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Studies on the Chemical and Photochemical Oxidation of Bacteriochlorophyll[1]

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Abstract

A simplified procedure is described for the preparation of crystalline bacteriochlorophyll from R. rubrum. The chemical dehydrogenation of bacteriochlorophyll with quinones is shown to give high yields of 2-desvinyl-2-acetyl-chlorophyll a, whereas the photooxidation of bacteriochlorophyll results in a mixture of products of which 2-desvinyl-2-acetyl-chlorophyll a is only a minor constituent. A number of interesting results have been observed spectrophotometrically during these oxidations under different reaction conditions. These observations are discussed and possible reaction mechanisms are outlined.

The proton magnetic resonance spectrum of 2-desvinyl-2-acetyl-chlorophyll a in deuteroacetone and the visible absorption spectra of this pigment and its magnesium-free derivative in acetone are reported. As expected, these spectra exhibit a marked resemblance to chlorophyll a and pheophytin a.

Introduction

Most of the recent research into the structure and physical and chemical properties of photosynthetic pigments has been limited to chlorophyll a and b, whereas bacteriochlorophyll, owing to its instability in organic solution, has largely been neglected. The degree of instability of bacteriochlorophyll varies enormously with the physical state of the pigment and with the conditions of storage; thus, crystalline bacterochlorophyll is stable when kept in the dark,[3] while the stability of solutions of this pigment can vary over a hundred-fold depending on the nature of the solvent.[4]

The changes that might arise during storage of bacteriochlorophyll can be classified under two general headings: first, the loss of magnesium; and secondly, oxidation, which includes allomerization of the isocyclic ring and dehydrogenation of the two reduced pyrrole rings. This work is concerned with the latter class of degradation and, in particular, the dehydrogenation of the tetrahydroporphyrin to a dihydroporphyrin or chlorin.

There have been reports that during the chromatographic purification of crude extracts of bacteriochlorophyll from photosynthetic bacteria a minor band of a green pigment appears on the column.[5] The origin and structure of this pigment have not been conclusively proved, but the evidence available suggests that it is probably 2-desvinyl-2-acetyl-chlorophyll a, formed possibly by photo-oxidation of bacteriochlorophyll in solution during the purification procedure. In confirmation of this

conclusion, both chemical and photo-oxidation of bacteriochlorophyll solutions have been reported to give a green chlorophyll-like pigment with an absorption spectrum similar to that of the chromatographic impurity.[5,6]

Results from a recent investigation on the selective chemical oxidative bleaching of bacteriochlorophyll in R. rubrum chromatophores indicated that a green chlorophyll-like pigment was formed which when extracted into organic solution had spectral properties apparently not identical to any previously reported pigment.[7] The similarity of the absorption spectrum of this product to that of the green pigment described earlier warranted a further investigation into the chemical and photochemical oxidation of bacteriochlorophyll solutions and the structure of the green pigment produced.

Experimental

The solvents acetone and ether were Baker and Adamson reagent grade, and were used without further purification. Commercial 2,3-dichloro-5,6-dicyanoquinone, o- chloranil, p-chloranil and p-benzoquinone were purified either by recrystallization from benzene or by sublimation. Polyethylene used for chromatography was of a low melt index (M.I. 0.044) [8] from Dow Chemical Co. Acetone d_6 was commercial material from Varian Assoc.

Spectrophotometric Studies. All visible and near infrared absorption spectra were recorded using a Cary 14R spectrophotometer. Nuclear magnetic resonance spectra were measured with a Varian A-60 Spectrometer.

Isolation of Bacteriochlorophyll. R. rubrum culture (10 liters) was centrifuged using a Sharples "super" continuous flow centrifuge (2.500rpm). The bacteriochlorophyll was extracted from the bacteria with acetone (200 ml) in a Waring Blendor. The acetone extract was diluted with distilled water to a 70:30, acetone:water mixture and chromatographed on a tightly packed polyethylene column (4 x 50 cm)[8] previously washed with acetone:water (60:40). Some vacuum was applied to the end of the column to increase the rate of percolation. The pigment was eluted with acetone:water (70:30) and collected in a Buchner flask (500 ml). As the pigment was collected, part of the acetone in the flask was pumped off by the vacuum, which increased the proportion of water and reduced the temperature of the solvent in the flask, causing the bacteriochlorophyll to crystallize. The solid was collected, re-crystallized from aqueous acetone and stored in the dark under vacuum. All operations described above were carried out either in the dark or under conditions of low light, to minimize photobleaching of the pigment.

(Analysis found: C, 70.99; H, 7.83; N, 5.93; Mg, 2.60. Calculated for $C_{55}H_{74}O_6N_4MgH_2O$: C, 71.06; H, 8.24; N, 6.03; Mg, 2.62.)

The crystalline material obtained, which gave a clear X-ray diffraction powder pattern,[9] was stable for more than six months, in agreement with the findings of Jacobs et al.[3]

Table I.

Absorptivities of bacteriochlorophyll in ether, ϵ (in Liter/m.mole./cm) and absorption maxima, λ (in $m\mu$).

ϵ	λ	ϵ	λ	ϵ	λ	ϵ	λ	Reference
96.0	(770)	22.0	(573)	47.1	(392)	73.4	(357)	This study
91.1	(773)	20.9	(577)	48.1	(391.5)	73.4	(358.5)	10
93.4	(767-70)	20.2	(574)	46.8	(392)	70.7	(357)	5
95.7	(772)	22.1	(575)	52.8	(391)	85.5	(358)	11

Preparation of 2-desvinyl-2-acetyl-chlorophyll a. A 10^{-2} M solution of 2,3-dichloro-5,6-dicyanoquinone (6 ml, 60 μmoles) was added to bacteriochlorophyll (45 mg, $\sim$50 μmoles) in acetone (100 ml). The absorption spectrum of this mixture showed that the bacteriochlorophyll had been completely oxidized. Ether (100 ml) was added and the acetone was washed out with distilled water. The ether solution was dried with magnesium sulphate, evaporated to dryness and the green residue was then dissolved in a minimum of acetone diluted with iso-octane (150 ml) and chromatographed on a sugar column (4 x 40 cm) previously washed with iso-octane. The mixture was developed with iso-octane containing 0.75% n-propylalcohol. The main green band which was preceded by a trace of a brown compound was collected and concentrated under vacuum, whereupon the green pigment precipitated giving 23 mg of dried material. A simpler method of purification of 2-desvinyl-2-acetyl-chlorophyll a, involving chromatography of the acetone solution of the reaction mixture directly on polyethylene,

was found to be equally satisfactory. The visible absorption spectrum is recorded in Figure 1, and the wavelengths and molar absorptivities of the principal absorption bands are summarized in Table II. (Analysis found: C, 71.60; H, 7.25; N, 5.85, Mg, 2.48. Calculated for $C_{55}H_{72}N_4O_6MgH_2O$: C, 71.22; H, 8.04; N, 6.04; Mg, 2.62.)

Table II

2-desvinyl-2-acetyl-chlorophyll a							
in acetone λ_{max} (mμ)	677	628	591	538	505	436	388
ε (liter/mmole/cm)							
for monohydrate	65.2	12.8	8.08	4.27	2.75	76.1	51.3

The compound 2-desvinyl-2-acetyl-pheophytin a was prepared by adding 1% of dilute hydrochloric acid to an acetone solution of 2-desvinyl-2-acetyl-chlorophyll a. Excess acid converted the pheophytin into the protonated form. The visible absorption spectrum of the pheophytin is recorded with the chlorophyll in Figure 1 and the spectral data of the magnesium-free derivative are described below Table III together with the values for 2-desvinyl-2-acetyl-pheophorbide a.[12]

Table III

2-desvinyl-2-acetyl-pheophorbide a							
in ether λ max. (mμ)	681	620	544	511	476		
2-desvinyl-2-acetyl-pheophytin a							
in acetone λ max. (mμ)	680	619	542	510	475	411	380
Band ratios	0.43	0.08	0.11	0.12	0.05	1.00	0.74

Preparation of Oxygen-free Solutions of Bacteriochlorophyll. Acetone was successively evacuated at liquid nitrogen temperature, sealed and thawed at room temperature until the final pressure obtained at liquid nitrogen temperature was less than 10^{-5} mm Hg. The deoxygenated acetone was then distilled under vacuum into a cuvette for spectrophotometric studies, or an NMR tube for magnetic resonance studies. The cuvette or NMR tube was sealed under vacuum.

Photo-oxidation of Bacteriochlorophyll Solutions. A tungsten filament bulb (300 watts) was employed to illuminate solutions of bacteriochlorophyll in a stoppered 1 cm cuvette. The light path between the bulb and the cuvette was maintained at 35 cm in all the experiments, and by means of a cut-off filter (Corning 2,600) only light with wavelength $>$ 700 mμ was used. The filter was attached to a light-proof box in the form of a window, thereby insuring that only light of wavelength $>$ 700 mμ reached the solution of bacteriochlorophyll. The extent of photo-oxidation of bacteriochlorophyll and production of the oxidized green pigment in the reaction mixture was measured spectrophotometrically. The intensity of the light in the Cary 14R spectrophotometer was found to be too low to induce photo-oxidation at a measurable rate. Thus errors that might have arisen from photo-oxidation during spectroscopic measurements could be neglected.

Results

Part I. Photo-oxidation

Storage of Bacteriochlorophyll. Crystalline bacteriochlorophyll

kept in the dark was found to be stable for more than six months, while acetone solutions of the pigment were unchanged after two weeks in the dark. No precautions were taken to degas or remove oxygen from the samples.

Illumination in the Presence and Absence of Air. A degassed solution of bacteriochlorophyll in acetone (2.8×10^{-5} M) was illuminated using the method described above. Spectrophotometric examination after two hours showed that the bacteriochlorophyll was not measurably destroyed, nor was any of the oxidized pigment formed. Air was then admitted into the cuvette, and after ten minutes illumination the bacteriochlorophyll was more than 70% destroyed and some of the green oxidized pigment was formed.

Photo-oxidation of Bacteriochlorophyll in the Presence of p-benzoquinone. Bacteriochlorophyll in acetone (2.15×10^{-5} M) was illuminated in a cuvette in the absence and presence of equimolar and excess p-benzoquinone (7.7×10^{-3} M) and the rate of photo-oxidation was recorded for the three solutions. No precautions were made to eliminate air from the reaction. Equimolar quantities of p-benzoquinone have little effect, but excess quinone can be seen to have a marked inhibiting effect on the photoreaction (Figure 2). Thus, in the absence of the quinone the bacteriochlorophyll was 25% destroyed in 1.0 min, and the time required for an equivalent oxidation in the presence of excess p-benzoquinone was 35 min.

The spectra of the reaction mixtures recorded at intervals during the photo-oxidation showed four unambiguous isosbestic points at 691,

605, 556 and 402 mμ (Figure 3).

Photo-oxidation of Bacteriochlorophyll in Ether. An air saturated ether solution of bacteriochlorophyll (2.15×10^{-5} M) was illuminated using the method described above. The unreacted bacteriochlorophyll was measured at intervals during the illumination. The rate of photo-oxidation in ether was found to be about forty times slower than the equivalent oxidation in acetone (Figure 2). Once again four isosbestic points were recorded at 695, 595, 558 and 402 mμ, respectively.

Chromatography of photo-oxidation products. The photo-oxidation of bacteriochlorophyll with oxygen produces a mixture of at least seven colored products, as determined by chromatography on a polyethylene column. The order of elution, using acetone:water (70:30) as the eluent, was first brown followed by purple, blue (bacteriochlorophyll) and three green bands leaving a residue of two brown bands. The yield of the main green product collected never exceeded 20% of the original bacteriochlorophyll used.

Part II. Chemical Oxidation of Bacteriochlorophyll

Oxidation Using Ferric Chloride. Solutions of bacteriochlorophyll in acetone and in methanol were oxidized with small quantities of a dilute solution of ferric chloride in methanol in a cuvette. The spectroscopic changes accompanying the oxidation indicated that ferric chloride oxidatively bleaches bacteriochlorophyll without producing any measurable amount of the green pigment.

Quinone Oxidation of Bacteriochlorophyll: 2,3-dichloro-5,6-dicyanoquinone. A 1.6×10^{-5} M solution of bacteriochlorophyll in acetone in a cuvette was examined spectrophotometrically to determine the changes in the spectrum that resulted on addition of 100 µl portions of 10^{-4} M 2,3-dichloro-5,6-dicyanoquinone. The bacteriochlorophyll was oxidized and a green pigment was formed, the reaction occuring in the dark. One mole equivalent of the quinone was required to oxidize one mole equivalent of bacteriochlorophyll (Figure 4). The rate of oxidation was too great to permit kinetic determinations with the experimental method employed here. Neither the addition of excess p-benzoquinone, nor the substitution of ether for acetone as solvent had any measurable effect on the rate of oxidation of bacteriochlorophyll by 2,3-dichloro-5,6-dicyanoquinone.

Optimum yields of the green pigment are obtained, as measured spectroscopically and chromatographically, by using equimolar amounts of 2,3-dichloro-5,6-dicyanoquinone and bacteriochlorophyll. Under these conditions the bacteriochlorophyll is oxidized completely and the major product (90%) is the green pigment. Excess of this quinone causes further oxidation, and a mixture of at least three green pigments results.

o-Chloranil. o-Chloranil behaves like 2,3-dichloro-5,6-dicyanoquinone and one mole equivalent induces a rapid oxidation of one mole equivalent of bacteriochlorophyll into the green pigment. Excess quinone results in a mixture of green pigments.

p-Chloranil. A 4.4×10^{-4} M acetone solution of bacteriochlorophyll (1 ml) was mixed with 10^{-2} M p-chloranil (50 µl) in acetone in the dark; 250 µl of this mixture was diluted to 5 ml with acetone and examined spectroscopically. The changes in absorption at 770 and 677 mµ with time were measured. The results from this and two other similar experiments were plotted to determine the kinetic order of the reaction. First and third order plots showed marked deviations from linearity whereas the second order plot of 1/conc. bacteriochlorophyll against time gave a good linear relationship (Figure 5). This would imply the disappearance of 1 mole of quinone for each mole of bacteriochlorophyll disappearing.

The spectra of the reaction mixture, which were recorded at different times throughout the oxidation, showed four isosbestic points at 698, 603, 535 and 391 mµ (Figure 6). These points correspond closely to the cross-over points in the complete oxidation of bacteriochlorophyll by 2,3-dichloro-5,6-dicyanoquinone (Figure 4) and by o-chloranil.

p-Benzoquinone. The oxidation of bacteriochlorophyll using excess p-benzoquinone is very slow; for example, an acetone solution of bacteriochlorophyll (8.8×10^{-5} M) was less than 35% oxidized after twelve days. Two of the isosbestic points in this oxidation are identical with those in the p-chloranil oxidation, and the third and fourth at 535 and 391 mµ are obscured by the end-absorption of p-benzoquinone. Since the quinone is in excess, the oxidation might be expected to obey pseudo-first order kinetics with respect to

bacteriochlorophyll, but a first order plot of $\log_{10}$(Bchl) against time does not give a straight line; the cause of this deviation is probably the instability of the p-benzoquinone in solution.

Nuclear Magnetic Resonance Studies. Spectra of the samples were recorded in the absence of oxygen using chlorophyll pigments (15-20 mg) dissolved in fully deuterated acetone (400 μl) with tetramethylsilane as an internal standard. The NMR spectrum of oxidized bacteriochlorophyll is recorded (Figure 7) and the major peaks are assigned to the proposed structure: 2-desvinyl-2-acetyl-chlorophyll a (Table IV).

(Note to editor) Place structure of 2-desvinyl-2-acetyl-chlorophyll a at this point.

Table IV. Chemical Shifts cps from TMS = 0 for 2-desvinyl-2-acetyl-chlorophyll a Proton Assignment

α	β	δ	10	phytyl	7 and 8	11	1	5	3	2	phytyl
598	587	527	376	309	265	232	219	216	200	291	87

The three methine protons cannot be unambiguously assigned, but by comparison with the reported spectra of chlorophyll a and b[13] the two peaks at 598 and 587 cps are probably the α and β protons. The α resonance would be expected to occur at lower field than the β, since the acetyl group in the 2-position should give rise to considerable deshielding of the former but the effect should be negligible on the

latter. Thus the α methine is assigned to the resonance at 598 cps and the β to 587 cps. The δ proton which is in the proximity of only one pyrrole ring should be the most shielded and is considered to be the resonance at 527 cps. The C_{10} proton is assigned to the line at 376 cps close to the value for this proton in chlorophyll a and b. Again by analogy with chlorophyll a and b the multiple resonance centered at 309 cps arises from the olefinic hydrogens in the phytyl chain. The weak resonances centered around 265 cps might arise from the 7 and 8 protons.

The five lines between 235 and 190 cps can be assigned to the ring methyls on carbons 1, 3 and 5, the ester methyl on C_{11} and the acetyl methyl on C_2. The peak at 232 cps is almost certainly the ester methyl on C_{11}, the resonance being very close to that of the same groups in chlorophyll a and b. The line at 216 cps is probably the C_5 methyl, since this group should be virtually unaffected by the 2-acetyl group and the resonance should therefore arise at the same position as in the spectra of chlorophyll a and b. The C_1 methyl resonance is shifted down field from the values obtained for chlorophyll a and b to 219 cps due to the close proximity of the carbonyl of the acetyl group. These last two assignments for C_1 and C_5 methyls are not unambiguous, and could be in the reverse order. The resonance at 200 cps most probably arises from the C_3 methyl: this value is down field from that quoted for chlorophyll a by 5 cps, and this effect might well arise from the deshielding of the carbonyl of the acetyl group on C_2. By elimination, the acetyl methyl resonance arises at 191 cps, 11 cps down field from the value found for this methyl resonance in bacteriochlorophyll

(180 cps).[14] The possible cause for this shift down field is the increased resonance in 2-desvinyl-2-acetyl-chlorophyll a over bacteriochlorophyll resulting in a greater deshielding of the methyl protons on the acetyl group of the former.

The remaining three large resonances can be assigned with a high degree of certainty. The multiplets at 87 cps and the quintet at 120 cps arise from the aliphatic methylene groups of the phytyl chain and the proton in the solvent impurity pentadeuteroacetone, respectively. The broad peak at 161 cps is assigned to traces of water associated with the pigment. Confirmation for this last assignment comes from two pieces of work: first, trace quantities of water added to the solvent hexadeuteroacetone cause a broad multiplet at 163 cps; and secondly, the results from a study on the temperature dependence of the NMR spectrum of bacteriochlorophyll showed that the only resonance to give an appreciable shift with temperature was a multiplet at 154 cps at 40° and which shifted down field to 184 cps at -45°. Thus this resonance is considered to be due to the water of crystallization of bacteriochlorophyll.[15]

Discussion

The original purpose of this research was to prepare the green oxidation product of bacteriochlorophyll reported by previous workers, and to elucidate its chemical structure. However, several interesting observations arose while trying to determine the optimum conditions for the oxidation. The conclusion from these observations and others regarding the structure of the oxidized pigment are discussed below.

The preparation of crystalline bacteriochlorophyll described above has the advantage of being both simpler and quicker than the methods previously described;[3-5,16] furthermore, acetone is used throughout in place of methanol as the organic solvent. These modifications in the experimental method reduce the possible extent of photo-oxidation and allomerization during purification.

Photo-oxidation of bacteriochlorophyll

Bacteriochlorophyll solutions are readily bleached when exposed to light in the presence of air. The active wavelength of the light extends into the near infrared where only the long wavelength band of bacteriochlorophyll can absorb radiation. In the absence of oxygen, the pigment is stable for periods of illumination which would completely destroy it in the presence of air. Similarly, air-saturated bacteriochlorophyll solutions are stable in the absence of light for several days.

The rate of the photo-oxidation of bacteriochlorophyll in air is very dependent on the nature of the solvent.[4] In this investigation the results from the photo-oxidation of bacteriochlorophyll in two solvents, ether and acetone, have been described. Further qualitative results not included in this paper became apparent during a recent study of the dimerization of bacteriochlorophyll in carbontetrachloride.[14] It was found that photo-oxidation in this solvent is concentration dependent: the rate of photo-oxidation decreases considerably as the concentration of the bacteriochlorophyll increases.[15] This last observation is of interest in connection with the concentration dependent aggregation of chlorophylls in non-polar solvents.[13,14,17] Finally,

the photo-stability of bacteriochlorophyll in acetone is markedly increased by the presence of excess p-benzoquinone; an observation first recorded by Goedheer.[4]

These results show that the rate of photo-oxidation of bacteriochlorophyll depends greatly on the environment of the chlorophyll molecules. In acetone the photo-oxidation rate is thirty five to forty-fold faster than it is in ether or in acetone in the presence of excess p-benzoquinone. Despite these large differences in photo-oxidation rates the visible absorption spectra of the reaction mixtures all show isosbestic points and the wavelengths of the points are barely affected by these environmental changes. The significance of the isosbestic points in the spectra of the reaction mixtures is twofold: first, the photo-oxidation does not involve any appreciable amounts of long-lived intermediate species; and secondly, the products of the photoreaction must be formed in a fixed ratio throughout the reaction. Further, since the positions of the isosbestic points are almost the same in acetone in the presence or absence of excess p-benzoquinone or in ether, the mechanism of the photo-oxidation is most probably the same under these three conditions. Thus, although the rate of photo-oxidation of bacteriochlorophyll is markedly altered by these changes in reaction conditions the direction of the reaction and the nature of the products formed seem unaffected.

At present too little is known about solvent-chlorophyll interactions and there is insufficient experimental evidence to propose a detailed reaction mechanism for the photo-oxidation of solutions of bacteriochlorophyll. The initial step is probably a light absorption by a bacteriochlorophyll-

solvent complex to give the electronically excited pigment. This excited bacteriochlorophyll is capable of reverting to the ground state by loss of energy or reacting with oxygen most probably by a radical mechanism to give a number of oxidation products. The relative importance and the nature of these two pathways and the effect of solvent changes and added quinone on them remains uncertain. The radical mechanism is indicated by the large number of products and the reaction conditions.

Since the photo-oxidation results in a mixture of several products of which the main green pigment is only a minor constituent this work was abandoned in favour of other more specific methods of oxidation

Chemical Oxidation of Bacteriochlorophyll The work of Linstead and his co-workers [18] on the oxidation of metal-free chlorophyll derivatives with high redox quinones suggested that these quinones might act as highly selective oxidants for bacteriochlorophyll. Initial studies showed that bacteriochlorophyll could in fact be oxidized by 2,3-dichloro-5,6-dicyanoquinone to a green chlorophyll pigment in high yield, the optimum conditions being one mole equivalent of the quinone to oxidize one equivalent of bacteriochlorophyll.

Three other quinones were examined, and of these o-chloranil was found to resemble 2,3-dichloro-5,6-dicyanoquinone in that equimolar quantities rapidly oxidized bacteriochlorophyll, p-chloranil induced a slower oxidation, and p-benzoquinone was the slowest. Similarly to the photo-oxidation of bacteriochlorophyll, the spectrum of the reaction mixtures during chemical oxidation showed clear isosbestic points. The wavelengths of these points from the quinone oxidation spectra were

almost the same for the four quinones studied. Several conclusions can be drawn from the quinone oxidations of bacteriochlorophyll: first, the reactions are highly selective, giving high yields of the oxidized green pigment; secondly, the rate at which the different quinones oxidize bacteriochlorophyll parallels the redox potentials of the quinones; and thirdly, the occurrence of isosbestic points in the absorption spectra of these reaction mixtures and the similarity of the wavelengths of the points from the different quinone oxidations suggest that the mechanism and the oxidation products are the same for all the reactions.

The most probable mechanism for the dehydrogenation is that proposed by Braude and Linstead and their co-workers for the dehydrogenation of di- and tetra-hydroaromatic compounds with quinones,[19] and involves a hydride transfer from the bacteriochlorophyll to the quinone. The intermediate hydroquinone anion and partially oxidized bacteriochlorophyll cation then react further by proton shift to give the hydroquinone and oxidized bacteriochlorophyll. However, the alternative homolytic reaction cannot be ruled out.

Two chlorins could theoretically be formed, one involving dehydrogenation of the 3,4 bond of bacteriochlorophyll and the other the 7,8 bond. All the data suggest that the major product results from the former dehydogenation It is of interest to note that Golden et al.[18(b)] found that bacteriochlorin e_6 trimethylester with 2,3-dichloro-5,6-dicyanoquinone gave only one product, the 2-desvinyl-2-acetyl-chlorin e_6 trimethylester, the other product involving dehydrogenation of ring IV was not formed. These workers concluded that the transition state for dehydrogenation of the IV ring involves considerable steric crowding between the methylene on C_7 and the groups on

C_{10} of the isocyclic ring V, whereas dehydrogenation of ring II does not involve this steric strain. The same argument can be applied to the dehydrogenation of bacteriochlorophyll itself.

Structure of Oxidized Bacteriochlorophyll The green pigment prepared by the oxidation of bacteriochlorophyll with 2,3-dichloro-5,6-dicyanoquinone has a visible absorption spectrum similar, if not identical, to the pigment reported by Holt and Jacobs. The evidence which is discussed below all points to it being 2-desvinyl-2-acetyl-chlorophyll a, which is the structure proposed by these workers.

The mode of preparation of the pigment indicates it is a dehydrogenation product of bacteriochlorophyll. This evidence, combined with the visible absorption spectrum, which is very similar to 2-desvinyl-2-formyl-chlorophyll a,[20] suggests that the oxidized bacteriochlorophyll is a chlorin and most probably a simple derivative of chlorophyll a. Of the two chlorins that could be formed, the data suggests that the one involving dehydrogenation of the 3,4 bond of bacteriochlorophyll is the most likely.

The magnesium-free derivative of the oxidized pigment has a spectrum similar to 2-desvinyl-2-formyl-pheophytin a,[20] but more important, the spectrum is almost identical to that of 2-desvinyl-2-acetyl-pheophorbide a[12] (Table III). It is well known that the phytyl chain of chlorophyll pigments has little effect on the absorption spectra of the pigments; thus it is not unreasonable to expect that 2-desvinyl-2-acetyl-pheophytin a would have a visible absorption spectrum almost identical to 2-desvinyl-2-acetyl-pheophorbide a, and further that the pheophytin of the green pigment is probably 2-desvinyl-2-acetyl-pheophytin a.

Confirmation that the green oxidized bacteriochlorophyll is 2-desvinyl-

2-acetyl-chlorophyll a comes from the NMR spectrum of this product (Figure 7). The peaks in the spectrum were assigned by comparison with the NMR spectra of chlorophyll a and b [13] and bacteriochlorophyll.[14] The positions of the methine hydrogen resonances indicate clearly that the pigment is a chlorin; this evidence combined with the bands arising from the phytyl group, the C_{11} ester methyl and C_{10} proton suggest that the pigment is a chlorophyll derivative.

The five sharp bands between 235 and 190 cps cannot be assigned unambiguously, but each is clearly equivalent and corresponds to one of the five lowfield methyl singlets expected from the compound 2-desvinyl-2 acetyl-chlorophyll a.

In conclusion, the major green oxidation product of bacteriochlorophyll is 2-desvinyl-2-acetyl-chlorophyll a, the structure proposed for the compound by Holt and Jacobs.[5] The biological importance of this pigment as a logical biosynthetic precursor for bacteriochlorophyll remains doubtful, since acetone extracts of R. rubrum bacteria when chromatographed in the dark show no sign of any green pigment,[21] perhaps the concentration is too low for detection by this method (less than $\sim$ 1% of the concentration of bacteriochlorophyll). Furthermore, it is unlikely that this pigment is the same as the one reported by Gould et al.,[7] for although the absorption spectra are similar they are not identical, and the visible spectrum of the magnesium-free derivative and the NMR spectrum in acetone[22] are clearly different from thos described above. The chlorophyll-like pigment of Gould et al. is probably one of the lesser pigments detected both in the photo - and chemical oxidations of bacteriochlorophyll.

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(21) M. Byrn, M. Calvin, and J. Lindsay Smith, (in press).

(22) E. S. Gould, unpublished observations.

Figure Captions

Fig. 1. Absorption spectra of 2-desvinyl-2-acetyl-chlorophyll a (————) and its pheophytin (----------) in acetone.

Fig. 2. Photodegradation of bacteriochlorophyll in non-degassed solutions: X————X, bacteriochlorophyll alone in acetone; ⊡.....⊡, bacteriochlorophyll with p-benzoquinone in equimolar quantities; O————O, bacteriochlorophyll with excess p-benzoquinone; and ⊙----------⊙, bacteriochlorophyll alone in ether.

Fig. 3. Photo-oxidation of bacteriochlorophyll in acetone. Time of illumination in seconds:---------- 0, —— - —— - 30, —— —— —— 90, ————270.

Fig. 4. Chemical oxidation of bacteriochlorophyll with an equimolar quantity 2,3-dichloro-5,6-dicyanoquinone in acetone:— — — — bacteriochlorophyll alone, ———— reaction mixture after the addition of an equal molar quantity of 2,3-dichloro-5,6-dicyanoquinone.

Fig. 5. Rate of oxidation of bacteriochlorophyll in the presence of an equimolar quantity of p-chloranil.

Fig. 6. Chemical oxidation of bacteriochlorophyll with an equimolar amount of p-chloranil. Time or reaction in minutes: —— - —— - 4, ····· 25, —— —— —— 50, ———— 102.

Fig. 7. The proton magnetic resonance spectrum of 2-desvinyl-2-acetyl-chlorophyll a in acetone d_6.

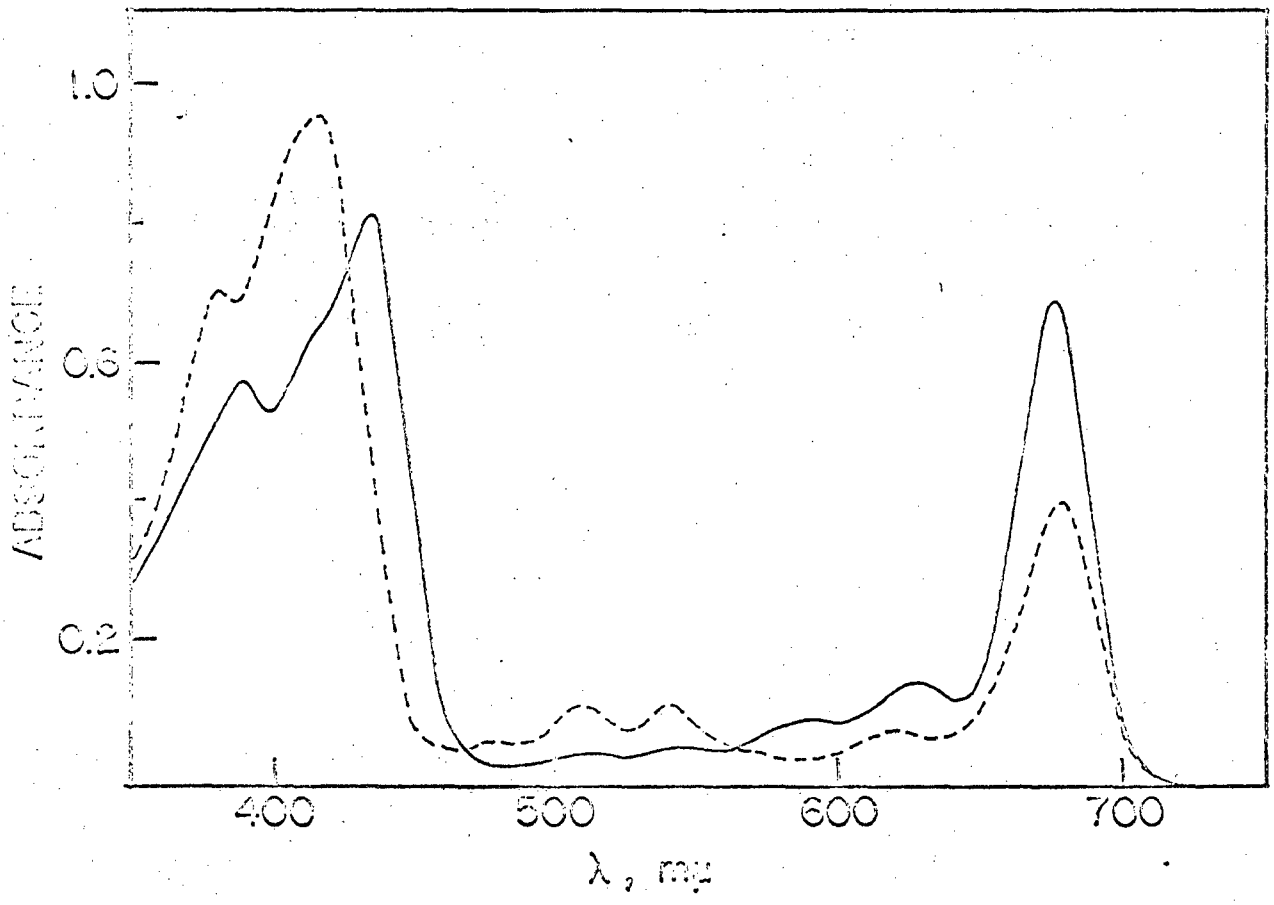

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Figure 1.
(Lindsay Smith & Calvin)

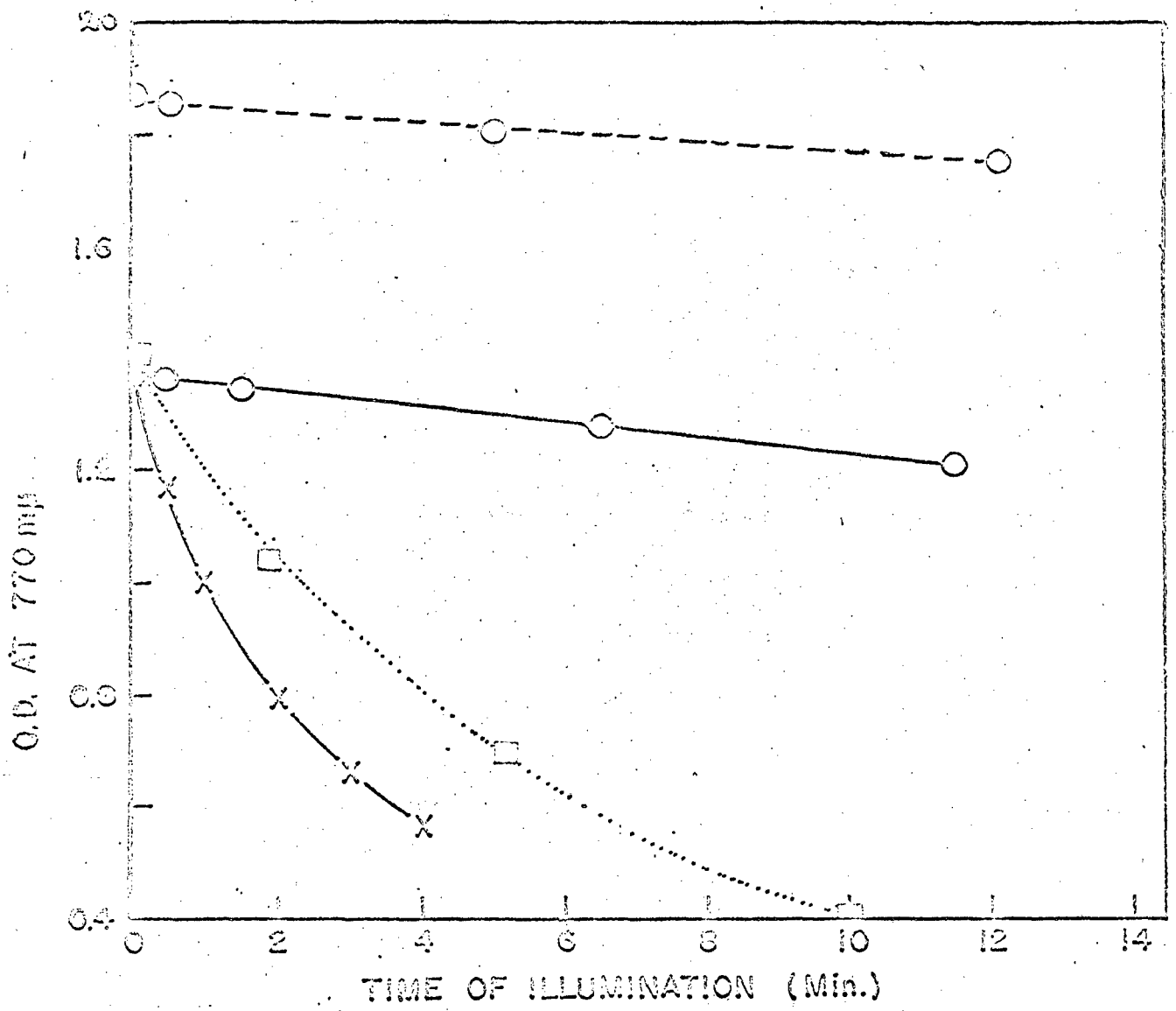

Figure 2.
(Lindsay Smith & Calvin)

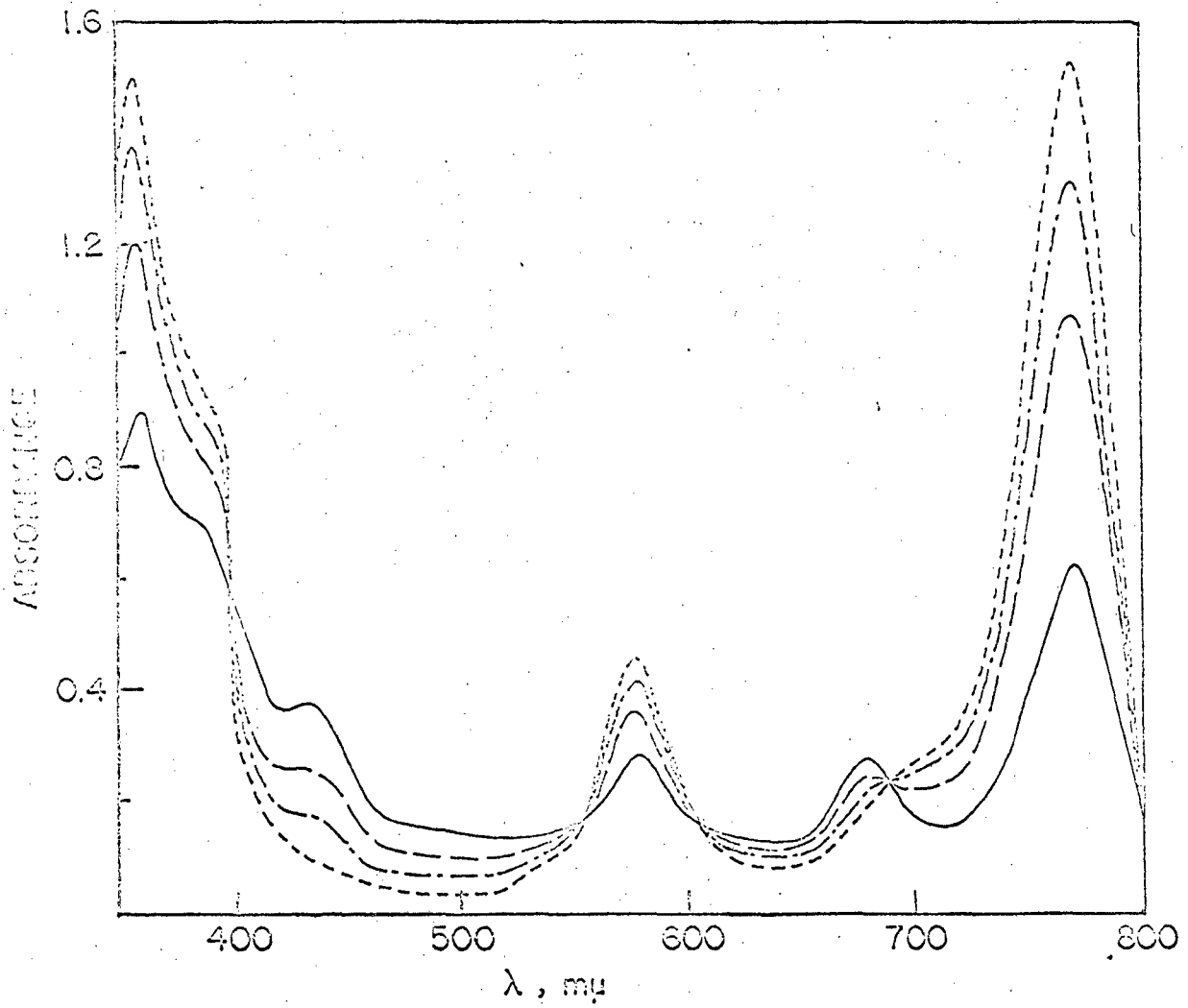

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Figure 3.
(Lindsay Smith & Calvin)

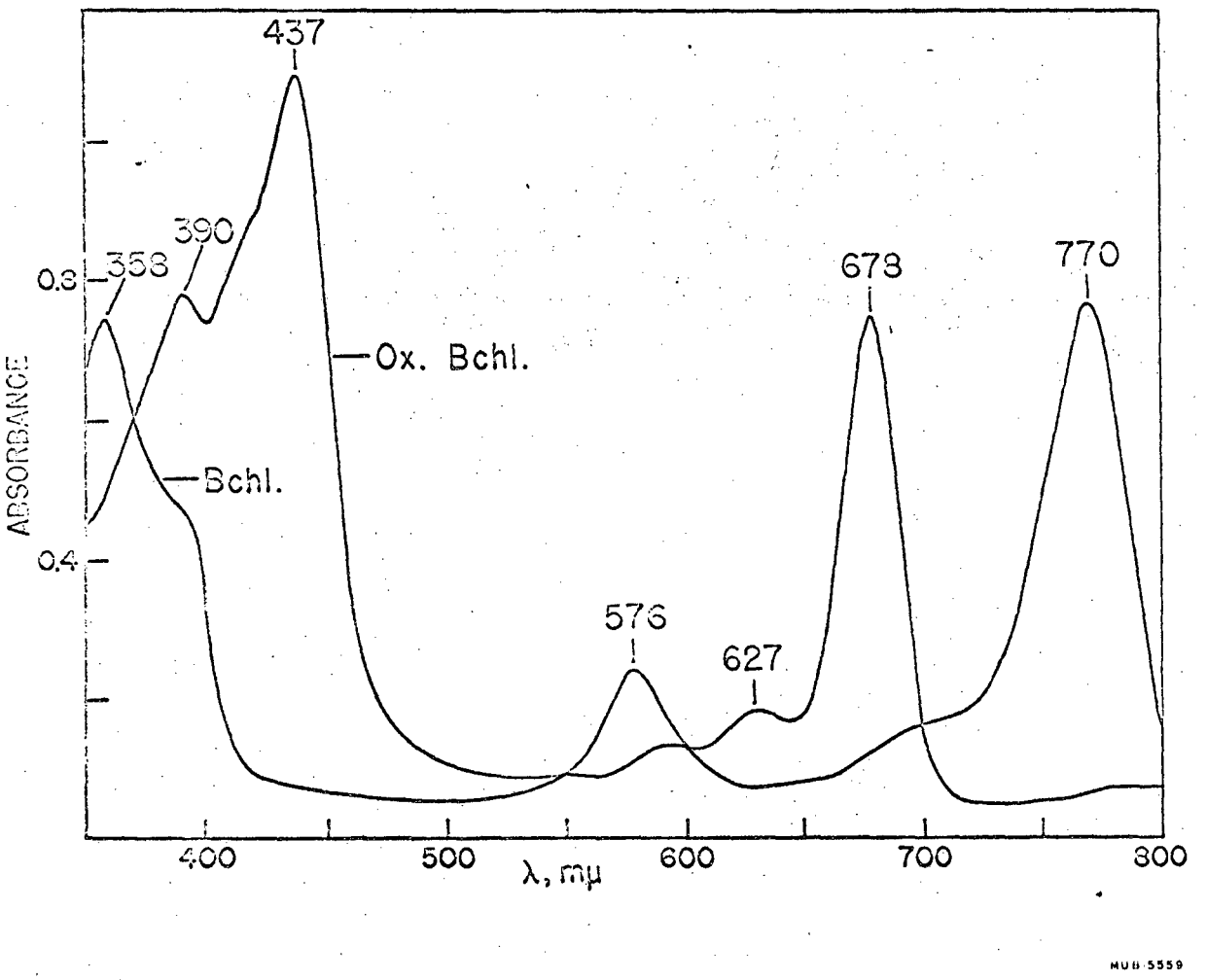


Figure 4.
(Lindsay Smith & Calvin)

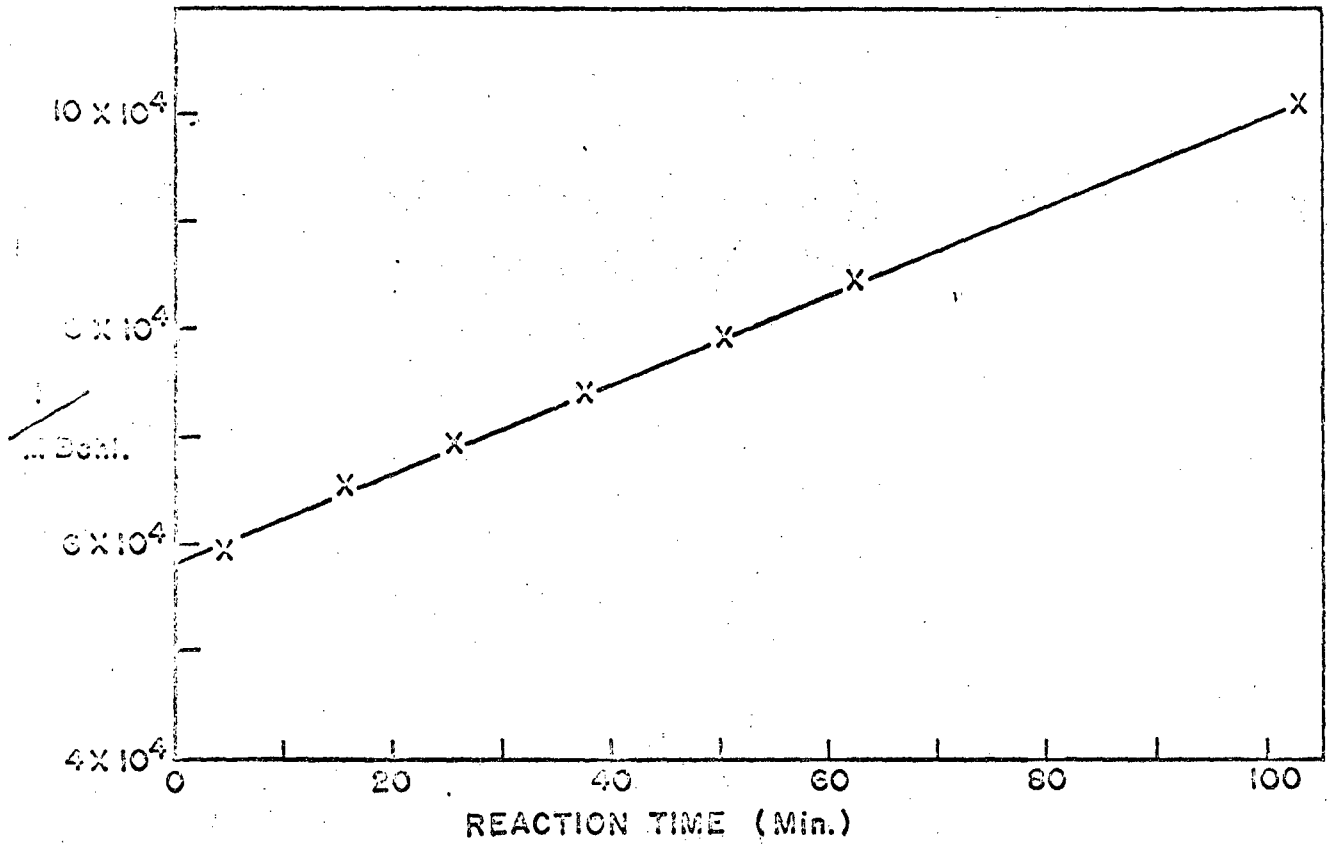


Figure 5.
(Lindsay Smith & Calvin)

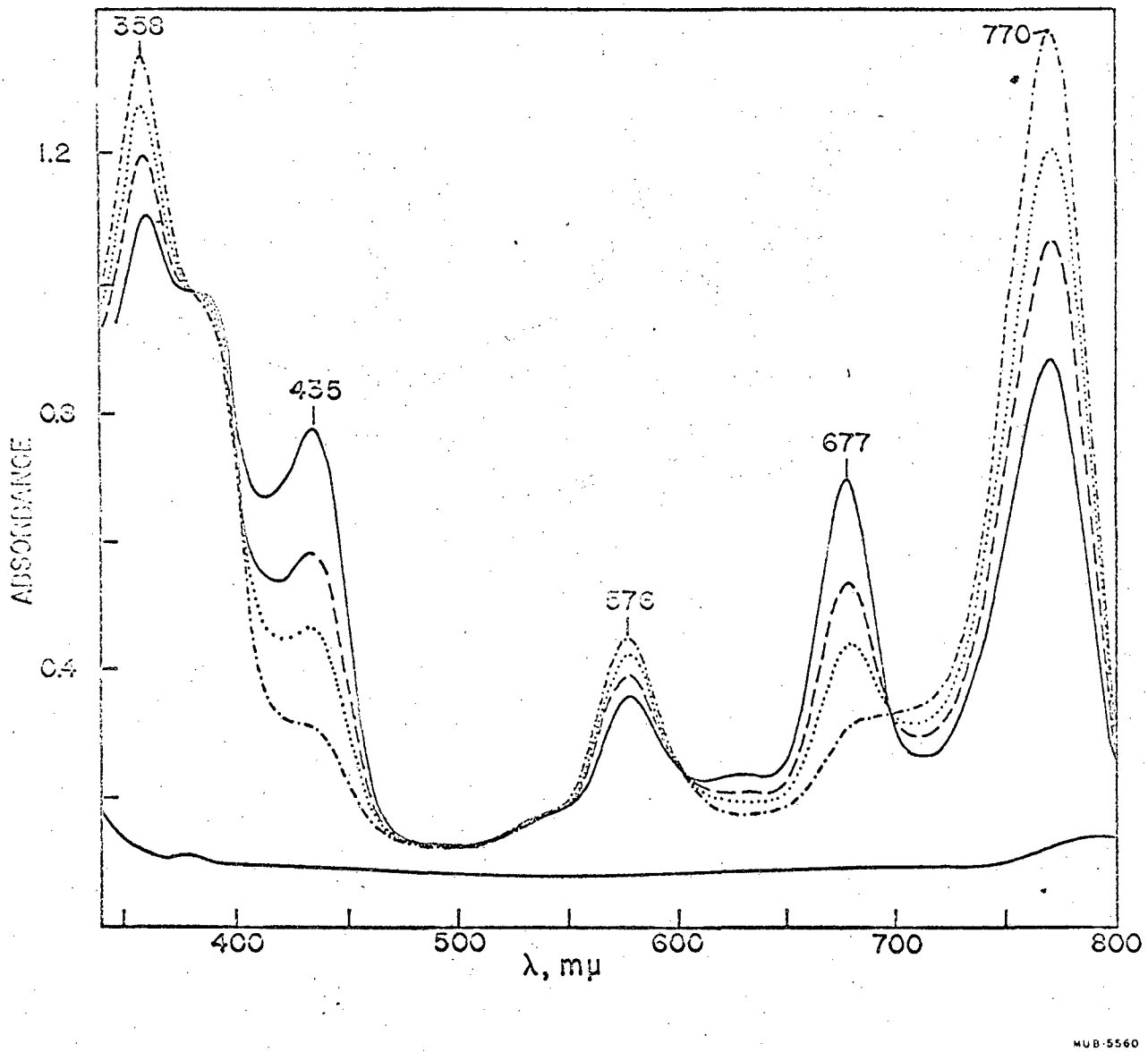


Figure 6.
(Lindsay Smith & Calvin)

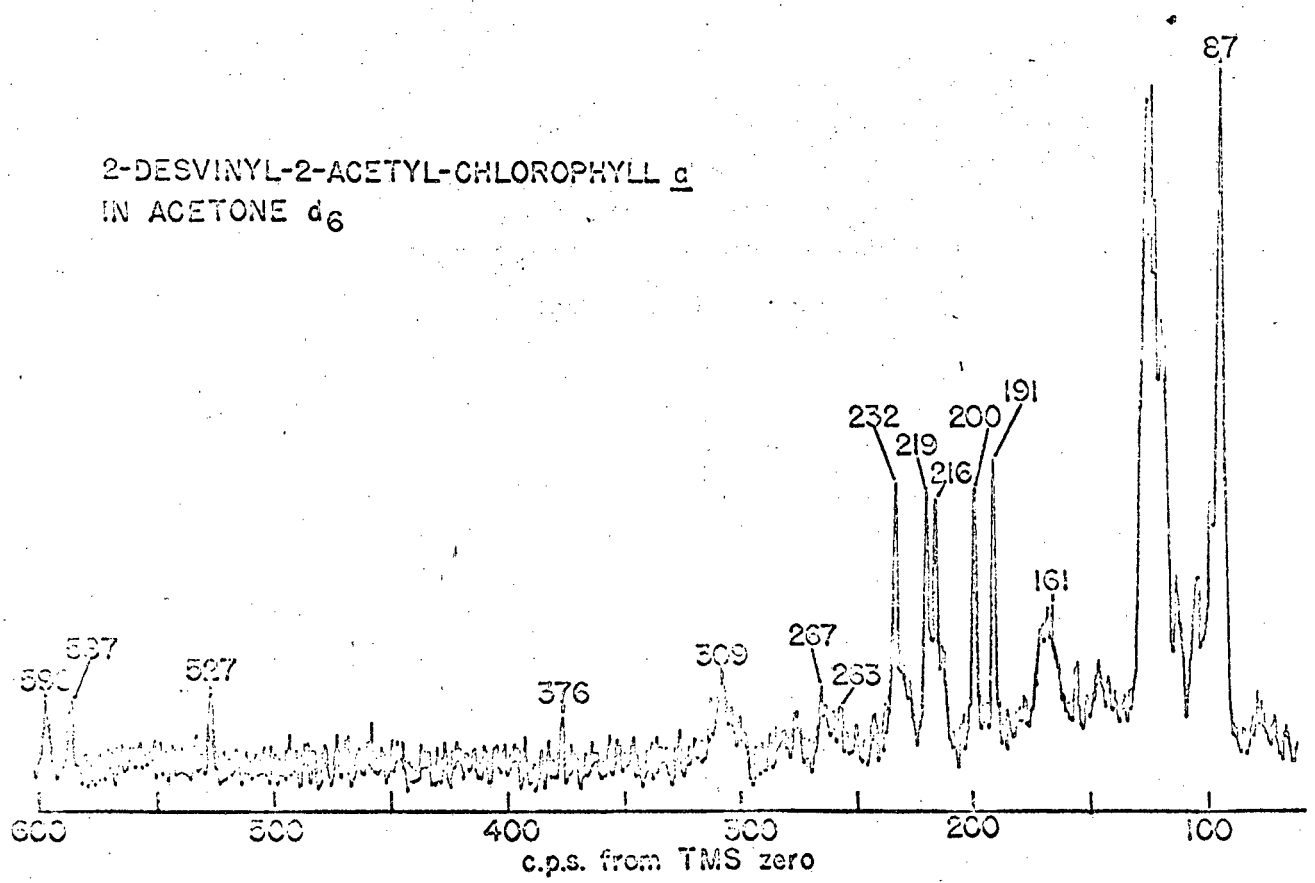
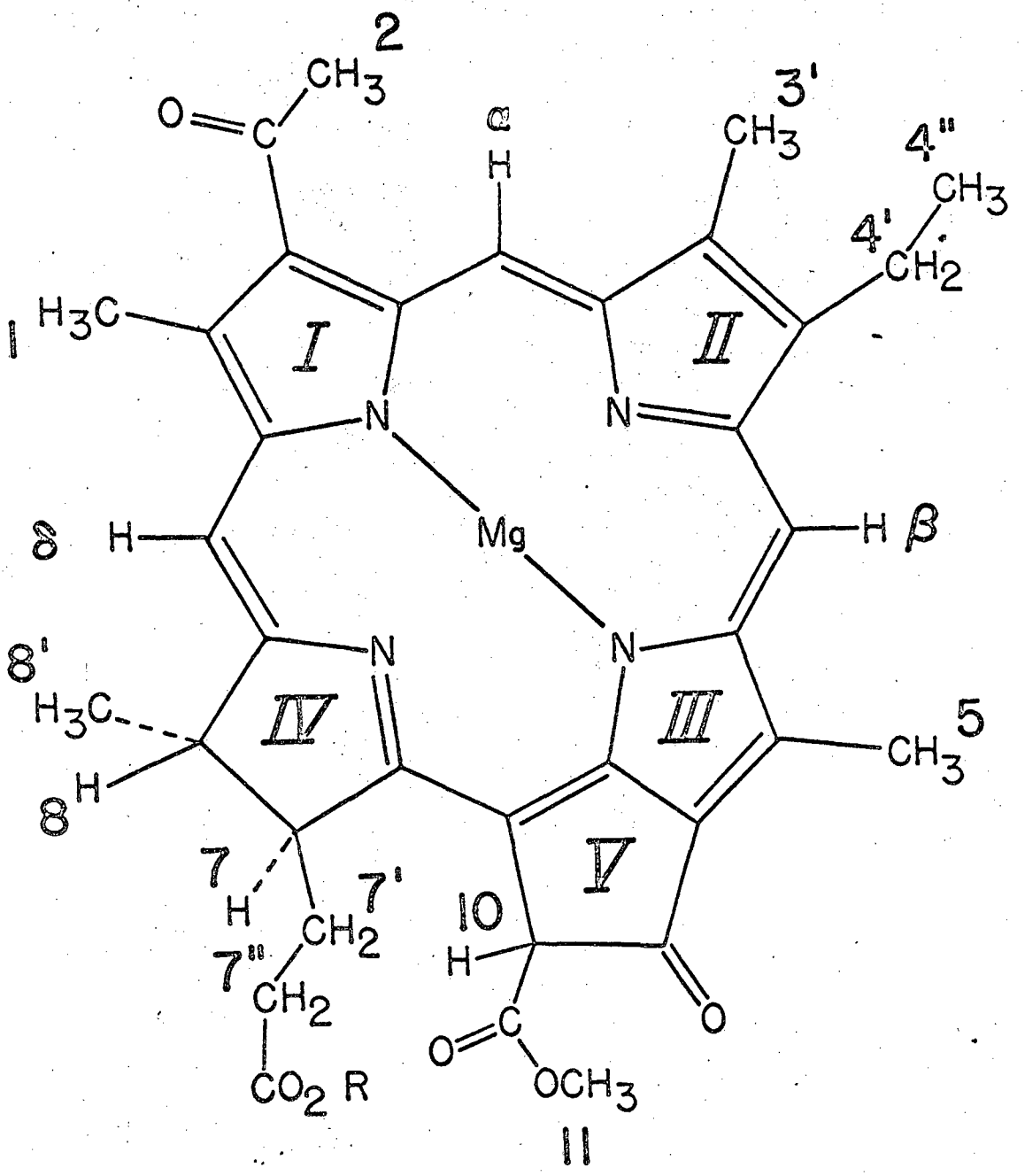


Figure 7.
(Lindsey Smith & Calvin)

Lindsay Smith & Calvin

Mss. #799

Structure of 2-desvinyl-2-acetyl chlorophyll a



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