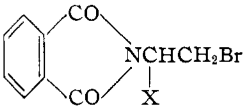
gave as sole product an N-tribromoethylphthalimide of undetermined structure.

The present note reports the peroxide-catalyzed reaction of N-(2-bromoethyl)-phthalimide (I) with N-bromosuccinimide in refluxing carbon tetrachloride to give the product of monobromination II in an 89% yield. The structure of II was established by analysis, by agreement of melting point with the known product prepared by addition of bromine to N-vinylphthalimide,[2] and by the fact that the product contains an active bromine atom which, conforming to expectation, reacts readily with ethanol at room temperature to give the corresponding ether III.



I, X = H
II, X = Br
III, X = $OC_2H_5$

Attempts to extend this bromination reaction to N-*n*-hexyl- and N-laurylphthalimides failed. Although consumption of the N-bromosuccinimide was complete under all conditions tried, mixtures of oils and low melting solids always resulted. That a non-specific substitution probably occurred throughout the length of the aliphatic chain was indicated by the fact that when N-ethylphthalimide was treated with N-bromosuccinimide, a low yield of the dibrominated product II could be isolated.

**Acknowledgment.**—The author is grateful to Mr. E. F. Shelberg for the microanalyses.

### Experimental

Bromination of **N-(2-Bromoethyl)-phthalimide** (I). **Preparation of N-(1,2-Dibromoethyl)-phthalimide** (II).—To a solution of 12.7 g. (0.05 mole) of N-(2-bromoethyl)-phthalimide (I), m.p. 82–84°, in 50 cc. of dry carbon tetrachloride was added 8.9 g. (0.05 mole) of colorless N-bromosuccinimide and 0.5 g. of benzoyl peroxide. After stirring and heating under reflux for 15 minutes, another portion (0.5 g.) of benzoyl peroxide was added and the reaction was continued for an additional 2.5 hours. At the end of this time, the color of the mixture had changed from deep orange to light yellow and a positive test for active bromine (starch–potassium iodide paper) was no longer present. After cooling in ice for one hour, the crystallized product was removed by filtration, dried, suspended in water, collected once more at the filter, washed with more water and dried again. There was obtained 11.0 g. of N-(1,2-dibromoethyl)-phthalimide (II), m.p. 122–123°. Two recrystallizations of a sample from carbon tetrachloride gave colorless prisms, m.p. 123–124°.

*Anal.* Calcd. for $C_{10}H_7Br_2NO_2$: C, 36.07; H, 2.12; Br, 48.00. Found: C, 36.24; H, 2.15; Br, 47.98.

From the filtrate of the reaction mixture an additional 2.9 g. of II could be obtained, bringing the total yield to 13.9 g. (89.5%).

**N-(2-Bromo-1-ethoxyethyl)-phthalimide** (III).—A suspension of 1.60 g. of the dibromo compound II in 50 cc. of absolute ethanol was stirred at room temperature overnight. The resulting solution was allowed to stand for another 24 hours and then was treated with 2 g. of solid sodium bicarbonate. After stirring for an hour, the mixture was filtered through a layer of charcoal and the colorless filtrate was concentrated to dryness *in vacuo*. Recrystallization of the residue from 10 cc. of hexane gave 0.90 g. (63%) of III, m.p. 78–81°. Two more recrystallizations gave analytically pure product, m.p. 81.5–82.5°.

*Anal.* Calcd. for $C_{12}H_{12}BrNO_3$: C, 48.34; H, 4.06. Found: C, 48.56; H, 3.94.

Bromination of **N-Ethylphthalimide**.—A solution of 17.5 g. (0.1 mole) of N-ethylphthalimide, m.p. 76.5–78°, in 100 cc. of carbon tetrachloride was refluxed and stirred for four hours with a finely powdered mixture of 17.8 g. (0.1 mole) of N-bromosuccinimide and 2 g. of benzoyl peroxide. After cooling to room temperature, insoluble succinimide was removed by filtration and the filtrate was concentrated to a volume of about 40 cc. Cooling in ice and removing crystallized product by filtration, and isolating more product from the filtrate as described above for the bromination of I, yielded a total of 4.5 g. (27% yield, based on the N-bromosuccinimide) of N-(1,2-dibromoethyl)-phthalimide (II), m.p. 121–124°, identified by mixed melting point with a sample of II, m.p. 123–124°.

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[2] M. Bachstez, *Ber.*, **46**, 3087 (1913).

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

# The Visible Adsorption Spectra of the Phase Test Intermediates of Chlorophyll-*a* and -*b*[1]

BY ALBERT WELLER[2]

RECEIVED JUNE 25, 1954

Accurate data on the visible absorption spectra of the phase test intermediates of chlorophyll-*a* and -*b* in pyridine have been obtained using a static system. The chlorophyll-*a* intermediate has a comparatively weak long wave length absorption band at 6830 Å., the major maximum at 5240 Å. and three others of comparable strength at 4860, 4280 and 3750 Å. The major maximum of the compound derived from chlorophyll-*b* is at 5580 Å. with two shoulders; one around 5600 Å. and the other around 5050 Å. Another maximum is at 4440 Å. and a very weak long wave length absorption band at 6300 Å. It is suggested that the intermediate compound is an ionized diradical formed by separation of the two electrons which are left after the acid ionization of the CH– group in position 10 of ring V.

The spectrum of the brownish colored intermediate which appears in the Molisch phase test[3] is of interest because of its relation to the reaction of chlorophyll with strong bases. An attempt to measure this spectrum in ethereal solution was made by Livingston and his co-workers,[4] using a flow system. More accurate results which are obtained with pyridine solutions, using a static system, are reported here.

It has been demonstrated by Conant and his

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[1] This work was made possible by the support of the Office of Naval Research (NR 051,028, Contract N 6ori-212.T.O.I) to whom the author is indebted.

[2] Post-doctoral Fellow 1951-1952 at the University of Minnesota. Present address: Laboratorium für physikalische Chemie, Wiederholdstr. 15, Stuttgart, Germany.

[3] H. Molisch, *Ber.*, *dtsch. bot. Ges.*, **14**, 16 (1896).

[4] B. Dunicz, T. Thomas, M. van Pee and R. Livingston, THIS JOURNAL, **73**, 3388 (1951).

co-workers[5] that the brownish colored intermediate which appears immediately when a chlorophyll solution is treated with alcoholic potash, disappears within a few seconds due to oxidative hydrolysis of ring V, thus yielding a green chlorin. Allomerization with oxygen leads to the same compound.[6]

It has been pointed out recently[7] that allomerization with oxygen is a slow phase test reaction (due to traces of base present) without detectable appearance of the intermediate, thus explaining the failure of allomerized chlorophyll to give the phase test. The fact that traces of acid inhibit allomerization is readily explained by neutralization.

Since the uptake of oxygen by chlorophyll is relatively slow in pyridine solutions[8] it was thought to be worthwhile to carry out the phase test in degassed pyridine, instead of ether. Under these conditions the color of the intermediate could be detected for more than one hour.

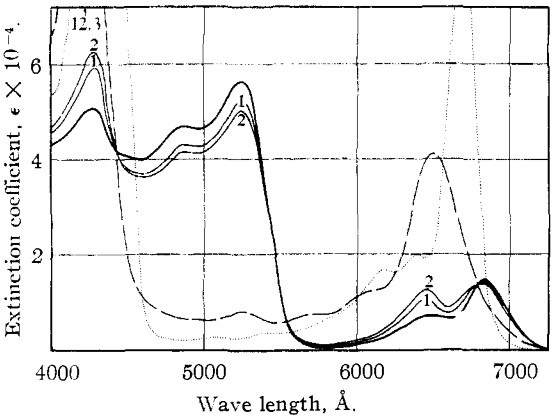

Fig. 1.—Chlorophyll-*a* derivative ($c_{\text{chl}}$ = 3.3 × $10^{-6}$ *m*; $c_{\text{OH}^-}$ = 2.1 × $10^{-3}$ *m*); cell length 8.1 cm.; . . . ., chlorophyll-*a* in pyridine; —, intermediate compound; — (1), starting at 8000 Å., 430 sec. after addition of base; — (2), starting at 8000 Å., 880 sec. after addition of base; – –, oxidation product.

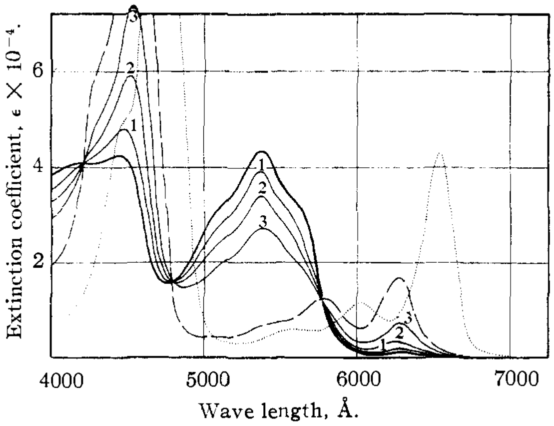

Fig. 2.—Chlorophyll-*b* derivative ($c_{\text{chl}}$ = 1.70 × $10^{-6}$ *m*; $c_{\text{OH}^-}$ = 5.0 × $10^{-3}$ *m*); cell length 0.76 cm. . . . ., chlorophyll-*b* in pyridine; —, intermediate compound; — (1), starting at 8000 Å., 320 sec. after addition of base; — (2), starting at 8000 Å., 980 sec. after addition of base; — (3), starting at 8000 Å., 2000 sec. after addition of base; – –, oxidation product.

## Experimental Materials and Methods

**Materials.**—The chlorophyll-*a* and -*b* were prepared and purified by a method described elsewhere.[9] Pyridine was dried with solid potassium hydroxide and distilled in a stream of dry helium.

**Apparatus.**—The absorption measurements were made with a Cary Recording Spectrophotometer using a tungsten lamp. The absorption cell was sealed to a tube which had a side-arm for the methanolic KOH solution and a side bulb in which the chlorophyll solution was chilled during evacuation. The spectrum of chlorophyll was taken before mixing, in order to determine the concentration. After mixing the spectra were successively run, using a chart speed of 10 Å./sec.

## Experimental Results

The brownish color of the intermediate appeared almost immediately after addition of the methanolic KOH from the side-arm to the chlorophyll solution in pyridine. Apparently the solution still contained some oxygen by which the intermediate was slowly oxidized to the green chlorin. Due to this reaction the spectra changed slowly. Some of these measured absorption spectra are represented by the numbered curves of Figs. 1 and 2, for chlorophyll-*a* and *b*, respectively. Depending on the length of the absorption cell used, the total concentration of chlorophyll or chlorophyll derivative was between 3 × $10^{-6}$ and 1.7 × $10^{-5}$ *m*. In addition to the intermediate curves the absorption spectra of the relative stable oxidation products, obtained after admittance of air, are given by the dashed curves.

From these and other similar absorption measurements which are not reproduced in this paper it was possible to determine accurately the extinction values of the intermediate compound. As indicated by the isosbestic points, only the intermediate compound and the final product are present in the solutions. Therefore

$$E = E_i \frac{C_i}{C_0} + E_f \frac{C_0 - C_i}{C_0} \qquad (1)$$

where $E$ is the extinction measured during the course of the oxidation reaction; $E_i$ and $E_f$ are the extinctions of intermediate and final product, respectively. $C_i/C_0$ is the fraction of intermediate present in the solution.

If it is assumed that the oxidation is a first-order reaction with respect to the intermediate, it follows

$$\log \frac{C_i}{C_0} = -0.434kt \qquad (2)$$

where $k$ is the first-order reaction rate constant (containing the concentration of oxygen). (1) and (2) give

$$\log (E - E_f) = \log (E_i - E_f) - 0.434kt$$

Thus plotting log ($E - E_f$) *versus* time and knowing $E_f$, $E_i$ can be determined from the intersection of the ordinate. The slopes, while being the same for all wave lengths, became smaller at longer times in some cases. This easily can be explained as due to

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[5] J. B. Conant, S. E. Kamerling and C. C. Steele, *ibid.*, **53**, 1615 (1931); J. B. Conant, J. F. Hyde, W. W. Moyer and E. M. Dietz, *ibid.*, **53**, 359 (1931); C. C. Steele, *ibid.*, **53**, 3171 (1931).

[6] H. Fischer and H. Pfeiffer, *Ann.*, **555**, 94 (1944).

[7] A. Weller and R. Livingston, THIS JOURNAL, **76**, 1575 (1954).

[8] H. Fischer and J. Riedmair, *Ann.*, **506**, 107 (1933).

[9] F. Zscheile and C. Comar, *Botan. Gaz.*, **102**, 463 (1941); R. Livingston, D. Sickle and A. Uchiyama, *J. Phys. Colloid Chem.*, **51**, 777 (1947).

the depletion of oxygen as the reaction progressed. The extinction coefficients obtained by this method for the intermediate compound are represented in Figs. 1 and 2 by the heavy line curves. The results are summarized in Table I.

TABLE I

WAVE LENGTHS AND MOLAR DECADIC EXTINCTION COEFFICIENTS OF THE MAXIMA OF THE PHASE TEST INTERMEDIATE OF CHLOROPHYLL-*a* AND -*b*

| Chlorophyll-*a* | | Chlorophyll-*b* | |
|---|---|---|---|
| λ (Å.) | $\epsilon \times 10^{-3}$ | λ (Å.) | $\epsilon \times 10^{-3}$ |
| 6830 | 14.5 ± 0.5 | 6300 | 0.9 ± 0.2 |
| 6450 | 7.0 ± 0.8 | 5370 | 43.5 ± 1.0 |
| 5240 | 56.5 ± 1.0 | 4440 | 42.2 ± 1.0 |
| 4860 | 46.6 ± 1.0 | 4200 | 41.2 ± 1.0 |
| 4280 | 50.7 ± 1.0 | | |

One experiment with chlorophyll-*b* was carried out without methanol. In this case the methanolic KOH solution in the side-arm was evaporated to dryness. After adding the KOH to the pyridine solution of chlorophyll-*b* no change in the absorption spectrum occurred even after 5 hours, thus indicating that at least a trace of alcohol is necessary for the appearance of the phase test intermediate.

At −78° in ethereal solution, the spectrum of chlorophyll-*a* and chlorophyll-*b* did not change after adding methanolic KOH; whereas at 0° the intermediate appeared within less than 5 sec.

No fluorescence of the intermediate could be detected visually.

## Discussion

In the case of chlorophyll-*b* the results given above for the absorption spectrum of the intermediate compound in pyridine agree quite well with those found by Livingston and co-workers[4] in ether. Some minor differences (*e.g.*, the long wave length shoulder at 5600 Å. and a slight spectral shift of the maxima and minima) may be due to the difference in solvent.

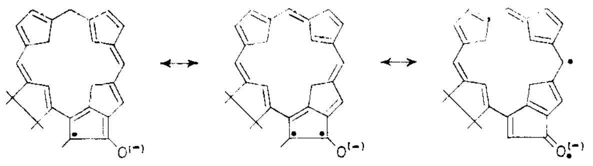
Generally the same holds for the chlorophyll-*a* derivative with the exception that the two maxima found between 4700 and 5400 Å. do not appear in Livingston's curve. It is not quite clear why no indication of these maxima was observed with the flow system.

The pale-yellow anaerobic product found in rigorously evacuated ethereal solutions never appeared in pyridine solutions.

It has been found by Freed and Sancier[10] that chlorophyll solutions in hydrocarbon–amine mixtures undergo a reversible reaction at low temperatures forming a brownish compound. In particular, the spectrum of the brownish compound derived from chlorophyll-*a* is almost identical with that given in Fig. 1 for the phase test intermediate. Apart from a slight spectral shift of the maxima and minima for which the difference in solvent and temperature may be responsible, it can be concluded from the relative height of the maxima that about 20% of the original green chlorophyll is still present at 160°K.

Chemical evidence indicates that ring V is involved in the phase test reaction. On account of the considerable difference in the effect of temperature on the reaction rates, a reaction mechanism similar to the amine reaction of chlorophyll can be ruled out.[7] This leaves the CH– group in position 10 as point of attack by $OH^-$ and favors the postulate[4] that the brownish color is due to a compound which is formed by the direct acid ionization of the CH group in position 10. The ionized dye thus formed would be expected to have a considerable charge resonance and consequently a high long wave length absorption band.[11]

However, the first absorption bands of the intermediate compounds are comparatively low. It has been suggested that the compound formed by splitting off the proton from carbon 10 could go over into the enolate ion having a double bond between carbon 9 and 10. Since, however, ring V is attached to the main system which has aromatic character considerable ring strain is involved forcing carbon 9 and 10 at least 1.8 Å. apart. Due to this distance the energy of the triplet state of the bond probably is low compared with that of the singlet state. It is suggested, therefore, that the intermediate compound exists as an ionized molecule in a triplet (or diradical) state, which is stabilized by the possibility of a great number of resonating structures. The high reactivity of the intermediate compound toward molecular oxygen is also explained by its diradical character.



It has been pointed out[12] that the absorption spectra of the phase test intermediate resemble the spectra of the phototropic product formed under steady illumination[12,13] of the reversibly reduced form of chlorophyll,[14] of the oxidation intermediate in a rigid solvent[15] and of the short-lived triplet state.[16] While these spectra do not appear to be identical, their similarity suggests at least common features of these chlorophyll intermediates and it can be assumed that they have radical or diradical character.

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(11) *Cf.* Th. Förster, *Z. Elektrochem.*, **45**, 548 (1939); R. S. Mulliken, *J. Chem. Phys.*, **7**, 14, 20 (1939).

(12) R. Livingston and V. A. Ryan, THIS JOURNAL, **75**, 2176 (1953).

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(14) A. Krasnovskii, *Doklady Akad. Nauk. S.S.S.R.*, **60**, 421 (1948).

(15) H. Linschitz and J. Rennert, *Nature*, **169**, 193 (1952).

(16) R. Livingston, G. Porter and M. Windsor, *ibid.*, **173**, 485 (1954).