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Inhibitory Effects of Lignans on the Activity of Human Matrix Metalloproteinase 7 (Matrilysin)

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Inhibitory effects of nine dibenzylbutyrolactone lignans on a human matrix metalloproteinase, matrilysin, were examined. All of the lignans examined inhibited matrilysin with the IC_{50} values ranging from 50 to >280 μM. Matairesinol, which has the basic structure of the other lignans, showed the weakest inhibition. Lignans with methylenedioxy ring(s) or a hydroxyl group at the C5-position inhibited matrilysin more strongly than matairesinol. 5-Hydroxypluviatolide, which has both a methylenedioxy ring and a hydroxyl group at the C5-position, was the most potent inhibitor (IC_{50} = 50 μM), suggesting that the introduction of these two elements might enhance synergistically the inhibitory activity of lignans. 5-Hydroxypluviatolide inhibited matrilysin in a competitive manner, and its inhibitory effect was greatly suppressed by the presence of another competitive inhibitor, dimethyl sulfoxide. The precursors of matairesinol, coniferyl alcohol and secoisolariciresinol, had no inhibitory activity, indicating that the dibenzylbutyrolactone structure is essential for the inhibition. It has been shown that lignans have the potential to inhibit matrilysin, and the knowledge of their structure−activity relationship might be beneficial to developing selective inhibitors for matrix metalloproteinases.

KEYWORDS: Competitive inhibitor; lignan; matairesinol; matrilysin; matrix metalloproteinase

INTRODUCTION

Matrix metalloproteinases (MMPs) comprise a family of zinc endopeptidases that are collectively capable of degrading almost all of the extracellular matrix (ECM) components. MMPs are believed to be responsible for both normal and pathological processes, including development, reproduction, maintenance, tissue destruction, and fibrotic diseases (*1*, *2*). Matrilysin (MMP-7) [EC 3.4.24.23] is one of the smallest MMPs composed only of a catalytic domain; its molecular mass is 19 kDa (*3*, *4*). X-ray crystallographic analysis of human MMP-7 demonstrated that it consists of a five-stranded β-sheet and three α-helices and contains a zinc ion essential for enzyme activity, as well as another zinc and two calcium ions that are regarded as necessary for enzyme stability (*5*). MMP-7 has been shown to degrade various ECM components such as collagen, fibronectin, vitronectin, proteoglycan, laminin, and elastin (*4*, *6*−*8*) and to be overexpressed in lesions of prostate (*9*), colon (*10*), brain (*11*), stomach (*12*), lung (*13*), and breast (*14*). These observations suggest that MMP-7 may play a critical role in tumor invasion and metastasis. In fact, there is enough evidence that MMP-7 enhances the metastatic potential of several tumor cells (*15*−*17*). From this viewpoint, developing and exploring selective inhibitors against MMP-7 should be therapeutically beneficial.

Lignans as secondary metabolites constitute an abundant class of phenylpropanoids that occur in many plants and have been receiving widespread interest because of their various biological activities such as antitumor, antimitotic, antioxidant, and antiviral activities (*18*−*21*). Lignans, phenylpropanoid dimers connected with a C8−C8′ bond, are structurally related to lignins, and many substructures in lignins contain the structural elements of isolated lignans. In 1990, the first example of an enzymatic reaction for producing an optically pure lignan from an achiral phenylpropanoid monomer was demonstrated with a cell-free extract of *Forsythia intermedia* (*22*). Since then, the stereochemical control of lignan biosynthesis has been studied in detail (*23*−*28*). On the other hand, lignans displaying antitumor activities, especially podophyllotoxin extracted from *Podophyllum* species, have attracted much attention for a long time (*29*), and the biotechnological production of these antitumor lignans has been studied for developing clinically useful anticancer drugs (*30*). The effects of podophyllotoxin and its derivatives on malignant tissues suggested that their antitumor activities were related to inhibiting cytochrome oxidase (*18*). The antitumor activities of lignans, however, have not yet been examined in regard to inhibiting MMPs.

In the present study, the inhibitory effects of nine dibenzylbutyrolactone lignans (**Figure 1**) on MMP-7 activity were

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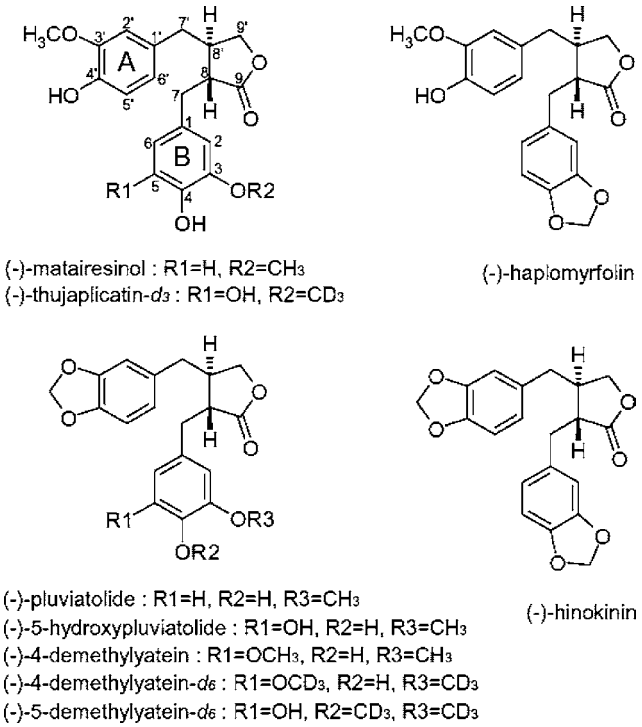

Figure 1. Molecular structures of dibenzylbutyrolactone lignans used in this study.

examined. To date, a number of lignans have been identified in humans and in several animals (*31*). Cereals such as rye, buckwheat, millet, soya, oats, and barley are rich in lignans and are the main source of the mammalian lignans. Their dietary precursors are thought to be secoisolariciresinol and matairesinol (*31*), which also serve as central precursors of numerous lignans in the plants (*32*−*34*). In this study, we focus on the structure of matairesinol, the basic structure of other lignans examined, and demonstrate their structure−activity relationship for the inhibition of MMP-7 activity. This is the first report referring to lignans as potent inhibitors against a member of the MMP family. The results obtained in this study suggest that lignan components taken with foods could play a role in the regulation of cell growth, tissue differentiation, tumor metastasis, and so forth.

MATERIALS AND METHODS

Materials. Recombinant human pro-MMP-7 was prepared according to the method previously reported (*35*). Pro-MMP-7 was incubated in 1 mM *p*-aminophenylmercuric acetate at pH 7.5, 37 °C, for 30 min and dialyzed against 50 mM HEPES buffer (pH 7.5) containing 10 mM $CaCl_2$ and 0.05% Brij-35 at 4 °C. The concentration of MMP-7 was determined using the molar absorption coefficient at 280 nm of 31.8 mM/cm calculated from the amino acid composition (*3*) with a Shimadzu UV-2200 spectrophotometer (Kyoto, Japan). A fluorescent substrate, MOCAc-PLGL(Dpa)AR, and MOCAc-PLG were purchased from the Peptide Institute (Osaka, Japan). MOCAc-PLGL(Dpa)AR is known to be cleaved at the peptide bond between glycine and leucine residues in the hydrolysis by MMP-7 (*36*). The concentrations of MOCAc-PLGL(Dpa)AR and MOCAc-PLG were determined using the molar absorption coefficients $\epsilon_{410} = 7.5$ mM/cm and $\epsilon_{324} = 12.9$ mM/cm, respectively (*36*, *37*). (±)-Matairesinol and (±)-secoisolariciresinol were synthesized according to the methods reported by Umezawa et al. (*23*, *38*). (±)-Hinokinin, (±)-pluviatolide, and (±)-haplomyrfolin were synthesized by the previously proposed procedures (*39*). The other lignans [(±)-4-demethylyatein, (±)-4-demethylyatein-d_6, (±)-5-demethylyatein-d_6, (±)-thujaplicatin-d_3, and (±)-5-hydroxypluviatolide] were prepared in a manner similar to the syntheses of (±)-matairesinol and (±)-secoisolariciresinol (*23*, *38*). All of the lignans used were racemic mixtures unless otherwise mentioned. The molar concentration of each lignan was calculated from its molecular weight. Coniferyl alcohol and 1,2-(methylenedioxy)benzene were from Fluka (Buchs, Switzerland). All other chemicals were of reagent grade and purchased from Nacalai Tesque (Kyoto). The high-performance liquid chromatography (HPLC) apparatus composed of a CCPM solvent delivery system, a UV-8010 monitoring system, a PX-8010 computer-controlled system, and a Chromatocorder 12 integrator was obtained from Tosoh (Tokyo).

Fluorometric Analysis of the MMP-7-Catalyzed Hydrolysis of MOCAc-PLGL(Dpa)AR. The hydrolysis of MOCAc-PLGL(Dpa)AR by MMP-7 was initiated by mixing 1232 μL of MMP-7 solution (7.61 nM) in 50.7 mM HEPES buffer containing 10.1 mM $CaCl_2$, 10 μL of lignan (0−35 mM) dissolved in DMSO, and 8 μL of MOCAc-PLGL-(Dpa)AR (234 nM) dissolved in DMSO at pH 7.5, 25 °C, and measured by following the increase in the fluorescence emission intensity at 393 nm by excitation at 328 nm with a JASCO FP-777 fluorescence spectrophotometer (Tokyo). The initial concentrations of MMP-7, MOCAc-PLGL(Dpa)AR, and DMSO were 7.5 nM, 1.5 μM, and 1.4%, respectively. In the case of hinokinin, 10.8% DMSO was added to the reaction mixture because of its sparing solubility. The hydrolysis was carried out under pseudo-first-order conditions, where the substrate concentration ([S]) is much lower than the Michaelis constant (K_m) in order to avoid its absorptive quenching effects. Under these conditions, the enzyme activity was evaluated by the specificity constant, k_{cat}/K_m, and the inhibitory effects of lignans were evaluated by IC_{50}, that is, the lignan concentration required to inhibit 50% of the enzyme activity observed in its absence at pH 7.5 and 25 °C.

Inner Filter Effect Correction for the Flurometric Analysis. The amount of the MOCAc-PLG produced in the MMP-7-catalyzed hydrolysis of MOCAc-PLGL(Dpa)AR has been estimated by comparing the fluorescence intensity with that of the authentic MOCAc-PLG solution (*35*, *37*, *40*, *41*). FRET molecules such as MOCAc-PLGL-(Dpa)AR have been extensively used as substrates for proteases especially, although the inner filter effects associated with these FRET substrates should be taken into consideration (*42*). We have prevented the inner filter effects due to the absorption of the excited and/or emitted light by the quenching groups on neighboring substrates or those separated from fluorophores (self-absorptive quenching effects) by lowering sufficiently the substrate concentration. In this study, however, some of the lignans (haplomyrfolin, hinokinin, thujaplicatin-d_3, and 5-demethylyatein-d_6) in the reaction mixture slightly absorbed the light excited at 328 nm, and consequently, they showed a weak emission at 393 nm. However, a linear relationship between the fluorescence intensity and the [MOCAc-PLG] was maintained even at the high concentration of these lignans (data not shown), indicating that the amount of the product can be estimated using the ratio of the increment in the fluorescence intensity to that in [MOCAc-PLG], $\Delta FI/\Delta$[MOCAc-PLG].

HPLC Analysis of the MMP-7-Catalyzed Hydrolysis of MOCAc-PLGL(Dpa)AR. The hydrolysis of MOCAc-PLGL(Dpa)AR by MMP-7 was initiated by mixing 950 μL of the MMP-7 solution (15.0 nM) in 53 mM HEPES buffer containing 10.1 mM $CaCl_2$, 10 μL of lignan (5-hydroxypluviatolide) (33.6 mM) dissolved in DMSO, and 40 μL of MOCAc-PLGL(Dpa)AR (0−3.5 mM) in DMSO at pH 7.5 and 25 °C. The initial concentrations of MMP-7, 5-hydroxypluviatolide, and DMSO were 14.3 nM, 336 μM, and 5.0%, respectively. The reaction was stopped at appropriate times by mixing 100 μL of the reaction solution with 400 μL of 1% trifluoroacetic acid (TFA). One hundred microliters of the reaction solution mixed with TFA was applied to HPLC performed on a 150 mm × 4.6 mm i.d. TSKgel ODS-80Ts column (Tosoh) equilibrated with 0.1% TFA. A linear gradient was generated from 20 to 70% acetonitrile from 5 to 20 min at a flow rate of 1.0 mL/min, and the absorption of the eluate was detected at 335 nm. The substrate and its two products, MOCAc-PLG and L(Dpa)AR, were separated by this condition (*41*). The initial reaction rate, v, was determined from the time course of the amount of the MOCAc-PLG produced, which was evaluated from its peak area. The kinetic parameters, the catalytic (k_{cat}) and Michaelis (K_m) constants, were

determined separately based on the Michaelis−Menten equation by using the nonlinear least-squares method (*43*).

RESULTS AND DISCUSSION

Inhibitory Potency of Lignans against MMP-7. The inhibitory effects of nine dibenzylbutyrolactone lignans on the MMP-7-catalyzed hydrolysis of MOCAc-PLGL(Dpa)AR were examined. All of the lignans inhibited MMP-7 activity with the IC_{50} values at 1.44% DMSO ranging from 50 to >280 μM. The order of the inhibitory effect was 5-hydroxypluviatolide > haplomyrfolin = hinokinin = thujaplicatin-d_3 > pluviatolide = 5-demethylyatein-d_6 > matairesinol = 4-demethylyatein = 4-demethylyatein-d_6. Matairesinol, which has two 4-hydroxy-3-methoxy aromatic rings (guaiacyl rings; designated A and B rings for the sake of convenience) and a lactone ring, inhibited MMP-7 weakly with an IC_{50} value higher than 280 μM. Sixty-five percent of the enzyme activity observed in its absence remained even at 280 μM matairesinol. Haplomyrfolin, hinokinin, thujaplicatin-d_3, and 5-hydroxypluviatolide showed relatively high inhibitory activities with IC_{50} values in the range of 50−100 μM.

Structure−Activity Relationship of Lignans for the Inhibition of MMP-7. The effect of introducing one or two methylenedioxy rings into A and B rings of matairesinol on MMP-7 activity was examined (**Figure 2A**). Pluviatolide, which has a methylenedioxy ring in the A ring, showed a slightly higher inhibitory activity than matairesinol; its IC_{50} value was determined to be 260 μM. On the other hand, haplomyrfolin, which has a methylenedioxy ring in the B ring, inhibited more strongly with an IC_{50} value of 100 μM. These results indicate that introducing a methylenedioxy ring into an aromatic ring of matairesinol enhances its inhibitory effect, although the introduction into the A ring appears to be less effective for the inhibition than that into the B ring. Hinokinin has two methylenedioxy rings in both the A and the B rings and showed a higher inhibitory activity than those of matairesinol and pluviatolide but similar to that of haplomyrfolin.

The effect of introducing a methoxy group into the B ring of matairesinol on MMP-7 activity was also examined (**Figure 2B**). The B ring of 4-demethylyatein is a syringyl ring (which has 4-hydroxy and 3,5-dimethoxy groups), and the A ring has a methylenedioxy ring. 4-Demethylyatein inhibited MMP-7 activity with an IC_{50} > 260 μM. The inhibitory effect of 4-demethylyatein was almost the same as that of matairesinol, although it has a methylenedioxy ring in the A ring. Taking the steric hindrance and/or the hydrophobicity of methoxy groups into consideration, the effect of the methylenedioxy ring in the A ring might be counteracted by introducing a methoxy group into the B ring. To evaluate the effect of the syringyl ring precisely, 4-demethylyatein-d_6, in which six hydrogen atoms of two methoxy groups are substituted by deuterium, was employed. As a result, the isotope effect due to the direct interaction of two methoxy groups in the B ring with the enzyme was not observed. On the other hand, 5-demethylyatein-d_6, which has two isotopically labeled methoxy groups at 3- and 4-positions and a hydroxyl group at the 5-position, showed a slightly higher inhibitory activity (IC_{50} = 220 μM) than 4-demethylyatein and 4-demethylyatein-d_6, suggesting the importance of a hydroxyl group at the 5-position in the B ring for the inhibitory activity of lignans.

The inhibitory effects of thujaplicatin-d_3 and 5-hydroxypluviatolide on MMP-7 activity were examined in comparison with that of matairesinol (**Figure 2C**). Thujaplicatin-d_3, which has a hydroxyl group at the 5-position and an isotopically labeled methoxy group at the 3-position of the B ring, inhibited MMP-7 activity more strongly than matairesinol; its IC_{50} value was 80 μM. Considering the results obtained with 4-demethylyatein-d_6 and 5-demethylyatein-d_6, the deuterium-labeled methoxy group at the 5-position of the B ring in thujaplicatin-d_3 might not have any influence on the inhibition. It is suggested that the introduction of a hydroxyl group into the 5-position of the B ring, as well as that of methylenedioxy rings into the A and B rings, might enhance the inhibitory activity of lignans. The most potent inhibitor among the lignans examined was 5-hydroxypluviatolide, which has both the methylenedioxy ring in

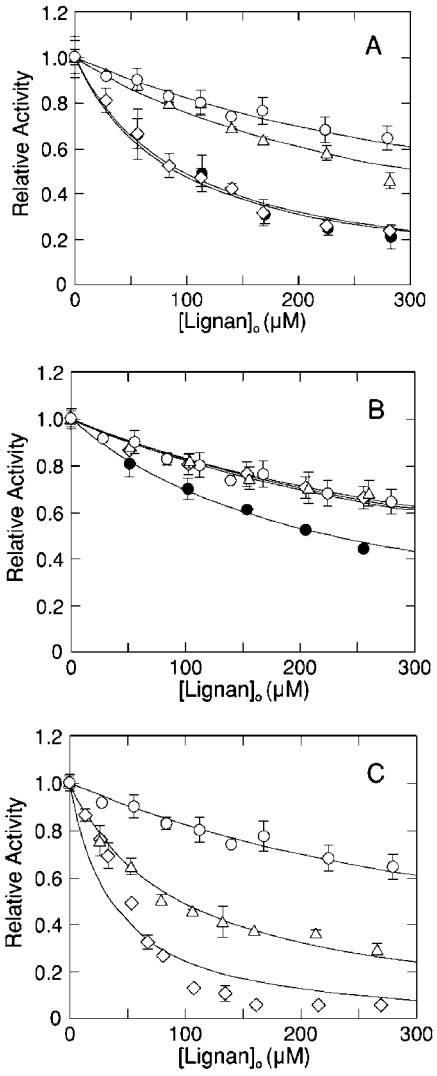

Figure 2. Inhibitory effects of lignans on the MMP-7-catalyzed hydrolysis of MOCAc-PLGL(Dpa)AR. Solid lines were drawn through data points on the basis of eq 1 by using the nonlinear least-squares method (*43*). (**A**) Inhibitory effect of lignans with methylenedioxy ring. Matairesinol, ○; pluviatolide, △; haplomyrfolin, ◇; and hinokinin, ●. The IC_{50} value of matairesinol in the presence of 1.4% DMSO was determined to be >280 μM, and those of pluviatolide, haplomyrifolin, and hinokinin were 260, 100, and 100 μM, respectively. (**B**) Inhibitory effect of lignans with the dimethoxy aromatic ring. Matairesinol, ○; 4-demethylyatein, △; 4-demethylyatein-d_6, ◇; and 5-demethylyatein-d_6, ●. The IC_{50} values of 4-demethylyatein and 4-demethylyatein-d_6 in the presence of 1.4% DMSO were >260 μM, and that of 5-demethylyatein-d_6 was 220 μM. (**C**) Inhibitory effect of lignans with hydroxyl group at the C5-position of the B ring. Matairesinol, ○; thujaplicatin-d_3, △; and 5-hydroxypluviatolide, ◇. The IC_{50} values of thujaplicatin-d_3 and 5-hydroxypluviatolide in the presence of 1.4% DMSO were 80 and 50 μM, respectively.

the A ring and the hydroxyl group at the 5-position of the B ring, and its IC_{50} value was 50 μM. The enzyme activity was almost completely inhibited on increasing the concentration of 5-hydroxypluviatolide to 160 μM. 5-Hydroxypluviatolide seems to inhibit MMP-7 activity according to the synergistic effect of the methylenedioxy ring in the A ring and the hydroxyl group at the 5-position of the B ring. These observations suggest that the combination of the substituents on the A and B rings could be important factors for determining the inhibitory potency of lignans. It is noted that 5-hydroxypluviatolide is a much more effective inhibitor than is 5-demethylyatein-d_6, although the structural difference between them is only a substitute group at the 4-position of the B ring except for deuterium substitution, suggesting that not only the hydroxyl group at the 5-position but also that at the 4-position of the B ring plays a role in the remarkable inhibition of MMP-7. One hypothesis is that the inhibitory activity of lignans might be enhanced by increasing the number of hydroxyl groups introduced into the B ring.

Effects of Precursor of Matairesinol and Related Compounds on MMP-7 Activity. Secoisolariciresinol, which is a dibenzylbutane lignan and lacks a lactone ring, is derived from two coniferyl alcohol molecules and converted into matairesinol in the biosynthetic pathway for lignans in many plants (*22*−*24*). We have focused on the relationship between the lignan biosynthesis and the inhibitory potency of lignans against MMP-7. Secoisolariciresinol had no inhibitory activity against MMP-7 in the concentration range up to 280 μM (data not shown). In addition, other closely related compounds to lignans, coniferyl alcohol and 1,2-(methylenedioxy)benzene, showed no inhibitory effects even at the concentration of 1.0 mM (data not shown). The structure of secoisolariciresinol is thought to be flexible owing to the possible rotation around the C8−C8′ bond, while that of matairesinol is fixed. A furofuran lignan, sesamin, which has two methylenedioxy rings, shows almost no inhibition against MMP-7 at concentrations up to 60 μM (unpublished data). These observations suggest that the rigid configuration of a lactone ring and the A and B aromatic rings, namely, the skeleton of dibenzylbutyrolactone lignan, is considered to be essential for the inhibition of MMP-7 and that the introduction of methylenedioxy ring(s) and hydroxyl group(s) contributes only in part to enhance the inhibitory activity of lignans.

Inhibitory Effect of 5-Hydroxypluviatolide on MMP-7 Activity. The inhibitory effect of 5-hydroxypluviatolide was precisely examined by HPLC to determine its inhibition behavior against MMP-7. In the HPLC analysis, 5.0% DMSO was added to the reaction mixture in order to dissolve enough substrate to separate k_{cat} and K_m. On the basis of the Michaelis−Menten equation, the k_{cat} and K_m values for the MMP-7-catalyzed hydrolysis of MOCAc-PLGL(Dpa)AR at 5.0% DMSO, pH 7.5 and 25 °C, in the absence of 5-hydroxypluviatolide were determined to be 5.9 ± 0.2 s and 60.1 ± 5.9 μM, respectively, and the corresponding values in the presence of 336 μM 5-hydroxypluviatolide were 5.9 ± 0.3 s and 119 ± 13 μM, respectively (**Figure 3A**). Upon addition of 336 μM 5-hydroxypluviatolide, the K_m value was doubled, while k_{cat} was unaltered. The plot of [S]/v vs [S] (Hanes−Woolf plot) gave two parallel lines (**Figure 3B**), indicating that the inhibition behavior of 5-hydroxypluviatolide is competitive. DMSO inhibits matrilyin activity competitively with the K_i value of 4.64 ± 0.28% at pH 7.5 and 25 °C (*41*). Assuming that 5-hydroxypluviatolide and DMSO are mutually exclusive as competitive inhibitors, the initial reaction rate, v, can be expressed as (*44*):

$$v = k_{cat}[E]_o[S]/\{K_m(1 + [L]/K_{iL} + [D]/K_{iD}) + [S]\} \quad (1)$$

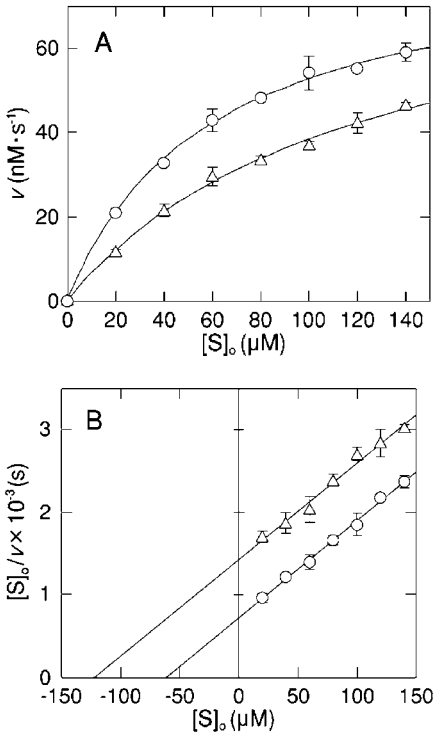

Figure 3. HPLC analysis of the inhibitory effect of 5-hydroxypluviatolide on the MMP-7-catalyzed hydrolysis of MOCAc-PLGL(Dpa)AR. 5-Hydroxypluviatolide concentrations were 0 (○) and 336 (△) μM. (**A**) Dependence of the reaction rate, v, of the hydrolysis of MOCAc-PLGL(Dpa)AR by MMP-7 on the substrate concentration, [S]. (**B**) Hanes–Woolf plot for the inhibition of MMP-7 activity by 5-hydroxypluviatolide.

where [L] and [D] are the initial concentrations of 5-hydroxypluviatolide and DMSO, respectively, and K_{iL} and K_{iD} are the inhibitor constants (K_i) of 5-hydroxypluviatolide and DMSO, respectively. Thus, the apparent K_m value (K_p) is expressed as:

$$K_p = K_m(1 + [L]/K_{iL} + [D]/K_{iD}) \quad (2)$$

On the basis of eq 2, the K_{iL} value for the inhibition of MMP-7 at pH 7.5 and 25 °C was determined to be 155 ± 10 μM from the intercepts of two lines on the x-axis in **Figure 3B**. On the other hand, the intrinsic K_m value, that is, the K_p value in the absence of inhibitors, was determined to be 29 ± 1 μM, which is consistent with the previously reported value (28 ± 3 μM) (*41*). The K_i value of 155 ± 10 μM is higher than the IC_{50} of 50 μM obtained by the fluorometric analysis in the presence of 1.4% DMSO (**Figure 2C**). In the case of competitive inhibition, K_i should be less than IC_{50} irrespective of the condition where the reaction is carried out. To account for this discrepancy, we examined fluorometrically the influence of DMSO on the inhibitory activity of 5-hydroxypluviatolide (**Figure 4**). As expected from the inhibition mechanism described above, the inhibitory activity of 5-hydroxypluviatolide was apparently suppressed at high concentrations of DMSO. The IC_{50} value increased from 50 to 110 μM with the increase in the DMSO concentration from 1.4 to 10.0%. A plot of IC_{50} vs $[DMSO]_0$ (%, v/v) gave a straight line, and the IC_{50} value at 0% DMSO was estimated to be 41 μM by extrapolating the IC_{50} values at various DMSO concentrations. The activity (k_{cat}/K_m) increased with increasing [DMSO] in the reaction mixture at high concentrations of 5-hydroxypluviatolide. The dielectric constant of DMSO is 45 (*41*, *45*), suggesting that DMSO decreases the hydrophobic interaction and increases the electrostatic interaction in the recognition between the MMP-7 and the lignan. It is

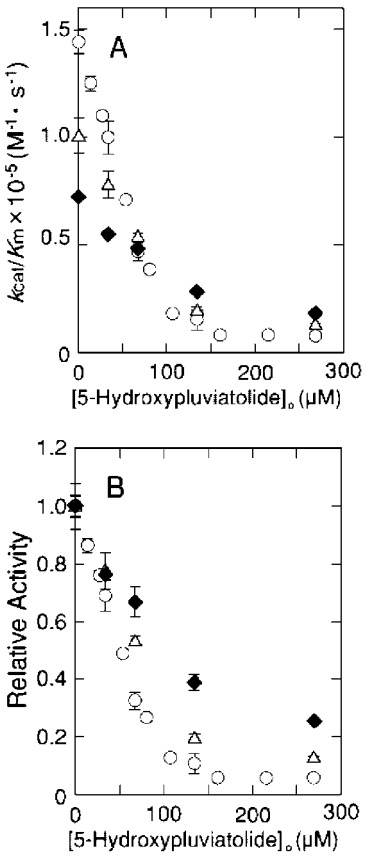

Figure 4. Effect of DMSO on the inhibitory effect of 5-hydroxypluviatolide on the MMP-7-catalyzed hydrolysis of MOCAC-PLGL(Dpa)AR. DMSO concentrations were 1.4 (○), 5.0 (△), and 10.0% (◆). (**A**) Inhibitory effect of 5-hydroxypluviatolide on MMP-7 activity (k_{cat}/K_m) at various DMSO concentrations. (**B**) Comparison of the inhibitory effect of 5-hydroxypluviatolide at various DMSO concentrations. The activities (k_{cat}/K_m) in the absence of 5-hydroxypluviatolide of 1.44×10^{-5} M^{-1} s^{-1} at 1.4% DMSO, 1.01×10^{-5} M^{-1} s^{-1} at 5% DMSO, and 0.73×10^{-5} M^{-1} s^{-1} at 10% DMSO were taken as relative activities of 1.0.

probable that DMSO decreases binding of 5-hydroxypluviatolide at the active site of MMP-7 not only by competing the active site with the lignan but also by altering the electrostatic and hydrophobic factors of the reaction solution. Accordingly, the peculiar phenomenon shown in **Figure 4A** suggests a possibility that if we could obtain the kinetic data in the absence of DMSO, the true K_i value for the inhibition of MMP-7 by 5-hydroxypluviatolide would be revealed to be much less than the value of 155 ± 10 μM determined in the presence of 5% DMSO. Considering the effect of DMSO on the inhibitory potency of lignans, hinokinin, which has two methylenedioxy groups, could be a much more potent inhibitor than that expected from the IC_{50} value of 100 μM determined at 11% DMSO, although its practical value as an inhibitor is thought to be low because of its sparing solubility. In this regard, the methylenedioxy ring may be unfavorable for enhancing the inhibitory activity.

Effects of Naturally Occurring Phenylpropane Derivatives on MMP-7 Activity. Phenylpropane derivatives such as lignan, flavonoid, and tannin are believed to be essential for biological protection in the plant kingdom, and their abundant biological activities to animal cells have been reported in recent years. In particular, green tea polyphenols, catechins, have attracted much attention due to their cancer preventive effects such as induction of apoptosis in cancer cells (*46*−*48*). As for the inhibitory potency against the members of the MMP family, we have examined the structure−activity relationship of catechins for the inhibition of MMP-7 precisely and demonstrated that the major constituent of green tea catechin, (−)-epigallocatechin gallate, inhibits MMP-7 activity noncompetitively with a K_i value of 1.65 ± 0.03 μM at pH 7.5 and 25 °C (*45*). In the present study, we demonstrate that lignans, matairesinol and its derivatives, competitively inhibit MMP-7 activity. Their inhibitory effects are not so strong (IC_{50} = 50 to >280 μM), but a series of inhibitry studies has provided us with insights into the structure−activity relationship of lignans for the inhibition of MMP-7 activity and valuable hints for designing potent inhibitors against MMP-7 for therapeutic use.

It is of great interest that the inhibition behavior of 5-hydroxypluviatolide, which was judged to be competitive, is completely different from that of (−)-epigallocatechin gallate. In the case of catechins, a galloyl group is likely to make a great contribution to the binding to the site other than the active site. There is currently no available data concerning how lignans such as 5-hydroxypluviatolide interact with the active site of MMP-7. Considering that the dibenzylbutyrolactone structure is essential for the inhibition, its lactone ring may play a role in the binding to the active site. The effects of methylenedioxy group and hydroxyl group at the 5-position of the B ring are thought to only reinforce the interaction probably by van der Waals interaction but not to directly interact with the active site by forming hydrogen bonds or hydrophobic interactions. It has been known that the S1′ subsite of MMP-7 shows a preference for hydrophobic residues, such as Leu, Phe, and Trp. In the case of the substrate used in this study, the Gly−Leu bond is cleaved by MMP-7, and here, the Leu residue is accommodated to the S1′ subsite. This is consistent with the competitive inhibition of the lignans against MMP-7 when we consider that the nonpolar character of the dibenzylbutyrolactone structure plays a critical role in the inhibition. On the other hand, the galloyl group of catechins seems to be essential in the inhibition against MMP-7. The galloyl group is much polar than the dibenzylbutyrolactone, and this character has catechins bind hydrophilic sites more favorably than the active site, which leads to noncompetitive inhibition.

To develop effective MMP-7 inhibitors for therapeutic use, the inhibitory potency of lignans should be enhanced more. It has been suggested that the hydroxyl groups introduced into the 4- and 5-positions of the B ring are of great benefit to enhancing the inhibitory activity, although the effect of hydroxyl groups on the A ring remains unclear. In humans and animals, enterodiol and enterolactone, which respectively belong to dibenzylbutane and dibenzylbutyrolactone lignans, are known as the major lignans (*31*). These two lignans possess phenolic hydroxyl groups at the 3- and 3′-positions of the aromatic rings, suggesting that enterolactone as a dibenzylbutyrolactone lignan might have potential for inhibiting MMP-7 in vivo. If more hydroxyl groups are added to its aromatic rings, the inhibitory potency could be enhanced. There is an interesting observation that the rate of breast cancer in women may be correlated with the presence of lignan precursors in their fiber-rich diets (*31*). Lignans are expected to play biologically significant roles in both plants and humans (*49*). In this study, the potential of lignans to inhibit MMP-7 has been suggested, and the knowledge of their structure−activity relationship might be of great use for developing selective inhibitors for MMPs. For the purpose of understanding inhibitory features of lignans more precisely, further investigations as to the effects of enantioselectivity of lignans and the other biologically significant lignans such as enterodiol and enterolactone are required.

ABBREVIATIONS USED

Brij-35, polyoxyethylene lauryl ether; DMSO, dimethyl sulfoxide; FRET, fluorescence resonance energy transfer; MOCAc-PLGL(Dpa)AR, (7-methoxycoumarin-4-yl)acetyl-L-Pro-L-Leu-Gly-L-Leu-[N^3-(2,4-dinitrophenyl)-L-2,3-diaminopropionyl]-L-Ala-L-Arg-NH_2; MOCAc-PLG, (7-methoxycoumarin-4-yl)acetyl-L-Pro-L-Leu-Gly.

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