BIOORGANIC CHEMISTRY 7, 409–420 (1978)

Synthesis and Properties of Chlorophyll-Derived Nitroxide Spin Labels[1]

G. BRUCE BIRRELL, TERRY D. LEE, O. HAYES GRIFFITH, AND JOHN F. W. KEANA

Department of Chemistry and Institute of Molecular Biology, University of Oregon, Eugene, Oregon 97403

Received February 8, 1978

A general synthesis of chlorophyll-derived spin labels is reported. The starting point is chlorophyll *a* and a long chain spin-labeled alcohol. The four-step synthetic route yields new spin labels in quantities practical for membrane spin-labeling studies. Two examples prepared are 12′-proxyltridecyl pyropheophorbide *a* and 12′-proxylhexadecyl pyropheophorbide *a*. The spin labels were purified and characterized by thin-layer chromatography, high-pressure liquid chromatography, mass spectroscopy, nmr and visible spectroscopy, and the number of unpaired spins per molecule. ESR spectral parameters are reported. The chlorophyll-derived spin labels intercalate into fluid phospholipid bilayers and are observed in both the bilayer phase and bound to membrane proteins in chromatophores of the purple photosynthetic bacterium, *Rhodopseudomonas sphaeroides*.

INTRODUCTION

The essential light-absorbing molecules of photosynthesis are the chlorophylls. Chlorophyll *a* is ubiquitous in plants and algae, and the closely related bacteriochlorophyll *a* or *b* is common to photosynthetic bacteria. These specialized lipid pigments are evidently associated with integral membrane proteins (*1*, *2*), although very little is known about the nature of the chlorophyll–protein interactions. Lipid–protein interactions in animal and bacterial membranes have been widely studied using phospholipid, fatty acid, and steroid spin labels (*3*, *4*). The spin labels are sensitive to molecular motion and orientation of the lipid chains in the bilayer and in contact with membrane proteins. One approach to the study of chlorophyll–protein interactions in photosynthetic membranes is to use spin labels derived from chlorophyll. We describe here a synthetic route to chlorophyll spin labels with the label at arbitrary positions along the hydrophobic lipid tail. The synthesis, purification, and characterization of two examples are reported along with preliminary ESR experiments on their behavior in lipid bilayers and photosynthetic membranes of *Rhodopseudomonas sphaeroides*.

RESULTS AND DISCUSSION

Synthesis of the chlorophyll-derived nitroxide spin labels. A reasonable approach to a nitroxide spin-labeled derivative of chlorophyll is to replace the phytyl alcohol grouping by a nitroxide alcohol grouping of similar size. The chlorophyll *a* molecule

[1] We join with the community of scientists worldwide to honor Bill Johnson on this, the occasion of his 65th birthday. JFWK, in particular, expresses his deep appreciation to Bill for the privilege of working with him and learning from him.

0045-2068/78/0074-0409$02.00/0

itself, however, is a somewhat labile molecule, largely owing to the presence of the magnesium in the center of the porphyrin ring and the carbomethoxy group attached to ring V (*5*). We therefore elected to attach the requisite nitroxide alcohol to pyropheophorbide *a*. This latter substance can be readily prepared from chlorophyll *a* using well-established procedures. The synthetic route to pyropheophorbide *a* from chlorophyll *a* via pheophytin *a* and pyropheophytin *a* is shown in Fig. 1.

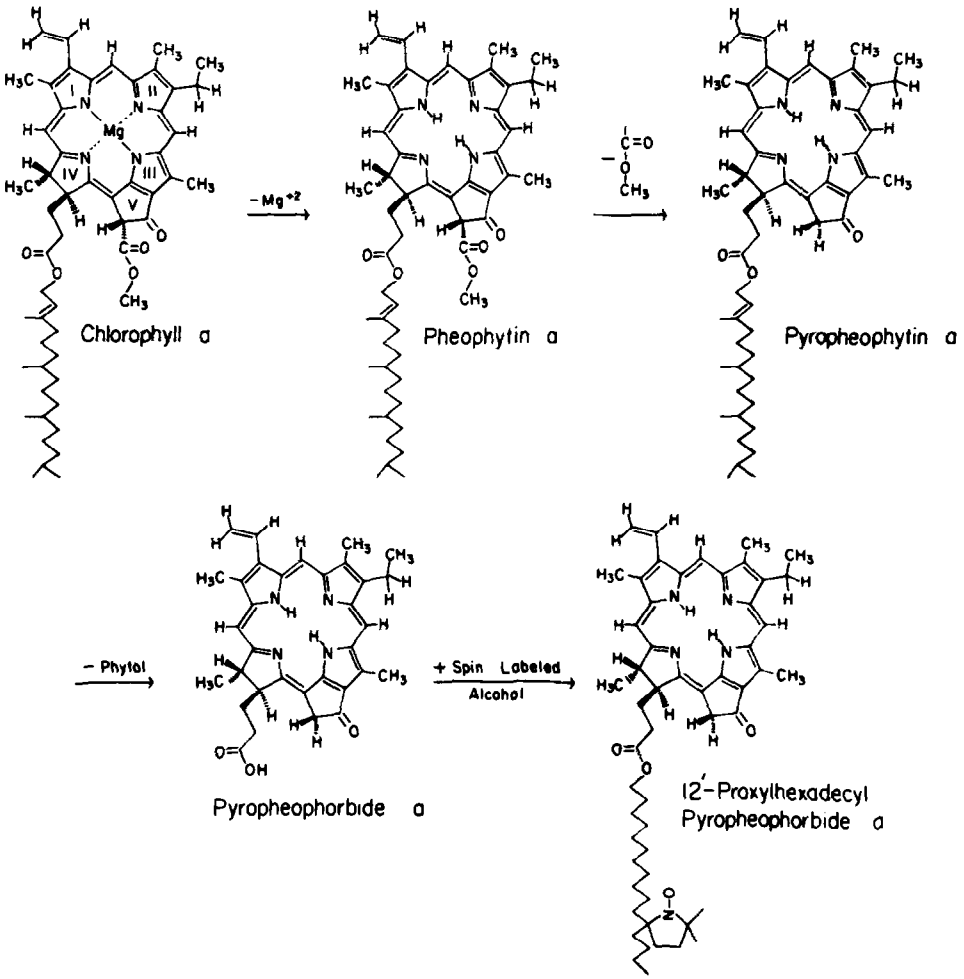


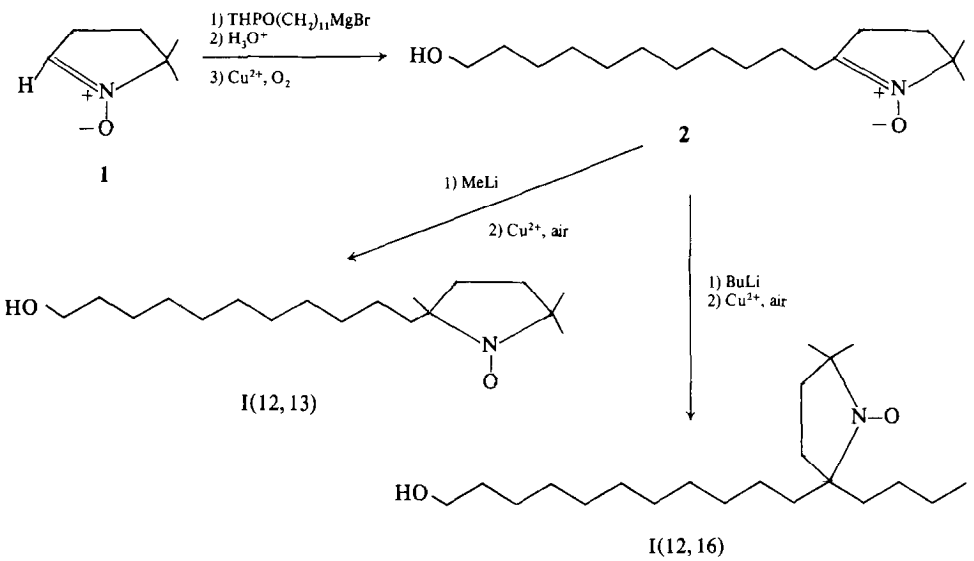
FIG. 1. Reaction sequence in the synthesis of the chlorophyll-derived spin labels. Shown here is the synthesis of 12′-proxylhexadecyl pyropheophorbide *a* II(12, 16).

The synthetic route to proxyl[2] nitroxide alcohols I(12, 13) and I(12, 16) used in this study parallels that introduced by Keana *et al.* (*6*, *7*) and is shown in Chart I. Thus, reaction of nitrone *1* with the Grignard reagent derived from 11-bromoundecanol tetrahydropyranyl (THP) ether followed by acid hydrolysis of the THP ether grouping

[2] Abbreviations used: proxyl, 5,5-dimethylpyrrolidine-*N*-oxyl; I(12, 13), 12-proxyltridecanol; I(12, 16), 12-proxylhexadecanol; II(12, 13), 12′-proxyltridecyl pyropheophorbide *a*; II(12, 16), 12′-proxylhexadecyl pyropheophorbide *a*; PC, phosphatidylcholine; BChl*a*, bacteriochlorophyll *a*.

and cupric-ion-catalyzed air oxidation of the resulting intermediate gave crystalline nitrone alcohol **2** in 79% yield. Reaction of **2** with methyllithium followed by oxidation of the intermediate after workup gave 12-proxyltridecanol I(12, 13). Alternatively, reaction of nitrone **2** with butyllithium in hexane led, after workup and oxidation, to 12-proxylhexadecanol I(12, 16).

CHART I



Next, the carboxyl group of pyropheophorbide *a* was activated by reaction with carbonyldiimidazole[3] and then allowed to react with nitroxide alcohol I(12, 13), affording 12′-proxyltridecyl pyropheophorbide *a* II(12, 13). The corresponding 16-carbon chain compound, 12′-proxylhexadecyl pyropheophorbide *a* II(12, 16) was prepared similarly using alcohol I(12, 16) (Fig. 1).

Characterization of the spin labels. The characterization of the final product rests on information from the following six sources: (1) the known coupling of alcohols to carboxyl groups via the mild carbonyldiimidazole reaction (*8*, *9*). (2) chromatography. By thin-layer chromatography with ether as the eluting solvent, the product migrates as a single spot with an R_f similar to the known compound, pyropheophytin *a*. The R_f value for the product is very different from either of the starting materials. Also, after final purification by high-pressure liquid chromatography the product was reinjected and shown to be a single component. (3) Field desorption mass spectroscopy. The field desorption mass spectrum of II(12, 13) showed the expected intense molecular ion at *m/e* 814. (4) The correspondence of the proton nmr spectra with the known spectra of pyropheophytin *a* and pyropheophorbide *a* (*10*). The 100-MHz nmr spectra of pyropheophytin *a*, pyropheophorbide *a*, and II(12, 16) are shown in Fig. 2. The spectra are quite complex and are dominated by the resonances from protons associated with the porphyrin ring. As expected, the methylene resonances of II(12, 16) are broadened

[3] Recently Boxer and Closs (*8*) have also reported the activation of pyropheophorbide *a* by carbonyldiimidazole.

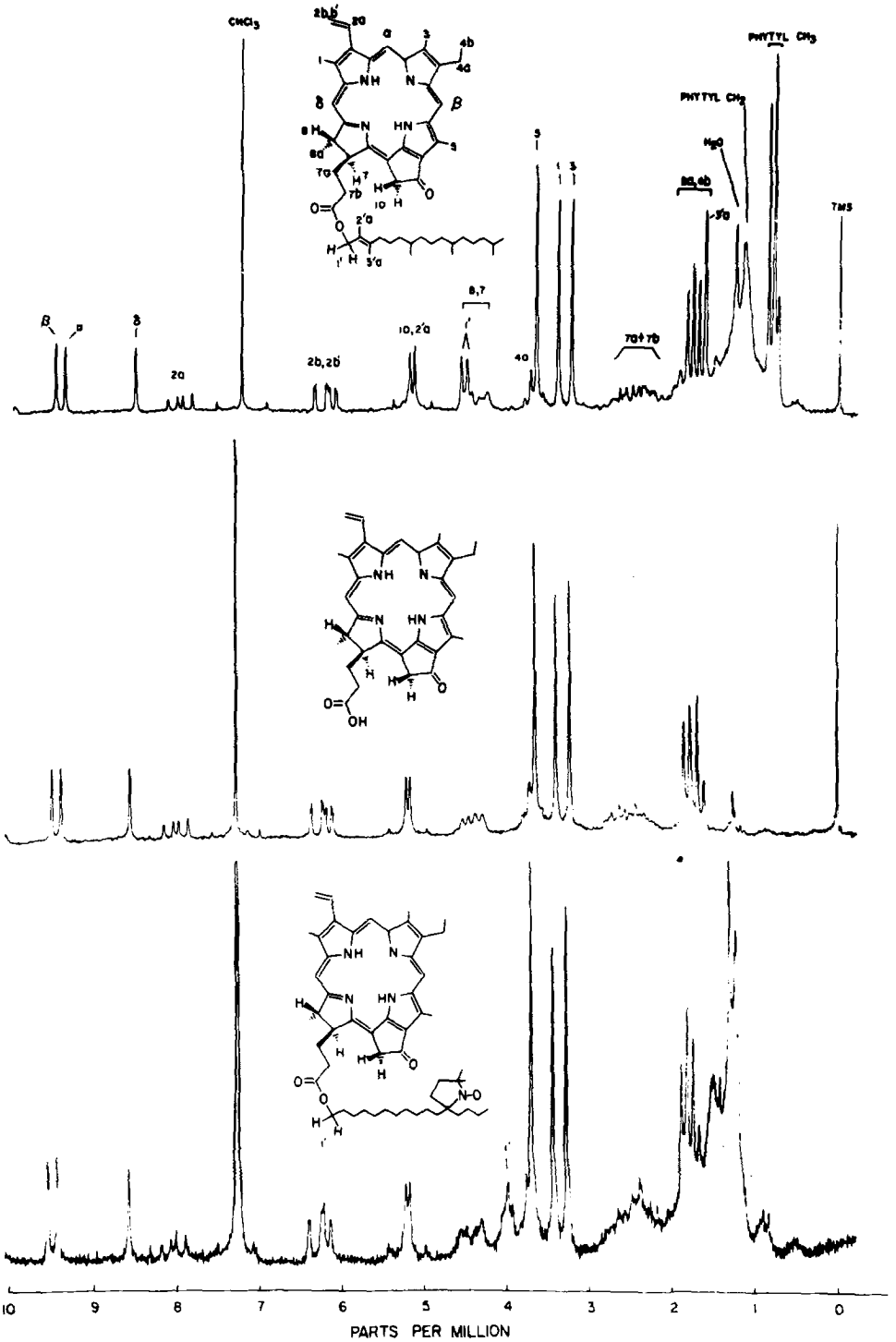


FIG. 2. The 100-MHz Fourier transform nmr spectra of pyropheophytin *a* (top), pyropheophorbide *a* (middle), and II(12, 16) (bottom) in $CDCl_3$. Spectral assignments for pyropheophytin *a* are those of Pennington *et al.* (*10*).

considerably by their proximity to the unpaired electron of the nitroxide moiety. Comparison of the spectrum of II(12, 16) with the others in the figure confirms that the porphyrin ring is still intact after the esterification reaction. The appearance of the 1'-methylene protons of the proxyl alcohol moiety at 4.0 ppm rather than 3.5 ppm (free alcohol) indicates that the alcohol has been esterified. (5) Visible spectroscopy. The maximum in the visible absorption spectrum of spin labels II(12, 13) and II(12, 16) is the same as pyropheophytin *a* (667 nm). The extinction coefficient of II(12, 13) is 47,500 which is nearly the same as pyropheophytin *a* [49,000 (*10*)]. The extinction coefficient of II(12, 16) is somewhat lower (39,000), suggesting that this spin label is of somewhat lower purity. (6) ESR data. Double integration of the ESR spectra indicates that one spin-labeled alcohol has been coupled to the pyropheophorbide *a* in the final product II(12, 13). While the purity of the corresponding II(12, 16) is somewhat lower, the ESR data are still consistent with the presence of one spin-labeled lipid chain in the

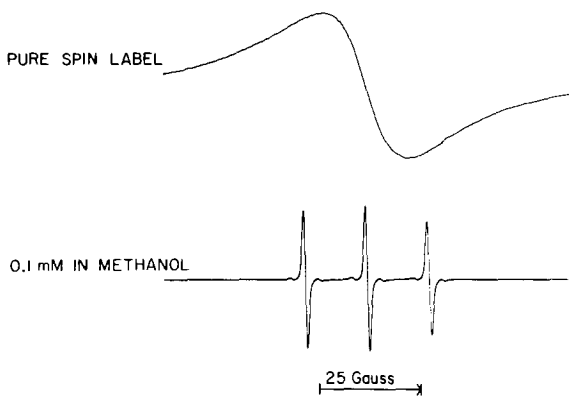


FIG. 3. The 25°C ESR spectra of neat II(12, 16) and this spin label dissolved in deoxygenated methanol (10^{-4} *M*).

final product. Regarding stability, there were no signs of signal loss during the ESR experiments. The labels were stable in the pure state indefinitely at −10°C.

ESR spectral parameters. The ESR spectrum of the undiluted spin label II(12, 16) is shown in Fig. 3. It consists of a single broad line and results from spin exchange between adjacent spin labels. The single line is evidence that the label is present in high concentrations and not largely destroyed or diluted by unlabeled compounds as sometimes happens in syntheses. On dilution to 10^{-4} *M* in deoxygenated methanol, the spin exchange effects disappear, leaving the sharp three-line (bottom) spectrum shown in Fig. 3. The spectrum results from the rapid tumbling of the label in a nonviscous solvent and can be characterized by the two solvent-dependent parameters, A_0 and g_0. A_0 is the distance (in gauss) between two adjacent spectral lines, and g_0 is determined as the center of the spectrum relative to a standard. The ESR spectral parameters of the two chlorophyll spin labels and the corresponding alcohols are given in Table 1. The g_0 values of all four spin labels are identical, and the A_0 value of each chlorophyll spin label is the same as that of the corresponding alcohol within experimental error. There is a small difference in A_0 values between the two pairs of spin labels which is not unexpected.

Spin-labeled vesicles and photosynthetic membranes. To test the behavior of the chlorophyll labels in phospholipid bilayers, aqueous dispersions of egg phosphatidylcholine (PC) were prepared containing the labels. Typical spectra at 25°C are shown in Fig. 4. These spectra are characteristic of isolated spins in a viscous fluid and indicate that there is no phase separation or clustering of the chlorophyll spin labels in lipid bilayers under these conditions. The ESR lines of II(12, 13) are narrower than those of II(12, 16) reflecting a greater degree of molecular motion. At low temperatures the ESR spectra of both spin labels approach the rigid glass limit (bottom spectrum, Fig. 4) and are very nearly superimposable.

The molecular motion can be characterized in terms of an order parameter, S. Values of S vary from 0 (rapid isotropic motion) to 1 (essentially no segmental motion of the

TABLE 1

ESR SPECTRAL PARAMETERS[a,b]

Spin label	A_0	g_0
I(12,13)	15.25	2.0057
I(12,16)	15.05	2.0057
II(12,13)	15.25	2.0057
II(12,16)	15.08	2.0057

[a] Spectra were recorded at 25°C for 10^{-4} M solutions in deoxygenated methanol.

[b] A_0 and g_0 values are relative to di-t-butyl nitroxide in water [$A_0 = 17.16$ G; $g_0 = 2{\cdot}0056$ (*25*)]. The A_0 and g_0 values are believed accurate to ± 0.1 G and ± 0.0001, respectively.

lipid chains) and are determined from the inner and outermost extrema of the ESR spectra (*11*). For the spin label II(12, 16) in egg phosphatidylcholine vesicles at 25°C, $S = 0.12$, and for the shorter chain spin label, II(12, 13), $S < 0.1$. It is interesting to note that the presence of the tetrapyrrole head group has very little influence on the segmental motion of the lipid chains as detected by spin labels. For both spin labels II(12, 13) and II(12, 16) the ESR spectra in egg phosphatidylcholine vesicles at 25°C are essentially superimposable with spectra of the corresponding spin-labeled long chain alcohols, I(12, 13) and I(12, 16), respectively.

The usefulness of these chlorophyll spin labels will lie in examining chlorophyll–protein interactions in photosynthetic membranes. We conclude with a preliminary experiment that tests whether the chlorophyll spin label reported does, in fact, bind to proteins in photosynthetic membranes (the question of specific binding is beyond the scope of the present paper).[4] The chlorophyll labels are essentially insoluble in water, and therefore it is troublesome to add these labels to biological preparations by simple diffusion. We have instead first formed vesicles of egg PC containing II(12, 16) at a relatively high spin label to PC ratio (1:20) and then fused these vesicles with photosynthetic membranes. For this experiment chromatophores from the nonsulfur

[4] It is quite possible, even likely, that the spin labels bind to nonspecific sites. This question can be addressed later by examining the relative binding of phospholipid and chlorophyll spin labels.

purple bacterium, *Rhodopseudomonas sphaeroides* strain Ga were used. These membrane vesicles contain bacteriochlorophyll *a* (BChl*a*), BChl*a* binding proteins, and a number of other proteins not associated with photopigments (*12*). The structure of BChl*a* is closely related to chlorophyll *a*.[5] The ESR spectra before and after fusion are shown in Fig. 5. Some of the spin label is clearly in fluid bilayer in the

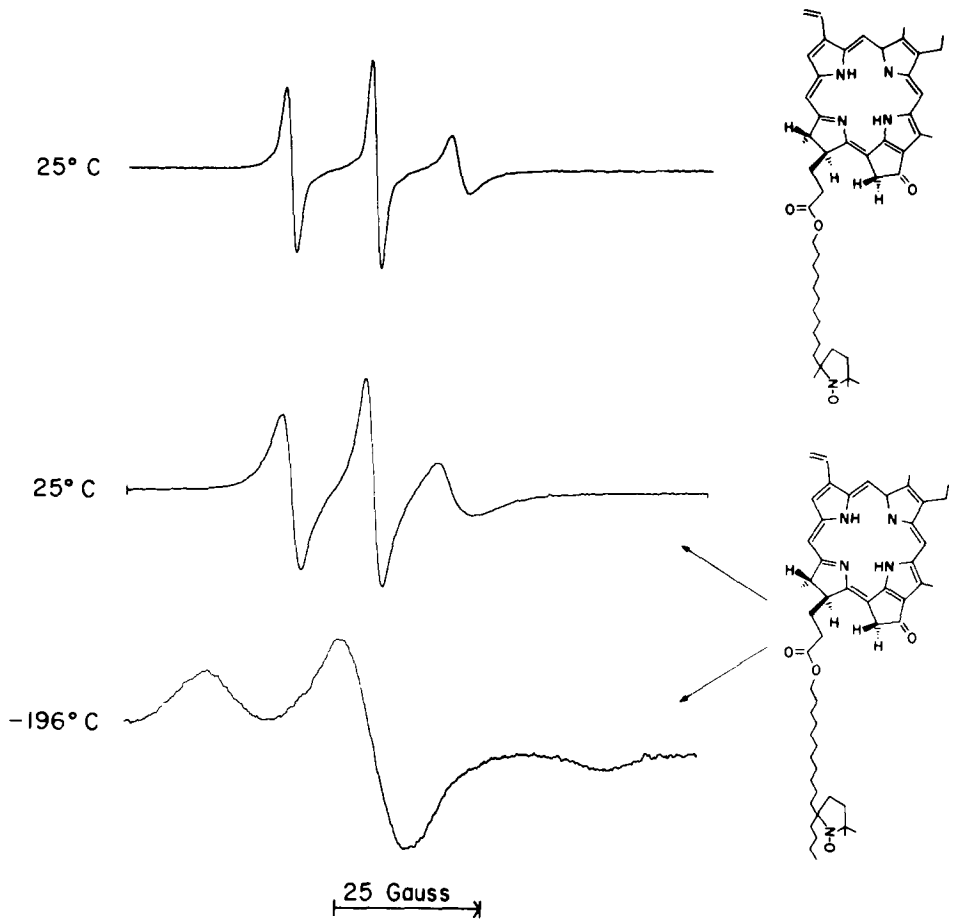


FIG. 4. ESR spectra of II(12, 13) and II(12, 16) in sonicated vesicles of egg phosphatidylcholine (PC) at a spin label : PC molar ratio of 1 : 160.

chromatophores. In addition, however, there is an immobilized component as marked by the arrow in Fig. 5. Similar results were obtained when pure spin label was introduced in a small amount of methanol instead of by fusion. We conclude that a significant number of the chlorophyll spin labels are immobilized on the hydrophobic surfaces of proteins in the chromatophore membranes.

[5] The structure of BChl*a* differs from chlorophyll *a* in that ring II is reduced (additional hydrogen atoms at the 3 and 4 positions) and an acetyl group replaces the ethylene at the 2 position in ring I.

There are a number of aspects of the structure and dynamics of photosynthetic membranes that are unknown. These include head group specificity, orientation effects, proximity of binding sites, and molecular motion of chromophore lipid chains. The synthesis of the chlorophyll-derived spin labels reported here is flexible and can be used to prepare labeled chromophores with varying chain length and head group characteristics. Judging from the stability and behavior in phospholipid vesicles and chromatophores, the new spin labels should be useful in exploring a number of these questions.

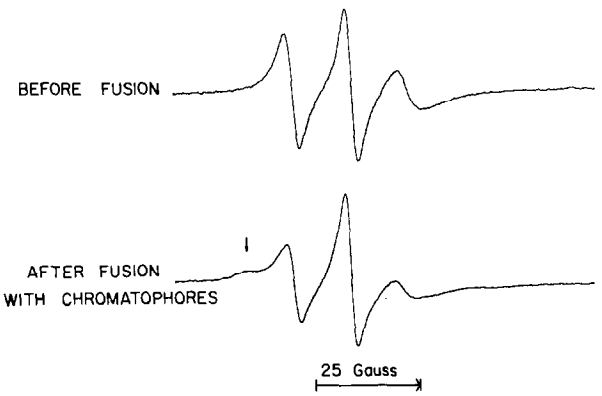


FIG. 5. Top spectrum: ESR spectrum of II(12,16) in egg PC vesicles at a spin label:PC molar ratio of 1:20. The bottom spectrum was obtained after fusion of these vesicles with chromatophores from *Rhodopseudomonas sphaeroides* strain Ga. The arrow indicates the immobilized component which is obtained after fusion. Both spectra were recorded at 25°C.

EXPERIMENTAL

The nmr spectra were recorded on a Varian XL-100 high-resolution spectrometer in $CDCl_3$, and only characteristic peaks are reported. Chemical shifts are reported in parts per million (δ) downfield from internal Me_4Si. Mass spectra (70 eV) *m/e* are given followed by the relative peak height in parentheses and were determined on a CEC 110-2B double-focusing mass spectrometer equipped with a direct-inlet. Elemental analyses were performed at the University of Oregon by Dr. R. Wielesek. Ultraviolet spectra were determined on a Cary 16 uv spectrophotometer. Solvents were routinely distilled. Commercial reagents were used as received. The 9.5-GHz ESR spectra were recorded on a Varian E-line ESR spectrometer, interfaced with a Varian 620L/100 computer. The number of spins per molecule was determined for the chlorophyll labels by comparing double integrated ESR spectra of the spin labels (10^{-4} *M* in ethanol) with doubly integrated spectra of analytically pure 2,2,6,6-tetramethyl-4-piperidinol-1-oxyl, also 10^{-4} *M* in ethanol. The field desorption mass spectrum of II(12,13) was determined by Dr. Douglas Barofsky, Oregon Graduate Center, Beaverton, Oregon.

Tetrahydropyranyl ether of 11-bromoundecanol. 11-Bromoundecanol (9.01 g, 35.9 mmol) and 2,3-dihydropyran (4.53 g, 53.9 mmol) were combined (N_2) in CH_2Cl_2 (60 ml) containing 5 mg of *p*-toluenesulfonic acid monohydrate. The solution was stirred at 21°C for 1 hr, then washed with saturated aqueous $NaHCO_3$ and brine, and dried over K_2CO_3. The solvent was evaporated, and the residue was distilled to give the

tetrahydropyranyl ether (11.7 g, 97%); bp 150–155° (0.05 mm); nmr δ 3.3–4.0 (6H, m, CH_2–O), 4.7 (1H, m, O–CH–O).

3,4-Dihydro-2,2-dimethyl-5-(11'-hydroxyundecyl)-2H-pyrrole-1-oxide **2**. To a stirred solution of 3.11 g (27.5 mmol) of nitrone **1** (*13*) in 35 ml of THF under N_2 at 0°C was added 34.8 ml of a 1.0 *M* THF solution of the Grignard reagent derived from the THP ether of 11-bromoundecanol. The bath was removed, and stirring was continued for 1 hr. The reaction was quenched with saturated aqueous NH_4Cl and filtered, and the filtrate was concentrated to an oil. This was dissolved in 95% EtOH (50 ml), acidified with conc. HCl (3.5 ml), and heated at 75°C for 30 min. The cooled solution was poured into water (100 ml) containing NaOH (2.0 g), extracted with $CHCl_3$, and dried over K_2CO_3, and then the solvent was evaporated. A solution of concentrated aqueous NH_4OH (5.0 ml) and $Cu(OAc)_2 \cdot H_2O$ (400 mg) in 95% EtOH (50 ml) was added to the crude mixture. The intensely blue solution immediately lost its color. Oxygen was bubbled slowly through the solution for 3 min, during which time the color was restored. Water was added, and the solution was extracted with chloroform. The extract was dried over K_2CO_3, the solvent was evaporated, and the crude product was recrystallized from toluene–hexane to provide nitrone alcohol **2** as colorless plates: mp 73–74.5° (6.19 g, 79%); nmr δ 1.97 (3H, m), 2.24 (1H, bs, exchangeable with D_2O), 2.56 (4H, m), 3.64 (2H, t, $J = 7$ Hz); uv λ_{max} (EtOH) 232–233 nm ($\varepsilon = 9900$). *Anal.* Calcd for $C_{17}H_{33}NO_2$: C, 72.04; H, 11.73; N, 4.94. Found: C, 71.90; H, 11.66; N. 4.54.

12-Proxyltridecanol, I(12,13). To 4 ml of a 2 *M* methyllithium solution in ether was added 850 mg (3.0 mmol) of nitrone **2** in 10 ml of ether under N_2 at a rate sufficient to maintain gentle reflux. Five minutes after the addition was complete, the solution was treated with saturated aqueous NH_4Cl. The ether was decanted and combined with two ether washings of the residue. The solvent was evaporated, and the residue was taken up in CH_3OH and stirred under air with 5 mg of $Cu(OAc)_2 \cdot H_2O$ for 15 min. Evaporation of the solvent followed by column chromatography ($CHCl_3$ elution) and preparative tlc on silica gel (ether) gave 280 mg (31%) of purified nitroxide I(12, 13): single spot by tlc (ether, $R_f = 0.4$); *m/e* 298.273 (7) (calcd for $C_{18}H_{36}NO_2$: 298.275), 284 (6), 268 (5), 128 (100), 112 (30), 69 (10), 55 (13), 41 (11).

12-Pyroxylhexadecanol, I(12,16). To a stirred solution of 1.60 g (5.65 mmol) of nitrone alcohol **2** in 8 ml of hexane at 25°C was added 88.3 ml of a 1.6 *M* hexane solution of *n*-butyllithium. The resulting solution was stirred for 5 min then cooled in an ice bath. Excess reagent was destroyed by cautious addition of 75 ml of H_2O. The organic phase was separated and combined with two 20-ml $CHCl_3$ washes of the aqueous phase. The organic phase was evaporated, and the residue was taken up in 50 ml of 95% ethanol containing 20 mg of $Cu(OAc)_2 \cdot H_2O$ and stirred under air for 1 hr. The solvent was evaporated, and the residue was dissolved in $CHCl_3$, washed with saturated aqueous $NaHCO_3$, and dried over K_2CO_3. After evaporation of the $CHCl_3$, 200 ml of hexane was added, and the mixture was heated to reflux on a steam bath. After a vigorous stir, the mixture was cooled to 4°C, affording crystalline starting nitrone **2**. Crude recovered **2** was treated with 200 ml of fresh boiling hexane (some remains undissolved), and then the mixture was allowed to cool to 4°C, affording 800 mg (50%) of recovered nitrone alcohol **2** suitable for reuse. The combined hexane mother liquors were evaporated, and the residue was chromatographed on silica gel [CCl_4:ethyl acetate (2:1), elutant] to yield 838 mg (43%) of proxyl alcohol I(12, 16) as

a yellow oil: *m/e* 340 (10), 284 (100), 268 (17), 154 (18), 127 (16). *Anal.* Calcd for $C_{21}H_{42}NO_2$: C, 74.06; H, 12.43; N, 4.11. Found: C, 74.00; H, 12.60; N, 3.98.

Pyropheophorbide a. Chlorophyll *a* was extracted from spinach leaves by the method of Strain and Svec (*14*) and was converted into pheophytin *a* by briefly shaking an ether solution of chlorophyll *a* with 6 *N* HCl at 25°C (*15*). The carbomethoxy group on ring V of pheophytin *a* was removed by refluxing a solution of pheophytin *a* in pyridine for 24 hr (*10*). Chlorphyll *a*, pheophytin *a*, and pyropheophytin *a* were in turn purified by sucrose column chromatography (*14*) before proceeding to the next step in the synthesis. The phytyl side chain was removed from pyropheophytin *a* by acid hydrolysis (*16*). The resulting pyropheophorbide *a* was purified by crystallization from hot ethanol. In a typical experiment 650 g (wet weight) of spinach leaves yielded 50 mg of black crystalline pyropheophorbide *a*. The nmr spectrum is shown in Fig. 2 (middle spectrum).

12'-Proxyltridecyl pyropheophorbide a, II(12,13). The procedure followed was that of Staab (*9*). To a solution of 13.6 mg (0.0254 mmol) of pyropheophorbide *a* in 0.2 ml of THF was added 7.9 mg (0.048 mmol) of carbonyldiimidazole. After a 1-hr stir under N_2, a solution of 14.3 mg (0.0479 mmol) of I(12, 13) in 0.1 ml of dry THF was added. Next, a solution of the catalyst was prepared by the reaction of 100 mg of imidazole and 15 mg of sodium metal in 1.0 ml of THF. Ten microliters of this solution were added to the original reaction solution. After an 18-hr stir at 21°C in the dark, ether was added, and the black solution was washed well with water. Evaporation of the solvent and column chromatography on silica gel ($CHCl_3$ elution) gave partially purified 12'-proxyltridecyl pyropheophorbide *a*. Final purification was accomplished using high-pressure liquid chromatography on a 4-ft × 0.25-in. Porosil-a column, eluting with $CHCl_3$:CH_3OH (97:3) to give 13.8 mg (67%) of 12'-proxyltridecyl pyropheophorbide *a* (retention time = 7.5 min), pure by silica gel tlc (ether, $R_f = 0.5$) and HPLC: λ_{max} (ether) = 667 nm; $\varepsilon = 47{,}500\ M^{-1}\ cm^{-1}$; unpaired spins per molecule, 1.0 ± 0.1. By way of comparison, pyropheophytin *a* shows λ_{max} (ether) = 667 nm, $\varepsilon = 49{,}000\ M^{-1}\ cm^{-1}$ (*10*). The field desorption mass spectrum of II(12, 13) showed an intense peak at *m/e* 814 corresponding to the molecular ion.

12'-Proxylhexadecyl pyropheophorbide a, II(12,16). Using the procedure for the preparation of the 12'-proxyltridecyl ester, 12'-proxylhexadecyl pyropheophorbide *a* was prepared from I(12, 16). Silica gel tlc R_f values and HPLC retention times were the same for both derivatives. For II(12, 16): λ_{max} (ether) = 667 nm; $\varepsilon = 39{,}100\ M^{-1}\ cm^{-1}$; unpaired spins per molecule, 0.8 ± 0.1.

Spin-labeled phospholipid vesicle preparation. Egg phosphatidylcholine was isolated from egg yolk by the method of Pangborn (*17*). Spin-labeled vesicles (molar ratio of spin label:PC = 1:160) were prepared by mixing together chloroform solutions of spin label (0.007 mg) and phosphatidylcholine (1 mg), evaporating the solvent under a stream of dry nitrogen gas, and dispersing the lipid spin label mixture by probe sonication in 0.1 ml of 0.1 *M* sodium phosphate (pH 7.6, containing 1 m*M* EDTA and 0.25 *M* sucrose).

Chromatophore preparation. Cells of the photosynthetic bacterium *Rhodopseudomonas sphaeroides* strain Ga (*18*) were grown on photosynthetic medium M22 of Sistrom (*19*) with malic acid replacing lactic acid and supplemented with 0.1% (*w/v*) casamino acids. The cells were grown under a light intensity of 100 fc and were

harvested according to the method of Fraker and Kaplan (*12*). The whole cells were passed through a French pressure cell twice at 20,000 psi; the resulting crude chromatophores were then purified by sucrose density gradient centrifugation (*12*) and dialyzed against 0.1 *M* sodium phosphate (pH 7.6, containing 1 m*M* EDTA and 0.25 *M* sucrose). The purified chromatophores contained 0.33 mg of phospholipid [determined by the method of Lowry and Tinsley (*20*)] per mg of protein [estimated by the method of Lowry *et al.* (*21*)]. Bacteriochlorophyll content [measured by the method of Cohen–Bazire *et al.* (*22*) and using a value of 59 for the millimolar extinction coefficient (*23*)] of the chromatophores was 75 nmol/mg of protein.

Fusion of chromatophores with spin-labeled egg PC vesicles. PC vesicles (molar ratio of spin label:PC = 1:20) prepared using 0.12 mg of egg PC (1.54×10^{-4} mmol) and 0.0066 mg of II(12,16) (7.7×10^{-6} mmol) were formed as described above. The vesicles were mixed (by bath sonication at 0°C for 15 min) with 1 ml of a chromatophore preparation containing 1.9 mg of chromatophore protein. The mixture was placed in a freezer overnight, thawed, and centrifuged through 20% sucrose (190,000*g*, 3 hr) to remove any unfused PC vesicles. The freezing and thawing procedure has previously been observed to promote fusion of membrane vesicles (*24*).

ACKNOWLEDGMENTS

We thank Dr. Douglas Barofsky of Oregon Graduate Center for determining the field desorption mass spectrum of II(12,13). We are pleased to acknowledge the help and useful discussions with Robert Benedict, Richard Roman, Michael Buccino, Edward Bernard, and Dr. William R. Sistrom. This work was supported by Public Health Service Grants GM-25698 and GM-24951.

REFERENCES

1. W. W. Parson, *Ann. Rev. Microbiol.* **28**, 41 (1974).
2. J. P. Thornber, *Annu. Rev. Plant Physiol.* **26**, 127 (1975).
3. O. H. Griffith and P. C. Jost, "Spin Labeling," Chap. 12 (L. J. Berliner, Ed.). Academic Press, New York, 1976.
4. O. H. Griffith and P. C. Jost, "Molecular Specialization and Symmetry in Membrane Function," Chap. 2 (A. K. Solomon and M. Karnovski, Eds.). Harvard University Press, Boston, 1978.
5. G. R. Seely, "The Chlorophylls," Chap. 3 (L. P. Vernon and G. R. Seely, Eds.). Academic Press, New York, 1966.
6. J. F. W. Keana, T. D. Lee, and E. M. Bernard, *J. Amer. Chem. Soc.* **98**, 3052 (1976).
7. T. D. Lee, G. B. Birrell, and J. F. W. Keana, *J. Amer. Chem. Soc.* **100**, 1618 (1978).
8. S. G. Boxer and G. L. Closs, *J. Amer. Chem. Soc.* **98**, 5406 (1976).
9. H. A. Staab, *Angew. Chem. Int. Ed.* **1**, 351 (1962).
10. F. C. Pennington, H. H. Strain, W. A. Svec, and J. J. Katz, *J. Amer. Chem. Soc.* **86**, 1418 (1964).
11. L. J. Berliner (Ed.), "Spin Labeling.' Academic Press, New York, 1976.
12. P. J. Fraker and S. Kaplan, *J. Bacteriol.* **108**, 465 (1971).
13. R. Bonnett, R. F. C. Brown, V. M. Clark, I. O. Sutherland, and A. Todd, *J. Chem. Soc.*, 2094 (1959); commercially available from Aldrich Co.
14. H. H. Strain and W. A. Svec, "The Chlorophylls." Chap. 2 (L. P. Vernon and G. R. Seely, Eds.). Academic Press, New York, 1966.
15. H. J. Perkins and D. W. A. Roberts, *Biochim. Biophys. Acta* **58**, 486 (1962).
16. J. L. Wickliff and S. Aronoff, *Anal. Biochem.* **6**, 39 (1963).

17. M. C. PANGBORN, *J. Biol. Chem.* **137**, 545 (1941); M. C. PANGBORN, *J. Biol. Chem.* **188**, 471 (1951).
18. M. GRIFFITHS AND R. Y. STANIER, *J. Gen. Microbiol.* **14**, 698 (1956).
19. W. R. SISTROM, *J. Bacteriol.* **131**, 526 (1977).
20. R. R. LOWRY AND I. J. TINSLEY, *Lipids* **9**, 491 (1974).
21. O. H. LOWRY, N. G. ROSEBROUGH, A. L. FARR, AND R. J. RANDALL, *J. Biol. Chem.* **193**, 265 (1951).
22. G. COHEN-BAZIRE, W. R. SISTROM, AND R. Y. STANIER, *J. Cell. Comp. Physiol.* **49**, 25 (1957).
23. G. COHEN-BAZIRE AND W. R. SISTROM, "The Chlorophylls," Chap. 10 (L. P. Vernon and G. R. Seely Eds.). Academic Press, New York, 1966.
24. P. C. JOST, K. K. NADAKAVUKAREN, AND O. H. GRIFFITH, *Biochemistry* **16**, 3110 (1977).
25. G. B. BIRRELL, S. P. VAN, AND O. H. GRIFFITH, *J. Amer. Chem. Soc.* **95**, 2451 (1973).