We isolated the highly potent attachment-inhibitors (three times more active than standard CuSO₄ in the blue mussel assay), trans-6-, 8-, and 10-shogaols, from a hexane extract of the roots of ginger, Zingiber officinale Roscoe. Trans-8-shogaol showed the highest antifouling activity comparable with that of tributyltin fluoride (TBTF), which is recognized as one of the most effective antifouling agents, in the conventional submerged assay.

Key words: Zingiber officinale Roscoe; shogaol; attachment-inhibiting activity; antifouling; Mytilus edulis galloprovincialis

Nature has provided us with a large pool of bioactive substances that exhibit a variety of biological activities which may be of considerable significance to humans. We have developed a biological assay which is quite useful as a first screening for evaluating antifouling substances. This assay uses the blue mussel assay to guide the isolation of attachment-inhibiting substances from a variety of natural sources, and we have earlier shown the attachment-inhibiting activity to be an important pre-requisite for a compound to exhibit antifouling activity. In our previous studies, we have isolated phloroglucinols from Eucalyptus spp. and a sesquiterpene from Tasmannia lanceolata as potent attachment-inhibitors against the blue mussel, Mytilus edulis galloprovincialis. In a continuous search for more antifouling compounds, we are screening the extracts from several plant species of the Zingiberaceae family for attachment-inhibiting activity against the blue mussel. We have evaluated the hexane, CHCl₃, EtOAc, BuOH, and H₂O extracts of the roots of Zingiber officinale, Curcuma zedoaria, C. aromatica, and C. longa for this activity and found that the hexane extract of these species, except for C. longa, and the ethyl acetate extract from C. aromatica showed very strong attachment-inhibiting activity against the blue mussel (Table 1). We have selected for further study Z. officinale from these species. Ginger is one of the most important spices throughout the world, its tuber having been utilized for treating headache, nausea, stomach ache, and colds as a traditional medicine and having also been used as a stimulant. It has also been shown to possess antioxidative properties, and there have been a few reports on its chemical constituents; however, there have been no reports on the antifouling activity of its constituents. In this paper, we report the isolation of attachment-inhibitors from the hexane fraction of the roots of ginger, Z. officinale.

Slices of the roots (6 kg) of Z. officinale were extracted by hexane (3l x 2). The combined hexane extract (1.64 g) was subjected to repeated silica gel
Shogaols as Antifouling Agents

1749

Fig. 1. Shogaols, Zingerone and Zingerols.

Table 2. Antifouling Activity of 1–6 and TBTF in the Conventional Submerged Assay

<table>
<thead>
<tr>
<th>Days</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>TBTF</th>
</tr>
</thead>
<tbody>
<tr>
<td>29*</td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>o</td>
</tr>
<tr>
<td>62</td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>o</td>
</tr>
<tr>
<td>101</td>
<td>x</td>
<td>o</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>o</td>
</tr>
<tr>
<td>125</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>o</td>
</tr>
</tbody>
</table>

* from 28 June 2001

Conventional submerged assay Zingerone (4), 6- and 8-zingerols (5 and 6) and trans-6-, 8- and 10-shogaols (1–3) were subjected to the conventional submerged assay at Mochimune fishing port in Shizuoka city. On a polyvinyl chloride plate (35 × 60 × 0.3 cm), sample zones (5 cm in diameter) were drawn, and each zone was coated with sample solutions made by mixing 15% polyvinyl butylated resin in methanol with each of these compounds. After the solvent had evaporated, the assay plate was set at a depth of one meter along the wharf at Mochimune port.

shogaol (2) was the most effective and maintained antifouling activity even after four months. The mechanism for the action of shogaols is assumed to be similar to that established for alkylphenols against the blue mussel. The antifouling action of these compounds is likely to be related to their membrane-perturbation potency, and an examination of the applicability of trans-8-shogaol (2) as an effective antifouling agent is now in progress.

column chromatography, using a hexane-diethyl ether gradient as the eluting solvent. HPLC (Daiso SP-120-s-ODS-BP, 20 × 250 mm) with MeOH–H₂O (9:1) gave three compounds, 1 (2 mg), 2 (3 mg), and 3 (3 mg), as attachment-inhibitors against the blue mussel, *M. edulis galloprovincialis*. These compounds (1–3) showed three times more attachment-inhibiting activity against the blue mussel (0.5 mg/4 cm in diameter for each compound) than standard CuSO₄ (1.0 mg/4 cm in diameter). Compounds 1, 2, and 3 were identified as trans-6-, 8-, and 10-shogaol by a comparison of the 1H-NMR and mass spectral data with those of authentic samples which had been synthesized by the aldol condensation of zingerone (4 from Givaudan-Roure K.K.) with hexanal, octanal or decanal and potassium tert-butoxide in tetrahydrofuran at −78°C.

These promising results prompted us to further evaluate these compounds, and trans-6- and 10-shogaol (1 and 3, 140 mg/5 cm in diameter for each compound) were then subjected to a conventional submerged assay at Mochimune fishing port in Shizuoka city. Trans-10-shogaol (3) showed only moderate activity (for 45 days) which was weaker than that of 1. Zingerone (4) and 6- and 8-zingerols (5 and 6) were also examined to find whether the α,β-unsaturated carbonyl moiety and alkyl chain was essential or not for the activity. After these initial experiments, zingerone (4), 6- and 8-zingerols (5 and 6) and trans-6- and 8-shogaols (1 and 2) (300 mg/5 cm in diameter for each compound) were resubjected to the conventional submerged assay. 6- and 8-zingerols (5 and 6) were also synthesized by the aldol condensation of zingerone with hexanal or octanal. The results of the conventional submerged assay are shown in Table 2. Of these compounds, trans-8-
References


