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Helical Tape Assemblies in Inclusion Crystals of Bile Acids and Their Derivatives

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Abstract

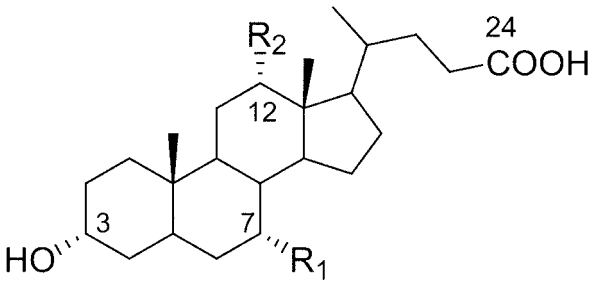
Helical molecular assemblies of bile acids and their derivatives were systematically investigated. These molecules have a common asymmetric structure with three different directions, and form characteristic inclusion crystals with the following hierarchical structure. First, such molecules arrange along their helical axes to yield the helical tapes with a definite direction. Second, the helical tapes are combined in a parallel fashion by using their side-chains through hydrogen bonds to produce chiral sheets. Third, the sheets stack together through van der Waals forces in a parallel or antiparallel fashion. Fourth, there exist chiral spaces for including guests among their sheets. Such hierarchical structure enables us to explain a role of the side-chains with different hydrogen bonding groups and length.

Introduction

The crystalline inclusion compounds of steroidal bile acids and their derivatives are widely known these days [1]. Figure 1 shows four representative bile acids; deoxycholic acid, cholic acid, chenodeoxycholic acid and lithocholic acid. Deoxycholic acid is known as one of the most classical hosts over one century [2]. In contrast, cholic acid has been recognized as a new host, since we reported on its channel-type inclusion compounds with a variety of organic substances in 1988 [3]. Chenodeoxycholic acid has large hexagonal channels for accommodating organic guests [4], while lithocholic acid does not seem to form inclusion compounds yet.

These acids can be transformed into many derivatives by various chemical methods. Focusing on their side-chains (Figure 2), their carboxyl groups can be changed to amide, ester, hydroxyl groups with different methylene number. Such chemical modification brought about a great amount of new host compounds with characteristic inclusion abilities. So far, lots of crystal structures of the inclusion compounds of the steroidal derivatives were determined by X-ray crystallographic analysis. When we compare a pair or a set of the hosts, we can acquire systematic information about hydrogen bonding networks, molecular assembly modes, inclusion spaces, reactions, and so on.

This paper concentrates on assembly modes of the host molecules. Steroidal molecules assemble in a right- or left-handed way around a crystallographic 2_1 axis to give a helical tape with directionality. The tapes associate in a parallel or antiparallel fashion to yield bilayered crystals.



R_1	R_2	
H	OH	Deoxycholic acid
OH	OH	Cholic acid
OH	H	Chenodeoxycholic acid
H	H	Lithocholic acid

Figure 1. Molecular structures of four bile acids.

Finally, we will examine the concept of molecular information and expression from a viewpoint of steroidal inclusion compounds.

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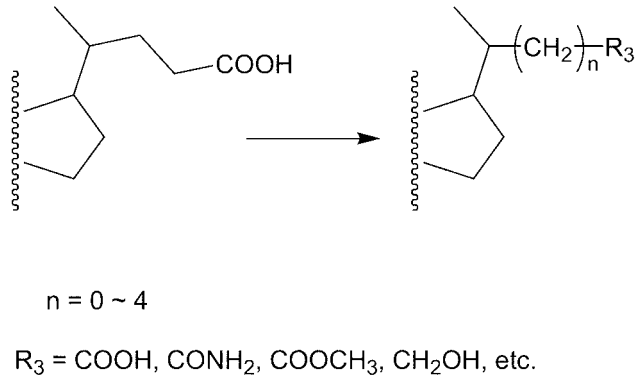

Figure 2. Acquisition of comprehensive host compounds by transformation of the side-chains of the four bile acids.

Experimental

Bile acids were purchased from Wako Co., and used without further purification. Their derivatives were prepared by the conventional methods. All other chemicals and solvents were of the commercially available purest grades. The hosts were recrystallized from neat organic guests or their solutions. The resulting inclusion crystals were filtered out and settled on a filter paper for some time to remove the adhering solvents and guests. The crystals were characterized by means of IR, ^{1}H- and ^{13}C-NMR spectroscopy, thermogravimetric analysis, X-ray powder diffraction methods. X-ray single crystal diffraction data were collected on a Rigaku RAPID diffractometer with 2D area detector with graphite-monochromatized Cu-$K\alpha$ radiation. Direct methods were used for the structure solution and the structure was refined by the full matrix least-squares procedure with the program TEXSAN. Non-hydrogen atoms were refined with anisotropic displacement parameters, and hydrogen atoms were placed in idealized positions.

Results and discussion

Hierarchical structure of inclusion crystals of bile acids and their derivatives

So far, we obtained over three hundreds of crystal structures of inclusion compounds of bile acids and their derivatives with a variety of organic substances. We compared the crystal structures from a view of molecular assembly modes due to noncovalent bonds, such as hydrogen bonds and van der Waals forces. Such comparison has led us to the conclusion that these crystals have a common hierarchical structure, as shown in Figure 3. It is noteworthy that highly chiral molecules cause chiral architectures through a series of association processes. Thus, the facially chiral molecules (Figure 3a) associate together to yield a helical tape-like assembly with directionality (Figure 3b). Such tapes combine together in various ways to produce helical tape assemblies. For example, when the tapes have hydrogen bonding groups on their surface, they are connected through hydrogen bonds to yield a two-dimensional sheet (Figure 3c). Such sheets stack together to make a bilayered assembly (Figure 3d). In most of the cases there are inclusion spaces among their tapes to accommodate guest components.

Asymmetric structure of bile acids and their derivatives

Chirality of the steroidal molecules plays an important role for understanding the helical assemblies of the hierarchical structure. Figure 4 schematically shows a steric structure of cholic acid. The asymmetric skeleton enables us to discriminate three directions of the steroidal molecule. The criterion for the directions is based on locations of its hydrogen bonding groups, side-chain, and methyl groups. The hydroxyl group (OH(3)) and the carboxyl group (COOH(24)) of the side-chain correspond to head and tail parts, respectively. Three hydroxyl groups constitute the hydrophilic belly site, while two methyl groups the lipophilic back site. The hydroxyl groups (OH(7) and OH(12)) function just like left and right hands. The OH(3) is closer to the OH(7) rather than the OH(12). The two methyl groups and the side-chain are located on the right side rather than on the left side.

Formation of a helical tape with a 2_1 axis

Most of the inclusion crystals of bile acids and their derivatives have crystallographic 2_1 or C2 axes. The consideration of molecular arrangements around the axes led us to the finding of a notable relation between their molecular structures and the assembly modes. Figure 5 exemplifies the relation in the case of deoxycholic acid. The deoxycholic acid molecules (Figure 5a) align around a crystallographic 2_1 axis, and their belly sites face the axis (Figure 5b). The direction of the axis can be defined so that their head parts are located at upper sites according to an arrow of the axis. Therefore, we can say that deoxycholic acid molecules arrange in a right-handed helical way (Figure 5c). Since the direction of the helical tape is defined toward the head end from the tail end, such an arrangement is schematically shown in a helical tape with an arrow (Figure 5d).

Figure 6 illustrates the resulting helical tapes of the four bile acids. As shown in Figure 6a, deoxycholic acid has the hydrogen bonding group (OH(12)) on the right side. Since the two groups (OH(3) and OH(12)) face the 2_1 axis, the head slightly leans to the right and the tail greatly separates from the axis. The neighbored molecules adhere through intermolecular hydrogen bonds between the OH(3) and OH(12) groups. The resulting assembly is a 2_1 right-handed helical tape, as shown in Figure 6a.

On the other hand, cholic acid has one hydroxyl group on the right side (OH(12)) and the other on the left side (OH(7)). As shown in Figure 6b, there exists an additional hydrogen bond between OH(3) and OH(7) of the neighbored molecules, leading to the situation that cholic acid molecules lie in a perpendicular position to the axis, and that the tail greatly separates from the axis. However, since the distance between OH(3) and OH(7) is shorter than that between OH(3) and OH(12), the head slightly leans to the upper as compared with the perpendicular position. The resulting assembly is a 2_1 right-handed helical tape.

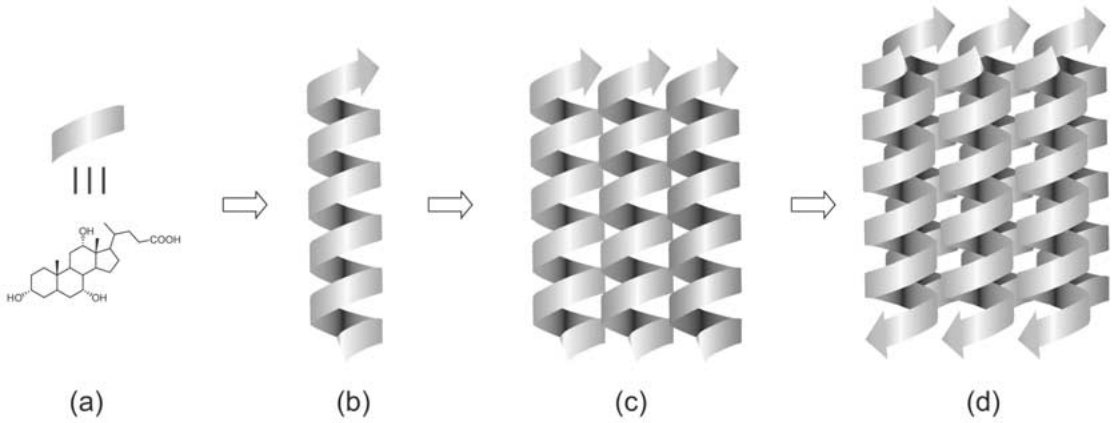

Figure 3. Schematic representation of the hierarchical structure of inclusion crystals of bile acids and their derivatives; (a) a chiral molecule, (b) a chiral tape, (c) an assembly of the tapes in a two-dimensional way, and (d) an assembly of the tapes in a three-dimensional way.

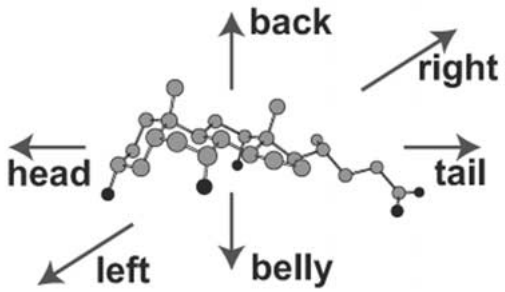

Figure 4. Chirality of a cholic acid molecule. The facial and amphiphilic chirality of the molecule enables us to discriminate three directions; head and tail, belly and back, right and leftt.

Chenodeoxycholic acid exhibits the opposite tendency as compared with deoxycholic acid, as shown in Figure 6c. Since two hydroxyl groups (OH(3) and OH(7)) are on the left side, the head leans to the left and the tail separates from the axis. The resulting assembly is a 2_1 left-handed helical tape. In contrast, lithocholic acid has only one hydroxyl group (OH(3)), and the molecules lie in a perpendicular position to the 2_1 axis. Since the head slightly leans to the upper, the resulting assembly is a 2_1 right-handed helical tape

Alignment of the helical tapes

Since the hydrogen bonding groups of the side-chains separate from the helical tapes, the groups function for adhering the helical tapes. Figure 7a shows the resulting tape assembly in the case of deoxycholic acid. It can be seen that the carboxyl groups (COOH(24)) just fit between the hydrogen bond (OH(3)–OH(12)) of their own tape and that of its neighbored tape. In other words, the resulting hydrogen bonding network consists of repeated bonds of –OH(3)–OH(12)–O=C–OH(24)–. The parallel combination of the tapes results in a chiral sheet with a hydrophilic inside and a lipophilic outside. Furthermore, such amphiphilic sheets stack together on the lipophilic surface through van der Waals forces, producing the known bilayered crystals. It does not seem that any changes of the side-chains in length will keep the tape assembly due to the just fitness of the chains. In fact, decrease of one methylene unit of the side-chain induced a change of the hydrogen-bonding network. Figure 7b shows the resulting cyclic network among the three hydrogen-bonding groups, as compared with the helical network in Figure 7a.

Figure 8a depicts the tape assembly in the case of cholic acid. The carboxyl group of a tape just fit into the incomplete hydrogen bond (OH(7)–OH(3)–OH(12)) of the neighbored tape, yielding the cyclic hydrogen-bonding network. The parallel combination of the tapes yields a chiral and amphiphilic sheet, as in the case of deoxycholic acid. The carboxyl groups lie in a perpendicular position to the crystallographic 2_1 axis and face toward the neighbored tape. Therefore, it is expected that the increase of two methylene units of the side-chain will keep the tape assembly. Figure 8b shows an example of the desired retention of the tape assembly [5].

The change of hydrogen-bonding groups induces a change of the donor–acceptor relationship among their groups. We carried out the transformation of the side-chain from carboxyl group to various hydrogen-bonding groups. Figures 9a-d show the resulting hydrogen-bonding network in the case of deoxycholic acid. The amide group with additional hydrogen replaces the original carboxyl group to result in the similar helical network (Figure 9b) [6]. Similarly, the hydroxyl group joins the helical network (Figure 9d) [7]. On the other hand, the ester group destroys the helical network (Figure 9c), inducing different networks. Figures 9e–h show a series of changes of the hydrogen-bonding network in the case of cholic acid. The amide group replaces the original carboxyl group to form the similar cyclic network (Figure 9f) [8], while the ester group (Figure 9g) and hydroxyl group (Figure 9h) destroy the network, yielding different networks [9].

Inclusion spaces for guest recognition

There are inclusion spaces among the helical assemblies, especially around the side-chains, for including various guests, as schematically shown in Figure 10. It is a reasonable idea that the different alignments of the tapes yield the corresponding characteristic inclusion spaces. As mentioned above, in the case of deoxycholic and cholic acids, the

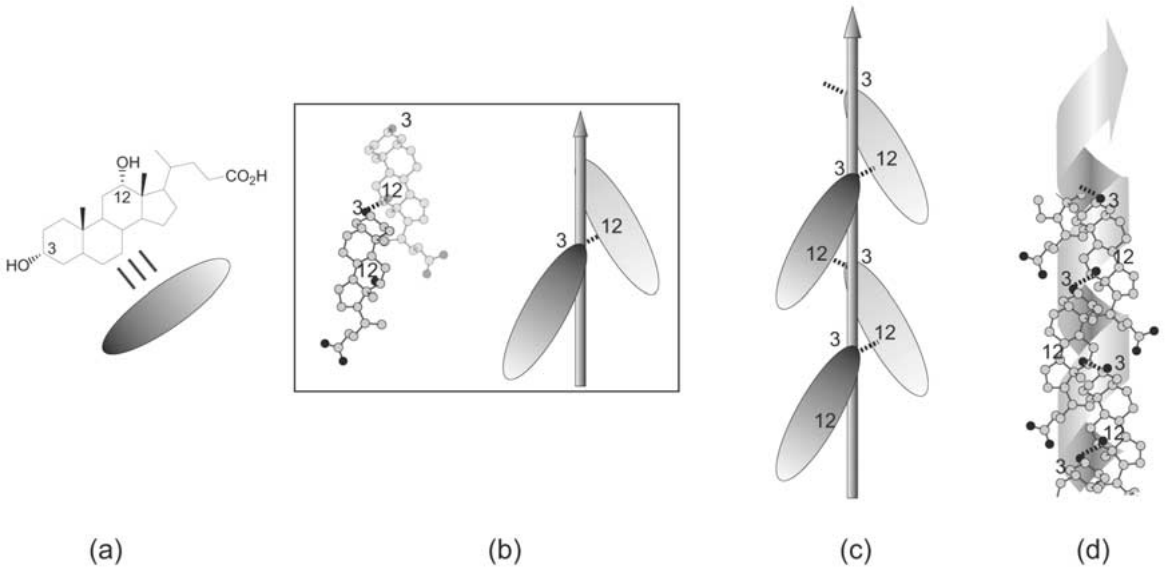

Figure 5. Schematic representation of the helical tape; (a) a chiral molecule of deoxycholic acid, (b) a bimolecular assembly along a crystallographic 2_1 axis, (c) a helical assembly along the axis, and (d) a helical tape with an arrow.

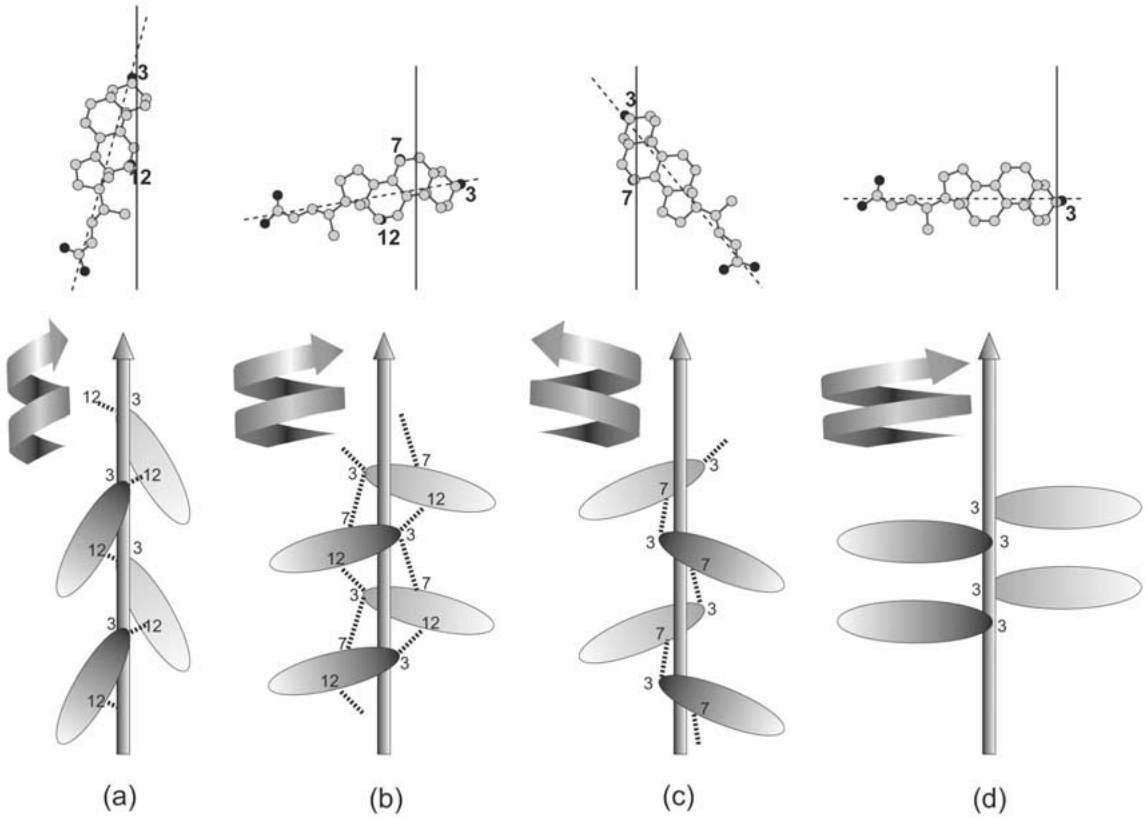

Figure 6. Schematic representation of the helical tapes of four bile acids; (a) deoxycholic acid, (b) cholic acid, (c) chenodeoxycholic acid, and (d) lithocholic acid.

carboxyl groups combine the tapes to yield the bilayered sheets involving channel-type inclusion spaces. Since various hydrogen-bonding groups can be arranged on the surface of the sheets, it is a challenging subject that we predict characteristic molecular recognition on the basis of molecular design.

Molecular information and expression by protein-like molecules

As mentioned above, our study made clear that the steroidal molecules have protein-like structures to form protein-like assemblies. The chiral molecular structures cause a series of chiral assembly modes. Therefore, it is considered that the steroidal molecules express their information through their

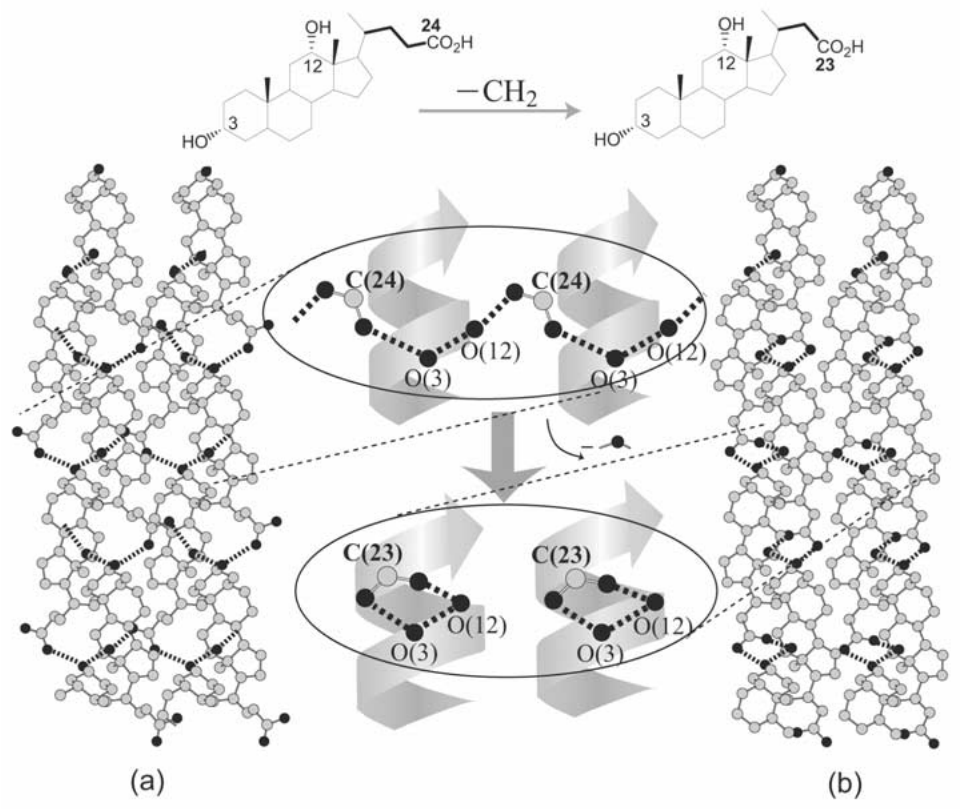

Figure 7. Tape assemblies of (a) deoxycholic acid, (b) a derivative of deoxycholic acid with one decreased methylene unit. Carbon and oxygen atoms are represented by gray and black circles, respectively.

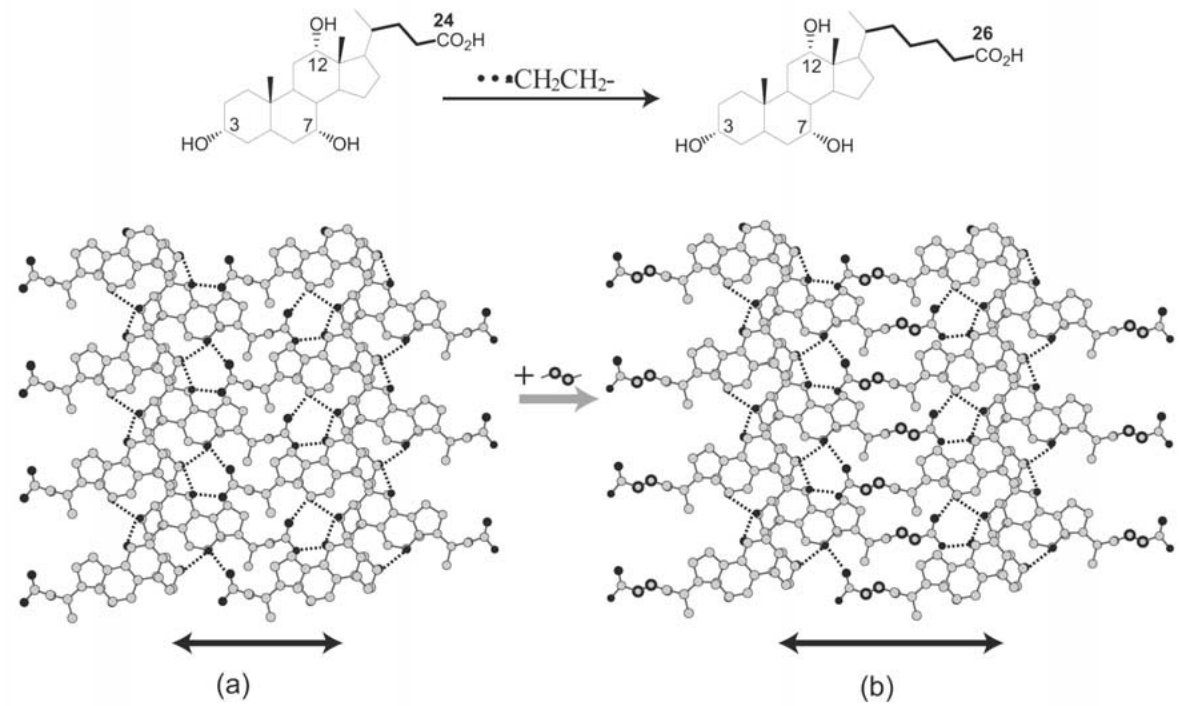

Figure 8. Tape assemblies of (a) cholic acid, (b) a derivative of cholic acid with two additional methylene units. Carbon and oxygen atoms are represented by gray and black circles, respectively.

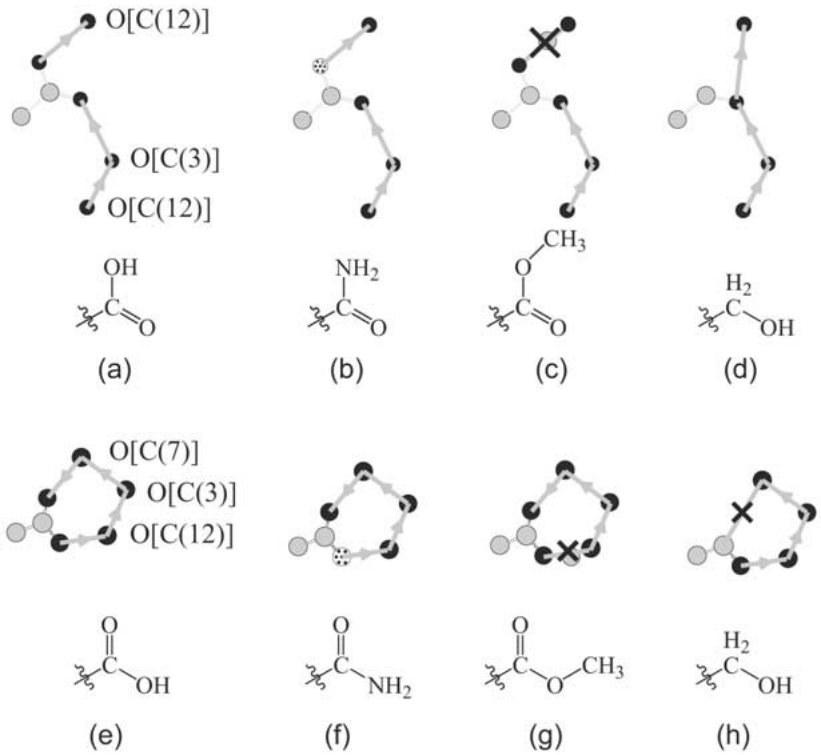

Figure 9. Hydrogen-bonding network; (a) deoxycholic acid, (b) amide, (c) ester, (d) alcohol derivatives of deoxycholic acid in the upper, (e) cholic acid, (f) amide, (g) ester, (h) alcohol derivatives of cholic acid in the lower.

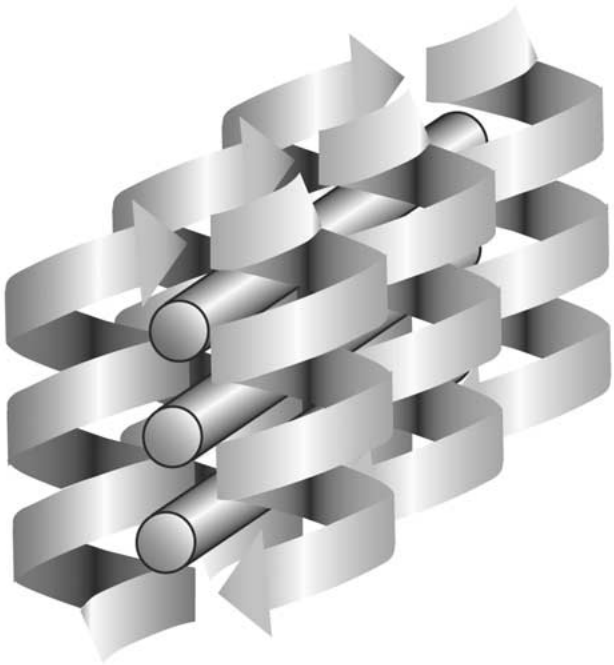

Figure 10. Schematic representation of chiral inclusion spaces in an assembly of the helical tapes.

assembly processes involving the hierarchical structure. So far, the concept of molecular information was used with proteins, nucleic acids and sugars. However, such a concept may be extended for chain compounds with many chiral carbons, such as the steroidal molecules. In other words, chiral carbon-chains theoretically constitute original information carriers. In this manner, the steroidal molecules tell us an exciting story about molecular information and expression [10].

Conclusion

We demonstrated the hierarchical structure of helical tape assemblies of bile acids and their derivatives. Such systematic research may clarify the relationship between their molecular structures and assembly modes, contributing greatly to the development of crystal engineering and prediction of crystal structures. Finally, it is noteworthy that these assemblies exhibit fascinating intercalation phenomena [11], molecular recognition [12], and polymerization control [13].

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