chromatography on Whatman No. 1 paper, in 1-butanol–acetic acid–water–pyridine[20]).

Anal. Calcd. for $C_{24}H_{34}O_9N_6 \cdot H_2O$: C, 50.69; H, 6.38; N, 14.78. Found: C, 51.09; H, 6.29; N, 14.76.

The above tetrapeptide was completely hydrolyzed to phenylalanine, glutamine, and glutamic acid on incubation with leucine aminopeptidase, as was demonstrated by paper chromatography of the digest. Therefore, no racemization had taken place during the synthesis of IV.

Hydrogen Exchange in Chlorophyll and Related Compounds, and Correlation with Molecular Orbital Calculations[1]

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Exchangeability of hydrogen at the C-10 and δ-methine positions in chlorophyll, bacteriochlorophyll, and some of their derivatives has been compared in several solvent systems. With methanol in tetrahydrofuran and in acetone, the hydrogen situated at both positions is labile, but exchange at C-10 is at least two orders of magnitude faster than at the δ-position. With methanol in pyridine, hydrogen exchange at C-10 is very rapid, whereas exchange at all methine carbon atoms is very slow. Exchange at the δ-position is influenced by the presence of magnesium; removal of the magnesium reduces the exchange rate at the bridge positions to a very low value. The experimental observations are discussed in the light of semiempirical molecular orbital calculations based on porphin and chlorin compounds.

Introduction

The photosynthetic role of chlorophyll as a hydrogen donor in a reversible cycle has long been a subject for speculation.[3] Such a hypothesis implies exchangeable hydrogen either in the ground state or excited states of chlorophyll. Consequently, a number of investigations have been carried out over the past 30 years in a search for labile hydrogen in chlorophyll. The first positive indication of "active" hydrogen in chlorophyll was provided by Fischer and Goebel[4] who used the Zerewitinoff reaction with methylmagnesium iodide in pyridine solution. They found one carbon-bound active hydrogen atom per mole in a large series of chlorophyll derivatives. Reaction conditions involving this use of the Grignard reagent are necessarily severe, and subsequent efforts to detect labile hydrogen in chlorophylls *a* and *b* and pheophytin *a* by exchange with tritium oxide[5] or deuterium oxide[6] were unsuccessful The more recent tritium studies of Vishniac and co-workers,[7] however, suggested that hydrogen exchange between water and chlorophyll occurs during photosynthesis, but no direct information about the site of exchange could be obtained. Russian investigators have been particularly concerned with hydrogen exchange in chlorophyll and have carried out extensive research in this area.[8-11] The reactivity of chlorophyll in hydrogen exchange has been clarified by the application of infrared and proton magnetic resonance spectroscopy, and, although the results by themselves neither support nor deny the hypothesis of a chemical role for chlorophyll in photosynthesis, the presence of labile hydrogen in chlorophyll has now been firmly established.

Chlorophylls *a* and *b* have previously been shown by an infrared procedure to exchange one proton with a stoichiometric equivalent of CH_3OD in carbon tetrachloride solution.[12] Preliminary nuclear magnetic resonance measurements suggested that this proton was located at the δ-methine position.[13] A detailed examination of the n.m.r. spectra of chlorophyll and its derivatives, however, indicated that the resonances from these compounds are subject to remarkable solvent and concentration effects.[14] The early n.m.r. observations, indicating that only the δ-hydrogen undergoes exchange, were made under conditions that virtually precluded observation of the C-10 proton resonance because of the "aggregation broadening" in pure $CDCl_3$ solutions. When exchange of hydrogen with chlorophyll was investigated under conditions that allowed direct and unambiguous observation of both the δ and C-10 resonances, it was found that both protons do in fact undergo exchange in neutral solutions, with the C-10 exchange about two orders of

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magnitude faster than the δ-methine exchange, as indicated in a preliminary report.[15] In view of these results, the infrared spectroscopic studies of hydrogen exchange in chlorophyll derivatives which were reported for particular conditions by Sidorov[8] indicate exchange of the C-10 proton only.

This communication reports in detail further studies on hydrogen exchange in chlorophyll and some related compounds. It summarizes attempts to correlate the experimental results with predictions of reactivity based on semiempirical molecular orbital theory.

Experimental

Chlorophyll. Chlorophylls *a* and *b*;[16] pheophytin *a*,[16] and pyrochlorophyll *a*[17] were prepared by standard procedures from spinach. Bacteriochlorophyll was prepared by the same procedure as chlorophyll[16] from cultures of *Rhodospirillum rubrum*. All the preparations were precipitated from petroleum ether and were dried *in vacuo*. They were substantially free of colorless impurities as shown by extinction coefficients.[18]

Deuterated Solvents. All deuterated solvents and exchange agents were obtained from Volk Chemical Co., Skokie, Ill., and were distilled before use.

Exchange Procedure. The pigments (*ca.* 10 mg.) were weighed into a small vial and then were quickly dissolved in 250 μl. of the previously prepared solvent mixture. The solution was transferred to a precision n.m.r. tube fitted with a ground joint, and the tube was affixed to a vacuum line. The solution was then degassed, and the tube was sealed off from the line at a pressure less than 5×10^{-6} mm. and at liquid nitrogen temperature. Removal of oxygen is essential if the samples are to be kept for any length of time. Our procedure made it possible to keep chlorophyll solutions in CH_3OH–$CDCl_3$ mixtures for months without significant change, as shown by absorption and n.m.r. spectra. All pigment solutions were stored in the dark, but no particular precautions against exposure to light were taken for the brief periods that were involved in manipulating the materials.

At the beginning of each experiment the solutions were removed from the liquid nitrogen in which they were stored, warmed quickly to 40°, and the n.m.r. spectrum was recorded. Subsequent spectra were taken at regular intervals. The total time of exchange at room temperature of the reaction mixture prior to the first n.m.r. measurement was in every instance less than 2 min. Integration of the spectra at the beginning of each experiment showed that no significant amount of exchange occurred in this period, and consequently this manipulation time was neglected in calculations of the half-times, $t_{1/2}$, for exchange. The half-time for exchange was found either by integration or by a comparison of the peak heights (C–10 or δ-proton resonance to either the α and β resonance) of the n.m.r. spectra. Both methods of estimation give the same results within the limit of the accuracy of the experiments.

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"Active" Hydrogen by Grignard Reagent. Chlorophyll *a* (80 mg.) was dissolved in anhydrous pyridine (5.0 ml.), 2.0 ml. of anhydrous benzene was added, and the solution cooled to 0°. Methylmagnesium bromide (5.0 ml. of a 3 *M* solution in diethyl ether) was then added dropwise to the stirred mixture in a nitrogen atmosphere. After the initial reaction subsided 20 ml. of dry diethyl ether was added, and the solution was warmed for 1.5 hr. while maintaining the nitrogen atmosphere. The reaction mixture was then cooled to 0°, and 60 ml. of D_2O (99.6%) was added. After stirring for 10 min., 4.0 g. of monobasic ammonium phosphate was added to break up the gel that had formed. The organic layer was separated, and the aqueous layer was extracted twice with 50-ml. portions of ether. The combined organic extracts were dried over magnesium sulfate and the solvents removed *in vacuo* and finally under high vacuum. The crude reaction product was dissolved in tetrahydrofuran-d_8, degassed, sealed off, and examined by n.m.r. spectroscopy. Column chromatography on powdered sugar was then used to characterize the product further.

When fully deuterated chlorophyll[18] was subjected to reaction with CH_3MgBr, H_2O rather than D_2O was used to quench the reaction mixture.

N.m.r. Spectra. The n.m.r. spectra in our first experiments were recorded with a Varian A-60 n.m.r. spectrometer using a sweep rate of 250 sec. (full scan) and a sweep width of 500 c.p.s. In all subsequent experiments spectra were obtained with a Varian HR-100 n.m.r. spectrometer. All spectral calibrations were obtained by use of the side-band technique.

Results and Discussion

Exchange at the C-10 Position. Tables I and II summarize the exchange data obtained under neutral conditions. The C-10 hydrogen atom in chlorophylls *a* and *b*, pheophytin *a*, and bacteriochlorophyll exchanged under these conditions at a rate approximately two orders of magnitude faster than the rate of exchange at the methine positions. On the other hand, exchange at the C-10 position in pyrochlorophyll *a* was so slow as to escape detection. For bacteriochlorophyll, exchange at C-10 appeared to proceed somewhat more slowly than the corresponding rates for chlorophylls *a* and *b*.

Table I. Pseudo-First-Order Rate Constants for Hydrogen Exchange at C-10 and δ-Positions in Methanol-d_4–Tetrahydrofuran Solutions at 38°

Compound	Concn.,[a] mole/l.	k[b] for C-10 exchange, sec.$^{-1}$	k[b] for δ exchange, sec.$^{-1}$	$\frac{k_{C\text{-}10}}{k_\delta}$
Chlorophyll *a*	0.185	3×10^{-4}	2×10^{-6}	150
Chlorophyll *b*	0.178	2×10^{-4}	2×10^{-4}	40
Pheophytin *a*	0.096	$>1 \times 10^{-3}$	$<10^{-9}$	$>10^6$

[a] CD_3OD concentration, in all cases, 9.1 moles/l. [b] k is calculated as a pseudo-first-order rate constant from k (sec.$^{-1}$) = ln $2/t_{1/2}$.

The rate of C-10 exchange was increased by a small but probably significant amount when the solvent system was changed from deuteriotetrahydrofuran (9.1 *M* CD_3OD) to deuterioacetone (*M*/*M* methanol-d_4, approximately 7.9 *M* in CD_3OD). Because of the dif-

Table II. Pseudo-First-Order Rate Constants for Hydrogen Exchange in Methanol-d_4–Acetone-d_6 (M/M) Solution at 40°

Compound	Concn., mole/l.	k^a for C-10, sec.$^{-1}$	k^a for δ, sec.$^{-1}$	k^a for β, sec.$^{-1}$	k^a for α, sec.$^{-1}$
Chlorophyll *a*	0.050	$>3 \times 10^{-3}$	2×10^{-6}	$\ll 5 \times 10^{-8}$	$\ll 5 \times 10^{-8}$
Chlorophyll *b*	0.050	5×10^{-4}	4×10^{-6}	$\ll 5 \times 10^{-8}$	$\ll 5 \times 10^{-8}$
Bacteriochlorophyll	0.050	7×10^{-5}	2×10^{-7}[b]	6×10^{-7}[b]	1×10^{-6}[a]
Pyrochlorophyll *a*	0.050	$<5 \times 10^{-5}$	6×10^{-6}	$\ll 5 \times 10^{-8}$	$\ll 5 \times 10^{-8}$
Pheophytin *a*	0.050	$\gg 3 \times 10^{-3}$	$\ll 5 \times 10^{-8}$	$\ll 5 \times 10^{-8}$	$\ll 5 \times 10^{-8}$

[a] k is calculated as a pseudo-first-order rate constant from k (sec.$^{-1}$) = ln $2/t_{1/2}$. [b] The assignment of these resonances in bacteriochlorophyll is not entirely settled. The lowest field proton (8.77 p.p.m.) is certainly that at the α-position (paramagnetic effect of the adjacent carbonyl) on the basis of methanol titrations in $CDCl_3$; the empirical observation that the δ-proton is closer to the carbonyl groups in the 5-ring ketone has led us to assign tentatively the intermediate resonance to this proton (8.44 p.p.m.). The remaining resonance (8.32 p.p.m.) is then assigned to the β-proton.

ference in methanol-d_4 concentration the rate constants in Tables I and II otherwise are not directly comparable. The requirement that the solvent system disaggregate the chlorophyll and the necessity for minimizing side reactions such as pheophytin formation and allomerization limits the choice of solvents to systems which have much the same solvation power. Hence, large solvent effects on the rate of C-10 exchange under neutral conditions are not to be expected.

Reaction of chlorophyll derivatives with exchange reagents under relatively basic conditions appears to cause exchange of only the proton at C-10. When chlorophyll *a* was treated with a 100-fold excess of methanol-d_4 in deuteriopyridine, the C-10 proton was completely exchanged in less than 10 min. at 40°, whereas the intensity of the signals from the α-, β-, and δ-methine protons was not significantly diminished after 3 months at the same temperature. Pheophytin *a* behaved in much the same way in the above solvent mixture. However, in this case the proton at C-10 was substantially exchanged during manipulation prior to the start of the run, and no further change in the n.m.r. spectrum was observed after 5 min. at 40°. The imino protons in pheophytin *a* were also completely exchanged prior to the beginning of the n.m.r. measurements. When pyrochlorophyll *a* was treated with pyridine-d_5–methanol-d_4 mixture, the C-10 protons were seen to exchange at an appreciable rate, and again the methine protons appeared to be inert.

The mechanism of the exchange reaction at C-10 has both an intrinsic interest and a bearing on possible photosynthetic exchange reactions. Our data indicate a significant variation in the rate of exchange at C-10: in acetone-d_6–methanol-d_4 the observed order of reactivity was pheophytin *a* > chlorophyll *a* > chlorophyll *b* > bacteriochlorophyll >> pyrochlorophyll *a*. Structural differences are important for bacteriochlorophyll and pyrochlorophyll *a* but do not appear sufficient to account entirely for the position of the other members of the series. It may be important that the order of reactivity is the same as the order of aggregate stability, and that even though the concentration of aggregates is low, the same factors involved in the formation of molecular aggregates are implicated in the reactivity of the C-10 proton.

Relative to neutral solution, proton exchange at C-10 is considerably accelerated under basic conditions, as described above, and in acidic solution (limited to the exchange with pheophytin *a* in acetic acid). Since enolization of the ring V carbonyl group would be facilitated by acidic or basic reagents, these results are in accord with Fischer and Goebel's original hypothesis.[4] That enolization provides the reaction pathway for exchange at C-10 is supported by the observation that hydrogen exchange between CH_3OD and 2-carbethoxycyclopentanone proceeds at comparable rates. It is possible that other mechanisms which do not involve the enol form of ring V may also be involved in the exchange reaction, but in the light of the evidence cited above this possibility seems unlikely.

We have not examined the effect of light on the rate of exchange of chlorophyll solutions because the pigment concentration necessary for n.m.r. measurements produces solutions which are virtually black to visible light even in layers a few microns thick. Because of the very high light absorption of chlorophyll, it will be difficult to introduce enough light into concentrated chlorophyll solutions to affect either the rate or locus of exchange. It is not surprising, therefore, that Mathewson, Richards, and Rapoport[19] found no change in the rate of methine exchange in chlorophyll when the reaction vessels were illuminated.

Exchange at Methine Positions. Woodward and Skarić[20] were the first to discover that the methine positions in chlorins were labile to electrophilic attack when they observed that the δ-hydrogen in chlorin-e_6 trimethyl ester was completely exchanged with deuterioacetic acid in 2 hr. at 80°. The magnesium-containing chlorophyll compounds undergo exchange at a significant rate with methanol in neutral solution. Under acid conditions the magnesium is very rapidly removed, and the exchangeability of the methine positions is sharply decreased. Exchange with neutral methanol at the δ-position in pheophytin *a* was so slow as to escape detection at 40°, whereas δ exchange in pyrochlorophyll, which still contains magnesium, is entirely comparable to the rate for chlorophylls *a* and *b*. In pure deuterioacetic acid the δ-proton in pheophytin *a* was seen to exchange, but the rate of exchange was relatively small compared to methine exchange of chlorophyll under neutral conditions. The exchange at the α-methine position in bacteriochlorophyll was quantitatively faster than exchange at the δ-position in chlorophylls *a* and *b*, as had previously been observed by Mathewson, Richards, and Rapoport.[19]

The solvent effects on the relative rates of exchange at the methine positions in the chlorophylls are relatively

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(20) R. B. Woodward and J. Škaric, *J. Am. Chem. Soc.*, **83**, 4676 (1961).

small, and the values obtained in acetone-d_6 or deuteriotetrahydrofuran are consistent with the relative rates of exchange at the methine positions observed by Mathewson, Richards, and Rapoport.[19]

The exchange of hydrogen at the α- and β-methine positions in chlorophylls *a* and *b* and pyrochlorophyll was so slow as to escape detection even after three months at 40° in all of the solvent systems used in this study. Thus, the rate of proton exchange at these positions must be at least two orders of magnitude smaller than the rate of exchange at the δ-position.

"Active" Hydrogen by Reaction with Grignard Reagent. The exchange of protons in chlorophyll treated with a Grignard reagent and then with deuterium oxide provided an interesting extension of this study, and a confirmation of Fischer's original study of the "labile" hydrogen in chlorophyll.[4] The reaction product was, as expected, very complex, and no less than five green pigments were detected by column chromatography on sucrose. This mixture showed extensive line broadening in the n.m.r., and the appearance of the separate methine resonances was questionable. To clarify this situation we treated deuteriochlorophyll *a* with the Grignard reagent and hydrolysed the product with H_2O. The n.m.r. spectrum of the dried reaction product clearly revealed the added methyl and alcohol groups as sharp lines above 5.0 p.p.m. (δ) in acetone-d_6 solution, and only one low-field resonance was detected. This resonance was centered near 6.2 p.p.m. and must be assigned to the protons located at C-10 in the products. It was more than 2 p.p.m. above the "methine region" for chlorophyll *a* in deuterioacetone, and the resonance coincided very closely with the location of the C-10 proton resonance in ordinary chlorophyll under similar conditions. The δ-hydrogen thus is not an "active" hydrogen with respect to Grignard reagent, whereas the C-10 hydrogen clearly is.

Exchange Reaction Mechanisms. The "active" character of the C-10 proton indicates that formation of the enolate anion of chlorophyll *a* is a relatively facile reaction. This supports all the previous suggestions of an enolization mechanism for exchange at C-10, the relative importance of acid, base, or concerted catalysis for the enolization being determined by the reaction conditions. The rate of exchange of the C-10 proton in neutral solution indicates that the keto–enol tautomeric equilibrium[10] must lie very far to the side of the keto form in chlorophyll *a* and its analogs, as earlier work had already suggested.[12]

The fact that the δ-proton showed no detectable exchange under the conditions used to detect "active" hydrogen indicates that nucleophilic localization or deprotonation at the δ-position could not substantially contribute to the rate of exchange of this proton in neutral solution. The δ-proton in chlorophyll *a* exchanges readily with methanol in acetone-d_6 but not in pyridine. This suggests that the exchange reaction is acid catalyzed. If the rate of exchange is a function of hydrogen ion concentration, a decrease in this factor by only two orders of magnitude would reduce the pseudo-first-order rate constant for exchange below the lower limits covered by our experiment, as was observed.

The relative reactivities of the compounds studied may be understood in terms of an acid-catalyzed exchange at the δ-position. Mesomeric interaction between the aldehyde group and the δ-position in chlorophyll *b* should be negligible as indicated by a valence bond analysis. Consequently, there should be little difference in electrophilic reactions at the δ-position in chlorophylls *a* and *b*. The experimentally observed order in acetone-d_6–methanol-d_4 was pyrochlorophyll *a* > chlorophyll *b* > chlorophyll *a* >> pheophytin *a*. The rate differences between the chlorophylls were not large and may be more dependent on solution interactions than on the small changes in electronic structure through the series. The fact that the δ-hydrogen in pyrochlorophyll *a* exchanged more rapidly than that in either chlorophyll *a* or *b* provides strong support for this hypothesis. The loss of the carbomethoxy group from chlorophyll *a* to form pyrochlorophyll should present only a small perturbation to the π-electronic structure since the carbomethoxy group is not in conjugation with the ring. However, there is a significant difference between the aggregation behavior, and thus the solvation of chlorophyll *a* and pyrochlorophyll *a*,[17] and this difference could account for the change in δ-proton reactivity in the above series. Thus the exchange of the δ-proton is probably a general electrophilic substitution, the small differences in rate in the chlorophyll series being primarily due to changes in solvation.

The relatively low reactivity of pheophytin *a* at the δ-position is also consistent with the hypothesis that the reaction is an electrophilic substitution. The fact that the reaction proceeds at an enhanced rate in acid solutions[19,20] supports this view, and in reality the problem is to explain why the magnesium-containing chlorophylls are so reactive. The partial ionic character of the magnesium–nitrogen bonds and the probable increased ring planarity and orbital overlap in the chlorophyll should, among other factors, increase its reactivity toward electrophilic reagents.

As an electrophilic reaction, the exchange of the δ-proton is remarkably facile. The equilibrium hydrogen ion concentration in acetone–methanol solutions must be no larger than 10^{-8} *M* which means that the second-order rate constant for deuterio deprotonation at the δ-position in chlorophyll must be of the order of 10^2.

Comparison of our rate data with the data obtained at 75° by Mathewson, Richards, and Rapoport[19] after correction for differences in concentration indicates as a rough estimate that the activation energy of deuterio deprotonation in chlorophyll *a* with methanol as the exchange agent is of the order of 6 kcal./mole. The above value suggests that the estimates of the localization energy in the δ-methine position given by Pullman[21] (1.93β) and the calculations described below are considerably too high. This would indicate that the transition state for deuterio deprotonation at the δ-position in chlorins which contain a central metal atom is more closely related to the starting materials than to the Wheland intermediate in which the δ-position would be tetracovalent; however, the fact that the temperature data were obtained in different solvents severely limits the reliability of these conclusions.

Predictions from Molecular Orbital Theory. Predictions of the relative reactivities of the C-10 and methine hydrogen atoms would be a highly uncertain and difficult task from the point of view of semiempirical mo-

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Table III. Hückel and SCF Charge Densities at the Methine Positions of Model Systems[24]

Model	Method	Position α	Position β	Position δ
Porphin metal complex[a]	Hückel	0.946	0.946	0.946
	SCF	0.910	0.910	0.910
7,8-Dihydroporphin metal complex[b]	Hückel	0.943	0.943	1.052
	SCF	0.843	0.843	0.992
Chlorophyll *a*[c]	Hückel	1.017	0.878	1.059
	SCF	1.351	0.804	0.949
Chlorophyll *b*[e]	Hückel	0.956	0.811	1.038
	SCF	0.722	0.816	0.940
Pheophytin *a*[f]	Hückel	0.798	0.712	0.929
	SCF	0.707	0.710	1.094

[a] Porphin metal complex: $\beta_{CC} = \beta = 1.7515$ e.v.; $\beta_{CN} = 0.9\beta$; $\alpha_N = 0.5\beta$; core charge N (all equivalent) = 1.50; effective nuclear charge of N = 3.72. [b] 7,8-Dihydroporphin metal complex: $\beta_{CC} = \beta = 1.7515$ e.v.; $\beta_{CN} = 0.9\beta$; $\alpha_N = 0.5\beta$; core charge N (all equivalent) = 1.5; effective nuclear charge N = 3.72. [c] Chlorophyll *a*: 7,8-dihydroporphin metal complex with vinyl substituent at position 2, carbonyl substituent at position 6, "vinyl-like" substituents at positions 1, 3, 4, 5, α, 17, and 18; $\beta_{CC} = \beta = 1.7515$ e.v.; $\beta_{CN} = 0.9\beta$; $\beta_{CO} = \beta$; $\alpha_N = 0.5\beta$; $\alpha_O = 2\beta$; β_{CC} (ring to "vinyl-like" substituents) = 0.9β[d]; β_{CC} ("vinyl-like" substituents) = 3β; effective nuclear charge N = 3.72; core charge N (all equivalent) = 1.50; effective nuclear charge O = 4.30; effective nuclear charge C_{26} (carbonyl carbon) = 3.55; effective nuclear charge C_6 = 3.30. [d] A. Streitwieser, Jr., "Molecular Orbital Theory for Organic Chemists," John Wiley and Sons, Inc., New York, N. Y., 1962. [e] Chlorophyll *b*: the same as footnote *c* except for substitution of a carbonyl group like that at position 6 for the "vinyl-like" substituent at position 3. [f] Pheophytin *a*: the same as footnote *c* except $\alpha_{N19,21} = 0.5\beta$; $\alpha_{N20,22} = 1.5\beta$; effective nuclear charge $N_{19,21} = 3.90$; effective nuclear charge $N_{20,22} = 4.25$; core charge $N_{19,21} = 1.00$; core charge $N_{20,22} = 2.00$.

MO[22] treatment and the self-consistent field (SCF) treatment which was developed by Pople[23] to calculate the charge densities and atom localization energies for several models of the chlorophyll system.[24] The pertinent results of these calculations are presented in Tables III and IV.

Calculated charge densities are probably the most accurate index to reactivity in large molecules because the charge density does not directly depend upon the total energy of the system. With one exception the predictions based on the calculated charge densities are that the δ-position should react the fastest in deuterio deprotonation, and chlorins should be much more reactive toward electrophilic reagents than the corresponding porphins. When all of the substituent effects were explicitly included in the calculation for chlorophyll *a*, both the Hückel and SCF calculations suggested that a high electrophilic reactivity should also be associated with the α-position, and indeed the latter calculations indicated that the α-position should be more reactive than the δ-position. Both of these predictions are in conflict with experiment. The reason for this discrepancy probably lies in the fact that the chosen substituent parameters do not reflect the actual interaction of the substituents with the macrocyclic ring. The chosen parameters are reasonable (Table III, footnote *d*), however, and a variation of the parameters seems pointless in these systems.

Table IV indicates that the predictions made from the calculated localization energies are not nearly so

Table IV. Electrophilic and Nucleophilic Localization Energies in Units of the Resonance Integral (β)[24,a]

Model	Method	Position α	Position β	Position δ
Porphin metal complex[b]	Hückel			
	Electrophilic	1.78β	1.78β	1.78β
	Nucleophilic	0.94β	0.94β	0.94β
	SCF			
	Electrophilic	−2.18β	−2.18β	−2.18β
	Nucleophilic	4.55β	4.55β	4.55β
	SCF (neglecting core repulsion)			
	Electrophilic	48.7 β	48.7 β	48.7 β
	Nucleophilic	56.1 β	56.1 β	56.1 β
7,8-Dihydroporphin metal complex[c]	Hückel			
	Electrophilic	1.79β	1.79β	2.19β
	Nucleophilic	2.13β	2.13β	2.36β
	SCF			
	Electrophilic	−2.09β	−2.09β	0.67β
	Nucleophilic	5.03β	5.03β	6.75β
	SCF (neglecting core repulsion)			
	Electrophilic	46.5 β	46.5 β	45.8 β
	Nucleophilic	53.7 β	53.7 β	52.8 β
Chlorophyll *a*[d]	Hückel			
	Electrophilic	1.92β	1.90β	1.94β
	Nucleophilic	1.78β	1.62β	1.86β
	SCF			
	Electrophilic	1.45β	1.40β	1.30β
	Nucleophilic	5.21β	5.38β	450.0 β
	SCF (neglecting core repulsion)			
	Electrophilic	73.8 β	74.4 β	72.7 β
	Nucleophilic	80.5 β	814. β	535.1 β

[a] Localization energy = (π-binding energy − core repulsion)$_{\text{parent system}}$ − (π-binding energy − core repulsion)$_{\text{Wheland intermediate}}$. [b] Same as Table III, footnote *b*. [c] Same as Table III, footnote *c*. [d] Same as Table III, footnote *e*.

lecular orbital theory. However, the theory should provide useful predictions of the relative reactivities of the methine hydrogens in the several systems which we have studied. We have used the classical Hückel–LCAO–

(22) R. Daudel, R. Lefebre, and C. Moser, "Quantum Chemistry," Interscience Publishers, Inc., New York, N. Y., 1959.
(23) J. A. Pople, *Trans. Faraday Soc.*, **49**, 1375 (1953).
(24) These calculations were performed on a Control Data 3600 Computer. We are grateful to Dr. Alice Chung and Professor M. J. S. Dewar for assistance in writing and compiling the programs.

clear. The calculated Wheland localization energy at the δ-position was lower for electrophilic substitution than nucleophilic substitution in all the chlorin models. This is in agreement with the earlier results of Pullman[21] and with our experimental findings. The electrophilic localization energies at the α- and β-positions were found to be still smaller, which directly conflicts with the results of the laboratory studies.

The localization energies calculated by the Hückel method involve the difference of two large numbers (the respective π-bonding energies). It is assumed solvation energies and entropies of reaction should be roughly the same at the several sites, and, therefore, localization energies should give at least the order of reactivity. This is clearly not the case. Probably the δ-position is less sterically hindered (because of the adjacent dihydropyrrole ring), and the inaccuracy associated with the computation of the π-bonding energies is almost as large as the differences in the π-energies.

With the SCF localization energies the situation is still more complicated and uncertain. The SCF method[23] explicitly takes account of the core repulsion. We have obtained this quantity by assuming that the core repulsion is equal to the attraction of nucleus *i* for an electron at atom *j*; *i.e.*, we have used the familiar integral (*ii*, *jj*). This approximation has been found to give more satisfactory results than the uniformly charged sphere approximation.[25] However, the uncertainty in the value of the core repulsion must still be at least 1% or more. When a 40-atom problem such as chlorophyll *a* is considered, this error corresponds to at least 20 e.v., which is larger than the anticipated localization energy. In these calculations we used the dimensions of nickeletioporphin[26] as an approximation for the chlorophyll skeleton.

If it is assumed that the changes in the core repulsion will be the same for each of the ions in the series, and only the difference in the SCF total energy is computed, then the associated errors should be similar to those found in the Hückel method. Of course the "localization energies" calculated by this method will be much too large, but their relative values should be a guide to the relative reactivities of the system. With these approximations it would appear that the δ-position should again be the most reactive, and the favorable steric situation should increase the difference between the δ-position and the other methine bridges. The fact that negative localization energies appear in Table IV clearly indicates the errors associated with the core-repulsion calculation.

(25) M. J. S. Dewar and A. Chung, *J. Chem. Phys.*, in press.
(26) E. B. Fleischer, *J. Am. Chem. Soc.*, **85**, 1216 (1963).

The most probable mechanism for nucleophilic attack at the methine bridges is ionization of the proton to form a carbanion. "Localization energies" for this mode of exchange could have been calculated by treating the negative center as a heteroatom; however, the number of highly questionable parameters which would have been necessary in these calculations prompted the conclusion that any agreement with experiment could only have been fortuitous.

The charge density at the δ-position in the model of pheophytin *a*, which we obtained by the SCF method, was considerably higher than the charge density for the corresponding position in the chlorophyll models where all four nitrogen atoms were considered equivalent. However, pheophytin *a* was found to be much less reactive in deuterio deprotonation reactions than chlorophyll *a*. This could follow from the fact that the four nitrogen atoms in chlorophyll *a* are not equivalent or from the possibility that the low value taken for the nitrogen coulomb integral in the chlorophyll model was not low enough to account entirely for the effect of the central magnesium atom.

Several of the differences between chlorophyll and pheophytin which were mentioned in the previous section, such as relative ring planarity, were not accounted for in the calculation and could also be responsible for sizable changes in charge density. The general agreement of the calculations with experimental results is gratifying. However, it is doubtful that calculations of this kind are of substantial predictive value. It is worth noting that the bond orders given by the SCF calculation appear to be more reasonable[26] than the corresponding values from the Hückel calculations.

Conclusions

The present results on the exchange behavior of chlorophyll, observed by p.m.r., provide the first unambiguous comparison of reactivity of the δ- and C-10 positions which infrared spectroscopy and other indirect methods could not provide.[9] Although the relation of these results, obtained with solutions of chlorophyll, to the behavior of pigments in the chloroplast is remote, the approaches that have been developed open the way to an understanding of the exchange of hydrogen and the role of chlorophyll in the intact photosynthesizing organism.

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