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Oriented Molecular Assemblies in Lipid Surfactant/Polymer Composite Films

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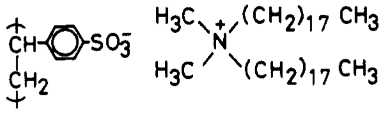
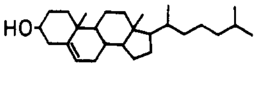
ABSTRACT: By casting a chloroform solution of cholesterol (CH) and a polyion complex (PIC), dimethyldioctadecylammonium/poly(styrenesulfonate), a nonbilayer film with a molecular orientation formed spontaneously in a composite film in the CH content range of 10–30 wt %. An usual multibilayer structure was maintained below 5 wt %. In order to clarify the nonbilayer structure, CH was removed selectively from the film by solvent extraction. The film changed from the nonbilayer structure to a multibilayer structure maintaining the macroscopic orientation of the original film. From the manner of the change in the X-ray diffraction by uniaxial stretching of the nonbilayer film and the CH-extracted film, it was assumed that the nonbilayer structure consisted of microrods oriented perpendicular to the film surface.

Introduction

An amphiphilic compound having two long alkyl chains usually forms a bilayer assembly in aqueous media. Drying of the aqueous dispersion of an amphiphile on a solid plate gives a multibilayer film, in which each plane of the original bilayer piles is arranged parallel to the film surface like a stratum. This film has been widely used to study the molecular orientation of a bilayer structure and intrinsic peptides incorporated into the bilayer by using various methods.[1] A solvent cast of the polyion complex (PIC) of an amphiphile also gives a well-oriented multibilayer film.[2]



PIC



CH

In general, a bilayer-forming substance seems to form a multibilayer film oriented parallel to the film surface as a stable structure. As described in a previous paper,[3] a series of small-angle meridional diffraction arcs and wide-angle equatorial diffraction arcs was observed by irradiation of an X-ray beam along the direction parallel to the film surface (edge view). The small-angle diffraction arcs corresponded to the spacing of the bilayer thickness and the wide-angle arcs the intermolecular distance between the long alkyl chains of the amphiphiles. On the other hand, a PIC film prepared by casting with cholesterol (CH) gave a completely different diffraction pattern, that is, a small-angle equatorial diffraction and a wide-angle meridional diffraction.[3]

An important effect of CH on the physical property of lipid bilayers is known as the condensing and fluidizing effect.[4] In the PIC system, pronounced effects of CH on the physical properties of the bilayers were also confirmed as changes in the mechanical properties and the ESR spectra of a spin probe in the film.[5,6] The X-ray diffraction patterns suggested that CH affected the molecular assembly as well as the physical properties of the PIC film. We assumed a nonbilayer structure from the change in the X-ray diffraction induced by uniaxial stretching of the film. However, X-ray diffraction measurement did not provide adequate information to clarify the structure probably due to the lack of regularity of the polymer (atactic polymer) and the low degree of crystallinity of the film. Therefore, it seemed difficult to establish the nonbilayer structure, especially within the microrod, directly from the X-ray study. Furthermore, as the film comprised multicomponents including a considerable amount of low molecular weight compounds, the decision regarding the structure from the alternation of the diffraction pattern in a similar manner to that of common polymeric materials should be cautiously made.

Since the diffraction pattern of the nonbilayer film may be ambiguous as a result of the disordered arrangement of the surfactant molecules, CH was removed from the film by extraction with solvents. If CH can be extracted selectively without disturbing the molecular orientation of the original film, a film with an unusual molecular orientation can be obtained. Furthermore, a comparison of the diffraction pattern of the nonbilayer film with that of the CH-extracted film may be informative to understand the supramolecular assembly in the nonbilayer film. In this paper, we report a further approach to clarifying the structure of the PIC film containing CH.

Experimental Section

Preparation of the Polyion Complex. The PIC having a stoichiometric composition was prepared from dimethyldioctadecylammonium bromide and sodium poly(styrenesulfonate) by a previously described method.[2,3,5]

Preparation of the Polyion Complex Films Containing Cholesterol (Film II). Commercially available extrapure-grade cholesterol purchased from Nakarai Chemical Co. and chloroform were used without further purification. Since the PIC cast film sometimes adheres to an untreated glass surface, the 5 wt/vol % chloroform solution of CH and the PIC was cast onto a Petri dish siliconized with trichlorooctadecylsilane. The solvent was slowly evaporated at 23 °C in an atmosphere of 60% relative humidity. The transparent film with a thickness of about 0.2 mm was dried under vacuum (<0.1 mmHg) and annealed in water at 60 °C for 10 min.

Removal of CH from the Composite Film (Film III). The PIC film containing 20 wt % CH (film **II**, 100 mg) was immersed in 100 mL of diethyl ether for 2 days at room temperature. The solvent was sometimes agitated slowly. The film was dried in vacuo and annealed in hot water.

Differential Scanning Calorimetry (DSC). The temperature and the enthalpy changes of the phase transition of the PIC from a gel state to a liquid-crystalline state were determined with a Du Pont 9900 differential scanning calorimeter at a heating rate of 10 K/min. Samples were hermetically sealed with the

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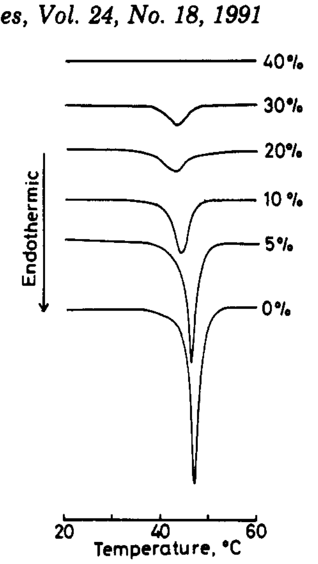

Figure 1. DSC thermograms of the PIC films containing 0–40 wt % cholesterol. Heating rate: 10 K/min. Enthalpy changes (kJ/mol of surfactant) from bottom to top: 46.0, 36.2, 22.3, 14.7, and 8.1.

same weight of water in aluminum pans and heated several times. The thermograms were reproducible upon scanning 3 times.

X-ray Diffraction Photographs of the Films. The X-ray photographs were obtained with a flat-film camera using Ni-filtered Cu Kα radiation for short spacings and V-filtered Cr Kα radiation for long spacings at 35 kV and 30 mA. The exposure times were 2 and 3 h, and the distances between the specimens and X-ray films were 80 and 110 mm, respectively. Each spacing was calculated from the diffraction position on the photographs.

Preparation of Uniaxially Stretched Film (Films Istr, IIstr, and IIIstr). A pure PIC film (film I), a film containing 20 wt % CH (film II), and a CH-removed film (film III) were stretched in water at 50 °C by clipping the film on a manual stretching device at a stretching speed of about 100%/min. The stretched films were allowed to cool and dried under ambient conditions on the device to prevent shrinkage of the films.

Results and Discussion

Differential Scanning Calorimetry of the PIC Film Containing Various Amounts of CH. Figure 1 shows the DSC thermograms of the pure PIC film (film I) and the PIC films containing 5–40 wt % CH. Film **I** showed a sharp endothermic peak centered at 43 °C due to the phase transition from a gel state to a liquid-crystalline state of the multibilayer. Addition of CH caused broadening of the peak and a decrease in the enthalpy change. With increasing CH content, and peak area decreased gradually and finally disappeared at 40 wt %.

X-ray Diffraction Photographs of the PIC Films. Figure 2 shows photographs taken by Cu Kα X-ray irradiation along the direction parallel and perpendicular to the film surface. The arrangement of the sample and X-ray films is also shown in Figure 2. The *X*, *Y*, and *Z* axes are defined for the sake of convenience. Sharp equatorial diffraction arcs at 4.1 Å of film **I** centered at 4.1 Å (Figure 2a) were ascribed to the spacing of the long alkyl chain packing of the surfactant molecules. Addition of CH caused a broadening of the diffraction pattern and a shift to a smaller angle (4.6 Å, Figure 2d–f and h). The results of X-ray diffraction and DSC suggested a broadening of the spacing and probably a disordering of the alkyl chains of the surfactant molecules. The same changes in the alkyl chains induced by added cholesterol are also known in aqueous dispersions of phospholipids.[7] An interesting feature of the X-ray diffraction pattern of the film containing more than 10 wt % CH is the meridional diffraction arcs at a wide angle, in contrast to the equatorial diffraction arcs of the multibilayer film.

The meridional diffraction of the 5 wt % CH film (Figure 2c) appearing at a small angle is caused by the bilayer structure, which is maintained at low CH content. However, above 10 wt %, the small-angle diffraction appeared in the equatorial direction (Figure 2e, f, and h). The film with 8 wt % CH showed an intermediate pattern (d). The longest spacing increased from 34 (a) to about 50 Å (f and h; maximum 53 Å) with increasing amounts of CH. Above 20 wt %, the spacing was almost constant. The diffraction pattern of the film containing 40 wt % CH was too complicated to analyze.

The X-ray diffraction patterns at a small angle for film **II** treated with hot water and untreated are shown in Figure 3 (edge view). In this case, the Cr Kα beam, which has a longer wavelength (2.291 Å) than the Cu Kα beam, (1.542 Å), was used to obtain much clearer large spacings at a small angle. Without annealing, the diffraction at a small angle had weak intensity and no directionality as can be seen in Figure 3a. The higher order structure grows by annealing the film in hot water. Therefore, all PIC films were annealed before testing.

Solvent Extraction of Cholesterol from the Non-bilayer Film. Diethyl ether was used as an extraction solvent owing to its high solubility for CH and poor solubility for the PIC. In diethyl ether, film **II** swelled (about 5% in length) and softened, but the shape did not change. After extraction, the theoretical amount of CH was recovered from the solvent (20.6%) and theoretical weight reduction of the film was observed (20.9%), while in the case of film **I** substantial weight loss did not occur (0.8%).

Figure 4 shows the DSC thermograms of the PIC films containing 20 wt % CH (film II), CH-extracted film **II** (film III), stretched film **III** (Film **IIIstr**), the pure PIC (film **I**), and stretched film **I** (Film **Istr**). Film **II** has a small endothermic peak (Figure 4a). However, by extracting CH from film **II**, the endothermic peak grew sharper (b) and had almost the same enthalpy change as film **I** (d).

Figure 5 shows X-ray diffraction photographs of film **III** and the stretched films, film **Istr**, film **IIstr**, and film **IIIstr**. Photographs a, d, f, and i were taken by irradiating a Cu Kα beam along the *X* direction of the films; b, e, g, and j were from the *Z* direction; and c, h, and k were from the *Y* direction as illustrated in the figure. Film **III** without annealing showed halo diffractions. By annealing film **III** in hot water, however, an equatorial diffraction at a small angle (37 Å) and sharp meridional diffraction arcs at a wide angle were observed (d). Retention of the macroscopic orientation through extraction of CH suggests that the polymer chain in the composite film plays an important role. Minor differences between film **III** and film **I** in the shape of the DSC peaks (Figure 4b and d) and the longest spacings (Figure 5d (37 Å) and Figure 2a (34 Å)) may arise from the polymer-like character of the PIC.

Stretching of the CH-Containing Film. The PIC film prepared from high molecular weight polymer is strong enough to withstand the stress applied during stretching. Since the film was too stiff to be stretched in the dried state, however, the film was softened in hot water and stretched. The elongation of the film was about 300%, but this was sufficient to change the structure of the PIC film. The stretched sample was released from the stretching device after cooling it to room temperature in order to avoid shrinkage of the film.

In Figure 5a–c, X-ray diffraction photographs of film **IIstr** are shown. A drastic change is observed in the diffraction pattern by stretching film **II** to 250% (see Figure 5a and c and Figure 2f). When the X-ray beam was

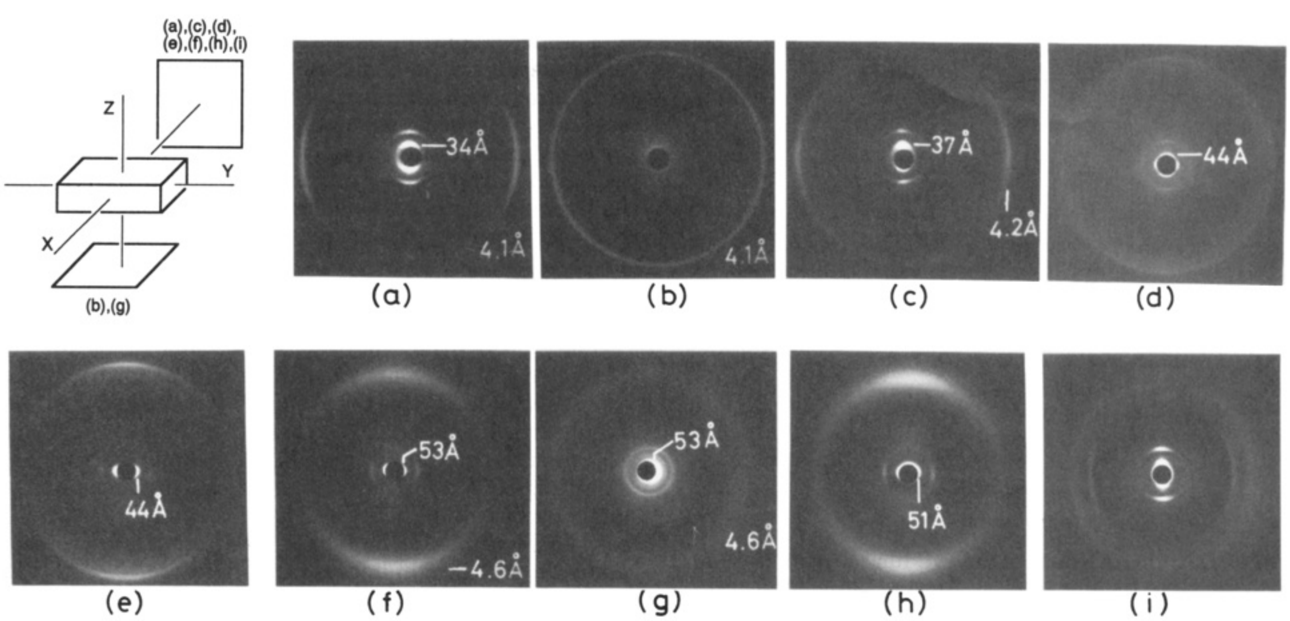

Figure 2. X-ray diffraction photographs of water-annealed PIC film containing various amounts of cholesterol by Cu Kα irradiation along the *Z* axis (b and g) and along the *X* axis (others). (a and b) 0 wt % (film **I**); (c) 5 wt %; (d) 8 wt %; (e) 10 wt %; (f and g) 20 wt % (film **II**); (h) 30 wt %; (i) 40 wt %. (a and b), Multibilayer pattern; (e, f, and h) the nonbilayer pattern.

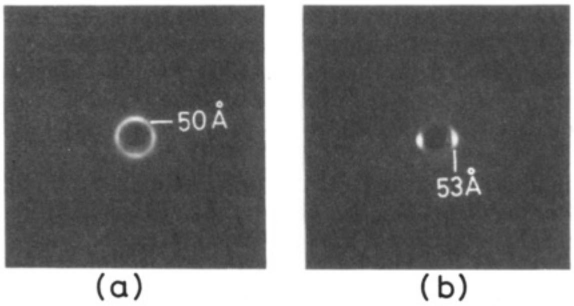

Figure 3. X-ray diffraction photographs of the PIC film containing 20 wt % CH before (a) and after (b) annealing in hot water. A Cr Kα beam along the *X* axis was used.

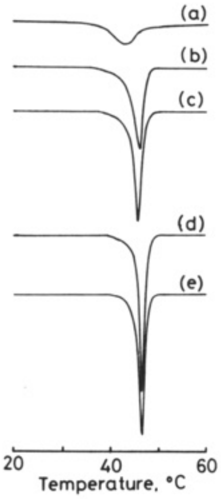

Figure 4. DSC thermograms of the PIC films (enthalpy change). Scanning rate: 10 K/min. (a) PIC film containing 20 wt % CH (film **II**) (14.7 kJ/mol); (b) CH-removed film by extraction with diethyl ether (film **III**) (42.4 kJ/mol); (c) 250% stretched film **III** (film **IIIstr**) (49.4 kJ/mol); (d) pure PIC film (film **I**) (46.0 kJ/mol); (e) 300% stretched film I (film **Istr**) (46.0 kJ/mol).

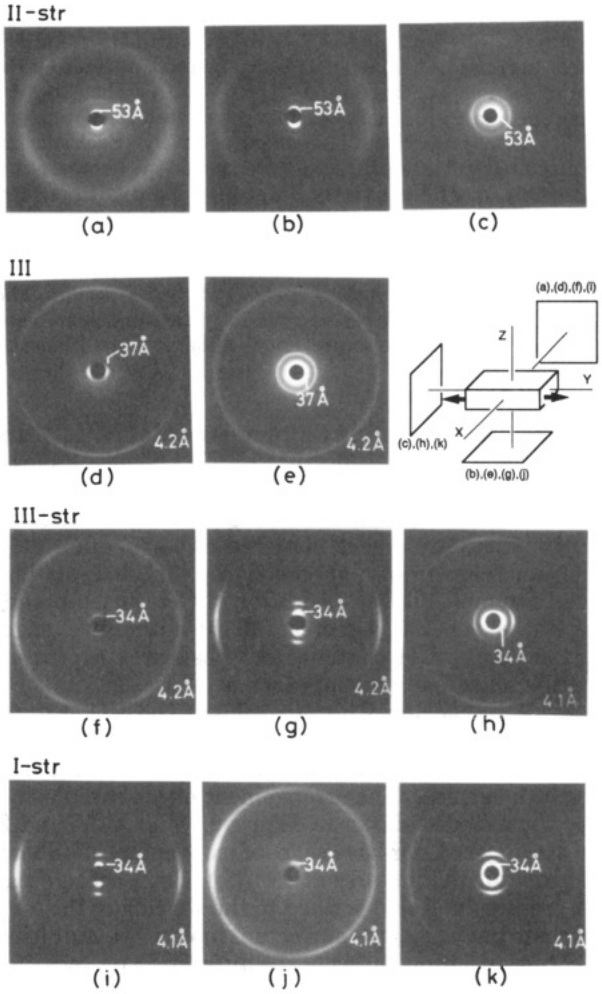

Figure 5. X-ray diffraction photographs of the PIC films by Cu Kα irradiation along three directions. (a, d, f, and i) Irradiation along the *X* axis; (b, e, g, and j) irradiation along the *Z* axis; (c, h, and k) irradiation along the *Y* axis. Bald arrows indicate the stretch direction. Stretch ratios of films **IIstr**, **IIIstr**, and **Istr** were 250%, 300%, and 300%, respectively.

irradiated along the stretching direction (*Y* direction), a ring pattern was observed (c). On the other hand, diffraction arcs with directionality were found in a and b, when the X-ray beam was irradiated along the directions perpendicular to the stretching direction (*X* and *Z* directions). The spacing of the small-angle diffractions (53 Å) was the same as that of unstretched film **II** (Figure 2f). This result can be explained by a model of the nonbilayer structure consisting of microrods as depicted in

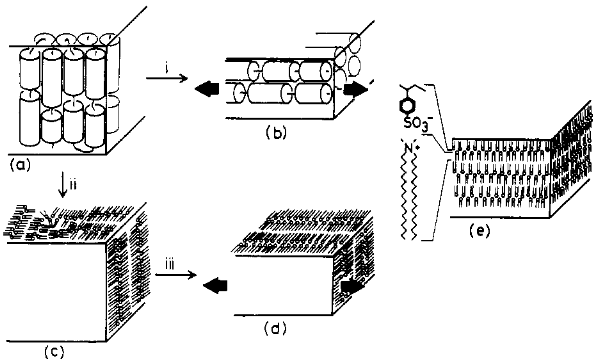

Figure 6. Schematic illustrations of the composite films. (i and iii) Uniaxial stretch; (ii) solvent extraction of cholesterol. (a) Film **II**: microrods are aligned perpendicular to the film surface. (b) Film **IIstr**: as the microrods were linked to each other with polymer chains, the rods are oriented along the direction of elongation. (c) Film **III**: a multibilayer structure is regenerated. (d) Film **IIIstr**: slipping at the lipophilic part of the bilayers results in a fully developed multibilayer with an unusual orientation. (e) Film **I** (the usual multibilayer film).

Figure 6a, rather than by the multibilayer model.

Film **III** was also stretched in water at 50 °C. The diffraction pattern of stretched film **III** taken by X-ray irradiation parallel to the stretching direction (Figure 5h) was almost the same as that of unstretched film **III** taken parallel to the *X* direction (d). However, the pattern of film **IIIstr** taken parallel to the *X* direction (f) was much different from that of film **III** (d). The pattern of film **IIIstr** taken parallel to the *Z* direction (g) was also different from that of film **III** (e). The intensity of the diffraction at a small angle in the photograph f was weak, in contrast to the case of film **IIstr** (a). The large spacing of film **IIIstr** coincided with the value of the pure PIC film (film **I**, 34 Å).

Figure 5i–k shows photographs of film **Istr**. The diffraction patterns were essentially unchanged, although weak diffractions at a small angle in photograph j (34 Å) were observed due to the disordered orientation of the multibilayer.

In the series of photographs for film **IIIstr** and film **Istr**, correlation between the diffraction patterns was found: photographs f, g, and h were similar to j, i, and k, respectively, although the directionality of the diffraction was different between h and k. The correlation of the diffraction pattern between film **IIIstr** and film **Istr** indicates that **IIIstr** had a multibilayer structure with an orientation that was completely different from that of film **I** (Figure 6d). As can be seen in Figure 4c, the sharp endothermic peak of film **IIIstr** parallel to that of film **I** (Figure 4d) shows that growth of the bilayer structure was induced by stretching the film. The molecular orientation of film **IIIstr** also means that the longitudinal axis of the surfactant molecule in film **II** was parallel to the film surface.

In this stage, on basis of the X-ray diffraction and DSC measurement, the following are concluded

1. Microrods[8] in the PIC film containing 10–30 wt % CH align perpendicular to the film surface (Figure 6a). The spacing of the diffraction at a small angle may correspond to the size of the rod (diameter), which depends on CH content. The longitudinal axis of the surfactant molecule in the microrods may be parallel to the film surface.

2. By stretching the film uniaxially, the microrods were reorganized and aligned parallel to the direction of elongation, since the rods are connected by polymer chains (Figure 6b).

3. Extraction of CH from the nonbilayer film by diethyl ether produces a disordered multibilayer film. However, the molecular orientation of the surfactant molecules in the nonbilayer film was maintained owing to the entanglement of the polymer chains. Reorganization of the surfactant molecules by annealing in hot water results in the developed multibilayer (Figure 6c). The normal line of the multibilayer in the film was parallel to the film surface.

4. When stress is applied to the CH-removed film, the bilayers slide at the lipophilic part of the bilayers and line up (Figure 6d). In this film, the normal line of the multibilayer is perpendicular both to the direction of elongation and to the film surface (*X* direction in Figure 5). The mode of the change in the diffraction pattern of film **III** by stretching was different from that of film **II**. This suggests that film **II** was not a lamellar structure.

The model of the nonbilayer structure consisting of microrods explains well the results of X-ray diffraction of the PIC films. To obtain further details on the structure, another approach may be required, for instance, a study of the relationship between the assembly of the film and the chemical structure of components in the film.[8] Investigation of the formation mechanism of the oriented film by self-organization should also be needed.

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(8) Addition of a conical-type amphiphilic compound, which has a relatively large hydrophobic volume, induces a bilayer phase of diacylphosphatidylethanolamine (PE) to a rodlike nonbilayer phase, which is called an inverted hexagonal phase. The nonbilayer structure, which has much hydrophobic volume, is supposed to accept the hydrophobic volume of the additive. Many nonbilayer-type PIC films were prepared with various conical-type additives including cholesterol. This implies the similarity of the structure of the nonbilayer film to the rodlike inverted hexagonal structure of PE.

(9) A PIC film prepared from poly(styrenesulfonate) labeled with a spin probe showed directionality in its ESR spectra. From the alternation of the spectra by stretching of the film, it was presumed that the axis of the polymer was perpendicular to the film surface in a similar manner as the microrod. If the film were lamellar structure, the polymer would not be oriented. The rodlike inverted hexagonal structure, in which the hydrophilic region including an ionic polymer would be located in the core, can best explain the data at the present stage. However, the presence of the endothermic peak of the nonbilayer films containing CH more than 10 wt % suggests that the bilayer structure may remain partly or in a disordered state. These data will be published elsewhere.

Registry No. CH, 57-88-5; PIC, 102265-23-6.