Alkylbenzyldimethylammonium Salts as Inhibitors for the Ice Nucleating Activity of Erwinia ananas

Michiko WATANABE, Takahiro MAKINO,* Katsuhide OKADA,** Morio HARA, Satoshi WATABE and Soichi ARAI

Department of Agricultural Chemistry, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan
* The Shizuoka Agricultural Experiment Station, Iwata-gun, Shizuoka 438, Japan
** Faculty of Education, Yamagata University, Yamagata 990, Japan

Received August 11, 1987

A variety of chemical compounds were examined as to their abilities to inhibit the ice nucleating activity of Erwinia ananas IN-10 cells and their outer membrane fraction. The nucleating activity of the outer membrane fraction was inhibited by many surface-active species among the compounds examined, whereas that of cells was inhibited only by amines and ammonium salts having amphiphilic structures. Ammonium salts with both an n-alkyl group having a carbon number of more than 8 and a benzyl group were particularly effective in inhibiting the nucleating activity of the bacterial cells. The inhibitory ability of one of the amphiphilic ammonium salts was greater at 15°C than at 4°C. When a tea plant was sprayed with one of the effective ammonium salts prior to being kept at −3°C overnight, it was possible to protect the plant from freeze-injury at the minimal concentration of 250 ppm.

Typical cryoinjury of crops in the field is frost damage caused by abnormally low temperatures in both late autumn and early spring. The frost in early spring often damages buds, shoots and immature fruits. In particular, cold-sensitive crops including tea plants, mulberry tress, fruit trees and vegetables suffer from frost injury to a greater or lesser extent every year. It has been reported that some strains of ice nucleation active (INA) bacteria such as Pseudomonas syringae, Pseudomonas fluorescens, Erwinia herbicola, Erwinia ananas and Xanthomonas campestris cause frost injury, as they act as heterogeneous ice nuclei in dewdrops.1~5) The ice crystals thus formed grow and propagate into the plant tissues where they injure the cells.

Several methods for preventing frost injury are available, such as covering of the crops with plastic film for agricultural use, sprinkling with water, air blowing with frost-protecting fans and smoking with burning heavy oil.6) These methods, however, have limitations as to effectiveness, material costs and expensive labor. Although the use of fans has long been the most common means of preventing frost in tea fields in Japan, it sometimes causes the promotion of frost injury when the atmospheric temperature happens to fall to −3°C.6) Anti-nucleating agents against the bacterial action were reported by some research groups7~10); they include bacteriosides, hydroxamic acid derivatives, heavy metals, cationic detergents and so on.

Two biotechnological approaches for reducing INA bacteria have been made on a laboratory scale. One is the use of bacteriophages to reduce the living cell number of INA bacteria11) and the other is the use of a bioantagonist which can reduce their population density.12)

The present study was a systematic approach for developing new types of antinucleating chemicals for practical use for the regulation of the INA bacterium, Erwinia ananas.
**MATERIALS AND METHODS**

**INA bacterium.** An INA bacterial strain identified as *Erwinia ananas*, from its bacteriological characteristics, was isolated from a tea germisphere grown in Shizuoka prefecture. This strain has been given the particular name of *E. ananas IN-102* and was used throughout this work. The bacterial cells were cultured in a medium comprising Bacto-Trypton (Difco, 1 g), Bacto-Proteose Peptone No. 3 (Difco, 1 g), dipotassium phosphate (0.15 g), magnesium sulfate (0.15 g), glycerol (1 g) and water (100 ml) at 20°C for 24 hr with vigorous shaking. The cells at the exponential growth phase were collected by centrifugation at 8,000 × g for 20 min and then washed twice with physiological saline (300 ml) at below 10°C.

**Outer membrane.** The cells were suspended in 10 mM Tris–HCl (pH 6.5) containing sucrose at a final concentration of 0.75 M and then their membranes were disrupted with a French Press (Otake Seisakusho) at a pressure of 1500 kg/cm². The lysate was separated from intact cells by centrifugation at 8,000 × g for 20 min. The outer membrane fraction was obtained from the lysate by sucrose density gradient ultracentrifugation at 4°C.

**Antinucleating agents.** Amino acid esters were synthesized by the C-activation method with p-toluenesulfonic acid. The esters were crystallized from acetone–petroleum ether and then recrystallized from the same solvent system. EMG-8 and EMG-12, enzymatically modified gelatins with octyl and dodecyl moieties, respectively, were produced as described. Each of EMG-8 and EMG-12 was purified by dialysis against running water for two days, prior to being freeze-dried. The powder thus obtained was washed with dichloromethane for further purification.

**Antinucleating activity test.** 1.8 ml of 0.1 M phosphate buffer (pH 6.5) was put in a test tube (10 mm inside diameter) and 10⁸ cells were suspended in it. The outer membrane fraction was similarly suspended at a concentration corresponding to an optical density at 280 nm of 10⁻². Each suspension was mixed with water (0.2 ml) containing a given amount of one of the test agents at 0°C and then kept at 0°C overnight, unless otherwise noted. The suspension was then cooled in an ethylene glycol bath kept at −5°C in order to examine the degree of supercooling (DS), which was determined by the method described in the previous paper and expressed as Tm − Ts, where Tm is the melting point and Ts the temperature at which freezing of the bulk water began. The agent concentration was expressed as ppm in the final test suspension.
Freezing prevention test. Young tea plants (Thea sinensis L) grown in pots were used for the test. Shortly before tea picking, the plants were sprayed with aqueous dispersions of n-octylbenzyldimethylammonium iodide at concentrations of 100, 250, 500 and 1000 ppm. Immediately after, each pot was coated with cotton to keep the soil and roots warm. The part above ground was covered with polyethylene film to prevent evaporation of water. The test plants were stored at -3°C overnight. The film was removed from the cold-treated plants, which were then warmed at 20°C under a mild flow of air. After 3hr, the effectiveness of the test agent was evaluated by 5 laboratory members and scored as follows; 1, all young leavesTable I. Antinucleating Abilities of Fatty Acids, Amino Acids and Amino Acid Esters for Bacterial Cells and Their Outer Membranes

<table>
<thead>
<tr>
<th>Agent</th>
<th>Conc. (ppm)</th>
<th>Degree of supercooling (°C)</th>
<th>Cell</th>
<th>Membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Av. ± S.E.</td>
<td>Av. ± S.E.</td>
<td>Av. ± S.E.</td>
</tr>
<tr>
<td>Fatty acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetate</td>
<td>1000</td>
<td>0.1±0.1</td>
<td>0.4±0.2</td>
<td></td>
</tr>
<tr>
<td>n-Butyrate</td>
<td>1000</td>
<td>0.1±0.1</td>
<td>0.3±0.2</td>
<td></td>
</tr>
<tr>
<td>n-Hexanoate</td>
<td>1000</td>
<td>0.2±0.1</td>
<td>0.7±0.3</td>
<td></td>
</tr>
<tr>
<td>n-Octanoate</td>
<td>1000</td>
<td>0.3±0.2</td>
<td>3.0±0.4</td>
<td></td>
</tr>
<tr>
<td>n-Decanoate</td>
<td>1000</td>
<td>0.3±0.2</td>
<td>2.5±0.5</td>
<td></td>
</tr>
<tr>
<td>n-Dodecanoate</td>
<td>1000</td>
<td>0.3±0.2</td>
<td>2.4±0.4</td>
<td></td>
</tr>
<tr>
<td>n-Octadecanoate</td>
<td>1000</td>
<td>0.1±0.1</td>
<td>1.2±0.4</td>
<td></td>
</tr>
<tr>
<td>Amino acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gly</td>
<td>1000</td>
<td>0.2±0.1</td>
<td>0.3±0.1</td>
<td></td>
</tr>
<tr>
<td>L-Ser</td>
<td>1000</td>
<td>0.3±0.1</td>
<td>0.3±0.1</td>
<td></td>
</tr>
<tr>
<td>L-Leu</td>
<td>1000</td>
<td>0.2±0.1</td>
<td>0.3±0.1</td>
<td></td>
</tr>
<tr>
<td>L-Phe</td>
<td>1000</td>
<td>0.1±0.1</td>
<td>0.4±0.2</td>
<td></td>
</tr>
<tr>
<td>L-Lys</td>
<td>1000</td>
<td>0.2±0.1</td>
<td>0.6±0.3</td>
<td></td>
</tr>
<tr>
<td>L-Arg</td>
<td>1000</td>
<td>0.2±0.1</td>
<td>0.6±0.2</td>
<td></td>
</tr>
<tr>
<td>L-Glu</td>
<td>1000</td>
<td>0.1±0.1</td>
<td>0.1±0.1</td>
<td></td>
</tr>
<tr>
<td>Amino acid ester</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gly-C₆</td>
<td>1000</td>
<td>0.6±0.2</td>
<td>0.7±0.3</td>
<td></td>
</tr>
<tr>
<td>Gly-C₁₂</td>
<td>1000</td>
<td>0.5±0.2</td>
<td>0.8±0.3</td>
<td></td>
</tr>
<tr>
<td>L-Phe-C₆</td>
<td>1000</td>
<td>0.3±0.3</td>
<td>0.6±0.2</td>
<td></td>
</tr>
<tr>
<td>L-Phe-C₁₂</td>
<td>1000</td>
<td>0.6±0.2</td>
<td>0.5±0.3</td>
<td></td>
</tr>
<tr>
<td>L-Leu-C₆</td>
<td>1000</td>
<td>1.5±0.5</td>
<td>2.4±0.4</td>
<td></td>
</tr>
<tr>
<td>L-Leu-C₈</td>
<td>1000</td>
<td>2.4±0.3</td>
<td>3.0±0.5</td>
<td></td>
</tr>
<tr>
<td>L-Leu-C₁₀</td>
<td>1000</td>
<td>1.8±0.5</td>
<td>2.0±0.4</td>
<td></td>
</tr>
<tr>
<td>L-Leu-C₁₂</td>
<td>1000</td>
<td>1.3±0.2</td>
<td>1.7±0.6</td>
<td></td>
</tr>
<tr>
<td>L-Lys-C₆</td>
<td>1000</td>
<td>0.6±0.1</td>
<td>0.8±0.2</td>
<td></td>
</tr>
<tr>
<td>L-Lys-C₈</td>
<td>1000</td>
<td>0.5±0.3</td>
<td>0.6±0.5</td>
<td></td>
</tr>
<tr>
<td>L-Lys-C₁₀</td>
<td>1000</td>
<td>0.2±0.2</td>
<td>0.5±0.4</td>
<td></td>
</tr>
<tr>
<td>L-Lys-C₁₂</td>
<td>1000</td>
<td>1.0±0.3</td>
<td>2.8±0.3</td>
<td></td>
</tr>
<tr>
<td>D-Nle-C₁₂</td>
<td>1000</td>
<td>0.9±0.4</td>
<td>1.9±0.5</td>
<td></td>
</tr>
</tbody>
</table>

Table II. Antinucleating Abilities of Surfactants for Bacterial Cells and Their Outer Membranes

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>Conc. (ppm)</th>
<th>Degree of supercooling (°C)</th>
<th>Cell</th>
<th>Membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Dodecylamine</td>
<td>1000</td>
<td>4.9±0.1</td>
<td>&gt;5a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>2.9±0.5</td>
<td>&gt;5a</td>
<td></td>
</tr>
<tr>
<td>EMG-8</td>
<td>1000</td>
<td>0.7±0.2</td>
<td>3.2±0.5</td>
<td></td>
</tr>
<tr>
<td>EMG-12</td>
<td>1000</td>
<td>0.7±0.4</td>
<td>2.9±0.5</td>
<td></td>
</tr>
<tr>
<td>Lecithin (soy)</td>
<td>1000</td>
<td>0.1±0.2</td>
<td>3.7±0.2</td>
<td></td>
</tr>
<tr>
<td>Polyvinyl pyrrolidone</td>
<td>1000</td>
<td>0.1±0.1</td>
<td>3.4±0.4</td>
<td></td>
</tr>
<tr>
<td>Na n-dodecanoate</td>
<td>1000</td>
<td>0.8±0.6</td>
<td>&gt;5a</td>
<td></td>
</tr>
<tr>
<td>Na dodecyl/sulfate</td>
<td>1000</td>
<td>0.1±0.1</td>
<td>&gt;5a</td>
<td></td>
</tr>
<tr>
<td>Tween-80</td>
<td>1000</td>
<td>0.7±0.7</td>
<td>3.0±0.5</td>
<td></td>
</tr>
</tbody>
</table>

Table III. Antinucleating Abilities of Amines for Bacterial Cells and Their Outer Membranes

<table>
<thead>
<tr>
<th>Amine</th>
<th>Conc. (ppm)</th>
<th>Degree of supercooling (°C)</th>
<th>Cell</th>
<th>Membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylamine</td>
<td>1000</td>
<td>0.5±0.5</td>
<td>0.2±0.1</td>
<td></td>
</tr>
<tr>
<td>Ethylamine</td>
<td>1000</td>
<td>0.3±0.3</td>
<td>0.1±0.1</td>
<td></td>
</tr>
<tr>
<td>n-Butylamine</td>
<td>1000</td>
<td>0.2±0.1</td>
<td>0.5±0.2</td>
<td></td>
</tr>
<tr>
<td>n-Hexylamine</td>
<td>1000</td>
<td>0.9±0.7</td>
<td>2.3±0.3</td>
<td></td>
</tr>
<tr>
<td>n-Octylamine</td>
<td>1000</td>
<td>4.4±0.2</td>
<td>&gt;5a</td>
<td></td>
</tr>
<tr>
<td>n-Decylamine</td>
<td>1000</td>
<td>4.9±0.1</td>
<td>&gt;5a</td>
<td></td>
</tr>
<tr>
<td>n-Dodecylamine</td>
<td>1000</td>
<td>4.9±0.1</td>
<td>&gt;5a</td>
<td></td>
</tr>
<tr>
<td>n-Tetradecylamine</td>
<td>1000</td>
<td>0.7±0.4</td>
<td>0.9±0.5</td>
<td></td>
</tr>
<tr>
<td>n-Hexadecylamine</td>
<td>1000</td>
<td>0.5±0.2</td>
<td>0.9±0.5</td>
<td></td>
</tr>
<tr>
<td>Benzylamine</td>
<td>1000</td>
<td>0.3±0.2</td>
<td>1.0±0.2</td>
<td></td>
</tr>
<tr>
<td>Cyclohexylamine</td>
<td>1000</td>
<td>0.3±0.2</td>
<td>0.2±0.1</td>
<td></td>
</tr>
<tr>
<td>Di-n-hexylamine</td>
<td>1000</td>
<td>0.5±0.1</td>
<td>1.1±0.1</td>
<td></td>
</tr>
<tr>
<td>Dibenzyline</td>
<td>1000</td>
<td>2.4±0.8</td>
<td>&gt;5a</td>
<td></td>
</tr>
<tr>
<td>Di-n-octylamine</td>
<td>1000</td>
<td>&gt;5a</td>
<td>&gt;5a</td>
<td></td>
</tr>
<tr>
<td>Glucosamine</td>
<td>1000</td>
<td>0.1±0.1</td>
<td>0.4±0.4</td>
<td></td>
</tr>
<tr>
<td>n-Hexyl-n-octylamine</td>
<td>1000</td>
<td>&gt;5a</td>
<td>&gt;5a</td>
<td></td>
</tr>
<tr>
<td>Hydroxyamine</td>
<td>1000</td>
<td>0.1±0.1</td>
<td>0.2±0.1</td>
<td></td>
</tr>
<tr>
<td>n-Octylbenzylamine</td>
<td>1000</td>
<td>&gt;5a</td>
<td>&gt;5a</td>
<td></td>
</tr>
<tr>
<td>Tri-n-butylamine</td>
<td>1000</td>
<td>0.1±0.1</td>
<td>0.2±0.1</td>
<td></td>
</tr>
<tr>
<td>Tri-n-hexylamine</td>
<td>1000</td>
<td>0.7±0.2</td>
<td>2.0±0.2</td>
<td></td>
</tr>
</tbody>
</table>

a Effective.

Gly-C₆, for example, denotes glycine hexyl ester.
RESULTS AND DISCUSSION

The screening of antinucleating agents was first performed by determining the degree of supercooling of bulk water in the presence of the INA bacterial cells or their outer membrane fraction. Table I shows that all the acids, amino acids and amino acid esters tested were ineffective for the cells, whereas some compounds with amphiphilic structures were effective for the membrane fraction. Assuming that an amphiphilic structure of a molecule may contribute to antinucleation, we tested several kinds of surfactants (Table II) and obtained the following results: (1) the nucleating activity of the outer membrane was inhibited by many kinds of surfactants, and (2) that of INA bacterial cells was inhibited only by a cationic surfactant. These results indicate that the ice-nucleation active site of the cell comprises an anionic environment, the conformation of which easily changes during the membrane preparation process.

These findings prompted us to examine systematically a series of amines as to their antinucleating ability. Table III shows that pri-

### Table IV. Antinucleating Abilities of Ammonium Salts for Bacterial Cells and Their Outer Membranes

<table>
<thead>
<tr>
<th>Ammonium salt</th>
<th>Conc. (ppm)</th>
<th>Degree of supercooling (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Av. ± S.E.</td>
</tr>
<tr>
<td>Tetramethylammonium</td>
<td>1000</td>
<td>0.2 ± 0.2</td>
</tr>
<tr>
<td>n-Butyltrimethylammonium</td>
<td>1000</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>n-Hexyltrimethylammonium</td>
<td>1000</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>n-Octyltrimethylammonium</td>
<td>1000</td>
<td>4.6 ± 0.3</td>
</tr>
<tr>
<td>n-Decyltrimethylammonium</td>
<td>1000</td>
<td>4.9 ± 0.1</td>
</tr>
<tr>
<td>n-Dodecyltrimethylammonium</td>
<td>1000</td>
<td>4.6 ± 0.4</td>
</tr>
<tr>
<td>n-Tetradecyltrimethylammonium</td>
<td>1000</td>
<td>3.6 ± 0.3</td>
</tr>
<tr>
<td>Benzyltrimethylammonium</td>
<td>1000</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>Di-n-hexyldimethylammonium</td>
<td>1000</td>
<td>1.5 ± 0.5</td>
</tr>
<tr>
<td>n-Hexyl-n-octyldimethylammonium</td>
<td>1000</td>
<td>&gt; 5a</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>&gt; 5a</td>
</tr>
<tr>
<td>n-Octylbenzyldimethylammonium</td>
<td>1000</td>
<td>&gt; 5a</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>&gt; 5a</td>
</tr>
<tr>
<td>n-Decylbenzyldimethylammonium</td>
<td>1000</td>
<td>&gt; 5a</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>&gt; 5a</td>
</tr>
<tr>
<td>n-Dodecylbenzyldimethylammonium</td>
<td>1000</td>
<td>&gt; 5a</td>
</tr>
<tr>
<td>n-Tetradecylbenzyldimethylammonium</td>
<td>1000</td>
<td>&gt; 5a</td>
</tr>
<tr>
<td>n-Hexadecylbenzyldimethylammonium</td>
<td>1000</td>
<td>&gt; 5a</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>&gt; 5a</td>
</tr>
<tr>
<td>n-Octadecylbenzyldimethylammonium</td>
<td>1000</td>
<td>&gt; 5a</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>&gt; 5a</td>
</tr>
<tr>
<td>n-Hexyloctadecylbenzyldimethylammonium</td>
<td>1000</td>
<td>&lt; 2.5</td>
</tr>
<tr>
<td>n-Heptyloctadecylbenzyldimethylammonium</td>
<td>1000</td>
<td>&lt; 2.5</td>
</tr>
<tr>
<td>n-Octyloctadecylbenzyldimethylammonium</td>
<td>1000</td>
<td>&lt; 2.5</td>
</tr>
<tr>
<td>n-Nonyloctadecylbenzyldimethylammonium</td>
<td>1000</td>
<td>&lt; 2.5</td>
</tr>
<tr>
<td>n-Decyloctadecylbenzyldimethylammonium</td>
<td>1000</td>
<td>&lt; 2.5</td>
</tr>
</tbody>
</table>

* Effective.
mary, secondary and tertiary amines whose hydrophobic groups are substituted with alkyl chains having carbon numbers of more than 8 were effective in increasing DS in the presence of cells. This suggests that development of the effect needs a structure containing at least both a hydrophobic group and a cationic group. In the case of amines, an increase in the carbon number of the alkyl group caused a decrease in water solubility.

In order to improve the water solubility we synthesized ammonium salts and tested their abilities as to the inhibition of the nucleating activity of the cells. As shown in Table IV, quarternary ammonium salts showed greater inhibitory abilities than the corresponding amines. As to an effective structure, it was thus suggested that one substitution should be a hydrophobic alkyl having a carbon number of more than 8. It was also found that a benzyl group acted as another favorable substituent. Alkylbenzyldimethylammonium salts (carbon number of the alkyl moiety: 8-18), even at 100 ppm, showed DS of more than 5°C. We also tested some agents having odd number carbon chains in their alkyl moieties, in a series of n-alkylphenyldimethylammonium salts, and found no particular difference in antinucleating effect between those with odd carbon numbers and those with even carbon numbers.

Reportedly, the nucleation activity of some strains of INA bacteria decreases over 26°C. \(^{18}\) This phenomenon suggests that the site involved in the development of the activity of the cells may change its original alignment at around 26°C and that, therefore, the compounds located at the site are more mobile at a higher temperature. The data in Table V show that some of the tested agents are more effective as to antinucleation at 15°C, supporting the above suggestion. The data also indicate that the spraying of antinucleating agents of this kind should be performed in the daytime.

In actuality, the freezing of water never occurs at a subzero temperature of higher than -3°C unless an effective heterogeneous nucleus is present. *E. ananas* is one of the most potent of such nuclei and causes frost injury to tea plants. Therefore, a required attribute of an antifrost agent for tea plants should be that a DS of more than 3°C is attained on spraying of the agent. It is also necessary that its concentration at the time of spraying should not be harmful to the plants. Table VI shows the results of pot tests. When tea plants were sprayed with the octylbenzyldimethylammonium salt at a concentration over 250 ppm and then stored at -3°C, cryoinjury hardly occurred.

**Acknowledgments.** We are greatful to Dr. T. Kitahara and Dr. T. Ebata of the Department of Agricultural

---

### Table V. Differences in Antinucleating Abilities of Various Types of Agents for INA Bacterial Cells at 4°C and 15°C\(^a\)

<table>
<thead>
<tr>
<th>Agent</th>
<th>Degree of supercooling (°C)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Av. ± S.E.</td>
<td>Av. ± S.E.</td>
<td></td>
</tr>
<tr>
<td>n-Octanoate</td>
<td>0.1 ± 0.2</td>
<td>0.2 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>L-Leu-C&lt;sub&gt;8&lt;/sub&gt;</td>
<td>0.1 ± 0.1</td>
<td>0.35 ± 0.3</td>
<td>&lt;5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Na dodecylsulfate</td>
<td>0.1 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>n-Octylamine</td>
<td>1.0 ± 0.4</td>
<td>&gt;5&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>n-Octylphenyldimethylammonium</td>
<td>3.8 ± 0.4</td>
<td>&gt;5&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>n-Octylbenzyldimethylammonium</td>
<td>&gt;5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&gt;5&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> The cells were treated with each of the agent dispersions at 1000 ppm for 3 hr.

### Table VI. Inhibition of Cryoinjury to a Tea Plant by Spraying an Aqueous Dispersion of n-Octylbenzyldimethylammonium Salt

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Number of times</th>
<th>Score&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>100</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>100</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>250</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>500</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>1000</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>1000</td>
<td>2</td>
<td>5</td>
</tr>
</tbody>
</table>

<sup>a</sup> See the text.
Chemistry, The University of Tokyo, for their technical guidance.

REFERENCES