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COMMUNICATION

Right-handed 2/1 Helical Arrangement of Benzene Molecules in Cholic Acid Crystal Established by Experimental and Theoretical Circular Dichroism Spectroscopy

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Heng Wang^a, Adriana Pietropaolo^{*b}, Wenbin Wang^{a,c}, Chen-Yi Chou^{a,d}, Ichiro Hisaki^c, Norimitsu Tohna^e, Mikiji Miyata^c, and Tamaki Nakano^{*a}

Right-handed two-fold helical (2/1 helical) arrangement of benzene included in the cavity of cholic acid (CA) crystal was directly established by experimental and theoretical circular dichroism (CD) spectral studies. Experimental spectra comprised chirality elements of benzene and CA and well agreed with the theoretical spectra which considered interactions between benzene and polar groups of CA.

Controlled supramolecular structures play important roles in artificial, biological, and medicinal functions,¹ and information on structural details is crucial in understanding properties. Identification of supramolecular structures, however, may not be as straightforward as that of covalently bound structures, which is especially true for solid-state supramolecules because conventional analytical techniques for solution cannot be applied. A typical example is inclusion crystals formed by cholic acid (CA). CA, an optically active, steroidal bile acid, can include a wide range of small molecules in its crystal lattice, including not only polar ones which can form intermolecular hydrogen bond with the hydroxyl and carboxyl groups of CA molecules, but also non-polar ones including benzene through hydrophobic interactions (Chart 1).²⁻⁷ Though the supramolecular structures of CA inclusion crystals are intriguing from a view of supramolecular chirality, chirality information cannot be obtained by conventional chiroptical measurements in solution because the supramolecular chirality can exist only in the crystal.

Benzene molecules form an inclusion complex with CA⁸ and have been proposed to be arranged as right handed 2/1 helix in the crystal cavity at ambient temperature without any directional non-covalent bonds such as hydrogen bonds or coordination bonds to support the helical structure.⁹ Chirality of aligned benzene molecules arises from single-handed inclination of their molecular planes along the helical axis leading to a slipped herringbone arrangement lacking glide plane symmetry.^{9,10} Such symmetry is commonly present in 2/1 symmetrical assemblies of benzene single crystal.¹¹ The discovery of benzene-CA complex has expanded the scope of supramolecular chirality.

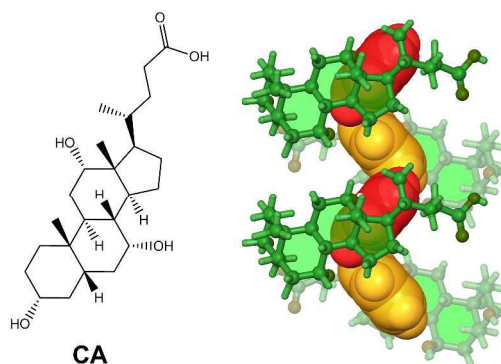


Chart 1. Structures of cholic acid (CA) (left) and 2/1 helical inclusion crystal of CA with benzene (right) where CA and benzene are indicated in green and red-yellow, respectively.

However, the single-handedness of benzene helical arrangement in CA crystal has not been directly identified through chiral property measurements; it has been only deduced from X-ray diffraction (XRD) results and the known absolute configuration of CA. Herein, we report the first, direct identification of the right-handed 2/1 helical arrangement of benzene molecules through a combination of experimental and theoretical solid-state circular dichroism (CD) spectral studies. CD spectroscopy detects chiroptical properties of materials whereas XRD requires anomalous scattering information

^a Institute for Catalysis (ICAT) and Graduate School of Chemical Sciences and Engineering, Hokkaido University, N 21, W 10, Kita-ku, Sapporo 001-0021, Japan. Email: tamaki.nakano@cat.hokudai.ac.jp; Fax: +81-11-7069156; Tel: +81-11-7069155.

^b Dipartimento di Scienze della Salute, Università di Catanzaro, Viale Europa, 88100 Catanzaro, Italy. Email: apietropaolo@unicz.it

^c On leave from College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China

^d On leave from Department of Chemical Engineering, National Cheng Kung University (NCKU), Tainan, Taiwan

^e Department of Material and Life Science, Graduate School of Engineering, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan.

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to determine handedness of chirality which is generally difficult for a system having no heavy atoms.

In this work, diffuse reflectance CD spectroscopy method was employed; this method allows measurements of solid-state samples.¹²⁻¹⁴ In addition, although chirality of aromatic ketones,¹⁵ *N*-aryl-*N*-nitrosamines¹⁶ and diaryl dichalcogenides¹⁷ in the inclusion crystals of CA has been studied by CD spectra, the observed chirality is due to chiral molecular conformation, *not supramolecular alignment*, of these compounds whose internal rotation may be hampered in the CA cavity. In sharp contrast, benzene cannot have a chiral conformation and can be chiral only through molecular arrangement of plural molecules. Although UV absorptions of CA and benzene significantly overlap,^{18,19} this point was carefully overcome in this work by resolving CD signals through curve fitting. Furthermore, theoretical spectra firmly supported the experimental results ruling out the possibility of anisotropic effects including linear dichroism (LD) which may contaminate CD spectra.

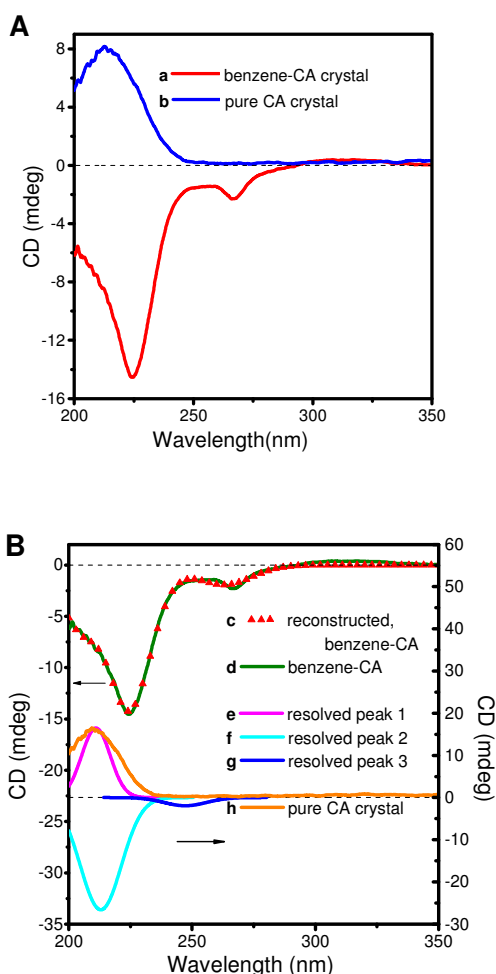


Fig. 1. CD spectra of benzene-CA inclusion crystal (a) and pure CA crystal (b) recorded [panel A] and peak resolution analysis of the benzene-CA spectrum by curve fitting [panel B]: reconstructed spectrum from the resolved peaks (c) and experimental spectrum (d) of benzene-CA crystal, resolved peaks obtained by Gaussian curve fitting (e, f, g), and experimental spectrum of pure CA crystal (h).

Benzene-CA inclusion crystal was prepared by adding an excess amount of benzene into a hot butanol solution of CA, which afforded needle-like crystals on standing at room temperature (ca. 20 °C). The inclusion crystal was characterized by ¹H NMR and thermal gravity analyses (ESI, as Figs. S1 and S2). The NMR spectrum indicated the molar ratio of CA to benzene in the inclusion crystal is approximately 1:1. TGA indicated endothermic transitions at 116 °C corresponding to release of benzene molecule which is much higher than its boiling point (80 °C). Also, the observed weight loss was 14.9%, which is close to the ideal value (16%) of 1:1 benzene-CA crystal. These results are in good agreement with those in the earlier report about benzene-CA inclusion crystal.⁸

Scattering CD spectra of benzene-CA inclusion crystal were measured using fine particles (particle size ca. 30~100 μm) obtained by grinding the crystal (ESI, Fig. S13). The spectrum is shown along with that of pure (guest-free) CA crystal as a reference sample in Fig. 1A.⁵ In order to minimize non-chiral anisotropic effects, four spectra were taken for each sample at different cell orientations at 90° interval of the cell quartz window positioned vertically to the incident light beam for measurement, and the spectra were averaged. Further, LD spectra of the two samples were compared with the CD spectra (ESI, Figs. S3-6); LD spectral patterns were completely different from the CD spectra patterns, indicating that the CD spectra reflect molecular chirality of the samples. On the contrary, crystals as obtained by recrystallization (particle size ca. 1~4 mm) led to a CD spectrum with a largely distorted baseline and to greater LD intensity compared with the finely ground sample (ESI, Fig. S9-12). Pure CA crystal showed a positive Cotton effect centered at 214 nm, which should reflect the configurational chirality of CA molecules and their 2/1 helical assembly. In a sharp contrast, the benzene-CA inclusion crystal showed an intense negative Cotton effect centered at 225 nm and a relatively weak negative Cotton effect at around 267 nm which correspond to the E2-band (200 nm) and B-band (260 nm) of benzene's absorption spectrum, respectively, indicating that the chiroptical properties detected by the spectrum are ascribed to benzene, not to CA. Because the high symmetry of each benzene molecule is maintained in the benzene-CA inclusion crystal, the Cotton effects are based on the chiral assembly of benzene molecules.

It is noteworthy that CD spectra of benzene-CA inclusion crystal and CA measured using nujol mulls were very similar in shape to those taken by the diffuse reflectance method (ESI, Figs. S22 and S23). These results support that the CD spectra obtained by the diffuse reflectance mode are reliable in establishing chiral alignment of benzene molecules in CA crystal.

In order to obtain more detailed information on the CD spectrum of the benzene-CA inclusion crystal, the spectrum was resolved into three fractions by Gaussian curve fitting (Fig. 1B). The CD curve of benzene-CA crystal can be resolved into three individual bands at 215 nm (Band 1, positive sign), 217 nm (Band 2, negative sign) and 263 nm (Band 3, negative sign), separately. As the peak position of Band 1 well matches that of the CA Cotton effect at 213 nm, Band 1 is considered to reflect chirality of CA in the benzene-CA crystal. On the other hand, Band 2 at 217 nm and Band 3 at 263 nm are assigned to 2/1 helical arrangement of benzene molecules included in CA crystal.

Further, the solvent accessible surface of the benzene-CA crystal tightly fits the arrangement of the benzene molecules inside the inclusion channel (ESI, Fig. S27), indicates that it is not possible for the included benzene molecules to move significantly enough within

the cavity to alter the handedness of the 2/1 helix. This aspect reinforces the above conclusion that CD bands based on benzene is ascribed to the single-handed helical arrangement and rules out the possibility that molecular motion of benzene molecules affects CD spectra.

Regarding CD spectra of aligned benzenes, those of *P*-(-)-hexahelicene²⁰ and a right-handed helical poly(*o*-phenylene) derivative²¹ have been reported. Although direct comparison between these two systems with benzene-CA is difficult since benzenes are conjugated in these two systems and isolated in the benzene-CA inclusion crystal, negative Cotton effects at around 250 nm seem common in the three systems (263 nm for the benzene-CA crystal, 244 nm for *P*-hexahelicene, and 265 nm for the poly(*o*-phenylene) derivatives). These negative Cotton effects may arise from the π - π^* transition individual of benzene rings and may be characteristic of right-handed helical arrangement of benzene molecules.

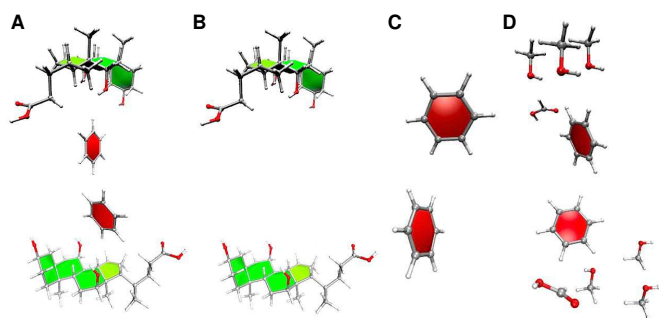


Fig. 2. Two benzene molecules in 2/1 helical arrangement in the cholic acid scaffold (A); two CA molecules without benzene (B); two benzene molecules in the 2/1 helical arrangement without CA (C); two benzene molecules in the 2/1 helical arrangement with methanols and formic acids as partial structures of CA (D).

In order to confirm the validity of the discussions described so far, CD spectra were theoretically calculated on model systems (Fig. 2). The calculation was performed in TDDFT framework using the Grimme's functional B97D.^{22, 23} The models used for calculations were extracted from the benzene-CA crystal X-ray structure coordinate (Fig. 2A), namely, two CA molecules (Fig. 2B), two benzene molecules (Fig. 2C), two benzene molecules with functional groups of CA which are represented by six methanol molecules and two formic acid molecules (Fig. 2D). The last model designed to save computer resources is closest to the real benzene-CA crystal system; omitting the aliphatic backbone of CA is considered to be reasonable as they would not significantly interact with π -electrons of benzene. Fig. 3 compares the theoretical spectra with the experimental spectra. The theoretical spectrum (Fig. 3A) of the two CA molecules model (Fig. 2B) has a relatively intense positive Cotton band at 215 nm which roughly matches the peak position of the positive Cotton band in the experimental spectrum of CA crystal while weaker negative Cotton bands were seen in the range of 225–250 nm in the theoretical spectrum which are absent in the experimental spectrum (Fig. 3A). The theoretical spectrum (Fig. 3B) of the model with two benzenes with functional groups of CA (Fig. 2D) well matched the experimental spectrum with two negative Cotton bands reproduced though the theoretical Cotton band at ca. 250 nm was of a higher energy compared with the corresponding experimental signal at ca. 270 nm (Fig. 3B). The “blue shift” effect

in theoretical spectra is commonly observed for most theoretical frameworks.^{24,25} In a sharp contrast, the theoretical spectrum (ESI, Fig. S24) of the two benzenes model (Fig. 2C) had a negative Cotton band at 235 nm and a positive band at 205 nm and the spectral shape did not match the experimental spectrum.

The fact that the theoretical spectra of the systems containing only CA molecules and only benzene molecules have noticeably different patterns from those of the experimental spectra indicates that electronic interactions in the ground state between CA and benzene contribute to the CD spectra. Further, the well-matching theoretical and experimental CD spectra in Fig. 3B strongly support that benzene molecules in the benzene-CA crystal are in 2/1 helical arrangement from a chiroptical spectral view.

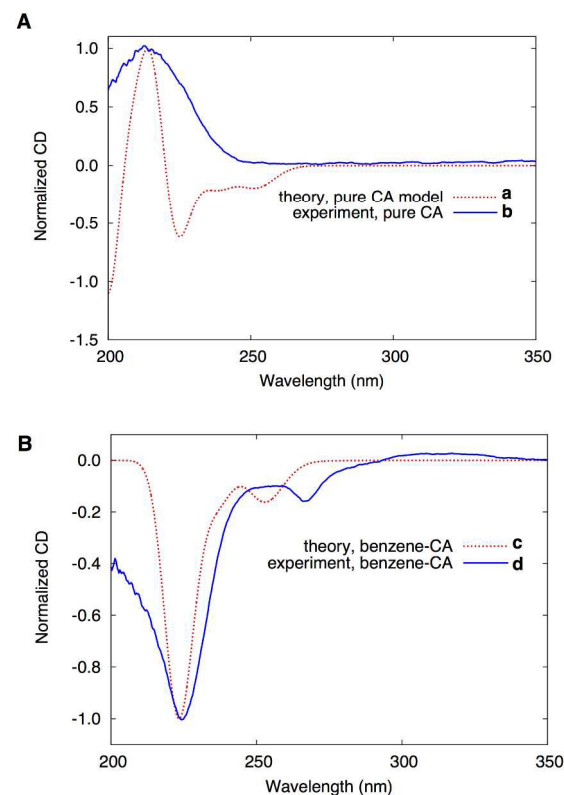


Fig. 3. Theoretical CD spectrum of two CA molecules model (Fig. 2B) (a) and experimental spectrum of pure CA crystal (b) [panel A], and theoretical spectrum of the benzene-CA crystal model (Fig. 2D) (c) and experimental spectrum of benzene-CA crystal (d) [panel B]. The experimental spectra are identical to those in Fig. 1 and are indicated here again for clarity in comparison.

Theoretical CD analysis also provided the following information. A model system with four benzene molecules with relevant functional groups of CA led to almost the same CD spectrum as that in Fig. 3B, indicating that the 2/1 helical arrangement in the real crystal can be sufficiently reproduced by the small system with only two benzenes and functional groups (ESI, Fig. S25). On the contrary, the theoretical CD spectrum of a model system with one benzene and functional groups was rather similar to the theoretical spectrum of the model with only CA molecules (ESI, Fig. S25). This result suggests that electronic interactions between benzene molecules in the 2/1 helical arrangement significantly contributes to the chiroptical properties.

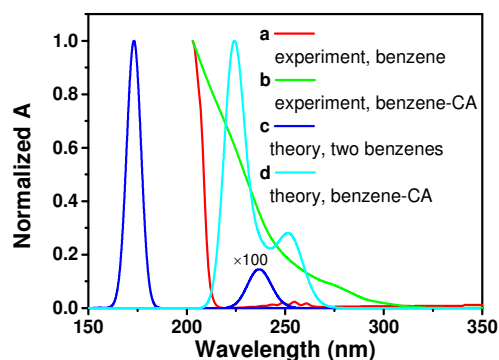


Fig. 4. Experimental absorbance spectra of benzene in a hexane solution (1×10^{-4} M, cell path 10 mm, ambient temperature) (a) and the benzene-CA crystal (scattering spectra obtained using CD spectrometer) (b), and theoretical absorbance spectra of the two benzenes model (Fig. 2C) (c) and the benzene-CA crystal model (Fig. 2D) (d).

The importance of CA-benzene and benzene-benzene electronic interactions mentioned above are further supported by theoretical absorption spectra of models C and D presented in Fig. 2 and experimental spectra of benzene in hexane solution and benzene-CA crystal (Fig. 4) and further by molecular orbital analyses (Fig. 5). As shown in Fig. 4, the two benzenes model (Fig. 2C) has a band centered at 238 nm corresponding to a HOMO-LUMO gap of 5.2 eV while the model with two benzenes with CA's partial structures (Fig. 2D) indicated a band at 258 nm corresponding to a HOMO-LUMO gap of 4.8 eV. These results are consistent with the experimental results that the benzene-CA crystal exhibited absorption bands in the longer wavelength range compared with benzene in hexane solution. Molecular orbital analyses (Fig. 5) suggested that the HOMO is localized in each benzene molecule while the LUMO is delocalized over two benzenes in both models presented Figs. 2C and 2D. Further, the "third-unoccupied" molecular orbital (LUMO+3) in the model in Fig. 2D is also delocalized. The delocalization in LUMO and LUMO+3 is consistent with the proposed benzene-benzene electronic interactions. The presence of methanols and formic acids raises the HOMO level in model D and decreases the level of LUMO and LUMO+3; the presence of the polar molecules breaks the symmetry of LUMO which is present in model C.

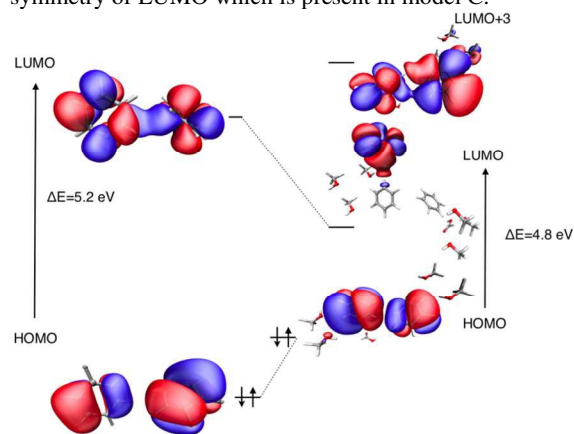


Fig. 5. Diagram of the frontier orbitals corresponding to the models in Fig. 2C (left) and Fig. 2D (right).

In conclusion, right handed helical assembly of benzene molecules in the cavity of CA crystal was directly established by experimental and theoretical CD spectra of the benzene-CA crystal for the first time, although the helical arrangement had been proposed only on the basis of crystal structure and absolute configuration of CA. A finely ground powder sample was indispensable in obtaining CD spectra with minimal anisotropy effects which do not arise from molecular chirality. The methodology combining experimental and theoretical chiroptical properties analyses presented here would be applicable to unveil chiral intermolecular structure of a variety of solid-state supramolecular materials.

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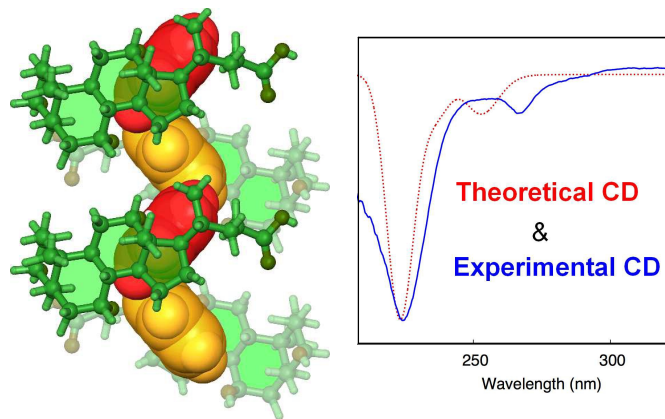
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TOC graphic



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