Inclusion Complexation of Cholestanol, Cholesterol, Sitosterol, Stigmasterol, and Ergosterol with Various Guest Compounds#

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Synopsis. The title steroids were found to form crystalline inclusion complexes with a wide variety of guest compounds. Crystal structures, interaction modes and molecular recognition features of selected host-guest systems have been characterized.

It has long been known that deoxycholic (**1a**)[1] and cholic (**1b**)[2] acids include various guest molecules and form crystalline inclusion complexes. Nevertheless, only a few reports dealing with the inclusion properties of other steroid compounds have appeared so far. Among those, earlier publications reported on the tendency of cholesterol (**3**) to form solvates,[3] and, more recently, the crystal structures of its hydrate[4] and ethanolate[5] have been analyzed. It has also been reported that *N,N,N',N'*-tetracyclohexylfumaramide cocrystallizes with **3** in the form of a 1:2 inclusion complex.[6] In a recent systematic study we have observed that steroids such as cholestanol (**2**), cholesterol (**3**), sitosterol (**4**), stigmasterol (**5**), and ergosterol (**6**) form, in fact, inclusion crystals with a wide variety of guest compounds, and that their inclusion properties can often be used for separation of guest structural isomers.

Results and Discussion

The variety of guest compounds included, and the stoichiometries of the corresponding complexes formed are listed in Table 1. Of the five steroids tested **2**—**6**, **2** and **3** showed a relatively high guest-inclusion ability. The host:guest ratio observed in the various complexes ranges from 1:1 to 4:1; however, a ratio of 2:1 characterizes the most common composition. This is in contrast with the dominant host:guest stoichiometry of 1:1 observed for the inclusion complexes of **1a** and **1b** (Chart 1).[1,2]

Crystal structure analysis of the 1:1 inclusioin complex of **1b** with acetophenone has shown that the guest moieties are accommodated in channels formed between one-dimensional arrays of host molecules, multiply hydrogen bonded to one another.[2] Formation of such an extended host-network is facilitated by the presence of three hydroxyl and one carboxyl functions in the molecular framework of **1b**. Clearly, a different pattern of association should be anticipated in the inclusion complexes of hosts **2**—**6**, as these molecules contain only a single hydrogen-bonding site. The previously described structures of cholesterol and its water and ethanol solvates were found to be particularly complex, containing a large number of independent molecules in the crystallographic asymmetric unit and features of local pseudosymmetry.[4,5,7] They exhibit a characteristic bilayer arrangements of the constituent species with a continuous hydrogen-bonding pattern linking the hydrophilic ends ("heads") of adjacent molecules in the crystal. Presumably, weak packing forces between the aliphatic tails of the cholesterol molecules allow for a significant population of several different conformational modes of this fragment in any of those structures.

The evident tendency of hosts **2** and **3** to incorporate polar guest species into the crystal lattice (Table 1) provides a good indication that inclusion complexation can be effective in facilitating the formation of simpler and more organized structures of these compounds.[8] Indeed, the present study has revealed several new crystalline forms of inclusion complexes involving these two steroids. Representative examples include the crystal structures of 2:1 (**2**)·2-naphthol and 1:1 (**3**)·2-methylpropenoic acid.

The crystal structure of the former complex is illustrated in Fig. 1. It consists of continuous chains of host and guest molecules linked to each other by hydrogen bonds along the *c*-direction of the unit cell. The 2-naphthol guest, lying approximately on the rotational symmetry axis, associates with two neighboring hosts related to each other by the twofold rotation; $[O(29)\cdots O(1)]_{av}=2.72(2)$ Å]. Each of the host species hydrogen bonds from the other side to another host molecule [at $O(1)\cdots O(1')=2.64(2)$ Å], thus forming a $(\text{-host-host-guest-})_\infty$ pattern. In view of the guest disorder about the twofold axis, the orientation of the hydrogen bonds cannot be well defined. It seems that, in any given chain, they point at either the $+c$ or $-c$ direction, in a random manner; a dynamic disorder of the hydrogen bonds is less probable. Packing of the hydrogen-bonded arrays is stabilized by van der Waals forces, the T-shaped fragments of each chain effectively interlocking between those of adjacent chains. The enviroment of every guest molecule thus consists of two strongly bound hosts, and two additional cholestanols approaching with their alkyl ends opposite sides of the naphthalene ring.

Isomorphous crystal structures are formed also by host **3** with *p*-cresol and with *cis*-$C_2H_5CH{=}CH(CH_2)_2OH$ as guests. On the other hand, with guest

#Dedicated to Professor Harold Hart on the occation of his 70th birthday.

Table 1. Host : Guest Ratios in the Inclusion Complexes of Hosts **2**—**6** with Various Guests [a]

Guest	Host : Guest	Guest	Host : Guest
(a) Complexes of **2**:			
MeOH	2 : 1	**8b**	2 : 1
EtOH	2 : 1	**8c**	2 : 1
n-PrOH	2 : 1	**7**	2 : 1[b]
Cyclohexanol	1 : 1	Acetone	4 : 1
9a	1 : 1[b]	Cyclohexanone	4 : 1
CH_2=$CHCH_2OH$	4 : 1	*rac*-2-Methylcyclohexanone[c]	4 : 1
CH≡CCH_2OH	4 : 1	*rac*-3-Methylcyclohexanone[c]	4 : 1
cis-C_2H_5CH=$CH(CH_2)_2OH$	4 : 1	4-Methylcyclohexanone	4 : 1
CH_2=$CH(CH_2)_2OH$	2 : 1	Benzaldehyde	2 : 1
trans-CH_3CH=$CHCH_2OH$	2 : 1	Acetophenone	2 : 1
trans-C_3H_7CH=$CH(CH_2)_2OH$	2 : 1	Benzophenone	2 : 1[b]
trans-CH_3CH=CHCOOH	2 : 1	DMF	2 : 1
CH_2=$C(CH_3)CH_2CH_2OH$	2 : 1	Pyridine	2 : 1
Phenol	2 : 1	*rac*-2-(Hydroxymethyl)pyran[c]	2 : 1
8a	2 : 1		
(b) Complexes of **3**:			
Cyclohexanol	1 : 1	$HOOC(CH_2)_2COOH$	2 : 1[b]
9a	1 : 1	CH_2=$C(CH_3)COOH$	1 : 1
Phenol	2 : 1	*cis*-C_2H_5CH=$CH(CH_2)_2OH$	2 : 1
8a	2 : 1	*rac*-$CH_3CHClCOOH$[c]	2 : 1
8b	2 : 1	*rac*-$CH_3CHBrCOOH$[c]	2 : 1
8c	2 : 1	*rac*-$C_2H_5CHClCOOH$[c]	2 : 1
7	2 : 1[b]	*rac*-$C_2H_5CHBrCOOH$[c]	2 : 1
CH_3COOH	2 : 1	$CH_3CHClCH_2COOH$	2 : 1
C_2H_5COOH	2 : 1	*trans*-CH_3CH=CHCOOH	2 : 1
C_3H_7COOH	2 : 1		
(c) Complexes of **4**:			
EtOH	2 : 1	Cyclohexanone	2 : 1
n-PrOH	2 : 1	2-Cyclohexenone	2 : 1
t-BuOH	2 : 1	γ-Butyrolactone	2 : 1
CH≡CCH_2OH	2 : 1	DMSO	2 : 1
cis-C_2H_5CH=$CH(CH_2)_2OH$	4 : 1	C_2H_5COOH	2 : 1
(d) Complexes of **5**:			
MeOH	2 : 1	CH≡CCH_2OH	2 : 1
DMF	2 : 1		
(e) Complexes of **6**:			
8a	4 : 1	Acetone	2 : 1
8b	4 : 1	CH≡CCH_2OH	2 : 1
8c	4 : 1		

a) Host : guest ratios were determined (unless stated otherwise) by thermogravimetry (TG) measurements. b) Host : guest ratios determined by elemental analysis. c) Racemic compound was included, and no optical resolution occurred.

species containing two hydrogen-bonding sites as 2-methylpropenoic acid, cholesterol forms a somewhat different structure type (Fig. 2). In the latter, the host and guest moieties associate in a 1 : 1 ratio, also forming hydrogen-bonded chains, but with an alternating arrangement of the two molecules along them. The hydrogen-bonding pattern is well ordered, all OH···O bonds pointing in the same direction along the polar screw axis parallel to *b*. The intermolecular packing in the primitive lattice appears to be slightly more efficient than that in the previous example.

In both crystals nonbonding dispersive interactions between the aliphatic end of the conformationally extended steroid and the molecular framework of the planar and unsaturated guest molecule, appear to contribute favorably to intermolecular organization and stability of the structure, while preserving the bilayer nature of the molecular arrangement. The crystal packing of the bilayers is dominated primarily by the shape of the large hosts, and is, therefore, nearly the same for complexes of either **2** or **3** with similarly sized monohydric alcohol guests. On the other hand, the crystal structure appears to be more significantly affected by differences in functional complementarity between the hydrogen-bonding sites in the various host-guest combinations.

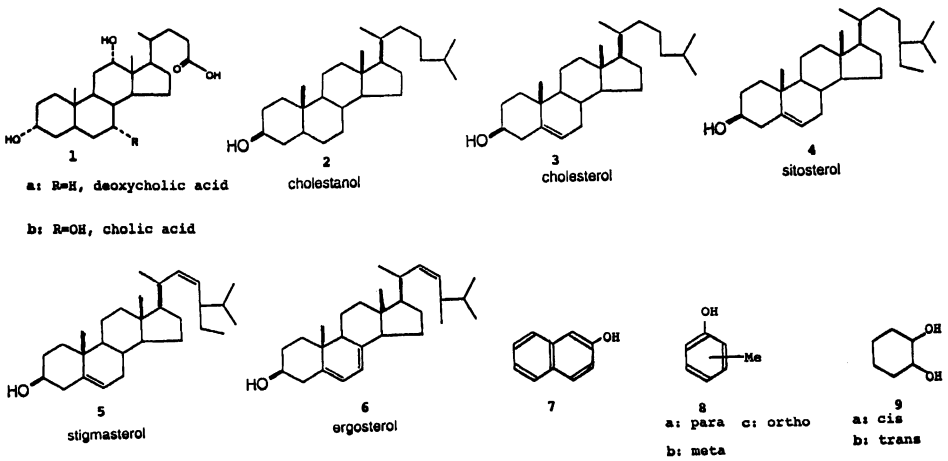

Chart 1.

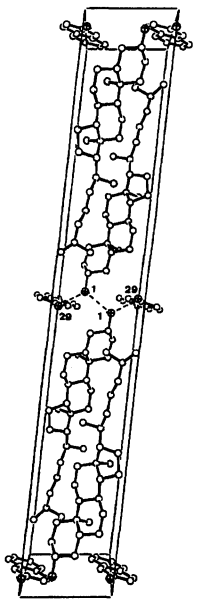

Fig. 1. The crystal structure of the 2:1 complex of cholestanol and 2-naphthol viewed down the *b*-axis (*c* is horizontal). Only one orientation of the disordered guest molecule is shown at each site, but guest species from adjacent unit-cells are also included to illustrate better the crystal packing. The hydroxyl O-atoms (numbered) are represented by crossed circles, and the hydrogen bonds by broken lines. The host-host and host-guest hydrogen-bonding distances are O(1)···O(1)=2.64(2) Å and $[O(1)\cdots O(29)]_{av}=2.72(2)$ Å, respectively.

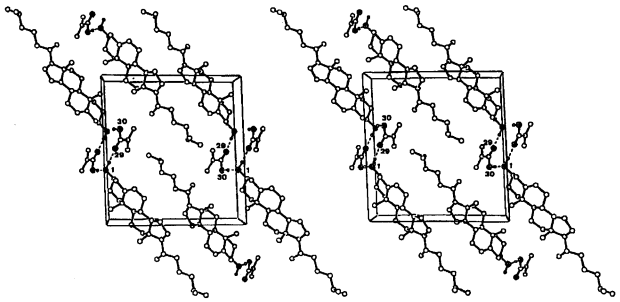

Fig. 2. The crystal structure of the 1:1 complex between cholesterol and 2-methylpropenoic acid stereoviewed down the short *b*-axis. Contents of more than one unit-cell are shown to illustrate better the various types of intermolecular interaction. The oxygen atoms (numbered) are repersented by crossed circles, and the hydrogen bonds by broken lines. The hydrogen-bonding distances are: OH(1)[host]···O(29)-[guest]2.75(1) Å and OH(30)[guest]···O(1)[host]2.61-(1) Å.

Molecular recognition ability of the steroid hosts associated with spatial complementarity is also quite high, and this feature can be used for separation of guest isomers. This is demonstrated by successful separation of *m*-cresol and *p*-cresol by inclusion complexation with cholestanol, as well as of *cis*-1,2-cyclohexanediol and *trans*-1, 2-cyclohexanediol by complexation with cholesterol (see Experimental Section). Nonetheless, chiral recognition ability of the steroid hosts is very poor, and the chiral guests listed in Table 1 could not be resolved by the inclusion complexation process. Further studies of the molecular recognition features, and their relation to structure, are in progress to account for the above observations.

Experimental

Preparation of the Inclusion Complexes (General Procedure). The inclusion complexes were obtained by recrystallization of the steroid host from neat liquid of the guest compound. For guests which are solid at ambient conditions, the two components were first dissolved in hot ethyl acetate, and then allowed to crystallize at room temperature. The crystals which formed were collected by suction filtration. The host:guest stoichiometry of the complexes was determined either by thermal gravimetric analysis or by elemental analysis. Data for each compound are given in Table 1.

Separation of *m*-and *p*-cresol. When a solution of an equimolar mixture of *p*-cresol and *m*-cresol (1.12 g, 10.4 mmol) and **2** (2.0 g, 5.2 mmol) dissolved in ethyl acetate (15 ml) was kept at room temperature for 6 h, a 2 : 1 complex of **2** and **8** was obtained as colorless prisms (1.93 g), which upon heating at 200°C and 20 Torr (1 Torr=133.322 Pa) gave by distillation a 72 : 28 mixture of **8a** and **8b** (0.13 g, 34% yield). The ratio of the two components was determined by gas chromatography.

Separation of *cis*-and *trans*-1,2-Cyclohexanediol. When a solution of a 79 : 21 mixture of *cis*-1,2-cyclohexanediol (**9a**) and *trans*-1,2-cyclohexanediol (**9b**) (0.6 g, 5.2 mmol) and **3** (2.0 g, 5.2 mmol) in ethyl acetate (10 ml) was kept at room temperature for 6 h, a 1 : 1 complex of **3** and **9a** was obtained as colorless prisms, which upon heating at 200°C and 1 torr gave 98% pure **9a** (0.3 g, 50% yield). The purity was determined by gas chromatography.

Crystallographic Analysis. Intensity data were measures at room temperature with a CAD4 diffractometer using Mo $K\alpha$ radiation to $2\theta_{max}=50°$.

Crystal data for (**2**)·2-naphthol (1 : 1/2): $C_{27}H_{48}O{\cdot}0.5(C_{10}H_8O)$, $M=460.76$, monoclinic, space group $C2$, $a=42.324(8)$, $b=10.557(6)$, $c=6.534(2)$ Å, $\beta=96.27(2)°$, $Z=4$, $D_c=1.055$ g cm^{-3}; for (**3**)·2-methylpropenoic acid (1 : 1): $C_{27}H_{46}O{\cdot}C_4H_6O_2$, $M=472.75$, monnoclinic, space group $P2_1$, $a=14.950(6)$, $b=6.186(7)$, $c=15.858(4)$ Å, $\beta=92.38(3)°$, $Z=2$, $D_c=1.072$ g cm^{-3}.

Both structure were solved by direct methods (SHELX-86),[9] and refined by full-matrix least-squares (SHELX-76).[10] In the cholestanol complex the 2-naphthol guest entities are located on, and (not containing such molecular symmetry) statistically disordered about, the crystallographic symmetry axes of twofold rotation. At each guest site in the crystal, the 2-naphthol occupies with 50% probability either one of two possible orientations with respect to the twofold axis. To avoid high correlations between parameters, the guest molecule was treated as geometrically constrained rigid group with only isotropic thermal parameters in the refinement calculations. At convergence, $R=0.085$ and $wR=0.085$ for 1415 observations above the threshold of $2\sigma(I)$ (out of 2264 unique data above zero); final $|\Delta\rho|<0.35$ e Å^{-3}. The diffraction data for this compound were collected initially in the Niggli reduced cell setting ($a=6.534$, $b=10.557$, $c=21.810$ Å, $\alpha=104.01$, $\beta=96.08$, $\gamma=90.09°$), and the structure was solved and refined in space group $P1$ with the asymmetric unit consisiting of 2 hosts and one guest. The results have indicated, however, that the guest species is similarly disordered, and that the two hosts have identical structures. These observations, and the evident $2/m$ symmetry of the diffraction pattern, confirmed the correctness of the higher-symmetry space group assignment.

Refinement of the cholesterol complex converged at $R=0.083$ and $wR=0.080$ for 1718 observations above the threshold of $2\sigma(I)$, out of 2507 unique data with positive intensities; final $|\Delta\rho|<0.33$ e Å^{-3}. Geometric restraints were applied to the carbon end of the guest molecule to avoid unreliable distortion of the covalent parameters (an artifact caused by excessive thermal motion) in this fragment. Poorly measured reflections at very low angles, and those suffering from apparent extinction effects [200, 400, 600, 510, $\bar{4}01$ in the cholestanol structure, and 100, $\bar{1}01$, $\bar{1}02$, 001, 002 in the cholesterol structure] were given zero weight in the calculations. Most hydrogen atoms were introduced in calculated positions; the hydroxyl H-atoms in the cholesterol complex were located from difference-Fourier maps. The loosely packed aliphatic tails of **2** and **3** exhibit a large-amplitude wagging motion, the three terminal methyl groups in the former and two in the latter being essentially disordered. Hydrogen atoms of these methyls were not included, and the correspnding C-atoms were assigned an isotropic U in the refinement. The hydrogen atoms in the cholestanol complex could not be located due to the orientational disorder of the 2-naphthol guest. As in the previous studies involving cholesterol,[4,5,7] the above features of the structure affected the amount of significant data that could be measured, as well as the precision of the structural analyses. The complete F_o-F_c data are deposited as Document No. 9053 at the Office of the Editor of Bull. Chem. Soc. Jpn.

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References

1) W. H. Herdon, *J. Chem. Educ.*, **44**, 724 (1967).

2) M. Miyata, M. Shibakami, W. Goonewardena, and K. Takemoto, *Chem. Lett.*, **1987**, 605; M. Miyata, M. Shibakami, and K. Takemoto, *J. Chem. Soc., Chem. Commun.*, **1988**, 655; K. Miki, A. Masui, N. Kasai, M. Miyata, and K. Takemoto, *J. Am. Chem. Soc.*, **110**, 6594 (1988).

3) E. Shefter, and T. Higuchi, *J. Pharm. Sci.*, **52**, 781 (1963).

4) B. M. Craven, *Acta Crystallogr., Sect. B*, **35**, 1123 (1979).

5) H. -S. Shieh, L. G. Hoard, and C. E. Nordman, *Acta Crystallogr., Sect. B*, **38**, 2411 (1982).

6) M. Segawa, K. Mori, and F. Toda, *Chem. Lett.*, **1988**, 1755.

7) H. -S. Shieh, L. G. Hoard, and C. E. Nordman, *Acta Crystallogr., Sect. B*, **37**, 1538 (1981).

8) I. Goldberg, *Top. Curr. Chem.*, **149**, 1(1988).

9) G. M. Sheldrick, "SHELX-86," in "Crystallographic Computing 3," ed by G. M. Sheldrick, C. Kruger, and R. Goddard, Oxford University Press, Oxford (1985), pp. 175—189.

10) G. M. Sheldrick, "SHELX-76, Program for Crystal Structure Determination," University of Cambridge, England (1976).