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Note

Inhibitory Activity of (9*R*,10*S*,12*Z*)-9,10-Dihydroxy-8-oxo-octadecenoic Acid, Its C-9 Epimer and Their Derivatives toward the Growth of Tea Pollen Tubes

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The inhibitory activity of (9*R*,10*S*,12*Z*)-9,10-dihydroxy-8-oxo-octadecenoic acid and its diacetate, acetonide and methyl ester toward tea pollen tube growth were different, the inhibition by the diacetate being the strongest. Each compound of the fatty acid and its derivatives exhibited more inhibition than its C-9 epimer. The fatty acid and its C-9 epimer showed the same toxicity against HeLa cells.

We have reported in a previous paper the isolation, relative stereochemistry, and biological activity of a novel fatty acid, (12*Z*)-9,10-dihydroxy-8-oxo-12-octadecenoic acid (**1**; Fig. 1) from the mushroom, *Hercium erinaceum*.[1] This compound exhibited cytotoxicity against HeLa cells and also inhibited tea pollen tube growth. The absolute configuration of the acid has recently been established as (9*R*,10*S*) by synthesis of the optically active form, and a large quantity of the compound was obtained.[2] We now describe a comparison of the inhibitory effects of the acid and its C-9 epimer (**5**) toward tea pollen tube growth and HeLa cells. The effects of the diacetate (**2** and **6**), acetonide (**3** and **7**), and methyl ester (**4** and **8**) derivatives of compounds **1** and **5** on the growth of tea pollen tubes were also compared to identify the functional inhibitory groups. Furthermore, the effects of compound **1** and linolenic acid on pollen tube growth were compared.

Inhibition of tea pollen tube growth

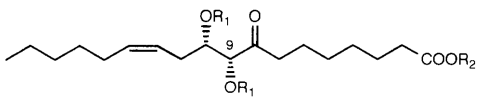
In the previous paper,[1] the inhibitory effect of compound **1** on tea pollen tube growth was shown by an inhibition zone in which pollen germination was completely inhibited by diffusion of the compound through an agar medium. In this experiment, tea (*Camellia sinensis* L.) pollen tubes were cultured on 10 ml of an agar medium containing various concentrations of compounds **1** and **5** and their derivatives in a Petri dish according to the methods of Iwanami (1957)[3] and Yokota and Konishi (1981)[4] in order to examine the effects of each compound in detail. The basal medium contained 8% sucrose and 1.2% agar. Pollen grains were placed six in a row on the medium, and incubated for 20 h at 25°C in the dark. The methyl esters (**4** and **8**) were dissolved in DMSO, and the other compounds in MeOH, and added to the medium after the agar had been liquefied by heating. The final concentration of MeOH or DMSO in the medium was 1.5%, at which concentration pollen tube growth was not inhibited. Pollen tube length was measured with calipers at 2 places per row to give 12 measurements per Petri dish. The mean values from 36 measurements in 3 separate experiments were used to express the relative growth against that of a control treatment.

The effects of compound **1** and derivatives **2**, **3**, and **4** on tea pollen tube growth are shown in Fig. 2. The growth of the tea pollen tubes was strongly inhibited by compound **1**. Among the derivatives of compound **1**, diacetate **2** showed the strongest inhibition. Inhibition by the acetate was stronger than that by compound **1**, while acetonide **3** and methyl ester **4** showed less inhibition than that of compound **1**. The concentrations at which pollen tube growth was depressed to 50% of the control value for compound **1**, diacetate **2**, acetonide **3**, and methyl ester **4** were 4.7, 2.5, 4.7, and 6.8 μg/ml, respectively. The inhibitory effect of compound **1** was stronger than that of linolenic acid, which depressed pollen tube growth to 50% of the control value at 7.9 μg/ml (data not shown).

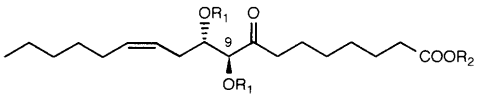
Iwanami (1979 and 1980)[5,6] has reported that, among monocarboxylic acids having 1 to 10 carbon atoms, inhibitory activity toward the pollen tube growth of *Camellia japonica* generally increased with increasing the number of carbon atoms, β-hydroxydecanoic acid and the other monocarboxylic acids having 8 to 10 carbon atoms having stronger inhibitory effects. Compound **1** with 18 carbon atoms showed stronger inhibitory activity than that of the monocarboxylic acids with 10 carbon atoms, which reduced pollen tube growth to 50% of the control value only at concentrations greater than 10 μg/ml. The stronger inhibitory activity of compound **1** may have been due to the greater number of carbon atoms than that in C-10 monocarboxylic acids.

The results also suggest that the carboxyl group was most related to toxicity, since the inhibitory effect of methyl ester **4** was less than that of compound **1**. The stronger inhibitory activity of diacetate **2** indicates that the functional groups of C-9 and C-10 may have affected the level of inhibitory activity.

The inhibitory effects of compound **5** and derivatives **6**, **7**, and **8** are also shown in Fig. 2. The concentrations at which the tube



	R_1	R_2
1	H	H
2	Ac	H
3	$C(CH_3)_2$	H
4	H	CH_3



	R_1	R_2
5	H	H
6	Ac	H
7	$C(CH_3)_2$	H
8	H	CH_3

Fig. 1. (9*R*,10*S*,12*Z*)-9,10-Dihydroxy-8-oxo-octadecenoic Acid, Its C-9 Epimer and Their Derivatives.

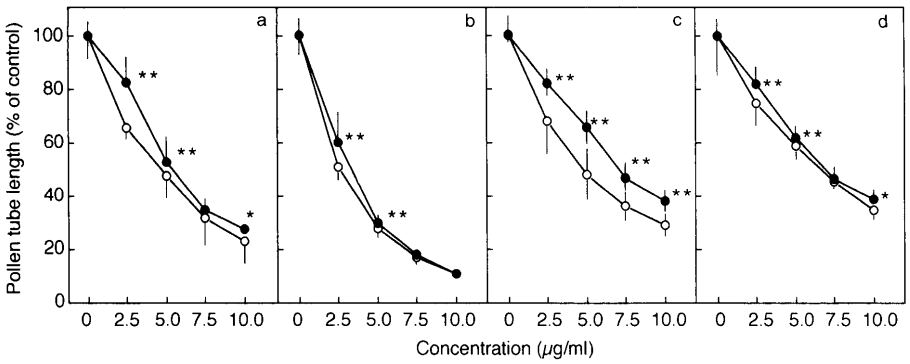


Fig. 2. Effects of (9*R*,10*S*,12*Z*)-9,10-Dihydroxy-8-oxo-octadecenoic Acid (a), Its Diacetate (b), Acetonide (c), and Methyl Ester (d) on the Growth of Tea Pollen Tubes.

The effects were compared between each compound (○) and its epimer (●). The vertical bars represent the standard deviation, significant differences being indicated by * ($p<0.05$) and ** ($p<0.01$) (*t*-test).

growth was reduced to 50% of the control value for compound **5**, diacetate **6**, acetonide **7**, and methyl ester **8** were 5.3, 3.3, 7.1, and 7.1 μg/ml, respectively. When the inhibitory effect is compared between each compound and its C-9 epimer, the epimers generally exhibited slightly less inhibition than the original compounds. The largest difference in inhibitory activity between both epimers was observed in the case of acetonides **3** and **7**, suggesting the possibility that stereochemistry may also have affected the inhibitory activity.

Cytotoxicity against HeLa cells

Compounds **1** and **5** showed the same toxicity against these cells: the minimum concentration giving 100% death of cells for **1** and **5** was 100 μg/ml. This result suggests that the stereochemistry of the dihydroxy groups was not involved in the toxicity against HeLa cells, unlike the effect on tea pollen tubes.

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