CALCIUM-BINDING TO NON-IONIC LIPIDS: CRYSTAL STRUCTURE OF A CALCIUM CHLORIDE COMPLEX OF GERANIOL

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SUMMARY: X-ray diffraction data were used to determine the crystal structure of a calcium chloride complex of geraniol. The geraniol molecules assume a bilayer arrangement, with channels of calcium and chloride ions separating the bilayers. Each calcium ion is coordinated to the hydroxyl groups of two symmetry-related geraniol molecules and to four chloride ions. Our results demonstrate that hydrophobic interactions within a lipid bilayer can lead to an arrangement of hydroxyl groups suitable for binding calcium ions. Similar interactions may be involved in the calcium-binding sites on membrane surfaces.

Calcium ions interact with the surfaces of many types of cells. These interactions appear to influence a number of cell surface and membrane properties, including adhesion (1), agglutination (2), membrane permeability (3), and membrane structure (4). Little is known about the types of interactions that may be involved, but they are generally presumed to be of the simple ionic type, in which calcium binds nonspecifically to such anionic residues as the phosphate groups of phospholipids or the sialic acid moieties of glycoproteins (5). However, investigations have shown that calcium ions can also bind to uncharged sites if the types of ligands required for calcium chelation are available in appropriate geometrical arrangements (6, 7). In this paper we describe the crystal structure of a calcium chloride complex of geraniol, which provides an example of how hydrophobic interactions can produce an arrangement of nonionic sites suitable for calcium binding.

METHODS

Clear, monoclinic plates were grown by slowly cooling a mixture of geraniol and anhydrous calcium chloride. The resulting complex crystallizes in space group C2/c with the following unit cell dimensions: $\underline{a} = 47.51(5)$, $\underline{b} = 4.188(1)$, $\underline{c} = 12.138(6)$ Å, and $\beta = 93.00(8)^\circ$. The crystals are extremely sensitive to moisture; therefore, they were mounted, along with mineral oil, in thin-walled glass capillary tubes. Weissenberg photographs displayed rather diffuse reflections, suggesting poor order within the crystals. Intensities for the 1242 unique reflections with $2\theta<100°$ were measured on an automatic diffractometer using nickel-filtered copper radiation. Three strong reflections ($\overline{10}$ 0 0, 0 0 4, and 3 1 2) were monitored periodically during data collection; the intensities of these reflections decreased by 16%, 11%, and 18%, respectively. The intensity values were scaled by a least-squares procedure in which the intensities of the standard reflections were used to calculate scale factors as a function of crystal exposure time. Intensities were corrected for crystal absorption, but absorption effects due to the capillary tube and mineral oil were ignored.

The observed density (1.16 g cm^{-3}) is consistent with a unit cell containing four $(C_{10}H_{18}O)_2 \cdot CaCl_2$ formula units, which indicates that the asymmetric unit contains half of one formula unit. Assuming this to be the case, coordinates for the calcium and chloride ions were obtained from Patterson maps. A Fourier map was calculated by using phase angles derived from the calcium and chloride ions. Examination of this map revealed that the geraniol molecule is disordered, with two predominant orientations. One is the orientation displayed in Figures 2 and 3. The second is approximately related to the first by a pseudo-mirror defined by atoms O, C(1), C(2), C(7) and C(8). Thus, there are two different positions assumed by atoms C(3), C(4), C(5), C(6), C(9) and C(10). In an attempt to resolve this disorder, both configurations were included simultaneously in space group Cc, but refinement of this model was unsuccessful. Therefore, the centric space group C2/c was used, and two positions were included for each of the six disordered atoms. These positions were assumed to have population parameters of 0.5. Coordinates of the nonhydrogen atoms, along with anisotropic temperature factors for the calcium and chloride ions, isotropic temperature factors for the oxygen and carbon atoms, a scale factor, and an extinction correction parameter were refined by full-matrix least-squares. The refined values for the isotropic temperature factors increase along the carbon chain (from a value of 3 $Å^2$ for atom C(1) to a value of 10 $Å^2$ for the terminal portion of the chain), indicating large thermal motion for the hydrocarbon chain and/or additional unexplained disorder. Hydrogen atoms could not be located. The final R index ($\Sigma||Fo|-|Fc||/\Sigma|Fo|$), which includes all reflections, is 0.169. This relatively high R index is attributable to several factors, including poor crystal quality, decomposition during data collection, uncorrected absorption effects of the glass capillary tube and mineral oil, and disorder of the hydrocarbon chains.

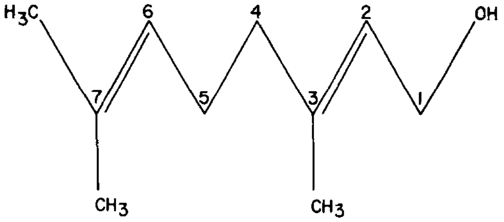

FIGURE 1. Structural formula of the geraniol molecule.

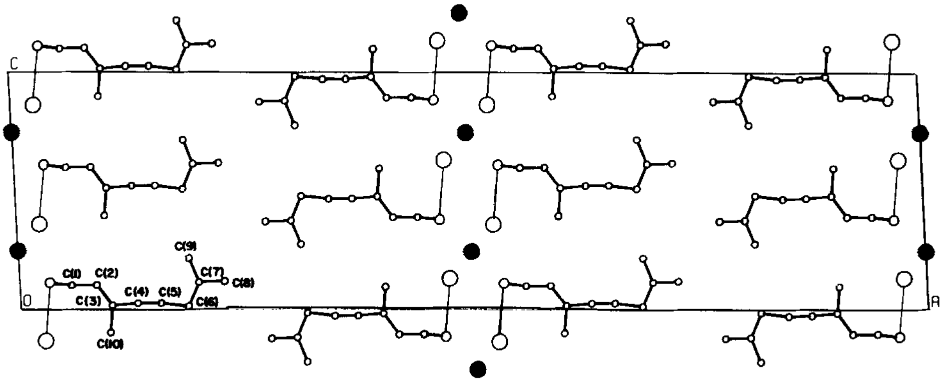

FIGURE 2. Crystal packing and hydrogen-bonding scheme as viewed along the b axis. Heavy lines represent covalent bonds, and fine lines represent hydrogen bonds from the oxygen atoms to the chloride ions. Calcium ions are represented by solid black circles and chloride ions by large open circles. Only one of the two possible orientations of the geraniol molecule is shown.

RESULTS AND DISCUSSION

The molecular conformation and crystal packing are depicted in Figure 2; the atom labeling corresponds to the parameters of Table 1. As expected, the geraniol molecules assume a bilayer arrangement, with the hydrophobic ends of neighboring molecules in close proximity. The bilayers are separated from each other by channels of calcium and chloride ions. These ions are joined to the geraniol array through

TABLE 1. Heavy-atom positional parameters and their standard deviations. Primes designate alternate positions for the disordered atoms.

ATOM	X	Y	Z
Ca	.0000	.387(1)	.250
Cl	.4744(1)	.390(1)	.135(1)
O	.0335(2)	.359(3)	.114(1)
C(1)	.0577(3)	.577(4)	.103(1)
C(2)	.0850(3)	.383(5)	.104(1)
C(3)	.1013(6)	.381(9)	.016(2)
C(3')	.1015(6)	.362(9)	.194(2)
C(4)	.1292(8)	.185(10)	.027(3)
C(4')	.1296(8)	.147(11)	.189(3)
C(5)	.1549(9)	.434(11)	.028(3)
C(5')	.1555(10)	.423(13)	.190(4)
C(6) [C(9')]	.1851(7)	.230(8)	.014(2)
C(7)	.1976(5)	.156(6)	.117(2)
C(8)	.2256(6)	-.022(6)	.122(2)
C(9) [C(6')]	.1880(7)	.234(8)	.220(2)
C(10)	.0977(8)	.565(9)	-.099(3)
C(10')	.0983(9)	.503(9)	.308(3)

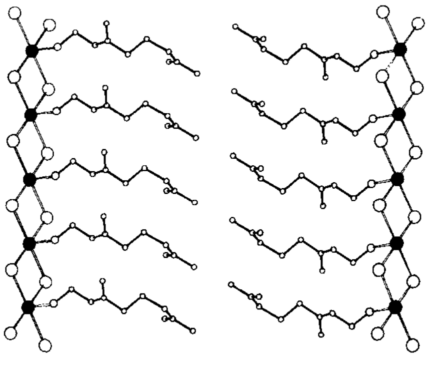

FIGURE 3. Drawing of the bilayer arrangement and calcium coordination, as viewed approximately along the *c* axis. The sixth member of each coordination shell, a hydroxyl group from a symmetry-related geraniol molecule, is not shown. Calcium ions are represented by solid black circles and chloride ions by large open circles. Only one of the two possible orientations of the geraniol molecule is shown.

the single hydroxyl groups, which hydrogen-bond to chloride ions and coordinate to calcium ions. The hydroxyl-chloride distance is 3.2 Å. Figure 3 is a more detailed view of the bilayer arrangement and calcium coordination. The calcium ion is coordinated to the hydroxyl groups of two symmetry-related geraniol molecules and to four chloride ions. The coordination polyhedron is an octahedron, with calcium-oxygen and calcium-chloride distances of 2.36 and 2.77 Å, respectively. These distances are in good agreement with the values expected from a consideration of the ionic and van der Waals radii.

The disorder and thermal motion within the hydrocarbon chains are typical properties found for various synthetic and natural membranes. Electron spin resonance studies of dipalmitoyl lecithin gels and liquid crystals indicate that, like the geraniol structure, the amount of disorder and/or thermal motion increases in going from the polar to the apolar end of the molecule (8). Similar disorder and excessive thermal motion were also noted in the crystal structure of phosphatidylethanolamine (9). This phenomenon is not surprising in view of the relatively weak van der Waals interactions that determine the geometry of the lipid bilayer.

Perhaps the most significant feature of this crystal structure is the relationship between hydrophobic interactions and the arrangement of calcium-binding sites. Van der Waals interactions between nonpolar hydrocarbon chains are accompanied by an alignment of hydroxyl groups into an orientation that permits interactions with the calcium and chloride ions. The resultant array of hydroxyl groups allows simultaneous hydrogen bonding to chloride ions and coordination to calcium ions, resulting in a tightly packed network of hydroxyl groups, cations and anions. This crystal structure thus provides an example of how hydrophobic interactions within a lipid bilayer can help stabilize an arrangement of nonionic sites suitable for calcium binding. Similar interactions involving cholesterol, glycolipids, and other non-ionic components should be considered when constructing models for the calcium-binding sites that are found on membranes.

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