086 0.293
G ₂	0.134 0.366	0.131 0.362	0.234 0.484	0.182 0.427
B	2.639 1.624	2.671 1.634	2.511 1.585	3.511 1.874
BS	1.234 1.111	1.462 1.209	1.344 1.160	
UV	1.175 1.084	1.278 1.130	1.651 1.285	0.898 0.948
	G ₁ , 0.042 0.205	G ₁ , 0.095 0.308	G ₃ , 0.079 0.281	G ₃ , 0.039 0.197
	G ₂ , 0.105 0.324	G ₂ , 0.108 0.329		UV ₃ , 0.580 0.762
	G ₃ , 0.100 0.316	G ₃ , 0.094 0.307		
Total D, A ²	6.390	6.819	6.844	5.990 (5.705) ^c
Total D ^{1/2} , A	6.793	6.996	6.583	5.763

Table 30 (Contd.)

Band, D, A^2 $D^{1/2}, A$	System		
	al ph <u>a</u> , bz-H ₂ O	ph <u>b</u> , E	ph <u>b</u> , py
R	0.634	0.387	0.408
	0.796	0.622	0.639
O	0.088	0.100	0.115
	0.297	0.316	0.339
Y	0.055	0.079	0.090
	0.235	0.281	0.300
G ₁	0.090	0.139	0.087
	0.300	0.373	0.295
G ₂	0.173	0.173	G ₂ +G ₃ , 0.330
	0.416	0.416	0.574
B	3.658	2.671	3.399
	1.912	1.634	1.844
BS		1.104	BS+UV ₁ , 1.064
		1.051	1.032
UV	0.784	0.760	0.979
	0.885	0.872	0.989

Table 30 (Contd.)

Band, D, A ² D ^{1/2} , A	System	al ph <u>a</u> , bz-H ₂ O	ph <u>b</u> , E	ph <u>b</u> , py
		G ₃ , 0.052 0.228	G ₃ , 0.099 0.315	
		UV ₃ , 0.517 0.719	B ₁ , 0.430 0.656	
			UV ₁ , 0.364 0.603	
Total D, A ²		6.048	6.310	6.474
Total D ^{1/2} , A		5.788	7.139	6.012

^a Additional bands, present in some systems, are tabulated after the UV band, and designated by the letter preceding the D value.

^b Total green band.

^c Total D with the last cut off at 28571.

Table 31

Changes in Oscillator and Dipole Strengths with Change
of Solvent in Reference to Ethyl Ether

Band, δf % Change δD % Change	Solvent and Compound	Ac, chl <u>a</u>	MeOH, chl <u>a</u>	1 1/2% py-pet, chl <u>a</u>	py, chl <u>a</u>	bz-D, chl <u>a</u>	bz-H ₂ O, chl <u>a</u>	cyclo- D, chl <u>a</u>	cyclo- H ₂ O, chl <u>a</u>	cyclo- D+bz, chl <u>a</u>
R + FR		0	+0.008	-0.011	0	+0.009	0	0	-0.011	+0.013
		0	+4.7	-6.5	0	+5.3	0	0	-6.5	+7.6
		0	+0.122	-0.054	0	+0.090	0	0	-0.058	
		0	+11.9	-5.3	0	+8.8	0	0	-10.7	
O ₁ + O		+0.006	+0.013	+0.010	+0.024	+0.014	0	+0.010	-0.004	+0.014
		+12.2	+26.5	+20.4	+49.0	+28.6	0	+20.4	-8.2	+28.6
		+0.045	+0.089	+0.069	+0.158	+0.096	0	+0.066	0	
		+17.1	+33.8	+26.2	+60.0	+36.5	0	+25.1	0	
Y		+0.004	+0.005	-0.009	-0.005	+0.008	0	0	-0.005	+0.003
		+14.8	+18.5	-33.3	-18.5	+29.6	0	0	-18.5	+11.1
		+0.030	+0.019	-0.038	0	+0.051	+0.012	+0.011	-0.018	
		+22.1	+14.0	-28.0	0	+37.5	+8.8	+8.1	-13.2	
G(Total)		0	-0.006	-0.003	+0.004	-0.008	-0.002	-0.004	-0.003	-0.004
		0	-25.0	-12.5	+16.7	-33.3	-8.3	-16.7	-12.5	-16.7
		+0.013	-0.017	0	+0.010	-0.025	-0.020	-0.006	0	
		+12.6	-16.5	0	+9.7	-24.3	-19.4	-5.8	0	

Table 31 (Contd.)

Band, δ % Change δD % Change	Solvent and Compound	Ac,	MeOH,	1 1/2%	py,	bz-D,	bz-H ₂ O,	cyclo-	cyclo-	cyclo-
		chl <u>a</u>	chl <u>a</u>	py-pet, chl <u>a</u>	chl <u>a</u>	chl <u>a</u>	chl <u>a</u>	D, chl <u>a</u>	H ₂ O, chl <u>a</u>	D+bz, chl <u>a</u>
B ₁ + B		-0.052	-0.147	0	0	-0.075	0	-0.081	-0.081	-0.074
		-10.1	-28.5	0	0	-14.6	0	-15.7	-15.7	-14.4
		-0.176	-0.510	0	0	-0.265	0	-0.284	-0.283	
		-8.7	-25.3	0	0	-13.2	0	-14.1	-14.1	
BS		0	-0.041	0	-0.074	0	-0.063	-0.082	-0.073	-0.065
		0	-10.0	0	-18.1	0	-15.4	-20.0	-17.8	-15.9
		0	-0.271	0	-0.414	0	-0.230	-0.295	-0.251	
		0	-17.8	0	-27.2	0	-15.1	-19.4	-16.5	
UV (Total) ^a		+0.032	+0.116	-0.041	+0.043	0	0	0	0	+0.040
		+7.2	+26.1	-9.2	+9.7	0	0	0	0	+9.0
		+0.173	+0.555	-0.143	+0.309	0	0	0	0	
		+11.4	+36.5	-9.4	+20.3	0	0	0	0	

Table 31 (Contd.)

Band, δf % Change δD % Change	Solvent and Compound	cyclo- H ₂ O + bz, chl <u>a</u>	Ac, chl <u>b</u>	py, chl <u>b</u>	bz-D, chl <u>b</u>	bz-H ₂ O, chl <u>b</u>	bz-H ₂ O, al chl <u>a</u>	py, ph <u>a</u>	bz-H ₂ O, ph <u>a</u>
R + FR		+0.016 +9.4	0 0 -0.032 -4.9	+0.006 +5.6	+0.016 +15.0 +0.089 +13.6	+0.006 +5.6 +0.022 +3.4	+0.008 +5.9 +0.051 +6.2	+0.010 +9.7 +0.069 +11.0	+0.012 +11.7 +0.078 +12.4
O ₁ + O		0 0	0 0 +0.010 +4.4	+0.008 +19.5	+0.009 +22.0 +0.052 +22.8	+0.003 +7.3 +0.014 +6.1	0 0 0 0	-0.003 -8.8 -0.018 -9.3	-0.008 -23.5 -0.041 -21.2
Y		0 0	0 0 0 0	Y+G ₁ +G ₂ -0.010 -19.6	0 0 +0.005 +6.2	0 0 +0.011 +13.6	+0.002 +11.1 +0.010 +10.5	0 0 -0.003 -5.3	-0.002 -18.2 -0.012 -21.0
G(Total)		+0.002 +8.3	0 0 0 0		0 0 -0.007 -4.1	0 0 -0.017 -10.0	0 0 0 +5.7	0 0 +0.026 +5.7	-0.012 -11.5 -0.023 -5.1
B ₁ + B		0 0	0 0 -0.184 -6.1	0 0	0 0 0 0	0 0 -0.301 -10.0	B+BS -0.051 -5.1 0 0	0 0 0 0	-0.048 -6.9 -0.128 -4.9

Table 31 (Contd.)

Band, δf % Change δD % Change	Solvent and Compound	cyclo- H ₂ O + bz, chl <u>a</u>	Ac, chl <u>b</u>	py, chl <u>b</u>	bz-D, chl <u>b</u>	bz-H ₂ O, chl <u>b</u>	bz-H ₂ O, al chl <u>a</u>	py, ph <u>a</u>	bz-H ₂ O, ph <u>a</u>
BS		-0.033 -8.1	BS+UV ₁ 0 0 0 0	0 0	BS+UV ₁ -0.031 -11.5 -0.321 -28.8	BS+UV ₁ -0.023 -8.5 0 0		+0.062 +17.3 +0.228 +18.5	0 0 +0.110 +8.9
UV (Total)		+0.064 +14.4	UV +0.015 +14.3 +0.053 +14.4	+0.100 +33.0	UV +0.037 +35.2 +0.137 +37.4	UV -0.017 -16.2 -0.061 -16.7	+0.027 +12.0 +0.105 +14.0	0 0 +0.103 +8.8	+0.094 +24.1 +0.476 +40.5

Table 31 (Contd.)

Band, δf % Change δD % Change	Solvent and Compound	bz-H ₂ O, al ph <u>a</u>	py, ph <u>b</u>
R + FR		+0.013 +14.4 +0.073 +13.0	+0.004 +6.3 +0.021 +3.4
O ₁ + O		0 0 -0.020 -18.5	0 0 +0.015 +15.0
Y		+0.004 +66.6 +0.021 +61.7	0 0 +0.011 +13.9
G (Total)		0 0 0 0	+0.003 +3.5 0 0

Table 31 (Contd.)

Band, δf % Change δD % Change	Solvent and Compound		
		bz-H ₂ O, al ph <u>a</u>	py, ph <u>b</u>
B ₁ + B		+0.094	+0.118
		+10.0	+15.6
		+0.147	+0.298
		+4.2	+9.6
BS			BS+UV ₁
			-0.093
			-24.6
			-0.404
UV(Total)			-27.5
		-0.045	UV
		-10.1	0
		-0.177	0
		-12.0	+0.219
			+28.8

^aComparisons are made with the last UV cut offs taken at the same wave length.

strength of the first band is about one half that of the second.

For chl a the strength of the B band is consistently greater than that of the BS band except in methanol where they become nearly equal. There is no corresponding change in the low energy band system in methanol; the strength of the UV band is significantly larger than in the other solvents. In solvents where it can be distinguished, the BS band of al chl a shows significantly less intensity than that of chl a. In solvents where the B band is presumably overlapping the BS band in al chl a, the combined intensity is greater than the sum of the B and BS band intensities of chl a. The B band intensity of chl b is significantly greater than that of chl a, while the BS band intensity is significantly smaller.

In the pheophytins the increased strengths in the G bands remarkably alter the appearance of the spectrum and give the following order of decreasing strengths in the low energy region: R , $G_2 + G'_2$, $G_1 \approx 0$, G_3 , and Y . The B band intensity of ph a is significantly larger than that of chl a, while the BS band intensity is smaller. The distances between the BS and B band peaks are about the same for both compounds. As a consequence there is not a well defined minimum between the B and BS bands in ph a as in chl a. In al ph a the BS band is either entirely overlapped by the B band or is of very low intensity, since there is no indication of a peak on the short wavelength side of the B band. The intensity of the B band of al ph a is less than the combined intensities of the B and BS bands of ph a in

ether, but nearly equal in wet benzene. The combined intensity of the B and B₁ bands in ph b is larger than that of the B band in chl b, and the BS band intensity is larger than that of chl b. Ph b shows a well defined minimum between the B and BS bands like chl b.

In EHCl, the strengths of the G bands of ph a and al ph a[†] decrease to the magnitude of those of chl a and al chl a respectively. The order of decrease of band strengths thus reverts to that of the magnesium containing compounds with the exception that the strength of the Y band is less than that of the G₁ band, as for chl b. A similar change for ph b in EHCl was not found, which corresponds to the relatively small changes in peak positions observed.

The variations of the total f and D values, given at the bottoms of Tables 29 and 30, reflect the overall effects of solvent or substituent change on intensity in the spectra. For chl a the most remarkable effect is the increase of total strength when a small amount of benzene is added to the dry and wet chclohexane solutions. It is interesting to note that the decrease of strength in going from chl a to al chl a is quite similar to the decrease in going from ph a to al ph a. In both instances nearly every band undergoes a significant change with the alteration of the isocyclic ring. Thus it appears that the

[†]The f and D values were not determined in EHCl. The data given in Tables 32 and 33 provide for the determination of the optical densities and show the decrease of intensities of the G bands.

perturbations arising from the isocyclic ring can influence transitions polarized along different axes in the chlorin plane.

The comparisons given in Table 31 show that the strengths of individual bands for a given compound can vary considerably with change of solvent. The R, O, Y, and G bands of chl a and the UV band of chl b, for example, undergo both increases and decreases of intensity. For a given solvent change, the bands of the different compounds, at corresponding positions in the spectrum, usually show differences in intensity changes. In some instances changes of the opposite sign occur. As example, for the BS bands of chl a, chl b, ph a, and ph b, in the change from ether to pyridine, the changes in f values are -0.074, 0, +0.062, and -0.093 respectively. In general, one or more of the bands in the upper energy region of each system show the largest absolute changes in intensity. In terms of per cent change, however, there are a number of systems in which bands in the lower energy region (predominantly the Y and O bands) show the largest change, for example, the Y band of al ph a in wet benzene, and the O bands of chl a in pyridine and dry benzene. The order of the sizes of band strength changes varies from system to system. These results give further indication of the differences in specific solvent interactions for each system.

The order of sizes of band strength changes is consistently different from the order of sizes of band peak shifts for a given system. While individual bands sometimes show the

same relative sizes of peak shift and intensity change, there are a number of instances where a large band peak shift is accompanied by little or no change in band strength, and vice versa. As example, the B bands of chl a and chl b show large red shifts in the change from ether to pyridine but the intensities remain unchanged. On the other hand, the BS band of chl a and the Y band of al chl a in the change to wet benzene, and the UV band of chl b in the change to acetone show changes in intensity but not in band position. These results suggest that solvent interactions can produce several types of perturbations. Where band position only is altered, a coulombic (or inductive) effect is probably predominant with little influence on the magnitude of the transition moment. Where band intensity only is altered, a conjugative (or migrational) effect is likely to be predominant with influence primarily on the magnitude of the transition moment. Finally, when position and intensity of a band are changed, both conjugative and coulombic effects are probably occurring.

As shown in Table 31 the intensity changes of neighboring bands and of next neighbor bands are, in most instances, different. For chl a, chl b, and ph a, where changes in two or more solvents can be compared, it can be seen that the differences in changes between neighboring bands and between next neighbor bands usually vary from solvent to solvent. These observations again are consistent with the notion that transitions of different polarization are present in both the upper

and lower energy systems. However, the lack of similarity in the intensity changes of neighboring bands, or of next neighbor bands, does not appear consistent with the suggestion that certain bands are related as vibrational sub-bands of the same transition. For example, the similarity of peak shifts for the R and O bands, and for the B and BS bands of ph a raised the possibility of relationships between these bands. However, the intensity changes of the R and the O bands are different in sign, and those of the B and BS bands differ in magnitude. This seems to be indicative of differences in band polarization.

For chl a, the B and BS bands consistently show fairly large decreases in intensity where changes occur. In pyridine and wet benzene the relative sizes of peak shifts and intensity changes for the B and BS bands show particularly poor correspondence. In dry and wet cyclohexane and benzene the UV band shows no intensity change, which correlates well with the absence of band peak shifts. When changes occur, the UV band shows predominantly small intensity increases which do not correspond well with the relative sizes of the peak shifts. The O band, where changes occur, shows large per cent increases in intensity except in wet cyclohexane where nearly all bands show intensity decreases. The relative sizes of the peak shifts and intensity changes for the O band correspond well in the dry inert solvents, but not in the others. The majority of the intensity changes of the R band are small increases which correspond fairly well with the relative sizes of the peak shifts.

The behavior of the Y band is perhaps the most erratic, both large increases and large decreases of intensity occurring. The total G intensity shows both increases and decreases, but the decreases are predominant.

For chl b, the larger intensity changes are consistently in the UV region, with both increases and decreases occurring. The B and BS bands show either no change or relatively small decreases of intensity. The relative sizes of the peak shifts and the intensity changes for the B band do not correspond, although there is correspondence for the BS band in wet and dry benzene. Where changes occur, the O band consistently shows increases of intensity, which are larger than the increases of the R band. This correlates with the relative sizes of the band shifts in dry benzene but not in the other solvents. Except in pyridine, where the G_1 band has apparently shifted to cover up the Y band, the Y and G bands show little or no change in intensity.

Although there is close correspondence in the orders of size of band shifts for chl a and al chl a in wet benzene, the intensity changes are not closely related. Al chl a shows intensity increases in the R and UV bands, and a decrease in the B band, while there is no change in those bands for chl a.

In comparing ph a vs al ph a in wet benzene significant differences are also observed. While the R bands show comparable increases, the Y, B, and UV bands of the two compounds change in opposite directions.

Comparison of the chlorophylls and the pheophytins in regards to the correlation of orders of sizes of band peak shifts and of intensity changes shows that the overall consistency (or lack of consistency) is about the same for both types of compounds. For the chlorophylls the O, BS, and UV bands tend to show the poorest correlation in relative sizes of changes, while for the pheophytins the Y and G bands tend to show the least correlation. The absolute magnitudes of intensity changes for corresponding bands of the chlorophylls and the pheophytins are about the same (although usually not for the same solvent change). There are some differences in direction of change however. For ph a, the O band consistently shows a decrease in intensity in contrast to the usual increases for the chlorophylls. Intensity increases occur in the BS band of ph a, and in the B bands of al ph a and ph b, while these bands show decreases in the chlorophylls.

Tables 32, 33, 34, and 35

Table 32 lists the peak molar extinction coefficients and the half-widths for the main red and blue bands, the absorbance ratio of the red band peak and the minimum in the green region, the wavelength of the green minimum, and the ratio of the absorbance of the red band peak and that at the wavelength corresponding to the position of the second green band of the pheophytin. These parameters are commonly used for characterization of the compounds. When there is a decrease in molar

Table 32

Spectroscopic Parameters of the Chlorophylls and Several
Derivatives in Various Solvents

System	$\epsilon_R \times 10^{-4}$, liters per mole cm	$\epsilon_B \times 10^{-4}$, liters per mole cm	$R_{1/2}$ m μ	$B_{1/2}$ m μ	$R/\min$	$\lambda_{\min}$ m μ	$R/505^a$
chl <u>a</u> , E	8.68	11.17	17.0	38.0	109	467.5	55
chl <u>a</u> , Ac	7.74	9.41	19.0	44.3	85	474.9	43
chl <u>a</u> , MeOH	6.72	6.40	22.7	87.1	95	476.7	44
chl <u>a</u> , 1 1/2% py-pet	8.52	9.96	16.6	41.0	94	476.3	47
chl <u>a</u> , py	7.97	10.62	19.4	39.5	114	483.0	57
chl <u>a</u> , bz-D	5.53	7.83	30.8	69.6	78	477.5	46
chl <u>a</u> , bz-H ₂ O	7.88	10.07	18.6	40.1	156	475.0	65
chl <u>a</u> , CCl ₄ -D	6.34	8.46	24.1	65.6	57	478.9	40
chl <u>a</u> , CCl ₄ -H ₂ O	7.30	9.14	18.6	45.0	72	476.9	45
chl <u>a</u> , cyclo-D	4.94	7.35	31.8	70.4	54	477.5	29
chl <u>a</u> , cyclo-H ₂ O	6.09	7.83	18.4	45.3	86	474.4	32
chl <u>a</u> , cyclo-D + bz	5.28	7.46	30.7	74.8	52	476.4	29
chl <u>a</u> , cyclo-H ₂ O + bz	7.67	9.99	17.6	42.1	51	475.0	32
chl <u>b</u> , E	5.09	14.31	16.1	21.2	23	497.5	18
chl <u>b</u> , Ac	4.24	12.01	20.1	25.9	17	510.0	17

Table 32 (Contd.)

System	$\epsilon_R \times 10^{-4}$, liters per mole cm	$\epsilon_B \times 10^{-4}$, liters per mole cm	$R_{1/2}$ $m\mu$	$B_{1/2}$ $m\mu$	$R/\min$	$\lambda \min$ $m\mu$	$R/505^a$
chl <u>b</u> , py	4.18	12.80	23.0	25.4	12	526.2	14
chl <u>b</u> , bz-D	3.37	9.79	37.1	34.9	10	519.5	10
chl <u>b</u> , bz-H ₂ O	5.18	13.71	17.2	21.8	20	512.1	19
al chl <u>a</u> , E	5.08	10.08	21.6	36.6	50	470.0	29
al chl <u>a</u> , py	4.74	10.63	24.7	29.1	30	480.00	22
al chl <u>a</u> , bz-D	4.21	9.11	23.8	38.0	42	473.6	26
al chl <u>a</u> , bz-H ₂ O	4.52	9.62	23.4	37.2	45	472.5	25
ph <u>a</u> , E	5.30	10.74	16.6	51.2	18	452.5	5.2
ph <u>a</u> , py	4.85	10.73	20.4	44.9	13	457.5	4.7
ph <u>a</u> , bz-H ₂ O	5.42	11.02	18.4	47.9	19	455.7	5.0
ph <u>a</u> , EHCl	4.14	12.29	27.9	37.8	28	474.2	10.4
al ph <u>a</u> , E	3.69	10.20	20.5	40.7	19	454.4	6.5
al ph <u>a</u> , py	3.19	9.66	24.4	39.6	8.3	458.7	4.5
al ph <u>a</u> , bz-H ₂ O	3.48	10.01	22.6	39.8	16	459.5	4.3
al ph <u>a</u> , EHCl	2.74	9.58	30.8	33.8	17	481.3	8.8
ph <u>b</u> , E	3.18	15.56	16.0	16.2	7.3	472.1	3.0
ph <u>b</u> , py	2.66	13.01	20.6	25.2	5.0	484.0	2.4
ph <u>b</u> , EHCl	2.65	13.54	23.0	29.2	5.5	487.5	3.0

^aFor the b compounds, the ratio R/520 is given.

Table 33

Ratios of Peak Absorbances (d), Oscillator Strengths (f),
and Dipole Strengths (D)

Ratio, d/d', f/f', D/D'	System	chl a, E	chl a, Ac	chl a, MeOH	chl a, 1 1/2% py-pet	chl a, py	chl a, bz-D	chl a, bz-H ₂ O	chl a, CCl ₄ -D	chl a, CCl ₄ -H ₂ O
B _T /R _T		1.29	1.22	0.95	1.17	1.33	1.42	1.28	1.33	1.25
		3.03	2.78	2.07	3.29	3.04	2.46	3.02		
		1.97	1.81	1.32	2.16	2.01	1.57	1.97		
B _T /BS		1.57	1.37	1.12	1.33	1.66	1.35	1.55	1.31	1.45
		1.26	1.12	1.00	1.31	1.54	1.14	1.46		
		1.33	1.20	1.21	1.43	1.90	1.21	1.57		
R _T /O _T		6.75	5.44	4.32	7.03	6.03	3.65	6.40	4.59	5.98
		3.47	3.04	2.87	2.70	2.33	2.84	3.50		
		3.89	3.29	3.25	2.92	2.48	3.10	3.85		
R _T /Y		12.5	9.8	9.4	16.5	13.9	6.85	11.3	9.00	11.5
		6.30	5.39	5.16	8.84	7.73	5.11	6.00		
		7.53	6.00	7.39	9.89	7.81	5.95	6.92		
R _T /G ₁		24.3	22.6	24.7	29.1	31.6	19.6	26.9	18.0	20.6
		11.3	11.1	9.9 ^a	12.2	8.95	11.2 ^a	7.64 ^a		
		14.6	13.0	13.3 ^a	15.1	13.8	14.3 ^a	12.3 ^a		
R _T /G ₂		56.8	44.6		46.9	41.3	19.9	28.3	38.6	46.6
		18.9	20.9		19.9	18.9				
		31.0	26.7		35.4	27.6				

Table 33 (Contd.)

Ratio, d/d' f/f' D/D'	System	chl a, cyclo-D	chl a, cyclo-H ₂ O	chl a, cyclo-D+bz	chl a, cyclo-H ₂ O+bz	chl b, E	chl b, Ac	chl b, py	chl b, bz-D	chl b, bz-H ₂ O
B_T/R_T		1.49	1.29	1.41	1.30	2.81	2.85	3.10	2.90	2.67
		2.58	2.74	2.41	2.81	6.38	6.48	5.86	5.69	6.05
		1.68	1.80			4.60	4.55		4.02	4.01
B_T/BS		1.36	1.33	1.30	1.44	2.71	2.52	2.54	2.48	2.81
		1.32	1.29	1.28	1.39	3.21	2.41 ^b	3.22	2.94 ^b	2.78 ^b
		1.42	1.37			3.56	2.61 ^b		3.77 ^b	2.41 ^b
R_T/O_T		3.54	5.69	3.52	5.88	5.42	4.40	3.68	3.21	6.36
		2.85	3.52	2.90	3.64	2.51	2.42	2.32	2.46	2.57
		3.14	3.79			2.46	2.61		2.65	2.79
R_T/Y		6.8	11.6	6.9	10.8	8.40	7.3		5.57	8.21
		6.00	7.22	6.10	6.65	6.69	6.93		7.69	6.65
		7.02	8.20			8.08	7.76		8.63	7.35
R_T/G_1		17.5	21.5	18.0	22.1	9.60	8.5	7.05	6.19	9.43
		14.0	13.3	15.2	13.3	3.06 ^a	2.97 ^a		4.73	4.35
		17.5	15.1			3.82 ^a	3.64 ^a		5.80	6.25
R_T/G_2		30.6	33.5	34.6	39.1	28.0	17.4	9.55	5.40	20.3
		21.0	17.7	22.9	15.5			12.5	15.4	12.6
		27.2	20.7						20.6	14.7

Table 33 (Contd.)

Ratio, d/d' f/f' D/D'	System									
	al chl E	a, py	al chl bz-D	a, bz-H ₂ O	al chl E	a, py	ph a, bz-H ₂ O	ph a, EHCT	al ph a E	
B_T/R_T	2.00	2.25	2.16	2.13	2.03	2.21	2.03	2.97	2.77	
	5.64			6.65 ^c	6.80	6.27	5.67		10.5	
	3.53			4.14 ^c	4.20	3.83	3.56		6.25	
B_T/BS	2.31	2.30			1.34	1.32	1.38	2.50		
	3.14				1.96	1.69	1.75			
	3.37				2.14	1.83	1.87			
R_T/O_T	5.66	4.10	5.3	5.3	6.56	5.46	6.8	5.20	8.90	
	3.78			4.12	3.03	3.64	4.42		5.62	
	4.12			4.47	3.26	3.99	4.66		5.20	
R_T/Y	10.8	7.59	10.2	9.3	17.5	12.6	20.7	10.3	24.3	
	7.55			7.20	9.36	9.40	12.8		15.0	
	8.69			8.35	11.1	12.9	15.7		16.5	
$R_T/G_1 + G'_1$	14.7	16.8	14.4	14.5	5.20	4.71	5.7	10.3	6.52	
	8.00			6.0 ^a	3.68	3.90	4.79		5.00	
	10.2			7.75 ^a	5.38	4.69	5.90		6.54	
$R_T/G_2 + G'_2$	29.4	52.2	20.8	28.0	4.52	4.26	4.98	18.6	4.56	
	17.0				1.98	2.22	2.30		2.25	
	24.2				2.63	2.92	3.02		3.09	

Table 33 (Contd.)

Ratio, d/d' f/f' D/D'	System	al ph a, py ⁻	al ph a, bz-H ₂ O	al ph a, EHCl ⁻	ph b, E ⁻	ph b, py ⁻	ph b, EHCl ⁻
B_T/R_T		3.02	2.87 10.0 5.77	3.48	4.89 12.00 8.02	4.90 13.10 8.33	5.12
B_T/BS				2.38	2.46 2.74 2.81	2.33 3.06 ^b 3.19 ^b	2.46
R_T/O_T		5.96	7.5 6.44 7.20	3.94	4.44 2.74 3.87	4.17 3.19 3.55	3.36
R_T/Y		9.30	18.2 10.3 11.5	5.34	5.08 3.32 4.90	3.82 3.72 4.53	3.75
R_T/G_1		4.45	4.86 5.72 7.04	8.78	3.03 2.52 2.79	2.74 3.72 4.69	3.01
R_T/G_2		3.47	3.60 2.78 3.66	13.6	2.98 1.66 2.24	2.46 0.96 ^d 1.24 ^d	3.12

^aThe ratio is R_T/G_T . ^bThe ratio is $B_T/BS + UV_1$.

^cThe ratio is $B_T + BS/R_T$. ^dThe ratio is $R_T/G_2 + G_3$

Table 34

The Change of Band Peak Wave Numbers with Change
of Substituent in the Same Solvent

Band, ν δ cm ⁻¹ % Change	Compound vs Compound Solvent			Compound vs Compound Solvent			Compound vs Compound Solvent			Compound vs Compound Solvent		
	chl b vs chl a, E ⁻	chl b vs chl a, py ⁻	chl b vs chl a, bz-H ₂ O	al chl a vs chl a, E ⁻	al chl a vs chl a, py ⁻	al chl a vs chl a, bz-H ₂ O	ph a vs chl a, E ⁻	ph a vs chl a, py ⁻	ph a vs chl a, bz-H ₂ O			
R	+434 +2.86	+340 +2.28	+431 +2.87	+163 +1.08	+201 +1.35	+212 +1.41	157 1.04	0 0	123 0.82			
O	+561 +3.45	+371 +2.29	+568 +3.52	+136 +0.83	117 0.72	+191 +1.18	+126 +0.77	+161 +1.0	+159 +0.98			
Y	+283 +1.63		+326 +1.89	+308 +1.77	88 0.51	+288 +1.67	+483 +2.78	+597 +3.48	+557 +3.23			
G ₁	350 1.86	595 3.21	450 2.40	+300 +1.60	+62 +0.33	+317 +1.69	58 0.31	140 0.76	113 0.60			
G ₂	392 1.96	240 1.22	50 0.25	+440 +2.20	+142 +0.72	+462 +2.32	300 1.50	35 0.18	196 0.98			
B	1268 5.42	1390 6.15	1390 5.65	+607 +2.60	+671 +2.97	+690 +2.98	+1079 +4.51	+1525 +6.75	+989 +4.26			
BS	1129 4.60	1270 5.35	1228 5.00	+1271 +5.18	+971 +4.09		+1121 +4.57	+1625 +6.85	+838 +3.42			
UV	0 0	+275 +1.09	300 1.13	+1088 +4.11	+1650 +7.06	+1040 +3.93	+703 +2.66	+1460 +5.75	+422 +1.60			

Table 34 (Contd.)

Band, δ cm ⁻¹ % Change	Compound vs Compound Solvent	al ph a	al ph a	al ph a	ph b	ph b	ph b	ph b	ph b-EHCl	al ph a
		vs chl a, E	vs chl a, py	vs chl a, bz-H ₂ O	vs chl a, E	vs chl a, py	vs chl b, E	vs chl b, py	vs chl b, E	vs chl a, E
R		232 1.53	37 0.25	190 1.26	+137 +0.91	+310 +2.08	297 1.91	30 0.20	279 1.79	395 2.58
O		0 0	+90 +0.56	+31 +0.19	+401 +2.46	+441 +2.73	160 0.95	+70 +0.42	100 0.60	125 0.76
Y		+383 +2.21	+546 +3.18	+473 +2.74	+543 +3.13	+672 +3.92	+260 +1.47		+352 +2.00	+75 +0.42
G ₁		+105 +0.56	+233 +1.26	+59 +0.31	0 0	+125 +0.67	+340 +1.84	+720 +4.00	+336 +1.82	195 1.02
G ₂		+30 +0.15	+181 +0.92	+24 +0.12	810 4.05	610 3.1	418 2.13	370 1.90	403 2.06	410 2.00
B		+1637 +7.0	+2050 +9.10	+1525 +6.58	298 1.28	+250 +1.11	+970 +4.39	+1640 +7.75	+958 +4.34	+1030 +4.30
BS					194 0.79	+480 +2.02	+935 +4.00	+1750 +7.80	+990 +4.23	
UV		+793 +3.0	UV ₃ +1138 +4.26	+1188 +4.49	+728 +2.76	+1700 +6.70	+675 +2.55	+1435 +5.60	+687 +2.60	295 1.07
							UV ₁ +910 +3.66	UV ₁ +1525 +6.35	UV ₁ +776 +3.12	

Table 34 (Contd.)

Band, $\delta \text{ cm}^{-1}$ % Change	Compound vs Compound Solvent	ph a-EHCl	al ph a	al ph a	al ph a	al ph a	ph b	ph b	ph b
		vs chl a E	vs chl a E	vs ph a E	vs ph a py	vs ph a EHCl	vs ph a E	vs ph a py	vs ph a EHCl
R		87 0.57	58 0.38	75 0.50	52 0.35	+29 +0.19	+294 +1.96	+295 +1.98	+242 +1.61
O		29 0.18	29 0.18	115 0.70	71 0.43	0 0	+275 +1.68	+280 +1.71	+490 +3.02
Y		+307 +1.77	51 0.29	100 0.56	51 0.29	358 2.03	+60 +0.34	+75 +0.42	+328 +1.86
G ₁		108 0.57	150 0.80	+163 +0.87	+373 +2.03	42 0.22	+48 +0.25	+265 +1.44	+94 +0.50
G ₂		0 0	48 0.24	+330 +1.67	+216 +1.10	48 0.24	510 2.59	575 2.93	795 3.97
B		+229 +0.98	+381 +1.63	+558 +2.28	+533 +2.21	+152 +0.64	1377 5.62	1275 5.29	539 2.28
BS		+1046 +4.26	+947 +3.86			97 0.39	1315 5.12	1100 4.35	1185 4.63
UV		+1000 +3.79	+814 +3.08	+90 +0.33		+186 +0.68	0 0	+240 +0.89	260 0.95
				G ₃ 50 0.23	G ₃ +99 +0.47		G ₃ 925 4.30		

^aChanges less than the estimated probable error are omitted. Changes tabulated without sign are negative, that is shifts to the red; changes with plus signs are positive.

Table 35

Changes in Oscillator and Dipole Strengths with
Change of Substituent in Ethyl Ether

Band, δf % Change δD % Change	Compound vs Compound Solvent	chl b	al chl a	ph a	al ph a	ph b	ph b	al ph a	ph b	al ph a
		vs chl E	vs chl E	vs chl E	vs chl E	vs chl E	vs chl E	vs ph E	vs ph E	vs chl E
R		-0.063	-0.034	-0.067	-0.080	-0.107	-0.044	-0.013	-0.040	-0.046
		-37.1	-20.0	-39.4	-47.0	-63.0	-41.1	-12.6	-38.8	-33.8
		-0.369	-0.197	-0.393	-0.461	-0.635	-0.266	-0.068	-0.242	-0.264
		-36.1	-19.2	-38.4	-45.0	-62.0	-40.7	-10.7	-38.5	-32.0
$O_1 + O$		-0.003	-0.013	-0.015	-0.033	-0.026	-0.018	-0.018	-0.011	-0.020
		-16.3	-26.5	-30.6	-67.3	-53.0	-44.0	-53.0	-32.3	-55.5
		-0.035	-0.063	-0.070	-0.155	-0.175	-0.128	-0.085	-0.093	-0.092
		-13.3	-24.0	-27.6	-59.0	-66.5	-56.0	-44.0	-48.4	-46.0
Y		-0.011	-0.009	-0.016	-0.021	-0.008	+0.003	-0.005	+0.008	-0.012
		-40.7	-33.3	-59.3	-77.8	-29.6	+18.7	-45.5	+72.8	-66.6
		-0.055	-0.041	-0.079	-0.102	-0.081	0	-0.023	+0.022	-0.061
		-40.5	-30.2	-63.9	-75.0	-59.5	0	-40.3	+38.6	-64.2
G(Total)		+0.011	0	+0.080	+0.044	+0.061	+0.050	-0.036	-0.019	+0.043
		+45.8	0	+333.0	+183.0	+252.0	+143.0	-34.6	-18.3	+172.0
		+0.068	+0.012	+0.353	+0.204	+0.308	+0.240	-0.149	-0.045	+0.192
		+66.0	+11.6	+343.0	+198.0	+299.0	+140.0	-32.6	-9.9	+167.0
$B_1 + B$		+0.168	+0.251	+0.185	+0.426	+0.241	+0.073	+0.241	+0.056	+0.175
		+32.6	+48.8	+36.0	+83.0	+46.8	+10.7	+34.4	+8.0	+22.8
		+0.990	+0.898	+0.624	+1.496	+1.086	0	+0.872	+0.462	+0.598
		+49.0	+44.5	+31.0	+105.3	+54.0	0	+33.1	+17.5	+20.6

Table 35 (Contd.)

Band, δf % Change δD % Change	Compound vs Compound Solvent	chl b	al chl a	ph a	al ph a	ph b	ph b	al ph a	ph b	al ph a
		vs chl a E	vs chl a E	vs chl a E	vs chl a E	vs chl a E	vs chl b E	vs ph a E	vs ph a E	vs chl a E
BS		-0.197	-0.166	-0.052		-0.134	+0.063		-0.082	
		-48.0	-40.5	-12.7		-32.7	+29.6		-22.9	
		-0.674	-0.654	-0.286		-0.416	+0.258		-0.130	
		-44.3	-43.0	-18.8			+30.5		-10.5	
UV (Total) ^a		-0.141	-0.181	-0.054	-0.075	-0.118	+0.023	0	-0.064	+0.134
		-31.8	-40.8	-12.2	-16.9	-26.6	+7.6	0	-16.4	+59.5
		-0.527	-0.773	-0.345	-0.327	-0.396	+0.131	0	0	+0.446
		-34.7	-50.9	-22.7	-21.5	-27.4	+13.2	0	0	+59.7
Total ^a		-0.245	-0.190	+0.060	-0.150	-0.093	+0.152	-0.210	-0.153	+0.040
		-14.9	-11.6	+3.7	-9.2	-5.7	+10.9	-12.3	-9.0	+3.8
		-0.607	-0.818	-0.190	-0.875	-0.270	+0.337	-0.685	-0.080	-0.057
		-9.2	-12.4	-2.9	-13.3	-4.1	+5.6	-10.7	-1.3	-1.0

^aComparisons are made with the last UV cut offs taken at the same wave length.

extinction coefficient of the peak with change of solvent, an increase in band half-width usually occurs. Only by examining Table 29 or Table 30 can it be determined if the actual strength of the transition is changed, or if the band is broadened thermally by the motions of loosely coupled solvent molecules. For example the B band of chl a has a smaller peak molar extinction coefficient and a greater half-width both in acetone and in wet benzene than in ether. In acetone the band strength is decreased while in wet benzene it is unchanged.

In Table 33 are given ratios of peak absorbances, oscillator strengths, and dipole strengths characterizing the relationships of band intensities for the lower energy band system and the B and BS bands. The subscript "1" with the letters designating the bands means that the total f or D value has been used either where band splitting occurred or where the separation between bands was not distinguished. With the peak absorbance ratios and the molar extinction coefficients of Table 32, the corresponding coefficients of the O, Y, G₁, G₂, and BS band peaks can be calculated.

The integrated intensity ratios provide an accurate appraisal of relative band intensity changes in comparison to the peak absorbance ratios. While these latter ratios tend to change in the same direction as the former, particularly where large changes of intensity occur, there are instances where the peak absorbance ratios are quite misleading. As example, for

chl a in going from ethyl ether to petroleum ether with 1 1/2 per cent pyridine the absorbance ratios for B_T/R_T and B_T/BS indicate decreases while the integrated intensity ratios show increases.

The B_T/R_T ratios undergo quite significant variations with change of solvent. These, in general, differ from compound to compound for a given solvent change, illustrating again the variations in specific solvent effects. Rather striking examples can be seen in comparing chl a and chl b in going from ethyl ether to pyridine where the ratio for chl a remains constant but decreases significantly for chl b, and in comparing chl a, ph a, and al ph a in the change from ethyl ether to wet benzene where the ratio remains essentially constant for chl a and al ph a but decreases significantly for ph a.

The variations of the sizes of the B_T/BS , R_T/O_T , and R_T/Y ratios with change of solvent again reflect the lack of relatedness between the corresponding bands. A more or less constant ratio would indicate an equivalency of perturbation change.

Table 34 shows the change of band peak energy with change of substituent in a given solvent. The plus signs refer to shifts to higher energies; otherwise the changes are to lower energies. The shifts and per cents are in reference to the second compound listed. While the comparisons reflect the patterns of band peak shifts with change of substituent, they also clearly emphasize the dependence of the pattern on solvent. As

example, for al chl a vs chl a in going from ether to pyridine the O and the Y bands shift to the red instead of to the blue, and for ph b vs chl a in going from ether to pyridine the B and BS bands shift to the blue instead of to the red.

In Table 35 are shown the changes in the f and D values of the individual bands with change of substituent pattern. The solvent in all cases is ethyl ether; the tabulated values were obtained by subtracting the particular value of the second listed compound from that of the first. The percentage changes are in reference to the second listed compound.

In comparing the bands of the chlorophylls and the pheophytins (Tables 34 and 35), in the red through the green region of the spectrum, correspondence between the various bands is assumed. This is not entirely in line with the present theory of the spectra. While the R bands always should correspond, it has been suggested for the chlorins (reference [58]) that, in comparing the free base with the metal substituted compound, the orange through green bands do not. Thus in the chlorophylls the O bands are assumed to represent transitions polarized along a different axis than that of the R bands, and presumably overlap the first vibrational sub bands related to the R bands. The Y and G bands would therefore represent overlapping vibrational sub bands associated with both the R and O transitions. As a consequence of the interdigitation of bands in the chlorophylls, absorption in the orange through the green region would be expected to be characterized by very little symmetry and

lack of distinctness of peak positions. The G_1 bands of the pheophytins are presumed to correspond to the O bands of the chlorophylls, and the G_2 bands are supposedly the first vibrational sub bands related to the G_1 bands. The O bands, and possibly the Y bands, of the pheophytins are presumably vibrational sub bands related to the R bands. Thus the substituent change of pheophytinization in essence is supposed to have shifted a band system from the orange to the green region of the spectrum.

The presently available data offer some support for the notion of the relatedness between the O bands of the chlorophylls and the G_1 bands of the pheophytins, however certain empirical difficulties appear. Comparison of the pheophytins with the corresponding chlorophylls in Table 35 shows that intensity increases of the G bands are always accompanied by intensity decreases of the O bands and in two instances by intensity decreases of the Y bands. However, intensity decreases of the O bands are accompanied also by decreases in the R bands. Further, intensity decrease of the O band occurs in other substituent changes (chl b vs chl a, al chl a vs chl a) with little or no increase of G band intensity. The G_2 bands of the pheophytins are always greater in intensity than the G_1 bands, whereas the O bands of the chlorophylls are always greater than the Y bands. Except for ph b, the peak positions of the G_1 and G_2 bands of the pheophytins are roughly equivalent to the

positions of the G_1 and G_2 bands of the corresponding chlorophylls. Thus the increase of G band intensity in the pheophytins could be visualized as intensification of already existing bands in the chlorophylls corresponding to the same transitions, rather than the shift of an entire band system from one spectral region to another. In general, the band symmetries for the chlorophylls, in the orange through the green region, did not give the appearance of complex interdigitation of band systems; the pheophytins, in fact, showed somewhat less symmetry in the O, Y, and G bands. The occurrence of splitting of the O bands of chl a and chl b might be interpreted as the partial resolution of band system interdigitation. However, there is a somewhat similar splitting of the G bands of ph a in a region where no interdigitation is supposed to occur. Such splittings are absent for al chl a and al ph a, which suggests that the intact cyclopentanone ring may play a role in band splitting.

The comparisons in Table 34 show the following characteristic variations in the lower (red through green) band system. For chl b vs chl a there is a general moving together of the bands, although an increase occurs between the R and the O bands. A similar moving together is observed in ph b vs ph a, but the distances between the R and the O bands remain about equal. These changes result from the change of substituent from a $-CH_3$ group (for the a compounds) to a $-CHO$ group (for the b compounds) at the 3-position on the chlorin ring.

According to the present theory of substituent effects

(reference [58]), the presence of a -CHO group at the 3-position is expected to reduce the relative intensity and the relative energy differences between the two band systems in the visible that are polarized along different axes in the chlorin ring. The observed moving together of the red through green bands seems to be in general agreement with theory. A difficulty would appear to be the separation of the R and the O bands for chl b vs chl a. Comparison of the R/O, R/Y, and R/G intensity ratios in Table 33 shows that there are substantial decreases (except in R/Y for chl b vs chl a) in going from the a to the b compounds, which is in general agreement with the prediction of reduction of intensity differences.

For al chl a vs chl a, the general shift to the blue is accompanied by a separation of the R and G bands and the O and Y bands, with the O-R and G_1 -Y distances showing relatively little change (except in pyridine). For al ph a vs ph a, while there is only a blue shift of the G bands, separations of the R and G bands, the Y and G bands, and the O and Y bands occur, with the O-R distance undergoing a decrease. These variations result from the alteration of the cyclopentanone ring that occurs with allomerization. The differences in changes of al chl a vs chl a and al ph a vs ph a suggest that the substituent effects of the magnesium atom and the isocyclic ring may not be entirely independent.

For ph a vs chl a there is separation between the R and the G_1 bands, the O and the R bands, and the O and the Y bands,

with a decrease in the G_1 -Y distance. Comparison between al ph a and al chl a shows a similar pattern of change. For ph b vs chl b similar variations are observed except that there is a slight increase in G_1 -Y rather than a decrease. These changes result from the replacement of the magnesium atom by two hydrogen atoms in the center of the chlorin ring.

In the present theory of the porphyrin spectra (references [57] and [58]) it is suggested that substituent perturbations can increase intensity in the lower band system by mixing the highly allowed states of the upper band system with those of the nearly forbidden lower band system. Thus, in effect, intensity is borrowed from the allowed transitions. Theory accounts only for the mixing of transitions polarized along the same axis. If transitions polarized along different axes are independent of each other, and if a given substituent change has effect predominantly along only one axis, a simple relationship between intensity changes in the upper and lower band systems might be observed. A possibility is that a certain band in the upper system might consistently show increases when a certain band in the lower system consistently decreases (or vice versa), while little change occurred in other sets of bands.

In Table 35 the following can be observed. Chl a has the strongest absorption for the R, O, Y, BS, and UV bands. The pheophytins have the strongest absorption in the G bands, and ph b has the strongest absorption in the B band. A change in substitution that results in decrease of intensity of the R band

also results in intensity decreases in the O and Y bands, except in comparing ph b with chl b, and ph b with ph a where there are increases in intensity in the Y band. The R band fairly consistently shows larger absolute (though not per cent) decreases in intensity than the Y and the O bands. Decreases of intensity in the R, O, and Y bands are consistently accompanied by an increase of intensity in the B band, and a decrease in intensity in the BS band, except in comparing ph b and chl b where an increase in the BS band as well as in the B band occurs. These concurrent changes may represent a correlation in support of present theory in that mixing of upper and lower states can occur. Because of the fact that none of the band intensities remain constant for a particular substituent change, no simple relationship between a given upper band and a given lower band is apparent. The concurrent changes in the various bands clearly indicates that the substituent changes considered have effects along more than one axis in the chlorin ring.

In Table 31, showing the effect of solvent change, there are several instances where an intensity increase in the R band is accompanied by a decrease in the B band. Examples are chl a-MeOH and ph a-wet benzene. These effects, though smaller than the effects of substituent change, may represent examples where solvent perturbations can influence the mixing of upper and lower states.

Referring to Tables 34 and 35 the following empirical relationships are observed. An intensity decrease of the R band

is accompanied by a blue shift for chl b vs chl a, al chl a vs chl a, ph b vs chl a, and ph b vs ph a, but by a red shift for ph a vs chl a, al ph a vs chl a, al ph a vs al chl a, ph b vs chl b, and al ph a vs ph a. Decreases of intensity in the O and Y bands are accompanied fairly consistently by blue shifts except for the O bands of ph b vs chl b and al ph a vs al chl a in ether. Increase of intensity of the B band and decrease of intensity of the BS band are accompanied by red shifts for chl b vs chl a and ph b vs chl a in ether, but by blue shifts in the other systems.

As shown in Table 33, change of substituent pattern that results in a relative increase (or decrease) of B_T/T_T is also accompanied, predominantly, by corresponding increases (or decreases) of B/BS and R/Y (R/O shows the same number of increases as decreases). For some of the solvent changes with respect to ethyl ether for a given compound, there is a similar relation between the changes of B_T/R_T , B/BS, and R/Y. The systems showing this are chl a-Ac, chl a-MeOH, chl a-1 1/2 per cent py-pet, chl a-bz-D, and ph b-py. This again suggests the possibility that changes in solvent perturbations can have a similar (though weaker) effect as change in substituent perturbation in mixing upper and lower energy states.

Table 36

In Table 36 are shown the distances, in cm^{-1} , between neighboring band peaks, and the distances between various bands

Table 36

Distances Between Band Peaks

Band- Band, cm ⁻¹	System								
	chl a, E	chl a, Ac	chl a, MeOH	chl a, 1 1/2% py-pet	chl a, py	chl a, bz-D	chl a, bz-H ₂ O	chl a, CCl ₄ -D	chl a, CCl ₄ -H ₂ O
O-R	1137	1125	1176	1184	1269	994	1124	1028	1128
Y-O	1078	1013	983	989	959	1117	1094	1176	1075
G ₁ -Y	1433	1479	1369	1451	1372	1561	1476	1610	1581
G ₂ -G ₁	1200	1280		1149	1135	1222	1162	1299	1299
B-G ₂	3378	3222		3174	2925	3221	3248	2952	3011
BS-B	1151	1177	1262	1185	1135	1060	1330	1169	1240
UV-BS	1868	1759	1651	1850	1630	2059	1982	2248	2118
B-R	8226	8119	8090	7947	7660	8115	8104	8065	8094
BS-R	9377	9296	9352	9132	8795	9175	9434	9234	9334
UV-R	11245	11055	11011	10982	10425	11234	11416	11482	11452
B-G ₁	4578	4502	4562	4323	4060	4443	4410	4251	4310
BS-G ₁	5729	5679	5824	5508	5195	5503	5740	5420	5550
B-O	7089	6994	6914	6763	6391	7121	6980	7037	6966
BS-O	8240	8171	8176	7948	7526	8181	8310	8206	8206
				O-O ₁ 382	O-O ₁ 569	R-FR 360		F-FR 409	
					UV ₃ -UV 1290	E-B ₁ 896			
					UV ₄ -UV ₃ 2560				

Table 36 (Contd.)

Band Band, cm ⁻¹	System	chl a, cyclo-D	chl a, cyclo-H ₂ O	chl a, cyclo-D+bz	chl a, cyclo-H ₂ O+bz	chl b, E	chl b, Ac	chl b, py	chl b, bz-D	chl b, bz-H ₂ O
O-R		968	1072	1000	1111	1264	1284	1300	1263	1261
Y-O		1155	1116	1134	1093	800	845		908	852
G ₁ -Y		1542	1420	1525	1455	800	684		674	700
G ₂ -G ₁		1170	1170	1308	1208	1158	1195	1490	1632	1562
B-G ₂		3359	3478	3223	3354	2502	2401	1775	1913	1989
BS-B		1051	1002	1063	1096	1340	1304	1255	1429	1411
UV-BS		2032	1950	2054	1866	3050	3170	3175	2872	2910
B-R		8164	8226	8190	8221	6524	6409	5930	6290	6364
BS-R		9215	9228	9253	9317	7864	7713	7185	7719	7775
UV-R		11247	11178	11307	11183	10864	10883	10360	10589	10685
B-G ₁		4499	4618	4531	4562	3660	3596	3265	3545	3551
BS-G ₁		5550	5620	5594	5658	5000	4900	4520	4974	4962
B-O		7196	7154	7190	7110	5260	5125	4630	5127	5103
BS-O		8247	8156	8253	8206	6600	6429	5885	6556	6514
	R-FR		R-FR	R-FR	R-FR	O-O ₁	O-O ₁	UV-UV ₁	R-FR	O-O ₁
	290		562	287	459	500	476	1650	448	465
	B-B ₁		B-B ₁	B-B ₁	B-B ₁	UV-UV ₁	UV-UV ₁	UV ₃ -UV	O-O ₁	UV-UV ₁
	1042		1048	1087	1132	1585	1625	1200	386	1460
	BS ₂ -BS		BS ₂ -BS	BS ₂ -BS	BS ₂ -BS	UV ₃ -UV	UV ₃ -UV	UV ₄ -UV ₃	B-B ₁	UV ₃ -UV
	350		421	466	395	1550	1275	1975	1163	1339
									UV ₃ -UV	
									1149	

Table 36 (Contd.)

Band- Band, cm ⁻¹	System								
	al chl a, E	al chl a, py	al chl a, bz-D	al chl a, bz-H ₂ O	ph a, E	ph a, py	ph a, bz-H ₂ O	ph a, EHCl	al ph a, E
O-R	1110	951	1069	1103	1420	1415	1406	1195	1380
Y-O	1250	988	1272	1191	1435	1395	1492	1414	1450
G ₁ -Y	1425	1522	1535	1505	892	635	806	1018	1145
G ₂ -G ₁	1340	1315	1312	1307	958	1240	1079	1308	1125
B-G ₂	3545	3454	3337	3476	4757	4485	4433	3607	4985
BS-B	1815	1435			1193	1190	1179	1968	
UV-BS	1685	2309			1450	1650	1566	1822	
B-R	8670	8130	8525	8582	9462	9170	9216	8542	10095
BS-R	10515	9565			10655	10360	10395	10510	
UV-R	12170	11874	12328	12244	12105	12010	11961	12332	12270
B-G ₁	4885	4669	4649	4783	5715	5725	5512	4915	6110
BS-G ₁	6700	6104			6908	6915	6691	6883	
B-O	7560	7183	7456	7479	8042	7755	7810	7347	8715
BS-O	9375	8614			9235	8945	8989	9315	
	UV ₃ -UV				O-O ₁	G' ₁ -G ₁	G ₃ -G ₂		G ₃ -G ₂
	805				675	500	1406		1370
					G' ₂ -G ₂	G' ₂ -G ₂			UV ₃ -UV
					465	310			1420
					G ₃ -G ₂	G ₃ -G ₂			
					1750	1525			

Table 36 (Contd.)

Band- Band, -1 cm	System	al ph a, py ⁻	al ph a, bz-H ₂ O ⁻	al ph a, EHCl ⁻	ph b, E ⁻	ph b, py ⁻	ph b, EHCl ⁻
O-R		1396	1345	1166	1401	1400	1443
Y-O		1415	1536	1056	1220	1190	1252
G ₁ -Y		1059	1062	1334	880	825	784
G ₂ -G ₁		1083	1227	1302	400	400	419
B-G ₂		4802	4749	3807	3890	3785	3863
BS-B				1719	1255	1365	1322
UV-BS				1733	2790	2850	2747
B-R		9755	9819	8665	7791	7600	7761
BS-R				10384	9046	8965	9083
UV-R			12304	12117	11836	11815	11830
B-G ₁		5885	5876	5109	4290	4185	4282
BS-G ₁				6828	5545	5550	5604
B-O		8359	8474	7499	6390	6200	6318
BS-O				9218	7645	7565	7640
		G ₃ -G ₂	G ₃ -G ₂		G ₃ -G ₂	UV-UV ₁	B-B ₁
		1408	1303		1335	1550	693
			UV ₃ -UV		B-B ₁		UV-UV ₁
			1329		745		1496
					UV-UV ₁		
					1350		

in the upper and lower energy systems which characterize their relative separation with variation of solvent. For the solvent change from ether to pyridine, the chlorophylls show greater variations in separations between the upper and lower bands than the pheophytins, while in going from ether to wet benzene ph a and al ph a generally show larger changes. Ph a and al ph a, in the change from ether to EHCl , show the largest changes in the B-R distance.

The distances between neighboring bands for a given compound in general show fairly significant variations with change of solvent. This is again indicative of lack of relatedness between bands, which for the R and O bands of the chlorophylls is in agreement with theory. Some exceptions are the O-R and BS-B distances for ph a, and the O-R and G_2-G_1 distances for ph b. It was pointed out above that the intensity change relationships for the O, R, and BS, B bands for ph a did not appear consistent for band relatedness. The R and O bands of ph b show nearly the same changes in intensity. This, with the relative constancy of O-R, suggests that the bands may be related, in agreement with theory. The magnitude of the G_2-G_1 distance for ph b, however, seems too small to represent a vibrational mode of the rigid chlorin ring. Similarly the $O-O_1$, R-FR, and BS_2-BS distances of the chlorophylls, and the $G'-G$ distances of ph a appear too small to pertain to vibrations of the chlorin ring. The G_2-G_1 distances for ph a and al ph a

show variations with change of solvent that are as large as those occurring for the chlorophylls, and differ significantly from each other in a given solvent. These observations indicate that the G_1 and G_2 bands are not related vibrational subbands as suggested by theory.

In an early interpretation of the chl a spectrum (reference [2], p. 630) it was suggested that the R, O, Y, G_1 , and G_2 bands were all related vibrational bands belonging to the same electronic transition. The differences in distances between successive neighboring band pairs for a given solvent, as well as the variations with change of solvent would clearly seem to rule out this interpretation. Similar observations, for the other compounds, on neighboring band pair relationships, offer support for the present theoretical interpretation in that the visible band systems must comprise at least two differently polarized transitions.

Comparison between different compounds shows that a given interband distance is usually characteristically different for each compound. In ethyl ether, the distances between neighboring band peaks show the following relative size relationships. For the O-R distance, $ph \underline{a} \approx ph \underline{b} > al \ ph \underline{a} > chl \underline{b} > chl \underline{a} \approx al \ chl \underline{a}$; for the O-Y distance, $al \ ph \underline{a} \approx ph \underline{a} > al \ chl \underline{a} \approx ph \underline{b} > chl \underline{a} > chl \underline{b}$; for the G_1 -Y distance, $chl \underline{a} \approx al \ chl \underline{a} > al \ ph \underline{a} > ph \underline{a} \approx ph \underline{b} > chl \underline{b}$; for the G_2 - G_1 distance, $al \ chl \underline{a} > chl \underline{a} > chl \underline{b} > al \ ph \underline{a} > ph \underline{a} > ph \underline{b}$; for the BS-B

distance, $al\ chl\ \underline{a} > chl\ \underline{b} > ph\ \underline{b} > ph\ \underline{a} \approx chl\ \underline{a}$: and for the UV-BS distance, $chl\ \underline{b} > ph\ \underline{b} > chl\ \underline{a} > al\ chl\ \underline{a} > ph\ \underline{a}$. As a consequence of the variations is specific solvent effects there is a marked dependency on the solvent in which the comparisons are made. For example, in comparing $al\ chl\ \underline{a}$ with $chl\ \underline{a}$ the difference in the UV-BS distances varies from $-183\ cm^{-1}$ in ether to $+679\ cm^{-1}$ in pyridine, and the difference in the O-R distances varies from $-27\ cm^{-1}$ to $-318\ cm^{-1}$.

Present theory of the effect of change of substituents suggests a correlation between intensity changes in the bands of the lower energy system and their separation from the bands of corresponding polarization in the upper energy system (reference [58]). Increase (or decrease) of intensity should be accompanied by increase (or decrease) of separation. It has been implied (reference [59]) that in the chlorophylls, the O band corresponds with the B band and the R band corresponds with the BS band. Therefore, in the pheophytins the G_1 band presumably should correspond to the B band in polarization. If substituent effects are independent, and if states of different polarization are not mixing, then the following simple relationships might be expected: 1) a decrease of the R band intensity should be accompanied by decrease of BS-R in both the pheophytins and the chlorophylls, 2) a decrease in O band intensity should be accompanied by a decrease in B-O for the chlorophylls, and 3) a decrease in G band intensity should be accompanied by a decrease in B- G_1 in the pheophytins.

Referring to Tables 36 and 35, the following are observed. For chl b vs chl a the intensities of the R and O bands decrease, and the BS-R and B-O distances decrease, in agreement with theory. For al chl a vs chl a the intensities of the R and O bands decrease, but BS-R and B-O increase which is not in agreement with theory. For ph a vs chl a R band intensity decreases, but BS-R increases, in disagreement with theory. However, the increase of G band intensity is accompanied by an increase of B-G₁ in agreement with theory. Similar relationships occur for ph b vs chl b and for al ph a vs al chl a. In the latter instance, it is assumed that the BS band of al ph a has essentially coalesced with the B band, and the B-R distance is found to be smaller than the BS-R distance of al chl a. For ph b vs ph a, the intensities of the R and G bands decrease and BS-R and G-G₁ decrease, in agreement with theory. For al ph a vs ph a, the intensities of the R and G bands decrease. The BS-R distance of ph a is larger than the B-R distance of al ph a, in agreement with theory. The B-G₁ distance of al ph a is greater than that of ph a which is in disagreement with theory. From these comparisons, it appears that the substituent change effects, corresponding to pheophytinization and allomerization, still pose problems for theoretical consideration.

Similar comparisons, referring to Tables 36 and 31, between changes in band intensities and interband distances arising from solvent changes for a given compound show that,

in 21 instances out of 28, intensity increases (or decreases) are accompanied by interband distance decreases (or increases). Empirically these changes, which are not in accord with theory, resemble those occurring for pheophytinization and allomerization. This suggests the possibility that specific solvent interactions may be predominant at the center of the chlorin ring and/or the isocyclic ring.

SUMMARY

Quantitative determination was made of the absorption spectra, in the visible through the near ultraviolet, of chl a, chl b, al chl a, ph a, al ph a, and ph b in a number of common solvents. Band peak energies and interband separations are given for 33 systems, the oscillator strengths of the individual bands for 25 systems, and the dipole strengths for 22 systems. These data characterize in detail the variations in absorption of the compounds, and reveal interesting differences in solvent effects. While the data are limited in scope, they provide information of value for the eventual detailed interpretation of the spectra. The current theoretical interpretations of the porphyrin spectra are briefly summarized and comparisons are made between certain predictions and experimental observations.

For each compound, the effects of solvent changes on the individual band positions and the integrated band intensities are examined. Comparisons are made with ethyl ether as the reference solvent. In almost every system, both the change in band position and the change in band intensity differ significantly for different bands. Thus the data, in general, do not provide evidence that any of the neighboring bands, or next

neighboring bands, are related as vibrational sub bands belonging to the same electronic transition. This is in partial agreement with present theory in the prediction that there are two independent transitions, polarized along different axes in the chlorin ring, in both the upper and lower energy band systems. The observations do not support present theory in that, for the lower energy system, certain pairs of bands have been assigned as companion vibrational bands belonging to the same electronic transitions. It may be that more than two electronic transitions are present in the lower band system, a possibility not accounted for in present theory.

For the different compounds in a given solvent, comparisons are made of peak positions and intensities of the various bands at corresponding positions in the spectrum. Each substituent change, corresponding to the intercomparison of two different compounds, shows significant effects, in nearly every case, on all of the bands, and thus on transitions polarized along different axes. The variations observed for the substituent change from a $-\text{CH}_3$ group to a $-\text{CHO}$ group at the 3-position on the chlorin ring (i.e. from the a derivative to the b derivative) are in general agreement with the effects predicted by theory. The substituent changes corresponding to allomerization and pheophytinization, however, produce some variations that are not in accord with present theory.

For the substituent changes studied, it is found that intensity decreases in the R, O, and Y bands (in the lower

energy system) are consistently accompanied by an increase of intensity in the B band (in the upper energy system), and, in most instances, by decrease of intensity in the BS band (in the upper energy system). These concurrent changes may represent a correlation in support of present theory in the prediction that intensity may be gained in the lower energy band system from the upper band system through the perturbational mixing effects of substituents. Several instances are observed where change of solvent causes an intensity increase of the R band accompanied by a decrease of intensity in the B band. These effects suggest that solvent perturbations can influence the mixing of upper and lower states.

The observed effects of substituent and solvent changes on band intensities and positions do not suggest definitive relationships between upper and lower bands in regards to the directions of polarization of the transitions in the chlorin ring. Thus, consistently similar variations of a certain upper band and a certain lower band, in relationship to the other bands, are not found. In the case of substituent effects, concurrent changes in nearly all the bands obscure a relationship. With different solvent changes for a given compound, the orders of sizes of band changes differ from system to system and a consistent pattern is not observed. The absence of definite relationships may arise from substituent effects not being independent of each other, or from the lack of independence of transitions polarized along different axes.

The ranges of per cent change observed are as follows: for solvent effects on band peak positions 0-5.1 per cent with an average of about 1.0 per cent, for solvent effects on band intensities 0-66.6 per cent with an average of about 10 per cent, for substituent effects on band peak positions 0-9.1 per cent with an average of about 2.1 per cent, and for substituent effects on band intensities 0-333.0 per cent with an average of about 50 per cent. As might be expected substituent effects are on the average significantly larger than solvent effects although both effects show wide variations in size.

The effect of the solvent, ethyl ether saturated with dry HCl gas, on ph a and al ph a was to make their spectra resemble those of chl a and al chl a, respectively. Fluorescence intensity was significantly reduced, as was observed for the chlorophylls in pure, dry nonpolar solvents. This indicates that protons were introduced into the centers of the chlorin rings of the pheophytins producing substituent patterns like those of the corresponding chlorophylls.

Small amounts of benzene in both dry and wet cyclohexane caused significant increases of intensity throughout the spectra of chl a. This indicates that specific solvent interactions are important in producing spectral changes, and suggests that a systematic study of the various compounds in mixtures of inert and active solvents would provide useful information toward understanding the various solvent effects.

Comparison of the effects of a given solvent variation for

al chl a vs chl a and for al ph a vs ph a shows that alteration of the isocyclic ring leads to significant differences in spectral changes. This indicates that specific solvent interactions occur at the isocyclic ring. The changes for al chl a vs chl a are different from those for al ph a vs ph a, suggesting that the substituent perturbations of the magnesium atom and of the isocyclic ring may not be independent.

In comparing the effects of a given solvent change on the chlorophylls and on the corresponding pheophytins, significantly different changes for corresponding bands are usually observed. With intensity variations, differences in direction of change occur in some instances. The changes in band peak positions for the chlorophylls are generally larger than for the pheophytins. These observations suggest that specific solvent interactions at the magnesium atoms in the chlorophylls are important.

In several aspects the effects of change of solvent were observed to be similar to the effects of substituent change. Thus there are a number of instances where solvent change resulted in an increase of the band intensity ratio B/R accompanied by increases in the intensity ratios B/BS and R/Y . In three-fourths of the observed solvent effects, increases (or decreases) of intensity of certain bands in the lower energy system (i.e. the R band for both the chlorophylls and the pheophytins, the O band for the chlorophylls, and the G_1 band for the pheophytins) were accompanied by decreases (or increases) in the distances between certain corresponding upper and lower

bands (i.e. BS-R for the chlorophylls and the pheophytins, B-O for the chlorophylls, and B-G₁ for the pheophytins). These concurrent changes resemble the variations occurring with pheophytinization and allomerization, and suggest the possibility that specific solvent interactions may be predominant at the center of the chlorin ring and/or the isocyclic ring.

Splitting of the R and O bands of chl a and chl b, and the G bands of ph a was observed in certain solvents. No splitting, however, was seen in the corresponding allomerized compounds. This suggests that the perturbations of the unaltered isocyclic ring play an essential role in the splitting.

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