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INHIBITION OF *CAMELLIA JAPONICA* POLLEN GERMINATION BY MYRMICACIN ANALOGS

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Key Word Index—*Camellia japonica*; Theaceae; pollen; germination; inhibition; myrmicacin; myrmic acids; hydroxyfatty acids.

Abstract—Pollen germination in *Camellia sinensis* was inhibited by a variety of organic acids with and without hydroxyl substitution at the 2- or 3-positions. Inhibition was greatest with the C_{10} and C_{11} acids.

The biological activity of myrmicacin (3-hydroxydecanoic acid) [1] and the analogous C_8 to C_{11} carboxylic acids on pollen, inhibition of germination, pollen tube elongation, pollen tube mitosis [2–6] and protoplasmic movement [7] has been reported. In 1979 Iwanami and Iwadare suggested that these "myrmic acids" should be regarded as a new group of growth inhibitors [4]. Further, the activity of myrmic acids on other substrates such as bacteria [8], fertilized sea urchin eggs [9, 10] and human erythrocytes [11] has been described.

An essential factor in the inhibitory effect of myrmic acids is the number of carbon atoms [4]. In addition, the presence of a hydroxyl group in the molecule seems to be essential [4–6], so it was of interest to investigate the effect of this hydroxyl group on the inhibitory activity. In this study, the effects of 13 carboxylic acids (Table 1) on pollen germination were compared.

The pollen grains of *Camellia japonica* were collected from freshly opened 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. The pH of the solution was adjusted to 5.5 with dilute sodium hydroxide. In each experiment, 100–150 pollen grains were sown on the plate. After leaving in a moist chamber at 25° for 1 hr, the percentage of germination was determined with a microscope. Germination percentage of control was between 99.6 and 98.2%. The experiment was repeated three times, and the mean values are presented in Fig. 1. The unhydroxylated acids were purchased from Wako Pure Chemical Industries Ltd. The synthesis of 2-hydroxy acids was carried out by modification of Seebach's method [12]. Myrmicacin was prepared by the known method [13].

Figure 1 represents the relationship between concentration of the acids and percentage of germination. The top diagram shows the results of treatment with unhydroxylated acids, and the bottom diagram the results of treatment with 2- and 3-hydroxy acids. The numbers on the curves are the numbers of carbon atoms in the acids and the numeral 3 in the parentheses by the numeral 10 in the bottom chart indicates the position of the hydroxyl group in myrmicacin. The position of the hydroxyl group of the 2-hydroxy acids is not indicated.

As shown in the top part of Fig. 1, the percentage of germinated pollen decreases with an increase in number of

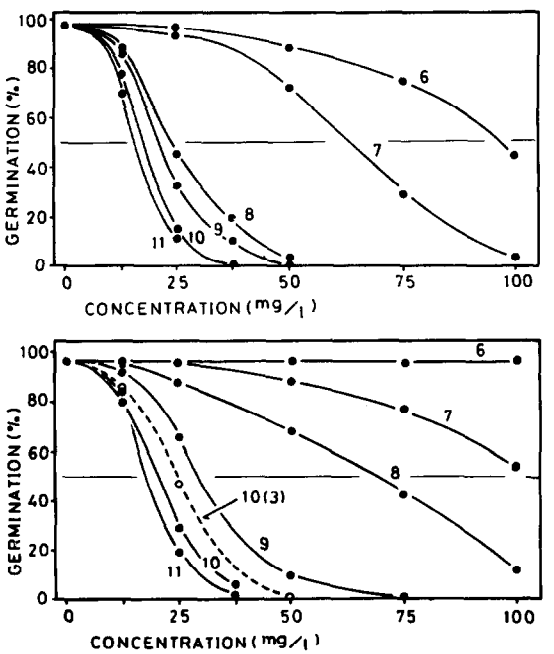

Fig. 1. Relationship between concentration of 13 carboxylic acids and percentage of pollen germination. Top chart: effect of unhydroxylated acids. Bottom chart: effects of hydroxy acids. The numbers along the lines indicate number of carbon atoms of the acids. 10(3): myrmicacin.

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Table 1. Carboxylic acids used in the experiments

Unhydroxylated acids	Number of carbon atoms	Hydroxy acids	Number of carbon atoms
Hexanoic acid	6	2-Hydroxyhexanoic acid	6
Heptanoic acid	7	2-Hydroxyheptanoic acid	7
Octanoic acid	8	2-Hydroxyoctanoic acid	8
Nonanoic acid	9	2-Hydroxynonanoic acid	9
Decanoic acid	10	2-Hydroxydecanoic acid	10
Undecanoic acid	11	2-Hydroxyundecanoic acid	11
		3-Hydroxydecanoic acid (myrmicacin)	10

carbon atoms of the acids. The germination percentage exceeded 40% when pollen was cultured on a medium containing 100 mg/l of hexanoic acid (C_6), whereas cultivation on media containing 50 mg/l of octanoic (C_8) or higher acids resulted in complete inhibition. The result is in agreement with previously reported behavior of myrmic acids [4].

Comparing with the unhydroxylated acids, 2-hydroxy acids showed moderate inhibitory effects, which are almost comparable to those of the unhydroxylated acids having one less carbon atom. The same relationship holds for 2- and 3-hydroxy acids. The germination curve of 3-hydroxydecanoic acid [10(3)] was very close to that of 2-hydroxynonanoic acid (9). The results suggest that the intensity of inhibition is related not to the carbon number of the acid but to the degree of unsubstitution of the carbon chain.

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