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Identification and Characterization of Sesaminol Metabolites in the Liver

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Sesame seeds contain a number of antioxidants, such as sesamin, sesamolin, sesaminol, and sesaminol glucosides. Sesaminol triglucoside was reported to suppress oxidative stress *in vivo*, but little is known about the metabolism of this potentially important compound. Therefore, we have studied the metabolites of sesaminol formed in the rat liver S9 mix and excreted in the liver of rats ingesting sesaminol triglucoside for 24 h. Nuclear magnetic resonance (NMR) and mass spectrometry (MS) analyses revealed that rat liver S9 mix transformed the sesaminol into a catechol-type metabolite. On the basis of a previous study with sesame lignans by culturing the genus *Aspergillus*, sesaminol-6-catechol was identified as the major metabolite. Sesaminol was further converted into 5″-methylated sesaminol-6-catechol by catechol-*O*-methyltransferase. Moreover, we successfully detected these metabolites in the liver of rats ingesting the sesaminol triglucoside.

KEYWORDS: Sesame lignans; sesaminol; metabolism; rat liver microsome; antioxidant

INTRODUCTION

For a long time, sesame has been categorized as one of the traditional healthy foods in East Asia. It contains linoleic-acid-rich oil and small quantities of lignans consisting of sesamin, sesaminol, sesamolin, and sesaminol triglucoside (**Figure 1**) (*1*). These lignans have been reported to have their own unique biological effects, including an antihypertensive effect (*2*), hypocholesterolemic activity (*3*), and antioxidative activities (*4*). In contrast, the information about the bioavailability of sesame lignans is limited mainly because of their extensive metabolism. The metabolism of sesame lignans appears to include a reduction reaction (*5*), but there is little systematic studies concerning the metabolites in humans. Nakai et al. (*6*) and Liu et al. (*7*) indicated that sesamin undergoes cleavage of methylenedioxyphenyl (MDP) groups to catechol or the methoxy catechol *in vitro* and in rats, respectively. Sesamin, having no phenolic hydroxyl group and little radical scavenging activity, prevents liver damage caused by alcohol or carbon tetrachloride (*8*), 7,12-dimethylbenz-[α]anthracene-induced rat mammary carcinogenesis (*9*), and hypertension (*2*, *10*), suggesting that sesamin metabolites might be at least in part involved in these effects. Recently, it has been reported that the incubation of sesaminol and sesamin with *Aspergillus* species resulted in the production of sesaminol-6-catechol and sesamin-6-catechol, respectively, both of which have antioxidant activities stronger than the parent compounds (*11*). Moreover, Kang et al. (*12*) have reported that sesaminol glucosides, which are also very weak antioxidant compounds present in defatted sesame flour, can decrease the susceptibility to oxidantive stress in hypercholesterolemia rabbits. Therefore, the bioavailability and metabolism of sesaminol glucosides need to be determined to clarify the function of the absorbed sesame lignans *in vivo*.

In this study, we investigated the *in vitro* metabolism of sesaminol, using the S9 fraction of the rat liver and identified sesaminol-6-catechol as the major metabolite. We also demonstrated that catechol-*O*-methyltransferase (COMT) can catalyze the conversion of sesaminol-6-catechol into the 5″-methylated derivative *in vitro*. This is the first report showing the successful detection of these metabolites in the liver of rats ingesting sesaminol triglucoside.

MATERIALS AND METHODS

Chemicals. Sesaminol (a mixture of sesaminol and 6-episesaminol) and sesaminol triglucoside (sesaminol-2′-*O*-*β*-D-glucopyranosyl-(1→2)-[*β*-D-glucopyranosyl-(1→6)]-*β*-D-glucopyranoside) were kindly supplied by Takemoto Oil and Fat Co., Ltd. (Aichi, Japan). The rat S9 mix was purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). Cofactor I consisted of $MgCl_2$ (8 μmol), KCl (33 μmol), D-glucose-6-phosphate (5 μmol), NADPH (4 μmol), NADH (4 μmol), Na_2HPO_4 (100 μmol), and NaH_2PO_4 (100 μmol), all of which were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other chemicals used were of the highest purity commercially available.

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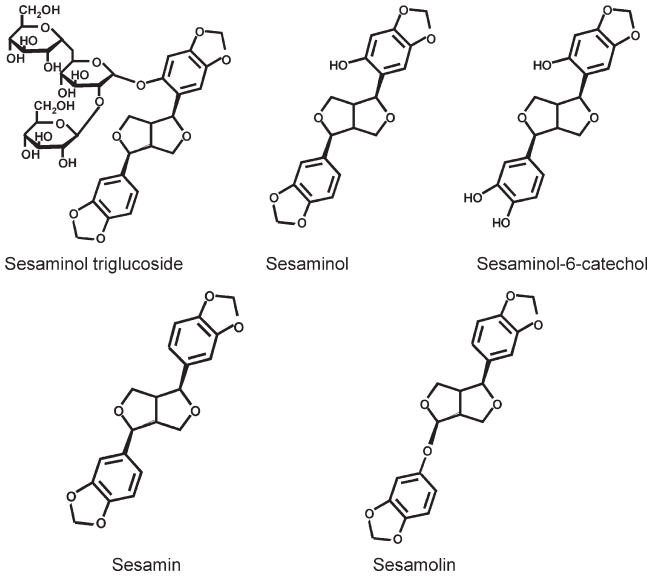


Figure 1. Chemical structures of (A) sesaminol triglucoside, (B) sesaminol, (C) sesaminol-6-catechol, (D) sesamin, and (E) sesamolin.

Enzymatic Reaction *in Vitro*. The incubation mixture contained the pooled rat S9 fraction (approximately 20 mg of protein) and 100 mM sesaminol [dissolved in dimethyl sulfoxide (DMSO), final concentration of 0.1%] in 9 mL of cofactor I, as desctibed above. After incubation at 37 °C for 24 h, the reaction was terminated by adding 10 mL of ethyl acetate (2 times). The control was incubated in the absence of sesaminol, analyzed by high-performance liquid chromatography (HPLC), and then characterized by liquid chromatography−mass spectrometry (LC−MS) (see below). The combined organic phase was evaporated to dryness and stored at −80 °C. Compound A for the HPLC analysis was fractionated from the sesaminol metabolites by preparative HPLC using a Develosil C30-UG-5 column (20 × 250 mm inner diameter, Nomura, Chemical Co., Ltd., Aichi, Japan) and UV detection at 300 nm. The column was eluted with a linear gradient from solvent A (water) to solvent B (methanol) at the flow rate of 7.0 mL/min in 45 min. The gradient program was as follows: initial (60% A), 40 min (20% A), and 45 min (0% A) (retention time of 25 min).

Methylation by the Rat Liver S9 Fraction. The methylation of sesaminol was examined using the rat S9 mix. The incubation mixture (final volume of 50 mL) consisted of cofactor I, 2000 units of COMT, 1 mM dithiothreitol (DTT), 1 mM $MgCl_2$, 2 mM *S*-adenosyl-L-methionine (SAM), approximately 20 mg/mL rat S9 fraction protein, and 100 mM sesaminol (dissolved in DMSO, final concentration of 0.1%). The incubation was conducted at 37 °C for 24 h. The reaction was quenched by extracting 2 times with 100 mL of ethyl acetate. The organic extract was combined, dried *in vacuo*, and stored at −80 °C. The sample was reconstituted with methanol assisted by centrifugation, and the supernatant was injected into the HPLC detection system. Compounds B1 and B2 were then fractionated by HPLC using a Develosil C30-UG-5 column and UV detection at 300 nm with a mobile phase of water/methanol (40:60, v/v) at the flow rate of 7.0 mL/min (retention times of 22 and 26 min). The analysis was carried out using LC−MS (see below) and nuclear magnetic resonance (NMR). The ^{1}H and ^{13}C NMR spectra were obtained using a Bruker ARX 400 instrument (400 MHz for ^{1}H and 100 MHz for ^{13}C) in dimethyl sulfoxide-d_6.

Animals and Diets. The female Wistar rats, obtained from Chubu Kagaku Shizai Co., Ltd. (Nagoya, Japan), were 6 weeks old (weighing 120−160 g). The animals were housed in an air-conditioned room under 12 h dark/12 h light cycles. After prefeeding with the control diet (CLEA Rodent Diet CE-2) for a week, the rats were randomly divided into two groups and fed the diet of each group [group 1, water; group 2, sesaminol triglucoside (sesame seed lees-derived) 0.9 mmol/kg with water] for 24 h. All of the animal protocols were approved by the Animal Experiment Committee of the Graduate School of Bioagricultural Sciences, Nagoya University. The samples were stored at −80 °C until used for the measurements of the sesaminol metabolites.

A portion of a liver (0.5 g) was homogenized with ethyl acetate (2 mL) containing 50 μM butylated hydroxytoluene (BHT) and 50 mM ethylenediaminetetraacetic acid (EDTA). After the centrifugation (3500 rpm) for 10 min at room temperature, the supernatants were evaporated to dryness and reconstituted in 100 μL of methanol. After centrifugation (15 000 rpm) for 5 min at 4 °C, the supernatants were used for the HPLC−electrochemical detection (ECD) analysis. The rats tissue extracts were analyzed by HPLC−ECD. The extracts were injected into a HPLC column (4.6 × 250 mm inner diameter, Develosil C30-UG-5 column, Nomura). The mobile phase was composed of water containing 50 mmol/L citric acid and 50 mmol/L lithium acetate (solvent A) and methanol containing 50 mmol/L citric acid and 50 mmol/L lithium acetate (solvent B). The gradient program was as follows: initial (60% A), 20 min (50% A), 40 min (43% A), 55 min (20% A), and 60 min (0% A) at the flow rate of 1.0 mL/min. The elution was monitored by a coulometric electrochemical detector (Coularray system, MC medical, Tokyo, Japan), with the working potentials set at +0, 100, 200, 450, and 500 mV.

HPLC−MS Analysis. The HPLC−MS analysis was carried out using a PLATFORM II (Micromass, Manchester, U.K.). Separation was performed using a Develosil ODS-HG column (250 × 4.6 mm inner diameter, Develosil ODS-HG-5 column). Solvent A was 5% acetic acid in 40% methanol at the flow rate of 1 mL/min. Operation was in MS mode, and electrospray ionization (ESI) was used. During the analysis, the ESI parameters were set as follows: the cone voltage was set at −35 V; the capillary voltage was set at −2.8 kV; and the source temperature was set at 70 °C. The select ion mode (SIM) was used for the detection of sesaminol (370) and sesaminol metabolites (358 and 372).

^{1}H NMR and ^{13}C NMR Analysis. Analysis of the sesaminol metabolites isolated from the rat S9 microsomal incubation was performed in a DMSO-d_6 solution using a Bruker APX 400.

The structure of the sesaminol metabolite collected by HPLC was assigned by NMR spectroscopy, incorporating ^{1}H NMR and ^{13}C NMR, heteronuclear multiple-bond correlation (HMBC), and heteronuclear multiple-quantum correlation (HSQC). The NMR experiments were performed by a Bruker ARX 400. The measurements were taken at 400 MHz (^{1}H) and 100 MHz (^{13}C) frequencies.

Assay for Radical Scavenging Activity. The sample was measured for antioxidative activity using the DPPH radical scavenging system by HPLC analysis according to the reported method (*13*). Sesamolin, sesamin, sesaminol, sesaminol triglucoside, and the isolated lignans were dissolved in DMSO and assayed at a final concentration of 150 μM. A 100 μL portion of the sample solution was mixed with 2 mL of 20 mg/mL DPPH in ethanol and 1 mL of a 100 mM Tris-HCl buffer (pH7.4). The mixture was vigorously shaken, left to stand for 40 min at room temperature in the dark, and then subjected to HPLC analysis. The analyses were performed using a TSKgel Octyl-80Ts column (4.6 × 150 mm, Tosoh, Ltd., Tokyo) at ambient temperature and a spectrophotometric detector (517 nm) with a mobile phase of methanol/water (70:30, v/v) at the flow rate of 1 mL/min. The activity was evaluated from the difference in the decreasing peak area of the DPPH radical detected between the blank and the sample. The values were reported as the mean of the three measurements.

RESULTS AND DISCUSSION

Identification of Sesaminol Metabolites Using Rat Liver S9 Mix. To identify the metabolites of sesaminol *in vivo*, we examined the metabolic conversion of sesaminol *in vitro* using the rat liver S9 mix. The HPLC chromatogram of the sample, obtained from a 3 h incubation of sesaminol with the rat liver S9 mix, revealed the formation of a major metabolite (peak A) with the retention time of 18 min (**Figure 2**). In the control group incubated without the NADPH generating system, we detected no significant peak, except for the parent compound (data not shown). The major metabolite was then isolated and purified using preparative HPLC. The ^{1}H NMR data for the metabolite is shown in **Table 1**. Assignment of the ^{1}H NMR has been achieved on the basis of the chemical-shift value and the splitting pattern. The data for the metabolite was quite similar to those of sesaminol, except for the methylene signal intensity. The proton signal of the methylendioxy

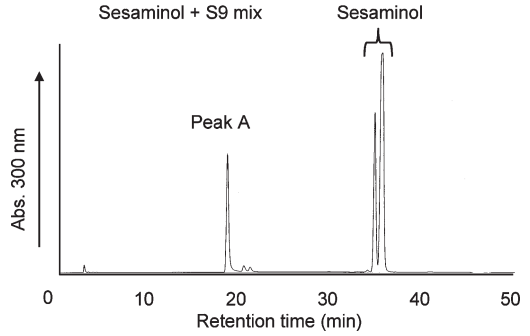
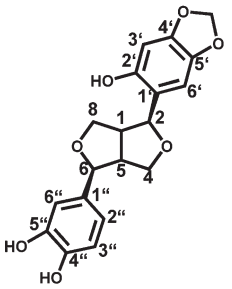


Figure 2. HPLC analysis of the product of the 3 h incubation of sesaminol with rat S9 mix. A chromatogram is shown using a photodiode array (PDA) detector at 300 nm. For identification of peak A, see **Table 1**.

Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data of Peak A Formed in the Incubation Mixture of S9 Mix with Sesaminol and Its Chemical Structure



position	δH (*J*,Hz)	δC
1	2.88 m	53.6
2	4.86 d (5)	80.9
4a	3.83 dd (3.8, 9.0)	70.9
4e	4.14 dd (6.4, 8.8)	
5	2.88 m	53.6
6	4.51 d (4.8)	84.5
8a	3.71 dd (4.2, 9.0)	71.5
8e	4.07 dd (6.8, 8.8)	
1'		120.2
2'		148.7
3'	6.43 s	97.5
4'		146.1
5'		139.6
6'	6.75 s	105.4
1"		132.3
2"	6.58 dd (1.8, 8.2)	116.9
3"	6.67 d (8.0)	115.2
4"		144.5
5"		145.0
6"	6.72 s	113.5
O-CH_2-O	5.87 d (10)	100.4
2'-OH	9.23 brs	
4"-OH	8.78 brs	
5"-OH	8.28 brs	

moiety in the metabolite was 8.8 ppm with a signal intensity of two protons, whereas that in sesaminol was 5.5 ppm with a signal intensity of four protons. As shown in **Figure 3**, the LC−MS analysis of the metabolite gave $[M - H]^-$ ions at *m*/*z* 357, suggesting that the metabolite has the same molecular weight as sesaminol-6-catechol, which have been isolated from the fermentation products of the sesaminol glucosides (*11*). Kang et al. reported that sesaminol aglycone was detected in abundant quantities in the serum and liver of rabbits fed the sesaminol glucosides. This indicates that sesaminol is the principal metabolite of sesaminol glucosides, because the dietary sesaminol glucosides are gradually hydrolyzed by β-glucosidase in the bacterial flora (*14*). In this study, a metabolic process that converts glucosides into an aglycone sesaminol may be necessary for further metabolism by the liver microsome enzymes. *In vitro*, sesaminol-6-catechol has been reported to show a strong antioxidative activity (*11*). Flavonoids, such as catechin and quercetin, show general antioxidative effects, because they have a catechol moiety in their structure (*15*). Therefore, the newly formed catechol structure of sesaminol-6-catechol is essential for the high antioxidative activity, such as a radical scavenging activity (*11*).

Methylation by the Rat Liver S9 Fraction. COMT is well-known to catalyze the *O*-methylation of a wide array of catechol-containing substrates, including exogeous flavonoids, using *S*-adenosyl-L-methionine as the methyl donor. We next examined the possibility that sesaminol-6-catechol could be

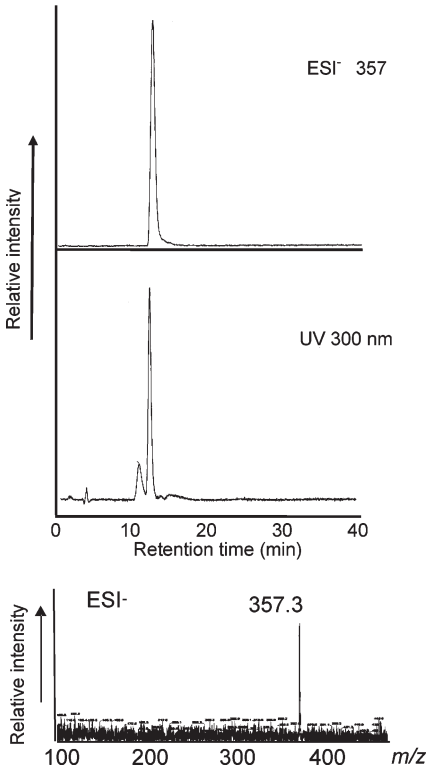


Figure 3. Mass spectra of peak A at *m*/*z* 356 identified in rat S9 mix.

further metabolized into the methylated ones by COMT. **Figure 4A** shows the HPLC chromatogram of the incubation of the rat liver microsome with sesaminol-6-catechol and the cofactor [phosphate buffer (pH 7.4), COMT, DTT, and SAM, as described in the Materials and Methods]. The HPLC chromatogram showed the formation of two major metabolites (peaks B1 and B2) with retention times of 22 and 26 min, respectively. The LC−MS analysis gave $[M - H]^-$ ions at *m*/*z* 371 for both peaks, clearly suggesting that the metabolites are the methylated derivatives of sesaminol-6-catechol. The NMR spectral data of these metabolites (peaks B1 and B2) are presented in **Table 2** and **Figure 5A**. Peak B1 of the ^{1}H NMR spectrum (**Figure 5A**) from these metabolites indicated that the methoxyl group (MeO) was assigned to 3.7 ppm (5″-MeO), and the other proton signals were almost identical to those of sesaminol-6-catechol. The signals in the ^{1}H and ^{13} C NMR spectra were assigned from the HMBC data (**Figure 5B**). The methoxyl proton signal at $^{\delta}H$ 3.76(s) was found to be correlated with C-5″ ($^{\delta}C$, 147.4), indicating that the methoxyl group was substituted at C-5″. Taken together with the LC−MS data (**Figure 4B**), it is concluded that its molecular formula was determined to be 6-(4″-hydroxy-5″- methoxyphenyl)-2-[2″-hydroxy-4′,5′-(methylenedioxy)phenyl-3,7-dioxabicyclo[3,3,0]-octane (5″-methylated sesaminol-6-catechol) . As for the ^{1}H and ^{13}C NMR analyses of peak B2, the chemical shifts of the proton and carbon signals were quite similar to those of the 5″-methylated metabolite, 5″-methylated sesaminol-6-catechol. Nevertheless, in the HMBC analysis, the correlation of the methoxyl proton signal in peak B2 was quite different from that of peak B1. We observed the HMBC correlations among the methoxyl group ($^{\delta}H$, 3.74), 2″ proton (dd, 6.72), and 4″ carbon ($^{\delta}C$, 147.4), suggesting that the methoxyl group was located at C-4″. The LC−MS analysis further suggested that those two compounds have similar features in their mass spectra (data not shown). We finally identified peak B2 as 4″-methylated sesaminol-6-catechol. Computer modeling showed that the energetically most favorable binding orientation of a catecholamine in the

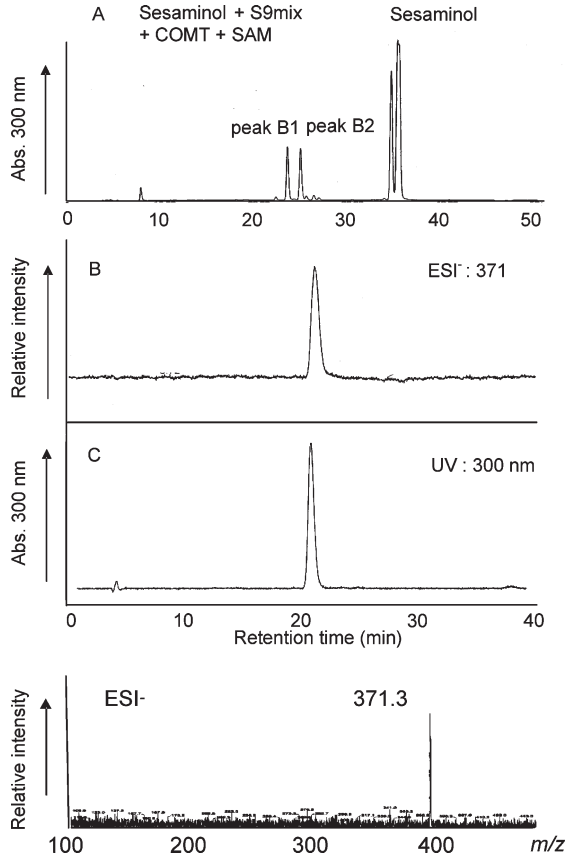
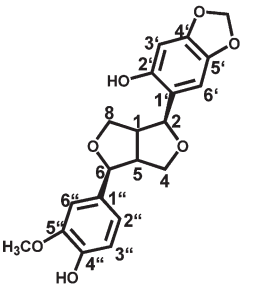


Figure 4. HPLC chromatogram of the incubation of the rat S9 mix, COMT, and SAM with sesaminol (mixture). (A) UV absorption at 300 nm. (B) LC−MS analysis of peak B1. The trace is an extracted ion profile [*m*/*z* 371 for methylated sesaminol-6-catechol] of a single linear scan. (C) Expanded section of the chromatogram showing the production of sesaminol metabolites from sesaminol (mixture). (D) Negative-ion ESI mass spectra of peak B1.

active site of COMT led to methylation of the hydroxyl group at the *meta* position. In the case of quercetin, the 3′-hydroxyl group is situated at the *meta* position with respect to the main part of the molecule (*16*). In this study, we suggested that the methylation of the 5″ position is more favorable than that of the 4″ position for the methylation of sesaminol-6-catechol.

Identification of Sesaminol Metabolites in Rat Liver. To demonstrate the physiological significance of sesaminol metabolites identified from the *in vitro* study, the metabolism of sesaminol was examined using the liver of rats fed the sesaminol glucosides. Representative HPLC−ECD chromatograms before and after the intake of the sesaminol triglucoside are shown in **Figure 6**. Besides sesaminol, significant amounts of sesaminol-6-catechol and 5″-methylated sesaminol-6-catechol (the three major rat liver S9 mix metabolites) were detected on the basis of their retention time and UV spectra of the standard samples. Kang et al. showed that dietary sesaminol glucosides could not be detected in the serum or liver, because sesaminol triglucoside is highly water-soluble and gradually hydrolyzed by β-glucosidase in the bacterial flora (*12*). The present study is consistent with the previous report showing that sesaminol is the principal metabolite of the sesaminol triglucoside (*12*). We concluded that the rat liver can metabolize sesaminol into not only sesaminol-6-catechol but also 5″-methylated sesaminol-6-catechol. However, we detected 5″-methylated sesaminol-6-catechol, whereas 4″-methylated sesaminol-6-catechol could not be in **Figure 6**. Recently, Jan et al. demonstrated that sesaminol triglucoside could be converted to enterolactone and enterodiol by rat intestinal microflora (*17*).

Table 2. ^{1}H and ^{13}C NMR Spectroscopic Data of Peak B1 Formed in the Incubation Mixture of S9 Mix, COMT, and SAM with Sesaminol and Its Chemical Structure



position	δH (*J*,Hz)	δC
1	2.93 m	53.5
2	4.87 d (5.2)	80.8
4a	3.84 dd (4.8, 9.2)	70.8
4e	4.12 m	
5	2.93 m	53.5
6	4.57 d (5.6)	84.6
8a	3.73 dd (4.8, 9.2)	71.6
8e	4.12 m	
1′		120.2
2′		148.7
3′	6.43 s	97.4
4′		146.1
5′		139.6
6′	6.76 s	105.4
1″		132.1
2″	6.70 m	118.5
3″	6.73 m	115.0
4″		145.8
5″		147.4
6″	6.87 s	110.3
O-CH_2-O	5.88 d (8)	100.4
2′-OH	9.29 brs	
4″-OH	8.90 brs	
OCH_3	3.76 s	55.5

On the other hand, sesamol, a phenolic compound having the partial structure of sesaminol, was partially metabolized into the catechol and 5-methylated catechol, possibly through the CYP-depended metabolism, while the glucuronide is the predominate form in the urine (*18*). Taken together, the ingested sesaminol might, at least in part, be demethylenated and then converted into 5″-methylated sesaminol-6-catechol by COMT and/or to the further conjugated form by the phase II metabolism in the liver.

Radical Scavenging Activity of the Sesame Lignans. It has been reported that sesame lignans, such as sesamin, sesamolin, and sesaminol glucosides, exhibited *in vivo* antioxidant activities when orally administered to animals (*12*, *19*−*22*). On the other hand, Suja et al. reported that sesamin and sesaminol glucoside showed no radical scavenging activity *in vitro* in contrast to that of sesaminol and sesamolin (*23*). We next examined the antioxidant potentials of the sesaminol triglucoside and the three major *in vivo* metabolites using the assay for the DPPH radical scavenging activity (**Figure 7**). In the assay, sesaminol, sesaminol-6-catechol, and 5″-methylated sesaminol-6-catechol showed significant radical scavenging activities, whereas the sesaminol triglucoside did not. The presence of adjacent hydroxyl groups on the flavonoids has been reported to play an important role in the potent antioxidative activity, because of their capacity for radical-scavenging or chelating metal ions (*24*). Compounds sesaminol, sesaminol-6-catechol, and 5″-methylated sesaminol-6-catechol, having hydroxyl groups, might be necessary for the structure to contain more than its group moieties for the scavenging effect against the hydroxyl radical or free radicals. In particular, sesaminol-6-catechol showed the most potent activity because of the unique *ortho*-dihydroxyl group.

In conclusion, this study identified the three related compounds, sesaminol, sesaminol-6-catechol, and 5″-methylated sesaminol-6-catechol, as the major metabolites *in vitro*. These metabolites were also identified, for the first time, as *in vivo* metabolites, which might be generated by the action of hepatic cytochrome P450 enzymes or COMT. Furthermore, these metabolites have significant antioxidant activities, strongly suggesting their physiological role in the *in vivo* antioxidant activity of the orally administered sesaminol glucosides. A recent report demonstrated that sesaminol triglucoside could be converted to

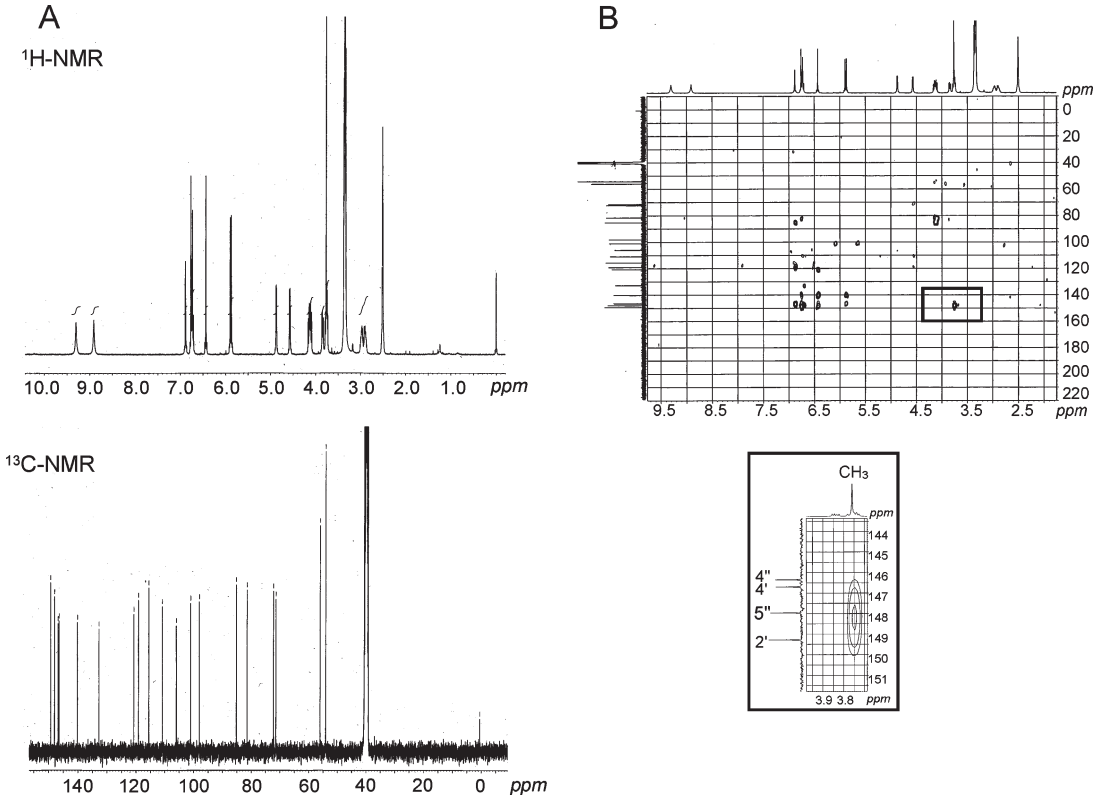


Figure 5. (A) ^{1}H NMR spectra (400 MHz) and ^{13}C NMR spectra (100 MHz). (B) HMBC spectra of peak B1.

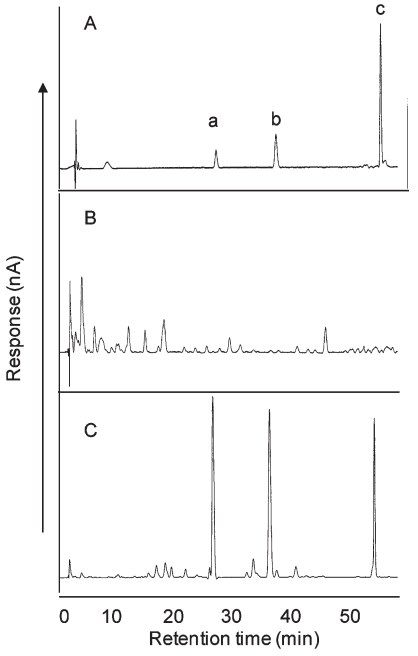


Figure 6. (A) HPLC−ECD profile of standard (a) sesaminol-6-catechol, (b) 5′′-methylated sesaminol-6-catechol, and (c) sesaminol. (B) Extract of liver from a control rat. (C) Extract of liver from rats administered sesaminol triglucoside.

enterolactone and enterodiol by rat intestinal microflora (*17*). Thus, it can be assumed that sesaminol triglucoside may be metabolized through the combination of several metabolic processes in the intestinal microbiota and liver. The present results encourage further investigation to identify the conjugated forms of the methylated metabolites and to determine the concentration−time profiles *in vivo* using the metabolite library of sesaminol triglucoside.

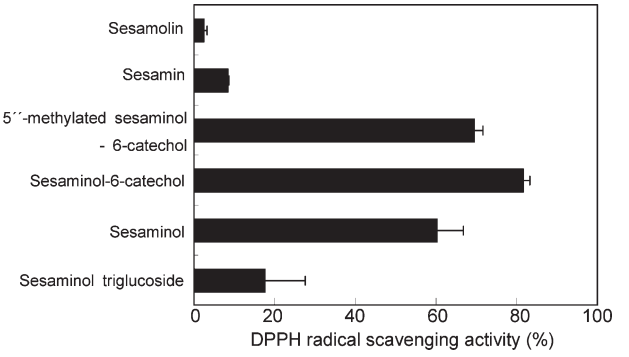


Figure 7. Antioxidantive activity of six sesame lignan compounds. The values are represented as the means ± standard deviation (SD) (N = 3).

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LITERATURE CITED

(1) Katsuzaki, H.; Kawakishi, S.; Osawa, T. Sesaminol glucosides in sesame seeds. *Phaytochemistry* **1994**, *35*, 773–776.

(2) Matsumura, Y.; Kita, S.; Morimoto, S.; Akimoto, K.; Furuya, M.; Oka, N.; Tanaka, T. Antihypertensive effect of sesamin. I. Protection against deoxycorticosterone acetate−salt-induced hypertension and cardiovascular hypertrophy. *Biol. Pharm. Bull.* **1995**, *18*, 1016–1019.

(3) Hirose, N.; Inoue, T.; Nishihara, K.; Sugano, M.; Akimoto, K.; Shimizu, S.; Yamada, H. Inhibition of cholesterol absorption and synthesis in rats by sesamin. *J. Lipid Res.* **1991**, *32*, 629–638.

(4) Fukuda, Y.; Nagata, M.; Osawa, T.; Namiki, M. Chemical aspects of the antioxidative activity of roasted sesame seed oil, and the effect of using the oil for frying. *Agric. Biol. Chem.* **1986**, *50*, 857–862.

(5) Jan, K. C.; Ho, C. T.; Hawang, L. S. Elimination and metabolism of sesamol, a bioactive compound in sesame oil, in rats. *Mol. Nutr. Food Res.* **2009**, *53*, 36–43.

(6) Nakai, M.; Harada, M.; Nakahara, K.; Akimoto, K.; Shibata, H.; Miki, W.; Kiso, Y. Novel antioxidantive metabolites in rat liver with ingested sesamin. *J. Agric. Food Chem.* **2003**, *51*, 1666–1670.

(7) Liu, Z.; Saarinen, N.; Thompson, L. Sesamin is one of the major precursors of mammalian lignans in sesame seed (*Sesamum indicum*) as observed *in vitro* and in rats. *J. Nutr.* **2006**, *136*, 906–912.

(8) Akimono, K.; Kitagawa, Y.; Akamatsu, T.; Hirose, N.; Sugano, M.; Shimizu, S.; Yamada, H. Protective effects of sesamin against liver damage caused by alcohol or carbon tetrachloride in rodents. *Ann. Nutr. Metab.* **1993**, *37*, 218–224.

(9) Hirose, N.; Doi, F.; Ueki, T.; Akazawa, K.; Chijiiwa, K.; Sugano, M.; Akimoto, K.; Shimizu, S.; Yamada, H. Suppressive effect of sesamin against 7,12-dimethylbenz[α]anthracene induced rat mammary carcinogenesis. *Anticancer Res.* **1992**, *12*, 1259–1266.

(10) Matsumura, Y.; Kita, S.; Tanida, Y.; Taguchi, Y.; Morimoto, S.; Akimoto, K.; Tanaka, T. Antihypertensive effect of sesamin. III. Protection against development and maintence of hypertension in stroke-prone spontaneously hypertensive rats. *Biol. Pharm. Bull.* **1998**, *21*, 469–473.

(11) Miyake, Y.; Fukumoto, S.; Okada, M.; Sakaida, K.; Nakamura, Y.; Osawa, T. Antioxidantive catechol lignans converted from sesamin and sesaminol triglucoside by culturing with *Aspergillus*. *J. Agric. Food Chem.* **2005**, *53*, 22–27.

(12) Kang, M.; Kawai, Y.; Naito, M.; Osawa, T. Dietary defatted sesame flour decreases susceptibility to oxidative stress in hypercholesterolemic rabbits. *J. Nutr.* **1999**, *129*, 1885–1890.

(13) Yamaguchi, T.; Takamura, H.; Matoba, T.; Terao, J. HPLC method for evaluation of the free radical-scavenging activity of foods by using 1,1-diphenyl-2-picrylhydrazyl. *Biosci., Biotechnol., Biochem.* **1998**, *62*, 1201–1204.

(14) Osawa, T.; Yoshida, A.; Kawakishi, S.; Yamashita, K.; Ochi, H. Protective role of dietary antioxidants in oxidative stress. In *Oxidative Stress and Aging*; Cutler, R. G., Packer, L., Eds.; Molecular and Cell Biology Updates: Basel, Switzerland, 1995; pp 367−377.

(15) Bors, W.; Heller, W.; Michel, C.; Saran, M. Flavonoids as antioxidants: Determination of radical-scavenging efficiencies. *Methods Enzymol.* **1990**, *186*, 343–355.

(16) van der Woude, H.; Boersma, M. G.; Vervoort, J.; Rietjens, I. M. Identification of 14 quercetin phase II mono- and mixed conjugates and their formation by rat and human phase II *in vitro* model systems. *Chem. Res. Toxicol.* **2004**, *17*, 1520–1530.

(17) Jan, K. C.; Hwang, L. S.; Ho, C. T. Biotransformation of sesaminol triglucoside to mammalian lignans by intestinal microbiota. *J. Agric. Food Chem.* **2009**, *57*, 6101–6106.

(18) Jan, K. C.; Ho, C. T.; Hwang, L. S. Elimination and metabolism of sesamol, a bioactive compound in sesame oil, in rats. *Mol. Nutr. Food Res.* **2009**, *53* (supplement 1), S36–S43.

(19) Nakano, D.; Itoh, C.; Ishii, F.; Kawagishi, H.; Takaoka, M.; Kiso, Y.; Tsuruoka, N.; Tanaka, T.; Matsumura, Y. Effect of sesamin on aortic oxidative stress and endothelial dysfunction in deoxycorticosterone acetate−salt hypertensive rats. *Biol. Pharm. Bull.* **2003**, *26*, 1701–1705.

(20) Ikeda, T.; Nishijima, Y.; Shibata, H.; Kiso, Y.; Ohnuki, K.; Fushiki, T.; Moritani, T. Protective effect of sesamin administration on exercise-induced lipid peroxidation. *Int. J. Sports Med.* **2003**, *24*, 530–534.

(21) Kang, M.; Naito, M.; Sakai, K.; Uchida, K.; Osawa, T. Mode of action of sesame lignans in protecting low-density lipoprotein of vascular superoxidative damage *in vitro*. *Life Sci.* **2000**, *55*, 161–171.

(22) Nakano, D.; Itoh, C.; Takaoka, M.; Kiso, Y.; Tanaka, T.; Matsumura, Y. Antihypertensive effect of sesamin. IV. Inhibition of vascular superoxide production by sesamin. *Biol. Pharm. Bull.* **2002**, *25*, 1247–1249.

(23) Suja, K.; Jayalekshmy, A.; Arumughan, C. Free radical scavenging behavior of antioxidative compounds of sesame (*Sesamum indicum* L.) in DPPH• system. *J. Agric. Food Chem.* **2004**, *52*, 912–915.

(24) Miyake, Y.; Yamamono, K.; Morimitsu, Y.; Osawa, T. Isolation of *C*-glucosylflavone from lemon peel and antioxidative activity of flavonoid compounds in lemon fruit. *J. Agric. Food Chem.* **1997**, *45*, 4619–4623.

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