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Molecular Interaction between Benzoic Acid and Florite and Complex Formation

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The molecular interaction between benzoic acid and florite R (porous calcium silicate) in solid state was studied by using powder X-ray diffraction analysis, infrared (IR)-spectroscopy, differential scanning calorimetry and thermogravimetric analysis. It was found that the mixing ratio, relative humidity and heating significantly affected the molecular behavior of benzoic acid in the physical mixture. In low mixing ratios (up to 30% benzoic acid), benzoic acid was almost transformed to amorphous calcium benzoate within a few hours after mixing. Storage of the physical mixtures containing benzoic acid in the ratio of 40—60% at high relative humidity, or heating them in sealed ampules, accelerated the rate of transformation of benzoic acid to amorphous calcium benzoate and also allowed the formation of a crystalline complex of the following composition: $(C_6H_5COO)_2\,Ca\cdot C_6H_5COOH\cdot H_2O$. The same complex was successfully prepared using benzoic acid and calcium benzoate trihydrate in a 1 : 1 molar ratio by coprecipitation from an aqueous solution, by mechanical grinding and by heating in sealed ampule at 100 °C.

Keywords benzoic acid; calcium benzoate; complex; florite; differential scanning calorimetry; grinding; solid state interaction; X-ray diffraction analysis

Many studies[2–11)] have indicated that excipients possibly interacted with drugs, leading to a significant alteration of the physicochemical properties of drugs. It seems very important to investigate the drug-excipient interaction as a preformulation step to permit the proper choice of additives and to achieve an optimum drug formulation. Florite[12,13)] is a special calcium silicate, with unique features originating from its characteristic porous structure. Florite is very effective as a dry binder and it has a good fluidity. It also has excellent liquid holding ability, so it has been used to formulate the oily vitamin E into solid dosage form.[14)]

In the present study, the mode of molecular interaction between benzoic acid and florite was investigated by using powder X-ray diffraction, thermal analysis and infrared (IR) spectroscopy. The complexed compound was isolated and identified as a calcium benzoate–benzoic acid complex.

Experimental

Materials Florite R was kindly supplied by Tokuyama Soda (Tokyo, Japan) and used after drying in a vacuum at 120 °C for 3 h. Benzoic acid (BA) (Koso Chemical Co., Tokyo, Japan) and calcium benzoate trihydrate (Wako Pure Chemical Industries Co., Tokyo, Japan) were used without further purification. Calcium benzoate anhydrate was prepared by drying calcium benzoate trihydrate at 120 °C in a vacuum. Other materials and solvents were of analytical reagent grade.

Determination of Water Content Water content was determined by the Karl Fischer method using an Aquacounter AQ-3 C (Hiranuma Sangyo, Japan).

Preparation of the Physical Mixtures BA which had a particle size of 90—150 μm and the dried florite which has a water content of 3.3% were uniformly mixed in various ratios using an agate mortar and pestle. The prepared mixtures were kept at room temperature at 0% relative humidity (RH).

Effects of Storage at Different RHs Samples of the physical mixtures containing 40, 50 and 60% BA were kept at 40 °C over P_2O_5 (0% RH) or over the saturated aqueous solution of KBr (79.6% RH). Samples of the stored mixtures were taken for analyses at specified time intervals.

Effects of Heating The physical mixtures containing 40, 50 and 60% BA were heated in sealed ampules (2 ml capacity) for 30 min at the following temperatures: 60, 80, 100 and 120 °C. After cooling of the heated ampules, the samples were taken off and subjected to analyses as soon as possible.

Isolation of the New Crystalline Compound After storage of the physical mixture of 60% BA at 40 °C and 79.6% RH for 2 weeks, the mixture was washed with ethyl ether to remove unreacted BA. The residue was dried at room temperature, then extracted with warm water (70 °C). The aqueous extract was left to crystallize at room temperature. The precipitated crystals were separated and dried at 40 °C over P_2O_5 for 3 d.

Powder X-Ray Diffraction Analysis Powder X-ray diffraction patterns were obtained by using a Rigaku Denki 2027 diffractometer. X-Ray generator (CuK_α, Ni-filtered, 30 kV, 5 mA), time constant: 0.5 s, scanning speed: 4 degree/min and count range: 400 or 2000 cps.

Differential Scanning Calorimetry (DSC) Analysis was performed utilizing a Perkin-Elmer DSC-2 by using a liquid sample pan. The instrument was operated under the following conditions: heating rate of 5 K/min, nitrogen gas flow, and a range of 1 mcal/s. The temperature was raised from 320 K to 399 K (1st run) then allowed to fall rapidly to 320 K, and the heating was repeated (2nd run).

Thermogravimetry (TG) TG was performed by using the Du-Pont 9900 thermal analysis system. The experiments were done under N_2 gas flow (50 ml/min) at a heating rate of 10 K/min.

IR-Spectroscopy IR-spectra were obtained by using a Hitachi 295 IR-spectrophotometer adopting the KBr disk method.

Preparation of Calcium Benzoate–BA Complex The following methods were applied to prepare the complex using equimolar amounts of calcium benzoate trihydrate and BA. The prepared complex was dried at 40 °C over P_2O_5 for 3 d.

(a) Coprecipitation: 3.364 g of calcium benzoate trihydrate and 1.221 g of BA were dissolved in 500 ml of water at about 70 °C. The solution was left to crystallize at room temperature. The deposited crystals were separated by filtration.

(b) Mechanical Grinding: A vibrational mill (Heiko TI 200) made from tungsten carbide was used. The weight of the total specimen was 2.29 g and the grinding time was 5 min.

(c) Heating in Sealed Ampule: About 2 g of the well mixed mixture of calcium benzoate trihydrate and BA was heated in a sealed ampule (2 ml capacity) at 100 °C for 1 h.

Results and Discussion

Effects of Storage and Heating on the Molecular Behavior of BA Figure 1 shows changes in the powder X-ray diffraction patterns of the physical mixtures of BA and florite after storage at 25 °C and 0% RH. Halo patterns were obtained within a few hours after mixing when the physical mixtures contained BA in a ratio of 10—30%. When the physical mixture contained 40% BA, the diffraction peaks of the BA crystals were still observed after storage for 5 h, though their intensities were clearly reduced. Figure 2 shows the IR-spectra of the various samples of BA and florite. It was noticed that the physical mixture of 40% BA showed both a carbonyl stretching band at 1700 cm^{-1} and a carbonyl anion band at about 1545 cm^{-1} after storage at 25 °C and 0% RH for 5 h. After storage under the same conditions, however, the IR-spectrum of the 30% BA physical mixture showed only the carbonyl

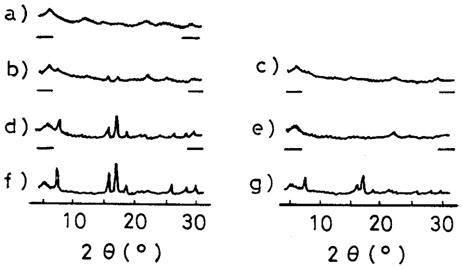

Fig. 1. X-Ray Diffraction Patterns of the Physical Mixtures (PMs) of BA and Florite Before and After Storage at 25 °C and RH 0%

a) florite; b) PM of 10% BA; c) (b) stored for 1 h; d) PM of 30% BA; e) (d) stored for 5 h; f) PM of 40% BA; g) (f) stored for 5 h.

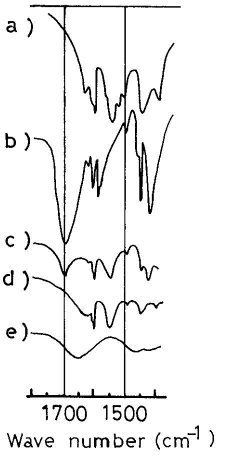

Fig. 2. IR-Spectra of BA–Florite Systems

a) calcium benzoate trihydrate; b) BA; c) PM of 40% BA stored at 25 °C and 0% RH for 5 h; d) PM of 30% BA stored at 25 °C, 0% RH for 5 h; e) florite.

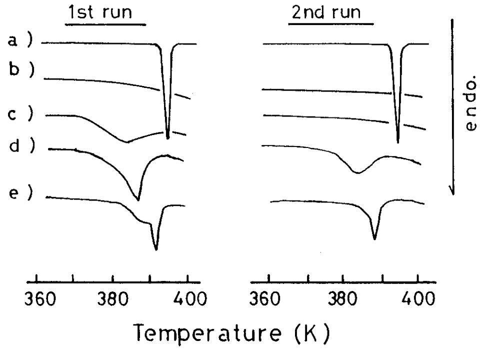

Fig. 3. DSC Curves of the Physical Mixtures of BA and Florite in Various Ratios

a) BA; b) PM of 45% BA; c) PM of 46% BA; d) PM of 60% BA; e) PM of 80% BA.

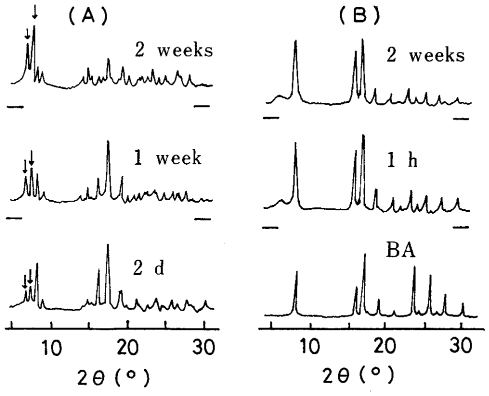

Fig. 4. Changes of the X-Ray Diffraction Patterns of the Physical Mixture of 60% BA during Storage

(A) stored at 40 °C and 79.6% RH; (B) stored at 40 °C and 0% RH (the arrows indicate the new peaks).

anion band. These results indicated that in the physical mixtures containing up to 30% BA, BA molecules almost transformed to an ionic amorphous form within a few hours after mixing. Application of the reported thin-layer chromatography (TLC) procedure[15] for the detection of calcium benzoate confirmed the presence of calcium benzoate in the stored mixtures.

It was suggested that the transformation of BA to amorphous calcium benzoate occurred as follows: BA molecules were transformed into a gaseous state with subsequent adsorption on the surface of florite. Concomitantly, the adsorbed BA molecules interacted with calcium cation, a component of florite, to form calcium benzoate. Konno *et al.*[6] reported that the phenomenon of transformation of organic crystalline medicinals to the amorphous state, when mixed with an adsorbent, took place *via* the gaseous phase induced by the medicinals themselves, and that high vapor pressure was necessary for the rapid amorphization.

Figure 3 shows the DSC curves of the physical mixtures of BA and florite in various mixing ratios. The physical mixture containing less than 45% BA showed no peak in either the 1st or 2nd DSC runs, as shown in curve b. The physical mixtures containing more than 60% BA showed two overlapped endothermic peaks between 370—389 K in the 1st runs. The first peak was broad and diffused, while the second peak was relatively sharp, becoming more prominent as the mixing ratio of BA increased. In the 2nd runs, the sharp peak disappeared while the broad peak was still observed.

The present DSC results indicated that BA took three phases in the physical mixtures. They are the crystalline state, disordered state and amorphous ionic state. It was obvious that heating accelerated the rate of transformation of BA molecules to the amorphous ionic state. Nakai *et al.* reported on the transformation of BA molecules to the ionic form, predominantly aluminum benzoate, by heating at 400 K with pillar interlayer montmorillonite.[9]

Figure 4 shows the changes in X-ray diffraction patterns of the physical mixture of 60% BA during storage at 40 °C and different RHs. Only a slight reduction of the intensities of the diffraction peaks of BA crystals was noticed in the case of storage at 0% RH. On the other hand, the physical mixture kept at 79.6% RH manifested a marked reduction in the intensities of BA diffraction peaks as storage time increased. It also showed new X-ray diffraction peaks at $2\theta = 6.8°$ and 7.3° which became more intense with storage time. The physical mixtures containing 40 or 50% BA kept at 79.6% RH also showed a similar behavior; even mixtures containing 10—30% BA stored at 79.6% RH showed only halo patterns.

Figure 5 shows the changes in X-ray diffraction patterns of the physical mixtures of BA and florite after heating in sealed ampules for 30 min. The heating of the physical mixtures of 40% and 50% BA resulted in a reduction in the intensities of BA diffraction peaks; this effect was more pronounced after heating at a high temperature. New

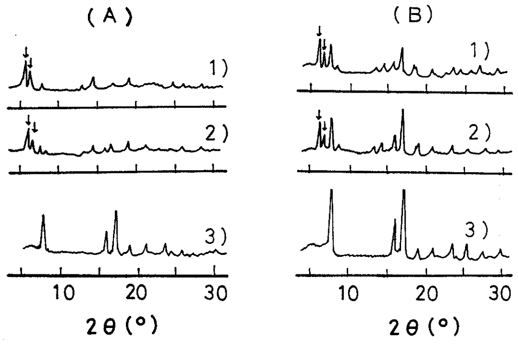

Fig. 5. Changes of the X-Ray Diffraction Patterns of the Physical Mixtures of BA and Florite after Heating in Sealed Ampules for 30 min

(A) PM of 40% BA; (B) PM of 50% BA; 1) heated at 100 °C; 2) heated at 60 °C; 3) before heating (the arrows indicate the new peaks).

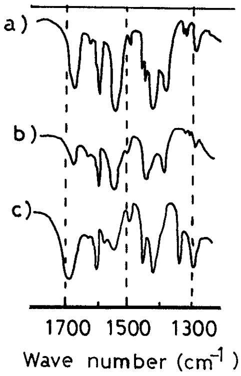

Fig. 6. IR-Spectra of the Physical Mixtures of BA and Florite after Storage at Different RHs or Heating in a Sealed Ampule

a) PM of 60% BA stored at 40 °C and 79.6% RH for 2 weeks; b) PM of 40% BA heated at 100 °C for 30 min in a sealed ampule; c) PM of 60% BA stored at 40 °C and 0% RH for 2 weeks.

diffraction peaks were observed at $2\theta = 6.8°$ and 7.3° in all diffractograms of the heated mixtures. The new diffraction peaks observed in Figs. 4 and 5 were in the same positions. These new diffraction peaks, however, could not be consistent with either calcium benzoate trihydrate, which had the main diffraction peak at $2\theta = 5.3°$ (Fig. 10), or anhydrous calcium benzoate, which had only one diffraction peak at $2\theta = 6.3°$ (Fig. 7).

Figure 6 shows the IR-spectra of the physical mixtures of BA and florite after storage at different RHs or heating in a sealed ampule. The IR-spectrum of the physical mixture stored at 0% RH (curve c) showed the carbonyl stretching band of BA at 1700 cm^{-1}, and it also showed the carbonyl anion band at about 1545 cm^{-1}. On the other hand, in the IR-spectra of the physical mixtures kept at 79.6% RH and 40 °C or heated in a sealed ampule at 100 °C, the strong carbonyl band was observed at 1685 cm^{-1}, which showed a marked low frequency shift of about 15 cm^{-1} compared to the carbonyl stretching band of dimerized BA. These also showed an obvious carbonyl anion band at about 1545 cm^{-1}.

The experimental results show that by keeping the physical mixtures containing BA in a ratio of 40—60% at 79.6% RH, or by heating them in a sealed ampule, the rate of transformation of BA was accelerated and a new crystalline compound was formed. In the mixtures of 10—30% BA, BA was only transformed to amorphous ionic state after the same treatment.

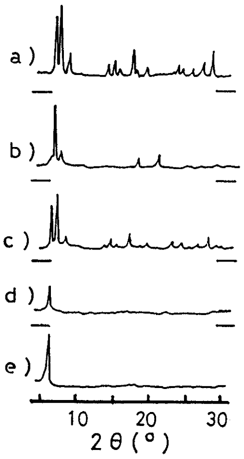

Fig. 7. X-Ray Diffraction Patterns of the Isolated Compound

a) as obtained; b) after heating at 100 °C for 1 h in a vacuum; c) (b) was stored at 40 °C and 79.6% RH for 1 week; d) after heating at 250 °C for 30 min; e) anhydrous calcium benzoate.

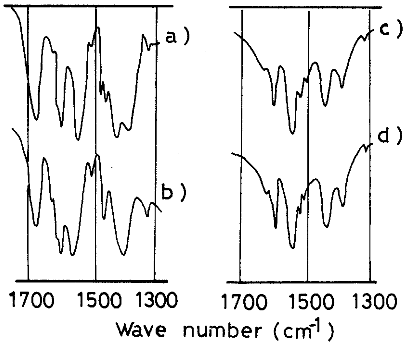

Fig. 8. IR-Spectra of the Isolated Compound

a) as obtained; b) after heating at 100 °C for 1 h in a vacuum; c) after heating at 250 °C for 30 min; d) anhydrous calcium benzoate.

Identification of the New Compound Figure 7 shows the powder X-ray diffraction patterns of the new isolated compound (a). The main diffraction peaks of the isolated compound were observed at $2\theta = 6.8°$ and 7.3°. The water content was determined by the Karl Fisher method as 4.87%. After the isolated compound was heated at 100 °C, it was dehydrated, and the X-ray diffraction pattern was different from that of the original hydrous sample as shown in Fig. 7b. When the anhydrous sample was stored at 40 °C and 79.6% RH for 1 week, it gave an X-ray diffraction pattern (c) almost the same as that of the orginal hydrous sample (a), indicating a reversible property of hydrous–anhydrous forms. On the other hand, after heating the isolated compound at 250 °C for 30 min, the X-ray diffraction pattern was similar to that of anhydrous calcium benzoate ((d) and (e)).

Figure 8 shows the IR-spectra of the new isolated compound. The IR-spectrum of the new isolated compound showed the carbonyl stretching absorption band, which appeared at a lower frequency of about 15 cm^{-1} compared to BA. It also showed the carbonyl anion absorption band at about 1545 cm^{-1}. The IR-spectrum of the isolated compound after heating at 100 °C still showed the shifted carbonyl band at the same wave number as the original sample. After heating the isolated compound at 250 °C for

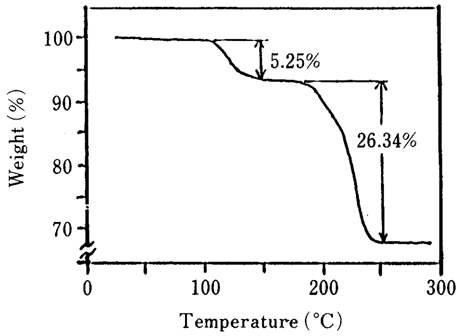

Fig. 9. TG Curve of the Isolated Compound

Heating rate: 10 K/min.

Table I. Elemental Analysis Data of the Isolated Complex and the Complex Prepared by the Coprecipitation Method

	Theoretical[a] (%)	Observed (%)	
		Isolated complex	Prepared complex
H	59.71	59.93	59.66
C	4.30	4.24	4.24

a) The theoretical values were based on the assumed formula of the complex.

30 min, the obtained spectrum was similar to that of anhydrous calcium benzoate (Fig. 8c, d).

Figure 9 shows the TG curve of the new isolated compound. It was noticed that the first weight loss occurred at about 100 °C and the weight loss percentage of 5.25% was comparable to the water content of the isolated compound (4.73%). This result indicates that the first weight loss was due to the loss of the crystal water. After the TG run, a condensed sublimate was observed on the periphery of the internal walls of the TG cell. Analyses of this sublimate by ultraviolet (UV), IR-spectroscopy and DSC indicated the presence of pure BA. IR-spectroscopy and powder X-ray analysis of the residue, which remained in the TG sample holder after heating, indicated that the residue was anhydrous calcium benzoate.

In view of the instrumental analysis data, the new isolated compound was speculated to be a complex composed of calcium benzoate anhydrate, benzoic acid and the water of crystallization. The observed lower frequency shift of the carbonyl band in the case of the isolated compound could be ascribed to the interaction of the carbonyl group of BA and calcium benzoate. When the complex was heated at 100 °C, it lost the water of crystallization and was transformed to the anhydrous form. After heating at 250 °C it was decomposed with subsequent sublimation of the bounded BA, leaving the stable calcium benzoate anhydrate.

Thermogravimetric analysis was successfully utilized for the determination of the molecular composition of the isolated complex. The averaged first weight loss at about 100 °C was estimated as 5.25%, that of the second step was 26.34% and the residual percentage at 250 °C was 68.41%. Water of crystallization, BA and calcium benzoate were assigned to each corresponding component. Based on the TG data, the following formula of the isolated complex was suggested: $(C_6H_5COO)_2\ Ca \cdot C_6H_5COOH \cdot H_2O$.

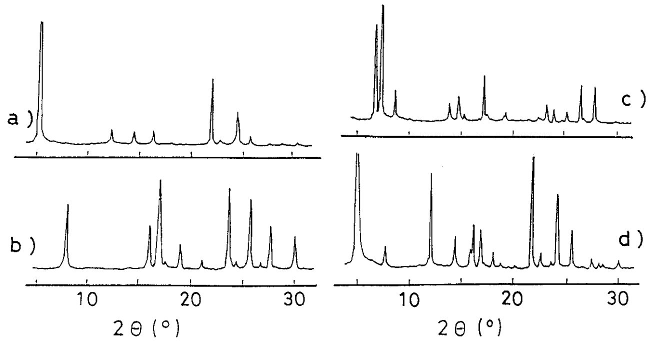

Fig. 10. X-Ray Diffraction Patterns of the Physical Mixture and the Coprecipitate of Calcium Benzoate Trihydrate and BA in a 1 : 1 Molar Ratio

a) calcium benzoate trihydrate; b) BA; c) the coprecipitate; d) the physical mixture.

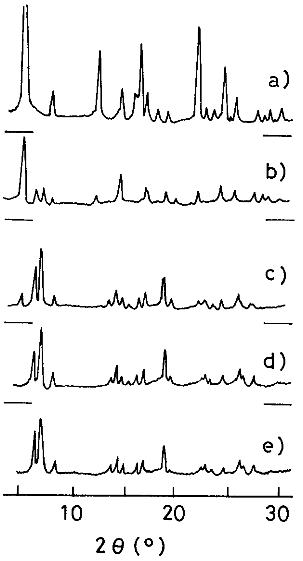

Fig. 11. X-Ray Diffraction Patterns of the Physical and the Ground Mixtures of Calcium Benzoate Trihydrate and BA in a 1 : 1 Molar Ratio

a) intact; b) ground for 1 min; c) ground for 3 min; d) ground for 5 min; e) ground for 10 min.

The elemental analysis data of the isolated complex (Table I) was comparable to the theoretical data calculated from the suggested formula of the complex. This confirmed the suggested formula of the complex.

Preparation of Calcium Benzoate–BA Complex by Coprecipitation, Grinding and Heating Figure 10 shows a comparison between the X-ray diffraction patterns of the physical mixture and the coprecipitate of calcium benzoate trihydrate and benzoic acid in a 1 : 1 molar ratio. The X-ray diffraction pattern of the coprecipitate was entirely different from that of the physical mixture. The X-ray diffraction pattern of the coprecipitate was almost the same as that of the isolated complex as shown in Fig. 7. However, when calcium benzoate trihydrate or BA were used in a different molar ratio (such as 1 : 2 or 2 : 1), the obtained complex was contaminated by the ingredient used in excess.

The variation of X-ray diffraction patterns of calcium benzoate trihydrate and BA in a 1 : 1 molar ratio with the elapse of grinding is shown in Fig. 11. The powder X-ray diffraction pattern of the mixture ground for 5 min coincided with the pattern of the complex. No further change could be observed after grinding for 10 min. From the IR measurements (Fig. 12), the maximum lower frequency shift

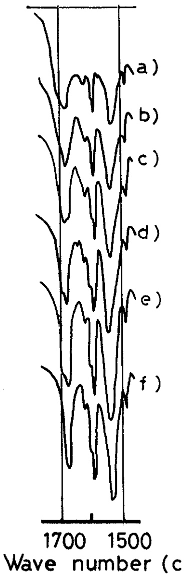

Fig. 12. IR-Spectra of the Physical and the Ground Mixtures of Calcium Benzoate Trihydrate and BA in a 1 : 1 Molar Ratio

a) intact; b) ground for 0.5 min; c) ground for 1 min; d) ground for 2 min; e) ground for 3 min; f) ground for 5 min.

of the carbonyl band of BA (from 1700 to 1685 cm^{-1}) was attained after grinding for 5 min, indicating that no free BA was included in the sample ground for 5 min. This result was in good agreement with the above X-ray diffraction results.

The powder X-ray diffraction pattern of the mixture of BA and calcium benzoate trihydrate in a 1 : 1 molar ratio after heating a in sealed ampule at 100 °C for 1 h (not shown) was also similar to that of the isolated complex. TG, IR-spectroscopy, water content and elemental analysis data of the complex prepared by any of the above described methods coincided with the corresponding data of the isolated complex.

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