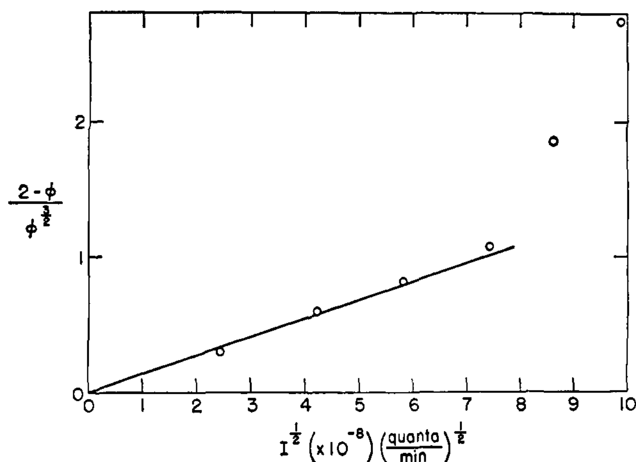


Figure 1.

The quantum yield of disappearance of benzophenone is defined as

$$\Phi_{-(C_6H_5)_2CO} = \frac{2k_1(T_1)}{I} \quad (6)$$

where I is the light intensity.

At steady state, the formation of T_1, II, and III will be equal to the disappearance of these species.

$$I = k_1(T_1) + k_4(II)(T_1) + k_5(III)(T_1) \quad (7)$$

$$2k_3(II)^2 = k_1(T_1) + k_2(III) \quad (8)$$

$$k_1(T_1) = k_2(III) \quad (9)$$

Combining (8) and (9) and solving for II, we have

$$(II) = [k_1(T_1)/k_3]^{1/2} \quad (10)$$

Substituting (6), (9), and (10) into (7) and solving in terms of I, we have

$$\frac{2 - \Phi}{\Phi^{3/2}} = k'I^{1/2} + k''I\Phi^{1/2}$$

where $k' = k_4/k_1(2k_3)^{1/2}$ and $k'' = k_5/2k_1k_2$

At low light intensity, the second term may be relatively insignificant as compared to the first term. Physically it means that the quenching is mainly due to the ketyl radical (II) at low light intensity. If our explanation is correct, by plotting $(2 - \Phi)/\Phi^{3/2}$ against $I^{1/2}$ we will obtain a straight line at low light intensity. At high light intensity, there will be an increase in 2-hydroxy-2-propyl radical (III) concentration, and the second term becomes increasingly more significant and the line will deviate with an increasing slope. Our experimental results are represented in Figure 1.

It has been shown that O-deuteration on 2-propanol has no measurable isotope effect on the quantum yield of photoreduction of benzophenone in 2-propanol at low light intensity.[10] It is reasonable to assume that O-deuteration will slow down reaction 2 and will increase the concentration of the solvent radical III. Therefore, the decrease in quantum yield with increasing light intensity will be more predominant in 2-propanol-*d*. A preliminary investigation was carried out in 2-propanol-*d*. We found that this was indeed the case (see Table I).

(10) W. M. Moore and M. D. Ketchum, *J. Phys. Chem.*, **68**, 214 (1964).

The generality of this phenomenon and a quantitative treatment on the rates of these processes[11] are being investigated.

Acknowledgment. The authors wish to thank the National Science Foundation for the support of this work, the Esso Research Foundation for a fellowship to one of us (S. M.), and Professor D. S. McClure for his helpful discussions.

(11) By using the values reported by C. Walling and M. J. Gibian (*J. Am. Chem. Soc.*, **87**, 3361 (1965)) that $k_1 = 8.7 \times 10^4$ and by A. Beckett, A. D. Osborne, and G. Porter (*Trans. Faraday Soc.*, **60**, 873 (1964)) that $k_3 = 5.9 \times 10^7$, the value for k_4 was estimated at the order of 10^{10}.

(12) National Science Foundation Fellow, 1962–1965; Esso Research Foundation Fellow, 1965–1966.

N. C. Yang, Steven Murov[12]
Department of Chemistry, University of Chicago
Chicago, Illinois 60637
Received April 2, 1966

Nuclear Magnetic Resonance Studies of Plant Biosynthesis. Bacteriochlorophyll[1]

Sir:

We wish to report experimental results, based upon the use of a deuterium-substituted substrate and proton magnetic resonance, that give new information about the biogenesis of bacteriochlorophyll in physiologically competent microorganisms.

Rhodospirillum rubrum (A.T.C.C. 11170) was grown in an ordinary water medium[2] with succinic acid-d_4 (>99 atom % D) as the only exogenous organic carbon source. Bacteriochlorophyll[3] was isolated from these organisms and converted to methyl bacteriopheophorbide,[4] which was purified by chromatography and crystallization. The nmr spectra of bacteriochlorophyll and methyl bacteriopheophorbide have been assigned[5] by conventional techniques;[6] hence we could accurately determine the relative abundance of hydrogen and deuterium at each position in the molecule.[7]

The ratio H/D at the hydrogen positions in the molecule reflects both the isotopic composition of the succinic acid and the exchange and hydrogen transfer reactions that occurred during biosynthesis. With deuterium decoupling, the hydrogen atoms at position 4'' (see **1** in Table I for numbering) in the H_2O–succinate-d_4 bacteriochlorophyll (0.12 M in C_3D_6O) ($\delta \sim 103$ cps) appeared as a sharp doublet ($J \sim 7.2$ cps). In the usual hydrogen bacteriochlorophyll, a triplet with the same coupling constant and chemical shift is ob-

(1) Based on work performed under the auspices of the U. S. Atomic Energy Commission.

(2) J. G. Ormerod, K. S. Ormerod, and H. Gest, *Arch. Biochem. Biophys.*, **94**, 449 (1961).

(3) H. H. Strain, M. R. Thomas, H. L. Crespi, M. I. Blake, and J. J. Katz, *Ann. N. Y. Acad. Sci.*, **84**, 617 (1960).

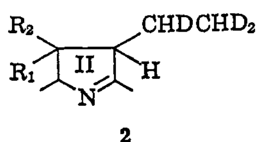
(4) H. Fisher and A. Stern, "Die Chemie des Pyrrole," Band III, Akademische Verlagsgesellschaft, Leipzig, 1940, p 317.

(5) J. J. Katz, R. C. Dougherty, and L. J. Boucher. "Chlorophyll," L. P. Vernon and G. R. Seely, Ed., Academic Press Inc., New York, N. Y., in press.

(6) G. L. Closs, J. J. Katz, F. C. Pennington, M. R. Thomas, and H. H. Strain, *J. Am. Chem. Soc.*, **85**, 3809 (1963).

(7) The nmr spectra were recorded on a Varian HA-100 nmr spectrometer in the field-sweep mode. Deuterium decoupling was carried out with an N.M.R. Specialties Hetero-Nuclear Decoupler adjusted for decoupling the deuterons in acetone-d_6 at "zero power."

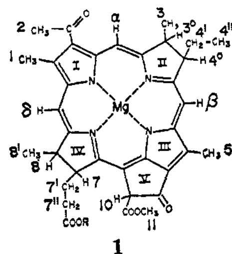
served. Further, the 4′ resonance ($\delta \sim 227$ cps) was a doublet of doublets ($J_1 \sim 7.2$ cps, $J_2 \sim 4.6$ cps). These data suggest that the principal isotopic species at locations 4^0, 4′, and 4″ in ring II of the molecule are as shown in **2**. The relative areas (Table I) and the



2

fact that none of these resonances are distinct without deuterium decoupling support this conclusion.

Table I. Relative Areas of Proton Resonances in Bacteriochlorophyll and Derivatives[a]



1

Position	H_2O medium, succinic acid-d_4 Bacterio-chlorophyll	Methyl bacterio-pheophorbide	H_2O medium, succinic acid-h_4 Methyl bacterio-pheophorbide
α	1.0 ± 0.03	1.0 ± 0.04	1.0 ± 0.08
δ	1.0 ± 0.05	1.0 ± 0.04	1.0 ± 0.08
β	1.0 ± 0.05	1.0 ± 0.04	1.0 ± 0.04
10	1.0 ± 0.10	0.9 ± 0.1	1.0 ± 0.05
8 + 3^0		2.1 ± 0.2	2.0 ± 0.3
7 + 4^0	2.0 ± 0.4	2.0 ± 0.1	2.0 ± 0.3
11	2.5 ± 0.09	2.6 ± 0.15	3.0 ± 0.08
12[d]		3.0 ± 0.1	3.0 ± 0.10
5	1.5 ± 0.1	} 3.1 ± 0.2[c]	3.1 ± 0.2
1	1.5 ± 0.1		3.0 ± 0.2
2	1.4 ± 0.06[b]	2.7 ± 0.2[b]	2.9 ± 0.2
7′ + 7″	0.0 ± 0.2	0.0 ± 0.1	} 6.2 ± 0.3[c]
4′	0.9 ± 0.2	1.1 ± 0.1	
8′		} 4.0 ± 0.15	3.0 ± 0.08
3			2.9 ± 0.15
4″	1.0 ± 0.06	1.0 ± 0.07	3.0 ± 0.20

[a] Values are the averages of four successive 2500-sec scans. The areas were obtained by manual integration with an Ott planimeter and internally standardized. The errors are standard deviations. Complete duplicate experiments are in close agreement. [b] The methyl group in the acetyl at position 2 undergoes exchange in strong acid, but not under neutral conditions. [c] These peaks overlap. They are sufficiently resolved to deduce chemical shifts, but are consolidated for integration. [d] The CH_3 protons at R in the propionic ester side chain.

The isotopic composition of the ethyl group at position 4 in ring II may be explained by the hypothesis that a deuteriopropionic acid side chain at that position underwent an oxidative decarboxylation (with abstraction of a deuteron from the methylene group adjacent to the ring) to give a vinyl group of the composition $-CD{=}CD_2$.[8] Direct reduction of this group with a reducing agent of the isotopic composition of the medium (see below) would then yield the observed product.

(8) *Cf.* S. Granick, "Proceedings of the Fifth International Congress of Biochemistry," The Macmillan Co., New York, N. Y., 1963, p 176.

If the acetyl group (at position 2) had a common origin with the ethyl group (at positions 4′, 4″) to the point of protoporphyrin IX[9] or a related divinyl compound,[10] the two reactions (hydration and oxidation) necessary for conversion of the vinyl to an acetyl group must have involved at least 20% of random exchange with the hydrogen of the medium. Exchange to this extent is considerably less than anticipated for equilibrium exchange but more than would be expected for the hydration and oxidation of an aliphatic double bond, or even a styrene-like double bond. There is reason to suspect that a cation center adjacent to a porphyrin nucleus may show an unusually long lifetime and thus contribute to hydrogen exchange. An alternative explanation for the differences in isotopic composition at positions 2 and 4′, 4″ is that these groups did not have a common chemical origin.

The data in Table I also suggest that the methyl groups at positions 1 and 5 had a different chemical history from the methyl groups at positions 3 and 8′. The individual resonances of the two methyl groups in each set (1 and 5, and 3 and 8′, respectively) were not sufficiently resolved to allow accurate individual integration. The line shapes strongly suggested that the two methyl groups within each set had approximately the same composition. Presumably, the decarboxylation at positions 1 and 5 preceded that at positions 3 and 8 by enough time to allow exchange with the medium.

Three further points that relate to bacteriochlorophyll biogenesis can be mentioned: (a) the methine protons, which presumably arise from glycine,[9] had the composition of the medium (100% hydrogen); (b) the hydrogen atoms that arose from reduction of carbon–carbon unsaturation (3^0, 4^0, 7, 8, and presumably 4′, 4″) all had the composition of the medium; and (c) the methyl ester function (at location 11) that has been shown to arise from S-adenosylmethionine[11] was probably synthesized directly from succinate by a series of reactions that did not allow complete exchange of the carbon-bound hydrogen of the succinate.

The observed isotope distribution pattern for the biogenesis of bacteriochlorophyll from succinate-d_4 was an average for *R. rubrum* in all stages of physiological development with continuously varying amounts of succinate-d_4 in the medium. The isotope discrimination factors, and possibly the biosynthetic pathway, may depend upon these or other factors. It must also be emphasized that the studies reported here are confined to one particular set of growth conditions. The experimental accuracy of the nmr measurements is such that processes that affected the composition to 5% or less would not be detected, and this is an upper limit to the utilization of endogenous succinic acid in this experiment.

(9) J. Lascelles, "Tetrapyrrole Biosynthesis and Its Regulation," W. A. Benjamin, Inc., New York, N. Y., 1964.

(10) O. T. G. Jones, *Biochem. J.*, **88**, 335 (1963); **89**, 182 (1963).

(11) K. D. Gibson, A. Neuberger, and G. H. Tait, *Biochem. J.*, **88**, 325 (1963).

Ralph C. Dougherty, Henry L. Crespi
Harold H. Strain, Joseph J. Katz
Argonne National Laboratory
Argonne, Illinois 60439
Received January 6, 1966