Relationship between Lipophylicity of Myrmicacin Homologs and Their Inhibitory Activity on *Camellia japonica* Pollens

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Relationship between lipophilicity of 3-hydroxy fatty acids and inhibitory activity on *Camellia japonica* pollen germination and pollen tube elongation was studied. The haf-maximum germination potential ($G_{0.5}$)s and half-maximum elongation ($E_{0.5}$)s of the pollens to the acids were obtained from the germination and elongation curves. Lipophilicity of the acids was evaluated by high performance liquid chromatography. Obvious relationship between lipophilicity and the biological activity of the tested acids was observed. The result is in agreement with our previous hypothesis, in which strength of the inhibitory activity of myrmicacin analogs is assumed to be related to accessibility of the inhibitors to lipid bilayer cell membrane.

Introduction

Studies on biological activity of myrmicacin (3-hydroxydecanoic acid)[1], an ant-origin herbicide, and related compounds, on various substrates such as pollens[2-6], bacteria[7], fertilized sea urchin eggs[8, 9] and human erythrocytes[10] have been reported. In 1979 Iwanami and Iwadare[3] demonstrated inhibitory activity of normal middle chain (C_{8-10}) aliphatic mono carboxylic acids on pollen growth was comparable to that of mymicacin, and they suggested that these "Myrmic Acids" shoud be regarded as a new group of growth inhibitors. Fur-

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ther study[11] revealed that intensity of inhibition of myrmicacin analogs on pollen germination was related to degree of unsubstitution of carbon chain of the molecules. We assumed that the degree of unsubstitution was related to lipophilicity of the substances, and it seemed of interest to investigate relationship between the inhibitory activity and lipophilicity.

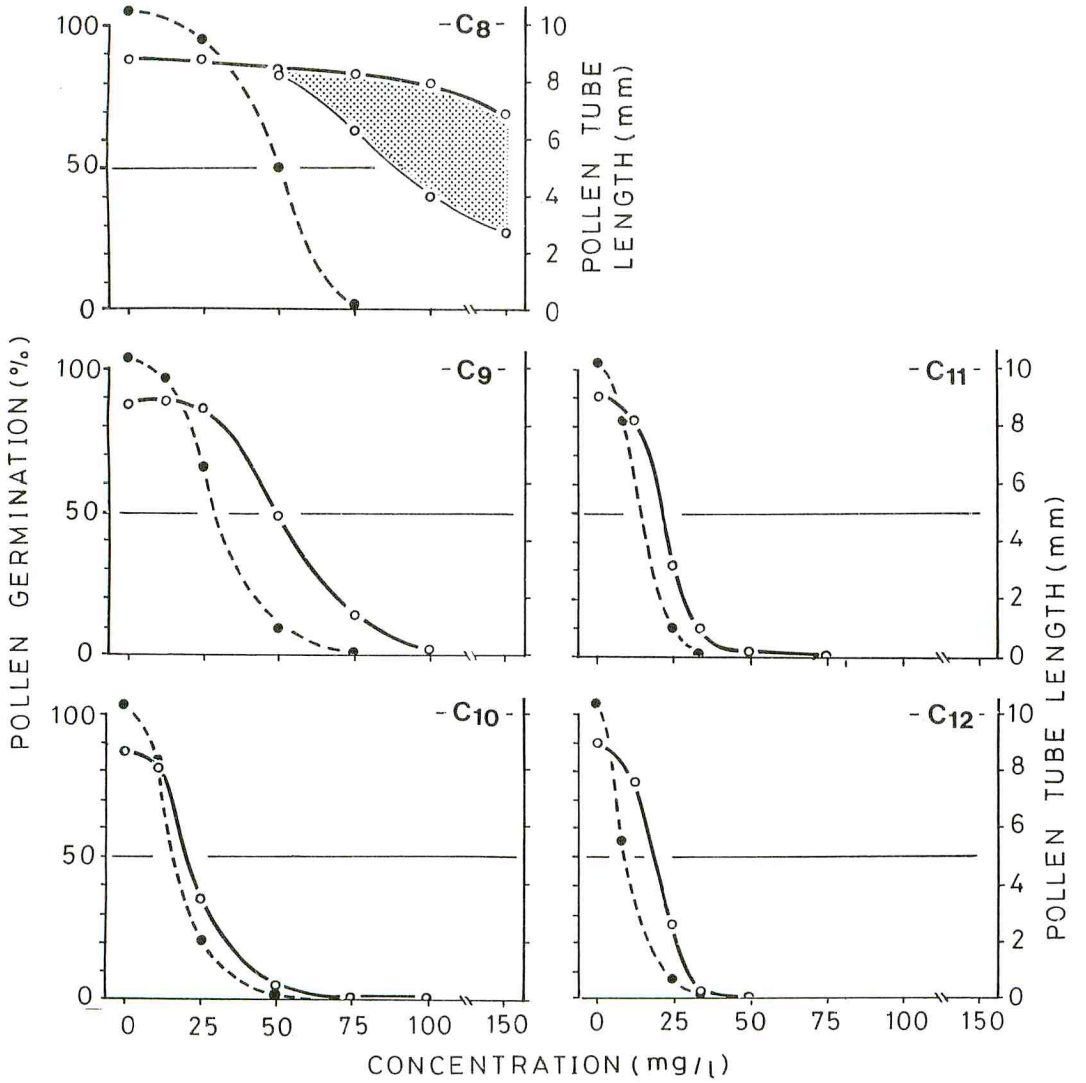

Fig. 1 Germination and pollen tube elongation of *Camellia japonica* pollens cultivated on 3-hidroxy acid-containing medium. —○— ; germination (%), ----●----- ; pollen tube elongation (mm). C_8 – C_{12} indicate number of carbon atoms of the acids.

Recently Butte et al.[12] have chromatographically evaluated $\log k^0$ value, which represents partition of substance between octadecylsylilated silica gel (stationary phase) and water (mobile phase), and demonstrated a good proportional relationship between $\log k^0$ value and conventionally defined lipophilicity ($\log P_{oct}$). They suggested that the chromatgraphic method should be used to measure lipophilicity. Their suggestion prompted us to employ the $\log k^0$ value as indication of liphilicity for the present work.

Materials and Methods

The acids employed in this study were normal C_8-C_{12} 3-hidroxy acids listed in Table 1. The acids were prepared by the known method[13]

The pollen grains of *Camellia japonica* were collected from freshly opend flowers. Sugar-agar plates with a constant thickness (1.5 mm) on a slide glass were prepared from sucrose (8%), agar (1%) and an aqueous solution of the acid at a concentrations of 12.5

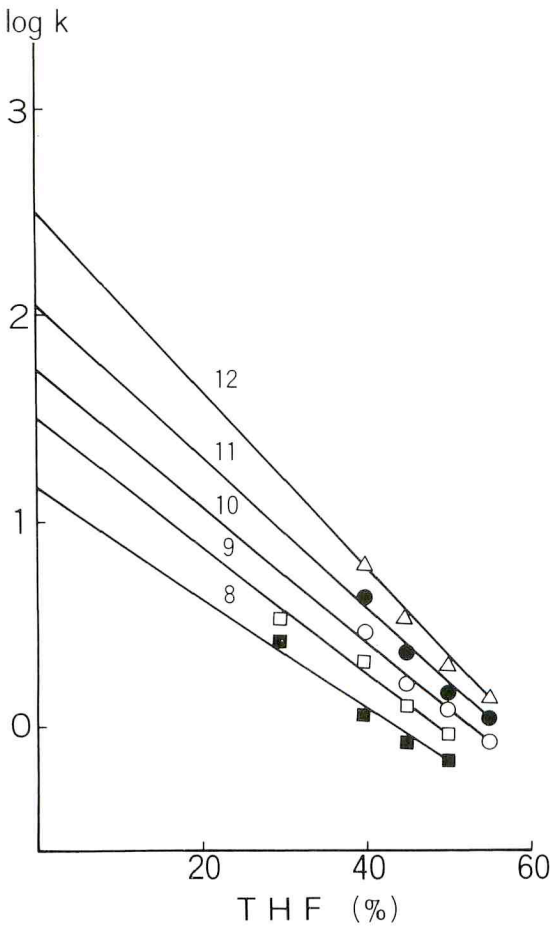

Fig. 2 Relationship between logk values of 3-hydroxy acids and THF concentration in the mobile phase. The numbers along the lines indicate number of the carbon atoms of the acids.

mg, 25 mg, 37.5 mg, 50 mg and 75 mg/L. The pH of the solution was adjusted to 5.5 with dilute sodium hydroxide solution. In each experiment, 100－150 pollen grains were sown on the plate. After leaving in a moist chamber at 25°C for 1 hr, the percentage of germination and length of pollen tubes were determined with a microscope. Each point appearing in Fig. 1 represents mean value of three determinations.

The high performance liquid chromatography was carried out on a Hitachi Model 655－15 equipped with 655A－30 differential refractometer as detector. The stationary phase was DuPont Zorbax ODS column (250 mm × 4.6 mm, 5－6 μm octadecylsilylated silica gel), and the mobile phase was mixture of tetrahydrofuran (THF) and water containing 0.01 mol/L phosphoric acid to suppress dissociation of the tested acids. The concentrations of THF in the mobile phase were 30%, 40%, 45%, 50% and 55%.

Results and Discussion

For evaluation of inhibitory activity of the tested acids, the half-maximum germination potential ($G_{0.5}$)s were obtained from the germination curves (Fig. 1) as points of intersection of the curves and 50% germination line. The half-maximum elongation ($E_{0.5}$)s were obtained from the elongation curves (Fig. 1) in the same manner. Then $PG_{0.5}$ ($-\log G_{0.5}$)s and $PE_{0.5}$ ($-\log E_{0.5}$)s were calculated. Incidentally $G_{0.5}$ of 3-hydroxyoctanoic acid (C_8) was evaluated by extrapolation of its germination curve because of its extremely weak activity (Fig. 1).

The $\log k^0$ values were carried out according to Butte's method[12,§] (Fig. 2).

Thus obtained $PG_{0.5}$s, $PE_{0.5}$s and $\log k^0$ values are shown in Table 1, and plots of $PG_{0.5}$ against $\log k^0$ value and those of $PE_{0.5}$ against $\log k^0$ values are shown in Fig. 3.

Table 1 Tested acids and their $\log k^0$ value, $PG_{0.5}$* and $PE_{0.5}$*

	Number of carbon atoms	$\log k^0$ value	$PG_{0.5}$	$PE_{0.5}$
3-Hydroxyoctanoic	8	1.17	3.056	3.530
3-Hydroxynonanoic	9	1.55	3.566	3.809
3-Hydroxydecanoic **	10	1.87	3.944	4.109
3-Hydroxyundecanoic	11	2.18	4.000	4.175
3-Hydroxydodecanoic	12	2.46	4.079	4.438

* $-\log$ (mol/L)
** myrmicacin

§ k (capacity ratio) = $(t_R - t_0)/t_0$ were t_R and t_0 are the retention times of a retained and an unretained peak respectively. $\log k^0$ value is calculated by extrapolation of logks for water-containing solvent mixtures to 100% water.

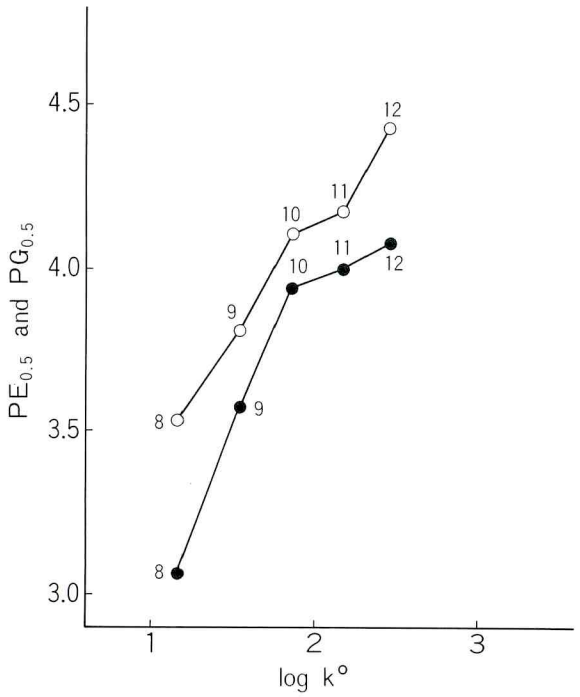

Fig. 3 Relationship between $\log k^0$ values and $PG_{0.5}$ and $PE_{0.5}$ of 3-hydroxy acids. —●—: $PG_{0.5}$, —○—: $PE_{0.5}$ The numbers along the lines indicate number of carbon atoms of the acids.

As seen in Fig. 3, $PG_{0.5}$s and $PE_{0.5}$s of the tested acids are related to $\log k^0$ values. The tested acids higher than 3-hydroxydecanoic acid (C_{10}) have considerably high $PG_{0.5}$s comparing with those of the lower acids. The similar tendency is observed in $PE_{0.5}$s. As described above, we have suggested "Myrmic Acids" as a new group of inhibitors, which are normal aliphatic mono carboxylic acids having 8 to 10 carbon atoms. Further, we have pointed out[11] that inhibitory activity of 2-hydroxy acids is almost comparable to that of unhydroxylated acids having one less carbon atom. On the basis of the facts, it seems reasonable to assume 3-hydroxy acids higher than 3-hydroxydecanoic acids (C_{10}) are regarded as "Myrmic Acids". Results of the present work are in agreement with the assumption.

Regarding to the mechanism of inhibition by myrmicacin and related compounds, we conceived[14] of that hydrocarbon chain of the inhibitors, which is lipophilic moiety of the compounds, is incorporated into the lipid bilayer cell membrane and that when the incorporated chain exceeds a critical length function of the cell membrane is impaired resulting the in inhibition. The results of this study give a support to the hypothesis.

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