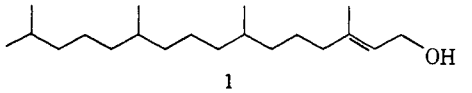
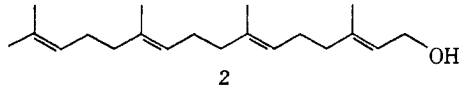
Esterifying Alcohols in the Chlorophylls of Purple Photosynthetic Bacteria. A New Chlorophyll, Bacteriochlorophyll (gg), *all-trans*-Geranylgeranyl Bacteriochlorophyllide a

Sir:

The pioneering studies of Fischer[1] resulted in the general impression that all the chlorophylls, including those of the photosynthetic bacteria, are esters of phytol. In 1961, Rapoport and Hamlow[2] discovered that the esterifying alcohol in the chlorophyll of green photosynthetic bacteria is *all-trans*-farnesol. We now report that the situation with respect to the esterifying alcohols in the bacteriochlorophylls from purple photosynthetic bacteria is likewise more complex than has previously been assumed. While the bacteriochlorophylls isolated[3] from the purple photosynthetic bacteria *Chromatium vinosum* (*Thiorhodaceae*) and *Rhodopseudomonas spheroides* (*Athiorhodaceae*) are indeed esters of phytol (**1**), the bacteriochlorophyll isolated from *Rhodospirillum rubrum* (*Athiorhodaceae*) is esterified at the propionic acid side chain by *all-trans*-geranylgeraniol (**2**).



1



2

The nmr spectrum of the bacteriochlorophyll isolated from *R. rubrum* is incompatible with the presence of a phytyl moiety because the strong resonances near 1 ppm characteristic of the terminal methyl groups of phytol are missing. Instead, prominent absorptions near 2 ppm and between 5 and 6 ppm are more compatible with the presence of an unsaturated acyclic terpene residue such as the farnesol detected by Rapoport and Hamlow.[2]

Treatment of bacteriochlorophyll from *R. rubrum* with alcoholic KOH gave an alcohol with proton resonances at (δ, ppm from internal TMS) 1.59 and 1.66 (CH_3), 2.01 (CH_2), 4.01 (CH_2OH), 5.08 and 5.36 (=CH), identical in all respects with the pmr spectrum obtained on an authentic sample of *all-trans*-geranylgeraniol. These chemical shifts are, of course, very similar to those of *all-trans*-farnesol, but a computer-assisted integration of the pmr spectrum of the esterifying alcohol from *R. rubrum* bacteriochlorophyll showed that the ratio of the areas of the vinyl proton resonances (δ 5.08 + 5.36) to the CH_2OH resonance (δ 4.01) is 2.1:1 (calcd, geranylgeraniol, 2:1; farnesol 1.5:1), indicative of the presence of four double bonds in the esterifying alcohol. The ratio of the areas of the low-field resonances (δ 4.01 + 5.08 + 5.36) to the high-field resonance (δ 1.59 + 1.66 + 2.01) is 5.0:1; for our sample of geranylgeraniol, 5.5:1; for *trans*-farnesol, 4.0:1 (calcd, geranylgeraniol, 4.5:1; farnesol, 4.0:1).

Mass spectroscopic comparisons of the chlorophylls and their esterifying alcohols were carried out in two instruments. First, the intact chlorophylls were heated in the source of an AEI MS-902 mass spectrometer. Fragments of the esterifying alcohol and a prominent ion corresponding in mass to the alcohol minus a molecule of water are observed. Authentic samples of chlorophylls a and b, in which the esterifying alcohol is phytol ($C_{20}H_{40}O$), show a peak at *m/e* 278 with the empirical formula $C_{20}H_{38}$. Fully deuterated chlorophyll a (^2H-chlorophyll a) yields an entirely analogous ion at *m/e* 316, for $C_{20}{}^2H_{38}$. To verify that these peaks do, in fact, originate from the esterifying alcohol at the propionic acid side chain, phytol, obtained by saponification of chlorophyll a with alcoholic KOH, was esterified with propionyl chloride in pyridine. Phytyl propionate gives a parent peak at *m/e* 352, $C_{23}H_{44}O$, and an intense peak at *m/e* 278, $C_{20}H_{38}$. Hence, fragments at *m/e* 278 observed in the mass spectra of the pyrolyzed chlorophylls a and b and bacteriochlorophyll from *R. spheroides* and *C. vinosum* originate in the ester moiety, confirmation that phytol is the principal esterifying alcohol in these chlorophylls. We have also found by the procedure of direct insertion in the spectrometer source that the esterifying alcohol in chlorobium chlorophyll from the green photosynthetic bacterium *Chloropseudomonas ethylica* is indeed farnesol.[2] This direct mass spectrometric examination of chlorophylls provides a sensitive and rapid screening procedure for ascertaining the nature of the esterifying alcohol.

With bacteriochlorophyll from *R. rubrum*, an intense ion at *m/e* 272 (exact mass, found 272.2495; calcd for $C_{20}H_{32}$, 272.2503) was observed, while the fully deuterated bacteriochlorophyll gave an ion at *m/e* 304. This unequivocally establishes the empirical formulas $C_{20}H_{32}$ and $C_{20}{}^2H_{32}$ for these ions. Thus, the esterifying alcohol in *R. rubrum* bacteriochlorophyll must have the empirical formula, $C_{20}H_{34}O$, corresponding to phytol minus 6 H, the same as that of geranylgeraniol.

The alcohol from *R. rubrum* has been compared to authentic *all-trans*-geranylgeraniol in the time-of-flight mass spectrometer. A prominent parent peak at *m/e* 290 is observed for both samples and is accompanied by a peak, half as intense, at *m/e* 272, evidently the parent minus H_2O. The fragmentation patterns of authentic *all-trans*-geranylgeraniol and the esterifying alcohols are virtually identical. Close examination of the spectrum reveals small additional peaks in the esterifying alcohol at higher masses at *m/e* 292, 294, 296, and 298, which can tentatively be interpreted to indicate that all possible C_{20} isoprenoid alcohols between the fully saturated 3,7,11,15-tetramethylhexadecan-1-ol and geranylgeraniol are present in small amounts or are formed in the mass spectrometer. There is also reason to suppose that some of the fully saturated alcohol is also present in the phytol from our chlorophyll a, and that even higher prenols[4] may be present in small amounts. We are, therefore, investigating the possibility that active center chlorophyll may have a different esterifying alcohol than does the bulk antenna chlorophyll.

The esterifying alcohol from *R. rubrum* bacteriochlorophyll exhibits chromatographic behavior, in the thin-layer chromatographic system of Schechter,[5] and

(1) H. Fischer and H. Orth, "Die Chemie Des Pyrrols," Vol. II, Part II, Akademische Verlag, Leipzig, 1940, p 305 ff.

(2) H. Rapoport and H. P. Hamlow, *Biochem. Biophys. Res. Commun.*, 6, 134 (1961).

(3) H. H. Strain and W. A. Svec in "The Chlorophylls," L. P. Vernon and G. R. Seely, Ed., Academic Press, New York, N. Y., 1966, pp 21-66.

(4) F. W. Hemming, *Biochem. Soc. Symp.*, No. 29, 105 (1970).

(5) I. Schechter, Thesis, University of California, Los Angeles, 1969; I. Schechter and C. A. West, *J. Biol. Chem.*, 244, 3200 (1969).

an infrared spectrum indistinguishable from those of authentic *all-trans*-geranylgeraniol. The nmr, mass spectrometric, and chromatographic data thus establish the principal esterifying alcohol in bacteriochlorophyll from *R. rubrum* as *all-trans*-geranylgeraniol. The corresponding alcohol from the fully deuterated bacteriochlorophyll is, therefore, fully deuterated geranylgeraniol. These findings are entirely compatible with the suggestion that geranylgeraniol pyrophosphate may be a precursor in the biosynthesis of phytol.[6]

It is now necessary to indicate the nature of the esterifying alcohol in naming the bacteriochlorophylls, and we have found it convenient to designate them bacteriochlorophyll (gg) and bacteriochlorophyll (phy). It should be noted that Wellburn has esterified chlorophyll a with geranylgeraniol by an *in vitro* enzymatic transesterification.[7] This product was called chlorophyll a (gg).

The observations reported here have obvious implications concerning photosynthesis, lipid and carotenoid metabolism, and the taxonomy of purple photosynthetic bacteria.[8]

Acknowledgment. Our interest in the bacteriochlorophylls[3] was stimulated by a report presented by Drs. David H. Dolphin and A. Gomez-Revilla at a meeting on the "Primary Photochemistry of Photosynthesis" held at Argonne National Laboratory in Nov 1971. At that time, Dr. Dolphin announced that the esterifying alcohol in bacteriochlorophyll from *R. rubrum* was not the expected phytol. Subsequent work at Argonne National Laboratory, reported here, establishes the identity of this alcohol. We thank Dr. Dolphin for reading our manuscript and for a sample of pure *all-trans*-farnesol. We are also deeply grateful to Drs. H. Rilling and L. Altman for a very pure sample of *all-trans*-geranylgeraniol.

Work at the Argonne National Laboratory was performed under the auspices of the U. S. Atomic Energy Commission.

(6) A. R. Wellburn, K. J. Stone, and F. W. Hemming, *Biochem. J.*, 100, 23C (1966).

(7) A. R. Wellburn, *Phytochemistry*, 9, 2311 (1970).

(8) NOTE ADDED IN PROOF. H. Brockmann, Jr., and G. Knobloch (*Arch. Mikrobiol.*, 85, 123 (1972)) have just published the finding that *R. rubrum* bacteriochlorophyll is a farnesyl ester. As no experimental data are given about either the nmr or mass spectroscopy on which the identification of Brockmann and Knobloch is based, the possibility that the esterifying alcohol in *R. rubrum* bacteriochlorophyll is strain dependent remains. We are investigating this possibility.

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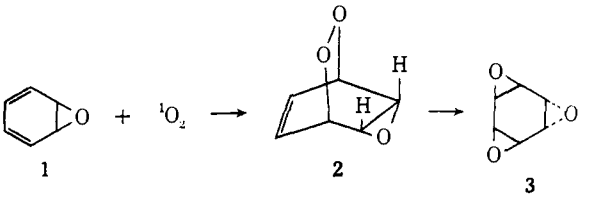
Received June 26, 1972

Synthesis of *trans*-Benzene Trioxide

Sir:

We wish to report a unique synthesis of *trans*-3,6,9-trioxatetracyclo[6.1.0.0^{2,4}.0^{5,7}]nonane (**3**) by thermal rearrangement of the 1,4-*endo*-peroxide (**2**) obtained from addition of singlet oxygen to oxepin—benzene oxide (**1**).



1 2 3

Reaction of **1**[1] with singlet oxygen generated from hypochlorite–hydrogen peroxide by the method of Foote,[2] extraction with ether, and evaporation yielded a semicrystalline residue that, on addition of a small amount of ether, gave 37% of pure, crystalline **2**.[3,4] The mass spectrum[5] of **2** shows a parent peak at *m/e* 126, the base peak at *m/e* 94 due to loss of O_2 from the molecular ion, and a peak at *m/e* 66 (relative intensity 42%) due to subsequent loss of CO.

Peroxide **2** undergoes *quantitative* rearrangement to **3**[3,6] in $CHCl_3$ at 45° with a half-life for the reaction of approximately 14 hr. The facile rearrangement of **2** to **3** is particularly interesting in view of the previously reported rearrangements of 1,4-*endo*-peroxides derived from 1,3-cyclohexadienes that require higher temperature and yield mixtures of hydroxy ketone or epoxy ketone in addition to bisepoxide.[7] The nmr spectrum of **3**[6] establishes that one epoxy group is trans to the other two epoxy groups and, consequently, establishes that the epoxy group and the endoperoxy group in **2** are trans.

endo-Peroxide **2** can also be prepared from the reaction of **1** with singlet oxygen generated from the adduct of ozone and triphenyl phosphite.[8] Attempts to prepare **2** by photosensitized oxygenation of **1** with Methylene Blue as sensitizer gave mainly phenol.

Further studies of **2** and **3** will be described at a later date.

Acknowledgment. We are indebted to the National Institutes of Health for financial support.

(1) E. Vogel and H. Günther, *Angew. Chem., Int. Ed. Engl.*, 6, 385 (1967).

(2) C. S. Foote, *et al.*, *J. Amer. Chem. Soc.*, 90, 975 (1968).

(3) Satisfactory elemental analyses have been obtained for **2** and **3**.

(4) Mp 91–92°; ir ($CHCl_3$) 3015, 1405, 1372, 1285, 973, 920, 882, and 847 cm^{-1}; nmr ($CDCl_3$) δ 3.75 (2 H, m, epoxy H), 5.1 (2 H, m, bridgehead H), and 6.35 ppm (2 H, t, J = 4 Hz, olefinic H).

(5) Direct inlet, source temperature <100°; we thank Mr. John Dolhun for the mass spectra.

(6) Mp 84–86°; ir ($CHCl_3$) 3020, 1450, 1239, 950, and 860 cm^{-1}; nmr ($CDCl_3$) δ 3.4 (4 H, s) and 3.5 ppm (2 H, s); mass spectrum[5] (70 eV) *m/e* (relative intensity) 126 (M, 5), 97 (26), 81 (21), 71 (52), 69 (75), 68 (61), 43 (22), 42 (21), 41 (100), and 39 (55).

(7) K. K. Maheshwari, P. de Mayo, and D. Wiegand, *Can. J. Chem.*, 48, 3265 (1970), and references cited therein.

(8) R. W. Murray and M. L. Kaplan, *J. Amer. Chem. Soc.*, 91, 5358 (1969).

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Received July 29, 1972