Degradation of Chlorophyll and Several Derivatives in Acid Solution

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The reaction rates of the forward reaction of the acid hydrolysis of chlorophylls *a* and *b* to pheophytins *a* and *b* and that of several chlorophyllides to their respective pheophorbides were studied at various concentrations by hydrochloric acid and pigment. The rate constants were determined spectrophotometrically at 25, 35, 45, and 55°. The energies of activation (ΔH_a) were determined for the chlorophylls and their derivatives from the above data.

The rates of conversion of chlorophylls *a* and *b* to their respective pheophytins were first studied by Joslyn and Mackinney[1] and Mackinney and Joslyn[2,3] and have never been substantiated by other investigations. Their data are somewhat confusing on whether the reactions follow first- or second-order kinetics and with respect to the temperature dependence of the reactions.

Additionally Borodin[4] and Lesley and Shumate[5] postulated a slower rate of conversion of the the chlorophyllides to their respective pheophorbides than for chlorophyll to pheophytin but offered no experimental proof.

This work reports on the kinetics of the reaction of chlorophylls *a* and *b*, and the ethyl, methyl, and free chlorophyllides with H^+, each in a system (80% acetone, 20% dilute hydrochloric acid) which is 10^{-4} *N* in hydrochloric acid.

Highly purified, freshly prepared pigments were used in all studies in contrast to the mixtures used by Joslyn and Mackinney in their first study. The chlorophyllides were prepared from a plant source found to be rich in the enzyme chlorophyllase.

Instrumental refinements accomplished during the interim appeared to offer the possibility of avoiding other difficulties encountered by the original workers, emphasizing the necessity for some of the repetitive investigations reported.

Data secured while establishing the technique described herein showed that the conversion reaction apparently followed first-order kinetics. The data obtained by the procedure outlined were therefore evaluated using first-order methods.

The specific reaction constants (k) for the *a* series of compounds are given in Table I. They were calculated from the data taken using least square mathematics.

The temperature dependency of the reaction is shown in Fig. 1 where the logs of the k values from Table I are plotted *vs.* the reciprocal of the absolute temperature. The regression equation for each compound was calculated and used in drawing the lines shown in the plot. The data obtained gave a series of approximately parallel sloping lines as is shown by the values of the activation energies (ΔH_a) of 10.4, 10.4, 10.6, and 10.8 $\times 10^3$ cal./mole for chlorophyll *a*, ethyl chlorophyllide *a*, methylchlorophyllide *a*, and free chlorophyllide *a*, respectively, calculated from the curves. The temperature quotient (Q_{10}) for this conversion varied from 1.73 to 1.77 with a mean value of 1.75. The lines were nearly equidistantly spaced from each other.

Thus a regular pattern apparently exists in the kinetics of this reaction with chlorophyll *a* having the slowest rate and with the ethyl, methyl, and free chlorophyllides each having a somewhat faster

TABLE I

SPECIFIC RATE CONSTANTS FOR CHLOROPHYLL *a* AND SOME OF ITS DERIVATIVES

	k (Observed), min.$^{-1}$			
	25°	35°	45°	55°
Chlorophyllide *a*	0.0171	0.0295	0.0573	0.0908
Methylchlorophyllide *a*	.0156	.0264	.0464	.0827
Ethylchlorophyllide *a*	.0127	.0231	.0422	.0630
Chlorophyll *a*	.0103	.0191	.0335	.0583

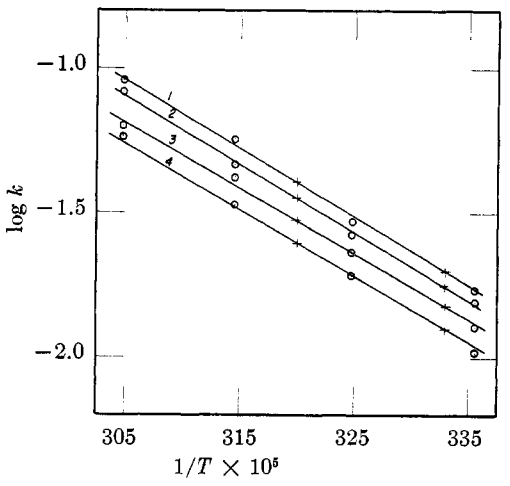

Fig. 1.—Plot of log k *vs.* reciprocal of absolute temperature for (1) chlorophyllide *a;* (2) methylchlorophyllide *a;* (3) ethylchlorophyllide *a;* and (4) chlorophyll *a*. The regression equations for the data shown are

(1) $E = 6.189 - 2370x$
(2) $E = 5.967 - 2318x$
(3) $E = 5.713 - 2262x$
(4) $E = 5.674 - 2279x$

(1) M. A. Joslyn and G. Mackinney, *J. Am. Chem. Soc.*, **60**, 1132 (1938).
(2) G. Mackinney and M. A. Joslyn, *ibid.*, **62**, 231 (1940).
(3) G. Mackinney and M. A. Joslyn, *ibid.*, **63**, 2530 (1941).
(4) J. Borodin, *Botan. Z.*, **40**, 608 (1882).
(5) B. E. Lesley and J. W. Shumate, U.S. Patent 2,097,198 (October 26, 1937).

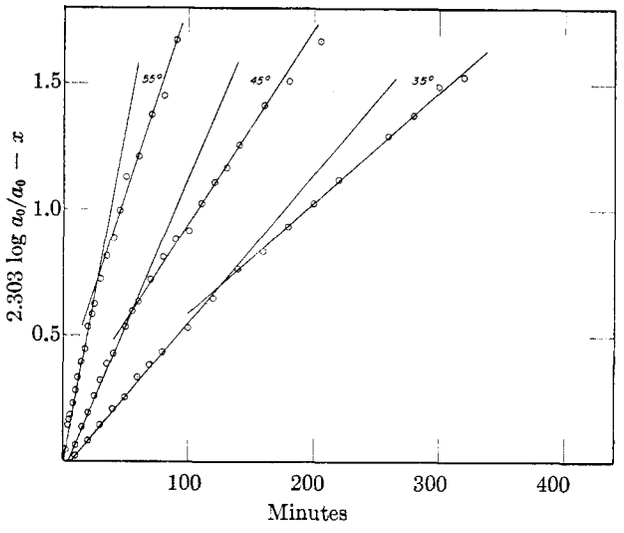

Fig. 2.—Rate of conversion of methylchlorophyllide *b* to methylpheophorbide *b* at 35, 45, and 55°.

rate. The difference in this regard is expressed by the intercept value in the regression equation, which expresses the magnitude of the frequency factor A in the equation: $k = Ae^{-E/RT}$.

The data obtained for chlorophyll *b* are similar in most respects to those obtained for chlorophyll *a*. The two compounds have about the same activation energies and temperature quotients. Chlorophyll *b* was found to react only 5.5 times slower than chlorophyll *a*, however, as compared to the 7 to 9 times slower rate reported by Mackinney and Joslyn.

Curves similar to those shown in Fig. 2 were obtained when 2.303 log $(a_0/a_0 - x)$ was plotted *vs.* t for the chlorophyllides *b;* where Fig. 2 shows this for the data obtained with methylchlorophyllide *b*. The striking difference in the behavior of chlorophyll *a* and its derivatives and the chlorophyll *b* derivatives lies in the slower rate of conversion. The data obtained for the latter compounds plot out with a slight curvature to which two lines can be fitted, one for the initial third and one connecting the last two thirds of the conversion. Repeated trials showed the phenomena to be real and not due to experimental error and different preparations using *Passiflora caerulea* instead of *A. altissima* reproduced the result.

It is thought that the initial rate is that of the conversion of allomerized pigment. Previous work by Mackinney and Joslyn[3] indicates that the rate of removal of the magnesium can be accelerated by oxidation of the isocyclic ring. Attempts were made to minimize the access of oxygen by blanketing the pigment solutions with nitrogen; this protection was lost, however, during the addition of acid and mixing. Once all the oxygen present in the reaction vessel was used up, the rate would become representative of the unoxidized starting material. The effect seems to be more noticeable in the *b* series, where the reaction occurs over a longer period of time.

The rate constants k and k^1, respectively, for each phase of the reaction as calculated from the data obtained, are reported in Table II. The values for the first-phase rate constant for chlorophyll *b* are left unreported, because some uncertainty exists in this respect. The break occurs much sooner in the course of this reaction than for the chlorophyllides and the calculated values would be based upon too few observed readings for accurate reporting.

The Arrhenius relationship for both phases of this reaction was evaluated graphically, with the second phase relationship shown in Fig. 3, assuming that it represents the reaction of the unaltered compounds. The regular pattern of reaction kinetics similar to that reported for chlorophyll *a* and its derivatives also is shown to exist for chlorophyll *b* and its derivatives. Replacing the phytol side chain with an ethyl or methyl group or hydrogen increases the rate in a nearly regular manner but has no effect on the temperature quotient or the activation energy required. Both phases of the reaction behave similarly in this regard. The mean of the temperature quotients for the first-phase rate for these compounds is 2.16 while a mean value of 1.86 is found to hold for the second-phase of the reaction. The mean activation energies for the first and second phases of the reaction are 14.5 and 11.7 $\times$ 10^3 cal./mole, respectively. The value for the activation energy for the second phase of the reaction corresponds rather closely with the mean activation energy reported for chlorophyll *a* and its derivatives.

The results of this work show that changing the chain length of the alcohol esterified with the propionic acid in the 7-position only slightly affects the ease with which the two hydrogen ions substitute for the magnesium ion in the center of the porphyrin ring. This result is not unexpected because the chain is essentially hydrocarbon in nature and is distant to the resonating part of the structure. The negligible influence of the substituents on the spectral characteristics of these compounds form the evidence that might be used as a basis for predicting the effects reported. The slight increase in rate of hydrolysis with shortening of the side chain is significant, however, and in the direction expected.

The small change in rate observed between the chlorophylls and the chlorophyllides would indicate that steric hindrance plays a minor role in the replacement reaction. It would also discount steric hindrance as a factor accounting for the difference in reaction rate between chlorophyll *a* and *b* since the C_{20} side chain should exert a greater steric effect than the 3-formyl group in *b*.

Steric or inductive effects caused by change in side chain length are small and therefore the re-

TABLE II

SPECIFIC RATE CONSTANTS FOR CHLOROPHYLL *b* AND SOME OF ITS DERIVATIVES

	k min.$^{-1}$				k^1 min.$^{-1}$			
	25°	35°	45°	55°	25°	35°	45°	55°
Chlorophyllide *b*	0.00293	0.00600	0.0139	0.0280	0.00260	0.00484	0.00945	0.0180
Methylchloro-phyllide *b*	...	.00573	.0119	.0269	...	.00443	.00780	.0152
Ethylchloro-phyllide *b*	...	.00444	.00910	.0182	...	.00394	.00690	.0126
Chlorophyll *b*	...	...	...	...	.00177	.00330	.00625	.0103

sults reported are significantly different but the total difference is nominal as might be expected.

Experimental

Preparation of Pigments.—Chlorophylls *a* and *b:* The procedure outlined by Mackinney[6] was used to extract these chlorophylls from frozen or fresh spinach. The drying steps and the alcohol washes were omitted, however, to avoid any possible allomerization. Separation and purification of the acetone extract was carried out by columnar chromatography on confectioners powdered sugar. The crude chlorophyll extract was applied to the column and developed with 4–9% acetone in petroleum ether. The zones containing the pigments were cut out and the pigments dissolved in acetone. Further purification of each pigment was accomplished by re-chromatography. Traces of sugar were removed by transferring the pigments to ether, washing them with water, and then transfering them back into acetone. The acetone solution of the pigments was dried with anhydrous sodium sulfate and stored at −26°.

Ethylchlorophyllides *a* and *b:* The compounds were prepared using the method of Holt and Jacobs[7] from fresh leaves of *Ailanthus altissima*. Preliminary investigations had shown this plant to be a rich source of chlorophyllase. Separation of the two chlorophyllides was accomplished by columnar chromatography on dry confectioners powdered sugar. A solution containing 15% chloroform plus 0.5% isopropyl alcohol in petroleum ether proved a better developing solvent than the solutions of chloroform or pyridine alone in petroleum ether used by Holt and Jacobs. Purification was accomplished by following the same procedures as outlined for the chlorophylls. Each pigment was then transferred to ether, washed free of sugar, transferred back into acetone and dried with anhydrous sodium sulfate.

Methylchlorphyllides *a* and *b:* These compounds were prepared by substituting methanol for the ethanol in the Holt and Jacobs procedure. All other steps were the same as those described in the preparation of the ethylchlorophyllides.

Chlorophyllides *a* and *b:* The method of Weast[8] was used in preparing these pigments. Fresh leaves of *A. altissima*, finely ground in a Waring blendor, were mixed with acetone to give an aqueous acetone solution of 66% acetone. The mixture was allowed to undergo enzyme reaction overnight at room temperature. The insolubility of the chlorophyllides in petroleum ether was used to check the completion of the reaction. As a further check, an ether extract of the pigments was underlayered with 0.001 *N* sodium hydroxide. All the green pigments transferred into the aqueous layer, indicating complete conversion of the ester to the free acid at the 7 position.

The separation and purification steps followed the same pattern used for the other pigments. Their visible spectra served as partial identification.

A Beckman DK2 recording spectrophotometer was used to determine the visible spectrum of each preparation in acetone solution. The spectra of the two parent compounds and each of their derivatives were found to be identical, which is in agreement with the findings of Holt and Jacobs[7] that the length of the side chain had no influence on the visible spectrum.

The ratio red peak/blue peak for each compound was used as criterium of purity. Only those preparations which had identical ratios, or exceeded the ratios reported by Mackinney[6] were used in the kinetic studies. Nearly all the preparations exceeded the purity ratios reported by Zscheile and Comar.[9]

Every preparation was checked for possible damage to the isocyclic ring by the Molisch phase test. The intermediate formed at the interface was yellow in the *a* series of compounds and reddish in the *b* series.

The hydrochloric acid number was used to differentiate between the chlorophylls and the chlorophyllides. All the chlorophyllides transferred into 22% hydrochloric acid.

Rate Studies.—The rates of conversion of the chlorophylls and the various prepared derivatives to pheophytin or their phenophorbides were determined at temperatures of 25.0, 35.0, 45.0, and 55.0°.

Rate studies on the *a* series of compounds were made by following the change in optical density (O.D.) at 660 mμ as a function of time in a Beckman Model B spectrophotometer. This instrument was selected because a modification could be made easily permitting the use of sealed reaction vessels. An aluminum block was constructed to accommodate two test tubes, one of which was fixed in the lightpath. Water from a constant temperature bath was circulated through the block, maintaining the desired temperature to within ±0.1° of the set temperature at all times. All parts were painted flat black to absorb stray light.

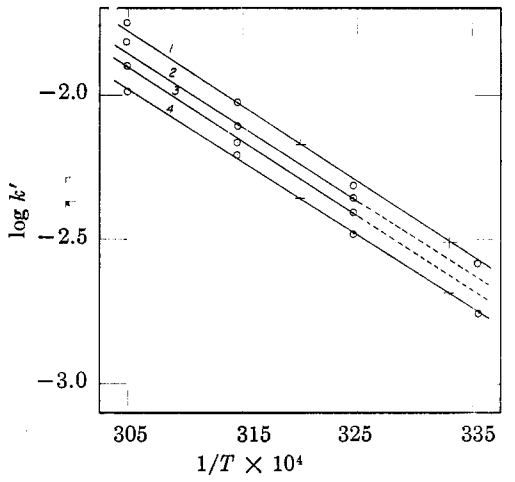

Fig. 3.—Plot of log k' *vs.* reciprocal of absolute temperature for (1) chlorophyllide *b;* (2) methylchlorophyllide *b.* (3) ethylchlorophyllide *b;* and (4) chlorophyll *b.* The regression equations for the data shown are

(1) $E = 6.234 - 2626x$
(4) $E = 5.778 - 2542x$

(6) G. Mackinney, *J. Biol. Chem.*, **132**, 91 (1940).
(7) A. S. Holt and E. E. Jacobs, *Am. J. Botany*, **41**, 710 (1954).
(8) C. A. Weast, M.S. thesis, University of California, May 1, 1939.
(9) F. P. Zscheile and C. L. Comar, *Botan. Gaz.*, **102**, 463 (1941).

O.D. readings at 645 mμ were followed in studying the rates of converstion of the *b* series of compounds. This wave length was selected because it was found to be the red-peak in the difference-spectra of chlorophyll *b* and pheophytin *b*. This finding has since been confirmed by Vernon.[10]

Trial runs were made with different buffer systems and aqueous hydrochloric acid solutions. All the buffer systems tried turned turbid when added to acetone solutions of the pigments to initiate the reaction. The aqueous hydrochloric acid solutions did not exhibit this difficulty and were therefore used in these studies. Acid was added to give a H^+ concentration of 1×10^{-4} in the 80% acetone reaction mixture. At this acid concentration the reaction rate was neither too fast at the elevated temperature nor too slow at the lower temperatures. The reduction in volume due to the hydration of the acetone molecules was taken into consideration in preparing this mixture. Hydrogen ions were in 10–100-fold excess of pigment concentration under the conditions used.

The concentration of pigment in the reaction mixture was adjusted to give an initial O.D. reading of about 0.43 in order to be within the range of greatest accuracy of the instrument and a range of concentration in which Beers law is followed, as indicated by series of tests. Preliminary trials where ΔOD was plotted *vs.* *t* for different chlorophyll concentrations indicated that the reaction was first order with respect to chlorophyll concentration.

Fifteen to twenty O.D. readings were taken during the course of each test, each of which was made in triplicate, to reduce the errors owing to instrumental inconsistencies on the rate factors. The conversion reaction was followed to at least 75% completion, following which it was completed by addition of a small crystal (8–10 mg.) of oxalic acid to the reaction vessel. The reaction was then assumed to be complete when the O.D. reacted a constant value.

(10) L. P. Vernon, *Anal. Chem.*, **32**, 1144 (1960).

The Baeyer–Villiger Condensation. I. *ortho*-Tritylation of Phenols

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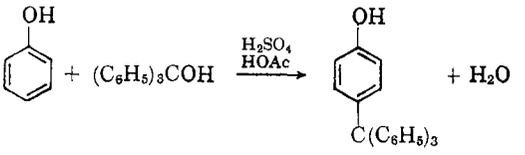
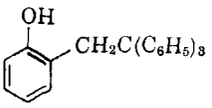
Procedures have been developed which permit the introduction of a trityl group *either ortho* or *para* to a phenolic hydroxyl group, within the limitation of steric influences of other substituents present. The generality of these specific procedures has been explored.

The introduction of the triphenylmethyl (trityl) group into the aromatic ring to form a triphenylarylmethane has often been referred to as the Baeyer-Villiger condensation, in recognition of the first observation and study of the reaction in 1902.[1] The initial observations were of the reaction between triphenylcarbinol and phenol in acetic acid, under the influence of sulfuric acid, to produce *para*-tritylphenol. Since that time, the reaction has been extended and found to embrace a large number of variations.



The triphenylcarbinol portion of the equation may be replaced with any equivalent that is capable of forming a stable carbonium ion, such as triphenylmethyl chloride[2] or the dichlorophosphonyl ether, $(C_6H_5)_3COPCl_2$.[3] In addition to alkylphenols, the reaction occurs with halophenols,[4] phenyl ethers,[1,3] anilines,[3c,4] and even unactivated alkyl-substituted benzenes.[5] Catalysts employed include sulfuric acid, hydrochloric acid,[5] perchloric acid,[6] zinc chloride[2a,7] or by direct fusion of the components without the intervention of any catalyst at all.[2a,2b,4] The presence of negative groups on a phenol, such as a nitro[3] or carbonyl,[8] prevents the reaction from occurring.

The structure of the tritylation products has been the subject of several investigations. Initial assignments as O-trityl ethers (to account for the lack of phenolic properties of the products) were disproven by the preparation of a true trityl phenyl ether by the direct action of triphenylmethyl chloride upon potassium phenolate.[2a] Aliphatic tritylation, exemplified by the suggested structure for the tritylation



product of *o*-cresol,[9] has been disproven both by the independent synthesis of the above ethane compound by a different route[10] (which proved to be a different material), as well as by the independent synthesis of *p*-trityl-*o*-cresol[11] (which proved to be identical with the *o*-cresol tritylation product).

(1) A. Baeyer and V. Villiger, *Ber.* **35**, 3013 (1902).

(2)(a) A. Baeyer, *Ber.* **42**, 2624 (1909). (b) M. Busch and R. Knoll, *Ber.*, **60**, 2243 (1927). (c) P. Schorigin, *Ber.*, **60**, 2373 (1927).

(3)(a) D. R. Boyd and G. Chignell, *J. Chem. Soc.*, 813 (1923). (b) D. R. Boyd and F. J. Smith, *ibid.*, 1477 (1924). (c) D. R. Boyd and D. V. N. Hardy, *ibid.*, 630 (1928).

(4) D. V. N. Hardy, *ibid.*, 1000 (1929).

(5) C. A. MacKenzie and G. Chuchani, *J. Org. Chem.*, **20**, 336 (1955).

(6) H. Burton and G. W. H. Cheeseman, *J. Chem. Soc.*, 832 (1953).

(7) J. van Alphen, *Rec. Trav. Chim.*, **46**, 288 (1927).

(8) N. P. Buu-Hoï, *J. Org. Chem.*, **22**, 666 (1957).

(9) P. Schorigin, *Ber.*, **59**, 2502 (1926).

(10) H. A. Iddles, K. S. French, and E. F. Mellon, *J. Am. Chem. Soc.*, **61**, 3192 (1939).

(11) H. A. Iddles and H. L. Minckler, *ibid.*, **62**, 2757 (1940).