Identification of the Metabolites of Episesamin in Rat Bile and Human Liver Microsomes

Namino Tomimori,* Masaaki Nakai, Yoshiko Ono, Yoshinori Kitagawa, Yoshinobu Kiso, and Hiroshi Shibata

Institute for Health Care Science, Suntory Wellness Ltd.; 1–1–1 Wakayamadai, Shimamoto-cho, Mishima-gun, Osaka 618–8503, Japan. Received December 18, 2011; accepted February 5, 2012; published online February 15, 2012

Episesamin is an isomer of sesamin, resulting from the refining process of non-roasted sesame seed oil. Episesamin has two methylendioxyphenyl groups on *exo* and *endo* faces of the bicyclic skeleton. The side methylendioxyphenyl group was metabolized by cytochrome-P450. Seven metabolites of episesamin were found in rat bile after treatment with glucuronidase/arylsulfatase and were identified using NMR and MS. The seven metabolites were (7*α*,7′*β*,8*α*,8′*α*)-3,4-dihydroxy-3′,4′-methylenedioxy-7,9′:7′,9-diepoxylignane (EC-1-1), (7*α*,7′*β*,8*α*,8′*α*)-3,4-methylenedioxy-3′,4′-dihydroxy-7,9′:7′,9-diepoxylignane (EC-1-2) and (7*α*,7′*β*,8*α*,8′*α*)-3,4:3′,4′-bis(dihydroxy)-7,9′:7′,9-diepoxylignane (EC-2), (7*α*,7′*β*,8*α*,8′*α*)-3-methoxy-4-hydroxy-3′,4′-methylenedioxy-7,9′:7′,9-diepoxylignane (EC-1m-1), (7*α*,7′*β*,8*α*,8′*α*)-3,4-methylenedioxy-3′-methoxy-4′-hydroxy-7,9′:7′,9-diepoxylignane (EC-1m-2), (7*α*,7′*β*,8*α*,8′*α*)-3-methoxy-4-hydroxy-3′,4′-dihydroxy-7,9′:7′,9-diepoxylignane (EC-2m-1) and (7*α*,7′*β*,8*α*,8′*α*)-3,4-dihydroxy-3′-methoxy-4′-hydroxy-7,9′:7′,9-diepoxylignane (EC-2m-2). EC-1-1, EC-1-2 and EC-2 were also identified as metabolites of episesamin in human liver microsomes. These results suggested that similar metabolic pathways of episesamin could be proposed in rats and humans.

Key words episesamin; metabolite; cytochrome P450

Sesamin is a major lignan in sesame seeds and oil, and is partially epimerized to episesamin during the refining process of non-roasted sesame seed oil.[1)] Epimerization to episesamin was also occurred under acid catalyzed conditions.[2)]

The mixture of sesamin and episesamin could be produced from refined sesame seed oil, and exhibits anti-oxidative activity,[3–5)] lowers serum cholesterol and lipid levels,[6–10)] decreases blood pressure,[11–16)] protects against alcohol-induced liver injuries,[17)] displays synergy with *α*-tocopherol,[8,18,19)] and elevates *γ*-tocopherol in plasma and the liver.[20,21)] Ide *et al.*[22)] reported that the change in the gene expression of hepatic fatty acid oxidation enzymes was greater with episesamin than with sesamin, and the differences in bioavailability appear to be important for their physiological activities.

Nakai *et al.*[3)] have shown that sesamin was metabolized to (1*R*,2*S*,5*R*,6*S*)-6-(3,4-dihydroxyphenyl)-2-(3,4-methylenedioxyphenyl)-3,7-dioxabicyclo[3,3,0]octane (SC-1) and (1*R*,2*S*,5*R*,6*S*)-2,6-bis(3,4-dihydroxyphenyl)-3,7-dioxabicyclo[3,3,0]octane (SC-2) through oxidization by demethylation by cytochrome P450.[23,24)] These compounds are further methylated by catechol-*O*-methyltransferase (COMT) to (1*R*,2*S*,5*R*,6*S*)-6-(4-hydroxy-3-methoxyphenyl)-2-(3,4-methylenedioxyphenyl)-3,7-dioxabicyclo[3,3,0]octane (SC-1m) and (1*R*,2*S*,5*R*,6*S*)-6-(4-hydroxy-3-methoxyphenyl)-2-(3,4-dihydroxyphenyl)-3,7-dioxabicyclo[3,3,0]octane (SC-2m) (Fig. 1). Moazzami *et al.* demonstrated that the major urinary metabolite of sesamin was SC-1 and the excretion of SC-1 ranged from 22.2 to 38.6% in humans.[25)] A recent paper has shown

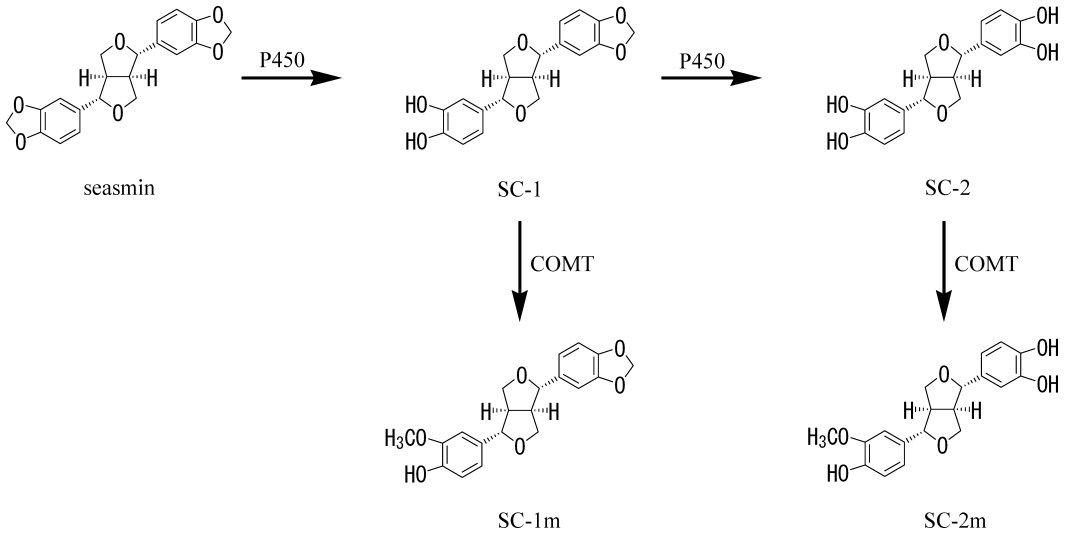

Fig. 1. Metabolic Pathways of Sesamin

The authors declare no conflict of interest.

* To whom correspondence should be addressed. e-mail: Namino_Sugiura@suntory.co.jp

that CYP2C9 is the most important CYP (cytochrome) isoform in the metabolism from sesamin to SC-1 in humans[26]; however, little is known about the metabolites of episesamin in rats and humans.

In this study, metabolites of episesamin were isolated from rat bile *in vivo* and from the reaction mixture with human liver microsomes *in vitro*, and then their structures were determined to estimate the metabolic pathway of episesamin.

MATERIALS AND METHODS

Chemicals A sesame lignan fraction containing sesamin and episesamin was prepared from refined sesame oil by crystallization and silica gel chromatography as described previously.[1] Episesamin was purified from this fraction by HPLC at an absorbance 280 nm, using a 250 mm×50 mm i.d. Develosil ODS-UG-5 reversed-phase HPLC column (Nomura Chemical Co., Japan).[3] Authentic standards of sesamin metabolites (SC-1 and SC-2) were prepared according to the method of Urata *et al.*[27] by Nemoto Science Co., Ltd.

Enzyme Source *β*-Glucuronidase from *Helix pomatia* (Type H-2) was purchased from Sigma-Aldrich Japan (Osaka, Japan). Pooled human hepatic microsomes were purchased from BD Gentest (Woburn, MA, U.S.A.).

Animals Sprague Dawley (SD) rats, weighing 280–330 g, were obtained from Oriental Bio Service (Kyoto, Japan). The animals were maintained in an air-conditioned room at 24°C with a 12 h light/dark cycle. Drinking water and regular feed were supplied *ad libitum*. The experiments were started after the animals had been acclimatized for at least 1 week. Experimental protocols were approved by the Animal Care and Use Committee of Suntory Holdings, Ltd., and followed the Guidelines for Animal Care and Use of Suntory Holdings, Ltd.

Preparation of (7*α*,7′*β*,8*α*,8′*α*)-3,4-Dihydroxy-3′,4′-methylenedioxy-7,9′:7′,9-diepoxylignane (EC-1-1), (7*α*,7′*β*,8*α*,8′*α*)-3,4-Methylenedioxy-3′,4′-dihydroxy-7,9′:7′,9-diepoxylignane (EC-1-2) and (7*α*,7′*β*,8*α*,8′*α*)-3,4:3′,4′-Bis(dihydroxy)-7,9′:7′,9-diepoxylignane (EC-2) According to the method of Urata *et al.*,[27] EC-1-1, EC-1-2 and EC-2 (metabolites of episesamin) were obtained by acid hydrolysis subsequent to acetoxylation of episesamin (Chart 1).

In detail, to a solution of episesamin (28.2 mmol) and dry chlorobenzene (282 mL) was added lead (IV) tetraacetate (42.4 mmol), and the mixture was heated at 70°C for 2 h. After cooling, the mixture was diluted with chlorobenzene (250 mL) and filtered through a Celite pad. The filtrate was washed with distilled water (3 times), dried with anhydrous sodium sulfate and concentrated under reduced pressure to give a mixture (11.1 g) of Epi-SAc-1-1, Epi-SAc-1-2 and Epi-SAc-2 as a pale yellow form.

The resulting residue from the above procedure was treated with 80% acetic acid (269 mL) at room temperature for 15 min. After evaporation of the solvents, residual acetic acid was removed by co-evaporation with water–ethanol (5 times), and then with ethanol. The residue was purified by silica gel column chromatography. Elution was performed with *n*-hexane–ethyl acetate (4 : 1 to 1 : 1, v/v) to obtain crude EC-1-1 and EC-1-2. Elution was also performed with *n*-hexane–ethyl acetate (1 : 2 to ethyl acetate only, v/v) to give crude EC-2. The crude EC-1-1 and EC-1-2 were purified by silica gel column chromatography with *n*-hexane–ethyl acetate (4 : 1 to 1 : 1, v/v). EC-1-1 was obtained as a yellow solid and EC-1-2 was obtained as a white solid. The EC-2 was purified by silica gel column chromatography with *n*-hexane–ethyl acetate (1 : 1 to 1 : 2, v/v) and was obtained as a yellow solid.

Structural Determination of Metabolites of Episesamin in Rat Bile Rats were fasted overnight with free access to water. The biliary duct of each rat was cannulated. Episesamin was suspended in olive oil and was orally administered at a dose of 500 mg/kg to rats. Bile was collected in a tube over 24 h, and bile samples were stored in a −80°C freezer until needed. Bile samples were treated with *β*-glucuronidase/arylsulfatase for hydrolysis, and extracted with ethyl acetate according to the method of Nakai *et al.*[3] After concentration *in vacuo*, the resultant extract was dissolved in methanol (MeOH) and then subjected to reversed-phase HPLC. The column was a Develosil ODS-UG-5 (250×20 mm, 5 *μ*m). The mobile phase consisted of (A) 0.05% aqueous trifluoroacetic acid (v/v) and (B) 0.05% trifluoroacetic acid/acetonitrile (v/v) using a gradient elution of 0–100% B at 0–100 min, and 100% B at 100–110 min. The flow rate was 5 mL/min. Detection wavelength was absorbance 280 nm.

Four colored fractions A to D were obtained and then subjected to reversed-phase HPLC again. The column was a Develosil C30-UG-5, (150×4.6 mm, 5 *μ*m), the flow rate was 1.0 mL/min, and detection wavelength was absorbance 280 nm. The isocratic conditions for further purification of fractions

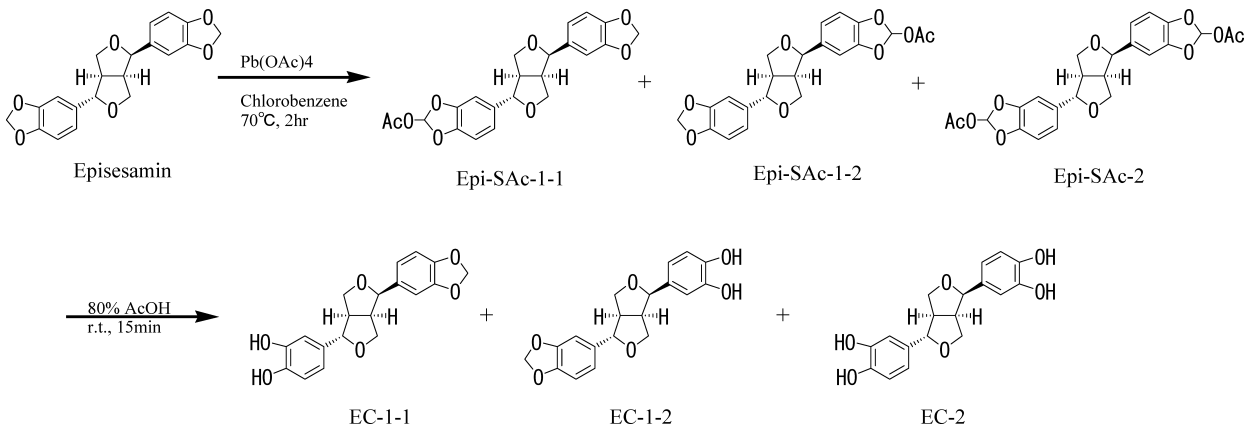

Chart 1. Acetoxylation and Acid Hydrolysis

B, C and D were 34% MeOH, 55% MeOH and 60% MeOH, respectively.

Metabolism of Sesamin or Episesamin with Human Liver Microsomes Sesamin or episesamin (final conc. 100 μM) was added to a mixture of pooled human liver microsomes (0.5 mg protein/mL), nicotinamide adenine dinucleotide phosphate (NADPH)-generating system (NADP 2.5 mM, glucose-6-phosphate 25 mM, 2 units of glucose 6-phosphate dehydrogenase, magnesium chloride 10 mM), and 250 mM potassium phosphate buffer (pH7.4) containing 0.25 mM ethylenediaminetetraacetic acid (EDTA) in a final volume of 0.5 mL. The mixtures were incubated at 37°C for 30 min and then stopped by adding 0.5 mL MeOH–acetonitrile (1 : 1, v/v). After centrifugation, the supernatant was filtered through a Millex-LH Filter (0.45 mm; Millipore, Billerica, MA, U.S.A.) before analysis.

Spectroscopy The structures of the metabolites were assigned by nuclear magnetic resonance spectroscopy, incorporating ^{1}H-NMR, ^{13}C-NMR, double quantum filtered correlation spectroscopy (DQF-COSY), total correlation spectroscopy (TOCSY), heteronuclear multiple bond correlation (HMBC), heteronuclear single quantum coherence (HSQC), and nuclear Overhauser effect (NOE), rotating frame Overhauser effect (ROE). The NMR experiments were performed with a Bruker DMX-750 spectrometer (Bruker Biospin, Germany). Samples were dissolved in DMSO-d_6. The residual proton peak and ^{13}C peak of DMSO-d_6 (δ 2.50 for ^{1}H and δ 39.43 for ^{13}C) were used as internal standard. The mass spectra were recorded with a Quattro micro MS system (Waters/Micromass, Manchester, U.K.), equipped with an electrospray ionization (ESI) ion source. Cone voltage and capillary voltage was 15 V and 3.5 kV, and source and desolvation temperatures were set at 80 and 150°C, respectively.

UPLC/MS Analysis of Metabolites The metabolites of sesamin and episesamin by human liver microsomes were analyzed using ACQUITY ultra performance liquid chromatography (UPLC) system (Waters Corp., Milford, MA, U.S.A.) coupled to a Quattro micro MS System. The column was an Acquity UPLC BHC C18, (100×2.1 mm, 1.7 μm, Waters Corp., Milford, MA, U.S.A.) maintained at a temperature of 40°C. A constant flow rate of 0.25 mL/min was used. The mobile phase was composed of solvents A (10 mM ammonium formate) and B (100% MeOH). The following gradient was used: 0–0.5 min: 80% solvent A/20% solvent B–65% solvent A/35% solvent B, 0.5–9 min: 65% solvent A/35% solvent B–15% solvent A/85% solvent B, and 9–10 min, 15% solvent A/85% solvent B. UV detection was carried out at 280 nm.

To separate EC-1-1 and EC-1-2, the column used was a COSMOSIL Cholester (100×2.0 mm, 2.5 μm, Nacalai Tesque Co., Japan) maintained at a temperature of 40°C and with a constant flow rate of 0.25 mL/min. The mobile phase was composed of solvents A (0.1% formic acid (v/v)) and B (100% MeOH). Elution was performed with an isocratic solvent mixture of 45% solvent A/55% solvent B.

The electrospray mass spectrometer was operated in negative ion mode. Cone voltage and capillary voltage was 45 V and 3.5 kV, and source and desolvation temperatures were set at 80°C and 250°C, respectively.

RESULTS

Preparation of Authentic Metabolites of Episesamin (EC-1-1, EC-1-2 and EC-2) Acetoxylated derivatives Epi-SAc-1-1, Epi-SAc-1-2 and Epi-SAc-2 were given by acetoxylation of episesamin with $Pb(OAc)_4$ in dry chlorobenzene. After acid hydrolysis, crude fractions of EC-1 (3.6 g) and EC-2 (2.1 g) were obtained by silica gel column chromatography. EC-1-1 (523 mg, 5.4% from episesamin), EC-1-2 (518 mg, 5.4% from episesamin) and EC-2 (334 mg, 3.6% from episesamin) were isolated by further purification using silica gel column chromatography. The structure of EC-1-1, EC-1-2 and EC-2 was confirmed by mass and ^{1}H-NMR spectra.

Identification of Metabolites of Episesamin in Rat Bile The episesamin-administrated rat bile extract was treated with glucuronidase/arylsulfatase and then subjected to reversed-phase HPLC. Four fractions were obtained as shown in Fig. 2. Metabolite **5** was isolated from fraction A. Metabolite **6** and **7**, metabolite **1** and **2**, metabolite **3** and **4** were purified from fractions B, C and D as described in "Materials and Methods," respectively.

Seven metabolites of episesamin were identified in rat bile by MS and NMR. The molecular weights of the metabolites were determinated as 342 (metabolites **1**, **2**), 356 (metabolites **3**, **4**), 330 (metabolite **5**) and 344 (metabolites **6**, **7**) by ESI-MS. The proton signal intensity of the methylenedioxy moiety (–O–CH_2–O–) in metabolites **1**–**4** was two protons at δ 6.0 by ^{1}H-NMR analysis. No proton signals of the methylenedioxy moiety in metabolites **5**–**7** were observed. The ^{1}H- and ^{13}C-NMR spectra of metabolites **3**, **4**, **6** and **7** showed a structure containing one methoxy moiety by δ 3.75 or 3.76. The difference of structures between metabolite **1** and **2**, **3** and **4**, **6** and **7** were confirmed by NOE correlation of H-2 to H-8. The location of the methoxy group at carbon atom C-3 of metabolite **3** was confirmed by HMBC from the methoxy group to C-3, from 4-hydroxyl group to C-3, C-4, C-5. The rotating frame Overhauser enhancement spectroscopy (ROESY) spectrum further confirmed the structure, showing interactions between the methoxy group and H-2. Similarly, the location of the methoxy group at carbon atom C-3′ of metabolite **4** was confirmed by the HMBC from the methoxy group to C-3′, NOE correlation between methoxy group and H-2′. The location of the methoxy group at carbon atom C-3 of metabolite **6** was confirmed by the HMBC from the methoxy group to C-3, NOE correlation between methoxy group and H-2. The location of the methoxy group at carbon atom C-3′ of metabolite **7** was confirmed by the HMBC from the methoxy group to C-3′, NOE correlation between methoxy group and H-2′.

The structures of metabolites **1**–**7** were identified by COSY, HSQC, HMBC, NOESY and ROESY experiments

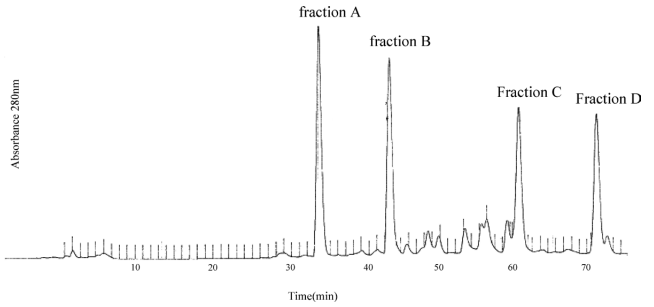

Fig. 2. HPLC Chromatogram of Rat Bile Extract after Treatment with Glucuronidase/Arylsulfatase

Table 1. The ^{1}H- and ^{13}C-NMR Spectra of Metabolites of Episesamin

	EC-1-1		EC-1-2		EC-1m-1		EC-1m-2		EC-2m-1		EC-2m-2		EC-2	
	δ_C	δ_H (J=Hz)	δ_C	δ_H (J=Hz)	δ_C	δ_H (J=Hz)	δ_C	δ_H (J=Hz)	δ_C	δ_H (J=Hz)	δ_C	δ_H (J=Hz)	δ_C	δ_H (J=Hz)
8′	54.1	2.75 m	53.9	2.76 m	54.1	2.78 m	53.7	2.82 m	53.8	2.76 m	53.8	2.80 m	53.8	2.74 m
7′	86.7	4.31 d (7)	86.9	4.23 d (7)	86.7	4.34 d (7)	86.8	4.30 d (7)	86.8	4.23 d (7)	86.8	4.27 d (7)	86.8	4.21 d (7)
9a	69.0	3.10 dd (9, 9)	68.7	3.03 dd (9, 9)	69.0	3.08 dd (9, 9)	68.6	3.05 dd (9, 9)	68.7	3.06 dd (9, 9)	68.7	3.10 dd (9, 9)	68.7	3.08 dd (9, 9)
9b		3.69 dd (8, 8)		3.68 dd (9, 9)		3.72 m		3.72 m		3.69 dd (9, 9)		3.70 m		3.66 dd (9, 9)
8	49.4	3.27 m	49.3	3.33 m	49.4	3.36 m	49.2	3.35 m	49.3	3.32 m	49.2	3.23 m	49.2	3.25 m
7	81.3	4.68 d (6)	81.2	4.76 d (6)	81.4	4.74 d (6)	81.1	4.78 d (6)	81.2	4.75 d (6)	81.2	4.69 d (6)	81.2	4.68 d (6)
9′a	70.2	3.69 dd (8, 8)	70.4	3.74 m	70.2	3.72 m	70.2	3.74 m	70.2	3.73 m	70.1	3.70 m	70.2	3.70 m
9′b		4.03 d (9)		4.00 d (9)		4.06 d (9)		4.04 d (9)		4.00 d (9)		4.01 d (9)		3.97 d (9)
1	135.6		132.6		130.0		132.5		129.5		129.5		132.3	
2	106.5	6.92 brs	106.2	6.90 brs	110.0	6.88 brs	106.1	6.91 brs	109.6	6.88 brs	113.1	6.75 brs	113.1	6.73 brs
3	147.4		147.1		147.3		147.0		147.1		144.7		145	
4	146.6		146.0		145.3		145.8		145.0		143.9		144.6	
5	108.0	6.85 d (6)	108.0	6.88 d (8)	115.1	6.73 brs	107.8	6.89 d (9)	113.3	6.73 brs	115.2	6.68 d (8)	115.1	6.67 m
6	119.4	6.85 d (6)	118.6	6.83 brd (8)	117.9	6.73 brs	118.5	6.84 brd (8)	117.8	6.73 brs	116.2	6.58 brd (8)	116.9	6.58 brd (8)
1′	129.5		132.4		135.6		132.1		132.3		132.2		129.5	
2′	113.2	6.74 brs	113.4	6.73 brs	106.5	6.92 brs	110.1	6.89 brs	115.0	6.73 brs	110.1	6.89 brs	113.3	6.75 brs
3′	144.9		144.7		147.4		147.4		145.1		147.4		144.7	
4′	144.0		145.1		146.6		145.8		144.6		145.8		143.9	
5′	115.3	6.68 d (8)	115.2	6.67 d (8)	108.0	6.86 d (4)	115.0	6.72 d (8)	115.1	6.67 d (8)	115	6.72 d (8)	115.1	6.67 m
6′	116.4	6.57 brd (7)	117.0	6.60 brd (8)	119.4	6.86 d (4)	118.5	6.76 brd (8)	116.9	6.61 brd (8)	118.5	6.75 broad	116.2	6.60 brd (8)
–O–CH_2–O–	100.9	6.00 s	100.8	6.00 s	100.9	5.99 s	100.7	6.00 s						
–OH		8.88 broad		8.83 s, 8.86 s		8.89 s		8.90 s		8.84 broad		8.85 broad		8.76 s, 8.81 s, 8.83 brs
CH_3					55.5	3.75 s	55.5	3.76 s	55.5	3.76 s	55.5	3.76 s		

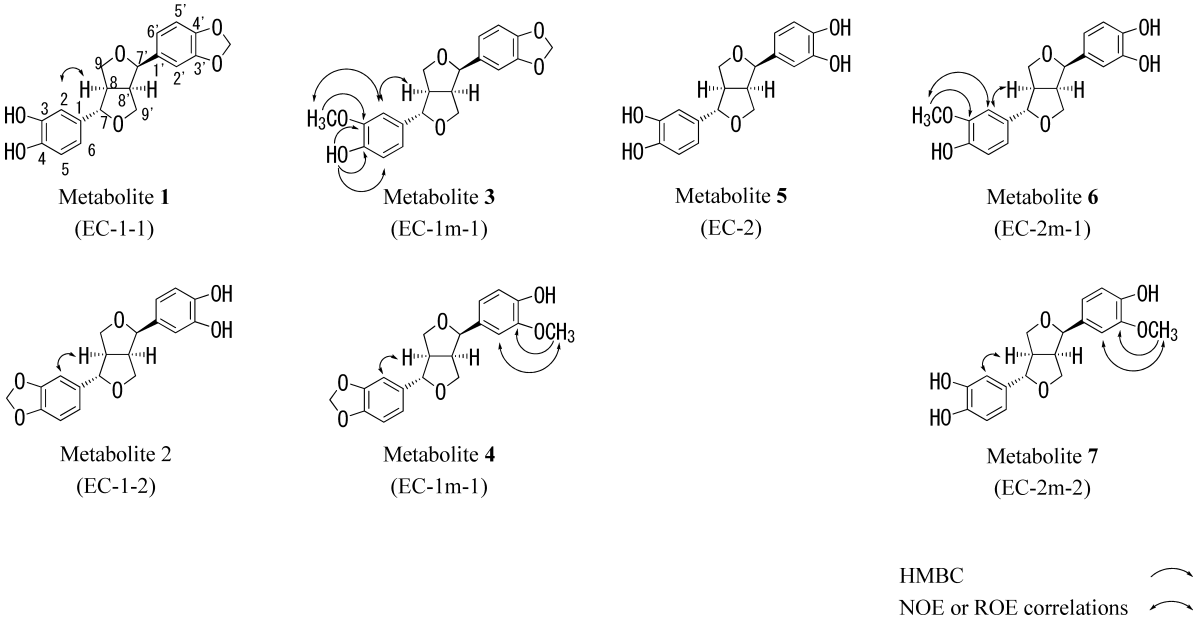

Fig. 3. Structures of Metabolites of Episesamin

as follows: (7*α*,7′*β*,8*α*,8′*α*)-3,4-dihydroxy-3′,4′-methylenedioxy-7,9′:7′,9-diepoxylignane (metabolite **1**), (7*α*,7′*β*,8*α*,8′*α*)-3,4-methylenedioxy-3′,4′-dihydroxy-7,9′:7′,9-diepoxylignane (metabolite **2**), (7*α*,7′*β*,8*α*,8′*α*)-3-methoxy-4-hydroxy-3′,4′-methylenedioxy-7,9′:7′,9-diepoxylignane (metabolite **3**), (7*α*,7′*β*,8*α*,8′*α*)-3,4-methylenedioxy-3′-methoxy-4′-hydroxy-7,9′:7′,9-diepoxylignane (metabolite **4**), (7*α*,7′*β*,8*α*,8′*α*)-3,4:3′,4′-bis(dihydroxy)-7,9′:7′,9-diepoxylignane (metabolite **5**), (7*α*,7′*β*,8*α*,8′*α*)-3-methoxy-4-hydroxy-3′,4′-dihydroxy-7,9′:7′,9-diepoxylignane (metabolite **6**) and (7*α*,7′*β*,8*α*,8′*α*)-3,4-dihydroxy-3′-methoxy-4′-hydroxy-7,9′:7′,9-diepoxylignane (metabolite **7**). The ^{1}H- and ^{13}C-NMR spectral data of these compounds are shown in Table 1. Metabolites **3** and **4** were previously reported as xanthoxylol and pluviathilol.[28–31] Analytical data of metabolite **5** was consistent with that reported for the products of episesamin cultured with *Aspergillus*.[32] Metabolite **7** was previously reported as 3′-*O*-demethylepipinoresinol.[33]

The structures of metabolites after treatment with glucuronidase/arylsulfatase are shown in Fig. 3.

Identification of Metabolites of Episesamin by Human Liver Microsomes A typical UV chromatogram after incubation of sesamin or episesamin with human liver microsomes is shown in Figs. 4a, b. Each metabolite peak (peak 1 or 2) was detected. The negative ion LC/MS spectrum of peak 1 or 2 showed deprotonated molecular ion *m/z* 341 $[M-H]^-$ (Figs. 5a, b). HPLC retention time of peak 1 was identical to that of the authentic standard SC-1 (Fig. 4c). HPLC retention time of peak 2 was identical to those of the authentic standard EC-1-1 and EC-1-2 (Fig. 4c). To determine whether peak 2 is EC-1-1, EC-1-2 or a mixture of the isomers, another HPLC was performed. Peak 2 was separated into two peaks and the retention time was identical to that of the authentic standard EC-1-1 or EC-1-2 (Figs. 6a, b). EC-1-1 and EC-1-2 were detected with single ion recording (SIR) at *m/z* 341; therefore, peak 2 was identified as a mixture of EC-1-1 and EC-1-2.

The other metabolites, except for SC-1 or EC-1, were not detected on the UV chromatogram after incubation of sesamin or episesamin with human liver microsomes; however, SC-2 was detected as a metabolite of sesamin by human liver microsomes with multiple reaction monitoring (MRM) at *m/z* 329.3>137.1, and the retention time was identical to that of the authentic standard SC-2 (Fig. 7b). Similarly, EC-2 was detected as a metabolite of episesamin (Fig. 7c).

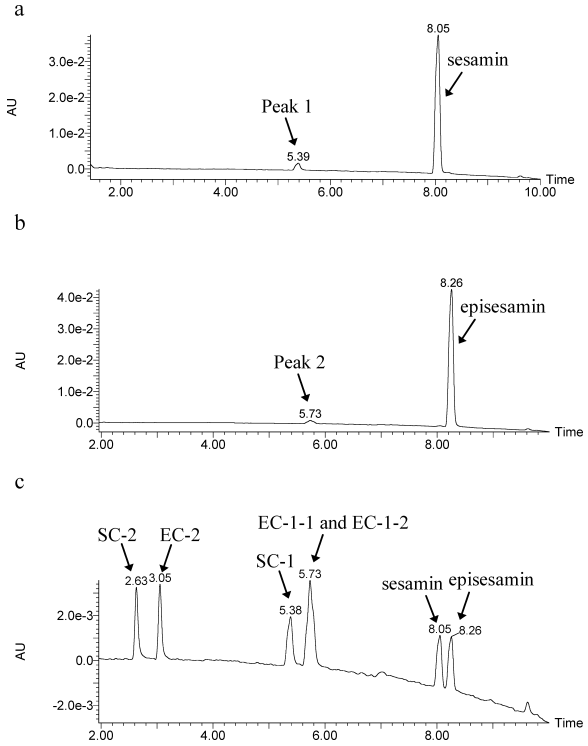

Fig. 4. HPLC Chromatogram Comparison of the Authentic Standard and the Metabolites of Sesamin or Episesamin

(a) Chromatogram after incubation of sesamin with human liver microsomes; (b) chromatogram after incubation of episesamin with human liver microsomes; (c) chromatogram of authentic standard SC2, EC-2, SC-1, EC-1-1, EC-1-2, sesamin and episesamin.

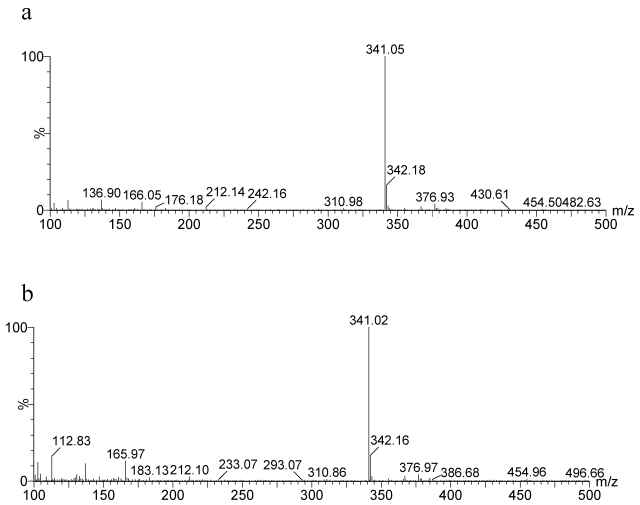

Fig. 5. Negative Ion LC/MS Spectrum of the Ion *m/z* 341 $[M-H]^-$

(a) Peak 1 metabolite of sesamin; (b) peak 2 metabolites of episesamin.

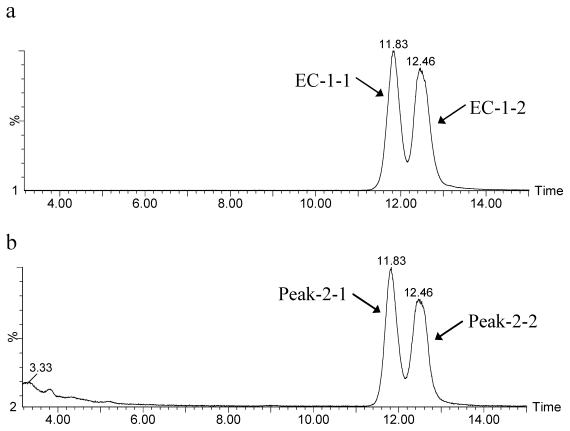

Fig. 6. MS Chromatogram (SIR Mode at *m/z* 341) Comparison of the Authentic Standard and the Metabolites of Episesamin

(a) Authentic standard EC-1-1 and EC-1-2; (b) chromatogram after incubation of episesamin with human liver microsomes.

DISCUSSION

Episesamin has two methylendioxyphenyl these groups on *exo* and *endo* faces, although sesamin has these groups on the exo face of the bicyclic skeleton.[2] Seven metabolites of episesamin were found in rat bile after oral administration, and were identified by MS and NMR. Orally ingested episesamin was oxidized to EC-1-1, EC-1-2 and EC-2 by demethylation by cytochrome P450,[23,24] and EC-1-1, EC-1-2 and EC-2 were methylated by COMT into EC-1m-1, EC-1m-2, EC-2m-1 and EC-2m-2, respectively. These metabolites were finally excreted into bile or blood by rapid conjugation of glucuronide by UDP-glucuronosyltransferase or sulfation by sulfotransferase.

Similarly, episesamin was metabolized to EC-1-1, EC-1-2 and EC-2 in human liver microsomes. Three metabolites of episesamin using human liver microsomes were identified by UPLC-MS/MS. No significant differences were observed in the yield of EC-1-1 and EC-1-2. As shown in Fig. 4b, EC-2 was not detected on the UV chromatogram, indicating that EC-2 was a minor metabolite in humans. The metabolic pathway of episesamin is considered to be similar in rats and humans. The predicted metabolic pathways of episesamin in rats and humans are illustrated in Fig. 8.

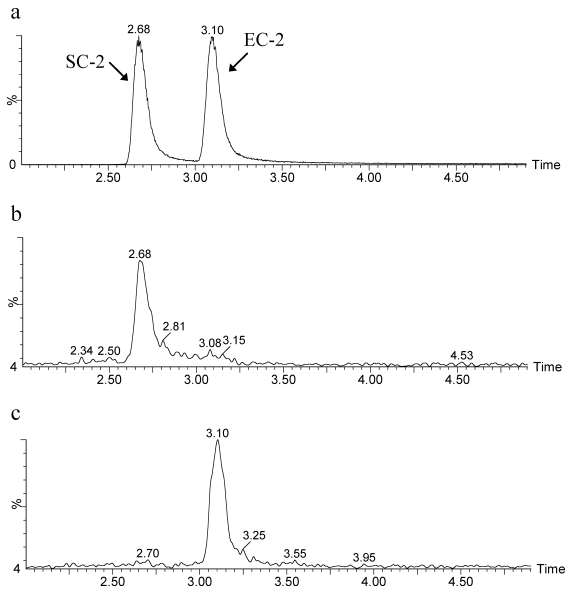

Fig. 7. MRM (*m/z* 329.3>137.1) Chromatogram of Comparison of the Authentic Standard and the Metabolites of Sesamin or Episesamin

(a) Authentic standards SC-2 and EC-2; (b) chromatogram after incubation of sesamin with human liver microsomes; (c) chromatogram after incubation of episesamin with human liver microsomes.

Moazzami *et al.* demonstrated that the major urinary metabolite of sesamin was SC-1 and the excretion of SC-1 ranged from 22.2 to 38.6% in humans.[25] Peñalvo *et al.* reported that SC-1 and SC-2 were found after fermentation with human microflora.[34] Yasuda *et al.* have reported the contribution of CYP1A2, 2C9, 2C19 and 2D6 to the metabolism of sesamin. In particular, CYP2C9 was most important in the metabolism from sesamin to SC-1 in humans.[26] SC-2 were identified as a metabolite of sesamin by human liver microsomes, but the peak of SC-2 was not detected on the UV chromatogram. So SC-2 is considered to be a minor metabolite in humans.

We demonstrated that SC-2 was formed by cytochrome P450 in human liver microsomes. Presumably, SC-1 and SC-2 are generated first by cytochrome P450 in the small intestine or the liver after absorption. Nakai *et al.*[3] have shown that SC-1 and SC-2 were further methylated by COMT to SC-1m and SC-2m in rats. After oral administration of a mixture of sesamin and episesamin, we found sesamin, SC-1, SC-2, SC-1m, SC-2m, episesamin, EC-1, EC-2, EC-1m, EC-2m in human plasma (unpublished data). The metabolic pathway of sesamin is also considered to be similar in rats and humans.

EC-1 might be also responsible for pharmacological activity, because SC-1 might be responsible for pharmacological activity such as anti-oxidative activity[3] and enhancement of endothelium-dependent vasorelaxation[13]. The gene expression of hepatic fatty acid oxidation enzymes was more strongly up-regulated by episesamin than sesamin.[22] The metabolism of sesamin and/or episesamin might be related to various physiological activities.

This study clearly demonstrated that seven metabolites of episesamin were isolated from rat bile, and that the metabolites of episesamin by cytochrome P450 were also identified using human liver microsomes. These results indicated that the metabolic pathway of episesamin is similar in rats and

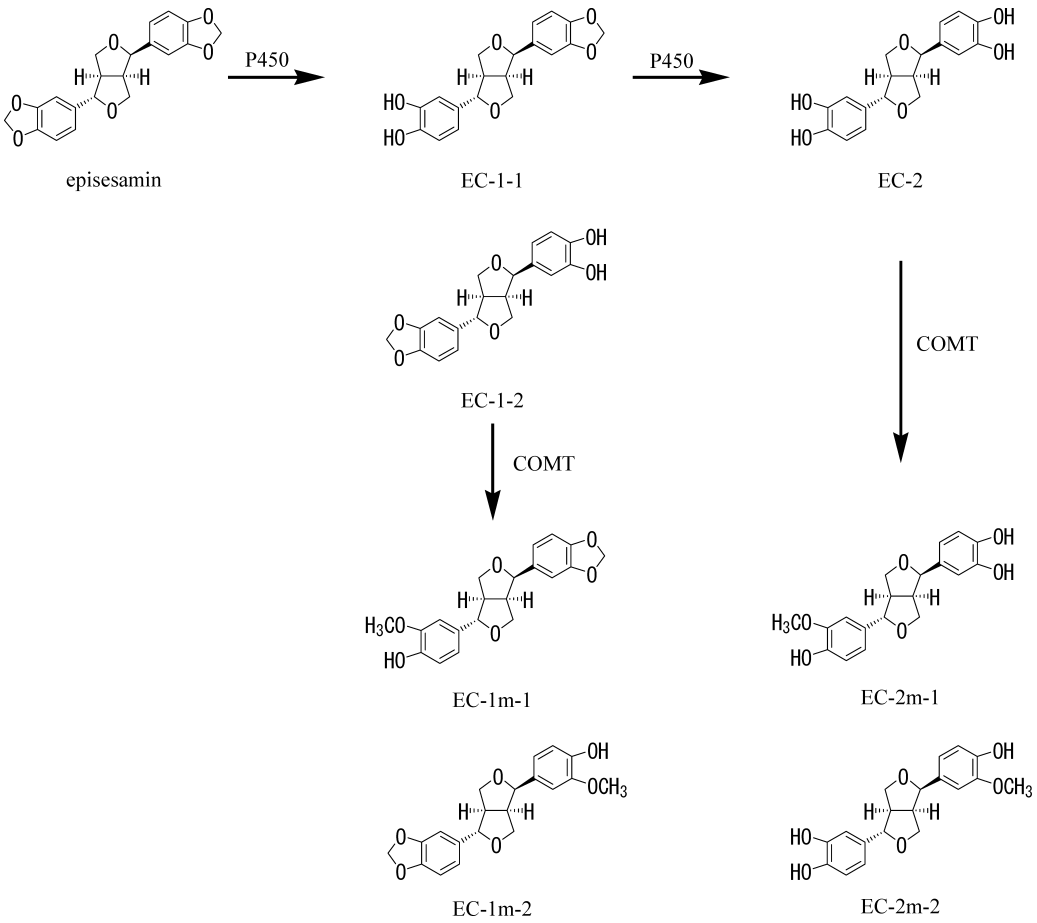

Fig. 8. The Predicted Metabolic Pathways of Episesamin in Rats and Humans

humans.

REFERENCES

1) Fukuda Y, Nagata M, Osawa T, Namiki M. Contribution of lignan analogues to antioxidative activity of refined unroasted sesame seed oil. *J. Am. Oil Chem. Soc.*, **63**, 1027–1031 (1986).
2) Li CY, Chow TJ, Wu TS. The epimerization of sesamin and asarinin. *J. Nat. Prod.*, **68**, 1622–1624 (2005).
3) Nakai M, Harada M, Nakahara K, Akimoto K, Shibata H, Miki W, Kiso Y. Novel antioxidative metabolites in rat liver with ingested sesamin. *J. Agric. Food Chem.*, **51**, 1666–1670 (2003).
4) 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.*, **24**, 530–534 (2003).
5) Kiso Y. Antioxidative roles of sesamin, a functional lignan in sesame seed, and it's effect on lipid- and alcohol-metabolism in the liver: a DNA microarray study. *Biofactors*, **21**, 191–196 (2004).
6) 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.*, **32**, 629–638 (1991).
7) Peñalvo JL, Hopia A, Adlercreutz H. Effect of sesamin on serum cholesterol and triglycerides levels in LDL receptor-deficient mice. *Eur. J. Nutr.*, **45**, 439–444 (2006).
8) Nakabayashi A, Kitagawa Y, Suwa Y, Akimoto K, Asami S, Shimizu S, Hirose N, Sugano M, Yamada H. alpha-Tocopherol enhances the hypocholesterolemic action of sesamin in rats. *Int. J. Vitam. Nutr. Res.*, **65**, 162–168 (1995).
9) Hirata F, Fujita K, Ishikura Y, Hosoda K, Ishikawa T, Nakamura H. Hypocholesterolemic effect of sesame lignan in humans. *Atherosclerosis*, **122**, 135–136 (1996).
10) Kiso Y, Tsuruoka N, Kidokoro A, Matsumoto I, Abe K. Sesamin ingestion regulates the transcription levels of hepatic metabolizing enzymes for alcohol and lipids in rats. *Alcohol. Clin. Exp. Res.*, **29** (Suppl), 116S–120S (2005).
11) Kita S, Matsumura Y, Morimoto S, Akimoto K, Furuya M, Oka N, Tanaka T. Antihypertensive effect of sesamin. II. Protection against two-kidney, one-clip renal hypertension and cardiovascular hypertrophy. *Biol. Pharm. Bull.*, **18**, 1283–1285 (1995).
12) 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.*, **18**, 1016–1019 (1995).
13) Nakano D, Takaoka M, Kiso Y, Matsumura Y. Antihypertensive effect of sesamin. *Vasc. Dis. Prev.*, **1**, 233–241 (2004).
14) Lee CC, Chen PR, Lin S, Tsai SC, Wang BW, Chen WW, Tsai CE, Shyu KG. Sesamin induces nitric oxide and decreases endothelin-1 production in HUVECs: possible implications for its antihypertensive effect. *J. Hypertens.*, **22**, 2329–2338 (2004).
15) Nakano D, Kwak CJ, Fujii K, Ikemura K, Satake A, Ohkita M, Takaoka M, Ono Y, Nakai M, Tomimori N, Kiso Y, Matsumura Y. Sesamin metabolites induce an endothelial nitric oxide-dependent vasorelaxation through their antioxidative property-independent mechanisms: possible involvement of the metabolites in the antihypertensive effect of sesamin. *J. Pharmacol. Exp. Ther.*, **318**, 328–335 (2006).
16) Miyawaki T, Aono H, Toyoda-Ono Y, Maeda H, Kiso Y, Moriyama K. Antihypertensive effects of sesamin in humans. *J. Nutr. Sci. Vitaminol.* (Tokyo), **55**, 87–91 (2009).
17) Akimoto 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.*, **37**, 218–224 (1993).
18) Yamashita K, Kagaya M, Higuti N, Kiso Y. Sesamin and alpha-tocopherol synergistically suppress lipid-peroxide in rats fed a high docosahexaenoic acid diet. *Biofactors*, **11**, 11–13 (2000).
19) Kamal-Eldin A, Pettersson D, Appelqvist LA. Sesamin (a compound from sesame oil) increases tocopherol levels in rats fed *ad libitum*. *Lipids*, **30**, 499–505 (1995).
20) Yamashita K, Nohara Y, Katayama K, Namiki M. Sesame seed lignans and gamma-tocopherol act synergistically to produce vitamin E activity in rats. *J. Nutr.*, **122**, 2440–2446 (1992).
21) 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[*a*]anthracene induced rat mammary carcinogenesis. *Anticancer Res.*, **12**, 1259–1265 (1992).
22) Ide T, Lim JS, Odbayar TO, Nakashima Y. Comparative study of sesame lignans (sesamin, episesamin and sesamolin) affecting gene expression profile and fatty acid oxidation in rat liver. *J. Nutr. Sci. Vitaminol.* (Tokyo), **55**, 31–43 (2009).
23) Kumagai Y, Wickham KA, Schmitz DA, Cho AK. Metabolism of methylenedioxyphenyl compounds by rabbit liver preparations. Participation of different cytochrome P450 isozymes in the demethylenation reaction. *Biochem. Pharmacol.*, **42**, 1061–1067 (1991).
24) Meyer MR, Peters FT, Maurer HH. The role of human hepatic cytochrome P450 isozymes in the metabolism of racemic 3,4-methylenedioxy-methamphetamine and its enantiomers. *Drug Metab. Dispos.*, **36**, 2345–2354 (2008).
25) Moazzami AA, Andersson RE, Kamal-Eldin A. Quantitative NMR analysis of a sesamin catechol metabolite in human urine. *J. Nutr.*, **137**, 940–944 (2007).
26) Yasuda K, Ikushiro S, Kamakura M, Ohta M, Sakaki T. Metabolism of sesamin by cytochrome P450 in human liver microsomes. *Drug Metab. Dispos.*, **38**, 2117–2123 (2010).
27) Urata H, Nishioka Y, Tobashi T, Matsumura Y, Tomimori N, Ono Y, Kiso Y, Wada S. First chemical synthesis of antioxidative metabolites of sesamin. *Chem. Pharm. Bull.*, **56**, 1611–1612 (2008).
28) Swain NA, Brown RC, Bruton G. A versatile stereoselective synthesis of *endo*,*exo*-furofuranones: application to the enantioselective synthesis of furofuran lignans. *J. Org. Chem.*, **69**, 122–129 (2004).
29) Gonzaléz MJTG. Pinto MMM., Kijjoa A., Anantachoke C., Herz W. Stilbenes and other constituents of *Knema austrosiamensis*. *Phytochemistry*, **32**, 433–438 (1993).
30) Pelter A, Ward RS, Rao EV, Sastry KV. Revised structures for pluviatilol, methyl pluviatilol and xanthoxylol: General methods for the assignment of stereochemistry to 2,6-diaryl-3,7-dioxabicyclo[3.3.0]-octane lignans. *Tetrahedron*, **32**, 2783–2788 (1976).
31) Corrie JFT, Green GH, Ritchie E, Taylor WC. The chemical constituents of Australian *Zanthoxylum* species. V. The constituents of *Z. pluviatile* Hartley; the structures of two new lignans. *Aust. J. Chem.*, **23**, 133–145 (1970).
32) Miyake Y, Fukumoto S, Okada M, Sakaida K, Nakamura Y, Osawa T. Antioxidative catechol lignans converted from sesamin and sesaminol triglucoside by culturing with *Aspergillus*. *J. Agric. Food Chem.*, **53**, 22–27 (2005).
33) Yang SJ, Fang JM, Cheng YS. Lignans, flavonoids and phenolic derivatives from *Taxus mairei*. *J. Chin. Chem. Soc.*, **46**, 811–818 (1999).
34) Peñalvo JL, Heinonen SM, Aura AM, Adlercreutz H. Dietary sesamin is converted to enterolactone in humans. *J. Nutr.*, **135**, 1056–1062 (2005).