7,8-Dihydro-1,3-dimethyl-7-phenylpyrimido[5,4-*d*]pyrimidine-2,4 (1*H*,3*H*)-dione (7a)——A suspension of **6** (100 mg) in toluene (20 ml) was refluxed for 3 h. The solution was allowed to stand at room temperature, then the precipitate was filtered off and dried to give 76 mg (89%) of **7a**, which was identical with the sample prepared above.

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Shape-transforming Action of Myrmicacin (3-Hydroxydecanoic Acid) and Some Related Compounds on the Membrane of Intact Human Erythrocytes

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The effects of the following compounds (most of which had been proved to inhibit mitotic progression of pollens) on the shape of the membrane of human erythrocytes were tested: myrmicacin (3-hydroxydecanoic acid) and its derivatives, even-numbered C_{4-10} fatty acids, and some C_{10} diols. They all induced a shape change of the membrane-exvagination (crenation) type at pH 7.4 to different extents, depending on their structures, but not at pH 6.0. The shape change induced was reversible. The structure-activity relationship and the mode of action were compared with those for the action of these compounds on pollen growth.

Keywords——membrane shape change; human erythrocytes; myrmicacin; fatty acids; transforming activity; crenation; mitotic progression

Introduction

One of the authors (Iwanami) found that 3-hydroxydecanoic acid (myrmicacin), present in secretions of a leaf-cutting ant, reversibly inhibits the mitotic progression of *Ornithogalum virens* pollens at any stage.[1] Further studies revealed that certain carboxylic acids structurally related to myrmicacin also have a similar effect on pollens from various plant species,[2-4]

and the effect appeared to be due to impairment of membrane function of the pollens as a result of their intercalation into the lipid bilayer of the plasma membrane, and not due to the direct inhibition of nuclear division.[4]

In view of the possibility that these compounds may also interact with plasma membrane of animal cells, attempts were made in this study to determine their perturbing effect on the plasma membrane of human erythrocytes by observing the induced shape change as a sensitive indicator of membrane perturbation,[5-7] and to compare the structure-activity relationship with that observed in the case of mitotic inhibition of pollens. The length of hydrocarbon chain of the compounds used here is limited to 10 carbon atoms or less, because only these compounds had been tested for effect on pollens and also because compounds with longer carbon chains are poorly soluble under the experimental conditions employed.

Materials and Methods

Erythrocytes——Human erythrocytes from freshly drawn ACD blood, kindly supplied by the Kyoto Prefectural Red Cross Blood Center, were washed three times with 140.5 mM NaCl containing 10 mM phosphate buffer, pH 7.4 (PBS), and resuspended in PBS.

Chemicals——Myrmicacin (3-hydroxydecanoic acid) was synthesized by the method of Myers and Temple.[8] Its acetyl derivative, methyl ester and decan-1,3-diol were synthesized from myrmicacin by the conventional procedures. Decan-1,4- and -1,5-diols were prepared from the corresponding decalactones by lithium aluminum hydride reduction. All the other fatty acids and derivatives were purchased from Wako Pure Chemical Industries, Ltd., except for 4-cyclohexylbutyric acid, which was purchased from Aldrich Chemical Co., Ltd.

Morphological Observation of Human Erythrocytes——Washed human erythrocytes were incubated with each of the test compounds in a 1% suspension at 37°C for 10 min and the shape of erythrocytes was observed under a JEOL JSM-35 scanning electron microscope, after fixation with 0.9% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4 and coating with carbon and gold.

In order to express the extent of shape change of a given cell population semiquantitatively, we used a morphological index:[7]

Morphological index $=\sum$(morphological score) $\times$[(number of transformed cells)/(total cell number)]

Positive morphological scores are assigned to each stage of the crenated shape, as described in Fig. 1.

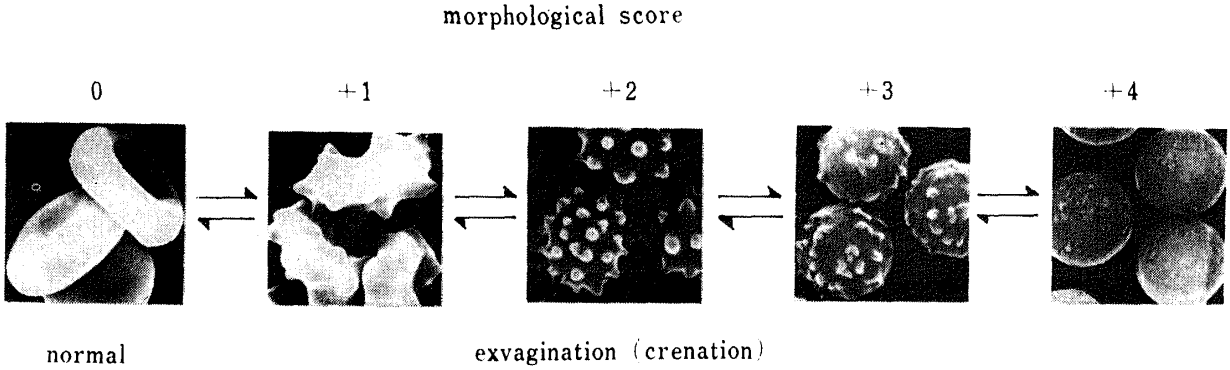


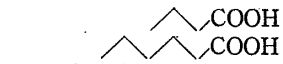
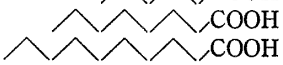
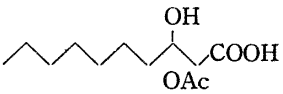
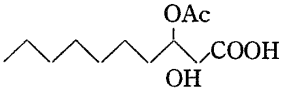
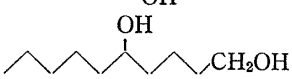
Fig. 1. Assignment of Morphological Score to Each Stage of the Shape Change of Human Erythrocytes

Results

Table I shows the membrane-exvaginating shape change of human erythrocytes in terms of the morphological index after treatment of a 1% cell suspension with 5 mM test compound at 37°C for 5 min.

The membrane-transforming activity of saturated fatty acids with straight carbon chains of C_{4-10} increased with increasing carbon number. However, C_{10} acids with a cyclic structure, such as 4-cyclohexylbutyric acid and 4-phenylbutyric acid, showed considerably weaker activity (+1.4 and +0.7 indices, respectively) than the corresponding straight chain acid, capric

Table I. Membrane Shape Change of Human Erythrocytes induced by Various Fatty Acids and Their Derivatives

Compound	Structure	Carbon number	Morphological index
None (control)		—	0
Unsubstituted acids			
Butyric acid		4	+0.05
Caproic acid		6	+0.05
Caprylic acid		8	+1.65
Capric acid		10	+2.90
4-Cyclohexylbutyric acid		10	+1.40
4-Phenylbutyric acid		10	+0.70
Hydroxy fatty acids and their derivatives			
3-Hydroxydecanoic acid (myrmicacin)		10	+1.40
3-Acetoxydecanoic acid		10	+1.80
Methyl 3-hydroxydecanoate		10	+1.00
Dibasic acid			
Sebasic acid		10	+0.05
Diols			
Decan-1,3-diol		10	+3.00
Decan-1,4-diol		10	+3.00
Decan-1,5-diol		10	+1.00

acid (+2.9 index).

Introduction of an OH group in the β-position of capric acid resulted in a moderate decrease in the activity. Acetylation of the OH group or methylation of the COOH group of the β-hydroxy fatty acid caused only a slight change in the activity. The activity was decreased drastically by the introduction of a COOH group in the ω-position of capric acid. On the other hand, decan-1,3- and -1,4-diols showed strong activity, comparable to that of capric acid, although decan-1,5-diol showed considerably weaker activity.

Table II shows that the alteration in shape of erythrocytes induced by capric acid and its derivatives could be reversed by washing the treated cells with 50 volumes of PBS.

As shown in Table III, echinocyte formation by the fatty acids was observed in the medium at pH 7.4 (for 1 mM capric acid and 5 mM myrmicacin, +2.0 and +1.4 indices, re-

Table II. Reversal of the Alteration in Shape of Erythrocytes upon Washing the Cells with Isotonic Saline

Compound	Morphological index	
	Before washing	After washing
None (control)	0	0
Capric acid (5 mM)	+2.90	0
3-Acetoxydecanoic acid (5 mM)	+1.80	0
Myrmicacin (5 mM)	+1.40	0
Decan-1,3-diol (5 mM)	+3.00	+0.05

Table III. Effect of Medium pH on the Erythrocyte Shape Change induced by Fatty Acids and Related Compounds

Compound	Morphological index	
	pH 6.0	pH 7.0
None (control)	0	0
Capric acid (1 mM)	0	+2.00
Myrmicacin (5 mM)	0	+1.40
Decan-1,3-diol (2 mM)	+1.05	+1.20

spectively), but not at pH 6.0, while decan-1,3-diol (2 mM) exerted an effect at both pH values. Changes of pH in the range of 6.0—7.4 did not affect the shape of untreated cells.

Discussion

The present results suggest that amphiphilic compounds possessing both a straight hydrocarbon chain of sufficient length (C_8 and C_{10}) to allow intercalation of the compound into the lipid bilayer of the membrane and a polar group or 2 polar groups at or near one end of the chain can exert a marked membrane-perturbing action on human erythrocytes, inducing a shape change of crenation type. Such an effect is similar to that of long-chain fatty acids already reported.[5] It was rather unexpected, however, that the neutral C_{10} compounds such as decan-1,3- and -1,4-diols have slightly stronger activity and 3-hydroxydecanoic acid (myrmicacin) rather weaker activity than decanoic acid. The reason for such variation in the activity remains to be clarified.

The structure-activity relationship of the test compounds acting on erythrocyte membrane was generally similar to that for the inhibitory action on pollens,[2-4] except that neutral compounds such as C_{10} diols and methyl 3-hydroxydecanoate showed considerable effects on erythrocytes but not on pollens. However, their mode of action on the erythrocyte membrane appears to be rather different from that on pollens; they are not active on erythrocytes in an acidic medium at pH 6.0, whereas they are very active on pollens at pH 4.0, and also the effect of all the active compounds on erythrocytes was reversible whereas a reversible effect on pollens was seen only with the C_{10} hydroxy acids and the acetoxy acid derivative. Therefore, the action of myrmicacin on erythrocyte membrane is not characteristically distinct from that of the related unhydroxylated fatty acids, in contrast to its unique effect on pollen growth.[1-4] These differences in the action on erythrocytes and pollens might result from differences in membrane structure, but we are unable to analyze the differences further at present because almost no information is available on the structure and conformation of pollen membranes.

References and Notes

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