Monolayers of Branched-Chain Fatty Acids I*

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Branched-chain fatty acids have received much attention from biological points of view. Some of them are found in the normal cell of several kinds of bacteria, while some of them are strongly bactericidal. The bactericidal activity of a number of branched-chain fatty acids has been studied extensively by Adams and his coworkers,(1) and it has been stated that there is parallelism between the bactericidal activity and the surface activity of these acids. However, the exact mechanism of the bactericidal activity is still obscure.

A number of new branched-chain fatty acids have been synthesized at the Chemical Department, Institute for Infectious Diseases, Tokyo University, and their biological and chemical properties have been thoroughly investigated.(2)

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* Part of this work was presented at the Meeting of the Chemical Society of Japan, held at Tokyo, Sept, 13 1947.

(1) W. M. Stanley and R. Adams, *J. Am. Chem. Soc.*, **54**, 1548 (1932).

(2) M. Asano, U. Kameta, T. Komai and I. Ishino, *Igakusōran (Japan)*, **1**, 41 (1945); M. Asano, U. Kameta and T. Wada, *J. Pharmaceut. Soc. Japan.*, **64**, 25 (1944); M. Asano and T. Yamakawa, *J. Pharmaceut. Soc. Japan*, **70**, 474 (1950); M. Asano, J. Ota and U. Ariyoshi, *Igakusōran (Japan)*, **1**, 42 (1945).

A detailed study of the monolayer behavior of these compounds may throw light on the mechanism of their action. In fact, it has been reported that there is a relation between the biological activity of alkyl substituted succinic acid half esters and the orientation of the molecules at air-water interface.(3) Unfortunately, at the present stage of our knowledge, it is not possible to correlate directly the surface properties of these acids with the biological activity. But, if we consider the surface behavior together with other properties of these acids, clearer understanding may be expected.

In this report, the measurements of force-area relations were made and the results were correlated with the structure and configuration of the molecules in the monolayer.

Techniques

The apparatus for measuring force-area curves was Langmuir-Adam type surface balance. It consisted of a nickel-plated brass trough and copper float with platinum ribbons. To avoid contamination, the whole surface of the trough, float system and glass barriers were heavily coated with solid paraffin in the usual manner, and they were rinsed with tap water about one hour before use.

The sample was dissolved with redistilled benzene and was spread on water by means of a micropipette. The volume of the micropipette was calibrated against the cross-sectional area of palmitic acid monolayer on distilled or acidic water substrates. The sensitivity of the torsion balance was 0.200 dyne per cm. per degree, and the surface pressure could be measured with the accuracy of ±0.05 dyne/cm.

Since these compounds formed more stable films on acid substrates than on neutral or alkaline solutions, and since it was necessary to minimize the influence of heavy metal ions, experiments were made on 0.01 N hydrochloric acid solution.

Measurement was started at about one minute after spreading the material at zero pressure, and the time interval between adjacent points on the force-area curves was about thirty seconds. If the pressure changed rapidly with time owing to the hysteresis, it was read when no appreciable change was observed on the value within one minute. Generally, the coincidence between curves obtained by compression and expansion was relatively poor. Whether this is due to the instability of the monolayer or to the true hysteresis is uncertain. Each point on the curve was the mean of at least three independent measurements and was accurate within 3 per cent.

Materials

The branched-chain fatty acids used in this experiment were obtained from the Chemical Department, Institute for Infectious Diseases, Tokyo University, by courtesy of the late Prof. Asano. Palmitic acid was purchased from Dr. Theodor Schuchardt G. M. B. H. Chemische Fabrik, and was recrystallized from methanol. Its melting point was 62°C.

Results and Discussions

1. Trialkylacetic Acids.—Fig. 1 shows the force-area curves for two trialkylacetic acids with two long hydrocarbon chains at 20°C. These films are liquid-expanded, the limiting area of which is 75~80 A.^{2} per molecule, and are compressible to 60~65 A.^{2} The film of methylundecyldodecylacetic acid (I) collapses at about 7 dynes per cm., whereas monolayer of ethyldecyldodecylacetic acid (II) withstands higher pressures of 12~13 dynes. The reason for this difference may be due to the fact that while I is solid, II is liquid in

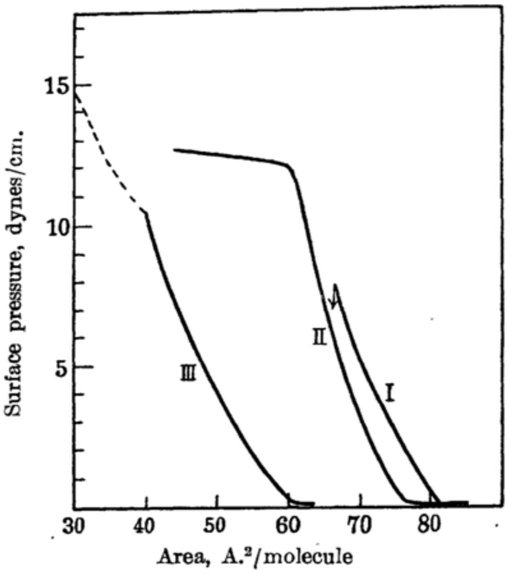

Fig. 1.—Force-area curves for methylundecyldodecylacetic acid (I) and ethyldecyldodecylacetic acid (II). Curve for phthioic acid (III) after Stenhagen is also shown in the figure. Substrate: 0.01 N HCl, 20°C.

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(3) J. H. Schulman and W. McD. Armstrong, "Surface Chemistry", Interscience Publishers Inc., (1949), p. 263.

bulk state at room temperature. Below 60 A.^{2}, the curve of II shows a flat portion which indicates the existence of equilibrium between the monolayer and three-dimensional liquid drops on the substrate.

In Fig. 1, the curve for phthioic acid at 20°C (III), reported by Stenhagen,(4) is also shown. Phthioic acid is one of the normal constituents of tubercle bacillus with the formula $C_{26}H_{52}O_2$, isolated by Anderson,(5) and its molecular structure has been assumed to be a trialkylacetic acid, especially ethyldecyldodecylacetic acid (II), by Stenhagen(4) and Robinson.(6) Later, Polgar and Robinson(7) investigated the structure of the acid extensively and concluded that it was 3, 13, 19-trimethyltricosanoic acid. But the exact constitution still remains to be solved. It may be concluded at least from our results that phthioic acid is not identical with I or II.

2. β-Methylpalmitic Acid.—β-Methylpalmitic acid (IV) forms liquid-expanded film, whose limiting area is 63 A.^{2}, and compressible to about 30 A.^{2} as shown in Fig. 2.

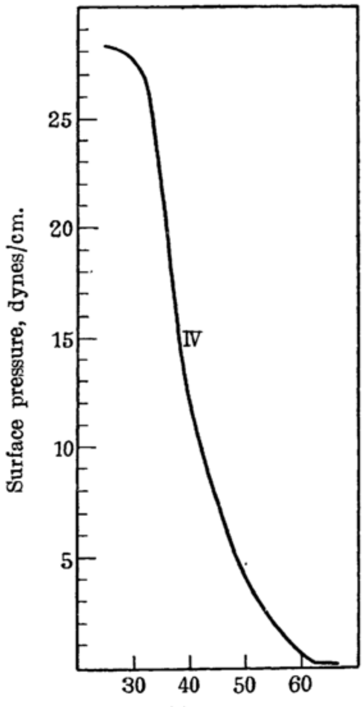

Fig. 2.—Force-area curve for β-methylpalmitic acid. Substrate: 0.01 N HCl, 25°C.

3. β, β′, β″ - Trialkylpropionic Acids Having Only One Long Chain.—Fig. 3 shows the curves for β-methyl-β′-ethyltridecanoic acid (V) and β-methyl-β′-ethylpentadecanoic acid (VI). Both films are liquid-expanded and compressible to about 35 A.^{2}

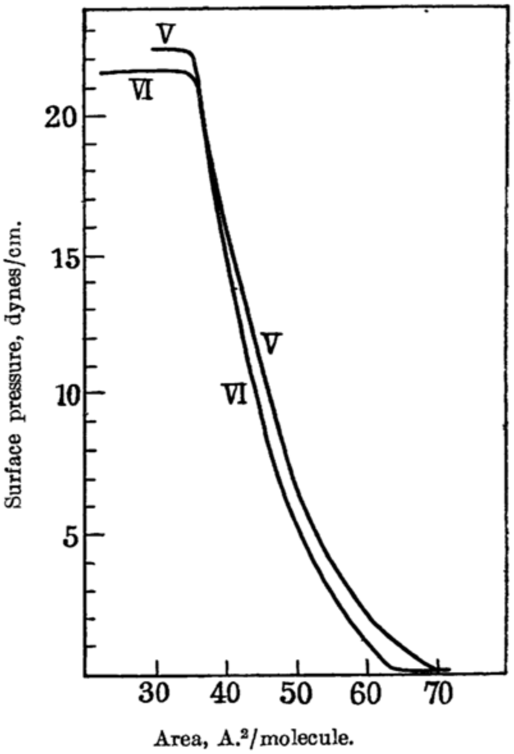

Fig. 3.—Force-area curves for β-methyl-β′-ethyltridecanoic acid (V) and β-methyl-β′-ethylpentadecanoic acid (VI). Substrate: 0.01 N HCl, 20°C.

4. Diheptylacetic Acid, β, β′-Diheptylpropionic Acid and γ, γ′-Diheptylbutyric Acid.—The effect of the position of diheptylmethyl group $[(C_7H_{15})_2-CH-]$ with respect to the carboxyl group of the acids was examined (VII, VIII and IX). As shown in Fig. 4, the limiting areas become smaller and the force-area curves steeper as the position of the substitution is separated from the carboxyl group. These monolayers collapse at about 50 A.^{2}

5. α-Ethyl Fatty Acids.—α-Ethylnonylic, α-ethylpalmitic (X), α-ethylstearic (XI) and α-ethylarachidic acids (XII) were examined (Fig. 5). α-Ethylnonylic acid does not form a stable monolayer, probably because of the high solubility of the compound. The monolayer of X is liquid-expanded, whereas monolayers of

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(4) E. Stenhagen and S. Ställberg, *J. Biol. Chem.*, **139**, 345 (1941).

(5) R. J. Anderson, *J. Biol. Chem.*, **97**, 639 (1932).

(6) R. Robinson, *J. Chem. Soc.* (*London*), 505 (1940).

(7) N. Polgar and R. Robinson, *J. Chem. Soc.*, (*London*), 389 (1945).

XI and XII show a transition from liquid-expanded to condensed states. The latter two substances show a marked temperature effect as shown in Fig. 6.

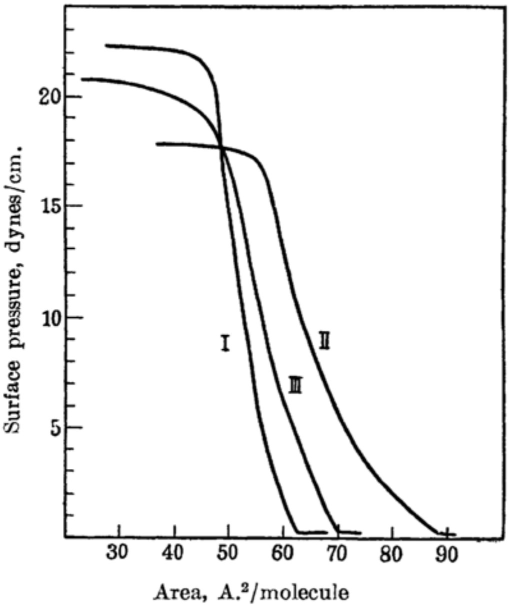

Fig. 4.—Force-area curves for diheptylacetic acid (VII), β,β'-diheptylpropionic acid (VIII) and γ,γ'-diheptylbutyric acid (IX). Substrate: 0.01 N HCl, 12°C.

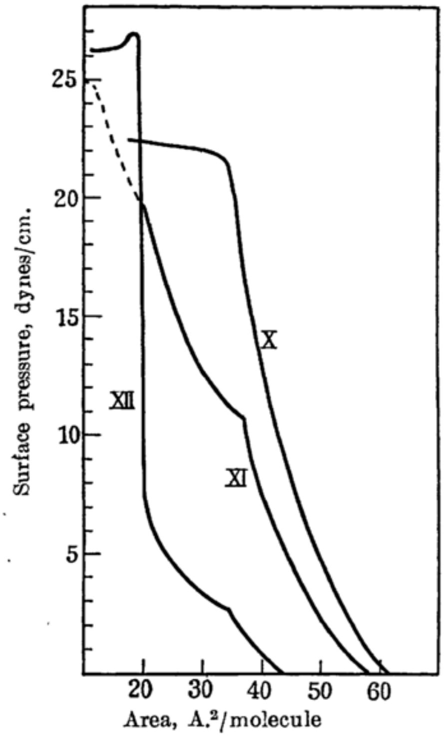

Fig. 5.—Force-area curves for α-ethylpalmitic acid (X), α-ethylstearic acid (XI) and α-ethylarachidic acid (XII). Substrate: 0.01 N HCl. 20°C.

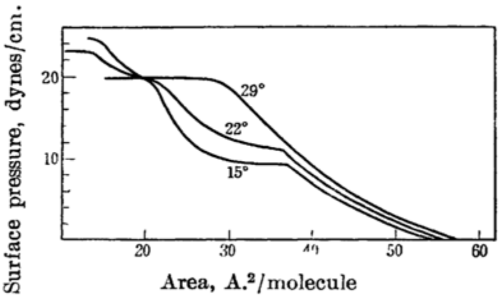

Fig. 6.—Temperature effect on the force-area curve for α-ethylstearic acid. Substrate: 0.01 N HCl.

6. α-Methyl-and α-Propylmyristic Acids.

—The effect of α-substituted groups of myristic acid on the force-area curve is shown in Fig. 7 (XIII and XV). As the side chain becomes longer, the limiting area is larger and the equilibrium pressure is lower. The curve for

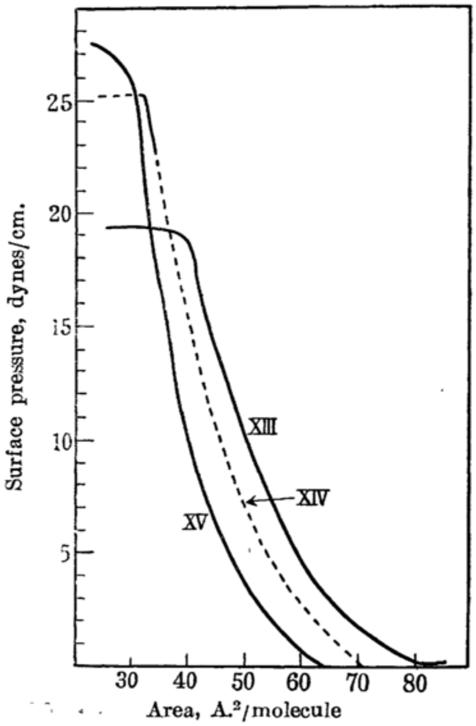

Fig. 7.—Force-area curves for α-propyl-(XIII) and α-methyl- (XV) myristic acids. XIV indicates the curve for α-methylpentadecylic acid after Stenhagen. Substrate: 0.01 N HCl, 20°C (XIV and XV) and 18°C (XIV).

α-ethylpalmitic acid (XIV) reported by Stenhagen⁽⁸⁾ falls between these curves as indicated by a dotted line in the figure.

The monolayers reported above are all of liquid-expanded type. In 1933, Langmuir proposed a theory for the liquid-expanded state.⁽⁹⁾ According to his theory, liquid-expanded films are composed essentially of two layers, the upper being an insoluble oil layer consisting of the hydrocarbon portion of the molecules, and the lower a layer of hydrophilic groups of the molecules which exists at the interface of oil and water phases and which acts as a gaseous film. The force-area relation for this state can be represented by the following equation:

$$(F-F_0)(a-a_0)=kT,$$

where F: surface pressure,

a: area per molecule,

k: Boltzmann constant,

T: absolute temperature,

F_0: a constant relating to the spreading coefficient of an assumed oil layer consisting of the hydrocarbon portion of the molecules in the film.

a_0: a constant relating to the actual cross-sectional area of one molecule on the surface.

The force-area relations for the branched-chain fatty acids examined fit this equation well. This theory has been opposed by several authors,⁽¹⁰⁾ but essentially it will be right, as was pointed out by Adam,⁽¹¹⁾ irrespective of minute difficulties.

Since two constants, F_0 and a_0, in the equation may be considered as relating to the structure of molecules in the monolayer, it may be reasonable for these constants as well as the limiting area or the area at collapse to be characteristic to the molecular structure. The values of F_0 and a_0 calculated from the experimental curves are listed in Table 1. From the table, it can be seen clearly that there are close relations between the structure of the molecules and the values of F_0 and a_0. These relations are summarized as follows:

i) When two long hydrocarbon chains exist in the molecule, constant a_0 becomes about 40 A.² This is shown in series 1 (except phthioic acid) and 4. Especially in series 4, the a_0 value of the three acids is determined by the diheptylmethyl radical [$(C_7H_{15})_2-CH-$] and is about 40 A.², which is twice the value of the cross-sectional area of one hydrocarbon chain, in spite of the quite different shapes of the force-area curves. Shereshefsky and Wall⁽¹²⁾ reported that dilauryl maleate forms a liquid-expanded film, the a_0 of which is 39.0 A.² Hence, two long hydrocarbon chains of these compounds consist of a hydrophobic portion of the molecules, and stand side by side in the monolayer. It may be mentioned here that a_0 is practically equal to the "area at collapse", because the force-area curves are nearly vertical at the collapse point.

ii) The a_0 of the films which possess only one long chain in the molecule is about 20 A.² Series 2, 3, 5 and 6 correspond to this structure. But as shown in series 6, the longer the side chain is, the larger is a_0, and, presumably, the smaller is $-F_0$. The value of the a_0 of tuberculostearic acid [$C_8H_{17}-CH(CH_3)-(CH_2)_8-COOH$] is also about 20 A.² if calculated from the published data.⁽⁴⁾

iii) An increase in the length of the long chain results in the increase in $-F_0$ value, but a_0 seems to be independent of it. Calculations were made on two other acids of similar structure (α-ethyltridecanoic and α-ethylpentadecanoic acids) based on the curves reported by Stenhagen,⁽⁴⁾ and they are included in Table 1. From these figures, the influence of the chain length on the F_0 value will be evident. It has been reported by Langmuir⁽⁹⁾ that, in normal chain fatty acids, there is a linear relation between the number of carbon atoms of the acids and F_0 as follows:

$$F_0=4.8-1.3\,n,$$

where n is the number of carbon atoms of the alkyl portion of the acid ($C_nH_{(2n+1)}COOH$). It can be concluded that there is a similar relation in the branched-chain fatty acids examined here at least qualitatively.

iv) As the branching shifts from the position of α, through β, to γ, with respect to the carboxyl group, $-F_0$ increases in this order. This is shown in series 4.

v) As the side chain in the α position becomes longer, a_0 increases and, apparently, $-F_0$ diminishes. Series 6 shows this regularity.

These relations are of a qualitative nature, and hold as long as films are of the liquid-expanded type. Using these relations, it should be expected that branched-chain fatty acids or other compounds newly synthesized or extracted from natural products can be identified, and in favorable cases, information about the

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(8) E. Stenhagen, *Trans. Faraday Soc.*, **36**, 597 (1940).

(9) I. Langmuir, *J. Chem. Phys.*, **1**, 756 (1933).

(10) For example, W. D. Harkins and E. Boyd, *J. Phys. Chem.*, **45**, 20 (1941).

(11) N. f. Adam, "The Physics and Chemistry of Surfaces", Oxford University Press, (1938), p. 67.

(12) J. L. Shereshefsky and A. A. Wall, *J. Am. Chem. Soc.*, **66**, 1072 (1944)

Table 1

The Values of F_0 and a_0 in the Langmuir Equation for Liquid-Expanded Films of Branched-Chain Fatty Acids

Series No.	Sample No.	Formula	Temperature	a_0	$-F_0$
1	I	$\begin{matrix}CH_3\\C_{11}H_{23}\\C_{12}H_{25}\end{matrix}\!\!>CHCOOH$	20°	45.0	11.8
	II	$\begin{matrix}C_2H_5\\C_{10}H_{21}\\C_{12}H_{25}\end{matrix}\!\!>CHCOOH$	20°	45.5	12.9
	III†	Phthioic acid	20°	20.	9.8
2	IV	$\begin{matrix}CH_3\\C_{13}H_{27}\end{matrix}\!\!>CHCH_2COOH$	25°	22.0	10.4
3	V	$\begin{matrix}CH_3\\C_2H_5\\C_{10}H_{21}\end{matrix}\!\!>C\ CH_2\ COOH$	20°	24.9	9.1
	VI	$\begin{matrix}CH_3\\C_2H_5\\C_{12}H_{25}\end{matrix}\!\!>C\ CH_2\ COOH$	20°	24.3	10.4
4	VII	$\begin{matrix}C_7H_{15}\\C_7H_{15}\end{matrix}\!\!>CHCOOH$	12°	42.0	8.6
	VIII	$\begin{matrix}C_7H_{15}\\C_7H_{15}\end{matrix}\!\!>CH\ CH_2\ COOH$	12°	38.5	12.5
	IX	$\begin{matrix}C_7H_{15}\\C_7H_{15}\end{matrix}\!\!>CH\ CH_2CH_2COOH$	12°	37.2	15.8
5	†	$\begin{matrix}C_2H_5\\C_{11}H_{23}\end{matrix}\!\!>CHCOOH$	18°	22	6.9
	X	$\begin{matrix}C_2H_5\\C_{14}H_{29}\end{matrix}\!\!>CHCOOH$	20°	22.2	9.9
	XI	$\begin{matrix}C_2H_5\\C_{16}H_{33}\end{matrix}\!\!>CHCOOH$	20°	20.2	10.1
	XII††	$\begin{matrix}C_2H_5\\C_{18}H_{37}\end{matrix}\!\!>CHCOOH$	20°	(13)	(11.9)
6	XIII	$\begin{matrix}C_3H_7\\C_{12}H_{25}\end{matrix}\!\!>CHCOOH$	20°	26.3	7.5
	XIV†	$\begin{matrix}C_2H_5\\C_{13}H_{27}\end{matrix}\!\!>CHCOOH$	18°	22	7.6
	XV	$\begin{matrix}CH_3\\C_{12}H_{25}\end{matrix}\!\!>CH\ COOH$	20°	19.1	9.8

† F_0 and a_0 were calculated from the $F-a$ curves reported by Stenhagen *et al.*⑷

†† The values of F_0 and a_0 are uncertain because the film shows a transition from liquid-expanded to condensed states.

structure of them may be obtained.

Summary

(1) Force-area relations for the monolayers of a number of branched-chain fatty acids have been investigated.

(2) These monolayers are of the liquid-expanded type, on acidic substrates, and force-area curves fit the Langmuir equation well.

(3) Constants F_0 and a_0 in the Langmuir equation have been calculated from the curves. It has been shown that there is a definite regularity between these constants and the structure of the molecules in the films.

(4) It was revealed that the structure of phthioic acid is not of the trialkylacetic acid type as postulated by Stenhagen and Robinson.

This work was started at the Department of Chemistry, Faculty of Science, Tokyo University under the direction of Prof. Sameshima. The author wishes to express his sincere appreciation to Prof. Sameshima for his kind direction, and also to Dr. Akamatsu and Dr. Tachibana of Tokyo University, and Dr. Umezawa of the National Institute of Health for their advice in the present study. He is also greatly indebted to the late Prof. Asano for a number of samples of branched-chain fatty acids.

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