carbon to which the cyano group is attached. If induction is of sufficient importance to shorten the distance R between the dipole center and carboxyl group by as little as 0.5 Å., ΔpK (calcd.) is increased by more than 50%.

It is probable that at least part of the general discrepancy between the calculated and found ΔpK values in Table VI is real and reflects the observation[23] that the Kirkwood–Westheimer treatment predicts too small a dependence of ΔpK values on solvent dielectric constant. If the differences between calculated and found figures for ΔpK are real, they indicate either the magnitude of the bond-transmitted inductive effect or the degree of inadequacy of the Kirkwood–Westheimer treatment.[24]

The electrostatic treatment is effective in accounting for the differences in ΔpK for 4-bromobicyclo[2.2.2]octane-1-carboxylic acid and δ-bromovaleric acid which are 0.67 and about 0.12,[25] respectively. The effective dielectric constant for the long, thin δ-bromovaleric acid is expected to be much larger than for the relatively fat bicyclo acid. Furthermore, free rotation in the aliphatic acid can result in orientations of the C–Br dipole which would not enhance the acidity of the carboxyl group. On the basis of the inductive effect, the only difference between the two acids is that in the bicyclic acid there are more chains of carbon atoms for the effect to travel from the substituent to the carboxyl group. At most, the inductive effect alone can account for about half of the difference in ΔpK between the aliphatic and bicyclic acids.

Electrostatic calculations of the effects of substituents on the rates of alkaline hydrolysis of ethyl 4-bromo- and 4-cyanobicyclo[2.2.2]octane-1-carboxylates give results similar to those for the ΔpK values for the corresponding acids. At present, we have no explanation for the difference between ρ and ρ' for the reactions of the acids with diphenyldiazomethane. It may be significant that ρ/ρ' for the reactions so far studied (Table V) bears an inverse relation to the solvent dielectric constant.

Acknowledgment.—We are grateful to Professor F. H. Westheimer for many suggestions and helpful discussions regarding the interpretation of the experimental results.

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[23] J. H. Elliott and M. Kilpatrick, *J. Phys. Chem.*, **45**, 472 (1941); M. Kilpatrick and W. H. Mears, THIS JOURNAL, **62**, 3047, 3051 (1940). See ref. 12b and F. H. Westheimer and J. G. Kirkwood, *Trans. Faraday Soc.*, **43**, 77 (1947), for discussions.

[24] (a) Professor F. H. Westheimer has offered the following comments with respect to the results of the present research. "Roberts' and Moreland's data for the bicyclooctane system clearly show that equations based on our cavity model[12,13] somewhat underestimate the effect of substituents on ionization constants. It should be noted, however, that whereas our equations underestimate the observed effects by a factor of about 2, the older Bjerrum–Eucken equation underestimates the observed effects by a factor of about 10; the newer approximation is therefore better than the older one by almost an order of magnitude. Furthermore, a logical extension of the electrostatic theory would lead to an approximation closer to the observed value of ΔpK than that obtained from the equations Kirkwood and I developed, since such an approximation might take into account the effect of electrical saturation in the solvent. This effect would necessarily decrease D_E and therefore increase the calculated value of ΔpK. Our equations are also limited by the mathematically convenient but physically unrealistic requirement that all charges or dipoles must lie at the foci of ellipsoidal cavities. Although the best approximation currently available underestimates the influence of dipoles in the bicyclooctane series, it is probable that a large part of the influence of substituents on ΔpK can be ascribed to simple electrostatic effects. It remains an open question whether any electrostatic theory which ignores the detailed structure of the substituted molecule can do better than yield a crude approximation to the observed results." (b) The relatively greater discrepancies between calculated and found ΔpK values for the aromatic acids suggests that a lower internal dielectric constant D should be used for such compounds.

[25] Computed from ΔpK for δ-bromovaleric acid in water at 25° which is 0.07, W. A. Waters, "Physical Aspects of Organic Chemistry," 4th Ed., D. Van Nostrand Co., Inc., New York, N. Y., 1950, p. 243, and the usual approximate proportionality of ΔpK to the reciprocal of the solvent dielectric constant.

[CONTRIBUTION FROM THE DEPARTMENT OF PHYSICS, THE OHIO STATE UNIVERSITY, AND THE SCHOOL OF CHEMISTRY, INSTITUTE OF TECHNOLOGY, UNIVERSITY OF MINNESOTA]

Infrared Spectra of Chlorophyll and Related Compounds[1]

BY JOHN W. WEIGL AND ROBERT LIVINGSTON

RECEIVED JANUARY 16, 1953

The infrared spectra of chlorophylls-a and -b, pheophytin-a, bacteriochlorophyll-a and allomerized chlorophyll-a are reported. A partial analysis of these complex spectra has been attempted.

In 1933, Stair and Coblentz[2] published infrared spectra of chlorophyll and certain related compounds, such as ethyl chlorophyllide, phytol and pheophytin. Since that time there has been sufficient progress in the fields of chlorophyll chemistry and infrared spectroscopy to warrant repetition of this work. While the new investigation provides substantially improved data, which permit more detailed interpretation, it also confirms the earlier, remarkably good, results.

Five compounds were examined: chlorophyll-a; chlorophyll-b, which differs in having a formyl, instead of a methyl, group at carbon 3; pheophytin-a, in which the central magnesium atom of the chlorophyll is replaced by two hydrogens; bacteriochlorophyll, in which ring II has been reduced and the vinyl radical at carbon 2 oxidized to an acetyl group; and finally, "allomerized" chlorophyll-a, which, according to Fischer[3] is chlorophyll-a whose

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[1] This work was made possible by the support of the Office of Naval Research (NR 051 028, Contract N6ori-212, T.O. I) to which the authors are indebted.

[2] (a) R. Stair and W. W. Coblentz, *J. Research Natl. Bur. Standards*, **11**, 703 (1933). (b) Compare E. I. Rabinowitch, "Photosynthesis," Vol. 2, Interscience Publ., Inc., New York, N. Y., 1951, pp. 610-612.

[3] H. Fischer and M. Strell, "FIAT" Rev. of German Science, Vol. 39 (1947), pp. 141-186; H. Fischer and H. Pfeiffer, *Ann.*, **555**, 94 (1944).

lone hydrogen at carbon 10 has been oxidized and replaced by an ether linkage.[4] Comparison of the spectra of these compounds with each other, and with new absorption curves for phytol and ethyl chlorophyllide made it possible to undertake a partial analysis of these complex spectra.

Experimental Methods and Materials

The pigments were prepared and purified as follows: Chlorophylls-a and -b were prepared by a modification[5] of a method of Zscheile. Residual traces of pheophytin and alcoholic impurities were removed by adsorbing a petroleum ether solution of the pigment on a column of paper pulp, washing with benzene and petroleum ether and eluting with anhydrous ether.

Pheophytin-a was prepared by an adaptation of the procedure of Mackinney and Joslyn[6]: a 2×10^{-4} *M* ether solution of chlorophyll-a was acidified (to 0.63×10^{-3} *M*) with sulfuric acid. After two hours, the acid was extracted with distilled water, the solution diluted with petroleum ether, and the pigments adsorbed at the top of a paper pulp column. The pheophytin was selectively eluted with benzene, the eluate concentrated under vacuum with petroleum ether, re-adsorbed on a similar column and eluted, free of chlorophyll and of benzene, with anhydrous ether.

Allomerized chlorophyll-a was prepared by a method used by Watson and Livingston[7]: 2.7×10^{-5} *M* chlorophyll-a was allowed to react with atmospheric oxygen 3.5 hours at room temperature, in a 0.7×10^{-1} *M* solution of lanthanum chloride in dry methanol. The product was adsorbed on paper pulp and eluted with anhydrous ether. It gave no phase reaction when tested with saturated methanolic KOH; its spectrum matched that observed by Watson.[7] The pheophytin of allomerized chlorophyll-a was prepared from the latter by a method similar to that used for pheophytin-a, again followed by chromatographic purification.

Bacteriochlorophyll was prepared from ***Rhodospirillum rubrum*** by a variation of the method of French.[8,9] It was not purified chromatographically.

The absorption curves of the pigments were checked in the visible region with a Beckman quartz spectrophotometer, before and after the infrared measurements. No significant decomposition was noted. The spectra matched those reported in the literature.[10,11]

A few samples were transferred from ether to CCl_4 and run in a 100 μ liquid cell. The others were deposited from a concentrated ether solution onto a salt window, dried under nitrogen and examined in the solid state. All carbon tetrachloride solutions and dry films were handled in dim light, to minimize photodecomposition. Aside from the solvent bands, there were no differences between the solid and solution spectra. At least two spectra were run on each compound; the best portions of these (as judged by the intensity and resolution of the sample bands and by the absence of impurity absorption) were combined to yield the curves shown in Figs. 1–3. These should contain no extraneous bands, other than those due to atmospheric CO_2 and traces of water vapor.

All spectra shown were run on a Perkin–Elmer Model 12-C spectrophotometer. In addition, samples of crystalline ethyl chlorophyllide were run between salt plates, with and without added mineral oil, and phytol was examined as the pure liquid and in CCl_4 solution.

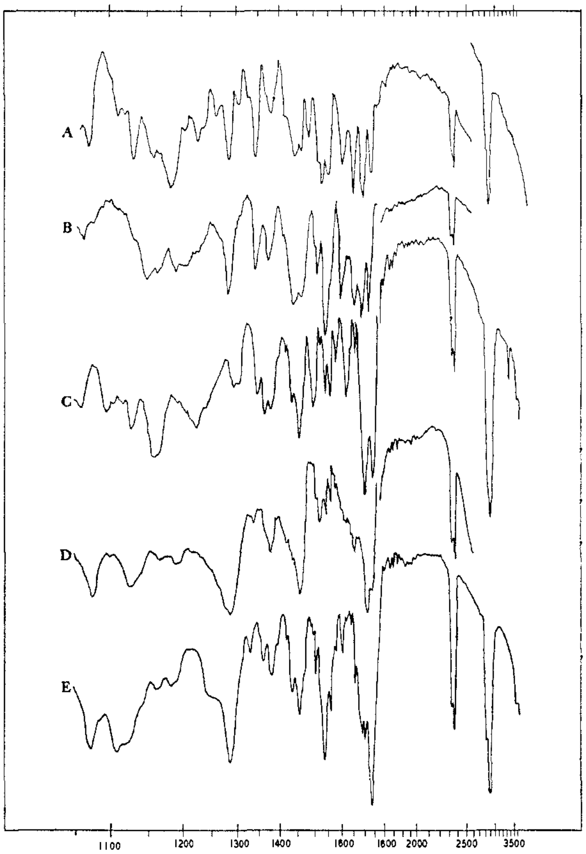

Fig. 1.—Infrared spectra of chlorophyll and related compounds; rock salt region, first half; standard slit drive: A, chlorophyll-a; B, chlorophyll-b; C, pheophytin-a; D, bacteriochlorophyll; E, allomerized chlorophyll-a.

Results and Discussion

Figures 1 and 2 show the spectra of chlorophyll-a and four related compounds in the rock salt region. Figure 3 shows higher-dispersion spectra of three compounds whose 3 μ region is of special interest. Table I summarizes the patterns in detail and Table II presents a few tentative assignments.

Although it was difficult to remove the last traces of alcohols (including phytol) and to avoid partial decomposition of the samples, the best chlorophyll preparations did not show the "N–H" or "O–H" band found near 3360 cm.$^{-1}$ by Coblentz.[2] Allomerized chlorophyll-a is seen to lack any absorption corresponding to the C_{10}-hydroxyl originally postulated by Fischer[12]; instead the ether band at 1105 cm.$^{-1}$ appears to confirm his more recent conclusion[3] that allomerization introduces an alkoxy group at position 10.

The imine bands of the pheophytins of native and allomerized chlorophyll-a are shown on Fig. 3. The N–H stretching frequency of the former was found to be 3400 cm.$^{-1}$, that of the latter, 3345 cm.$^{-1}$. Although both bands are displaced from the "free" N–H frequency of pyrrole (3500 cm.$^{-1}$ in dilute solution, 3535 cm.$^{-1}$ in the vapor[13]), the

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(4) For structural formulas see Rabinowitch, ref. 2b, Vol. I, 1945, pp. 442–445.

(5) R. Livingston, D. Sickle and A. Uchiyama, *J. Phys. Colloid Chem.*, **51**, 777 (1947).

(6) G. Mackinney and M. A. Joslyn, THIS JOURNAL, **60**, 1132 (1938).

(7) Unpublished data, see Rabinowitch, ref. 2b, Vol. II, p. 614.

(8) C. S. French, *J. Gen. Physiol.*, **23**, 483 (1940).

(9) For details, see J. W. Weigl, THIS JOURNAL, **75**, 999 (1953).

(10) See Rabinowitch, ref. 2a, Vol. II, pp. 603 ff.; for bacteriochlorophyll, see reference 9.

(11) In neutral ether solution, the pheophytin of allomerized chlorophyll-a exhibits the following peak wave lengths and relative extinction coefficients in the region 320–700 mμ: 400 mμ (3.62), 500 mμ (0.32), 530 mμ (0.22), 615 mμ (0.15), 672 mμ (1.23). The spectrum of an acidic methanol solution of this compound matched that obtained by R. Pariser (Ph.D. Thesis, University of Minnesota, 1950).

(12) H. Fischer and G. Spielberger, *Ann.*, **510**, 159 (1934).

(13) N. Fuson and M. L. Josien, *J. Chem. Phys.*, **20**, 145, 1043 (1952).

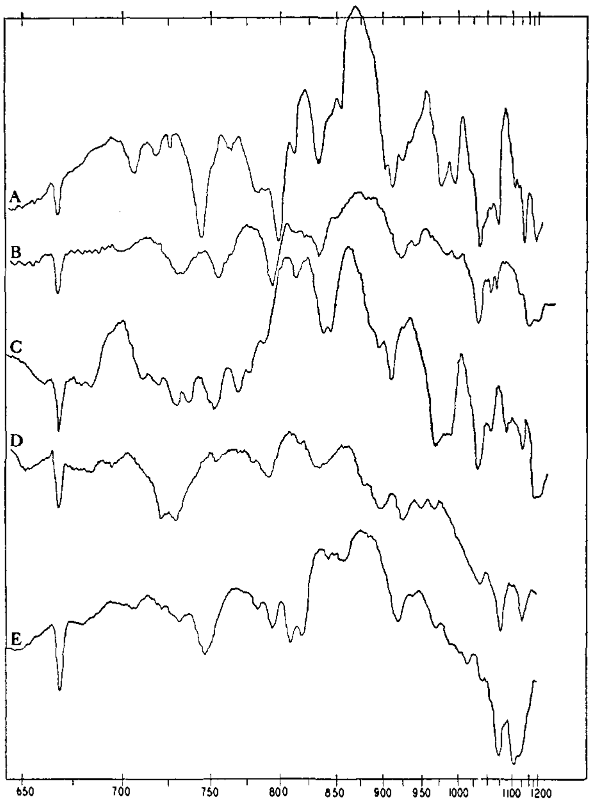

Fig. 2.—Infrared spectra of chlorophyll and related compounds; rock salt region, second half; standard slit drive: A, chlorophyll-a; B, chlorophyll-b; C, pheophytin-a; D, bacteriochlorophyll; E, allomerized chlorophyll-a.

shifts are smaller than that observed by Vestling and Downing[14] for etioporphyrin I (to 3300 cm.$^{-1}$). While these displacements are somewhat greater than that caused by association in liquid pyrrole (3395 cm.$^{-1}$),[13] these N–H bands are sharp, and the shifts may be due to changes in the electron density on the nitrogens, rather than to hydrogen-bonding. The imine stretching bands in porphyrins and related compounds appear to be remarkably weak; in fact, in two recent investigations of porphyrin infrared spectra these bands were not detected at all.[15,16]

No evidence was found for carbonyl stretching bands corresponding either to the formyl group of chlorophyll-b or the acetyl group of bacteriochlorophyll. Presumably such bands were obscured by others in the 6 μ region.

Coblentz[1] observed that even a smooth-looking layer of ethyl chlorophyllide shows hardly any infrared absorption bands. We were able to duplicate, as well as to explain this result; it is due to strong light scattering by the microcrystals. When the layer was covered with a drop of mineral oil, the absorption bands appeared clearly. While our spectra of ethyl chlorophyllide and phytol do not merit publication, they are consistent with the identification in Tables I and II.

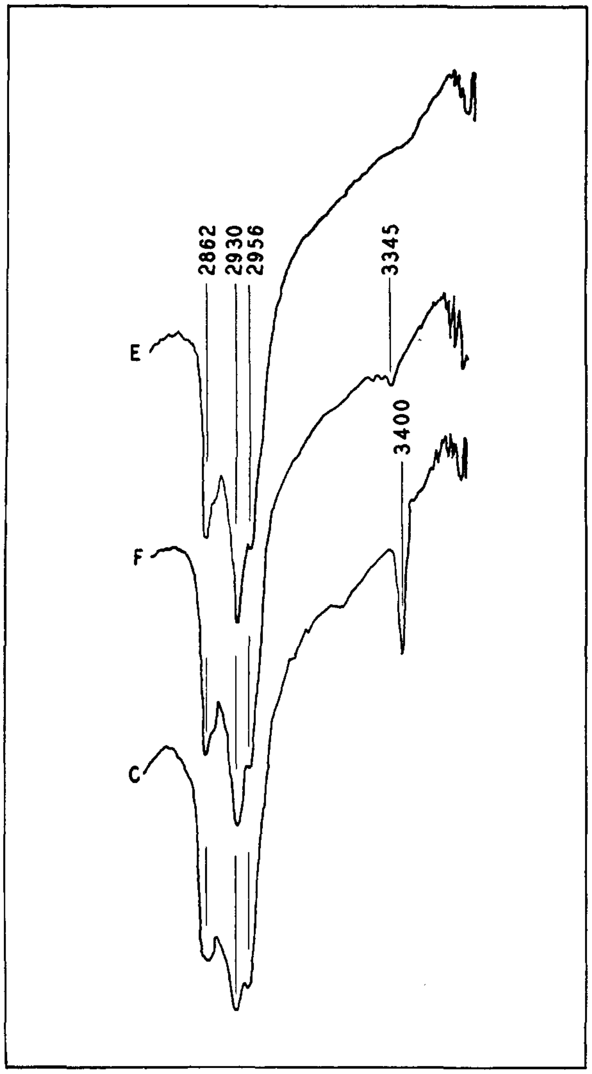

Fig. 3.—Infrared spectra of chlorophyll and related compounds; lithium fluoride region; slit fixed at about 0.06 mm.; E, allomerized chlorophyll-a; F, pheophytin of allomerized chlorophyll-a; C, pheophytin-a.

We confirmed the results of Stair and Coblentz[2] and of Mecke[17] that chlorophyll-a is practically transparent between 0.7 and 2.5 μ. Similar results have been reported by Pruckner[18] for a bacteriochlorin, a chlorin and a porphin. No bands were found in the KBr region (15 to 27 μ) which

TABLE I

SUMMARY OF BANDS, BY GROUPS

Frequencies in cm.$^{-1}$

Bands common to all five compounds

Bands corresponding to phytol bands: 2900 and vicinity, 1740, 1660, 1460–1470, 1445, 1380–1385, 1165, 1035, 728–732

Bands not appearing in phytol[a]

1700, 1290 (in pheo.-a at 1300); 1110–1130; 968–980 (980 in chl.-a and -b, 968–970 in others); 840–845; 815; 743 (chl.-a and allom. chl.-a) to 752–755 (others); 720; 694–708 (different for each[c]).

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[14] C. S. Vestling and J. R. Downing, THIS JOURNAL, **61**, 3511 (1939).

[15] M. Kraemer, University of Minnesota, 1950 (private communication).

[16] C. W. Craven, K. R. Reissman and H. I. Chinn, *Anal. Chem.*, **24**, 1214 (1952).

[17] R. Mecke and W. C. G. Baldwin, *Naturwissenschaften*, **25**, 305 (1937).

[18] F. Pruckner, *Z. physik. Chem.*, **A187**, 257 (1940).

TABLE I (*Continued*)

Bands common to several of the compounds

All of the chlorophylls, but not pheo.-a: 1190–1195, 1070, 792–796
All compounds except bacteriochlorophyll: 1590–1620[a], 1545, 1350–1355, 985–995
All compounds except chlorophyll-b: 780–782
All compounds except allomerized chlorophyll-a: 832–835
Chl.-a and pheo.-a: 1560, 1495 (chl.-a) to 1505 (pheo.-a), 1310, 910[b]
Chl.- and allom chl.-a: 1330, 1245–1265, 855, 743[d]
Chl.-b and bact. chl.: 945, 885
Pheo.-a and bact. chl.: 896, 683
Chl.-a, chl.-b and pheo.-a: 1228, 1205–1210, 1060, 763 (in pheo.-a. at 770)
Chl.-a. chl.-b and allo. chl.-a: 1610[a]
Chl.-a, chl.-b and bact. chl.: 1525, 925[b]
Chl.-b, pheo.-a and bact. chl.: 752–755[d]

Bands unique to single compounds

Chl.a: 902, 800, 705[e]
Chl.-b: 1155, 700[e]
Pheo.-a: 3400; 1620 and 1590 (these may be due to split of 1610[a]); 1365, 1095, 736, 708[e]
Bact. chl.: 694,[c] 652
Allom. chl.-a: 1105, 1015, 918[b], 808, 703[e]

[a] Chl.-a, chl.-b and allomerized chl.-a have a band at 1610, which in pheo.-a appears to be split to 1590 and 1620. [b] Chl.-a has a doublet at 910 and 925; 910 is shared with pheo.-a; 925 appears also in chl.-b and bact. chl.; allom. chl.-a has a band in between, at 918; all these appear to be related. [c] These appear to be closely related: 694–708. [d] These appear to be closely related: 743–755. [e] All these bands were also found in ethyl chlorophyllide, except for 1110–30 and 720, which may have been obscured by the oil.

TABLE II

TENTATIVE BAND ASSIGNMENTS

Frequencies in cm.$^{-1}$

1740 ester groups at C_7 and C_{10}[a]
1700 ketone in ring V
1660 phytol C=C[b]
1610 semi-isolated, or possibly vinyl, C=C[c]
1460–1470, 1445 C–H bending (mainly phytol)
1380 C–CH_3 stretching (phytol)
1105 C–OCH_3 at C_{10}
985–995 vinyl C–H bending (at C_2)
780–782 methyl C–H rock (at C_3)
736 N–H rocking

[a] Although both phytol spectra show a strong band at 1730–45 cm.$^{-1}$, this apparently is due to an impurity, such as a phytol ester. [b] For some reason, relatively weak in pheo.-a, bact. chl. and allom. chl.-a; in phytol at 1675-80. [c] Note that in pheophytin-a this band appears to be split to 1590 and 1620; this might be evidence for two isomers of pheophytin having semi-isolated double bonds in different positions.

could be attributed to the N–Mg–N oscillation. Either the absorption due to this very interesting vibration is too weak to be observed, or else it lies at longer wave lengths.

Acknowledgments.—The authors are greatly indebted to Professor B. L. Crawford, Jr., (Univ. of Minnesota) for the use of the infrared spectrophotometer and to Mr. B. Zaslow for operating it, to Mr. James Johnson (Univ. of Minnesota) for a contribution of *R. rubrum*, to Professor Farrington Daniels (Univ. of Wisconsin) for a sample of ethyl chlorophyllide, and to Professor Paul Rothemund (Antioch College) for a sample and a spectrum of phytol.

MINNEAPOLIS, MINN.

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY, INSTITUTE OF TECHNOLOGY, UNIVERSITY OF MINNESOTA]

The Phototropy of Chlorophyll in Fluid Solutions[1,2]

BY ROBERT LIVINGSTON AND VICTOR A. RYAN

RECEIVED OCTOBER 4, 1952

Oxygen-free chlorophyll solutions in methanol undergo reversible color changes when they are illuminated. Steady illumination (at an "intensity" of about 5 × 10^{19} quanta absorbed per liter per second) produced the following changes (measured with bands isolated by interference filters). For chlorophyll-b, the absorption increased at λ 4030 and 5245 Å. and decreased at λ 4680, 5880 and 6450 Å. For chlorophyll-a, the absorption increased at λ 4680, 5020 and 5245 Å. and decreased at λ 4030, 4395, 5880 and 6450 Å. Similar solutions, illuminated with a flash of high intensity and short duration, undergo color changes whose temporal course can be followed by photographing a transient trace on an oscilloscope. Using scanning light of λ 4680, 4705 or 4775 Å. and a 2 × 10^{-6} *m* chlorophyll-b solution, a partial bleaching, which had a half-life of about 5 × 10^{-4} sec., was observed. When the wave length of the scanning light was λ 5245 Å., an increase in absorption was observed. The decay of this change appears to follow a rate law different from that which governs the disappearance of the bleaching observed with λ 4680 Å., etc. A similar, although less marked, phototropic response was exhibited by a chlorophyll-a solution, scanned with λ 5245 Å. Oxygen inhibits both the flash and the steady-state phototropic changes. These results are consistent with the following postulates. In addition to the short-lived, singlet fluorescent state, excited chlorophyll can exist in a metastable state (perhaps the lowest triplet state) and as a radical. The radical is formed by the reaction of the metastable molecule with a molecule of the solvent. Under steady, relatively weak, illumination only the radical and normal chlorophyll are present in detectable amounts. Both the radical and the metastable molecule are formed (in comparable concentrations) when the solution is illuminated with a flash of high intensity. The metastable molecules disappear by reacting with normal chlorophyll molecules (or possibly by a simple, first-order decay), while the radicals reform normal chlorophyll molecules by a second-order reaction.

Solutions of chlorophyll, in air-free methanol or similar solvents, undergo a reversible color change when they are illuminated. In pure methanol at ordinary temperatures, the original color of the chlorophyll returns so rapidly when the light is extinguished, that its rate of return cannot be determined by those methods which have been previously used to study the

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[1] This work was made possible by the support of the Office of Naval Research (NR 051,028, Contract N6ori-212, T.O. I) to which the authors are indebted.

[2] This paper is based in part upon a dissertation submitted by V. Ryan to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy, December, 1951.