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Effect of Guest Species on Inclusion Compound Formation of Deoxycholic Acid by Co-Grinding

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Deoxycholic acid (DCA) was ground with a guest compound by using a vibrational mill. Powder X-ray diffraction, differential scanning calorimetry, and fluorescence spectroscopic data indicated the formation of the inclusion complex in the ground mixture of DCA–phenanthrene and DCA–naphthalene systems. The DCA–naphthalene ground mixture was found to have higher crystallinity than the DCA–phenanthrene ground mixture, and this was assumed to relate to the difference in the crystallization temperature of the inclusion complex. When low-temperature grinding was performed for DCA and other aromatic hydrocarbons along with naphthalene and phenanthrene, all the ground mixtures were obtained in amorphous state. Crystallization of inclusion compounds from the amorphous ground mixtures was observed to occur at a higher temperature as the stoichiometric ratio (DCA/guest) of the compound increased. With regard to the formation of crystalline complex between DCA and a guest, such as naphthalene, by grinding at room temperature, a two-step mechanism was proposed: i.e., (i) amorphization of both DCA and guest crystals, and (ii) crystallization of the inclusion complex induced above a certain temperature.

Deoxycholic acid (DCA) has interesting properties of forming inclusion compounds with a variety of compounds.[1—7] Viewing the crystal structures, we can find guest molecules accommodated in the channel-like spaces which DCA molecules provide in the crystal structure.[8—20] Inclusion compounds of DCA and guest compounds have conventionally been prepared by coprecipitation or slow evaporation method. Stoichiometric ratios (DCA : guest) were found to vary according to the size of guest molecules.[1]

Nakai et al. demonstrated that co-grinding caused inclusion complex formation between cyclodextrins and guest compounds.[22—25] A cyclodextrin molecule looks like a molecular capsule, and the inclusion complex was obtained in an amorphous state by co-grinding. On the other hand, the formation of a DCA inclusion complex is considered to require maintenance of crystal structure by specific stacking of DCA molecules. Therefore, the grinding process, which tends to destroy crystal structures, seems not to be able to provide the inclusion complex for DCA–guest systems. However, the crystalline inclusion compounds were previously found to be formed by grinding process for DCA–salicylic acid[26] and DCA–menadione systems.[27] This study is designed to investigate the mechanisms of inclusion compound formation by means of the co-grinding method and to clarify the effect of guest species on the phenomena.

Experimental

Materials. DCA, phenanthrene, and dibenz[*a*,*h*]anthracene were obtained from Nacalai Tesque, Inc. (Kyoto, Japan). Naphthalene and benz[*a*]anthracene were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan), respectively. All the chemicals were reagent grade.

Coprecipitation Method for Preparing Inclusion Compound. Adequate amounts of DCA and a guest compound were dissolved in methanol at 55 °C. The solution was cooled down to room temperature and then kept at 4 °C to crystallize the inclusion compound. The inclusion compound was filtrated out on a filter paper and dried at room temperature. Thermogravimetric data confirmed complete removal of the solvent from the inclusion compound.

Preparation of Ground Mixture. A physical mixture of DCA and a guest compound was prepared using a vortex mixer at the stoichiometric ratio of the inclusion compound. Three grams of each physical mixture was loaded in a 50 mL mill chamber made of aluminum oxide and ground using a vibrational mill TI-200 (CMT, Tochigi, Japan). Low-temperature grinding was performed by TI-500ET (CMT, Tochigi, Japan) with a 10 mL stainless steel mill chamber using liquid nitrogen as a cooling medium.

Powder X-Ray Diffractometry (XRD). Powder X-ray diffraction was carried out on a Rigaku Miniflex diffractometer (Tokyo, Japan). The measurement conditions were as follows: target Cu, filter Ni, voltage 30 kV, current 15 mA.

Solid-State Fluorescence Spectroscopy. An FP-770F spectrofluorometer equipped with an attachment FP-1060 for solid sample measurement (Japan Spectroscopic Co., Ltd., Tokyo, Japan) was used. Fluorescence emission spectra of phenanthrene was obtained by excitation at 310 nm.

Differential Scanning Calorimetry (DSC). DSC curves were obtained on a TA 9900 thermal analysis system (TA Instruments, Delaware, USA). About 2 mg of a sample was sealed in an aluminum pan and measurement was performed at a heating rate of 5 °C min^{-1} under a nitrogen gas flow.

Results and Discussion

Co-Grinding of DCA–Naphthalene System. It was reported that inclusion complex crystals of DCA and naphthalene were obtained at the 2 : 1 (DCA : naphthalene) molar ratio by means of coprecipitation from methanol.[2] Figure 1 shows the powder X-ray diffraction (XRD) patterns of DCA, naphthalene, and the inclusion compound crystals. The inclusion compound showed characteristic peaks at $2\theta = 6.8$, 7.3, 9.3, 13.8, and 18.0° (indicated by arrows) which were not observed in the XRD pattern of DCA nor naphthalene crystals. After grinding the inclusion compound for 10 min, the peaks were broadened and the background level of the diffractogram was increased, indicating some lowering of the crystallinity of the sample. Since the diffraction peaks characteristic of the inclusion compound at $2\theta = 13.8$ and 18.0° were clearly observed, however, the crystal structure of the inclusion compound was considered to remain still after grinding.

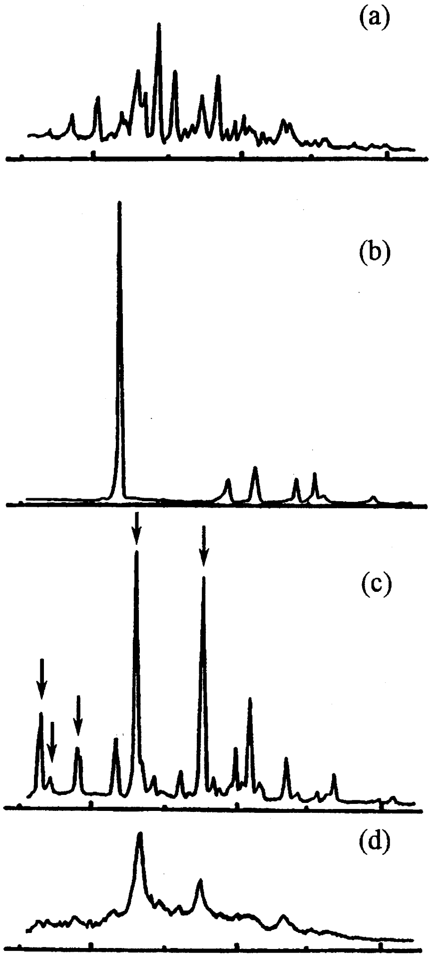


Fig. 1. Powder X-ray diffraction patterns of (a) deoxycholic acid (DCA) crystals, (b) naphthalene crystals, (c) inclusion compound (2 : 1 stoichiometry), and (d) after grinding of the inclusion compound (c) for 10 min.

Figure 2 shows changes in the XRD pattern of the physical mixture of DCA–naphthalene by grinding at 2 : 1 (DCA : naphthalene) molar ratio, which was the same as the stoichiometry of the coprecipitate. New peaks corresponding to the inclusion compound were observed after grinding for 3 min, and after grinding for 10 min the XRD pattern was identical to that of the ground sample of the inclusion compound shown in Fig. 1(d). This indicated that the inclusion compound between DCA and naphthalene could be formed by grinding.

Co-Grinding of DCA–Phenanthrene System. Figure 3 shows the XRD patterns of DCA, phenanthrene and the DCA–phenanthrene inclusion compound (3 : 1 combining molar ratio) as well as the ground sample of the inclusion compound. Similarly to the DCA–naphthalene system, the inclusion compound of DCA and phenanthrene showed the characteristic peaks at $2\theta = 6.6$, 7.4, and 13.9° (indicated by arrows). In the XRD pattern of the ground sample of the inclusion compound, the diffraction peak due to the inclusion compound at $2\theta = 13.9°$ was still observed but the crystallinity of the sample was more decreased in compari-

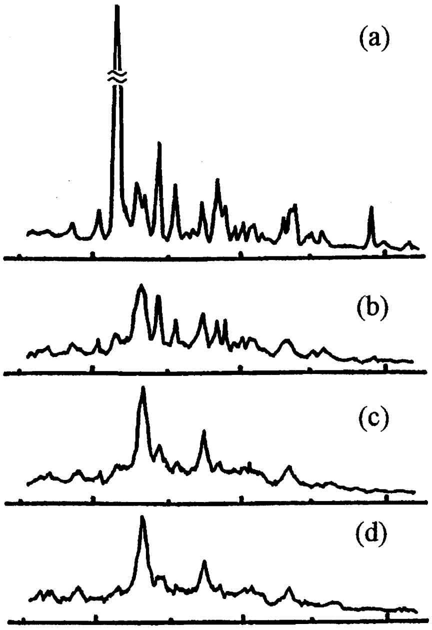


Fig. 2. Change of the powder X-ray diffraction pattern of the 2 : 1 physical mixture of DCA and naphthalene by grinding for (a) 0 min, (b) 3 min, (c) 5 min, and (d) 10 min.

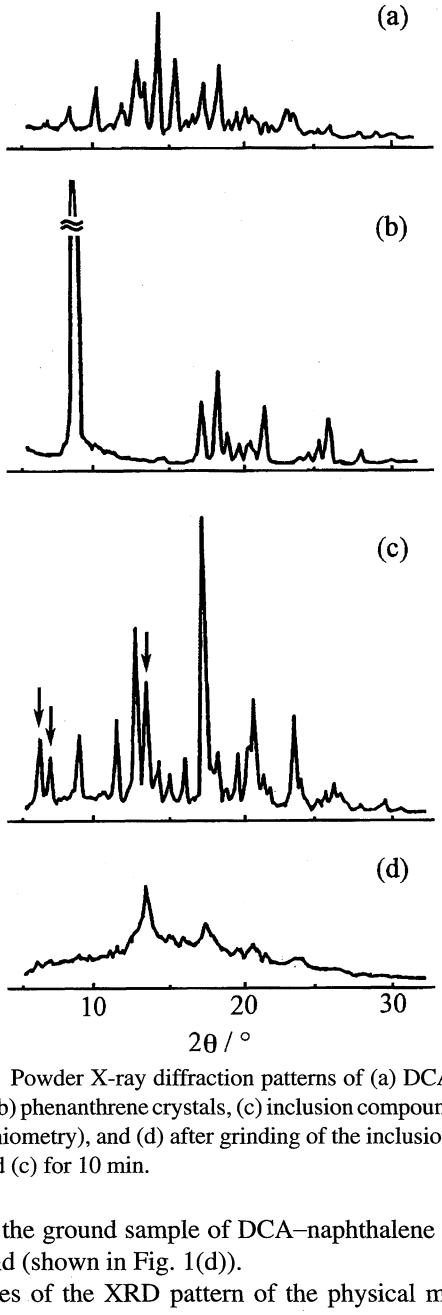


Fig. 3. Powder X-ray diffraction patterns of (a) DCA crystals, (b) phenanthrene crystals, (c) inclusion compound (3 : 1 stoichiometry), and (d) after grinding of the inclusion compound (c) for 10 min.

son with the ground sample of DCA–naphthalene inclusion compound (shown in Fig. 1(d)).

Changes of the XRD pattern of the physical mixture of DCA and phenanthrene (3 : 1 molar ratio) by grinding are shown in Fig. 4. Elongation of grinding time decreased the intensities of the diffraction peaks due to DCA and phenanthrene crystals. After 10 min grinding, the XRD pattern was almost identical to that of the ground sample of the inclusion compound (Fig. 3(d)). These data suggested that grinding could cause inclusion compound formation between DCA and phenathrene, similarly to the DCA–naphthalene system.

Solid-state fluorescence measurements[28] were carried out in order to investigate the molecular states of phenanthrene in the ground mixture with DCA. Figure 5 shows the fluorescence emission spectra of DCA–phenanthrene systems which were measured at an excitation wavelength of 310 nm. The physical mixture of phenanthrene–DCA gave almost the same spectrum as that of phenanthrene crystals, while the emission spectrum of the inclusion compound was significantly different from that of the phenanthrene crystals. It is noteworthy that the 10 min ground mixture showed the same spectral pattern as the inclusion compound. This indicated that phenanthrene molecules in the ground sample existed in a similar state to those in the inclusion compound, so the inclusion formation by grinding was confirmed in DCA–phenanthrene system.

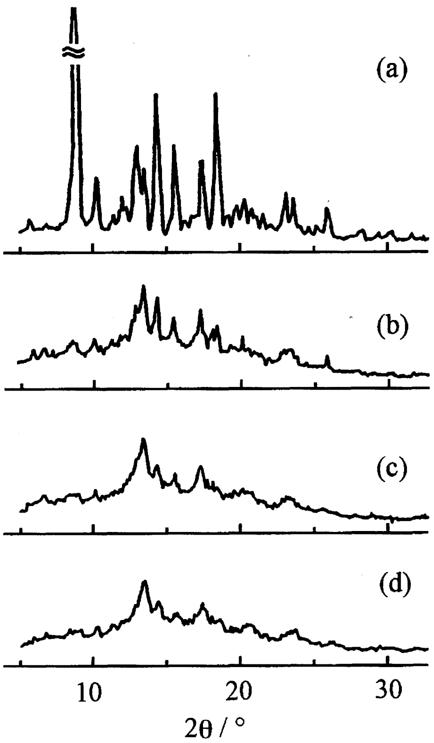


Fig. 4. Change of the powder X-ray diffraction pattern of the 3 : 1 physical mixture of DCA and phenanthrene by grinding for (a) 0 min, (b) 4 min, (c) 7 min, and (d) 10 min.

It was reported that highly pure solid phenanthrene showed fluorescence emission peaks at 355, 370, and 390 nm at room temperature.[29] However, commercially available phenanthrene usually contains a trace amount of impurity.[30] In the presence of an impurity such as anthracene (> 10^{-6}%), the peak intensities at 355 and 370 nm were decreased, whereas those of peaks around 410 and 430 nm were increased because of energy transfer between phenanthrene and impurity molecules.[30] Therefore, the phenanthrene crystals used in this study were considered to contain some impurities which affected the fluorescence spectrum of phenanthrene. Phenanthrene molecules in the DCA inclusion complex were assumed to be isolated not only from other phenanthrene molecules but from impurity molecules. This may explain that the inclusion compound gave a fluorescence spectrum like pure phenanthrene.

Thermal Behavior of Ground Mixture. Figure 6 shows the DSC curves of DCA–phenanthrene systems. On the DSC curves of DCA crystals, phenanthrene crystals, and the inclusion compound, endothermic peaks of melting were observed at 175, 100, and 186 °C, respectively. The ground mixture showed an exothermic peak at ca. 65 °C as well as an endothermic peak at 186 °C. Since the crystallinity of

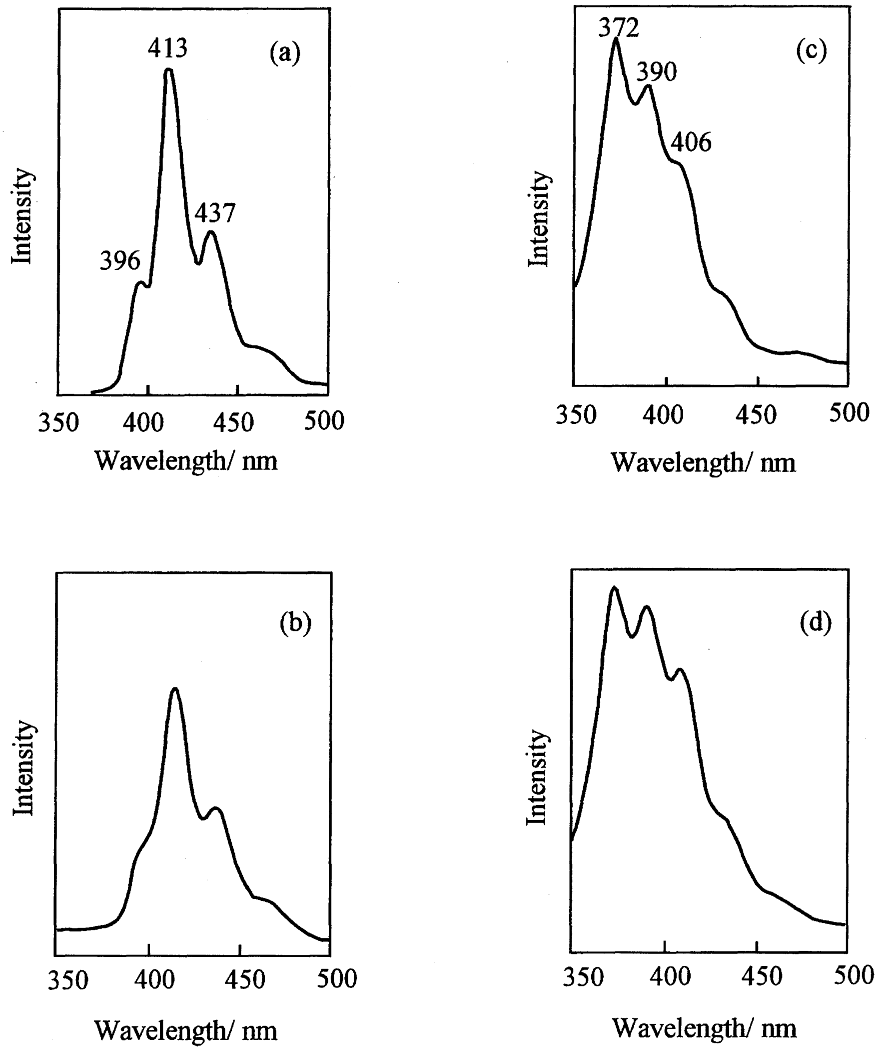


Fig. 5. Solid-state fluorescence emission spectra of phenanthrene in (a) phenanthrene crystals, (b) physical mixture with DCA (DCA : phenanthrene=3 : 1), (c) inclusion compound with DCA (3 : 1), and (d) ground mixture with DCA (3 : 1). The excitation wavelength was 310 nm.

the ground mixture was found to be enhanced by heating at a temperature above the exothermic peak, the exothermic event was considered to be due to the rearrangement of the inclusion complex toward the stable crystalline state.

In order to clarify the difference in the crystallinity between DCA–naphthalene and DCA–phenanthrene ground mixtures, we investigated the crystallization behavior of inclusion complexes from the ground mixtures. When co-grinding was carried out at a low temperature by cooling with liquid nitrogen, the samples were obtained in almost an amorphous state in both systems, as shown in Fig. 7(a) and (b). DSC measurements were performed for the amorphous ground mixtures of DCA–naphthalene and DCA–phenanthrene obtained by low-temperature grinding. On the DSC curve of the ground mixture of DCA–phenanthrene (Fig. 8(b)), an exothermic peak due to crystallization was observed at 71.0 °C, and the enthalpy change was greater than that of the ground sample prepared at an uncontrolled temperature (Fig. 6(d)). On the other hand, the ground mixture of DCA–naphthalene also showed an exothermic peak at 47.8 °C. Since the temperature of mill chamber was assumed to be elevated to 40—50 °C by the grinding process without cooling, the inclusion complex of DCA–naphthalene could be crystallized during the process of grinding. In the DCA–phenanthrene system, however, the elevated temperature of mill chamber could not be high enough for inducing crystallization of DCA–phenanthrene inclusion complex. Namely, the crystallization temperatures of inclusion compound were considered to differentiate the crystallinity of the ground mixtures between DCA–naphthalene and DCA–phenanthrene systems.

Crystallization of DCA Inclusion Complex from Ground Mixture. In order to investigate the effect of guest species on the inclusion compound formation, anthracene, benz[*a*]anthracene, and dibenz[*a*,*h*]anthracene were also employed as guest compounds. As listed in Table 1, DCA formed inclusion compounds with these guest compounds and the combining molar ratios varied from 1 : 1 to 4 : 1 (DCA : guest) which was generally assumed to be dependent upon the molecular size of the guest compound.[1]

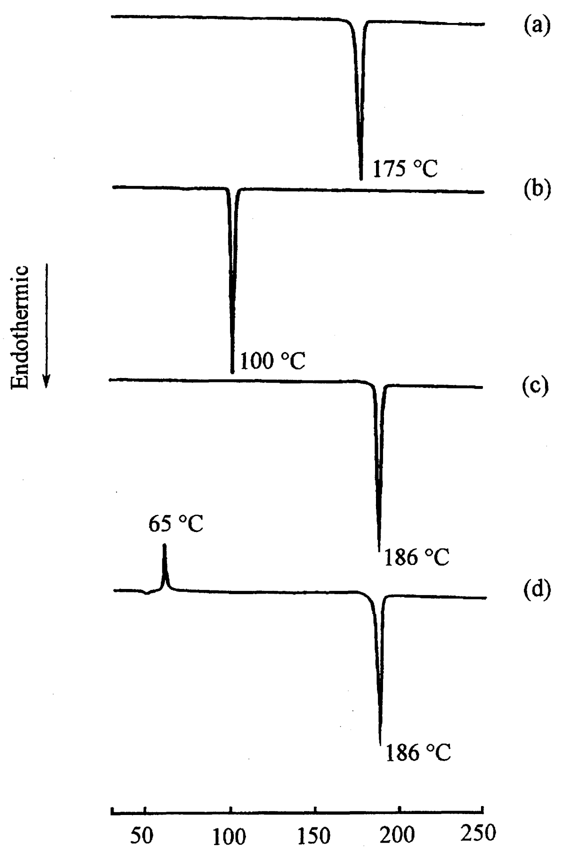
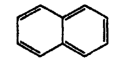
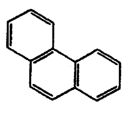
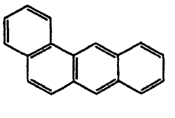
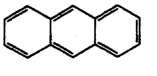
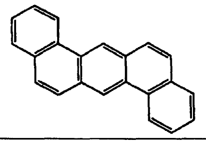


Fig. 6. DSC curves of (a) DCA crystals, (b) phenanthrene crystals, (c) inclusion compound of DCA and phenanthrene (3 : 1), and (d) ground mixture of DCA and phenanthrene (3 : 1).

Low-temperature grinding was applied for physical mixtures of DCA with the guest compounds at the mixing molar ratio which was fixed to the stoichiometry of each inclusion complex. Powder X-ray diffractometry indicated that all the ground samples were obtained in amorphous state (left column in Fig. 7). DSC measurements were performed for the ground samples; the results are shown in Fig. 8. Two characteristic thermal events were observed as an exothermic peak and an endothermic peak on each DSC curve. By heating at a temperature above the exothermic peak, the amorphous XRD pattern of each ground mixture was changed to the crystalline pattern which was identical to that of the inclusion compound crystals prepared by coprecipitation (right column in Fig. 7). Therefore, the exothermic peak should result from the crystallization of inclusion complex. Endothermic peaks observed around 200 °C were considered to be due to the melting of the crystalline inclusion compounds.

The temperatures of the endothermic peaks were not significantly different from each other. Since the inclusion compounds gave similar XRD patterns as independent of guest compounds (Fig. 7), this result could be explained in terms of the similarity in the crystal structure of host DCA molecules in all the systems. More remarkable is the variation in crystallization temperature of the inclusion complex, depending upon the guest species. For each guest, the stoichiometry of the inclusion compound and the thermal properties are summarized in Table 1. From viewing the values in Table 1, one finds the crystallization temperatures of inclusion compound from ground mixture to have a correlation with the stoichiometry of the inclusion compound. Namely, the crystallization temperature could be elevated with an increase in the combining molar ratio (DCA/guest) of the inclusion compound.

Table 1. Physicochemical Properties of Guest and the Inclusion Compound with DCA

Guest	Structure of guest compound	Maximum length in the space filling model of guest molecule (Å)	Stoichiometric ratio (DCA : guest)[1]	Melting point of guest compound (°C)	Crystallization temperature of inclusion compound (°C)	Melting point of inclusion compound (°C)
Naphthalene		8.8	2:1	80.2	47.8	185.9
Phenanthrene		11.1	3:1	100.0	71.0	184.7
Benz[*a*]anthracene		13.3	3:1	156.0	71.5	198.9
Anthracene		11.3	4:1	218.0	87.2	191.1
Dibenz[*a*,*h*]anthracene		15.5	4:1	110.0	94.2	222.4

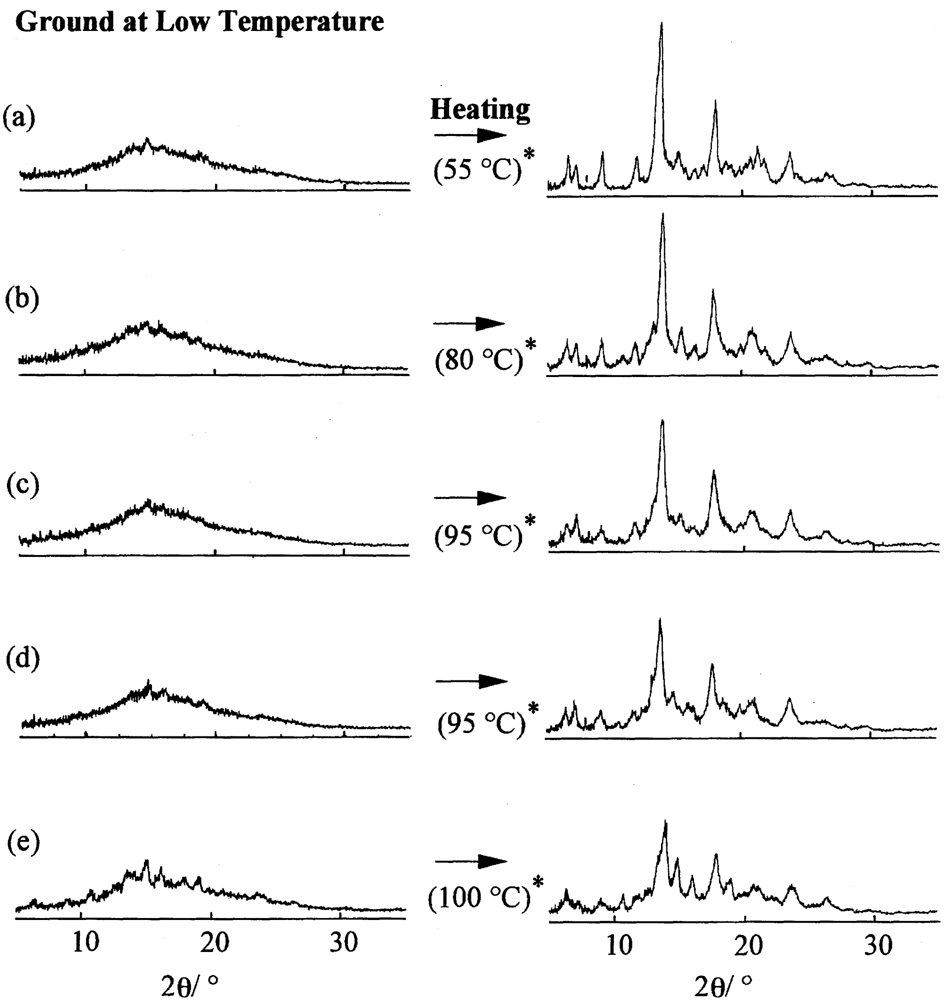


Fig. 7. Powder X-ray diffraction patterns of the ground mixtures, prepared at low temperature, of DCA– (a) naphthalene (2 : 1), (b) phenanthrene (3 : 1), (c) benz[*a*]anthracene (3 : 1), (d) anthracene (4 : 1), and (e) dibenz[*a*,*h*]anthracene (4 : 1) before and after crystallization by heating.

Conclusions

During the co-grinding process in DCA–guest systems, the formation of crystalline complex was assumed to take place in a two-step mechanism. At the first step, the crystal structures of both DCA and guest were broken up by mechano-chemical force. The second is a relaxation step to establish a stable crystal structure of the inclusion compound. The second step was assumed to require thermal activation of molecular movement, because ground samples were obtained in amorphous state by a low-temperature grinding process. On the DSC curves, the amorphous ground mixtures showed an exothermic peak due to crystallization of the inclusion compound, and the temperature of crystallization of the inclusion complex was not dependent on the melting point of the guest compound, but seemed to be dependent on the stoichiometric ratio, as was reported in connection with the molecular size of the guest compound.1) The crystallization of the DCA–naphthalene (2 : 1) ground mixture was observed at a temperature as low as 47.8 °C on the DSC curve. Since the temperature of mill chamber was supposed to be elevated to 40—50 °C during the grinding process, the ground mixture of the DCA–naphthalene system could be obtained in the crystalline state. Consequently, the molecular size of the guest compound, via the stoichiometry, could be one important factor determining the crystallization temperature of the inclusion compound.

However, there were considered to be other factors affecting the crystallization step of inclusion compound. Camphor and menadione formed the crystalline inclusion complexes with DCA at the combining molar ratio of 2 : 1 (DCA : guest),27) which is the same as that of the DCA–naphthalene system. Crystallization of inclusion complexes from the amorphous samples prepared by low-temperature grinding was found to occur at 71.5 and 69.0 °C for DCA–camphor and DCA–menadione systems, respectively.31) These temperatures were obviously higher than the crystallization temperature of the DCA–naphthalene inclusion complex. Differently from aromatic hydrocarbons, camphor and menadione molecules have carbonyl groups in the structures. This carbonyl group, which has the ability to form hydrogen bonds, may affect the molecular diffusion required for establishing

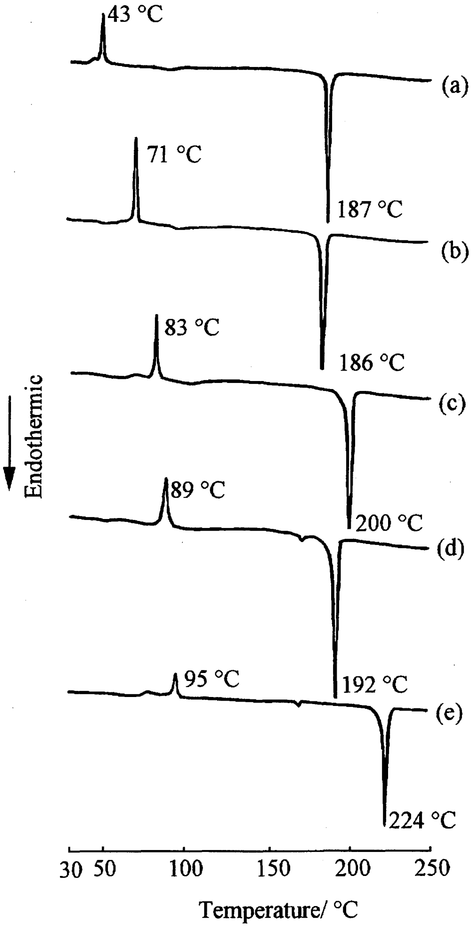


Fig. 8. DSC curves of the ground mixtures, prepared at low temperature, of DCA- (a) naphthalene (2 : 1), (b) phenanthrene (3 : 1), (c) benz[*a*]anthracene (3 : 1), (d) anthracene (4 : 1), and (e) dibenz[*a*,*h*]anthracene (4 : 1).

crystal structure.

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