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Specific Inclusion of Water by *N*-Methyldeoxycholanamide Revealed by the Crystal Structure of the Hemihydrate

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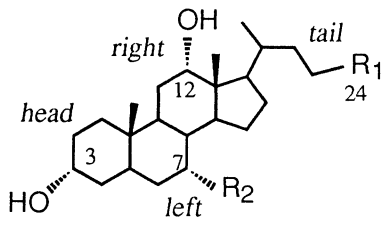
N-Methyldeoxycholanamide specifically includes water instead of organic substances, which is in contrast to deoxycholic acid and deoxycholanamide. Such a specific inclusion behaviour is reasonably explained by the crystal structure of the hemihydrate. The *N*-methyl group induces a ladder-like hydrogen bonding scheme involving water. The resulting molecular arrangement inhibits an inclusion of organic guests.

Bile acids and their derivatives (Fig. 1) are considered to express their own molecular information through crystalline assemblies by various noncovalent bond. In order to clarify the expression of the information, our attention is focused on a correlation among the chemical structures, crystal structures and inclusion behaviours.[1] Transformations of the steroidal side chains provide us an efficient method for detecting such a correlation. Deoxycholic acid (**1**),[2] cholic acid (**2**),[3a] their amides (**3**, **4**)[3b,c] as well as their conjugated acids (**5**, **6**)[3d] were already established to include various organic substances. In contrast, we have found that *N*-methyldeoxycholanamide (**7**) specifically includes water, although the other hosts predominantly include organic substances. This paper concerns with the inclusion behaviour of *N*-substituted deoxycholanamides and a reasonable explanation for the behaviour on the basis of comparison of crystal structures of **3** and **7**.



R_1=	R_2= H	R_2=OH
COOH	Deoxycholic acid (**1**)	Cholic acid (**2**)
$CONH_2$	Deoxycholanamide (**3**)	Cholanamide (**4**)
$CONHCH_2COOH$	Glycodeoxycholic acid (**5**)	Glycocholic acid (**6**)
$CONHCH_3$	*N*-Methyldeoxycholanamide (**7**)	*N*-Methylcholanamide (**8**)

Fig. 1. Bile acids and their derivatives.

Compound **7** was prepared from the parent acid by the method described in literature.[4] The amide was recrystallized from over fifty different organic liquid substances, such as alcohols, esters, carboxylic acids, nitriles, and so on, as in the cases of **1**-**6**. The resulting crystals proved to include no organic substances, but to include semimolar water by means of thermal analysis and infrared spectroscopy. The thermal diagram had only one endothermic peak at 170-180 °C (a peak top at 175 °C), accompanied by a decrease of the corresponding weight. This indicates that water molecules are tightly fixed in the crystals. At present only one exception was observed in the case of ethylene glycol, which was included together with water in an equimolar ratio.

X-ray crystal structure analysis gave a direct evidence for such a specific inclusion of water by **7**. Single crystals suitable for the analysis were grown at room temperature by slow evaporation of alcoholic solutions of **7**.

The crystal of a 2:1 complex of **7** with water belongs to the monoclinic space group $C2$,[5] while the one of a 1:1 complex of **3** with 1-butanol belongs to the monoclinic space group $P2_1$.[3b] The observed bond distances and angles are the normal values for the standard steroidal compounds within experimental errors. The side chains adopt *gauche* conformations in both crystals. Fig. 2 shows the crystal packing of **7** viewed down along the crystallographic *b* axis. This top view is similar to that of the complex of **3**.[3b] The asymmetric host molecules associate in a *head*-to-*tail* and *right*-to-*left* fashion to give amphiphilic sheets, which are stacked in an antiparallel fashion to form a bilayered structure. The bilayers of **7** are closely stacked to leave no channels among the bilayers and to include water on the hydrophilic sites. On the other hand, the bilayers of **3** slide each other on the lipophilic sites to leave channels where organic guests are included.

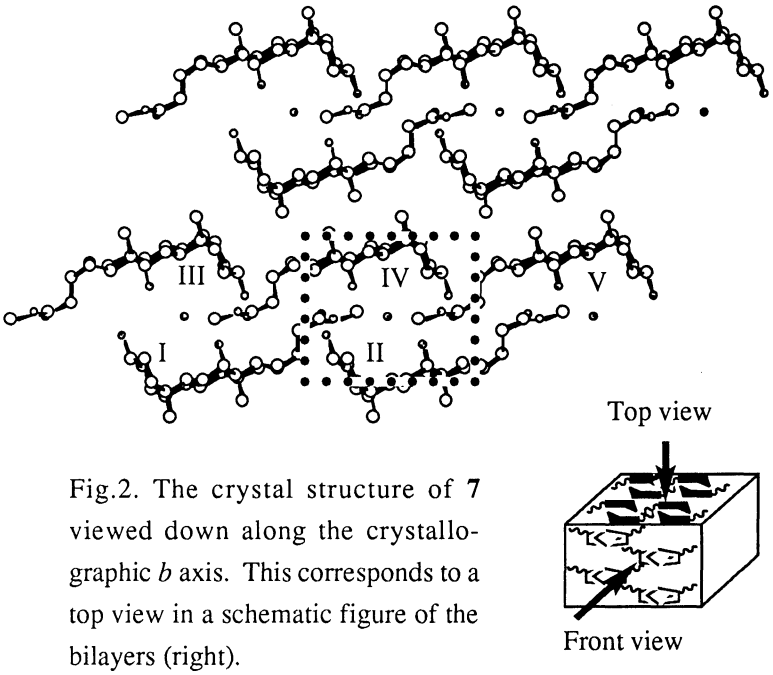


Fig.2. The crystal structure of **7** viewed down along the crystallographic *b* axis. This corresponds to a top view in a schematic figure of the bilayers (right).

However, front views (see Fig. 2) of each hydrophilic layer exhibit a great difference in molecular arrangements. Figs. 3 and 4 show the views of **3** and **7**, respectively. Fig. 3 shows no overlapping of steroidal skeletons on the basis of a rectangular arrangement, which is common in the crystals of the inclusion compounds of **1**, **2**, **3** and **4**. In contrast, Fig. 4 shows overlapped skeletons on the basis of a rhombic arrangement. The latter arrangement does not bring about channels between the bilayers, leading to disappearance of the inclusion abilities against organic substances. Similar phenomenon was observed in the case of 5β-petromyzonol (R_1= CH_2OH; R_2=OH in Fig. 1).[3c]

Hydrogen bonding networks of **3** and **7** are compared in Fig. 5, where only the participating groups framed in Figs. 2, 3 and 4 are designated. Each number (I-V) indicates the identical molecule in each figure. It can be seen that **3** forms cyclic networks which are bridged by alcoholic molecules. The one cycle has the sequence of OH[C(3)]---OH[C(12)]---O=C(24)-NH---OH[C(3)], where the distances are 2.904(6), 2.738(6) and 3.058(8) Å, respectively. The alcoholic molecule is connected by double hooks of NH---OH(Guest)---OH[C(12)], where the distances are 2.88(1) and 3.03(1) Å, respectively. On the other hand, **7** forms a ladder-like network where water molecules connect bars of both sides. The network has the sequences of NH---OH[C(3)]---O=C(24) and OH(H_2O)---OH[C(12)]---O=C(24), where the distances are 3.045(9), 2.960(8), 3.063(7) and 2.798(7) Å, respectively.

Such a difference of the networks can be correlated by the following transformation of molecular arrangements. Thus, the introduction of *N*-methyl group inhibits the cyclic hydrogen bonding employed in the case of **3**. So, another combinations among hydroxyl groups of the host molecules are required for a favourable donor-acceptor relationship. It can be seen from comparison between Figs. 3 and 4 that when a series of molecules (I and I', IV and IV') are fixed, another series of the molecules (II and II', III and III', V and V') slide each other by half

unit of the crystal along the crystallographic *b* axis. This sliding causes a replacement of guest alcohols by hydroxyl groups at C-3 positions of the latter molecules (II and II', III and III', V and V'). Another hydroxyl groups at C-12 positions of the identical molecules simultaneously moves near hydroxyl groups at C-12 positions of the former molecules (I and I', IV and IV'). These proximate hydroxyl groups at C-12 positions are connected by water molecules. In this way the comparison of the two crystal structures gives us a reasonable explanation for the correlation among the chemical structures, the molecular assemblies and the inclusion behaviours.

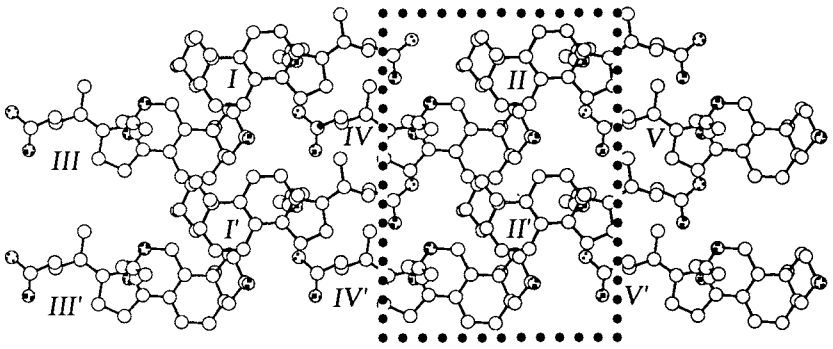


Fig.3. Stacking of amphiphilic sheets of **3** on hydrophilic sides. This corresponds to a front view in a schematic figure of the bilayers of Fig. 2.

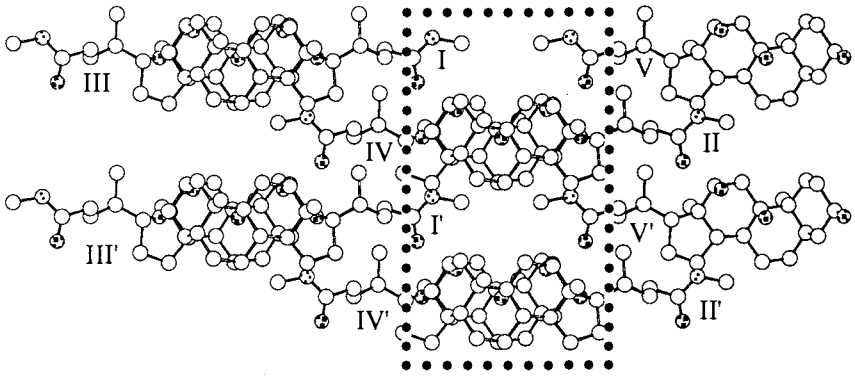


Fig.4. Stacking of amphiphilic sheets of **7** on hydrophilic sides. This corresponds to a front view in a schematic figure of the bilayers of Fig. 2.

Compounds **1**[6] and **2**[7] are known to form hydrates together with or without organic substances. However, we usually obtain the inclusion compounds with alcoholic guests instead of the hydrates, when we recrystallize the hosts as well as **3** and **4** from various alcohols containing

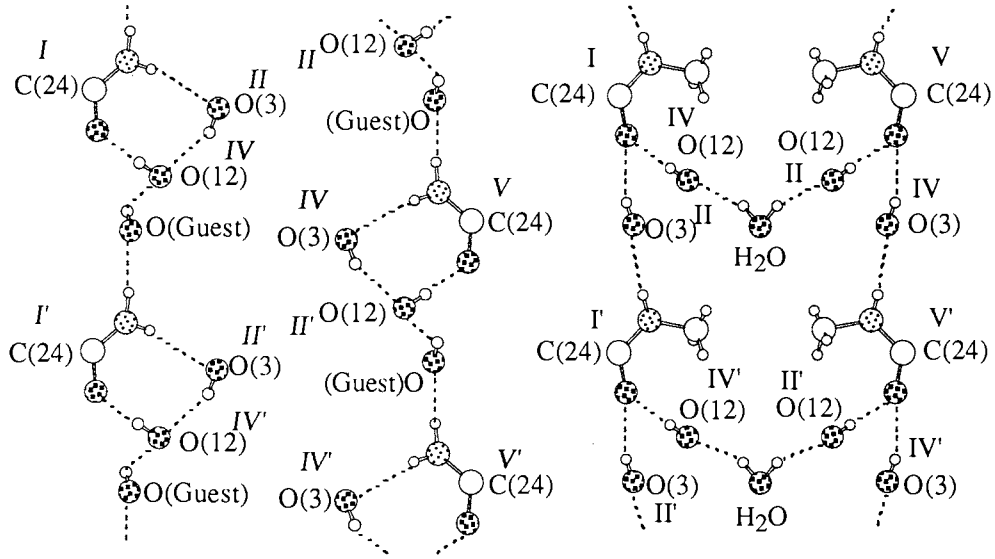


Fig.5. Schematic representation of hydrogen bonding networks of **3** (left) and **7** (right).

a relatively large amount of water. This indicates that these hosts form the inclusion compounds with organic substances rather than water. In contrast, it is noteworthy that 5β-petromyzonol forms the inclusion compounds with neither organic substances nor water.[3e] Further studies[8] display that *N*-methylcholanamide (**8**) also includes water rather than organic substances, and that *N*, *N*-dimethyldeoxycholanamide includes neither organic substances nor water.

In conclusion, this study demonstrates that the inclusion behaviours of bile acids and their derivatives vary from one case to another, depending on the functional groups of the steroidal side chains. The acids and amides are flexible hosts, while *N*-methylamides are inflexible hosts. Finally, such behaviours of the hosts remind us of proteins, which have three-dimensional structures sensitive to pendent groups of amino acids.

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5) Crystal data for **7**-H_2O(2:1): $C_{25}H_{43}NO_3$+0.5H_2O, M=414.63, monoclinic $C2$, a=25.019(4), b=7.565(4), c=14.215(3) Å, β =118.71(1)°, V=2359(1) Å^3, Z=4, Dc=1.167 g cm^{-3}. Intensity data were collected by the ω-2θ scan mode with 2θ up to 55.0° on a Rigaku AFC-7R automated four-circle diffractometer using graphite-monochromatized Mo-Kα radiation. The structure was solved by direct methods (SHELXS-86) and refined by the full-matrix least-squares method to the final R value of 0.049 for 1811 [|Fo|>3σ(|Fo|)] reflections. Atomic coordinates, bond lengths and angles will be deposited at the Cambridge Crystallographic Data Centre.

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8) **8** as well as *N*, *N*-dimethyldeoxycholanamide have similar bilayered structures in their crystals, but have different hydrogen bonding networks and molecular arrangements from **7**. Details will be published.

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