[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY, INSTITUTE OF TECHNOLOGY, UNIVERSITY OF MINNESOTA]

An Attempt to Detect Hydrogen–Deuterium Exchange between Chlorophyll and Water[1]

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Neither chlorophyll-a nor pheophytin-a undergo detectable hydrogen–deuterium exchange when they are allowed to stand in the dark in neutral organic solvents containing an excess of deuterium oxide.

Chlorophyll-a contains 72 hydrogen atoms, at least one of which has generally been considered to be "labile." Fischer and Goebel[2] found that in pyridine solution ethyl chlorophyllide has one hydrogen which reacts with Grignard reagent. By examining a number of related compounds they were able to demonstrate that it is the one located at carbon 10, for it turned out to be replaceable only when there was also a carboxyl substituent at position 10 and a ketone group at carbon 9. These conditions were considered to favor enolization of the ketone and consequent labilization of hydrogen 10 in the presence of base. In those compounds in which the central metal ion had been replaced with amine hydrogens, the latter were likewise found to give a positive Zerewitinoff test; here again the strongly basic conditions undoubtedly aided the dissociation of hydrogens. Hydrogen 10 appears to be readily lost in oxidative reactions, such as the allomerization[3] of chlorophyll.

The only published experiment on the lability of the hydrogens of chlorophyll in *neutral* solvents appears to be one described by Norris, Ruben and Allen[4]: a mixture of purified chlorophylls-a and -b was dissolved in 80% ethanol labeled with tracer amounts of tritium, and illuminated for 30 minutes. The fact that the chlorophyll took up less than 5% of the tritium expected for one exchangeable hydrogen per molecule could be explained either by a lack of exchangeable hydrogens or by a twenty-fold isotope discrimination in the equilibrium. The authors proposed repetition of the experiments with deuterium oxide of sufficient concentration to make isotope effects insignificant. Prior to the present communication, no such experiments have been reported.[5]

Experimental

Both purified chlorophyll-a and pheophytin-a were investigated. Since only a limited quantity of concentrated D_2O was available, the pigment was dissolved in an aprotic solvent and brought into contact with an excess of heavy water. Table I lists the experimental conditions. During subsequent operations the samples were kept at or below room temperature. In those experiments in which the organic solvent was immiscible with water, good phase contact was established by a large interface, as well as by periodic shaking of the vessel. The solubility of water in these solvents was sufficient to ensure a large molal excess of exchangeable deuterium over pigment present in the organic phase.

All samples were handled in dim light. Contact with atmospheric moisture was minimized by keeping the vessels glass-stoppered and wrapped with Parafilm, and by using hypodermics, instead of pipets. Volume reductions were carried out by rapid boiling on a vacuum still; a fine stream of helium bubbles assured continuous boiling. Suction was applied by aspirator through a Drierite tube, or, more usually, by a mechanical pump through a Dry Ice or liquid nitrogen trap. Volumes less than about 1 ml. were reduced under a jet of tank helium.

After exchange, sample 1 was transferred to 0.5 ml. of benzene by a series of evaporations and solvent additions. Samples 5b and 6a were similarly transferred to CCl_4. Samples 2, 3, 4, 5a and 6b were dried by pumping through a cold trap; all but sample 4 were evacuated further for periods of 14 to 24 hours. Each was then dissolved in a small volume of CCl_4.

In experiments 1, 2 and 3 the chlorophyll solution was distributed directly into combustion boats, dried under a stream of helium gas, and swept with helium for an additional 15–30 minutes. Due care was taken to prevent contact with the atmosphere before the material was dry and to minimize it afterwards. After experiments 4, 5 and 6 it was necessary to store the dyes for about six months prior to combustion; concentrated CCl_4 solutions were drawn into capillary tubes and the latter sealed off and stored in the dark at 0°. Absorption spectra of all samples were checked on a Beckman quartz spectrophotometer: even the spectra of pigments from runs 5a, 5b and 6 stored six months in CCl_4, showed no band shifts and only minor relative intensity changes. The bright red fluorescence of the dyes remained qualitatively unchanged. No effort was made to account for all material quantitatively, but evidently only minor decomposition took place. It is, hence, not likely that a significant fraction of hydrogen was lost selectively from the material prior to combustion.

The samples were burned in a combustion train which will be described in detail elsewhere.[6] Each sample of about 1 mg. yielded approximately 1 cc. (S.T.P.) of hydrogen.

After each exchange run, a sample of pigment was transferred to about 0.3 ml. of CCl_4 and pipetted into a 200-micron thick cell with rocksalt windows. The hydrogen-stretching region of the infrared spectrum was examined by means of a Perkin-Elmer 12 C spectrophotometer with a NaCl prism. The solution could be recovered without contact with the atmosphere. The spectra of samples 1–6 revealed no bands ascribable to deuterium or to decomposition products.

Reagents and Solvents.—The heavy water used contained 81.3% D/(D + H), as shown by mass analysis. Acetone was treated with NaI and redistilled,[7] dioxane was purified with HCl, neutralized and distilled with sodium,[8] ether was redistilled from sodium, and reagent grade benzene had been dried with sodium wire. Other chemicals were of "reagent grade." The preparation of chlorophyll has been described elsewhere.[9] Normal pheophytin-a was prepared by the action of dilute H_2SO_4 on chlorophyll-a in ether[10] and purified by adsorption on a column of paper pulp followed by selective elution with benzene.

Deuterated pheophytin-a was prepared by allowing 0.221 mmole of 0.114 *N* HCl containing 78.8% D/(D + H) to stand in contact with 3.07×10^{-2} mmole of chlorophyll-a

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(2) H. Fischer and S. Goebel, *Ann.*, **522**, 168 (1936).

(3) E. I. Rabinowitch, "Photosynthesis," Vol. I, Interscience Publishers, Inc., New York, N. Y., 1945, p. 459.

(4) T. H. Norris, S. Ruben and M. B. Allen, THIS JOURNAL, **64**, 3037 (1942); Table II, experiment 1.

(5) A. W. Frenkel (private communication) has performed two such experiments: chlorophylls-a and -b were allowed to exchange for 30 minutes at 23° with acetone-D_2O (4:1). No significant uptake of deuterium was noted.

(6) A. H. Brown, to be published.

(7) R. S. Livingston, THIS JOURNAL, **69**, 1220 (1947).

(8) K. Hess and H. Frahm, *Ber.*, **71**, 2627 (1938).

(9) R. Livingston, W. Watson and J. McArdle, THIS JOURNAL, **71**, 1541 (1949).

(10) By a procedure based on that of G. Mackinney and M. A. Joslyn, *ibid.*, **60**, 1132 (1938).

TABLE I

EXPERIMENTAL CONDITIONS AND RESULTS

Expt. No.	Compound	Mmoles cpd. × 10^3	Organic solvent	Volume, ml.	D_2O added, ml.	Exchg. time, hours	% D calcd. for one exchg. H/mol.	% D found ± av. dev. of mass analyses	No. of mass analyses[d]
1	Chl.-a	27.2	Ether	10.0	2.0	3.25	1.13	0.0333 ± 0.0022	5
2	Chl.-a	10.4	Acetone[a]	23.0	2.6	2	1.13	.0817 ± .0069	4
3	Chl.-a	7.3	Dioxane[a]	11.0	2.0	2	1.13	.1045 ± .019	4
4	Chl.-a	5.6	Dioxane[a]	8.5	2.0	3	1.13	.100 ± .010	4
5	Chl.-a	11.0	Acetone[b]	11.0	0.50	24	0.92	[a] .190 ± .030	3
								[b] .190 ± .015	3
6	Pheo.-a	18.3	Benzene	133.0	1.0	5	1.13	[a] .031 ± .005	3[e]
								[b] .0235 ± .003	3[e]
7*a*	Pheo.-a	(Not exchanged)						.635 ± .02	4[e]
7*b*	Pheo.-a	3.7	Ether	1.0	2.0[c]	2.5	0.017[f]	.667[g] ± .02	4[e]
7*c*	Pheo.-a	3.7	Acetone[a]	7.0	2.0[c]	3	0.017[f]	.598 ± .03	4[e]
7*d*	Pheo.-a	(Not exchanged)						.593 ± .05	5
7 (average)								.623 ± .037	

[a] Plus 5 to 10% of dry ether. [b] Plus 10% ether and 0.1 ml. of concd. NH_3 (1.5×10^{-2} *N*). [c] *Normal* water in experiment 7. [d] In addition, other samples were combusted and discarded prior to those collected, in order to clean out the combustion train. [e] Includes duplicate analysis of one sample; in runs 7b and 7c, of two samples. [f] D content of normal water; calcd. for 2 exchangeable H's per molecule pheophytin. [g] Two subsequent combustions yielded only 0.408; however, the combustion temperature was accidentally too low and the conversion to hydrogen probably incomplete.

in 5 ml. of ether. No pheophytin was formed even after 18 hours at 38°; however, the reaction went to completion in less than 15 minutes after 3 ml. of acetone had been added to merge the phases. After an additional hour standing, the solution was neutralized with 0.40 mmole of anhydrous K_2CO_3 and centrifuged to permit separation of the organic layer from the water and excess salt.

The sample was tested for loss of deuterium by exchange with *normal* water. The solvent was pumped off through a liquid air trap, and the dry pheophytin was evacuated for 5 hours. It was taken up in 5.0 ml. of ether and the solution was divided into four portions: (a) 1.0 ml. was evaporated to 0.2 ml. under helium; the pheophytin was dried onto a NaCl plate and examined by means of the infrared spectrometer. The sample was recovered in CCl_4 without opportunity to exchange with H_2O. (b) A second 1.0-ml. portion was allowed to stand in contact with 2.0 ml. of *normal* distilled water at 31°. After 2.5 hours the layers were separated and the pheophytin was transferred to 1 ml. of CCl_4. (c) 1.0 ml. of the ether solution was evaporated to small volume; 2.0 ml. of *normal* distilled water and 7.0 ml. of acetone was added, forming a single phase. After three hours exchange at 31° the solution was evaporated under helium until the pheophytin began to precipitate in the residual water. Four ml. of CCl_4 was added; the remaining acetone was extracted with distilled water. The phases were finally separated by centrifugation. (d) The remaining 2.0 ml. of solution was analyzed without exchange. Each of these samples was evaporated onto combustion boats, burned, and analyzed for deuterium.

Results

Table I shows that when chlorophyll-a or pheophytin-a, dissolved in aprotic solvents, was allowed to stand in contact with heavy water for from two to five hours, the pigments retained only 2 to 10% of the deuterium expected from the equilibration of one hydrogen per molecule with the water. This means that, within the error of our analysis, no deuterium was taken up. Contamination of the pigment with 0.02 to 0.1 mole per cent. of a deuterated impurity would account for the small amounts of tracer which were found in the samples.[11]

The results for pheophytin were checked by allowing imine-deuterated pheophytin to stand in contact with an excess of normal water (experiment 7). No significant fraction of the isotope was lost. This result confirms the validity of the preceding experiments, for here there was no opportunity for spurious gain of deuterium, as there had been previously for accidental losses. At the same time, it rules out isotope discrimination as the explanation for the observed lack of exchange.

It is interesting to compare the isotopic composition of the deuterated pheophytin, as originally prepared, with that of the acid used to form it. A total of 154.5 mmoles of D and 41.6 mmoles of H(78.8% D) was added to 3.07×10^{-2} mmole of chlorophyll. Isotope effects neglected, the resultant pheophytin should have contained 2 × 78.8/74, or 2.13% D. The 0.62% actually found was only 30% of the expected equilibrium concentration. This value appears too small to be due to an *equilibrium* isotope effect: it must rather arise from *kinetic* discrimination during pheophytinization.[12] This implies that the pheophytin, once formed, cannot exchange its imine hydrogens, even with an acidic medium. Unfortunately we had no opportunity to confirm this point.

Discussion

The fact that chlorophyll in neutral solution has no exchangeable hydrogens bears not only on the chemistry of the pigment itself but also on reactions photosensitized by it. Tracer experiments on photosynthesis[4,13] and on the chlorophyll-sensitized reduction of an azo dye by ascorbic acid in dioxane[14] have shown that chlorophyll fixes no isotopic hydrogen in *non-labile* positions while participating in these reactions. We may now generalize these results by adding that hydrogen cannot have been picked up in *labile* positions and subsequently lost.[15] Therefore, for at least one photo-

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[11] The 0.19% D fixed in experiment 5 may be significant, indicating that there is a slow exchange in basic solution.

[12] This explanation is consistent with the finding of Mackinney and Joslyn (THIS JOURNAL, **60**, 1132 (1938), **62**, 230 (1940), **63**, 2530 (1941)) that the rate of pheophytinization is proportional to (H^+) and that the rate determining step is probably the entrance of the first hydrogen ion (see Rabinowitch, ref. 3, p. 467).

[13] S. Aronoff, *Bot. Reviews*, **16**, 525 (1950).

[14] J. W. Weigl and R. Livingston, THIS JOURNAL, **74**, 4211 (1952).

[15] For the case of photosynthesis we must assume that the hydrogens of chlorophyll are not "activated" by its protein or other linkages. The similarity of its spectrum *in vivo* and in solution makes this assumption reasonable.

sensitized reaction in solution and probably for photosynthesis *in vivo*, it is possible to rule out one mode of action: primary oxidation of the chlorophyll by the oxidant, followed by its reformation by the reductant.

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Reactions of Hydrocarbons Induced by Alkyl Fluoride Boron Trifluoride. IV. Stoichiometry of the Self-alkylation of Isobutane

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The self-alkylation of isobutane at −80°, as promoted by isopropyl fluoride and boron trifluoride, has been quantitatively investigated. The equation is: $BF_3 + 2i\text{-}C_3H_7F + 4i\text{-}C_4H_{10} \xrightarrow{-80^\circ} 2C_8H_{18} + 2C_3H_8 + BF_3\cdot 2HF$. More than 0.5 mole of boron trifluoride per mole of isopropyl fluoride produces no additional reaction. The octanes produced are principally 2,2,4-trimethylpentane plus other trimethylpentanes. Very small amounts of hydrocarbons other than octanes are formed. A possible explanation of the observed stoichiometry is given.

Introduction

Under the influence of an alkyl fluoride and boron trifluoride, isoparaffins undergo a self-alkylation reaction in which two or more molecules of isoparaffin unite to form a higher molecular weight saturated hydrocarbon.[1] The experiments described here were performed to study the reaction quantitatively and to elucidate some points concerning the mechanism of the reaction. In order to eliminate side reactions, these experiments were performed at −80°. The particular reactants chosen were boron trifluoride, isopropyl fluoride and isobutane.

The sequence of reactions which occurs is believed to be[1,2]

$$i\text{-}C_3H_7F + BF_3 \longrightarrow i\text{-}C_3H_7^{\oplus} + BF_4^{-} \tag{1}$$

$$i\text{-}C_3H_7^{\oplus} + i\text{-}C_4H_{10} \longrightarrow C_3H_8 + t\text{-}C_4H_9^{\oplus} \tag{2}$$

$$t\text{-}C_4H_9^{\oplus} \rightleftarrows i\text{-}C_4H_8 + H^{+} \tag{3}$$

$$t\text{-}C_4H_9^{\oplus} + i\text{-}C_4H_8 \longrightarrow C_8H_{17}^{\oplus} \tag{4}$$

$$C_8H_{17}^{\oplus} + i\text{-}C_4H_{10} \longrightarrow C_8H_{18} + t\text{-}C_4H_9^{\oplus} \tag{5}$$

i-Propyl fluoride was chosen for this investigation rather than *t*-butyl fluoride so that it would be possible to distinguish the paraffin formed in equation 2 by saturation of the carbonium ion from the original isobutane charged. If *t*-butyl fluoride were used, there would be no way to prove conclusively whether the alkyl fluoride participated directly in the reaction, or not. Reaction 2 is not reversible because the propane formed contains no tertiary hydrogen to undergo hydrogen exchange. Carbonium ions do not react with normal paraffins under the conditions of these experiments.[1]

This conception of the mechanism leaves unexplained the relation between the amount of boron trifluoride charged and the total amount of reaction obtained. It was primarily to investigate this aspect of the reaction that the present work was undertaken.

Experimental

Apparatus.—The apparatus used consisted of a gas handling system in calibrated sections of which the reagents and gaseous products were measured, a low-temperature reactor in which the reaction was conducted and a low-temperature fractionating column for separation of the gaseous products from the normally liquid products.

All of the reactions at low temperatures were performed in a glass reactor which consisted of a cylindrical vessel surrounded by a jacket in which liquid nitrogen was vaporized. By this means the temperature could be reduced well below −100°. This jacket in turn was completely surrounded by an evacuated jacket for heat insulation. The reactor was attached by means of a 45/50 standard taper joint to the top, through which a thermocouple well, a stirrer and a gas inlet tube entered the reactor. A vacuum-jacketed reflux condenser, cooled with solid carbon dioxide, was sealed to the vent from the reactor and was followed by a trap which could be cooled with solid carbon dioxide or liquid nitrogen and an absorption tube filled with sodium calcium hydrate.

The reactor was also used as the stillpot on the low-temperature distillation column in which the gaseous products were distilled from the liquid reaction products. For this purpose, hot air was passed through the jacket of the reactor to boil the charge.

Reagents.—The isobutane used was supplied by the Matheson Company and was C.P. grade, 99% pure or better. It was used without further purification. The boron trifluoride was also supplied by the Matheson Company and was claimed to be 97% pure or better. It was used as received.

The isopropyl fluoride was prepared by adding hydrogen fluoride to propylene according to the procedure described by Grosse and Linn.[3] The product was distilled, discarding the first and last 10%. No propylene could be detected by infrared analysis in the fraction retained for use.

Technique.—The reactor was attached to the high-vacuum system and the isobutane and *i*-propyl fluoride were measured as gases in calibrated sections of the apparatus and were then condensed, in the reactor. The boron trifluoride was measured as a gas and was forced by mercury displacement into the liquid reaction mixture at −80°.

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(1) A. Schneider and R. M. Kennedy, THIS JOURNAL, **73**, 5017 (1951).

(2) P. D. Bartlett, F. E. Condon and A. Schneider, *ibid.*, **66**, 1531 (1944).

(3) A. V. Grosse and C. B. Linn, *J. Org. Chem.*, **3**, 26 (1939).