[CONTRIBUTION FROM CHEMISTRY DEPARTMENT, BROOKHAVEN NATIONAL LABORATORY]

Solvates of Chlorophylls and Related Substances and Their Equilibria[1]

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The spectrum of chlorophyll-*b* zero-solvate in dry hydrocarbon showed evidence of a thermally excited state about 1400 cal./mole above the ground state in agreement with the thermodynamic value derived from the variation of relative intensities of components with temperature. Chlorophyll-*a* and possibly bacteriochlorophyll also exhibited this phenomenon. Solvation of chlorophyll-*b* corresponding to equilibria between zero- and mono-solvate and between mono- and di-solvate has been found by varying temperature and concentration of such polar substances as water, *n*-propyl ether, pyridine and diisopropylamine in the solvents 1:1 propane–propene, *n*-propylbenzene and 1:1 methylcyclohexane–methylcyclopentane. The constant for the equilibrium between mono- and dipyridinate of chlorophyll-*b* was approximately 10 at 275°K. In seeking to determine at what part of the chlorophyll molecule solvation took place, it was found that allomerized chlorophyll-*a*, bacteriochlorophyll, pheophytin-*a* and -*b* and metalloporphins also formed solvates. In addition to forming this type of solvate diisopropylamine interacted with chlorophyll-*b* probably at the enol–keto couple of carbon-10. Pheophytin-*b* when in polar solvents exhibited changes in its spectrum resembling those of chlorophyll-*b*. Hence, if solvation occurred at the magnesium atom of the chlorophyll, a similar process probably occurred at the corresponding hydrogen atoms of pheophytin. However, additional equilibria appeared to be taking place in the solutions of the latter. Two preparations of pheophytin-*a*, one made by exhaustive purification by S. Aronoff and E. Kmetec and the other in the usual way, gave decidedly different spectra at 75°K. even though their spectra were practically the same at room temperature. The spectrum of the former preparation was more refined at 75°K. and could be imagined to be a component of that of the other preparation. The spectrum of chlorophyll-*b* in diisopropylamine in 1:1 propane–propene made it evident that solvation processes were rapid even at about 75°K.

Our investigations[2] which revealed species of chlorophylls in equilibrium in ether–hydrocarbon solvents led to indications that the species were solvates.

Zero-solvates

To begin with we shall recall the work of Livingston, Watson and McArdle[3] who in their studies of the fluorescence of chlorophylls found that careful drying of the solvent such as benzene reduced the fluorescence almost to zero and that the fluorescence could be activated by a polar solvent which formed a 1:1 molecular addition compound with the chlorophyll.

The absorption spectrum of chlorophyll-*b* in carefully dried benzene, which we shall call the "dry" spectrum, may be roughly described as differing from that of chlorophyll-*b* in ordinary benzene, the "wet" spectrum, by the appearance of an additional spectrum shifted toward longer wave lengths. For example, the prominent band which appears at 6450 Å. is accompanied by a new component at 6650 Å. when the benzene was dry. We repeated the work on the absorption spectra of the chlorophylls[4] in benzene which we took great pains to dry. Chlorophyll-*b* was about 10^{-5} *M* so that minute concentrations of polar impurities such as water from the vacuum line may have been enough to form the 1:1 molecular compound. Finally calcium hydride as powder was introduced directly into the solution itself. Rather quickly the intensity of the dry spectrum increased and its continued growth could be followed easily. The greatest value of *f*, the ratio of the height of the maximum at 6650 Å. to that at 6540 Å. was observed to be 0.76 at 300°K. in methylcyclopentane and methylcyclohexane. Even though a solution of chlorophyll-*b* in *n*-propylbenzene in contact with calcium hydride had been heated to 360°K. for three hours, it continued to give the same *f* value at 300°K. On reducing the temperature, the intensities of the components of the bands at short wave lengths increased at the expense of those at long wave lengths; the process was an apparent intensification of the so-called wet spectrum. In Fig. 1 are reproduced the changes of the band in the red. Figure 2 shows the refinement at 180°K. in the spectrum of chlorophyll-*b* effected by the presence of calcium hydride by comparison with the spectrum of a solution in which calcium hydride was absent. In the latter instance the solvent *n*-propylbenzene had been distilled in a vacuum line from calcium hydride.

It is convenient to define a characteristic temperature which we shall call the transit temperature, Tr, as one at which equal heights are reached by corresponding absorption maxima of two forms of chlorophyll in equilibrium in polar solvents.

The fact that calcium hydride does not alter the chlorophyll molecule is illustrated by the reproducibility of the Tr after forming successive solutions with other solvents. In Table I, the Tr values are given in parentheses. Each successive solution was prepared from the previous one by pumping off the solvent in a vacuum leaving the chlorophyll-*b* as a dry film and then condensing the new solvent

TABLE I

THE TRANSIT TEMPERATURE OF CHLOROPHYLL-*b* IN VARIOUS SOLUTIONS WITH AND WITHOUT CALCIUM HYDRIDE

(213°K.)	(183°K.)	(None)
	Dry	
10% *n*-Propyl ether + Methylcyclohexane	*n*-Propyl-benzene →	*n*-Propylbenzene + CaH_2

(213°K.)	(186°K.)
10% *n*-Propyl ether + Methylcyclohexane + CaH_2	10% *n*-Propyl ether + *n*-Propylbenzene + CaH_2

(1) Research performed under the auspices of the U. S. Atomic Energy Commission.

(2) S. Freed and K. M. Sancier, *Science*, **114**, 275 (1951); **116**, 175 (1952).

(3) R. Livingston, W. F. Watson and J. McArdle, THIS JOURNAL, **71**, 1542 (1949).

(4) Kindly prepared for us by Professor Robert Livingston and his associates at the University of Minnesota (ONR Project N60 ri-212 Task Force I).

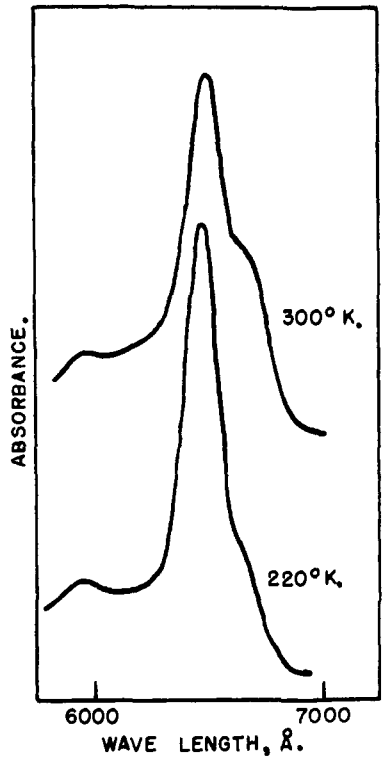

Fig. 1.—Absorption band in the red of chlorophyll-*b* in dry *n*-propylbenzene at 220° and 300°K.

on it. We observed no perceptible change in the spectrum after the series of operations as was indicated also by the return to the same value of Tr.

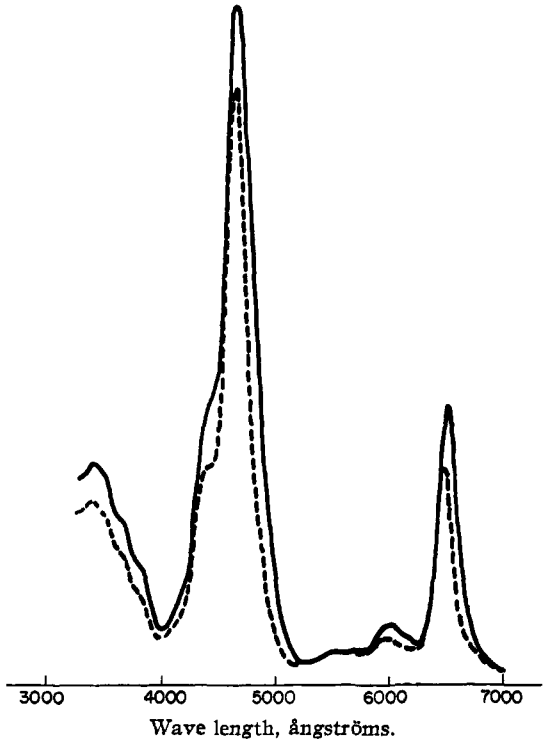

Fig. 2.—Absorption spectra of chlorophyll-*b* in dry *n*-propylbenzene at 180°K.: --- with, and — without calcium hydride added.

Pumping the chlorophyll dry in a high vacuum for several hours seemed not to affect the spectra of subsequent solutions.

In the presence of calcium hydride the chlorophyll exists as the zero-hydrate as is deduced from the fact that there is no sign of a Tr and that a solution of chlorophyll-*b* in a hydrocarbon in the presence of calcium hydride does not fluoresce, together with the proof of Livingston, Watson and McArdle that a 1:1 solvate is required for fluorescence.

Figure 2 shows that it was possible to distinguish at low temperatures the spectrum of chlorophyll-*b* in dry hydrocarbons from that of chlorophyll-*b* in the same solvent that actually contained traces of water. Figure 2 gives the absorption curves which at room temperature could scarcely be told apart. The *f* value of the solution in contact with calcium hydride was 0.59 at room temperature and that of the other solution was roughly equal to 0.55. Supposedly, the degrees of dryness differed little. However, a reduction in temperature revealed decided differences. The solid line represents in good approximation the zero-hydrate of chlorophyll-*b*, the dashes primarily the monohydrate.

We shall now consider the nature of the transformations with temperature of chlorophyll-*b*, zero-solvate, in a hydrocarbon solvent, in this instance 1:1 methylcyclohexane–methylcyclopentane in contact with calcium hydride. The readjustments in equilibrium were more easily followed in the red region of the spectrum but they proceeded in the blue region also. The ratio *f* of the relative heights of the bands at 6650 Å. and 6450 Å., a quantity proportional to the equilibrium constant, was measured as a function of the temperature. When the logarithm of *f* was plotted against the reciprocal of the absolute temperature, $1/T$, Fig. 3, the difference in heat content $\Delta H = -1400 \pm 200$ cal./mole. (The *f* value at 216°K. is off the line because the degree of overlapping of the components of the band was considerable and uncertain in magnitude.) The fact that as the temperature was lowered the intensity of the component toward short wave lengths increased at the expense of the one at long wave lengths was consistent with the

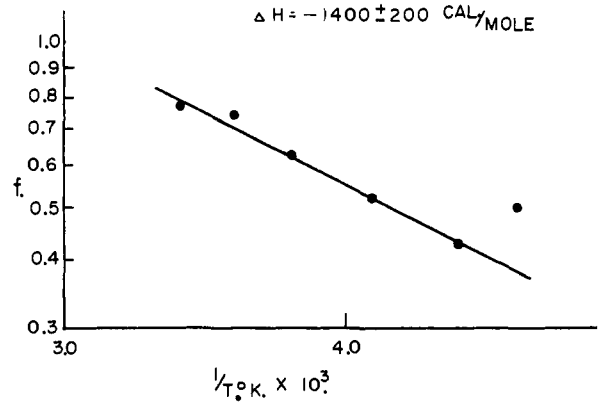

Fig. 3.—*f*, the ratio of the intensities of the bands 6650 Å./6450 Å., as a function of $1/T$, °K. for a solution of chlorophyll-*b* in 1:1 methylcyclopentane–methylcyclohexane in contact with calcium hydride.

idea of thermal distribution in population between quantum levels provided that the difference in energy between the thermally excited state and the ground state computed from the energy interval at 6650 and 6450 Å. was also 1400 cal./mole. Actually, this interval was 460 cm.$^{-1}$ or 1320 cal./mole. The corresponding energy difference estimated from the band in the blue was 495 cm.$^{-1}$ or 1420 cal./mole. Since the components of this band were not resolved, the approximate value of the wave number difference was obtained from the width of the band at half-height at 300°K. and at 200°K.; at 200°K. the contribution from the component at long wave length was negligible as judged by the disappearance of the corresponding component in the red region of the spectrum. The agreement between the thermodynamic value 1400 cal./mole and the spectroscopic values 1320 and 1420 cal./mole was well within the precision of measurement. In Fig. 3 extrapolation of the line, $\log f$ *vs.* $1/T$, to infinite temperature gave $f = 7 \pm 1$ which depends on the ratio of the statistical weights of the two states and the oscillator strengths of their transitions.

Chlorophyll-*b* dissolved in *n*-propylbenzene with a trace of water, the spectrum of which is represented by the dashed curve of Fig. 2, under the same analysis as above yielded $\Delta H \cong -2500$ cal./mole. That is, the match with the spectroscopic energy interval no longer held. It appears then that $\Delta H \cong -2500$ cal./mole includes the energy of some solvation process. This is confirmation that two processes are associated with the reciprocating intensities of the components. The coincidence which exists at higher temperatures between the spectrum of the monohydrate of chlorophyll-*b* and that arising from the basic state of its zero-hydrate can be distinguished then by the behavior of the relative intensities with temperature.

Of all the substances that we are discussing in this paper only chlorophylls-*a* and -*b* and possibly bacteriochlorophyll have shown evidence of thermally excited states.

Zero-, Mono- and Disolvates and Their Equilibria

Evidence of progressive solvation can be followed by the addition of *n*-propyl ether to a solution of chlorophyll-*b* dissolved in 1:1 methylcyclopentane-methylcyclohexane with calcium hydride. When the mole fraction of *n*-propyl ether was 0.004, the *f* value changed from 0.76 to 0.43 and then Tr = 190°K. When the mole fraction was 0.1, $f = 0$, *i.e.*, no band at 6650 Å. was visible and Tr = 213°K. A higher concentration of the ether than water was required for equal degrees of solvation as Livingston, Watson and McArdle had found in the relative concentrations required for full activation of fluorescence. As was to be expected the lower the concentration of the polar substance the lower must be the temperature to achieve the same degree of solvation.

Higher concentrations of a polar solvent bring about transformations between the monosolvate of chlorophyll and the disolvate. The determination of an equilibrium constant for such a transformation at 275°K. was made by varying the concentration of pyridine in a solution of chlorophyll-*b* dissolved in *n*-propylbenzene. Pyridine was used rather than the ether because the latter did not form sufficient concentrations of the disolvate. The intensity at 4800 Å. was followed as successive quantities of pyridine were added. At first about enough pyridine was added (6.7×10^{-4} *M*) to convert all the dry spectrum to the wet (monopyridinate), and this constituted the base upon further addition of pyridine for the computation of the next equilibrium, mono- to dipyridinate. In view of the fact that the spectra of the mono- and dipyridinate were virtually identical but shifted 130 Å., the normalization of the maximum intensity of the dipyridinate at 4800 Å. was taken to be that of the monopyridinate at 4670 Å. in a solution in which practically all the chlorophyll was in the form of the monopyridinate. This procedure was resorted to in order to obtain the limiting intensity of the dipyridinate at this temperature without the otherwise necessary high concentration of pyridine and its excessive effect on the refractive index of the solvent. The wave lengths 4670 and 4800 Å. were selected in order to avoid overlapping from the shoulder on the short wave length side of the Soret band.

Table II summarizes the data. The equilibrium constant at 275°K. was fairly constant over the range in molarity of pyridine from 8.25×10^{-3} to 2.22×10^{-1}. It fell off at higher concentrations indicating that further solvation was occurring, perhaps at other sites of the chlorophyll-*b* molecules. The assumption was made that the equilibrium constant of the zero- to monosolvate was much greater than that of the mono- to disolvate since it was found that at extremely small concentrations, roughly equal to that of chlorophyll, the dry spectrum was eliminated. It should be recalled that Miller and Dorough[5] found that magnesium tetraphenylchlorin formed mono- and dipyridinates at room temperature.

TABLE II

EQUILIBRIUM AT 275°K. BETWEEN CHLOROPHYLL-*b* AND PYRIDINE: $\text{Chl} \cdot \text{Py} + \text{Py} \rightleftarrows \text{Chl} \cdot 2\text{Py}$

Concn. pyridine, mole/liter	Relative intensity at 4800 Å.	$K = \frac{(\text{Chl}\cdot 2\text{Py})}{(\text{Chl}\cdot\text{Py})(\text{Py})}$
6.7×10^{-4}	0	
8.25×10^{-3}	9.5	11
2.05×10^{-2}	22	10
8.13×10^{-2}	58	12
2.22×10^{-1}	76	8.7
8.54×10^{-1}	94	5.3
1.76	112	4.5

Varying concentrations of pyridine affected the Tr of chlorophyll-*b* in *n*-propylbenzene in much the same way as did ether except that a smaller concentration of pyridine was required. For example, corresponding to $\sim 10^{-6}$ *M*, 6×10^{-4} *M*, 0.03 *M* and 3 *M* pyridine in *n*-propylbenzene the Tr's were 163°, 212°, 243° and 300°K., respectively. When the solvent consisting of 3 *M* pyridine was removed by pumping at high vacuum and methylcyclohexane was condensed on the sample in the presence of calcium hydride, $f = 0.4$ at 300°K. rather

(5) J. R. Miller and G. D. Dorough, THIS JOURNAL, **74**, 3977 (1952).

than 0.76 indicating that a small amount of pyridine was retained by the chlorophyll. Addition of 10% *n*-propyl ether resulted in a Tr = 220°K. instead of the initial value of 213°K. The small difference was also probably due to retained pyridine.

In Fig. 4 is shown the effect on Tr of chlorophyll-*b* by different concentration of *n*-propyl ether in *n*-propylbenzene and in aliphatic solvents. There is an approximately linear relation between Tr and the fraction of ether in the benzene. However, there is little difference in the Tr value between 0.1 and 1.0 mole fraction *n*-propyl ether in the non-aromatic hydrocarbon; only when the ether is reduced to at least 0.004 mole fraction is there an appreciable change in Tr.

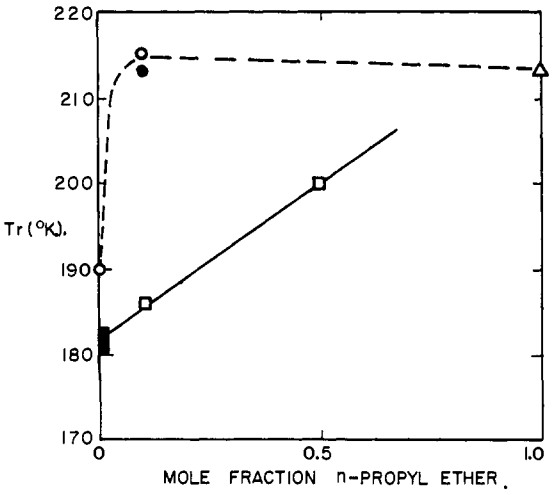

Fig. 4.—The effect of concentration of *n*-propyl ether on the transit temperature, Tr, of chlorophyll-*b* in hydrocarbons.

Mole fraction *n*-propyl ether	Solvent
■ 0 (trace H_2O)	*n*-Propylbenzene
□ .1, 0.5	*n*-Propylbenzene + CaH_2
○ .004, 0.1	Methylcyclohexane + CaH_2
● .1	1:1 Propane–propene
△ 1.0	.....

There appears to be a competition between ether and benzene for chlorophyll; that benzene does react weakly with chlorophyll is suggested by the fact that it is a much better solvent than aliphatic hydrocarbons and that the maximum value f = 0.6 that we obtained was less in benzene than in saturated hydrocarbons. Chlorophyll-*a* appears to be altogether similar to chlorophyll-*b* in these solvation processes.

In the interaction of mono- and of diisopropylamine with chlorophyll-*b*, we have examples of solvation at two sites, at the magnesium presumably and at the enol–keto couple. For isopropylamine the latter type of solvation or interaction led to the intermediate[6] of the Molisch phase test and ultimately to irreversible products. In Fig. 5 is shown the spectra of chlorophyll-*b* in 15% diisopropylamine in 1:1 propane–propene at three temperatures. The equilibria were more complex than those which occurred in solutions of ether. No band appeared in the region of the green corresponding to the intermediate characteristic of the Molisch phase test found in solutions of isopropylamine at low temperature. Instead, two strong bands appeared at low temperature at about 4300 and 4800 Å. at the expense of the main blue band which was present at high temperature at about 4500 Å. Spectra representing solvates stable at low temperatures have always appeared only toward longer wave lengths and closely resembled those at the high temperatures. This drastic difference in shape and position of the components of the blue band induced by the secondary amine resembled some features of the spectra of the intermediate formed by the primary amine in the phase test. Also reminiscent of the phase test was the occurrence of an irreversible reaction with the secondary amine. It seemed probable then that the secondary amine also reacted at the enol–keto couple. At low temperatures the band in the red behaved in a manner which has proved to be characteristic of the solvation process; the band representing the solvate stable at low temperature made its appearance toward long wave lengths. It may be of interest to note that the solvation processes took place rapidly even at about 75°K.

(6) S. Freed and K. M. Sancier, *Science*, **117**, 655 (1953).

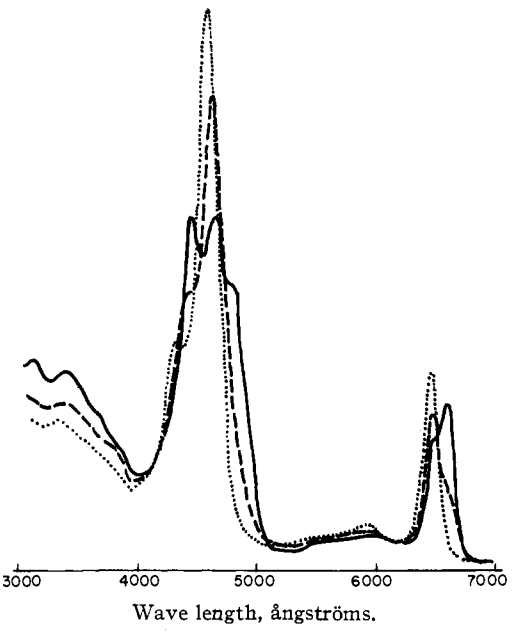

Fig. 5.—Absorption spectra of chlorophyll-*b* in diisopropylamine in 1:1 propane–propene at ····. 230°K., - - - 170°K.. — 75°K.

Solvates of Substances Related to Chlorophyll

Pheophytins.—Just what sites on the chlorophyll molecule were active in solvation was to be indicated by a process of elimination by observing whether various chlorophyll derivatives participate in equilibria such as we have previously described. The choice of pheophytin where the magnesium atom of chlorophyll is replaced by two hydrogen atoms might serve to indicate whether the solvation occurred at the magnesium atom or ion. Pheophytin-*b* did not exhibit a dry band characteristic of the zero-solvate of chlorophyll-*b* and in dry *n*-propylbenzene exhibited no equilibrium of species.

However, in 10% *n*-propyl ether in methylcyclopentane the transformation in the spectra seemed as striking as those of the chlorophylls, also with some resemblance to them but with some difference, Fig. 6. The changes in the blue band suggested an equilibrium between solvates with Tr = 218°K. It is within the intense band at about 4000 Å that the changes in equilibrium can most clearly be seen. In addition the band at 3800 Å. becomes more intense at the expense of the band at 4000 Å. as the temperature is lowered. Hence, two equilibria seem to be active at the same time.

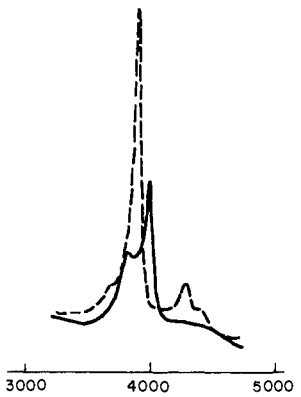

Fig. 6.—Absorption spectra of pheophytin-*b* in 10% *n*-propyl ether in methylcyclopentane and added calcium hydride: - - -, 190°K.; —, 130°K.

Pheophytin-*a* was studied in the same way. Two preparations were available, one furnished by Professor Livingston and the other, the outcome of an exhaustive series of chromatographic processes on paper, by S. Aronoff and E. Kmetec of Iowa State College. At room temperature the absorption spectra of both preparations were practically indistinguishable. In dry *n*-propylbenzene, the spectra of both samples remained almost unchanged as the temperature was reduced to 170°K. In 10% *n*-propyl ether in 1:1 propane–propene enormous changes occurred which were particularly evident at the Soret band (Fig. 7) between 225° and 190°K.; the bands became less intense and broader. Between 190° and 75°K. the spectra changed little but there was some sharpening and a reciprocity in the intensities of two peaks suggesting equilibria. The method of purification followed by Aronoff and Kmetec led to a spectrum which appeared distinctly simpler at the low temperature than that prepared in the usual way, Fig. 8.

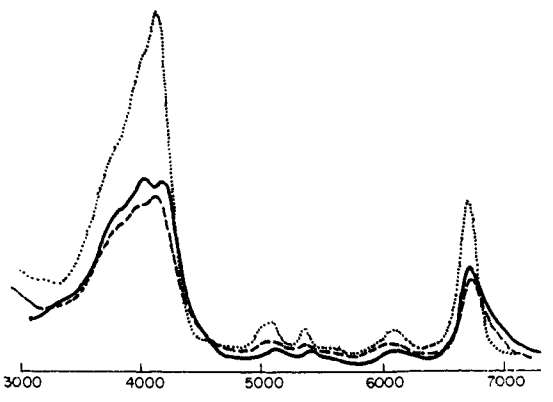

Fig. 7.—Absorption spectra of pheophytin-*a* in 10% *n*-propyl ether and 1:1 propane–propene: · · · · ·, 225°K.; - · - · -, 190°K.; —, 75°K.

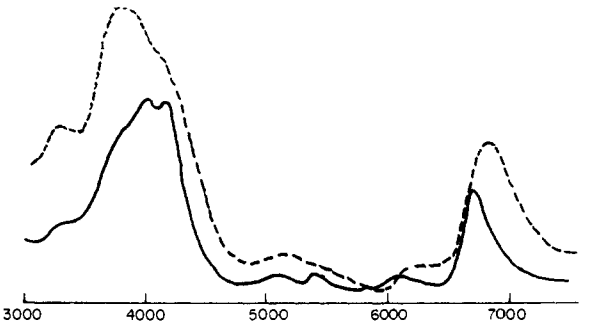

Fig. 8.—Absorption spectra of two samples of pheophytin-*a* in 10% *n*-propyl ether in 1:1 propane–propene at 75°K.: —, especially purified by Aronoff and Kmetec; - - - -, usual preparation.

It would appear that the decision as to whether or not solvation occurs at the central magnesium atom cannot be definitively made by using pheophytin because it seems that if solvation occurs at magnesium it also occurs at or near the two corresponding hydrogen atoms.

Pheophytin, a free chlorin base, did not appear to be a mixture of tautomers in *n*-propylbenzene such as Dorough and Shen[7] reported to be present in tetraphenylporphin dissolved in methylcyclohexane. It is just possible that the Soret band examined by us is not as sensitive an indicator for such a mixture as the bands toward the red which these investigators employed. Also, we found evidence of equilibria in chlorophylls and in some but not in all metalloporphins dissolved in *n*-propyl ether, which those authors[7] failed to find among their metalloporphins in a mixture of ethyl ether, isopentane and ethyl alcohol. The non-existence of several species of metalloporphins was regarded by these authors as confirmation that the free base existed as tautomers since hydrogen atoms might link to either vicinal or diagonal nitrogen atoms of the pyrrole groups while the metallic atom being supposedly in the form of a central spherically symmetrical ion could not do so. Our work suggests the need of re-examining chlorophylls and pheophytins with the techniques of fluorescence which these authors employed.

Allomerized Chlorophyll-*a*.—To test whether the enol–keto isomerism may be an important factor in the solvation processes of chlorophyll, we examined allomerized chlorophyll-*a* in which a methoxy group replaces the labile hydrogen atom on carbon-10 of chlorophyll so that enol–keto isomerism is non-existent. Figure 9 shows the spectra of allomerized chlorophyll-*a* in 10% *n*-propyl ether in 1:1 propane–propene at 180° and 253°K. The Tr is 213°K. Allomerized chlorophyll-*a* underwent transformations similar to those of chloro-

(7) G. D. Dorough and K. T. Shen, THIS JOURNAL, 72, 3939 (1950).

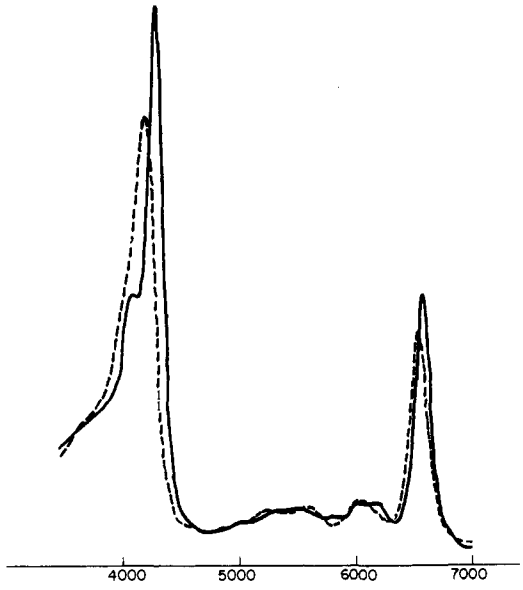

Fig. 9.—Absorption spectra of allomerized chlorophyll-*a* in 10% *n*-propyl ether and 1:1 propane–propene: —, 180° K.; - - - -, 253°K.

phyll-*a* and of chlorophyll-*b* when the solvent was 10% *n*-propyl ether in hydrocarbons.

Evidence for zero-, mono- and disolvates of allomerized chlorophyll-*a* was obtained using different amounts of pyridine in *n*-propylbenzene in contact with calcium hydride. In *n*-propylbenzene there was no dry spectrum. 3×10^{-5} *M* pyridine in *n*-propylbenzene, about equivalent to the concentration of the chlorophyll, was sufficient to shift the wave length of the band in the red from 6567 Å. in pure *n*-propylbenzene to 6588 Å. This shift very probably represented the formation of the monopyridinate. The spectrum appearing as the temperature is lowered, giving rise to a Tr = 228°K., represented the dipyridinate. In 2 *M* pyridine at 300°K. the band in the red shifted from 6588 to 6596 Å. due to the formation of the dipyridinate and the Tr representing the equilibrium of mono- and dipyridinate was now at 300°K. In summary, the Tr increases with the concentration of pyridine. These data and the wave lengths of the red band at 300°K. are given in Table III.

TABLE III

ALLOMERIZED CHLOROPHYLL-*a* IN PYRIDINE SOLUTIONS IN *n*-PROPYLBENZENE WITH CALCIUM HYDRIDE

Concn. of pyridine, *M*	Tr, °K.	Wave length of the band in the red at 300°K. (Å.)
0	None	6567
3×10^{-5}	228°	6588
0.03	263°	..[a]
3	300°	6596

[a] This solution contained methylcyclohexane instead of *n*-propylbenzene, and because of the difference in refractive indices the wave lengths of the maxima are not strictly comparable.

It appears then that the possibility of enol-keto isomerism does not change the solvation process in chlorophylls qualitatively. The number of equilibria seems to be the same and their transit temperatures are in the same range. In allomerized chlorophyll, however, there appears to be no evidence for a thermally excited state.

Methyl Bacteriochlorophyllide.—Methyl bacteriochlorophyllide has but one possible resonance configuration, that is, the central magnesium atom can bond only to diagonally opposite pyrrole nitrogens. This compound then offers a means of checking whether resonance hybrids may have an important qualitative influence on the solvation process. The possibility of enol–keto isomerization complicates the interpretation.

In Fig. 10 is shown the spectra of methyl bacteriochlorophyllide-*a* plus -*b* in *n*-propyl ether at three temperatures. The band at 7750 Å. seems to be insensitive to temperature while most of the others engage in obvious changes. The 5750 Å. band at 300°K. exhibits enormous changes. Relative to this band, there is a Tr = 228°K., and the abrupt wave length shift and decrease in intensity of the main band in the blue is in agreement with this observation. This behavior is similar to that of the chlorophylls, and hence the single resonance configuration in methyl bacteriochlorophyll reacts in the same way as the three possible resonance hybrids of chlorophyllide. However, there still remains the possibility that there are three chlorophylls-*b*, for example, and that each chlorophyll is really only one of the configurations separated by an energy barrier from the other.

Fig. 10.—Absorption spectra of methyl bacteriochlorophyllide in *n*-propyl ether at —, 300°K., - - - -, 228°K., and —, 160°K. The band at 5300 Å. is probably due to the presence of spirilloxanthin.

There are indications that methyl bacteriochlorophyllide in *n*-propylbenzene in contact with calcium

hydride exhibits some sort of thermal activation similar to that of chlorophyll-*b*, zero-solvate.

Other Metalloporphins. Copper Etioporphin II.—There is no evidence of species in equilibrium in the spectra of this substance in *n*-propyl ether down to as low a temperature as 138°K. Evidently the copper porphin does not readily solvate with ether.

Zinc Tetraphenylporphin.—Figure 11 shows the spectrum of zinc tetraphenylporphin in *n*-propylbenzene with added calcium hydride, and it also shows the effect on the spectrum produced by the addition of a quantity of pyridine about equivalent to that of the porphin. In the presence of the pyridine the 5500 Å. band is noticeably double at 300°K., and only the long wave length component is present at 250°K. The main band in the blue also shows the same kind of equilibrium reminiscent of behavior of the spectrum of the chlorophylls.

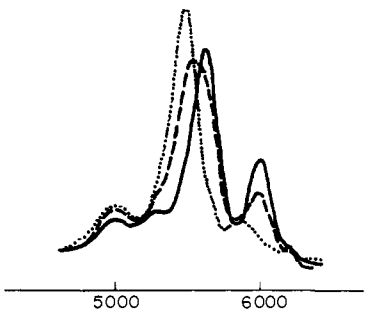

Fig. 11.—Part of the absorption spectra of zinc tetraphenylporphin in *n*-propylbenzene with calcium hydride added; · · · · ·, 300°K. Pyridine added about equivalent to porphin concentration: - - - -, 300°K.; —, 250°K.

The spectra are in agreement with those reported by Miller and Dorough[5] and correspond to the equilibrium between the porphin and the porphin monopyridinate. The changes in spectra that we observed with temperature are entirely consistent with changes brought about by varying concentration which they reported.

Experimental

All spectra were determined by means of a Cary recording spectrophotometer (Model 11). We concerned ourselves principally with the visible region.

In order to study spectra from 75° to 370°K., a Pyrex glass dewar vessel was designed for use in the Cary as is shown schematically in Fig. 12. At the bottom of the dewar vessel, the inner and outer walls terminate in fused quartz cells (A) with plane windows attached to the Pyrex (F) by means of graded seals (B). At the bottom of the sample tube (E) is attached a quartz cell with plane windows spaced about 1 to 3 mm. along the optic axis and about 12 mm. wide and 20 mm. high. The top (K) of the sample tube is closed with a tapered-ground joint, a stopcock and spherical joint. A large rubber stopper (J) is placed on the sample tube so that it rests on the flat lip at the top of the dewar. A section from a rubber inner-tube I serves to form a vacuum seal between the rubber stopper and the dewar. The top of the dewar is fitted with about 3″ of unsilvered single-walled glass to keep the stopper warm and two large ball joints (H) are attached. These ball joints are used to pump on liquid nitrogen to lower its temperature, through which to pass thermocouple wires, and to connect the liquid nitrogen supply to the cooling coil (D) at other times. The top of the copper cooling coil is made so that it can be pushed through one of the ball joints (H). The copper tubing is threaded or snug-fitted using grease to the exit and entrance tubes of the copper coil. Liquid nitrogen is forced through the cooling coil from a dewar reservoir by means of nitrogen gas.

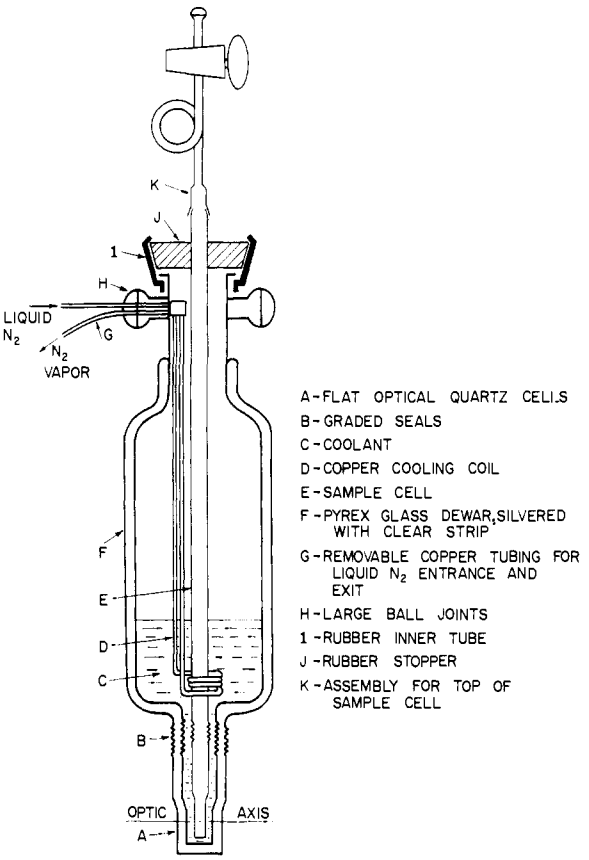

Fig. 12.—Pyrex glass dewar vessel with optical path of fused quartz.

This arrangement is used with a coolant of isopentane for temperatures in the range of 300° to 130°K. and 1:1 propane–propene for the range 230° to 75°K. A thermocouple with its junction at the bottom of the dewar, near the sample, is used to determine temperature. In our set-up the temperature rose about 1 deg./min. When the temperature of liquid nitrogen is desired, the cooling coil is removed. In order to eliminate bubbling the pressure of nitrogen is reduced to about 0.5 atmosphere, incidentally reducing the temperature below 77°K., and then dry nitrogen gas, or better helium, is supplied at a pressure greater than the vapor pressure of the liquid nitrogen. In this way a body of about 500 cc. of liquid nitrogen could be maintained quiet for about 10 minutes. For temperatures greater than 300°K., water was used.

The multipot of the Cary was set for complete compensation while the dewar vessel with coolant, optical cell and solvent was in the sample path and an approximate optical equivalent of quartz in the reference path. No correction was made for the small changes of this setting for temperature of the solvents.

The solvents employed were purified in the following way: *n*-propyl ether, C.P., distilled on 30-plate column over calcium hydride and stored over calcium hydride; methylcyclopentane and methylcyclohexane, pure grade, distilled from calcium hydride, on a 10-plate column; pyridine, C.P., distilled on a 10-plate column over barium oxide; isopropylamine and diisopropylamine distilled on a 10-plate column over calcium hydride; propane and propene, Phillips research grade, distilled in a vacuum line at least twice from one vessel at 193°K. to another at 77°K.

In addition for the samples of the chlorophylls and derivatives supplied by Robert Livingston and co-workers at the University of Minnesota, we are grateful for the methyl

bacteriochlorophyllide prepared by Earl Jacobs of the University of Illinois and supplied to us by Henry Linschitz from the University of Syracuse; for pheophytin-*a* and -*b* supplied by Sam Aronoff and E. Kmetec of Iowa State College; for the copper etioporphin II supplied by Alsoph H. Corwin of the Johns Hopkins University; and for the zinc tetraphenylporphin prepared by G. D. Dorough at Washington University.

Conclusions

1. The prominent bands of the spectrum of chlorophyll-*b* as the zero-solvate in dry hydrocarbons are composed of two components. As the temperature is lowered the short wave length component of bands increases in intensity at the expense of the long wave length component suggesting depopulation of thermally excited states. The thermodynamic value of the heat of this transformation is $\Delta H = -1400 \pm 200$ cal./mole, which compares favorably with the spectroscopic value 1320 cal./mole measured from the energy interval of the two components of the band in the red; confirmation is obtained from the spectroscopic interval in the blue region. Chlorophyll-*a* and possibly methyl bacteriochlorophyllide exhibit this phenomenon; however, there is no evidence that allomerized chlorophyll-*a*, pheophytin-*a* or -*b*, or zinc tetraphenylporphin do so.

2. Solvation of chlorophyll-*b* corresponding to the equilibria between zero- and monosolvate and between mono- and disolvate have been studied by varying temperature and concentration of such polar substances as water, *n*-propyl ether, pyridine and diisopropylamine in the solvents 1:1 propane–propene, *n*-propylbenzene and methylcyclohexane. There is evidence of interaction between chlorophyll-*b* and *n*-propylbenzene. The constant for the equilibrium between mono- and dipyridinate of chlorophyll-*b* in *n*-propylbenzene at 275°K. is about 10 at from 6.7×10^{-4} *M* to 0.1 *M* pyridine and then drops to about 4.5 at 1.76 *M*. Chlorophyll-*a*, allomerized chlorophyll-*a* and methyl bacteriochlorophyllide in a solvent of 20% *n*-propyl ether and 1:1 propane–propene all have transit temperatures representing the equilibrium between the mono- and dietherate in the same range of temperature as does chlorophyll-*b*.

3. The absence of possible enol–keto tautomerism as in allomerized chlorophyll-*a* does not prevent the formation of mono- and disolvates as was shown by the work with pyridine. In ether solutions there is evidence of but one degree of solvation, but this is an artifact caused by the indistinguishability of the spectra of the zero- and monoetherates. It was the presence of the thermally excited level in chlorophyll-*b* which made easy the distinction between the spectra of the zero- and monoetherate.

4. The components of the bands due to different solvates of chlorophyll-*b* in diisopropylamine are very well separated, and in addition there are indications that solvation at the enol–keto couple is occurring. The secondary amine in contrast to the primary amine does not produce the brown color characteristic of the Molisch phase test but nevertheless there is evidence in the spectrum at low temperature that suggests an intermediate. There is rapid equilibration of solvates even at about 75°K.

5. The changes in the spectra of pheophytin-*a* and -*b* in ether solutions at low temperatures indicate that a solvation occurs somewhat similar to that of chlorophyll, and if in the latter the magnesium is the site of solvation it is inferred that the hydrogens of the pyrroles of pheophytin are probably the sites of solvation. In pheophytin-*b* the spectra are simple enough to indicate that another equilibrium is active in addition to the solvation process described above.

6. In 20% *n*-propyl ether and 1:1 propane–propene two samples of pheophytin-*a*, the one prepared by ordinary chromatography and the other extensively purified by paper chromatography, can be easily distinguished by the greater simplicity of the spectrum of the latter at 75°K. while at 300°K. their spectra are almost indistinguishable. At low temperature the simpler spectrum may be imagined to be included within the spectrum of the other substance.

7. The solvation properties of methyl bacteriochlorophyllide as a mixture of *a* and *b* are qualitatively the same as those of chlorophyll-*b* and seem not to be affected by its limitation to only one resonance configuration compared to three possible ones in chlorophyll.

8. Some solvation properties of two metalloporphins were examined. Copper etioporphin II showed no tendency to solvate in *n*-propyl ether. Zinc tetraphenylporphin readily formed a pyridinate, and the changes in spectra with temperature reflecting the shifts in equilibrium between the zero- and monopyridinate are entirely analogous to those brought about by varying pyridine concentration as reported by Miller and Dorough.[5]

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