*trochem.*, **50**, 31 (1974).
(14) T. Kakutani, H. Kinoshita, and M. Senda, *Rev. Polarogr.*, **20**, 15 (1974).
(15) N. Koizumi and S. Aoyagi, *J. Electroanal. Chem. Interfacial Electrochem.*, **55**, 452 (1974).
(16) D. E. Smith, *Crit. Rev. Anal. Chem.*, **2**, 247 (1971).
(17) J. M. Hale, "Reactions of Molecules at Electrodes", N. S. Hush, Ed., Wiley-Interscience, New York, N.Y., 1971, p 229.
(18) M. E. Peover, ref 17, p 259.
(19) R. A. Marcus, *J. Chem. Phys.*, **43**, 679 (1965).
(20) J. P. Brenet and K. Traore, "Transfer Coefficients in Electrochemical Kinetics", Academic Press, London, 1970.
(21) M. E. Peover and B. S. White, *J. Electroanal. Chem. Interfacial Electrochem.*, **13**, 93 (1967).
(22) A. E. J. Forno, M. E. Peover, and R. Wilson, *Trans. Faraday Soc.*, **65**, 1322 (1970).
(23) R. A. Copenhafer, Ph.D. Thesis, University of North Carolina, 1971.
(24) R. D. Rieke, R. A. Copenhafer, A. M. Aguiar, M. S. Chattha, and J. C. Williams, Jr., *J. Electroanal. Chem. Interfacial Electrochem.*, **42**, 309 (1973).
(25) R. D. Rieke, R. A. Copenhafer, A. M. Aguiar, M. S. Chattha, and J. C. Williams, Jr., *J. Chem. Soc., Chem. Commun.*, 1130 (1972).
(26) C. K. White, Ph.D. Thesis, University of North Carolina, 1974.
(27) To be published.
(28) L. Horner and H. Hoffman, *Ber.*, **91**, 45 (1958).
(29) A. Zaki and H. Fahin, *J. Chem. Soc.*, 2701 (1942).
(30) R. D. Rieke, W. E. Rich, and T. H. Ridgway, *J. Am. Chem. Soc.*, **93**, 1962 (1971).
(31) T. Tomes, *Collect. Czech. Chem. Commun.*, **9**, 12 (1937).
(32) R. S. Nicholson, *Anal. Chem.*, **37**, 1351 (1965).
(33) J. Heyrovsky and J. Kuta, "Principle of Polarography", Academic Press, New York, N.Y., 1966.
(34) D. E. Smith, "Electroanalytical Chemistry", Vol. 1, A. J. Bard, Ed., Marcel Dekker, New York, N.Y., 1966.
(35) P. Dalahay, "Double Layer and Electrode Kinetics", Interscience, New York, N.Y., 1965.
(36) A. N. Frumkin, *Z. Phys. Chem.*, **164**, 121 (1933).
(37) J. N. Brown and L. M. Trefonas, *J. Heterocycl. Chem.*, **9**, 187 (1972).
(38) R. L. R., Towns, R. Majeste, J. N. Brown, and L. M. Trefonas, *J. Heterocycl. Chem.*, **7**, 835 (1970).
(39) J. M. Hogan, R. A. Engel, and H. F. Stevenson, *Anal. Chem.*, **42**, 249 (1970).
(40) M. Senda and P. Delahay, *J. Phys. Chem.*, **65**, 1580 (1961).
(41) R. D. Rieke and R. A. Copenhafer, *Tetrahedron Lett.*, 4097 (1971).
(42) R. D. Rieke and R. A. Copenhafer, *Electroanal. Chem. Interfacial Electrochem.*, **56**, 409 (1974).
(43) Personal communication, Professor A. M. Aguiar.
(44) J. H. Stocker, R. M. Jenevein, A. M. Aguier, G. W. Prejean, and N. P. Portnoy, *J. Chem. Soc., Chem. Commun.*, 1478 (1971).

# The Crystal and Molecular Structure and Absolute Configuration of Ethyl Chlorophyllide *a* Dihydrate. A Model for the Different Spectral Forms of Chlorophyll *a*

**Hsi-Chao Chow, Robert Serlin, and Charles E. Strouse***

*Contribution No. 3419 from the Department of Chemistry, University of California, Los Angeles, California 90024.*
*Received December 10, 1974*

**Abstract:** A single crystal x-ray structural determination of ethyl chlorophyllide *a* has provided the first detailed structural data for a magnesium-containing chlorophyll derivative. It has also furnished a model for chlorophyll aggregation in vivo. Ethyl chlorophyllide *a*, prepared by the chlorophyllase catalyzed transesterification of chlorophyll *a*, crystallizes from acetone-water mixtures in the form of the dihydrate. The crystals are trigonal with space group $P3_1$ and unit cell parameters $a = 8.859$ (1), $c = 38.119$ (5) Å, $Z = 3$, $d_{\text{calcd}} = 1.31$, $d_{\text{obsd}} = 1.28$ (3). The crystal structure was determined by standard methods and refined to a conventional $R$ of 0.053. Refinement of the complete data set, including Friedel pairs, in space groups $P3_1$ and $P3_2$ provided an absolute configuration consistent with that determined chemically for chlorophyll *a*. The magnesium atom in this structure is five coordinate with a water molecule in the fifth coordination site. The second water molecule is hydrogen bonded to the coordinated water molecule and to the methyl ester carbonyl oxygen atom of one chlorophyllide molecule and to the carbonyl oxygen atom of the ethyl ester of another. A layered crystal structure is formed in which the two-dimensional aggregate that makes up each layer is composed of cross-linked one-dimensional polymers. These one-dimensional polymers provide the basis for a model of chlorophyll aggregation in photosynthetic organisms. Estimates of the positions of the red absorption maxima of short one-dimensional polymers show a striking correspondence to those observed for chlorophyll in vivo.

Chlorophyll *a* is the principal photosynthetic pigment in algae and higher plants. In the process of photosynthesis this pigment serves both as a collector of photoexcitation and as the electron donor in the primary photosynthetic reaction. A knowledge of the molecular structure of the photosynthetic apparatus depends upon the detailed elucidation of the distribution and organization of this pigment in the cell.

As a basis for such an elucidation, x-ray structural studies of a number of chlorophylls and chlorophyll derivatives have been undertaken in this laboratory. The structure of ethyl chlorophyllide *a* dihydrate reported herein provides the first detailed structural data for a magnesium-containing chlorophyll derivative. The mode of aggregation observed in this structure furnishes a reasonable model for chlorophyll aggregates in vivo. Detailed structural parameters of both the isolated molecule and the aggregate provide a basis for further theoretical studies. A preliminary report of this work has appeared.[1]

## Experimental Section

**Preparation of Ethyl Chlorophyllides *a* and *b*.** The chlorophyllides were prepared by a modified version of the procedure described by Holt and Jacobs.[2] Fresh leaves (1 kg) of a plant rich in chlorophyllase, *Ailanthus altissima*, were blended in a Waring blender with 3 l. of 95% ethanol. The mixture was allowed to stand for 4–5 hr in the dark under a nitrogen atmosphere. The course of the conversion to the chlorophyllides was followed with the basicity test described by Willstätter and Stoll.[3] After filtration under vacuum, the leaf meal was washed with 1 l. of acetone. Fifty grams of talc was then added to the combined filtrate and it was diluted with 4 l. of distilled water. This mixture was allowed to stand for several hours at 5°C and then filtered under vacuum. The layer of talc containing the crystalline chlorophyllide was washed alternately with petroleum ether and 50% aqueous acetone until it had a light gray color. Very thorough washing at this point is necessary for best results. The crystalline chlorophyllides were removed from the talc by extraction with 1 l. of absolute ethanol and recrystallized by dropwise addition of 1 l. of water. The crystals were collected on Whatman No. 50 filter paper and washed with petroleum ether and aqueous acetone. This preparation yields about 1 g of mixed chlorophyllides.

Ethyl chlorophyllides *a* and *b* were separated by column chromatography on powdered sugar. Three glass columns (45 × 8 cm) were packed with sifted powdered sugar with a vacuum (15 cm) applied to the bottom of each column. Crystalline ethyl chlorophyllide (0.35 g) was dissolved in 70 ml of pyridine and diluted to 2.5 l. with petroleum ether. About 100 ml of petroleum ether was applied to each of the columns and one-third of the sample solution was then added to each. The columns were eluted with 0.5% pyridine and 0.5% *n*-propyl alcohol in petroleum ether. Chlorophyllide *a* was eluted from the column first. In this preparation maximum yield was sacrificed for improved purity; the leading and trailing edges of the band were discarded. The eluate was transferred to a separatory funnel and washed exhaustively with water. Upon removal of the pyridine, the chlorophyllide precipitated at the petroleum ether–water interface and was easily collected by filtration. The chlorophyllide *b* band was treated in the same way.

Single crystals were prepared by very slow addition of water containing 100 ppm $Ca^{2+}$ to an acetone solution of the chromatographed chlorophyllide. A motorized syringe was used to add 0.5 ml of water to 3 ml of the acetone solution over a period of days at 0°C. Elemental analysis of the crystalline chlorophyllides prepared by Holt and Jacobs[2] indicated the presence of two water molecules per pigment molecule.

All solvents and reagents were reagent grade and used without further purification. The powdered sugar was C & H brand which contains 3% cornstarch. In early preparations reagent grade petroleum ether (bp 20–40°) was used for the chromatography but in later preparations technical grade pentane was substituted with no apparent pigment degradation.

**X-Ray Measurements.** The crystal of ethyl chlorophyllide *a* dihydrate used for the structure determination was a thin hexagonal plate bounded by faces of the form {01.0}, {10.0}, and {00.1}. The crystal had an average edge length of 0.12 mm and a thickness of 0.05 mm. It was mounted on a Syntex $P\bar{1}$ autodiffractometer with the symmetry axis of the crystal roughly perpendicular to the $\varphi$ axis of the instrument. The diffractometer was equipped with a Cu x-ray source ($\lambda_{\mathrm{Cu}\,K\bar{\alpha}}$ 1.5418), a scintillation counter, a pulse height analyzer, and a graphite monochromator (take-off angle = 4°). The space group ($P3_1$ or $P3_2$) had been established in a preliminary structural determination with a limited data set.[1] Lattice parameters obtained by a least-squares fit of 15 carefully centered reflections were $a = 8.859$ (1) Å and $c = 38.119$ (5) Å. These lattice parameters give a calculated density of 1.31 g/cm$^3$ in agreement with an observed density of 1.28 (3) g/cm$^3$ obtained by flotation in zinc chloride solution. Intensity data were collected at room temperature with the $\theta$–$2\theta$ scan technique for all reflections with $h \geq 0$ and $k \leq 0$. Bisecting mode data collection was used to a maximum $2\theta$ of 115°, and all accessible reflections in the range 115 to 155° were collected in the parallel mode. Each reflection was scanned from 1.0° below the $K\alpha_1$ reflection to 1.0° above the $K\alpha_2$ reflection at a scan rate of 2.0°/min. Total time spent counting background was equal to eight-tenths of the scan time for each reflection. The intensities of three reflections were remeasured every 97 reflections to monitor the tube efficiency and possible crystal deterioration. No significant deviations were observed.

The intensity of a reflection, $I(h,k,l)$, and its estimated standard deviation, $\sigma[I(h,k,l)]$, were calculated[4] according to the equations $I = \mathrm{CT} - (t_c/t_b)(B_1 + B_2)/2$ and $\sigma(I) = [\sigma_s{}^2 + (0.04I)^2]^{1/2}$, where $I$ is the net integrated intensity, CT is the total integrated count, $t_c$ and $t_b$ are the times employed for counting the scan and background, respectively, $B_1$ and $B_2$ are the background counts on the low and high sides of the reflection, and $\sigma_s$ is the contribution to the standard deviation in the intensity from counting statistics. The usual Lorentz-polarization corrections were applied to the data.

Two sets of data were prepared for subsequent refinement. The first set included all reflections with $I > 3\sigma(I)$ while in the second set the intensities of the Friedel pairs were averaged and those reflections for which the averaged $I$ was smaller than 3 times its estimated standard deviation were discarded. The second data set, containing 1919 reflections, was used in the initial least-squares refinements and Fourier difference syntheses while the first data set, with 3335 reflections, was used in the determination of the absolute configuration and final refinement.

**Refinement.** Initial atomic positions used in the refinement were those obtained from a preliminary investigation[1] in which the solution of the structure was obtained by a trial and error technique. Least-squares refinement of these initial positions along with isotropic temperature factors for all carbon and nitrogen atoms and anisotropic thermal parameters for oxygen and magnesium atoms converged to $R = 0.082$.[5] An electron density difference synthesis at this point revealed most of the hydrogen atoms. Reliable positions were not located, however, for hydrogen atoms attached to C37 and for one of the hydrogen atoms attached to O7. In all subsequent structure factor calculations, hydrogen atoms attached to carbon atoms were included in calculated positions. The configuration of each methyl group was based on the best hydrogen position from the electron density difference map. Methylene, methine, and vinyl hydrogen positions were based on only the carbon atom positions. In all cases a C–H distance of 1.0 Å was used. The positions of H17, H18, and H29 were obtained directly from the difference map.

At this stage a small absorption correction was applied to all the intensity data ($\mu = 9.65$ cm$^{-1}$, $T_{\min} = 0.86$). Further refinement with fixed hydrogen atom positions and anisotropic thermal parameters for all nonhydrogen atoms reduced $R$ to 0.069. Another electron density map furnished a somewhat more reliable orientation for the C37 methyl group and for the hydrogens on O7. In both electron density maps two maxima were located for the hydrogen on O7 that is coordinated to O4. These two peaks are separated by about 1.0 Å and lie on either side of a straight line between O7 and O4. A vector between these two maxima is roughly parallel to the longest principal axis of the thermal ellipsoid of O7. These two maxima probably are a consequence of the large thermal motion of the interstitial water molecule.

In subsequent least-squares refinement the real part of the anomalous dispersion correction was included for all nonhydrogen atoms, and the positions of the four water hydrogen atoms were refined along with the positions and anisotropic thermal parameters for all nonhydrogen atoms. The initial position for HC was chosen 1.0 Å along the O4–O7 vector, roughly between the two peaks in the electron density map. In this refinement the thermal parameters for the water hydrogen atoms were fixed at the values obtained for the oxygen atoms to which they were bonded. This refinement converged to $R = 0.050$ and $R_w = 0.048$ for the averaged data set.

The absolute configuration was determined by two parallel refinements in the space groups $P3_1$ and $P3_2$. In these two calculations 3335 reflections were used with anomalous dispersion corrections for the scattering factors of all nonhydrogen atoms. These calculations gave $R_w = 0.0630$ and 0.0637 for the space groups $P3_1$ and $P3_2$, respectively. Application of Hamilton's $R$ factor ratio test[6] indicated that $P3_1$ could be accepted as the correct space group at the 0.995 confidence level. This configuration is in complete accord with that determined chemically by Fleming[7] for chlorophyll *a*. A final refinement in $P3_1$ with recalculated hydrogen coordinates converged to $R = 0.053$ and $R_w = 0.062$. A tabulation of the final atomic parameters is provided in Table I and a listing of observed and calculated structure factors is available.[8]

Scattering factors of Stewart, Davidson, and Simpson[9] were used for hydrogen and those of Hanson, Herman, Lea, and Skillman[10] for all nonhydrogen atoms. The anomalous dispersion corrections were those obtained by Cromer and Liberman.[11] The function minimized in all least-squares refinements was $\Sigma w(|F_o| - |F_c|)^2$ where $w = 1/(\sigma(F))^2$.

## Description of the Structure

The molecular structure of ethyl chlorophyllide *a* dihydrate is shown in Figure 1. The five-coordinate magnesium atom is displaced 0.39 Å from the N1–N2–N3 plane in the same direction as the methyl ester substituent. A water molecule occupies the fifth coordination site. A second "interstitial" water molecule is hydrogen bonded both to the coordinated water molecule and to the carbonyl oxygen

Table I. Final Atomic Parameters for Ethyl Chlorophyllide *a* Dihydrate[a]

| Atom | $x/a$ | $y/b$ | $z/c$ | $\beta$ | Atom | $x/a$ | $y/b$ | $z/c$ | $\beta$ |
|---|---|---|---|---|---|---|---|---|---|
| H5 | 0.3081 | -0.1930 | -0.2472 | 4.00 | H27A | -0.5604 | 0.0678 | -0.1612 | 4.00 |
| H10 | -0.1635 | 0.1367 | -0.2163 | 4.00 | H27B | -0.4479 | 0.1053 | -0.1974 | 4.00 |
| H17 | -0.3529 | -0.5208 | -0.0439 | 4.00 | H27C | -0.3582 | 0.2272 | -0.1628 | 4.00 |
| H18 | -0.2292 | -0.7296 | -0.0792 | 4.00 | H29 | -0.6212 | -0.4507 | -0.0847 | 4.00 |
| H20 | 0.0238 | -0.6582 | -0.1127 | 4.00 | H30A | -0.5346 | -0.8018 | -0.0937 | 4.00 |
| H21A | 0.3811 | -0.6284 | -0.1582 | 4.00 | H30B | -0.6224 | -0.7033 | -0.0728 | 4.00 |
| H21B | 0.1992 | -0.7828 | -0.1752 | 4.00 | H31A | -0.4643 | -0.8695 | -0.0345 | 5.00 |
| H21C | 0.2022 | -0.7181 | -0.1348 | 4.00 | H31B | -0.6693 | -0.9797 | -0.0461 | 5.00 |
| H22 | 0.3868 | -0.5651 | -0.2282 | 5.30 | H32A | -0.0387 | -0.4155 | -0.0379 | 6.00 |
| H23A | 0.5963 | -0.3359 | -0.2603 | 7.00 | H32B | 0.0475 | -0.5331 | -0.0500 | 6.00 |
| H23B | 0.5312 | -0.1986 | -0.2418 | 7.00 | H32C | -0.1070 | -0.6078 | -0.0205 | 6.00 |
| H24A | 0.3818 | 0.0184 | -0.2844 | 5.00 | H34A | -0.5690 | -0.1524 | 0.0072 | 6.00 |
| H24B | 0.3465 | 0.1736 | -0.2977 | 5.00 | H34B | -0.5511 | -0.3158 | 0.0222 | 6.00 |
| H24C | 0.2203 | -0.0265 | -0.3112 | 5.00 | H34C | -0.7434 | -0.3339 | 0.0186 | 6.00 |
| H25A | 0.1382 | 0.2693 | -0.2921 | 5.00 | H36A | -0.6744 | -0.7877 | 0.0605 | 6.00 |
| H25B | 0.0129 | 0.2829 | -0.2622 | 5.00 | H36B | -0.5558 | -0.5886 | 0.0459 | 6.00 |
| H26A | -0.0943 | 0.0527 | -0.3221 | 7.00 | H37A | -0.3083 | -0.6355 | 0.0657 | 7.00 |
| H26B | -0.1655 | 0.1837 | -0.3103 | 7.00 | H37B | -0.4603 | -0.7729 | 0.0923 | 7.00 |
| H26C | -0.2249 | 0.0041 | -0.2883 | 7.00 | H37C | -0.4036 | -0.5667 | 0.0930 | 7.00 |

| Atom | $x/a$ | $y/b$ | $z/c$ | $\beta_{11}$ | $\beta_{22}$ | $\beta_{33}$ | $\beta_{12}$ | $\beta_{13}$ | $\beta_{23}$ |
|---|---|---|---|---|---|---|---|---|---|
| HA | 0.2191 (91) | -0.0952 (90) | -0.1125 (17) | 180 | 194 | 79 | 115 | -73 | -38 |
| HB | 0.0933 (90) | -0.0053 (90) | -0.1046 (18) | 180 | 194 | 79 | 115 | -73 | -38 |
| HC | 0.0345 (136) | 0.1051 (156) | -0.0520 (27) | 215 | 432 | 134 | 116 | 12 | -299 |
| HD | -0.1112 (115) | -0.0303 (138) | -0.0638 (24) | 215 | 432 | 134 | 116 | 12 | -299 |
| MG | -0.0395 (3) | -0.2339 (3) | -0.1590 | 175 (5) | 158 (5) | 60 (2) | 100 (5) | 7 (8) | 31 (9) |
| O1 | -0.6747 (7) | -0.1692 (7) | -0.0927 (1) | 195 (11) | 270 (14) | 66 (4) | 166 (11) | 5 (18) | 13 (19) |
| O2 | -0.3509 (7) | -0.1819 (8) | -0.0339 (2) | 140 (12) | 366 (17) | 75 (5) | 67 (12) | -33 (19) | -130 (23) |
| O3 | -0.6375 (7) | -0.3464 (7) | -0.0267 (1) | 166 (11) | 287 (14) | 53 (4) | 97 (11) | 66 (17) | 11 (19) |
| O4 | -0.7400 (9) | -0.7630 (9) | -0.0085 (2) | 345 (18) | 519 (22) | 81 (5) | 315 (18) | 78 (25) | 59 (27) |
| O5 | -0.5185 (7) | -0.7672 (7) | 0.0192 (2) | 212 (12) | 253 (13) | 79 (5) | 139 (11) | 22 (20) | -45 (20) |
| O6 | 0.1244 (7) | -0.0623 (7) | -0.1226 (1) | 178 (12) | 192 (12) | 77 (5) | 107 (10) | -70 (19) | -43 (18) |
| O7 | -0.0031 (10) | 0.0432 (13) | -0.0665 (2) | 232 (16) | 489 (28) | 124 (8) | 149 (18) | 17 (30) | -265 (34) |
| N1 | 0.0837 (7) | -0.3688 (7) | -0.1741 (1) | 141 (12) | 142 (12) | 49 (4) | 82 (10) | 20 (19) | 21 (19) |
| N2 | 0.0234 (7) | -0.1069 (7) | -0.2076 (2) | 169 (13) | 141 (12) | 49 (4) | 90 (10) | -3 (20) | -4 (18) |
| N3 | -0.2271 (7) | -0.1677 (7) | -0.1570 (2) | 145 (12) | 146 (12) | 57 (5) | 84 (10) | 31 (19) | 61 (20) |
| N4 | -0.1809 (7) | -0.4338 (7) | -0.1200 (1) | 141 (12) | 137 (12) | 43 (4) | 79 (10) | -23 (18) | 11 (18) |
| C1 | 0.0876 (9) | -0.4995 (9) | -0.1553 (2) | 161 (16) | 136 (15) | 59 (6) | 87 (13) | -52 (25) | -90 (25) |
| C2 | 0.2080 (9) | -0.5420 (9) | -0.1724 (2) | 131 (15) | 140 (15) | 70 (6) | 81 (13) | -110 (25) | -113 (26) |
| C3 | 0.2732 (9) | -0.4415 (10) | -0.2011 (2) | 128 (15) | 164 (16) | 63 (6) | 86 (13) | 6 (24) | -25 (26) |
| C4 | 0.1955 (9) | -0.3281 (9) | -0.2024 (2) | 117 (14) | 144 (15) | 53 (5) | 54 (12) | 26 (23) | -30 (24) |
| C5 | 0.2216 (9) | -0.2079 (10) | -0.2277 (2) | 142 (15) | 172 (16) | 57 (6) | 92 (14) | 10 (24) | 9 (25) |
| C6 | 0.1424 (9) | -0.1034 (9) | -0.2307 (2) | 129 (15) | 131 (15) | 50 (6) | 31 (12) | -23 (24) | -38 (24) |
| C7 | 0.1705 (9) | 0.0161 (9) | -0.2597 (2) | 164 (16) | 142 (15) | 50 (6) | 77 (14) | 42 (24) | 13 (24) |
| C8 | 0.0625 (9) | 0.0813 (9) | -0.2538 (2) | 161 (16) | 134 (15) | 52 (6) | 55 (13) | 4 (24) | 71 (23) |
| C9 | -0.0277 (9) | 0.0070 (9) | -0.2207 (2) | 156 (16) | 156 (15) | 35 (5) | 67 (13) | 23 (22) | 67 (23) |
| C10 | -0.1502 (10) | 0.0390 (9) | -0.2056 (2) | 184 (17) | 126 (15) | 54 (6) | 78 (13) | -21 (25) | 51 (24) |
| C11 | -0.2492 (10) | -0.0424 (9) | -0.1752 (2) | 170 (15) | 130 (14) | 61 (6) | 93 (13) | -41 (25) | -20 (25) |
| C12 | -0.3816 (9) | -0.0195 (9) | -0.1595 (2) | 153 (16) | 152 (15) | 53 (6) | 102 (13) | -41 (24) | 2 (24) |
| C13 | -0.4422 (9) | -0.1376 (9) | -0.1313 (2) | 125 (14) | 133 (14) | 56 (6) | 59 (12) | -9 (24) | -3 (24) |
| C14 | -0.3438 (9) | -0.2236 (9) | -0.1306 (2) | 130 (14) | 143 (15) | 53 (5) | 93 (12) | -21 (23) | 24 (23) |
| C15 | -0.3852 (9) | -0.3444 (9) | -0.1036 (2) | 122 (14) | 141 (14) | 45 (5) | 55 (12) | -11 (22) | -5 (23) |
| C16 | -0.3091 (8) | -0.4432 (8) | -0.0977 (2) | 134 (14) | 126 (13) | 34 (5) | 72 (12) | 17 (21) | -11 (21) |
| C17 | -0.3486 (9) | -0.5742 (9) | -0.0683 (2) | 145 (15) | 116 (13) | 47 (5) | 62 (12) | 10 (22) | 48 (21) |
| C18 | -0.1990 (9) | -0.6168 (9) | -0.0720 (2) | 173 (16) | 161 (16) | 62 (6) | 107 (14) | 37 (26) | 93 (25) |
| C19 | -0.1236 (9) | -0.5392 (9) | -0.1078 (2) | 142 (14) | 117 (14) | 51 (5) | 57 (13) | -1 (23) | 9 (22) |
| C20 | -0.0041 (9) | -0.5716 (8) | -0.1245 (2) | 142 (15) | 120 (14) | 60 (6) | 76 (12) | 0 (25) | 11 (23) |
| C21 | 0.2509 (10) | -0.6753 (10) | -0.1593 (2) | 194 (18) | 179 (17) | 87 (7) | 139 (15) | 7 (28) | -30 (28) |
| C22 | 0.3979 (11) | -0.4445 (11) | -0.2263 (2) | 192 (19) | 176 (18) | 96 (8) | 92 (16) | 49 (31) | 52 (32) |
| C23 | 0.5150 (13) | -0.3197 (14) | -0.2444 (3) | 278 (24) | 350 (28) | 110 (10) | 197 (24) | 44 (38) | -3 (42) |
| C24 | 0.2912 (11) | 0.0521 (11) | -0.2901 (2) | 251 (20) | 227 (19) | 55 (6) | 126 (16) | 89 (28) | 97 (28) |
| C25 | 0.0322 (10) | 0.1974 (10) | -0.2769 (2) | 223 (19) | 204 (18) | 68 (7) | 116 (16) | 92 (29) | 170 (28) |
| C26 | -0.1220 (13) | 0.1058 (12) | -0.3006 (2) | 362 (27) | 295 (23) | 77 (7) | 168 (21) | -141 (37) | 98 (35) |
| C27 | -0.4380 (10) | 0.1050 (10) | -0.1712 (2) | 182 (17) | 165 (16) | 65 (6) | 114 (14) | -38 (25) | -5 (25) |
| C28 | -0.5635 (9) | -0.2038 (9) | -0.1029 (2) | 141 (15) | 143 (15) | 50 (6) | 80 (13) | -32 (23) | -47 (23) |
| C29 | -0.5310 (8) | -0.3395 (9) | -0.0828 (2) | 134 (14) | 128 (14) | 53 (5) | 80 (12) | 2 (23) | 0 (22) |
| C30 | -0.5285 (9) | -0.7381 (9) | -0.0716 (2) | 141 (15) | 142 (15) | 58 (6) | 55 (12) | 29 (24) | 65 (23) |
| C31 | -0.5711 (10) | -0.8590 (10) | -0.0398 (2) | 206 (18) | 188 (17) | 71 (7) | 115 (15) | 111 (27) | 71 (26) |
| C32 | -0.0639 (11) | -0.5374 (14) | -0.0436 (2) | 180 (19) | 532 (34) | 69 (7) | 204 (21) | 3 (29) | 211 (38) |
| C33 | -0.4904 (11) | -0.2798 (10) | -0.0454 (2) | 171 (17) | 180 (17) | 59 (6) | 98 (15) | -14 (28) | 29 (26) |
| C34 | -0.6252 (11) | -0.2836 (12) | 0.0086 (2) | 267 (21) | 370 (26) | 51 (7) | 179 (20) | 72 (30) | 9 (31) |
| C35 | -0.6176 (11) | -0.7883 (11) | -0.0087 (2) | 178 (18) | 195 (19) | 83 (8) | 104 (16) | 78 (30) | 131 (29) |
| C36 | -0.5587 (13) | -0.7014 (12) | 0.0511 (2) | 304 (24) | 296 (24) | 83 (7) | 185 (20) | 43 (37) | -74 (35) |
| C37 | -0.4255 (14) | -0.6676 (13) | 0.0776 (3) | 342 (28) | 278 (23) | 97 (9) | 132 (21) | -102 (41) | -158 (38) |

[a] Standard deviations, shown in parentheses, refer to the last digit of the preceding number. The anisotropic temperature factor $T$ is defined as $T = \exp[-(\beta_{11}h^2 + \beta_{22}k^2 + \beta_{33}l^2 + 2\beta_{12}hk + 2\beta_{13}hl + 2\beta_{23}kl)]$.

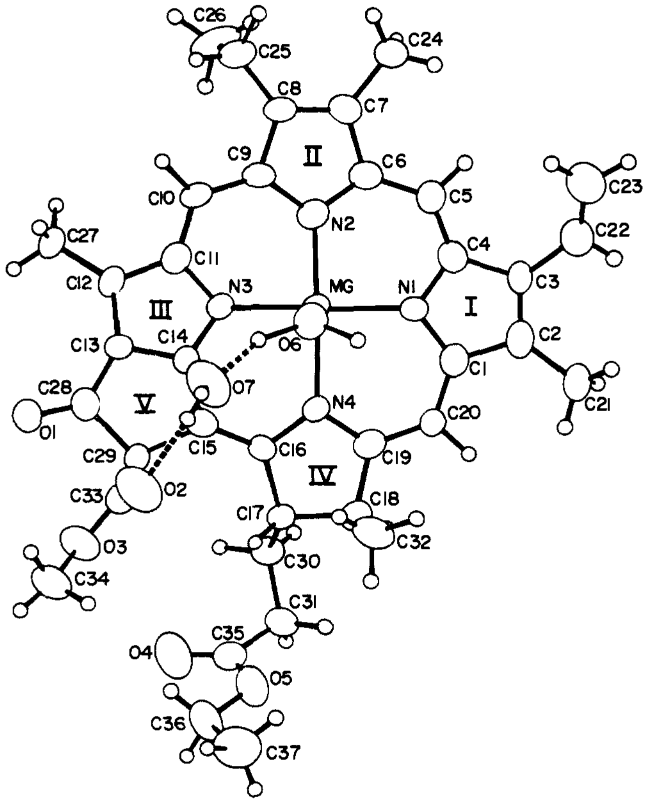

**Figure 1.** The molecular structure of ethyl chlorophyllide *a* dihydrate. For nonhydrogen atoms 50% probability ellipsoids are shown.

atom of the methyl ester. Intermolecular hydrogen bonds result in the formation of a two-dimensional aggregate consisting of cross-linked one-dimensional polymers. The one-dimensional polymers (Figure 2) result from the formation of a hydrogen bond between the coordinated water molecule and the ketone oxygen atom of an adjacent molecule. In the crystal, these polymers are cross-linked by a longer hydrogen bond between the interstitial water molecule and the carbonyl oxygen atom of the ethyl ester (Figure 3). The nature of these one-dimensional polymers is of special significance since they provide a structural model for chlorophyll aggregation in photosynthetic organisms (vide infra).

Intramolecular distances and angles in ethyl chlorophyllide *a* dihydrate are tabulated in Table II. The magnesium–nitrogen bonding in ethyl chlorophyllide *a* is distinctly asymmetric with Mg-N bond lengths ranging from 2.021 (6) Å for Mg-N3 to 2.167 (6) Å for Mg-N4. The average distance of 2.086 Å is in good agreement with the value of 2.07 ± 0.02 Å predicted for the Mg-N distance in a hydrated magnesium porphine complex on the basis of the structure of hydrated magnesium phthalocyanin.[12]

Several of the bond lengths in the $\beta$ substituents of ethyl chlorophyllide *a* appear to be affected by thermal motion. In particular, the C-C distances in the two ethyl groups are 1.49 (1) and 1.47 (1) Å, significantly shorter than a normal C-C single bond length of 1.54 (1) Å. Similarly the C=C distance in the vinyl group is 1.27 Å compared to a normal double bond distance of 1.34 (1) Å.[13] Thermal motion correction with a riding model gives bond distances of 1.52 and 1.48 Å for the ethyl groups and 1.29 Å for the vinyl group.

As observed in methyl pheophorbide *a*,[14] and in nickel deoxophylloerythrin methyl ester,[15] the C28-C29 distance, 1.57 Å, is longer than an ordinary C-C single bond. This long distance is probably a manifestation of the strain in the isocyclic ring.

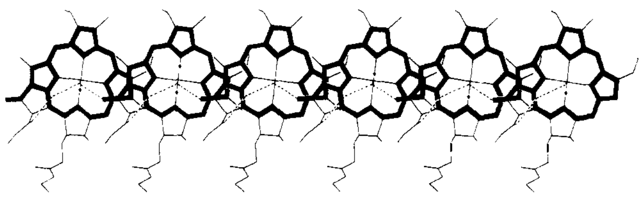

**Figure 2.** A fragment of the one-dimensional polymer found in crystalline ethyl chlorophyllide *a* dihydrate. The heavy lines indicate the conjugated portion of each molecule. Dashed lines indicate hydrogen bonds.

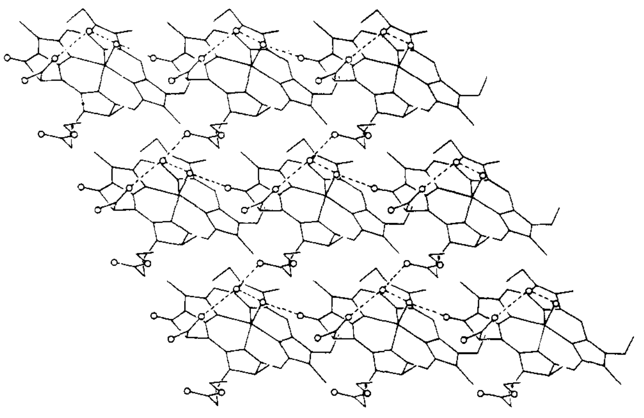

**Figure 3.** The packing of one layer in the structure of ethyl chlorophyllide *a* dihydrate viewed down the $3_1$ axis.

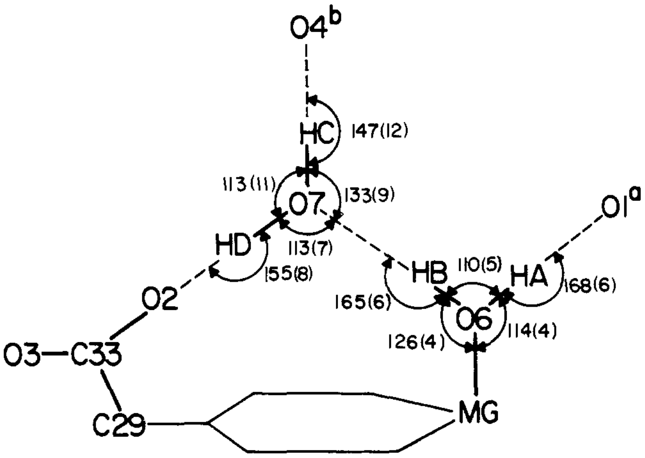

**Figure 4.** The hydrogen bonding network in ethyl chlorophyllide *a* dihydrate.

Distances and angles in the hydrogen bonding network are presented in Figure 4 and Table III. Deviations from linearity for the O-H- - -O angles are probably not significant. The coordination of each of the water oxygen atoms is roughly tetrahedral.

Short intermolecular interatomic distances between nonhydrogen atoms are listed in Table IV. The only unusually short distance involving hydrogen is the 2.01 Å distance between H5 and H23B. The configurations of the methyl groups, particularly those at C37 and C21, obtained from the electron density maps are subject to some uncertainty. In most cases the difference map showed a torus of electron density with two or three distinct maxima which were assigned as hydrogen positions.

Distances of the nonhydrogen atoms from the N1-N2-N3 plane are shown in Figure 5. It can be seen in this figure that each of the unsaturated pyrrole rings is rotated significantly out of the N1-N2-N3 plane with the $\beta$-carbon atoms below this plane and the magnesium atom above.

Table II. Bond Distances and Angles between Nonhydrogen Atoms

Distances

| | | | | | |
|---|---|---|---|---|---|
| Mg | N1 | 2.063 (6) | C2 | C3 | 1.346 (9) |
| Mg | N2 | 2.094 (6) | C7 | C8 | 1.361 (9) |
| Mg | N3 | 2.021 (6) | C12 | C13 | 1.404 (9) |
| Mg | N4 | 2.167 (6) | C17 | C18 | 1.555 (9) |
| Mg | O6 | 2.035 (6) | | | |
| | | | C4 | C5 | 1.369 (9) |
| N1 | C1 | 1.377 (8) | C5 | C6 | 1.418 (9) |
| N1 | C4 | 1.384 (8) | C9 | C10 | 1.377 (9) |
| N2 | C6 | 1.361 (8) | C10 | C11 | 1.414 (9) |
| N2 | C9 | 1.388 (8) | C14 | C15 | 1.397 (9) |
| N3 | C11 | 1.402 (8) | C15 | C16 | 1.364 (9) |
| N3 | C14 | 1.347 (8) | C19 | C20 | 1.383 (9) |
| N4 | C16 | 1.387 (8) | C20 | C1 | 1.388 (9) |
| N4 | C19 | 1.348 (8) | | | |
| | | | C2 | C21 | 1.495 (9) |
| O1 | C28 | 1.232 (8) | C3 | C22 | 1.475 (10) |
| O2 | C33 | 1.183 (8) | C7 | C24 | 1.500 (9) |
| O3 | C33 | 1.336 (8) | C8 | C25 | 1.478 (9) |
| O3 | C34 | 1.442 (9) | C12 | C27 | 1.489 (9) |
| O4 | C35 | 1.212 (9) | C13 | C28 | 1.430 (9) |
| O5 | C35 | 1.330 (9) | C15 | C29 | 1.534 (9) |
| O5 | C36 | 1.469 (9) | C17 | C30 | 1.533 (9) |
| | | | C18 | C32 | 1.502 (11) |
| C1 | C2 | 1.451 (9) | C22 | C23 | 1.274 (11) |
| C3 | C4 | 1.476 (9) | C25 | C26 | 1.493 (11) |
| C6 | C7 | 1.464 (9) | C28 | C29 | 1.568 (9) |
| C8 | C9 | 1.463 (9) | C29 | C33 | 1.500 (9) |
| C11 | C12 | 1.420 (9) | C30 | C31 | 1.535 (9) |
| C13 | C14 | 1.416 (9) | C31 | C35 | 1.494 (10) |
| C16 | C17 | 1.524 (8) | C36 | C37 | 1.466 (13) |

Angles

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| N1 | Mg | N2 | 89.3 (2) | Mg | N1 | C1 | 125.8 (4) |
| N2 | Mg | N3 | 85.0 (2) | Mg | N1 | C4 | 125.7 (5) |
| N3 | Mg | N4 | 89.4 (2) | Mg | N2 | C6 | 125.3 (5) |
| N4 | Mg | N1 | 88.6 (2) | Mg | N2 | C9 | 127. 7 (4) |
| N1 | Mg | N3 | 158.2 (3) | Mg | N3 | C11 | 132.2 (5) |
| N2 | Mg | N4 | 159.4 (2) | Mg | N3 | C14 | 120.8 (4) |
| N1 | Mg | O6 | 103.7 (2) | Mg | N4 | C16 | 126.1 (4) |
| N2 | Mg | O6 | 107.7 (2) | Mg | N4 | C19 | 122.6 (4) |
| N3 | Mg | O6 | 98.1 (2) | | | | |
| N4 | Mg | O6 | 92.7 (2) | | | | |

Angles

| | | | |
|---|---|---|---|
| C1 | N1 | C4 | 108.0 (6) |
| C6 | N2 | C9 | 106.5 (6) |
| C11 | N3 | C14 | 105.9 (5) |
| C16 | N4 | C19 | 109.2 (5) |
| N1 | C1 | C2 | 108.4 (6) |
| N1 | C4 | C3 | 108.3 (6) |
| N2 | C6 | C7 | 110.7 (6) |
| N2 | C9 | C8 | 109.4 (6) |
| N3 | C11 | C12 | 111.0 (6) |
| N3 | C14 | C13 | 110.7 (6) |
| N4 | C16 | C17 | 111.4 (5) |
| C1 | C2 | C3 | 108.7 (6) |
| C4 | C3 | C2 | 106.6 (6) |
| C6 | C7 | C8 | 106.3 (6) |
| C9 | C8 | C7 | 107.1 (6) |
| C11 | C12 | C13 | 104.5 (6) |
| C14 | C13 | C12 | 107.9 (6) |
| C16 | C17 | C18 | 102.6 (5) |
| C19 | C18 | C17 | 101.9 (5) |
| N1 | C1 | C20 | 124.9 (6) |
| N1 | C4 | C5 | 124.9 (7) |
| N2 | C6 | C5 | 124.4 (6) |
| N2 | C9 | C10 | 125.2 (6) |
| N3 | C11 | C10 | 120.8 (6) |
| N3 | C14 | C15 | 133.9 (6) |
| N4 | C16 | C15 | 121.0 (6) |
| N4 | C19 | C20 | 125.3 (6) |
| C4 | C5 | C6 | 128.1 (7) |
| C9 | C10 | C11 | 126.1 (6) |
| C14 | C15 | C16 | 125.9 (7) |
| C19 | C20 | C1 | 128.7 (6) |
| C20 | C1 | C2 | 126.6 (7) |
| C20 | C19 | C18 | 121.8 (6) |
| C5 | C4 | C3 | 126.8 (7) |
| C5 | C6 | C7 | 124.8 (7) |
| C10 | C9 | C8 | 125.3 (7) |

| | | | |
|---|---|---|---|
| C10 | C11 | C12 | 128.3 (6) |
| C15 | C14 | C13 | 115.4 (6) |
| C15 | C16 | C17 | 127.6 (6) |
| C3 | C2 | C21 | 126.8 (7) |
| C1 | C2 | C21 | 124.5 (7) |
| C2 | C3 | C22 | 126.6 (7) |
| C4 | C3 | C22 | 126.8 (7) |
| C8 | C7 | C24 | 127.9 (7) |
| C6 | C7 | C24 | 125.7 (7) |
| C7 | C8 | C25 | 127.5 (7) |
| C9 | C8 | C25 | 125.4 (7) |
| C11 | C12 | C27 | 125.9 (6) |
| C13 | C12 | C27 | 129.6 (6) |
| C12 | C13 | C28 | 144.4 (7) |
| C14 | C13 | C28 | 107.7 (6) |
| C14 | C15 | C29 | 105.2 (6) |
| C16 | C15 | C29 | 128.9 (6) |
| C16 | C17 | C30 | 113.2 (5) |
| C18 | C17 | C30 | 111.8 (6) |
| C17 | C18 | C32 | 113.4 (6) |
| C19 | C18 | C32 | 111.5 (6) |
| C23 | C22 | C3 | 128.9 (8) |
| C8 | C25 | C26 | 114.6 (7) |
| C13 | C28 | C29 | 107.3 (6) |
| C15 | C29 | C33 | 116.3 (6) |
| C15 | C29 | C28 | 104.5 (5) |
| C33 | C29 | C28 | 107.3 (5) |
| C17 | C30 | C31 | 111.8 (6) |
| C35 | C31 | C30 | 111.0 (6) |
| C33 | O3 | C34 | 117.3 (6) |
| C35 | O5 | C36 | 116.4 (6) |
| O1 | C28 | C13 | 131.9 (7) |
| O1 | C28 | C29 | 120.9 (6) |
| O2 | C33 | O3 | 123.9 (7) |
| O2 | C33 | C29 | 126.1 (7) |
| O3 | C33 | C29 | 110.0 (6) |
| O4 | C35 | O5 | 123.9 (8) |
| O4 | C35 | C31 | 122.8 (8) |
| O5 | C35 | C31 | 113.2 (7) |
| O5 | C36 | C37 | 108.8 (7) |

Table III. Hydrogen Bonding Distances and Angles in Ethyl Chlorophyllide $a$ Dihydrate

| Distances | | Angles | |
|---|---|---|---|
| O6–O1$^a$ | 2.65 (1) | O7–O6–Mg | 121.1 (3) |
| O6–O7 | 2.79 (1) | O1$^a$–O6–O7 | 104.3 (2) |
| O7–O4$^b$ | 3.04 (1) | O1$^a$–O6–Mg | 112.4 (2) |
| O7–O2 | 2.98 (1) | O4$^b$–O7–O6 | 114.9 (3) |
| HA–O1$^a$ | 1.58 (7) | O4$^b$–O7–O2 | 108.6 (2) |
| HA–O6 | 1.09 (7) | O2–O7–O6 | 123.5 (3) |
| HB–O6 | 0.97 (7) | | |
| HB–O7 | 1.84 (7) | | |
| HC–O4$^b$ | 2.40 (10) | | |
| HC–O7 | 0.73 (9) | | |
| HD–O2 | 2.18 (9) | | |
| HD–O7 | 0.85 (9) | | |

$^a x + 1, y, z$. $^b x + 1, y + 1, z$.

Table IV. Intermolecular Interatomic Distances Less than 3.5 A between Nonhydrogen Atoms

| Atom 1 | Atom 2 | Distance |
|---|---|---|
| O1 | O6$^a$ | 2.65 (1) |
| O1 | C31$^b$ | 3.15 (1) |
| O1 | C30$^b$ | 3.46 (1) |
| O2 | O7 | 2.98 (1) |
| O2 | C26$^c$ | 3.50 (1) |
| O3 | C32$^a$ | 3.34 (1) |
| O4 | C24$^d$ | 3.41 (1) |
| O6 | C21$^b$ | 3.34 (1) |
| O7 | O4$^e$ | 3.04 (1) |
| O7 | O6 | 2.79 (1) |
| C6 | C36$^f$ | 3.41 (1) |
| C10 | C23$^a$ | 3.41 (1) |
| C11 | C23$^a$ | 3.50 (1) |
| C23 | C34$^f$ | 3.35 (1) |

$^a x - 1, y, z$. $^b x, y + 1, z$. $^c -y, x - y, \frac{1}{3} + z$. $^d -1 - y, -1 + x - y, \frac{1}{3} + z$. $^e x + 1, y + 1, z$. $^f y - x, -1 - x, z - \frac{1}{3}$.

While the magnesium atom is 0.39 Å above the N1–N2–N3 plane, it is 0.23, 0.21, and 0.32 Å above the planes of rings I, II, and III, respectively. Each of the unsaturated pyrrole rings is planar and the maximum deviation from the planes of pyrroles of atoms attached at the $\alpha$ or $\beta$ positions is 0.11 Å. Each of the atoms in the ketone carbonyl group is less than 0.02 Å from the plane of ring III. This allows the possibility of significant conjugation between the carbonyl double bond and the chlorin $\pi$ system. In contrast, while C22 of the vinyl group is 0.01 Å from the plane of ring I, C23 is displaced 0.51 Å from the plane toward the nearest neighboring chlorophyllide molecule.

## Discussion

X-ray structural data are available for a number of pigments related to chlorophyll.[12,14-18] Of particular interest are the structures of magnesium phthalocyanindipyridine hydrate[12] and methyl pheophorbide *a*.[14] The latter compound differs from ethyl chlorophyllide *a* in the nature of the alcohol which has replaced the phytol of chlorophyll *a*, and by substitution of two hydrogen atoms for the magnesium atom.

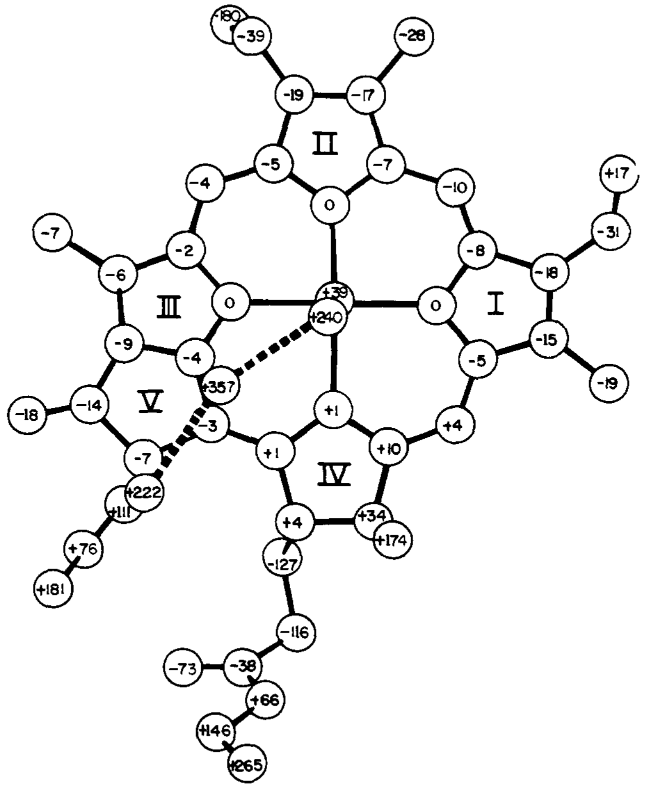

Figure 5. Distances (×100 in Å) of nonhydrogen atoms from the N1-N2-N3 plane.

As might be expected, the geometries of the chlorophyllide and pheophorbide molecules are very similar. It is somewhat surprising, however, that one-dimensional "polymers" similar to those found in ethyl chlorophyllide structure are also observed in the pheophorbide crystals (Figure 6). The existence of these "polymers", even in the absence of any hydrogen bonding, demonstrates the stability of this mode of aggregation.

A detailed comparison of the structural parameters of ethyl chlorophyllide and methyl pheophorbide reveals a few significant differences. The orientation of the vinyl substituents in the two structures differs by an approximate twofold rotation about the C3-C22 bond. In both cases, however, the plane of the vinyl group is rotated significantly out of the chlorin plane with displacement of C23 toward the nearest neighboring molecule. The dihedral angle between the vinyl plane and the plane of ring I is 31° in the chlorophyllide and 15° in the pheophorbide.

In the chlorophyllide structure the displacement of C23 from the plane of ring I results from a strong intramolecular interaction between H23B and H5. Even with C23 displaced 0.51 Å from the ring I plane, the H23B-H5 distance is only 2.01 Å. The large dihedral angle which results from this interaction prevents appreciable conjugation between the double bond of the vinyl group and the chlorin $\pi$ system. On the other hand the overlap of $\pi$ orbitals of the vinyl group with those of the neighboring molecule may contribute significantly to the stability of the aggregate. The only intermolecular C-C distances less than 3.5 Å in the one-dimensional polymer are the C23-C10 distance, 3.41 Å, and the C23-C11 distance, 3.50 Å.

Most of the available information related to the function and organization of chlorophyll in photosynthetic organisms has been based on studies of the visible absorption, fluorescence, and circular dichroism spectra of intact chloroplasts and various fractionated chloroplast preparations. From the observation that in all condensed forms of chlorophyll (e.g., monolayers, crystals, and colloidal solutions) the red absorption maximum occurs at lower energy than that of monomeric chlorophyll in solution, and from the fact that chlorophyll in vivo also exhibits a red shift, it has been suggested that the chlorophyll in green plants occurs in an aggregated state. While other interactions (e.g., chlorophyll-protein interactions) could give rise to the observed red shift, the existence of aggregated chlorophyll in vivo seems to have been widely accepted and numerous models for the structural nature of the aggregates have appeared.[14,19]

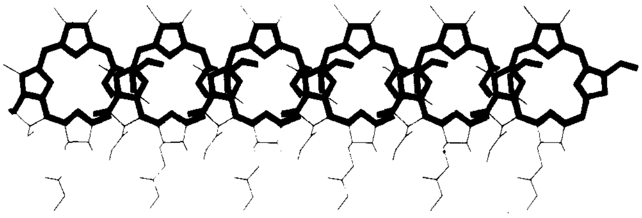

Figure 6. A fragment of the pheophorbide polymer found in crystalline methyl pheophorbide *a*.[14] The heavy lines indicate potentially conjugated portions of each molecule.

French and coworkers[20] have provided a more detailed description of the absorption spectra of photosynthetic organisms. From low-temperature spectra, four different spectral forms of chlorophyll *a* have been resolved which appear to occur in a wide variety of different photosynthetic species. These four "universal forms" of chlorophyll *a* have also been observed in two different chlorophyll-protein complexes.[21] Several interpretations of these observations are possible. First, the different spectral forms could result from specific interactions between chlorophyll and other components in the membrane structure. If the same or similar components are present in the photosynthetic apparatus of various species, similar absorption spectra would be observed. If, on the other hand, the observed red shifts result from chlorophyll-chlorophyll interactions, there are still several alternate interpretations of the spectroscopic data.

The various spectral forms may correspond to different exciton components in the absorption spectrum of one or more chlorophyll aggregates. Katz and coworkers[19] have proposed that the bulk of the chlorophyll in the cell is present in the form of anhydrous oligomers, and they have shown that exciton spliting in the absorption spectra of oligomers of this type could account for the various components in the red portion of the absorption spectrum. The model requires, however, that the chlorophyll resides in an environment in which "access of water must be strictly limited".[19] While the phytyl portion of the chlorophyll molecule is probably associated with a hydrophobic portion of the membrane structure, it seems unlikely that the polar chlorin portion of the molecule is in an environment that strictly excludes water. It is also difficult to reconcile this model with the existence of well-characterized chlorophyll-protein complexes which make up a significant portion of the photosynthetic antenna system.[21]

The preliminary structural data for ethyl chlorophyllide *a* furnished an alternate structural model for chlorophyll aggregation.[1] With the hypothesis that chlorophyll polymers have structures similar to that of the ethyl chlorophyllide *a* polymer, application of the exciton model of molecular interaction[22] can provide an estimate of the magnitude of the spectral shift that occurs upon polymer formation. Polymers of this type, in which the component molecules are related by a simple translation, give rise to one dominant exciton component in the absorption spectrum. In the case of the red band in the chlorophyll spectrum, this component is at the low-energy edge of the exciton band. Thus,

Table V. A Comparison of the Predicted Wavelengths (nm) of the Red Absorption Maxima of Chlorophyll *a* Polymers of Different Lengths with Those of the Universal Forms Observed by French et al.[a]

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Half monopoles | 662 | 675 | 681 | 684 | 686 | 688 | 689 | 690 |
| Monopoles on each atom | 662 | 671 | 676 | 679 | 681 | 682 | 683 | 683 |
| Dipole model | 662 | 674 | 679 | 682 | 684 | 686 | 687 | 687 |
| Universal forms of chlorophyll *a*[b] | 662, 670, 677, 683 | | | | | | | |

[a] In all cases the wavelength of the lowest energy exciton component is tabulated. In linear polymers of this type, this component has a much larger oscillator strength than any other. [b] Reference 19.

the principal effect of polymerization on this band is a simple red shift. In an earlier paper,[23] exciton calculations in the dipole approximation for one-dimensional chlorophyll polymers of different lengths predicted values of this red shift that showed a striking similarity to those observed for various forms of chlorophyll in vivo.

In an effort to obtain a more reliable prediction of these red shifts than that provided by the dipole approximation, a new series of calculations has been carried out with a point monopole model. The transition monopoles used in these calculations are those obtained by Philipson, Tsai, and Sauer[24] from the molecular orbital calculations of Weiss.[25] In the point monopole approximation, the interaction energy takes the form

$$V = \sum_i \sum_j \frac{Q_i Q_j}{r_{ij}}$$

where $Q_i$ and $Q_j$ are transition monopoles on the $i$th and $j$th atoms of neighboring molecules and $r_{ij}$ is the distance between them. In their calculations, Philipson, Tsai, and Sauer[24] divided each of the transition monopoles in half and located the two halves 1.0 Å above and below each conjugated atom.

Three different calculations have been carried out for the chlorophyll polymers. The first was a monopole calculation with "half monopoles" 1.0 Å above and below the chlorin plane, the second a monopole calculation with monopoles located at the nuclear positions, and the third a dipole calculation based on the transition dipole moment derived from the point monopoles. The results of these three calculations are tabulated in Table V. The differences observed in the three calculations are only slightly larger than the uncertainty which results from the experimental determination of the transition dipole moment upon which all three calculations are based.

Although the monopole model represents a higher level of approximation than the dipole model, there are still a number of important assumptions implicit in this treatment. Probably the most important is the neglect of the change in the van der Waals interaction upon polymerization.[22] It can be argued that this neglect is justified by the insensitivity of the position of the absorption maximum under consideration to a change in solvent, and by the fact that in the formation of a one-dimensional polymer there is a relatively minor change in solvation. These arguments are not, however, readily amenable to experimental verification.

The organization of chlorophyll molecules in the photosynthetic membrane is probably designed to provide for efficient transfer of photoexcitation from the photosynthetic antenna to the reaction center. Formation of relatively short polymer fragments with structures similar to the ethyl chlorophyllide *a* polymers could enhance the rate of transfer to the reaction center in two ways. First, the relatively strong interaction between molecules in a given polymer results in very efficient excitation transport within that polymer. Appropriate spatial arrangement of these polymers could further enhance the rate of excitation transport. In two recent papers, Seely[26] has studied the rate of excitation transport in hypothetical photosystems with various distributions of the different spectral forms of chlorophyll. This work demonstrates that an ordered distribution of the various spectral forms, with the lowest energy form closest to the reaction center and the highest energy form the farthest away, could enhance the rate of excitation transport to the reaction center. The hypothesis that the different spectral forms are simply different length polymers could easily be accomodated in this model.

While the occurrence of relatively short one-dimensional polymers of chlorophyll of the type described above could account for the various "universal" forms of chlorophyll *a* in vivo, it cannot account for the large red shift observed in the absorption spectrum of the reaction center chlorophyll, P700. This shift may result in part from the chlorophyll–electron acceptor interaction that makes this chlorophyll unique.

The model of chlorophyll aggregation developed in this paper is consistent with the observation that a large fraction of the antenna chlorophyll occurs in discrete chlorophyll–protein complexes, and with the fact that several universal forms of chlorophyll *a* are observed in many different photosynthetic species. Quantitative estimates of the red shift in the absorption spectra of chlorophyll polymers provide a tentative identification of the different spectral forms. It is clear, however, that structural assignments based primarily on visible spectra are not unambiguous and that further experimental data are necessary to substantiate this hypothesis. An x-ray structural determination of a photosynthetically active chlorophyll–protein complex would provide the information needed, but more indirect evidence may prove sufficient. Spectroscopic studies of chlorophyll polymers in vitro are now underway in this laboratory.

**Acknowledgment** is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, to the National Science Foundation (BMS74-12596), and to the UCLA Campus Computing Network for support of this work.

**Supplementary Material Available.** A listing of observed and calculated structure factor amplitudes will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for papers in this issue may be obtained from the Business Office, Books and Journals Division, American Chemical Society, 1155 16th Street. N.W., Washington, D.C. 20036. Remit check or money order for $4.50 for photocopy or $2.50 for microfiche, referring to code number JACS-75-7230.

## References and Notes

(1) C. E. Strouse, *Proc. Natl. Acad. Sci U.S.*, **71**, 325 (1974).
(2) A. S. Holt and E. E. Jacobs, *Am. J. Bot.*, **41**, 710 (1954).
(3) R. Willstätter and A. Stoll, "Investigations of Chlorophyll", F. M. Schertz and A. R. Merz, The Science Press Printing Co., Lancaster, Penn., 1973, p. 160.
(4) The programs used in this work included locally written data reduction programs; JBPATT, JBFOUR, and PEAKLIST, modified versions of Fourier programs written by J. Blount; local versions of ORFLS (Busing, Martin, and Levy), structure factor calculations and full-matrix least-squares refinement; ORTEP (Johnson), figure plotting; ABSN (Coppens), absorption correction; and ORFFE (Busing, Martin, and Levy), distance, angle, and error computations. All calculations were performed on the IBM 360-91 KK computer operated by the UCLA Campus Computing Network.
(5) $R = [\Sigma||F_o| - |F_c||/\Sigma|F_o|]$; $R_w = [\Sigma w(|F_o| - |F_c|)^2/\Sigma w|F_o|^2]^{1/2}$; $w = 1/(\sigma_F)^2$.
(6) W. C. Hamilton, *Acta Crystallogr.*, **18**, 502 (1965).
(7) I. Fleming, *Nature* (*London*), **216**, 151 (1967).

(8) See paragraph on supplementary material.
(9) R. F. Stewart, E. R. Davidson, and W. T. Simpson, *J. Chem. Phys.*, **42**, 3175 (1965).
(10) H. P. Hanson, F. Herman, J. D. Lea, and S. Skillman, *Acta Crystallogr.*, **17**, 1040 (1964).
(11) D. T. Cromer and K. Liberman, *J. Chem. Phys.*, **53**, 1891 (1970).
(12) M. S. Fischer, D. H. Templeton, A. Zalkin, and M. Calvin, *J. Am. Chem. Soc.*, **93**, 2622 (1971).
(13) A. D. Mitchell and L. C. Cross, Ed., "Tables of Interatomic Distances and Angles", The Chemical Society, London, 1958.
(14) M. S. Fischer, D. H. Templeton, A. Zalkin, and M. Calvin, *J. Am. Chem. Soc.*, **94**, 3613 (1972).
(15) R. C. Pettersen, *J. Am. Chem. Soc.*, **93**, 5629 (1971).
(16) J. L. Hoard, *Science*, **174**, 1295 (1971).
(17) W. Hoppe, G. Will, J. Gassman, and H. Weichselgartner, *Z. Kristallogr., Kristallgeom., Kristallphys., Kristallchem.*, **128**, 18 (1959).
(18) R. C. Pettersen, *Acta Crystallogr., Sect. B*, **25**, 2527 (1969).
(19) J. J. Katz and J. R. Norris, *Curr. Top. Bioenerg.*, **5**, 41 (1973).
(20) C. S. French, J. S. Brown, and M. C. Lawrence, *Plant Physiol.*, **49**, 421 (1972).
(21) J. P. Thornber, *Annu. Rev. Plant Physiol.*, **26**, 127 (1975).
(22) M. Kasha, H. R. Rawls, and M. A. El-Bayoumi, *Pure Appl. Chem.*, **11**, 371 (1965).
(23) C. E. Strouse, *Prog. Inorg. Chem.*, in press.
(24) K. D. Philipson, S. C. Tsai, and K. Sauer, *J. Phys. Chem.*, **75**, 1440 (1971).
(25) C. Weiss, *J. Mol. Spectrosc.*, **44**, 37 (1972).
(26) G. R. Seely, *Theor. Biol.*, **40**, 173 (1973); *ibid.*, **40**, 189 (1973).

# The Crystal and Molecular Structure of Ethyl Chlorophyllide *b* Dihydrate at −153°

**Robert Serlin, Hsi-Chao Chow, and Charles E. Strouse***

*Contribution No. 3420 from the Department of Chemistry, University of California, Los Angeles, California 90024.* *Received December 10, 1974*

**Abstract:** Enzyme-catalyzed transesterification of chlorophyll *b* with ethanol provides a derivative whose chemical and spectroscopic properties differ little from the parent pigment. Ethyl chlorophyllide *b* dihydrate crystallizes from acetone-water mixtures in the trigonal space group $P3_1$ with lattice parameters $a$ = 8.760 (2) Å and $c$ = 37.673 (8) Å at −153°. A Syntex $P\bar{1}$ diffractometer equipped with a Cu x-ray source (λ 1.5418) was used to collect a complete set of low-temperature intensity data. Ethyl chlorophyllides *a* and *b* are isomorphous; cross-linked one-dimensional polymers composed of translationally equivalent chlorophyllide molecules are observed in both structures. Estimates of the position of the red absorption maxima for short one-dimensional chlorophyll polymers were obtained from exciton calculations and are compared with those observed in vivo.

Chlorophyll *b* occurs as an accessory photosynthetic pigment in most higher plants where it appears to function solely as a light harvester. Studies of the fluorescence spectra[1] of intact plants have shown that the excitation produced when chlorophyll *b* absorbs light is very rapidly transferred to chlorophyll *a*. The secondary role of the pigment in higher plants is demonstrated by the fact that there exists a mutant strain of barley which does not produce chlorophyll *b* but which is still capable of photosynthesis.[2] The occurrence of this pigment in the plant is probably related to its ability to absorb red light at slightly higher energy than chlorophyll *a* thus making more efficient use of the incident light.

As a part of a program designed to provide the structural basis for a deeper understanding of the molecular architecture of the photosynthetic apparatus, x-ray structural investigations of a number of chlorophylls and their derivatives have been undertaken. The accompanying[3] paper provides a description of the structure and aggregation of ethyl chlorophyllide *a* dihydrate and its possible relationship to the various forms of chlorophyll *a* which occur in the cell. The x-ray structural determination of ethyl chlorophyllide *b* dihydrate is reported in this paper.

## Experimental Section

Ethyl chlorophyllide *b* was prepared by a modified version of the procedure of Holt and Jacobs.[4] Details of this procedure are discussed in the accompanying paper.[3] Crystals of ethyl chlorophyllide *b* dihydrate were grown by slow addition of water to an acetone solution at 0°. Crystals grew predominantly in the form of truncated trigonal pyramids bounded by the (001), $(\bar{1}1\bar{3})$, $(0\bar{1}\bar{3})$, and $(10\bar{3})$ faces. A small well-formed crystal with maximum edge length 0.15 mm and thickness 0.06 mm was glued to a glass fiber with the [001] axis parallel to the fiber axis, and covered by a Lindemann glass capillary. The crystal was then mounted on a Syntex $P\bar{1}$ autodiffractometer equipped with a copper x-ray source, graphite monochromator, scintillation counter, pulse height analyzer, and a locally constructed low-temperature device[5] which maintained the sample at a temperature of −153°. Lattice parameters were determined by the careful centering of 15 reflections both at room temperature and at −153° ($a$ = 8.760 (2) Å, $c$ = 37.673 (8) Å at −153° and $a$ = 8.855 (2) Å, $c$ = 38.059 (7) Å at 25°; $d_c^{25}$ = 1.34 g/cm³ for $Z$ = 3, $d_o^{25}$ = 1.30 (3) by flotation in aqueous zinc chloride).

Intensity data were collected at −153° in order to maximize the amount of observable data, to obtain more accurate hydrogen atom positions, and to minimize the effect of thermal motion on the bond lengths in the ethyl and vinyl groups that was observed in the chlorophyllide *a* structure. The intensities were measured with the $\theta$–$2\theta$ scan technique for all reflections with $h \leqslant 0$, $k \geqslant 0$, and $l \geqslant 0$. Bisecting mode data collection was used to a maximum $2\theta$ of 99°, and all accessible reflections in the range 98 to 125° were collected in the parallel mode. Each reflection was scanned from 1.0° below the $K\alpha_1$ reflection to 1.0° above the $K\alpha_2$ reflection at a scan rate of 2.0°/min. Total time spent counting background was equal to eight-tenths of the scan time for each reflection. The intensities of three reflections were remeasured every 97 reflections to monitor the tube efficiency and possible crystal deterioration. No significant deviations were observed.

The intensity of a reflection $I(h,k,l)$ and its estimated standard deviation $\sigma[I(h,k,l)]$ were calculated[6] according to the equations $I = \mathrm{CT} - (t_c/t_b)(B_1 + B_2)/2$ and $\sigma(I) = [\sigma_s^2 + (0.04I)^2]^{1/2}$, where $I$ is the net integrated intensity, CT is the total integrated count, $t_c$ and $t_b$ are the times employed for counting the scan and background, respectively, $B_1$ and $B_2$ are the background counts on the low and high sides of the reflection, and $\sigma_s$ is the standard deviation in the intensity due to the counting statistics. The usual Lorentz-polarization corrections were applied to the data. A trial absorption correction ($\mu$ = 9.65 cm⁻¹) gave transmission factors in the range 0.94 to 0.96 and therefore no absorption correction was used.