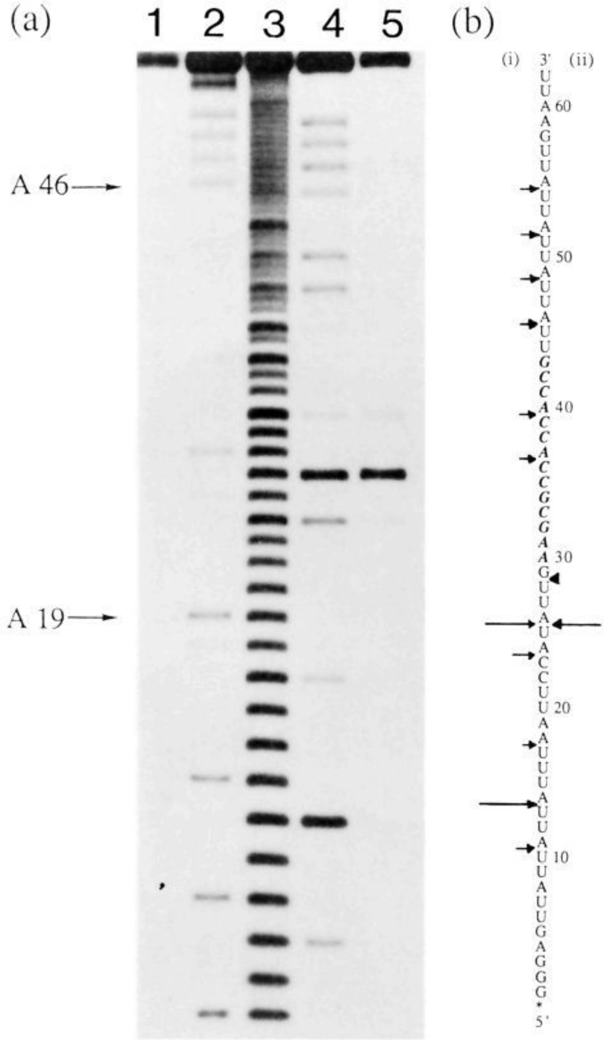

Figure 2. (a) Autoradiogram of a 25% denaturing polyacrylamide gel showing site-selective cleavage of a 5′ end-labeled 62mer of RNA. Lane 1, RNA only; lane 2, partial RNase U2 digest (A specific); lane 3, hydroxide cleavage ladder; lane 4, RNase S digest (in 20% formamide buffer); lane 5, hybrid enzyme digest: to 7 μL of a solution of end-labeled RNA (1.6 pmol) containing 2 μL deionized formamide in 50 mM Tris·Cl, 50 mM NaCl, 0.1 mM EDTA, pH 7.0, was added 3 μL of the hybrid enzyme solution (1.0 pmol) at 0 °C. The reaction was quenched after 3 s by the addition of 12 μL of formamide preheated to 95 °C. (b) Histogram indicating the cleavage pattern of (i) RNase S and (ii) the hybrid enzyme. The heights of the arrows indicate the relative cleavage intensities at the indicated bases, and the italicized sequence designates the oligonucleotide binding site.

by runoff transcription.[2] The transcript was end-labeled on the 5′ terminus with γ-^{32}P ATP and T4 polynucleotide kinase and purified on a 15% denaturing polyacrylamide gel. Cleavage of the end-labeled RNA was analyzed by high resolution denaturing polyacrylamide gel electrophoresis (Figure 2a).

Inspection of the histogram of the cleavage patterns produced by the hybrid enzyme (Figure 2b) reveals that the oligonucleotide binding domain site-selectively delivers the catalytic activity of RNase S to RNA. Cleavage occurs primarily at one pyrimidine–purine site adjacent to the site of oligonucleotide hybridization. In contrast, cleavage of RNA by RNase S, in the presence or absence of the free 14 nucleotide oligomer, occurs relatively nonselectively at 5′-pyrimidine–purine-3′ sites, UpA being the preferred cleavage site. These results demonstrate that the rate of hybridization and subsequent cleavage by the hybrid ribonuclease is more rapid than the rate of nonselective RNase S cleavage. High cleavage selectivity has thus been obtained by combining the specificity of the oligonucleotide with the inherent specificity of RNase S. Addition of S-protein to a preformed *S*-peptide–oligonucleotide RNA complex did not afford selective cleavage. Unfortunately, hybridization and cleavage by the hybrid enzyme at elevated temperatures (>37 °C) leads to diminished cleavage efficiency and specificity, most likely due to dissociation of the S-protein from the peptide–oligonucleotide adduct. Direct fusion of an oligonucleotide binding site to RNase A either via a bifunctional crosslinker or by construction of a Cys1 RNase A mutant may lead to hybrid enzymes capable of catalytically cleaving RNA's at any predefined site.

In conclusion, this work not only helps define chemical strategies for rationally altering enzymic properties but also may lead to a new class of sequence-specific ribonucleases for studying RNA structure and function.

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New Channel-Type Inclusion Compound of Steroidal Bile Acid. Structure of a 1:1 Complex between Cholic Acid and Acetophenone

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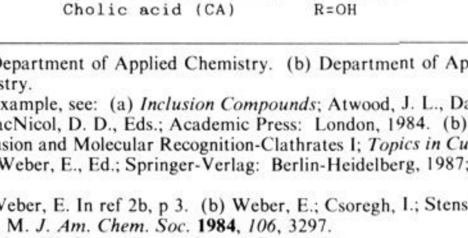
Special attention has been drawn recently to crystal inclusion chemistry which involves a variety of viewpoints.[2,3] Among lattice-type inclusion compounds,[3] typical steroidal bile acids, deoxycholic acid (DCA), and apocholic acid (ACA) are classical compounds in host–guest chemistry.[4] The crystal structures of DCA and ACA with guest molecules provide tunnel-like spaces usually called "channels".[5,6] Many crystallographic studies have indicated that the DCA channels can accommodate a wide variety of organic molecules[5] and also an organometallic compound such as ferrocene.[7] It is interesting to use the host–guest interaction in this channel as a model for reaction space in the solid state such as novel host–guest reactions[8] and inclusion polymerizations.[9]



Deoxycholic acid (DCA) R=H
Apocholic acid (ACA) R=H (8,14 en)
Cholic acid (CA) R=OH

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(1) (a) Department of Applied Chemistry. (b) Department of Applied Fine Chemistry.

(2) For example, see: (a) *Inclusion Compounds*; Atwood, J. L., Davies, J. E. D., MacNicol, D. D., Eds.; Academic Press: London, 1984. (b) Molecular Inclusion and Molecular Recognition-Clathrates I; *Topics in Current Chemistry*; Weber, E., Ed.; Springer-Verlag: Berlin-Heidelberg, 1987; Vol. 140.

(3) (a) Weber, E. In ref 2b, p 3. (b) Weber, E.; Csoregh, I.; Stensland, B.; Czugler, M. *J. Am. Chem. Soc.* **1984**, *106*, 3297.

(4) Herndon, W. C. *J. Chem. Educ.* **1967**, *44*, 724.

(5) For a review, see: Giglio, E. in ref 2a, Vol. II, p 207.

(6) Popovitz-Biro, R.; Tang, C. P.; Chang, H. C.; Lahav, M.; Leiserowitz, L. *J. Am. Chem. Soc.* **1985**, *107*, 4043.

(7) Miki, K.; Kasai, N.; Tsutsumi, H.; Miyata, M.; Takemoto, K. *J. Chem. Soc., Chem. Commun.* **1987**, 545.

(8) (a) Chang, H. C.; Popovitz-Biro, R.; Lahav, M.; Leiserowitz, L. *J. Am. Chem. Soc.* **1987**, *109*, 3883. (b) Aoyama, H.; Miyazaki, K.; Sakamoto, M.; Omote, Y. *J. Chem. Soc., Chem. Commun.* **1983**, 333.

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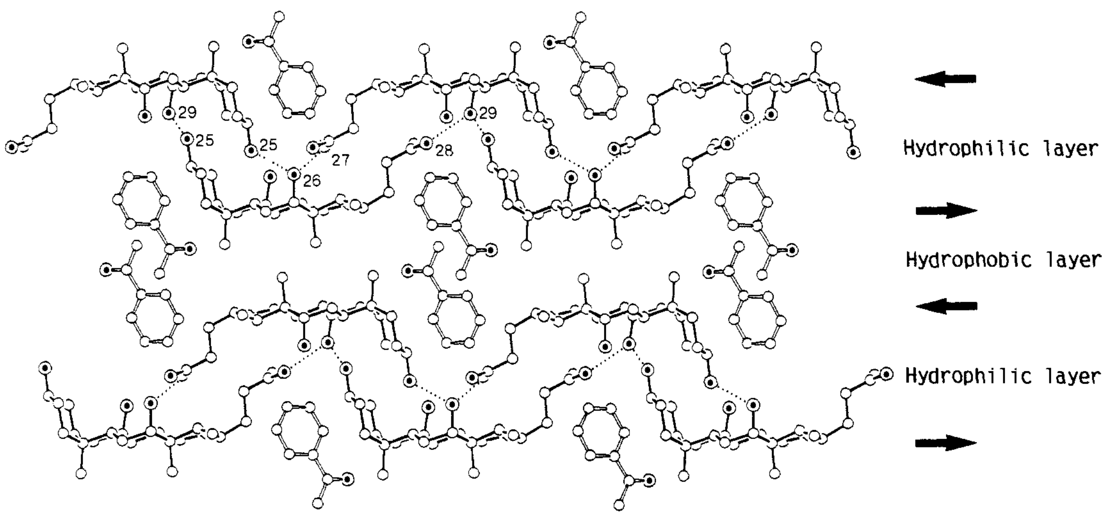

Figure 1. The crystal structure of the inclusion compound between cholic acid (CA) and acetophenone as viewed down along the crystallographic *b* axis. Carbon and oxygen atoms are represented by empty and half-filled circles, respectively. The hydrogen bond network, together with the numbering scheme of atoms concerned, is shown by dotted lines. The arrows indicate the direction from the *head* to *tail* (see text) in the layered CA molecules.

We have been studying a series of inclusion compounds of bile acids and their derivatives to develop new types of host molecules which provide new inclusion spaces for molecular recognition and reaction.[10] Here we report the structure of an inclusion compound between cholic acid (CA) and acetophenone established by X-ray crystallography, which demonstrates that a new channel-type inclusion phenomenon is provided by CA molecules for acetophenone and breaks down a "myth" that such channel-type inclusion phenomena of bile acids are a property of DCA and ACA alone.[4]

The needle-like crystals of the present inclusion compound were obtained from a solution of CA in acetophenone. The crystal structure is depicted in Figure 1.[11] The most striking structural feature is that the acetophenone molecules are accommodated into the channels formed by the CA host molecules. The CA molecules are arranged in a one-dimensional array so that the OH and CH_3 groups associate to form hydrophilic and hydrophobic layers, respectively. Such amphiphilic layered structures are observed also in the DCA and ACA crystal structures, but the direction of parallel layers formed by DCA and ACA is different from those of CA in the present structure; i.e., the direction from *head* to *tail* is the same in the two layers of host molecules which form the hydrophilic array of DCA and ACA. The two sides of the molecule, that near the steroidal A ring and that of methylene and COOH chains, are distinguished as the *head* and *tail*, respectively. In contrast, the CA molecules are arranged in *antiparallel* fashion to form hydrophilic layers as shown by the arrows in Figure 1. Within the hydrophilic layers, four kinds of hydrogen bonds are linked to form three-dimensional rigid networks with the cyclic sequence of $-\mathrm{O}(26)^{\mathrm{I}}-\mathrm{H}\cdots\cdots\mathrm{O}(27)^{\mathrm{II}}=\mathrm{C}(24)^{\mathrm{II}}-\mathrm{O}(28)^{\mathrm{II}}-\mathrm{H}\cdots\cdots\mathrm{O}(29)^{\mathrm{III}}-\mathrm{H}\cdots\cdots\mathrm{O}(25)^{\mathrm{IV}}-\mathrm{H}\cdots\cdots\mathrm{O}(26)^{\mathrm{I}}$.[13] The O···O distances are 2.870 (5), 2.700 (5), 2.727 (4), and 2.881 (4) Å, respectively. This network is in contrast to that in the DCA or ACA structures where three kinds of hydrogen bonds are formed.[5] In addition, the present crystal structure is different from that of the complex between CA and ethanol, the only other known structure of CA including an organic guest,[14] where no channel or layered arrangement is formed.[15] In comparing the channel properties of the present CA structure with those of DCA with acetophenone,[17] the molar ratio of host/guest of the latter is 5:2, while that of the

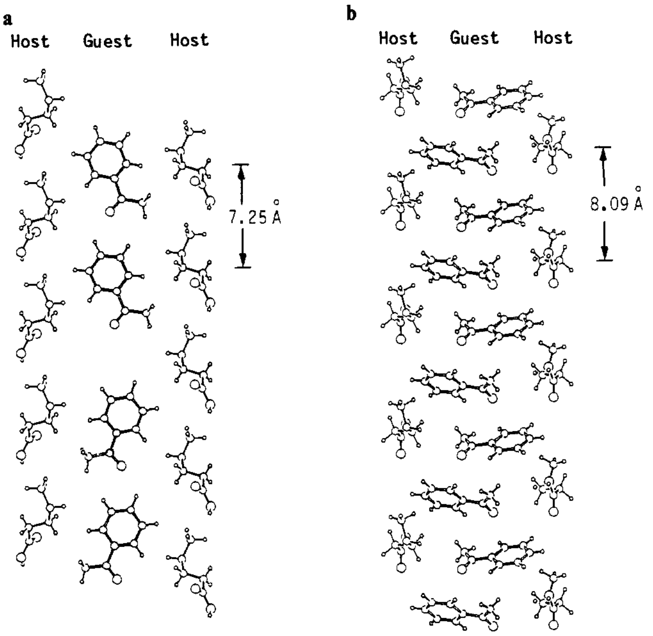

Figure 2. Schematic drawings of the channels of deoxycholic acid (DCA) and cholic acid (CA) including acetophenone molecules as guests. The channel walls are represented as the steroidal side chains: (a) DCA–acetophenone 5:2 complex[17] and (b) CA–acetophenone 1:1 complex (this work).

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(9) For example, see: (a) Takemoto, K.; Miyata, M. *J. Macromol. Sci.-Rev.* **1980**, *C18*, 83. (b) Miyata, M.; Noma, F.; Okanishi, K.; Tsutsumi, H.; Takemoto, K. *J. Inclusion Phenom.* **1987**, *5*, 249. (c) Farina, M. ref 2a, Vol. III, p 297.

(10) (a) Miyata, M.; Goonewardena, W.; Shibakami, M.; Takemoto, K.; Masui, A.; Miki, K.; Kasai, N. *J. Chem. Soc., Chem. Commun.* **1987**, 1140. (b) Miyata, M.; Shibakami, M.; Goonewardena, W.; Takemoto, K. *Chem. Lett.* **1987**, 605. (c) Miyata, M.; Shibakami, M.; Takemoto, K. *J. Chem. Soc., Chem. Commun.* **1988**, 655.

(11) Crystal data: $C_{24}H_{40}O_5 + C_8H_8O$, fw = 528.73, monoclinic, space group $P2_1$, $a = 13.719$ (2) Å, $b = 8.093$ (1) Å, $c = 14.229$ (2) Å, $\beta = 113.689$ (7)°, $V = 1446.7$ (3) $Å^3$, $Z = 2$, $D_x = 1.214$ g cm^{-3}. The structure solved by the direct method (MULTAN-78[12]) was refined to $R = 0.045$ for 2175 reflections collected up to sin $\theta/\lambda = 0.55$ $Å^{-1}$ (Cu Kα radiation) on a diffractometer. All the computations were done on an ACOS 850 computer at the Crystallographic Research Center, Institute for Protein Research, Osaka University.

(12) Main, P.; Hull, S. E.; Lessinger, L.; Germain, G.; Declercq, J. P.; Woolfson, M. M. MULTAN-78: *A System of Computer Programs for the Automatic Solution of Crystal Structures from X-ray Diffraction Data*; University of York, England, and Louvain, Belgium, 1978.

(13) Key for equivalent positions; I: $(x, -1 + y, z)$, II: $(1 - x, -{}^1/_2 + y, 1 - z)$, III: (x, y, z), IV: $(2 - x, -{}^1/_2 + y, 2 - z)$.

(14) Johnson, P. L.; Schaefer, J. P. *Acta Crystallogr.* **1972**, *B28*, 3083.

(15) Other than the CA structure with ethanol,[14] only a structure without any organic guests has been reported, where CA is crystallized in a monohydrated form providing a layered arrangement of CA,[16] but both hydrogen bonds and hydrophobic contacts between layers are different from those found in the present crystal structure.

(16) Lessinger, L. *Cryst. Struct. Commun.* **1982**, *11*, 1787.

(17) Tang, C. P.; Chang, H. C.; Popovitz-Biro, R.; Frolow, F.; Lahav, M.; Leiserowitz, L.; McMullan, R. K. *J. Am. Chem. Soc.* **1985**, *107*, 4058.

former is 1:1. As shown in Figure 2, the periodic depths of the channels, which correspond to one of crystallographic axes, are 8.09 and 7.25 Å for the former and latter, respectively. These facts, together with the qualitative comparison of channel sizes for acetophenone molecules, indicate that the channel space formed by the CA molecules is much larger than that of DCA and also slightly larger than that of ACA. As shown also in Figure 2, the acetophenone molecules are accommodated in the channel of the host molecules so that their phenyl planes are parallel to the channel direction in DCA, whereas they are almost perpendicular to this direction in CA. This shows that the host–guest interaction in the CA channels is somewhat different from those of hitherto investigated DCA and ACA channels.

In conclusion, contrary to the long-accepted concept that it is a unique ability of DCA and ACA to form stable channel-type inclusion compounds,[4] the present study demonstrates that a new channel-type inclusion phenomenon which is different in the channel size and host–guest interaction character can be provided also by CA.

Acknowledgment. The present study was partially supported by the Grant-in-Aids for Scientific Research from the Ministry of Education, Science and Culture.

Supplementary Material Available: Tables of fractional atomic coordinates and interatomic bond distances and angles (5 pages). Ordering information is given on any current masthead page.

Bis((pentamethylcyclopentadienyl)metal)pentalenes. A New Class of Highly Delocalized, Fused Metallocenes

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There is considerable interest in bridged metallocenes arising from the unusual properties of mixed-valence molecules[1] and the potential for new types of catalytic reactions involving multielectron transfer. However, a generally applicable, rational synthesis of such compounds has not yet been developed. With the exception of derivatives of fulvalene[2] and *as*-indacene,[3] most bridged ferrocenes have been obtained directly from $(\eta^5\text{-}C_5H_5)_2Fe$. Moreover, examples of binuclear metallocenes of other transition elements are limited to the fulvalene-bridged compounds reported by Smart and co-workers,[4] $(\eta^3\text{-}C_3H_5)Ni(\text{pentalenyl})Ni(\eta^3\text{-}C_3H_5)$,[5] bis(pentalenyl)dinickel,[6] and bis(pentalenyl)dicobalt.[7]

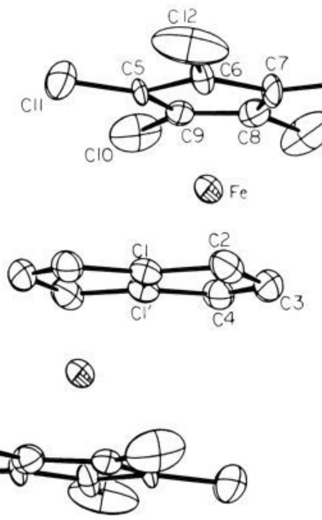


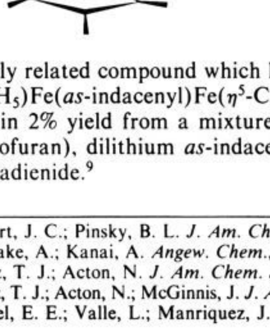
Figure 1. Molecular geometry and atom labeling scheme for **1a**. Selected bond distances (Å) are as follows: Fe–C(1) = 2.120 (4), Fe–C(1)′ = 2.117 (3), Fe–C(2) = 2.025 (3), Fe–C(3) = 2.016 (4), Fe–C(4) = 2.023 (5), C(1)–C(1)′ = 1.464 (5), C(1)–C(2) = 1.436 (4), C(2)–C(3) = 1.421 (7), C(3)–C(4) = 1.408 (5).

Table I. Cyclic Voltammetry Data[a]

compd	$E_{1/2}$[b]	$E_{1/2}$[c]	ΔE[d]
1a	−0.665	0.335	1.011
1b	−1.545	−0.660	0.890
1c	−1.245	−0.405	0.830

[a] Supporting electrolyte is 0.1 M NBu_4ClO_4. All potentials in volts referenced to $Ag/AgNO_3$. Temperature 20 °C. Sweep rate 20 mv/s. Solvent CH_2Cl_2. [b] For the process $M(II)/M(II) \rightarrow M(II)/M(III) + e^-$. [c] For the process $M(II)/M(III) \rightarrow M(III)/M(III) + e^-$. [d] Obtained from differential-pulse voltammetry.

We recently reported the synthesis of (pentamethylcyclopentadienyl)acetylacetonate compounds of iron(II), cobalt(II), and nickel(II) and showed that they are convenient starting materials for the preparation of some mixed $(\eta^5\text{-}C_5Me_5)/(\eta^5\text{-}C_5H_5)$ derivatives.[8] We now report that they can also be used as starting materials in the synthesis of binuclear metallocenes of type **1**.



1a M=Fe
1b M=Co
1c M=Ni

The only related compound which has been reported to date is $(\eta^5\text{-}C_5H_5)Fe(as\text{-indacenyl})Fe(\eta^5\text{-}C_5H_5)$. This compound is obtained in 2% yield from a mixture of $FeCl_2\cdot THF$ (THF = tetrahydrofuran), dilithium *as*-indacenide, and excess lithium cyclopentadienide.[9]

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(1) Cowan, D. O.; Le Vanda, C.; Park, J.; Kaufman, F. *Acc. Chem. Res.* **1973**, *1*, 1.

(2) Mueller-Westerhoff, U.; Eilbracht, P. *J. Am. Chem. Soc.* **1972**, *94*, 9272.

(3) Gitany, R.; Paul, I. C.; Acton, N.; Katz, T. J. *Tetrahedron Lett.* **1970**, 2773.

(4) Smart, J. C.; Pinsky, B. L. *J. Am. Chem. Soc.* **1977**, *99*, 956.

(5) Miyake, A.; Kanai, A. *Angew. Chem., Int. Ed. Engl.* **1971**, *10*, 801.

(6) Katz, T. J.; Acton, N. *J. Am. Chem. Soc.* **1972**, *94*, 3281.

(7) Katz, T. J.; Acton, N.; McGinnis, J. *J. Am. Chem. Soc.* **1972**, *94*, 6205.

(8) Bunel, E. E.; Valle, L.; Manriquez, J. M. *Organometallics* **1985**, *4*, 1680.

0002-7863/88/1510-6596$01.50/0