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ORIGINAL CONTRIBUTION

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Biodetergent
IV. Monolayers of corynomycolic acids at the air-water interface

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Abstract The effects of alkyl chain length and of differences in the length of the two alkyl chains on the formation of a monolayer of chemically synthesized corynomycolic acid (2-alkyl-3-hydroxy fatty acid) at the air-water interface were examined. Hydrophobic interactions between the two alkyl chains are required for the formation of a condensed film, which is most stable when the total number of carbon atoms in the two alkyl chains is 25 or more and the difference in their lengths is one. *Syn*-isomers form condensed films but usually not *anti*-isomers. However, films may also be formed by the *anti*-isomer when the alkyl chain at the carboxy group (the 2-position) is longer than the alkyl chain at the hydroxy group (the 3-position). That is, the contribution of *anti*-isomers to condensed film formation depends on the polar carboxy group which has greater involvement in this formation. The extrapolated area for the condensed film of corynomycolic acid was 40 $Å^2$ per molecule, thus confirming that both the carboxy and hydroxy groups are present on the water surface when a bipolar monolayer is formed.

Key words Biomimetic surfactant – Corynomycolic acid – Monolayer – Condensed film – Alkyl chain length

Introduction

The microbial biosurfactants produced by microorganisms have been attracting interest as new functional and ecological materials, since they have special properties such as a wide variety of possible structures, high biodegradability, low toxicity and biological activity.

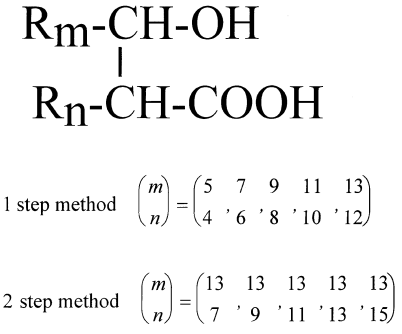
We previously reported the surface activities and detergency of polycarboxylic biosurfactants, such as spiculisporic acid (4,5-dicarboxy-4-pentadecanolide) [1] and agaricic acid (2-hydroxy-1,2,3-nonadecanetricarboxylic acid) [2], as a replacement for fatty acid soap. Natural corynomycolic acid (2-alkyl-3-hydroxy fatty acid) produced by *Corynebacterium lepus* is also a biosurfactant of the same type. It is known to have a mixture of homologies with different lengths of alkyl chain [3]. Therefore, it is not a suitable material for studying surface active properties and other basic properties. So, we chemically synthesized corynomycolic acid to study the relationship between its chemical structure and its surface active properties [4]. As biosurfactants characteristically behave like biological membranes [5], we further expect corynomycolic acid to form a stable monolayer at the air-water interface, since the acid has a structure with two lipophilic alkyl chains and two hydrophilic groups.

In this paper, we study the effects of alkyl chain length and differences in the lengths of the two alkyl chains on the formation of a monolayer of chemically synthesized corynomycolic acid at the air-water interface. We further discuss the hydrophobic interaction between alkyl chains on the formation of a monolayer and the possible conformation of the molecules in the monolayer.

Experimental

Synthesis of corynomycolic acid

Ten kinds of corynomycolic acids with different alkyl chain lengths shown in Scheme 1 were synthesized by one-step and two-step methods. The structures were determined by fourier transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR), and mass spectroscopy (MS) measurements. FTIR spectra were determined using a Perkin Elmer FTIR spectrometer, Model 1720, on a KBr disk, ^{1}H-NMR spectra were recorded on a JEOL EM-360 (60 MHz) with tetramethylsilane (TMS) as the internal standard and $CDCl_3$ as a solvent. Mass spectra were taken using a Hitachi M-2000 mass spectrometer. The corynomycolic acids are described briefly as $CM_{m\text{-}n}$, where m is the number of carbons in the alkyl chain at the 3-position and n is that of the alkyl chain at the 2-position.



Scheme 1 Schematic representation of corynomycolic acids

Synthesis of corynomycolic acid by the one-step method

Corynomycolic acids with different alkyl chain lengths were synthesized according to the method of Ishigami et al. [6] with some modification. The method consists of Claisen condensation to an oxo-ester, reduction to a hydroxyester with sodium borohydride, and hydrolysis with alkali to corynomycolic acid [4]. We used fatty acid ethyl esters with alkyl chain lengths of 6, 8, 10, 12 and 14 (Tokyo Kasei, reagent special grade) as a starting material to obtain $CM_{5\text{-}4}$, $CM_{7\text{-}6}$, $CM_{9\text{-}8}$, $CM_{11\text{-}10}$, $CM_{13\text{-}12}$, respectively. $CM_{11\text{-}10}$, and $CM_{13\text{-}12}$ were successfully separated into two isomers, *syn* and *anti*, by column chromatography of the hydroxyesters on silica, eluting with benzene.

Synthesis of corynomycolic acid (2-alkyl-3-hydroxyhexadecanoic acid) by the two-step method

According to Solowag and LaForge [7], 3-hydroxyhexadecanoic acid with the desired length of alkyl chain at the 2-position was synthesized as follows.

To a suspension of sodium hydride (NaH in oil 60%, 4.00 g) in 100 ml of dry benzene, ethyl acetoacetate (13.6 g 0.1 mol) was added dropwise with stirring. The suspension was stirred for 30 min, cooled in an ice bath, and myristoyl chloride (25 g) added over a period of 15 min with stirring. The mixture was refluxed for 15 min, cooled, poured onto crushed ice and acidified with hydrochloric acid. Ethanol (70 ml) was added and the two layers separated. The benzene layer was washed three times with 10% ethanol, dried over sodium sulfate and solvent evaporated in vacuo.

To the residue was added 40 ml of methanol and 17.6 g sodium methylate (as a 28% methanol solution). After keeping it at room temperature for 90 min, the mixture was poured onto crushed ice and acidified with hydrochloric acid (10 ml). The organic material was extracted with ether; the ether solution was washed with water, dried over sodium sulfate and the ether removed under reduced pressure. The oily residue was recrystallized from hexane to give 18.3 g of methyl 3-oxohexadecanoate, a colorless solid, at a 63% yield: m.p. 35–36 °C, IR (KBr) 1740, 1715, 1180 cm^{-1}, H-NMR ($CDCl_3$) δ 0.88 (t, 3H), 1.25–1.58 (m, 22H), 2.52 (t, 2H), 3.43 (s, 2H), 3.73 (s, 3H), MS m/e 288 (M^+). Anal. found (calcd): C, 71.35 (71.78); H, 11.52 (11.34).

All the 2-alkyl-3-hydroxyhexadecanoic acids were synthesized using similar techniques; the synthesis of 2-pentadecyl-3-hydroxyhexadecanoic acid is described here. To a solution of sodium ethoxide (prepared from 0.23 g sodium metal and 20 ml dry ethanol), 2.84 g methyl 3-oxohexadecanoate (0.01 mol) was added. After stirring for 30 min, 1-bromopentadecane (0.01 mol) in 5 ml of ethanol was added dropwise over a period of 15 min. The mixture was refluxed for at least 6 h, during which time sodium bromide began to precipitate. The mixture was poured onto crushed ice, acidified with hydrochloric acid, extracted with benzene, dried over sodium sulfate and the solvent removed under reduced pressure. The residue was purified by column chromatography on silica, eluting with benzene. After recrystallization from methanol, ethyl 2-pentadecyl-3-oxohexadecanoate was obtained as a colorless solid with a 51% yield: mp. 55 °C, IR (KBr) 1734, 1714, 1181 cm^{-1}, H-NMR ($CDCl_3$) δ 0.91 (t, 9H), 1.26–1.84 (m, 50H), 2.52 (dt, 2H), 3.43 (t, 1H), 4.20 (q, 2H), MS m/e 508 (M^+). Anal. found (calcd): C, 78.11 (77.89); H, 12.55 (12.68). Hexane was used to recrystallize the other 2-alkyl-3-oxohexadecanoates.

Ethyl 2-pentadecyl-3-oxohexadecanoate (0.10 mol) was dissolved in 300 ml ethanol. To this solution, cooled in an ice bath, sodium borohydride (0.13 mol) was added in portions with stirring. After stirring for 20 min, the solution was kept at room temperature for 24 h with stirring. The reaction mixture was poured onto crushed ice and acidified with hydrochloric acid. The product was extracted with methylene chloride and dried over sodium sulfate. After the solvent had been removed under reduced pressure, the residue was separated by column chromatography on silica, eluting with benzene, to give the *syn*- and *anti*-isomers of ethyl 2-pentadecyl-3-hydroxyhexadecanoate at 17% and 26% yields, respectively.

syn-Ethyl 2-pentadecyl-3-hydroxyhexadecanoate: m.p. 65–66 °C, IR (KBr) 3327, 1729, 1178 cm^{-1}, H-NMR ($CDCl_3$) δ 0.88 (t, 9H), 1.25–1.68 (m, 52H), 1.57 (br s, 1H), 2.41 (m, 1H), 3.77 (dt, 1H), 4.17 (dq, 2H), MS m/e 490 (M^+-H_2O). Anal. found (calcd): C, 77.32 (77.58); H, 13.45 (13.02).

anti-Ethyl 2-pentadecyl-3-hydroxyhexadecanoate: m.p. 58–60 °C, IR (KBr) 3514, 1708, 1181 cm^{-1}, H-NMR ($CDCl_3$) δ 0.88 (t, 9H), 1.26–1.73 (m, 52H), 1.58 (br s, 1H), 2.41 (m, 1H), 3.68 (dt, 1H), 4.18 (dq, 2H), MS m/e 492 (M^+-H_2O). Anal. found (calcd): C, 77.40 (77.58); H, 13.33 (13.02).

Each 2-alkyl-3-hydroxyhexadecanoate (0.05 mole) was further dissolved in 100 ml of mixed solvent (ethanol:water = 5:1). Potassium hydroxide (0.1 mol) was added and the solution refluxed for 2 h to give a clear solution. The solvent was removed under reduced pressure, the residue neutralized with hydrochloric acid, extracted with methylene chloride, dried over sodium sulfate and concentrated. The products were recrystallized from hexane to afford 2-alkyl-3-hydroxyhexadecanoic acids at almost quantitative yields from the hydroxy esters.

syn-2-Heptyl-3-hydroxyhexadecanoic acid (*syn*-$CM_{13\text{-}7}$): m.p. 75.5–77 °C, IR (KBr) 3298, 3200-2450, 1736 cm^{-1}, H-NMR ($CDCl_3$) δ 0.88 (t, 6H), 1.2–1.7 (m, 36H), 1.45 (s, 1H), 2.49 (q, 1H), 3.88 (q, 1H), MS m/e 352 (M^+-H_2O). Anal. found (calcd): C, 73.99 (74.54); H, 12.69 (12.51).

anti-2-Heptyl-3-hydroxyhexadecanoic acid (*anti*-$CM_{13\text{-}7}$): m.p. 62.0–64.5 °C, IR (KBr) 3532, 3280-2480, 1685 cm^{-1}, H-NMR ($CDCl_3$) δ 0.88 (t, 6H), 1.2–1.72 (m, 36H), 1.49 (s, 1H), 2.45 (q, 1H), 3.72 (q, 1H), MS m/e 352 (M^+-H_2O). Anal. found (calcd): C, 74.85 (74.54); H, 12.31 (12.51).

syn-2-Nonyl-3-hydroxyhexadecanoic acid (*syn*-CM_{13-9}): m.p. 55.0–57.0 °C, IR (KBr) 3330, 3250-2450, 1698 cm^{-1}, H-NMR ($CDCl_3$) δ 0.88 (t, 6H), 1.2–1.71 (m, 40H), 1.49 (s, 1H), 2.48 (q, 1H), 3.87 (q, 1H), MS m/e 380 (M^+-H_2O). Anal. found (calcd): C, 75.14 (75.32); H, 13.11 (12.64).

anti-2-Nonyl-3-hydroxyhexadecanoic acid (*anti*-CM_{13-9}): m.p. 62.5–68.0 °C, IR (KBr) 3530, 3200-2400, 1736 cm^{-1}, H-NMR ($CDCl_3$) δ 0.88 (t, 6H), 1.2–1.74 (m, 40H), 1.50 (s, 1H), 2.45 (q, 1H), 3.72 (q, 1H), MS m/e 380 (M^+-H_2O). Anal. found (calcd): C, 75.08 (75.32); H, 12.97 (12.64).

syn-2-Undecyl-3-hydroxyhexadecanoic acid (*syn*-CM_{13-11}): m.p. 70.0–72.0 °C, IR (KBr) 3402, 3250-2440, 1697 cm^{-1}, H-NMR ($CDCl_3$) δ 0.89 (t, 6H), 1.2–1.72 (m, 44H), 1.47 (s, 1H), 2.49 (q, 1H), 3.86 (q, 1H), MS m/e 408 (M^+-H_2O). Anal. found (calcd): C, 75.96 (76.00); H, 13.18 (12.76).

anti-2-Undecyl-3-hydroxyhexadecanoic acid (*anti*-CM_{13-11}): m.p. 58.5–61.0 °C, IR (KBr) 3529, 3250-2480, 1686 cm^{-1}, H-NMR ($CDCl_3$) δ 0.89 (t, 6H), 1.2–1.7 (m, 44H), 1.51 (s, 1H), 2.46 (q, 1H), 3.72 (q, 1H), MS m/e 408 (M^+-H_2O). Anal. found (calcd): C, 75.45 (76.00); H, 13.22 (12.76).

syn-2-Tridecyl-3-hydroxyhexadecanoic acid (*syn*-CM_{13-13}): m.p. 69.0–70.5 °C, IR (KBr) 3402, 3300-2480, 1705 cm^{-1}, H-NMR ($CDCl_3$) δ 0.87 (t, 6H), 1.2–1.71 (m, 48H), 1.48 (s, 1H), 2.49 (q, 1H), 3.85 (q, 1H), MS m/e 436 (M^+-H_2O). Anal. found (calcd): C, 76.22 (76.59); H, 13.11 (12.86).

anti-2-Tridecyl-3-hydroxyhexadecanoic acid (*anti*-CM_{13-13}): m.p. 68.0–70.0 °C, IR (KBr) 3513, 3300-2500, 1710 cm^{-1}, H-NMR ($CDCl_3$) δ 0.88 (t, 6H), 1.2–1.75 (m, 48H), 1.51 (s, 1H), 2.46 (q, 1H), 3.70 (q, 1H), MS m/e 436 (M^+-H_2O). Anal. found (calcd): C, 76.41 (76.59); H, 12.98 (12.86).

syn-2-Pentadecyl-3-hydroxyhexadecanoic acid (*syn*-CM_{13-15}): m.p. 69.0–70.5 °C, IR (KBr) 3392, 3280-2470, 1698 cm^{-1}, H-NMR ($CDCl_3$) δ 0.88 (t, 6H), 1.2–1.71 (m, 52H), 1.45 (s, 1H), 2.48 (q, 1H), 3.86 (q, 1H), MS m/e 464 (M^+-H_2O). Anal. found (calcd): C, 77.33 (77.12); H, 13.15 (12.94).

anti-2-Pentadecyl-3-hydroxyhexadecanoic acid (*anti*-CM_{13-15}): m.p. 80.5–82.0 °C, IR (KBr) 3535, 3290-2430, 1684 cm^{-1}, H-NMR ($CDCl_3$) δ 0.88 (t, 6H), 1.2–1.74 (m, 52H), 1.49 (s, 1H), 2.46 (q, 1H), 3.71 (q, 1H), MS m/e 464 (M^+-H_2O). Anal. found (calcd): C, 77.01 (77.12); H, 13.26 (12.94).

Surface pressure versus area isotherms

Surface pressure versus area isotherms were determined by a fully automated, continuously recording, surface film balance (San-esu Keisoku Model FSD-20). The film balance system consists of a Teflon coated trough of 506 mm (length) by 150 mm (width) and a microprocessor (NEC PC-9801). The temperature of the subphase (water distilled twice) was controlled by circulating thermostated water within the trough. A benzene or benzene-ethanol solution (30–45 μl) of synthesized corynomycolic acids (2–3 mg/ml) was placed on the surface of the subphase; then, a Teflon barrier was swept to compress the film at the speed of 0.4 mm/s. The pressure was calibrated using an octadecanoic acid monolayer, and the pressure-area isotherm was taken as an indication of purity for the preparation.

Results and discussion

Separation of *syn*- and *anti*-corynomycolic acids

As shown in the experimental section, we synthesized various 2-alkyl-3-hydroxyhexadecanoic acids by a two-step method; the number of m in C_{m-n} was fixed at 13. *Syn*- and *anti*-isomers were successfully separated, by column chromatography on silica, at the stage of ethyl 2-alkyl-3-hydroxyhexadecanoates. According to the patent by Kamada et al. [8], the first fraction was identified as the *syn*-isomer, and the second fraction as the *anti*-isomer. This is reasonable because the *syn*-isomer can form intramolecular hydrogen bonding between 3-OH and COOEt and would be less polar than the *anti*-isomer. Further, such intramolecular hydrogen bonding would affect the O—H and C=O bond stretching in the IR spectra. In fact, the C=O stretching of *syn*-ethyl 2-pentadecyl-3-hydroxyhexadecanoate (1729 cm^{-1}) was higher than that of the *anti*-isomer (1708 cm^{-1}). Therefore, we identified the first fraction as the *syn*-isomer and the second fraction as the *anti*-isomer.

Effects of the alkyl chain lengths of corynomycolic acid on the formation of a monolayer

Figure 1 shows the pressure-area isotherms at 25 °C for five kinds of corynomycolic acids with different alkyl chain lengths, synthesized by the one-step method. The monolayer isotherm of CM_{5-4}, which has the shortest alkyl chains used in this work, was not determined due to its solubility in water. The isotherms of CM_{7-6} and CM_{9-8} exhibited only an expanded state. The isotherm of CM_{11-10} exhibits a gaseous region at low pressure and a phase change to an expanded film at about 25 $mN \cdot m^{-1}$, but it did not form a condensed film. The corresponding isotherm for CM_{13-12} exhibited a phase transition from an expanded to a condensed film on compression.

According to Ogino et al. [9], the hydrophobic interaction between the alkyl chains is essential for the

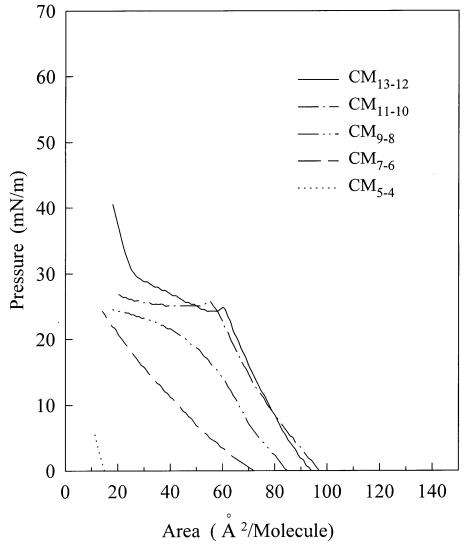


Fig. 1 Pressure-area isotherms of corynomycolic acids synthesized by one step at 25 °C

formation of a condensed film; this interaction depends on the alkyl chain length. In the case of $CM_{m\text{-}n}$ where $n = m - 1$, a condensed film can be formed when m exceeds 13 (i.e. 2-dodecyl-3-hydroxyhexadecanoic acid). It is also known that these acids form a stable condensed film in which the molecules are completely erect.

Effects of the *syn*- and *anti*-isomers of corynomycolic acid on the formation of a monolayer

Corynomycolic acid has two isomers, that is *syn*- and *anti*-isomers. To study the effect of these isomers on the formation of a monolayer, pressure-area isotherms for *syn*- and *anti*-isomers of both $CM_{13\text{-}12}$ and $CM_{11\text{-}10}$ were determined at 15 °C and 25 °C. Typical isotherms for $CM_{13\text{-}12}$, measured at 15 °C, are shown in Fig. 2. The *syn*-isomer of $CM_{13\text{-}12}$ forms a condensed film at both 15 °C and 25 °C, while *syn*-$CM_{11\text{-}10}$ can only form a condensed film at 15 °C. However, *anti*-isomers of both $CM_{13\text{-}12}$ and $CM_{11\text{-}10}$ form only expanded films and not condensed films at 15 °C and 25 °C.

This monolayer behavior may be explained as follows. When the interaction between the hydrophobic groups of two alkyl chains is strongest, the *syn*-isomer can form intramolecular hydrogen bonds, between the two polar groups (OH and COOH), at the water surface as shown in Scheme 2a. However, under the same situation the *anti*-isomer cannot form intramolecular hydrogen bonds. When hydrogen bonding is dominant between *anti*-isomers, the two alkyl chains are positioned as shown in Scheme 2b and the hydrophobic interaction between the alkyl chains is not strong enough to form a condensed film.

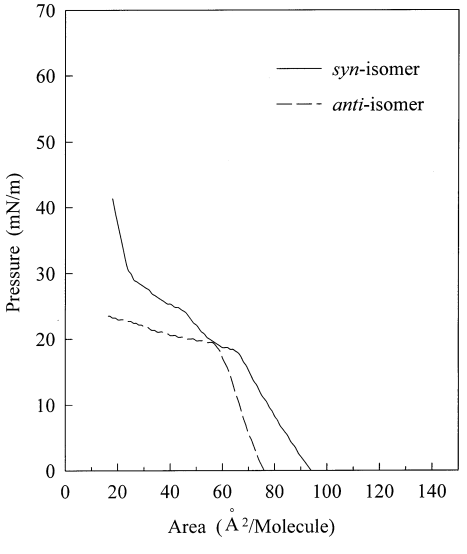
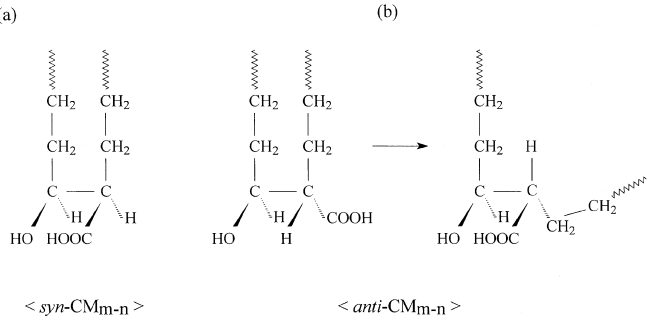


Fig. 2 Pressure-area isotherms of *syn*- and *anti*-isomers of $CM_{13\text{-}12}$ at 15 °C



Scheme 2 Schematic illustration of the molecular conformations of *syn*- and *anti*-$CM_{m\text{-}n}$

Molecular conformation of corynomycolic acid in the monolayer

The isotherms of hexadecanoic acid 3-hydroxyhexadecanoic acid and $CM_{13\text{-}12}$, which all have the same effective chain length and fatty acid structure, were determined in order to study the molecular conformation of corynomycolic acid in the monolayer. Figure 3 shows that hexadecanoic acid, which is a straight alkyl chain with only COOH as a polar group, forms a typical condensed film and gives an extrapolated area of about 24 $Å^2$ per molecule. Since this value is equivalent to the cross-sectional area of COOH, it is thought that the molecules are oriented so that the straight alkyl chains are erect and close-packed with the carboxy groups on the water surface.

There is an inflection point in the isotherm of 3-hydroxyhexadecanoic acid, which has one alkyl chain and two polar groups. The pressure at which the inflection point appears increases as the temperature is raised. The extrapolated area for the molecule finally observed is about 25 $Å^2$ per molecule. This suggests that 3-hydroxyhexadecanoic acid may be oriented so that only COOH exists on the water surface, or even that the hydroxy groups are immersed in the water phase forming a monopolar condensed film. In other words, the effective polar group in the acid consists of the entire HOOC-CH_2-CHOH moiety, leaving a 13-carbon alkane chain as the hydrophobic portion, just as for tetradecanoic acid [10].

In the case of *syn*-$CM_{13\text{-}12}$, which has two polar group and two alkyl chains, there was a phase transition from an expanded film to a condensed one at a surface pressure of about 30 $mN \cdot m^{-1}$. The extrapolated area is about 40 $Å^2$ per molecule, which is equivalent to the total area when both COOH and OH groups exist on the water surface. Therefore, *syn*-$CM_{13\text{-}12}$ forms a bipolar monolayer. Consequently, the formation of a condensed

film depends strongly on both the interaction between the two polar groups and the hydrophobic interaction between the two alkyl chains.

Effects of a difference in the lengths of the alkyl chains on the formation of a monolayer

It has been considered that the interaction between the alkyl chains, which is essential for the formation of a monolayer [9], depends not only on the alkyl chain length but also on the difference in the lengths of the two alkyl chains [11]. Therefore, pressure-area isotherms for 2-alkyl-3-hydroxyhexadecanoic acids, $CM_{13\text{-}n}$, with the desired length of alkyl chain at the 2-position, were determined at 25 °C (shown in Fig. 4).

The $CM_{13\text{-}n}$ *syn*-isomers, $CM_{13\text{-}7}$ and $CM_{13\text{-}9}$ did not form a condensed film, while $CM_{13\text{-}11}$, $CM_{13\text{-}12}$, $CM_{13\text{-}13}$ and $CM_{13\text{-}15}$ formed condensed films. However, the only *anti*-isomer of $CM_{13\text{-}n}$ to form a condensed film was $CM_{13\text{-}15}$, as shown in Fig. 5.

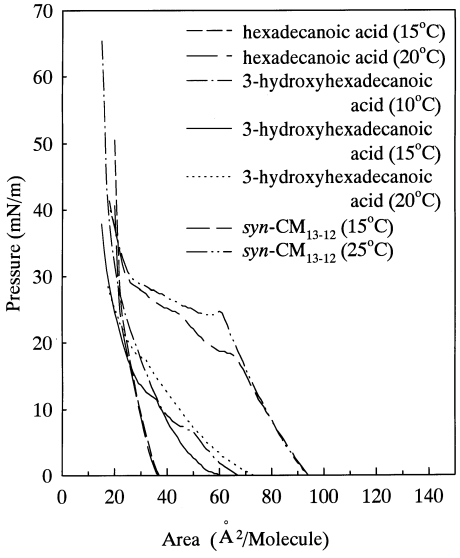


Fig. 3 Temperature dependence of the pressure-area isotherms of hexadecanoic acid, 3-hydroxyhexadecanoic acid and *syn*-$CM_{13\text{-}12}$

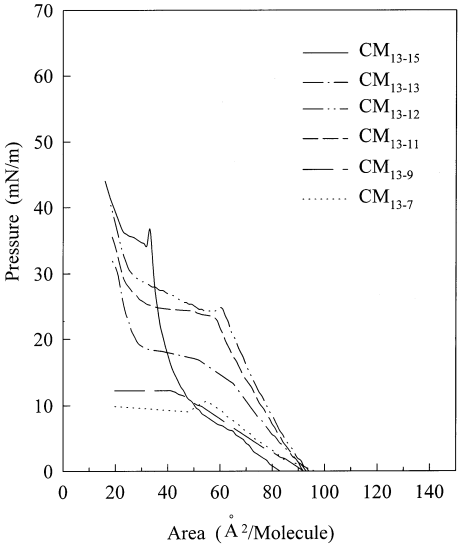


Fig. 4 Pressure-area isotherms of *syn*-$CM_{13\text{-}n}$ ($n = 7$–15) at 25 °C

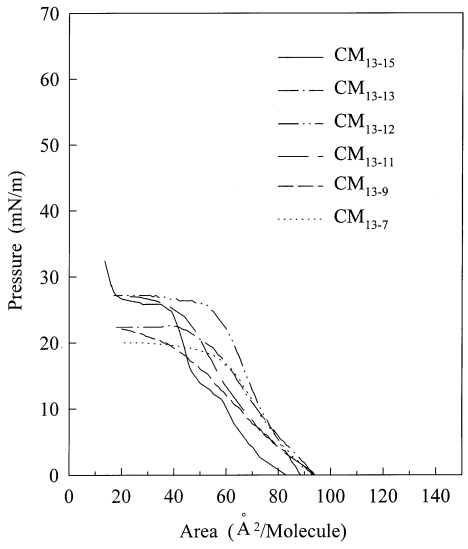
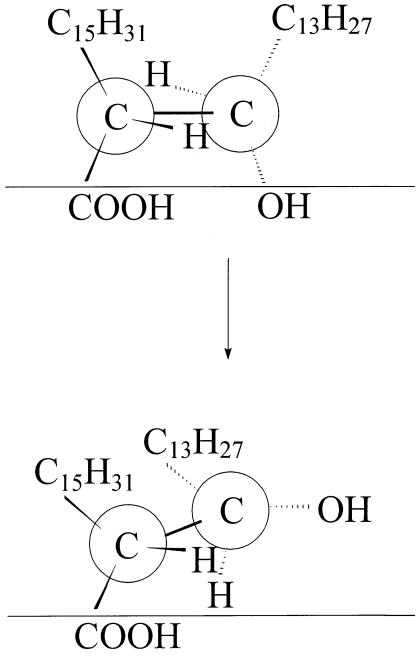


Fig. 5 Pressure-area isotherms of *anti*-$CM_{13\text{-}n}$ ($n = 7$–15) at 25 °C



Scheme 3 Schematic representation of the molecular conformation of *anti*-$CM_{13\text{-}15}$

Abe et al. [11] reported that monolayers of sodium 2-sulfonatofatty acid alkyl esters were more readily converted to condensed films as the difference between the two alkyl chain lengths decreased. In our case, the highest value in pressure was obtained for $CM_{13\text{-}12}$ and not $CM_{13\text{-}13}$ (Fig. 4). When the water solubility of alcohols and acids in the same length alkyls are compared, acids generally have higher solubility. Therefore, when *syn*-$CM_{m\text{-}n}$ is situated on the water surface, it can be supposed that COOH would enter into water more deeply than OH. Assuming that COOH is further from the water surface than OH, we carried out model calculations and obtained the value of $1.3 \sim 1.5$ as the difference between the two alkyl chain lengths (*m*-*n*).

Thus, the monolayer is most stabilized by the interaction between two alkyl chains when the difference between *m* and *n* is about 1.3. When $m = 13$, *n* should be $\sim$11.7, and $CM_{13\text{-}12}$ is the closest molecule to this figure. Therefore, it can be considered that the most stable condensed film, with the strongest cohesive force, is formed when the difference in the lengths of the two alkyl chains (*m*-*n*) is 1.

As shown in Fig. 5, only the *anti*-isomer of $CM_{13\text{-}15}$ formed a condensed film, behaving differently from the other *anti*-isomers of corynomycolic acid. This is due to the fact that the alkyl chain at the 2-position is longer in comparison with the other corynomycolic acids. As illustrated in Scheme 3, due to the longer alkyl chain of the COOH side (at 2-position), the less polar OH group is further from the interface, so the interaction between the polar groups (OH and COOH) is weakened and the interaction between the alkyl chains initiated. The *anti*-isomer finally shows behavior similar to that of the *syn*-isomer, which is consistent with our suppositions.

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