Sesamin Is One of the Major Precursors of Mammalian Lignans in Sesame Seed (*Sesamum indicum*) as Observed In Vitro and in Rats[1,2]

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ABSTRACT Plant lignans occur in many foods, with flaxseed presently recognized as the richest source. Some plant lignans can be converted by intestinal microbiota to the mammalian lignans, enterodiol and enterolactone, which may have protective effects against hormone-related diseases such as breast cancer. This study determined whether plant lignans in sesame seed, particularly sesamin, could be metabolized to the mammalian lignans. The total plant lignan concentration in sesame seed (2180 μmol/100 g) was higher than that in flaxseed (820 μmol/100 g). In vitro fermentation with human fecal inoculum showed conversion of sesamin to the mammalian lignans, although at a lower rate (1.1%) compared with that of secoisolariciresinol diglucoside (57.2%). However, when fed to female Sprague-Dawley rats for 10 d, sesamin (15 mg/kg body weight) and a 10% sesame seed diet resulted in greater ($P < 0.05$) urinary mammalian lignan excretion (3.2 and 11.2 μmol/d, respectively), than the control (< 0.05 μmol/d). We conclude that sesame seed is a rich source of mammalian lignan precursors and sesamin is one of them. From intermediate metabolites of sesamin identified in rat urine by GC-MS, a tentative metabolic pathway of sesamin to mammalian lignans is suggested. J. Nutr. 136: 906–912, 2006.

KEY WORDS: • *sesamin* • *sesame lignans* • *mammalian lignans* • *metabolism* • *sesame seed*

The mammalian lignans, enterolactone (EL)[5] and enterodiol (ED) were shown to possess weak estrogenic and antiestrogenic activities that may protect against hormone-dependent diseases such as breast and prostate cancer (1,2). They are produced from dietary plant lignans such as secoisolariciresinol diglucoside (SDG) or its aglycone secoisolariciresinol (SECO), matairesinol, 7-hydroxymatairesinol, lariciresinol, and pinoresinol (3,4) by the action of intestinal microbiota of humans and animals (5–9). Flaxseed is currently known as the richest source of mammalian lignan precursors compared with other plant foods (10,11).

Sesame seed (*Sesamum indicum*), a traditional health food in Asian countries, contains large amounts of the plant lignans sesamin, sesamolin, and sesaminol glucosides (12,13). Sesamin, the major fat-soluble lignan in sesame seed, affects lipid metabolism (14–16) and has antihypertensive (17–20), and anticancer activities (21,22). However, only a limited number of studies have examined the metabolism of sesame seed lignans (23–28). Nakai et al. (23) reported that sesamin undergoes cleavage of methylenedioxyphenyls to catechol or methoxy catechol structures in rat liver. In a recent study, Penalvo et al. (26) demonstrated the conversion of sesamin to mammalian lignans ED and EL by in vitro fecal fermentation and the increased plasma EL and ED levels in healthy volunteers after sesame seed ingestion. In our previous study in postmenopausal women (27), a significant increase in urinary mammalian lignan excretion similar to that after ingestion of flaxseed was detected after the ingestion of sesame seed.

In this study, we investigated for the first time whether sesamin, a lignan isolated from sesame seed, is converted to the mammalian lignans ED and EL in vivo in rats and compared the lignan values with those after sesame seed ingestion. In addition, we compared the conversion of sesamin to ED and EL with that of SDG using in vitro fermentation with human fecal inoculum.

MATERIALS AND METHODS

Chemicals. Sesamin (a mixture of sesamin and episesamin, 54:46, wt:wt) was from Takemoto Oil & Fat. SDG was isolated and purified from flaxseed as described previously (29). ED and EL (>99.5%) were synthesized in the laboratory of Dr. M. Lautens (Department of Chemistry, University of Toronto, ON, Canada). Sesamin (>98%) and sesamolin (>98%) standards were isolated and purified in our laboratory from sesame oil, and sesaminol (>96%) from defatted

[1] Presented in part at Experimental Biology 05, April 2005, San Diego, CA [Liu Z, Coulman KD, Saarinen NM, Thompson, LU. Sesame seed is a richer source of mammalian precursors than flaxseed (abstract). FASEB J. 2005;19:A449].

[2] Supported in part by Natural Sciences and Engineering Research Council of Canada Grant A9995, National Technology Agency of Finland (TEKES) Grant 40285/02, and Robin Hood Multifoods Company. The authors have no conflict of interest.

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[5] Abbreviations used: ED, enterodiol; EL, enterolactone; SDG, secoisolariciresinol diglucoside; SECO, secoisolariciresinol; d_9-BSA, deuterated *N,O*-bis(trimethyl-d_9-silyl)acetamide; TMS, trimethyl silylation.

Manuscript received 29 September 2005. Initial review completed 18 October 2005. Revision accepted 17 January 2006.

sesame flour extracts by HPLC (27). β-Glucuronidase (*Helix pomatia*) and *N*,*O*-bis(trimethyl-d_9-silyl) acetamide (BSA) were from Sigma Aldrich and Tri-Sil reagent (TMS; hexamethyldisilazane:trimethylchlorosilane in pyridine, 2:1:10) was purchased from Pierce. Deuterated *N*,*O*-bis(trimethyl-d_9-silyl) acetamide (d_9-BSA) was from C/D/N Isotopes. The other reagents were of analytical grade, and all solvents used were of HPLC grade.

Lignan analysis of sesame seed and flaxseed. Sesame seed (Grain Process Enterprises) was extracted with hexane, and the sesame oil and defatted sesame flour were analyzed for lignan content. Sesamin, sesamolin, and sesaminol were analyzed by HPLC with photodiode array detector; other lignans were analyzed by GC-MS as described previously (27). For comparison, flaxseed (Pizzey's Milling) was also analyzed for its lignans in a similar manner.

In vitro fermentation with human fecal inoculum. Samples (~2 mg) of sesamin or SDG, and blank samples, all in duplicate, were subjected to in vitro fermentation with fresh human fecal inoculum collected from 1 healthy volunteer as described previously for food fermentation (10,30,31). The centrifuged (10,000 × *g*; 10 min) fermentation digesta were analyzed for mammalian lignans using GC-MS methods as previously described (30).

Animals and diets. Female 10-wk-old Sprague-Dawley rats (Charles River) were housed 2/cage in a temperature-controlled facility maintained at 22–24°C and 50% humidity with a 12-h light:dark cycle. Drinking water and diets were consumed ad libitum during the whole experimental period. The basal diet, AIN 93G, (32) was purchased from Dyets. The 10% (wt:wt) sesame seed diet was prepared by mixing ground sesame seed with the basal diet. Animal care and use conformed to the Guide to the Care and Use of Experimental Animals (33), and the experimental protocol was approved by the University of Toronto Animal Care Committee.

Animal study design. After acclimatization to the basal diet for 4 d, 18 rats were randomly assigned to 3 groups ($n = 6$), and fed for 10 d either the basal diet (control), 10% sesame seed diet, or the basal diet plus a daily gavage of sesamin (15 mg/kg body weight). The vehicle used for sesamin administration was corn oil and the volume administered was 200 μL/100 g body weight, representing the estimated oil intake in rats consuming a sesame seed diet. The controls were also gavaged with similar volumes of corn oil. Individual 72-h urine collections in metabolic cages were started after 7 d of treatment; during the collection period, food intake was also recorded. The urine collection containers contained 120 μL of 0.56 mol/L ascorbic acid and 120 μL of 0.15 mol/L sodium azide solutions as preservatives. The centrifuged (10,000 × *g*; 10 min) urine volumes were measured and stored at −20°C until used for lignan analyses.

Lignan analyses of urine samples. Urinary lignan analysis was performed using a previously described method (10) with slight modifications. Briefly, 2 mL of 0.1 mol/L sodium acetate buffer (pH 4.5) and 60 μL of 50% β-glucuronidase in 0.1 mol/L sodium acetate buffer were added to 1 mL urine sample, and the mixture was incubated in a 37°C water bath overnight to hydrolyze the lignan conjugates. The samples were then passed through Sep-ed SPE Cartridges (Octadecyl C18:14%, 200 mg:3 mL) that were preconditioned with 5 mL of chloroform:methanol (1:1, v:v), 5 mL of methanol, and 5 mL of distilled water. They were then washed with 5 mL of distilled water, and the polyphenolic fractions were eluted with 4 mL of methanol. The samples were completely evaporated using a vacuum rotary evaporator at <60°C. The residue was dissolved in 1 mL of methanol before derivatization for GC-MS analysis.

TMS derivatization and GC-MS analyses. The above extracted urinary lignan samples were derivatized by Tri-Sil reagent or BSA or deuterated BSA (d_9-BSA). Derivatization with d_9-BSA was used to determine the number of hydroxyl groups (34,35); each hydroxyl group increases the mass units by 9 when d_9-BSA is used instead of nonlabeled TMS reagent. A total of 75 μL of internal standard solution (5α-androstane-3β, 17β-diol and stigmasterol in methanol, each at 100 mg/L) was added to the above samples in methanol They were then dried under nitrogen gas flow and derivatized with 100 μL of Tri-sil reagent for 0.5 h at 60°C or with 100 μL of BSA or d_9-BSA overnight at room temperature. The silylating agents were removed under nitrogen gas flow, and the trimethylsilylated samples were dissolved in 100 μL of hexane before GC-MS.

GC-MS analyses were carried out with an Agilent 5973 Network Mass Selective Detector coupled to an Agilent 6890 Series GC System, equipped with a HP 5-ms capillary column (25 m × 0.12 mm i.d. × 0.25-μm film thickness) (Agilent Technologies). The flow rate of helium carrier gas was 1 mL/min. The oven temperature was programmed as follows: 100°C (for 1 min) rising at 25°C/min to 280°C (for 17 min). The temperatures of the injection port, ion source, and interface were 250, 230, and 280°C, respectively. The injection volume was 1 μL and a splitless insert was adapted. A mass range from 50 to 700 amu was scanned using an electron ionization energy of 70 eV. Lignans were quantitated on the basis of selected mass fragments.

Statistical analysis. Urinary lignan data were $\log_{10}$ transformed and analyzed by 1-way ANOVA followed by Tukey's test using SigmaStat (Jandel Scientific, San Rafael, CA). Differences were considered significant at $P < 0.05$. Values in the text are means ± SEM.

RESULTS

Lignan concentrations of sesame seed and flaxseed. The lignans sesamin, sesamolin, sesaminol, sesamolinol, SECO, pinoresinol, lariciresinol, piperitol, and matairesinol, were identified in the defatted sesame seed portion by comparing their mass spectra and retention times with those of authentic compounds and the mass spectra database. Sesamin and sesamolin were the only lignans detected in the sesame oil portion. There were other minor lignans in the defatted sesame seed portion but they cannot yet be identified due to lack of authentic compounds or related spectral data.

Sesame seed contains sesamin, sesamolin, and sesaminol as its major lignans and SECO, matairesinol, pinoresinol, and lariciresinol as minor lignans (**Table 1**). Flaxseed with SECO as the major lignan, also contains minor amounts of matairesinol, pinoresinol, and lariciresinol but no sesame lignans (Table 1).

In vitro mammalian lignan production by human fecal fermentation. The mammalian lignans ED and EL were produced from SDG and sesamin after incubation with fecal inoculum. However, the conversion of sesamin to mammalian lignans after the 24-h incubation was very low compared with that of SDG (**Table 2**). SDG was converted mainly to ED, whereas sesamin was converted to both ED and EL in similar amounts.

Urinary mammalian lignan excretion in rats. Body weight and food intake did not differ among the control, sesamin, and sesame seed treatment groups (data not shown). Based on the daily food intake (15.1 ± 0.58 g) and sesamin concentration of sesame seed (15.2 μmol/g), the sesamin intake in the sesame seed group was estimated to be 22.9 μmol/d, which was ~2 times the level of sesamin provided in the sesamin group (11.7

TABLE 1

Lignan concentrations of whole sesame seed and flaxseed[1]

Lignan	Sesame seed	Flaxseed
	μmol/100 g	
Sesamin	1520 ± 6.8	ND[2]
Sesamolin	362 ± 4.1	ND
Sesaminol	278 ± 2.5	ND
Secoisolariciresinol	0.2 ± 0.1	800 ± 15.2
Matairesinol	0.3 ± 0.0	2.6 ± 0.1
Pinoresinol	22.3 ± 0.5	9.9 ± 0.5
Lariciresinol	0.8 ± 0.0	7.0 ± 0.1
Total	2180 ± 12.9	820 ± 15.7

[1] Values are means ± SEM, $n = 2$.
[2] ND, not detected.

TABLE 2

Mammalian lignan production after in vitro fermentation of sesamin and SDG with human fecal inoculum[1]

Mammalian lignan	Sesamin	SDG
	μmol/mmol[2]	
ED	5.8 ± 0.0	554 ± 2.4
EL	4.6 ± 3.3	18.1 ± 4.8
ED+EL	10.5 ± 3.3	572 ± 2.4
ED+EL, % converted	1.1	57.2

[1] Values are means ± SEM, $n = 2$.
[2] μmol ED or EL/mmol lignan precursor (sesamin or SDG).

μmol/d). The urinary excretions of ED and EL were higher ($P < 0.05$) in the sesamin and sesame seed groups than in the control, which did not excrete lignans (<0.05 μmol/d); in both cases, the amounts of ED were greater than EL (**Table 3**). The total excretion of ED and EL in the sesame seed group was 3.5 times that in the sesamin group.

In vivo metabolites of sesamin in rat urine. No sesamin or other sesame lignans were detected in the urine of the treated rats. However, a number of sesamin metabolites (**Fig. 1**) were detected and identified or tentatively identified based on their mass spectra (**Fig. 2** and **Table 4**).

Metabolite M1 was the major metabolite in the urine of the sesamin and sesame seed groups followed by metabolite M2 (Fig. 1 and 2). They were identified as ED and EL, respectively, by comparing their mass spectra and GC retention times with those of authentic compounds and literature data (36–38).

Metabolite M3 (Fig. 1) exhibited a molecular ion peak at *m/z* 486, and derivatization with d_9-BSA revealed the presence of 2 hydroxyl groups as its molecular ion gave rise to *m/z* 504, an 18 mass shift higher than the nonlabeled molecule (Fig. 2 and Table 4). There was no fragment of $[M\text{-}90]^+$ in the mass spectrum, indicating that both hydroxyl groups were located in the aromatic moiety of the molecule (39). The molecular weight of M3 was 12 mass units less than that of sesamin, indicating that M3 is possibly an oxidative demethylenation product of the methylenedioxyphenyl ring of sesamin. Thus, the structure of M3 was deduced as 6-(3,4-dihydroxyphenyl)-2-(3,4-methylenedioxyphenyl)-3,7-dioxabicyclo[3,3,0]-octane, previously reported as dimethylpiperitol (23,40).

Metabolite M4 had a molecular ion of *m/z* 428, and fragments $[M\text{-}CH_3]^+$ of *m/z* 413 and $[M\text{-}OCH_3]^+$ of *m/z* 397 (Fig. 2 and Table 4). The deuterated TMS derivative of M4 showed only 1 hydroxyl group in the structure because its molecular ion gave rise to *m/z* 437, a 9 mass shift higher than the nonlabeled molecule. Its molecular weight was 14 mass units (CH_2) more than that of M3. The mass spectrum and GC retention time of M4 was exactly the same as the component in the defatted sesame seed that was identified as piperitol, which is possibly a methylation product of M3.

Metabolite M5 was detected as a further oxidative demethylenation product of the methylenedioxyphenyl ring of M4. Its molecular ion peak was at *m/z* 560 and there were 3

TABLE 3

Urinary excretion of mammalian lignans in rats after consumption of sesamin, sesame seed, or control diets for 10 d[1]

Item	Sesamin	Sesame seed	Control
		μmol/d	
Sesamin consumption	11.7 ± 0.2[b]	22.9 ± 2.0[c]	0.0 ± 0.0[a]
Urinary excretion			
ED	2.3 ± 0.1[b]	11.1 ± 1.9[c]	0.0 ± 0.0[a]
EL	0.9 ± 0.1[c]	0.1 ± 0.0[b]	0.0 ± 0.0[a]
ED+EL	3.2 ± 0.2[b]	11.2 ± 1.9[c]	0.0 ± 0.0[a]

[1] Values are means ± SEM, $n = 6$. Means in a row without a common letter differ, $P < 0.05$.

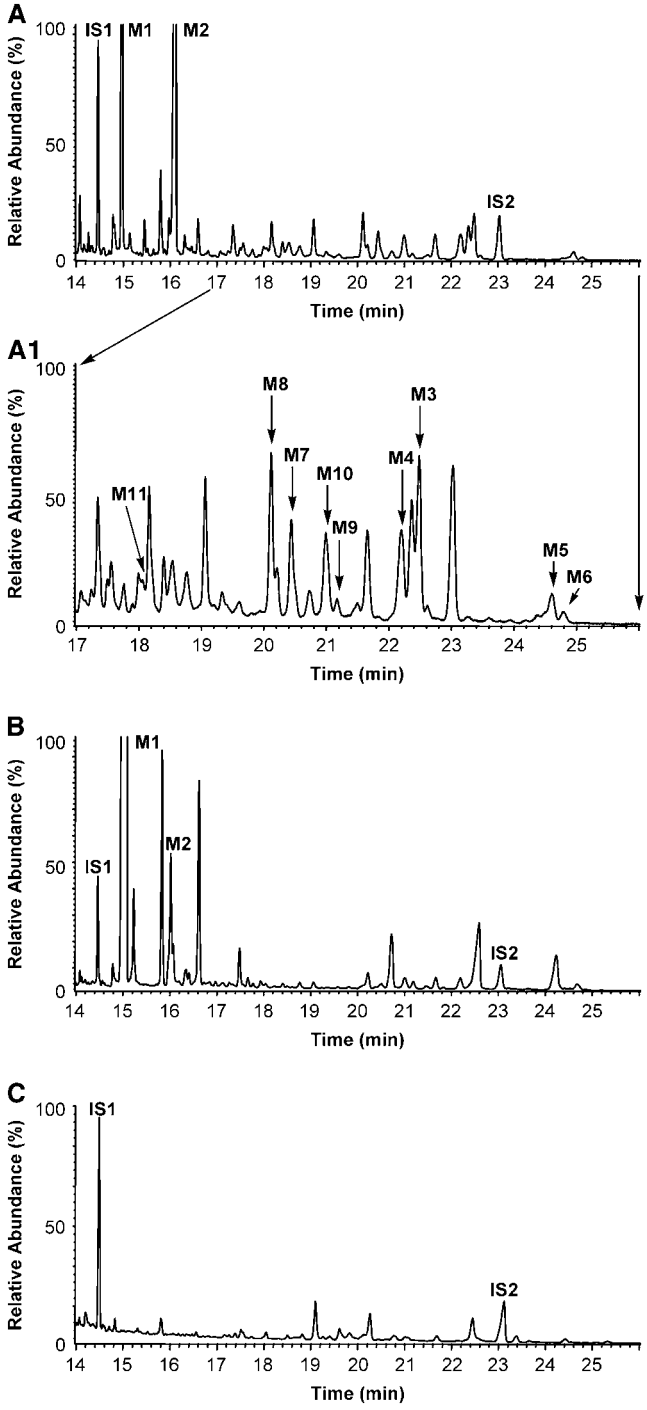

FIGURE 1 GC-MS total ion chromatogram profiles of urine of rats fed (*A*) sesamin, (*B*) sesame seed, and (*C*) control diets. (*A1*) expanded (*A*) from retention time 17–26 min, (IS1) internal standard 1: 5α-androstane-3β, 17β-diol, (IS2) internal standard 2: stigmasterol, (M1–M11) metabolites 1–11.

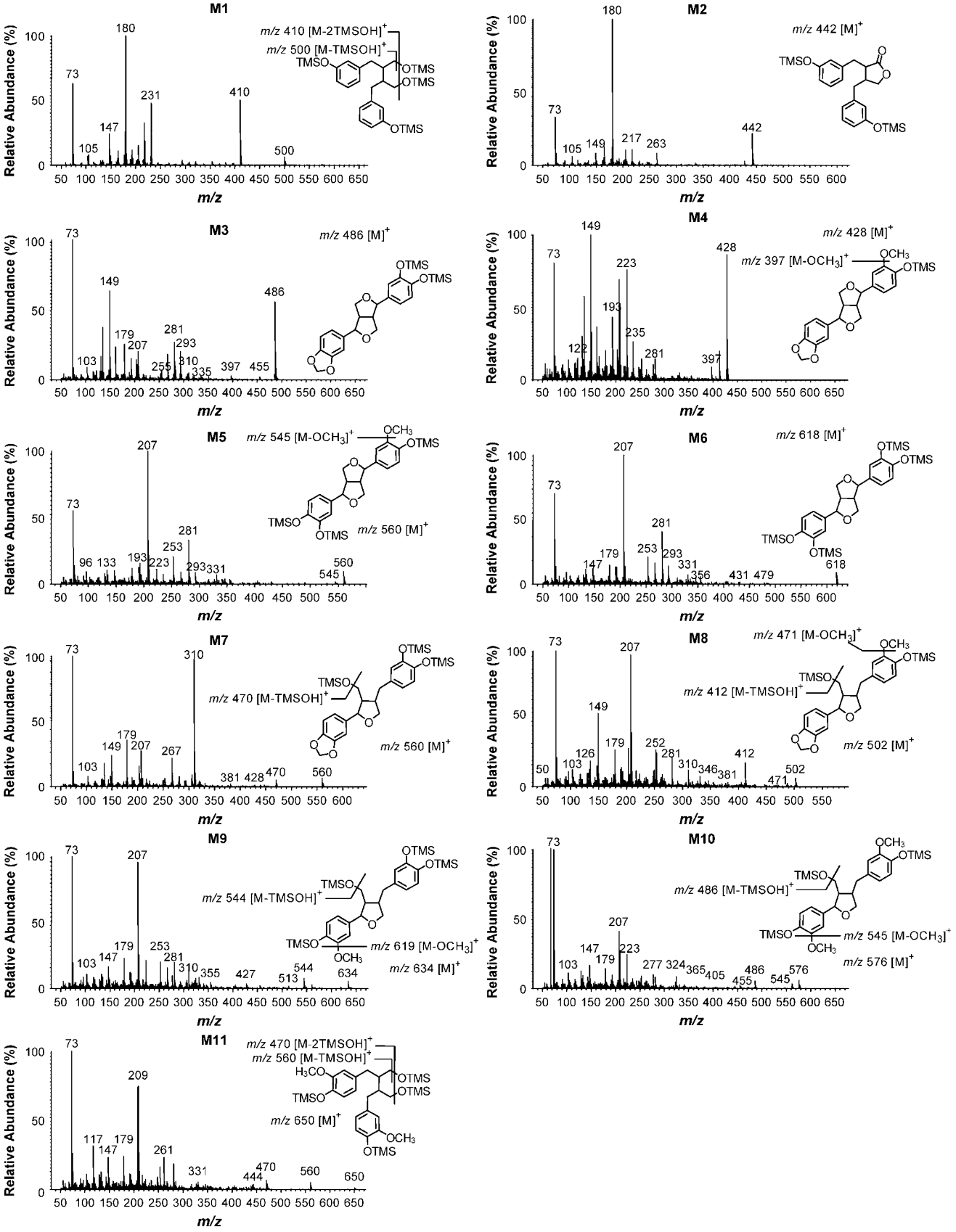

FIGURE 2 Mass spectra of sesamin metabolites in the urine of rats fed sesamin.

TABLE 4

Mass spectra of sesamin metabolites in rat urine after derivatization with d9-BSA[1]

Compound	Ion
	m/z
M1	527(500), 428(410), 399(381), 372(354), 339(321), 312(294), 240(231), 226(217), 214(205), 202(193)
M2	460(442), 272(263), 226(217), 214(205), 189(180)
M3	504(486), 473(455), 406(397), 353(335), 328(310), 311(293), 299(281), 285(267), 273(255)
M4	437(428), 406(397), 244(235), 232(223), 218(209)
M5	587(560), 569(545), 556(529), 311(293), 285(267), 232(223), 218(209), 211(193)
M6	654(618), 328(310), 311(293), 299(281), 285(267)
M7	587(560), 488(470), 328(310), 285(267)
M8	520(502), 489 (471), 421(412), 328(310), 261(252), 218(209)
M9	670(634), 655(619), 571(544), 342(324), 328(310), 232(223)
M10	603(576), 571(545), 504(486), 473(455), 462(444), 342(324), 286(277), 261(252), 232(223), 218(209)
M11	686(650), 587(560), 488(470), 270(261), 256(247), 218(209)

[1] The corresponding TMS derivatives are given in parentheses.

hydroxyl groups in the structure based on the mass spectrum of the deuterated TMS derivative of this compound (Fig. 2 and Table 4). There was also no fragment of $[M\text{-}90^{1\sim3}]^+$, suggesting that all hydroxyl groups are in the aromatic moieties of the molecule. The peak of the $[M\text{-}15]^+$ fragment at *m/z* 545 was observed as $[M\text{-}CH_3]^+$. The molecular weight of this compound was 12 mass units less than that of M4. Therefore, the structure of M5 was inferred to be 6-(4-hydroxy-3-methoxyphenyl)-2-(3,4-dihydroxyphenyl)-3,7-dioxabicyclo[3,3,0]octane, which has a structure similar to that of 3′-*O*-demethylepipinoresinol (23,41).

The GC retention time of metabolite M6 (24.8 min) was relatively longer than that of all other metabolites (Fig. 1). Its mass spectrum shows a molecular ion of *m/z* 618 and its deuterated TMS derivative demonstrated 4 hydroxyl groups in the aromatic position, which were confirmed by the absence of fragments of $[M\text{-}90^{1\sim4}]^+$ (34) (Fig. 2 and Table 4). M6 was identified as a further oxidative demethylenation product of the methylenedioxyphenyl ring of M3, which was 2,6-bis(3,4-dihydroxyphenyl)-3,7-dioxabicyclo[3,3,0]octane (23).

Metabolite M7 had a molecular ion at *m/z* 560 and a fragment $[M\text{-}TMSOH]^+$ at *m/z* 470 (M-90), indicating only 1 aliphatic hydroxyl group (Fig. 2 and Table 4). Furthermore, its deuterated TMS derivative confirms 3 hydroxyl groups in the molecule, suggesting that there are 2 hydroxyl groups at the aromatic position. The molecular weight of M7 is 2 mass units (2H) more than that of M3. Therefore, the structure of M7 was tentatively deduced to be 2-(3,4-dihydroxytolyl)-4-(3,4-methylenedioxyphenyl)-3-hydroxylmethylene-furan, which is possibly a reductive cleavage product of the furofuran ring of M3.

Metabolite M8 had a molecular ion of *m/z* 502 and a fragment $[M\text{-}TMSOH]^+$ of *m/z* 412 (Fig. 2 and Table 4). Its deuterated TMS derivative demonstrated 2 hydroxyl groups, implying 1 hydroxyl in the aliphatic position and the other in the aromatic position. A fragment of $[M\text{-}CH_3]^+$ at *m/z* 487 indicated a methoxy group in the structure. The molecular weight of this metabolite was 2 mass units (2H) more than that of M4 and 14 mass units more than that of M7, suggesting that M8 is a reductive cleavage product of the furofuran ring of M4 or a methylation product of M7. M8 was tentatively determined to be 2-(4-hydroxy-3-methoxytolyl)-4-(3,4-methylenedioxyphenyl)-3-hydroxylmethylene-furan.

Metabolite M9 showed a molecular ion at *m/z* 634, a fragment of $[M\text{-}TMSOH]^+$ at *m/z* 544, and no further fragments of $[M\text{-}2TMSOH]^+$ and $[M\text{-}3TMSOH]^+$ (Fig. 2 and Table 4). Its deuterated TMS derivative suggests 4 hydroxyl groups in the molecule, indicating that 3 of them are at the aromatic position and one in the aliphatic position. The molecular weight of M9 is 346, 2 mass units (CH_2) more than that of M5, indicating that it is likely a reductive cleavage product of the furofuran ring of M5. Therefore, the structure of M9 was inferred to be 3′-demethyl-lariciresinol (42).

Metabolite M10 was detected as lariciresinol and metabolite M11 as secoisolariciresinol (SECO) by comparing their mass spectral data (Fig. 2 and Table 4) and GC retention times with the authentic compounds and those reported (34,35,42).

Additional minor peaks were also detected but were not identified. They may be intermediate metabolites of sesamin or further metabolites of mammalian lignans.

DISCUSSION

A previous study (28) showed that a portion of dietary sesamin and its isomer episesamin is absorbed via the lymph, reaches the liver, and is then transported to other tissues, but is removed from the body within 24 h after consumption. A portion of sesamin (15–35%) was excreted in feces but none was excreted in the urine, indicating that sesamin was metabolized to other compounds in either the intestine or liver. The liver was shown to produce hydroxylated metabolites of sesamin that are excreted in bile (23). In this study, we observed very limited conversion of sesamin to ED and EL by in vitro fermentation with human fecal microbiota; we also did not detect any sesamin or episesamin in the urine of rats fed sesamin or sesame seed. Therefore, we surmise that sesamin is only partially metabolized in the colon to ED and EL; it is absorbed and metabolized in the liver to hydroxylated metabolites, which are then excreted in bile. These metabolites that are excreted in bile undergo enterohepatic circulation and are further metabolized to the mammalian lignans by the intestinal microbiota. Hence, the primary site of sesamin metabolism may differ from that of the more hydrophilic lignan precursors such as SDG, which is more readily metabolized by the intestinal microbiota to ED and EL before absorption. This is supported by the previous observation that plasma ED and EL peak at 9–12 h after flaxseed (43) or SDG (44) ingestion and were much more delayed at 24 h after sesame seed intake (26).

Sesamin can be classified as a furofuran lignan with methylenedioxyphenyls. The transformation of furofuran lignan such as pinoresinol and its glucosides to mammalian lignans by intestinal microbiota involves hydrolysis of glucoside, demethylation of a methoxy group, elimination of a 4-hydroxy group in the 3,4-dihydroxyphenyl moiety, oxidation of dibenzylbutanediol to dibenzylbutyrolactone, and reductive cleavage of furofuran rings (42). Sesamin and some other sesame lignans that have methylenedioxyphenyl moieties in their structures may require additional oxidative demethylenation of the methylenedioxyphenyl ring for conversion to mammalian lignans. Methylenedioxyphenyl structures undergo oxidative demethylenation of the methylenedioxy ring, resulting in the formation of the corresponding catechol by cytochrome P450–catalyzed oxidation (45). Nakai et al. (23) identified 4 oxidative demethylenated and corresponding products of sesamin in the rat liver. In the present study, we

identified 11 potential metabolites of sesamin in rat urine after ingestion of sesamin; this total includes those 4 liver metabolites of sesamin found by Nakai et al. (23). These liver metabolites of sesamin could have undergone enterohepatic circulation, reached the colon, and been metabolized further by the colonic microbiota to other metabolites including ED and EL. Therefore, a tentative metabolic pathway of sesamin is proposed (**Fig. 3**), based on identified urinary metabolites of sesamin. Sesamin may first be demethylenated into dimethylpiperitol (M3) and then further demethylenated to M6 or methylated in its catechol moiety to piperitol (M4), possibly by liver catechol-*O*-methyl transferase. M4 could be further demethylenated to 3′-*O*-demethylepipinoresinol (M5). The formation of M3–M6 may occur predominantly in the liver (23), whereas intestinal microbiota may also play a part in their production (26). M3–M5 could further undergo reductive cleavage of their furofuran rings to M7–M9, respectively, similar to pinoresinol (42). Further elimination of the furan ring, demethylation, and dehydroxylation of intermediate metabolites may be carried out by intestinal microbiota in the same way as for furofuran lignans such as pinoresinol, and common lignans such as lariciresinol and SECO.

Although ED and EL were detected when sesamin was fermented with human fecal inoculum, the conversion was relatively small compared with SDG. Because SDG is more hydrophilic than sesamin, the bacterial conversion of plant lignans to mammalian lignans may also depend on the solubility of these compounds in a hydrophilic medium phase. Penalvo et al. (26) also observed the formation of ED and EL along with 2 other metabolites after sesamin fermentation. Those 2 other metabolites were not observed in this in vitro study, perhaps due to the low rate of conversion in our fermentation system.

Epi-sesamin, an isomer of sesamin produced from sesamin during the refining of unroasted sesame seed oil (46), was not detected in the urine of rats fed a 1:1 mixture of sesamin and epi-sesamin. Umeda-Sawada et al. (28) found sesamin and epi-sesamin in rat liver after their administration, but both were lost from the body within 24 h after administration, indicating that they are absorbed and metabolized. In the urine of the sesamin group, we observed a number of putative intermediate metabolites of epi-sesamin (data not shown) that had the same mass spectra as the sesamin metabolites but different retention times. These metabolites were absent in the urine of rats fed sesame seed, which does not contain epi-sesamin. Because the metabolites of sesamin and epi-sesamin did not differ after cleavage of the furofuran rings, we presume that both sesamin and epi-sesamin might have been converted to the same mammalian lignans ED and EL, despite enantiomorphism.

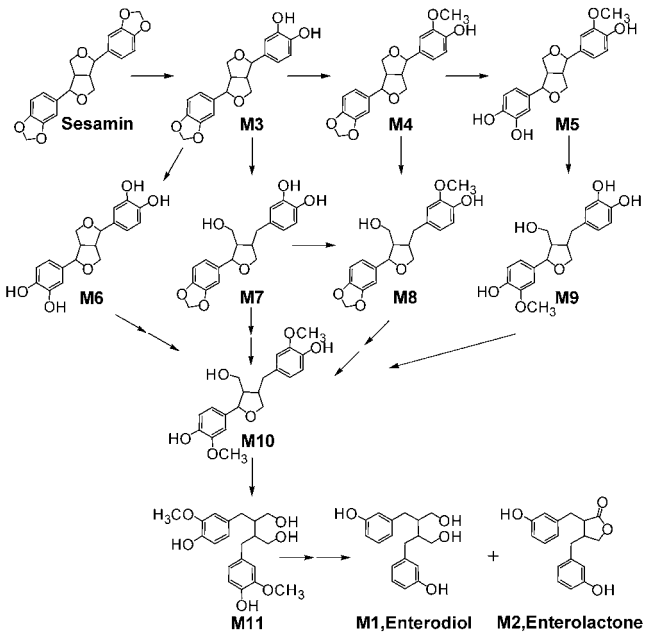

FIGURE 3 Tentative metabolic pathway of sesamin to mammalian lignans.

The urinary excretion of mammalian lignans was ~2.50 times higher in the sesame seed group than in the sesamin group. This could be due to a slower conversion rate of epi-sesamin to ED and EL compared with sesamin, or to the presence of other mammalian lignan precursors in sesame seed. If the conversion rates of sesamin to urinary mammalian lignans are the same in both groups, the amount of sesamin consumed in the 10% sesame seed group (22.9 μmol/d) could produce only ~1.96 times more urinary mammalian lignans than that in the sesamin group (11.7 μmol sesamin intake/d). Obviously, a large number of other precursors of mammalian lignans exist in the sesame seed. There are other known precursors of mammalian lignans in sesame seed such as SECO, pinoresinol, lariciresinol, and matairesinol but their total (0.24 μmol/g sesame seed) still could not account for the rest of the urinary mammalian lignan excretion. Hence, other compounds, including the other major sesame lignans such as sesaminol, glycosides, and/or sesamolin, may also potentially be precursors of mammalian lignans. Studies are currently in progress to determine their metabolism to mammalian lignans.

Flaxseed was reported to be the richest source of mammalian lignan precursors (10,11). Our previous study (27) showed that the increase in urinary mammalian lignan excretion of postmenopausal women after the intake of 25 g sesame seed (81.0 μmol/d) did not differ significantly from that after the intake of 25 g flaxseed (65.1 μmol/d). In this study, the urinary excretion of rats fed 10% sesame seed (11.2 μmol/d) was also similar to previously reported mammalian lignan excretion after the intake of 10% flaxseed (12.8 μmol/d) (47). Hence, sesame seed may be an alternative rich dietary source of mammalian lignan precursors although, as observed previously (48), lignan concentrations may vary in different batches of seeds.

As was estimated for a 10% flaxseed diet (49), the intake of a 10% sesame seed diet by rats is equivalent to an intake of 25–50 g sesame seed by humans, depending on the amount of other foods consumed. Sesame seed at 25 g/d can provide ~379 μmol sesamin/d and produce amounts of mammalian lignans similar to that produced by 25 g of flaxseed (27). Sesame seed is commonly consumed in Asian countries especially China, Japan, and India where there is a low incidence of breast cancer compared with Western countries (50). If sesame seed is found to be effective in reducing the risk of breast cancer in experimental studies as was seen with the intake of 10% flaxseed in animal studies (2) or 25 g flaxseed by postmenopausal breast cancer patients (51), the intake of sesame seed as a rich source of mammalian lignan precursors may provide an additional explanation for the difference in breast cancer incidence in Asian and Western countries.

In conclusion, these studies showed that rats administered dietary sesame seed excreted large amounts of urinary ED and EL compared with the control. Sesamin, a major lignan of sesame seed, was converted to mammalian lignans in vitro by human fecal microbiota and increased the urinary mammalian lignan excretion in rats. These results demonstrate that sesame seed is one of the richest dietary sources of mammalian lignan precursors and sesamin is one of them. A tentative metabolic

pathway for the conversion of sesamin to the mammalian lignans was also suggested.

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