(particularly π,π^* states since their solvent shifts are unpredictable both in terms of direction and magnitude) are in order. With our technique we hope to help empirically obtain some of this information.

Acknowledgment. We wish to thank the Army Research Office, Durham, for financial support of this research.

Lawrence D. Weis, Ben W. Bowen, Peter A. Leermakers
Hall Laboratory of Chemistry, Wesleyan University
Middletown, Connecticut 06457

Received April 13, 1966

The Role of Bacteriochlorophyll in Photosynthetic Hydrogen Transfer[1]

Sir:

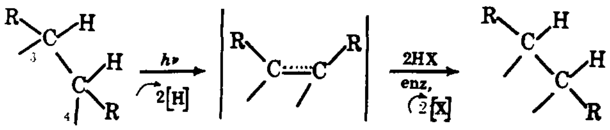
The participation of chlorophyll as a hydrogen transfer agent in the water-splitting reaction of photosynthesis has been the subject of considerable speculation. Some of the chemical mechanisms that have been proposed for green plant photosynthesis involve a reversible photochemical oxidation–reduction cycle of the following type for either one- or two-electron transfers.[2]

$$\text{chlorophyll a} \longrightarrow \text{dehydrochlorophyll a} + [\text{H}]$$

$$\text{dehydrochlorophyll a} + \text{HOH} \longrightarrow \text{chlorophyll a} + 0.5\text{O}_2$$

Proposals of this type have been centered on the hydrogens in the 7,8 positions, the δ hydrogen, and the C_{10} hydrogen of chlorophyll a. Investigations designed to evaluate such mechanisms, using tritium as a hydrogen tracer, have been inconclusive; the results show small amounts of incorporation, but these cannot be considered as evidence for or against a hydrogen donor mechanism.[3–5] Deuterium experiments have eliminated δ-hydrogen participation, but not the C_{10}, C_7, or C_2 positions.[6]

We wish to introduce the notion that the 3,4 positions of ring II are photosynthetically important. If this is true, then in the oxygen-producing green plants and algae, which contain the chlorin type of pigments, it is possible that chlorophyll is reduced and then oxidized in the 3,4 positions. In the anaerobic photosynthetic organisms, such as the purple bacteria, which contain reduced chlorin pigments, the bacteriochlorophyll could be oxidized and then reduced in the 3,4 positions as shown in the following scheme for 2H transfer.



It would be equally feasible to formulate the transfer in terms of a single hydrogen atom and a partially oxidized bacteriochlorophyll. The immediate impetus for such a proposal came from the work of Holt, *et al.*,[7] and Olson and Romano,[8] who demonstrated the presence of both chlorin and dihydrochlorin pigments in the green photosynthetic bacteria, *Chlorobium*. This proposal cannot be tested on the green algae, since the pigment is isolated as chlorophyll and would not contain the photosynthetically important hydrogens. However, with anaerobic purple bacteria, the bacteriochlorophyll is isolated in the reduced state and changes in the 3,4 positions can be evaluated. The recent discovery of the specific oxidation of bacteriochlorophyll[9] in the 3,4 positions using 2,3-dichloro-5,6-dicyanobenzoquinone, converting the dihydrochlorin to the chlorin 2-desvinyl-2-acetylchlorophyll a, makes it possible for us to determine how much of the incorporated tritium is in the 3,4 positions.

In order to obtain a sufficient amount of bacteriochlorophyll for measurement, the experiment was performed using an extremely dense suspension (0.5-ml packed cells suspended in 3 ml of medium) of 4-day-old *Rhodospirillum rubrum*. Tritiated water (0.92 curie) was added to give a molar specific activity of 1.09×10^{13} dpm/mole; the suspension was placed in a flat cell with a path length of 5 mm. It was then illuminated from both sides with 23,000 foot-candles of white light for 15 min. The bacteriochlorophyll was isolated by acetone extraction, purified on powdered polyethylene tlc, and counted. The bacteriochlorophyll was then oxidized to give the 3,4-dehydro compound and purified on mannitol tlc, and its radioactivity was again determined.[10]

From the specific activity of the initial bacteriochlorophyll it was determined that approximately 2% of the bacteriochlorophyll molecules had incorporated tritium, and that from 62 to 96% (five separate photoexperiments, three of them in duplicate) of the incorporated radioactivity was in the 3,4 positions. Since we have no independent way of determining the rate of photosynthesis, we cannot yet relate this 2% labeling to an electron turnover rate, or a bacteriochlorophyll synthesis rate. However, because of the relatively great age of the cell culture and the extremely high density of the suspension in which the experiment was performed, and the fact that the dark control showed less than 0.03% tritium incorporation, we believe that, for the most part, this incorporation does not represent new bacteriochlorophyll synthesis. Under optimal growth conditions for dilute suspensions (one-twentieth of those of the experiment) the rate of bacteriochlorophyll increase is 1.7% for a 15-min period. A succeeding exposure of the tritium-labeled bacteria to light in a nontritiated medium for 15 min removes approximately 20% of the initially incorporated tritium. The fact that so much activity is removed requires a photoinduced hydrogen turnover. The fact that it is not all removed seems to necessitate a migratory active site.

In addition, a tritium isotope effect has not been included in the calculation of the reported 2% incorporation. It seems probable that this incorporation does indeed represent photoinduced electron turnover in the bacteriochlorophyll. However, the experiments

---

[1] The work described in this paper was sponsored, in part, by the U. S. Atomic Energy Commission.

[2] E. I. Rabinowitch, "Photosynthesis," Vol. I, Interscience Publishers, Inc., New York, N. Y., 1945, p 548; M. Calvin, "Horizons in Biochemistry," M. Kasha and B. Pullman, Ed., Academic Press Inc., New York, N. Y., 1962, p 34.

[3] M. Calvin and S. Aronoff, University of California–Lawrence Radiation Laboratory Report, UCRL-263, Dec 1948.

[4] T. H. Norris, S. Ruben, and M. B. Allen, *J. Am. Chem. Soc.*, **64**, 3037 (1942).

[5] W. Vishniac and I. A. Rose, *Nature*, **182**, 1089 (1958).

[6] J. J. Katz, *et al.*, *J. Am. Chem. Soc.*, **86**, 4220 (1964).

[7] A. S. Holt, D. W. Hughes, H. J. Mende, and J. W. Purdie, *Plant Cell Physiol.*, **4**, 49 (1963).

[8] J. M. Olson and C. A. Romano. *Biochim. Biophys. Acta*, **59**, 726 (1962).

[9] J. R. Lindsay Smith and M. Calvin, submitted for publication.

[10] M. Byrn, Ph.D. Thesis, University of California, Berkeley.

necessary to determine independently photosynthetic electron turnover rate, as well as new chlorophyll synthesis, have yet to be performed. Beyond this, a double isotope tracer (deuterium and tritium) experiment is required to estimate the isotope effect both for magnitude and direction. Finally, the possible ambiguity introduced by cochromatography of a highly labeled photoproduced contaminant, itself of intrinsic interest if such exists, must be evaluated.

In view of the time required to complete these various operations, it seems wise to make known these preliminary results.

(11) Charles F. Kettering International Fellow, 1964–1965.

Marianne Byrn, John R. Lindsay Smith,[11] and Melvin Calvin
Department of Chemistry, Laboratory of Chemical Biodynamics and Lawrence Radiation Laboratory
University of California, Berkeley, California
Received April 18, 1966

A Four-Membered Arsenic Ring[1]

Sir:

The homocyclic compounds $(CH_3As)_5$ and $(C_6H_5As)_6$ have been demonstrated by X-ray crystallography.[2,3] We wish now to report the synthesis and properties of $(CF_3As)_4$, which seems to be the first clearly recognized example of an As_4 ring compound.

Synthesis and Purification. Mercury acted upon CF_3AsI_2 (2-day shaking, sealed tube at 25°) to produce a liquid–solid mixture from which the solid was separated by a magnetic separatory funnel[4] and purified by crystallization from hexane at −78°, in a filtering Λ-tube.[5] Recrystallization yielded a sample melting in a 0.1° range at 98.2°, under autogenous pressure. Further evidence of purity was the normal behavior of the vapor-tension data, which determined the equation $\log P = 12.4514 - 4003/T$. Averages of $\log P$ and $1/T$ for pairs or triplets of closely adjacent points are shown in Table I. Included are points observed after cooling from higher temperatures, all in good agreement.

Table I. Volatility of Solid $(CF_3As)_4$

Temp, °C	43.65	53.1	60.8	67.7	74.95	81.1
P_{obsd}, mm	0.65	1.51	2.93	5.15	9.00	13.9
P_{calcd}, mm	0.65	1.52	2.92	5.11	8.95	14.2

Table II. Infrared Comparison of $(CF_3As)_4$ with $(CF_3P)_4$[a]

Suggested assignments	$(CF_3As)_4$	$(CF_3P)_4$
C–F, 2 × ν_2	2277 (0.38)	2302 (0.53)
C–F, $\nu_1 + \nu_2$	2246 (0.60)	2285 (0.45)
C–F, 2 × ν_1	2225 (0.73)	2256 (1.2)
C–F, ν_2 + CF_3, δ–e	1865 (0.19)	1893 (0.20)
C–F, ν_1 + CF_3, δ–e	1842 (0.27)	1871 (0.36)
C–F, ν_2 + CF_3, δ–a	1665 (0.17)	1685 (0.12)
C–F, ν_1 + CF_3, δ–a	1643 (0.07)	1665 sh (0.06)
C–F, ν_2 + E–CF_3, ν_2	1455 (0.08)	1624 (0.06)
C–F, ν_1 + E–CF_3, ν_2	1430 (0.11)	1607 (0.05)
C–F, ν_2 + E–CF_3, ν_1	1425 (0.11)	1580 (0.04)
C–F, ν_1 + E–CF_3, ν_1	1400 (0.09)	1565 sh (0.03)
2 × CF_3, δ–e	1438 (0.11)	1461 (0.10) (or 308 + 1154?)
308 + 1135	...	1444 (0.08)
CF_3, δ–a + δ–e	1257 (1.6)	1277 (1.6)
CF_3, δ–e + E–CF_3, ν_2	1039 (2.6)	1213 (2.2)
CF_3, δ–e + E–CF_3, ν_1	1024 (1.8)	1173 (24)
C–F, ν_2	1140 (500)	1154 (270)
C–F_1, ν_1	1117 (250)	1135 (118)
CF_3, δ–e	728 (23)	742 (9)
CF_3, δ–a	527 (0.17)	534.5 (0.55)
E–CF_3, ν_2	310 (5)	471 (0.5)
E–CF_3, ν_1	295 (2)	436 (9.0) 432 (8.0)
P–CF_3, ρ	...	308 (2.0)

[a] Relative intensities are in parentheses after the frequencies (cm^{-1}). Abbreviations: sh = shoulder; ν = stretching (arbitrary subscripts show different phase patterns); δ = deformation; ρ = rocking; a = asymmetric; e = symmetric; E = As or P.

Formula. The molecular formula $(CF_3As)_4$ agrees with the observed vapor-phase molecular weight: found, 579; calcd, 576. The 27 strongest mass spectrum peaks[6] all were assignable to fragments containing no atoms other than As, C, and F. The three highest *m/e* ratios (with parenthetical relative intensities on an arbitrary scale) were assignable as $(CF_3As)_4^+$ = 576 (58), $(CF_3)_3As_4^+$ = 507 (107), and $(CF_3)_2As_4F^+$ = $(CF_3)_3As_2(CF_2)_2^+$ (?) = 457 (30), confirming the As_4 group. Others were 362 (10), 324 (34), 318 (20), 312 (4), 299 (29), 274 (37), 268 (10), 262 (12), 243 (11), 231 (10), 225 (42), 219 (57), 219 (57), 181 (29), 175 (8), 169 (17), 150 (81), 144 (13), 137 (8), 125 (12), 113 (55), 106 (16), 94 (30), 75 (40), 69 (60), and many weaker peaks.

Infrared Spectrum. The infrared spectrum of $(CF_3As)_4$ vapor is compared with that of $(CF_3P)_4$ in Table II, showing some remarkable similarities; even the assignments of overtones and combinations are parallel. The samples were in heated cells with very thin KBr windows, permitting observation by the Beckman IR7 instrument (with NaCl or CsI optics) down to 275 cm^{-1}, at pressures as high as 100 mm.

Other peaks, possibly assignable when the lower range fundamentals are known, were as follows: for $(CF_3As)_4$, 1376 (0.05), 1353 (0.04), 1322 (0.06), 1285 (0.21), 1226 (1.2), 1197 (2.5), 1090 (12), 1057 (3.3), 1039 (2.6), 1024 (1.8), 993 (0.17), 939 (0.02), 904 (0.02), 850 sh (0.05), 820 (0.09), 785 (0.05), 606 (0.13), and 557 (0.07); for $(CF_3P)_4$, 1485 sh (0.06), 1406 (0.07), 1395 (0.08), 1381 (0.10), 1313 (0.22), 1289 (1.1), 1251 (0.8), 1213 (2.2), 1194 (3.6), 1106.5 (5), 1085 (3), 1069 sh (1.0), 1035 sh (0.12), 1017 (0.13), 994 (0.25), 973 (0.15), 915 (0.02), 901 (0.07), 879.5 (0.09), 862 (0.07), 844 (0.04), 810 (0.03), 785 (0.04), 760 sh (0.05), 702 (0.10), 680 (0.07), 584 (0.08), 569 (0.12), 510 (0.09), and 493 (0.13).

The ultraviolet spectrum of $(CF_3As)_4$ showed broad and equally high peaks at 2240 (ε 3900) and 1970 A

---

(1) The major part of this research was performed at the University of Southern California by A. H. Cowley, who wishes to thank the University Research Institute of the University of Texas for financial support during the Fall Semester, 1965. The experimental facilities and supplemental work by Burg were supported by the National Science Foundation through Grant No. GP-3812. The initial synthesis from CF_3AsI_2 and mercury was done by Cullen at the University of Cambridge in 1958; a sample of the mixed product which he made from CF_3AsCl_2 and mercury at the University of British Columbia in 1961 was proved to be the same as that obtained independently at the University of Southern California.

(2) J. H. Burns and J. Waser, *J. Am. Chem. Soc.*, **79**, 859 (1957).

(3) K. Hedberg, E. W. Hughes, and J. Waser, *Acta Cryst.*, **14**, 369 (1961); S. E. Rasmussen and J. Danielson, *Acta Chem. Scand.*, **14**, 1862 (1960).

(4) R. I. Wagner and A. B. Burg, *J. Am. Chem. Soc.*, **75**, 3870 (1953).

(5) Similar in principle to that described by A. B. Burg and R. Kratzer, *Inorg. Chem.*, **1**, 726 (1962).

(6) Observed by means of the CEC 21-102 mass spectrometer at the University of Texas. The range scanned was *m/e* 15–600, with the heated-inlet system left at room temperature.