New Chiral Recognition Ability of a Steroidal Bile Acid; Direct Evidence for Efficient Optical Resolution of Racemic Lactones by Cholic Acid Inclusion Crystals

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A new chiral recognition ability has been found for cholic acid inclusion crystals, in which only one enantiomer of a guest lactone can be accommodated in the channels of the host steroidal molecules by crystallization from racemic solution; direct evidence for this phenomenon was obtained from the crystal structure of the 1:1 inclusion compound between cholic acid and γ-valerolactone.

The formation of a crystalline inclusion compound between cholic acid (CA) and acetophenone demonstrated that a new channel type of inclusion phenomenon is provided by CA molecules,[1] which countered the belief that such channel-type inclusion phenomena of bile acids are properties exclusively of deoxycholic acid (DCA) and apocholic acid (ACA) alone.[2] Many crystallographic studies had indicated that the channels formed by DCA and ACA can accommodate a wide variety of organic molecules,[3] whereas only a few crystal structures had been reported for CA inclusion compounds and these did not show such channels.[4–6] The guest-free CA crystals,[7] have also been shown to have the unusual property whereby guest

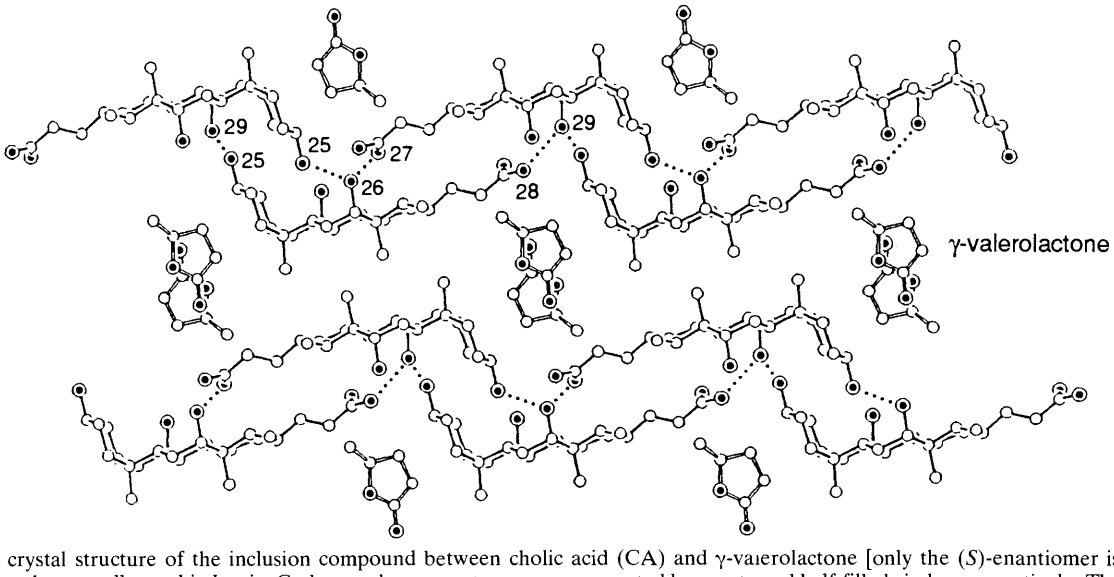

Fig. 1 The crystal structure of the inclusion compound between cholic acid (CA) and γ-valerolactone [only the (*S*)-enantiomer is included] viewed down the crystallographic *b* axis. Carbon and oxygen atoms are represented by empty and half-filled circles, respectively. The hydrogen bond network, together with the numbering scheme of the atoms concerned, is shown by dotted lines; $-O(26)^{I}-H\cdots O(27)^{II}$ = $C(24)^{II}-O(28)^{II}-H\cdots O(29)^{III}-H\cdots O(25)^{IV}-H\cdots O(26)^{I}$, where the four O···O distances are 2.842(7), 2.738(7), 2.672(7) and 2.912(7) Å and the symmetry codes I–IV are $(x, -1+y, z)$, $(1-x, -1/2+y, 1-z)$, (x, y, z) and $(2-x, -1/2+y, 2-z)$, respectively.

molecules such as acetophenone can be added, removed or exchanged without any intervention of an amorphous state.[8] This phenomenon can be interpreted as the first example of intercalation in organic crystals.[8]

The CA assembly is also unusual in that inclusion crystals lead to optical resolution of racemic lactones.[9] Here we report direct crystallographic evidence for this chiral recognition ability of the CA crystalline state demonstrated by the crystal structure of the 1 : 1 inclusion compound between cholic acid and γ-valerolactone.

Needle-like crystals were obtained by recrystallization of a solution of CA in racemic γ-valerolactone. The crystal structure of CA with γ-valerolactone is depicted in Fig. 1.† It can be seen that only the (*S*)-enantiomer of γ-valerolactone is tightly included in the hydrophobic channels parallel to the crystallographic *b* axis with neither hydrogen bonding nor statistical disorder in orientation. This is clear structural evidence for the efficient optical resolution by an inclusion crystal of a steroidal bile acid; it is noteworthy that such ability has never been observed in DCA.[3]‡ Crystal structures of compounds which indicate an ability for optical resolution in a crystalline assembly have been reported:[10] tri-*o*-thymotide,[11] strychnine and brucine,[12] 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose,[13] (−)-sparteine,[14] bis-β-naphthol[15] and 1-phenyl-1-(*o*-chlorophenyl)prop-2-yn-1-ol.[16] In all these cases except for tri-*o*-thymotide, the most important interaction between the host and guest is hydrogen bonding, by which one enantiomer is selectively trapped.§ Although tri-*o*-thymotide does not hydrogen bond to the guest molecules accommodated in hydrophobic atmosphere, the guests are disordered, their orientation not being determined with certainty.[11] Therefore, the present study provides the first example in which one enantiomer is efficiently selected from a racemic mixture and accommodated in a hydrophobic channel without hydrogen bonding or disorder, with the results being confirmed crystallographically.

As seen in Fig. 2,[17] the wall of the CA channel is composed of five units derived from five different CA molecules to form a deformed pentagonal channel space in which the guest lactone molecules are accommodated. The steroidal tail units play a dominant role in determining the shape of the channel. The deep side pocket of the channel is formed by two tail units,[1] by which the methyl group of the lactone is fixed to this channel. The hydrogen atoms attached to the asymmetric carbon atom of the lactone are contacted by this tail unit, preventing the lactone molecule from exchanging its methyl group with its hydrogen atom. This provides a rationale for the selective accommodation of the (*S*)-enantiomer by the assembly of naturally chiral CA molecules.

As far as we know, such a specific feature of a particular molecular unit is unknown in lattice-type inclusion compounds,[3,10] although lariat crown ethers provide well known examples among macrocyclic compounds.[18] Therefore, we can classify cholic acid as a natural lariat in lattice type inclusion compounds.

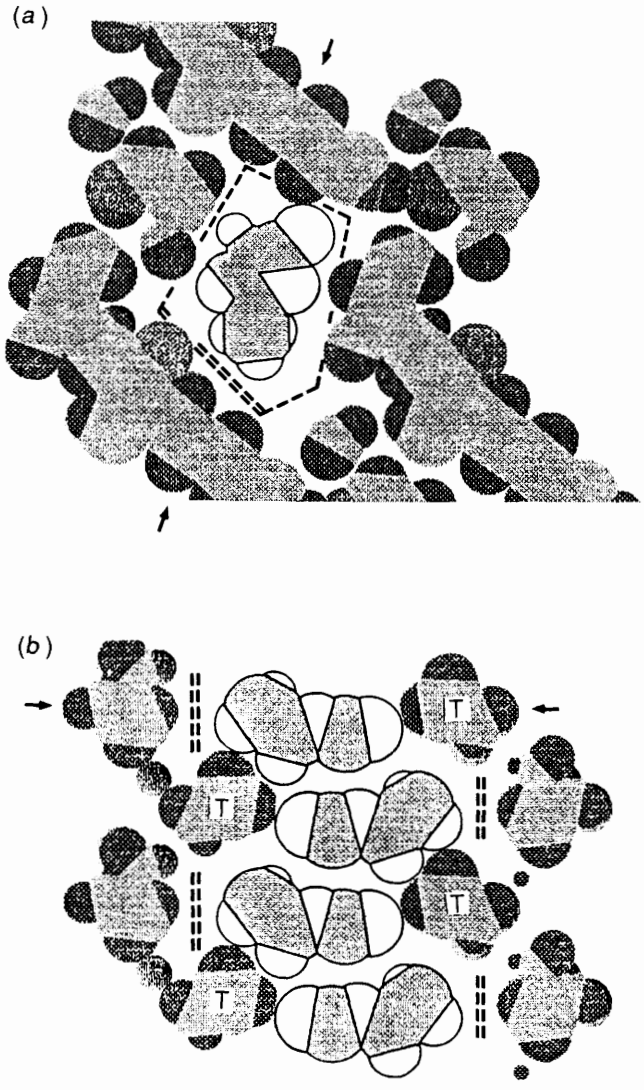

Fig. 2 Schematic drawings[17] of cross-sections of the CA channels (0.2 Å thickness) using space-filling models. The host (CA) and guest (γ-valerolactone) molecules are shown by filled and empty circles, respectively. (*a*) A horizontal section view of the CA deformed pentagonal channel as viewed along the crystallographic *b* axis. The five walls are shown by broken lines. The fifth wall, which is not present in DCA[3] but appears only in CA channels, is shown by the double broken line. The two arrows correspond to the cross-section shown in (*b*). (*b*) A perspective view of a vertical section of the channel. The two arrows show the position of the section of (*a*). The double broken lines are the same as in (*a*). 'T' indicates the tail parts which play an important role in determining the channel side pockets (see text).

† *Crystal data* for CA–γ-valerolactone (1 : 1), $C_{24}H_{40}O_5 + C_5H_8O_2$, monoclinic, $P2_1$, a = 13.010(3), b = 8.003(2), c = 14.049(4) Å, β = 104.76(2)°, V = 1414.4(6) Å^3, Z = 2, D_c = 1.194 g cm^{-3}. As the crystals were found to be essentially isomorphous with those of the inclusion compound of CA with acetophenone,[1] its atomic coordinates were employed as an initial model. The guest molecule was located in a difference Fourier map. Electron densities of the guest lactone were carefully checked to seek possible disorder, but significant electron densities which would imply disorder could not be detected; we conclude that only one enantiomer is included in this channel. The structure was refined to R = 0.069 for 1921 reflections collected to $\sin\theta/\lambda$ = 0.60 Å^{-1} (Mo-Kα radiation) on a diffractometer. All computations were performed on an ACOS 930 computer at the Research Center for Protein Engineering, Institute for Protein Research, Osaka University. The crystal structure is similar to that of CA with acetophenone including the hydrogen bond network pattern, but there is a difference in the conformation about the C(22)–C(23) bond. A detailed comparison of the structures will be published elsewhere. Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Notice to Authors, Issue No. 1.

‡ It has been reported that DCA accommodates (+)-camphor to form an inclusion compound (J. G. Jones, S. Schwarzbaum and L. Lessinger, *Acta Crystallogr., Sect. B*, 1982, **38**, 1207). However, DCA was crystallized from a solution containing only (+)-camphor (not racemic) and the guest camphor molecules were spherically disordered in the DCA channel.

§ In macrocyclic compounds, for example, β-cyclodextrin shows chiral recognition for racemic fenoprofen. However, this resolution is not perfect but both enantiomers are present in a 3 : 1 ratio as a disordered structure (J. A. Hamilton and L. Chen, *J. Am. Chem. Soc.*, 1988, **110**, 5833).

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