Two-dimensional Thermodynamic Properties of Sterols

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Abstract : 5 α -cholestan-3 β -ol (II) is similar to 5-cholesten-3 β -ol (cholesterol) (I) except the absence of the Δ^5 double bond, whereas 5 β -cholestan-3 α -ol (III) is remarkably different from I in its skeletal structure and in the direction of its OH moiety. Two-dimensional thermodynamic properties such as surface pressure-molecular area (π -A) relationship, equilibrium spreading pressure (ESP) and enthalpy of compression of monolayer at air-water interface were studied for these sterols. II shows similar π -A relationships to I, whereas III gives the different ones. ESP value, increasing with increasing temperature, followed the order of II > I > III. Enthalpy of elution, ΔH_{ESP} , from the crystals onto water surface at ESP and also enthalpy of compression, ΔH_{comp} , of the monolayers from $\pi=0$ to ESP were obtained from the temperature dependence of ESP and of π -A relationship, respectively. The sum of ΔH_{ESP} and $-\Delta H_{\text{comp}}$, which means the two-dimensional enthalpy of fusion, is approximately proportional to the ordinary enthalpy of fusion. II and III molecules gave a 1:1 compound in the mixed monolayer on water surface as well as in solid state. The ESP value for the 1:1 compound was also determined.

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1 Introduction

Cholesterol (5-cholesten-3 β -ol) (I), one of the most familiar sterols, is widely distributed in animal bodies: It is one of the most important components in cell membrane or organelle films. It plays an important role in the metabolism of lipids and controls the fluidity of the membrane. In bilayer constituting the membrane, cholesterol causes a decrease in the energy content of the host lipid phase transition. It does so by liquefying phospholipid at temperatures below the phase transition in the gel phase and by restricting the hydrocarbon chains above the phase transition in the liquid crystalline phase. Concomitantly, cholesterol increases the permeability properties of phospholipid systems below the

phase transition and reduces them above it.

By the way, as shown in Fig.1, there are two additional sorts of sterol species resemble to cholesterol, i.e., 5 α -cholestan-3 β -ol (cholestanol) (II) and 5 β -cholestan-3 α -ol (epicoprostanol) (III). These two kinds of sterols are isomers to each other (1). One human body contains about 140-145 g of I accompanying about 5-10 g of II, which is generally produced in the livers of animals. III was also detected in the animal bodies. In the molecule of the above cholesterol family, each A ring in the steroid rings directly links an OH moiety. The four steroid rings (cyclopentano-phenanthrene nucleus) of I and also of II are linked in an all *trans*-type form. Namely, II is similar to I in its skeletal structure; the molecules of I and II look just like planks. Furthermore, II is similar to I in the direction of OH moiety.

On the other hand, A and B rings of III are linked in a *cis*-type form. Consequently, the A-ring part is steeply bend against the molecular axis of III (1). Furthermore,

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5-Cholesten-3 β -ol (I)

(Cholesterol)

5 α -Cholestan-3 β -ol (II)

(Cholestanol)

5 β -Cholestan-3 α -ol (III)

(Epicoprostanol)

Fig.1 Molecular Structures of Three Kinds of Sterols: 5-cholesten-3 β -ol (I), 5 α -cholestan-3 β -ol (II) and 5 β -cholestan-3 α -ol (III). II and III have OH moieties that are in opposite direction to each other.

the direction of the OH moiety of the III is different from that of I or II. Thus, the physical properties of the aggregate of III at the air-water interface should be largely different from those for I and II as well as those in the solid state. As examples of the thermodynamic properties in the solid state, melting points and enthalpies of fusion, ΔH_{fusion} , of I, II and III (2) are listed in Table 1.

In the present study, two-dimensional thermodynamic properties such as surface-pressure-molecular area (π - A) relationship, enthalpy of compression, and equilibrium spreading pressure (ESP) were studied for I, II and III as a function of temperature. Especially, ESP is defined as the surface pressure of the monolayer being in equilibrium with crystals spread on water surface and

reflects the situation of the bulk crystal. Namely, the ESP is a bridge to study the relationship between crystals and monolayers and, one of the most important thermodynamic properties in the surface chemistry (3-6).

The ESP and the enthalpy change in the eluting process of the sterol molecules from their crystals into their monolayers on the water surface were discussed in conjunction with their molecular structures. In addition, the binary mixture of II and III, whose OH moieties are in opposite directions to each other, was also examined in two-dimensional aspect. As a result it has become clear that II and III molecules in the binary mixed monolayer on the water surface make a 1:1 compound as well as in the solid state (2).

2 Experimental

2.1 Samples

Samples of three kinds of sterols, (I) 5-cholesten-3 β -ol (purity >99 %), (II) 5 α -cholestan-3 β -ol (>98 %) and (III) 5 β -cholestan-3 α -ol (>95 %), were purchased from Aldrich Co. and used without further purification. Chloroform (spectroscopic grade, Dojin Laboratories) was also used as a spreading solvent. Water (pH 6.8; conductivity, 0.04 $\mu\text{S}/\text{cm}$) was used for subphase solution after being purified through ion-exchange filters and a reverse osmosis membrane.

2.2 Surface Pressure and π - A Relationship Measurements

After the crystals of sample were placed on a clean water surface, surface pressure was measured with a platinum Wilhelmy plate on an automatic surface tension apparatus (Kyowa Kaimen Kagaku Co. Ltd., model CBVP-Z); the surface pressure was recorded until being constant to the sensitivity of the apparatus (0.02 mNm^{-1}). The temperatures in the measurements were maintained within ± 0.1 K. On the other hand, π - A relationships at various temperatures for the samples were obtained using a temperature-programmable Langmuir trough made by Kato (7). The compression rate for the monolayer was 10 % of area /min.

3 Results and Discussion

3.1 Time Courses of Surface Pressure and ESP Values for Sterols

Figures 2-I, -II and -III show the time courses of the surface pressures of the monolayers co-existing with the

Table 1 Thermodynamic Properties for Sterols.

	(I) 5-cholesten-3 β -ol	(II) 5 α -cholestan-3 β -ol	(III) 5 β -cholestan-3 α -ol	1:1 compound of II and III
Molar Weight	386.68	388.68	388.68	777.36
Melting point/K	422.5*	413.5*	385.8*	418.3*
$\Delta H_{\text{fusion}}/\text{kJ mol}^{-1}$	26.5*	22.6*	15.8*	48.4*
$\Delta H_{\text{ESP}}/\text{kJ mol}^{-1}$	16.5	9.6	6.3	19.3
$\Delta G_{\text{comp}}/\text{kJ mol}^{-1}$				
293.1 K	0.181	0.270	0.056	0.160
298.1 K	0.214	0.280	0.059	0.206
303.1 K	0.243	0.287	0.069	0.256
308.1 K	0.253	0.292	0.077	0.265
313.1 K	0.283	0.331	0.082	0.298
$\Delta H_{\text{comp}}/\text{kJ mol}^{-1}$	-1.2	-0.5	-0.4	-1.8
$\Delta H_{\text{spread}}/\text{kJ mol}^{-1}$	17.7	10.1	6.7	21.1

*reference (2)

crystals of I, II and III. As shown in Fig.2-I, after a yield time the surface pressure for the crystals of I under the condition of a constant temperature increased rapidly and gradually approached a constant value. This indicates that the sterol molecules elute spontaneously from the crystals onto water surface and finally produce a monolayer being in equilibrium with the excess bulk phase. The constant value of the surface pressure of the monolayer was adopted as ESP (3,4).

The yield time decreased with increasing temperature. Similar results were obtained for the samples of II and III as shown in Figs.2-II and-III. As can be seen from the mutual comparison of Figs.2-I, -II and -III, the yield time for each sterol is considerably different from those for others at various constant temperatures: The yield time increases in order of I < II < III. Namely, III needs a longer time to start eluting from its crystals onto the water surface than I or II in spite of its low melting point and enthalpy of fusion. The yield time is thought to be attributable to the properties of the crystal surface being directly in contact with the water. Therefore, the surface of the crystal of III seems to be more hydrophobic than that for the crystal of I or II. Hydrophilic OH moieties of III molecules would be located inside of the crystal. Unfortunately, X-ray diffraction analyses for the single crystals of II and III have not been in success because of the difficult preparation of their single crys-

tals suitable for X-ray diffraction analysis. Therefore, the structure details in the crystal of III as well as that of II are not still unknown. On the other hand, the two crystal forms for I were confirmed by means of calorimetric and X-ray diffraction measurements (8-10); the transition temperature between the two forms is about 312 K. The crystal structures of both forms of I in low and in high temperatures have been also confirmed by using their single crystals (11-13): The both crystals are quite similar to each other (13). As our ESP experiments were carried out below 313 K, whose temperature is very close to the transition point between the two solid forms, the crystal form at higher temperature would not need to be seriously considered. In addition, I itself forms bilayers of ca. 30 Å thickness with the polar region at the outer crystalline surface (11,14). Furthermore, as described in the previous paper (2), X-ray diffraction analysis for the powder samples indicated that the I and II crystals would have almost same packing mode with each other. Consequently, the yield time for I and II should have been almost equal with each other. However, yield time for I is shorter than that for II. Namely, the surface of the crystal of I is more hydrophilic than that of II. This is probably because of the existence of the double bond in I molecule.

The ESP values obtained for the three kinds of sterols are plotted in Fig.3 as a function of temperature. The

Fig.2 Time Courses of the Surface Pressures of 5-cholesten-3 β -ol (I), 5 α -cholestan-3 β -ol (II), and 5 β -cholestan-3 α -ol (III) Symbol ■ denotes 293.1 K; □, 298.1 K; ●, 303.1 K; ○, 308 K; ▲, 313.1 K.

ESP value for each sterol increases almost linearly with increasing temperature. It should be noted that the ESP values for II are, as a whole, close to those for I, compared with those for III.

3.2 ESP for Fully Hydrated, Amorphous Solids of Sterols

Collapsed monolayers (i.e., fully hydrated, amorphous solids) of I and II were collected and picked up with a spatula and transferred onto water surface; the constant surface pressures (ESP) for the monolayers of I and II, being in equilibrium with the solids, were

measured with the surface tension apparatus. The obtained ESP value was $25.5 \pm 0.5 \text{ mNm}^{-1}$ at 293.9 K for the solid of I and $26.5 \pm 0.5 \text{ mNm}^{-1}$ at 295.2 K for that of II, respectively. They are far below the ESP values for the ordinary crystals of I and II (see Fig.3). This indicates that the fully hydrated, amorphous solid is thermodynamically stable and has a lower ESP value than the anhydrous crystals.

3.3 Enthalpy of Elution from Crystals into Monolayer

The variation of ESP with temperature was used to obtain the enthalpy of elution, ΔH_{ESP} , from the crystals into the monolayer at ESP using the following two-dimensional Clausius-Clapayron's Equation (15,16) which neglects the contribution of the area due to the molecules in the crystal (16-18).

$$\frac{d\pi_{\text{ESP}}}{dT} = \frac{\Delta H_{\text{ESP}}}{TN_A A_{\text{ESP}}} \quad [1]$$

where the left side of the equation denotes the slope of the ESP vs. T relationship, N_A , Avogadro's number, and A_{ESP} , the molecular area of the sample at its ESP. The A_{ESP} values were obtained from the π - A relationships for the sterols shown in Figs. 4-I, -II and -III. The ΔH_{ESP} values obtained for the three kinds of sterol crystals are also listed in Table 1. The magnitudes of ΔH_{ESP} values decrease in the order of I > II > III. The order is same to that for the melting points and the enthalpies of fusion of their crystals.

3.4 π - A Relationships and Enthalpies of Compression and Spreading

Figures 4-I, -II and -III show the π - A relationships at various temperatures for the three kinds of sterols. The π - A relationships for II, whose OH group is in a same direction to that for I, are almost same to those for I. On the other hand, the π - A profiles for III, whose OH group is in opposite direction to that for I, are obviously different from those of I or II. The π - A curves for III at

various constant temperatures obviously expand and the collapsed pressures for the monolayers of III are lower than those for the monolayers of I and II.

From the temperature dependence of the π - A relationship, we evaluated the enthalpy of compression, ΔH_{comp} , of the monolayer from $\pi=0$ to ESP. Namely, the area integral of the π - A curve at a constant temperature from $\pi=0$ to ESP gives the work of compression, i.e., the Gibbs energy of compression, ΔG_{comp} . The ΔG_{comp} values obtained at various constant temperatures are listed in Table 1.

$\Delta G_{\text{comp}}/T$ vs. $1/T$ relationships for the monolayers of I, II and III gave relatively good straight lines. Thus, application of the Gibbs-Helmholtz's equation to the temperature dependence of the ΔG_{comp} gives the ΔH_{comp} for the sterol monolayer. The obtained ΔH_{comp} values are listed in Table 1. The sum of ΔH_{ESP} and $-\Delta H_{\text{comp}}$ means the enthalpy of spreading, ΔH_{spread} , of the sterol molecules from the crystal into the disordered monolayer at $\pi=0$. The ΔH_{spread} values for the three kinds of sterols are also listed in the Table 1. The spreading from crystal into disordered monolayer means the two-dimensional fusion of crystal into the two-dimensional disordered liquid. Therefore, the two-dimensional fusion should be correlated to the ordinary three-dimensional fusion. In fact, as shown in Fig. 5, an approximately linear relationship exists between the ΔH_{fusion} and the ΔH_{spread} ; its slope is almost one and the value of its interception at the ΔH_{fusion} axis is almost equal to heat of hydration.

Fig. 3 Temperature Dependence of ESP for the Crystals of 5-cholesten-3 β -ol (I), 5 α -cholestan-3 β -ol (II) and 5 β -cholestan-3 α -ol (III).

Fig.4 The π - A Relationships for 5-cholesten-3 β -ol (I), 5 α -cholestan-3 β -ol (II) and 5 β -cholestan-3 α -ol (III) over a Temperature Range of 293.1-343.1 K with an increment of 5 K. The π - A curves expand with increasing temperature.

3.5 Mixed Monolayers of II and III

The sterols, II and III, whose OH moieties are in opposite directions to each other, make a strong 1:1 compound in the solid state (2) : The existence of the 1:1 compound was confirmed by a phase diagram which shows the highest melting point at 1:1 molar ratio. The

formation of 1:1 compound in the mixed monolayer of II and III on water surface was also confirmed by the DSC analysis of the collapsed monolayers having 1:1 molar ratio. Namely, the collapsed monolayers were collected from water surface and dried in vacuum, and the DSC analysis was carried out. The dried film mate-

Fig. 5 ΔH_{fusion} vs. ΔH_{spread} Relationship for Three Kinds of Sterols.

rial having 1:1 molar ratio indicated the exactly same melting point (418.3 K) to that for the ordinary 1:1 compound of II and III in solid state (2). On the other hand, a simple mixing in a mortar of the crystallites of II and III did not show such the high melting point.

From the π - A relationships for the mixed monolayers of II and III, we obtained average molecular area as a function of composition under a constant surface pressure at 293.1 K. As a representative example, Fig. 6 shows the average molecular area, A_{av} , at $\pi = 20 \text{ mNm}^{-1}$ vs. mole fraction of III, x_{III} , in the mixed monolayers of II and III. The A_{av} plot at 1:1 in molar ratio ($x_{\text{III}} = 0.5$) lies just on a linear line satisfying ideal additivity rule of average molecular area (19), whereas other A_{av} plots at various mole fractions positively deviate from the line: Positive deviations of the A_{av} from the ideal additivity rule are observed at both sides of $x_{\text{III}} = 0.5$. At $x_{\text{III}} = 0.5$, 1:1 compound would be only one species, whose molecular area is just the sum of the original molecular areas for II and III: The molecules of 1:1-compound would coexist with the molecules of II and III at the ranges below and above $x_{\text{III}} = 0.5$, respectively. That is, Fig. 6 consists of two phase-diagrams: one phase-diagram is for the mixture of II and 1:1 compound and the other one, for 1:1 compound and III. Each diagram shows that the molecules of 1:1 compound in the mixed monolayers would repulse II and III molecules and would expand the mixed monolayer.

In order to know the degree of expansion for all components (i.e., II, III and 1:1-compound) in the mixed film, we evaluated their partial molecular areas

Fig. 6 Average Molecular Area, A_{av} , as a Function of Composition in Monolayers of II and III at a Constant Surface Pressure (20 mNm^{-1}) at 293.1 K. The plot at 1:1 molar ratio is just on the ideal mixing linear line. The existence of the 1:1 compound in the monolayer was actually confirmed by the DSC analysis for the film material collected at 1:1 molar ratio.

from the A_{av} vs. x_{III} curve. Namely, as shown in Fig. 7, the partial molecular areas for II, 1:1-compound as one molar sterol unit and III were determined by the respective intercepts at $x_{\text{III}} = 0$, $x_{\text{III}} = 0.5$ and $x_{\text{III}} = 1$ of a tangential line drawn at any given point on the A_{av} vs. x_{III} curve, respectively. The partial molecular areas for II and III in the mixed film are large especially at abundant regions of 1:1-compound (i.e., near $x_{\text{III}} = 0.5$), whereas the partial molecular area for 1:1-compound is almost constant regardless of mole fraction, x_{III} . Thus, positive deviations of A_{av} from the ideal additivity rule at both sides of $x_{\text{III}} = 0.5$ in the phase diagram in Fig. 6 would be caused mainly from the expansion of the partial molecular areas for II and III in the mixed film.

Figure 8 shows π - A relationships for the 1:1 compound at various temperatures. The collapsed pressures for the monolayers of the 1:1 compound are slightly higher than that for III and largely lower than that for II at various temperatures.

Figure 9 shows the time course of the surface pressure for the crystals of the 1:1 compound prepared by evaporating the solvent (chloroform) from a 1:1-molar ratio solution of II and III. The yield time also decreased with increasing temperature. The relatively long yield time at 298 K suggests that the surface of the

Fig.7 Partial Molecular Areas at $\pi=20 \text{ mNm}^{-1}$ at 293.1 K for all components (i.e., II, III and 1:1 compound) in the Mixed Film, Determined from the Curve of A_{av} vs. x_{III} (Fig.6). Symbol $\triangle$ denotes partial molecular area for II; $\square$, for III; $\bullet$, for 1:1 compound.

Fig.8 π - A Relationships for the 1:1 Compound of II and III at Various Temperatures.

crystal of the 1:1 compound is hydrophobic, compared with that for II. However, at higher temperatures, the surface of the crystal of the 1:1 compound becomes to be hydrophilic, compared with that of II. The obtained

ESP values for the 1:1 compound at various constant temperatures are also plotted in Fig.3. The ESP values for the 1:1 compound are rather close to those for III. From the inclination of the ESP vs. temperature curve

Fig.9 Time Course of the Spreading Pressure of the Crystals of the 1:1 Compound of II and III at Various Constant Temperatures. The crystal sample was prepared by the evaporation of chloroform from the 1:1 mixture of II and III in chloroform. Symbol ■ denotes 293.1 K; □, 298.1 K; ●, 303.1 K; ○, 308 K; ▲, 313.1 K.

and the π - A relationships at various constant temperatures, we obtained ΔH_{ESP} and ΔH_{comp} for the 1:1 compound. The obtained thermodynamic data for the 1:1 compound are listed in the last column of Table 1. The data are corresponding to two molar sterols; the thermodynamic properties of the 1:1 compound, as one molar sterol unit, are close to those for II.

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