JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Intestinal Distribution and Excretion of Sesaminol and Its Tetrahydrofuranoid Metabolites in Rats

Kuo-Ching Jan,[†] Kuo-Lung Ku,[‡] Yan-Hwa Chu,[†] Lucy Sun Hwang,[*,§] and Chi-Tang Ho[*,§,#]

[†]Food Industry Research and Development Institute, Hsinchu, Taiwan

[‡]Department of Applied Chemistry, National Chiayi University, Chiayi, Taiwan

[§]Graduate Institute of Food Science and Technology, National Taiwan University, Taipei, Taiwan

[#]Department of Food Science, Rutgers University, 65 Dudley Road, New Brunswick, New Jersey 08901, United States

ABSTRACT: Sesame seeds (*Sesamum indicum* L.) are unique because of potent and various physiological activities imparted by their bioactive lignans. This investigation studied the intestinal distribution and excretion of sesaminol in Sprague−Dawley (SD) rats. To investigate the distribution of sesaminol (per oral 220 mg/kg), the changes in concentration of sesaminol and its metabolites were determined in the intestines and plasma within the 24 h period after tube feeding of sesaminol to SD rats. Results show that the epimerization of sesaminol appeared to be catalyzed by acid in the simulated gastric fluids. The major sesaminol epimer was characterized as 2-episesaminol using 2D-NMR. These findings indicate that sesame sesaminol and its epimer are poorly absorbed prior to reaching the rectum and that substantial amounts pass from the small to the large intestine, where they are metabolized by the colonic microflora to tetrahydrofuranoid metabolites. Sesaminol in plasma was largely present as phase II conjugates, and the seven metabolites were detected as the 2-episesaminol, sesaminol-6-catechol, methylated sesaminol-catechol, *R,R*-hydroxymethyl-sesaminol-tetrahydrofuran, *S,R*-hydroxymethylsesaminol-tetrahydrofuran, enterolactone, and enterodiol. Excretions of sesaminol in urine and feces within the 24 h period were equivalent to 0.02 and 9.33% of the amount ingested, respectively.

KEYWORDS: sesaminol, episesaminol, catechol metabolites, tetrahydrofuranoid lignans, tissue distribution

INTRODUCTION

Sesame seed contains furofuran lignans with beneficial physiological activities, which are mostly related to lignans and their metabolites, such as sesamin, sesaminol, sesaminol glucosides, enterolactone, and enterodiol. Sesame lignans undergo transformations, including epimerization and hydrolysis, during oil refining. Sesaminol was reported to be formed from sesamolin during the bleaching process of unroasted sesame oil, and the antioxidant activity of refined unroasted sesame seed oil is mainly attributed to sesaminol. Sesamolin is largely transformed to sesaminol and to a lesser extent hydrolyzed to sesamol.[1,2] Theoretically, four stereoisomers of sesaminol can be present, and sesaminol isomers have been reported in commercial sesame oil,[3,4] but the transformation of sesaminol to its stereochemical epimer in vivo has not been investigated. Despite the structural diversity of plant lignans from different sources, most of them undergo conversion to enterodiol (END) and enterolactone (ENL) by gut microbiota.[5−8] Sesame lignans have fewer bioactivity properties in vitro, but they have been reported to be converted to bioactive phenolic compounds after oral administration.[9−11] In a recent study with sesame lignans, sesaminol has been reported to be metabolized in vivo. Sesaminol-6-catechol was identified as a major metabolite in rat liver enzymes. Sesaminol-6-catechol was further converted to methylated sesaminol 6-catechol via a liver enzyme, catechol-*O*-methyltransferase.[12] Our previous studies reported that sesaminol triglycoside was metabolized via intestinal microflora to a biologically active catechol moiety and then absorbed into the body in rats.[13,14] These metabolites of sesaminol triglycoside have been reported to have their own unique biological effects, including antioxidative activities,[12] as well as anti-inflammatory[14,15] and estrogenic activities.[14] However, sesaminol has methylenedioxyphenyl moieties in its structure, which may require additional oxidative demethylenation of the methylenedioxyphenyl ring for conversion to tetrahydrofuranoid sesaminol [4-(((3*R*,4*R*)-5-(6-hydroxybenzo[*d*][1,3]dioxol-5-yl)-4-(hydroxymethyl)tetrahydrofuran-3-yl)methyl)benzene-1,2-diol (*R,R*-hydroxymethylsesaminol-tetrahydrofuran, ST-2), 4-(((3*S*,4*R*,5*S*)-5-(6-hydroxybenzo[*d*][1,3]dioxol-5-yl)-4-(hydroxymethyl) tetrahydrofuran-3-yl)-methyl)benzene-1,2-diol (*S,R*-hydroxymethylsesaminol-tetrahydrofuran, ST-3), sesaminol-6-catechol, methylated sesaminol-6-catechol, ENL), and END] (Figure 1). Sesaminol has chiral centers in tetrahydrofuran rings; the bioavailability of sesaminol needs to be determined to clarify the function of the absorbed sesame lignans in vivo. However, few studies have attempted to measure the distribution of sesaminol and its metabolites in the intestines following gastric exposure.

In this study, we investigated the in vitro epimerization of sesaminol, using simulated gastric and intestinal fluids, and measured the time-dependent appearance and disappearance of sesaminol and its epimer in the digestive system. We also investigated the stability of sesaminol in a simulated gastric and intestinal tract and the distribution of sesaminol and its main transformation products in various parts of the intestines. In addition, tetrahydrofuranoid type metabolites of sesaminol present in the intestines were measured by LC-MS/MS. This paper reports on

Received: December 31, 2010
Revised: March 8, 2011
Accepted: March 8, 2011
Published: March 08, 2011

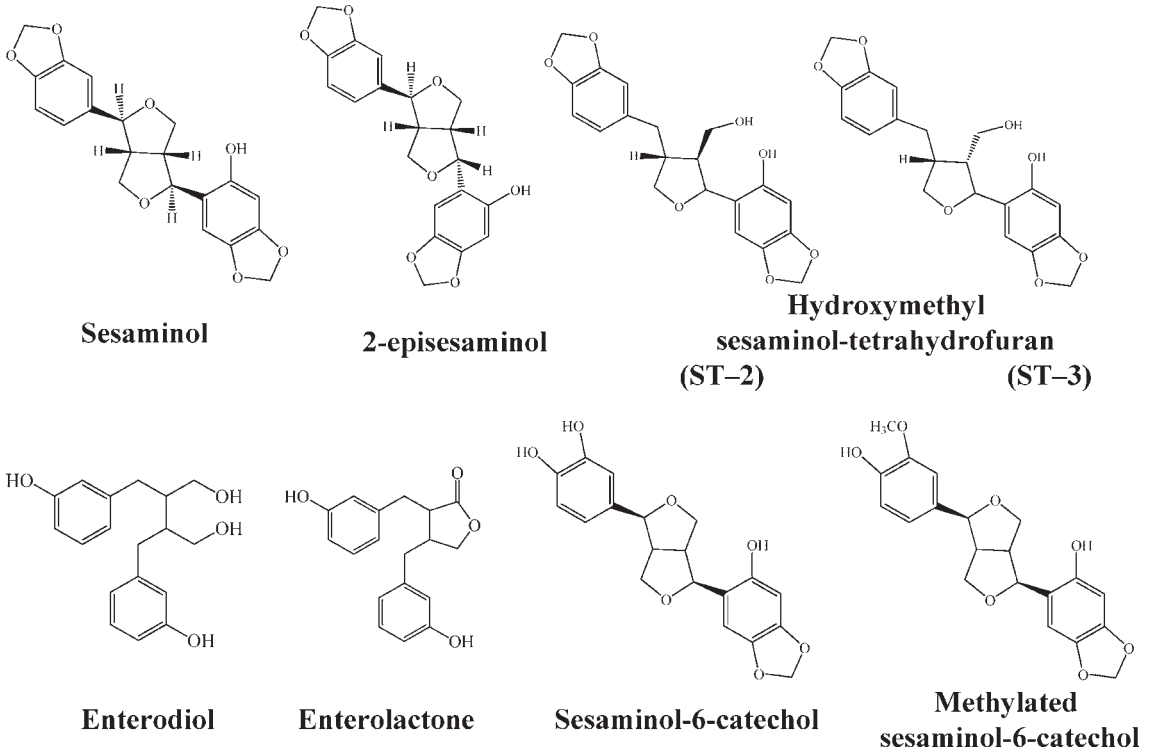


Figure 1. Chemical structures of sesaminol and its metabolites.[12]

a study of the detection of these metabolites in the intestinal tissues of rats ingesting sesaminol.

MATERIALS AND METHODS

Materials and Chemicals. Acetic acid, cellulase (from *Aspergillus niger*), β-glycosidase (from almonds), and sulfatase (type H-1, from *Helix pomatia*, containing sulfatase and β-glucuronidase) were obtained from Sigma-Aldrich (Poole, Dorset, U.K.). XAD-2 gel was purchased from Aldrich (Milwaukee, WI). All other chemicals used were of analytical grade. Liquid chromatographic grade solvents were obtained from Mallinckrodt Baker (Phillipsburg, NJ). Triply deionized water (Millipore, Bedford, MA) was used for all preparations. Simulated gastric fluids (SGF) and intestinal fluids (SIF) were freshly prepared on the day of experiment according to United States Pharmacopoeia specification.[16]

Extraction and Isolation of Sesaminol. For the isolation of sesaminol triglucoside (STG), sesame seeds were defatted with *n*-hexane and extracted with 80% MeOH. The 80% MeOH extract was charged into an Amberlite XAD-2 column and eluted with H_2O, 20% MeOH, 40% MeOH, and 60% MeOH. The 60% MeOH fraction was then purified by preparative HPLC. For the isolation of sesaminol, the 60% MeOH fraction (1 g) was incubated at 50 °C for 48 h with β-glucosidase (0.6 g) and cellulase (0.7 g) in 50 mM acetate buffer (pH 4.5). The reaction mixture was extracted with EtOAc. The EtOAc extract was fractionated into sesaminol with 98% purity using preparative HPLC.[17]

Preparation of Sesaminol Metabolites by Intestinal Microflora. Sesaminol metabolites were prepared according to the method of Jan et al.[13] The incubation of sesaminol with human intestinal bacterial mixture and isolation of sesaminol metabolites were described previously. An HIB mixture (300 mL) and sesaminol (1 g) dissolved in 10 mL of PBS were added to 500 mL of GAM broth and anaerobically incubated at 37 °C for 7 days. A 125 mL portion of reaction mixture was taken out at intervals and then extracted three times with *n*-BuOH. The *n*-BuOH extracts were combined, and solvent was removed in vacuo to give a residue. The *n*-BuOH extracts was chromatographed on a Diaion HP-20 column and eluted with H_2O, 50% aqueous MeOH, and MeOH in a sequential manner. The 50% aqueous MeOH and MeOH fractions were combined and applied to a Sephadex LH-20 column and eluted with MeOH/H_2O (6:4). Fractions containing metabolites were combined and chromatographed again on another Sephadex LH-20 column and eluted with MeOH/H_2O (1:1). The metabolites of sesaminol were chromatographed on a preparative LC and purified by ODS column. Similarly, the major metabolite ST-2 (*R*,*R*-hydroxymethylsesaminol-tetrahydrofuran) and its isomer, ST-3 (*S*,*R*-hydroxymethylsesaminol-tetrahydrofuran), were isolated and identified.

Stability of Sesaminol in Simulated Gastric and Intestinal Fluids. The stability of sesaminol was investigated in SGF and SIF, according to the method described previously.[17,18] Briefly, to pre-incubated media of either simulated gastric (19 mL, pH 1.2) or intestinal fluids (19 mL, pH 6.8) containing DMSO (1 mL) was added a solution of sesaminol in a final concentration of 0.5 mM. DMSO was required to solubilize the compounds under experimental conditions. The resulting mixtures were incubated at 37 °C on a thermostat orbital mixer (Ratek Instruments, Australia). Samples were taken at specified time intervals and analyzed by HPLC. Sesaminol and episesaminol were prepared by HPLC. The HPLC system consisted of a Hitachi L-2130 pump (Tokyo, Japan) and a Hitachi L-2400 UV detector (Tokyo, Japan). Data calculations were performed using a SISC program (Taipei, Taiwan). The Astec CHIROBIOTIC T Chiral Column (100 mm × 3.0 mm, 5 μm pore size; Advanced Separation Technologies, USA) was maintained at 35 °C. The detector

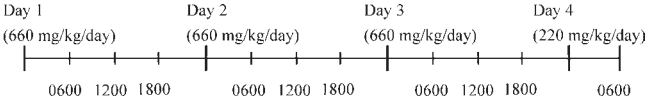
wavelength was 290 nm. The solvent system used was (A) water and (B) methanol. The linear gradient program used was the following: flow rate = 1.0 mL/min; 0−25 min, 60% A and 40% B; after 25 min, 0% A and 100% B. Injection volume was 200 μL. UV−visible spectra were obtained using a Helios Alpha UV−vis spectrophotometer (Thermo Electron Co., Waltham, MA). The ^{1}H NMR and ^{13}C NMR (500 MHz) spectra were recorded using a Bruker Avance-500 MHz FT-NMR spectrometer (Bruker Co., Rheinstetten, Germany).

Sesaminol. UV λ^{MeOH} = 292; MW = 370; ESI$^-$-MS, *m*/*z* 369 [M − H]$^-$; ^{1}H (500 MHz, $CDCl_3$) δ 3.14 (m, 2H), 3.87 (dd, *J* = 3.2, 8.9 Hz, H), 3.91 (dd, *J* = 2.7, 8.9 Hz, H), 4.33 (dd, *J* = 1.8, 7.2 Hz, H), 4.15 (dd, *J* = 6.6, 9.7 Hz, H), 4.75 (d, *J* = 3.3 Hz, 2H), 5.88 (s, H, O−CH_2−O), 5.87 (s, H, O−CH_2−O), 5.94 (s, 2H, O−CH_2−O), 6.43 (s, 1H), 6.49 (s, 1H), 6.78 (d, *J* = 10.5 Hz, 1H), 6.79 (dd, *J* = 1.4, 8.5 Hz, 1H), 6.85 (d, *J* = 1.4 Hz, 1H); ^{13}C (125 MHz, $CDCl_3$) δ 119.6, 150.6, 99.4, 140.9, 148.07, 105.6, 85.3, 53.4, 72.4, 134.5, 106.1, 150.6, 148.0, 108.2, 119.3, 86.5, 52.9, 70.6, 101.2 (O−CH_2−O), 101.1 (O−CH_2−O).

2-Episesaminol. UV λ^{MeOH} = 292; MW = 370; ESI$^-$-MS, *m*/*z* 369 [M − H]$^-$; ^{1}H (500 MHz, $CDCl_3$) δ 2.87 (m, 1H), 3.34 (m, 1H), 3.47 (t, *J* = 9.4 Hz, 1H), 3.84 (dd, *J* = 7, 10 Hz, 1H), 3.98 (t, *J* = 8.9 Hz, 1H), 4.12 (dd, *J* = 3.2, 9.7 Hz, 1H), 4.40 (d, *J* = 7 Hz, 1H), 4.94 (d, *J* = 6 Hz, 1H), 5.87 (s, 2H, O−CH_2−O), 5.94 (s, 2H, O−CH_2−O), 6.39, (d, *J* = 8.7, 1H), 6.43 (s, 1H), 6.79 (d, *J* = 1.4 Hz, 2H), 6.85 (s, 1H); ^{13}C (125 MHz, $CDCl_3$) δ 119.6, 150.7, 99.5, 141.0, 148.1, 105.6, 84.3, 50.5, 71.7, 134.6, 106.1, 150.7, 148.1, 108.2, 119.3, 87.5, 53.5, 69.9, 101.1 (O−CH_2−O), 101.1 (O−CH_2−O).

Animals and Diets. The experimental protocol was approved by the National Laboratory Animal Center (Taipei, Taiwan). Inbred male Sprague−Dawley (SD) rats (body weight = 275 ± 25 g, mean ± SD) were housed in pairs in cages in a room with controlled temperature (20−22 °C) and relative humidity (50−70%) and a 12 h light/dark cycle (lights on at 7:00 a.m.). The rat diet was AIN 93 M diet (Purina Mills, St. Louis, MO). Rats consumed their food ad libitum and had unlimited access to water; their weight and food consumption were recorded weekly.

Distribution Experiment. Five groups (1, 3, 6, 9, and 24 h) of animals (*n* = 6) were administered, via gastric gavage, 220 mg/kg body weight sesaminol dissolved in 50% propylene glycol for 4 days in three daily doses (660 mg/kg/day), for the following dosing schedule:



After consuming the sesaminol diet for 4 days, rats were anesthetized in the morning of the fourth day (2200 mg/kg, 10 doses), without overnight fasting, using CO_2 as a carrier. The plasma, small intestine, cecum, colon, and rectum were collected at 24 h on the fourth day after administration. The intestines of the rats were rinsed free of their contents, which is standard procedure for estimating intestinal tissue concentrations. Rats were fully bled via the abdominal aorta. Blood (8−12 mL) was collected in heparin tubes, and plasma was subsequently prepared in centrifuge tubes by centrifuging for 20 min at 1000*g* and 4 °C. After blood collection, the tissues were dissected, weighed, and immediately frozen in liquid nitrogen.

The plasma and intestinal samples were prepared and analyzed according to a previously described method.[19,20] The contents of sesaminol conjugated metabolites were processed and analyzed as plasma and tissues. Plasma and intestinal tissues of rat were homogenized (Polytron) with ACN. The extracts were transferred to a 25 mL tube and centrifuged for 10 min at 10000*g* and 4 °C. The organic solvent from the supernatant was evaporated at 50 °C. Subsequently, the residues were dissolved with 1 mL of 0.1 N NaOAc (pH 5.0, with 200 mg/mL ascorbic acid) buffer. Each sample (100 μL) was hydrolyzed with 50 μL of enzyme [1000 U of sulfatase activity in 0.1 N NaOAc buffer (pH 5.0, with 200 mg/mL ascorbic acid)] for incubation at 37 °C. At the termination of hydrolysis, the pH was adjusted to 7.4. All samples were deproteinized with 250 μL of internal standard (hesperetin, 5 μg/mL in ACN) solution and centrifuged for 10 min at 10000*g* and 4 °C. After centrifugation, 20 μL of supernatant was injected into the LC-MS system.

Excretion Experiment. Rats (*n* = 6) were administered, via gastric gavage, 220 mg/kg body weight sesaminol in 50% propylene glycol for 4 days. The provided time of day was the same as in the distribution experiment. Urine and feces from rats housed in metabolic cages were collected at the following time periods: 0−4, 4−8, 8−12, and 12−24 h after administration of sesaminol. After consuming the sesaminol diet for 4 days, rats were anesthetized in the morning, without overnight fasting, using CO_2 as a carrier. Ascorbic acid (200 mg/mL) was added to urine samples, which were then stored at −80 °C until analysis.

Urinary and fecal sesaminol conjugated metabolite analysis was performed using a previously described method with slight modifications in rat excreta.[19,20] Briefly, 2 mL of 0.1 mol/L sodium acetate buffer (pH 4.5) and 50 μL of 1000 U sulfatase activity in 0.1 mol/L sodium acetate buffer were added to urine or feces extracts, and the mixture was incubated in a 37 °C water bath overnight to hydrolyze the sesaminol conjugates. At the termination of hydrolysis, the pH was adjusted to 7.4. Subsequently, all samples were deproteinized by centrifuging for 10 min at 10000*g* and 4 °C. Two hundred and fifty microliters of internal standard (hesperetin, 2.0 μg/mL in ACN) solution was added to each sample before deproteinization. After centrifugation, 20 μL of supernatant was subjected to LC-MS analysis.

Determination and Identification of Sesaminol and Its Metabolites in Tissues and Excreta. Identification of sesaminol metabolites was carried out by LC-MS/MS analysis. These analyses were performed on a Thermo HPLC system equipped with an electrospray-ionization (ESI) ion trap mass spectrometer (ThermoFinnigan LXQ Advantage, San Jose, CA). The separation was achieved using a YMC Hydrosphere C18 column (2.0 × 150 mm i.d.; 5 μm, YMC, Tokyo, Japan). For operation in the MS/MS mode, a mass spectrometer with ESI was used. During the analyses, the ESI parameters were set as follows: capillary voltage, −49 V for negative mode; source voltage, 4.5 kV; source current, 100 μA; sheath gas flow rate, 35 au; capillary temp, 350 °C; tube lens voltage, −110 V. The collision energy of the tetrahydrofuranoid metabolites was adjusted to 30% to maximize the intensity of the deprotonated molecular ion (precursor). Collision energy was also adjusted to optimize the product ion signals as 30% for sesaminol metabolite analysis.

MS/MS product ions were produced by the collision-activated dissociation of selected precursor ions in the collision cell of a two-dimensional linear ion trap mass spectrometer. The mass was analyzed using the second analyzer of the instrument.

Selected reaction monitoring, the method of choice because it has the highest selectivity and sensitivity in quantitative LC-MS/MS, was used to monitor six transitions for each analysis: sesaminol and episesaminol, m/z 369 → 271; hydroxymethylsesaminol-tetrahydrofuran m/z 359 → 221; enterodiol, m/z 301 → 253; enterolactone, m/z 297 → 253; sesaminol-6-catechol, 357 → 207; methylated sesaminol-6-catechol, 371 → 191. All LC-MS/MS data were processed by Xcalibur version 2.0 data acquisition software.

The analytical methods of determining sesaminol and its metabolites (sesaminol glucuronide/sulfate, hydroxymethylsesaminol-tetrahydrofuran, ENL, and END) in tissues were developed and validated in this study. The coefficients of variation of intraday and interday assays were <5% and in the concentration range of 0.1−20.0 μg/mL. For the analysis of sesaminol, hydroxymethylsesaminol-tetrahydrofuran (ST-2 and ST-3), ENL, and END, the lower limits of quantitation were 0.2, 0.3, 0.1, and 0.3 μg/mL and the limits of detection were 0.05, 0.07, 0.03, and 0.04 μg/mL, respectively.

Statistical Analysis. All samples were extracted in triplicate. Sesaminol and its metabolites concentrations were expressed in the concentrations of intestines, feces, plasma, and urine. Intestines of six rats were pooled before analysis. Data were analyzed by t test and ANOVA analysis of variance, and differences were considered to be statistically significant at $P < 0.05$.

RESULTS

Stability of Sesaminol in Simulated Gastric and Intestinal Fluids. In simulated gastric and intestinal fluids at 37 °C sesaminol underwent transformation to 2-episesaminol (Figure 2). Sesaminol appears to be catalyzed by acid, which is transformed to an epimer under gastric conditions. Sesaminol showed approximately 35% epimerization in SGF within the first hour of incubation. There was no significant difference in the transformation of sesaminol triglycoside with SGF. Sesaminol is most stable in SIF at neutral pH.

In SGF, sesaminol and its transformation product gave the same molecular ion peak, $[M - H]^-$ 369, in mass spectra and had the same fragmentation patterns. Using the results of mass spectroscopy, they were presumed to be the stereoisomers of sesaminol. The differences in their heteronuclear single quantum correlation (HSQC) and heteronuclear multiple bond correlation (HMBC) were thought to be caused by stereochemical changes of the protons and carbon on the tetrahydrofurofuran ring. To confirm their stereochemical configuration, ^{1}H NMR with homo-decoupling analysis was applied.

The spectrometric property of peak 1 (Figure 2A) was identical with those of the authentic sesaminol previously isolated from sesame seeds[4] using both the ^{1}H NMR spectrum and mass spectrum. The stereochemical structure of the tetrahydrofurofuran ring of sesaminol was consequently determined to be H-2 axial and H-6 axial. The structure of episesaminol (peak 2) isolated from simulated gastric fluid can be clearly established by ^{1}H and ^{13}C NMR spectral data. In the ^{1}H NMR spectrum, episesaminol showed a pattern identical to that of sesaminol except for H-l, which was shifted downfield by the phenolic OH group being structurally closer to H-l. Each proton on the tetrahydrofurofuran ring can be assigned (Table 1). A methine proton at H-5 appeared as a multiplet at δ 2.87 and was observed to couple with H-4a (1H, dd, J = 10, 7) at δ 3.84, H-4e (1H, dd, J = 3.2 and 9.7) at δ 4.12, H-6 (1H, d, J = 7) at δ 4.40, and H-l (1H, m) at δ 3.34. Another multiplet at δ 3.34 was H-l, which was coupled with H-8a (1H, t, J = 9.4) at δ 3.47, H-8e (1H, t, J = 8.9) at δ 3.98, H-2 (1H, d, J = 6) at δ 4.94, and H-5 (1H, m) at δ 2.87 (Table 1). From these data, peak 2 was assigned to be an epimer of sesaminol.

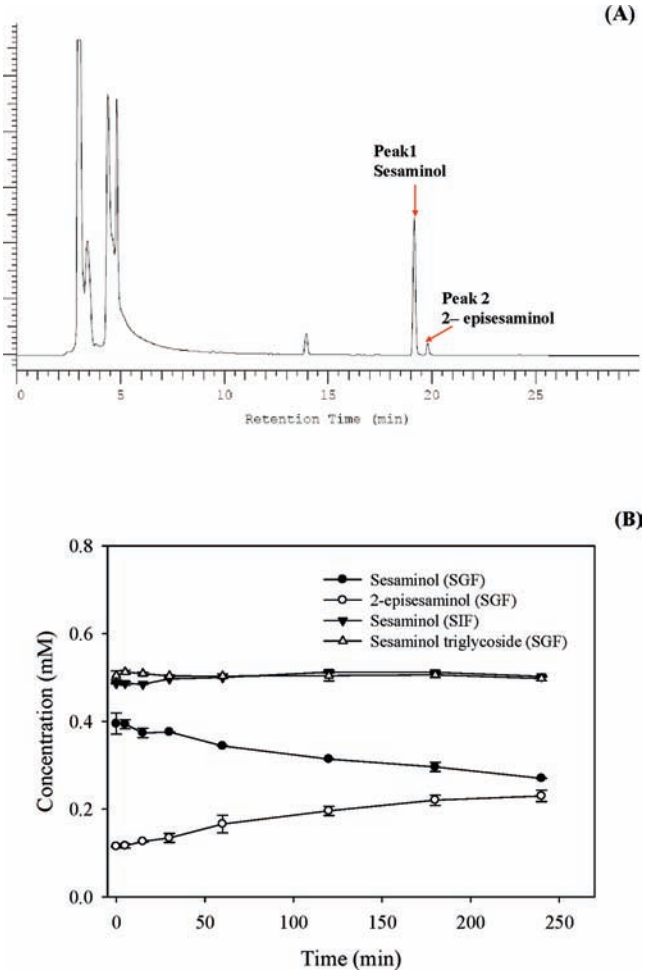


Figure 2. HPLC chromatograms (A) and time-dependent changes of concentration of sesaminol and sesaminol triglycoside (B) in digestive stability assay [simulated gastric fluid (●, △, and ○) and intestinal fluids (▼)].

The presence of H-2 equatorial and H-6 axial on this tetrahydrofurofuran ring caused upfield shifts in positions C-2 (δ 86.5 → 84.3), C-4 (δ 72.4 → 71.7), and C-8 (δ 70.6 → 69.9) and a downfield shift in position C-6 (δ 85.4 → 87.5) compared with the corresponding positions. ^{1}H NMR showed *meta*-coupling between H-8/H-5 and H-4/H-1 (J = 3.2 Hz) and *ortho*-coupling between H-6/H-5 and H-8/H-1 (J = 8.9 Hz) on this tetrahydrofurofuran ring (Table 1). On the tetrahydrofurofuran ring of the molecule, HMBC showed coupling between H-6 (δ 4.40)/H-1 (δ 3.34) and C-4 (δ 71.7). The position of C-8 (δ 69.9) was assigned from couplings with H-2 (δ 4.94) and H-1 (δ 3.34). The position of C-2 (δ 84.3) was assigned from couplings with H-4e (δ 4.12), H-8e (δ 3.98), and H-8a (δ 3.47), and position of C-6 (δ 87.5) was assigned from couplings with H-8e (δ 3.98), H-4e (δ 4.12), and H-4a (δ 3.84) (Figure 3). The aromatic carbons were assigned from couplings to attached protons in HSQC. Thus, the structure of episesaminol was established as 2-episesaminol.

Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data of Sesaminol and 2-Episesaminol Formed in the Incubation Mixture of Simulated Gastric Fluid

sesaminol				2-episesaminol			
δ_C		δ_H		δ_C		δ_H	
C-5	53.4	H-5, H-1	3.14 (2H, m)	C-5	53.5	H-5	2.87 (1H, m, H-5)
C-1	52.9			C-1	50.5	H-1	3.34 (1H, m, H-1)
C-8	70.6	H-8a	3.91 (1H, dd, J = 2.7, 8.9)	C-8	69.9	H-8a	3.47 (1H, t, J = 9.4)
C-4	72.4	H-4a	3.87 (1H, dd, J = 3.2, 8.9)	C-4	71.7	H-4a	3.84 (1H, dd, J = 10, 7)
C-8	70.6	H-8e	4.15 (1H, dd, J = 6.6, 9.7)	C-8	69.9	H-8e	3.98 (1H, t, J = 8.9)
C-4	72.4	H-4e	4.33 (1H, dd, J = 1.8, 7.2)	C-4	71.7	H-4e	4.12 (1H, dd, J = 3.2, 9.7)
C-2	86.5	H-6, H-2	4.75 (2H, d, J = 3.3)	C-2	84.3	H-2	4.94 (1H, d, J = 6)
C-6	85.4			C-6	87.5	H-6	4.40 (1H, d, J = 7)

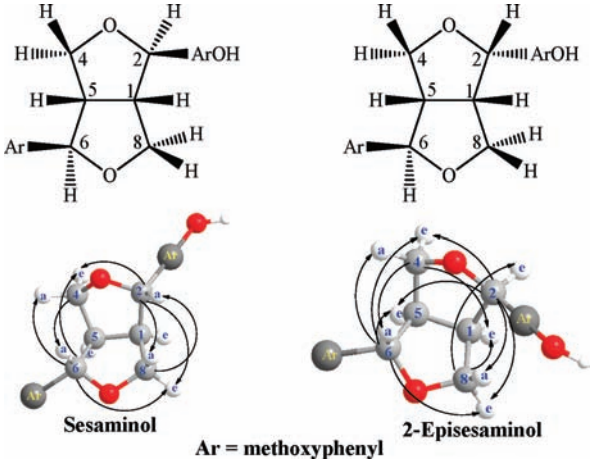


Figure 3. HMBC correlations of sesaminol and 2-episesaminol.

Distribution Study. To investigate the distribution of sesaminol in rats, the concentrations of sesaminol and its metabolites were determined in intestinal tissues and plasma within 9 h after administration of sesaminol to rats. The plasma concentrations of sesaminol and its metabolites were analyzed by LC-MS/MS (Figure 4). In the intestines, the concentration of sesaminol and its conjugated metabolites reached a maximum at 180−540 min after administration and were rarely found after 9 h. However, the concentrations of sesaminol were significantly greater than those of sesaminol conjugated metabolites in intestinal tissues. Sesaminol was widely distributed in rat intestines, with the highest concentration in the large intestine and cecum and the lowest in the small intestine (Figure 5). In the plasma, sesaminol conjugated metabolites concentrations were significantly greater than that of sesaminol, and the highest concentration was found 60 min after administration.

After administration, sesaminol was converted to hydroxymethylsesaminol-tetrahydrofuran (ST-2 and ST-3), sesaminol-6-catechol, methylated sesaminol-6-catechol, END, and ENL by intestinal microflora.[12,13] From LC-ESI-MS analysis of rat plasma, sesaminol was found to be converted to other tetrahydrofuranoid lignans (sesaminol-6-catechol and methylated sesaminol-6-catechol) by liver enzymes (Figure 4). After 24 h, the 2-episesaminol and tetrahydrofuranoid lignans (hydroxymethylsesaminol-tetrahydrofuran, END, and ENL) were found to be higher in the cecum than in other intestines in the rat (Table 2).

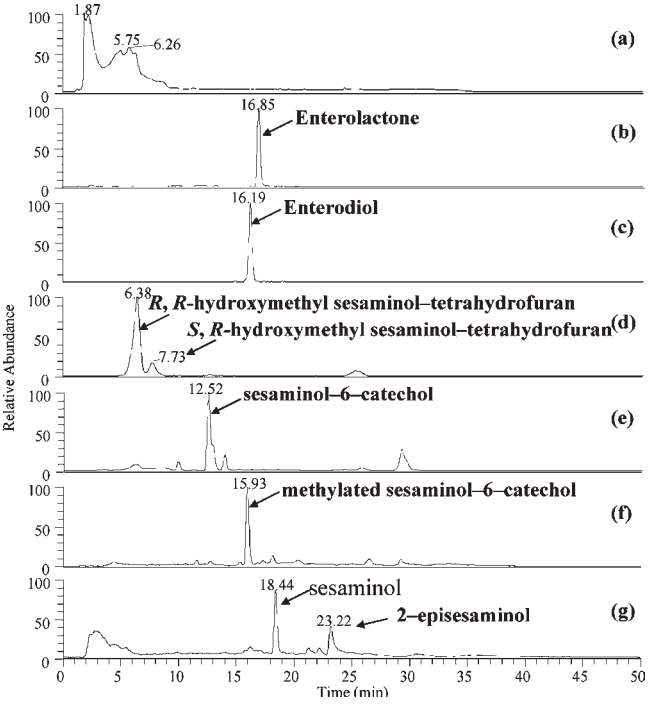


Figure 4. HPLC and SRM chromatograms of the analyzed sesaminol and its metabolites in rat plasma: (a) A280; (b) ENL; (c) END; (d) hydroxymethylsesaminol-tetrahydrofuran; (e) sesaminol-6-catechol; (f) methylated sesaminol-6-catechol; (g) sesaminol and 2-episesaminol.

In the cecum, the concentrations of hydroxymethylsesaminol-tetrahydrofuran, END, and ENL were found to be 18.3 ± 3.40, 3.24 ± 0.33, and 5.62 ± 0.69 μmol/g, respectively. Oral ingestion of sesaminol by rats resulted in the metabolism of the respective sesaminol stereoisomer (2-episesaminol and *S,R*-hydroxymethylsesaminol-tetrahydrofuran) from the stomach and distal intestine and its subsequent appearance in plasma. Analyzing plasma samples demonstrated that the content of the sesaminol was higher than that of its stereoisomer in circulation. After the administration of sesaminol, the resulting plasma levels of sesaminol and its metabolites differed significantly, ranking as follows: sesaminol > ENL > END > 2-episesaminol > *R,R*-hydroxymethylsesaminol-tetrahydrofuran > *S,R*-hydroxymethylsesaminol-tetrahydrofuran. This study shows that sesaminol is modified by gastric conditions and that a small amount of

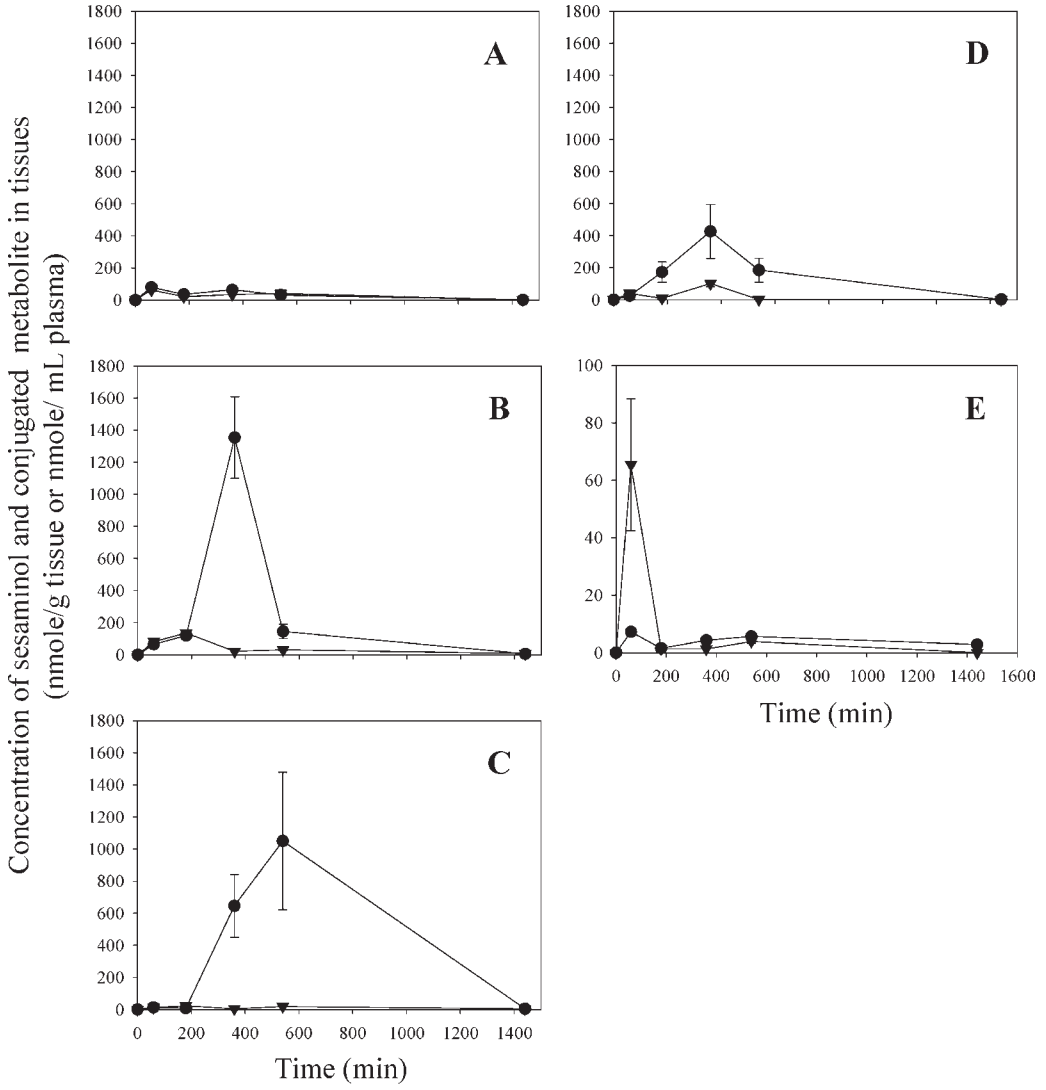


Figure 5. Time-dependent changes of sesaminol (●) and sesaminol glucuronide/sulfate (▼) concentrations in different rat tissues after oral administration of sesaminol (220 mg/kg): (A) small intestine; (B) cecum; (C) large intestines; (D) rectum; (E) plasma.

Table 2. Intestine Concentration of Tetrahydrofuranoid Metabolites in Rat at 24 h[a]

	concn (μmol/mL or μmol/g)				
	plasma	small intestine	cecum	large intestine	rectum
sesaminol	2.85 ± 0.51a	0.71 ± 0.20ab	5.76 ± 1.81b	4.56 ± 0.60a	1.63 ± 0.05b
2-episesaminol	0.81 ± 0.15b	0.24 ± 0.08d	2.61 ± 0.80de	1.69 ± 0.30d	0.44 ± 0.01f
ST-2	0.19 ± 0.02e	0.66 ± 0.13ab	18.3 ± 3.40a	2.63 ± 0.25bc	2.87 ± 0.27a
ST-3	0.12 ± 0.01f	0.19 ± 0.04de	2.50 ± 0.30de	0.36 ± 0.05e	0.52 ± 0.06e
enterodiol	0.39 ± 0.02d	0.44 ± 0.04c	3.24 ± 0.33d	1.55 ± 0.40d	0.84 ± 0.06d
enterolactone	0.57 ± 0.09c	0.85 ± 0.19a	5.62 ± 0.69bc	3.71 ± 1.11ab	1.28 ± 0.09c

[a] Data are expressed as the mean ± SD. Data in the same row with different letters are significantly different at $p < 0.05$.

sesaminol epimer is absorbed into systemic circulation. Furthermore, the microbial populations, in particular in the colon, are likely to modify the molecular structure of sesaminol (*R,R*-hydroxymethylsesaminol-tetrahydrofuran, ENL, and END).

Excretion Study. To investigate the excretion of sesaminol in rats, the concentrations of sesaminol were determined in urine and feces within 24 h after administration to rats. Sesaminol conjugated metabolites were mainly excreted in rat excreta. In urinary excretion (Table 3), the concentrations of sesaminol conjugated metabolites were significantly higher than the concentrations of sesaminol. The highest concentrations of sesaminol conjugated metabolites were found at 0−8 h after administration.

Table 3. Individual Urinary Excretion of Sesaminol and Its Conjugated Metabolites during Each Time Interval after Oral Administration of Sesaminol 220 mg/kg

	concn[a] (μmol/mL)	
time (h)	sesaminol	sesaminol glucuronide/sulfate
0−4 (4 h)	1.00 ± 0.07	66.2 ± 8.40*
4−8 (4 h)	15.6 ± 1.38	43.4 ± 3.87*
8−12 (4 h)	27.2 ± 3.14	29.9 ± 4.02
12−16 (4 h)	1.28 ± 0.05	16.0 ± 1.61*
16−24 (8 h)	2.12 ± 0.25	18.8 ± 1.38*
% of dose	0.02 ± 0.01	0.08 ± 0.01

[a] Data are expressed as the mean ± SD ($n = 6$). *, $p < 0.05$ compared with group sesaminol.

Table 4. Individual Fecal Excretion of Sesaminol and Its Conjugated Metabolites during Each Time Interval after Oral Administration of Sesaminol 220 mg/kg

	concn[a] (mmol/g)	
time (h)	sesaminol	sesaminol glucuronide/sulfate
0−4 (4 h)	1.32 ± 0.01	3.09 ± 0.32*
4−8 (4 h)	2.59 ± 0.43	4.81 ± 0.72*
8−12 (4 h)	3.09 ± 0.35	11.8 ± 1.65*
12−16 (4 h)	11.3 ± 1.54	0.64 ± 0.05*
16−24 (8 h)	2.85 ± 0.48	0.15 ± 0.02*
% of dose	9.33 ± 1.24	9.03 ± 1.22

[a] Data are expressed as the mean ± SD ($n = 6$). *, $p < 0.05$ compared with group sesaminol.

The majority of sesaminol conjugated metabolites was excreted in the urine. The percentage of conjugated metabolites excreted in urine in rats during 0−24 h accounted for 0.08 ± 0.01% of the total excretion. Urinary amounts of sesaminol and sesaminol conjugated metabolites increased in response to sesaminol ingestion, reaching a peak at 0−12 h. The unchanged sesaminol was present in very low concentrations and represented only 0.02% up to 24 h after administration.

In the feces (Table 4) the concentrations of sesaminol and its conjugated metabolites reached maxima at 12−16 and 8−12 h, respectively. The percentages of sesaminol and sesaminol conjugated metabolites excreted in feces (Table 4) in rats were highest in the first 24 h, accounting for 9.33 ± 1.24 and 9.03 ± 1.22% of the total excretion for sesaminol and sesaminol conjugated metabolites, respectively. From LC-MS-MS analysis of rat excreta, sesaminol was found to be converted to tetrahydrofuranoid lignans (*R,R*-hydroxymethylsesaminol-tetrahydrofuran, ENL, and END) by rat intestinal microflora or liver enzymes. After 24 h, the tetrahydrofuranoid lignans were found in higher concentration in the feces than in the plasma in rat (Table 5). In the feces, the concentrations of hydroxymethylsesaminol-tetrahydrofuran, END, and ENL were 2.97 ± 0.47, 3.44 ± 0.22, and 4.13 ± 0.35 μmol/g, respectively. Paralleling the results from plasma analyses, the stereochemical configuration of the sesaminol consumed influenced the levels of metabolites detected in urine (Table 5). The stereoisomer that was found in

Table 5. Excreta Concentration of Tetrahydrofuranoid Metabolites in Rat at 24 h

	concn[a] (μmol/mL or μmol/g)	
metabolite	urine	feces
2-episesaminol	0.36 ± 0.19	0.76 ± 0.13*
ST-2	0.27 ± 0.04	2.97 ± 0.47*
ST-3	0.12 ± 0.01	1.52 ± 0.24*
enterodiol	0.32 ± 0.01	3.44 ± 0.22*
enterolactone	0.33 ± 0.01	4.13 ± 0.35*

[a] Data are expressed as trhe mean ± SD. *, $p < 0.05$ compared with group urine.

the lowest urinary levels postingestion was 2-episesaminol and *S,R*-hydroxymethylsesaminol-tetrahydrofuran in 24 h. It is concluded that sesaminol and its metabolites could be either incorporated into the liver and then transported to the kidney or excreted in bile and go into enterohepatic circulation to be eventually excreted in feces or urine. Only trace quantities of tetrahydrofuranoid lignans were detected in the cecum, large intestine, and feces, but they were absent in extracts of urine. Overall, however, absorption and excretion were associated with extensive metabolism of sesaminol.

DISCUSSION

The results from this study demonstrate that the stereochemical characteristics of a compound markedly influence its action on the digestive system. Many studies report the dependence of stereochemical configuration on the absorption and excretion of flavanols, and factors contributing to the observed variation have been identified. The stereochemical configuration of flavanols, via differences in absorption, distribution, metabolism, and excretion, could be a key factor, partially accounting for these disparities. In previous studies reporting oral absorbability of flavanol stereoisomers following their oral intake, the ranking of flavanols was found to be (−)-epicatechin > (+)-epicatechin = (+)-catechin > (−)-catechin.[21−23] Catechins were subjected to in vitro gastric and small intestinal digestion. EGCG, EGC, and ECG were significantly degraded, with losses of 71−91, 72−100, and 60−61%, respectively.[24] Catechins are prone to epimerization, oxidative degradation, and polymerization under high-pH conditions. In alkaline conditions, the catalytic epimerizations of (−)-epicatechin to (−)-catechin and of (+)-catechin to (+)-epicatechin have been observed.[25,26] On the other hand, the synthesized flavan-3-ol *C*-glycosides were found to be stable in simulated gastric fluid, but underwent degradation in colostomy fluid.[27] Uniquely, episesaminol was absorbed more poorly than sesaminol in SD rats. The results from this study demonstrate that stereochemical characteristics of lignan markedly influence its plasma and intestine levels. Our results show that there are significant dissimilarities between sesaminol and the tetrahydrofuranoid metabolites with regard to their absorption fate in rats, as reflected by their concentrations present in plasma and intestines. The major sites of absorbability are stomach and proximal intestines for sesaminol and 2-episesaminol. Additionally, the tetrahydrofuranoid metabolites need to be metabolized and absorbed in the distal intestines. Moreover, sesaminol and 2-episesaminol differed significantly from each other with regard to the levels of conjugated metabolites present in plasma, strongly suggesting that sesaminol affects phase II metabolic

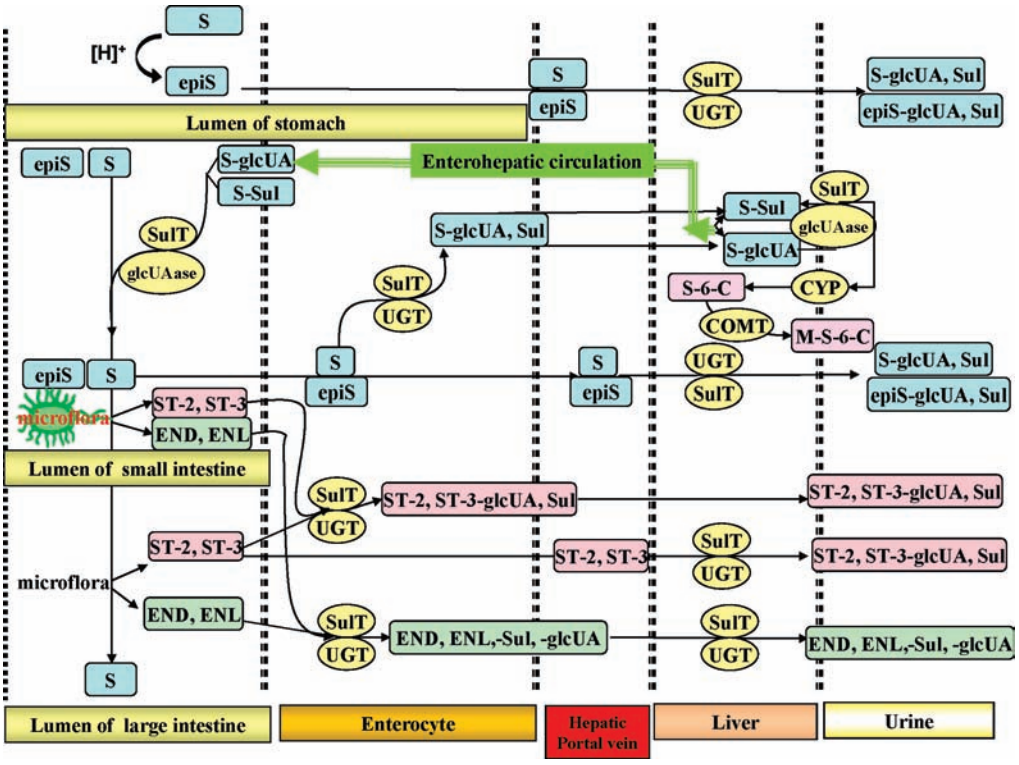


Figure 6. Schematic of the proposed metabolic fate of sesaminol and its metabolites as they pass from the lumen of the intestine into the hepatic portal vein, where they are further metabolized before returning to the bloodstream and being excreted in urine via the kidneys or in feces via enterohepatic circulation. S, sesaminol; episS, 2-episesaminol; S-6-C, sesaminol-6-catechol; M-S-6-C, methylated sesaminol-6-catechol; ST-2, *R,R*-hydroxymethylsesaminol-tetrahydrofuran; ST-3, *S,R*-hydroxymethylsesaminol-tetrahydrofuran; END, enterolactone; ENL, enterodiol; glcUA, glucuronide; Sul, sulfate; UGT, glucuronyltransferase; SulT, sulfotransferase; glcUAase, glucuronidase; Sulase, sulfatase; CYP, cytochrome P450; COMT, catechol-*O*-methyltransferase.

pathways (sulfation and glucuronidation) more than its 2-epimer in vivo. These results showed the epimerization of sesaminol present in the gastrointestinal tract, due to low-pH condition. The levels of 2-episesaminol were very low in plasma, whereas it had poor bioavailability. We also observed that sesaminol triglucoside was stable under acidic conditions, and no epimerization was found. This may be due to the stabilization of the lignin structure by sugar moiety.

Figure 6 is a representation of the absorption, distribution, metabolism, and excretion of sesaminol based on current knowledge. Sesaminol can be transformed to epimer in the stomach after ingestion and enter the systemic circulation after passing through the liver. Absorbed sesaminol enters the systemic circulation after passage through the liver and being metabolized. Sesaminol was metabolized to sesaminol-6-catechol and methylated sesaminol-6-catechol. A portion of the sesaminol and its metabolites go a step further to be conjugated by sulfation and glucuronidation. These conjugated metabolites were transported to the intestine as bile via enterohepatic circulation. Events that occur following ingestion are discussed; activities of colonic microbiota have been shown to generate other metabolites. It has also been shown that deconjugations catalyzed by intracellular enzymes can occur during gastrointestinal absorption, thereby releasing sesaminol. Another portion of sesaminol that is not absorbed from the small intestine moves into the distal small intestine, where it is metabolized to hydroxymethylsesaminol-tetrahydrofuran (ST-2 and ST-3), END, and ENL by microflora. Further absorption appears to take place in the distal intestine (cecum, large intestine, and rectum), where they undergo very limited absorption. Although there have been studies investigating the absorption/bioavailability of lignan, compared with sugar moieties of the lignans, comparatively little is known about absorption and metabolism of lignans. Sesaminol triglycoside are large, highly water-soluble molecules that have been considered less likely to be absorbed in the gastrointestinal tract.[19] However, the distribution data discussed above demonstrate that sesaminol was apparently absorbed from the small intestines into the circulatory system and excreted in urine. Nevertheless, sesaminol was not dispersed and metabolized to any extent in water-soluble conditions (gastrointestinal fluids). Comparison between sesaminol triglycoside and sesaminol with the metabolites (*R,R*-hydroxymethylsesaminol-tetrahydrofuran, END, and ENL) shows that lignan glycosides have higher metabolized efficiencies. However, the absorption efficiency of aglycone from sesaminol triglycoside was low in the distal intestine, and variations in absorption were observed between different intestines. Moreover, the aglycone from sesaminol triglycoside that reached the colon was exposed to a substantial microbial population and might be metabolized to hydroxymethylsesaminol-tetrahydrofuran (ST-2 and ST-3), with the tetrahydrofuranoid lignan further metabolized by disruption of the tetrahydrofuran structure to yield ENL and END. In contrast to the previous studies, this study recovered 4−10 times less enterolignan in feces and urine than was expected from the intake of precursors.[14,19] The tetrahydrofuranoid metabolites derived from the ingested sesaminol may contribute to comprehend the pathway of absorption and metabolism.

AUTHOR INFORMATION

Corresponding Author

*E-mail: (C.-T.H.) ho@aesop.rutgers.edu or (L.S.H.) lshwang@ntu.edu.tw.

Funding Sources

This study was supported by research grants NSC 94-2313-B-002-016 from the National Science Council and 100-EC-17-A-03-04-0332 from the Ministry of Economic Affairs, Taiwan, Republic of China.

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