times measured at various concentrations of 6-methylpurine. (3) This procedure for interpreting the relaxation data is very sensitive to the various parameters of association, diffusion geometry, and relaxation vectors which yields very unambiguous fits of parameter sets.

A comparison between measured and calculated values is shown in Table II.

Comparison between Deuterated and Undeuterated 6-Methylpurine. In a mixture composed of 70% 8-deuterio-6-methylpurine and 30% undeuterated 6-methylpurine the relaxation times of H(2) and H(8)[28] are longer than the values in the undeuterated compound (Table I). From these results we conclude that neither head-to-head nor head-to-tail alignment of purine molecules is exclusively formed. With the assumption that the stack is arranged vertically, the number of head-to-head and head-to-tail alignments should be approximately equal. In comparison to the various proposed stack structures of purine, our results for 6-methylpurine are in agreement with those of Jardetzky[1,8] and with the "parallel model" proposed by Cheng.[2] Our results, however, do not support models which assume an intermolecular H_2–H_2 distance greater than 0.35 nm.[2,7]

Summary

We believe that relaxation time measurements of undeuterated self-associating compounds and the mathematical treatment of the relaxation and chemical shift data described in this paper are useful tools in studying stacking phenomena. Use of both relaxation time measurements and chemical shift measurements eliminates uncertainties in the thermodynamic calculations. In addition, from the relaxation behavior of the stacks, the structure of the molecular stacks can be deduced.

Experimental

6-Methylpurine (Sigma M6502) was used without further purification. All measurements were carried out at 33 °C in ^2H_2O (Ega 99,8% 15, 188-2)[29] unless otherwise stated. The 8-deuterio-6-methylpurine was prepared by using the method in reference 13 and twice recrystallized from water. The T_1 relaxation times were measured by using the inversion recovery method;[30] the mean deviation of the T_1 values was 5%. The T_2 relaxation times were measured by the Carr–Purcell–Meiboom–Gill method;[31] the mean deviation of the T_2 values was 10%. Me_2SO was used as an external chemical shift standard, and subsequently the chemical shifts were corrected to internal Me_4Si. The temperature of the sample in the probe was measured by a thermocouple placed in the solution. Most measurements were performed on a Varian XL 200 NMR spectrometer, put to our disposal by the "Fonds zur Föderung der wissenschaftlichen Forschung", which is thankfully appreciated by us. The ^1H and ^{13}C measurements at 100 and 25.1 MHz were performed on a Varian HA 100 D modified for FT mode by Digilab Inc. The ^{13}C measurements at 90.5 MHz were done on a Bruker WH 360 NMR spectrometer by Dr. N. Müller, Institut für Organische Chemie, J.K. Universität Linz, to whom we are indebted.

Acknowledgment. Our warmest thanks for stimulating discussions are due to Dr. Oleg Jardetzky. We are very grateful to Dr. M. Westler for his interest and his critical revision of the manuscript.

Registry No. 6-Methylpurine, 2004-03-7; 8-deuterio-6-methylpurine, 13479-71-5.

(28) The difference in the relaxation times of H(8) and H(2) is due to the fact that proton H(8) can be relaxed by the neighboring N–H group by the scalar coupling relaxation mechanism. The existence of this additional mechanism can be demonstrated by measuring the T_1 of 6-methylpurine dissolved in ^1H_2O (1 M, 33 °C). The relaxation time of H(2) is slightly reduced to 7.1 s, whereas the T_1 of H(8) changes to 5.7 s. Addition of HCl (pH 2 in the solution) leads to a further increase of the H(8) relaxation rate (T_1 = 5.6). There is little effect on the relaxation time of H(2).

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Photoprocesses in Photosystem I Model Systems†

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Abstract: Photoprocesses in covalently linked bis(pyrochlorophyllide *a*) ethylene glycol diester molecules have been investigated by laser absorption and fluorescence techniques. Solvent interactions significantly alter the photochemical properties of the linked macrocycles. In toluene/ethanol solution the linked pairs fold into a configuration with optical properties that resemble those of the special pair chlorophyll (P700) of photosystem I of green plants. Photoexcitation of the model system results in the formation of an S_1 state. This excited state returns to the ground state primarily by fluorescence and internal conversion. Under appropriate excitation conditions stimulated (laser) emission is observed. Long (30 ns) excitation pulses produce significant triplet populations. The configuration and possible reactions involving this triplet are discussed. In contrast, the folded pairs in methylene chloride/ethanol solution exhibit an unusually short fluorescence lifetime and a correspondingly low quantum yield. Some of the anomalous behavior of the folded pairs photoexcited in methylene chloride are shown to result from differences in ground-state structure and composition. Rapid internal conversion processes prevent the build-up of a significant excited-state population in this solvent system. It is suggested that the internal conversion process responsible for the lifetime shortening involves the formation of charge-transfer states.

The primary light energy conversion events of photosynthesis begin with the absorption of a photon by one of a large assemblage of light-harvesting (antenna) chlorophyll molecules. This is then followed, on a short time scale, by transfer of the light-induced electronic excitation energy through the antenna to a photoreaction center where the excitation energy is trapped by a very few chlorophyll molecules in which charge separation occurs.[1] The photoreaction center and its associated pigment system in green

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plants has been termed photosystem I (PS I). The photoreactive chlorophyll in PS I absorbs light at 700 nm, hence it has been designated P700 (P for pigment) by Kok[2] who discovered it. P700 is widely considered to be the primary electron donor in green plant photosynthesis. From comparisons of the EPR signal line widths of chlorophyll *a* (Chl *a*) monomer radical cation, Chl $a^{\cdot+}$, with that of the free radical produced in photoexcited Chl *a*/water aggregates (P740) it has been postulated that the unpaired spin remaining after charge separation in $P700^{\cdot+}$ is shared by a special pair of Chl *a* molecules.[3-6] This hypothesis has received additional support from ENDOR experiments.[7-9] The magnetic resonance evidence for a special pair, which is convincing for the photoreaction center in purple photosynthetic bacteria, is somewhat more equivocal for green plants, but even here recent ESR studies support a "dimeric" chlorophyll structure.[10] All things considered, however, a special-pair formulation for the primary electron donor in both bacterial and green plant photosynthesis gives a better overall account for the properties of PS I reaction center chlorophyll than does any other model so far suggested.[11]

The special-pair model of Boxer and Closs[18] and Shipman et al.[19] has been in recent times the point of departure for most of

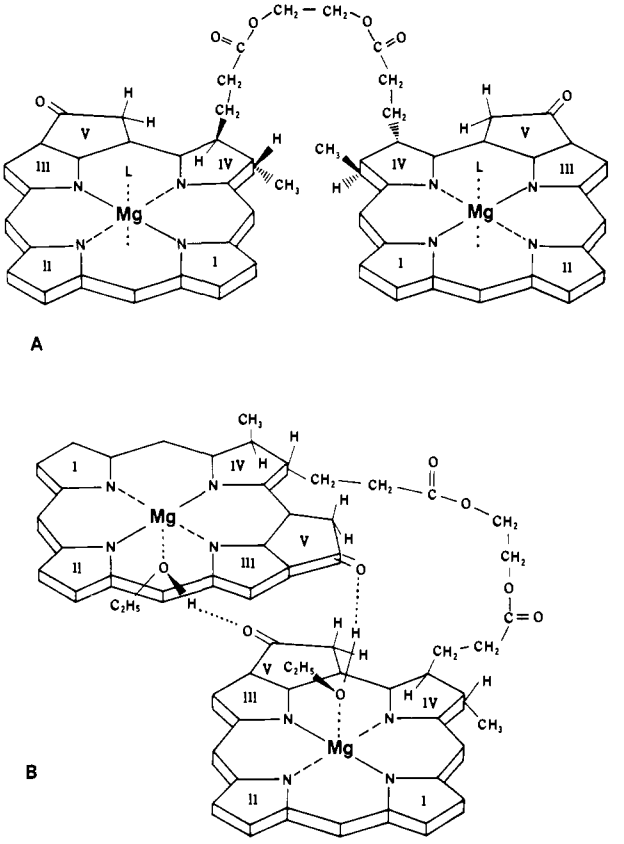

Figure 1. Structure of covalently linked pyrochlorophyllide *a* macrocycles in (A) the open configuration and (B) the folded configuration. The two macrocycles are linked by ethylene glycol through their propionic acid side chains. The central Mg atoms in A are shown with coordination number 5 i.e., with one each of the axial positions occupied by a nucleophilic ligand L. In the presence of strong nucleophilic bases such as pyridine, all four of the axial positions may be occupied and the Mg atoms then are each six coordinated. The hydrogen-bonding nucleophile responsible for folding in structure B is ethanol.

the model systems for P700 chlorophyll.[20] In this model, two Chl *a* molecules are arranged in a parallel configuration with the π systems of the two macrocycles in contact at their van der Waals radii.[19] This model has been realized in the laboratory either by (a) covalent linkage of the two macrocycles through their propionic acid side chains by a dihydric alcohol such as ethylene glycol[18,26] or (b) self-assembly at low temperatures.[27,28] To simplify the synthetic procedure and to facilitate subsequent folding, the pyrochlorophyllide (Pchlide *a*) macrocycle has commonly been used in place of the chlorophyllide *a* macrocycle, although linked pairs of the latter have also been synthesized.[26] The Pchlide *a* macrocycle is identical with that of chlorophyllide *a* except for the replacement of the carbomethoxy group normally present at

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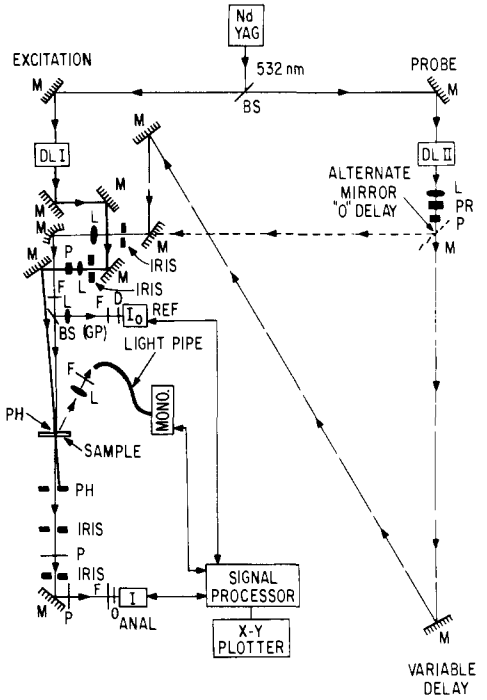

Figure 2. Experimental arrangement for photoexcitation experiments. DLI and DLII and the excitation and probe dye lasers, respectively. BS, beam splitter; M, mirror; L, lens; P, polarizer; PR, polarization rotor; F, filter; PH, pinhole; D, diffusor; I_0, reference detector; I, analytical detector; and M, monochromator.

position 10 on Ring V by an H atom. The elimination of the carbomethoxy groups greatly reduces the steric barrier to folding of the linked pair into the desired parallel configuration, but the redox properties of the macrocycle are changed to a significant extent. All of the covalently linked pair experiments described in this paper were carried out with Pchlide *a* pairs (Figure 1A). More complex covalently linked model compounds have also been synthesized. Boxer and Bucks[29] linked two pyrochlorophyllide (Pchlide *a*) macrocycles and then attached a Mg-free pyropheophorbide macrocycle to one of the linked Pchlide macrocycles, thus providing a configuration where there is the possibility of electron transfer from the special pair to an electron acceptor. A rudimentary antenna–special-pair model that consists of three macrocycles, containing either Mg or Zn, esterified to a trihydric alcohol, has also been prepared. Under conditions where two of the macrocycles are in a special-pair configuration, the third macrocycle is free (i.e., monomeric) and functions as an antenna to the special pair.[30]

Recently, Bucks et al.[31] have argued that covalently linked pyrochlorophyllide *a* macrocycle pairs are not likely to duplicate the excited-singlet-state electron-transfer reactions characteristic of the primary photochemistry, although these worker's picosecond absorption kinetic measurements do not rule out the possibility of electron transfer. While the question of electron transfer from photoexcited singlet-state covalently linked pairs is still an open one, the optical and magnetic resonance properties of the covalently linked pairs are remarkably faithful replications of P700. The

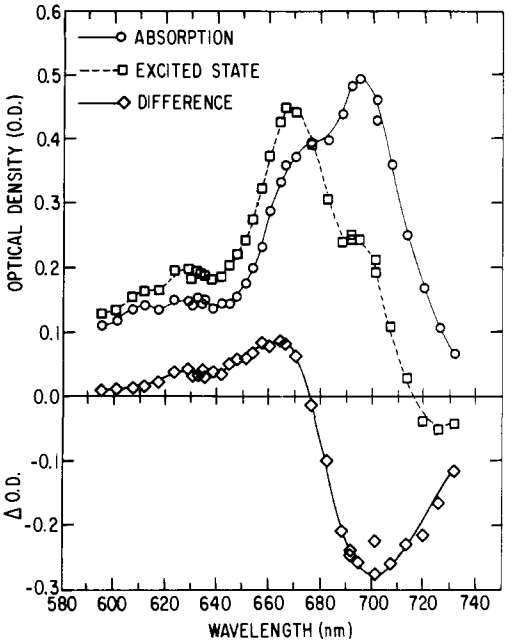

Figure 3. Absorption, photoexcitation, and difference spectra of a 2×10^{-4} M solution of linked Pchlide *a* pairs in toluene containing 0.024 M ethanol. The optical pumping was at 695 nm with no time delay between the pump and probe beams.

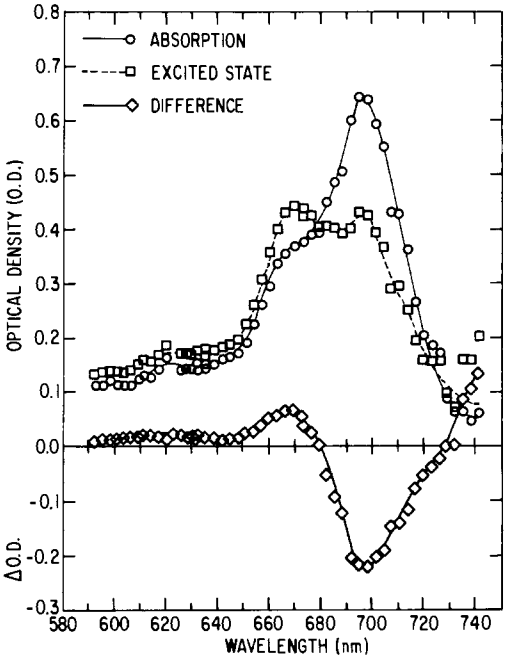

Figure 4. Absorption, photoexcitation, and difference spectra of a 2×10^{-4} M solution of linked Pchlide *a* pairs in toluene containing 0.024 M ethanol. Optical pumping was at 695 nm with a 15-ns time delay between pump and probe beams.

study of the optical properties of these synthetic systems provide a valid point of departure for understanding energy-transfer mechanisms of the primary electron donor in photosynthesis, particularly for green plant P700. The principal objective of this work is to clarify the excited-state characteristics of the covalently linked pair that bear on its application as a model for the electron donor of photosystem I (P700).

In the present manuscript we are primarily interested in two questions. First, what photoprocesses occur in excited folded linked pairs formed by addition of a nucleophile to the covalently linked pairs in a nonnucleophilic solvent. The second question we address is the nature of the photoprocess (and photoproducts) occurring when the folded pairs are excited in the presence of the solvent methylene chloride.

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Experimental Section

The apparatus used for the measurements of the excited-state absorption spectra is very similar to that of Hammond[36,37] (Figure 2). A polarization rotor is used to ensure that the polarizations of the probe beam and excitation beam are orthogonal. Additional polarizers are inserted in the probe beam after passage through the sample to exclude pickup of any residual excitation beam by the detector. Pinholes are used to eliminate interference from fluorescence. The sample cell is placed at an ~4° angle to the probe beam to reduce the probability for gain in the cavity of the 1-mm sample cell. The intensity of the probe beam is kept at a level such that the ground-state population is not significantly altered. This is confirmed by comparing visible absorption spectra produced with the probe beam (Figures 3 and 4) with spectra recorded on a Cary 14 spectrophotometer. The excited-state spectra were recorded with a weak tunable probe beam in the presence of a strong pump laser exciting the molecule at its absorption maximum.[37] This technique has the advantage that the excited-state absorption spectrum can be obtained in the emission region of the dye.

Zero time delay corresponds to coincidence of the probe and excitation pulses (pulse widths 4–5-ns fwhm). For measurement of triplet-state spectra, an optical delay line is used to delay the probe pulse with respect to the excitation pulse. For measurements at wavelengths longer than 725 nm, a dye-laser-pumped high-pressure hydrogen Raman cell was used to generate the probe pulse.

To measure the fluorescence as a function of excitation wavelength, the output of the dye laser used to excite the solution was channeled through a PTR F-100 tunable grating filter to eliminate contributions from dye fluorescence. The sample solution was excited at an angle of 30° at a repetition rate of 10 Hz. The emitted light was detected after passing through a monochromator by a Hamamatsu 928 photomultiplier. Output from the photomultiplier and reference photodiode was normalized, averaged, and displayed on an x-y recorder. Details of the technique for measuring the fluorescence lifetimes have been described elsewhere.[38]

Visible absorption spectra for the solutions described in Table II were recorded on a Cary 14 spectrophotometer equipped with a cryostat for low-temperature measurements. NMR spectra were collected on a 200-MHz (^{1}H) Nicolet spectrometer. The authors are indebted to Arthur G. Kostka and Professor Gerhard L. Closs for providing these data. The chlorophyll concentrations were typically (1–2) × 10^{-4} M. Halogenated hydrocarbon solvents obtained from Aldrich Chemical Co. were purified on a column of Woelm basic alumina activity grade I and dried over activated molecular sieves prior to use. All chlorophyll compounds were freed from volatile nucleophiles by codistillation with CCl_4.[38] Chlorophyll solutions were prepared in a N_2-purged drybox.

Results and Discussion

Linked-Pair Configurations. The state of aggregation of the covalently linked pair and the geometry of its aggregates is expected to depend on the nucleophilicity of the solvent system, the presence or absence of extraneous nucleophiles, the number of nucleophilic centers in the extraneous nucleophile, whether the extraneous nucleophiles are capable of hydrogen bonding, the nucleophile and chlorophyll concentrations, and the temperature.[21] From NMR data, covalently linked pairs dissolved in pyridine, tetrahydrofuran, or other nucleophilic (Lewis base) solvents appear to be present in an open configuration,[18,26] with one or two molecules of solvent (depending on its basicity) in the axial sites of each of the Mg atoms (Figure 1A). The visible absorption spectrum of the covalently linked pairs in such solvents is essentially identical with those of the monomeric macrocycles, but in contrast to the monomeric Chl *a* species no laser emission can be obtained from the open linked pair.[33] In the nonpolar, nonnucleophilic solvents benzene and carbon tetrachloride, the coordination unsaturation of the central Mg atoms of the linked pair with coordination number 4 can be alleviated by coordination interactions between the keto C=O function in one macrocycle and the Mg atom of another.[21] In species formed by intramolecular keto C=O···Mg interactions, the two macrocycles that form the coordination bond would then be (roughly) orthogonal to each other. This type of interaction may be intra- or intermolecular. Intermolecular keto C=O···Mg coordination interactions between two linked pairs in their open configuration to form a dimer of linked pairs has not been previously discussed in the literature.[39] There is, however, evidence from our data that such intermolecular dimerization may occur in pure methylene chloride solutions of the covalently linked pairs (see below). Nonnucleophilic solvent systems (e.g., toluene or methylcyclohexane) that contain hydrogen-bonding nucleophiles such as water or ethanol may at low temperatures self-assemble the macrocycles of the linked pair into the parallel configuration of Figure 1B.[19] Again, the possibility of intermolecular self-assembly between two linked pairs in their open configuration cannot be excluded. It should be recognized that the linked pyrochlorophyllide species generated by coordination interactions do not have fixed geometry. Rather, the various species must be related to each other and their precursors by a complex set of dynamic equilibria.

Covalently Linked Pairs in Toluene/Ethanol Solution. We will first discuss the properties of the covalently linked pairs in toluene/ethanol solution since the interpretation of these experimental observations are straightforward. In toluene solution the linked pair assumes the folded configuration of Figure 1B in the presence of a hydrogen-bonding nucleophile such as ethanol. The principal species present has a characteristic visible absorption maximum near 695 nm.[18,26] Because formation of the folded pair is an equilibrium process, the concentration of nucleophile required for folding is a function of the covalently linked pair concentration. A 10- to 100-fold molar excess of the folding agent was used in our experiments. Complete folding is not attained, and the residual open pair can be seen as a shoulder in the 665-nm region of the absorption spectrum (Figure 3).

The results of photoexcitation experiments for a toluene/ethanol solution are illustrated in Figures 3 and 4. The spectra of Figure 3 (simultaneous excitation and probe pulses) show bleaching of the ground state of the folded pair and formation of transient excited species that absorb in the 620–675-nm region. The spectra of Figure 4 show that when the probe pulse is delayed by 15 ns relative to the excitation pulse the maximum of the transient absorption is close to 670 nm. Transient absorption at this wavelength was reported by Periasamy et al.[32a] However, our results are different from Periasamy et al. in that our data show that two transient species are formed on excitation. First, there is a rapidly decaying transient, which has significant absorption below 670 nm, and which we assign to the excited singlet of the folded pair. The decay of this transient parallels the reappearance of the 695-nm ground-state absorption. Our present results are in accord with those of Pellin et al.[34a] who observed the simultaneous bleaching at 700 nm and formation of a 660-nm transient in folded synthetic linked pairs. Periasamy et al[32a] had previously interpreted the 700-nm bleaching to be caused by opening of the folded pair and the increase in absorbance near 660 nm to originate in the monomer-like open macrocycles formed in this process. However, Pellin et al[34a] noted that the rapid decay of the 700-nm bleaching indicated that the ground state of the folded pair was repopulated at the same rate as the excited state was decaying. Pellin[34b] further observed that the rate of decay of fluorescence for a folded pair in ethanol/toluene solutions matched the decay of the 660-nm transient absorption, supporting the view that this absorption is associated with the S_1 state of the folded pair.

To obtain further information about the transient species, 665-nm radiation was used for excitation. This wavelength is close to the absorption maximum of the open form of the linked pair. We observe transients absorbing maximally at 656 nm (0 time delay) and 665 nm (15-ns time delay). Bleaching occurs only at 695 nm. The extent of bleaching for excitation at either 665 or 695 nm is proportional to the optical density of the folded form of the linked pair at these wavelengths. There is no evidence for the involvement of the open fraction of the linked pair in the photoprocess. Both sets of excitation experiments support the assignment of the approximately 660-nm absorbing transient to the S_1 state of the folded pair and the approximately 665–670-nm

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Table I. Photobleaching of Covalently Linked Pyrochlorophyllide *a* Pairs Folded with Ethanol in Toluene[a]

probe time delay, ns	% bleaching (695 nm)	estimated populations, % S_0	S_1	T_1
0	78–83	12	72	16
15	46–48	54	4	42

[a] 2.2×10^{-4} M linked pair; 0.024 M ethanol. Excitation frequency, 695 nm; pulse width (FWHM), 5 ns.

absorption to a triplet state formed by intersystem crossing from the S_1 state of the folded pair. Approximate estimates of the ground- and excited-state populations have been calculated from the experimental difference spectra determined at the varying time delays. The results for our toluene/ethanol solution of the Pchlide *a* linked pair are shown in Table I. We conclude that under our experimental conditions, the primary and principal photoproduct formed is the S_1 state of the folded pair. Regeneration of the ground state can be correlated with the rate of fluorescence or, at high S_1 concentrations, by the rate of stimulated emission.

Excited-State Populations as a Function of Excitation Conditions. To see how our observations can be reconciled with those of Periasamy *et al.*,[32] we consider the interpretation of their experimental results. In their original communication[32a] Scheme I was proposed, where (Pchlide *a*)$_p$ is a folded pair, (^{3}Pchlide

Scheme I

$$(\text{Pchlide } a)_p \xrightarrow{h\nu} (^3\text{Pchlide } a\text{–Pchlide } a)_u \xrightarrow{\text{I}} (\text{Pchlide } a\text{–Pchlide } a)_u \xrightarrow{\text{II}} (\text{Pchlide } a)_p$$

a-Pchlide *a*)$_u$ the linked pair in the open configuration with one ground-state and one triplet-state macrocycle formed in the photoprocess, and (Pchlide *a*–Pchlide *a*)$_u$ the linked pair in the open configuration. According to the authors, reaction I, Scheme I, has rate constants very similar to those for monomeric triplet Chl *a* and the refolding reaction II, Scheme I, is very fast.[32a] As a result of a later study on folded systems formed by a self-assembly process, they suggested a revised scheme,[32b] which we will write as Scheme II, for the linked pair. Here the formation of

Scheme II

$$(\text{Pchlide } a)_p \xrightarrow{h\nu} {}^1(\text{Pchlide } a)_p^* \xrightarrow{\text{I}} (^3\text{Pchlide } a\text{–Pchlide } a)_u$$

the excited singlet S_1 state is postulated to be the first step in the photoexcitation. They conclude that the unfolding, reaction I, Scheme II, is slow enough so that fluorescence from the folded state can be observed.[32b]

A computer simulation can be used to show that the conclusions reached about the photoproducts produced depend on the properties of the exciting pulse and the time intervals involved in the experiment.[40] We have calculated the time evolution of the populations of the ground, S_0, excited singlet, S_1, and triplet, T_1, states for Gaussian pulses of 5-ns (Figure 5) and 30-ns (Figure 6) (fwhm) 695-nm radiation. To obtain these results we used a rate of fluorescence, k_{21F}, obtained from our experimental values for the quantum yield and fluorescence lifetime.[33] We found that to reconcile our calculations to the experimental values for the populations obtained from photoexcitation experiments (Figures 3 and 4, Table I), it was necessary to assume that there are two nonradiative pathways for energy loss from the S_1 state, i.e., internal conversion, k_{ic}, and intersystem crossing, k_{isc}, to the triplet. If we do not include internal conversion and derive an approximate intersystem crossing rate from the reported values for the quantum

(40) Kugel, R.; Hunt, J. E.; Wagner, A.; Katz, J. J.; Hindman, J. C., manuscript in preparation.

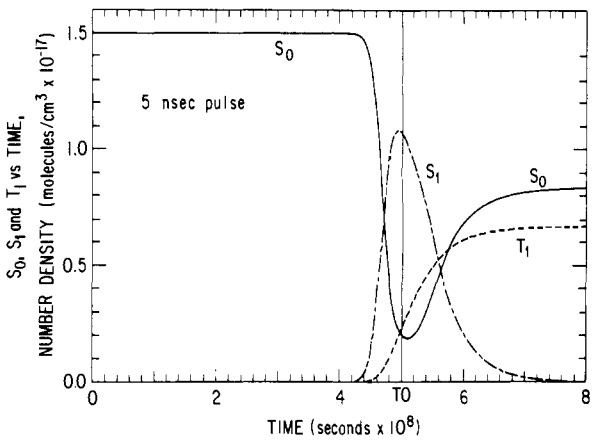

Figure 5. Time evolution of excited-state populations produced by a 5-ns pulse. The excitation pulse power is 16 MW/cm^2. The pulse is arbitrarily centered at time = 50 ns. S_0, ground state, S_1, first excited singlet state, and T_1, lowest lying triplet state.

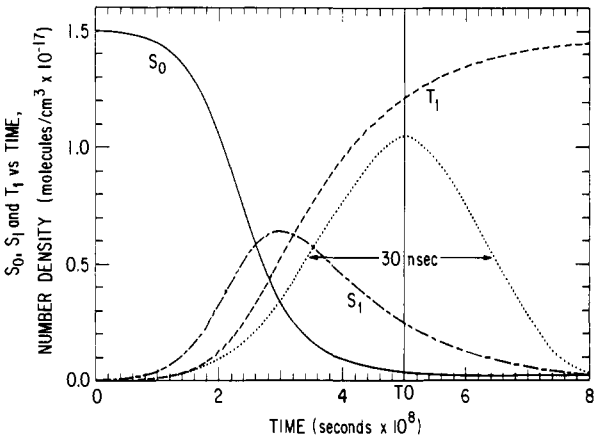

Figure 6. Time evolution of excited-state populations produced by a 30-ns pulse. The excitation pulse power is 16 MW/cm^2. The pulse is arbitrarily centered at time = 50 ns. The dotted linke represents the Gaussian excitation pulse. Note that the maximum in the excited singlet populations, S_1, is reached before the maximum in the excitation pulse. If the excited-state population is sampled after the excitation pulse, triplet will predominate.

yield and fluorescence lifetime,[33] the calculated values for the triplet yields are significantly higher than those observed experimentally. These results lead to Scheme III for describing the

Scheme III

$$(\text{Pchlide } a)_p \xrightarrow{h\nu} {}^1(\text{Pchlide } a)_p^* \xrightarrow{k_{21F}} (\text{Pchlide } a)_p + h\nu_1$$

$$(\text{Pchlide } a)_p \xrightarrow{h\nu} {}^1(\text{Pchlide } a)_p^* \xrightarrow{k_{ic}} (\text{Pchlide } a)_p + \text{heat}$$

$$(\text{Pchlide } a)_p \xrightarrow{h\nu} {}^1(\text{Pchlide } a)_p^* \xrightarrow{k_{isc}} {}^3(\text{Pchlide } a)_p + \text{heat}$$

results of photoexcitation of the folded covalently linked Pchlide *a* pair. To fit our experimental data, we used the following values for the rates shown in Scheme III: $k_{21F} = 0.68 \times 10^8$, $k_{ic} = 1.0 \times 10^8$, and $k_{isc} = 0.6 \times 10^8$. It is clear from our results that the primary photoproduct is the S_1 state of the folded linked pair (as in Scheme II). It is also clear that the bulk of the excited, S_1, state reverts to the ground state either by fluorescence or internal conversion. Intersystem crossing to form a triplet occurs at a rate that is very close to that observed for S_1 of monomeric Pchlide *a* in pyridine. We conclude from our observations that breaking of the hydrogen bonds and unfolding of the linked pairs is not a primary pathway for deactivation of the excited, S_1, state.

To see why Periasamy et al.[32] concluded that unfolding and formation of entity having a triplet- and ground-state macrocycle

Table II. Absorption and Emission Properties of Chlorophyll *a* and Pyrochlorophyllide *a* Linked Pairs[a] in Various Solvents at Room Temperature

species[a]	solvent	λ_a, nm	λ_F, nm (λ_e = 337 nm)	integral quantum yield, ϕ_f	fluorescence lifetime, τ_f, ns
Pchlide *a* pairs (10^{-4} M)	methylene chloride (dry)	669[b]	670 730	~0.016	<1.2[c]
Pchlide *a* pairs (5×10^{-4} M)	pyridine	669[d]	685[d] 735	0.13[d]	5.7 ± 0.2[d]
Chl *a* (1.2×10^{-4} M)	pyridine (dry)	669[d]	685[d] 736	0.35[d]	7.3 ± 0.1[d]
Pchlide *a* pairs (2×10^{-4} M)	toluene	678	737.5 668		6.7
Pchlide *a* pairs (5×10^{-4} M)	toluene (0.1 M ethanol)	695[d]	730[d]	0.3[d]	4.4 ± 0.3[d]
Pchilide *a* pairs (2.6×10^{-3} M)	methylene chloride (satd H_2O)	668 691	667 723	~0.3	6.2 <1.2 and 4.8[c]
Pchlide *a* pairs (2×10^{-3} M)	methylene chloride (0.03 M ethanol)	691 669	725 669	~0.1	<1.2 and 5.9 5.0 ± 0.5

[a] Pchlide *a* pairs, covalently linked bis(pyrochlorophyllide *a*) ethylene glycol diester. [b] At 10^{-3} M the absorption maximum shifts to 675 nm. [c] Shorter lifetime observed at 730 nm and longer lifetime at 680 nm. [d] Values from ref 27.

were important, we have used a computer simulation to calculate the population distributions under the experimental conditions employed by these authors, i.e., a 30-ns exciting pulse, the intensity of which is sufficient to completely bleach the ground state, and examination of the populations after the pulse. The results of this calculation are shown in Figure 6. It is clear from Figure 6 that Periasamy et al.[32] would not have seen the formation of the S_1 state of the folded pair or been able to observe the major pathways for the $S_1 \rightarrow S_0$ conversion. At the pump powers used, the maximum of the S_1 state population occurs before the maximum of the exciting pulse. By the time the pulse is over, both the ground state S_0 and the first excited singlet state S_1 are depopulated. At the time these authors started their measurements the folded linked pair would be largely converted to triplet. Under these conditions no conclusions about the initial photoproducts can be reached. On the other hand, their observations could yield information about species that are formed from the fraction of the molecules that proceed to the triplet state via intersystem crossing. If we accept their conclusion that the transient species present has an open configuration with one ground-state and one triplet-state macrocycle, the overall photoprocess could be represented by Schemes III and IV followed by refolding of the linked pair. k_{31} in Scheme

Scheme IV

$$^3(\text{Pchlide } a)_p \xrightarrow{k_{bb}} (^3\text{Pchlide } a\text{–Pchlide } a)_u \xrightarrow{k_{31}} (\text{Pchlide } a\text{–Pchlide } a)_u$$

IV is the rate of decay of the triplet-ground-state moiety to the ground state.[32a] If the rate of hydrogen bond breaking, k_{bb}, and internal electronic rearrangement is fast, then we can account for the fact that these authors would not observe the triplet of the folded pair. Additional experiments are needed to confirm the validity of the structure proposed by Periasamy et al.[32] for the transient triplet state.

Covalently Linked Pairs in Methylene Chloride Solution. As a preliminary to our discussion of the photoproducts formed on photoexcitation of the folded covalently linked pair in methylene chloride/ethanol solution, we have examined the optical properties of the linked pair in pure methylene chloride solution. Bucks et al.[31] have reported observations on the linked pair in methylene chloride containing 0.5 M pyridine. Under these conditions pyridine is the ligand for Mg and the differences between the quantum yield, and fluorescence lifetimes observed as compared with the linked pair in pyridine could be correlated with the differences in dielectric constant.[31] However, we find that the properties of the linked pair in pure methylene chloride solution are markedly different from those in pyridine or methylene chloride/pyridine solution.

The maximum of the red absorption band of a dilute (~10^{-4} M) solution of the covalently linked pair in methylene chloride is at 669 nm, very close to that of the open linked pair or of monomer Chl *a* itself in pyridine (Table II). This fact taken by itself suggests that the linked pair in methylene chloride is in an open configuration. However, the fluorescence properties of the linked pair in dry methylene chloride and in pyridine differ significantly (Table II). A detailed examination of the absorption spectra shows that there is a difference in the coordination number of the Mg in the two solvents. In pyridine, the linked pair has a spectrum resembling that of a pyridine solution of monomeric Chl *a*, where the central Mg is hexacoordinated. In methylene chloride, the absence of a Q_x (0,0) transition in the 640-nm absorption region of the spectrum suggests a coordination number of five.[41]

The fact that there is a difference in coordination number cannot by itself account for the difference in fluorescence properties of the linked pairs in the two solvents. The central Mg of the linked macrocycles in diethyl ether or ethanol solution are also five-coordinated, but the solutions are strongly fluorescent.

We find that an increase in the concentration of the covalently linked pairs (to 10^{-3} M) results in a shift of the absorption maximum to ~675 nm. The ^{1}H NMR spectrum of such a 10^{-3} M solution in carefully dired CD_2Cl_2 is considerably distorted and contains broad, diffuse peaks. This behavior is reminiscent of that observed when dimers with C=O···Mg linkages are formed by monomeric Pchlide *a* molecules in dry benzene, toluene, or carbon tetrachloride.[21] There is evidence for the formation of similar dimers by intramolecular interaction of the covalently linked Pchlide *a* pairs in dry benzene.[18] Since the formation of intramolecular dimers is concentration independent,[42] we would have to conclude that in the present case the dimers are formed by interaction of two covalently linked pairs. It appears that methylene chloride, although it is presumably a relatively weak nucleophile, interferes with the formation of intramolecular pairs in the covalently linked pairs.

In contrast to its behavior in solvents such as pyridine, the Stokes shift between absorption and fluorescence maxima is very small, less than 1 nm. The small Stokes shift implies that the nuclear configurations are little changed in going from the ground to the excited (S_1) state. This is the most favorable configuration for a fast radiationless transition from the excited (S_1) to ground (S_0) state.[43] Two observations support the conclusion that internal conversion is the primary pathway for energy loss from the excited singlet in methylene chloride (and in 1,2-dichloroethane). One is the low value for the fluorescence quantum yield (Table II). The second is that optical excitation of the linked pair in pyridine bleaches approximately 30% of the ground state with about half of this converted to triplet after 15 ns. Under the same conditions,

(41) Evans, T. A.; Katz, J. J. *Biochim. Biophys. Acta* **1975**, *396*, 414–426.
(42) DiPaolo, T.; Sandorfy, C. *Can. J. Chem.* **1974**, *52*, 3612–3622.
(43) Turro, N. J. "Modern Molecular Photochemistry"; The Benjamin/Cummings Publishing Co., Inc.: Menlo Park: CA, **1978**; Chapter VI, pp 153–198.

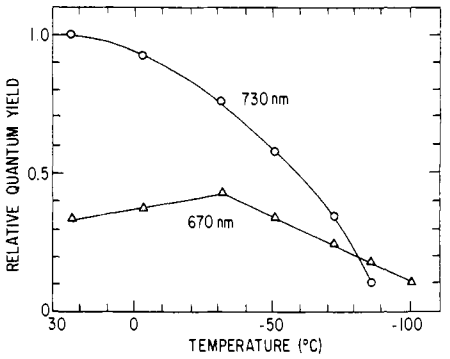

Figure 7. Temperature dependence of the relative fluorescence quantum yield of a 2×10^{-4} M solution of linked Pchlide *a* pairs in methylene chloride. Excitation wavelength was 337 nm. Normalized to unity for the relative quantum yield at 730 nm and 23 °C. The integral quantum yield is 0.016 at 25 °C.

only about 3% of the ground state can be bleached in methylene chloride.

Evidence for the presence of more than one fluorescent species in methylene chloride solution is obtained from measurements of the temperature dependence of the quantum yield for a 2×10^{-4} M solution of the linked pair (Figure 7). A particularly interesting feature of these results is the difference in the temperature dependence of the fluorescence yields at the two fluorescent maxima, 670 and 730 nm. At 730 nm the fluorescence quantum yield steadily diminishes with decreasing temperature. The quantum yield at 670 nm, although only a third of that at 730 nm at 23 °C, shows a slightly increase with decreasing temperature down to −32 °C. Below this temperature the quantum yield at 670 nm falls with decreasing temperature (Figure 7).

Lifetime measurements also indicate the presence of at least two ground-state species. Fluorescence decay of a 4×10^{-4} M methylene chloride solution photoexcited by a short pulse (<1 ns) dye laser at 451 nm is biphasic monitored at 680 and 725 nm at room temperature. The shorter lifetime component (730 nm) decays with a <2.5-ns lifetime, and the longer component decays (670 nm) with a lifetime of 4.5–5.2 ns. As the temperature is lowered, the short-lifetime component disappears. At 77 K the fluorescence lifetime is 5.2 ns at 680 nm and 6.5 ns at 725 nm. One interesting aspect of these results in the fact that at room temperature the primary emission is near 723 nm, with a less intense 670-nm band, although the principal absorption band is at 669 nm with little evidence for the presence of a long-wavelength absorbing species. This behavior is similar to that found for Chl *a* in toluene/ethanol and methylcyclohexane/ethanol solutions where the predominent fluorescence emission was found in the 719–730-nm region; again the principal absorption at room temperature was in the monomer region at 665 nm.[28] These observations have been interpreted in terms of energy transfer from excited monomeric species to long-wavelength absorbing and emitting self-assembled pairs. We believe a similar explanation can be given for the present observations. The fact that the long-wavelength emitting species is short-lived indicates that the folded pairs formed in methylene chloride are asymmetric. The marked decrease in the fluorescence quantum yield for the long-wavelength emission with decreasing temperature, without a concomitant increase of the short-wavelength quantum yield, would be consistent with an increase in the concentration of linked species containing intermolecular keto C=O···Mg bonds that act as energy traps with decreasing temperature.

Multiple-Ground-State Species in Methylene Chloride/Nucleophile Systems. We have obtained results significantly different from those reported by Pellin et al.[34a] in a methylene chloride/ethanol system and have, as a consequence, come to quite different conclusions about the photophysics of the folded covalently linked pairs. As is the case of methylene chloride, methylene chloride/nucleophile systems show fluorescence behavior indicative of more than one ground state (Table III and Figures 8 and 9). The quantum yields monitored at 670 and 730 nm (Figure 8) show

Table III. The Effect of Excitation Wavelength on Fluorescence Emission for Covalently Linked Pyrochlorophyllide *a* Pairs in Various Solvent Systems

solvent	λ_e, nm	relative amplitude of emission, 670 nm: 723 nm	fluorescence lifetimes, ns[a] 686 nm	727 nm
methylene chloride (satd H_2O)	337	2.9:1	5.0	⩽1
	438	5.7:1		
	451	1.4:1		⩽1
	527	3.2:1		⩽1
	665	6.9:1		⩽1 and 5.22[b]
	696			⩽1
toluene (0.024 M ethanol)	451	0:1		
	624	0.06:1		
	660	0.29:1		
	692	0:1		
	696			4.27
methylene chloride	451		4.6[c]	⩾1.2[d]
	527			⩽1.2
	665		5.5[b]	⩽1.2
	696			⩽1

[a] The lifetimes indicated by ⩽1 ns represent upper limits determined by the width of the exciting pulse (⩽1 ns) and the response time of the detection system. [b] Two components, biphasic. [c] Concentration of covalently linked Pchlide *a* pairs, 2×10^{-4} M. [d] Concentration of covalently linked Pchlide *a* pairs, 1×10^{-3} M.

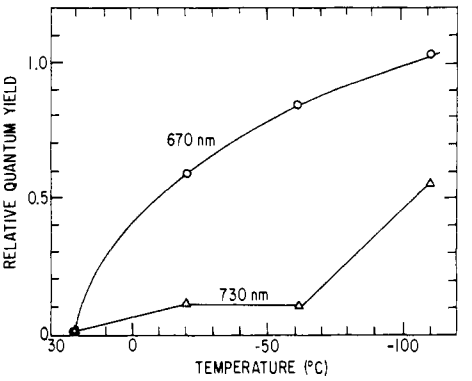

Figure 8. Temperature dependence of the relative fluorescence quantum yield of a 2×10^{-4} M solution of linked Pchlide *a* pairs in methylene chloride solution containing 0.034 M ethanol. Excitation wavelength was 667 nm. Monitor wavelengths are indicated on the figure.

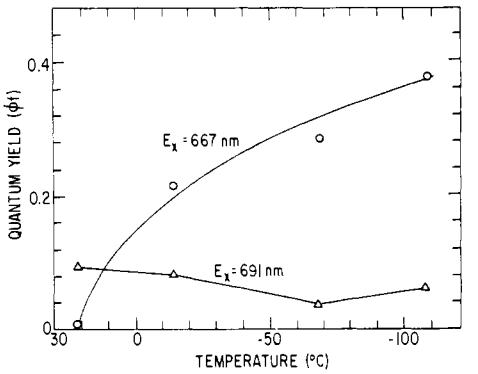

Figure 9. Temperature dependence of the (integral) fluorescence quantum yield as a function of excitation wavelength for a 2×10^{-4} M solution of linked Pchlide *a* pairs in methylene chloride solutions containing 0.034 M ethanol (on the basis of a quantum yield of 0.35 for Chl *a* in pyridine solution).

different temperature dependences. A comparison of Figures 7 and 8 shows that both the quantum yields at a given temperature and their temperature dependencies differ in these solutions, which suggest that different equilibrium conditions exist in methylene chloride/ethanol and in pure methylene chloride. This behavior

is also exhibited in the absorption spectra. Whereas the maximum absorption at room temperature is 669 nm, in methylene chloride/ethanol mixtures the absorption maximum is at 691 nm (Table II), indicating that formation of a folded pair in the latter case is essentially complete. The optical spectrum in a methylene chloride/water mixture is very similar, although the extent of folding is not as complete. In both solvent systems the fluorescence decay curves are biphasic (Tables II and III). As in the case for pure methylene chloride solutions, the short lifetime is associated with the species emitting at long wavelengths (730 nm). Selective excitation[28] has been used to confirm the heterogeneity of the solution composition and the assignment of fluorescence emission wavelengths (Table III).

We find that the emission associated with "monomer" or open linked pair is at a maximum when the excitation wavelength is 665 nm. On the other hand, if we excite into the absorption band of the folded species (692 nm), we only observe a short fluorescence lifetime. This is in marked contrast to what is observed in the linked-pair solutions in toluene/ethanol. The selective excitation experiment supports the conclusion that the folded species in methylene chloride/ethanol, although its absorption maximum at 691 nm is close to that of the folded species in toluene/ethanol, nevertheless has significantly different excited-state properties.

A curious aspect of the fluorescence quantum yield measurements in methylene chloride/ethanol solutions is the increase in the quantum yield at 680 nm relative to the quantum yield at 727 nm with decreasing temperature (Figure 9). This suggests increased emission from monomer-like species as the system is cooled. That the quantum yield at 727 nm does not increase with decreasing temperature, although the concentration of long-wavelength absorbing species is known to increase, is consistent with the view that the long-wavelength species formed is essentially nonfluorescent. Spectral observations do not indicate any increase in the amount of linked pair in an open configuration as the temperature is lowered. The increase in fluorescence quantum yield at 680 nm with decreasing temperature may therefore indicate a decrease in the number of species at low temperature that can act as energy traps, i.e., that quench the fluorescence. We have shown in an earlier communication[28] that the optical properties of the Chl *a* species formed by self-assembly in methylcyclohexane/ethanol solution are best explained on the assumption that at low temperatures formation of monomers and nucleophilically folded pairs are favored at the expense of intermolecular species formed by keto C=O···Mg interactions.

According to Pellin et al.,[34a] the fluorescence lifetime increases monotonically with decreasing temperature; no changes in the fluorescence spectra are observed as the temperature is changed, and no wavelength dependence of the fluorescence lifetime is found. These results led them to the conclusion that there were two excited levels in the same molecule. In contrast, our results lead to the conclusion that there is heterogeneity in the ground-state solution composition and that there are at least two ground-state species that have excited states with different fluorescence properties. Our results (Table II and III) agree with those of Bucks et al.[31] who first reported that the fluorescence decay of Pchlide *a* pairs folded in CH_2Cl_2 is biphasic; the short-lived (100 ps) species, constituting 90% of the photoproducts, have excitation spectra corresponding to the folded linked Pchlide *a* pairs, whereas the long-lived species (3 ns) have spectra characteristic of the open pairs.

The Role of Methylene Chloride. It is clear from the preceding discussion that methylene chloride plays a significant role in determining the properties of covalently linked pyrochlorophyllide *a* pairs, both in the presence and absence of added nucleophiles. There is evidence in the literature that methylene chloride can function in more than one way. A study of the covalently linked pyrochlorophyllide *a* pairs in toluene, methylene chloride, and acetonitrile solutions, where the macrocycles were coordinated to pyridine, showed that the fluorescence lifetimes and quantum yields depend inversely on the static dielectric constant of the solvents.[31] No specific interactions of the methylene chloride and the pyrochlorophyllide *a* macrocycles are indicated by these observations. Other modes of interaction nevertheless are possible. There is evidence in the literature, for example, that both hydrogen-bonding[44] and charge-transfer complexes[45,46] can form in halocarbon solvents. It has also been shown that fluorocarbons containing chlorine and bromine are able to disrupt a variety of different types of hydrogen bonds, e.g., O—H···O and N—H···O=C.[42,47] DiPaolo and Sandorfy[42] have reported that chlorine-containing fluorocarbons become strong hydrogen-bond breakers if they contain in addition a hydrogen atom, as, for example, CF_3CHCl_2. These workers suggest the complexes formed in these solvent systems may be of the charge-transfer type. The fact that we do not observe the formation of intramolecular complexes involving keto C=O···Mg bonds in dilute solutions of the covalently linked Pchlide pairs could be interpreted in terms of such bond breaking by the methylene chloride molecules. Anomalous behavior in the proton-donating ability of methylene chloride has also been noted in a study of organic halide/alcohol mixtures, where it has been suggested that both protons in methylene chloride can form hydrogen bonds and thus bind two alcohol molecules.[48]

We have suggested elsewhere[49] that the symmetry of the excited state has a strong effect on fluorescence behavior. Where the excited state of the linked pair has two fully equivalent macrocycles, a radiative channel for relaxation is available, but where the excited state has a asymmetric configuration, nonradiative pathways for energy dissipation become important. The operation of a nonradiative channel results in a reduction in the observed fluorescence quantum yield and a shortening of the fluorescence lifetime. From models, the size of the methylene chloride molecule is such as to prevent formation of a folded species in which the macrocycles have the parallel orientation shown in Figure 1B.

One pathway of nonradiative relaxation is molecular motion of the linked pair, e.g., bending or folding motions. Where molecular motions provide a relaxation pathway, the fluorescence quantum yields and lifetimes may become temperature dependent.[50] According to Katraro et al.[50] such temperature-dependent processes are associated with low-lying vibrational levels in S_1, corresponding to frequencies of the order of a few hundred reciprocal centimeters. From the temperature dependence of the fluorescence lifetime of the linked pair in pyridine solution, we have calculated a value of $\Delta E = 230 \pm 15$ cm^{-1}, which corresponds to a vibrational frequency of $\sim 0.7 \times 10^{13}$ s^{-1}.[40] We can compare this frequency with approximate rates of internal conversion of the linked pairs in methylene chloride solution. The internal conversion rate on the linked pairs can be calculated by assuming that the intrinsic fluorescence lifetime of the Pchlide *a* pair, in the absence of internal conversion, is (approximately) equal to that of Chl *a* in pyridine (15 ns). We partition the integral quantum yield ($\phi_f = 0.016$, Table II) between the two fluorescing species on the basis of their relative quantum yields (Figure 7). We employ the approximation[43] that these quantum yields reflect the ratio of the intrinsic fluorescence rate and the rate of internal conversion. We find, for the 670-nm emitting species, a rate of internal conversion $k_{ic} = 1.5 \times 10^{10}$ s^{-1}, $\tau \cong 70$ ps. For the 730-nm emitting species, $k_{ic} = 5 \times 10^9$ s^{-1}, $\tau_{ic} \cong 200$ ps. An internal conversion rate of this order of magnitude can also be calculated for the 730-nm emitting species in methylene chlroide/ethanol

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solutions from the results of Pellin et al.[34] and the data in Figures 8 and 9. The magnitude of these internal conversion rates for the linked pair in its folded configuration in methylene chloride suggests that charge transfer (CT) rather than molecular motion is involved in the relaxation process. In this connection it is of interest to note that Netzel et al.[51,52] postulated the formation of charge-transfer states on photoexcitation of the asymmetric species, cofacial diporphyrins, in solutions of methylene chloride and methylene chloride + 0.1 M $[N(C_2H_5)_4]Cl$. For the latter solution the charge-transfer state was represented as $Cl^-Mg^{+\cdot}H_2^{-\cdot}$,[51] with a lifetime for this state of 630 ± 20 ps. It was suggested that complexation of the central Mg by Cl^- reduced the electrostatic attraction between the $Mg^{+\cdot}$ and $H_2^{-\cdot}$ relative to the CT product formed when the Cl^- ion is absent. The lifetime of the CT product in methylene chloride has been reported to be τ = 380 ± 40 ps, corresponding to $k_{ic} \cong 2.6 \times 10^9$ s^{-1};[51] and $\tau \cong 200$ ps, corresponding to $k_{ic} = 5 \times 10^9$ s^{-1}.[52,53] No specific role was assigned to methylene chloride by these workers. It is difficult to account for our observations without concluding that there are strong specific interactions between the methylene chloride and the linked Pchlide *a* macrocycles.

Registry No. P700, 53321-11-2; bis(pyrochlorophyllide *a*)ethylene glycol diester, 67582-80-3.

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A Microwave Study of Perfluoroethylene Oxide

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Abstract: Assignment of the microwave spectra of the normal, oxygen-18, and carbon-13 isotopic species of perfluoroethylene oxide has given a complete structure. The molecular parameters are r(CO) = 1.391 (2) Å, r(CC) = 1.426 (4) Å, r(CF) = 1.329 Å (2), θ(CCO) = 59.17 (8)°, and θ(FCF) = 109.01 (19)°. The dipole moment was found to have a value of 0.576 (5) D. A vector analysis of a number of fluorinated oxiranes indicated that the oxygen atom is at the negative end of the molecule. Microwave spectra of three low-lying vibrational states were assigned, and their energies were determined from relative intensity measurements to be 226 (17) and 306 (17) cm^{-1} for the symmetric vibrations and 182 (23) cm^{-1} for the asymmetric vibration. The shortening effect of fluorine substituents upon the ring bonds which was established earlier for *cis*-1,2-difluoroethylene oxide is also found with perfluoroethylene oxide. However, the vicinal fluorine–fluorine nonbonded distance is almost 0.2 Å longer than the value of 2.7 Å consistently observed for other fluorocarbons. Structural trends established both theoretically and experimentally for the oxiranes and other fluorocarbons are used to predict unknown structures of fluorinated oxiranes.

A number of experimental and theoretical investigations into the structure of substituted three-member ring systems have been conducted.[1-14] Microwave spectroscopy has been used to determine the structures of some fluorinated three-member ring systems, including cyclopropanes[1-5] and oxiranes.[6-9] These systems provide data for testing the various theoretical approaches used to predict substituent effects on structure.

In their work on cyclopropanes, Deakyne, Allen, and Craig[10] found that charge density difference plots could be used to qualitatively predict the changes in ring geometry with fluorine substitution. This technique was found to be useful since Mullikan overlap populations did not predict bond lengths well and correlation diagrams are complicated for large systems. Their treatment explained for the first time several observed changes in the cyclopropane ring of 1,1-difluorocyclopropane and *cis,cis*-1,2,3-difluorocyclopropane. In addition, they proposed a simple additivity principle that allows the prediction of ring bond changes for multiply substituted systems from the changes for less substituted systems.

By this method the effect of 1,1,2,2-tetrafluoro substitution was determined from the experimentally observed changes upon 1,1-difluoro substitution. No change in the C(1,2)–C(3) bond is predicted. The C(1,2)–C(3) bond of *cis*-1,2-difluorocyclopropane was also predicted to remain unchanged. Skancke and Boggs[12] used ab initio calculations to predict the structures of *cis*- and *trans*-1,2-difluorocyclopropane, and they also predict a virtually unchanged C(1,2)–C(3) bond length. This is contrary to the commonly observed shortening of carbon–carbon bonds adjacent to the site of fluorine substitution. Only a partial structure of 1,1,2,2-tetrafluorocyclopropane has been reported,[3] and the question of C(1,2)–C(3) bond length change has not been resolved.

The structure of *cis*-1,2-difluoroethylene oxide was determined by microwave spectroscopy and compared with the structure of ethylene oxide.[6] It was found that all the ring bonds shorten and

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