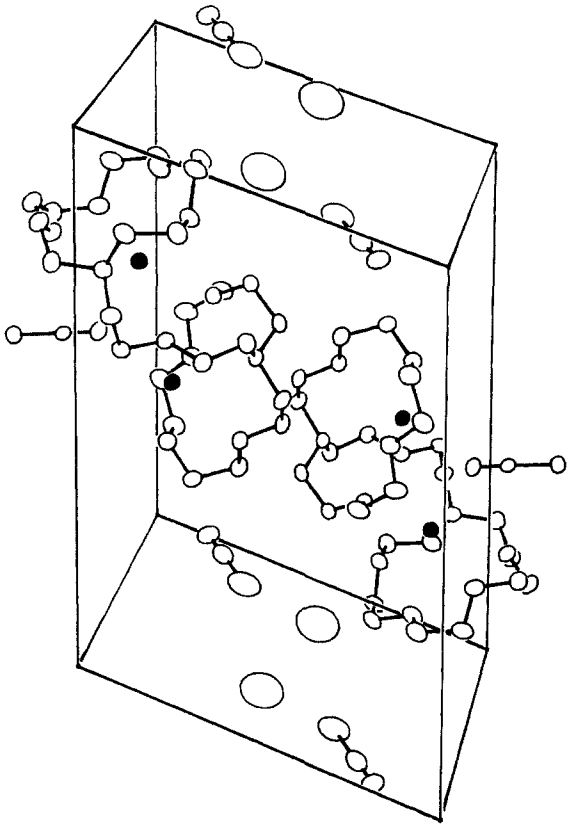

Figure 2. Unit-cell packing diagram for BOA15C5·2KSCN·H_2O (●, potassium cations).

of BOA15C5 encapsulates two K^+ cations instead of one. The two cations are mainly bound by each crown ring, respectively, and are bridged by two oxygens (O4 and O9) and one anionic nitrogen (N3). Another SCN^- anion forms only a hydrogen bond with a water molecule present in the crystal. The anion is not in the coordination sphere of the cations and therefore is not pictured in Figure 1 but can be found in the unit-cell packing diagram (Figure 2). A similar bridge-binding was reported by Truter et al. in the KSCN complex of dibenzo-24-crown-8 (DB24C8-2KSCN),[6] in which two K^+ cations are held in the large crown ring and bridged by two ring oxygens and two anionic nitrogens. However, the complex is much more symmetric than our complex here, and the distance between the two K^+ in DB24C8-2KSCN (3.41 (1) Å) is shorter than that in BOA15C5-2KSCN (3.925 (2) Å).

The arrangement of donor atoms in each crown ring around one K^+ cation is very similar to that in the K^+ complex of N-pivot lariat monoaza-15-crown-5.[4c] The five donor atoms of the macroring are disposed in a half-chair-like arrangement, and each cation resides significantly out of the average plane. The oxygen atom in the bridging chain (O9) coordinates to both cations like a lariat donor atom. However, the coordination sphere for the two cations is not identical. One of the right ring oxygen atoms, O4 in Figure 1, interacts with both cations (K1 and K2), and the donation of the right ring to K1 does not occur with the whole ring but with only one of the ring oxygens. Interestingly the oxygen atom, O4, is even closer to K1 than to K2, and K1–O4 is slightly shorter than K1–O9 although O9 is the first lariat donor atom for K1. Thus, K1 is octacoordinated while K2 is still heptacoordinated. In the complexes of N-pivot lariat monoaza-15-crown-5, it has been reported that the potassium cation can be heptacoordinated or octacoordinated according to the number of the donor atoms in the sidearm.[4c]

The crystallographic result here is in line with the observations in the study of the stability constants and ^{13}C NMR in homogeneous solutions,[2c] i.e., under the conditions of high $[K^+]/[\text{crown unit}]$ most of the complexes may exist as a 2:2 crown unit–cation complex, and the oxygen atom in the bridging chain interacts specifically with the cation held in the cavity. A similar lariat-ether effect was also observed in other bis(crown ether) systems which contain a monoazacrown unit.[7] The structure of the complex also gives the explanation why BOA15C5 can extract most of alkali-metal cations efficiently without the selectivity expected from its ring size.[2c] As pointed out by Gokel et al. for the flexible ligands the guest cation organizes the host's donor group array.[4c]

Acknowledgment. This work was partly supported by a Grant-in-Aid for Scientific Research (no. 61470088) from the Ministry of Education, Science and Culture of Japan. All the measurements and calculations were performed at the Center of Advanced Instrument Analysis, Kyushu University.

Supplementary Material Available: Details of X-ray analyses and tables of distances and angles, positional parameters, and thermal parameters (7 pages). Ordering information is given on any current masthead page.

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Enantioselective Enzymatic Sterol Synthesis by Ultrasonically Stimulated Bakers' Yeast

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Received July 16, 1987

Studies on the in vitro enzymatic formation of lanosterol from 2,3-oxidosqualene (**1** → **2**) have utilized the sedimented microsomes from various mammalian (e.g., rat and hog) liver cells as the typical source of the cyclizing enzyme 2,3-oxidosqualene lanosterol-cyclase.[1-5] However, the use of the microsomal cyclase has severely limited the enzymatic process to only small scale (1–3 mg or less) substrate conversions. Consequently, this enzymatic process has not been used previously for preparative purposes. We now wish to report that an "ultrasonically stimulated" suspension of bakers' yeast (*Saccharomyces cerevisiae*) is an *inexpensive*, *convenient*, and *direct* source of a sterol cyclase[6] which can be

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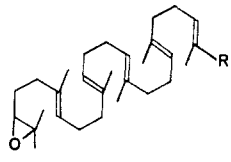
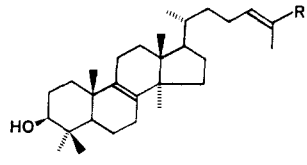
employed for the *gram*-scale enzymatic cyclization of squalene oxide and squalene-like substrates. Additionally, the enzymatic method is completely enantioselective as exemplified by a synthesis of lanosterol (**2**) and the cytotoxic agent, ganoderic acid Z (**4a**).

When 1.00 g of 2,3-oxidosqualene (**1**) was incubated anaerobically at 37 °C for 12 h in the presence of 14 g of Triton X-100[7] with an ultrasonically pretreated[8] suspension of bakers' yeast (100 g, Sigma type II) in 1 L of 0.10 M phosphate buffer (pH 7.4), lanosterol was isolated chromatographically,[9] after filtration and extractive workup with ether, in 41.9% yield (*83.8% conversion* based on one enantiomer of *d,l*-**1**). A control incubation using a boiled yeast preparation afforded 96% of recovered squalene oxide and 3% (0.014 g) of lanosterol. In the absence of substrate **1**, a control afforded 0.008 g of lanosterol which corresponds to the native lanosterol from 100 g of the yeast. The use of a yeast suspension that was not ultrasonically stimulated prior to incubation gave approximately 19% conversion under identical conditions. Also, consonant with the expectations that the cyclase operates on a single enantiomer of racemic **1**, the enzymatically formed lanosterol displayed an $\alpha^{20}_D = +61.8°$ [10b] ($CHCl_3$, c = 1, lit.[10] $\alpha^{20}_D = +62.0$). The recovered (*R*)-squalene oxide (56.5%) displayed an $\alpha^{23}_D = +1.4°$ (MeOH, c 2, lit.[11] $\alpha^{23}_D = +2.0$) which corresponds to approximately a 68% resolution of the racemate.



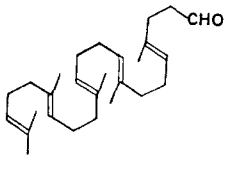
1 R = CH_3



2 R = CH_3

3 R = CO_2CH_3

4a R = CO_2H
b R = CO_2CH_3



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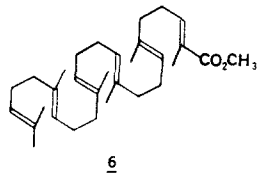

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In order to determine whether lanosterol production was limited by the transformation of **2** to ergosterol via the normal biogenetic pathway, we incubated lanosterol for 24 h by using the ultrasonically treated yeast and recovered 98% of the lanosterol. This result suggests that the synthesized lanostereol is not significantly assaulted by other enzymes.

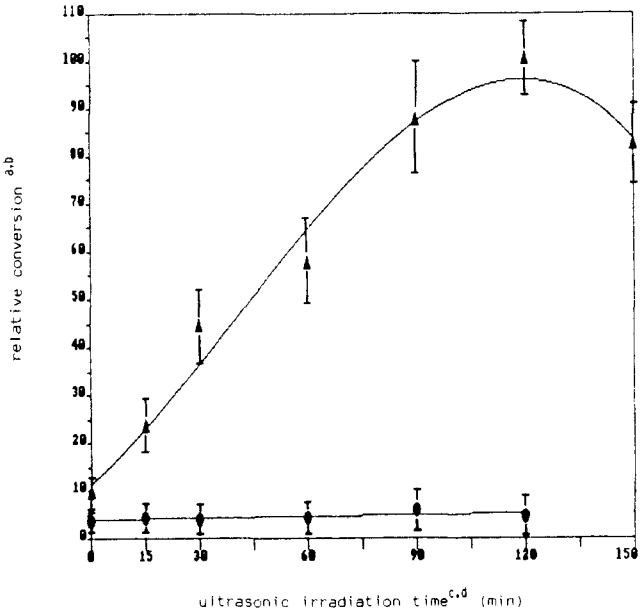

Figure 1. (a) Conversions are listed relative to maximum conversion at 2 h, and each point is an average of three separate trials. (b) Incubations were performed anaerobically for 6 h at 37 °C with 2.0 mg of substrate **3** and 28 mg of Triton X-100 with use of either 2.0 mL of a whole cell yeast suspension (see ref 8) in 0.10 M buffer (▲) or 2.0 mL of a cell-free cyclase system (see ref 6a) in phosphate buffer (●). (c) Cyclase systems were sonicated prior to incubation. (d) Incubations were terminated by addition of 2 mL of 15% KOH/MeOH, heated to 80 °C for 10 min, then shaken with 6 mL of hexane, and centrifuged. A 5.0-mL aliquot of extract was diluted with 1.0 mL of a solution of estrone methyl ether and analyzed by HPLC on silica gel eluting with 98.5:1.5:0.5 hexane–CH_2Cl_2–*i*-PrOH.

The utility of this enzymatic cyclization method was demonstrated by an enantioselective synthesis of the cytotoxic sterol, ganoderic acid Z,[12] from epoxide **3**. Substrate **3** was prepared by condensation of $Ph_3{=}C(CH_3)CO_2CH_3$[13] with aldehyde **5**[14] in benzene at 60 °C to afford **6** (66% yield, after separation of *E*/*Z* isomers), followed by conversion of **6** to the epoxide **3**[15] via the bromohydrin as described for the preparation of squalene oxide.[14] Incubation of 0.500 g of epoxide **3** and 7 g of Triton X-100 with the ultrasonicated yeast mixture (50 g of yeast, 500 mL of 0.1 M phosphate buffer, pH 7.4) at 37 °C for 28 h afforded 0.157 g (62% conversion based on one antipode of **3**) of the methyl ester of ganoderic acid Z (**4b**), $\alpha^{23}_D = +55.7°$ (lit.[12] $\alpha^{23}_D = +56°$), whose ^1H NMR, ^{13}C NMR, IR, and mass spectral data were consistent with an authentic sample of **4** prepared by the method of Ourisson.[12] Alkaline hydrolysis gave ganoderic acid Z (**4a**).

To determine the effect of ultrasound on the enzymatic reaction, we briefly examined the time course of sterol production as a function of ultrasonic irradiation (Figure 1). For incubations conducted with whole cells, there was a significant increase in sterol formation when cells were first sonicated for at least 0.5 h, reaching maximum conversion efficiency at ~2 h. By contrast, sterol production with a cell-free cyclase system[6a] was completely insensitive to ultrasonic irradiation, suggesting that the ultrasonic effect is most likely associated with facilitating substrate diffusion by removing the obstructing outer membrane rather than activating the cyclase or preventing its inhibition. However, we cannot exclude the possible enhancement of cyclase activity caused by liberating membrane-associated sterol carrier protein factors. Experiments designed to probe this aspect in greater detail and

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(8) For the typical ultrasonic pretreatment; 25 g of bakers' yeast (Sigma type II) was suspended in 125 mL of 0.1 M phosphate buffer, pH 7.4, and irradiated ultrasonically at 0 °C for 2 h at intensities ≈40 W/cm by using a Branson Sonifier 185 cell-disruptor with a titanium immersion tip. The resulting dispersion was diluted with 0.1 M phosphate buffer to a total of 250 mL.

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to explore the cyclization of other unnatural substrates by this technique are in progress.

Acknowledgment. Support of this work by the American Cancer Society, Florida Affiliation, and the American Heart Association is gratefully acknowledged. R.G. is the recipient of an NIH-MARC scholarship award.

On the Red Shift of the Bacteriochlorophyll-*b* Dimer Spectra

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Received April 20, 1987

The UV-vis spectroscopy of chlorophylls often differs greatly from in vitro to in vivo. In particular, the long wavelength transition of bacteriochlorophyll-*b* (BChlb) in reaction centers of the photosynthetic bacterium *Rhodopseudomonas viridis* exhibits a large red shift (790 nm in vitro, 960 nm in vivo). The 960-nm absorption maxinum has been assigned to a BChlb dimer, "special pair", which was recently shown to exist in the X-ray crystal structure of *R. viridis*.[1,2] The origin of this red shift has been a subject of intense interest, and a number of reasons have been suggested to explain it including the following: solvent effects, electrostatic effects of amino acid side chains, dimer formation, and exciton interactions.[3-7]

We present here the results of the calculated UV-vis spectra, employing the quantum chemical INDO method,[8-10] of the BChlb dimer based on the X-ray coordinates seen in *R. viridis*. We observe a large red shift and intensification of the long wavelength transition that agrees well with experiment. While there may be many contributing factors to the spectral shift of BChlb dimers in vivo, the formation of the dimer alone appears to be sufficient to induce a large red shift.

Figure 1 shows the structure of the BChlb dimer we employed which is based on the X-ray crystal coordinates.[1] The monomers are labeled as to their major association with either the L or M subunits of the membrane protein in which they are embedded.[1,2] The average distance between atomic centers of the two overlapping pyrrole rings is roughly 3 Å, and the Mg-Mg distance is about 7 Å. The macrocycles are skewed in the plane of the figure about an axis which is roughly perpendicular to the figure and passes through the two overlapping rings and are tilted nearly 15° relative to one another. The monomer MOs shown in Figure 2 are those that would constitute the four-orbital model as described by Gouterman.[11] The monomers, while not geometrically identical, have similar MOs. As well, their Qy absorptions (and oscillator strengths) are similar with BChlb-L and BChlb-M at 12993 cm^{-1} (0.555) and 12864 cm^{-1} (0.590), respectively.[12] The

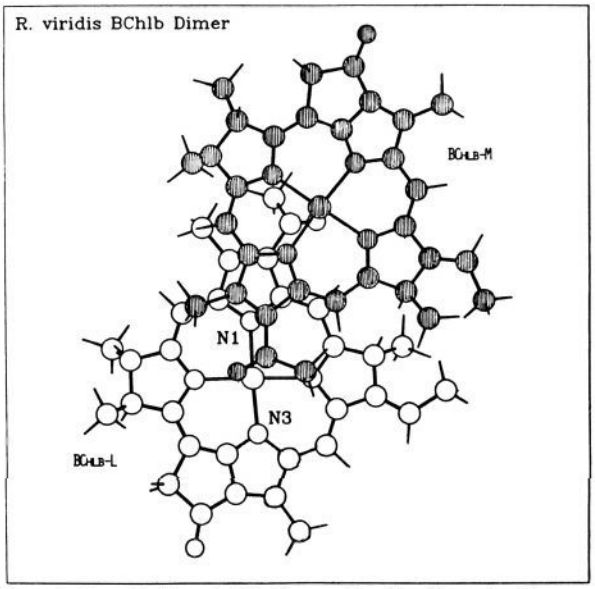

Figure 1. Bacteriochlorophyll-*b* dimer utilized in this study. The macrocycles are labeled BChlb-L or BChlb-M corresponding to their association with the L or M subunits of the photosynthetic membrane protein (ref 2 and 3).

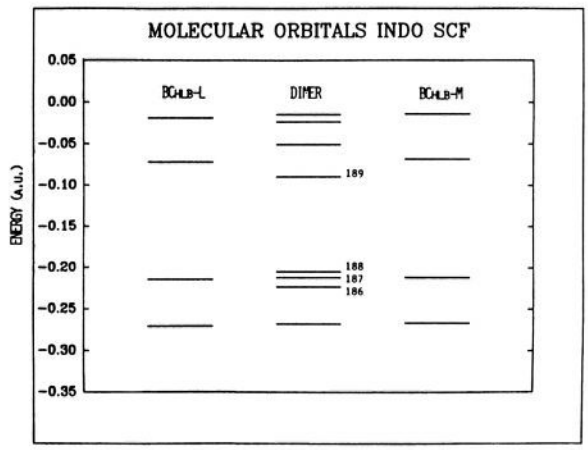

Figure 2. The molecular orbitals of the BChlb dimer and its constituent monomers were calculated separately. All calculations were closed shell restricted and converged to 10^{-6} in energy.

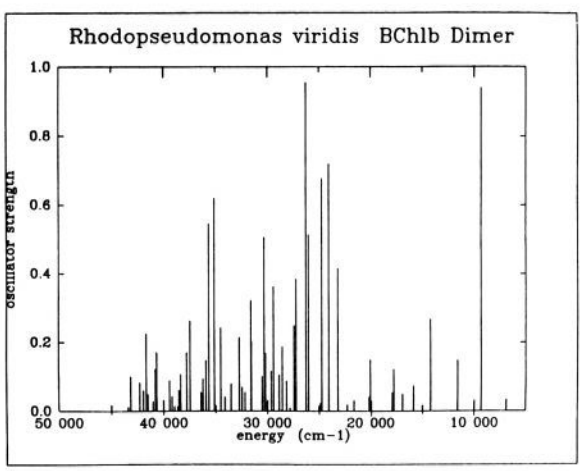

Figure 3. The calculated INDO/CI spectrum for bacteriochlorophyll-*b* dimer. The calculation includes the lowest 120 singly excited configurations from the self-consistent field ground state.

MOs of the BChlb dimer are split significantly and, except for dimer MOs 189 and 190, do not exhibit obvious monomer par-

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