The cytotoxic activity for colon 26 cell line of matairesinol, oxidized matairesinol, 9,9'-epoxylignan and oxidized 9,9'-epoxylignan were examined. (−)-Matairesinol (Mat 1) showed greatest cytotoxic activity \( (LC_{50} = 9 \mu g/ml) \) of the lactone-type lignans. 7,7'-Oxomatairesinol having same steric configuration as that of (−)-matairesinol showed greater activity \( (LC_{50} = 25 \mu g/ml) \) than hydroxy or mono-oxomatairesinol. The activities of 9,9'-epoxylignan and 7,7'-oxo-9,9'-epoxylignan having same steric configurations as (−)-matairesinol were weaker than that of corresponding matairesinols. Different activity levels were observed between enantiomers.

Key words: matairesinol; cytotoxic activity; oxidized matairesinol

Many kinds of lignans are biosynthesized by plants and are often contained in dietary plants. It has been shown that the degree of oxidation on the benzylic position of tetrahydrofuran and lactone-type lignans affects their antioxidant activity. \(^1\,\,2\) The benzylic position may be oxidized during an oxidation process \(^3\) or in biosynthesis. \(^4\) A relationship between the antioxidant activity of lignan compounds and defense to certain adult diseases has been suggested. \(^5\) This report describes the relationship between the oxidation degree of the benzylic position of lignans and their cytotoxic activity for colon 26 cell line. The effect of the stereochemistry of matairesinol and oxidized matairesinol on cytotoxic activity is also described. Both (−)\(^6\) and (−)\(^7\)-matairesinol have been isolated from plants, and the optical purity of each natural product was estimated as 99%ee. \(^8\) However, it has been reported that the (−)-matairesinol was not present in the assay system used, but that (−)-matairesinol was produced. \(^9\) Though the cytotoxic activity of (−)-matairesinol has been reported, \(^10\) a comparison of activities of (−) and (−)-matairesinol has not been reported. To clarify the important factor in the activity of (−)-matairesinol, the cytotoxic activities of (+)-matairesinol, 9,9'-epoxylignan and 7,7'-oxo-9,9'-epoxylignan are examined for the first time in this article. The experiment of activity of 9,9'-epoxylignan is important to estimate the contribution of carbonyl group on lactone ring to biological activity. It is known that lignans display many kinds of biological activities, \(^4\) however, some lignans have only been isolated as mixtures of enantiomers \(^14\) and most biological research has been done using these isolated compounds from plants. Previously, the antifungal activity of matairesinol has been reported \(^15\) and the binding affinity of Lio-7 to human sex hormone-binding globulin has been described. \(^16\) The dietary toxicity of 7-hydroxymatairesinol has also been reported. \(^17\) Podophyllotoxin is well known \(^4\) as cytotoxic lignan and research about its antitumor properties has been done. \(^18\) The relationship between structure and biological activity of lignan is very complicated. \(^4\) Continuing research efforts on the biological activity of lignan by using synthesized optically pure lignans is very important. The characteristic of this research is that synthetic optically pure lignans were used for biological research. The results of this research will contribute to the utilization of lignans and plant materials containing lignans to industry. There is a possibility that a new function of dietary plants will be discovered. New information about the relationship between structure and activity will also be provided and will contribute to determining the structural factor of lignan that is most important to biological activity. The 4-hydroxy-3-methoxyphenyl group, which is one of the main phenyl group of lignans was used in this research as an aromatic structure (Fig. 1).

Results and Discussion

(−)-Matairesinol (Mat-1), Mat-2–Mat-4, Lio-1–Lio-5, and Lio-7 were synthesized from L-glutamic acid by the same method as previously described. \(^1\) (−)-matairesinol (Mat-8), Mat-7, Lio-10, and Lio-11 were
Cytotoxic Activity of Lignan 2943

(-)-Matairesinol (Mat-1): R_1=R_2=R_3=R_4=H
Mat-2: R_1=R_2=O, R_3=R_4=H
Mat-3: R_1=R_2=R_3=H, R_4=O
Mat-4: R_1=R_2=R_3=R_4=O

(+)-Matairesinol (Mat-8): R_1=R_2=R_3=R_4=H

Mat-7: R_1=R_2=O, R_3=R_4=O

Lio-1: R_1=R_2=R_3=R_4=H
Lio-2: R_1=R_2=R_3=H, R_4=O
Lio-3: R_1=R_2=O, R_3=R_4=O
Lio-4: R_1=OH, R_2=H, R_3=OH, R_4=O
Lio-5: R_1=H, R_2=OH, R_3=OH, R_4=H
Lio-6: R_1=R_2=OH, R_3=OH
Lio-8: R_1=R_2=H, R_3=R_4=O

Lio-9

Lio-10: R_1=R_2=O, R_3=R_4=O
Lio-11: R_1=R_2=H, R_3=R_4=H

Fig. 1. (-) and (+)-Matairesinol. Oxidized Matairesinols, 3,4-Dibenzyltetrahydrofuran, and Oxidized 3,4-Dibenzyltetrahydrofuran.

Since the activity disappeared with the presence of a benzylic hydroxy group in Lio-type lignans, dibenzyl and benzoyl Mat compounds were tested. The tendency of activity of butyro lactone lignan (Mat type of lignans) was same as that of Lio type of lignans (Fig. 3). (-)-Matairesinol (Mat-1), which has two free benzylic positions, showed strongest activity. The activity of dibenzoyl type (Mat-4, LC_{50}=25\mu g/ml) was weaker than that of (-)-matairesinol (Mat-1). However, the activity of Mat-4 was stronger than that of mono ketone compounds (Mat-2, LC_{50}=45\mu g/ml; Mat-3, LC_{50}=49\mu g/ml). The activities of butyro lactone lignans (Mat type of lignans) were stronger than that of corresponding tetrahydrofuran lignans (Lio type of lignan). It was shown that the oxidation on the benzylic positions of (-)-matairesinol (Mat-1) decreased activity, however, the compound bearing two benzoyl groups had stronger activity than that of the compounds bearing one benzoyl group. It could be assumed that a long conjugated system such as Mat-4 is important for cytotoxic activity and that the biological mechanism of (-)-matairesinol (Mat-1) having no carbonyl group on benzylic position was different from that of Mat-4. In the case of radical scavenging activity, the higher oxidation degree on the benzylic position decreased the activity, and (-)-matairesinol (Mat-1) which has free benzylic positions...
had the strongest radical scavenging activity. In this experiment, the compound having highest radical scavenging activity, (−)-matairesinol (Mat-1), showed strongest cytotoxic activity. The reduction of the lactone ring to a tetrahydrofuran ring reduced the activity. This fact means that the carbonyl group of the lactone ring is important for the higher activity.

The comparison of activity between enantiomers was achieved (Fig. 4). The activity of (−)-matairesinol (Mat-1, LC₅₀ = 9 µg/ml) was stronger than that of (+)-matairesinol (Mat-8, LC₅₀ = 37 µg/ml). Except for the activities of Lio-7 and Lio-11, the compounds having same steric configuration as that of (−)-matairesinol (Mat-1) had higher activity. The difference of activity between Mat-4 and Mat-7, and Lio-3 and Lio-10 was smaller than that between (−)-matairesinol (Mat-1) and (+)-matairesinol (Mat-8). It could be assumed that one reason for this is enolization and racemization of Mat-4, Mat-7, Lio-3 and Lio-10 in cells. The fact that the activities of the different stereoisomers showed higher activity in the case of Lio-7 and Lio-11 means that the receptor for these compounds is different from that of (−)-matairesinol (Mat-1). It was found that stereochemistry affected the activity.

Figures 5 and 6 show the relationship between activity and concentration of compounds. In the case of Lio-7, Lio-11, Lio-3, and Lio-10, the tendency of activity at the highest concentration was same as that of LC₅₀ value (Fig. 5). In the case of butyrolactone type of compounds (Mat compounds), only (−)-matairesinol (Mat-1) showed a different curve (Fig. 6). At the lower

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mat-1</th>
<th>Lio-1</th>
<th>Lio-2</th>
<th>Lio-3</th>
<th>Lio-4</th>
<th>Lio-5</th>
<th>Lio-7</th>
<th>Lio-8</th>
<th>Lio-9</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC₅₀ (µg/ml)</td>
<td>9</td>
<td>ND</td>
<td>ND</td>
<td>46</td>
<td>ND</td>
<td>ND</td>
<td>37</td>
<td>50</td>
<td>34</td>
</tr>
</tbody>
</table>

ND = Not Detected

Fig. 2. Cytotoxic Effect on Colon 26 Cells of (−)-Matairesinol and Lio Compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mat-1</th>
<th>Lio-1</th>
<th>Lio-2</th>
<th>Lio-3</th>
<th>Lio-4</th>
<th>Lio-5</th>
<th>Lio-7</th>
<th>Lio-8</th>
<th>Lio-9</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC₅₀ (µg/ml)</td>
<td>9</td>
<td>45</td>
<td>49</td>
<td></td>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Ar = 4-hydroxy-3-methoxyphenyl

Fig. 3. Cytotoxic Effect on Colon 26 Cells of (−)-Matairesinol and Oxidized Matairesinols.
concentration, only (−)-matairesinol showed activity. The other compounds showed activity only at higher concentration. The activity level at the highest concentration differed from that of the LC50 value. (−)-Matairesinol (Mat-1) showed higher activity than Mat-4, Mat-7, and Mat-8 at the lower concentration, however, Mat-4 which has same stereochemistry as that of (−)-matairesinol and highest oxidation degree on benzylic position showed higher activity than that of (−)-matairesinol (Mat-1), Mat-7, and Mat-8 at the highest concentration (100 µg/ml). This fact means that the mechanism of cytotoxic activity of Mat-1 is different from that of other compounds.

This experiment provided important information on the cytotoxic activity of (−)-matairesinol (Mat-1). The different activities of (−) and (+)-matairesinol was demonstrated for the first time. The lactone ring is important for the higher activity. The oxidation on the benzylic position decreased the activity, showing higher LC50 values, however, the activity of dicarbonyl compound (Mat-4) was higher than that of mono-carbonyl compounds. Though the LC50 value of Mat-4 was higher than that of (−)-matairesinol (Mat-1), the activity of Mat-4 at 100 µg/ml is higher than that of (−)-matairesinol (Mat-1). Different mechanisms for the cytotoxic activities of Mat-1 and Mat-4 can be assumed. It was first found that oxidized matairesinol, 9,9'-epoxylignan, and oxidized 9,9'-epoxylignan had cytotoxic activity.

**Experimental**

Optical rotation values were measured on a Horiba SEPA-200 instrument. NMR data were obtained using a JNM-EX400 spectrometer. EIMS data were measured with a JMS-MS700V spectrometer. The silica gel used was Wakogel C-300 (Wako, 200–300 mesh). HPLC analysis were performed with Shimadzu LC-6AD and SPD-6AV instruments.

**Determination of the optical purity of the compounds.**

Lio-7 and Lio-10: >99%ee, HPLC, DAICEL chiral column OD-H, detected at 280 nm, 1 ml/min, 30% EtOH/hexane, tR 8.5 min (Lio-7), tR 9.7 min (Lio-10).

Lio-3 and Lio-11: >99%ee, HPLC, DAICEL chiral column OD-H, detected at 280 nm, 1 ml/min, 20% EtOH/hexane, tR 25.7 min (Lio-3), tR 22.2 min (Lio-11).

(−)-Matairesinol (Mat-1) and (+)-matairesinol (Mat-8): >99%ee, HPLC, DAICEL chiral column OD-H, detected at 280 nm, 1 ml/min, 30% EtOH/hexane, tR 16.6 min (Mat-1), tR 14.2 min (Mat-8).

Mat-4 and Mat-7: >99%ee, HPLC, DAICEL chiral column OD-H, detected at 280 nm, 1 ml/min, 40% EtOH/hexane, tR 8.5 min (Mat-4), tR 12.6 min (Mat-7).

(8R,8'R)-3,3',4,4'-Tetramethoxy-7,7'-dioxo-9,9'-epoxylignane (Lio-9). A reaction mixture of phenol Lio-3 (25 mg, 0.07 mmol), Me2SO4 (0.02 ml, 0.21 mmol), K2CO3 (39 mg, 0.28 mol), and dibenzo-18-crown-6 (2 mg) in CH3CN (20 ml) was heated under refluxing for 16 h. After additions of EtOAc and H2O, the organic solution was separated, washed with brine, and dried (Na2SO4). Concentration followed by silica gel column chromatography (EtOAc/hexane, 1:1) gave dimethyl ether (10 mg, 0.02 mmol, 29%) as a colorless oil; [α]D 29.0 +27° (c 0.5, CHCl3); 1H NMR (CDCl3, 400 MHz) δ:
**Fig. 5.** Relationship between the Concentration and Activity of Lio Compounds.
Each value is the mean ± S.D. of three independent experiments.

**Fig. 6.** Relationship between the Concentration and Activity of Mat Compounds.
Each value is the mean ± S.D. of three independent experiments.
3.92 (6H, s), 3.94 (6H, s), 4.02–4.04 (2H, m), 4.15–4.18 (1H, d, J = 3.92 Hz), 4.82 (6H, s), 3.94 (6H, s), 4.02–4.04 (2H, m), 4.15–4.18 (1H, d, J = 3.92 Hz).  

Cell and cell culture. Colon26 colon adenocarcinoma cells derived from BALB/c mouse were kindly provided by Institute of Development, Aging and Cancer, Tohoku University (Sendai, Japan). Colon26 cells were subcultured in ERDF medium (Kyokuto Pharmaceutical, Tokyo, Japan) supplemented with 5% of fetal bovine serum (FBS) at 37°C in a humidified atmosphere of 5% CO2.

Cytotoxic assay. The colon26 cells were inoculated in 96-well culture plate at 1.0 x 104 cells/ml suspended in the ERDF medium with 2% FBS and various concentrations of different compounds. After cultivation for 48 h, the cell viability was assessed by a WST-8 reduction assay kit (Dojin Laboratories, Japan). The WST-8 reduction activity of the cells is presented as the ratio of cell activity. Briefly, a WST-8 solution was added to the culture medium at 10% concentration and incubated for 3 h at 37°C prior to colorimetry at 450 nm.

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