entirely to the optimum affinity of cyclohexaamylose for the activated complex. This is equivalent to saying that, in the ground state, a portion of the free energy gained from association of cyclohexaamylose with **1** is used to impose the orientational restriction, thereby decreasing the stability of the inclusion complex by an amount equal to the rate acceleration.

In conclusion, orientational catalysis by cyclohexaamylose supports the suggestion that binding forces between an enzyme and its substrate can be used to overcome part of the free-energy barrier to activation.[8] The cyclohexaamylose-induced rate acceleration, however, is much smaller than rate accelerations which can be achieved by converting intermolecular to intramolecular reactions.[9] Consequently, when the reacting groups in an intramolecular reaction can assume a mutually favorable orientation without introducing strain elsewhere in the system, the imposition of rigid orientational restrictions apparently leads to only a small additional rate acceleration.

Acknowledgment. Financial support from the National Science Foundation is gratefully acknowledged.

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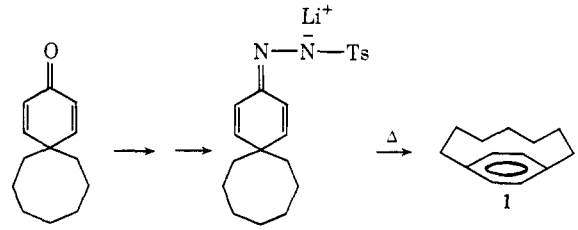
[7]Paracyclophane[1]

Sir:

The smallest known [*m*]paracyclophane[2] is the $m = 8$ isomer, first described over 11 years ago.[3] The synthesis of [8]paracyclophane was an indirect one and not obviously extended to the lower homologs.[4] A more conventional ring contraction route succeeded in providing [8]paracyclophanecarboxylic acid,[5,6] but [7]paracyclophane (**1**) has evaded synthesis for over a decade.[4]

We report here a simple, one-step synthesis of **1** and a few of the properties of this smallest of the known [*m*]paracyclophanes.

Our route was suggested by the observation that 4,4-dimethylcyclohexadienylidene[7] rearranged to *p*-xylene on generation in the gas phase.[8] Accordingly, we synthesized[9] spiro[5.7]trideca-1,4-dien-3-one (**2**) and converted it to the lithium salt of the corresponding tosylhydrazone. Flash pyrolysis of this material at 360–380° (0.1 Torr) gave a material which was resolved by gas chromatography into two peaks in the ratio 1.4/1. The yield was approximately 20%.[11] The first product was a mixture of 1-phenylheptane and 7-phenylheptene-1 (nmr analysis), and the second was the anticipated **1**.



2 1

A precise mass measurement established the formula as $C_{13}H_{18}$ (calcd, 174.14084; found, 174.14078). The nmr spectrum, which closely resembled that of [9]paracyclophane[12] (CCl_4, singlet, τ 2.93, 4 H; triplet, τ 7.36, 4 H, $J = 6.5$ Hz; sym mult, τ 8.5–9.5, 8 H; sym mult, τ 10.3–10.9, 2 H), is consistent only with **1**. Benzocyclononene is eliminated by a comparison of nmr spectra,[13] and one would not expect a singlet for the aromatic protons of [7]metacyclophane.[14] Further, the ultraviolet spectrum reported for [8]metacyclophane (266 nm, log ϵ 2.4)[15] does not compare well with that of **1**.

The ultraviolet spectrum of **1** (EtOH, nm (log ϵ), 216 (4), 245 (4), 283 (3)), does match well with that predicted by Allinger and coworkers,[4,5] 210 (4), 247 (3), 288 (2), and thus the aromatic ring is probably substantially deformed. A precise determination of the amount of bending must await the determination of the structure of **1** or a derivative, however.

Speculation on the mechanism of formation of **1** is premature, but leading possibilities include direct ring migration or carbon–carbon insertion to give a bridged Dewar benzene that subsequently opens to **1**.

(1) Support for this work by the National Science Foundation through Grant GP-30797X and by the donors of the Petroleum Research Fund, administered by the American Chemical Society, is gratefully acknowledged (5528 ACl,4).
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Electron Nuclear Double Resonance of Bacteriochlorophyll Free Radical *in Vitro* and *in Vivo*[1]

Sir:

This is a preliminary account of the first electron nuclear double resonance (endor)[2] studies of bacterio-

(1) Work performed under the auspices of the U. S. Atomic Energy Commission.
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chlorophyll free radicals generated both *in vitro* and *in vivo* in photosynthetic purple bacteria.

The bacteriochlorophyll free radical generated by oxidation *in vitro* has a line width ($\Delta H \simeq 13$ G) significantly different from the free radical associated with photosynthesis *in vivo* ($\Delta H \simeq 9$ G). This discrepancy has been widely observed[3-11] but only rarely discussed.[4-7] In a recent proposal to explain the narrowing of the *in vivo* electron paramagnetic resonance (epr) signal, it has been suggested[4] that the primary photochemical oxidation product of photosynthesis is a special pair of chlorophyll molecules over which the unpaired electron is delocalized. Studies on ^{13}C-labeled algae[5] support this hypothesis, as does the recent work of McElroy, *et al.*[6] Delocalization of the unpaired electron spin over two molecules reduces the spin density at each site by a factor of ~ 2 and thus halves the coupling constants, *i.e.*, effectively narrows the line width of the signal. The endor technique provides a direct test for the "special pair" model.

We have recorded endor signals at $\sim -170°$ for bacteriochlorophyll (ex. *Rhodospirillum rubrum*), *Rhodopseudomonas spheroides* and *Rhodospirillum rubrum* cells, and *Rhodospirillum rubrum* chromatophores with a Varian E-700 endor spectrometer. Monomeric bacteriochlorophyll dissolved in 12% methanol in methylene chloride (v/v) was oxidized by elemental iodine to BChl·+.[12] The free-radical signal of intact *R. rubrum* or chromatophores prepared from this organism[13] was generated by oxidation with $K_3Fe(CN)_6$, a standard technique used to produce an epr signal that is generally agreed to be identical with that associated with the primary act of photosynthesis.[6,14-16]

Figure 1a shows a typical *in vitro* BChl·+ endor spectrum. It has a pronounced peak near 13.7 MHz, the so-called matrix endor signal.[17] The endor spectrum of oxidized *R. rubrum* chromatophores has a stronger signal, but is otherwise essentially identical with that from intact *R. rubrum* and *R. spheroides*. These *in vivo* spectra (Figure 1b) exhibit a weak matrix endor signal. The lack of intensity for the matrix endor signal from *R. rubrum* chromatophores is significant because our experience has been that the matrix endor signal at $-170°$ is commonly the most intense signal observed. We, therefore, conclude that as there appear to be relatively few protons near the unpaired electron, active center bacteriochlorophyll in the intact organism is in an essentially anhydrous environment, with perhaps only a few water molecules present. The delocalization of the unpaired electron over a "special pair" of chlorophyll molecules is also expected to decrease significantly the intensity of the matrix endor signal, and probably both effects are involved in the diminution of the *in vivo* matrix endor signal.

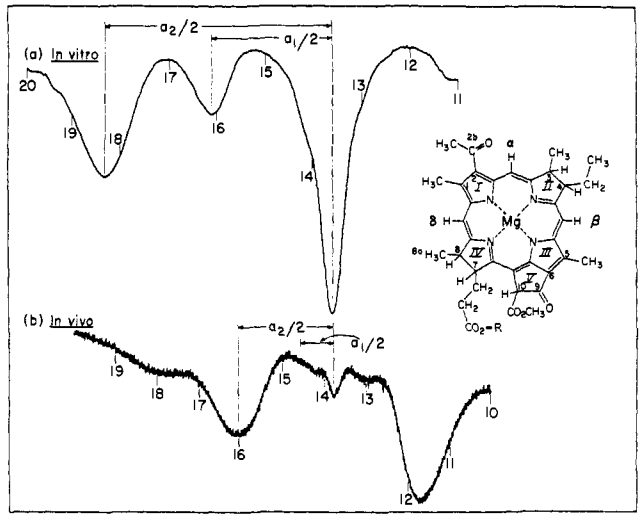

Figure 1. Endor spectra from protons in BChl *in vitro* (2.5 ml of 10^{-3} *M* BChl) and *in situ* at $-170°$. The two peaks, $a_1/2$ and $a_2/2$, from the matrix signal are assigned to two methyl groups. The inset is the BChl molecule, where R the esterified alcohol stands for geranylgeraniol in the case of *R. rubrum* (and phytol in *R. spheroides*). (a) Endor spectrum from BChl oxidized by I_2 (0.75-to 4 mequiv) in CH_2Cl_2 with 12% CH_3OH (v/v). (b) Endor spectrum from *R. rubrum* chromatophores. The BChl has been oxidized *in situ* by a minimal amount of $K_3Fe(CN)_6$. The coupling constants are about $^1/_2$ those in a. The peak assigned to $a_1/2$ is very sensitive to magnetic field strength, a common phenomenon in high power endor studies. Features of spectra from intact cells are virtually identical but the signals are weaker.

Table I. Endor and Esr Parameters Observed for BChl·+ (ex. *R. rubrum*) and *R. rubrum*

	Epr ΔH_{pp}, G	a_1,[a] MHz	a_2,[a] MHz
In vitro BChl·+	13	4.6	9.4
In situ *R. rubrum*	9	1.7 (3.4)[b]	4.3 (8.6)[b]

[a] See Figure 1 for significance of a_1 and a_2. Only isotropic methyl coupling constants are listed. [b] Values in parentheses are twice the observed hyperfine coupling constants.

We observe symmetrical displacement of all endor resonances about the free proton frequency ($\sim$13.7 MHz), indicating such signals arise from protons. We assign the peaks indicated by the coupling constants a_1 and a_2 in Figure 1 to methyl groups. The skewed bell shape of these signals is characteristic for methyl group interactions,[17] and rotating methyl groups are known to be the most readily observed endor signals under the conditions of our experiments.[17] The coupling constants determined by the endor experiments (Table I) show that the *in vivo* coupling constants are slightly less than one-half the coupling constants observed in the *in vitro* system, in accord with predictions from the "special pair" model. The assignments are also consistent with experimental and theoretical work on

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methyl hyperfine coupling constants of dimer cations.[18,19]

As yet, we have not explained the significant intensity in the wings (17–19 MHz) of the *in vivo* spectrum. If a small amount of monomeric BChl·$^+$ were present in the "*in vivo*" systems, it could account for these wings. An alternate interpretation seems more tenable. *R. rubrum* cultured in ^2H_2O with [^{1}H]succinic acid as substrate gives similar endor spectra to those in Figure 1, except that the wings are absent. The bacteriochlorophyll isolated from organisms grown in this way contains no ^{1}H at the methine positions.[20,21] The endor spectra from bacteriochlorophyll of this unusual isotopic composition indicate that only methyl groups give rise to the peaks associated with a_1 and a_2. In addition, [^{2}H]BChl in which the α- and β-methine protons have been exchanged for ^{1}H[22] gives weak endor resonances between 16 and 20 MHz. Furthermore, a weak endor signal can be observed from [^{1}H]BChl itself from 18 to 21 MHz when signal-to-noise is optimal. These observations support the view that the weak broad wings in the endor spectra arise in part from anisotropic methine protons and the intense narrow peaks arise from isotropic methyl groups. The special pair model predicts sharpening of these broad weak peaks such that they would be more intense for *in vivo* systems.

As we have been unable to devise any other simple model that appears to be consistent with both the endor and epr spectra, we conclude that the 9-G epr signal associated with photosynthesis in purple bacteria has a line width consistent with delocalization of the unpaired electron over two active-center bacteriochlorophyll molecules.[23] We have carried out similar experiments on chlorophyll a free radicals in algae and have arrived at essentially similar conclusions.

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Electron Spin Resonance of [^{25}Mg]Chlorophyll a[1]

Sir:

The electron spin resonance (esr) signal from oxidized chlorophyll is widely accepted to originate in a π-cation radical.[2,3] The details of the π cation are still obscure. While it has been assumed that the unpaired electron is fully delocalized over the entire chlorophyll macrocycle as a true π-cation radical, the possibility that spin on ^{25}Mg (10.13% natural abundance)[4] might contribute to the esr spectrum has not been previously investigated. Esr studies of *in vivo* chlorophyll systems[2,5] or some fraction of the photosynthetic apparatus[6–11] have been interpreted in terms of photooxidation of chlorophyll in the photosynthetic reaction center.[12] Whereas the reversible photo-esr signal from green plants,[13] signal I, is characterized by a Gaussian peak, a g value of 2.0025, and a line width of about 7 G, the signals recorded from *in vitro* chlorophyll a monomer systems are approximately 9 G in width.[3] The long-known discrepancy between the signal from Chl a·$^+$[14] and the *in vivo* signal has recently been explained as arising from spin delocalization over two adjacent chlorophyll molecules positioned in a special way.[15]

Analysis of esr spectra of π-electron radicals is facilitated by application of the theory of second moments,[16,17] particularly if applied to radicals of different isotopic composition. Since in our experiments we encounter only Gaussian line shapes, it is proper to apply eq 1 to obtain the second moment, $\langle \Delta H^2 \rangle$, where

$$\langle \Delta H^2 \rangle = {}^1/_4(\Delta H_{pp})^2 \quad (1)$$

ΔH_{pp} is the first derivative, peak-to-peak, absorption line width. Second moment analysis of esr signals from chlorophyll of unusual isotopic composition may then be formulated as follows[15]

$$\langle \Delta H^2 \rangle_{^1H} = 15.91 \langle \Delta H^2 \rangle_{^2H} \quad (2a)$$

$$\langle \Delta H^2 \rangle_{\text{total},\,^1H} = \langle \Delta H^2 \rangle_x + (0.1013)\langle \Delta H^2 \rangle_{^{25}Mg} + \langle \Delta H^2 \rangle_{^1H} \quad (2b)$$

$$\langle \Delta H^2 \rangle_{\text{total}\,^2H} = \langle \Delta H^2 \rangle_x + (0.1013)\langle \Delta H^2 \rangle_{^{25}Mg} + \langle \Delta H^2 \rangle_{^2H} \quad (2c)$$

$$\langle \Delta H^2 \rangle_{\text{total},\,^1H,\,^{25}Mg} = \langle \Delta H^2 \rangle_x + (0.99)\langle \Delta H^2 \rangle_{^{25}Mg} + \langle \Delta H^2 \rangle_{^1H} \quad (2d)$$

where $\langle \Delta H^2 \rangle_x$ is the contribution to the second moment from atoms other than hydrogen and ^{25}Mg, *i.e.*, ^{14}N, ^{13}C, and g anisotropy. The other subscripts identify

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