to a decrease in the vertical dipole moment. The dipole moment of calcium stearate in the monolayer is not known and so this decrease cannot be calculated. It is worth noting, however, that since the monolayer plates as calcium stearate monohydrate, it is likely that some water molecules must be strongly bound by the monolayer. The contribution of the dipole moment of these bound water molecules might play an important role in reducing the over-all vertical dipole moment and thus the surface potential.

The finding that calcium stearate monohydrate is present in the plated multilayer is of great interest in accounting for some aspects of the behavior of multilayer electrodes. Calcium stearate multilayers have been used as electrodes which are specific for calcium ions under certain conditions.[25] These multilayers have a specific conductance of 10^{-4} ohm.$^{-1}$ cm.$^{-1}$ at 25°, one which is much higher than would be anticipated. Sodium stearate,[26] for example, has a specific conductance of 7.2×10^{-11} ohm^{-1} cm.$^{-1}$ at 170°. However, the presence of 1% water in sodium stearate[27] increases the specific conductance by a factor 10^3. It seems very likely, therefore, that the presence of water of hydration in the calcium stearate multilayer is in part responsible for the specific conductance observed.

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(26) G. Manecke, *Z. physik. Chem.* (Leipzig), **195**, 354 (1950).

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Photoreduction of Ethyl Chlorophyllide *a* by Ascorbic Acid in Ethanol–Pyridine Solutions

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Quantum yields for the photochemical reduction of ethyl chlorophyllide *a* by ascorbic acid in ethanol–pyridine solutions have been determined. According to a proposed mechanism, photoreaction occurs principally between the pyridinium ascorbate ion pair and the chlorophyllide solvates Chl(eth)$_2$ and Chl(eth)(py). Pyridine acts not only to neutralize ascorbic acid, but also, in the form of pyridinium ion, to stabilize the immediate products of photoreaction by transferring hydrogen. The photoreaction is inefficient, the maximum quantum yield of about 0.02 being attained only when the ascorbic acid concentration approaches 0.1 *M*. When ethanol is the solvent component in excess, the reduced chlorophyllide first formed is converted by acid into a mixture of two reduced pheophorbides in equilibrium, one absorbing maximally near 525, the other near 620 mμ. The rate of regeneration of chlorophyllide or pheophorbide in the dark is apparently limited by the availability of an active form of oxidized ascorbic acid. Structures are proposed for the reduced pheophorbides and chlorophyllide. As aids to understanding the reaction the distribution of chlorophyllide among the three possible solvate species was investigated by spectral methods, and the extent of ionization and ion pair formation in ascorbic acid solutions was investigated by conductivity and indicator methods.

Introduction

The reversible photoreduction of chlorophyll and its derivatives by ascorbic acid has attracted much interest because of its possible relationship to the primary process of photosynthesis.[1,2] It is known from qualitative work that for efficient reduction to occur, an organic base such as pyridine and an "activator" such as ethanol or water must be present,[3–5] but their precise roles have not been established. Chlorophyll or pheophytin is regenerated in the dark, the latter if the reduced chlorophyll loses magnesium.[4]

We wish to present quantum yields for photoreduction at various ascorbic acid concentrations in solvents of composition varying from pure ethanol to pure pyridine and to propose a mechanism to account for the necessity of both ethanol and pyridine. Most previous work has employed water–pyridine mixtures; ethanol–pyridine mixtures have the advantages that the entire solvent composition range may be employed, and magnesium loss from reduced chlorophyll is slower. For incidental reasons ethyl chlorophyllide *a* was used instead of chlorophyll; replacement of the phytyl group of chlorophyll by an ethyl group is not expected to affect the solution photochemistry significantly, and it should be possible to make valid comparison with chlorophyll reduction as studied by others.

Experimental

Materials.—Ethyl chlorophyllide *a* was prepared from *Datura stramonium* by the method of Holt and Jacobs[6] and purified chromatographically. Ethyl pheophorbide *a* was prepared in solution as needed by treating ethyl chlorophyllide in ethanol with 0.01 ml. of concentrated HCl, excess acid being neutralized on addition of pyridine.

Ascorbic acid (Eastman) was at first used directly but later recrystallized from 1:1 ethanol–water. Some runs with unrecrystallized ascorbic acid gave evidence of the presence of a retarder; others did not, but gave the same yields as with recrystallized acid. Other organic reagents were usually recrystallized, but dehydroascorbic acid (Mann) and β-carotene (Nutritional Biochemicals) were used as received. Absolute ethanol was further dried by distillation from $Mg(OC_2H_5)_2$; pyridine was dried over BaO and distilled.

Air was removed from the reaction cells by bubbling through them prepurified nitrogen (Matheson) which had passed through

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(1) A. A. Krasnovskii, *Dokl. Akad. Nauk SSSR*, **60**, 421 (1948).

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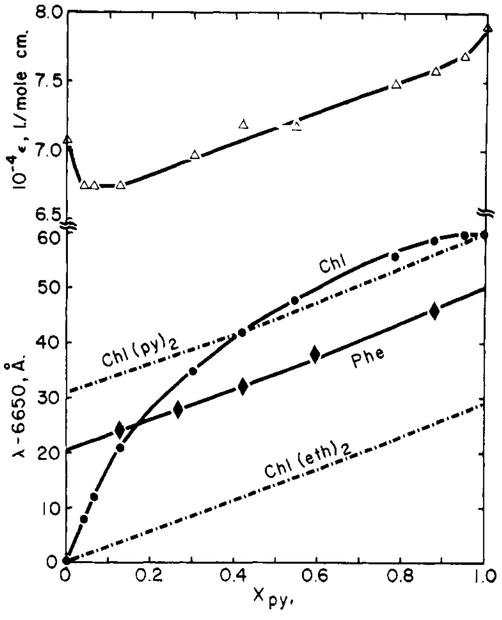

Fig. 1.—Lower part: variation of wave length of maximum absorption in the red of ethyl chlorophyllide *a* and ethyl pheophorbide *a* with mole fraction in ethanol–pyridine mixtures. Dashed lines indicate the presumed variation for particular solvate species. Upper part: variation of extinction coefficient at wave length of maximum absorption in the red of ethyl chlorophyllide *a* with solvent composition.

a solution of the sodium ketyl of benzophenone in anisole. In general, induction periods and side reactions indicative of oxygen were insignificant or absent.

Apparatus and Procedure.—Progress of the reduction was followed qualitatively on a Cary Model 14 recording spectrophotometer, adapted so that the spectrum could be recorded while the sample was irradiated. When quantum yields were determined, the progress of the reaction was followed intermittently with a Beckman DU spectrophotometer and light was measured with an Eppley thermopile. Light from a 750-watt projector lamp was made nearly monochromatic by a Baird–Atomic 6600 Å. interference filter, of band pass 90 Å.; the rate of light absorption was generally around 5×10^{-6} einstein/l. sec. All runs were made at 25°.

The quantum yield was calculated as the ratio of the rate of decrease of chlorophyllide concentration to the rate of light absorption. In runs at low pyridine concentration, pheophorbide is eventually formed by magnesium loss from reduced chlorophyllide and back oxidation. Since pheophorbide retards the photoreduction of chlorophyllide, the quantum yields recorded are for only the first one or two light intervals, before a significant amount of pheophorbide has accumulated. At high pyridine concentration, pheophorbide is not formed, but the back oxidation of reduced chlorophyllide becomes important, and therefore only the quantum yield for the first interval is reported.

All extinction coefficients for chlorophyllide and its derivatives are relative to the value 7.66×10^4 reported for chlorophyllide in acetone.[6]

Conductivity of ascorbic acid was measured with an Industrial Instruments Model RC-16B bridge.

Units and Nomenclature.—The abbreviations Chl, Phe, and AH_2 are used in equations for ethyl chlorophyllide *a*, ethyl pheophorbide *a*, and undissociated ascorbic acid; Asc represents ascorbic acid without regard to its state of ionization; $ChlH_2$ is reduced chlorophyllide; $PheH_2$ refers to reduced pheophorbide in general, while $PheH_2525$ and $PheH_2620$ are the forms with absorption maxima near these wave lengths.

The concentrations of the solvent components ethanol, pyridine, and water are expressed as mole fractions x_{eth}, x_{py}, and x_w; concentrations of solutes such as chlorophyllide and ascorbic acid are expressed as mole per liter.

Results

Solvation of Chlorophyllide.—In view of the activator effect, it seemed plausible that the formation by chlorophyllide of complexes with 1 or 2 moles of solvent[7,8] would affect its reactivity. If the magnesium of chlorophyllide is coordinated octahedrally, three solvate species are possible in ethanol–pyridine solutions: $Chl(eth)_2$, Chl(eth)(py), and $Chl(py)_2$. The distribution of chlorophyllide among these three species was estimated from the shift in the position of the red band as the solvent varies from ethanol to pyridine.

The variation of the positions of the red absorption maxima of chlorophyllide and pheophorbide with x_{py} is shown in Fig. 1. The gradual increase in peak wave length for pheophorbide reflects the increase in refractive index of the medium with increasing x_{py}, whereas in the chlorophyllide spectrum, the effect of changing complex species is superimposed on the effect of changing refractive index.[9,10]

The extinction coefficient of the red band of chlorophyllide is shown in the upper part of Fig. 1. The drop in intensity near $x_{py} = 0$ and $x_{py} = 1$ is explicable if the red band splits into two, belonging to different solvates. From $x_{py} = 0.125$ to 0.88 the extinction coefficient of pheophorbide (not shown) increases gradually from 4.70×10^4 only to 4.89×10^4, rather smaller proportionally than the increase for chlorophyllide.

The distribution of chlorophyllide among the three species was analyzed under the assumption, valid for small differences, that if a_1, a_2, and a_3 represent the fractions of chlorophyllide in the forms $Chl(eth)_2$, Chl(eth)(py), and $Chl(py)_2$, the absorption peaks of which, in a particular mixture of ethanol and pyridine, are at λ_1, λ_2, and λ_3, then the observed peak wave length λ is

$$\lambda = a_1\lambda_1 + a_2\lambda_2 + a_3\lambda_3 \qquad (1)$$

Upon introducing equilibrium constants K_1 and K_2 for the formation of Chl(eth)(py) from $Chl(eth)_2$ and of $Chl(py)_2$ from Chl(eth)(py)

$$K_1 = a_2x_{eth}/a_1x_{py} \qquad (2)$$

$$K_2 = a_3x_{eth}/a_2x_{py} \qquad (3)$$

and rearranging terms one obtains

$$\lambda - \lambda_1 = K_1(x_{py}/x_{eth})[(\lambda_2 - \lambda_1) - (\lambda - \lambda_1) + K_2(x_{py}/x_{eth})(\lambda_3 - \lambda)] \qquad (4)$$

Equation 4 was solved under the assumption that the variation of λ_1, λ_2, and λ_3 with x_{py} parallels that for pheophorbide, on grounds that the magnesium is expected to contribute little to the magnitude of the red shift with refractive index. The dashed curves in Fig. 1 give estimates for the variation of λ_1 and λ_3 with x_{py}, and with their aid the following values were obtained for the remaining unknowns.

$$\lambda_2 - \lambda_1 = 38.0 \text{ Å}. \qquad (5)$$

$$K_1 = 4.85 \qquad (6)$$

$$K_2 = 0.243 \qquad (7)$$

Evidently, the mixed solvate is the predominant species, except at the extremes of solvent composition.

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[7] S. Freed and K. M. Sancier, *J. Am. Chem. Soc.*, **76**, 198 (1954).

[8] J. R. Miller and G. D. Dorough, *ibid.*, **74**, 3977 (1952).

[9] N. S. Bayliss and E. G. McRae, *J. Phys. Chem.*, **58**, 1002 (1954).

[10] E. G. McRae, *ibid.*, **61**, 562 (1957).

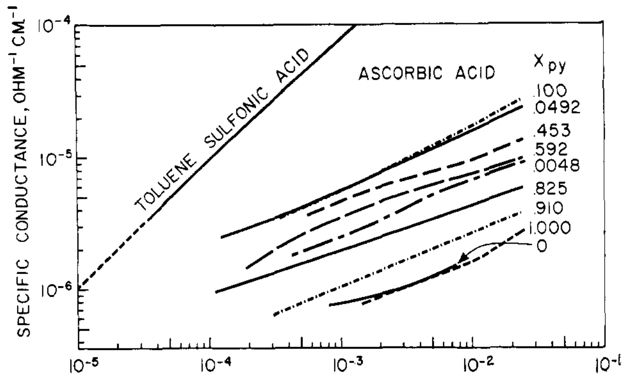

Fig. 2.—Conductance of ascorbic acid dissolved in ethanol–pyridine mixtures, compared with that of a strong electrolyte, toluenesulfonic acid. For toluenesulfonic acid, $x_{py} = 0.05$ or 0.10.

Ionization of Ascorbic Acid.—It has been claimed[11] that the role of pyridine is to make ascorbate ion, which is the true reductant. If so, there ought to be a correlation between the rate of photoreduction and the extent of ionization of ascorbic acid in ethanol–pyridine mixtures. Since ascorbic acid and pyridine are both weak, ionization is not complete and conductivity measurements were undertaken to estimate the extent of it in ethanol–pyridine mixtures.

Figure 2 shows the conductivity of ascorbic acid solutions in various mixtures of pyridine and ethanol. In all mixtures conductivity varied with concentration as a power somewhat less than one-half, typical of electrolytes that are only slightly dissociated. Since determination of the mobility of pyridinium and ascorbate ions was precluded, estimates of ion concentration were obtained from comparison with the conductivity of toluenesulfonic acid in ethanol–pyridine solutions. Toluenesulfonate ion has a size and a mass similar to those of ascorbate, and presumably a similar mobility too. The conductivity of toluenesulfonic acid solutions was the same at $x_{py} = 0.05$ and $x_{py} = 0.10$, and was nearly linear in concentration, indicating complete ionization. For the accuracy desired, it was felt sufficient to take the concentration of free pyridinium or ascorbate ions to be the same as the concentration of the toluenesulfonic acid solution having the same conductivity.

The extent of dissociation of ascorbic acid into ions capable of carrying current is plotted against x_{py} in Fig. 3. As x_{py} increases, the mobile ion concentration rises rapidly to a maximum between $x_{py} = 0.05$ and 0.10, then falls steadily to a rather low value in pure pyridine. Apparently, pyridinium ascorbate ion pairs predominate when $x_{py} > 0.1$ and become more stable relative to the free ions as the dielectric constant decreases from 24.3 in ethanol to 12.3 in pyridine.

The fraction of ascorbic acid present as ion pairs as opposed to undissociated ascorbic acid was estimated from the spectrum of methyl red in ethanol–pyridine mixtures containing ascorbic acid. For methyl red (MRH) in ethanol containing half-neutralized ascorbic acid

$$K_{\mathrm{Ind}} = \frac{[\mathrm{MRH}][\mathrm{AH}^-]}{[\mathrm{MR}^-][\mathrm{AH}_2]} = 3.11 \qquad (8)$$

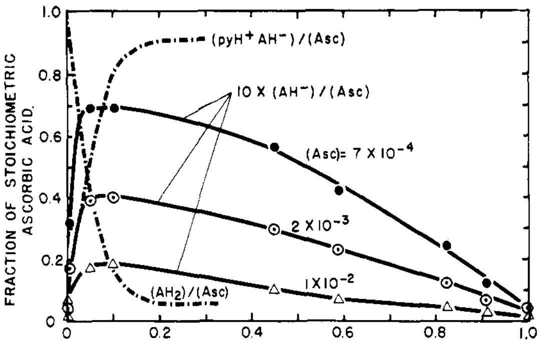

FIG. 3.—Relative amounts of undissociated ascorbic acid $[AH_2]$, free ions $[AH^-]$, and ion pairs $[pyH^+AH^-]$ in ethanol–pyridine mixtures as fractions of the total concentration [Asc]. $[AH_2]/[Asc]$ and $(pyH^+AH^-)/[Asc]$ curves calculated for $[Asc] = 2 \times 10^{-3}$ *M*. Scale for $[AH^-]/[Asc]$ is enlarged 10×.

In Fig. 3 are plotted the fractions of undissociated ascorbic acid and ion pairs, calculated under the assumption that K_{Ind} retains its value in solutions containing pyridine. Although an indicator method such as this is subject to specific solvent effects of unknown magnitude, conversion of ascorbic acid into ion pairs appears to be nearly complete at $x_{py} = 0.15$.

Course of the Reaction.—In dry ethanol, reduction of chlorophyllide to reduced pheophorbide was observed, but with a quantum yield of only about 10^{-4} initially, falling to about 10^{-5} at 5% reduction. It is possible that trace impurities are responsible.[12] There is no back reaction in the dark and the presence of a small amount of water has no effect. Neutralization of the ascorbic acid by tetrabutylammonium hydroxide diminishes even this reaction, showing that the presence of ascorbate ion alone is not sufficient for reduction.

Pheophorbide seems definitely to be reduced in wet ethanol, though more slowly than when pyridine is present, and limited regeneration occurs in the dark.[13]

There has been some dispute over whether chlorophyllide is reduced in absolutely dry pyridine.[1,3-5,11] With our dry pyridine, reduction indeed occurred, but at a rate much slower than when ethanol or water is present. The back reaction is rapid and a photostationary state is soon reached. In a run on the Cary only 5% of the chlorophyllide was in the reduced form at the stationary state, as compared with nearly 100% in similar runs in which some water or ethanol was present.

Dark regeneration of chlorophyllide in pyridine is not complete; instead, on continued illumination there is formed a compound whose longest wave length band is at 631 mμ. The compound is formed only in the light from $ChlH_2$ in the presence of oxidized products of ascorbic acid; it is stable in the dark, and its reversible photoreduction is sensitized by chlorophyll. A run made with a yellow-tinged solution of ascorbic acid in pyridine gave this product exclusively after a transitory formation of $ChlH_2$. Normally this compound is only a minor by-product, but probably accounts for most of the net chlorophyllide loss in pyridine-rich solutions.[14]

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(11) T. T. Bannister, "Photochemistry in the Liquid and Solid States," John Wiley and Sons, Inc., New York, N. Y., 1960, p. 110.

(12) R. Livingston and D. Stockman, *J. Phys. Chem.*, **66**, 2533 (1962).

(13) M. S. Ashkinazi, I. A. Dolidze, and V. E. Karpitskaya, *Biofizika*, **6**, 294 (1961).

(14) There seems to be no reference to the formation of this compound in the literature, but it probably appears in the spectrum of regenerated chlorophyll in Fig. 2 of ref. 5.

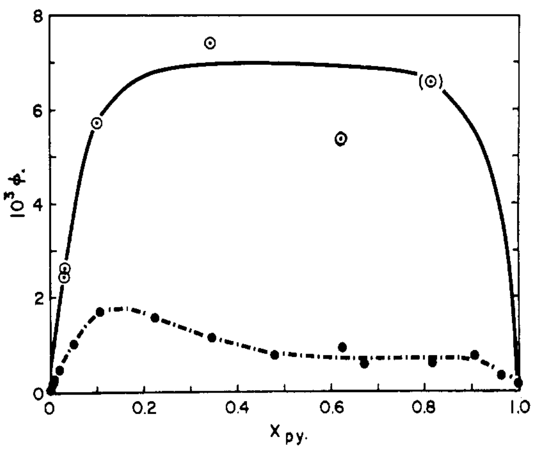

Fig. 4.—Variation of the quantum yield (ϕ) for chlorophyllide reduction with solvent composition in ethanol–pyridine mixtures; [Chl] ~ 10^{-5} M, [Asc] ~ 1.8×10^{-3} M, I_a ~ 5×10^{-6} einstein/l. sec.; point at (0.81, 6.6) interpolated from data of Fig. 5. Runs providing points for lower dashed curve apparently contained an adventitious retarder.

In pyridine–ethanol mixtures with $x_{py} > 0.4$ and [Asc] = 2×10^{-3}, the product of reduction is the red reduced chlorophyllide ($ChlH_2$) described by Krasnovskii,[1] and in the dark chlorophyllide is regenerated almost exclusively. At higher ascorbic acid concentrations or in the presence of water, regeneration of pheophorbide becomes important.

In mixtures with $x_{py} < 0.3$, the initial product is $ChlH_2$, but most of this loses Mg in a reaction examined by Bannister[4,11,15] to give a form of reduced pheophorbide absorbing maximally near 620 mμ, from which pheophorbide is regenerated in the dark.

Oxidation by iodine confirmed the identities of $ChlH_2$ and $PheH_2620$, and also showed that $PheH_2620$ is much more readily oxidized than $ChlH_2$.

There is an aspect of the matter which so far has escaped notice. The product of reduction of pheophytin in pyridine has its highest absorption in the visible near 525 mμ.[5] In ethanol[13] or 30% aqueous pyridine[11] the visible maximum is near 620 mμ, and the possibility that there is more than one reduced pheophytin has been noted.[5] When pheophorbide was reduced in a series of ethanol–pyridine mixtures and the spectra of the products were corrected for absorption by residual pheophorbide, the ratio of the optical density at 525 to that at 590 (where $PheH_2620$ was actually measured to minimize interference from pheophorbide) increased steadily from 0.80 in ethanol to 1.44 at x_{py} = 0.8, indicating increased formation of the 525 form as the pyridine content increased. During the slow back reaction, the optical densities at both wave lengths went down, but in each solution maintained their original ratio. Evidently there are two forms of reduced pheophorbide, $PheH_2525$ and $PheH_2620$, existing in equilibrium, with an equilibration time short compared to the time required for back reaction.

Spectra.—Spectra of the reduced compounds are available in the literature.[5,11] The following extinction coefficients were derived by detailed analysis of spectral changes during forward and back reactions, and are believed accurate within ± 1000 l./mole cm.

$ChlH_2$: In ethanol (extrapolated to x_{py} = 0), (λ, 10^{-3} ε) = (522, 47.5); shifts to (524, 42.5) at x_{py} = 0.6 and to (525, 39.0) at x_{py} = 1.0; the far violet peak remains near (411, 32.0). The values in pyridine are the same as Zieger and Witt's,[5] corrected for background absorption.

$PheH_2620$: In ethanol and ethanol–pyridine mixtures up to x_{py} = 0.2 at least, peaks are at (620, 19.0) and (380, 48.0). The red peak is very broad and the position of maximum absorption is hard to determine.

$PheH_2525$: Spectrum appears to resemble that of $ChlH_2$, but extinction coefficients could not be determined accurately.

The spectrum of the derivative absorbing at 631 mμ can be calculated on the assumption that it was the only product in the run in which it was formed. In pyridine–ethanol solution of x_{py} = 0.8: peak at (631, 36.4); shoulders at (610, 19.5) and (584, 13.5); peak (?) at (510, 4.4); peaks close together at (416, 48) and (406, 41) but with unknown background absorption.

Quantum Yield.—The quantum yield for reduction of chlorophyllide at fixed ascorbic acid concentration and light intensity varies with x_{py} as shown in Fig. 4. The lower of the two curves represents a long and internally consistent series of runs which gave quantum yields lower than those obtained later under ostensibly identical conditions. Since the course of the photoreaction and the rate of back reactions were the same in the two series, the difference is attributed to a quenching impurity, probably in the ascorbic acid stock solution. The lower curve illustrates the x_{py} dependence in more detail and is shown for that purpose. It was generally harder to get consistent results in the pyridine-rich region than in the ethanol-rich region.

The quantum yield increases linearly with x_{py} up to about x_{py} = 0.05. The increase could be attributed to the increasing concentration either of pyH^+AH^- or of Chl(eth)(py), but not both. Correlation is much better with pyH^+AH^-, and addition of a strong base (tetrabutylammonium hydroxide) to a solution already containing pyridine completely suppresses reduction, as it should if pyH^+AH^- is required. Therefore, formation of the ion pair, not the solvate, is responsible for reduction in this region. There is, however, no correlation with the concentration of free ascorbate ions.

The decline in yield with increasing x_{py} in the high pyridine region is probably associated with the conversion of Chl(eth)(py) into $Chl(py)_2$, since presumably no further change is occurring in the state of the ascorbic acid. Although the decline in yield in the high pyridine region is more abrupt than the decline in Chl(eth)(py) concentration, exact proportionality of the two is not necessarily to be expected if solvate exchange occurs in the triplet excited state of chlorophyllide.

At x_{py} = 0.8 the quantum yield depends on the ascorbic acid concentration according to

$$\phi = \phi_\infty [\mathrm{Asc}]/(K_A + [\mathrm{Asc}]) \qquad (9)$$

with ϕ_∞ = 0.021 and K_A = 4.2×10^{-3} M (Fig. 5).[16]

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[15] T. T. Bannister and J. E. Bernardini, *Biochim. Biophys. Acta*, **59**, 188 (1962).

[16] We prefer a log–log plot of ϕ against [Asc] to the more usual plot of $1/\phi$ against 1/[Asc] because the former is better able to suggest proper

At $x_{py} = 0.1$, nearly the same curve seems to be followed, but it could not be extended reliably to high concentration because of rapid formation of reduced pheophorbide.

In the usual interpretation of eq. 9, $K_A = k_d/k_1$ is the ratio of rate constants of excited state decay and reaction with ascorbic acid. If k_d for triplet excited chlorophyllide be taken as 750 sec.$^{-1}$,[17-19] then $k_1 = 1.8 \times 10^5$ l./mole sec.

An independent evaluation of the rate of reaction of excited chlorophyllide with ascorbate was obtained by carotene inhibition. β-Carotene quenches the triplet state of chlorophyll with a rate constant $k_Q = 1.3 \times 10^9$ l./mole sec.[20,21] The quantum yield (ϕ_Q) in the presence of carotene is related to that (ϕ) in its absence by

$$(\phi - \phi_Q)/\phi_Q = k_Q[\text{carotene}]/(k_d + k_1[\text{Asc}]) \qquad (10)$$

From the data of Table I an average value of 1100 sec.$^{-1}$ for $k_d + k_1[\text{Asc}]$ is derived, and upon again taking $k_d = 750$ sec.$^{-1}$, the value $k_1 = 1.9 \times 10^5$ l./mole sec. is obtained.

TABLE I

CAROTENE INHIBITION OF CHLOROPHYLLIDE REDUCTION

$x_{py} = 0.030$, [Asc] $= 1.84 \times 10^{-3}$ *M*

10^6[Chl], *M*	$10^6 I_a$, einsteins/l. sec.	10^6[carotene], *M*	$10^3\phi$	$k_d + k_1$[Asc], sec.$^{-1}$
8.6	4.8	0	2.63	..
8.6	4.5	0	2.46	..
7.6	3.9	2.9	0.56	1085
7.4	4.2	5.9	.40	1460
10.4	5.5	11.6	.12	760
			Av.	1100

The values derived from carotene inhibition and ascorbic acid concentration dependence are in excellent agreement with that ($k_1 = 1.5 \times 10^5$ l./mole sec.) found recently[19] by flash photolysis for quenching of triplet state chlorophyll by ascorbic acid in aqueous pyridine. Evidently the process that leads to quenching of the triplet state is the same process that leads to reduction.

It was not practicable to vary light intensity over a wide range, but an upward trend of quantum yield with light intensity, perhaps approaching the square root, is discernible in a series of comparable runs (Table II). During the later stages of a reduction of pheophorbide increasing the light intensity by a factor of 1.71 increased the quantum yield by a factor of 1.30.[22]

TABLE II

EFFECT OF LIGHT INTENSITY ON QUANTUM YIELD OF CHLOROPHYLLIDE REDUCTION

$x_{py} = 0.10$, [Asc] $= 1.95 \times 10^{-3}$ *M*

10^6[Chl], *M*	$10^6 I_a$, einsteins/l. sec.	$10^3\phi$
7.3	2.2	1.58
7.7	5.2	1.59
9.0	5.6	1.73
7.2	10.4	2.39

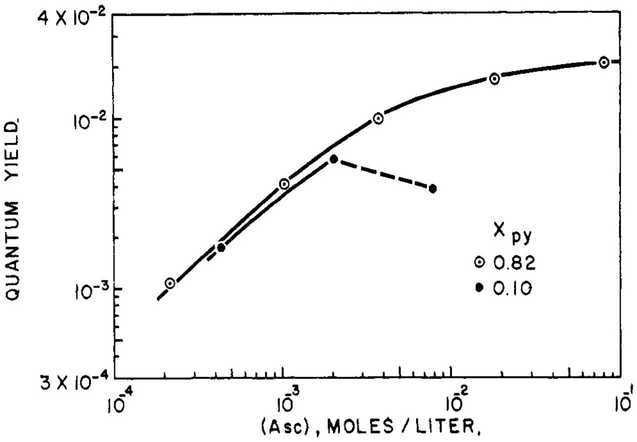

Fig. 5.—Variation of quantum yield for chlorophyllide reduction with ascorbic acid concentration; [Chl] $\sim 8 \times 10^{-6}$ *M*, $I_a \sim 4 \times 10^{-6}$ einstein/l. sec. Curve through points for $x_{py} = 0.82$ calculated from eq. 9 with $\phi_\infty = 0.021$ and $K_A = 4.2 \times 10^{-3}$ *M*.

One obtains the impression from the literature that water is a more potent "activator" of photoreduction than ethanol. Although this is true when concentration is expressed as volume fraction, the difference is much less marked when concentration is expressed as mole fraction. The difference that remains is partly attributable to the noticeably[23] greater power of water to form the reactive complex Chl(w)(py). The results of a few water runs and a comparable ethanol run are collected in Table III.

TABLE III

COMPARISON OF WATER AND ETHANOL AS ACTIVATORS FOR PHOTOREDUCTION OF CHLOROPHYLLIDE

[Chl] $\sim 7.5 \times 10^{-6}$ *M*, $I_a \sim 3.5 \times 10^{-6}$ einstein/l. sec.

Activator	x_{py}	10^3[Asc], *M*	$10^3\phi$	% Phe regenerated initially
None	1.00	1.9	0.18	0
Ethanol	0.82	3.7	9.9	3
Water	.89	4.2	13.3	17
Water	.75	3.8	15.5	28
Water	.58	4.1	21.1	60

The extent of conversion to reduced pheophorbide (estimated by comparing initial rates of disappearance of $ChlH_2$ and appearance of chlorophyllide immediately after the photoreaction) increases steadily with water content and much exceeds that with ethanol.

In Fig. 6 the rate of photoreduction of pheophorbide is compared with that of chlorophyllide. Under similar conditions the rates are about the same initially, but that of pheophorbide reduction soon falls to a much lower value as the concentration of $PheH_2$ builds up. The quantum yield in the run shown declined hyperbolically with time. The generation of pheophorbide by oxidation of reduced pigment ultimately retards the reduction of chlorophyllide; this effect is visible in the run with $x_{py} = 0.03$ of Fig. 6.

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weighting of points over a wide range of concentration. In these coordinates curves of (9) have an invariable shape, and the parameters K_A and ϕ_∞ are obtained by inspection of the superimposed theoretical curve.

(17) H. Linschitz and K. Sarkanen, *J. Am. Chem. Soc.*, **80**, 4826 (1958).

(18) R. Livingston, *Quart. Rev.* (London), **14**, 174 (1960).

(19) R. Livingston and P. J. McCartin, *J. Am. Chem. Soc.*, **85**, 1571 (1963).

(20) E. Fujimori and R. Livingston, *Nature*, **180**, 1036 (1957).

(21) H. Claes, *Z. Naturforsch.*, **16b**, 445 (1961).

(22) Bannister, in ref. 11, reports quantum yields up to 0.07 in 40% aqueous pyridine, with 0.1 *M* ascorbic acid, and with light intensities about ten times as much as ours. These conditions all favor increased yields over those reported here.

(23) The intensity of a peak at 638 mμ is apparently a measure of the amount of $Chl(py)_2$.[3,24] In water-pyridine mixtures this peak is smaller than it is in ethanol-pyridine mixtures of the same molar composition.

(24) P. J. McCartin, *J. Phys. Chem.*, **67**, 513 (1963).

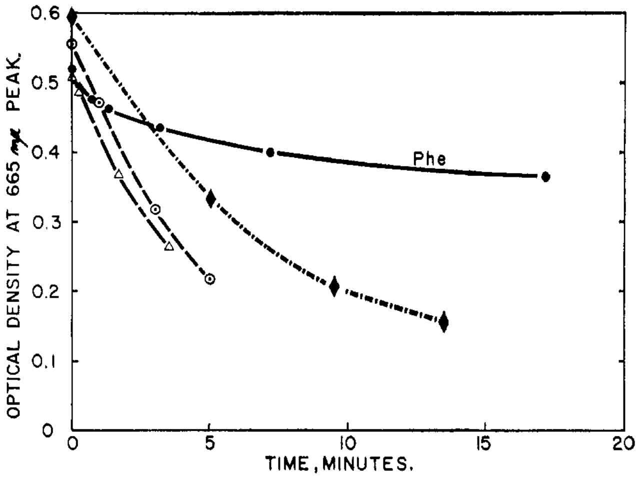

Fig. 6.—Rate of photoreduction of chlorophyllide and pheophorbide in ethanol–pyridine solution; [Asc] $\sim 2 \times 10^{-3}$ M, $I_a \sim 4.5 \times 10^{-6}$ einstein/l. sec.: ●, [Phe] $= 1.1 \times 10^{-5}$ M, $x_{py} = 0.105$; ◆, [Chl] $= 0.86 \times 10^{-5}$ M, $x_{py} = 0.031$; △, [Chl] $= 0.76 \times 10^{-5}$ M, $x_{py} = 0.099$; ⊙, [Chl] $= 0.82 \times 10^{-5}$ M, $x_{py} = 0.103$, like above but in presence of 8.6×10^{-5} M dehydroascorbic acid.

Dehydroascorbic acid in the amount of 4% of the ascorbic acid concentration and 10 times the chlorophyllide concentration has little effect on the quantum yield (Fig. 6 and Table IV). It did not matter whether commercial acid was added or whether it was prepared *in situ* just before the beginning of the run by oxidation with 2,6-dichloroindophenol.

TABLE IV

EFFECT OF VARIOUS SUBSTANCES ON THE RATE OF PHOTOREDUCTION OF CHLOROPHYLLIDE

[Chl] $= 7\text{–}10 \times 10^{-6}$ M, $I_a = 4\text{–}6 \times 10^{-6}$ einstein/l. sec., [Asc] $= 1.8\text{–}2.1 \times 10^{-3}$ M

Substance added	Concn., M	$10^4\phi$
$x_{py} = 0.10$		
None	. . .	5.7
Dehydroascorbic acid (commercial)	7.8×10^{-5}	4.7
Dehydroascorbic acid, prepared *in situ*	8.6×10^{-5}	4.6
None, [Asc] $= 1.86 \times 10^{-3}$		7.0
Tetrabutylammonium hydroxide to above to neutralize Asc	1.86×10^{-3}	0.06
Benzoic acid to above in equal amount	1.86×10^{-3}	1.74
Malic acid	1.11×10^{-2}	0.84
None	. . .	1.73
Benzoic acid	3.73×10^{-3}	1.70
$x_{py} = 0.030$		
None	. . .	2.63
None	. . .	2.46
Water	$x_w = 0.044$	1.97
Benzoic acid	1.94×10^{-2}	0.90
NaCNS	8.7×10^{-3}	3.08
$MgCl_2 \cdot 6H_2O$	8.0×10^{-3}	0.29

The effects of other added substances are also set out in Table IV. Benzoic acid[25] partially restores the reducing activity lost by titrating pyridinium ascorbate with tetrabutylammonium hydroxide. Malic acid, benzoic acid in excess, and magnesium chloride depress the quantum yield; thiocyanate enhances it slightly.

Back Reaction.—After each photoreduction run, the dark regeneration of chlorophyllide or pheophorbide was followed for 2 or 3 hr., though return of pigment was usually not complete until the next day. The results are best explained if the rate of regeneration is controlled by the availability of an active form of oxidized ascorbic acid.

The regeneration never followed the simple second-order rate law that might be expected from direct reaction between reduced pigment and dehydroascorbic acid. In aqueous pyridine, the regeneration of chlorophyllide followed a $^3/_2$ order rate law, and was generally faster than in ethanolic pyridine. In pyridine-rich mixtures with ethanol, regeneration of chlorophyllide followed a first-order rate law, with rate constant decreasing from 5×10^{-4} sec.$^{-1}$ in pure pyridine to 1×10^{-4} sec.$^{-1}$ at $x_{py} = 0.6$. The rate of regeneration of pheophorbide from reduced pheophorbide was comparable (1.6×10^{-4} sec.$^{-1}$ at $x_{py} = 0.9$). In the high pyridine region the regeneration rate was independent of the ascorbic acid concentration.

In ethanol-rich mixtures the conversion of $ChlH_2$ to $PheH_2$ is first order in the $ChlH_2$ concentration and in the ascorbic acid concentration. The rate constant in the expression

$$-d[ChlH_2]/dt = k[Asc][ChlH_2] \qquad (11)$$

decreases from 0.24 l./mole sec. in pure ethanol to 0.12 at $x_{py} = 0.1$ and 0.072 at $x_{py} = 0.2$ as a consequence of the change in the state of ionization of ascorbic acid and probably in the state of solvation of $ChlH_2$ also. The values for k include a relatively small contribution from the direct oxidation of $ChlH_2$ to chlorophyllide.

The regeneration of pheophorbide in ethanol-rich solutions is irregular and obeys no rate law. There is sometimes found an initial fast stage, an intermediate slow stage, and a final fast stage, apparently in response to changes occurring in the form of the oxidized ascorbic acid.

Discussion

Perhaps the most significant finding is that the ion pair pyridinium ascorbate is an active reducing agent while ascorbate ion by itself is not. An explanation for this follows from the endothermicity of the reaction.

If an electron jumps from ascorbate ion to photoexcited chlorophyllide, there is nothing to prevent an almost immediate return jump of an electron from $Chl\cdot^-$ to $AH\cdot$. Ascorbate would then act only as a quencher. However, if within a few vibrational times after the electron jump to Chl, a proton is transferred also, the products $ChlH\cdot$ and $AH\cdot$ may be stabilized long enough to drift apart and react further to give the final products of reduction.[26] To the pyridine may be assigned not only the role of neutralizing ascorbic acid but also, in the form of pyridinium ion, the role of stabilizing the immediate product of reduction by protonating it.[27]

Even when the ascorbate concentration is high, the quantum yield barely exceeds 2%. Again, the reason for such a low ceiling on the yield is probably the endothermicity of the reaction. Immediately after reaction the radicals $ChlH\cdot$ and $AH\cdot$ are close together in a solvent cage. If they diffuse back together instead of escaping into the bulk of the solution, they will

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(25) Benzoic acid has nearly the same pK (4.01) as ascorbic acid (4.10), but has no reducing activity toward chlorophyllide.

(26) The existence of what are apparently radical products of photoreduction of chlorophyll has been demonstrated in flash photolysis experiments.[5,19]

(27) It may be in this step that the deuterium isotope effect operates (A. A. Krasnovskii and G. P. Brin, *Dokl. Akad. Nauk SSSR*, **96**, 1025 (1954))

react to give starting materials. Only by disproportionation of ChlH· radicals that have escaped the solvent cage can the stable product $ChlH_2$ be formed. The fraction (β) of radicals escaping is hard to estimate but probably does not exceed 0.1,[28] and this accounts for most of the reduction in quantum yield.

Mg^{+2} probably inhibits the reaction by complexing with ascorbate; malic and benzoic acids by suppressing the ionization of ascorbic acid.

Mechanism.—The more important reactions involved in reduction of chlorophyllide are summarized below, with appropriate rates or rate constants.

$$\text{Chl} \xrightarrow[665\ \text{m}\mu]{\text{light}} \text{Chl}' \quad \text{rate} = I_a \tag{12}$$

(Chl′ = singlet excited chlorophyllide, Chl* = triplet)

If a steady state is assumed for Chl′

$$\text{Chl}' \longrightarrow \text{Chl} \quad \text{rate} = (1 - \alpha)I_a \tag{13}$$

(includes fluorescence and internal conversion)

$$\text{Chl}' \longrightarrow \text{Chl}^* \quad \text{rate} = \alpha I_a,\ \alpha \leq 0.7^{29} \tag{14}$$

$$\text{Chl}^* \longrightarrow \text{Chl} \quad k_d = 750\ \text{sec.}^{-1} \tag{15}$$

$$\text{Chl}^* + \text{AH}^- \longrightarrow \text{Chl} + \text{AH}^- \quad \sim k_l \tag{16}$$

$$\text{Chl}^* + \text{pyH}^+\text{AH}^- \longrightarrow [\text{ChlH}\cdot + \text{AH}\cdot] + \text{py} \tag{17}$$

$$k_1 = 1.8 \times 10^5\ \text{l./mole sec.}$$

(Chl* may be $\text{Chl}^*(\text{eth})_2$ or Chl*(eth)(py), and [ChlH· + AH·] represents the radicals in a solvent cage. If Chl* is $\text{Chl}^*(\text{py})_2$, $k_1 = 1 \times 10^4$.[19]) If a steady state is assumed for {ChlH· + AH·}

$$\{\text{ChlH}\cdot + \text{AH}\cdot\} \longrightarrow \text{Chl} + \text{AH}_2 \quad (1 - \beta)k_1 \tag{18}$$

$$\{\text{ChlH}\cdot + \text{AH}\cdot\} \longrightarrow \text{ChlH}\cdot + \text{AH}\cdot \quad \beta k_1,\ \beta \sim 0.1 \tag{19}$$

$$2\text{AH}\cdot \longrightarrow \text{AH}_2 + \text{A} \quad k_{t1} \tag{20}$$

$$\text{AH}\cdot + \text{ChlH}\cdot \longrightarrow \text{AH}_2 + \text{Chl} \quad k_{t2} \tag{21}$$

$$2\text{ChlH}\cdot \longrightarrow \text{ChlH}_2 + \text{Chl} \quad k_{t3} \tag{22}$$

In the reduction of pheophorbide, the steps are analogous but in addition it is necessary to include

$$\text{AH}\cdot + \text{PheH}_2 \longrightarrow \text{AH}_2 + \text{PheH}\cdot \quad k_2 \tag{23}$$

At high acid concentration or after extensive conversion this reaction becomes important in the photoreduction of chlorophyllide.

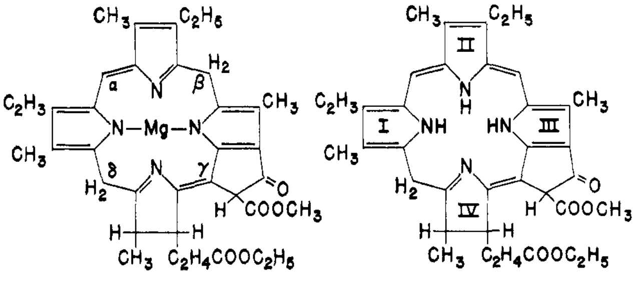
Without the last reaction and with neglect of quenching by AH^- the usual steady-state assumptions lead to the following expression for chlorophyllide reduction

$$\frac{d(\text{ChlH}_2)}{dt} = \alpha I_a \frac{k_1(\text{pyH}^+\text{AH}^-)}{k_d + k_1(\text{pyH}^+\text{AH}^-)} \frac{\beta}{2 + \dfrac{k_{t2}}{\sqrt{k_{t1}k_{t3}}}}$$

$$= \frac{R}{2 + \dfrac{k_{t2}}{\sqrt{k_{t1}k_{t3}}}} \tag{24}$$

where R is the rate of liberation of pairs of radicals from solvent cages.

If the radical disproportionation reactions are about equally fast, $k_{t1} = k_{t2} = k_{t3}$ and $d(\text{ChlH}_2)/dt = R/3$. With the estimates given for α and β, the maximum quantum yield $\phi_{\max} = \alpha\beta/3 = (0.7)(0.1)/3 = 0.023$, and it is not surprising that the observed quantum yield is no higher than this.



I II

Fig. 7.—Proposed structures for reduced ethyl chlorophyllide *a* (I) and reduced ethyl pheophorbide *a* (II).

The self-limited reduction of pheophorbide is typical of a number of reactions involving chlorophyll we have observed. It is interesting to try to interpret the curve of Fig. 6 for pheophorbide reduction to yield a value for k_2.

When reaction 23 is included, the steady-state approximation leads to a quartic expression for (AH·)

$$R = 2k_{t1}(\text{AH}\cdot)^2 + k_2(\text{AH}\cdot)(\text{PheH}_2) + k_{t2}(\text{AH}\cdot) \times \{(k_{t1}/k_{t3})(\text{AH}\cdot)^2 + (k_2/k_{t3})(\text{AH}\cdot)(\text{PheH}_2)\}^{1/2} \tag{25}$$

The equation becomes tractable if the assumption is made that $k_{t2} = 2k_{t1} = 2k_{t3} = 2k_t$. Then

$$(\text{AH}\cdot) = R/\{k_2(\text{PheH}_2) + 2(k_tR)^{1/2}\} \tag{26}$$

and the ratio of the quantum yield after $PheH_2$ has accumulated to the yield at the beginning is

$$\phi/\phi_0 = 1/\{1 + k_2(\text{PheH}_2)/2(k_tR)^{1/2}\}^2 \tag{27}$$

From this $k_2/k_t^{1/2}$ may be calculated.

Applied to the pheophorbide run of Fig. 6, eq. 27 gives values of $k_2/k_t^{1/2}$ increasing from 350 to 650 while ϕ/ϕ_0 decreases from 0.39 to 0.06. If $k_t \approx 10^9$, $k_2 \approx 10^7$ l./mole sec. The result is reasonable in view of the approximations that have been made.

Structure of the Reduced Products.—It is possible to propose structures for the reduced products based on their spectra and the reactions of which they are capable.

The spectrum of $ChlH_2$ is more characteristic of a porphodimethene structure with opposite bridge carbons reduced than of a structure with three or four pyrrole rings in conjugation,[30,31] such as mesobiliviolin[30] or biliverdin.[32] Because reduction of the γ-bridge carbon would appear to introduce considerable strain into the cyclopentenone ring, the β,δ-reduced structure is favored (I of Fig. 7). The structure of $PheH_2525$ is presumably analogous.

$PheH_2620$ is in equilibrium with $PheH_2525$ so is presumably a tautomer. Since no Mg-containing analog of $PheH_2620$ has appeared, it is reasonable to suppose that at least one of the added hydrogens is on a nitrogen. Woodward's criterion for reactivity of bridge carbons suggests that a proton at the β-carbon would be more labile than one at the δ-carbon.[33] Since the only N-tautomer for the β-hydrogen is the struc-

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[28] D. Booth and R. M. Noyes, *J. Am. Chem. Soc.*, **82**, 1868 (1960).

[29] Approximately (1 − fluorescence yield); see ref. 18.

[30] C. H. Gray, A. Kulczycka, and D. C. Nicholson, *J. Chem. Soc.*, 2276 (1961).

[31] D. Mauzerall and S. Granick, *J. Biol. Chem.*, **232**, 1141 (1958); D. Mauzerall, *J. Am. Chem. Soc.*, **84**, 2437 (1962).

[32] C. H. Gray, A. Lichtarowicz-Kulczycka, D. C. Nicholson, and Z. Petryka, *J. Chem. Soc.*, 2264 (1961).

[33] R. B. Woodward and V. Skaric, *J. Am. Chem. Soc.*, **83**, 4676 (1961).

ture with the hydrogen on the nitrogen of ring II, structure II of Fig. 7 is proposed for $PheH_2620$. The spectrum of $PheH_2620$ is furthermore almost identical with that given by Woodward for a phlorin base[34]; the proposed structure differs from the phlorin structure only in that the β-pyrrole positions of ring IV are reduced.

Acknowledgment.—We are indebted to Mr. John Miller for the preparation of ethyl chlorophyllide *a* from Jimson weed and for its chromatographic purification.

(34) R. B. Woodward, *Ind. Chim. Belge*, **27**, 1293 (1962).

[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT, UNIVERSITY OF SOUTHERN CALIFORNIA, LOS ANGELES 7, CALIFORNIA]

The Thermodynamics of the Dehydrogenation of Propane by Iodine Vapor[1a]

BY PRAKASH S. NANGIA AND SIDNEY W. BENSON[1b]

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In the range 308 to 340° the gas phase reaction, $I_2 + C_3H_8 \rightarrow 2HI + C_3H_6$, proceeds quantitatively to equilibrium with no side reactions. From the measured compositions at equilibrium it is possible to calculate values of K with an estimated precision of $\pm 3\%$ and hence $\Delta F°$ with a precision of ± 0.04 kcal. In the short range given, the points fall on an Arrhenius-type plot with $\Delta H° = 27.88 \pm 0.40$ kcal./mole and $\Delta S° = 37.0 \pm 0.69$ gibbs/mole. Using the spectroscopically known and very precise thermodynamic data for I_2 and HI, it is possible to obtain $\Delta F°$, $\Delta H°$, and $\Delta S°$ for the reaction, $C_3H_8 \rightleftarrows C_3H_6 + H_2$. These agree with combinations of data known from independent sources to within 0.02 kcal./mole, 0.12 kcal./mole, and 0.24 gibbs/mole, respectively. This agreement constitutes a very strong support for the assignments of frequencies and internal barriers in C_3H_8 and C_3H_6.

Introduction

In some recent studies in this laboratory, attention has been focused on the gas phase studies of the iodides. The main reason is that the iodides are well-behaved kinetic systems and consequently lend themselves to a quantitative evaluation of the thermodynamic and kinetic parameters of alkyl radicals and related molecular species. Many studies relating to the reactions of I_2 + olefins and HI + olefins as well as RI + HI have been reported recently from our laboratory.[2]

From the equilibrium study of reactions of I_2 + olefins, it has been possible to deduce thermodynamic values for variously C–I bonded groups,[3] which in turn permits the calculation of thermodynamic properties for various primary, secondary, and tertiary iodides. The products of the pyrolysis reactions of most organic iodides are usually iodine + olefin + alkane.[4] It was of interest therefore to explore the possibility of reverse processes under suitable conditions of temperature and pressures of reactants. The importance to the equilibrium studies stems from the findings, in this laboratory, of the existence of a discrepancy in thermodynamic quantities, *e.g.*, $S°$ of butene-1, from I atom catalyzed reversible isomerization of butene-1 ⇄ butene-2.[5]

Two recent publications[6] from our laboratory have dealt with isobutane as the hydrocarbon. As a natural extension of these studies, the present work describes the equilibrium measurements for the dehydrogenation of propane; the reaction system involved is $C_3H_8 + I_2 \rightleftarrows C_3H_6 + 2HI$. From the experimentally measured quantities, $\Delta H°$ and $\Delta S°$ of the above reaction, and precisely known values of $\Delta H_f°$ and $S°$ for both I_2 and HI, the differences in these latter quantities for the dehydrogenation part may be deduced or, *vice versa*, using the values known for all reactants and products, $\Delta H°$ and $\Delta S°$ of the reaction may be computed and compared with the observed values.

A detailed investigation of the kinetics of the above system has also been carried out with an objective of finding the bond energy of a secondary H atom and will be communicated in due course.

Inasmuch as the reaction system involves a mole change, it can be simply studied by measuring the increase in pressure with time. Most of the work has been carried out by this method while a few runs were made by studying spectrophotometrically the depletion of I_2 with time.

Experimental

Reagents.—C.P. grade propane, supplied by Matheson Co., Inc., was purified by bulb-to-bulb distillations and finally stored in a flask. The small samples taken for reaction purposes were again purified a few times shortly before use. Analysis of these samples by g.l.c. (gas–liquid partition chromatography) on a Perkin–Elmer J-column showed an impurity to the extent of 1% with a retention time corresponding to C_2H_6. Due corrections were made to determine the actual initial pressures of C_3H_8 throughout the work.

Iodine was of resublimate quality supplied by Allied Chemical Co.

Apparatus.—The apparatus consisted of the standard glass vacuum line, including all stopcocks lubricated with silicone grease. A cylindrical Pyrex glass vessel packed with glass tubes having a volume of *ca.* 900 cc. served as the reaction vessel. Surrounding the reaction vessel, with small air gap in between, was an electrically heated aluminum core furnace which was insulated with glass wool and mounted in a transite box. The temperature of the furnace was controlled to within ±0.2° over a period of a few hours by an Electronotherm controller in conjunction with a Wheatstone bridge which had a platinum wire resistance (50 ohms) as one of its arms for sensing the furnace temperature. The temperature of the furnace was determined by using a copper–constantan thermocouple with the fused end fixed deep into a well created in the reaction vessel, a cold junction in ice–water, and a Leeds and Northrup millivolt potentiometer No. 8686. Calibrations of thermocouple against N.B.S. certified metals (zinc, lead, and tin) showed the temperature to be correct to within ±0.2° of the absolute value. As the equilibrium runs were kept for a few days, the temperature did change slightly.

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(2) See D. B. Hartley and S. W. Benson, *J. Chem. Phys.*, **39**, 132 (1963), for references to many previous publications.

(3) S. W. Benson and A. Amano, *ibid.*, **36**, 3464 (1962).

(4) S. W. Benson, *ibid.*, **38**, 1945 (1963).

(5) S. W. Benson and A. N. Bose, *J. Am. Chem. Soc.*, **85**, 1385 (1963).

(6) H. Teranishi and S. W. Benson, *ibid.*, **85**, 2887 (1963); **85**, 2890 (1963).