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-acetylchlorophyll a, whereas the photooxidation of bacteriochlorophyll results in a mixture of products of which 2-desvinyl-2-acetylchlorophyll a is only a minor constituent. Kinetic measurements on these oxidations have been recorded spectrophotometrically, and the rates are found to be very dependent on changes in the reaction conditions, although the products are hardly affected. Possible mechanisms to explain the observed effects are discussed. The proton magnetic reasonance spectrum of 2-desvinyl-2-acetylchlorophyll a in deuterioacetone 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.

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 bacteriochlorophyll is stable when kept in the dark,[3] while the stability of solutions of this pigment can vary over 100-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-acetylchlorophyll a, formed possibly by photooxidation of bacteriochlorophyll in solution during the purification procedure. In confirmation of this conclusion, both chemical and photooxidation 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 with 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 Section

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 (MI 0.044)[8] from Dow Chemical Co. Acetone-d_6 was commercial material from Varian Associates.

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 l.) was centrifuged using a Sharples "super" continuous flow centrifuge (2500 rpm). 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 × 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 Büchner 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, recrystallized 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.

Anal. Calcd for $C_{55}H_{74}O_6N_4MgH_2O$: C, 71.06; H, 8.24; N, 6.03; Mg, 2.62. Found: C, 70.99; H, 7.83; N, 5.93; Mg, 2.60.

The crystalline material obtained, which gave a clear X-ray diffraction powder pattern,[9] was stable for more than 6 months, in agreement with the findings of Jacobs, *et al.*[3] The wavelengths and molar absorptivites are given in Table I.

Preparation of 2-Desvinyl-2-acetylchlorophyll a. A 10^{-2} *M* solution of 2,3-dichloro-5,6-dicyanoquinone (6 ml, 60 μmoles) was

(1) This work was carried out under the auspices of the U. S. Atomic Energy Commission.
(2) Charles Kettering Research Foundation Fellow.
(3) E. E. Jacobs, A. E. Vatter, and A. S. Holt, *Arch. Biochem. Biophys.*, **53**, 228 (1954).
(4) J. C. Goedheer, *Biochim. Biophys. Acta*, **27**, 478 (1958).
(5) A. S. Holt and E. E. Jacobs, *Am. J. Botany*, **41**, 718 (1954).
(6) E. Schneider, *Z. Physiol. Chem.*, **226**, 221 (1934).
(7) E. S. Gould, I. D. Kuntz, and M. Calvin, *Photochem. Photobiol.*, **4**, 483 (1965).
(8) A. F. H. Anderson and M. Calvin, *Nature*, **194**, 285 (1962).
(9) L. H. Vogt, personal communication.

Table I. Absorptivities of Bacteriochlorophyll in Ether[a]

ε	λ	ε	λ	ε	λ	ε	λ	Ref
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	*b*
93.4	767–770	20.2	574	46.8	392	70.7	357	5
95.7	772	22.1	575	52.8	391	85.5	358	*c*

[a] ε in l./mmole cm and absorption maxima, λ, in mμ. [b] J. H. C. Smith and A. Benitez, "Modern Methods of Plant Analysis," Vol. IV, K. Peach and M. V. Tracey, Ed., Springer-Verlag, Berlin, 1955, p 142. [c] J. W. Wiegl, *J. Am. Chem. Soc.*, **75**, 999 (1953).

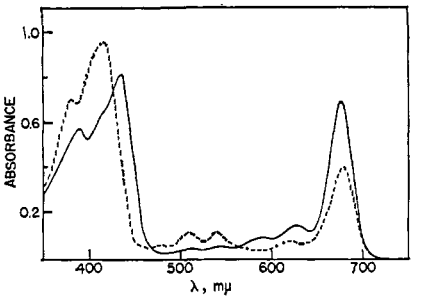


Figure 1. Absorption spectra of 2-desvinyl-2-acetylchlorophyll a (———) and its pheophytin (---------) in acetone.

added to bacteriochlorophyll (45 mg, ~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 sulfate and evaporated to dryness, and the green residue was then dissolved in a minimum of acetone diluted with isooctane (150 ml) and chromatographed on a sugar column (4 × 40 cm) previously washed with isooctane. The mixture was developed with isooctane containing 0.75% *n*-propyl alcohol. 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-acetylchlorophyll 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.

Table II. Wavelengths and Molar Absorptivities for 2-Desvinyl-2-acetylchlorophyll a

λ_{max}, mμ (in acetone)	677	628	591	538	505	436	388
ε (l./mmole cm) for monohydrate	65.2	12.8	8.08	4.27	2.75	76.1	51.3

Table III.

	2-Desvinyl-2-acetylpheophorbide a						
λ_{max}, mμ (in ether)	681	620	544	511	476		
	2-Desvinyl-2-acetylpheophytin a						
λ_{max}, mμ (in acetone)	680	619	542	510	475	411	380
Band ratios	0.43	0.08	0.11	0.12	0.05	1.00	0.74

Anal. Calcd for $C_{55}H_{72}N_4O_6MgH_2O$: C, 71.22; H, 8.04; N, 6.04; Mg, 2.62. Found: C, 71.60; H, 7.25; N, 5.85; Mg, 2.48.

The compound 2-desvinyl-2-acetylpheophytin a was prepared by adding 1% of dilute hydrochloric acid to an acetone solution of 2-desvinyl-2-acetylchlorophyll 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 in Table III together with the values for 2-desvinyl-2-acetylpheophorbide a.[10]

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. 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.

Photooxidation of Bacteriochlorophyll Solutions. A tungsten filament bulb (300 w) 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 2600) only light with wavelength >700 mμ was used. The filter was attached to a light-proof box in the form of a window, thereby ensuring that only light of wavelength >700 mμ reached the solution of bacteriochlorophyll. The extent of photooxidation 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 photooxidation at a measurable rate. Thus errors that might have arisen from photooxidation during spectroscopic measurements could be neglected.

(10) (a) A. Stern and F. Pruckner, *Z. Physiol. Chem.*, **185**, 140 (1939); (b) O. T. G. Jones, *Biochem. J.*, **91**, 572 (1964).

Results

Part I. Photooxidation. Storage of Bacteriochlorophyll. Crystalline bacteriochlorophyll kept in the dark was found to be stable for more than 6 months, while acetone solutions of the pigment were unchanged after 2 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 2 hr 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 a 10-min illumination the bacteriochlorophyll was more than 70% destroyed and some of the green oxidized pigment was formed.

Photooxidation 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 photooxidation 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

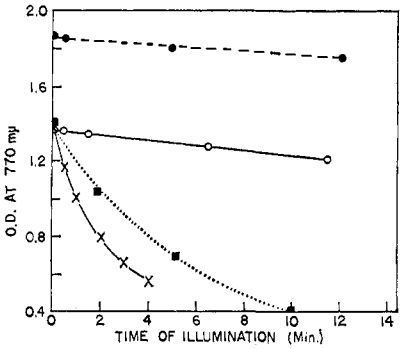


Figure 2. Photodegradation of bacteriochlorophyll in nondegassed solutions: ×—×, bacteriochlorophyll alone in acetone; ■...■, bacteriochlorophyll with *p*-benzoquinone in equimolar quantities; O—O, bacteriochlorophyll with excess *p*-benzoquinone; and ●---●, bacteriochlorophyll alone in ether.

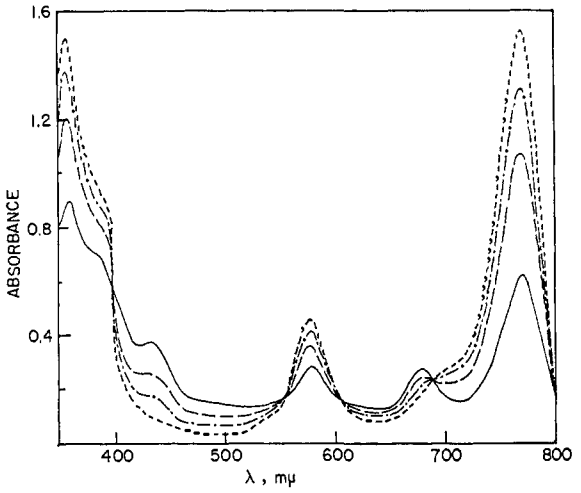


Figure 3. Photooxidation of bacteriochlorophyll in acetone. Time of illumination in seconds: ·····, 0; — — —, 30; - - -, 90; ———, 270.

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 photooxidation showed four unambiguous isosbestic points at 691, 605, 556, and 402 mμ (Figure 3).

Photooxidation 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 photooxidation in ether was found to be about 40 times slower than the equivalent oxidation in acetone (Figure 2). Once again four isosbestic points were recorded at 695 mμ, 595, 558, and 402, respectively.

Chromatography of Photooxidation Products. The photooxidation 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.

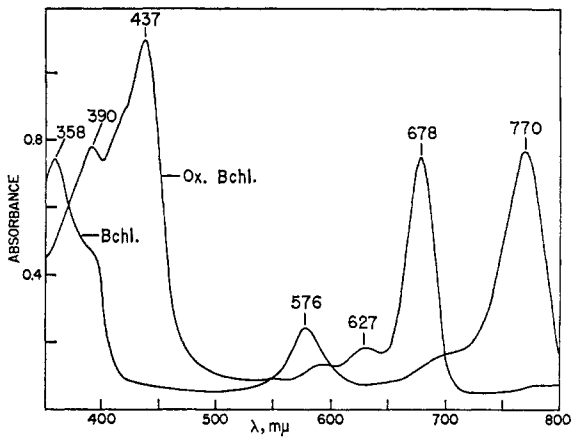


Figure 4. Chemical oxidation of bacteriochlorophyll with an equimolar quantity of 2,3-dichloro-5,6-dicyanoquinone in acetone: - - - -, bacteriochlorophyll alone; ———, reaction mixture after the addition of an equimolar quantity of 2,3-dichloro-5,6-dicyanoquinone.

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 occurring in the dark. One molar equivalent of the quinone was required to oxidize 1 molar equiv 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 1 molar equiv induces a rapid oxidation of 1 molar equiv 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

Table IV. Chemical Shifts (cps) from TMS = 0 for 2-Desvinyl-2-acetylchlorophyll 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	191	87

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/(concentration of 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.

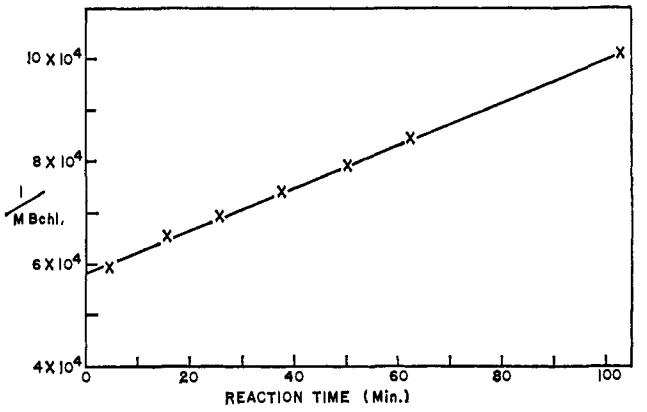


Figure 5. Rate of oxidation of bacteriochlorophyll in the presence of an equimolar quantity of *p*-chloranil.

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.

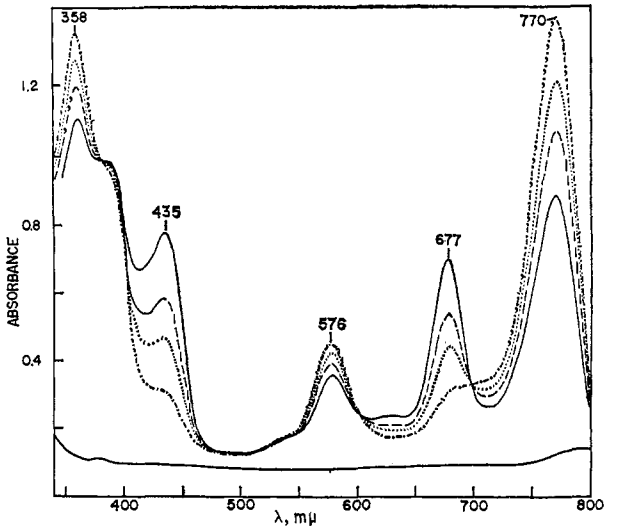
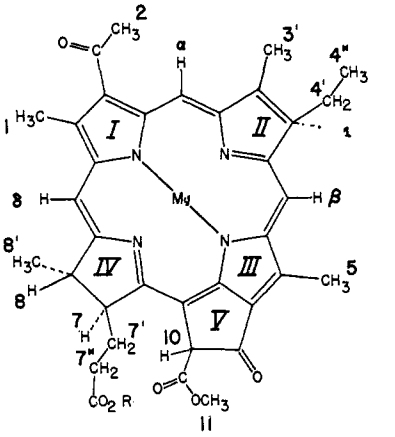


Figure 6. Chemical oxidation of bacteriochlorophyll with an equimolar amount of *p*-chloranil. Time of reaction in minutes: — — —, 4; ·····, 25; — — —, 50; ———, 102.



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 12 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 (bacteriochlorophyll) against time does not give a straight line; the cause of this deviation is probably the instability of the *p*-benzoquinone in solution.

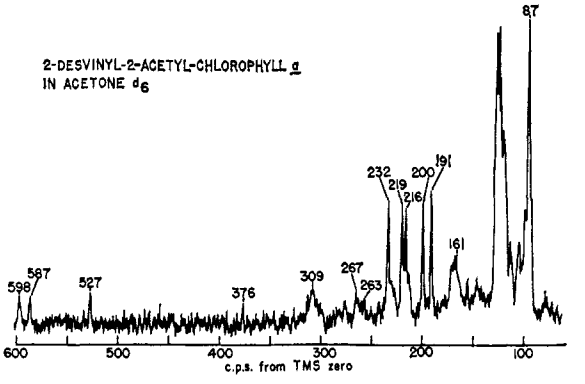


Figure 7. The proton magnetic resonance spectrum of 2-desvinyl-2-acetylchlorophyll a in acetone-d_6.

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-acetylchlorophyll a (Table IV).

The three methine protons cannot be unambiguously assigned, but by comparison with the reported spectra

of chlorophyll a and b[11] 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 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 downfield 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 downfield 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 downfield from the value found for this methyl resonance in bacteriochlorophyll (180 cps).[12] The possible cause for this shift downfield is the increased resonance in 2-desvinyl-2-acetylchlorophyll 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 and the quintet at 120 cps arise from the aliphatic methylene groups of the phytyl chain and the proton in the solvent impurity pentadeuterioacetone, 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 hexadeuterioacetone 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 of 154 cps at 40° and which shifted downfield to 184 cps at −45°. Thus this resonance is considered to be due to the water of crystallization of bacteriochlorophyll.[13]

(11) G. L. Closs, J. J. Katz, F. C. Pennington, M. R. Thomas, and H. H. Strain, *J. Am. Chem. Soc.*, **85**, 3809 (1963).
(12) K. Sauer, J. R. Lindsay Smith, and A. Schultz, *ibid.*, **88**, 2681 (1966).
(13) J. R. Lindsay Smith, unpublished observations.

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 conclusions 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;[3-5,14] furthermore, acetone is used throughout in place of methanol as the organic solvent. These modifications in the experimental method reduce the possible extent of photooxidation and allomerization during purification.

Photooxidation 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 photooxidation of bacteriochlorophyll in air is very dependent on the nature of the solvent.[4] In this investigation the results from the photooxidation 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 carbon tetrachloride.[12] It was found that photooxidation in this solvent is concentration dependent: the rate of photooxidation decreases considerably as the concentration of the bacteriochlorophyll increases.[13] This last observation is of interest in connection with the concentration-dependent aggregation of chlorophylls in nonpolar solvents.[11,12,15] Finally, the photostability 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 photooxidation of bacteriochlorophyll depends greatly on the environment of the chlorophyll molecules. In acetone, the photooxidation rate is 35- to 40-fold faster than it is in ether or in acetone in the presence of excess *p*-benzoquinone. Despite these large differences in photooxidation 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 photooxidation 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. Fur-

(14) (a) C. S. French, *J. Gen. Physiol.*, **23**, 483 (1940); (b) A. Jensen, O. Aasmundrud, and K. E. Eimjellen, *Biochim. Biophys. Acta*, **88**, 466 (1964).
(15) (a) J. J. Katz, G. L. Closs, F. C. Pennington, M. R. Thomas, and H. H. Strain, *J. Am. Chem. Soc.*, **85**, 3801 (1963); (b) A. F. H. Anderson and M. Calvin, *Arch. Biochem. Biophys.*, **107**, 251 (1964).

ther, 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 photooxidation is most probably the same under these three conditions. Thus, although the rate of photooxidation 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 photooxidation 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 remain uncertain. The radical mechanism is indicated by the large number of products and the reaction conditions.

Since the photooxidation results in a mixture of several products of which the main green pigment is only a minor constituent, this work was abandoned in favor of other more specific methods of oxidation.

Chemical Oxidation of Bacteriochlorophyll. The work of Linstead and his co-workers[16] on the oxidation of metal-free chlorophyll derivatives with high oxidation–reduction 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 1 mole equiv of the quinone to oxidize 1 equiv 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 photooxidation 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 oxidation–reduction 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 which was proposed by Braude and his co-workers for the dehydrogenation of di- and tetrahydroaromatic compounds with quinones,[17] 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 dehydrogenation. It is of interest to note that Golden, *et al.*,[16b] found that bacteriochlorin e_6 trimethyl ester with 2,3-dichloro-5,6-dicyanoquinone gave only one product, the 2-desvinyl-2-acetylchlorin e_6 trimethyl ester; 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 to, if not identical with, the pigment reported by Holt and Jacobs. The evidence which is discussed below all points to its being 2-desvinyl-2-acetylchlorophyll 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-formylchlorophyll a,[18] 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 suggest 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-formylpheophytin a,[18] but more important, the spectrum is almost identical with that of 2-desvinyl-2-acetylpheophorbide a[10] (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-acetylpheophytin a would have a visible absorption spectrum almost identical with 2-desvinyl-2-acetylpheophorbide a, and further that the pheophytin of the green pigment is probably 2-desvinyl-2-acetylpheophytin a.

Confirmation that the green oxidized bacteriochlorophyll is 2-desvinyl-2-acetylchlorophyll 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;

(16) (a) U. Eisner and R. P. Linstead, *J. Chem. Soc.*, 3749 (1955); (b) J. H. Golden, R. P. Linstead, and G. H. Whitam, *ibid.*, 1725 (1958).

(17) (a) E. A. Braude, L. M. Jackman, and R. P. Linstead, *ibid.*, 3548 (1954); (b) E. A. Braude, L. M. Jackman, R. P. Linstead, and G. Lowe, *ibid.*, 3133 (1960).

(18) A. S. Holt and H. V. Morley, *Can. J. Chem.*, 37, 507 (1959).

this evidence combined with the bands arising from the phytyl group, the C_{11} ester methyl, and the 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 low-field methyl singlets expected from the compound 2-desvinyl-2-acetylchlorophyll a.

In conclusion, the major green oxidation product of bacteriochlorophyll is 2-desvinyl-2-acetylchlorophyll 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;[19] perhaps the concentration is too low for detection by this method (less than ~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[20] are clearly different from those 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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(19) M. Byrn, M. Calvin, and J. Lindsay Smith, *J. Am. Chem. Soc.*, **88**, 3178 (1966).

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

Communications to the Editor

Stereochemically Nonrigid Organometallic Compounds. II. 1,3,5,7-Tetramethylcyclooctatetraenemolybdenum Tricarbonyl

Sir:

There is a class of organometallic compounds of the transition elements—perhaps a very large class—in which one or more internal rearrangement processes, carrying the molecule from one to another of two or more equivalent configurations, may occur with an activation energy of such a magnitude that the temperature dependence of the rate may be observed. In some cases the rate at room temperature is such that nuclei which are, presumably, not instantaneously in identical environments appear to be equivalent on the time scale of an nmr measurement; sometimes,[1,2] low-temperature measurements are capable of revealing the probable instantaneous configuration (*i.e.*, the structure of lowest free energy) due to the appearance of a well-resolved, nonaveraged spectrum. In one case[2] so far, it has even been possible, by careful examination and interpretation of the behavior of the nmr spectrum at temperatures between the low- and high-temperature limiting spectra, to determine in some detail the path by which the rearrangement process occurs. In other cases, *e.g.*, $(C_5H_5)_2Hg$[3] and $C_8H_8Fe(CO)_3$,[4,5] even at the lowest accessible temperatures (~−160°) complete development of a "static" spectrum including fine structure is not observed. There are still other cases[6,7] in which time-averaged spectra have been reported at room temperature, but low-temperature data are as yet lacking, at least in detail.

One example of the latter type was recently reported by Winstein, *et al.*,[8] who observed that $C_8H_8Mo(CO)_3$ exhibits only a single resonance at room temperature, whereas structure can be resolved at −30°.

In order to determine the influence of attaching heavy groups in place of protons to the rings in these systems, with the particular hope of reducing to a more convenient range the rates of some of the faster and hence not readily investigated systems, we have studied, *inter alia*, the effect of replacing C_8H_8 by 1,3,5,7-tetramethylcyclooctatetraene in some molecules.[9]

We report here our results on the analog of the molybdenum complex, *viz.*, $(CH_3)_4C_8H_4Mo(CO)_3$.[10]

(1) R. B. King and A. Fronzaglia, *J. Am. Chem. Soc.*, **88**, 709 (1966).

(2) M. J. Bennett, F. A. Cotton, A. Davison, J. W. Faller, S. J. Lippard, and S. M. Morehouse, *ibid.*, **88**, 4371 (1966).

(3) G. G. Dvoriantseva, K. F. Turchin, R. B. Materikova, U. N. Sheinker, and A. N. Nesmeyanov, *Dokl. Akad. Nauk SSSR*, **166**, 868 (1966).

(4) H. P. Fritz and C. G. Kreiter, *J. Organometal. Chem.* (Amsterdam), **4**, 313 (1965).

(5) F. A. Cotton, A. Davison, and J. W. Faller, to be published; F. A. L. Anet and S. Winstein, private communication.

(6) J. E. Mahler, D. A. K. Jones, and R. Pettit, *J. Am. Chem. Soc.*, **86**, 3589 (1964).

(7) While the systems specifically mentioned here all contain cyclic unsaturated organic groups, what are possibly intramolecular environment averaging processes also occur in some noncyclic systems, notably allyl complexes.

(8) S. Winstein, H. D. Kaesz, C. G. Kreiter, and E. C. Friedrich, *J. Am. Chem. Soc.*, **87**, 3267 (1965).

(9) The synthesis of 1,3,5,7-tetramethylcyclooctatetraene was accomplished by the method of P. de Mayo and R. W. Yip, *Proc. Chem. Soc.* (London), 84 (1964). We are grateful to Professor de Mayo for providing much information beyond that given in the published note.

(10) Air-sensitive red crystals of $1,3,5,7\text{-}(CH_3)_4C_8H_4Mo(CO)_3$, melting at 90–93° dec, were prepared by refluxing equal weights of tetramethylcyclooctatetraene and diglyme–molybdenum tricarbonyl in hexane for 16 hr. *Anal.* Calcd for $C_{15}H_{16}MoO_3$: C, 52.95; H, 4.73. Found: C, 51.99; H, 4.89. The infrared spectrum in cyclohexane showed three strong carbonyl stretching frequencies at 1987, 1930, and 1903 cm^{-1} (± 3 cm^{-1}).

(11) An X-ray crystallographic study has been undertaken to clarify the structural details.