Three novel lipoxygenase inhibitors, tetrapetalone B (2, C_{28}H_{35}NO_{9}), C (3, C_{28}H_{34}NO_{8}), and D (4, C_{28}H_{36}NO_{10}), were isolated from a culture broth of *Streptomyces* sp. USF-4727 that produced a lipoxygenase inhibitor tetrapetalone A (1) simultaneously. Each chemical structure was revealed by spectroscopic evidence, this suggests that these three compounds are structurally related to 1. They had a tetracyclic skeleton and a β-D-rhodinosyl moiety. Tetrapetalone B, C, and D inhibited soybean lipoxygenase with IC_{50}: 320, 360, and 340 μM respectively.

Key words: soybean lipoxygenase inhibitor; *Streptomyces*; tetrapetalone

Human lipoxygenase (HLO) and cyclooxygenase (COX) catalyze the first step of the arachidonic pathway. The resultant leukotrienes, lipoxins, and prostaglandins are important classes of signaling molecules that may be involved in a variety of human diseases.1–3) Soybean lipoxygenase (SBL) catalyzes the oxidation of linoleic acid, arachidonic acid, and other unsaturated fatty acids. Therefore, SBL is often used for lipoxygenase inhibitory assay. We have studied lipoxygenase inhibitors from soil microorganisms using SBL3) and we reported recently that we have designed a simple new screening assay system and succeeded in structure determination of a novel lipoxygenase inhibitor, tetrapetalone A (1).5–7) Our further investigations have also succeeded in isolating other active compounds, tetrapetalone B (2), C (3), and D (4) from the culture extract of *Streptomyces* sp. USF-4727.

In this report, we describe the fermentation of USF-4727 and the isolation and structure elucidation of three novel lipoxygenase inhibitors, 2, 3, and 4, and also describe inhibitory activity against SBL.

**Materials and Methods**

*Chemicals.* SBL (type 1) and linoleic acid were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA), and other chemicals were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

**Instruments.** Spectroscopic measurement was taken with the following instruments: NMR, JEOL Alpha-400 spectrometer (tetramethylsilane as internal reference at 0 ppm for 1H and 13C NMR); FAB-MS, JEOL JMS-700 spectrometer; UV and visible spectra, Shimadzu UV-160 A spectrometer; melting point, Yanagimoto MP-J3; IR, Jasco FT/IR-550; HPLC for analysis, Jasco PU-2080 Plus, Jasco UV 2077 Plus, Jasco MX-2080-32, and HPLC for preparation; Jasco 880-PU, Jasco UV-970.

**SBL assay.** We evaluated the SBL inhibitory activity of each compound using the authentic conjugated diene method at 234 nm as described in our previous report.4)

**Cultivation of a *Streptomyces* sp. USF-4727 strain.** The strain USF-4727 was inoculated into 800 ml of the medium (0.4% glucose, 0.4% yeast extract, 1.0% malt extract, pH 7.3) in a 2-liter Erlenmeyer flask and cultivated at 30°C for 10 days on a rotary shaker (130 rpm).

**Isolation of tetrapetalone B (2), C (3), and D (4).** The culture filtrate (7.2 liters) was applied to a Diaion HP-20 (1 kg) column. After washing with 2 liters of water, the crude extract was eluted with 2 liters of MeOH and 2 liters of aceton. The MeOH and the acetone elutants were mixed and evaporated under reduced pressure, and then applied to a silica gel column (Wakogel C-200, 50 g), and eluted with n-hexane, n-hexane/EtOAc = 3/7, n-hexane/acetone = 3/7, and acetone, in that order.

Tetrapetalone B (2) was included in the n-hexane/acetone = 3/7 and acetone fraction. These fractions were charged onto a Sephadex LH-20 (MeOH) column to yield a crude powder. Finally, the powder was recrystallized from the n-hexane-acetone system to yield 10 mg of tetrapetalone B (2) from 7.2 liters of culture broth.

Tetrapetalone C (3) and D (4) were found in the n-
hexane/EtOAc = 3/7 fraction. Further purification of this fraction was done by Sephadex LH-20 (MeOH) and preparative HPLC (35% CH$_3$CN/10 mM phosphate buffer (pH 2.6), Capcell Pak C18 SG120, 150 μm × 250 mm, UV 254 nm). Continuously, 3 and 4 were separated by preparative HPLC (50% MeOH/H$_2$O, Capcell Pak C18, SG120, 150 μm × 250 mm, UV 254 nm). Then 13 mg of 3 and 20 mg of 4 were obtained each from 7.2 liters of a culture filtrate of USF-4727.

**Tetrapetalone B (2).** Pale yellow amorphous powder; melting point, 191–193 °C. HRFAB MS [M + H]$^+$, m/z 546.2352 (546.2339 calcd. for C$_{38}$H$_{36}$NO$_5$). UV/VIS $\lambda_{\max }$ (MeOH): 217 nm (ε 9310), 336 nm (ε 4190). IR $\nu_{\max }$ (KBr) cm$^{-1}$: 3420, 1580, 1570, 1560, 1540, 1250. The $^1$H- and $^{13}$C-NMR data in CD$_3$OD are shown in Table 1.

**Tetrapetalone C (3).** Colorless amorphous powder; melting point, 154–157 °C. HRFAB MS [M + H]$^+$, m/z 488.2286 (488.2284 calcd. for C$_{38}$H$_{36}$NO$_5$). UV/VIS $\lambda_{\max }$ (MeOH): 206 nm (ε 10350), 250 nm (sh. ε 5890), 311 nm (ε 3190), 350 nm (sh. ε 2900). IR $\nu_{\max }$ (KBr) cm$^{-1}$: 3400, 1720, 1630, 1380, 1280, 1170, 1060, 1020. The $^1$H- and $^{13}$C-NMR data in CD$_3$OD are shown in Table 1.

**Tetrapetalone D (4).** Colorless amorphous powder; melting point, 148–151 °C. HRFAB MS [M + H]$^+$, m/z 546.2352 (546.2339 calcd. for C$_{38}$H$_{36}$NO$_5$). UV/VIS $\lambda_{\max }$ (MeOH): 208 nm (sh. ε 9920), 247 nm (sh. ε 4470), 312 nm (ε 1720), 335 nm (sh. ε 1440). IR $\nu_{\max }$ (KBr) cm$^{-1}$: 3420, 1720, 1630, 1380, 1240, 1160, 1120, 1060, 1020. The $^1$H- and $^{13}$C-NMR data in CD$_3$OD are shown in Table 1.

**Treatment of tetrapetalone A (1) with diazomethane.** An ethereal diazomethane solution was added slowly to the MeOH (5 ml) solution of 1 (50 mg) until the color changed to yellow at room temperature. After evaporation under reduced pressure, crude products were purified by preparative HPLC (35% CH$_3$CN/H$_2$O, Capcell Pak C18, SG120, 150 μm × 250 mm, UV 254 nm). Then 5 mg of 1-methoxytetrapetalone A and 17 mg of 3-methoxytetrapetalone B were obtained. The $^1$H- and $^{13}$C-NMR data in CD$_3$OD are shown in Table 1.

**1-Methoxytetrapetalone A.** Colorless amorphous pow-

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Table 1. $^1$H-NMR (400 MHz) and $^{13}$C-NMR (100 MHz) Spectral Data for Tetrapetalone B (2), C (3), and D (4) in CD$_3$OD

<table>
<thead>
<tr>
<th>Position</th>
<th>Tetrapetalone B (2) $^1$H</th>
<th>Tetrapetalone C (3) $^1$H</th>
<th>Tetrapetalone D (4) $^1$H</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.41 (3H, d, 6.8)</td>
<td>0.85 (3H, t, 6.4)</td>
<td>1.28 (3H, d, 6.8)</td>
</tr>
<tr>
<td>2</td>
<td>1.80 (3H, s)</td>
<td>0.85 (3H, t, 6.4)</td>
<td>0.84 (3H, s)</td>
</tr>
<tr>
<td>3</td>
<td>3.50 (1H, br. d, 9.2)</td>
<td>3.50 (1H, br. d, 9.2)</td>
<td>3.50 (1H, br. d, 9.2)</td>
</tr>
<tr>
<td>4</td>
<td>ca. 1.7 (1H, m), ca. 1.7 (1H, m), ca. 1.7 (1H, m)</td>
<td>ca. 1.7 (1H, m), ca. 1.7 (1H, m), ca. 1.7 (1H, m)</td>
<td>ca. 1.7 (1H, m), ca. 1.7 (1H, m), ca. 1.7 (1H, m)</td>
</tr>
<tr>
<td>5</td>
<td>ca. 3.6 (1H, m)</td>
<td>ca. 3.6 (1H, m)</td>
<td>ca. 3.6 (1H, m)</td>
</tr>
</tbody>
</table>

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der; HRFAB MS [M + H]⁺, m/z 486.2469 (486.2492 calcd. for C₂₇H₃₆NO₇). ¹H-NMR (CD₂OD, 400 MHz) δ_H: 0.77 (3H, t, J = 7.2 Hz, 18-H), 1.25 (3H, d, J = 6.8 Hz, 6'-H), 1.37 (3H, d, J = 6.8 Hz, 20-H), ca. 1.7 and ca. 1.9 (each 1H, m, 3'-H), 2.17 (1H, m, 8'-H), 3.21 (1H, br. d, 7-H, 7.6 Hz), 3.47 (1H, br. s, 4'-H), 3.64 (1H, q, J = 6.8 Hz, 5'-H), 4.10 (3H, s, 1-OCH₃), 4.61 (1H, br. d, J = 9.2 Hz, 1'-H), 4.87 (1H, dd, J = 8.8 and 2.0 Hz, 9-H), 5.53 (1H, br. s, 5-H), 5.65 (1H, d, J = 2.0 Hz, 13-H), 5.99 (1H, t, J = 2.0 Hz, 11-H). ¹³C-NMR (CD₂OD, 100 MHz) δ_C: 5.5 (q, C-16), 8.0 (q, C-18), 17.6 (q, C-6’), 20.6 (q, C-20), 22.8 (q, C-19), 26.7 (t, C-2’), 28.0 (t, C-17), 30.9 (t, C-3’), 41.8 (d, C-8), 56.1 (d, C-7), 60.3 (q, 1-OCH₃), 67.2 (d, C-4’), 75.0 (s, C-15), 75.5 (d, C-5’), 75.7 (s, C-4), 83.7 (d, C-9), 103.0 (s, C-2), 103.6 (d, C-1’), 116.6 (d, C-13), 117.5 (d, C-11), 124.6 (d, C-5’), 142.7 (s, C-6), 157.9 (s, C-14), 167.7 (s, C-10), 178.4 (s, C-1), 188.6 (s, C-12), 203.4 (s, C-3).

Results and Discussion

Three novel lipoxigenase inhibitors, tetrapetalone B (2), C (3), and D (4), were found in a culture broth of Streptomyces sp. USF-4727 strain that produced tetrapetalone A (1) simultaneously.

The purification of 2 was carried out in a way similar to that of 1, 6, 10 as described in Materials and Methods. In this purification procedure, Sephadex LH-20 was useful for the separation of 2 from 1.

The structure elucidation of 2 was done by the spectroscopic method. Tetrapetalone B (2) was isolated as a yellow amorphous powder. The HRFAB MS data gave a molecular formula, C₂₇H₃₆NO₇, for 2. The ¹H- and ¹³C-NMR spectra of 2 in CD₂OD resembled those of 1, indicating that 2 is structurally related to 1 (Table 1).

Tetrapetalone A, C₂₆H₃₅NO₇, was constructed with a tetracyclic skeleton and a β-rhodinosyl moiety. The ¹H-, ¹³C-NMR, ¹H–¹H COSY, HMOC, and HMBC data for the sugar moiety of 2 were consistent with the spectral data for the sugar moiety of 1, suggesting the presence of a β-rhodinosyl moiety in 2 as well as 1. The spectral data for the tetracyclic skeleton moiety in 2 were also similar to the data for the skeleton moiety in 1. In the ¹H-NMR spectra of these two compounds, however, the signals were significantly different, as follows: (1) A doublet methyl signal at 1.41 ppm was observed in the spectrum of 2 instead of a triplet methyl signal at 0.62 ppm shown in the spectrum of 1. (2) The methylene signals at C-17 (δ_H: 1.85 and 3.14, each 1H, m) in 1 did not exist in the spectrum of 2. (3) A 1H quartet signal at 6.65 ppm and a singlet methyl proton at 1.89 ppm were newly observed.

In the HMBC spectrum of 2, a carbonyl carbon signal at 172.4 ppm showed a cross peak with a singlet methyl proton at 1.89 ppm and a quartet 1H signal at 6.65 ppm. These results suggest an additional acetoxy group at C-17 in 2. The difference in the molecular formula (C₂₂H₂O₂) between 1 and 2 also indicates the existence of an acetoxy group instead of a hydrogen atom. We determined the planar structure of 2. This structure was also supported by ¹H–¹H COSY, HMOC, and HMBC spectral data (Fig. 2).

The stereochemistry of 2 was estimated by the chemical shift and coupling constant in the ¹H-NMR spectrum and NOE correlations. Considering the β-rhodinosyl moiety of 2, 1'-H indicated a large coupling constant (8.8 Hz) with 2'-Hax and a small coupling constant (<1 Hz) with 2'-Heq. The coupling constant between 4'-H and 5'-H was also small (<1 Hz) in the ¹H-NMR spectrum. In the NOESY spectrum of this moiety, a cross peak was observed between 1'-H and 5'-H. These data indicate that the stereochemistry of the β-rhodinosyl moiety in 2 is consistent with this moiety in 1. On the tetracyclic skeleton of 2, two large coupling constants, 9.2 Hz and 10.0 Hz, appeared at 7-H/8-H and at 8-H/9-H in the ¹H-NMR spectrum, respectively. Strong NOE correlations at 7-H/9-H, 7-H/17-H, and 8-H/19-H were observed and a very weak correlation appeared between 19-H and 20-H. Because these data were consistent with those for 1, we concluded that the relative stereochemistry of the tetracyclic skeleton and the β-rhodinosyl moiety of 2 is consistent with that of 1, as shown in Fig. 1.

The relative stereochemistry of C-17 in 2 was elucidated by NOE correlations. In the NOESY spectrum of 2, cross-peaks were observed at 5-H/18-H and 7-H/17-H. On the other hand, cross-peaks at 7-H/18-H and 5-H/7-H did not appear in this spectrum. On the basis of these results, we constructed a stereochemistry of C-17 as shown in Fig. 3. In this proposed stereochemistry of C-17, 5'-H/17-H and 7-H/17-H are assumed to be close to each other. Another stereochemistry of C-17 was not in agreement with the result of the NOESY experiment described above. Therefore we established the relative stereochemistry of C-17 as shown in Fig. 3, and all the relative stereochemistry of 2 was estimated as shown in Fig. 1.

The chemical structures of 3 and 4 were also investigated by ¹H- and ¹³C-NMR, ¹H–¹H COSY, HMOC, HMBC data, and HRFAB MS, suggesting that these compounds are similar to 1 and 2. The HRFAB MS gave a molecular formula, C₂₇H₃₅NO₇ and C₂₇H₃₅NO₁₀, for 3 and 4 indicating that these compounds included one additional oxygen atom as compared to 1 and 2.

The ¹H- and ¹³C-NMR, ¹H–¹H COSY, HMOC, and HMBC spectra indicated that a part of the tetracyclic skeleton (C-2 and C-3) of 3 was different from that of 1. In the HMBC spectrum of 3, the methyl proton at C-16 (δ_H: 1.59) showed a correlation with C-1, C-2, and C-3. Furthermore, C-3 showed a cross peak with 17-H and another with 5-H (Fig. 3) in this spectrum. Judging from the chemical shift, C-2 (δ_C: 72.2) was a carbon atom
next to an oxygen atom and C-3 ($\delta_C$: 210.2) was a ketone carbon. Considering these results and the chemical formula of 3 (C$_{26}$H$_{33}$NO$_8$), a hydroxy group is probably connected to C-2 (Fig. 4). The planar structure of 3 was constructed as shown in Fig. 1. In a similar way, the planar structure of 4 was determined (Fig. 1). A summary of the $^1$H- and $^{13}$C-NMR data and the $^1$H–$^1$H COSY, and HMBC data for 2, 3, and 4 is shown in Table 1 and Fig. 2.

The relative stereochemistry of 3 and 4 was estimated by the coupling constant in the $^1$H-NMR spectrum and NOE correlations. Because these data were consistent with those for 1 and 2, we considered the stereo-

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**Fig. 1.** Structures of Tetrapetalone A (1), B (2), C (3), and D (4).

**Fig. 2.** 2D-NMR Analysis of Tetrapetalone B (2), C (3), and D (4).

**Fig. 3.** Stereochemistry of C-17 in Tetrapetalone B (2).

**Fig. 4.** Partial Structures of Tetrapetalone C (3).
chemistry of 3 and 4, except for C-2, to be as shown in Fig. 1. The relative stereochemistry of C-2 was elucidated by NOE correlations. The protons at C-16 showed NOE correlations to 17-H and 18-H with high intensity. On the other hand, a very weak correlation was observed to 5-H, indicating a methyl group at C-2 as shown in Fig. 1.

Tetrapetalone B (2), C (3), and D (4) inhibited SBL as effectively as 1 did (Table 2). In our previous report,\(^5\) we explained that a hydroxy group at C-3 is important for this activity. The fact that 3 and 4 indicated this activity suggests that another factor besides the C-3 hydroxy group might contribute to this inhibitory activity in the cases of 3 and 4.

We have already obtained 3-methoxytetrapetalone A by treating 1 with diazomethane, which is called 'tetrapetalone A-Me' in our previous paper.\(^4\) Recently, it was found that 1-methoxytetrapetalone A was also contained in this reaction mixture as a minor product (Fig. 5). These results suggest that 1 exists as a keto-enol tautomer, form (i) and form (iii), through an intermediate (ii) under certain conditions, though it takes form (i) in CD\(\text{OD}_3\) and DMSO-\(d_6\), solvents for NMR measurement (Fig. 6). This tautomerization, which might also occur in 2, is thought to impart certain chemical properties to 1 and 2, and the properties may play a role in the bioactivity.

Four tetrapetalones, 1, 2, 3, and 4, possess a similar tetracyclic skeleton and \(\beta\)-\(D\)-rhodinosyl moiety, suggesting that these compounds are related in the biosynthetic pathway. Tetrapetalone B (2) is thought to be synthesized biologically through 1. Tetrapetalone C (3) and tetrapetalone D (4) are considered to be derived from 1 and 2 respectively.

The relative stereochemistry of 2, 3, and 4 was determined by the coupling constant in the \(^1\text{H-NMR}\) spectrum and NOE correlations. The absolute stereochemistry of the \(\beta\)-rhodinosyl moiety and of the

| Table 2. Inhibitory Activities of Tetrapetalones against Soybean Lipoxigenase |
|-----------------------------|---|
| I.C\(_{50}\) (\(\mu\)M)         |   |
| Tetrapetalones A (1)         | 190 |
| Tetrapetalones B (2)         | 320 |
| Tetrapetalones C (3)         | 360 |
| Tetrapetalones D (4)         | 340 |
| Kojic Acid (positive control)| 110 |
| NDGA (positive control)      | 290 |

Fig. 5. Methylation of Tetrapetalone A (1) with Diazomethane.

Fig. 6. Proposed Keto-enol Tautomerization of Tetrapetalone A (1).
tetracyclic skeleton in each of the three compounds is considered to be consistent with those of 1, as shown in Fig. 1. Further investigation is, however, needed to determine the accurate absolute stereochemistry of 2, 3, and 4. A study of the biosynthetic relationship for the four tetrapetalones and of the mode of lipoxygenase inhibitory activity is now in progress.

References


