N-Chloroacetylmescaline. Chloroacetyl chloride (1.68 g, 15 mmol) was added dropwise over a period of 3 min to a stirred solution of 2.78 g (5 mmol) of mescaline sulfate dihydrate in 30 ml of 1.0 N aqueous sodium hydroxide, cooled in an ice bath. After stirring for 10 min the ice bath was removed and stirring continued for 30 min. The mixture was cooled in an ice bath and the precipitated solid collected to yield 1.8 g (62.7%) of a colorless solid. Recrystallization from water gave long fine colorless needles, mp 78–79°.

Anal. Calcd for $C_{13}H_{18}ClNO_4$: C, 54.26; H, 6.30; N, 4.87. Found: C, 54.46; H, 6.46; N, 4.67.

The ir spectrum showed $\lambda_{max}^{CHCl_3}$ 2.85 (w), 3.38 (m), 6.00 (s), 6.30 (s), 6.60 (m), 6.88 (s), 7.10 (w), 7.40 (w), 7.52 (w), 7.65 (w), 8.90 (s), and 10.0 (m) μ. The ultraviolet spectrum showed λ_{max}^{EtOH} 269 mμ (ϵ 700).

N-Bromoacetylmescaline. The same procedure as described for N-chloroacetylmescaline, *i.e.*, the reaction of 2.78 g (5.0 mmol) of mescaline sulfate dihydrate with 3.02 g (15 mmol) of bromoacetyl bromide, yielded 1.77 g (53.3%) of N-bromoacetylmescaline as a colorless solid. Recrystallization from water gave long fine colorless needles, mp 83–84°.

Anal. Calcd for $C_{13}H_{18}BrNO_4$: C, 47.00; H, 5.46; N, 4.22. Found: C, 47.05; H, 5.34; N, 4.16.

The uv spectrum showed λ_{max}^{EtOH} 268 mμ (ϵ 760).

Attempted Photocyclization of N-Bromoacetylmescaline. A solution of 540 mg (1.62 mmol) of N-bromoacetylmescaline in 100 ml of water and 70 ml of alcohol was irradiated with a 200-W Hanovia mercury discharge tube (Vycor filter) under nitrogen for 1 hr. The pale yellow solution was stirred with 500 mg of silver carbonate for 10 min and the silver salts were then removed by filtration through a Büchner funnel containing a matting of Celite filter aid. The filtrate was concentrated to a volume of 50 ml and then lyophilized to leave an orange oil which failed to crystallize. The oil was taken up in 5 ml of ethyl acetate and placed on a chromatography column containing 40 g of alumina (Merck). Ethyl acetate (100 ml) was passed through the column to elute the product. Evaporation of the ethyl acetate left a colorless crystalline solid, mp 87–89°. Recrystallization from isopropyl ether gave 125 mg (30.8%) of colorless needles, mp 90–91°. The compound was identified as N-acetylmescaline (lit.[17] mp 93–94°).

(17) E. Spath and J. Bruck, *Chem. Ber.*, 71B, 1275 (1938).

Anal. Calcd for $C_{13}H_{19}NO_4$: C, 61.65; H, 7.56; N, 5.53. Found: C, 61.54; H, 7.51; N, 5.51.

The nmr spectrum showed a singlet at 1.98 (three protons), multiplets centered at 2.80 and 3.50 (two protons each), a singlet at 3.90 (nine protons), a broad peak at 5.95 (one proton), and a singlet at 6.50 (two protons) ppm.

Attempted Photocyclization of N-Chloroacetylmescaline. A solution of 2.09 g (7.28 mmol) of N-chloroacetylmescaline in 600 ml of alcohol was irradiated with a 200-W Hanovia mercury discharge tube (Vycor filter), under nitrogen for 24 hr. Treatment of the solution with silver carbonate as described above, filtration, and evaporation left a yellow oil. The oil was taken up in 10 ml of ethyl acetate and filtered through 50 g of Merck alumina using 250 ml of ethyl acetate as eluent. Evaporation left a pale yellow crystalline solid which was taken up in 50 ml of hot isopropyl ether, treated with Norit, filtered, and cooled to yield 400 mg of colorless needles, mp 70–86°. Thin layer chromatography (silica gel; 1-propanol–water, 70:30) showed that the solid was mostly N-acetylmescaline, slightly contaminated with some unchanged N-chloroacetylmescaline.

N-Chloroacetyl-3,5-dibromotyrosine. Chloroacetyl chloride (2.69 g; 24 mmol) was added dropwise over a period of 5 min to a stirred solution of 5.7 g (16.8 mmol) of 3,5-dibromotyrosine in 35 ml of 2.0 N aqueous sodium hydroxide, cooled in an ice bath. After removing from the ice bath and stirring for 1 hr longer the solution was adjusted with dilute hydrochloric acid to pH 2 and the precipitated solid collected to yield 4.5 g (64.6%) of the N-chloroacetyl derivative as a pale yellow solid. Recrystallization from water gave fine colorless needles, mp 159–161°.

Anal. Calcd for $C_{11}H_{10}Br_2ClNO_4$: C, 31.80; H, 2.43; N, 3.37. Found: C, 32.00; H, 2.57; N, 3.37.

The infrared spectrum showed λ_{max}^{KBr} 2.91 (s), 5.70 (s), 6.00 (s), 6.45 (s), 6.65 (s), 7.00 (m), 7.50 (w), 7.95 (m), and 8.45 (m) μ. The uv spectrum showed λ_{max}^{EtOH} 285 mμ (ϵ 2680) and 292 mμ (ϵ 2810).

Attempted Photocyclization of N-Chloroacetyl-3,5-dibromotyrosine. Photolysis was conducted with a 200-W Hanovia mercury discharge tube (Vycor filter) under nitrogen in both absolute ethanol and in ethanol–water solution. In all cases only dark brown resinous material was isolated and was not further investigated.

Acknowledgment. We are indebted to Dr. C. M. Foltz for the titration data presented in Figure 1.

Chlorophyll–Ligand Interactions from Nuclear Magnetic Resonance Studies[1]

Joseph J. Katz, Harold H. Strain, Daniel L. Leussing,[2] and Ralph C. Dougherty[2]

Contribution from the Chemistry Division, Argonne National Laboratory, Argonne, Illinois 60439, and the Department of Chemistry, The Ohio State University, Columbus, Ohio 43210. Received September 28, 1967

Abstract: Chlorophyll–ligand coordination interactions, revealed by nmr spectra, have been studied by observing ring-current effects on proton chemical shifts of the ligands bound to chlorophyll. The use of deuteriochlorophyll simplifies interpretation of the nmr spectra. The equilibrium constant for the disaggregation of chlorophyll *a* dimers by methanol in CCl_4 to form chlorophyll monosolvate is found to be $K_1 = 56$ l. mol^{-1}. Equilibria involving the disolvate are less important, $K_2 = 1.3$ l. mol^{-1}. Pentacoordinate magnesium(II) appears to dominate the equilibria. Data are given for the interaction of other aliphatic alcohols and deuteriochlorophylls *a* and *b* and deuteriobacteriochlorophyll. Solvent and temperature dependence were examined. The Mg–O bond distance deduced from ring-current considerations for alcohol–chlorophyll interactions in halocarbon solvents is 3.1 Å.

$\mathbf{I}$t has long been known that chlorophyll interacts with nucleophilic ligands. Chlorophyll coordinates water[3,4] strongly. Livingston[5] found that chlorophylls

a and *b* form stable monosolvates with nucleophiles such as water, alcohols, amines, ketones, and ethers and

(2) The Ohio State University, Columbus, Ohio 43210.
(3) E. Rabinowitch, "Photosynthesis," Vol. I, Interscience Publishers Inc., New York, N. Y., 1945, p 450.
(4) E. E. Jacobs and A. S. Holt, *J. Chem. Phys.*, 20, 1326 (1952).

that chlorophylls are strongly fluorescent when solvated and are nonfluorescent in the unsolvated state. Although Evstigneev, et al.,[6] adduced evidence to show that magnesium was involved in some way in the solvation and fluorescence phenomena, it was only from recent infrared[7,8] and nuclear magnetic resonance (nmr)[9,10] studies that the role of magnesium in these phenomena was recognized explicitly. It now appears well established that magnesium in chlorophyll with coordination number 4 is coordinately unsaturated. It is the coordination unsaturation of the magnesium that leads to chlorophyll–nucleophile interactions, and in the absence of extraneous nucleophiles, to self-interactions[10] to form dimers. Thus, in nonpolar solvents, the coordination unsaturation of the magnesium is relieved by coordination of the ketone oxygen function of one chlorophyll molecule with the magnesium atom of a second chlorophyll molecule to form a dimer. Chlorophyll monomer–dimer equilibria have been studied by a number of workers who have used changes in electronic absorption and fluorescence spectra as an index to the state of aggregation of the chlorophyll.[11–15] Spectrophotometry in the visible has also been used to study stability constants of the related magnesium porphyrins.[16,17]

Unfortunately, quantitative measurements on chlorophyll–chlorophyll or chlorophyll–ligand interactions by absorption spectroscopy in the visible are attended by very serious difficulties. Chlorophyll has a large extinction coefficient, and this necessitates the use of very dilute (10^{-4}–10^{-6} M) solutions, or, at slightly higher concentrations, the collection of difference spectra. In either case the photoreactivity of the pigments is a serious problem. Further, at the high dilutions required for spectrophotometry in the visible it is very difficult to exclude equimolar quantities of water or other adventitious nucleophile ligands from the solutions. Not only are reliable data difficult to obtain by visible absorption spectroscopy, but treatment of the data itself is not at all clear. The application of the Benesi–Hildebrand method to the calculation of association constants from visible absorption spectra is almost certain to give erroneous results because of self-association of the chlorophyll.[18] The magnitude of the difficulty can be judged by a comparison of the results of Sauer, et al.,[13] who studied the dimerization of chloro-

phyll in carbon tetrachloride, and Amster and Porter,[14] who investigated dimer equilibria in dry hydrocarbons. Whereas Sauer, et al.,[13] deduced equilibrium constants for the dimerization of chlorophylls a and b and bacteriochlorophyll, Amster and Porter,[14] using essentially the same experimental procedure, were unable to deduce any equilibrium constant for chlorophyll a. Because a solution containing only dimeric chlorophyll cannot be prepared, it is necessary to deduce the absorption maxima and extinction coefficients of the dimer by indirect means. The values deduced by Sauer, et al.,[13] and Amster and Porter[14] for chlorophyll b dimer are so different as to cast considerable doubt on the whole procedure.[19]

Nmr techniques have been successfully used to elucidate the mode of aggregation of the chlorophylls and the approximate geometry of the chlorophyll dimers.[9,10] Nmr spectroscopy, moreover, offers also a direct technique for examining the interaction of chlorophyll with ligands other than itself. Closs[9] first observed that at approximately equimolar concentrations of methanol and methyl chlorophyllide a in $CDCl_3$ solution, the methyl proton resonance of the methanol is displaced by 1.7 ppm to higher field. This is taken to indicate that the protons of the methanol coordinated to the magnesium atom of the chlorophyllide are subject to a large diamagnetic shift arising from the ring current of the chlorin macrocycle. No significant chemical shifts of ligand protons are observed in the presence of the magnesium-free pheophytins. Storm, Corwin, and coworkers[17] have used the same principle for studying magnesium porphyrin–pyridine interactions by nmr. The ring-current effect makes it possible to detect coordination by hydrogen-containing ligands to chlorophyll by observing the chemical shifts of the ligand in the absence and then in the presence of chlorophyll. Such observations are considerably facilitated by using deuteriochlorophylls,[20] for the ligand chemical shifts can be observed without interference from resonances arising from other hydrogen atoms in the chlorophyll molecule. Further, the C-10 proton in chlorophyll is exchangeable;[21] thus the nmr experiments can be carried out using deuteriochlorophyll with ordinary hydrogen at the C-10 position. The value of the C-10 chemical shift then can be used to determine the relative amounts of monomeric and aggregated chlorophyll, while the ligand proton resonances can be used to determine the relative amounts of bound and free ligand.[22] The chemical shift data in principle allow the calculation of equilibrium constants. Our ultimate objective is to learn something about coordination of chlorophyll with important chloroplast components

(5) R. Livingston and S. Weil, *Nature*, 170, 750 (1952); R. Livingston, W. F. Watson, and J. McArdle, *J. Amer. Chem. Soc.*, 71, 1542 (1949); R. Livingston, *Quart. Rev.* (London), 14, 174 (1960).

(6) V. B. Evstigneev, V. A. Gavrilova, and A. A. Krasnovskii, *Dokl. Akad. Nauk SSSR*, 70, 261 (1950).

(7) J. J. Katz, G. L. Closs, F. C. Pennington, M. R. Thomas, and H. H. Strain, *J. Amer. Chem. Soc.*, 85, 3801 (1963).

(8) A. F. H. Anderson and M. Calvin, *Arch. Biochem. Biophys.*, 107, 251 (1964).

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

(10) J. J. Katz, R. C. Dougherty, and L. J. Boucher in "The Chlorophylls," L. P. Vernon and G. R. Seely, Ed., Academic Press Inc., New York, N. Y., 1966, pp 185–251.

(11) S. Freed and K. M. Sancier, *J. Amer. Chem. Soc.*, 76, 198 (1954).

(12) G. R. Seely, *Spectrochim. Acta*, 21, 1835, 1847 (1965).

(13) K. Sauer, J. R. L. Smith, and A. J. Schultz, *J. Amer. Chem. Soc.*, 88, 2681 (1966).

(14) R. L. Amster and G. Porter, *Proc. Roy. Soc.* (London), A296, 38 (1967).

(15) S. Aronoff and P. Kirk, *Nature*, 214, 722 (1967).

(16) J. R. Miller and G. D. Dorough, *J. Amer. Chem. Soc.*, 74, 3997 (1952).

(17) C. B. Storm, A. H. Corwin, R. R. Arellano, M. Matz, and R. Weintraub, *ibid.*, 88, 2525 (1966).

(18) D. W. Tanner and T. C. Bruice, *J. Phys. Chem.*, 70, 3816 (1966).

(19) For chlorophyll b dimer, Sauer, et al.,[13] deduce λ_{max} 648 nm (ϵ 45,600) and 645 nm (ϵ 59,200) (in CCl_4), whereas Amster and Porter[14] deduced values for the same dimer species of λ_{max} 659 nm (ϵ 33,000) and 641 nm (ϵ 33,000) (in dry 3-methylpentane).

(20) H. H. Strain, M. R. Thomas, and J. J. Katz, *Biochim. Biophys. Acta*, 75, 306 (1963).

(21) R. C. Dougherty, H. H. Strain, and J. J. Katz, *J. Amer. Chem. Soc.*, 86, 4220 (1964).

(22) The nmr line widths of ligands bound to chlorophyll in partially aggregated solutions are generally small compared to the magnitude of the chemical shifts observed on coordination of the ligand to the central magnesium atom of the chlorophyll.[10] This indicates that ligand exchange is rapid on the nmr time scale. The ligand chemical shift is thus the weighted average between bound and free ligand, and complex equilibria can be examined by varying concentrations and measuring chemical shifts. The C-10 chemical shift is similarly a weighted average.[9,10]

such as carotenoids, lipids, quinones, and the like, and with water. To this end, we have now examined the effects of variation of solvent, temperature, and nucleophile on chlorophyll–ligand interactions for a number of aliphatic alcohols with the chlorophylls *a* and *b* and bacteriochlorophyll.

Initial efforts by Closs and coworkers[9] to evaluate equilibrium constants for chlorophyll–methanol interaction suggested that more than simple disaggregation of the chlorophyll dimer is involved. If the reasonable assumption is made that the ketone and ester carbonyl oxygen atoms of the chlorophyll will compete with exogenous ligands for coordination with magnesium, then many energetically permitted species can exist in equilibrium. Thus, we can expect monomeric chlorophyll species such as Chl, Chl-L$_1$, and Chl-L$_2$, dimeric species such as Chl$_2$, Chl$_2$-L$_1$, and Chl$_2$-L$_2$, and perhaps oligomers such as Chl$_n$, Chl$_n$-L$_1$, Chl$_n$-L$_2$, and the like. Reliable chemical shift data can readily be obtained for chlorophyll in the monomeric chlorophyll species and for the free ligand in chlorophyll solutions. The remaining chemical shifts and the equilibrium constants for the principal equilibria can then be obtained by a self-consistent analysis of the nmr data aided by computer methods. In addition to the possibility of quantitative evaluation of the equilibria, nmr spectra provide a convenient and certain qualitative guide to the interaction of ligands with the fifth and sixth (axial) coordination sites of the central magnesium atom.

The size of the chlorin ring makes it possible to map directly the coordination interaction of chlorophyll monomers in two dimensions.[9] With small ligands such as methanol, this kind of aggregation map is no longer possible, because the coordinated ligand must be totally encompassed by the diamagnetic cone of the macrocyclic ring current. Fortunately, considerable work has been directed toward the understanding of macrocyclic ring currents,[23–26] and ring-current approximations have been successfully applied to the study of π–π stacking in porphyrin solutions.[24] Application of ring-current calculations to chlorophyll–ligand interactions should thus provide a reasonable approximation of the geometries involved in the coordination interaction.

Experimental Section

Materials. The chlorophylls used in this study were prepared and purified by our usual procedures.[27] Deuteriochlorophyll[20] was extracted from algae cultured in 99.8% D$_2$O.[28] Traces of coordinate water were removed by repeatedly dissolving the chlorophyll in rigorously dried CCl$_4$ and distilling away the solvent under high vacuum with warming immediately before each experiment.

Because this drying procedure is effective in removing water from chlorophyll (no water can be detected in dried deuteriochlorophyll either by infrared or nmr spectroscopy) and because our solutions are between 10^{-2} and 10^{-1} *M* in chlorophyll as compared to the 10^{-4}–10^{-6} *M* solutions normally used for visible absorption spectroscopy, we can be quite certain that adventitious ligands

(23) C. E. Johnson and F. A. Bovey, *J. Chem. Phys.*, **29**, 1012 (1958).
(24) R. J. Abraham, P. A. Burbidge, A. H. Jackson, and D. B. MacDonald, *J. Chem. Soc., Sect. B*, 620 (1966).
(25) R. J. Abraham, *Mol. Phys.*, **4**, 145 (1961).
(26) J. W. Emsley, J. Feeney, and L. H. Sutcliffe, "High Resolution Nuclear Magnetic Resonance Spectroscopy," Vol. I, Pergamon Press, Oxford, 1965, pp 141, 595.
(27) H. H. Strain and W. A. Svec, in ref 10, pp 21–66.
(28) H. F. DaBoll, H. L. Crespi, and J. J. Katz, *Biotech. Bioeng.*, **4**, 281 (1962). See also A. J. Williams, A. T. Morse and R. S. Stuart, *Can. J. Microbiol.*, **12**, 1167 (1966).

are present to the extent of only a few per cent at most of the nominal ligand, and that exogenous ligand–ligand competitions are not an important factor in our experiments.

The ligands were highest purity commercial materials and were distilled on the vacuum line before use.
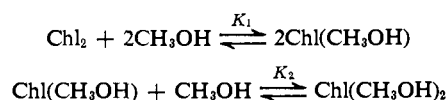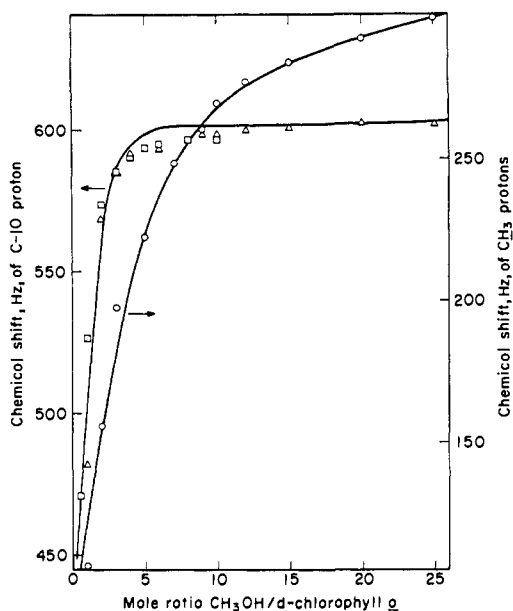
Nmr Samples. All nmr samples were prepared quickly from freshly dried chlorophyll and distilled solvent, thoroughly degassed, and sealed under high vacuum. Titration experiments were carried out in a nitrogen atmosphere, with incremental additions of ligand from a micropipet. No deterioration of the chlorophyll in any of the experiments could be detected from nmr or visible spectra, or by chromatography.

Nmr Spectra. All spectra were obtained with a Varian HA-100 nmr spectrometer equipped with an electronic scan for time-averaging collection of data. All spectra were collected in frequency-sweep mode. Chemical shifts were obtained from measurement of the difference in frequency between the control and analytical channels in the HA-100 system. Chemical shifts are reported in cycles per second (cps) from internal hexamethylsiloxane (HMS), with shifts to lower field positive. In a few instances measurements were made with an external reference, typically benzene. Such chemical shifts have been empirically corrected for bulk susceptibility differences and adjusted to the HMS reference scale.

Results

Equilibrium Constants. Figure 1 is a plot of the observed and calculated chemical shifts for the resonances due to the C-10 proton in deuteriochlorophyll *a* and the CH$_3$ group of methanol as it was added in increments to 0.059 *M* deuteriochlorophyll *a* in dry carbon tetrachloride. The calculated curves were obtained in the following way. We assumed that the chemical shift of methanol in 0.06 *M* deuteriopheophytin *a* in carbon tetrachloride (3.27 ppm) accurately reflected the chemical shift of uncomplexed methanol in the chlorophyll solution. The chemical shift of the C-10 proton in monomeric chlorophyll *a* was obtained by extrapolation of the titration data. The chemical shifts for both C-10 and bound methyl protons, respectively, were assumed to be equal in the Chl(CH$_3$OH) and Chl(CH$_3$OH)$_2$ species.

A set of constants were then assumed for the following equilibria

$$\text{Chl}_2 + 2\text{CH}_3\text{OH} \underset{}{\overset{K_1}{\rightleftharpoons}} 2\text{Chl}(\text{CH}_3\text{OH})$$

$$\text{Chl}(\text{CH}_3\text{OH}) + \text{CH}_3\text{OH} \underset{}{\overset{K_2}{\rightleftharpoons}} \text{Chl}(\text{CH}_3\text{OH})_2$$

Using each set of constants and the total concentrations of chlorophyll and alcohol, it was possible to calculate theoretical equilibrium concentrations of Chl$_2$, Chl(CH$_3$OH), and Chl(CH$_3$OH)$_2$ for each data point. From these calculations and the chemical shifts for the uncomplexed methanol and the chlorophyll C-10 proton, least-squares values of the complexed methanol and chlorophyll dimer C-10 proton chemical shift were calculated. Simultaneously, the sum square of the residuals between the observed and theoretical chemical shifts was obtained. Each equilibrium constant was then varied to determine the effect on the sum square. Additional variations were made in the directions which gave the best fit. This process was repeated until the region of the minimum sum square was mapped. The initial variations in the constants were made over a factor of 2. If the data warranted it, the region of the minimum was scanned using a finer network, to locate the minimum with greater accuracy. In all cases true minima were obtained. The programs in IBM PUFFT language are available on request.

Figure 1. Chlorophyll a-methanol interaction in CCl_4 solution. Chemical shifts of CH_3 (O) and C-10 ($\triangle$) protons as a function of methanol–deuteriochlorophyll (0.064 M) ratio. Additional C-10 points ($\square$) are derived from a CD_3OD titration of ordinary chlorophyll a (0.059 M). The solid lines are the calculated curves.

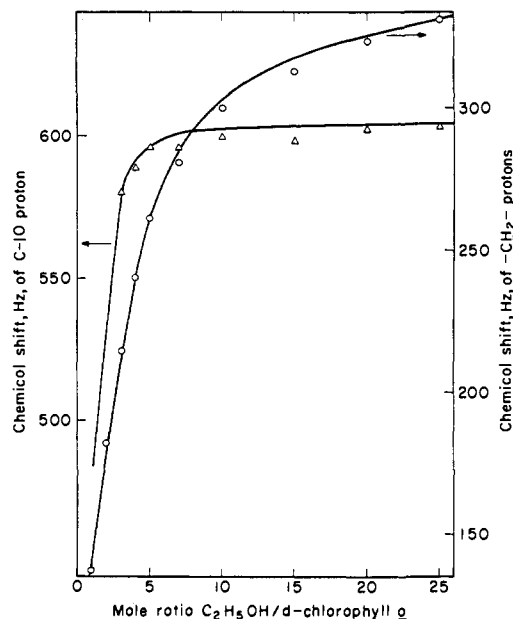Figure 2. Chlorophyll a-ethanol interaction in CCl_4 solution. Chemical shifts of CH_3CH_2OH (O) and C-10 ($\triangle$) protons as a function of ethanol–deuteriochlorophyll a ratio. Deuteriochlorophyll a, 0.068 M. The solid lines show the calculated values.

In the early states of the development of the computer programs for these calculations we also considered the following equilibria

$$Chl_2 + CH_3OH \xrightleftharpoons{K_3} Chl_2(CH_3OH)$$

$$Chl_2 + Chl \xrightleftharpoons{K_4} Chl_3$$
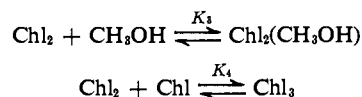
As it became clear that K_1 and K_2 described the predominant equilibria in the solutions, we did not further examine other equilibria. The scatter in the experimental data would allow for a number of minor equilibria in this system. However, at present we have no reasonable method for sorting these out.

Figure 2 is an experimental and calculated disaggregation plot for deuteriochlorophyll a and ethanol in carbon tetrachloride. Figure 3 is a similar plot of deuteriochlorophyll b and t-butyl alcohol in destabilized chloroform. Figures 1–3 are representative of the agreement between the experimental and calculated curves. The agreement would no doubt be improved if the methanol chemical shifts in the $Chl(CH_3OH)$ and $Chl(CH_3OH)_2$ species were allowed to be independent and if more species were included in the calculation. We feel, however, that the agreement is at present as close as our confidence in the data. The major source
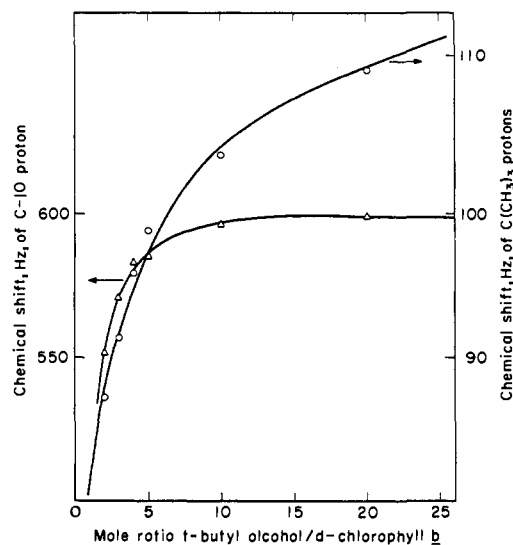

Figure 3. Chlorophyll b-t-butyl alcohol interaction in (destablized) $CHCl_3$ solution. Chemical shifts of $(CH_3)_3COH$ (O) and C-10 ($\triangle$) protons as a function of ligand–deuteriochlorophyll b ratio. Deuteriochlorophyll b, 0.056 M. The solid lines are the calculated values.

of error in the data came from measurement of the small volumes of added nucleophiles.

Table I lists the equilibrium constants that were obtained in the calculations. The only significant variation in K_1 in the series is with t-butyl alcohol.

The close similarity between the interaction of chlorophyll a and chlorophyll b with methanol in chloroform is indicated in Figure 4. Both the C-10 and methyl resonance curves are indistinguishable within the error limits of the experiment. This is not the situation, however, for a comparison of chlorophyll a and bacteriochlorophyll binding.

Table I. Equilibrium Constants for Chlorophyll–Alcohol Interactions

Chlorophyll	Ligand	Solvent	Log K_1	Log K_2
Deuteriochlorophyll a	CH_3OH	CCl_4	1.7	0.1
	CH_3OH	$CHCl_3{}^a$	1.6[b]	0.1[b]
	CH_3CH_2OH	CCl_4	1.6	−1.4
Deuteriochlorophyll b	CH_3OH	$CHCl_3{}^a$	1.6	<−1
	$(CH_3)_3COH$	$CHCl_3{}^a$	1.0	0.0

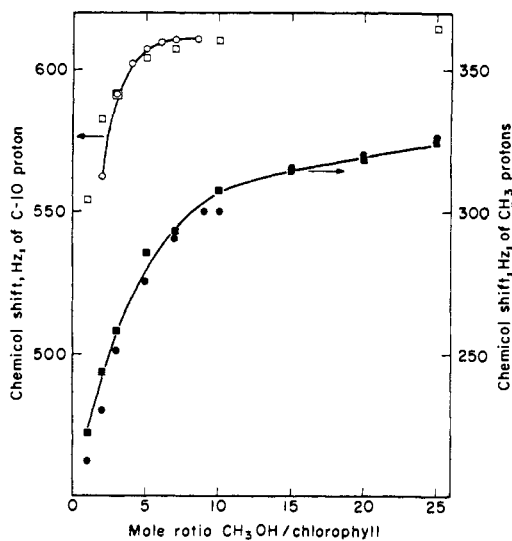[a] In freshly destabilized and dried $CHCl_3$. [b] Obtained from C-10 data only.

Figure 4. Comparison of chemical shifts for methanol interactions with deuteriochlorophylls *a* and *b* as a function of ligand–chlorophyll ratio in CDCl₃ solution. Deuteriochlorophyll *a* experiment: C*H*₃OH (●), C-10 proton (○), deuteriochlorophyll *a* concentration, 0.07 *M*; for deuteriochlorophyll *b* experiment: C*H*₃OH (■), C-10 proton (□), deuteriochlorophyll *b* concentration, 0.056 *M*.
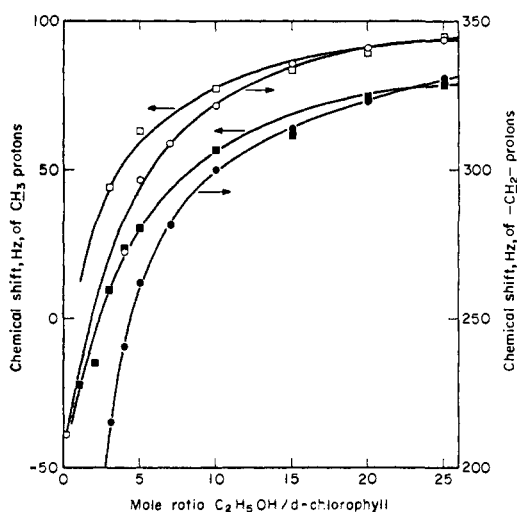

Figure 5. Comparison of methyl and methylene proton resonances of ethanol as a function of ligand–chlorophyll ratio in CCl₄ solution for deuteriochlorophyll *a* (0.068 *M*) and deuteriobacteriochlorophyll (0.056 *M*). Deuteriochlorophyll *a* experiment: C*H*₃ (□), C*H*₂ (○). For deuteriobacteriochlorophyll experiment: C*H*₃ (■), C*H*₂ (●).

Figure 5 compares the ethanol chemical shifts for disaggregation of deuteriochlorophyll *a* and bacteriochlorophyll in carbon tetrachloride. The two sets of curves are similar, but it is obvious that either K_1 is substantially smaller for bacteriochlorophyll than for chlorophyll *a*, or the chemical shift of complexed ethanol is smaller in bacteriochlorophyll than in chlorophyll *a*. The latter would appear to be the more likely of the two possibilities in view of the very close agreement in values of K_1 for chlorophylls *a* and *b*.

Solvent Effects. It is difficult to compare the aggregation behavior of the chlorophylls in a wide range of solvents because most of the solvents that effectively dissolve the chlorophylls are also nucleophilic and thus coordinate with the central magnesium atom.
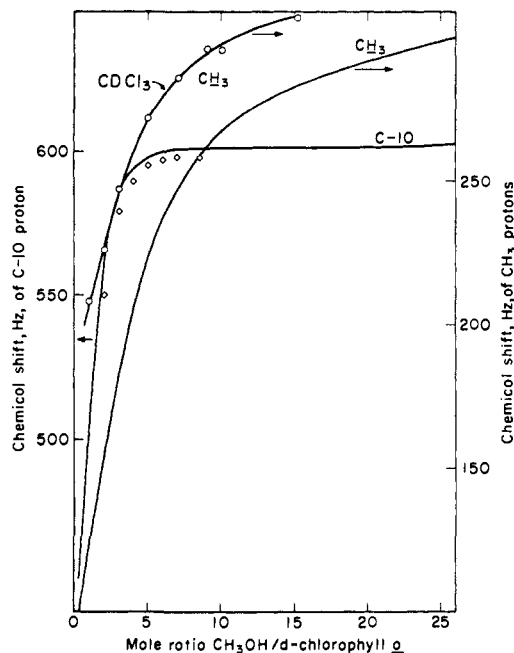

Figure 6. Comparison of methanol–chlorophyll interactions in CCl₄ and CDCl₃ solutions. The two curves (without data points) are the calculated curves for methanol–chlorophyll *a* interactions in CCl₄ solution shown in Figure 1. The curve marked CDCl₃ shows the chemical shifts of C*H*₃OH (○) and of the C-10 proton (◇) in CDCl₃ solution. For the CDCl₃ experiment, the concentration of the deuteriochlorophyll *a* was 0.07 *M*. The C-10 chemical shifts are nearly the same in CCl₄ and CDCl₃, but the C*H*₃ chemical shifts are distinctly larger for a given ligand–chlorophyll ratio in CDCl₃ solution.

We are therefore limited to studies in halocarbons or other relatively nonpolar or nondonor solvents. Surprisingly, substantial solvent effects can be noted even in the limited range of acceptable solvents. Figure 6 compares the disaggregation plots for deuteriochlorophyll *a*–methanol in carbon tetrachloride and destabilized chloroform. Similar plots for deuteriochlorophyll *b* are precisely analogous. The C-10 curves are virtually identical, which requires similar values for K_1 for both systems. However, the methanol chemical shifts are dissimilar in the two solvents, with the chloroform curve showing considerably more curvature at low mole ratios. The proton resonances for uncomplexed methanol were virtually the same in chloroform as in carbon tetrachloride (3.27 ppm). Even at a mole ratio of 35:1, the carbon tetrachloride system showed considerable curvature in the methanol plot, with a chemical shift of 3.06 for the CH₃ resonances being observed. At a mole ratio of 35:1, the chloroform–methanol system disaggregation plot was already quite flat. This can be interpreted by examining the effect of K_2 on the disaggretation curves: if the value of K_2 increases, the mole ratio at which the methanol chemical shift becomes nearly constant also increases. Conversely, the smaller the value of K_2 the more the methanol plot will follow the curvature of the C-10 plots. Thus the data in Figure 6 indicate that the equilibrium constant for formation of Chl(CH₃OH)₂ must be smaller in chloroform than in carbon tetrachloride.

A limited number of studies of chlorophyll *a* disaggregation in tetrachlorethylene indicated that there was no qualitative difference between this solvent and carbon tetrachloride *vis-a-vis* the C-10 resonances. This sug-

gests that K_1 was probably close to the same value in the two solvents. However, there were substantial differences for even large mole ratios of added alcohols. This result is in line with the comparison between chloroform and carbon tetrachloride, K_2 being distinctly larger in the latter solvent.

Temperature Effects. The volatility of most of the solvents used for these studies, as well as the volatility of the ligands, limits the upper range of any study of thermal effects on ligand–chlorophyll interaction. The low solubility of chlorophyll in cold nonpolar solvents limits the low range.

The temperature of a deuteriochlorophyll a–n-propyl alcohol solution in trichlorethylene was increased in 10° increments from ambient to 60°. Over this temperature range the O-methylene shift (from internal HMS) of the n-propyl alcohol increased by 0.2 ppm. The change was in the direction anticipated from the equilibrium constants and from considerations of the change in entropy on coordination. That is, increase in temperature decreases the coordination interactions. The changes in chemical shift were completely reversible on cooling. When chlorophyll a was heated with methanol in tetrachloroethylene to 100°, irreversible changes in the nmr spectrum occurred presumably because of reaction between the chlorophyll and methanol.

Effects of Ligand Structure. The effect of changing the added alcohol from methanol to t-butyl alcohol has been indicated in Figures 3 and 6. The dominant effect of increasing the steric requirements of the alcohol was to decrease the value of K_1 (Table I). We have examined other simple aliphatic alcohols including n- and isopropyl alcohols and n-butyl alcohol. As expected, there appeared to be only a small quantitative gradation in the behavior of these alcohols. All three resembled more closely the behavior of methanol than of t-butyl alcohol.

Phytol, the lipophile ester residue of chlorophylls a and b and bacteriochlorophyll, is, as expected, a disaggregating ligand for chlorophyll a in chloroform. The effects of coordination were seen in upfield shifts for the O-methylene and vinyl proton resonances of the phytol molecule.[29]

Interaction Geometries from Ring-Current Calculations. We have used space-filling molecular (Courtauld) models to estimate the geometries of the chlorophyll–ligand complexes. The estimates of molecular geometry made from models were then used as the basis for calculating the chemical shifts of the complex ligands by the methods of Abraham and his co-workers.[24,25] Direct magnetic susceptibility measurements[30,31] have shown that $\Delta\chi_{\text{porphyrin}}/\Delta\chi_{\text{benzene}}$ equals 16.7, where $\Delta\chi$ is the difference in magnetic susceptibility perpendicular and parallel to the plane of the ring. The relative anisotropy of a porphyrin $vs.$ a benzene ring is thus 16.7. By Pople's[32] equivalent dipole approximation, in which the current loop is replaced by an equivalent dipole at the center of the ring, $\mu = Ai/c$, where A is the area of the ring, i the ring current, and c the velocity of light. Then

(29) The nmr spectrum of phytol is listed in "High Resolution NMR Spectra Catalogue," Varian Associates, Palo Alto, Calif., 1962, Spectrum No. 346, and additional nmr data may be found in ref 10, p 234.
(30) R. Havemann, W. Haberditzl, and P. Grzegorzewski, Z. Physik. Chem. (Leipzig), 217, 91 (1961).
(31) G. Wagniere and M. Gouterman, Mol. Phys., 5, 621 (1962).
(32) J. A. Pople, J. Chem. Phys., 24, 1111 (1956).

$$\mu_{\text{porphyrin}}/\mu_{\text{benzene}} = \Delta\chi_{\text{porphyrin}}/\Delta\chi_{\text{benzene}} = 16.7$$

Taking 5.02 and 37.96 Å² for the areas of the benzene and porphyrin rings, $i_{\text{porphyrin}}/i_{\text{benzene}}$ is found to be 2.21. The factor k, by which the values in Johnson and Bovey's table[23] for benzene[26] must be multiplied to convert benzene to porphyrin values, is

$$k = (i_{\text{porphyrin}}/i_{\text{benzene}})(r_{\text{benzene}}/r_{\text{porphyrin}})$$

Taking the values 1.39 and 3.48 Å for the radii of a benzene and a porphyrin ring, k is calculated to be 0.88.

That chlorophyll a is a chlorin rather than a porphyrin is neglected for several reasons. A rigorous treatment of the difference would have to introduce so many more approximations that the anticipated improvement in the values appears small. Neglecting the chlorin protons will probably introduce only a small error for chemical shifts calculated near the middle of the ring. The loss of two electrons will decrease the ring current and the decrease in ring size will reduce the effective ring radius. The two factors will operate in opposite directions and may nearly cancel each other for a probe near the center of the ring. Further, the complexed ligand will tend to lay away from the chlorin side of the molecule because of the unfavorable steric interactions at the 7 and 8 positions. The approximations we have made would probably not be justified for calculations of chemical shifts in the plane of the ring and near the γ, 7, 8, or δ positions.

Table II compares the theoretical shifts obtained by calculation (as above) from measurements of space-

Table II. Chemical Shifts of Methyl Groups Calculated from Model Geometry by the Method of Abraham[24,25]

Alcohol	Coordinates (Å) Z^a	ρ^b	Chemical shifts, ppm Calcd	Obsd
Methanol	5.0	1.0	4.2	4.3[c]
Ethanol	5.5	1.8	2.8	3.0[d]
n-Propyl alcohol	6.5	2.4	1.7	2.0[d]
n-Butyl alcohol	6.5	5.5	0.5	1.0[d]

[a] Average distance of terminal methyl protons above plane of ring. [b] Average distance of terminal methyl protons from center of ring. [c] Obtained from least-squares analysis of equilibrium. [d] Obtained by linear extrapolation of O-methylene shift vs. methyl shift.

filling models with those obtained by extrapolation of chemical shift data. The experimental values for methanol and ethanol were obtained from the equilibrium constant calculation, and are the best least-squares values for those parameters. The values for the terminal methyl groups of n-propyl alcohol and n-butyl alcohol were obtained by assuming (1) that the shift of the O-methylene groups in these alcohols would be the same as that observed for methanol, because the geometries should be quite similar, and (2) that the equilibria for methanol and the higher alcohols were sensibly identical. The first of these assumptions holds for methanol vs. ethanol, where the shift for the O-methylene protons obtained from the equilibrium constant calculation was 4.4 ppm as compared to the 4.3-ppm shift for methanol. The comparison between methanol and ethanol also lends weight to the second assumption above (see Figures 1 and 2). If the equilibria are the same, then the ratio of the shifts of a given proton at
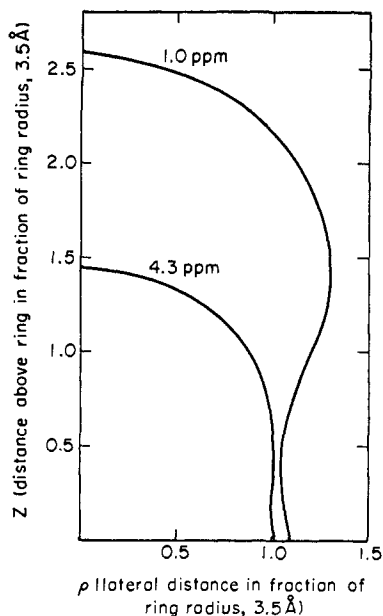
Figure 7. Isoshielding coordinates of protons corresponding to chemical shift of coordinated methanol (4.3 ppm) and the methyl group of n-butyl alcohol (1.0 ppm). Proton distances above ring, Z, and in the plane of the ring, ρ, are given in units of ring radius, 3.5 Å.

two sets of concentrations should be the same as the ratio of the shifts of a different proton at the same two concentrations. This allows us to obtain the experimental values for Table II without doing a complete analysis of every system.

The calculations can also be run backwards to obtain interaction geometries from the experimental chemical shifts. Figure 7 shows the isoshielding coordinates for the experimental shifts of the methyl groups of methanol and n-butyl alcohol. The average coordinates reflected in Figure 7 are very reasonable when space-filling models are used as a basis. These calculations require a magnesium–oxygen distance of 3.1 Å in the Chl(CH₃OH) complex.

Discussion

The values for the equilibrium constants found in this investigation appear to be considerably smaller than the literature values for similar equilibria, but are in surprisingly good agreement with the earlier estimate made by Closs, et al.[9]

Sauer, Smith, and Schultz[13] report a K_d of 10^4, 0.8 × 10^4, and 2.2 × 10^4 for chlorophyll a and b and bacteriochlorophyll, respectively, in the dimerization equilibrium in carbon tetrachloride

$$2\text{Chl} \underset{}{\overset{K_d}{\rightleftharpoons}} \text{Chl}_2$$
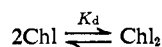
Amster and Porter[14] have reported a value of $K = 1 \times 10^3$ for the following equilibrium between chlorophyll b and pyridine in 3-methylpentane.

$$(\text{Chl-}b)_2 + 2\text{py} \underset{}{\overset{K}{\rightleftharpoons}} 2\text{Chl-}b(\text{py})$$
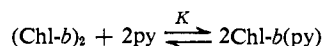
The equilibrium studied by Amster and Porter is precisely analogous to the study reported here. The larger value for K_1 obtained by these workers is probably due to the following: (1) a change in solvent from CCl₄ or CHCl₃ to 3-methylpentane should destabilize

the dimers; (2) because pyridine is a stronger base than the aliphatic alcohols, the pyridine–chlorophyll complex may have a substantially larger enthalpy of formation than the alcohol–chlorophyll complexes, although qualitative nmr results with pyridine and chlorophyll a do not appear to demand this conclusion; (3) the intrinsic difficulties of examining chlorophyll–ligand interactions, which have led to widely divergent estimates of the dimer visible spectrum,[13,14,33] may be as important in this discrepancy as all of the other factors combined.

Storm, Corwin, and their coworkers[17] have reported stability constants for porphyrin–pyridine complexes

$$\text{Mg-porphyrin(py)} + \text{py} \underset{}{\overset{K}{\rightleftharpoons}} \text{Mg-porphyrin(py)}_2$$
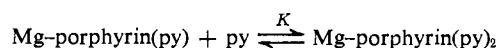
that varied from 0.07 to 0.2, depending on the porphyrin. The changes in solvent and macrocyclic ring and the probable increase in the strength of the first complex easily account for the small differences between the porphyrin–pyridine values and the present values of K_2.

The comparison between disaggregation plots for deuteriochlorophylls a and b indicates that the formyl residue at position 3 in chlorophyll b has apparently very little effect on the primary coordination interactions of the molecule. It is clear that chlorophyll b[11] and bacteriochlorophyll[13] aggregate through their additional carbonyl residues even at low concentrations or high concentrations of added nucleophiles. However, this coordination at sites other than the C-9 carbonyl must be in competition with C-9 aggregation and not in addition to it. The formation of trimeric species at high concentrations of chlorophyll b[7] in nonpolar solvents is probably related to the primary dimer interaction in the same way that K_2 is related to K_1 for exogenous ligands.

The obvious differences among the ethanol titration plots for chlorophyll a and bacteriochlorophyll (Figure 5) are probably related to changes in the magnetic anisotropy of the macrocyclic rings. There is no intuitive way to assess the relative effects on the macrocyclic ring current of a porphyrin on going to first a chlorin and then a dihydrochlorin. However, the effect of the ring-current change should be smaller in the chlorin than the dihydrochlorin because the ligand will tend to occupy the space over pyrrole rings I, II, and III in the chlorin whereas in the dihydrochlorin the ring current will be symmetrically diminished. The added steric interaction of the 3 and 4 positions in bacteriochlorophyll with a coordinated ligand may also account for a small reduction in K_1 in this system.

The relatively minor importance of K_2 in the coordination interactions of chlorophyll a with oxygen nucleophiles is demonstrated by the effect of a change of solvent from carbon tetrachloride to chloroform (Figure 6). In the latter solvent, K_2 virtually disappears. That is, Chl(CH₃OH)₂ is no longer a major species in this solution. This change must be due to the more effective solvation of Chl(CH₃OH) by the somewhat polar chloroform molecules.

There is also evidence to indicate that the coordination number of magnesium(II) is 5 when it is coordinated to

(33) S. S. Brody and M. Brody, *Biochim. Biophys. Acta*, **54**, 495 (1961).

nitrogen. Thus, Swift and Lo[34] find the primary solvation number of magnesium nitrate or perchlorate in liquid ammonia to be 5.0. In chlorophyll, magnesium is coordinated to four nitrogen atoms, which should reduce the Lewis acid character of the magnesium with respect to further coordination. This view appears to be consistent with the calculations of Zerner and Gouterman[35] on the positive charge of metals in metalloporphyrins. The large difference between K_1 and K_2 in our experiments, which strongly implies a preference for pentacoordination of the magnesium, is thus consistent with other evidence.

The fact that K_1 is changed by a factor of 4 when the added alcohol was changed from methanol to t-butyl alcohol suggests that there is a definite steric requirement for coordination of nucleophiles with the central magnesium of chlorophyll. Steric interaction would favor coordination of the low molecular weight alcohols, enol phosphates, or amines with chlorophyll as compared to the bulkier phospho- or sulfolipids or sugar phosphates. Preliminary measurements show plant sulfolipid to be a weak coordinating agent for chlorophyll a in CCl_4 solution.[36]

The calculations of ligand–chlorophyll interaction geometries and theoretical chemical shifts for the Chl-(ROH) complexes show surprisingly close agreement in both directions. This close agreement can be taken as support of the assumptions in the calculations, as it is difficult to see how a broadly self-consistent pattern would emerge from these calculations if the assumptions were very much out of line. The Mg–O bond distance

required by our calculations (3.1 Å) is somewhat longer than would have been anticipated for a strong Mg–O coordination bond. The chlorin π-electron cloud, which has a half-width of 2.5 Å, probably presents a substantial steric barrier to a shorter Mg–O distance.

Conclusion

We believe the experiments described here provide direct evidence for chlorophyll–ligand interactions in halocarbon solvents. The nmr method in conjunction with deuteriochlorophylls makes possible the direct examination of all of the macro species involved in the equilibria, for not only are the nmr spectra simplified, but the C-10 proton, which is now clearly visible, can be used simultaneously to follow the state of aggregation of the chlorophyll itself. The principal species in the equilibrium between aggregated chlorophyll and alcohol are the chlorophyll dimer and the chlorophyll monomer monosolvate. The equilibrium constants for disaggregation of chlorophyll dimer by 2 mol of alcohol to form chlorophyll monosolvate appear to be of the order of 60 l. mol^{-1}. Both chlorophyll dimer and monosolvate contain pentacoordinate magnesium(II). Chlorophyll disolvate species, in which magnesium is hexacoordinate, appear much less important in the solvent systems we have used. The procedure described here appears to be broadly applicable, and other classes of ligands are under investigation.

(34) T. J. Swift and H. H. Lo, *J. Amer. Chem. Soc.*, **89**, 3988 (1967).
(35) M. Zerner and M. Gouterman, *Theoret. Chim. Acta*, **4**, 44 (1966).
(36) J. J. Katz, *Progr. Appl. Spectry.*, in press.

Communications to the Editor

Stabilities of Carbonium Ions in Solution. I. Calorimetric Heats of Formation in Fluorosulfonic Acid–Antimony Pentafluoride Mixtures at −60°[1]

Sir:

We report herein a method for calorimetric estimation of the heats of formation, ΔH_{C^+}, of simple carbonium ions in solution. Until recently the evidence for aliphatic and alicyclic carbonium ions rested mainly on strong inference from kinetic data and analogy to the well-characterized triarylcarbonium salts. Lately, Olah,[2,3] Deno,[4] and Gillespie[5] have demonstrated that

many kinds of aliphatic and alicyclic compounds are converted quickly and completely to stable, well-characterized ions in very strong acid solutions. The carbonium ions are most stable and formed most cleanly in fluorosulfonic acid–antimony pentafluoride mixtures (SbF_5–HSO_3F) at low (−40 to −90°) temperatures where their structures are demonstrated by nmr. With good evidence in hand for their existence in super acid, there is a strong incentive to determine the energy changes for formation of the ions. In this report we describe a calorimeter capable of measuring the heat resulting from rapid formation of 10^{-3} M carbonium ion at temperatures down to −65° with solvents as viscous as 15 mole $\%$ SbF_5 in HSO_3F.

The instrument is basically that described earlier.[6] Ethanol replaces mineral oil as the liquid in the heater. The thermistor (Teflon coated, 2000 ohms at −60°) was

(1) We gratefully acknowledge support of this work by National Science Foundation Grant GP-6550X.
(2) G. A. Olah and C. U. Pittman, Jr., *Advan. Phys. Org. Chem.*, **4**, 305 (1966).
(3) P. von R. Schleyer, W. E. Watts, R. C. Fort, Jr., M. B. Comisarow, and G. A. Olah, *J. Am. Chem. Soc.*, **86**, 5679 (1964).
(4) N. C. Deno, *Progr. Phys. Org. Chem.*, **2**, 129 (1964).
(5) T. Birchall and R. J. Gillespie, *Can. J. Chem.*, **42**, 502 (1964).

(6) E. M. Arnett, W. G. Bentrude, J. J. Burke, and P. McC. Duggleby, *J. Am. Chem. Soc.*, **87**, 1541 (1965).