C_2F_4N, 121 (1.3) $C_3F_3N_2$, 145 (1.6) C_3F_5N, 164 (17.8) C_3F_6N, 171 (1.3) $C_4F_5N_2$, 209 (3.0) $C_4F_7N_2$, and 214 (1.5) C_4F_8N.

Photolysis with Octafluorobutene-2. Tetrafluoro-2,3-diaza-1,3-butadiene (0.15 g, 1.17 mmoles) and octafluorobutene-2 (0.234 g, 1.17 mmoles) were condensed under vacuum into a silica tube fitted with a Fischer-Porter polytetrafluoroethylene valve and photolyzed for 12 hr at room temperature using the BH-6 lamp. Octafluorobutene-2 (0.58 mmole, 50%), hexafluoro-2,4-diaza-1,4-pentadiene (0.2 mmole, 15%), and dodecafluoro-3,4-dimethyl-2,5-diaza-1,5-hexadiene, $CF_2{=}N{-}CF(CF_3){-}CF(CF_3){-}N{=}CF_2$ [0.35 mmole, 30% (*Anal.* Calcd for $C_6F_{12}N_2$: F, 69.5; mol wt, 328. Found: F, 67.6; mol wt, 324)], were isolated by fractional distillation-condensation and vapor phase chromatography. The mass spectrum of $CF_2{=}N{-}CF(CF_3){-}CF(CF_3){-}N{=}CF_2$ is summarized as follows: 31 (17.4) CF, 50 (16.8) CF_2, 69 (100) CF_3, 76 (10.6) C_2F_2N, 95 (9.3) C_2F_3N, 100 (2.1) C_2F_4, 114 (75) C_2F_4N, 121 (2.4) $C_3F_3N_2$, 126 (2.8) C_3F_4N, 145 (3.1) C_3F_5N, 164 (85.2) C_3F_6N, 176 (1.4) C_4F_6N, 190 (1.0) $C_4F_6N_2$, 221 (2.6) $C_5F_7N_2$, and 259 (1.7) $C_5F_9N_2$.

Photolysis with Chlorotrifluoroethylene. Tetrafluoro-2,3-diaza-1,3-butadiene (0.26 g, 2.0 mmoles) and chlorotrifluoroethylene (0.15 g, 1.0 mmole) were condensed under vacuuum into a 5-l. flask fitted with a water-cooled quartz insert and irradiated for 35 min with a Hanovia 450-w, low-pressure ultraviolet light.

Hexafluoro-2,4-diaza-1,4-pentadiene (0.12 mmole, 12%) and heptafluoro-2,5-diaza-3-chloro-1,5-hexadiene (0.23 mmole, 23%) were isolated from the crude products by vapor phase chromatography and characterized by their infrared, mass, and F^{19} nmr spectra.

The mass spectrum of $CF_2{=}N{-}CF_2{-}CFCl{-}N{=}CF_2$ is summarized as follows: 31 (33.1) CF, 35 (2.5) Cl, 45 (1.4) CFN, 50 (35.6) CF_2, 57 (1.2) C_2FN, 66 (1.7) CFCl, 69 (100) CF_3, 76 (21.1) C_2F_2N, 81 (1.0) C_2F_2, 85 (17.7) CF_2Cl, 92 (1.2) C_2FClN, 95 (17.4) C_2F_3N, 111 (1.1) C_2F_2ClN, 114 (83.4) C_2F_4N, 121 (5.2) $C_3F_3N_2$, 130 (60.2) C_2F_3ClN, 145 (3.1) C_3F_5N, 159 (3.1) $C_3F_5N_2$, 180 (2.4) C_3F_5ClN, and 209 (14.4) $C_4F_7N_2$.

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Reaction of Chlorophylls a and b with Amines. Isocyclic Ring Rupture and Formation of Substituted Chlorin-6-amides[1]

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Abstract: Spectral studies of the reaction of amines with chlorophylls a and b confirm the fact that, with primary and secondary amines, cleavage of ring V occurs. With chlorophyll b reaction also occurs at the formyl group when primary amines are used. Kinetic data indicate that alcohol and water have a marked effect on the rate of the reaction in pure amines. Chemical and chromatographic studies support the spectral observations.

The β-keto ester system in ring V of chlorophylls a and b (**1a**) and related compounds is particularly sensitive to nucleophilic attack by alkoxides and by amines.[3] Nomenclature and proton numberings are shown in Figures 1 and 2. Ring V has frequently been the focus of attention because of the special role it may play in photosynthesis. It is the site of oxidation reactions leading to 10-hydroxy derivatives by enzymatic reactions[4a] and by the allomerization reaction.[4b] The keto group in ring V coordinates with magnesium to give chlorophyll aggregates in nonpolar solvents.[5] This group is also the site of attack by amines.

Fischer and co-workers[6] investigated the reactions of magnesium-free derivatives of chlorophyll such as methyl pheophorbide, *meso*-pheophorbide, and methyl pheoporphyrin with bases such as ammonia, piperidine, methylamine, hydrazine, and phenylhydrazine. Fischer concluded that ring V was cleaved and that chlorin-6-amide derivatives were formed.

Weller and Livingston[3b] extended Fischer's work to include chlorophylls a and b. On the basis of analogy they proposed that ring V was cleaved and that a substituted chlorin-6-amide was formed, apparently with retention of the magnesium. With chlorophyll b (**1b**) it was not clear from their data whether or not reaction also occurred at the formyl group. The spectral data also made it difficult to be certain of ring V

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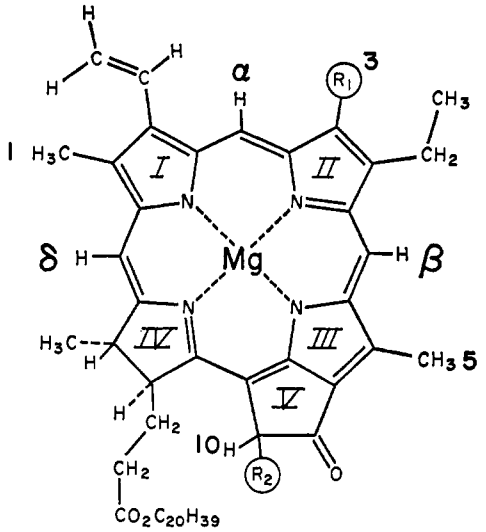
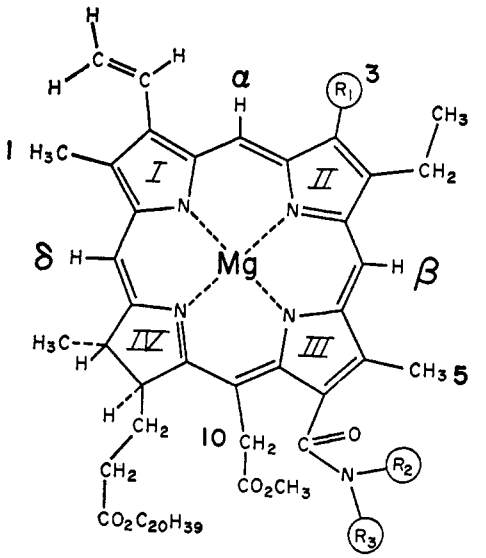


1a, $R_1 = CH_3$; $R_2 = CO_2CH_3$
1b, $R_1 = CHO$; $R_2 = CO_2CH_3$
2a, $R_1 = CH_3$; $R_2 = H$
2b, $R_1 = CHO$; $R_2 = H$
2c, $R_1 = CH_3CH_2CH_2N{=}CH$; $R_2 = H$

Figure 1. Proton designations for compounds with intact ring V.



3a, $R_1 = CH_3$; $R_2 = H$; $R_3 = CH_3CH_2CH_2$
3b, $R_1 = CH_3CH_2CH_2N{=}CH$; $R_2 = H$; $R_3 = CH_3CH_2CH_2$
4a, $R_1 = CH_3$; $R_2 = H$; $R_3 = (CH_3)_2CHCH_2$
5a, $R_1 = CH_3$; $R_2, R_3 = (CH_2)_5$
5b, $R_1 = CHO$; $R_2, R_3 = (CH_2)_5$

Figure 2. Proton designations for chlorin-6-amides.

cleavage; in Fischer's investigations there was very little shift in the λ_{max} of the major red peak while the shifts observed by Weller and Livingston were about 20 mμ. There have been numerous studies on the photochemistry of chlorophyll in the presence of amines and hydrazines.[7] Recently, Seely[8] has used the chlorophyll–amine reaction with ethyl chlorophyllides and chlorophyll to compare them with the reactivity of "hypochlorophylls" toward amines. His visible spectral data of the products derived from chlorophyll and chlorophyllides are comparable to those we have observed but his cited preparative procedures[6] were used by Fischer to prepare magnesium-free products. These reports, together with the possible interaction of organic bases, such as ethanolamine[9] or basic groups on associated protein,[10] with chlorophyll in the plant, made it desirable to explore these reactions more thoroughly, making use of infrared and nmr spectra[5] for structural determinations and for following the course of the reaction.

We first investigated the amine–chlorophyll reaction by injecting ethereal solutions of chlorophyll into the amine using Weller and Livingston's procedure[3b] and observing the spectral changes by rerunning the spectrum periodically. The major red peak of chlorophyll a (**1a**) occurred at about 660 mμ, while the major peak of the product occurred at about 640 mμ. We treated the data in the same way as Weller and Livingston (see Experimental Section), but our rate data differed considerably from theirs (Table I). It was soon apparent that the differences might be due to trace amounts of water or alcohols, since we found that the reaction rates

Table I. Logarithm of Velocity Constants for the Reaction of Chlorophylls a and b with Amines

Compound	Amine	pK_b	Log k', sec^{-1} at 26°		
			Weller and Livingston[a]	In anhydrous amine	In amine plus ethanol (% EtOH, v/v)
Chlorophyll a	Propylamine	3.41	...	−3.84	−3.09 (1.0) −2.84 (0.10) −2.81 (0.010)
Chlorophyll a	Isobutylamine	3.51	−2.82	−3.95	...
Chlorophyll a	Piperidine	2.79	−1.88	−2.92	−2.63 (1.0) −2.49 (0.10) −2.37 (0.010)
Chlorophyll a	Ethanolamine	4.56	...	−1.42	−1.47 (1.0) −1.47 (0.10)
Chlorophyll b	Propylamine	3.41	...	−3.11	...
Chlorophyll b	Isobutylamine	3.51	−2.31	−3.32	...
Chlorophyll b	Piperidine	2.79	−1.40	−2.76	−2.82 (0.010)

[a] See ref 3b.

(7) G. R. Seely in "The Chlorophylls," L. P. Vernon and G. R. Seely, Ed., Academic Press Inc., New York, N. Y., 1966, p 558.
(8) G. R. Seely, *J. Am. Chem. Soc.*, **88**, 3417 (1966).
(9) B. Lerch and H. Stegemann (*Z. Naturforsch.*, **21b**, 216 (1966)) have recently reported that free ethanolamine is widely distributed in the leaves of higher plants.
(10) Reference 7: J. C. Goedheer, p 399; J. M. Olson, p 413; B. Ke, p 427.

were especially sensitive to the presence of these solvents (Table I). The reaction rate was greatest for the chlorophyll a–piperidine reaction, when the molar ratio of alcohol to chlorophyll was about 1:1.

In general, the reactions of chlorophyll b with amines were more rapid, and the kinetics were not as cleanly first order as they were with chlorophyll a. In the case of primary amines this was very probably due to reaction at both ring V and at the formyl group. We did not observe as rapid a reaction with piperidine as Weller and Livingston had, but the order of reactivity was the same as it was in the a series: piperidine > propylamine > isobutylamine.

In our studies we isolated solid chlorophyll by Strain's procedure,[11] freed it of water and alcohols, and made anhydrous ethereal solutions of the solid. The nmr spectrum of our chlorophyll a in chloroform-*d* was virtually identical with the aggregated spectrum previously reported.[5] Chlorophyll with trace amounts of disaggregating solvent exhibits significant shifts in certain nmr resonances. Weller and Livingston, it may be observed, used ethereal eluents to recover chlorophyll from chromatographic columns for their studies.

Drying of the amine proved to be the most critical factor in the rate studies. Reactions with amine taken directly from a stock bottle were relatively fast. After drying the amine over potassium hydroxide and distilling it, either in a stream of dry nitrogen or helium, as Weller and Livingston had done, the reaction rate was reduced. But the rate was still faster than the rate obtained with amine dried over molecular sieves. The amine thus treated showed no evidence of water either in gas chromatograms or in nmr spectra.

The variations of the reaction rate with solvent impurities indicate that a complex is involved. It is known that in nonpolar solvents, such as chloroform-*d*, small amounts of methanol lead to disaggregation of associated chlorophyll molecules.[5] Seely and Folkmanis[12] have reported that in ethanol–pyridine solutions of chlorophyll a, mixed solvate, Chl(eth)(py), is apparently the predominant species, except in the extremes of solvent composition. Thus, although one might expect that the amines would be effective in disaggregating and in coordinating, our kinetic data indicate that interaction of chlorophyll with alcohol is significant even in the presence of large concentrations of amine. The maximum rate is observed when the molar ratio of chlorophyll to alcohol is about 1:1 and increasing the amount of alcohol retards the reaction. These unusual effects may imply interaction of the alcohol with some other proton of the chlorophyll molecule in addition to the interaction with magnesium, and it is possible that the great hydrogen-bonding propensity of the hydroxyl group may be involved.

Our experiments with ethanolamine were of special interest, since the ethanolamine possesses both hydroxyl and amino groups. The reaction of ethanolamine with chlorophyll a proved to be very fast, contradicting Weller and Livingston's conclusion that the reaction rates were dependent on the base strengths of the amines. The effects of added ethanol were slightly inhibiting, within experimental error, for such a rapid reaction.

The change of about 20 mμ in the red peak λ_{max} occurs only when magnesium is present. A similar shift is reported in the conversion of chlorophyll a to chlorophyllin a[3a] by means of methanolic potassium hydroxide, so that the amide grouping is not an essential structural feature for the large observed shift in absorption. With pheophytin a and propylamine there was very little change in λ_{max} of the red peak, but reaction was observable in the infrared. Chromatographic analysis showed that removal of magnesium from the propylamide **3a** prepared from chlorophyll a gave the same product that was obtained from reaction of pheophytin a with propylamine.

The spectral changes in the visible were correlated with significant changes in the infrared and with chromatograms by following the reaction using all three measurements simultaneously. Chlorophyll was dissolved in 3–10% solution (v/v) of amine in tetrahydrofuran. As the reaction occurred, there was no appreciable decrease in the ester carbonyl absorption whereas the ketone carbonyl decreased. At the same time a new absorption, assumed to be due to the amide carbonyl, appeared at about 1650 cm^{-1}. Visible spectra and chromatographic analysis of the samples used for infrared study showed that with each amine a single product was being formed whose visible spectrum was the same as that observed in our kinetic studies.

Reactions involving chlorophyll b and primary amines showed a clear indication, in the infrared spectra, of reaction with the formyl group. Both the ketone and formyl carbonyl absorption decreased in intensity when the primary amines, propylamine and isobutylamine, were used. With piperidine the same reaction conditions did not affect the intensity of the formyl carbonyl absorption.

Comparable reactions with amines did not occur with pyrochlorophyll a (**2a**),[13] establishing the fact that the β-keto ester system is essential for ring V cleavage, just as it is for the phase test. With pyrochlorophyll b (**2b**) and propylamine, however, there was infrared evidence of reaction with the formyl group, but this reaction did not lead to any major changes in the visible spectrum. Our reported velocity constants for chlorophyll b did not follow first-order kinetics as cleanly as those reported for chlorophyll a, partly because of these formyl reactions with primary amines.

The amine reactions were also readily followed by means of nmr spectra in tetrahydrofuran-d_8. Significant shifts were observed for the α, β, δ, OCH_3, and 5-CH_3 (Table II). The C-10 proton was eliminated during the course of the reaction, and a new resonance associated with two protons appeared upfield (0.35–1.00 ppm), thus confirming the deduction that ring V had been opened and that a chlorin-6-amide had been formed.

The resonance associated with the new C-10 protons is not a sharp single peak but appears broadened by about 5 cps. In the case of the piperidine compounds this resonance is a poorly defined multiplet in the tetrahydrofuran-d_8 reaction mixture. The chlorophyll a–piperidine product in chloroform-*d* with added

(11) H. H. Strain, M. R. Thomas, and J. J. Katz, *Biochim. Biophys. Acta*, 75, 306 (1963).

(12) G. R. Seely and A. Folkmanis, *J. Am. Chem. Soc.*, **86**, 2763 (1964).

(13) F. C. Pennington, H. H. Strain, W. A. Svec, and J. J. Katz, *ibid.*, **86**, 1418 (1964).

Table II. Comparison of Nmr Chemical Shifts of Chlorophylls and Chlorophyll Amine Products[a] (δ, ppm from tetramethylsilane)

Compd	CHO	CHN	α	β	δ	C-10 H	CO_2-CH_3	5-CH_3
1a	...	...	9.36	9.60	8.45	6.21	3.73	3.61
3a	...	...	9.53	9.55	8.55	5.46	3.65	3.36
4a	...	...	9.53	9.55	8.57	5.46	3.65	3.36
5a	...	...	9.54	9.57	8.58	5.31[b]	3.75[c] 3.71	
1b	11.20	...	10.09	9.65	8.25	6.05	3.66	3.50
3b	...	9.60	10.66	9.37	8.15	5.30	3.56	3.25
5b	11	...	10.11	9.40	8.25	5.05	3.68[c] 3.64	3.20

[a] Spectra in tetrahydrofuran-d_8 containing about 10 moles of amine to 1 mole of pigment. These chemical shifts were very close to those obtained in pure tetrahydrofuran-d_8. [b] This is a multiplet which is a singlet in chloroform-d with added methanol-d_3. [c] There are two resonances associated with CO_2CH_3 in this solvent system.

methanol-d_4 exhibits a much sharper 10-CH_2 resonance. These data may be explained by steric and aggregation factors, as the ester group would be expected to be affected by the 6-carboxamide group. The OCH_3 resonance shifts about 0.08–0.10 ppm upfield after reaction with propyl- and isobutylamine.

In both chlorophylls a and b treated with piperidine, the original OCH_3 is split into two peaks. As the reaction proceeds in tetrahydrofuran-d_8 the C-10 proton disappears rapidly, and the associated shifts of α, β, and δ proton resonances also occur rapidly. The splitting of the OCH_3 is a slower process. Only one major product is isolated by column chromatography. The chlorophyll a piperidide also exhibits only a single OCH_3 resonance in chloroform-d with added methanol-d_4. A methanol titration[5] of this product in chloroform-d shows that both the C-10 protons and the OCH_3 group are markedly affected by small amounts of methanol, which is known to act as a disaggregating agent under these conditions. The data point to the fact that the protons in the new 10-CH_2 group are not equivalent. With the piperidides the relatively large piperidino group accentuates this nonequivalence and two conformers are observed.

In the chlorophyll b reactions propylamine and piperidine show marked differences. The shifts of the low-field protons are very much greater in the case of propylamine, and the formyl proton is converted to an aldimine proton. Assignment of the resonance at δ = 9.60 ppm to the aldimine proton is based on the fact that it is not as sharp as the other resonances at low field. The observed shift of 1.60 ppm is comparable to the shift of 1.85 ppm observed for the conversion of the formyl proton in benzaldehyde to the propylaldimine.

In Table II we have used the assignments of Closs and co-workers.[5] In our chlorophyll a–amine experiments, however, the methine proton designated α shifts downfield 0.183 ppm, while the β proton resonance shifts only slightly upfield, 0.033 ppm. Since opening ring V would be expected to have a greater effect on the chemical shift of the β proton, these observations reopen the question as to whether the lower field proton resonance in chlorophyll a should be assigned to the α proton.

At the completion of the nmr studies the reaction mixtures were chromatographed. The chlorophyll a–isobutylamine reaction and the chlorophyll a– and b–piperidine reaction mixtures were free of even trace amounts of colored by-products. With the propylamine reactions a trace amount of by-product was observed with chlorophyll a, while with the chlorophyll b a minor component, estimated at 5–10%, was present above the major component zone on the column.

Because of the similarity of methyl 2-cyclopentanonecarboxylate to the ring V in chlorophyll, we also investigated its reaction with amines.[14] We found that ring opening does not occur as readily as it does with chlorophyll, and that when the ring does open, diamides of adipic acid are formed. The initial reaction between methyl 2-cyclopentanonecarboxylate and amines such as propylamine and isobutylamine results in the formation of 1:1 addition compounds, probably with carbinolamine structures. In a much slower reaction in tetrahydrofuran, over a period of days at room temperature, primary amines were found to form enamines. Analogous reactions or ketimine formation may occur with chlorophyll to a minor extent, but we were not able to detect them in our experiments. Ring opening appears to be the rule when chlorophyll reacts with primary and secondary organic bases.

Experimental Section

Isolation of Chlorophylls and Chlorophyll Derivatives. Chlorophylls a and b were isolated from spinach essentially by the procedure reported by Strain, Thomas, and Katz.[11] Two separations were made on powdered sugar columns using 0.5–1% propanol in petroleum ether (bp 20–40°) for development. The pigments were precipitated twice from petroleum ether and thoroughly dried under vacuum.

The amine derivatives were also purified by powdered sugar column chromatography. The pigments were dissolved in ether which was then dissolved in petroleum ether. They formed greenish blue bands on the columns. The columns were washed with 0.5–1% propanol in petroleum ether. The bands were carefully cut out of the columns and the pigments eluted with anhydrous ether. The ether solutions were washed thoroughly with water, dried (Na_2SO_4), and concentrated under vacuum. The residual waxy solids were frequently precipitated by adding water to methanol solutions. The derivative was usually purified by dissolving it in a small amount of ether and precipitating it with petroleum ether.

Magnesium Isochlorin e_4 6-Carboxypropylamide Phytyl Methyl Ester (3a). This compound was precipitated from a concentrated ether solution by addition of petroleum ether: λ_{max}^{ether} 640 and 413 mμ; $\lambda_{max}^{pyridine}$ 646 and 429 mμ; ν_{max}^{THF} 1740 (CO_2R), no 1700 (CO), and 1647 cm^{-1} (CON). *Anal.* Calcd for $C_{58}H_{81}N_5O_5Mg$: N, 7.35. Found: N, 7.50.

Magnesium Isochlorin e_4 6-Carboxyisobutylamide Phytyl Methyl Ester (4a). This compound was recovered by removing the solvent from purified ether solutions: λ_{max}^{ether} 641 and 414 mμ; $\lambda_{max}^{pyridine}$ 647 and 430 mμ; ν_{max}^{THF} 1740 (CO_2R), no 1700 (CO), and 1647 cm^{-1} (CON). *Anal.* Calcd for $C_{59}H_{83}N_5O_5Mg$: C, 73.31; H, 8.65; N, 7.25. Found: C, 73.25; H, 8.84; N, 7.12.

Magnesium Isochlorin e_4 6-Carboxypiperidide Phytyl Methyl Ester (5a). This compound was precipitated from a concentrated ether solution by adding petroleum ether: λ_{max}^{ether} 641 and 414 mμ; $\lambda_{max}^{pyridine}$ 647 and 430 mμ; ν_{max}^{THF} 1740 (CO_2R), no 1700 (CO), and 1629 cm^{-1} (CON). *Anal.* Calcd for $C_{60}H_{83}N_5O_5Mg$: N, 7.16. Found: 7.12.

Magnesium Isorhodin g_5 6-Carboxypropylamide-3-propylaldimine Phytyl Methyl Ester (3b). This compound formed very gelatinous precipitates in petroleum ether: λ_{max}^{ether} 622 and 441 mμ; ν_{max}^{THF} 1740 (CO_2R), no 1700 (CO), no 1663 (CHO), 1653 (CON), and 1634 cm^{-1} (CHN). *Anal.* Calcd for $C_{61}H_{86}N_6O_5Mg$: N, 8.34. Found: 8.24.

Magnesium Isorhodin g_5 6-Carboxypiperidide Phytyl Methyl Ester (5b). This compound was precipitated from concentrated ether solution by adding petroleum ether: λ_{max}^{ether} 621 and 441 mμ; ν_{max}^{THF} 1740 (CO_2R), no 1700 (CO), 1663 (CHO), and 1626 cm^{-1}

(14) F. C. Pennington and W. D. Kehret, in preparation.

(CON). *Anal.* Calcd for $C_{60}H_{81}N_5O_6Mg$: N, 7.06. Found: N, 6.94.

Pyrochlorophyll b (2b). This compound was prepared from chlorophyll b following the procedure for the preparation of pyrochlorophyll a. It was purified by means of powdered sugar chromatography: λ_{max}^{ether} 644 and 451 mμ, ratio blue:red 2.97; ν_{max}^{THF} 1737 (CO_2R), 1694 (CO), and 1661 cm^{-1} (CHO).

Pheophytin a and isochlorin e_4 6-carboxypropylamide were prepared from the corresponding magnesium complexes by treating ethereal solutions of **1a** and **3a** with 1:1 hydrochloric acid for several seconds. Water was used to wash out the acid. The ether solutions were dried (Na_2SO_4) and evaporated, and the residue was chromatographed on powdered sugar. Isochlorin e_4 6-carboxypropylamide (λ_{max}^{ether} 662 and 441 mμ), prepared in this way, was chromatographically identical with that prepared from pheophytin a and propylamine.

Procedures for Following the Reaction of Chlorophylls with Amines. A. Visible Spectrum Investigations. Ethyl ether solutions (about 40–70 μl) of chlorophyll were injected into quartz or Pyrex cells containing 3.5 ml of redistilled dry amine. A Beckman DK-2A spectrophotometer was used for the measurements with the cell compartment thermostated at 26°. The first spectrum was determined as soon after mixing as possible, and further measurements were made at appropriate intervals, depending on the rate of the reaction. The equation derived by Weller and Livingston[3b] was used for the rate constant calculations with E_0 taken as an extrapolated value.

$$\frac{\text{concn of chlorophyll at time } t}{\text{initial concn of chlorophyll}} = \frac{(E_{obsd} \text{ at time } t) - E_\infty}{E_0 - E_\infty}$$

For $k' = k$(concn amine), where k' is the pseudo-first-order rate constant

$$k't = \log \frac{(E - E_\infty)}{(E_0 - E_\infty)}$$

For those reactions in which methanol, ethanol, or water were added, amine solutions containing varying amounts of the solvent were prepared, and the procedure above was followed. The 0.01% (v/v) ethanol in piperidine gave a mole ratio of chlorophyll to alcohol of about 1:1.

B. Nmr Spectrum Investigations. (1) Nmr Spectrum of Chlorophyll Used in Kinetic Studies. The chlorophyll (40 mg) was dissolved in chloroform-*d* (*ca.* 0.3 ml) and the spectrum determined. It proved to be virtually the same as that previously reported.[5]

(2) Reaction of Chlorophyll with Amines in Tetrahydrofuran. The chlorophyll (*ca.* 35 mg) was dissolved in about 250 ml of tetrahydrofuran. The amine (20–25 ml) was added and the solution sealed on a vacuum line after degassing. The reaction was usually complete after 1 day. The tube was opened, the contents transferred, and solvent and excess amine were removed *in vacuo*. Chromatography on powdered sugar as described above gave the amides.

Preparation and Properties of 10-Hydroxychlorophylls a and b[1]

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Abstract: Green substances produced by enzymatic oxidation of chlorophylls a and b are shown by chemical analysis, chromatography, nmr, and infrared spectroscopy to be 10-hydroxychlorophylls. These compounds are also shown to be major products of the allomerization reaction of the chlorophylls in CH_3OH. Similar allomerization of the fully deuterated chlorophylls, with H substituted at C-10 by normal exchange, provides the corresponding fully deuterated oxidation products with the C-10 H replaced by OH, as shown by nmr. These results provide critical evidence for the molecular structure of the oxidized chlorophylls and strong support for the structure of the methoxy lactones that are also formed in the allomerization reactions.

In the course of previous work on the nuclear magnetic resonance and infrared spectroscopy of the chlorophylls,[3–5] we tried to prepare the methyl chlorophyllides by enzymatic methanolysis, using a procedure similar to the one used by Holt and Jacobs.[6] The plant material available to us, however, contained an active oxidative enzyme in addition to the chlorophyllase required for the *trans* esterification and we isolated a product, different from both chlorophyll and methyl chlorophyllide, which we decided after brief study must be an oxidized chlorophyll, probably formed by the introduction of an hydroxyl group at C-10.[7] The compounds corresponding to both the a and b series were obtained.[8] Another green compound that appeared to be closely related to the oxidized chlorophylls was also encountered by Barrett and Jeffrey[9] in the extracts of diatoms. Although their substance has not been definitely characterized, it seemed to be an hydroxy derivative of the free acid (chlorophyllide a).

On the basis of further work, we now believe we have definitely established the structure of the "oxidized" chlorophylls prepared enzymatically as 10-hydroxychlorophylls a and b and have shown them to be structurally identical with major products of the allomerization reaction of chlorophylls in methanol. The allomerization of the ordinary chlorophylls and of the fully deuterated chlorophylls thus becomes a practical procedure for the preparation of 10-hydroxychlorophylls.

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