STUDIES ON INCLUSION ABILITIES AND MOLECULAR RECOGNITION OF BILE ACIDS HAVING MULTIPLE HYDROXY GROUPS

（複数の水酸基をもつ胆汁酸の包接能と分子認識に関する研究）

1998

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Preface

The studies described in this thesis have been carried out under the direction of Professor Mikiji Miyata at Material and Life Science, Graduate School of Engineering, Osaka University.

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January, 1998

Contents

General Introduction

Although biopolymers, such as proteins or nucleic acids are commonly considered to have molecular information, the information is only designated by the sequence of the unit. For example, only the sequence of amino acids fix the information of the proteins.. In other words, these biopolymers could be hold the information owing to the units that arranged under fixed sequence.[1,2] To expand this idea, there is no nessensity to stick to biopolymers. Even oligomeric carbon-chain compounds also hold informations. In this case, the atoms that composed the molecule is the 'unit' of the molecule. So the information can be fixed as the molecules by using strong covalent bond.

On the other hand, the fixed information expressed through the weak non-covalent interactions. So For example, reactivility, color, smell or taste are one of the expression of the information. Moreover, the functions that is caused by the molecule is also the expression

To clear this problem, steroidal bile acids are the typical molecules for this study. Because they are composed of highly asymmetric bodies and have multiple functional groups. Furthermore, these compounds commomly form good single crystals so easily. And it has been known that some bile acid can form inclusion crystals with other organic substances. So there are good posibility to clear the relationship between the molecular structure and the molecular assemblies or inclusion abilities.

This thesis consists of following five chapters. Chapter 1 describes the Inclusion abilities and crystal structure of steroidal bile acids In Chapter 2, the author show inclusion abilities and molecular recognition of bile acids having 6-positioned hydroxy group. Chapter 3 deals with Inclusion abilities and molecular recognition of bile acids that have 3- and 7-positioned hydroxy groups. Chapter 4 describes Inclusion abilities and molecular recognition of bile acids that have 3- and 12-positioned hydroxy groups. And Chapter 5 the author show Inclusion Abilityies and Molecular Recognitions of bile Acid that have 3-, 7- and 12-positioned hydroxy groups.

Chapter 1
Inclusion Abilities and Crystal Structure of Steroidal Bile Acids

1-1 Introduction

Steroidal bile acids, such as cholic acid (3α, 7α, 12α-trihydroxy-5β-cholan-24-oic acid, CA) and deoxycholic acid (3α, 12α-dihydroxy-5β-cholan-24-oic acid, DCA), are naturally-occurring steroids formed in livers of vertebrate animals from cholesterol and exist as the main part of the bile. .General molecular formula is depicted in Figure 1-1 They have unique and fascinating molecular structure due to following characteristics: The first is a rigid 'thick' steroidal plane with a flexible 'thin' side chain. The second is the wavy shape due to the *cis* fusion of the A and B rings and the *trans* fusion of the B, C and D rings. The third is asymmetric structure having more than thirteen chiral centers. The forth is multifunctionality with the carboxylic acid group at the end of the side chain and a few hydroxy groups in the steroidal plane.

These bile acids are distinguished by number, position and direction of hydroxy groups in the steroidal skeleton. There are four substituted positions at 3, 6, 7, and 12 of the steroidal skeleton and two attached directions α face and β face. α face is the opposite side of the two methyl groups and the β face is the same side of two methyl groups, as shown in Figure 1-2. For example, CA has three hydroxy groups at 3, 7,and 12 position whole directed to *a* face and DCA has two at 3-, and 12-directed to α, and ursodeoxycholic acid (3α, 7α-dihydroxy-5β-cholan-24-oic acid, UDCA)

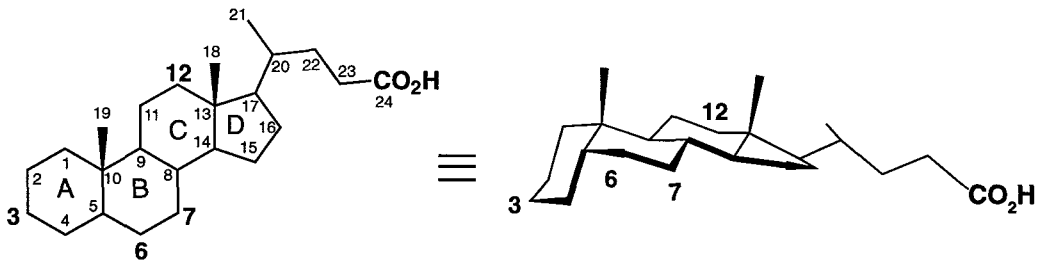


Figure 1-1. General molecular formula of steroidal bile acids

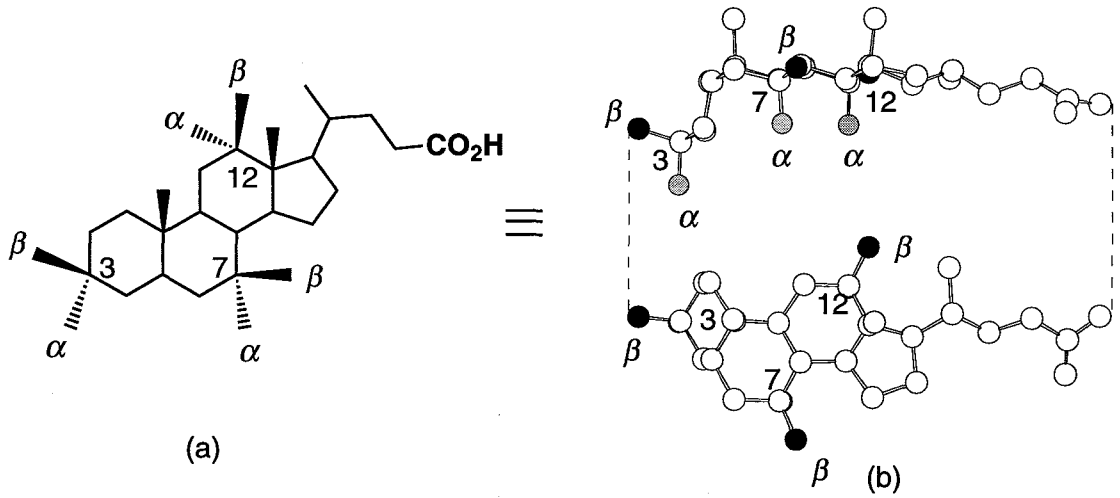


Figure1-2. Directions of steroidal bile acid (a) rational formula of steroid, (b) ball & stick model of steroid; upper is top view and lower is the front view. a and b directional hydroxy groups are represented shared and filled circle, respectively.

has two hydroxy groups at 3-position directed to α and at 7-position to β.

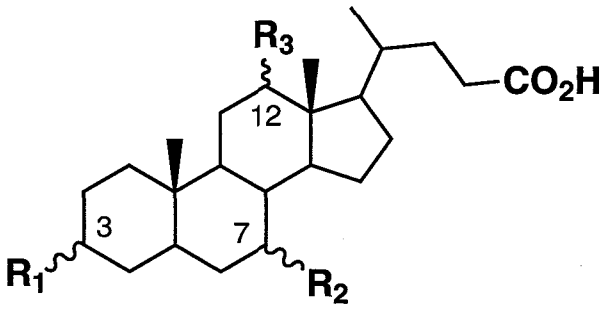
Among these steroidal bile acids, DCA have been known to form inclusion crystals with other organic substances since the discovery of molecular complexes by Wieland in 1928.[3] Successive studies of inclusion phenomena have extended the guest molecules to a wide variety of organic substances, such as aliphatic, alicyclic and aromatic hydrocarbons, alcohols, fatty acids, esters, ketones, ethers, nitriles and etc. In 1972, Craven and DeTitta revealed the first crystal structure of the 1:1 compound of DCA with acetic acid.[4] Subsequent X-ray studies in the past two decades established that nearly all the inclusion compounds have the cumulated bilayered structure with channels where the guest molecules are included in. The inclusion ability have been believed as unique property of DCA.

CA is also an classical compound and its molecular complexes with some alcohols had been discovered since the end of last cencturey.[5] However, CA has not been believed to form inclusion compounds at all except them, while DCA has versatile inclusion ability. In 1979, Miyata found that CA also formed inclusion crystals with various organic substances. The subsequent crystallographic studies revealed that inclusion crystals of CA has cumulated bilayer structure with molecu-

Table 1-1. Approximate inclusion abilities of steroidal acids towards organic substances. The acids are designated by numbers in parentheses in the text.

R_1	R_2			R_3
	H	α-OH	β-OH	
α-OH	—	— —	*	H
	— —	— —	nd	α-OH
	+	*	nd	β-OH
β-OH	+ +	+	+ & —	H
	+	+ +	+	α-OH
	+ & —	+	nd	β-OH

(+): hydrophilic guests included; (++): more hydrophilic guests included;
(-): lipophilic guests included; (- -) : more lipophilic guests included;
(*) : guest-free crystals obtained; (nd): more research needed.



lar channels. This structure was similar to those of inclusion compounds of DCA. Successive works by Miyata, and other research groups in South Africa and Tsukuba revealed that CA includes as many guest molecules as DCA such as aliphatic ester, lactones, ketones, aromatic compounds, and etc.

Although DCA and CA have paid much attention as host compounds, inclusion compounds of other bile acids have been never studied. The author had started systematic investigation of inclusion abilities and crystal structures of whole stereo isomers of CA, DCA and hyodeoxycholic acid (3α, 6α-dihydroxy-5β-cholan-24-oic acid, HDCA). This chapter summaries overview of the inclusion ability of a series of isomers of 3-hydroxy-, 3,7-dihydroxy-, 3,12-dihydroxy-, and

3,7,12-trihydroxy-cholanoic acids including DCA and CA.

1-2 Inclusion Abilities of Various Bile Acids Derivatives.

3-Hydroxycholanoic acids have two isomers[(3α), (3β)], 3,7-dihydroxycholanic acids have four isomers [(3α, 7α), (3α, 7β), (3β, 7α), (3β, 7β)], 3, 12-dihydroxycholanic acids have four isomers [(3α, 12α), (3α, 12β), (3β, 12α), (3β, 12β)], and 3, 7, 12 trihydroxycholanic acids have eight isomers [(3α, 7α, 12α), (3β, 7α, 12α), (3α, 7β, 12α), (3α, 7α, 12β), (3β, 7β, 12α), (3β, 7α, 12β), (3α, 7β, 12β), (3β, 7β, 12β)], respectively. Table 1 summaries inclusion abilities of these bile acids. Most of bile acids with various hydroxy groups form the inclusion compounds. This means that steroidal bile acids are good source for host molecules of lattice inclusion compounds. The wavy and complex molecular structure enables to form cavities in crystalline state.

Among commercially available naturally-occurring bile acids [(3α), (3α, 7α), (3α, 7β), (3α,12α), (3α, 7α, 12α)], ursodeoxycholic acid [(3α, 7β), UDCA] does not have inclusion abilities at all , whereas CA, DCA, and chenodeoxycholic acid [(3α, 7α), CDCA] form inclusion compounds with various organic compounds. Guest molecules of LCA has strictly limited and other organic compounds form guest-free crystals. 3α-series (3α), (3α, 7α), (3α, 12α), (3α, 7α, 12α) include lipophilic guest molecules, while 3β-series (3β), (3β, 7α), (3β, 12α), (3β, 7α, 12α) include hydrophilic compounds such as alcohols and ethers. This results shows that directions of 3 position plays an important role for guest preference.

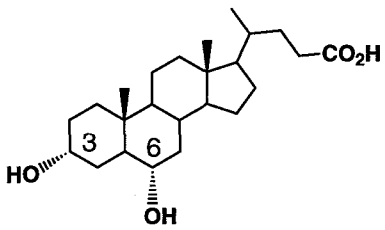
1-3 Summary

In this chapter, the author showed that steroidal bile acids tend to form inclusion crystals with organic substances. And only two bile acids could not include any organic compounds. Among these acids, steroids that have the 3α-hydroxy group tend to include lipophilic guest molecule, whereas 3β-isomers like hydrophilic organic substances. This result shows that 3-positioned hydroxy group plays an important role to form stable inclusion crystals.

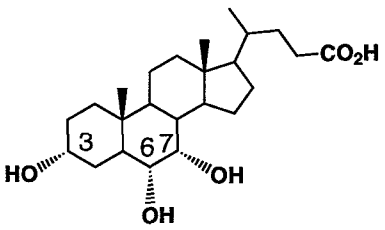
Chapter 2
Inclusion Abilities and Molecular Recognition of Bile Acids Having 6-Positioned Hydroxy Group

2-1 Introduction

Two naturally-occuring steroidal bile acids that have 6-positioned hydroxy group have been known. One is hyodoxycholic acid (3α, 6α-dihydroxy-5β-cholan-24-oic acid, HDCA) and another is hyocholic acid (3α, 6α, 7α-trihydroxy-5β-cholan-24-oic acid, HCA). In this chapter, the author report the inclusion abilities and the molecular assembly modes of the derivatives.



HDCA



HCA

2-2 Experimantal

General Method. IR spectra were taken on a JESCO IR-810 or a JESCO IR Report 100 grating spectrometer using KBr disk. ^{1}H-NMR spectra were recorded on a JOEL 270 MHz or 400 MHz FT-NMR spectrometer, and chemical shifts are reported in parts per million (ppm) from tetramethylsilane. Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) were mesured by a Rigaku Thermoflex TG 8110 instrument or a Rigaku Thermo Plus TG 8120 instrument. X-ray powder diffraction pattern were mesured by Rigaku X-ray diffractometer RINT 2000 with Cu-Ka radiation.

Materials. HDCA and HCA were purchased from Tokyo Chemical Industry Co., Ltd. and

SIGMA Chemical Company, respectively. These bile acids were used without further purification. Pyridine, aniline, 1-butanol and other solvents were all regent grade quality, perchased commeracially and used without further purification.

Preparation of Inclusion Crystals. Preparation of inclusion crystals usually consists of re-crystallization of HDCA or HCA from organic substances. A typical procedure is as follows; recrystallization experiment involves dissolving of HDCA (30 mg) in 0.8 mL of pyridine with heating at 100 °C. The solution were allowed to be cool at room temperature untill crystals began to form. Crystals were collected and air-dried on a filter paper.

X-ray Crystallographic Study. X-ray diffraction measurements were performed on a Rigaku RAXIS-IV diffractometer with a graphite-monochromated Mo-$K\alpha$ radiation at 203 K. Data were corrected for Lorenz and polarization effects. The structures were solved by direct methods (SIR92 for the crystals of HDCA with pyridine and HCA with 1-butanol; SHLEX86 for the guest-free crystal of HCA; SnB for the crystal of HCA with 3-methyl-1-butanol) and refined against the *F*o data by full-matrix least-squares methods. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms attached to carbon were placed in calculated position while O-H positions were obtained from difference Fourier synthesis. All calculations were performed using the TEXSAN crystallographic software package of the Molecular Structure Corporation.

2-3 Results and Discussion

Hyodeoxycholic acid

HDCA is one of the steroidal bile acids that have 3- and 6-positioned hydroxy groups. Although its discovery dates back to 1923, its inclusion ability has never been reported so far.[6] In fact, Rheinboldt concluded that HDCA did not form inclusion compounds on the basis of its melting diagram.[7] More recently, Hall et al. determined a crystal structure of guest-free HDCA.[8] Over

Table 2-1. Guest-release temperatures and molar ratios of inclusion crystals of HDCA

Guests	Guest release temperature/°C [a, b]	Molar ratio host:guest [a]
Pyridine	78, 95	1:1
Aniline	83, 128	1:1
o-Toluidine	107, 122	1:1
m-Toluidine	107	1:1
p-Toluidine	97, 126	1:1
Methyl benzoate	84, 113	2:1
Ethyl benzoate	96, 125	2:1

[a]Determined by TGA-DSC. [b]Endothermic peak top temperature for guest-release.

the past ten years we continue to reexamine the inclusion abilities of deoxycholic acid (3α, 12α-dihydroxy-5β-cholan-24-oic acid, DCA) and related compounds.[1] This research broke down the conventional thought about the inclusion ability of HDCA. Here the author wish to report the inclusion compounds of HDCA with specific guest molecules and the crystal structure of a 1:1 compound of HDCA with pyridine.

Commercially available HDCA was recrystallized from over one hundred organic substances, such as alcohols, ketones, esters, amines, and so on. The resulting crystals were characterized by thermogravimetric analysis (TGA), solid state IR and ^{1}H-NMR spectroscopy. Most of the substances employed yielded guest-free crystals as expected. But only several aromatic amines, such as pyridine, aniline and toluidines gave the inclusion crystals at a 1:1 molar ratio of host to guest, as shown in Table 2-1. In the inclusion crystals except for *m*-toluidine, the author observed three endothermic peaks in the thermal scanning calorimeter diagram. One peak at the highest temperature (202°C) corresponds to the melting point of HDCA itself. The other two peaks at lower temperature are based on the release of the guest molecules from the host lattice, as indicated by a mass loss in the simultaneous TG measurement.

Slow evaporation of pyridine solutions of HDCA at room temperature afforded single crystals suitable for X-ray crystallographic study. The resulting crystals belong to orthorhombic, $P2_12_12_1$, similar to those of guest-free HDCA.[3] Figure 2-1(a) depicts the crystal packing of the 1:1 inclusion compound of HDCA with pyridine, viewed along the crystallographic *c* axis. It can be seen

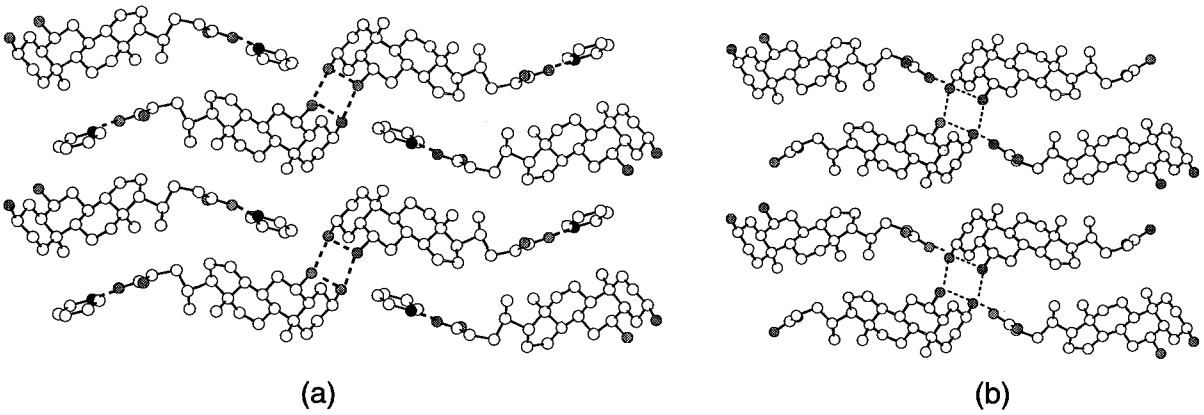


Figure 2-1. The crystal structures of HDCA viewed down the crystallographic *c* axis. (a) inclusion compound of HDCA with pyridine. (b) guest-free crystal of HDCA.[3] Carbon, oxygen and nitrogen atoms are represented by empty, shaded and filled circles, respectively. Dotted line that connect between O atoms shows hydrogen bonding networks. Hydrogen atoms are omitted for clarity.

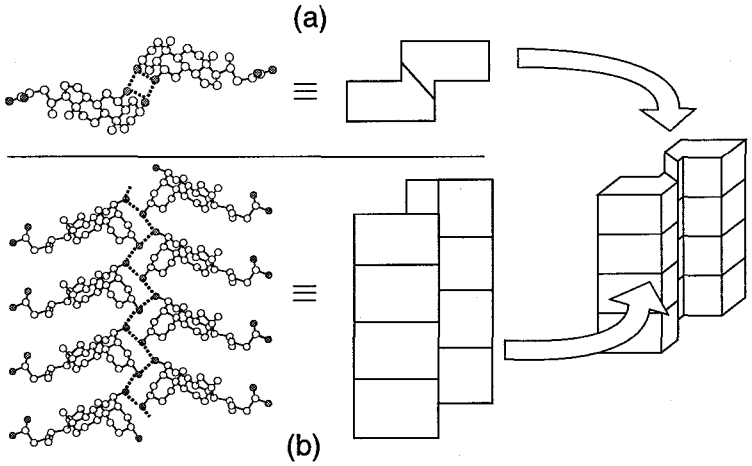


Figure 2-2. Schematic representation of a column formed by HDCA. (a) top view, (b) front view.

from the figure that the host molecules stack along the *c* axis in a head-to-head fashion to form a helical column (Figure 2-2). The column is retained by a helical hydrogen bonding network in a sequence of O(3)H···O(6)H···O(3)H···, where the distances are 2.882(8) and 2.912(7) Å, respectively (Figure 2-3(a)).

The bond distances between carbon and oxygen atoms of a carboxylic group are different. Namely, C-O(24a) is 1.327(9) Å, while C-O(24b) is 1.209(10) Å. A hydrogen atom is located at O(24a) by difference Fourier synthesis. The distance between O(24a) and nitrogen atom is 2.703(9)

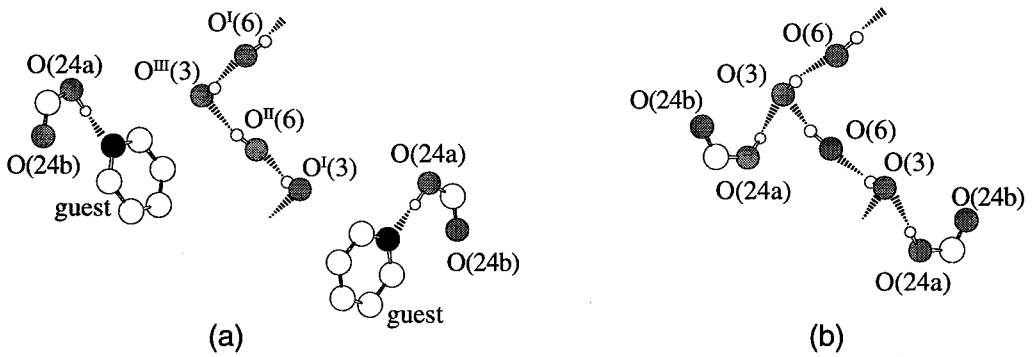


Figure 2-3. Schematic representation of the hydrogen bonding networks : (a) HDCA - pyridine. The helical hydrogen bonding network, together with the numbering scheme of the atoms concerned, is shown: O(3)^{I}H···O(6)^{II}H···O(3)^{III}H···O(6)^{I}H··· where O···O distances are 2.882(8) and 2.912(7)Å and the symmetry code I-III are (x, y, z), (- x + 1/2, - y, z - 1) and (- x + 1/2, - y, z), respectively. (b) guest-free HDCA.[3]

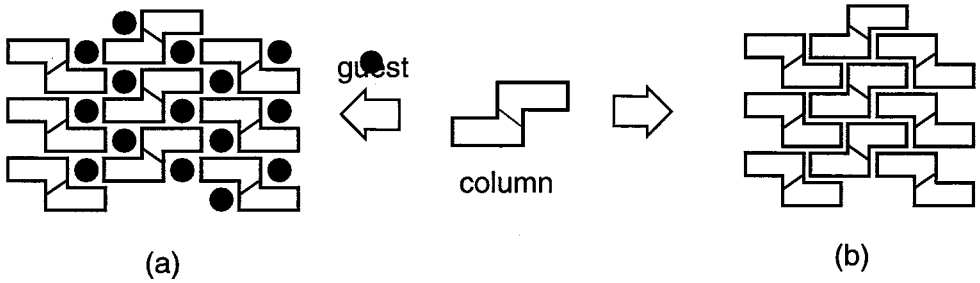


Figure 2-4. Schematic representation of the crystal structures by using the top view of the column in Figure 2-2. (a) HDCA - pyridine, (b) guest-free HDCA.

Å. In addition, the IR spectrum of the crystal has only a C=O stretching band at 1710 cm^{-1} for COOH instead of COO^{-}. These results indicate that the guest molecules are caught through hydrogen bondings between the carboxylic groups and the nitrogen atoms of the guest.

Next we compared this structure with that of the guest-free crystal (Figure 2-1(b)).[8] Both the crystals have columnar structures and very similar hydrogen bonding networks among the host molecules in common. It is noteworthy that the side chains play an important role in determining flexibilities of the assemblies. Namely, the carboxylic groups are attached to O(3) in the network through hydrogen-bondings in case of the guest free crystal (Figure 2-3(b)), while the groups leave the network to bind the guest molecules in case of the inclusion crystal. This transformation accompanies a conformational change of the side chain. Thus, the dihedral angle C(20)-C(22)-

C(23)-C(24) of the former takes a value of -98°, while that of the latter takes a value of 67°.

Figure 2-4 schematically illustrates the correlation between the inclusion crystal and guest-free crystal, viewed along crystallographic *c* axis. In the absence of the suitable guest molecules, the carboxylic groups bridge the columns to yield no void space between them. Introduction of the guest molecules forces the columns to open inclusion spaces for them. This implies the guest specificity of HDCA.

On the other hand, the compounds with aromatic esters, such as methyl benzoate and ethyl benzoate, seem to have different crystal structures from those with the aromatic amines by means of their powder X-ray diffraction patterns.

Hyocholic acid

HCA was discovered in 1956.[9,10] So far, there were neither reports about its inclusion ability nor those about its crystal structure. However, the author have found that HCA has an inclusion ability towards specific organic substances. This paper concerns with structural analyses of both guest-free and inclusion crystals of HCA, accompanied by an interesting role of water.

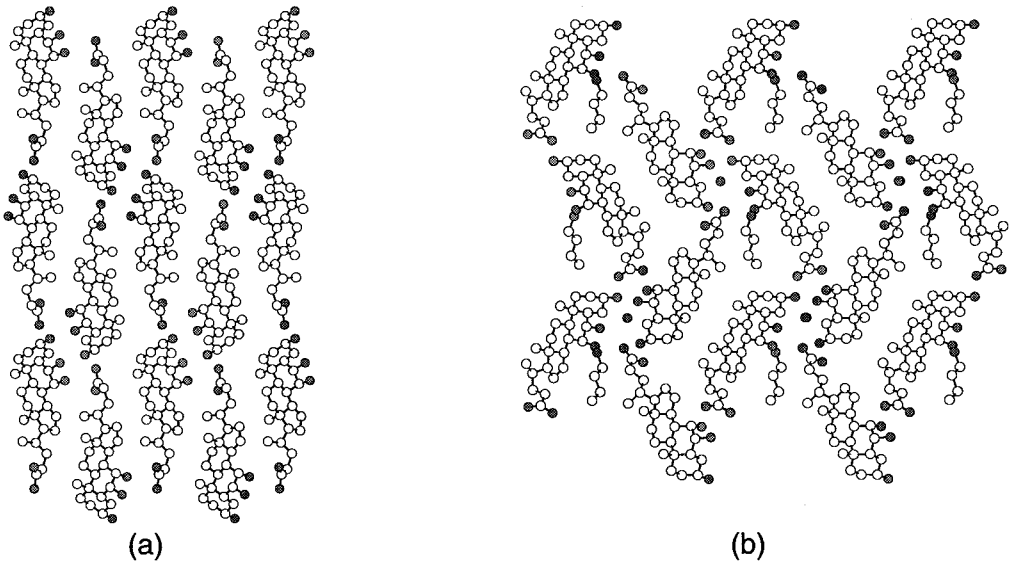


Figure 2-5. Schematic representation of the crystal structures of HCA; (a) guest-free crystal veiwed down from crystallographic *c* axis, (b) inclusion crystal between HCA and 1-butanol veiwed down from crystallographic *a* axis. Carbon and oxygen atoms are represented by empty and shaded circles, respectively. Hydrogen atoms are omitted for clarity.

Recrystallization of HCA from various organic substances mostly yielded prismatic crystals, which were characterized by thermogravimetric analysis, IR and ^{1}H-NMR spectroscopy, powder X-ray diffractometer, and so on. It was found that the resulting crystals were guest-free except for the cases of 1-butanol and 3-methyl-1-butanol. The detailed analyses clarified that these crystals also included water molecules, and that their stoichiometry (host:alcohol:water) were 2:1:1 for 1-butanol, and 4:2:1 for 3-methyl-1-butanol. It is noteworthy that use of dried 3-1-butanol or 3-methyl-1-butanol gave only guest-free crystals. This result indicates that the inclusion crystals were formed in the presence of water.

Next the author analyzed their single crystals by means of X-ray diffraction method. The guest-free crystals belong to orthorhombic, $P2_12_12_1$. Figure 2-5(a) depicts the crystal structure viewed down along the crystallographic *c* axis. It can be seen from the figure that HCA arranges almost linearly each other on the *ab* plane. And the host molecules constitute a 2_1 column along the *c* axis with a hydrogen-bonding network, which consists of helical main chains with pendent carboxyl groups. The main chain has a sequence of O(3)H···O(6)H···O(3)H···, where the hydrogen bonding distances are 2.809(4) and 2.998(4) Å, respectively. The side chain is composed of O(7)H···O(24a)=C-O(24b)H (the distance is 2.951(5) Å), and the O(24b)H is connected to the main chain at O(3) by hydrogen bonding (2.661(5) Å).

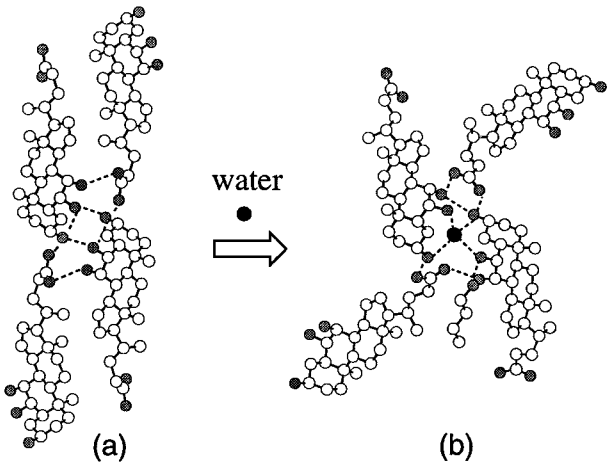


Figure 2-6. Schematic representation of the crystal structures of HCA. (a) guest-free crystal of HCA. (b) inclusion crystal of HCA and 1-butanol. Dotted line that connect between O atoms shows hydrogen bonding networks. Hydrogen atoms are omitted for clarity.

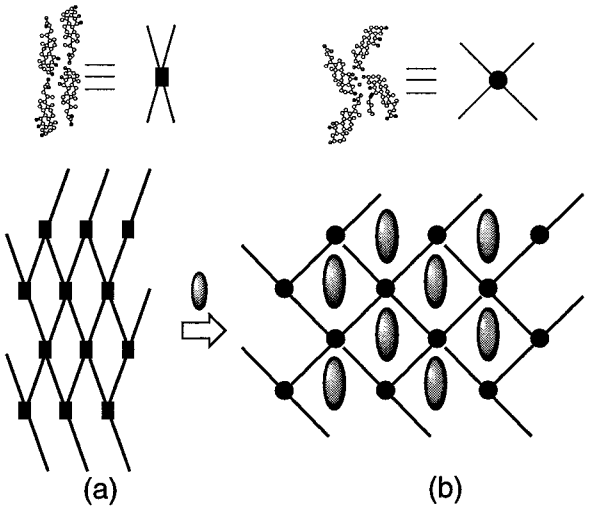


Figure 2-7. Schematic representation of the crystal structures of HCA; (a) guest-free crystal (b) inclusion crystal of HCA and 1-butanol. Lines, filled circles and shadowed circles shows host molecules, hydroben bonding parts and guest molecules, respectively.

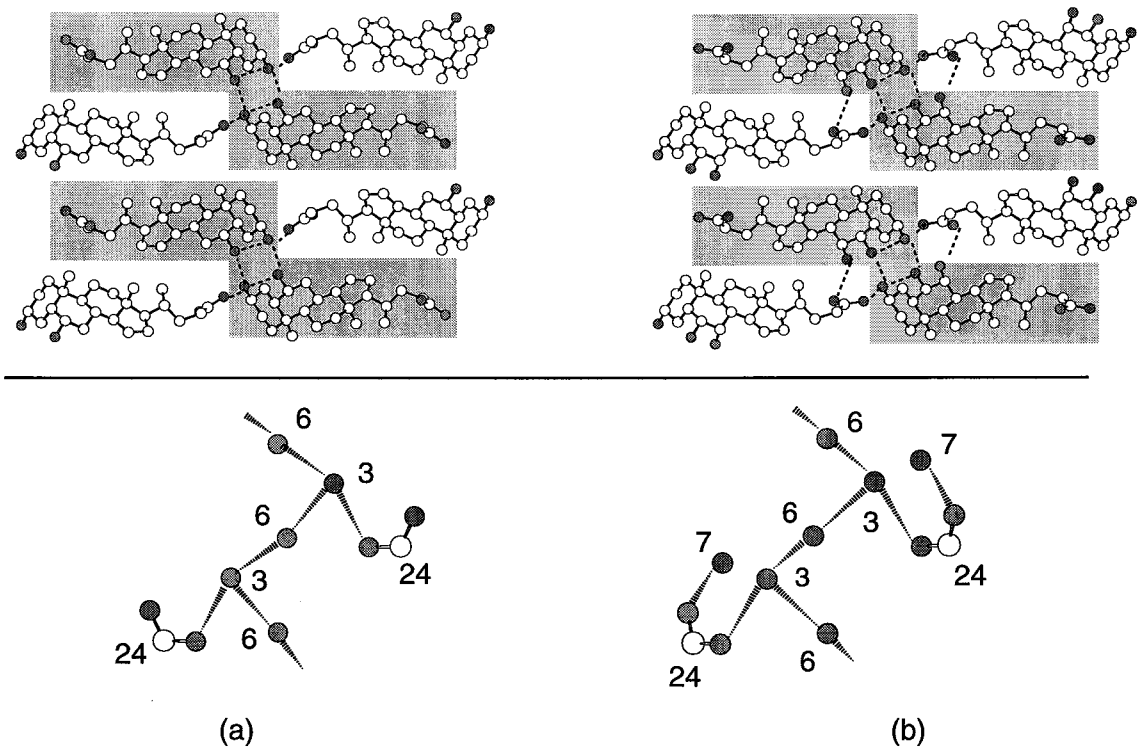


Figure 2-8. Comparison (upper) the molecular asembly modes and (low) the hydrogen bonding networks between (a) HDCA and (b) HCA. Dotted line shows the hydrogen bonding networks. upper; Viewed down from crystallographic c axis. lower; Viewed down from crystallographic b axis, respectively.

On the other hand, the crystals of the inclusion compound of HCA with 1-butanol belong to a monoclinic system, space group $P2_1$, which is different from that of the guest-free crystal. Figure 2-5(b) draws the crystal structure of the compound, viewed along the crystallographic *a* axis. It can be seen that host molecules arrange in a zig-zag fashion so as to leave inclusion spaces for guests. Figure 2-6 shows that the relationship between guest-free HCA and the inclusion crystal of HCA and 1-butanol. Both of molecular assembly modes have a similar arrangement that are composed of four molecules of HCA. In the guest-free crystals, the four molecules arrange in linear, while in inclusion crystal of HCA and 1-butanol, the four molecules arrange in cross manner by insertion of water molecules.

Figure 2-7 schematically illustrates the correlation between the guest-free and inclusion crystals of HCA. The structures are compared to "pantograph". Namely, hydrogen bonding networks in the guest-free crystals correspond to closed "pantograph", while the networks in the inclusion crystals correspond to open "pantograph". This change takes place in the presence of water molecules. So we can say that water molecules function as "joints" to open the "pantograph", leading to a formation of inclusion spaces for accommodating guest molecules.

In addition, the crystal structure of the inclusion compound of HCA with 3-methyl-1-butanol is similar to that of HCA with 1-butanol from a viewpoint of crystal structures. In fact, both crystals belong to the same space group (monoclinic, $P2_1$). And the guest molecules are included in the same cavity. However, additional methyl group of the guest molecule expel half of water to give a different hydrogen-bonding network. This result indicates that water plays an important role in modifying inclusion spaces for specified guests.

On the other hand, recrystallization from ethanol and water, HCA formed dihydrate crystal. X-ray crystallographic study illustrated that the hydrate crystal is similar to the guest-free crystal of HCA.

2-4 Comparison between HDCA and HCA

The crystal structure of guest-free crystal of HCA is quite similar to that of HDCA (Figure 2-

8). Both crystals belong to orthorhombic, $P2_12_12_1$. The difference is only the hydrogen bonding network. In the case of HDCA, the molecular assembly was composed of the units that were connected by the helical hydrogen bonding network. Between the units, the carboxylic group at the end of the side chain attached the helical hydrogen bonding network. On the other hand, HCA also form the unit that was composed of helical hydrogen bonding network just like HDCA. In the case of HCA, however, the additional hydroxy group at 7-position caught the carboxylic group at the end of the side chain, just as a 'hook'. Thus it may say that the crystal structure of HCA is stabilized by the additional hydroxy group.

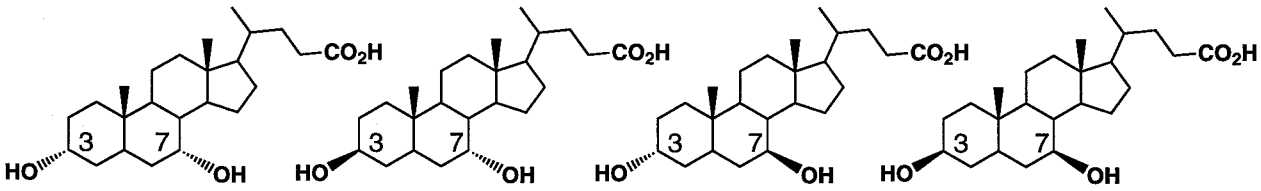
2-5 Summary

In this chapter, the author clarified that both HDCA and HCA form inclusion compounds with specific organic substances. HDCA tends to include aromatic amines, whereas HCA includes spacific alcohols in the presence of water. From the view point of crystallographic study, the guest-free crystals of HCA and HDCA were composed of the same 'unit' that were connected by helical hydrogen bonding networks (between the hydroxy groups at 3- and 6-position). And the 'unit' is also available both in the inclusion crystal of HDCA and in the hydrate crystal of HCA. Thus, the 6-positioned hydroxy group plays an interesting role to build molecular assemblies.

Chapter 3
Inclusion Abilities and Molecular Recognition of Bile Acids Having 3- and 7-Positioned Hydroxy Groups

3-1 Introduction

Bile acids that have 3- and 7-positioned hydroxy groups have four isomers, such as chenodeoxycholic acid (3α, 7α-dihydroxy-5β-cholan-24-oic acid, CDCA), 3-epichenodeoxycholic acid (3β, 7α-dihydroxy-5β-cholan-24-oic acid, 3ECDCA), ursodeoxycholic acid (3α, 7β-dihydroxy-5β-cholan-24-oic acid, UDCA) and 3-epiursodeoxycholic acid (3β, 7β-dihydroxy-5β-cholan-24-oic acid, 3EUDCA). The diffences among them are only the directionality of the 3- and 7-positioned hydroxy groups. Among these isomers, CDCA and UDCA are naturally-occuring steroidal bile acids. And these compounds have been to believed to yield only guest-free crystals in the presence of any organic substances. In this chapter, the author will report the inclusion abilities and molecular assembly modes of the isomers.



CDCA 3ECDCA UDCA 3EUDCA

3-2 Experimantal

General Method. IR spectra were taken on a JESCO IR-810 or a JESCO IR Report 100 grating spectrometer using KBr disk. ^{1}H-NMR spectra were recorded on a JOEL 270 MHz or 400 MHz FT-NMR spectrometer, and chemical shifts are reported in parts per million (ppm) from

tetramethylsilane. Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) were mesured by a Rigaku Thermoflex TG 8110 instrument or a Rigaku Thermo Plus TG 8120 instrument. X-ray powder diffraction pattern were mesured by Rigaku X-ray diffractometer RINT 2000 with Cu-Kα radiation.

Materials. CDCA and UDCA were purchased from Tokyo Chemical Industry Co., Ltd. These bile acids were used without further purification. 3ECDCA and 3EUDCA were prepared from CDCA and UDCA by using Mitsunobu reaction as described in the literature, respectively.[13] 3-Methyl-2-pentanol, 2'-acetonaphthone and other organic substances were all regent grade quality, perchased commeracially and used without further purification.

Preparation of Inclusion Crystals. Preparation of inclusion crystals usually consists of recrystallization of the bile acids from organic substances. A typical procedure is as follows; recrystallization experiment involves dissolving of 3ECDCA (30 mg) in 0.5 mL of 3-methyl-2-pentanol with heating at 120 °C. The solution were allowed to be cool at room temperature untill crystals began to form. Crystals were collected and air-dried on a filter paper.

X-ray Crystallographic Study. X-ray diffraction measurements were performed on a Rigaku AFC7R diffractometer at Gifu University or a Rigaku RAXIS-IV diffractometer with a graphite-monochromated Mo-*Kα* radiation. Data were corrected for Lorenz and polarization effects. The structures were solved by direct methods (SIR92 for the crystals of 3EUDCA with 2'-acetonaphthone and 3EUDCA with 1'-hydroxy-2'-acetonaphthone; SHLEX86 for the guest-free crystal of 3ECDCA and the crystal of 3ECDCA with 3-methyl-2-pentanol) and refined against the *F*o data by full-matrix least-squares methods. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms attached to carbon were placed in calculated position while O-H positions were obtained from difference Fourier synthesis. All calculations were performed using the TEXSAN crystallographic software package of the Molecular Structure Corporation.

3-3 Results and Discussion

Chenodeoxycholic Acid

CDCA is one of the naturally-occuring steroidal bile acid. And it is known that CDCA forms a helical assembly (hexagonal, $P6_5$) with a large channel (Figure 3-1).[11] Therefore, CDCA may include a wide range of organic guests. However, there are only several reports about the inclusion abilities so far.[12] Recently, we have found that CDCA forms inclusion crystals with wide range of organic substances. And powder X-ray diffraction pattern of the inclusion crystals show that every guest molecules are included in the helical assemblies.

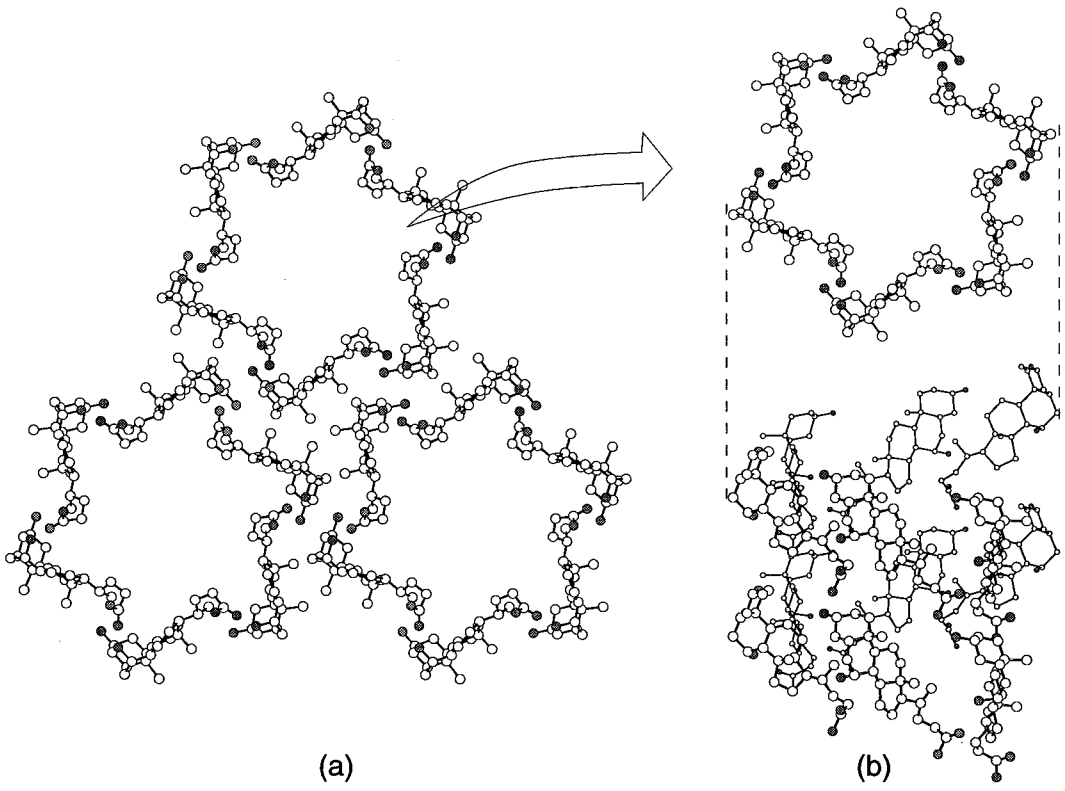


Figure 3-1. Molecular assembly mode of CDCA (a) viewed down the crystallographic *c* axis. (b) *upper*, one helical tube, *lower*, the side view of the helical tube. Carbon and oxygen atoms are represented by empty and filled circles, respectively.

Ursodeoxycholic Acid

UDCA, one of naturally-occuring bile acids, has been well-known as a medicine,[15] originally isolated from gall of bear.[16] The compound has been believed to yield only guest-free crytsals in the presence of any organic substances.[17,18] Although UDCA was recrystallized from over one hundred organic substances, resulting crystals were all guest-free crystals as expected. Thus UDCA is the rare compound that could not form inclusion crystals. Figure 3-2 shows the molecular assembly mode of the guest-free crystal of UDCA.[17]

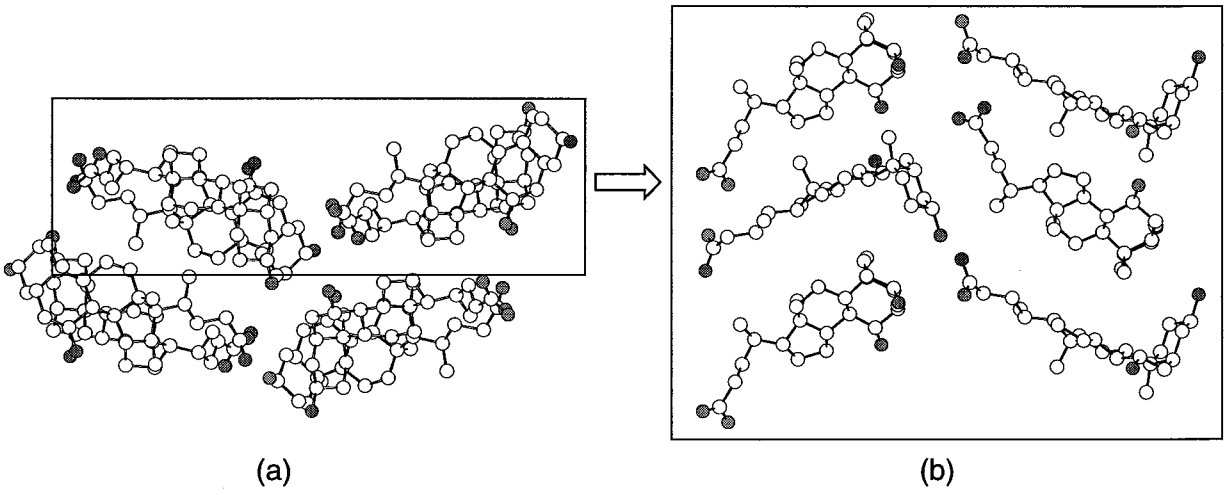


Figure 3-2. Molecular assembly mode of UDCA viewed down the crystallographic (a) *a* axis, and (b) *b* axis.

3-Epichenodeoxycholic Acid

3ECDCA is 3β-isomer of CDCA and it was prepared from CDCA as described in the literature.[13] The compound was recrystallized from over one hundred organic substances, such as alcohols, ketones, esters, ethers, and so on. The resulting crystals were characterized by thermogravimetric analysis (TGA), solid state IR and ^{1}H-NMR spectroscopy. All the crystals that was obtained were guest-free crystals. But in the case of 3-methyl-2-pentanol, 3ECDCA fromed an inclusion crystal with the alcohol at 1:1 host to guest ratio (Table3-1).

X-ray crystallographic study illustrated the molecular assembly mode of the crystal between 3ECDCA and 3-methyl-2-pentanol (Figure 3-3). The crystal belongs to orthorhombic, $P2_12_12_1$,

quite differ from CDCA. In the crystal, guest molecules were hardly disordered in the inclusion space and only oxygen atoms of the alcoholic molecule were found. The hydroxy group of the guest molecule connected between host molecules by hydrogen bonds. The cyclic hydrogen bonding network has the sequence of O(3)H···O(guest)H···O(7)H···O(24a)=C-O(24b)H···O(3)H, where the hydrogen bonding distances are 2.681, 2.887, 2.779 and 2.597Å, respectively.

The crystal structure of the guest-free crystal has been reported.[14] Although the crystal system and the space group are same to the inclusion crystal (orthorhombic, $P2_12_12_1$), 3ECDCA arranges just like herringbone in the guest-free crystals (Figure 3-4). Between the compounds,

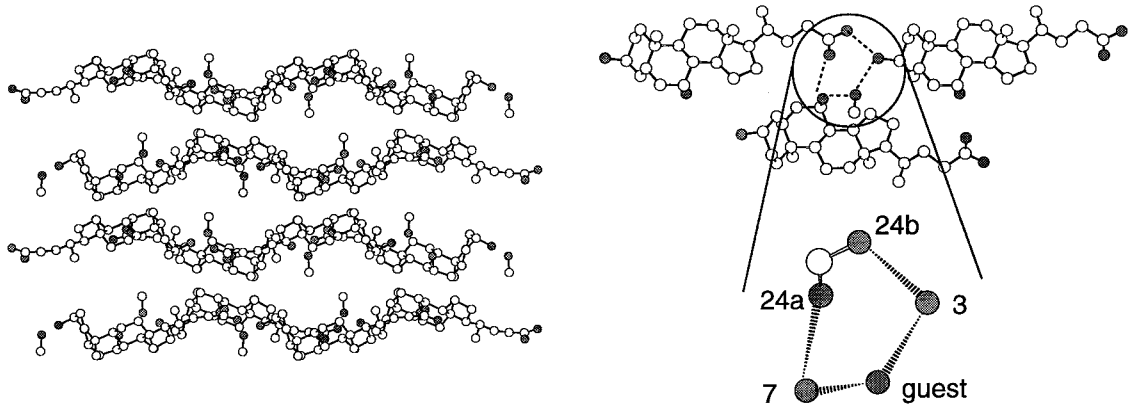


Figure 3-3. Molecular assembly mode of the crystal between 3ECDCA and 3-methyl-2-pentanol. (a) Viewed down from the crystallographic *c* axis, (b) hydrogen bonding networks.

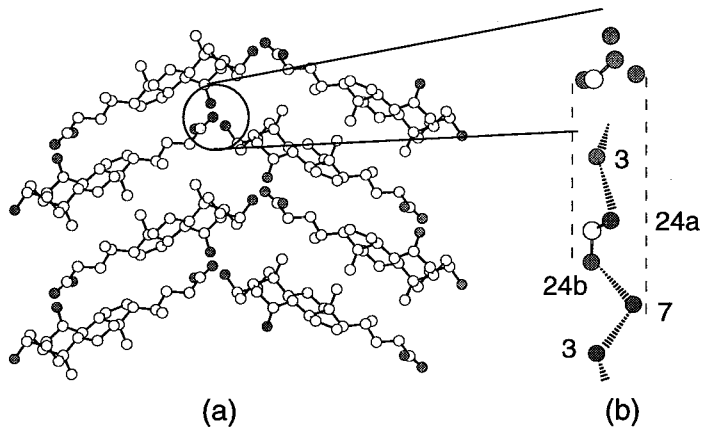


Figure 3-4. Molecular assembly mode of the guest-free crystal of 3ECDCA. (a) Viewed down from the crystallographic *c* axis, (b) hydrogen bonding networks.

helical hydrogen bonding networks connect with O(3)H···O(24a)=C-O(24b)H···O(7)H···O(3)H···, where the distances are 2.812, 2.662 and 2.700Å, respectively.

Table3-1. Inclusion ability of 3ECDCA.

Included	3-methyl-2-pentanol
Not Included	

Methanol
Ethanol
1-Propanol
2-Propanol
2-Methyl-2-propanol
1-Butanol
3,3-Dimethyl-1-butanol
2-Butanol
2-Methyl-2-butanol
3,3-Dimethyl-2-butanol
2-Methyl-1-propanol
2-Methyl-1-butanol
3-Methyl-1-butanol
2-Ethyl-1-butanol
3-Methyl-2-butanol
2,3-Dimethyl-2-butanol
2-Methyl-1 -pentanol
2-Pentanol
1-Pentanol
3-Methyl-1-pentanol
4-Methyl-1-pentanol
2-Methyl-2-pentanol
4-Methyl-2-pentanol
2-Methyl-3-pentanol
3-Methyl-3-pentanol
3-Pentanol
1-Hexanol
2-Hexanol
3-Hexanol
2-Methyl-1-hexanol
2-Methylcyclohexanol
3-Methylcyclohexanol
4-Methylcyclohexanol
1-Phenylethanol
2-Phenylethanol
1-Heptanol
2-Heptanol
3-Heptanol
4-Heptanol
1-Octanol
Benzylalchol
Phenol

THF
1,4-Dioxane
Anisole
Phenetole
Diethyl ether
1,3-THF
4-Methyl-1,3-THF
THP
1,3-Dioxane
4-Methyl-1,3-dioxane
2,4-Dimethyl-1,3-dioxane
2-Phenyl-1,3-dioxane
4-Phenyl-1,3-dioxane
Phthalan

β-Butyrolactone
γ-Butyrolactone
γ-Valerolactone
δ-Valerolactone
γ-Heptalactone
γ-Hexalactone
γ-Decanolactone

Acetonitrile
Propionitrile
Chloroacetonitrile
β-Chloropropionitrile
Butyronitrile
Isobutyronitrile
Valeronitrile
4-Methyl-*n*-valeronitrile
Heptyl cyanide
Acrylonitrile
Crotononitrile
Allyl cyanide
2-Methyl-2-butenenitrile
2-Methyl-3-butenenitrile
Benzonitrile
m-Tolunitrile
o-Tolunitrile

Methyl formate
Ethyl formate
Propyl formate
i-Propyl formate
Methyl acetate
Ethyl acetate
i-Propyl acetate
Butyl acetate
i-Butyl acetate
Heptyl acetate
Vinyl acetate
Phenyl acetate
Benzyl acetate
Methyl propionate
Butyl propionate
i-Butyl propionate
Methyl benzoate
Ethyl benzoate
i-Propyl benzoate

Acetone
2-Butanone
3-Methyl-2-butanone
3,3-Dimethyl-2-butanone
2-Pentanone
4-Methyl-2-pentanone
3-Pentanone
2,4-Pentadione
3-Hexanone
3-Me thylcyclohexanone
4-Methylcyclohexanone
Acetophenone
p-Methylacetophenone
Propiophenone
p-Methylpropiophenone
Butyrophenone
p-Methylbutyrophenone
Dibenzylketone
Cyclopentanone
2-Methylcyclohexanone

Chloroform
Nitroethane
Dichloromethane
Benzene
Toluene
Ethylbenzene
n-Propylbenzene
n-Butylbenzene
n-Amylbenzene
o-Xylene
m-Xylene
p-Xylene
4-*t*-Butyltoluene
o-Fluorotoluene
m-Fluorotoluene
p-Fluorotoluene
1,2-Diethylbenzene
2-Chloro-*m*-xylene
1,2-Dichlorobenzene
1,2,3-Trimethylbenzene
1,2,3,4-Tetramethylbenzene
Phenyl cyclohexane
Aniline
o-Toluidine
m-Toluidine
p-Toluidine
Indan
Indene

3-Epiursodeoxycholic Acid

3EUDCA also have been believed to yield only guest-free crystals in the presence of any organic substances,[14] although other bile acids tend to form inclusion crystals.[1] In contrast, our recent research has revealed that 3EUDCA includes a wide range of organic compounds. Here the author report that 3EUDCA forms a layered assembly with large channels where naphthalene derivatives included in at 1:1 host-to-guest ratio.

Compound 3EUDCA was prepared from commercially available UDCA as described in literature.[13] The acid was recrystallized from neat organic liquids, or from alcoholic solvents dissolving the third solid components.[19] The resulting crystals were characterized by TGA, solid state IR and ^{1}H-NMR spectroscopy. A wide variety of organic compounds, such as aromatic and aliphatic alcohols, ketones, ethers, esters and so on, were included by 3EUDCA as a host, whereas they were not included in DCA. Table 3-2 shows a part of the inclusion compounds. It is noteworthy that 3EUDCA includes both small ketones such as 3-pentanone and naphthalene derivatives such as 2'-acetonaphthone in a 1:1 molar ratio of host to guest. The author did not observe such an inclusion in the case of other bile acids and their derivatives. For example, deoxycholic acid formed inclusion compounds with 2'-acetonaphthone in a 3:1 molar ratio of host to guest.

X-ray powder diffraction study showed that the inclusion compounds have very similar patterns, indicating that the inclusion crystals consisted of a similar molecular assembly mode. This

Table 3-2. Inclusion ability of 3EUDCA with various organic compounds.

guest	host-guest ratio	guest	host-guest ratio
4-Methyl-2-pentanol	1 : 1[a, b]	Anisole	1 : 1 [a, b]
2-Methyl-1-pentanol	1 : 1[a, b]	Ethylbenzene	1 : 1 [a, b]
Isobutyl acetate	1 : 1[a, b]	*n*-Propylbenzene	1 : 1 [a, b]
Propyl propionate	1 : 1[a, b]	Stylene	1 : 1 [a, b]
Ethyl benzoate	1 : 1[a, b]	*o*-Xylene	1 : 1 [a, b]
Pentan-2-one	1 : 1[a, b]	2'-Acetonaphthone	1 : 1 [b]
Pentan-3-one	1 : 1[a, b]	1'-Hydroxy-2'-acetonaphthone	1 : 1 [b]
4-Methylcyclohexanone	1 : 1[a, b]	2-Methylnaphthalene	1 : 1 [b]
Acetophenone	1 : 1[a, b]	1-Methoxynaphthalene	1 : 1 [b]

[a] Determined by TGA-DSC. [b] Determined by ^{1}H-NMR.

was confirmed by a single X-ray structural analysis. Single crystals suitable for the analysis were obtained by recrystallization of 3EUDCA with 2'-acetonaphthone. Fig.3-5(a) depicts the crystal packing of the inclusion compound viewed along the crystallographic *c* axis. It can be seen that the host molecules arrange just like the "chicken-wire" network with large channel-type inclusion spaces. The molecular assembly mode is quite unique because inclusion crystals of other bile acids tend to form bilayered structures.[1] All the host molecules is connected by hydrogen bonds. The helical network has a sequence of O(3)H···O(7)H···O(24a)=C-O(24b)H···O(3)H, where the hydrogen bonding distances are 2.744(5), 2.894(5) and 2.595(4) Å, respectively.

The channel is analyzed by space filling model, leading that the channel can be described as a straight pillar with a rectangular cross section of approximate dimensions 7.4 x 11.5 Å. The guest molecules are accommodated in the channels only by van der Waals contact. It is interesting that 2'-acetonaphthone arranges perpendicular to the channel and forms a column along the crystallographic *c* axis. In the column, 2'-acetonaphthone stacks at 3.51 Å distance which corresponds to half of the crystal *c*-dimension (7.020 Å). The space filling model of one column is depicted in

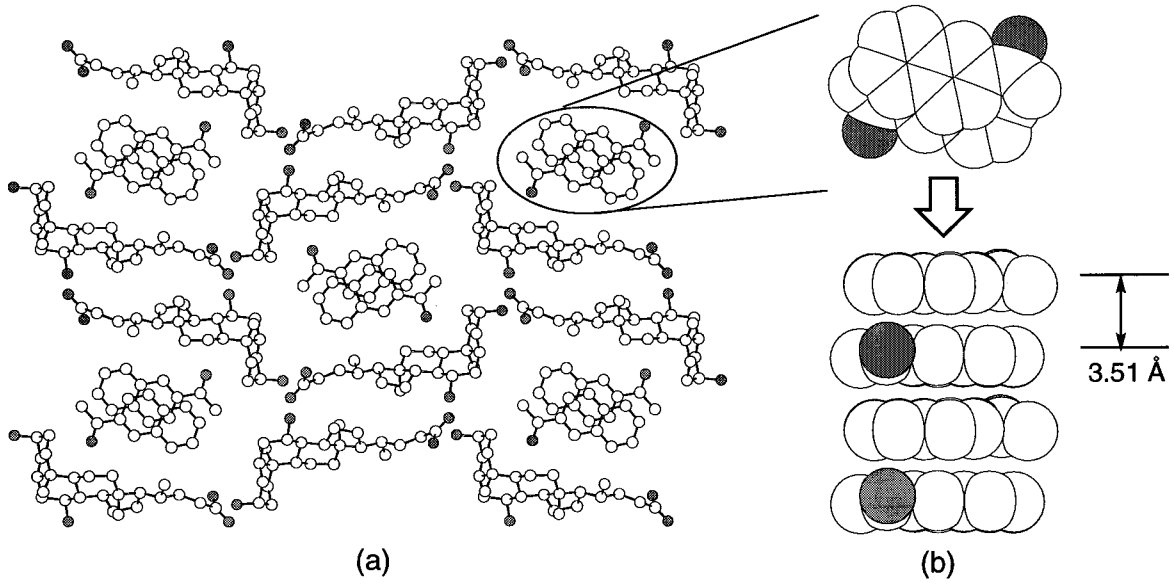


Figure 3-5. (a) The crystal structure of a 1:1 complex between 3EUDCA and 2'-acetonaphthone viewed down crystallographic *c* axis. Carbon and oxygen atoms are represented by empty and filled circle, respectively. Hydrogen atoms are omitted for clarity. (b) The space filling model of one column of 2'-acetonaphthone in inclusion space.

Fig.3-5(b).

In addition, we obtained single crystals by recrystallization of 3EUDCA from 3-pentanone. The X-ray measurement at 193 K indicated that guest molecules were disordered in the channels. This indicates that the guest molecules are too small in size to provide thermally stable inclusion compounds.

In conclusion, this study demonstrates inclusion compounds of 3EUDCA with a wide range of organic compounds. 3EUDCA form a new channel-type large inclusion space as compared with any other bile acids, enabling to include naphthalene derivatives with 1:1 stoichiometry.

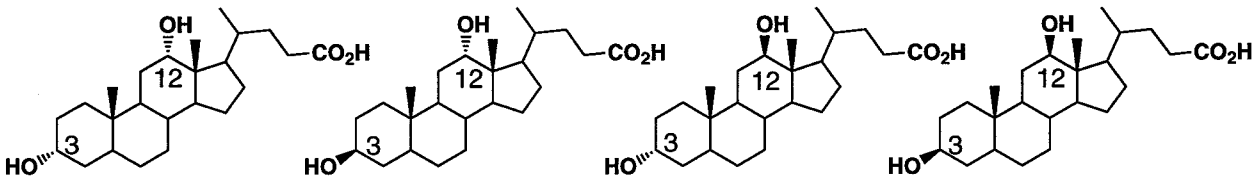
3-4 Summary

In this chapter, the author clarified the inclusion abilities and molecular recognition of bile acids that have the hydroxy groups at 3- and 7-position. Among the four isomers, UDCA and 3ECDCA could hardly form inclusion crystals. 3EUDCA, however, included various organic substances. Furthermore 3EUDCA also included naphthalene derivatives, such as 2'-acetonaphthone with 1:1 host to guest ratio. In the inclusion space, the guest molecules stacked each others with a distance at 3.51Å. So this is the typical example to control the arrangement of aromatic compounds.

Chapter 4
Inclusion Abilities and Molecular Recognition of Bile Acids Having 3- and 12-Positioned Hydroxy Groups

4-1 Introduction

3, 12-Dihydroxylcholanoic acids have four isomers, such as deoxycholic acid (3α, 12α-dihydroxy-5β-cholan-24-oic acid, DCA), 3-epideoxycholic acid (3β, 12α-dihydroxy-5β-cholan-24-oic acid, 3EDCA), 12-epideoxycholic acid (3α, 12β-dihydroxy-5β-cholan-24-oic acid, 12EDCA) and 3,12-epideoxycholic acid (3β, 12β-dihydroxy-5β-cholan-24-oic acid, 3,12EDCA). Among these isomers, DCA has been known to form inclusion crystals with various organic substances.[20] But there is no report about inclusion abilities or molecular structures of other steroidal bile acids. In this chapter, the author report the inclusion abilities and molecular assembly modes of the isomers.



DCA 3EDCA 12EDCA 3,12EDCA

4-2 Experimantal

General Method. IR spectra were taken on a JESCO IR-810 or a JESCO IR Report 100 grating spectrometer using KBr disk. ^{1}H-NMR spectra were recorded on a JOEL 270 MHz or 400 MHz FT-NMR spectrometer, and chemical shifts are reported in parts per million (ppm) from tetramethylsilane. Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) were mesured by a Rigaku Thermoflex TG 8110 instrument or a Rigaku Thermo Plus TG 8120

instrument. X-ray powder diffraction pattern were mesured by Rigaku X-ray diffractometer RINT 2000 with Cu-Kα radiation.

Materials. DCA was purchased from Tokyo Chemical Industry Co., Ltd. and the bile acid was used without further purification. 3EDCA was prepared from DCA by inversion of the hydroxy group at 3-position via Mitsunobu condensation reaction as described in the literature.[13] 12EDCA was prepared by selective reduction of the corresponding 12-keto derivative of DCA as described in the literature.[21] And 3,12EDCA was prepared from 12EDCA by inversion of the hydroxy group at 3-position via Mitsunobu condensation reaction as described in the literature.[13] Organic substances used for recrystallization were all regent grade quality, perchased commeracially and used without further purification.

Preparation of Inclusion Crystals. Preparation of inclusion crystals usually consists of recrystallization of the bile acids from organic substances. A typical procedure is as follows; recrystallization experiment involves dissolving of 3EDCA (30 mg) in 0.5 mL of 3-methyl-2-pentanol with heating at 120 °C. The solution were allowed to be cool at room temperature untill crystals began to form. Crystals were collected and air-dried on a filter paper.

X-ray Crystallographic Study. X-ray diffraction measurements were performed on a Rigaku AFC7R diffractometer at Gifu University or a Rigaku RAXIS-IV diffractometer with a graphite-monochromated Mo-*Kα* radiation. Data were corrected for Lorenz and polarization effects. The structures were solved by direct methods (SHLEX86, SIR92 and MALTAN88) and refined against the *F*o data by full-matrix least-squares methods. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms attached to carbon were placed in calculated position while O-H positions were obtained from difference Fourier synthesis. All calculations were performed using the TEXSAN crystallographic software package of the Molecular Structure Corporation.

4-3 Results and Discussion

3-epideoxycholic acid

3EDCA is the 3β-epimer of DCA. The inclusion ability was checked by recrystallization method from over one hundred organic substances. Table 4-1 shows the resulting inclusion compounds of 3EDCA. 3EDCA tends to include hydrophilic substances such as alcohols, lactones, and so on, whereas DCA forms inclusion crystals with a wide range of organic substances. In the case of 3EDCA, recrystallized from others yield only guest-free crystals.

X-ray crystallographic study illustrated that 3EDCA formed bilayered structure like DCA. Figure 4-1(b) depicts the crystal structure of the inclusion crystal of 3EDCA and benzyl alcohol. In the crystal, 3EDCA arranges in the parallel on the hydrophilic site and anti-parallel on the lipophilic site, just similar to that of DCA. Between host molecules, 3EDCA forms channel-like inclusion spaces where the guest molecules included in.

Next, the author compared the crystal structures between DCA and 3EDCA to clear the differences of their molecular recognitions (Figure 4-1(a)). DCA forms helical hydrogen bonding networks by using three hydogen bonding groups which belong to different molecules. So there is no chance for the guest molecules to bind for host molecules with hydrogen bonds. Although 3EDCA also forms helical hydrogen bonding networks like DCA, the networks comprises of the

Table 4-1. Inclusion ability of 3EDCA with various organic compounds[a)].

guest	host-guest ratio	guest	host-guest ratio
Methanol	1 : 1	THF	1 : 1
Ethanol	1 : 1	1,4-Dioxane	1 : 1
2-Methyl-1-propanol	1 : 1	Acetone	1 : 1
2-Methyl-1-butanol	1 : 1	β-Butyrolactone	1 : 1
3-Methyl-1-butanol	1 : 1	γ-Butyrolactone	1 : 1
2-Methyl-1-pentanol	1 : 1	γ-Valerolactone	1 : 1
Benzyl alcohol	1 : 1	δ-Valerolactone	1 : 1
2-pentanol	1 : 1	Propionitrile	1 : 1
2-Ethyl-1-butanol	1 : 1	Chloroform	1 : 1
3-Methyl-2-pentanol	1 : 1	Nitroethane	1 : 1

[a] Determined by TGA-DSC.

hydroxy groups of guest molecules. The sequence is O(3)H···O(guest)H···O(12)H···O(24a)=C-O(24b)H···O(3)H···, where the hydrogen-bonding distances are 2.766, 2.741, 2.752, 2.647Å, respectively. So 3EDCA tends to form inclusion crystals with alcohols.

Powder X-ray diffraction patterns show that 2-methyl-1-butanol, 3-methyl-1-butanol, 2-methyl-1-pentanol and lactones were included in the similar inclusion lattice of 3EDCA with benzyl alcohol. Other aliphatic alcohols, however, included in other inclusion lattice. Figure 4-1(c)

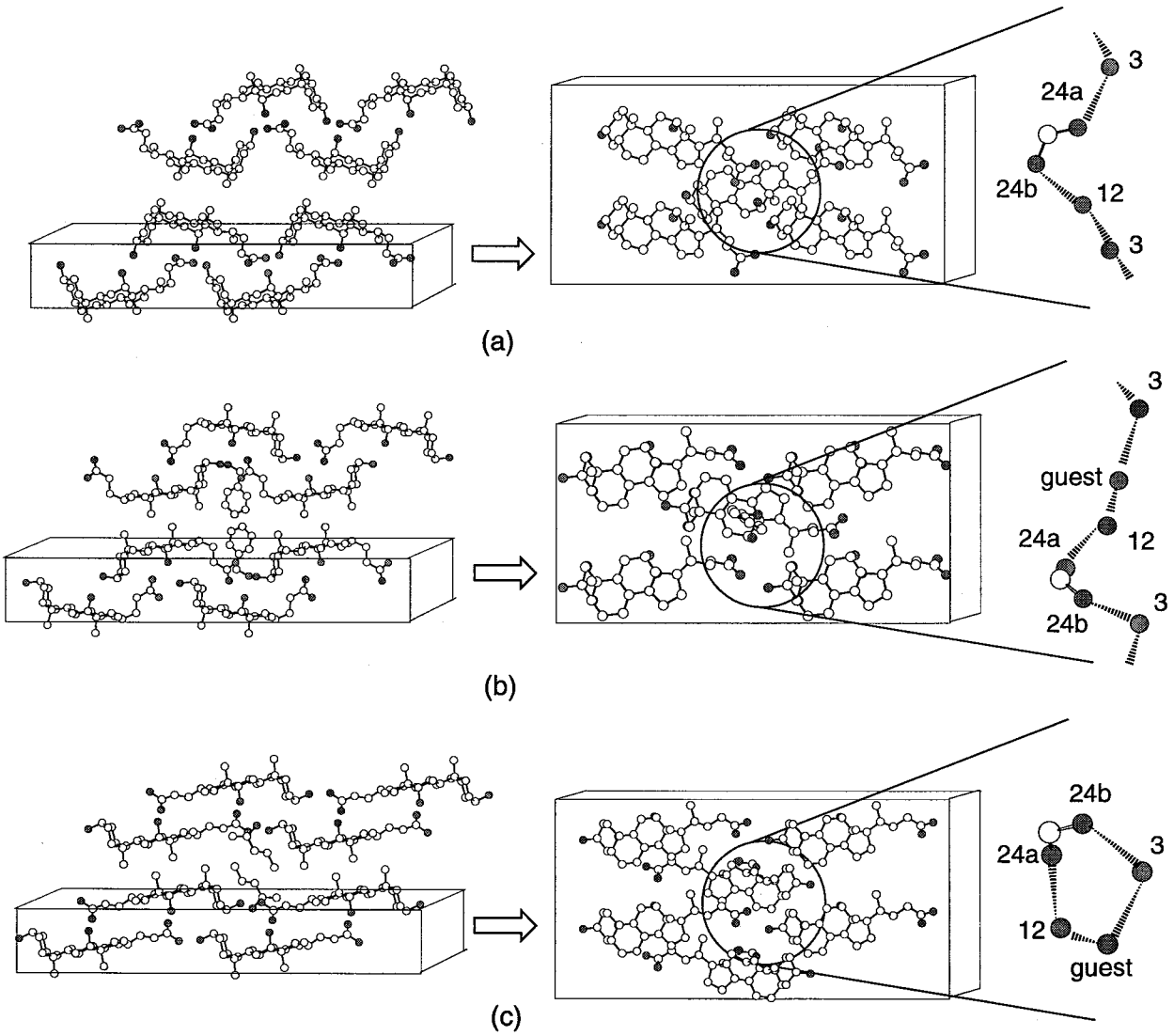


Figure 4-1. Comparison of molecular assembly modes. (a) DCA / acetophenone. (b) 3EDCA / benzyl alcohol. (c) 3EDCA / 2-pentanol. Carbon and cxygen atoms are represented in enpty and filled circles, respectively.

depicts the crystal structure of 3EDCA and 2-pentanol viewed along the crystallographic *b* axis. This crystal belongs to different space group (monoclinic, $P2_1$) from those of compounds of 3EDCA and benzyl alcohol. Although 3EDCA forms bilayered structure, the host compound arranges in anti-parallel fashion both hydrophilic and lipophilic sites. In the crystal structure, host molecules are connected by cyclic hydrogen bonding networks of O(3)H···O(guest)H···O(12)H···O(24a)=C-O(24b)H···O(3)H, where the hydrogen-bonding distances are 2.743, 2.796, 2.746, 2.570Å, respectively. Powder X-ray diffraction patterns of 2-methyl-1-propanol, 2-ethyl-1-butanol and 3-methyl-2-pentanol are quite similar to that of 2-pentanol. So these alcohols are included just like 2-pentanol. Thus 3EDCA forms guest-dependent polymorphism to include guest mlecules.

12-pideoxycholic Acid

12EDCA is the 12β epimer of DCA. This compound was prepared by selective reduction of the corresponding 12-keto derivative of DCA as described in the literature.[21] 12EDCA was recrystallized from organic substances, most of the resulting crystals were guest-free crystals. But in the case of 2-methyl-2-butanol, 12EDCA fromed inclusion crystal with the alcohol at 1:1 host to guest stoichiometry.

Slow evaporation of ethanol solution of 12EDCA at room temperature afforded single crystals suitable for X-ray crystallographic study. Figure 4-2 depicts the crystal structure of the guest-free crystal of 12EDCA. In the molecular structure, there was a characteristic change at the conformation of the side chain. The conformation of the side chain is quite different from other bile acids. Here, the four dihedral angles, C(13)-C(17)-C(20)-C(21), C(17)-C(20)-C(22)-C(23), C(20)-C(22)-C(23)-C(24) and C(22)-C(23)-C(24)-O(24a) are indicated as Ψ1, Ψ2, Ψ3 and Ψ4, respectively. Although Giglio concluded that the Ψ1 is always confined within a very narrow range around -60° from the calculation of the intramolecular van der Waals energy,[20,22] the Ψ1 of 12EDCA is -164.2(4)°. This was caused by the inversion of the hydroxy group at 12-position. If the Ψ1 were still -60° in the case of 12EDCA, the inverted O(12) would be very close to the C(21). This result was also supported by the calculation of the van der Waals energy (Figure 4-3). This is the

first example to change the conformation of Ψ1.

The diheadral angle of Ψ2 is 177.7(4)°, that indicates a 'trans' conformation. And the angle of Ψ3 is 62.7(7)°. These values correlate well with the values calculated from a van der Waals energy map.

Thus inversion of 12-positioned hydroxy group induces the conformational change at the side chain. Consequently, the side chain turns toward β-face of the steroidal plane.

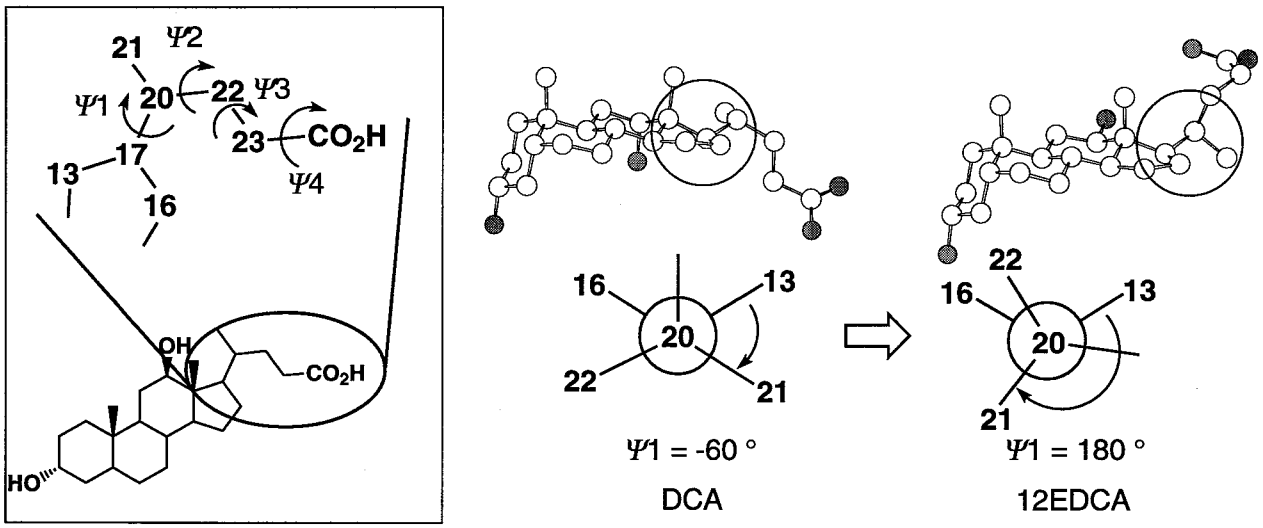


Figure 4-2. Comparison the conformation of the side chain between DCA and 12EDCA. Carbon and oxygen are represented by empty and shaded circle, respectively.

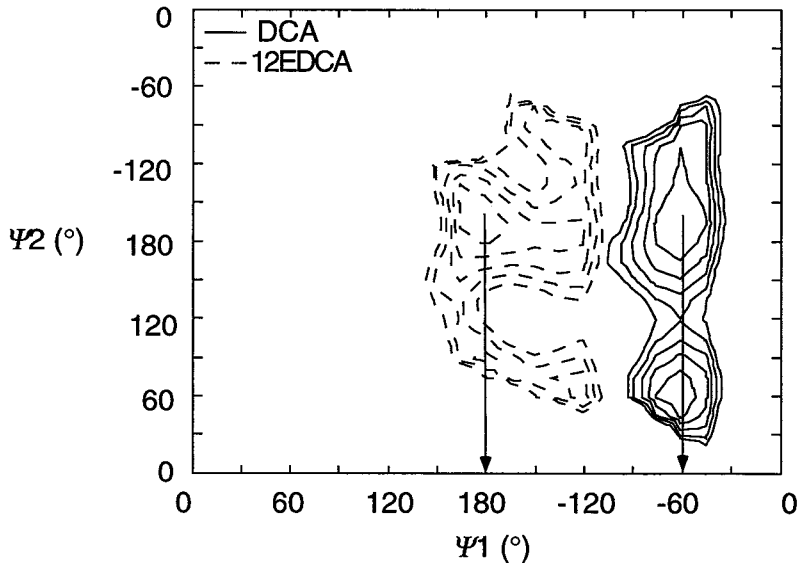


Figure 4-3. Van der Waals energy of DCA and 12EDCA side chain.

3,12-Epideoxycholic Acid

3,12EDCA is a double epimer of DCA. Recrystallization of 3,12EDCA from various organic substances afforded the inclusion compounds, which were characterized by TGA, solid state IR and ^{1}H NMR spectroscopy. Table 4-2 shows the parts of resulting inclusion compounds of 3,12EDCA. 3,12EDCA included wide variety of organic substances, such as aliphatic alcohols, ketones, ethers, esters, nitriles, and so on. But their host to guest ratio varied widely.

Powder X-ray diffraction pattern shows that all inclusion compounds are similar molecular assembly modes. This was confirmed by single crystal X-ray structural analysis. Single crystals suitable for the analysis were obtained by recrystallization of 3,12EDCA and chloroform. Figure 4-4 depicts the crystal structure of the inclusion crystal. 3,12EDCA forms a helical arrangement (hexagonal, $P6_1$) and guest compounds are included in the helical tubuland. In the case of 3,12EDCA, the host molecule could build only the fixed inclusion space. So the host to guest ratios of inclusin compounds due to the size of guest molecules.

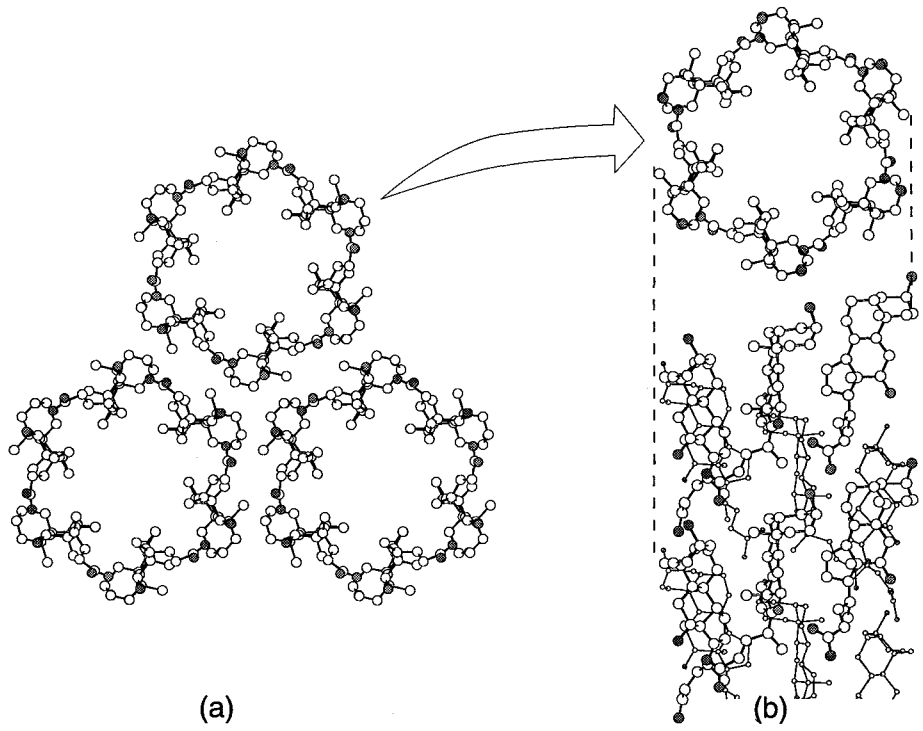


Figure 4-4. Molecular assembly mode of 3,12EDCA (a) viewed down the crystallographic *c* axis. (b) *upper*; one helical tube, *lower*; the side view of the helical tube.

Table 4-2. Inclusion ability of 3,12EDCA with various organic compounds.

guest	host-guest ratio	guest	host-guest ratio
Methanol	3: 2 [a, b]	3-Pentanone	4 : 1 [a, b]
Ethanol	5 : 2 [a, b]	Acetophenone	6 : 1 [a, b]
2-Propanol	3 : 1 [a, b]	i-Propyl acetate	6 : 1 [a, b]
2-Methyl-1-propanol	5 : 2 [a, b]	Methyl propionate	6 : 1 [a, b]
3-Methylcyclohexanol	6 : 1 [a, b]	Ethyl propionate	4 : 1 [a, b]
THF	5 : 2 [a, b]	g-Valerolactone	3 : 1 [a, b]
1,4-Dioxane	3 : 1 [a, b]	Acetonitrile	2 : 1 [a, b]
2-Butanone	6 : 1 [a, b]	Benzonitrile	3 : 1 [a, b]
2-Pentanone	5 : 1 [a, b]	Chloroform	2 : 1 [a, b]

[a] Determined by TGA-DSC. [b] Determined by ^{1}H-NMR.

4-4 Pseudo-mirror Imaged Molecular Assembly Modes

Molecular assemblies or higher dimensional structures of chiral compounds reflect their own molecular structures. Tartaric acid is the typical molecule for this case, because this compound has the enantiomer and the enantiomers form just mirror-imaged molecular assemblies each other.[23] Here, however, the author would like to show that even stereoisomers may form pseudomirror-imaged molecular buildings using steroidal bile acids. They are composed of a identical rigid steroidal skeleton and a flexible side chain, furthermore they have two hydroxyl groups and a carboxyl group. The differences among them are only the position or directionality of hydroxyl groups. So the relationship of these compounds are only stereoisomers each others.

First,the author show that pseudomirror-imaged molecular assemblies using 3EDCA and 3ECDCA. Both 3EDCA and 3ECDCA have a steroidal skeleton and same number or kinds of functional groups. The difference between them is only the position of one hydroxyl group, that is, 3ECDCA has the 7-Positioned hydroxyl group instead of 12-positioned one in 3EDCA. Both these bile acids form guest-free crystals by recrystallization method. X-ray crystallographic study illustrated that the molecular arrangements of the guest-free crystals are just pseudomirror-imaged to each others (figure 4-5). It could be thought that the molecules, 3ECDCA and 3EDCA, caused this results. These compounds are surely composed of the identical body, so these are, that to say,

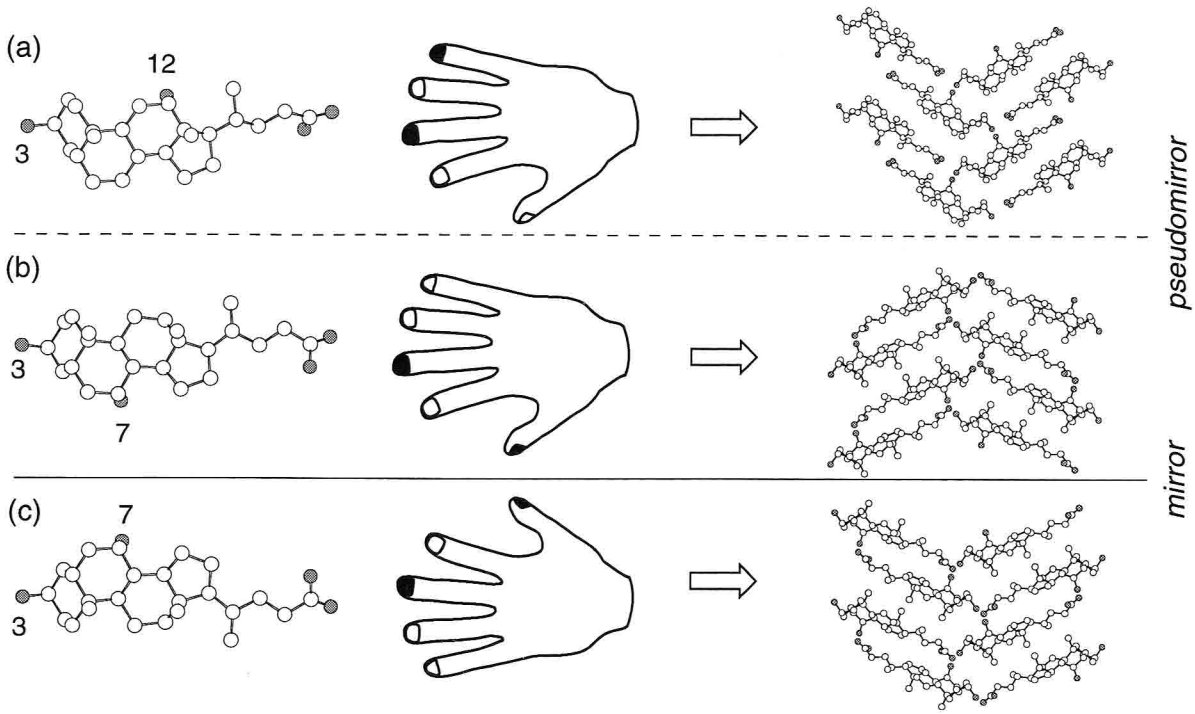


Figure 4-5. Pseudomirror imaged molecular assembly modes by using stereoisomers. (a) 3EDCA/guest-free crystal. (b) 3ECDCA / guest-free crysta. (c) enantiomer of 3ECDCA(imaginary)

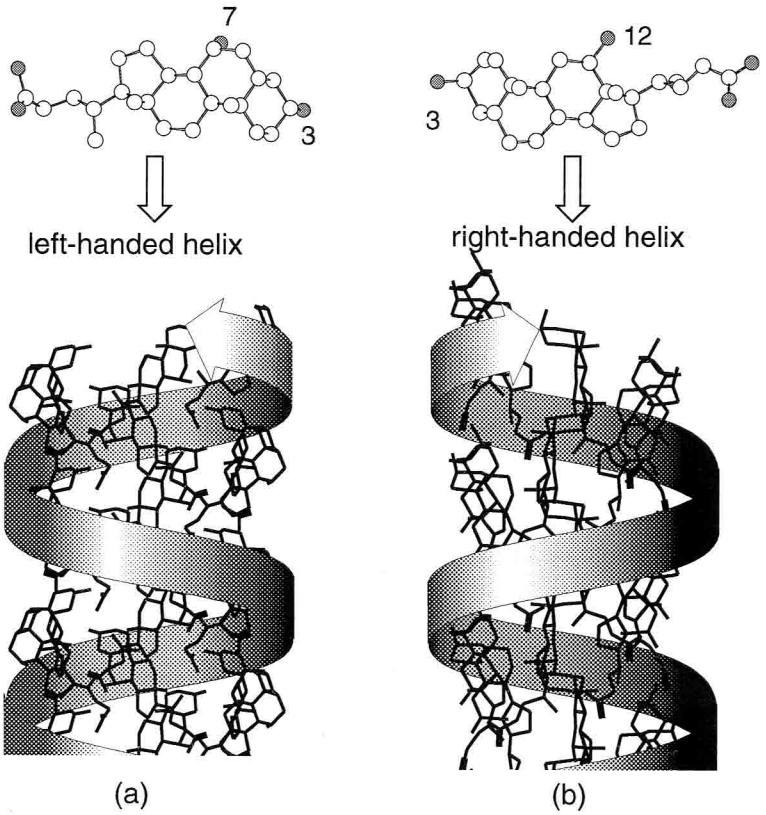


Figure 4-6. Reversal helical sences by using stereoisomers. (a) Molecular structure of CDCA (b)Molecular structure of 3,12EDCA

only the right hand. But the 7-positioned and 12-positioned hydroxy groups correspond to thumb and little finger, respectively. So the molecular structures of the bile acids could form pseudomirror-imaged molecular assemblies.

Next, the author show the reversal helical sences that are built with stereoisomers, using CDCA and 3,12EDCA (Figure 4-6). CDCA forms a helical arrangement as shown in Capter 3.[11,12] And the helical sence is left-handed one (hexagonal, $P6_5$). On the other hand, 3,12EDCA also forms a helical arrangement. But pay attention to that helical columns, the helical sense is right-handed one along the crystallographic *c* axis (hexagonal, $P6_1$), just reversal sense of CDCA. Here, both CDCA and 3,12EDCA are steroidal bile acids. And the differences are only the position of hydroxy group or their directionalities. 3,12EDCA has 3-positioned hydroxyl group like CDCA, but another hydroxyl group is stands on 12-positioned carbon whereas it stands on 7-positioned one in the case of CDCA. So these compounds are stereoisomers each other.

Thus, even stereoisomers could build very interesting molecular assembly modes. This might be a new method to design supramolecules.

4-5 Summary

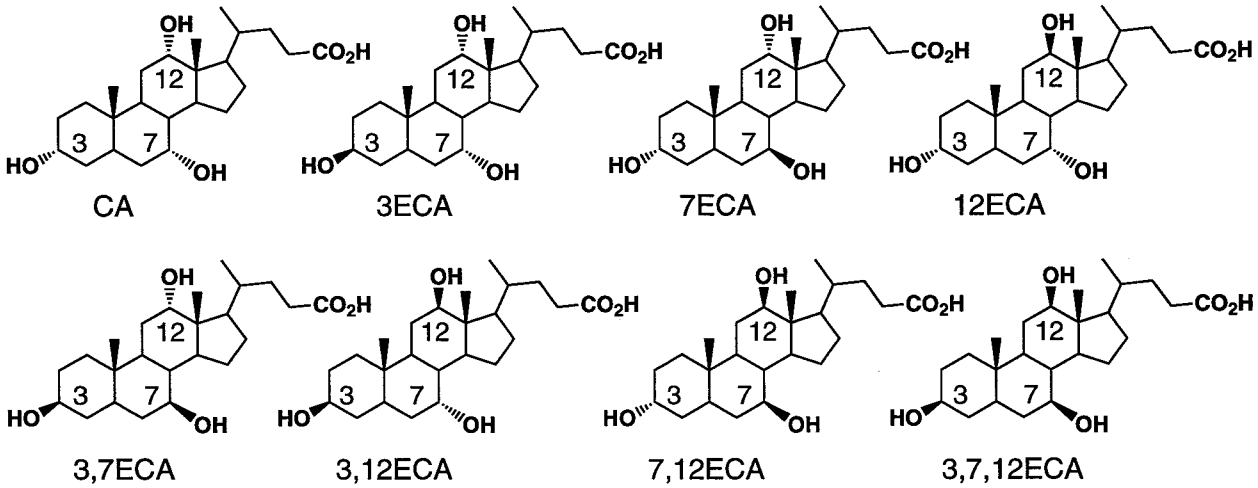
In this chapter, the author reported the inclusion abilities and molecular assembly modes of bile acids that have 3- and 12-positioned hydroxy groups. Among the four isomers, 3EDCA included hydrophilic substances, especially alcohols. The compound was also able to form guest-dependent polymorphism. On the other hand, inversion of the hydroxy group at 12-position induces the conformational change of the side chain.

In the case of 3,12EDCA, novel type of a inclusion crystal was obtained. This is the first example for bile acids to build such a right-handed helical tubuland. And this result enanbled the author to realize pseudomirror imaged molecular assemblies.

Chapter 5
Inclusion Abilities and Molecular Recognitions of Bile Acid Having 3-, 7- and 12-Positioned Hydroxy Groups

5-1 Introduction

3, 7, 12-trihydroxylcholanoic acids have eight isomers: CA (3α, 7α, 12α-trihydroxy-5β-cholan-24-oic acid), 3-epicholic acid (3β, 7α, 12α-trihydroxy-5β-cholan-24-oic acid, 3ECA), 7-epicholic acid (3α, 7*b*, 12α-trihydroxy-5β-cholan-24-oic acid, 7ECA), 12-epicholic acid (3α, 7α, 12β-trihydroxy-5β-cholan-24-oic acid, 12ECA), 3,7-epicholic acid (3β, 7β, 12α-trihydroxy-5β-cholan-24-oic acid, 3,7ECA), 3,12-epicholic acid (3β, 7α, 12β-trihydroxy-5β-cholan-24-oic acid, 3,12ECA), 7,12-epicholic acid (3α, 7β, 12β-trihydroxy-5β-cholan-24-oic acid, 7,12ECA) and 3, 7,12-epicholic acid (3β, 7β, 12β-trihydroxy-5*b*-cholan-24-oic acid, 3,7,12ECA). Among them, CA is a naturally-occurring steroidal bile acid and isolated from the bile of animals in the last century. Inclusion compounds of CA with some alcohols have been known since last century.[5] More recently, Miyata revealed that CA also form channel-type inclusion compounds with a variety of organic substances.[1] However, inclusion compounds and crystal structures of the other isomers of 3, 7, 12-trihydroxylcholanoic acids have never been reported. Therefore in this chapter,



the author shows the inclusion abilities and the molecular assembly modes of a series of the isomers of CA.

CA is only a commercially available 3, 7, 12-trihydroxylcholanoic acid. Therefore, the author prepared these isomers by partial syntheses from CA. They were prepared by selective reduction from corresponding keto derivatives[21] or by the inversion condensation of Mitsunobu reaction[13].

5-2 Experimantal

General Method. IR spectra were taken on a JESCO IR-810 or a JESCO IR Report 100 grating spectrometer using KBr disk. NMR spectra were recorded on a JOEL 270 MHz or 400 MHz FT-NMR spectrometer, and chemical shifts are reported in parts per million (ppm) from tetramethylsilane. Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) were mesured by a Rigaku Thermoflex TG 8110 instrument or a Rigaku Thermo Plus TG 8120 instrument. X-ray powder diffraction pattern were mesured by Rigaku X-ray diffractometer RINT 2000 with Cu-Kα radiation.

Materials. CA was purchased from Tokyo Chemical Industry Co., Ltd. and the bile acid was used without further purification. 3ECA was prepared from CA by inversion of the hydroxy group at 3-position via Mitsunobu condensation reaction as described in the literature.[13] 7ECA or 12ECA were prepared by selective reduction of the corresponding 7-keto or 12-keto derivative of CA as described in the literature, respectively.[21] And 3,7ECA or 3,12ECA were prepared from 7ECA or 12ECA by inversion of the hydroxy group at 3-position via Mitsunobu condensation reaction.[13] Organic substances used for recrystallization were all regent grade quality, perchased commeracially and used without further purification.

Preparation of Inclusion Crystals. Preparation of inclusion crystals are usually carried out

with recrystallization method and suspension method.[28] The recrystallization method consists of recrystallization of the bile acids from organic substances. A typical procedure is as follows. Recrystallization experiment involves dissolving of 3ECA (30 mg) in 0.5 mL of 3-methyl-2-pentanol with heating at 120 °C. The solution were allowed to be cool at room temperature untill crystals began to form. Crystals were collected and air-dried on a filter paper. The suspension method consists of suspension of the bile acids and organic substances in *n*-hexane. The typical procedure is as follows; 3ECA (0.730 g, 1.79 mmol) was suspended in the solution of the racemic 2-pentanol (0.315 g, 3.57 mmol) in hexane (10 ml). After standing 12 hours at room temperature, the suspended solution was filtered and air-dried on a filter paper. In the case of the alcohol that have two asymmetric carbons such as 3-methyl-2-pentanol, the alcohol was used double the molar quantity of 3ECA.

Optical Resolution. The enantiomeric purity of alcohols were established by ^{13}C-NMR, using the camphorsulfonated darivatives of alcohols. The typical procedure is as follows. 3-methyl-2-pentanol that was included in the host lattice was recovered by a micro-distillation. To 3-methyl-2-pentanol (38 mg, 0.372 mmol) and triethylamine (112 mg, 1.12 mmol) in ether (1 mL) was added (1S)-camphorsulfonyl chloride (93.2 mg, 1.12 mmol). The mixture was stirred at room temperature for 24 h and then concentrated. The rasidue was chromatographed to give 115 mg (98 %) of 3-methyl-2-pentyl camphorsulfonate.[24]

X-ray Crystallographic Study. X-ray diffraction measurements were performed on a Rigaku AFC7R diffractometer at Gifu University or a Rigaku RAXIS-IV diffractometer with a graphite-monochromated Mo-$K\alpha$ radiation. Data were corrected for Lorenz and polarization effects. The structures were solved by direct methods (SHLEX86, MALTAN88 or SIR92) and refined against the *F*o data by full-matrix least-squares methods. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms attached to carbon were placed in calculated position while O-H positions were obtained from difference Fourier synthesis. All calculations were performed using the

TEXSAN crystallographic software package of the Molecular Structure Corporation.

5-3 Results and Discussion

3-Epicholic Acid

3ECA is the 3*b*-epimer of CA. This compound was prepared from CA by inversion of the hydroxy group at 3-position via Mitsunobu condensation reaction as described in the literature.[13] Recrystallization from various organic substances, 3ECA forms inclusion crystals with hydrophilic organic substances, such as ethers, ketones, and alcohols. Especially, 3ECA includes a series of aliphatic alcohols involving one to six carbon atoms, while CA includes only methanol, ethanol and 1-propanol. (Table 5-1). Therefore, only the change of the direction of one hydroxy group gives rise to drastic change of the molecular recognition.

Single crystals suitable for X-ray structural analysis were obtained from recrystallization of 3ECA from 2-pentanol. Figure 5-1(b) depicts the crystal structure of the inclusion crystal. In the crystal, 3ECA forms bilayered structures similar to that of CA (Figure 5-1(a)). And the arrange-

Table 5-1. Inclusion ability for alcoholic guest molecules using 3ECA and CA.

guest	3ECA	CA
Methanol	1 : 1	1 : 1
Ethanol	1 : 1	1 : 1
1-Propanol	1 : 1	1 : 1
2-Propanol	1 : 1	GF
2-Methyl-1-propanol	1 : 1	GF
2-Butanol	1 : 1	GF
1-Pentanol	1 : 1	GF
2-Methyl-1-butanol	1 : 1	GF
3-Methyl-1-butanol	1 : 1	GF
2-Pentanol	1 : 1	GF
3-Methyl-2-butanol	1 : 1	GF
2-Methyl-1-pentanol	1 : 1	GF
3-Methyl-1-pentanol	1 : 1	GF
2-Ethyl-1-butanol	1 : 1	GF
3-Methyl-2-pentanl	1 : 1	GF

[a] Determined by TGA-DSC. [b] Determined by ^{1}H-NMR.

ment of the molecules are also similar to CA (anti-parallel in each layers). Between the host molecules, 3ECA forms channel-like inclusion spaces where the guest molecules were included in.

Next, the author compared the hydrogen bonding networks between CA and 3ECA to clarify the differences of the molecular recognition. CA forms cyclic hydrogen bonding networks by using four hydrogen bonding groups which belong to different molecules. In this case, the alcoholic guests do not join the hydrogen bonding network, explaining the limited inclusion ability of CA. On the other hand, 3ECA forms branched networks, since the directional change forces 3-positioned hydroxy groups to combine with carboxyl groups of the side-chains. The alcoholic guest is inserted between the 3-positioned hydroxy group and the 12-positioned one, enabling us to explain the inclusion of the alcohols.

This effect of the inversion of the hydroxy group at 3-position to the crystal structures and the inclusion abilities is observed in those of 3EDCA described in chapter 4. Both hosts have 3β and 12α hydroxy groups and the carboxylic acid construct hydrophilic layer by the hydrogen bonds

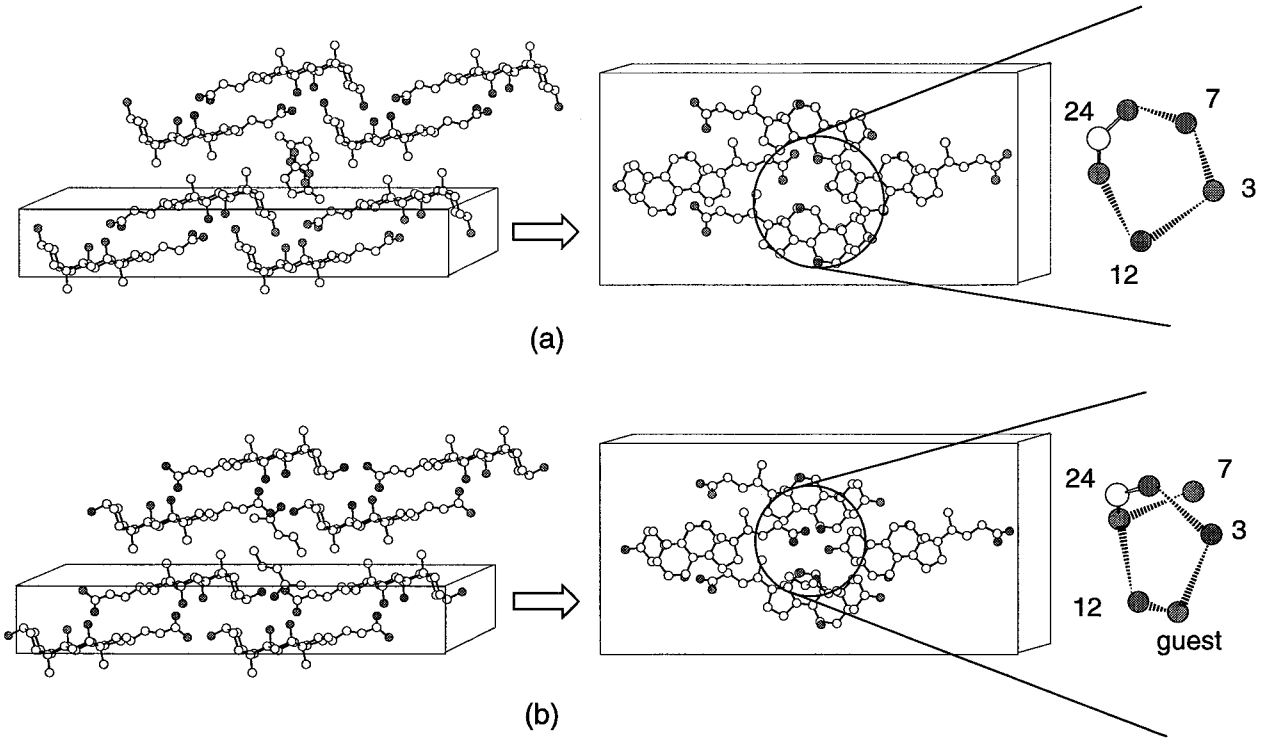


Figure 5-1. Molecular assembly modes of (a) CA / γ-valerolactone and (b) 3ECA / 2-pentanol. *left*; Viewed down along the crystallographic *b* axis. *right*; Depict the hydrogen bonding networks.

between the hydrophilic faces. 7α-Hydroxy groups, one more addition hydroxy group in 3ECA, hardens the hydrogen bond network between hydrophilic layer (Figure 5-2). On the other hand, 3β-hydroxy group in both hosts catches the hydrogen bond accepting and donating guest molecules.

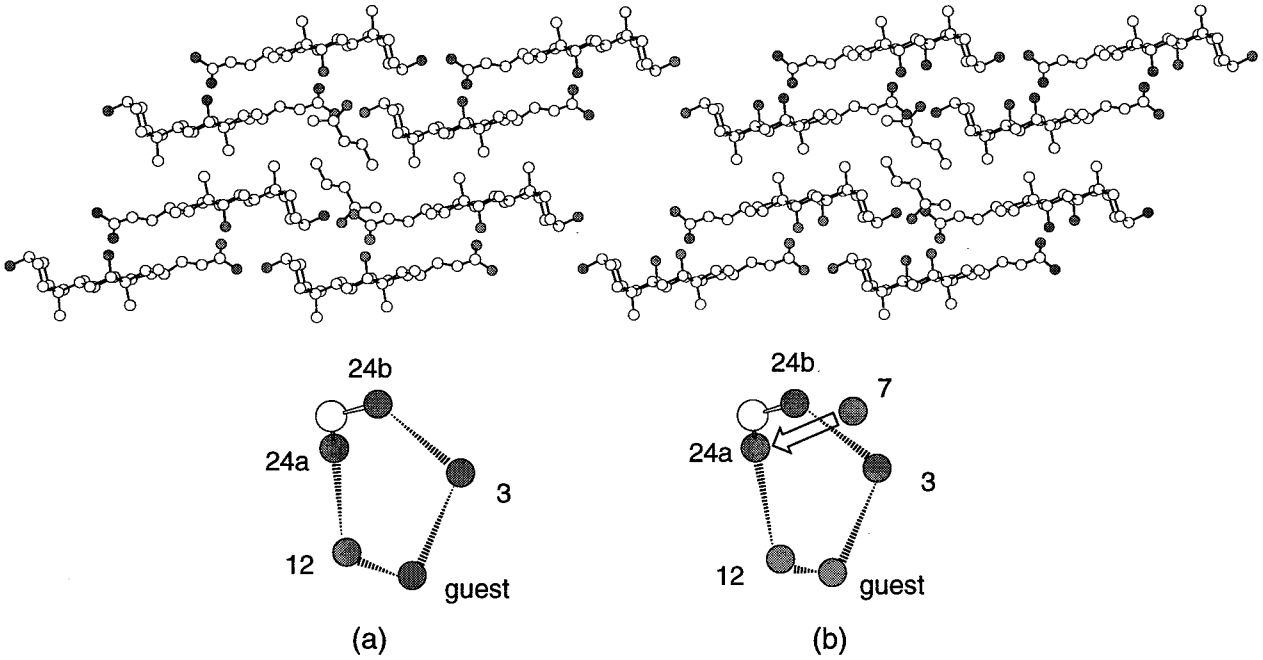


Figure 5-2. Comparison of hydrogen bonding networks. (a) 3EDCA / 2-pentanol (b) 3ECA / 2-pentanol

12-Epicholic acid

12ECA is the 12β epimer of CA. Recrystallization form more than eighty organic solvents yields only guest-free crystals. Until now, the author can not find any guest molecules for 12ECA. Therefore, the author concluded that 12ECA has no inclusion ability at all. The molecular structure of 12ECA was shown in Figure 5-3. The most interesting structural feature is the conformation of the side chain of 12ECA. The equatorial hydroxy group at 12-position turns the side chain toward β-face of the steroidal plane. This conformational change is identical to that of 12EDCA described in Chapter 4, leading to the drastic change of the molecular assemblies.

12ECA has a characteristic monolayer structure as shown in Figure 5-4. Whole molecules

in crystalline state are linked by hydrogen bonds between diverse hydrogen bond functional groups of 12ECA, consisting of 3 and 7 hydroxyl groups directed to α face, carboxylic acid directed to β-face and 12 hydroxy group to equatorial direction of the steroidal plane. Cyclic hydrogen bonding network between α-directioned two hydroxy groups and a carboxylic group yields monolayer assembly of 12ECA. For the hydrogen bonding network has a sequence of O(3)H···O(7)H···O(24a)=C-O(24b)H···O(3)H, where the hydrogen bonding distances are 2.791, 2.867 and 2.688Å, respectively. The equatorial-directioned hydroxy groups at 12-position connected each others with a distance of 3.337Å. Three dimensional hydrogen bond network and close packing between lipophilic faces enables to form not inclusion compounds but stable guest-free crystals.

3,12-Epicholic acid

3,12-Epicholic acid (3,12ECA) is a double epimer of CA. This compound was prepared from 12ECA by inversion of the hydroxy group at 3-position via Mitsunobu condensation reaction as described in the literature.[13] The resulting compounds were subjected to recrystallization from

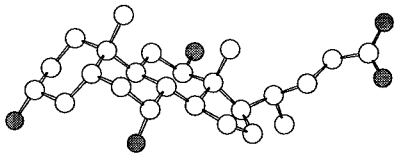


Figure 5-3. Molecular structure of 12ECA

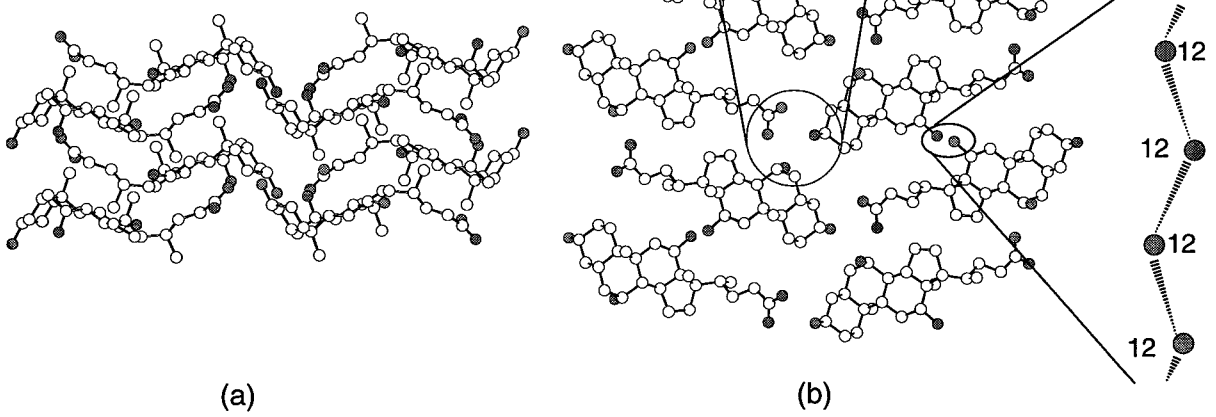


Figure 5-4. Molecular assembly mode of 12ECA. (a) Viewed along the crystallographic *b* axis. (b) Viewed along the crystallographic *c* axis.

various organic solvents. However, 3,12ECA form an inclusion crystal with acetonitrile only. The host-guest ratio is 1:1 form thermal analysis and solution ^{1}H-NMR. Survey of other guest compounds failed. Other organic solvents do not give inclusion crystal of 3,12 ECA, but form guest-free crystals. X-ray crystallography of the guest free crystal reveals that 3,12 ECA form a monolayer structure without any inclusion cavities.

The crystal structure of the guest-free 3,12ECA is depicted in Figure 5-5. The molecular structure and crystal structure is quite similar to those of 12ECA (Figure 5-6). And the cyclic hydrogen bonding network that connects between 3,12ECA also exist. For the crystal, the networks have a sequence of O(3)H···O(7)H···O(24a)=C-O(24b)H···O(3)H, where the hydrogen-bonding distances are 2.768, 2.847 and 2.615Å, respectively. The hydrogen bonds between the hydroxy groups at 12-position are also existence with a distance of 3.286Å.

The equatorial hydroxy group at 12-position turn the carboxylic acid to β-face and the divergent hydrogen bond functional group in the molecular structure of 3,12ECA yields the three dimensional hydrogen bond network without any host cavities.

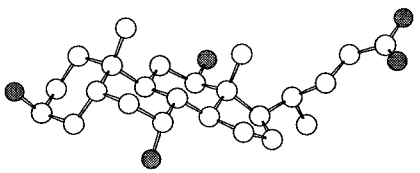


Figure 5-5. Molecular structure of 3,12ECA

Figure 5-6. Molecular assembly mode of 3,12ECA. (a) Viewed along the crystallographic *b* axis. (b) Viewed along the crystallographic *c* axis.

3,7-Epicholic acid

3,7-Epicholic acid (3,7ECA) is a double epimer of CA and this has one additional hydroxy group at 12-position of 3EUDCA. This compound was prepared from 7ECA by inversion of the hydroxy group at 3-position via Mitsunobu condensation reaction as described in the literature.[13,25] Recrystallization from various organic solvents yields inclusion compounds of 3,7ECA. Especially, aliphatic ketones such as acetone and 3-pentanone were included in 3,7ECA. X-ray crystallography of the inclusion crystal of 3-pentanone reveals that 3,7ECA forms channel-type structure with large inclusion cavities (Figure 5-7). The geometry of the guest molecule except the oxygen atom could not find from the electron density map due to the disordered structure of the guest molecule. The oxygen atom of the guest molecule are caught by a hydrogen bond with the hydroxy group at 12-position of 3,7ECA. In spite of the large cavity of 3,7ECA, small ketones form stable inclusion crystals because of the hydrogen bonds between host molecules and guest molecules.

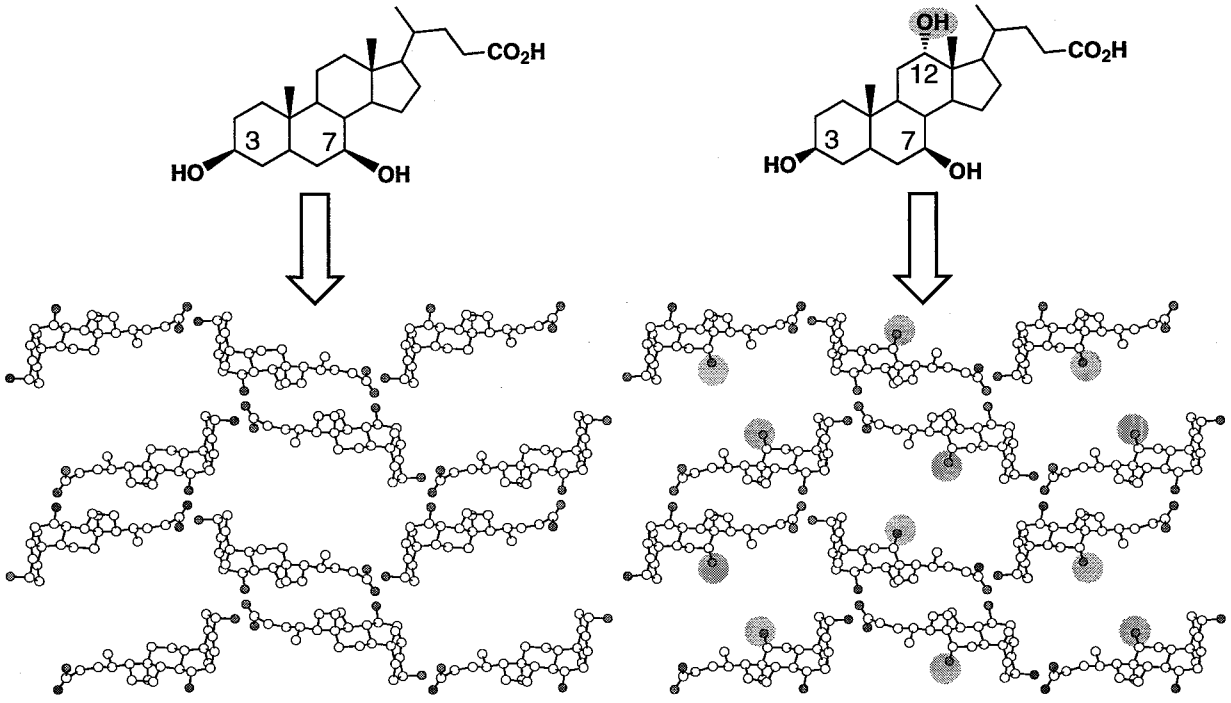


Figure 5-7. Molecular assembly modes of (a) 3EUDCA and (b) 3,7ECA.

This characteristic chicken wire structure of 3,7ECA clathrate is quite similar to those of 3EUDCA. Divergent hydrogen bond functional group yield three dimensional network with large inclusion cavities. The presence of hydroxy group at 12-position in the molecular structure of 3,7ECA plays an important role for inclusion properties. This hydroxy group protrudes from the wall of the channel and acts as a hydrogen bond hook to the guest molecules. Accordingly, 3,7ECA have hydrogen bond functional group on the wall and 3EUDCA does not. Although steric dimensions of the host cavities are quite similar to each other, 3,7ECA forms stable inclusion compounds with small ketones that 3EUDCA does not include by host-guest hydrogen bond. This result indicates that the host pair of 3,7ECA and 3EUDCA must be a good example to fine tuning of the inclusion cavity.

5-4 Optical Resolution of Alcohols by Selective Enclathration of 3ECA and 3EDCA

Much attention is being donated to the resolution of neutral organic substances by lattice inclusion compounds because of highly efficient and easy procedure.[26] However, the use of the compounds for optical resolution of aliphatic alcohols have been limited and their enantioselectivities are relatively low.[27] This is because steric differences between the alkyl substituents are too small for the large cavities of the inclusion compounds. Here, the author report the optical resolution of four racemic alcohols, such as 2-butanol, 3-methyl-2-butanol, 2-pentanol and 3-methyl-2-pentanol by using 3ECA or 3EDCA as a host molecule(Figure 5-8). Among these secondary alcohols, 2-

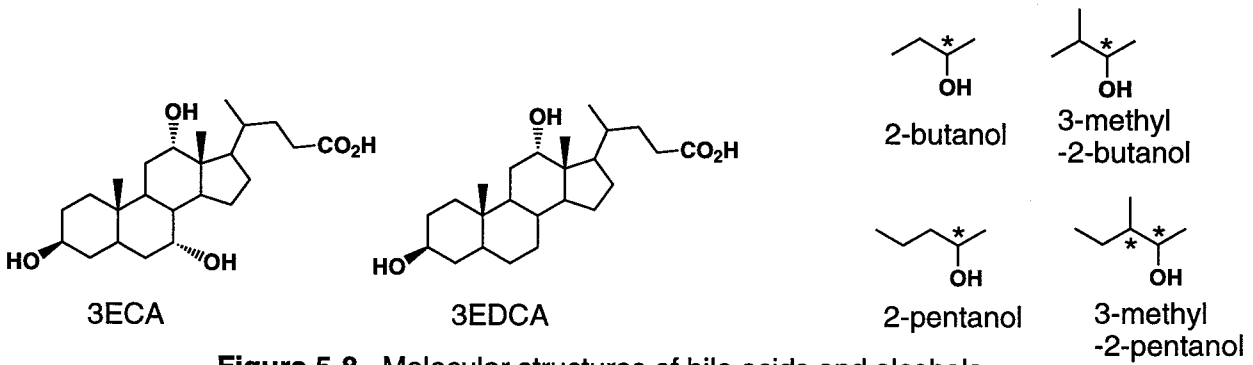


Figure 5-8. Molecular structures of bile acids and alcohols

butanol, 3-methyl-2-butanol and 2-pentanol have one asymmetric carbon in their own bodis. Another has two asymmetric carbon, so the alcohol has four stereoisomers.

3ECA was treated with all of the four racemic alcohols by recrystallization method and suspension method.[28] Table 5-2 and Figure 5-9 shows their enentiomer excess(%e.e.), respectively. By using recrystallization method, racemic 2-butanol and 3-methyl-2-butanol yielded 31 % and 29

Table 5-2. Optical resolution of aliphatic alcohol by using 3ECA and 3EDCA as a host.[a)]

alcohols	3ECA		3EDCA		Predominant Configuration
	recrys [c)]	susp [d)]	recrys	susp	
2-butanol	31	25	GF [b)]	GF	R-(-)
3-methyl-2-butanol	29	39	GF	GF	R-(-)
2-pentanol	79	94	90	GF	R-(-)
3-methyl-2-pentanol	67	99<	99<	GF	(2R, 3S)

[a)]Determined by ^{13}C-NMR. [b)] Obtained guest-free crystal. [c)] Recrystallization method. [d)] Suspension method

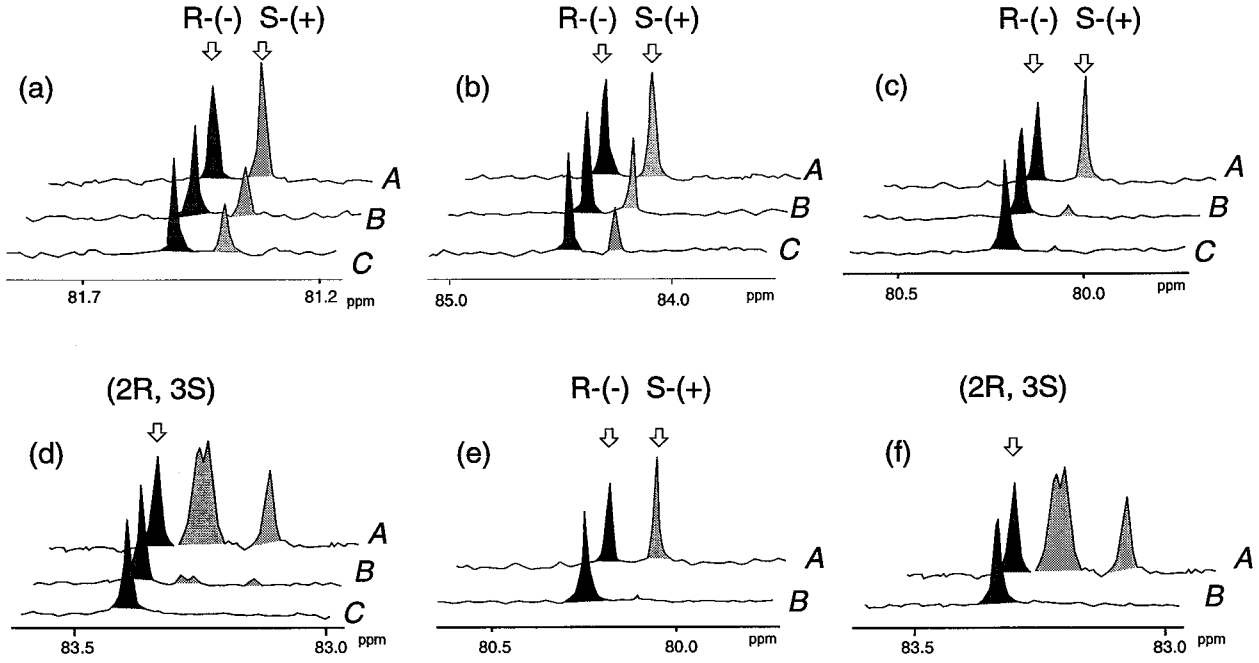


Figure 5-9. Optical resolution of aliphatic alcohols using 3ECA and 3EDCA as hosts. (a) 2-butanol with 3ECA, (b) 3-methyl-2-butanol with 3ECA, (c) 2-pentanol with 3ECA, (d) 3-methyl-2-pentanol with 3ECA, (e) 2-pentanol with 3EDCA, (f) 3-methyl-2-pentanol with 3EDCA.
A : before optical resolution, *B*: recrystallization method, *C*: suspension method.

% e.e., respectively. But in the case of 2-pentanol, one cycle of recrystallization yielded 79 % e.e. Predominant configurations of the included alcohols are all R-isomers. On the other hand, (2R, 3S)-3-methyl-2-pentanol are preferentialy included in the inclusion lattice of 3ECA. In this case, 3ECA recognized one isomer from four isomers.

Recrystallization from the four racemic alcohols, the author obtained single crystals suitable for X-ray crystallographic study, respectively. Table 5-3 shows their crystallographic data. All inclusion crystals of 3ECA belong to the same space group (monoclinic, $P2_1$). In these crystals, each alcohols were included in the same inclusion spaces with same hydrogen bonding networks. The hydroxy group of the guest molecule is a member of the cyclic hydrogen-bonding network. Therefore the configuration of the asymmetric oxygenated carbon is determined. Further, it can be seen that the size and shape of the inclusion space is suitable for 3-methyl-2-pentanol. So 3ECA recognizes 3-methyl-2-pentanol finer than 2-Butanol. Although 2-Pentanol and 3-methyl-2-butanol are stereoisomers each others, the enantiomeric purity are hardly different. This means that the inclusion space has side pockets suitable for the *n*-propyl group than the *i*-propyl one.

The suspension method increased the enantiomeric purity for 2-pentanol from 79 to 94 %

Table 5-3. Crystallographic data for inclusion crystals of 3ECA.

guest	2-butanol	3-methyl-2-butanol	2-pentanol	3-methyl-2-pentanol
formula	$C_{28}H_{50}O_6$	$C_{29}H_{52}O_6$	$C_{30}H_{54}O_6$	$C_{30}H_{54}O_6$
formula weight	482.70	496.73	510.75	510.75
crystal system	monoclinic	monoclinic	monoclinic	monoclinic
space group	$P2_1$	$P2_1$	$P2_1$	$P2_1$
a, Å	12.240(1)	12.596(1)	12.590(2)	12.947(2)
b, Å	7.9457(9)	7.9424(8)	7.866(2)	7.953(2)
c, Å	14.442(1)	14.459(5)	14.468(3)	14.332(2)
β, deg	100.218(7)	101.63(1)	102.95(2)	103.12(3)
V, Å^3	1382.2(2)	1416.8(4)	1396.4(6)	1437.1(5)
Z	2	2	2	2
R	0.056	0.059	0.044	0.055
temperature, K	298	218	193	298
instrument	AFC7R	RAXIS-IV	AFC7R	AFC5R

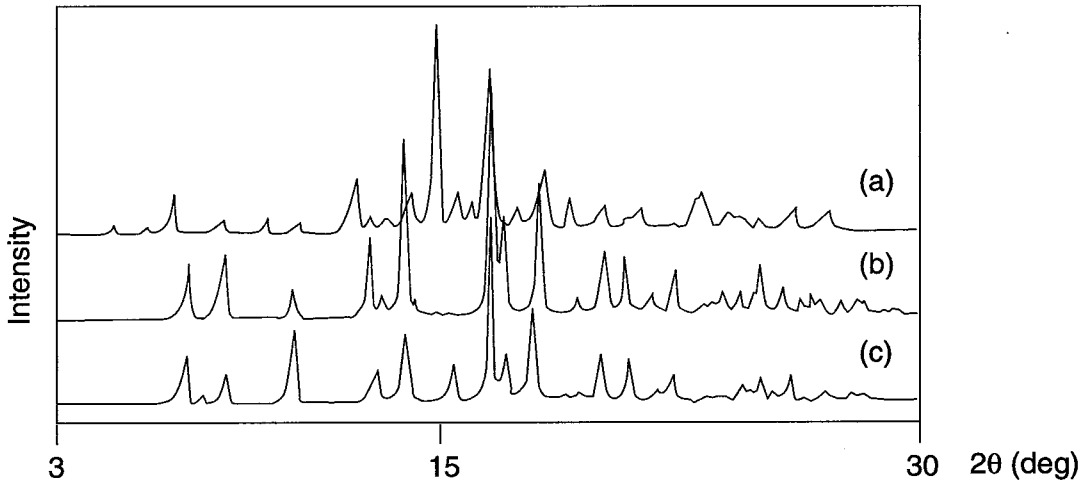


Figure 5-10. X-ray powder diffraction pattern of the crystals of 3ECA. (a) Guest-free crystal of 3ECA. (b) Inclusion crystal with 2-pentanol obtained from the recrystallization method. (c) Inclusion crystal with 2-pentanol obtained from the suspension method.

e.e., and for 3-methyl-2-butanol from 29 to 39 % e.e., while for 2-butanol it decreased from 31 to 25 % e.e. This means that the suspension method is very useful one to resolute enantiomers. Furthermore, the suspension method had a high selectivity to 3-methyl-2-pentanol. Only one cycle of this procedure yielded over 99 % e.e. of (2R, 3S)-3-methyl-2-pentanol.

Powder X-ray diffraction patterns of the inclusion crystals obtained by the suspension method are similar to that obtained by a recrystallization method. Figure 5-10 (a) shows the X-ray diffraction pattern of the guest-free crystal that was prepared by heating inclusion crystals for several hours *in vacuo*. And the diffraction pattern of inclusion crystals obtained from the recrystallization method is shown in Figure 5-10 (b). The diffraction pattern (c) belongs to the crystals obtained from the suspension method. This means that the host compound 3ECA forms inclusion crystals in the suspension.

Next, the author tried to resolute racemic secondary alcohols by using 3EDCA as a host molecule. Although a recrystallization method yielded inclusion crystals with 2-pentanol and 3-methyl-2-pentanol, the author could not obtain any inclusion crystals by using a suspension method. The enentiomeric purity of 2-pentanol and 3-methyl-2-pentanol are 90 and 99 < % e.e., respectivety. So 3EDCA also has high enantioselectivity to secondary alcohols.

The crystal structure of the inclusion compound of 3EDCA with racemic 2-pentanol is similar to that of 3ECA with 2-pentanol, as shown in Figure 5-2. The difference point is only the hydrogen-bonding network pattern because of a lack of the hydroxy group at 7-position, as mentioned at 5-3. Although the lack of one hydroxy group weakened the hydrogen bonding network in 3EDCA, 3EDCA could recognize the chirality finer than 3ECA.

In conclusion, 3ECA and 3EDCA recognize the chirality of aliphatic secondary alcohols, and has versatility in their resolution. And these bile acids are superior to other host compounds in enantioselectivity.

5-5 Summary

In this chapter, the author clarified the inclusion abilities and molecular recognition of bile acids that have the hydroxy groups at 3-, 7- and 12-position. Among them, 3ECA could form inclusion crystals with wide range of alcohols. This means that inversion of the hydroxy group at 3-position controls the environment of the inclusion space. Moreover, 3ECA recognized the chirality of aliphatic secondary alcohols, and has varsatility in their resolution. 12β-Isomers, such as 12ECA and 3,12ECA build very similar molecular assemblies each others.

Summary

This thesis deals with systematic studies of inclusion abilities and molecular recognition of steroidal bile acids having multiple hydroxy groups.

In Chapter 1,the author showed that steroidal bile acids tend to form inclusion crystals with organic substances. And only two bile acids could not include any organic compounds. Among these acids, steroids that have the 3α-hydroxy group tend to include lipophilic guest molecule, whereas 3β-isomers like hydrophilic organic substances. This result shows that 3-positioned hydroxy group plays an important role to form stable inclusion crystals. describes the inclusion abilities of steroidal bile acids.

In Chapter 2, the author clarified the inclusion abilities and molecular recognition of bile acids that have 3- and 6-positioned hydroxy groups. Hyodeoxycholic acid and hyocholic acid form inclusion compounds with suitable organic substances, respectively. And the crystal structures of the acid that have the hydroxy groups at 3- and 6-positions form 'unit' that were connected by hydrogen bonds.

In Chapter 3, the author clarified the inclusion abilities and molecular recognition of bile acids that have the hydroxy groups at 3- and 7-position. Among the four isomers, ursodeoxycholic acid and 3-epichenodeoxycholic acid could hardly form inclusion crystals. 3-Epiursodeoxycholic acid (3EUDCA), however, included various organic substances. Furthermore 3EUDCA also included naphthalene derivatives, such as 2'-acetonaphthone with 1:1 host to guest ratio. In the inclusion space, the guest molecules stacked each others. So this is the typical example to control the arrangement of aromatic compounds.

In Chapter 4, the author clarified the inclusion ability and molecular assemblies of bile acids that have the hydroxy groups at 3- and 12-position. Among the isomers, 3-epideoxycholic acid tends to include hydrophilic organic substances, especially alcohols. The compound was also able to form guest-dependent polymorphism. On the other hand, inversion of the hydroxy group at 12-position induces the conformational change of the side chain. In the case of 3,12EDCA, novel

type of a inclusion crystal was obtained. This is the first example for bile acids to build such a right-handed helical tubuland. And this result enanbled the author to realize pseudomirror imaged molecular assemblies.

In Chapter 5, the author clarified the inclusion abilities and molecular recognition of bile acids that have the hydroxy groups at 3-, 7- and 12-position. Among them, 3ECA could form inclusion crystals with wide range of alcohols. This means that inversion of the hydroxy group at 3-position controls the environment of the inclusion space. Moreover, 3ECA recognized the chirality of aliphatic secondary alcohols, and has varsatility in their resolution. 12β-Isomers, such as 12ECA and 3,12ECA build very similar molecular assemblies each others.

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List of Publications

1. Combinatorial Chemistry of Inclusion Compounds by Using Steroids
   Mikiji Miyata, Kazuki Sada and Yasuhito Miyake
   Mol. Cryst. Liq. Cryst., in press.

2. Novel Channel-type Inclusion Compounds of 3-Epiursodeoxycholic Acid
   Yasuhito Miyake, Junji Hirose, Yasuhiro Hasegawa, Kazuki Sada and Mikiji Miyata
   J. Chem. Soc. Chem. Commun., in press.

3. Inclusion Compounds of Hyodeoxycholic Acid and the Crystal Structure of a 1:1 Complex between the Acid and Pyridine
   Yasuhito Miyake, Yoshiaki Matsuura, Kazuki Sada and Mikiji Miyata
   Chem. Lett., **1997**, 1263.

4. Inclusion Compounds of Hyocholic Acid with Specific Alcohols
   Yasuhito Miyake, Kazuki Sada and Mikiji Miyata
   Supramolecular Chemistry, in contribution.

5. A Crystal Structure of 12-Epideoxycholic Acid
   Yasuhito Miyake, Kazuki Sada and Mikiji Miyata
   Acta Cryst., Sec.C, in contribution.

6. Pseudo-Mirror Imaged Molecular Assembly Modes of Bile Acids
Yasuhito Miyake, Kazuki Sada and Mikiji Miyata
J. Chem. Soc. Chem. Commun., in preparation.

7. Optical Resolution of Aliphatic Alcohols by using 3-Epicholic Acid
Yasuhito Miyake, Kazuki Sada and Mikiji Miyata
in preparation.

8. Molecular Recognition and the Assembly Modes of 3b-Isomers of Bile Acids
Yasuhito Miyake, Kazuki Sada and Mikiji Miyata
in preparation.

Acknowledgment

The author wish to express his sincerest gratitude to Professor Mikiji Miyata for his constant guidance, helpful suggestions and hearty encouragement thoughout this work.

The author also deeply grateful to Dr. Kazuki Sada for his powerful and helpful discussions.

The author desires to express his sincere thanks to Associate Professor Yoshiaki Inaki and Associate Professor Takehiko Wada for their helpful advices.

Grateful acknowledgment are made to Mr. Kazunori Nakano, Mr. Yoshihiro Nakahata, Ms. Tomoko Yamaguchi, Mr. Yukio Hishikawa, Mr. Norimitsu Tohnai, Mr. Sugahara, Mr. Naotaka Shiomi, Mr. Junji Hirose , Mr. Yasuhiro Haegawa, Mr. Yoshiaki Matsuura and all other members of Miyata laboratory for their encouragement and helps.

Sincere thanks are due to Professor Takashi Kawamura and Dr. Masahiro Ebihara at Gifu University for the instrument of the X-ray Crystallography.

Finally, the author is very thankful to his father (Yutaka Miyake), mother (Toyoko Miyake), brothers (Yoshifumi Sato and Takuji Miyake) for their understanding and supports.