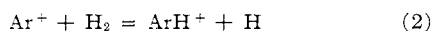


taken together with the ratio^{11,12} $k_1^{\text{Ar}}/k_1^{\text{O}_2} \cong 0.6$, give $k_1 = 0.7$ to 1.0×10^{14} (cc. mole⁻¹)² sec.⁻¹. Recent direct measurements by Kaufman and Kelso¹² give $k_1 = 1.5 \times 10^{14}$ (cc. mole⁻¹)² sec.⁻¹ and by Clyne *et al.*,¹³ give $k_1 = 2.0 \times 10^{14}$ (cc. mole⁻¹)² sec.⁻¹. The value obtained in these preliminary pulse radiolysis investigations is in reasonable agreement.

Reaction 1 is also being studied in the system Ar-N₂O-O₂. The uncertainties are presently greater in this case, and a value for k_1 has not yet been determined. A complete account of all the work involving molecular oxygen will be presented in the near future.

The method offers considerable promise as a fast reaction technique for the investigation of atomic reactions in the gas phase. Thus, in a manner similar to the oxygen study, a microsecond burst of hydrogen atoms may be obtained by reaction 2 and the



subsequent kinetics followed where optically absorbing products are formed.

Acknowledgment.—We are grateful to Dr. F. Kaufman for his comments, for providing us with his own recent unpublished results for k_1 , and for bringing to our attention other recent studies which have not yet appeared in print.

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Azo Coupling of Aryldiazonium and B₁₀H₁₀⁻² Ions

Sir:

Recent studies have shown the polyhedral B₁₀H₁₀⁻² ion to be a very reactive substrate in apparent electrophilic substitution reactions. Examples of halogenation,¹ alkylation,^{1a} nitrosation,² and deuteration³ have been presented. We wish to report the extremely facile azo coupling reaction which occurs between a variety of aryldiazonium ions and the B₁₀H₁₀⁻² ion. The resulting products are highly colored dyes formally related to the well-known azo dyes and as such are monosubstituted B₁₀H₁₀⁻² ions. Crystalline products have been isolated and characterized using unsubstituted, *p*-CH₃-, *p*-Br-, *p*-CH₃O-, *p*-NO₂-, *m*-NO₂-, *m*-CH₃O-, and *m*-CF₃-benzenediazonium tetrafluoroborates or hexafluorophosphates. In a typical preparation, 0.050 mole of *p*-BrC₆H₄N₂BF₄ dissolved in 50 ml. of acetonitrile was added to 0.050 mole of K₂B₁₀H₁₀ dissolved in 1 l. of acetonitrile at -35°. A yellow solution resulted which rapidly became purple. This solution was warmed to room temperature and concentrated to about 25 ml. *in vacuo*, whereupon 0.052 mole of KBF₄ was isolated by filtration. The filtrate was passed through a column of acid-washed alumina, concentrated to near dryness, and dissolved in dilute

(1) (a) W. H. Knoth, H. C. Miller, D. C. England, G. W. Parshall, E. L. Muettterties, and J. C. Sauer, *J. Am. Chem. Soc.*, **84**, 1056 (1962); (b) W. H. Knoth, H. C. Miller, and E. L. Muettterties, *Inorg. Chem.*, **3**, 159 (1964).

(2) W. H. Knoth, J. C. Sauer, H. C. Miller, and E. L. Muettterties, *J. Am. Chem. Soc.*, **86**, 115 (1964).

(3) E. L. Muettterties, J. H. Balthis, Y. T. Chia, W. H. Knoth, and H. C. Miller, *Inorg. Chem.*, **3**, 444 (1964).

aqueous mineral acid. The addition of an aqueous solution of tetramethylammonium chloride precipitated the tetramethylammonium salt of the monoprotonated azo coupling product. *Anal.* Calcd. for C₁₀H₂₆N₃B₁₀Br: C, 31.90; H, 6.96; N, 11.16; B, 28.74; Br, 21.23; equiv. wt., 376. Found: C, 32.14; H, 7.05; N, 11.05; B, 28.60; Br, 21.20; equiv. wt., 370. When the precipitation with tetramethylammonium chloride was carried out in basic solution, the tetramethylammonium salt of the substituted B₁₀H₁₀⁻² ion was obtained. *Anal.* Calcd. for C₁₄H₃₇N₄B₁₀Br: C, 37.40; H, 8.30; N, 12.46; B, 24.07; Br, 17.78; equiv. wt., 450. Found: C, 37.14; H, 8.25; N, 12.16; B, 23.92; Br, 17.54; equiv. wt., 446.

Attempts to carry out the coupling reactions in aqueous solution led to the rapid precipitation of yellow solids upon mixing the two reactants. After isolation and drying, these yellow solids detonated with considerable brisance. At the present time these materials are considered to be water-insoluble aryldiazonium salts which contain the B₁₀H₁₀⁻² anion. When added to acetonitrile or acetone, these salts dissolved and generated the corresponding protonated azo coupling products which were isolated as their tetramethylammonium salts in the usual way.

The ArN=NB₁₀H₉⁻² species and their conjugate acids are reversibly interconverted by the addition of acid or base, respectively. The pK_A's associated with this acid-base equilibrium were determined for the eight available compounds by a spectrophotometric method in aqueous buffer solutions.⁴ A fairly linear function of the Hammett σ -constant was obtained from these data with a ρ -value of 1.7 for dissociation of the conjugate acids.

The ¹¹B n.m.r. spectra of the ArN=NB₁₀H₉⁻² species and their conjugate acids were obtained at 19.3 and 32 Mc./sec. In both series only apical substitution was observed. In the conjugate acid form, the additional proton is not coupled to the substituted boron atom. This, coupled with the similarity in the ρ -value determined in this study to the ρ -value obtained in the corresponding equilibria with monosubstituted azobenzenes⁵ (2.2) and with the similar shifts observed in the ultraviolet absorption spectra of azobenzene⁶ (321 to 420 m μ) and C₆H₅N=NB₁₀H₉⁻² (341 to 453 m μ) upon acidification, suggests that protonation of species of the latter type occurs on nitrogen. The greater basicity of ArN=NB₁₀H₉⁻² species relative to azobenzene (by about 8 pK units) is consistent with the enhanced basicity of B₁₀H₉N(CH₃)₂^{-2(1a)} relative to N,N-dimethylaniline.⁷

The coupling reaction is probably best described as an electrophilic substitution reaction on B₁₀H₁₀⁻² to form ArN=NB₁₀H₉⁻² and 1 equiv. of hydrogen ion followed by protonation of the coupling product. Of additional interest is the fact that the B₁₀H₁₀⁻² and B₁₀D₁₀⁻² ions show no kinetic isotope effect in a competitive coupling reaction with *p*-methoxybenzenediazonium ion. Apparently some step prior to B-H bond-

(4) A. Albert and E. P. Serjeant, "Ionization Constants of Acids and Bases," John Wiley and Sons, Inc., New York, N. Y., 1962, Chapter 4.

(5) H. H. Jaffé and R. W. Gardner, *J. Am. Chem. Soc.*, **80**, 319 (1958); see also S. J. Yeh and H. H. Jaffé, *ibid.*, **81**, 3279 (1959); J. H. Collins and H. H. Jaffé, *ibid.*, **84**, 4708 (1962).

(6) H. H. Jaffé, S. J. Yeh, and R. W. Gardner, *J. Mol. Spectry.*, **2**, 120 (1958).

(7) N. F. Hall and M. R. Sprinkle, *J. Am. Chem. Soc.*, **54**, 3469 (1932).

breaking is irreversible⁸ or, in the case of a single transition-state process, bond breaking has not occurred to an extent which may be detected by this means.

In contrast to the extremely rapid reactions of $B_{10}H_{10}^{-2}$, $B_{12}H_{12}^{-2}$ does not react with aryldiazonium ions in acetonitrile.

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(8) L. Melander, "Isotope Effects on Reaction Rates," The Ronald Press Co., New York, N. Y., 1960, p. 103.

(9) Alfred P. Sloan Research Fellow.

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Exchangeable Hydrogen in Chlorophyll and the Path of Hydrogen in Photosynthesis¹

Sir:

Whether labile hydrogen atoms in chlorophyll are involved in photosynthesis is an important question that has attracted much attention but which has not received an unambiguous answer.² The recent demonstration by the method of proton magnetic resonance that chlorophylls *a* and *b* in solution have readily exchangeable hydrogen atoms at the C-10 and δ -methine positions³ has aroused interest in the possibility that an exchange of hydrogen between water and chlorophyll, particularly at the δ position, occurs during photosynthesis. The availability of fully deuterated photosynthetic organisms⁴ now permits a new approach to these questions. Hydrogen incorporated into the chlorophyll of fully deuterated organisms either by chemical exchange or by metabolism can be readily detected by proton magnetic resonance and, because the proton resonances in chlorophyll have been assigned,⁵ the location of the incorporated hydrogen can be determined.

In a typical experiment, fully deuterated *Scenedesmus obliquus* grown in 99.7% D_2O ⁶ with CO_2 as the sole carbon source was harvested by centrifugation and immediately resuspended in fresh H_2O medium.⁴ The resuspended cells were then allowed to continue photosynthesis, receiving 5% CO_2 in nitrogen and a light intensity of 1100 ft.-candles. After 16 hr., the

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

(2) B. Coleman and W. Vishniac, *Natl. Acad. Sci.-Natl. Res. Council, Publ.*, No. 1145, 213 (1963); W. Vishniac and I. A. Rose, *Nature*, **182**, 1089 (1958); W. Vishniac, "Chemical Participation of Chlorophyll in Photosynthesis," in "Comparative Biochemistry of Photoreactive Systems," M. B. Allen, Ed., Academic Press, New York, N. Y., 1960, pp. 377-386; J. W. Weigl and R. Livingston, *J. Am. Chem. Soc.*, **74**, 4211 (1952); T. H. Norris, S. Ruben, and M. B. Allen, *ibid.*, **64**, 3037 (1942).

(3) J. J. Katz, R. C. Dougherty, F. C. Pennington, H. H. Strain, and G. L. Closs, *ibid.*, **85**, 4049 (1963); R. B. Woodward and V. Škarić, *ibid.*, **83**, 4676 (1961); J. H. Mathewson, W. R. Richards, and H. Rapoport, *Biochem. Biophys. Res. Commun.*, **13**, 1 (1963).

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

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

(6) H. F. DaBoll, H. L. Crespi, and J. J. Katz, *Biotech. Bioeng.*, **4**, 281 (1962).

algae were again harvested, and the chlorophylls extracted and purified by conventional means.⁴ Each chlorophyll was dissolved in deuterioacetone (0.05 *M*), and the proton magnetic resonance spectrum was recorded.⁷ The p.m.r. spectra showed only a sharp resonance at 6.24 p.p.m. for chlorophyll *a* and 6.22 p.p.m. (relative to TMS as an internal standard) for chlorophyll *b*. These resonances correspond exactly to those assigned to the C-10 proton in ordinary chlorophyll *a* and *b*.⁵ No other proton resonances could be detected, and, in particular, no evidence could be found for the introduction of hydrogen at the δ position. The resonances of the 7 and 8 protons are broad and would be broadened even more by deuterium coupling. Small amounts of hydrogen at these sites are therefore difficult to detect. However, the spectra show no evidence of hydrogen at the 7 and 8 positions. As shown by the n.m.r. observations, chlorophyll synthesis *de novo* in a period of 16 hr. was less than 1%.

Algae allowed to stand 16 hr. in the dark also show hydrogen at the C-10 position. However, the normal extraction and purification procedure is found to introduce hydrogen into this position. Pending the development of other separation procedures, it cannot be decided whether exchange at C-10 is a light or a dark reaction, or whether it is a result of induced exchange during purification. It appears quite certain, however, that none of the other hydrogen atoms in the tetrapyrrole nucleus is involved in a reversible dehydrogenation-hydrogenation cycle with water. Specifically, the present results appear to be inconsistent with recent suggestions that δ -nucleophilicity is involved in photosynthesis.⁸ Furthermore, if chlorophyll undergoes any chemical reaction in photosynthesis, it must do so without affecting the exchangeability of the hydrogen atoms.

When algae are transferred from H_2O to D_2O , severe, if not complete, inhibition of photosynthesis occurs. It is important for the present purpose to demonstrate that a similar inhibition does not occur when the algae are transferred from D_2O to H_2O .

Three-milliliter aliquots of stock cultures of deuterio *S. obliquus* were centrifuged, resuspended in H_2O medium, and allowed to photosynthesize in a Warburg apparatus under the same conditions as used for the exchange experiments. In a typical run the rate of oxygen evolution during the first hour was 4.7 μ moles/min. as compared to 2.1 μ moles/min. for the same culture in D_2O medium. By the 18th and 24th hours the rate of oxygen evolution had dropped to 1.3 μ moles/min., and oxygen production at approximately this rate continued for more than 2 days. Thus, photosynthesis was not significantly slowed or interrupted by transfer of these organisms from D_2O to H_2O , and the number of moles of oxygen produced in 16 hr. per mole of chlorophyll in the sample was roughly 2×10^3 as a lower limit. The closely related green alga *Chlorella vulgaris* suffers severe inhibition of photosynthesis when transferred from D_2O to H_2O , and consequently our experiments were restricted to *S. obliquus*.

Proton magnetic resonance is not a very sensitive procedure, and hydrogen turnover in less than 5% of the chlorophyll would probably not be detected. If hydro-

(7) Measured on a Varian HR-100 n.m.r. spectrometer.

(8) J. H. Mathewson, Abstracts, 146th National Meeting of the American Chemical Society, Denver, Colo., Jan., 1964, p. 16A.