An organosulfur compound was isolated from oil-macerated garlic extract by silica gel column chromatography and preparative TLC. From the results of NMR, IR, and MS analyses, its structure was determined as E-4,5,9-trithiodeca-1,7-diene-9-oxide (iso-E-10-devinylajoene, iso-E-10-DA). This compound was different from E-4,5,9-trithiodeca-1,6-diene-9-oxide (E-10-devinylajoene, E-10-DA) only in the position of a double bond. Iso-E-10-DA had antimicrobial activity against Gram-positive bacteria, such as Bacillus cereus, B. subtilis, and Staphylococcus aureus, and yeasts at the concentration lower than 100 µg/ml, but Gram-negative bacteria were not inhibited at the same concentration. The antimicrobial activity of iso-E-10-DA was inferior to those of similar oil-macerated garlic extract compounds such as E-ajoene, Z-ajoene, and Z-10-DA. From these results, it was suggested that trans structure and/or the position of double bond of iso-E-10-DA reduce the antimicrobial activity.

Key words: organosulfur compound; garlic; antimicrobial effect; oil-macerated garlic extract; Allium sativum

Garlic (Allium sativum L.) is used world-wide as a spice, food, and folk medicine. Oil-maceration is one method for processing garlic. This type of garlic product is common as a health food in Europe but rare in the United States and Japan.1) The products are prepared by mixing mashed or chopped garlic and a vegetable oil. Bordia2) and Bordia et al.3) reported that the consumption of oil-macerated garlic products or ether-extracted garlic oil, which is nearly identical in quantitative composition to the oil macerates, decreased serum cholesterol in humans. Lawson et al.4) reported that oil-macerated garlic products contained mainly vinyl-dithiins (2-vinyl-4H-1,3-dithien and 3-vinyl-4H-1,2-dithien), ajoene5,6) [(E, Z)-4,5,9-trithiodeca-1,6,11-triene-9-oxide], and a small amount of sulfides.

In previous studies, we isolated Z-10-devinylajoene7) (Z-10-DA, Z-4,5,9-trithiodeca-1,6-diene-9-oxide) from an oil-macerated garlic extract. At that time, we found an unknown peak eluted out just behind of Z-10-DA. In the preliminary study, the unknown peak-containing fractions showed antibacterial activity against Bacillus subtilis. Therefore, we attempted to isolate these compounds from oil-macerated garlic extract.

Oil-macerated garlic extract was prepared by the method of Yoshida et al.7) i.e., 5 kg of garlic was mashed and mixed with 5 kg of middle chain fatty acids triglyceride (MCT, Panacete 810, Japan Oil and Fat Co. Ltd., Tokyo, Japan), then the mixture was left at room temperature overnight. The oil layer was separated and filtered after drying by anhydrous sodium sulfate. The oil was put onto a silica gel (Wakogel Q63, Wako Pure Chemical Industries Ltd., Osaka, Japan) column (3 × 50 cm) equilibrated with hexane, and then equilibrated with hexane to remove the oil. After washing the column with 1,500 ml of 20% 2-propanol in hexane, the unknown peak-containing fractions were eluted with 1,500 ml of 40% 2-propanol in hexane. Further purification was done by preparative Si-TLC (20 × 20 cm, 0.5 mm thickness, Kieselgel 60, E. Merck, Darmstadt, Germany) developed in ethyl acetate. The UV254 visible band (RF=0.23) was extracted by ethyl acetate.

After the purification, yields for the compound from 5 kg of garlic were 20 mg. To identify the chemical structure of the compound, several analyses were done. NMR spectra were measured by a JEOL Alpha 500 spectrometer with tetramethyl silane (TMS) as an internal standard. Infrared spectra (IR) were measured by a Shimadzu IR-740. EI-HRMS was done with a Hitachi MZ-80B.

From the result of 1H-1H COSY analysis, the linkage pattern, –CH=CH–CH2–, was determined. The position of double bond in the structure was determined by HMBC analysis, which showed a correlation between carbon (C-10; δC 40.4) and proton (H-8; δH 5.6). In the 1H-NMR spectrum, the coupling constant values for H-7 (J=7.6 Hz, 14.6 Hz) and H-8 (J=14.6 Hz) were similar to the values of the trans double bond from E-4,5,9-trithiodeca-1,6-diene-9-oxide (E-10-devinylajoene, E-10-DA).6) Therefore, this double bond was determined as the trans form. From the results of UV, IR, NMR, and HRMS analyses, the compound was determined as E-4,5,9-trithiodeca-1,7-diene-9-oxide (iso-E-10-DA). Spectral data of these compounds are as follows. E-4,5,9-TRithiodeca-1,7-diene-9-oxide, Oil; UV λmax (EtOH) (nm (logε)): 235 (3.46); IRmax (CDCl3 cm−1) 2950 (s), 1740 (s), 1620 (m), 1420 (m), 1070 (s), 980 (s),

1 To whom correspondence should be addressed. Tel: 81-52-836-4367; Fax: 81-52-834-6714; E-mail: futsuchi@kb3.so-net.ne.jp
Organosulfur Compound from Garlic

\[ \begin{align*}
\text{S-(+)-Alk(en)yl-L-cysteine sulfoxides} & \quad \text{Allinase} \\
\text{NOS} & \quad \text{Allilin} \\
\text{HN} & \quad \text{Allyl methyl thiosulfinate} \\
\text{O} & \quad \text{Oil maceration} \\
\text{S} & \quad \text{Iso-E-10-devinylajoene} \\
(\text{E-4,5,9-Trithiadeca-1,7-diene-9-oxide}) & \quad \text{3-Vinyl-4H-1,2-dithilin} \\
\text{S} & \quad \text{Z-10-devinylajoene} \\
(\text{Z-4,5,9-Trithiadeca-1,6-diene-9-oxide}) & \quad \text{2-Vinyl-4H-1,3-dithilin}
\end{align*} \]

Fig. Chemical Structure and Proposed Formation Mechanism for Compounds Found in the Oil-macerated Garlic Extract.

Allilin and allyl methyl thiosulfinate were formed from S-alk(en)yl-L-cysteine sulfoxides by allinase.\(^5,\text{10}\) Z-10-Devinylajoene\(^7\) and iso-E-10-devinylajoene are presumed to have been formed from allyl methyl thiosulfinate. Vinyldithiins are formed by dimerization of thiaoacrolein.\(^3\) Solid and broken arrows indicate major and minor reactions, respectively.

950 (s), 920 (s), 660 (m); \(^1\)H-NMR (\(\text{CDCl}_3\)): \(\delta\): 6.1 (1H, dt, \(J=7.6\) Hz, 14.6 Hz, H-7), 5.6 (1H, d, \(J=14.6\) Hz, H-8), 5.3 (2H, m, H-2), 4.77 (1H, dd, \(J=17\) Hz, 0.9 Hz, H-1a), 4.69 (1H, dd, \(J=11\) Hz, 0.9 Hz, H-1b), 2.74 (2H, d, \(J=7.6\) Hz, H-3), 2.65 (2H, d, \(J=1.3\) Hz, H-6), 1.7 (3H, s, H-10); \(^1\)C-NMR (\(\text{CDCl}_3\)): \(\delta\): 138.4 (C-8), 133.5 (C-2), 132.2 (C-7), 118.7 (C-1), 42.5 (C-3), 40.4 (C-10), 39.3 (C-6); HRMS \(m/z\) \((\text{M}^+)\): Calcd. for C\(_{13}\)H\(_{17}\)O\(_{2}\): 208.0049, Found: 208.0042. Although this compound is strictly similar to E-10-DA, it has never been reported up to date, therefore, it is a novel compound. Quantitative analysis of oil-macerated garlic extract by HPLC showed that the compound was contained at 10 to 15 \(\mu\)g/g (data not shown). Block et al. have reported that (E,Z)-10-DA was produced from allyl methanethiosulfinate by S-allylthiolation of allyl methanethiosulfinate with subsequent \(\beta\)-elimination and readdition of methanesulfenic acid.\(^6,\text{8}\) Iso-E-10-DA might be produced by a similar procedure from allyl methanethiosulfinate (Fig.).

The minimum inhibitory concentrations (MICs) of iso-E-10-DA against several microorganisms were measured by the method described in previous papers.\(^7,\text{10}\) To measure the MIC, a culture containing \(10^5\) cells was plated onto the solid medium including various concentrations of iso-E-10-DA and cultivated under the conditions shown in Table for 3 days. The surviving cells were detected on the plate as colonies, and the MIC was evaluated by the detection of no survivors. The MICs of iso-E-10-DA for each microorganism are shown in Table. For Gram-positive bacteria, MICs against two strains of Bacillus spp. were between 50 and 60 \(\mu\)g/ml. The growth of S. aureus was inhibited at 70 \(\mu\)g/ml. These results were much higher than those of E- and Z-ajoene and Z-10-DA. The MIC of iso-E-10-DA for Micrococcus luteus was higher than 100 \(\mu\)g/ml. The growth of Gram-negative bacteria, such as Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Xanthomonas maltophilia, was not inhibited below 100 \(\mu\)g/ml. Two strains of yeast, i.e., Saccharomyces cerevisiae and Schizosaccharomyces pombe were inhibited in growth at 80 and 40 \(\mu\)g/ml, respectively. These results
Table. MIC of Microbial Growth by the Compound Isolated from Oil-macerated Garlic Extract

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Cultivation condition</th>
<th>MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gram-positive bacteria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacillus cereus IAM 12605</td>
<td>a, d</td>
<td>60</td>
</tr>
<tr>
<td>Bacillus subtilis IFO 13719</td>
<td>a, d</td>
<td>50</td>
</tr>
<tr>
<td>Staphylococcus aureus IFO 14462</td>
<td>b, d</td>
<td>70</td>
</tr>
<tr>
<td>Micrococcus luteus IFO 12708</td>
<td>a, d</td>
<td>&gt;100</td>
</tr>
<tr>
<td><strong>Gram-negative bacteria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escherichia coli IFO 3301</td>
<td>b, d</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Klebsiella pneumoniae IAM 1063</td>
<td>b, d</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa IFO 12689</td>
<td>a, d</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Xanthomonas maltophilia IAM 12423</td>
<td>a, d</td>
<td>&gt;100</td>
</tr>
<tr>
<td><strong>Yeasts</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saccharomyces cerevisiae IFO 2347</td>
<td>a, e</td>
<td>80</td>
</tr>
<tr>
<td>Schizosaccharomyces pombe IFO 0347</td>
<td>c, e</td>
<td>40</td>
</tr>
</tbody>
</table>

a IAM, Institute of Applied Microbiology, University of Tokyo, Tokyo, Japan; IFO, Institute for Fermentation, Osaka, Japan.
b a, b, and c, cultivation temperatures were 30, 37, and 28°C, respectively. d, cultivated in PY medium (0.5% peptone, 0.3% yeast extract, 0.3% NaCl, pH 7.0). e, cultivated in YM medium (1% glucose, 0.5% polypeptide, 0.3% malt extract, 0.3% yeast extract, pH 6.0).

Values represent the mean of five measurements.

showed a moderate antimicrobial activity of iso-E-10-DA for Gram-positive bacteria and yeasts.

Iso-E-10-DA has a strictly similar structure with E-10-DA, and they are different only in the position of a double bond. E-10-DA has not been isolated from oil-macerated garlic extract, therefore, its antimicrobial activity has not been demonstrated. In a previous study, we compared antimicrobial activity of the oil-macerated garlic extract constituents having structures consisting of a disulfide bond and sulfanyl group i.e., E- and Z-ajoene and Z-10-DA. From the results, Z-ajoene and Z-10-DA showed similar levels of antimicrobial activity, and were slightly higher than those of E-ajoene. This suggested that the cis double bond between disulfide bond and sulfanyl group is effective for antimicrobial activity. Iso-E-10-DA was different from Z-10-DA in the position of a double bond and isomeric structure. These differences might reduce the antimicrobial activity.

References


