

[CONTRIBUTION FROM THE DIVISION OF FRUIT PRODUCTS, UNIVERSITY OF CALIFORNIA]

## The Photo-oxidation of Chlorophyll

BY S. ARONOFF<sup>1</sup> AND G. MACKINNEY

When chlorophyll in acetone or benzene solution is exposed to light in the presence of oxygen, it is irreversibly bleached. Pink intermediates are formed, the absorption spectra of which are shown in Fig. 1. They are quite stable if kept in

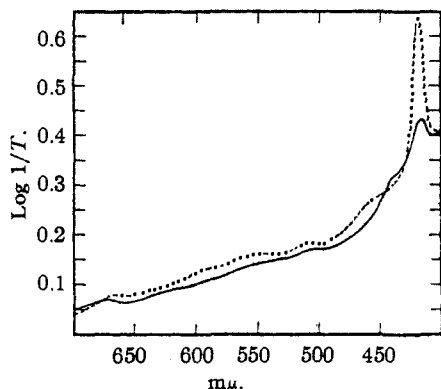


Fig. 1.—Photodecomposition products of chlorophyll *a* —; chlorophyll *b* - - -.

the dark for several days, and exhibit an orange fluorescence. On continued exposure, the solutions become colorless and devoid of fluorescence. Experiments on photo-bleaching of chlorophyll differ in kind. The often-quoted experiments of Willstätter and Stoll<sup>1a</sup> dealt with colloidal chlorophyll in aqueous solution in the presence of carbon dioxide. Pheophytin was formed, with pure carbon dioxide, whether the solutions were illuminated or not. Their subsequent experiments are less easily understood. Their reported increase in color strength, "Farbstärke" refers to the increase in olive-green, owing to pheophytin formation.<sup>2</sup> The products of oxidation which were noted, in addition to the pheophytin itself, are described only with respect to their solubility, and appearance as a brownish flocculent precipitate in petroleum ether. No recognizable degrada-

(1) Present address, Department of Chemistry, University of Calif.

(1a) Willstätter and Stoll, "Assimilation der Kohlensäure," Julius Springer, Berlin, 1919, p. 413.

(2) In our experience, the formation of pheophytin is dependent on the presence of acid, not on oxygen or light. One is tempted to suggest a misprint<sup>1a</sup> p. 413, line 17, where it is stated that on irradiating a solution of chlorophyll *a* in CO<sub>2</sub>-free air, for four hours, the solution becomes more olive green owing to pheophytin. In line 23, it is stated that the increase in color strength was noticeable on "mehrstündiger Belichtung bei Sauerstoff Zutritt." The results are more understandable if it is assumed that the atmosphere was originally O<sub>2</sub>-free, not CO<sub>2</sub>-free, and that pheophytin formation under such conditions is a complicating factor, unconnected with the direct photo-oxidative breakdown of chlorophyll.

tion products of chlorophyll have yet been identified *in vivo*, as a result of photo-oxidation,<sup>2a</sup> and it seems clear that the breakdown products have not followed the natural course of chlorophyll disappearance. (One must exclude here the effect of mechanical injury, caused for example by frost on leaves, where as a result of cellular disorganization, pheophytin may be found.)

Gaffron<sup>3</sup> was primarily concerned with establishing the course of oxygen transport, whether a pigment- or acceptor-peroxide was formed. Chlorophyll in methanol solution was slowly bleached in the absence of an acceptor, and for each quantum absorbed, one mole of oxygen is taken up. Weber<sup>4</sup> preferring the acceptor theory to the metastable states postulated by Kautsky<sup>5</sup> obtained rapid bleaching and yellow to brown oxidation products with chlorophyll in methanol solution. It is even more difficult to follow these experiments because his chlorophyll and that of Baur<sup>6</sup> are products stated to contain 40% pure natural chlorophylls *a* and *b*. It is obviously impossible, therefore, to determine whether any genuine relation exists between fluorescence yields and photo-oxidation.

In view of the uncertainties as to the products hitherto used, no extensive discussion of the mechanism is warranted. In this paper we report results of kinetic studies on the photo-oxidation of pure chlorophylls *a* and *b* in benzene at certain wave lengths. Some quantum yield estimates are also given. Finally, the effect of carotene on the bleaching of chlorophyll is considered.

**Kinetic Studies.**—Chlorophylls *a* and *b* were prepared<sup>7</sup> as in previous work, and solutions approximately 10<sup>-5</sup> to 10<sup>-6</sup> *M* were exposed to the filtered light from a 200-watt mercury lamp for stated periods of time. Destruction of chlorophyll was estimated spectrophotometrically, by measurement of the decrease in transmission at the red maximum, *ca.* 6600 for chlorophyll *a*, and 6425 Å. for chlorophyll *b*. It is to be noted that no intermediate, except the products shown in

(2a) Mackinney, *Ann. Rev. Biochem.*, **9**, 471 (1940).

(3) Gaffron, *Ber.*, **60**, 755, 2229 (1927).

(4) Weber, *ibid.*, **69B**, 1026 (1936).

(5) Kautsky, Hirsch and Fleisch, *ibid.*, **68**, 152 (1935).

(6) Baur, *Helv. Chim. Acta*, **18**, 1157 (1935).

(7) Mackinney, *J. Biol. Chem.*, **133**, 91 (1940).

Fig. 1, was to be detected, with any appreciable absorption in that region of the spectrum. Wave lengths selected were 4358, 5461 and 5770-5790 Å. for the incident light. Their respective intensities, as measured by an 80-junction Moll-type thermopile were 1.00:2.19:1.75 after passage through the filters used to isolate these lines. The reaction cell was of quartz, with sufficient head-space to provide oxygen.

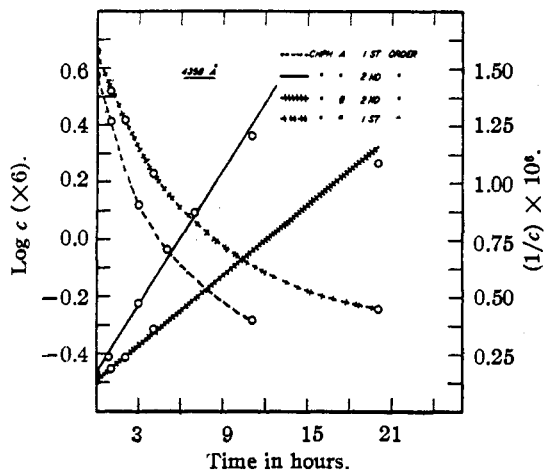


Fig. 2a.

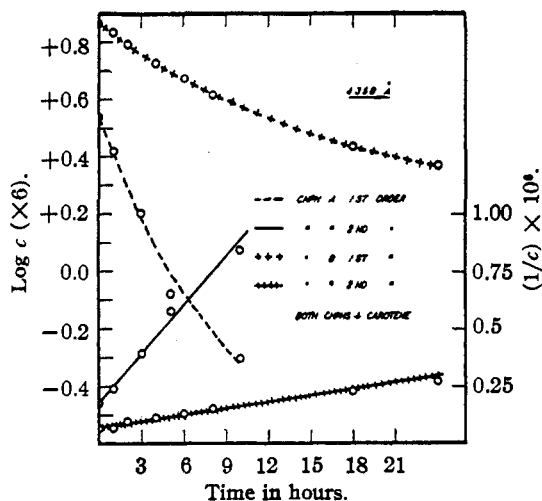


Fig. 2b.

The data were then plotted at all three wave lengths, with log *c* and 1/*c* as a function of time. In each case, the plot favors a second order reaction, although with carotene present at 4358 Å. the data are less unfavorable to a first order than in all the other cases (see Figs. 2a, b). It is possibly, therefore, permissible to summarize the results briefly in Table I.

It is difficult to compare the rates at which the

TABLE I  
SECOND ORDER RATE CONSTANTS FOR CHLOROPHYLLS *a* AND *b*<sup>a</sup> (TIMES 10<sup>-5</sup>)

Wave length in Å.	4358	5461	5770-5790
Chlorophyll <i>a</i>	1.54	0.62	0.85
Chlorophyll <i>a</i> + carotene	1.29		
Chlorophyll <i>b</i>	0.83	0.50	0.25
Chlorophyll <i>b</i> + carotene	0.12		

<sup>a</sup> *t* in hours, *c* in moles per liter.

two chlorophylls are photo-oxidized. The peak for chlorophyll *a* is closer to the 4358 line, than is the case with chlorophyll *b*, and the ratio of the two absorption coefficients in benzene is *ca.* 1.5:1 at this point. Nevertheless, the intermediate (Fig. 1) with a maximum at 4200 Å. absorbs a greater portion of the light in the one case than the other. When all three wave lengths of incident light are adjusted for equal intensity, the rates at 4358 should be more than ten times the values at 5461 Å. on the basis of the absorption coefficients, and this is not apparently the case. In view of the uncertainties as to the intermediates, a more precise comparison of the values at the three wave lengths is not possible.

**Quantum Yields.**—A Bureau of Standards lamp was then substituted for the Hg lamp, in the same optical system, with the thermopile in place of the reaction cell, with the same galvanometer. Quantum yields were estimated of the order  $5 \approx 5 \times 10^{-4}$ . An example of one calculation is given in detail. At 4358 Å. initial concentration chlorophyll *a*)  $0.37 \times 10^{-5}$  mole per liter. In 25 ml. of solution, there were initially  $5.6 \times 10^{16}$  molecules. After eleven hours, the concentration was  $0.052 \times 10^{-5}$  mole per liter. In 25 ml. there were finally  $0.78 \times 10^{16}$  molecules. Loss =  $(5.6 - 0.78)$  or  $4.8 \times 10^{16}$ . Radiant flux from standard lamp on absorption cell is equivalent to 1580 ergs per sec. (2.44-cm. galvanometer throw). With neutral screen (*T* = 0.1), response to Hg lamp is 3.07 cm., equivalent to  $1.99 \times 10^4$  ergs per sec. The number of quanta per second are therefore  $0.44 \times 10^{16}$ , and in eleven hours,  $1.74 \times 10^{20}$ . The initial transmission at this wave length was 0.02, and the final transmission was 0.59. On the basis of calculations for shorter periods, from one to three hours of exposure, we may here assume 50% absorption, and the quantum efficiency becomes

$$\frac{4.8 \times 10^{16}}{0.87 \times 10^{20}} = 5 \times 10^{-4} \text{ mole per quantum.}$$

In spite of the numerous refinements which might

TABLE II  
EFFECT OF CAROTENE ON PHOTO-OXIDATION OF CHLOROPHYLL

Sample	Chph:carotene (mole ratio)	% T 6600 Å.	Mole chph.	Mole Carotene		Ratio, (final/original)
				Original	Final	
0	...	4.2	$6.00 \times 10^{-6}$	...	...	...
1	...	21.8	2.90	...	...	...
2	30	14.5	3.00	$0.20 \times 10^{-6}$	0	0
3	15	12.8	3.40	.40	0	0
4	7.5	11.9	3.68	.80	$0.14 \times 10^{-6}$	0.17
5	5.0	11.7	3.74	1.20	.73	.61
6	3.8	11.2	3.92	1.60	1.3	.81
7	3.0	11.5	3.82	2.00	1.6	.80

be made in these measurements, the efficiency is of an unquestionably low order of magnitude.

**The Effect of Carotene.**—Carotene absorbs strongly in the blue-violet region, and since at 4358 Å. in benzene, chlorophyll *a* has a higher absorption coefficient than chlorophyll *b*, the effect of carotene, Table I, *ca.*  $10^{-6}$  *M* particularly on the *b* component might be explained on the basis of absorption and dissipation of the incident energy in non-utilizable form. However, previous experiments in acetone, using red filters, showed that carotene had a similar effect in a region where the proportion of light absorbed by this pigment was negligible. This was, therefore, investigated further. Tubes containing chlorophyll (*a* and *b*), and varying amounts of carotene were simultaneously exposed to sunlight, submersed in an acid bichromate bath of sufficient depth that the transmission below 5500 Å was nil. By dilution, with no large deviation from Beer's law, the original bichromate solution was estimated to have a transmission of 0.001% at 5300 Å. The addition of carotene to the chlorophyll gave increasing protection to the latter until a mole ratio of 1:8 was reached. At this point, the rate had been decreased by *ca.* 25%. The addition of carotene beyond this ratio gave no further protection. The oxidation of the carotene was considerable, as calculated from spectroscopic measurements at 4800 Å., and results are summarized in Table II. The transmission of sample O, kept in the dark, showed no change during the period of exposure, forty-five minutes.

A carotene control, irradiated in the bichromate bath, showed less than 0.5% change in composition, demonstrating the effectiveness of the filter.

**Discussion.**—The rate-governing step in the photo-decomposition of chlorophyll by visible light is apparently of the second order. It is impossible to deduce any mechanism from the rate

measurements as such. An apparently low quantum efficiency was possibly to be anticipated, since numerous products formed, or likely to be formed, would in large measure be a result of photo-oxidation. The 8:1 chlorophyll carotene ratio might suggest possible compound formation. As a result of several determinations at 50 Å. intervals on a Beckmann spectrophotometer from 3000 to 7000 Å., there appears to be complete additivity of the two separate spectra, when mixed, within the experimental error. Compound formation is, therefore, not shown spectroscopically. It would seem that the extent to which chlorophyll is protected, appears to approach a value of roughly 0.25. The same may be true for carotene destruction, but the solubility of carotene in acetone limits the extent to which the mole ratio can be lowered, at this concentration. Acetone has the advantage, however, of easy purification. An attempt was made to determine whether as found by Weber<sup>4</sup> for other acceptors, an accelerating effect could be determined at very low carotene concentrations, but this was not found within the experimental error. Qualitatively, we have noted that the bleaching action may be greatly retarded by addition of hydroquinone, and accelerated by such compounds as ascorbic acid, to the solution. In view of the numerous experiments suggested by these results, it seems wise to postpone more detailed discussion.

### Summary

The photo-oxidation of chlorophylls *a* and *b* has been studied kinetically. The reaction appears to be of the second order, and to have a very low quantum efficiency. A protective action of carotene on the destruction of chlorophyll has been noted, attaining a constant value at a mole ratio of approximately 1:8.