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

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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 spectra¹ 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 accompanying³ 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 $\bar{1}3$), 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 Lin-

demann 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 device⁵ 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° ; $d_0^{25} = 1.34$ g/cm³ for $Z = 3$, $d_0^{25} = 1.30$ (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 θ - 2θ scan technique for all reflections with $h \leq 0$, $k \geq 0$, and $l \geq 0$. Bisecting mode data collection was used to a maximum 2θ 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 $2.0^\circ/\text{min}$. 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 calculated⁶ according to the equations $I = CT - (t_c/t_b)(B_1 + B_2)/2$ 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 σ_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.

Table I. Final Atomic Parameters for Ethyl Chlorophyllide *b* Dihydrate

Atom	<i>x/a</i>	<i>y/b</i>	<i>z/c</i>	β	Atom	<i>x/a</i>	<i>y/b</i>	<i>z/c</i>	β
HA	0.1955 (149)	-0.1091 (136)	-0.1127 (28)	4.00	Mg	-0.0550 (5)	-0.2467 (4)	-0.1590	1.80 (7)
HB	0.0610 (146)	-0.0635 (140)	-0.1033 (30)	4.00	O6	0.1043 (10)	-0.0745 (10)	-0.1221 (2)	2.16 (15)
HC	0.0368 (147)	0.0982 (150)	-0.0415 (29)	4.00	O1	-0.6987 (9)	-0.1870 (9)	-0.0916 (2)	2.43 (16)
HD	-0.1293 (154)	-0.0445 (163)	-0.0598 (31)	4.00	N1	0.0713 (11)	-0.3806 (11)	-0.1741 (2)	1.87 (17)
H5	0.3026	-0.2009	-0.2489	3.00	N2	0.0152 (11)	-0.1121 (11)	-0.2077 (2)	1.93 (18)
H10	-0.1854	0.1287	-0.2168	3.00	N3	-0.2449 (11)	-0.1828 (10)	-0.1580 (2)	1.71 (18)
H17	-0.3866	-0.5325	-0.0383	4.00	N4	-0.1961 (11)	-0.4501 (10)	-0.1198 (2)	1.43 (16)
H18	-0.2524	-0.7625	-0.0791	4.00	C1	0.0761 (13)	-0.5122 (13)	-0.1557 (3)	1.60 (21)
H20	0.0140	-0.6757	-0.1122	3.00	C2	0.1977 (14)	-0.5556 (14)	-0.1732 (3)	2.13 (22)
H21A	0.3468	-0.6331	-0.1435	4.00	C3	0.2678 (14)	-0.4491 (14)	-0.2018 (3)	2.05 (22)
H21B	0.1397	-0.7864	-0.1470	4.00	C4	0.1889 (14)	-0.3374 (13)	-0.2025 (3)	1.70 (21)
H21C	0.2739	-0.7432	-0.1806	4.00	C5	0.2181 (14)	-0.2108 (14)	-0.2277 (3)	1.94 (23)
H22	0.3898	-0.5724	-0.2283	3.00	C6	0.1399 (13)	-0.1036 (13)	-0.2304 (3)	1.72 (22)
H23A	0.5922	-0.3437	-0.2627	3.00	C7	0.1684 (13)	0.0189 (13)	-0.2585 (3)	1.75 (21)
H23B	0.5211	-0.2070	-0.2452	3.00	C8	0.0552 (13)	0.0819 (14)	-0.2533 (3)	1.74 (21)
H24	0.2961	0.1344	-0.3149	3.00	C9	-0.0399 (14)	0.0014 (14)	-0.2211 (3)	2.01 (23)
H25A	0.0038	0.2895	-0.2607	3.00	C10	-0.1685 (13)	0.0290 (13)	-0.2058 (3)	1.78 (22)
H25B	0.1373	0.2852	-0.2906	3.00	C11	-0.2685 (14)	-0.0547 (14)	-0.1756 (3)	1.90 (21)
H26A	-0.1092	0.0224	-0.3166	3.00	C12	-0.4047 (13)	-0.0321 (13)	-0.1594 (3)	1.56 (22)
H26B	-0.2414	0.0346	-0.2875	3.00	C13	-0.4609 (14)	-0.1478 (13)	-0.1315 (3)	1.78 (22)
H26C	-0.1461	0.1879	-0.3177	3.00	C14	-0.3636 (14)	-0.2377 (14)	-0.1318 (3)	1.83 (22)
H27A	-0.5918	0.0420	-0.1659	3.00	C15	-0.4039 (14)	-0.3590 (14)	-0.1036 (3)	1.74 (22)
H27B	-0.3973	0.2097	-0.1567	3.00	C16	-0.3269 (13)	-0.4629 (13)	-0.0982 (3)	1.76 (21)
H27C	-0.4382	0.1226	-0.1964	3.00	C17	-0.3705 (13)	-0.5928 (13)	-0.0675 (3)	1.33 (20)
H29	-0.6495	-0.4792	-0.0791	3.00	C18	-0.2225 (13)	-0.6418 (13)	-0.0723 (3)	1.79 (21)
H30A	-0.6476	-0.7252	-0.0751	3.00	C19	-0.1415 (13)	-0.5579 (14)	-0.1081 (3)	1.90 (21)
H30B	-0.5536	-0.8241	-0.0945	3.00	C20	-0.0186 (14)	-0.5917 (14)	-0.1255 (3)	1.99 (22)
H31A	-0.7119	-1.0017	-0.0458	3.00	C21	0.2384 (13)	-0.6944 (13)	-0.1604 (3)	1.79 (21)
H31B	-0.5035	-0.9081	-0.0351	3.00	C22	0.3944 (14)	-0.4510 (14)	-0.2259 (3)	2.00 (22)
H32A	-0.0346	-0.4362	-0.0417	3.00	C24	0.2956 (16)	0.0702 (15)	-0.2875 (3)	2.73 (25)
H32B	-0.1391	-0.6211	-0.0194	3.00	C25	0.0290 (13)	0.2057 (14)	-0.2765 (3)	1.84 (22)
H32C	0.0132	-0.5923	-0.0481	3.00	C27	-0.4616 (15)	0.0933 (14)	-0.1707 (3)	2.02 (23)
H34A	-0.5767	-0.1632	0.0101	3.00	C28	-0.5844 (15)	-0.2192 (14)	-0.1023 (3)	2.08 (23)
H34B	-0.7593	-0.3402	0.0218	3.00	C29	-0.5544 (13)	-0.3602 (13)	-0.0827 (3)	1.67 (22)
H34C	-0.5704	-0.3338	0.0260	3.00	C30	-0.5497 (14)	-0.7576 (14)	-0.0719 (3)	1.92 (22)
H36A	-0.5619	-0.5892	0.0441	3.00	C31	-0.6002 (14)	-0.8800 (14)	-0.0394 (3)	2.21 (23)
H36B	-0.6842	-0.7880	0.0600	3.00	C35	-0.6434 (15)	-0.8099 (15)	-0.0077 (3)	2.28 (23)
H37A	-0.2998	-0.5911	0.0665	3.00					
H37B	-0.4305	-0.6086	0.0992	3.00					
H37C	-0.4286	-0.7834	0.0846	3.00					

Atom	<i>x/a</i>	<i>y/b</i>	<i>z/c</i>	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
O7	-0.0215 (10)	0.0366 (11)	-0.0657 (2)	115 (18)	167 (21)	45 (7)	58 (16)	-67 (31)	-124 (31)
O2	-0.3643 (10)	-0.1906 (11)	-0.0333 (2)	50 (16)	221 (22)	35 (7)	27 (16)	-27 (27)	-70 (30)
O3	-0.6556 (10)	-0.3670 (10)	-0.0253 (2)	144 (19)	159 (18)	18 (6)	73 (16)	59 (27)	-40 (27)
O4	-0.7702 (10)	-0.7886 (10)	-0.0065 (2)	132 (18)	182 (19)	43 (7)	97 (16)	44 (29)	14 (30)
O5	-0.5278 (10)	-0.7751 (10)	0.0185 (2)	137 (17)	138 (17)	27 (6)	84 (15)	45 (26)	-7 (25)
O8	0.4101 (10)	0.0282 (10)	-0.2900 (2)	143 (17)	147 (18)	39 (7)	74 (15)	92 (28)	100 (28)
C23	0.5122 (16)	-0.3237 (17)	-0.2460 (3)	134 (27)	165 (31)	62 (12)	98 (26)	-5 (48)	14 (48)
C26	-0.1289 (15)	0.1064 (16)	-0.3018 (3)	137 (28)	153 (28)	50 (11)	94 (24)	-38 (45)	82 (47)
C32	-0.0870 (15)	-0.5677 (17)	-0.0432 (3)	111 (26)	185 (30)	42 (11)	96 (24)	-58 (41)	29 (43)
C33	-0.5067 (17)	-0.2932 (16)	-0.0443 (3)	146 (30)	168 (30)	26 (11)	123 (26)	92 (50)	68 (47)
C34	-0.6423 (15)	-0.3014 (15)	0.0095 (3)	153 (29)	154 (29)	41 (12)	103 (25)	83 (47)	119 (43)
C36	-0.5637 (15)	-0.7045 (15)	0.0507 (3)	101 (24)	142 (27)	41 (10)	64 (22)	-58 (40)	-26 (42)
C37	-0.4214 (16)	-0.6733 (16)	0.0776 (3)	143 (28)	136 (28)	51 (12)	56 (24)	7 (46)	42 (45)

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

Refinement of the Structure. The structural investigation of the isomorphous ethyl chlorophyllide *a* dihydrate³ provided a trial structure for least-squares refinement of ethyl chlorophyllide *b* dihydrate. Three cycles of refinement of the nonhydrogen atom positions from ethyl chlorophyllide *a* with isotropic temperature factors gave a conventional *R* factor of 0.127. The distortion of the C24 position in the refinement indicated the configuration of the formyl group. The position for O8 was estimated and refined in further least-squares cycles. A difference electron density map revealed the positions of most of the hydrogen atoms. In all subsequent structure factor calculations, hydrogen atoms were included in computed positions. The configuration of each methyl group was based on the most reasonable hydrogen position from the electron density map. The positions of methylene, methine, and vinyl hydrogen atoms 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.

Initial positions for the water hydrogen atoms were chosen on vectors from the water oxygen atoms toward the oxygen atoms to which they are hydrogen bonded. An O-H distance of 1.0 Å was used. In the final refinement, positional parameters for the water hydrogen atoms and nonhydrogen atoms were refined along with anisotropic thermal parameters for the carboxyl and formyl oxygen atoms, O7, C23, C26, C32, C33, C34, C36, and C37. Because of the low data to parameter ratio, isotropic temperature factors were refined for all other nonhydrogen atoms. The real part of the anomalous dispersion correction was applied to the structure factors of magnesium and oxygen. Scattering factors of Stewart, Davidson, and Simpson⁷ were used for hydrogen and those of Hanson, Herman, Lea, and Skillman⁸ for all nonhydrogen atoms. The anomalous dispersion corrections were those obtained by Cromer and Liberman.⁹ The refinement converged to *R* = 0.048, *R_w* =

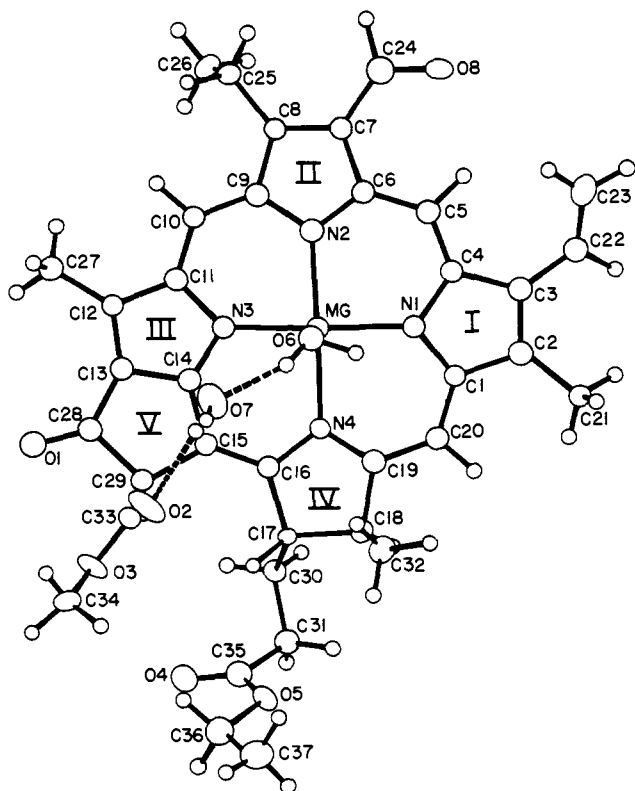


Figure 1. The molecular structure of ethyl chlorophyllide *b* dihydrate. For nonhydrogen atoms 50% probability ellipsoids are shown.

0.050¹⁰ for 1293 reflections with $I > 3\sigma(I)$. In the final cycles three reflections with anomalously large ΔF 's were omitted from the refinement.

A tabulation of final positional and thermal parameters appears in Table I. The molecular structure is illustrated in Figure 1 along with the numbering system used in this investigation. Intramolecular bond distances and angles are tabulated in Table II and short intermolecular interatomic distances are listed in Table III.

Results and Discussion

The overall molecular geometries of ethyl chlorophyllides *a* and *b* are very similar. The only significant bond length differences between the two structures were those in the ethyl and vinyl groups. The C25-C26, C36-C37, and C22-C23 distances of 1.49 (1), 1.47 (1), and 1.27 (1) Å in the ethyl chlorophyllide *a* structure are significantly shorter than ordinary single and double C-C bonds. On the other hand, distances of 1.54 (1), 1.52 (1), and 1.32 (1) Å, observed in the ethyl chlorophyllide *b* structure, are within experimental error the same as normal single bond and double bond distances of 1.541 (3) and 1.337 (6) Å.¹¹ The apparent improvement in these bond distances results from the collection of the chlorophyllide *b* data at low temperature. As observed in other systems^{3,12} which contain the isocyclic ring, ring V, the C28-C29 distance is unusually long (1.570 (13) Å).

The distances of all nonhydrogen atoms from the N1-N2-N3 plane are shown in Figure 2. Rings I and II are tipped significantly out of the plane. Each of the unsaturated pyrrole rings is essentially planar and, with one exception, the atoms attached at the α and β positions are within 0.05 Å of the pyrrole planes; C5 is 0.16 Å below the plane of ring II. The magnesium atom is 0.22, 0.21, and 0.37 Å above the planes of rings I, II, and III, respectively. The ketone oxygen, O1, is displaced 0.06 (3) Å below the plane of ring III while the formyl oxygen, O8, is 0.18 (3) Å above the ring II plane. These relatively small displacements allow

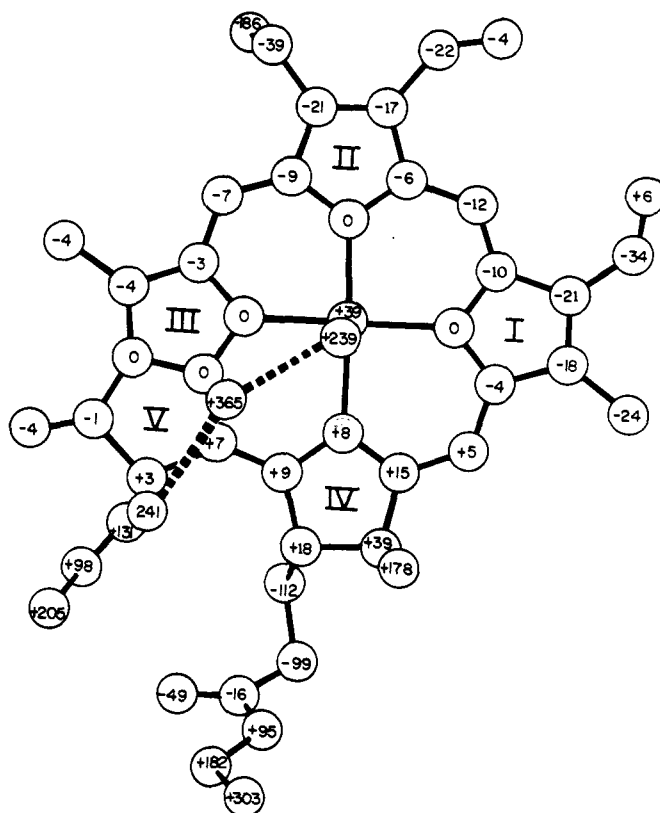


Figure 2. Distances ($\times 100$ in Å) of nonhydrogen atoms from the N1-N2-N3 plane.

the possibility of significant conjugation between the carbonyl orbitals and the chlorin π system. On the other hand, the 0.43 Å displacement of C23 from ring I limits the possible extent of conjugation between the vinyl group and the molecular π system. The dihedral angle between the plane of the vinyl group and the plane of ring I is 26°.

The observation of one-dimensional polymers in the crystal structure of ethyl chlorophyllide *a* dihydrate provided a model for chlorophyll *a* aggregation. Substitution of the methyl group of ethyl chlorophyllide *a* with the formyl group of ethyl chlorophyllide *b* has very little influence on molecular packing or polymer formation (see Figure 3). The most significant difference in the structures of the polymers is a reduction in the interplanar distance of the N1-N2-N3 planes from 3.54 Å in ethyl chlorophyllide *a* to 3.39 Å in ethyl chlorophyllide *b*. This reduction is probably a result of the difference in temperature rather than the chemical difference. The change in interplanar spacing is of roughly the same magnitude as the change in the RMS thermal motion of atoms in the overlapping portion of the two rings. Distances and angles in the hydrogen bonding network that forms and cross-links the one-dimensional polymers are provided in Figure 4 and Table III.

Short intermolecular interatomic distances between nonhydrogen atoms are listed in Table IV. The only unusually short distance involving hydrogen is the 1.94 Å intermolecular distance between H5 and H23B. This interaction prevents the vinyl group from rotating into the plane of ring I. With the exception of the C21 methyl group, the configurations of the methyl groups in this structure are very similar to those of ethyl chlorophyllide *a*. Careful examination of the electron density maps in this region for both chlorophyllides *a* and *b* revealed no reasonable alternatives for the C21 configurations. However, because of the large thermal motion of these groups, especially in the room-temperature

Table II. Bond Distances and Angles between Nonhydrogen Atoms

Distances				Angles									
Mg	N1	2.054 (8)	C16	C17	1.531 (13)	C1	N1	C4	106.7 (8)	C10	C9	C8	125.8 (10)
Mg	N2	2.101 (9)	C18	C19	1.532 (14)	C6	N2	C9	106.7 (8)	C10	C11	C12	128.1 (10)
Mg	N3	2.004 (9)	C2	C3	1.353 (13)	C11	N3	C14	103.7 (9)	C15	C14	C13	114.5 (9)
Mg	N4	2.164 (8)	C7	C8	1.370 (13)	C16	N4	C19	109.3 (9)	C15	C16	C17	125.1 (9)
Mg	O6	2.012 (7)	C12	C13	1.372 (13)								
			C17	C18	1.566 (12)	N1	C1	C2	109.1 (9)	C3	C2	C21	127.0 (10)
N1	C1	1.362 (12)				N1	C4	C3	109.6 (9)	C1	C2	C21	124.4 (9)
N1	C4	1.402 (12)	C4	C5	1.384 (13)	N2	C6	C7	109.8 (9)	C2	C3	C22	125.5 (10)
N2	C6	1.359 (12)	C5	C6	1.415 (13)	N2	C9	C8	109.4 (9)	C4	C3	C22	128.6 (10)
N2	C9	1.399 (12)	C9	C10	1.392 (13)	N3	C11	C12	111.9 (9)	C8	C7	C24	125.4 (10)
N3	C11	1.406 (12)	C10	C11	1.397 (13)	N3	C14	C13	112.5 (9)	C6	C7	C24	127.0 (10)
N3	C14	1.335 (11)	C14	C15	1.418 (13)	N4	C16	C17	113.5 (9)	C7	C8	C25	127.7 (10)
N4	C16	1.364 (12)	C15	C16	1.393 (13)	N4	C19	C18	112.8 (9)	C9	C8	C25	125.9 (9)
N4	C19	1.328 (12)	C19	C20	1.411 (13)					C11	C12	C27	126.3 (9)
			C20	C1	1.377 (14)	C1	C2	C3	108.6 (9)	C13	C12	C27	129.8 (10)
O2	O3	2.247 (10)				C4	C3	C2	106.0 (9)	C12	C13	C28	144.9 (10)
O4	O5	2.273 (10)	C2	C21	1.508 (14)	C6	C7	C8	107.6 (9)	C14	C13	C28	107.1 (9)
O1	C28	1.235 (11)	C3	C22	1.440 (14)	C9	C8	C7	106.4 (9)	C14	C15	C29	106.4 (9)
O2	C33	1.189 (12)	C7	C24	1.460 (14)	C11	C12	C13	103.9 (9)	C16	C15	C29	128.1 (9)
O3	C33	1.339 (12)	C8	C25	1.498 (13)					C16	C17	C30	112.2 (8)
O3	C34	1.410 (12)	C12	C27	1.477 (13)	C14	C13	C12	108.0 (9)	C18	C17	C30	109.3 (8)
O4	C35	1.216 (12)	C13	C28	1.447 (13)	C16	C17	C18	100.6 (8)	C17	C18	C32	112.4 (9)
O5	C36	1.463 (12)	C15	C29	1.531 (14)	C19	C18	C17	102.7 (8)	C19	C18	C32	111.1 (9)
O5	C35	1.336 (12)	C17	C30	1.520 (13)					C23	C22	C3	129.5 (10)
O8	C24	1.233 (12)	C18	C32	1.505 (14)	N1	C1	C20	125.6 (9)	C8	C25	C26	111.8 (9)
			C22	C23	1.317 (14)	N1	C4	C5	123.9 (9)	C13	C28	C29	108.3 (9)
C1	C2	1.456 (13)	C25	C26	1.541 (14)	N2	C6	C5	124.2 (10)	C15	C29	C33	114.4 (9)
C3	C4	1.453 (13)	C28	C29	1.570 (13)	N2	C9	C10	124.8 (10)	C15	C29	C28	103.6 (8)
C6	C7	1.439 (13)	C29	C33	1.538 (14)	N3	C11	C10	120.0 (9)	C33	C29	C28	105.2 (9)
C8	C9	1.438 (13)	C30	C31	1.539 (13)	N3	C14	C15	132.9 (10)	C17	C30	C31	112.0 (8)
C11	C12	1.439 (13)	C31	C35	1.478 (14)	N4	C16	C15	121.4 (10)				
C13	C14	1.420 (13)	C36	C37	1.521 (14)	N4	C19	C20	125.5 (10)	C35	C31	C30	113.5 (9)
										C33	O3	C34	116.0 (9)
										C35	O5	C36	114.2 (8)
						C4	C5	C6	128.6 (10)				
						C9	C10	C11	126.6 (10)	O1	C28	C13	131.6 (10)
						C14	C15	C16	125.4 (10)	O1	C28	C29	120.1 (9)
						C19	C20	C1	127.4 (10)	O2	C33	O3	125.3 (10)
										O2	C33	C29	126.8 (10)
						C20	C1	C2	125.3 (9)	O3	C33	C29	107.9 (10)
						C20	C19	C18	121.8 (9)	O4	C35	O5	125.8 (11)
						C5	C4	C3	126.4 (10)	O4	C35	C31	122.4 (11)
						C5	C6	C7	125.8 (10)	O5	C35	C31	111.8 (10)
										O5	C36	C37	107.3 (9)
										O8	C24	C7	125.1 (11)

Table III. Hydrogen Bonding Distances and Angles in Ethyl Chlorophyllide *b* Dihydrate

O6-O1 ^a	2.64 (1)	O7-O6-Mg	122.8 (4)
O6-O7	2.78 (1)	O1 ^a -O6-O7	104.2 (3)
O7-O4 ^b	2.97 (1)	O1 ^a -O6-Mg	111.0 (4)
O7-O2	2.92 (1)	O4 ^b -O7-O6	115.7 (3)
HA-O1 ^a	1.61 (12)	O4 ^b -O7-O2	106.4 (3)
HA-O6	1.05 (12)	O2-O7-O6	122.3 (4)
HB-O6	0.83 (11)		
HB-O7	1.98 (11)		
HC-O4	1.98 (11)		
HC-O7	1.05 (11)		
HD-O2	2.06 (11)		
HD-O7	0.88 (11)		

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

structure, there is considerable uncertainty associated with these configurations.

The wavelengths of the absorption maxima of one-dimensional chlorophyll *a* polymers predicted by a simple exciton model³ show a striking resemblance to those observed for the different spectral forms of chlorophyll *a* that occur in photosynthetic organisms.¹³ Similar calculations for chlorophyll *b* provide some insight into the organization of this accessory pigment in the cell. In the absence of any molecular orbital calculations for chlorophyll *b*, exciton calcula-

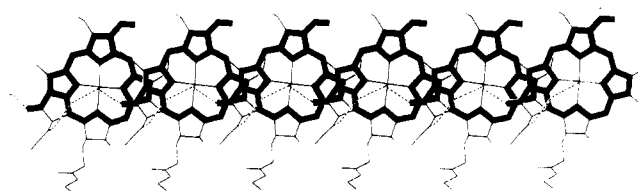


Figure 3. A fragment of the one-dimensional polymers found in crystalline ethyl chlorophyllide *b* dihydrate. The heavy lines indicate the conjugated portion of each molecule and the dashed lines indicate hydrogen bonds.

tions have been carried out in the dipole limit. In the case of chlorophyll *a* polymers, the monopole and dipole calculations gave very similar results.

In the dipole limit, the interaction energy between two translationally equivalent molecules is given by¹⁴

$$E = \left(\frac{\mu^2}{R^3} \right) (1 - 3 \cos^2 \theta)$$

where μ is the transition dipole moment which can be obtained from integration of the absorption spectrum, R is the distance between the point dipoles, and θ is the angle between the translation vector \mathbf{R} and the transition dipole moment μ . On the basis of molecular orbital calculations,¹⁵ the

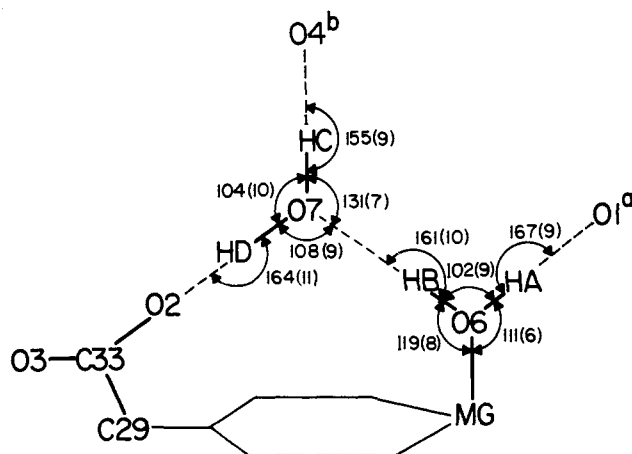


Figure 4. The hydrogen bonding network in ethyl chlorophyllide *b* dihydrate.

red band in the absorption spectrum of chlorin molecules has been assigned to a transition polarized along the long axis of the conjugated system. For the purposes of these calculations this direction is taken to correspond to the N1–N3 vector.

If 643 nm, the position of the absorption maximum in ether solution,⁴ is used as an estimate of the position of the absorption maximum of monomeric chlorophyll *b* in vivo, an exciton calculation for the dimer predicts an absorption maximum at 650 nm. The red shift is smaller than that predicted for the chlorophyll *a* dimer because of the smaller transition moment of chlorophyll *b*.

The isomorphism of the ethyl chlorophyllide *a* and *b* structures suggests the possibility of mixed chlorophyll *a*–chlorophyll *b* polymers. A further exciton calculation was carried out to estimate the magnitude of the chlorophyll *a*–chlorophyll *b* interaction in such a polymer. The interaction energy can be estimated as

$$E = \left(\frac{\mu_a \mu_b}{R} \right) (1 - 3 \cos^2 \theta)$$

where μ_a and μ_b are the transition moments of chlorophyll *a* and chlorophyll *b*, R is the distance between interacting molecules, and θ is the angle between μ and R . The values of R and θ were obtained from the ethyl chlorophyllide *a* structure (structural parameters from ethyl chlorophyllide *b* are not significantly different). Solution of the secular equation for an *a*–*b* “dimer” predicts a weakened “chlorophyll *b*” peak at 640 nm and an enhanced “chlorophyll *a*” absorption at 665 nm.

While French, Brown, and Lawrence¹³ originally assigned a weak band at 640 nm and a strong band at 650 nm in the low-temperature spectra of photosynthetic organisms to chlorophyll *b*, the occurrence of a 640 nm peak in spectra of photosynthetic organisms lacking chlorophyll *b* has cast doubt on the assignment of this absorption.¹⁶ Thus, the only band unambiguously assigned to chlorophyll *b* occurs at the wavelength predicted for a chlorophyll *b* dimer. It is premature to conclude from this coincidence that all chlorophyll *b* present in vivo occurs in the form of dimers. The progression of absorption maxima for chlorophyll *a* which suggests the correspondence between the different spectral forms and different polymer lengths is not apparent for chlorophyll *b*. If the 650 nm peak corresponds to chlorophyll *b* dimers, the concentration of monomeric chlorophyll *b* must be relatively small. On the other hand, absorptions from mixed *a*–*b* polymers could be effectively masked by the chlorophyll *a* absorptions.

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

O6	O1 ^a	2.64 (1)	O3	C32 ^d	3.34 (1)
O6	O7	2.78 (1)	O4	C24 ^f	3.34 (1)
O6	C21 ^a	3.26 (1)	O8	C32 ^g	3.36 (1)
O6	C30 ^c	3.47 (1)	O8	C34 ^h	3.37 (1)
			O8	C37 ^g	3.48 (1)
O7	O2	2.92 (1)	C1	C28 ^a	3.44 (1)
O7	O4 ^c	2.97 (1)	C5	C36 ^h	3.44 (1)
O1	C31 ^b	3.09 (1)	C5	C27 ^a	3.48 (1)
O1	C30 ^b	3.39 (1)	C6	C36 ^h	3.35 (1)
O1	C20 ^d	3.48 (1)	C9	C21 ^b	3.43 (1)
O1	C1 ^d	3.50 (1)	C10	C23 ^d	3.32 (1)
O2	C26 ^e	3.48 (1)	C11	C23 ^d	3.43 (1)
			C23	C34 ^h	3.24 (1)

^a $x + 1, y, z$. ^b $x, y + 1, z$. ^c $x + 1, y + 1, z$. ^d $x - 1, y, z$. ^e $-y, x - y, 1/3 + z$. ^f $-1 - y, -1 + x - y, 1/3 + z$. ^g $y - x + 1, -x, z - 1/3$. ^h $y - x, -x - 1, z - 1/3$.

A comparison of the predicted absorption maxima with those observed for chlorophyll in vitro is also of value. If an acetone solution of chlorophyll is diluted quickly with water, a colloidal solution is produced which has a broad red absorption shifted to lower energy. As was the case for chlorophyll *a*, the observed absorption maximum of colloidal chlorophyll *b* occurs at somewhat lower energy, 655 nm,¹⁷ than that predicted for the dimer. It should be noted that the solvent system in these experiments is the same as that used to crystallize the chlorophyllides.

Much of the chlorophyll *b* in green plants is present in the form of a light harvesting chlorophyll–protein complex. Recent data¹⁸ indicate that the complex contains three chlorophyll *a* and three chlorophyll *b* molecules per protein subunit. The low-temperature absorption spectrum of this complex shows intense bands at 650, 662, 670, and 677 nm and weaker bands at 640 and 683 nm. It is clear that while spectroscopic predictions developed in this and the accompanying paper are suggestive, they do not provide an unambiguous interpretation of this spectrum. Further structural and spectroscopic studies of these pigment systems are now underway in this laboratory.

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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, 24X 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 St., N.W., Washington, D.C. 20036. Remit check or money order for \$4.00 for photocopy or \$2.50 for microfiche, referring to code number JACS-75-7237.

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The Crystal and Molecular Structure of the Triclinic and Monoclinic Forms of Valinomycin, $C_{54}H_{90}N_6O_{18}$

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Abstract: Two forms of uncomplexed valinomycin have been crystallized and their structures determined by X-ray diffraction. The monoclinic form crystallizes in space group $P2_1$ with the following cell dimensions: $a = 23.144 \text{ \AA}$, $b = 10.347 \text{ \AA}$, $c = 14.526 \text{ \AA}$, $\beta = 99.57^\circ$, and $Z = 2$. The other form crystallizes in the triclinic space group $P1$ with cell dimensions $a = 22.281 \text{ \AA}$, $b = 10.333 \text{ \AA}$, $c = 14.507 \text{ \AA}$, $\alpha = 90.05^\circ$, $\beta = 105.28^\circ$, $\gamma = 93.31^\circ$, and $Z = 2$. The two structures were refined by full-matrix least-squares to residuals of 0.16 and 0.14, respectively. An analysis of the conformations of the three independent molecules as determined in this study indicates that the gross features of all three are the same. However, minor differences in the conformations, as much as 12° in the torsion angles, are observed. Two different kinds of hydrogen bonds are found in all three molecules. Each molecule has four hydrogen bonds involved in β turns (one amino acid residue hydrogen bonding to a carbonyl group of an ester residue three groups removed). Each molecule also has two previously undescribed hydrogen bonds in which the amino acid is hydrogen bonded to a carbonyl group of an amino acid residue four groups away. A mechanism of coordinating potassium ion is postulated based on the observed conformation.

Valinomycin is a cyclic dodecadepsipeptide that selectively transports potassium ion across natural and synthetic membranes.^{1,2} Extensive studies of this transport process have been conducted to determine, if possible, the basis for the potassium ion selectivity of valinomycin and to ascertain whether it acts as a channel or a carrier in membranes.² Knowledge of the conformation of complexed and uncomplexed valinomycin facilitates the development of models for its complexation, transport, and release mechanisms.

In valinomycin the sequence (L-Val-D-HyIv-D-Val-L-Lac) is repeated three times giving a cyclic molecule with the possibility of threefold symmetry (Figure 1). Infrared, NMR, Raman, and X-ray crystallographic studies have shown that the potassium complex exists in solid and solution as a doughnut shaped molecule possessing threefold symmetry in which all amide protons are hydrogen bonded to neighboring residues in β -turn type conformations.³⁻¹⁴ The potassium ion in the center of the doughnut has sixfold coordination to the amino carbonyls (Figure 2).^{13,14}

The conformation of uncomplexed valinomycin is considerably more complex. By means of ir, NMR, and ORD, it has been found that the hydrogen bonding scheme in valinomycin is a function of solvent polarity.^{4,9,10,12,15} It has been proposed that the conformation in nonpolar solvents is similar to that found for the potassium ion complex, i.e., six intramolecular amide-carbonyl hydrogen bonds. An open conformation has been proposed in which there is no intramolecular hydrogen bonding, but rather an interaction between solute and solvent when an aqueous-dioxane solvent system was used. For solvent polarities between these two extremes, it has been suggested that there are three intramolecular amide-carbonyl hydrogen bonds, the nitrogens

belonging to either the D-Val or to the L-Val residues, so that threefold symmetry is retained.

Two different crystals, obtained from different solvent systems, have been studied and a single conformation has been found for the three independent molecules whose structure was determined in this study. This conformation is quite different from that proposed on the basis of spectral data.

Experimental Section

Two forms of uncomplexed valinomycin have been crystallized. Modification A was obtained from *n*-octane and has disordered solvent present in the monoclinic lattice. Modification B is a triclinic form obtained from ethanol-water solutions. Relevant crystal data are presented in Table I.¹⁶

Modification A. The monoclinic crystals were mounted in glass capillaries to prevent solvent loss. Data suitable for least-squares refinement of both positional and thermal parameters were collected by the θ - 2θ scan technique on an Enraf Nonius CAD-4 diffractometer using Ni-filtered $Cu K\alpha$ radiation. No significant changes were observed in the intensities of two standard reflections which were measured after every 96 intensities were recorded. Intensities were corrected for Lorentz and polarization factors but not for extinction or absorption. Atomic scattering factors were taken from the literature; both real and imaginary dispersion corrections were applied.¹⁷ Weights were calculated according to a modified Hughes scheme:^{18,19} $w = a/|F_d|$ for $a < |F_d|$; $w = |F_d|/a$ for $|F_d| < a$; the value of "a" was 27.0.

The 214.1 \AA^3 difference in the unit cell volumes of the two crystal forms is in very good agreement with the volume reported for one *n*-octane molecule (208.5 \AA^3).²⁰ Because of the symmetry requirements in space group $P2_1$, this one solvent molecule must be disordered.

Modification B. The triclinic cell is nearly congruent with the