Monolayers of Branched-Chain Fatty Acids. II. α-Aromatic Substituted Fatty Acids

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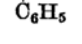
In our previous report, force-area curves for a number of branched-chain fatty acids having aliphatic side chains have been studied.⁽¹⁾ It was concluded that there are close relations between the force-area curves and the position and the length of the side chains. In this report, experiments were carried out for four series of branched-chain fatty acids having aromatic side chains in the α-position, namely, 2-phenyl-, 2-(α-naphthyl)-, 2-benzyl- and 2-(α-naphthomethyl)-*n*-fatty acids,* which were shown to have antibacterial activities in vitro.⁽²,³⁾

Asano and his co-workers⁽²⁾ and Ukita and his co-workers⁽³⁾ have postulated that a good antibacterial effect might be expected if a substance occupied a large area when adsorbed on a bacterial cell surface. This assumption led them to syntheses and examination of the antibacterial activities of aromatic ring-substituted compounds mentioned above. Contrary to their expectation, no influence of substituting groups on the antibacterial activity has been found, though substitution of naphthyl and naphthomethyl groups had a little better effect against *S. aureus* than substitution of phenyl group. They assumed implicitly that a naphthalene ring occupies a much larger area on the cell surface than a benzene ring, but this assumption should be verified experimentally. From force-area relation on water substrate, one can estimate the orientation of the molecules of these compounds at the cell surface, and this is one of the reasons for which this study was done.

Materials and Methods

Twenty-three substances were used in this experiment:

α-phenyl series:



$$C_nH_{2n+1}\underset{\displaystyle C_6H_5}{\underset{|}{C}}HCOOH \quad n=2,\ 8,\ 10,\ 12,\ 13,\ 14, 16.$$

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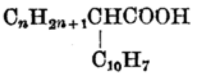
* For simplicity, the names of these compounds were referred in this paper to α-phenyl-, α-naphthyl-, α-benzyl- and α-naphthomethyl-series respectively.

(1) M. Izawa, This Bulletin, **25**, 182 (1952).

(2) M. Asano, H. Motomatsu and O. Tamemasa, *J. Pharmaceut. Soc. Japan*, **70**, 622 (1950). (In Japanese)

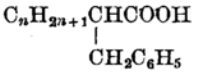
(3) T. Ukita, O. Tamemasa and H. Motomatsu, *J. Pharmaceut. Soc. Japan*, **71**, 289 (1951).

α-naphthyl series:



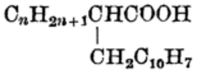
$C_nH_{2n+1}\underset{\substack{|\\ C_{10}H_7}}{C}HCOOH$ $n=4, 6, 8, 10, 12.$

α-benzyl series:



$C_nH_{2n+1}\underset{\substack{|\\ CH_2C_6H_5}}{C}HCOOH$ $n=4, 6, 8, 10, 12.$

α-naphthomethyl series:



$C_nH_{2n+1}\underset{\substack{|\\ CH_2C_{10}H_7}}{C}HCOOH$ $n=0, 4, 6, 8, 10, 12.$

All these compounds were synthesized by Dr. Tamemasa of the Chemical Department, Institute for Infectious Diseases, Tokyo University. As to their synthetic methods, constants and bactericidal properties, reference should be made to the original papers.(2,3)

Techniques employed were the same as those of the previous report.(1) Langmuir-Adam surface balance was made by Suzuki Scientific Instrument Works, Tokyo. The torsion wire of 0.278 dyne per cm. per degree was used. All experiments were carried out at 20°C. in an air-thermostat, which can be kept closed during the period of all operations.

Results and Discussion

1. Monolayers on 0.01 N Hydrochloric Acid Solution.—a. α-Phenyl series (Fig. 1).—Force-area curves begin to rise at about 60 A.^{2} per molecule, and collapse at 42 A.^{2}, with the equilibrium surface pressure of 27.5 dynes per cm. The effect of the length of the aliphatic portion of the molecules on the force-area curves is not large. Films of $n=8$ or lower members of the series are unstable.

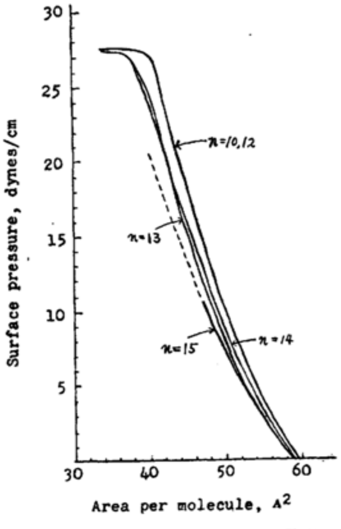


Fig. 1.—Force-area curves for $\left.\begin{array}{l}C_nH_{2n+1}\\C_6H_5\end{array}\right\rangle CH\cdot COOH$ series on 0.01 N HCl solution at 20°C.

b. α-Naphthyl series (Fig. 2).—The limiting area of the monolayers is about 60 A.^{2}, but the curves are steeper than those for α-phenyl series. These monolayers reach equilibrium pressure of 23 dynes at 45 A.^{2} In this case, also, the length of the alkyl chain exhibits a minor effect on the force-area curves, and the members lower than $n=6$ give no stable film.

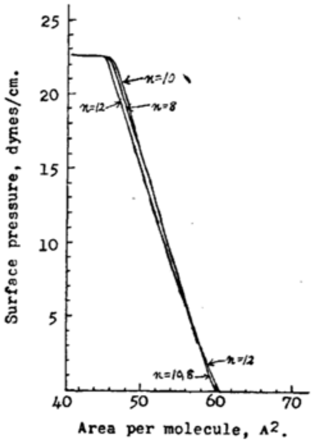


Fig. 2.—Forse-area curves for $\left.\begin{array}{l}C_nH_{2n+1}\\C_{10}H_7\end{array}\right\rangle CH\cdot COOH$ series on 0.01 N HCl solution at 20°C.

c. α-Benzyl series (Fig. 3).—Limiting area of this series is about 67 A.^{2}, and the films collapse at 45 A.^{2} and 24 dynes. Acids of $n=6$ or lower members give unstable films, and $n=8$ acid seems to dissolve considerably.

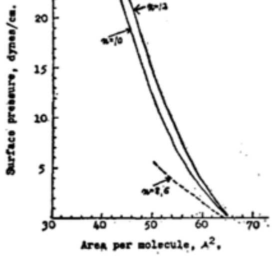


Fig. 3.—Force-area curves for $\left.\begin{array}{l}C_nH_{2n+1}\\C_6H_5\cdot CH_2\end{array}\right\rangle CH\cdot COOH$ series on 0.01 N HCl solution at 20°C.

d. α-Naphthomethyl series (Fig. 4).—Limiting area is about the same as that of α-benzyl series, namely, 66 A.², but the force-area curves are steeper, and the monolayers collapse at about 50 A.² and 19 dynes. The effect of the length of the aliphatic chain is negligibly small, as shown in the figure. Acids with $n=6$ or lower do not give stable films.

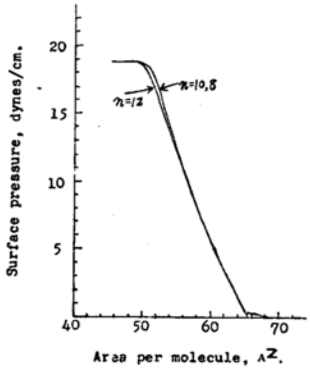


Fig. 4.—Force-area curves for $\begin{matrix}C_nH_{2n+1}\\ C_{10}H_7\cdot CH_2\end{matrix}\Big> CH\cdot COOH$ series on 0.01 N HCl solution at 20°C.

These results can be represented schematically as the curves shown in Fig. 5. In every series, the length of the alkyl chains in the molecules has only minor effect upon the force-area curves. Therefore, it was concluded that the force-area curves are determined mainly by the aromatic portion of the molecules. In the case of α-alkyl substituted fatty acids, if the length of the longer aliphatic chain becomes longer, the cross-sectional area per molecule becomes larger at low pressure region, and smaller at higher pressures.(1,4) The fact that there exists no such relation in α-aromatic substituted acids may be due to the stronger interaction between adjacent molecules, probably because of the higher molecular weight of these substances.

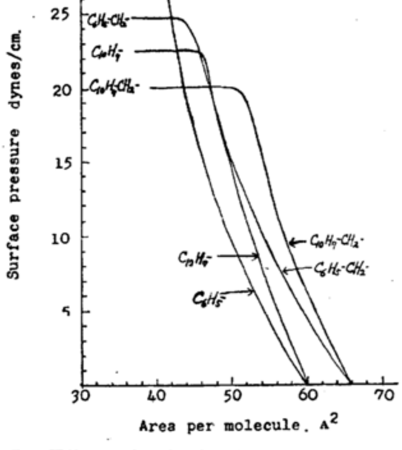


Fig. 5.—Effect of substituting groups on the force-area curves of α-substituted n-fatty acids.

Limiting areas per molecule of α-phenyl and α-naphthyl acids and those of α-benzyl and α-naphthomethyl acids are nearly equal, and are 60 A.² and 66 A.² respectively. Accordingly, phenyl and naphthyl groups are considered to have such an orientation as having equal cross-sectional areas on the surface. From the molecular models, it is shown that phenyl or naphthyl group can orient vertically on the surface of water, that is, the plane of the aromatic ring can be placed perpendicular to the water surface, and that phenyl and α-naphthyl groups are able to have an orientation having nearly equal cross-sectional areas. This is in accordance with the result of the experiment.

Stenhagen has reported that the collapse area for α-cyclohexyl-fatty acids (50 A.²) equals the sum of the area of p-alkylcyclohexanol (30 A.²) and that of hydrocarbon chain(20 A.²). (4)If the additivity of the cross-sectional areas can be applied to any films exhibiting the liquid expanded state, the area for phenyl, naphthyl, benzyl or naphthomethyl group is 22, 25, 25, and 30 A.² respectively. The collapse area for ω-phenyl-n-fatty acids reported by Ställberg and Stenhagen(5) is 21 A.², which is in agreement with the figure from the molecular models (ca. 20 A.²), and this area corresponds to the experimental data given above for phenyl group (22 A.²). For the figures other than that for phenyl, comparison was not practicable because of the lack of experimental data.

2. Influence of pH of the substrate on the force-area curves (Fig. 6).—Fig. 6 shows the force-area curves for α-phenylpalmitic acid on 1/30 M phosphate buffer substrate, pH of which being 5.5, 6.0, 7.0 and 8.0, together with the curve on 0.01 N hydrochloric acid solution.

The curve on the substrate of pH 5.5 coincides well with that on 0.01 N HCl solution but the equilibrium pressure is higher. At pH 6.0 and 7.0, curves are somewhat expanded, and become unstable at high pressure region.

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

(5) S. Ställberg and E. Stenhagen, *Svensk Kem. Tid.*, **53**, 355, (1941); *C. A.*, **36**, 1536-2 (1942).

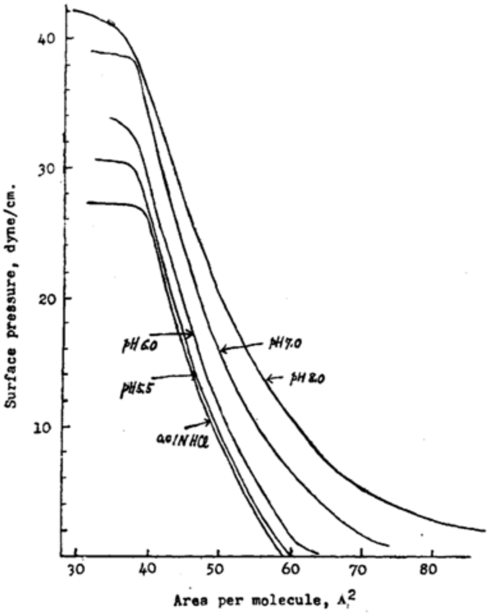

Fig. 6.—Effect of *p*H on the force-area curves of $\begin{matrix}C_{14}H_{29}\\ C_6H_5\end{matrix}\!\!>\!CH\cdot COOH$ on M/30 phosphate buffer.

The equilibrium pressure obtained by rapid compression is higher than the pressure at acidic region. At *p*H 8.0, the film expands much more, and shows a gaseous state, and becomes unstable at 20 dynes per cm. or higher. These findings are in agreement with the data for other fatty acids at different *p*H.

Effect of *p*H on other substances examined here was similar to the effect on α-phenylpalmitic acid shown in figure 6, though the stability region was considerably varied from one compound to another, and, therefore, was not shown in this report.

3. Surface behavior and antibacterial properties.—It has been well known that a peak will be found when antibacterial activity is plotted against the total number of carbon atoms of substances of a homologous series. It was reported that for the four series mentioned above the highest activity was found in the member having about 20 carbon atoms.(2, 3) It has not been possible to interpret the existence of the activity maximum in terms of the surface behavior only, and there is no direct relation between film properties and antibacterial activity of any one of the four series, nor for the aliphatic substituted fatty acids reported previously.(1) Daniels concluded that the increase in activity in an ascending series paralleled the decrease in water solubility, the increase in lipid solubility, and to a certain extent, the increase in surface activity, and that the rapid decrease beyond the activity maximum was attributable to a limiting water solubility.(6) On the other hand, Work and Work(7) suggested that the bactericidal activity of surface-active compounds should be interpreted solely in terms of their surface activity. They believed that the existence of activity maximum was due to the micelle formation at higher concentrations. Whether Daniels' or Work and Work's conclusion is correct must be examined further in other lines of experiments in the case of aromatic substituted fatty acids.

The effect of substitution upon bactericidal activity was shown to be very small.(3) Considering the relatively large experimental errors of bactericidal tests, it should be concluded from the film experiment that these four series of compounds have equal cross-sectional areas on the surface. Provided that substances which occupy larger area at the cell surface have stronger antibacterial activity than those which possess smaller area, it is not unreasonable to say that the four series have almost equal antibacterial activity. It would be of interest to examine the activity of substances which lie flat on the surface.

Summary

(1) Force-area relations for 2-phenyl-, 2-(α-naphthyl)-, 2-benzyl- and 2-(α-naphthomethyl)-*n*-fatty acids have been investigated.

(2) Force-area curves are characteristic to each of the four series, showing that the length of alkyl chains in the molecules has only a minor effect upon the curves.

(3) Influence of *p*H on the force-area curves, so far examined, is shown to be similar to other fatty acids.

(4) It was concluded that the aromatic ring in the α-position of the fatty acids has such an orientation that the plane of the aromatic ring is placed perpendicular to the water surface.

(5) Film properties seem to have no direct relation to the antibacterial activity of these compounds, and some of the regularities for the activity are discussed.

The author wishes to thank Dr. Hamao Umezawa, the chief of the Division of Anti-

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(6) T. C. Daniels, *Ann. Rev. Biochem.*, **12**, 447 (1943).

(7) T. S. Work and E. Work, "The Basis of Chemotherapy", Interscience Publishers, Inc., New York, N. Y., 1948, p. 319.

biotics of this Institute, for helpful discussions, and is especially grateful to Dr. Osamu Tamemasa for a number of branched-chain fatty acids.

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