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Mechanochemical Complexation between Deoxycholic Acid and Salicylic Acid

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Abstract. A complex between deoxycholic acid (DCA) and salicylic acid (SA) was prepared by grinding and coprecipitation methods. The resultant complex was characterized by means of powder X-ray diffractometry, IR spectroscopy and thermal analysis. The stoichiometry (DCA : SA 1 : 1) of the complex obtained by grinding was identical to that obtained by coprecipitation. The powder X-ray diffraction pattern of the DCA–SA complex differed from the typical pattern of DCA–guest complexes such as DCA–camphor and DCA–phenanthrene complexes. IR spectra suggested that a different kind of hydrogen bonding was formed in the crystal of the DCA–SA complex, compared with the other DCA–guest complexes. This was in good agreement with data from the crystal structure.

Key words: deoxycholic acid, salicylic acid, complex, mechanochemistry, grinding.

1. Introduction

Deoxycholic acid (DCA), 3α,12α-dihydroxy-5-β-cholan-24-oic acid, is one of the bile acids which can be found in various types of mammals. It is well known that it plays an important role in the absorption of lipid by micellar formation. The DCA molecule contains hydrophilic and hydrophobic moieties. The hydrophilic moiety consists of one carboxyl group and two hydroxyl groups. This unique molecular structure is not only responsible for the micellar formation but also affects other chemical properties, including complexation. DCA possesses an ability to form complexes with many guest compounds, such as: fatty acids [1], aromatic compounds [2], ketones [3] and organometallic compounds [4]. These complexes have different stoichiometry and crystal forms, depending on the size, shape, and polarity of the guest molecules. Giglio reported that DCA crystal structures were divided into three groups: orthorhombic, tetragonal and hexagonal [5]. The orthorhombic structure seems to be the most appropriate for forming channel-type complexes with various guests. In this system, intermolecular hydrogen bonding between the carboxyl and hydroxyl groups of adjacent DCA molecules resulted in the formation of a pleated bilayer structure. The outer surface of the bilayer, covered with

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methyl groups of DCA, provides a hydrophobic channel in which nonpolar guest molecules can preferentially be accommodated. The channel can be adjusted to the size of guest molecules to form complexes with many compounds.

Mechanochemistry is associated with chemical reactions triggered by means of mechanical force. Mechanical force (grinding or compression forces) causes a shear strain which leads to a change in the symmetry of molecules. This change breaks and destabilizes the electronic structure of bonding and makes the solid prone to chemical reaction [6]. The grinding process can also induce solid state complexation through the breaking of the crystal structure followed by molecular rearrangement. Toda et al. [7] reported that charge-transfer complexes between tetrathiafulvalene (donor) and tetracyanoquinodimethane (acceptor) were formed by grinding. Nakai et al. [8–10] reported that cyclodextrins could form an amorphous complex with a variety of guests during the grinding process. In our previous study [11], we investigated the complexation between DCA and menadione by grinding, and illustrated that menadione molecules would be accommodated in the bilayer structure of DCA molecules.

In a preceding paper we also reported that salicylic acid (SA) formed a complex with DCA whose crystal structure differed significantly from other orthorhombic crystals [12]. There was no clear evidence for the bilayer. The purpose of this study is to characterize the DCA–SA complex and to compare its properties with those of other DCA–guest complexes by means of X-ray diffractometry, thermal analysis and IR spectroscopy. The complex formation between DCA and SA by the grinding method was also investigated.

2. Experimental

2.1. MATERIALS

Deoxycholic acid, salicylic acid, *d*-camphor (CAM) and phenanthrene (PHE) were purchased from Nacalai Tesque, Kyoto. All other chemical compounds were of analytical reagent grade.

2.2. PREPARATION OF DCA–GUEST COMPLEXES BY COPRECIPITATION

An equimolar mixture of DCA and SA was dissolved in ethanol at 55 °C and filtered through a membrane filter. The solution was cooled down and concentrated by evaporation at room temperature until the coprecipitate was formed. The coprecipitate was filtered out and dried over phosphorus pentoxide at room temperature. In the cases of the DCA–PHE and DCM–CAM complexes, the samples were prepared according to the methods reported by Candeloro De Sanctis et al. [2] and Jones et al. [13], respectively. The content of SA, PHE and CAM in each of the complexes was determined by UV measurement at wavelengths of 304, 250 and 290 nm, respectively, after dissolving the DCA–guest complexes in ethanol.

2.3. PREPARATION OF PHYSICAL AND GROUND MIXTURES

Physical mixtures of DCA–SA (molar ratio 2 : 1, 1 : 1 and 1 : 2) were prepared by mixing DCA and SA in a bottle using a vortex mixer for 5 min. A ground mixture was prepared by grinding the physical mixture (3.0 g) using a vibrational mill (CMT, Tokyo)

2.4. POWDER X-RAY DIFFRACTOMETRY

A Rigaku Denki powder X-ray diffractometer (Miniflex, Tokyo) was used. The measurement conditions were as follows: target Cu, filter Ni, voltage 30 kV, current 15 mA, scanning speed 4 degree/min.

2.5. THERMAL ANALYSIS

Differential scanning calorimetry (DSC) was carried out on a Du Pont thermal analysis system (TA 9900, USA). A sample (about 3 mg) was sealed in an aluminum hermetic pan and measured at a scanning speed of 5 °C/min from 30 to 250 °C under flowing nitrogen gas. Thermogravimetry–differential thermal analysis (TG-DTA) was performed with a Mac Science thermal analysis system (TG-DTA 2010, Tokyo). A sample was placed in a platinum pan and measured at a scanning speed of 5 °C/min from 30 to 300 °C under flowing nitrogen gas.

2.6. SUBLIMATION EXPERIMENT

The sublimation behaviour of SA from the coprecipitate, physical mixture and ground mixture were compared. Samples (about 10 mg) were heated isothermally at 80 °C in a vacuum. The weight loss was recorded up to 6 h using a thermal gravimetric module of the Du Pont TA 9900 apparatus.

2.7. INFRARED (IR) SPECTROSCOPY

The measurement was carried out by the KBr method using a Jasco FT-IR spectrophotometer (Model 230, Tokyo).

3. Results and Discussion

3.1. COGRINDING IN THE DCA–SA SYSTEMS

Figure 1 shows the powder X-ray diffraction patterns of DCA–SA 1 : 1 physical and ground mixtures. The physical mixture showed characteristic diffraction peaks at 2θ = 11.0 and 16.0° due to SA crystals and DCA crystals, respectively. After grinding for 5 min, the new diffraction peaks due to the DCA–SA complex appeared at 2θ = 11.4 and 18.5° (indicated by arrows). These peak intensities

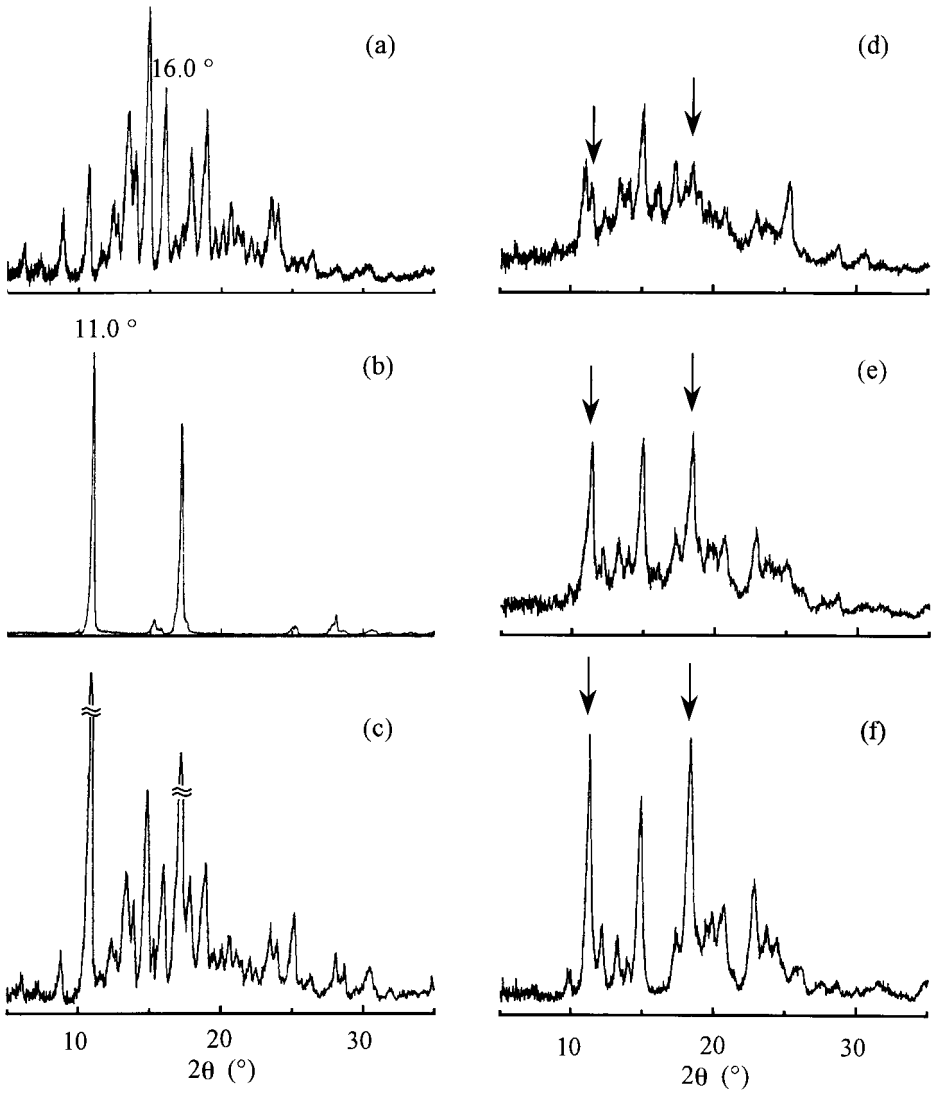

Figure 1. Effect of grinding on the powder X-ray diffraction pattern of a DCA–SA physical mixture (molar ratio 1 : 1). (a) DCA crystals, (b) SA crystals, (c) physical mixture, (d) after grinding for 5 min, (e) after grinding for 7 min, (f) after grinding for 15 min.

increased with prolonged grinding time, whereas those of DCA and SA decreased. After grinding for 15 min, the ground mixture showed an identical powder X-ray diffraction pattern to that of the DCA–SA 1 : 1 complex, which was prepared by the coprecipitation method. This result indicated the gradual formation of the complex by the grinding method.

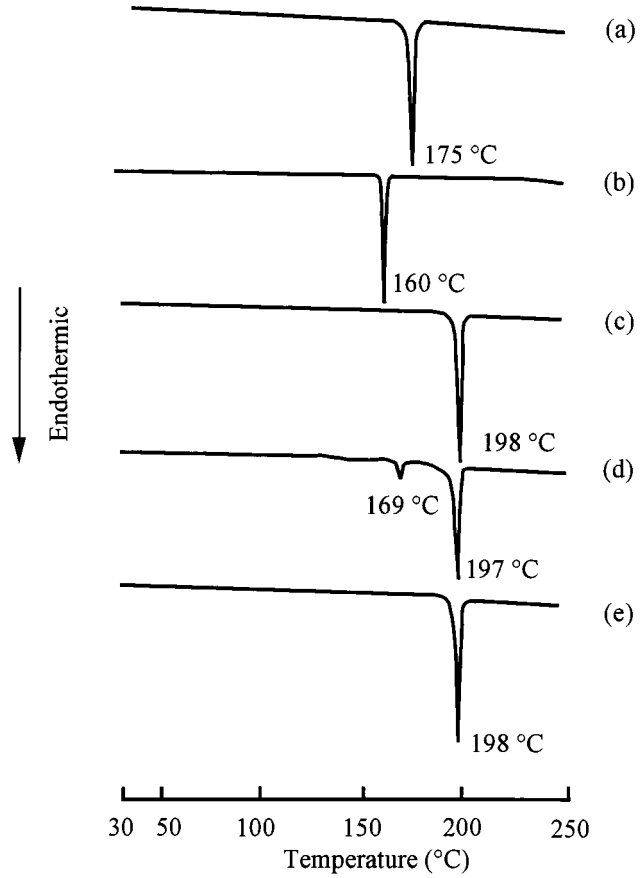

Figure 2. DSC curves of DCA : SA 1 : 1 systems (closed pan, heating rate 5 °C/min). (a) DCA crystals, (b) SA crystals, (c) DCA–SA complex, (d) DCA–SA physical mixture, (e) DCA–SA ground mixture (ground for 15 min).

3.2. Assessment of complexation by thermal method

Figure 2 shows DSC curves of DCA–SA systems. DCA (a), SA (b) and the complex (c) showed melting peaks at 175 °C, 160 °C and 198 °C, respectively. The ground mixture (e) showed an identical curve to the complex, having only one endothermic peak at 198 °C, whereas the physical mixture (d) demonstrated two sharp endothermic peaks at 169 and 197 °C. The first endothermic peak should be due to melting of DCA, while the second peak was melting of the complex, suggesting that complexation had occurred during the heating process. Since no appreciable endothermic peak due to the melting of SA was observed, most of the SA seemed to be consumed in complexation with DCA below the melting temperature of SA. A similar heating effect on complex formation between DCA and menadione has been reported previously [11].

TG-DTA curves of DCA–SA systems are illustrated in Figure 3. SA crystals (b) exhibited a weight loss which began at 85 °C, while in the case of the complex (d) and ground mixture (e) the weight loss started around 135 °C. This result suggests that sublimation of SA was suppressed by the complexation. The SA molecules must be trapped in the channel of DCA. The ground mixture and complex showed respective weight losses of 25.2 and 26.2%, which was in good agreement with the calculated SA content (26.0%) in a 1 : 1 DCA–SA mixture. In the TG curve of the physical mixture, the weight loss due to SA sublimation was observed in two steps. The first step began at 85 °C and was assigned to SA loss from the SA crystals. This step corresponds to the broad endothermic peak around 131 °C in DTA curve (c). The second step started at 135 °C, i.e., the same temperature as the complex, suggesting again that the complexation had occurred during the heating process. DTA curves of all samples correlated well with DSC results, although the former peaks are broader.

From DSC and TG–DTA, it was confirmed that the complexation occurred by grinding or heating the physical mixture of DCA and SA.

To determine the stoichiometry of the complex formed by the grinding process, we performed a sublimation experiment. Figure 4 shows sublimation curves of the DCA–SA system at 80 °C in a vacuum. Under these conditions, SA was confirmed to be completely sublimed within 1 h from the intact crystals (data is not shown). Since the weight losses from the complex (a) and equimolar ground mixture (b) were as low as about 0.5%, the sublimation of SA was suppressed by the complexation. The ground mixture (molar ratio 1 : 2) (c) exhibited a weight loss of 19.6%. This value is in good agreement with the theoretical value (20.6%) calculated from the excess amount of SA. This result indicates that free SA contained in the ground mixture (molar ratio 1 : 2) was completely lost during the sublimation experiment, and after sublimation the complex had a stoichiometry of 1 : 1. The stoichiometry of the ground mixture was also confirmed by the results of powder X-ray diffractometry. The powder X-ray diffraction patterns of the 1 : 2 ground mixture was changed to the same pattern as the 1 : 1 ground mixture (Figure 1f) after sublimation for 6 h. The 1 : 1 physical mixture (d) exhibited a weight loss of 19.9%, which was lower than the SA content (26.0%). This result is well understood in terms of partial complexation of SA with DCA during the sublimation experiment.

3.3. Molecular state of the DCA–SA complex

IR spectroscopy was employed to investigate the molecular state of SA in the complex. The IR spectra of the DCA–SA system are shown in Figure 5. The intact SA crystals (b) showed carbonyl stretching and O—H stretching bands at lower wave numbers of 1659 and 3236 cm^{-1}, respectively due to the intra- and intermolecular strong hydrogen bonding [14, 15]. The spectrum of the physical mixture (c) was a simple superimposition of the spectra of DCA crystals and SA crystals. On the other hand, the ground mixture (ground for 15 min) (e) showed the same IR

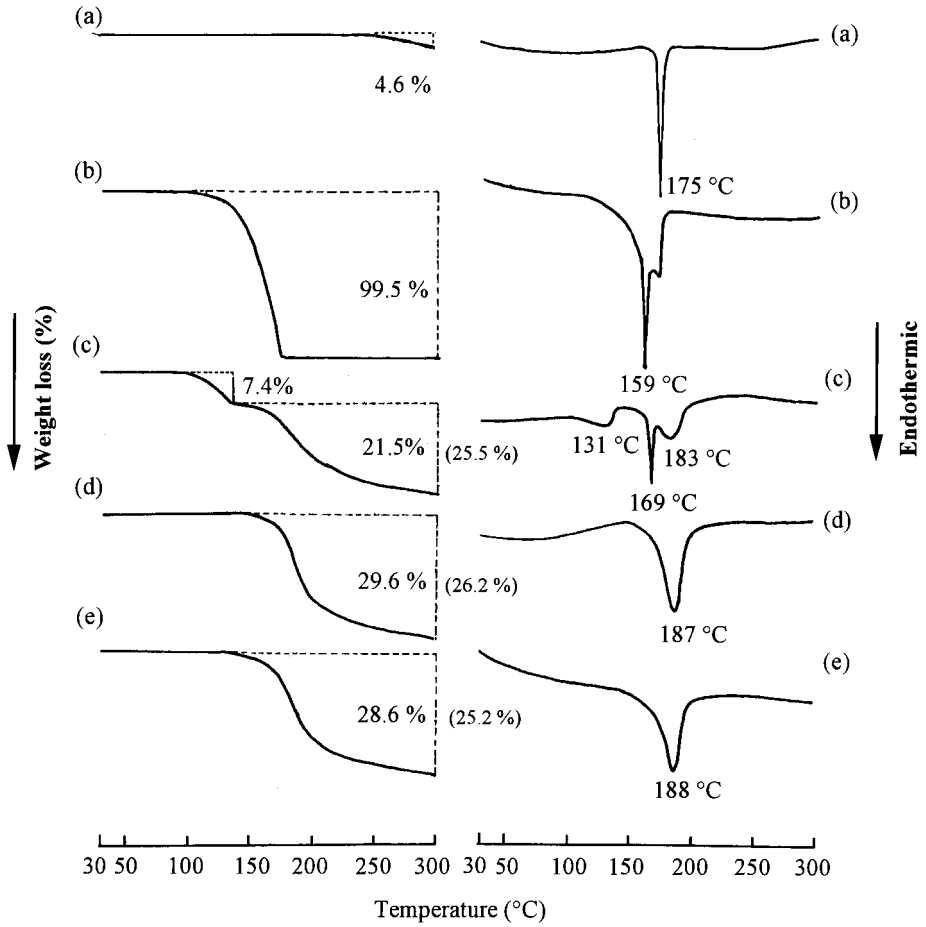

Figure 3. TG–DTA curves of DCA : SA 1 : 1 systems (heating rate 5 °C/min). (a) DCA, (b) SA, (c) DCA–SA physical mixture, (d) DCA–SA complex, (e) DCA–SA ground mixture (ground for 15 min). *Note:* The data in parentheses indicate the actual weight loss of SA, which is calculated from correct weight loss of DCA as observed in curve a.

spectrum as the DCA–SA complex (f) in which the carbonyl stretching and O—H stretching bands were observed at 1664 and 3373 cm^{-1}. These higher stretching frequencies of O—H and carbonyl groups were consistent with molecular structure of the DCA–SA complex, as shown in Figure 6b, in which intermolecular hydrogen bonding was not observed between SA molecules.

The powder X-ray diffraction patterns of DCA complexes with other guests are shown in Figure 7 and compared with those calculated from the crystal structure using the CRYSTALLOGRAPHICA V 1.21 program (Oxford Cryosystems, UK). The experimentally obtained powder X-ray diffraction patterns (a, b, and c) compared well with the calculated powder patterns (d, e and f), respectively, although some differences in relative peak intensities were observed. In the calculated powder pat-

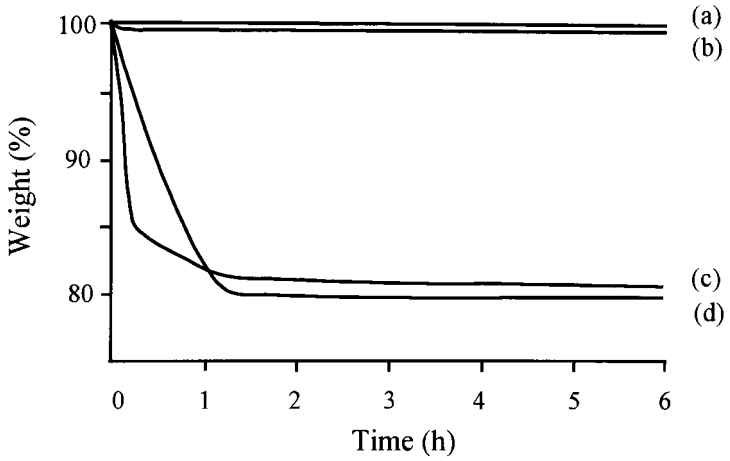

Figure 4. Sublimation curves of DCA–SA systems (isothermal at 80 °C). (a) DCA : SA 1 : 1 complex (0.3%), (b) DCA : SA 1 : 1 ground mixture (0.5%), (c) DCA : SA 1 : 2 ground mixture (19.6%), (d) DCA : SA 1 : 1 physical mixture (19.9%). *Note:* The data in parentheses were SA weight loss after sublimination for 6 h.

Table I. Crystal data of DCA complexes.

Guests	DCA : guest	Space group	*a* (Å)	*b* (Å)	*c* (Å)	Ref.
Phenanthrene	3 : 1	$P2_12_12_1$	26.81	13.60	21.66*	2
d-camphor	2 : 1	$P2_12_12_1$	27.37	13.81	7.23	13
Salicylic acid	1 : 1	$P2_12_12_1$	15.87	17.32	10.32	12

* The translation period of the DCA molecules along *c* is 7.22 (Å).

tern of the DCA–PHE complex, the diffraction peak due to the (210) plane was not clearly observed. With regard to the difference in relative intensities between the calculated and the experimental patterns, our investigations lead us to believe that these might be due to the imperfection of the crystals or to the preferred orientation. It is noteworthy that both the DCA–PHE and DCA–CAM complexes showed characteristic diffraction peaks due to (200), (110), (210) and (310) planes, while the DCA–SA complex showed different diffraction peaks. This result indicates that the crystal structure of the DCA–PHE and DCA–CAM complexes are quite distinct from that of the DCA—SA complex (Table I).

Figure 8 shows the IR spectra of DCA–guest complexes. DCA exhibited sharp peaks due to free O—H stretching vibrations (3567 and 3553 cm^{-1}) and two carbonyl stretching peaks (1716 and 1699 cm^{-1}). In the DCA–CAM and DCA–PHE complexes, the free O–H stretching peaks disappeared, whereas the broad band resulting from hydrogen-bonded O—H stretching was observed in the range of 3500–3200 cm^{-1}. The carbonyl stretching band due to DCA was shifted to a lower wave number of 1693 cm^{-1} (the peak at 1743 cm^{-1} in the DCA–CAM complex

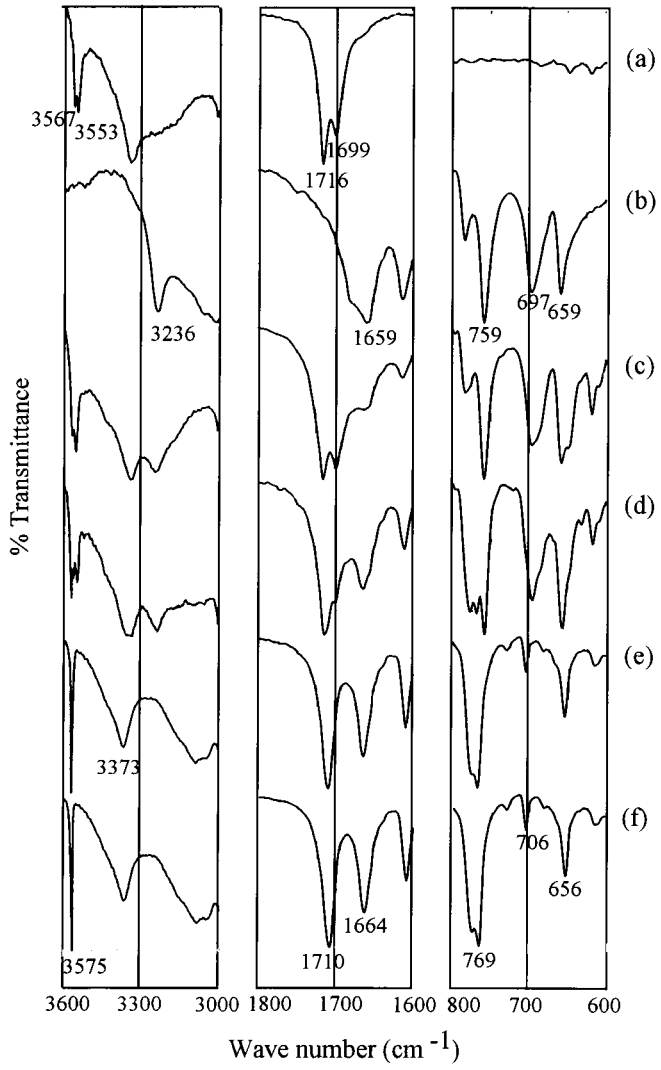

Figure 5. IR spectra of DCA : SA 1 : 1 systems. (a) DCA crystals, (b) SA crystals, (c) DCA : SA 1 : 1 physical mixture, (d) DCA : SA 1 : 1 ground mixture (ground for 1 min), (e) DCA : SA 1 : 1 ground mixture (ground for 15 min), (f) DCA : SA 1 : 1 complex.

is due to carbonyl stretching of CAM). This result can be explained by the crystal packing of DCA–PHE complex, which is shown in Figure 6a. Candeloro de Sanctis et al. reported that DCA undergoes intermolecular hydrogen bonding (as indicated by broken line) between the O—H and carbonyl groups of DCA molecules to build up a DCA bilayer sheet [2]. In comparison with other DCA complexes, the DCA–SA complex showed sharp O—H stretching at 3575 cm^{-1} and a different broad band assigned as hydrogen-bonded O—H stretching in the range of 3500–3200 cm^{-1}. The peak of DCA carbonyl stretching was observed at a higher value

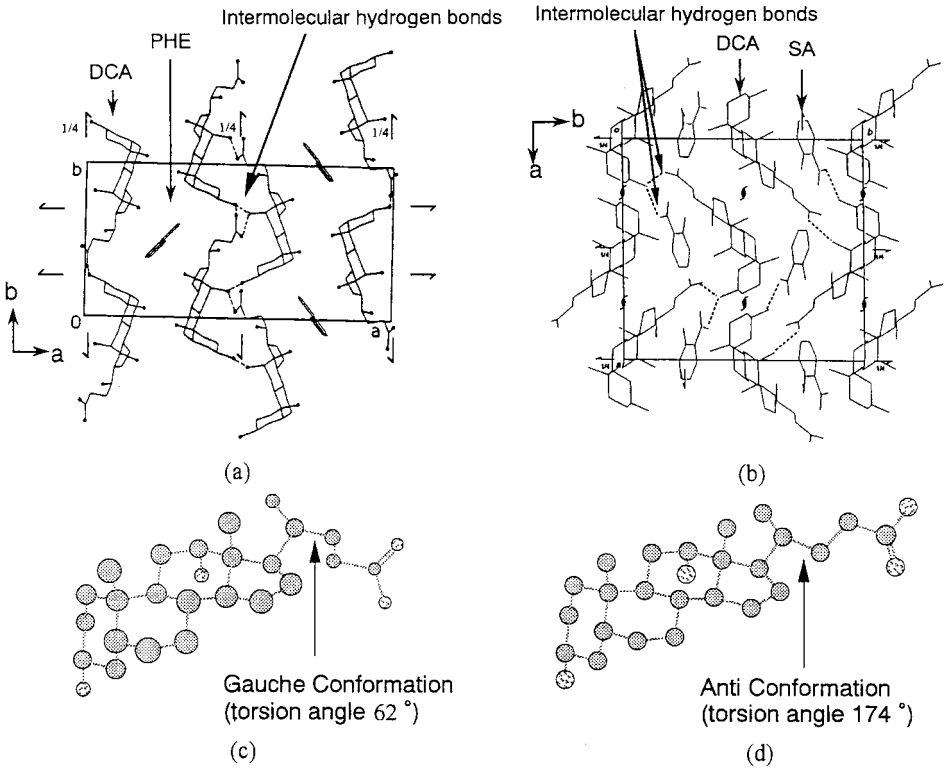

Figure 6. Molecular packing of DCA–guest complexes and molecular conformation of DCA in channel of complexes. (a) Molecular packing of DCA : PHE 3 : 1 complex, (b) molecular packing of DCA : SA 1 : 1 complex, (c) molecular conformation of DCA in DCA : PHE 3 : 1 complex, (d) molecular conformation of DCA in DCA : SA 1 : 1 complex. *Note:* Molecular packing of DCA–PHE 3 : 1 complex and DCA–SA 1 : 1 were redrawn using data from *Acta Crystallogr.* **B28**, 3656 (1972) and *Acta Crystallogr.* **C53**, 803 (1997).

of 1710 cm^{-1} in comparison with other DCA complexes. These results agree well with the molecular packing of the DCA–SA complex, as depicted in Figure 6b, indicating the existence of a different kind of hydrogen bonding between DCA and either DCA or SA. In the DCA–SA complex, an *anti* conformation in the DCA side chain was observed (Figure 6d), whereas the conformation was *gauche* in the other DCA complexes, as exemplified by the molecular structure of DCA in the DCA–PHE complex (Figure 6c). The *gauche* conformation seemed to facilitate hydrogen bond formation between the carboxyl group and the hydroxyl group of adjacent DCA molecules.

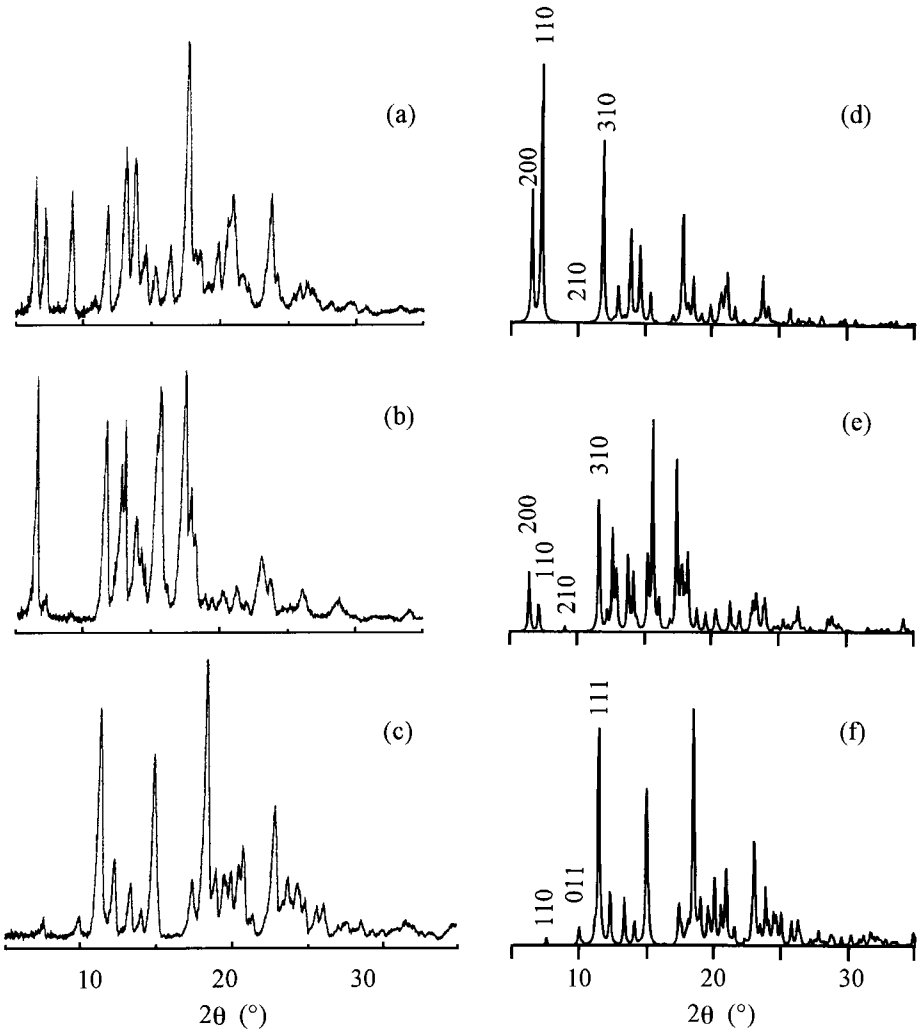

Figure 7. Powder X-ray diffraction pattern of DCA–guest complexes. (a) DCA : PHE 3 : 1 complex, (b) DCA : CAM 2 : 1 complex, (c) DCA : SA 1 : 1 complex, (d) calculated powder X-ray diffraction pattern of (a), (e) calculated powder X-ray diffraction pattern of (b), (f) calculated powder X-ray diffraction pattern of (c).

4. Conclusion

In conclusion, the arrangement of DCA molecules in the DCA–SA complex is clearly distinct from that in other DCA complexes. IR spectra suggested that a different kind of hydrogen bonding was formed in the crystal of the DCA–SA complex, compared with the other DCA–guest complexes. The similarity in powder X-ray diffraction of orthorhombic crystals, such as DCA–CAM and DCA–PHE complexes, may be helpful in assessing the undetermined crystal structure of DCA

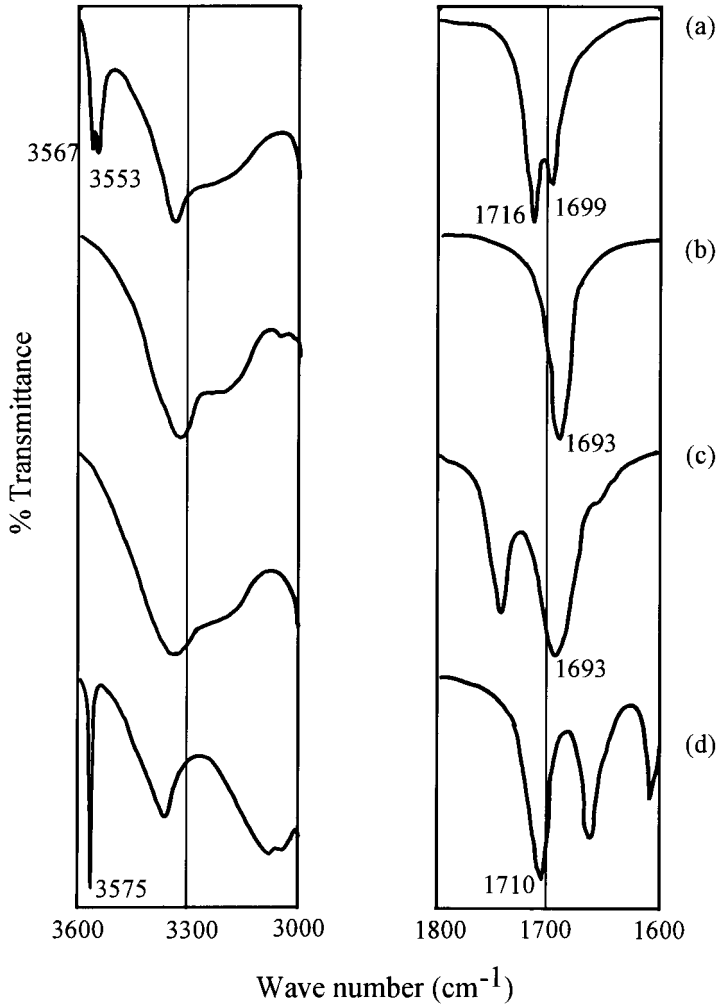

Figure 8. IR spectra of DCA–guest complexes. (a) DCA crystals, (b) DCA : PHE 3 : 1 complex, (c) DCA : CAM 2 : 1 complex, (d) DCA : SA 1 : 1 complex.

complexes. Mechanochemistry (grinding) was shown to be a useful means for preparing DCA complexes.

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