Octaethylisobacteriochlorin, a Model of the Sirohydrochlorin Ring System of Siroheme Enzymes: Preparation and Spectroscopic and Electrochemical Properties of the Free Base and Its Zn(II) Complex

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Abstract: As the first step in developing synthetic analogues of the siroheme prosthetic group of nitrite and sulfite reductases, octaethylisobacteriochlorin (H_2(OEiBC)) and its Zn(II) complex (Zn(OEiBC)) have been prepared and certain physicochemical properties investigated. Details of the synthesis and purification of H_2(OEiBC) are presented. From 360-MHz ^1H nuclear magnetic resonance evidence, the free base as obtained is most probably a mixture of two nearly equally abundant isomers with trans-reduced pyrroline rings. Quantitative spectral data for H_2(OEiBC) and Zn(OEiBC) are reported. By absorption and fluorescence emission spectral criteria, H_2(OEiBC) proves to be a viable model of sirohydrochlorin, the metal-free form of siroheme whose complete structure has recently been elucidated. H_2(OEiBC) and Zn(OEiBC) undergo two one-electron oxidations to the radical cations and dications. Comparison of potentials for the two primary oxidations with those for corresponding octaethylporphyrin (OEP) and octaethylchlorin (OEC) species reveals that the ease of oxidation follows the order OEiBC > OEC > OEP. Successive potential intervals are 0.2–0.3 V. The electron paramagnetic resonance spectra of H_2(OEiBC)$^{+\cdot}$ and Zn(OEiBC)$^{+\cdot}$ exhibit no resolved hyperfine splittings. Absorption spectra of the radical cations were measured by a spectroelectrochemical technique using an OTTLE cell. When electrolyzed at potentials sufficient to generate the cation radical, solutions of H_2(OEiBC) in acetonitrile and dichloromethane eventually develop an intense royal blue chromophore, whose formation is more rapid at potentials sufficient to generate H_2(OEiBC)$^{2+}$. The blue species was also obtained by reaction of H_2(OEiBC) in dichloromethane with 2 equiv of the strong one-electron oxidant (p-BrC$_6$H$_4$)$_3$N$^{+\cdot}$. This species was identified as the protonated chlorin H_3(OEC)$^+$ and is proposed to be formed by dehydrogenation of H_2(OEiBC)$^{2+}$.

Introduction

The great majority of heme-containing proteins and enzymes possess prosthetic groups in which an iron atom is bound to a fully unsaturated porphyrin macrocycle **1** and is further coordinated by one or two axial ligands from protein side chains. Familiar examples include myoglobin, hemoglobin, cytochromes *b* and *c*, cytochrome P-450, and cytochrome oxidase. Prosthetic groups in which an iron atom is bound to a reduced porphyrin macrocycle are less common.

Porphyrins and certain of their metal complexes are subject to reduction reactions leading to a variety of isolable products.[2] Among these are dihydroporphyrins (chlorins, **2**) and tetrahydroporphyrins (bacteriochlorins, **3**; isobacteriochlorins, **4**) resulting from saturation of peripheral double bonds of the parent porphyrin. Structures of macrocycles **1–4** are set out in Figure 1. Biological interest in reduced porphyrins has centered principally on chlorins and bacteriochlorins inasmuch as the magnesium complexes of these macrocycles are the essential chromophoric units of algal and plant chlorophylls and bacteriochlorophylls, respectively.[3]

Two synthetic isobacteriochlorins, H_2(TPiBC) and H_2(OEiBC),[4] were isolated over 20 years ago.[5,6] Although these macrocycles and several of their metal complexes have since been produced by both chemical[2,7,8] and photochemical[2,9-12] reactions, species containing ring system **4** remain substantially less thoroughly investigated than those with macrocyclic structures **2** and **3**. Only recently has the X-ray study of Zn(TPiBC)(py)[13] provided definitive structural characterization of ring system **4**.

The initial demonstration of the natural occurrence of an isobacteriochlorin structure is that of Murphy et al.,[14-16] who showed that the siroheme prosthetic group is common to several assimilatory and dissimilatory sulfite and nitrite reductases. Subsequent research has provided further evidence substantiating the presence of siroheme in nitrite reductases from several different sources[17-19] and has culminated in proof of the structure of the metal-free prosthetic group, sirohydrochlorin, as **5**.[20] Further, sirohydrochlorin has been identi-

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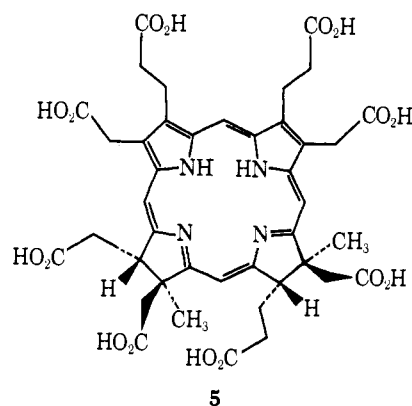
fied[20-24] on the biosynthetic pathway to vitamin B_{12} and has been proposed as an evolutionary link between sulfite and nitrite reductases and B_{12}.[24] Because of the emerging biological significance of isobacteriochlorins and our current interest in the multielectron reductions catalyzed by nitrite and sulfite reductases ($NO_2^- + 8H^+ + 6e^- \rightarrow NH_4^+ + 2H_2O$; $SO_3^{2-} + 8H^+ + 6e^- \rightarrow H_2S + 3H_2O$), in which siroheme is the substrate binding and activating site,[17-19,25-27] we have commenced a study of macrocycle **4** and certain of its metal complexes. Of the two most readily accessible synthetic isobacteriochlorins, H_2(TPiBC) and H_2(OEiBC), we have selected the latter as a model for sirohydrochlorin and, ultimately, siroheme because it[6] and several of its metal complexes[6,11] bear the closer absorption spectral relationship to sirohydrochlorin and its complexes.[14,28] As a necessary prelude to an investigation of the iron complexes of H_2(OEiBC), we have carried out a detailed examination of the spectroscopic and electrochemical characteristics of the free base and its zinc complex. The results of this study are reported here.

Experimental Section

Preparation of Compounds. H_2(OEP),[29] Fe(OEP)Cl,[8] *trans*-H_2(OEC),[8] and Zn(OEC)[30] were prepared by published methods.

Octaethylisobacteriochlorin (H_2(OEiBC)). The procedure is based

on the reduction of Fe(III) porphyrins by sodium in isoamyl alcohol.[6,31] A suspension of 200 mg (0.320 mmol) of Fe(OEP)Cl in 38 mL of isoamyl alcohol was degassed and placed under a dinitrogen atmosphere. All subsequent manipulations were performed using degassed solutions under a dinitrogen atmosphere in subdued light. The mixture was heated to 135 °C and 2.4 g of sodium was added. During addition, the reaction mixture changed in color from brown to red, to green, and finally to dark blue. After stirring for 90 min at 135 °C, the mixture was cooled to 5 °C, and 3 mL of methanol followed by 12 mL of ice-cold concentrated hydrochloric acid was added. This mixture was stirred at 5 °C for 30 min and then transferred to a separatory funnel containing 100 mL of benzene and 35 mL of concentrated aqueous ammonia. Any remaining portions of solid in the reaction flask were washed with benzene until the washings were no longer highly colored. The combined benzene solution was extracted with the aqueous ammonia, resulting in an eventual color change in the organic phase from blue to red. The organic phase was washed with four 50-mL portions of water or until the aqueous phase was neutral. After drying over sodium sulfate, the organic phase was evaporated to dryness in vacuo, affording 154 mg of solid, which from its absorption spectrum is a mixture of H_2(OEP), H_2(OEC), H_2(OEBC), and H_2(OEiBC). Typically this material contains about 75% H_2(OEiBC). Product separation was achieved by medium pressure liquid chromatography using a 25 × 500 mm Altex preparative column packed with magnesium oxide (Mallinkrodt, heavy powder). The crude product (100 mg) dissolved in 10 mL of benzene was injected onto the column. Elution was initiated with 10:1 v/v petrolem ether/benzene. A small yellow-brown band, containing H_2(OEBC), was first eluted. At this point a 1:1 v/v petroleum ether/benzene eluant was employed, resulting in the elution of a purple band of H_2(OEiBC). (Initial fractions of this band should be checked for contamination with H_2(OEBC), λ_{max} 721 nm.[6]) A small green band containing H_2(OEC) remained on the column; it eluted slowly, even with pure benzene. A pink band of H_2(OEP) and a black band of degraded material remained near the top of the column. Removal of solvent from the purple eluate gave a purple oil on the walls of the flask, which is highly soluble in most organic solvents. After several days, crystals formed (mp 129–131 °C) but could not be obtained free of the remaining uncrystallized oil. Attempts at recrystallization from methanol[6] afforded a solid (mp 136–138 °C) only with great difficulty and considerable loss of material. Microscopic examination of this solid revealed it to be an aggregation of amorphous or at best microcrystalline particles. The loss of material and the questionable value of the recrystallization step led to our isolation of the product in a tractable solid form by lyophilization. The purple film was dissolved in a minimal quantity of benzene and the solution was frozen at −15 °C. Evacuation to ~10^{-5} atm resulted in removal of benzene and isolation of pure H_2(OEiBC) as a fluffy purple powder in ca. 40% yield from crude product: mp 117–119 °C (sealed tube) (lit. mp 138 °C,[6] 130–133 °C,[8] 142 °C,[7] 135–140 °C[32]); MS m/e (principal peaks) 538 (M^+), 509, 480, 451, 269 (M^{2+}). Ratios of $(M + 1)^+$ and $(M + 2)^+$ to M^+ agreed with predictions from isotopic abundancy. Anal. Calcd for $C_{36}H_{50}N_4$: C, 80.25; H, 9.35; N, 10.40. Found: C, 80.18; H, 9.53; N, 9.52. Low nitrogen analyses were also encountered by Eisner.[6]

M(OEiBC), M = Zn(II), Cu(II). Under a dinitrogen atmosphere, a solution of H_2(OEiBC) in chloroform was treated with an excess of a methanolic metal(II) acetate solution. After 5-min reaction time, the chloroform solution was extracted several times with water. Evaporation of the chloroform solutions afforded the Cu(II) and Zn(II) complexes as intensely colored solids, which were identified by their absorption spectra.[6] ^{1}H NMR (CDCl$_3$), Zn(OEiBC): δ 7.03 (1), 7.67 (2), 8.70 (1), meso-H. EPR (CHCl$_3$, ~25 °C), Cu(OEiBC): $\langle g \rangle$ = 2.096, $|a_{Cu}|$ = 90 G, $|a_N|$ = 15 G. These parameters are virtually identical with those of Cu(OEP) measured under the same conditions, as are the spectra in frozen solution at 85 K. Similar observations have been made for Cu(OEP)/Cu(OEC)[33a] and for Cu(sirohydrochlorin)/Cu(protoporphyrin IX dimethyl ester).[33b]

Physical Measurements. Owing to the sensitivity of the majority of compounds to dioxygen, especially in solution, critical manipulations and measurements were performed under a pure dinitrogen or argon atmosphere and with minimal exposure to light. Electronic absorption spectra of solutions were recorded on a Cary Model 17 or Cary Model 219 spectrophotometer. Proton magnetic resonance spectra were determined on either a Brüker MXS 360-MHz spectrometer with an Oxford Instrument Magnet or a Varian XL-100-15 spectrometer. Data collection and processing were done by a Nicolet
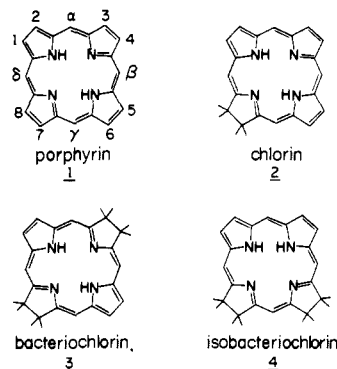

Figure 1. Ring structures and numbering scheme of porphyrin (1), chlorin (2), bacteriochlorin (3), and isobacteriochlorin (4).

Model 1180 Fourier transform computer with a TT 1010 A pulser unit and a Diablo Model 31 magnetic disc storage system. Electron paramagnetic resonance spectra were obtained with a Varian E-12 spectrometer. Mass spectra were recorded on a Varian MAT low resolution spectrometer. Electrochemical experiments were performed with Princeton Applied Research equipment which included a Model 173 potentiostat-galvanostat with a Model 179 digital coulometer or Model 176 current-to-voltage converter and a Model 175 universal programmer. A Pt disk (Beckman 39273) was used as the working electrode in cyclic voltammetry, and a Pt gauze electrode in bulk electrolysis. Potentials were measured vs. a saturated calomel electrode.

Electronic spectra of the isobacteriochlorin and zinc isobacteriochlorin monocation radicals were obtained with an optically transparent thin-layer electrode cell (OTTLE). The OTTLE was of a gold minigrid (500 lines/in.)-quartz microscope slide variety similar to that described by Norris et al.[34] An important modification was that a 0.002-in. gold wire was sandwiched between the quartz slides and the gold minigrid outside the light path. This gold wire lead facilitated rapid and uniform electrolysis. A miniature sodium chloride saturated calomel electrode[35] was used as the reference electrode for the OTTLE experiments.

Acetonitrile for electrochemical measurements was distilled from CaH_2, from Na_2CO_3-$KMnO_4$, and again from CaH_2 taking only the middle 60% portion; the purified solvent was stored over Linde 3A molecular sieves. Butyronitrile for electrochemical measurements was flash distilled from Na_2CO_3-$KMnO_4$ and then slowly distilled from CaH_2 taking only the middle portion, which was stored over neutral activated alumina. Dichloromethane for spectroelectrochemical studies was distilled from CaH_2 and stored over Linde 3A molecular sieves. All electrochemical measurements were performed under an argon atmosphere using thoroughly degassed solutions containing 0.05 M (n-Bu$_4$N)ClO$_4$ as the supporting electrolyte. The OTTLE cell was loaded in an inert atmosphere box.

Results and Discussion

Following the initial preparation by Eisner[6] of H_2(OEiBC) and its Cu(II) complex, several subsequent reports of the free base and certain of its properties,[7-9,32] and of the photosensitized formation of a Sn(IV) complex,[11] have appeared. However, the physicochemical properties of the free base have not been delineated at a level of detail consistent with complete characterization and assessment of purity. H_2(OEiBC) was synthesized from Fe(OEP)Cl by the sodium/isoamyl alcohol procedure,[6,31] which affords predominantly trans reduction of porphyrin pyrrollic rings.[8] Purification of the crude reduction product, which contained ca. 75% of H_2(OEiBC), resembled the procedure briefly outlined by Eisner[6] but was modified by the use of medium pressure liquid chromatography. H_2(OEiBC) was typically obtained in ca. 40% yield from the crude mixture. The disparities in melting points between that of our lyophilized product and literature values are possibly due to differences in the relative amounts of diastereomers present and/or to the different methods (lyophilization vs. crystallization[6,8]) used to isolate the final purified product. The

Table I. Quantitative Absorption Spectral Data for H_2(OEiBC) and Zn(OEiBC), and Comparison with Sirohydrochlorins

H_2(OEiBC)[a]		sirohydrochlorin[d,e]		Zn(OEiBC)[f]		Zn sirohydrochlorin[d,g]	
λ_{max}[b]	ϵ_M[c]	λ_{max}	A_λ/A_{588}	λ_{max}	ϵ_M	λ_{max}	A_λ/A_{597}
355 (sh)	49 000	362 (sh)	2.2				
370	87 900			368	55 000	374	1.0
380	78 300	378	3.3	386	85 000	392	2.0
402	44 800			400	91 000	404	2.4
480	5 540	480 (sh)	0.24	490	3 900		
510	8 500	510	0.37	524	4 800		
545	15 100	545	0.67	553	9 200	553	0.24
586	27 300	588	1.0	597	44 000	597	1.0
635	8 280	638	0.15	642	1 200		

[a] 10^{-4} M benzene solution. [b] nm. [c] M^{-1} cm^{-1}. [d] Methyl ester. [e] Chloroform solution; data from ref 14 (see also ref 28). [f] 9×10^{-5} M in 10:1 v/v chloroform/methanol. [g] Methanol solution; data from ref 28 (see also ref 14).
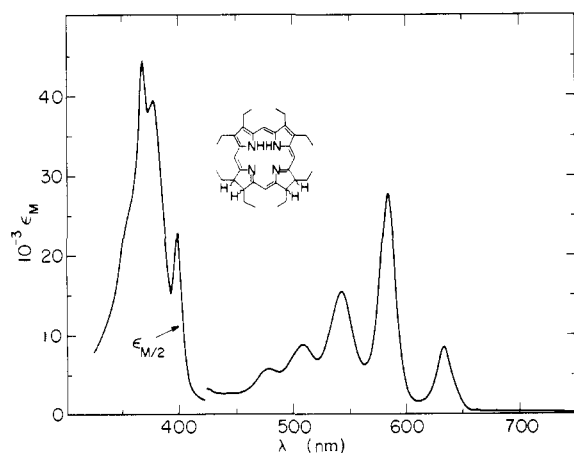

Figure 2. Absorption spectrum of 10^{-4} M H_2(OEiBC) in benzene. Note that $\epsilon_M/2$ values are plotted at $\lambda < 420$ nm.

lyophilized product was used throughout this investigation.

Absorption Spectra. Previous reports of the UV/vis spectrum of H_2(OEiBC) have been confined to a tabulation of λ_{max} and ϵ_M data in benzene solution and presentation of a partial spectrum.[6] The experimental spectrum in benzene is shown in Figure 2, and quantitative spectral data are recorded in Table I. Band maxima and relative absorbancies agree closely with earlier results,[6] but our extinction coefficients (determined in triplicate) are ca. 15% lower. The spectrum consists of multiple bands in the Soret region followed by a progression of four bands of increasing intensity and terminates in a less intense band at 635 nm. The spectrum is somewhat sensitive to solvent, particularly in terms of relative intensities of the lower energy features, as is shown by the results in dichloromethane (Figure 3). Zn(OEiBC) previously has been generated in benzene and has been reported to exhibit maxima at 553 and 598 nm.[6] These features have been observed in two other solvents. Quantitative spectral data for a chloroform/ methanol solution are given in Table I, and the spectra of Zn(OEiBC) and H_2(OEiBC) in dichloromethane are compared in Figure 3. The principal differences between the two species are alteration in relative intensities, especially in the Soret region, and small red shifts of bands in Zn(OEiBC) at $\lambda_{max} > 400$ nm.

Spectral data for H_2(OEiBC), Zn(OEiBC), sirohydrochlorin methyl ester, and its Zn(II) complex are compared in Table I. Although there are differences in the Soret region (possibly due to solvent effects), the principal bands of the free bases and Zn(II) complexes are in good agreement in terms of band maxima and, for the most part, relative intensities. The intensity ratios of the most intense to the lowest energy band in the visible for H_2(OEiBC) ($A_{635}/A_{586} = 0.30$) and sirohydrochlorin methyl ester ($A_{638}/A_{588} = 0.15$) do not
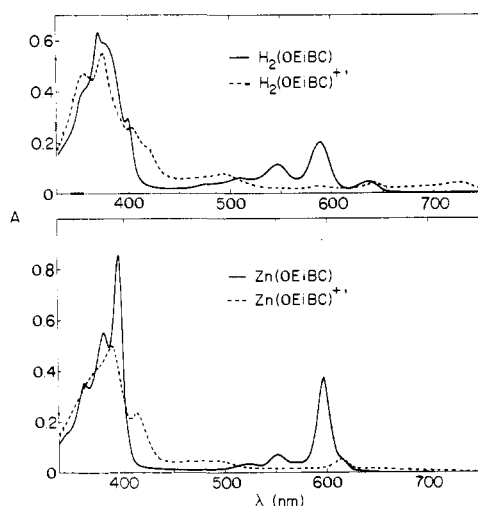
Figure 3. Spectra of H_2(OEiBC) and H_2(OEiBC)[+.] (upper) and Zn(OEiBC) and Zn(OEiBC)[+.] (lower) in dichloromethane containing 0.05 M (n-Bu$_4$N)ClO$_4$ recorded in the OTTLE cell. The shoulder and peak at 614 nm in the lower spectra are due to a slight Zn(OEC) impurity. The 642-nm band in the H_2(OEiBC)[+.] spectrum arises from H_2(OEC) produced in the oxidation (see text).

closely agree, so the presence of contaminating chlorin ($\lambda_{max} \cong 646$ nm, $\epsilon \gtrsim 70\ 000$[7,8,31]) might be suspected. However, the symmetrical nature of 635 nm band (Figures 1 and 3), together with a nearly identical ratio (0.28) found by Eisner,[6] indicates that chlorin contamination is not significant, thus solvent effects and intrinsic spectral differences between the synthetic and natural bases are responsible. A further spectral comparison is afforded by fluorescence emission spectra. A benzene solution of H_2(OEiBC) exhibits an orange fluorescence; the most intense emission band occurs at 589 nm and a minor band is found at 638 nm. In comparison, the major and minor emission bands of sirohydrochlorin occur at 596 and 639 nm, respectively.[15] The collective spectral results for H_2(OEiBc) and Zn(OEiBC), together with related comparisons given earlier,[14] demonstrate that compounds in the OEiBC series serve as viable models for sirohydrochlorin chromophores. By this criterion, these compounds are preferable to TPiBC species whose bands at $\lambda > 400$ nm are red-shifted[8,36] compared with the corresponding sirohydrochlorins.

^{1}H NMR Spectra. In an attempt to determine the number and relative amounts of isomers formed in our preparation of H_2(OEiBC), spectra have been determined in CDCl$_3$ and C$_6$D$_6$ at 360 MHz. Spectral data are collected in Table II and the complete spectrum in CDCl$_3$ is presented in Figure 4. Earlier ^{1}H NMR investigations have been performed at 60[32] and 100 MHz[7] but complete spectra have not been published. However, these results did provide evidence for splitting of

Table II. ^{1}H NMR Data for $H_2(OEC)$ and $H_2(OEiBC)$ Obtained at 360 MHz

trans-$H_2(OEC)$		$H_2(OEiBC)$		
position	δ (CDCl$_3$), ppm	position	δ (CDCl$_3$), ppm	δ (C$_6$D$_6$), ppm
α-, β-H	9.71	α-H	8.52, 8.50	8.78, 8.75
γ-, δ-H	8.87	β-, δ-H	7.47, 7.45	7.64, 7.62
		γ-H	6.88, 6.86	6.85, 6.76
7,8-H	4.55 (m, X)[a]	5,6,7,8-H	3.69 (m)[c]	3.52 (m)[c]
3,4-CH$_2$	3.99 (q)	2,3-CH$_2$	3.42 (q)	3.39 (q)
2,5-CH$_2$	3.94 (q)	1,4-CH$_2$	3.30 (q)	3.34 (q)
1,6-CH$_2$	3.91 (q)	5,8-CH$_2$	2.06 (m)[d]	1.90 (m)
7,8-CH$_2$	2.19 (m, A)[a,b] 2.44 (m, B)[a,b]	6,7-CH$_2$	1.89 (m)[d]	1.74 (m)
3,4-CH$_3$	1.86 (t)	2,3-CH$_3$	1.52 (t)	1.56 (t)
2,5-CH$_3$	1.83 (t)	1,4-CH$_3$	1.48 (t)	
1,6-CH$_3$	1.81 (t)	5,8-CH$_3$	1.04 (t)	0.89 (t)
7,8-CH$_3$	1.06 (t)	6,7-CH$_3$	0.99 (t)	
N-H	-2.51 (br)	N-H	2.98	3.65

[a] ABX multiplet: $J_{AX} = 7$, $J_{BX} = 4$, $J_{AB} = 14$ Hz. [b] $J_{A\text{-}CH_3} = J_{B\text{-}CH_3} = 7$ Hz. [c] Multiplet of two coupled non-equivalent H's, also coupled to 5,8- and 6,7-CH$_2$. [d] Multiplet collapsed to AB quartet upon irradiation of coupled CH$_3$ and 5,6,7,8-H signals.

meso-H signals, suggesting the possibility of formation of more than one isomer by the sodium/isoamyl alcohol reduction procedure. The meso signals have been assigned previously[32] on the basis of kinetically selective H/D exchange experiments.[32,38] In both CDCl$_3$ and C$_6$D$_6$ three principal signals of 1:2:1 intensity ratio are observed in the meso-H region (6–9 ppm). Each of these is split into a doublet with nearly equally intense components. The splittings were not collapsed by irradiation in any other region of the spectrum and decreased when observed at 100 MHz. The splitting was largest for γ-H (between the pyrroline rings) in C$_6$D$_6$ solution, being 32 Hz at 360 MHz. The latter changed to 8.8 Hz at 100 MHz. We conclude that our preparation of $H_2(OEiBC)$ contains at least two diastereomers[39] and, very probably, only two. In view of the preparative method and the observed instability of cis-$H_2(OEC)$,[8] it is entirely likely that the two diastereomers are those of overall C_2 and C_s symmetry containing only trans-reduced pyrroline rings.

The remainder of the spectrum of $H_2(OEiBC)$ was assigned (Table II) on the basis of decoupling experiments and chemical shift arguments.[40] These assignments agree with those of Bonnett et al.[32] obtained at 60 MHz. The decoupling experiments also demonstrated the diastereotopic nature of the 5,8 and 6,7 methylene protons by the emergence of two AB quartets at 1.89 and 2.06 ppm ($J \sim 11$ Hz) upon simultaneous irradiation at $\sim$3.7 (5,6,7,8-H) and $\sim$1.0 ppm (5,6,7,8-CH$_3$). A similar result has been observed with $H_2(OEC)$[41] and has been confirmed at 360 MHz. Also included is the spectrum of trans-$H_2(OEC)$ in CDCl$_3$ at 360 MHz (Figure 4, previously presented at 100 MHz[8]) and a spectral assignment (Table II), which confirms those made from spectra recorded at lower frequencies.[32,40,41]

Voltammetry. The redox properties of a wide variety of porphyrins and metalloporphyrins have been established,[42,43] but there have been relatively few investigations of chlorins and their complexes,[43] and none involving isobacteriochlorins. An electrochemical study has been undertaken in order to define the primary redox processes of $H_2(OEiBC)$ and $Zn(OEiBC)$ and to provide comparative redox potentials for corresponding members of the OEP, OEC, and OEiBC series of compounds under closely similar experimental conditions.[44] Typical cyclic voltammograms for oxidation of OEC and OEiBC species in the 0 to +1 V interval are presented in Figure 5, and potentials are collected in Table III. Also included in the table are potentials from earlier cyclic voltammetry (OEP)[45] and potentiometric titration (OEC)[30] studies. Under our conditions, well-defined reductions were not observed for $Zn(OEP)$ and
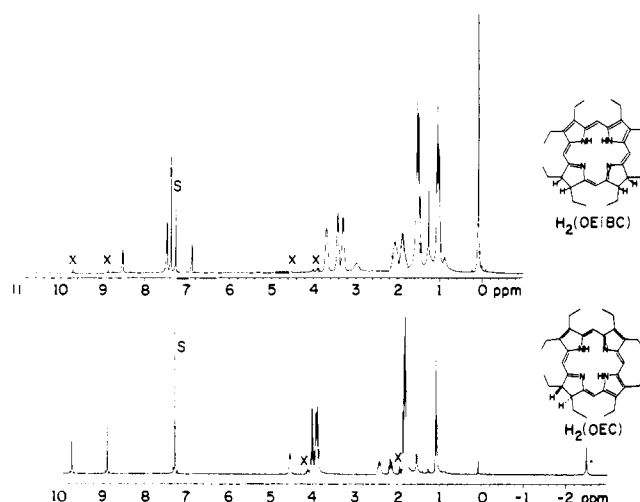
Figure 4. ^{1}H FT NMR spectra (360 MHz) of 10^{-4} M solutions of $H_2(OEiBC)$ (a nonlyophilized sample, upper) and trans-$H_2(OEC)$ (lower) in CDCl$_3$ solution (S = CHCl$_3$). In the upper spectrum, X indicates signals from $\sim$1% $H_2(OEC)$ impurity (other samples have been obtained free of this impurity); signals due to residual benzene (δ 7.4) and petroleum ether (δ 0.95, 1.2) from the purification procedure are also present. In the bottom spectrum, X indicates signals from a <1% $H_2(OEP)$ impurity.

$Zn(OEC)$; the reduction of $Zn(OEiBC)$ appears irreversible with an estimated potential of -1.9 V.

As is typical of many porphyrins and metalloporphyrins,[42,43] $H_2(OEC)$, $Zn(OEC)$, and $Zn(OEiBC)$ undergo two one-electron oxidations to the cation radical and dication. Their first oxidations, particularly if scanned separately, conform more closely to strict reversibility criteria ($\Delta_{p-p} = 59$ mV, $i_{p,a}/i_{p,c} = 1$), as does the first oxidation of $H_2(OEiBC)$. The second oxidation of the free base is irreversible and is described in a following section. The data in Table III establish that the ease of oxidation of the three free bases and their Zn(II) complexes follows series 1, in which successive potential intervals are 0.2–0.3 V. Other results[30,43] generalize the order $M(OEC) > M(OEP)$ for cases in which oxidation is confined

$$OEiBC > OEC > OEP \qquad (1)$$

to the ligand. The trend presumably reflects decreasing negative charge on the ring and increasing metal–ligand covalency, as suggested by Felton.[43] Two regularities in potentials, $\Delta_{ox} = E_{1/2}{}^{ox}(2) - E_{1/2}{}^{ox}(1) = 0.3 \pm 0.1$ V and $\delta = E_{1/2}{}^{ox}(1) - E_{1/2}{}^{red}(1) = 2.25 \pm 0.15$ V, have been observed for many

Table III. Potentials of Free Bases and Zn(II) Complexes in the OEP, OEC, and OEiBC Series

compd	solvent	$E_{1/2}$, V^a ($\Delta_{\text{p-p}}$, mV), for various couples			
		$2^+/1^+$	$1^+/0$	$0/1^-$	$1^-/2^-$
H$_2$(OEP)^g	n-PrCN	+1.40^b	+0.89 (57)	−1.44 (73)	−1.90 (>100)
H$_2$(OEC)^h	n-PrCN	+1.18 (77)	+0.64 (62)	−1.47 (79)	−1.95 (120)
	MeCN	+1.11 (70)	+0.59 (59)	−1.46 (68)	∼−1.9^c
H$_2$(OEiBC)	MeCN	+0.88 (130)	+0.34 (60)	−1.70 (84)	d
Zn(OEP)^i	n-PrCN	+1.03 (52)	+0.65 (54)	d	d
Zn(OEC)^j	MeCN	+0.83 (71)	+0.35 (58)	d	d
Zn(OEiBC)	MeCN	+0.64 (60)	+0.050 (65)	∼−1.9^c	d
H$_3$(OEC)$^+$	CH$_2$Cl$_2$^{f}		+1.12 (36)^e		

^{a} $E_{1/2}$ = 1/2 ($E_{\text{p,a}}$ + $E_{\text{p,c}}$); at 25 °C vs. SCE. ^{b} $E_{\text{p,a}}$ (irreversible). ^{c} $E_{\text{p,c}}$ (irreversible). ^{d} Well-defined reduction waves out to −2.0 V not observed. ^{e} Probable 2e$^-$ process. ^{f} 0.01 M in ClCH$_2$COOH. $^{g-j}$ Lit. data: ^{g} +1.30, +0.81, −1.46, −1.86 V;[45] ^{h} ∼+0.6 V;[30] ^{i} +1.02, +0.63, −1.61 V;[45] ^{j} +0.20 V.[30]
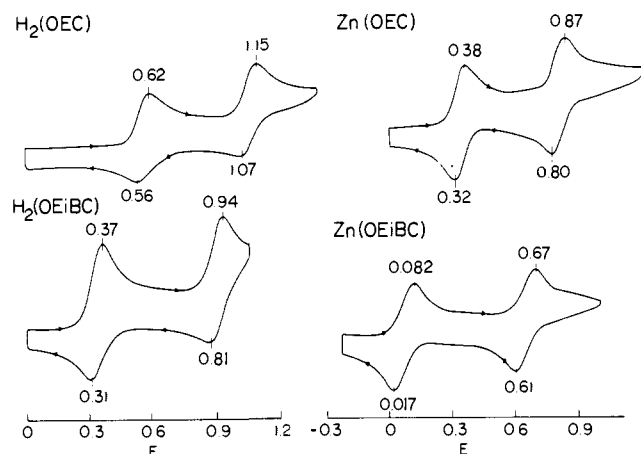
Figure 5. Cyclic voltammograms of 1$^+$/0 and 2$^+$/1$^+$ processes of H$_2$(OEC), Zn(OEC), and Zn(OEiBC) recorded at 100 mV/s and H$_2$(OEiBC) at 200 mV/s, in acetonitrile solution. Peak potentials vs. SCE are indicated.

M(II)(OEP) complexes[42,43,45,46] and serve as criteria for ligand-based oxidation and reduction. Corresponding regularities may reasonably be expected for M(OEC) and M(OEiBC) species but with different mean potential differences. Here we observe that Δ_{ox} and δ for Zn(OEiBC) are 0.59 and ∼1.9 V, respectively, which are outside the proscribed OEP limits, while Δ_{ox} = 0.38 V for Zn(OEC), within the specified limits in the OEP series. Oxidation of Zn(OEiBC) with (ZnTPP)(ClO$_4$)[46] ($E_{1/2}$ ∼ 0.7 V) in dichloromethane afforded the cation radical Zn(OEiBC)$^+$· whose EPR signal (g = 2.003, $\Delta H_{\text{p-p}}$ = 10 G) is devoid of resolved hyperfine structure. Measurements below room temperature revealed diminution of signal intensity reflecting formation of a diamagnetic dimer, a behavior well characterized for M(OEP)$^+$· (M = Mg,[46] Zn[47]). H$_2$(OEiBC)$^+$· was generated by electrolysis in the EPR cavity at room temperature. Its signal (g = 2.002, $\Delta H_{\text{p-p}}$ = 10 G) also exhibited no resolved hyperfine splittings.

Oxidation of OEiBC Species: Spectroelectrochemistry. In order to provide spectral characterization of hitherto unreported isobacteriochlorin cations, the first oxidation products of Zn(OEiBC) and H$_2$(OEiBC) have been examined spectroelectrochemically using the OTTLE cell. This technique has also been employed to identify the product of the second, irreversible oxidation of the free base.

(a) **Zn(OEiBC).** Bulk electrolysis of Zn(OEiBC) in acetonitrile at +0.15 V resulted in a color change from intense red-purple to pale green. Back-electrolysis at −0.15 V with simultaneous coulometry indicated essentially complete reduction of Zn(OEiBC)$^+$· to Zn(OEiBC). The spectrum of the cation radical was obtained by oxidation at +0.25 V in dichloromethane and is shown in Figure 3. It consists of three

features (373 (sh), 389, 413 nm) in the Soret region, which is much reduced in intensity compared with the neutral complex, a broad band at ∼490 nm, and a very weak and diffuse band in the 630–750-nm region. Reduction at −0.25 V resulted in near total recovery of the Zn(OEiBC) spectrum. Several additional oxidation–reduction cycles afforded no spectral degradation, further demonstrating the reversibility of the first Zn(OEiBC) oxidation. The second oxidation, although reversible by cyclic voltammetry, was not well behaved on the longer spectroelectrochemical time scale. Oxidation at +0.75 V gave a rather featureless spectrum with broad bands at 347 and 373 nm. Reduction of this solution at −0.25 V did not yield the Zn(OEiBC) spectrum but instead one without appreciable visible absorption.

(b) **H$_2$(OEiBC).** The first oxidation of H$_2$(OEiBC) was examined in acetonitrile and dichloromethane with similar results. Spectra of the free base and its radical cation in dichloromethane are given in Figure 3. The spectrum of H$_2$(OEiBC) exhibits features at 356 (sh), 370, 375 (sh), 401, 476 (sh), 510, 548, 588, and 636 nm. Electrolysis at +0.45 V for ≤2 min gave a final spectrum with bands at 357, 374, 404, 418 (sh), 492, 588, 642, and 725 nm. Reduction at 0 V, after maintaining the cation radical at +0.45 V for 10 min, gave a 95% recovery of the H$_2$(OEiBC) spectrum, as judged from the 588-nm band intensity. However, the 642-nm feature persisted. Further cycles produced like results except for a gradual increase in intensity at 642 nm. By spectral comparison, the 642-nm species was identified as H$_2$(OEC), which was also found to form, but at a faster rate, in oxidation–reduction cycles carried out in acetonitrile.

Bulk electrolysis in acetonitrile (Pt electrode) at +0.45 V for times appreciably longer (∼30 min) than required for oxidation in the OTTLE cell yielded different results. A series of spectral changes was observed, terminating in formation of a royal blue solution with intense Soret bands at 394 and 406 nm and a prominent visible feature at 619 nm. Cyclic voltammetry of this solution over the range 0 to +2 V revealed processes with $E_{1/2}$ ∼ +1.1 and +1.6 V and no peaks associated with H$_2$(OEiBC) and H$_2$(OEiBC)$^+$· (Figure 5). These processes were also observed in repetitive scans of H$_2$(OEiBC) solutions over the same potential range at scan rates of ≤500 mV/s. The blue species can be produced with greater efficiency by electrolysis at potentials more positive than that (+0.88 V) required for oxidation of H$_2$(OEiBC)$^+$· to H$_2$(OEiBC)$^{2+}$. Thus, oxidation of an H$_2$(OEiBC) solution at +1.1 V for 5 min gave the royal blue solution with the following spectral properties (λ_{max}, A_λ/A_{619}): 394, 6.4; 406, 6.0; 522, 0.27; 619 nm, 1.0.

The blue species was identified as follows. The ^{1}H NMR spectrum of a CDCl$_3$ solution of the residue obtained after evaporation of the blue acetonitrile solution from bulk electrolysis contained two equally intense meso-H signals (9.16, 10.01 ppm), suggestive of a chlorin structure. Related signals

(8.80, 10.05 ppm) were observed upon treatment of $H_2(OEC)$ with trifluoroacetic acid.[32] Chromatography of the blue solution on alumina afforded $H_2(OEC)$, identified spectrally. Cyclic voltammetry of $H_2(OEC)$ in acetonitrile in the presence of increasing amounts of chloroacetic acid resulted in a decrease in peaks associated with the chlorin and its cation radical (Figure 5) and an increase in the features with $E_{1/2} \sim +1.1$ and $+1.6$ V. Treatment of $H_2(OEC)$ in dichloromethane containing 0.05 M $(n\text{-}Bu_4N)(ClO_4)$ (the supporting electrolyte employed in electrolysis experiments) with chloroacetic acid resulted in an immediate blue solution essentially identical with that obtained by high potential electrolysis in the same medium.[48a] These spectra are convincingly similar to the published spectrum of the monoprotonated chlorin, $H_3(OEC)^+$ (λ_{max} 391, 403, 516, 618 nm), but not $H_4(OEC)^{2+}$ (λ_{max} 406, 636 nm), given by Fuhrhop.[30,48b] Separate spectral experiments demonstrated that the blue species is not a protonated form of $H_2(OEP)$ or $H_2(OEiBC)$. These findings collectively demonstrate that the final, stable electrolytic oxidation product of $H_2(OEiBC)$ is $H_3(OEC)^+$.
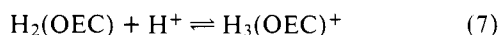
Lastly, in order to ensure that chlorin formation is indicative of the reactivity of isobacteriochlorin unaffected by electrode surface effects or illumination in the spectrophotometer, $H_2(OEiBC)$ was titrated in dichloromethane with the strong oxidant $[(p\text{-}BrC_6H_4)_3N](SbCl_6)$ ($E_{1/2} \sim +1.0$ V),[49] whose reduced form is nonbasic. Titration with 2 equiv of oxidant (reaction 2) gave a solution visually identical with those above. Spectrophotometric examination of the reaction revealed no intermediates and a final spectrum with λ_{max} 394, 406, 522, and 628 nm. The 619→628-nm shift of the low energy band presumably arises from cation–anion interaction.

$$H_2(OEiBC) + 2[(p\text{-}BrC_6H_4)_3N](SbCl_6) \rightarrow H_3(OEC)^+$$
$$+ 2SbCl_6^- + H^+ + (p\text{-}BrC_6H_4)_3N \quad (2)$$
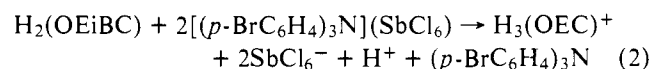
The conversion of isobacteriochlorin to chlorin is represented by the overall reaction 3, which has been found to be electro-

$$H_2(OEiBC) \rightarrow H_3(OEC)^+ + H^+ + 2e^- \quad (3)$$
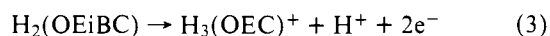
chemically irreversible and to require either 2 C or 2 equiv of a strong oxidant (reaction 2) to proceed to completion. Chlorin can be generated at potentials just sufficient to oxidize the free base to its radical cation. Under these conditions, reactions 4–7 are suggested, leading to net reaction 8. (Reaction 6 might be

$$H_2(OEiBC) \rightarrow H_2(OEiBC)^{+\cdot} + e^- \quad (4)$$

$$2H_2(OEiBC)^{+\cdot} \rightleftharpoons H_2(OEiBC) + H_2(OEiBC)^{2+} \quad (5)$$

$$H_2(OEiBC)^{2+} \rightarrow H_2(OEC) + 2H^+ \quad (6)$$

$$H_2(OEC) + H^+ \rightleftharpoons H_3(OEC)^+ \quad (7)$$

$$H_2(OEiBC)^{+\cdot} \rightarrow H_3(OEC)^+ + H^+ + e^- \quad (8)$$

subject to catalysis by solvent impurities.) A questionable step is the disfavored disproportionation reaction 5 ($K \sim 10^{-9}$) of the initially predominant cation radical. However, if this equilibrium is established rapidly, a fast irreversible reaction of the dication, whose occurrence is indicated by the irreversibility of the process at ca. $+0.9$ V, could displace the equilibrium and provide a reasonable pathway to chlorin. The detection of very small quantities of $H_2(OEC)$ in OTTLE experiments after rapid generation of the radical cation (Figure 3) shows that free chlorin is first formed and not protonated until concentrations are sufficient to displace reaction 7 to the right. At potentials sufficient to generate $H_2(OEiBC)^{2+}$ ($\gtrsim +0.9$ V), chlorin formation is greatly accelerated and reaction 5 need not be invoked; reactions 6 and 7 suffice. Redox processes at $+1.1$ and $+1.6$ V are associated with $H_3(OEC)^+$, indicating that protonation of chlorin causes an anodic potential shift of ca. 0.5 V; these processes have not been further studied.

Summary

This research provides a detailed synthesis and purification of $H_2(OEiBC)$ which, from 360-MHz ^{1}H NMR evidence, is obtained most probably as a mixture of two nearly equally abundant isomers with trans-reduced pyrroline rings. By absorption and fluorescence emission spectral criteria, $H_2(OEiBC)$ serves as an entirely acceptable model for the metal-free prosthetic group, sirohydrochlorin, of sulfite and nitrite reductases. $H_2(OEiBC)$ has proven to be stable in subdued light in the absence of dioxygen. When subjected to nonbasic oxidizing conditions, as in acetonitrile or dichloromethane, $H_2(OEiBC)$ undergoes eventual oxidative dehydrogenation to the protonated chlorin $H_3(OEC)^+$. Of the various physicochemical properties investigated, the marked shifts to less positive potentials of the two primary oxidation processes of $H_2(OEiBC)$ and $Zn(OEiBC)$, compared with the corresponding chlorins and porphyrins (Table III), are the most striking. In ongoing work, $Fe(OEiBC)$, $Fe(OEC)$, and $Fe(OEP)$ species are currently being examined in order to ascertain whether macrocyclic structural differences affect the potentials of metal-centered redox processes and the ability to bind, and mediate the reduction of, physiological substrates (sulfite, nitrite) of siroheme-containing enzymes.

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(48) (a) λ_{max} (A_λ/A_{619}): 394 (6.9), 406 (6.3), 522 (0.28), 619 nm (1.0). (b) This spectrum (in chloroform containing HCl/methanol) differs somewhat in absorbance ratios from those obtained in this work in different media. λ_{max} (A_λ/A_{618}): 391 (5.0), 403 (4.0), 516 (0.20), 618 (1.0).[30]

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Communications to the Editor

Lifetime of Chlorophyll *a* Radical Cation in Water-Containing Acetonitrile

Sir:

Recently, in vitro properties of chlorophylls (Chl's) have drawn the attention of many workers in relation to modeling of photosynthetic primary processes as well as to construction of artificial solar conversion systems.[1-6] One of the most important, yet the least understood, processes in plant photosynthesis is the oxidation of water taking place within the photosystem II (PS II).[7] Here the primary oxidant, capable of initiating a chain of enzymic reactions leading finally to water splitting, is generally supposed to be a photooxidized reaction center ($P_{680}^+\cdot$) consisting of Chl *a*. Such an apparent complexity of PS II processes suggests an inefficiency of a reaction between *monomeric* Chl $a^+\cdot$ and H_2O *without assistance* by an enzyme. However, to our knowledge, no experimental verification for this presumption has been reported to date. In the present work we measured the lifetime of electrochemically generated Chl $a^+\cdot$ in water-containing acetonitrile. The experimental results enabled an estimation of the rate constant for a possible reaction Chl $a^+\cdot$ + H_2O.

Chl *a* was isolated from fresh spinach and purified chromatographically.[8] Acetonitrile (AN), which is sufficiently inert to most organic radical cations, was used as the solvent after dehydration and distillation treatments.[9] An optically transparent thin-layer electrochemical cell of conventional design[10,11] was fabricated by sandwiching a Pt grid working electrode (80 mesh, 20 × 10 mm, 0.15 mm thick, optical transmittance 55%) with a pair of Pyrex slide glasses (25 × 40 × 1 mm) using a Teflon spacer 0.16 mm thick. The potential of the working electrode was controlled potentiostatically against an Ag layer (~20 mm²) vacuum deposited onto the inner face of a slide glass of the thin-layer cell. About 5 mL of Chl *a* solution (10^{-4}–10^{-3} M) was poured into a glass cup in which a Pt wire counterelectrode was immersed. The meniscus of the solution rose well above the Pt grid part when the thin-layer cell was set vertically and its lower end was dipped into the solution in the cup. The whole assembly was placed in the sample compartment of a Shimadzu spectrophotometer Model MPS-5000, and one could measure the spectral changes of the Chl *a* solution during and after electrolysis. The Karl Fischer titration was used to determine [H_2O] in AN, and all the measurements were conducted at room temperature (20 ± 3 °C).
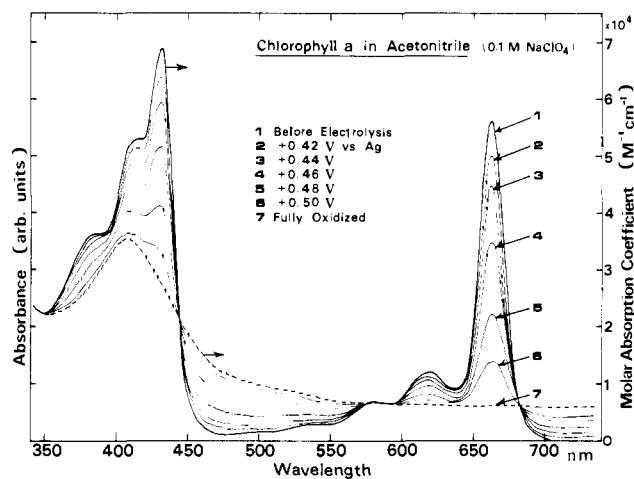

Figure 1. Change of the absorption spectrum of Chl *a* (original concentration, 5 × 10^{-4} M) in the course of electrochemical oxidation in AN. The supporting electrolyte is 0.1 M $NaClO_4$. The values of molar absorption coefficient (right ordinate) for Chl *a* and Chl $a^+\cdot$ in AN were calculated referring to the value ϵ_{662} = 8.63 × 10^4 M^{-1} cm^{-1} for Chl *a* in ether.[13] The electrooxidation of water does not proceed at potentials below +1.0 V vs. Ag.

Figure 1 shows the spectral changes of a Chl *a* solution during potential-controlled electrolysis ([H_2O] = 0.04 M). With rising anodic potential, the main peaks (432 and 663 nm) tended to decrease, while the absorbance in the 445–580- and >683-nm regions showed an increase, with isobestic points at 340–350, 445, 580–595, and 683 nm. This spectral change was reversible with respect to the electrode potential. At potentials higher than +0.50 V vs. Ag, the spectrum in the range of 650–730 nm became more and more flat against the wavelength.[12] Thus we assumed a flat absorbance for the oxidized Chl *a* in the above range and calculated the concentration ratio of oxidized to unoxidized Chl *a* at each potential, based on the peak absorbance (663 nm). The slope of the Nernstian plot[11] for this oxidation reaction was ~51–52 mV per decade change in the concentration ratio, being close to the theoretical one-electron value of 58 mV. This shows that the electrochemical process involved is a one-electron oxidation leading hence to the radical cation, Chl $a^+\cdot$. The broken curve in Figure 1 corresponds to the absorption spectrum of Chl $a^+\cdot$ thus determined in this particular solvent.

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