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Biotransformation of Sesaminol Triglucoside to Mammalian Lignans by Intestinal Microbiota

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Plant lignans occur widely in foods, with flaxseed recognized as their 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 sesaminol triglucoside (STG), could be metabolized to mammalian lignans. STG is a furofuran lignan with methylenedioxyphenyls. The transformation of furofuran lignans to mammalian lignans by intestinal microbiota involves the hydrolysis of glucoside, demethylenation of a methylene group, oxidation of dibenzylbutanediol to dibenzylbutyrolactone, and reductive cleavage of furofuran rings. STG has methylenedioxyphenyl moieties in their structures that may require additional oxidative demethylenation of the methylenedioxyphenyl ring for conversion to mammalian lignans. However, STG is metabolized, via intestinal microbiota, to a catechol moiety. The major STG metabolite was characterized as 4-[((3*R*,4*R*)-5-(6-hydroxybenzo[*d*][1,3]dioxol-5-yl)-4-(hydroxymethyl)tetrahydrofuran-3-yl)methyl]benzene-1,2-diol using NMR and mass spectrometry, and STG could be converted to enterolactone and enterodiol by rat intestinal microflora.

KEYWORDS: Sesaminol triglucoside; mammalian lignans; biotransformation; intestinal microbiota

INTRODUCTION

Sesame exhibits many beneficial physiological effects, which are mostly related to its lignan compounds, such as sesaminol glucosides. Sesaminol glucosides have no antioxidative properties in vitro, but they have been reported to be converted to phenolic compounds after oral administration and showed antioxidative activity (*1*−*3*). Plant lignans, such as secoisolariciresinol, matairesinol, lariciresinol, and pinoresinol, are converted to enterolactone (ENL) and enterodiol (END) (*4*, *5*) by the intestinal microbiota of humans and animals (*6*−*9*). Nakai et al. (*10*) and Liu et al. (*11*) reported that sesamin undergoes cleavage of methylenedioxyphenyl (MDP) groups to catechol or methoxycatechol in humans and in rats, respectively. Our previous studies reported that sesaminol triglucoside might be deglycosylated to form sesaminol, by intestinal microbiota, and then incorporated via lymphatic absorption into the cardiovascular system. LC-MS/MS analysis of rat organs suggested that sesaminol triglucoside could be converted to mammalian lignans by rat intestinal microbiota (*12*).

In this study, we investigated whether sesaminol triglucoside, a lignan isolated from sesame seed, is converted to the mammalian lignans in rats. In addition, we used incubation of sesaminol triglucoside with intestinal bacteria to identify major sesaminol triglucoside metabolites.

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MATERIALS AND METHODS

Materials and Chemicals. Acetic acid was obtained from Sigma-Aldrich (Poole, Dorset, U.K.). XAD-2 gel was purchased from Aldrich (Milwaukee, WI). General anaerobic medium (GAM) broth was provided by Nissui (Tokyo, Japan). All other chemicals used were of analytical grade. Liquid chromatographic grade solvents and reagents were obtained from Mallinckrodt Baker (Phillipsburg, NJ). Triply deionized water (Millipore, Bedford, MA) was used for all preparations.

Extraction and Isolation of Sesaminol Triglucoside (STG). Black sesame (*Sesamum indicum*) was supplied by Yuan-Shun Food Co. (Yun-Ling County, Taiwan). For the isolation of 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 under the following conditions: column, Cosmosil ODS (250 × 20 mm i.d.); solvent, MeOH; flow rate, 4 mL/min (*13*). The purity of STG was 99%.

Animals and Diets. Our experimental protocol was approved by the National Laboratory Animal Center (Taipei, Taiwan). Inbred male Sprague−Dawley rats (body wt = 275 ± 25 g, mean ± SD) were housed in pairs in cages in a room with controlled temperature (20−22 °C), relative humidity (50−70%), and a 12 h light/dark cycle (lights on at 7:00 a.m.). The rat diet was an 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 determined weekly.

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Time Course for the Transformation of STG to Its Metabolites by Rat Intestinal Bacteria. Fecal samples were collected in plastic boxes, kept under anoxic conditions using an AnaeroGen Compact (Oxoid, Hampshire, U.K.). Fresh feces (5 g) were homogenized in 50 mL of sterile anaerobic salt medium at room temperature in an anaerobic tent to give a rat intestinal bacterial (RIB) mixture. The sterile anaerobic salt medium comprised the following constituents in distilled water (g/L): $NaHCO_3$, 5.0; NaCl, 0.9; $(NH_4)_2SO_4$, 0.9; KH_2PO_4, 0.45; $K_2HPO_4 \cdot 3H_2O$, 0.45; $CaCl_2 \cdot 2H_2O$, 0.03; $MgCl_2$, 0.02; $MnSO_4 \cdot 4H_2O$, 0.01; $CoCl_2 \cdot 6H_2O$, 0.01; $FeSO_4 \cdot 7H_2O$, 0.01; and cysteine, 0.25. STG (200 mg) was cultured with RIB mixture (5 mL) at 37 °C under anaerobic conditions. The culture mixtures were periodically sampled during a culturing period of 1−9 days (*14*, *15*). Transformation of STG metabolites was monitored by HPLC: a 5 mL portion of the sample was extracted with *n*-BuOH (saturated with H_2O, containing 0.1% acetic acid). After evaporation of *n*-BuOH in vacuo, the residue was dissolved in 5 mL of MeOH. The MeOH solution was diluted with a mixed solvent of MeOH and H_2O (1:1) to a volume of 2.5 mL, which was filtered through a 0.2 μm membrane filter, and a 5 μL portion was subjected to HPLC analysis. Substrate and metabolites were well separated and detected under the conditions mentioned above. Concentrations of STG metabolites were calculated according to calibration curves of respective authentic samples.

Preparation of a Human Intestinal Bacterial (HIB) Mixture. In a pilot study, subjects maintained a diet low in lignans for 2 weeks. Feces (5 g) were obtained from two healthy subjects (28−35 years old, one man and one woman) who ingested a low-lignan diet and did not receive antibiotics for at least 3 months. Fresh feces were homogenized in 100 mL of GAM broth under anaerobic conditions, and the sediments were removed by decantation to give a 5% supernatant HIB mixture.

Incubation of STG with HIB Mixture and Isolation of the Metabolites. An HIB mixture (300 mL) and STG (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 (saturated with H_2O, containing 0.1% acetic acid). The *n*-BuOH extracts were combined, and solvent was removed in vacuo to give a residue. The residue was dissolved in H_2O. The H_2O solution 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). STG metabolites were chromatographed on a preparative LC and purified by ODS column (*16*).

Characterization of STG Metabolites. The structure of the sesaminol triglucoside metabolite collected by HPLC was assigned by UV−visible spectra, mass spectra, and nuclear magnetic resonance spectroscopy, incorporating 1H NMR, correlation (COSY), heteronuclear multiple bond correlation (HMBC), heteronuclear multiple quantum correlation (HSQC), and nuclear Overhauser effect (NOESY). UV−visible spectra were obtained using a Helios Alpha UV−vis spectrophotometer (Thermo Electron Co., Waltham, MA). MS analyses were performed using a ThermoFinnigan LXQ Advantage mass spectrometry (San Jose, CA). Operation was in MS mode, and electrospray ionization (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 temperature, 350 °C; tube lens voltage, −110 V (*17*). The ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded using a Bruker Avance-500 MHz FT-NMR spectrometer (Bruker Co., Rheinstetten, Germany).

ST-1. *(sesaminol):* UV λ_{max}^{MeOH} = 292; MW = 370; ESI$^-$-MS, *m*/*z* 369 [M − H]$^-$; ^{1}H (500 MHz, *d*-$CDCL_3$) δ 2.88 (m, 1H, H-8), 3.14 (m, 1H, H-8′), 3.87 (dd, *J* = 3.2, 8.9 Hz, 2H, H-9), 4.33 (dd, *J* = 1.8, 7.2 Hz, 2H, H-9), 3.91 (dd, *J* = 2.7, 8.9 Hz, 2H, H-9′), 4.15 (dd, *J* = 6.6, 9.7 Hz, 2H, H-9′), 4.75 (d, *J* = 3.3 Hz, 1H, H-7′), 4.94 (d, *J* = 6 Hz, 1H, H-7), 5.88 (s, 2H, O−CH_2−O), 5.94 (s, 2H, O−CH_2−O), 6.43 (s, 1H, H-3), 6.49 (s, 1H, H-6), 6.78 (d, *J* = 10.5 Hz, 1H, H-5′), 6.79 (dd, *J* = 1.4, 8.5 Hz, 1H, H-6′), 6.85 (d, *J* = 1.4 Hz, 1H, H-2′); ^{13}C (125 MHz, *d*-$CDCl_3$) δ 119.6 (C-1), 150.6 (C-2), 99.4 (C-3), 140.9 (C-4), 148.07 (C-5), 105.6 (C-6), 84.3 (C-7), 50.5 (C-8), 72.4 (C-9), 134.5 (C-1′), 106.1 (C-2′), 150.6 (C-3′), 148.0 (C-4′), 108.2 (C-5′), 119.3 (C-6′), 87.5 (C-7′), 53.5 (C-8′), 71.7 (C-9′), 101.2 (O−CH_2−O), 101.1 (O−CH_2−O).

ST-2. *[4-(((3R,4R)-5-(6-hydroxybenzo[d][1,3]dioxol-5-yl)-4-(hydroxymethyl)tetrahydrofuran-3-yl)methyl)benzene-1,2-diol]:* UV λ_{max}^{MeOH} = 295; MW = 360; ESI$^-$-MS, *m*/*z* 359 [M − H]$^-$; ^{1}H (500 MHz, *d*-CD_3OD) δ 1.74 (m, 1H, H-8), 1.95 (m, 1H, H-8′), 2.68 (d, *J* = 7.7 Hz, 2H, H-7′), 3.66 (dq, *J* = 5.6, 6.0 Hz, 2H, H-9′, H-9), 3.62 (d, *J* = 6.0 Hz, 2H, H-9′), 4.19 (d, *J* = 10.3 Hz, 1H, H-7), 5.76 (s, 2H, −O−CH_2−O−), 6.20 (d, *J* = 10.5 Hz, 1H, H-5′), 6.31 (s, 1H, H-6), 6.39 (d, *J* = 8.4 Hz, 1H, H-6′), 6.41 (s, 1H, H-3), 6.53 (s, 1H, H-2′); ^{13}C (125 MHz, *d*-CD_3OD) δ 125.2 (C-1), 151.0 (C-2), 98.4 (C-3), 147.4 (C-4), 142.3 (C-5), 109.8 (C-6), 40.8 (C-7), 47.6 (C-8), 63.2 (C-9), 132.5 (C-1′), 115.7 (C-2′), 144.1 (C-3′), 144.0 (C-4′), 117.1 (C-5′), 129.4 (C-6′), 33.3 (C-7′), 40.6 (C-8′), 66.05 (C-9′), 101.89 (O−CH_2−O).

ST-3. *[4-(((3S,4R,5S)-5-(6-hydroxybenzo[d][1,3]dioxol-5-yl)-4-(hydroxymethyl)tetrahydrofuran-3-yl)methyl)benzene-1,2-diol]:* UV λ_{max}^{MeOH} = 294; MW = 360; ESI$^-$-MS, *m*/*z* 359 [M − H]$^-$; ^{1}H (500 MHz, *d*-DMSO) δ 1.81 (m, 1H, H-8′), 1.99 (m, 1H, H-8), 2.73 (d, *J* = 5.5 Hz, H, H-7′), 2.76 (d, *J* = 5.5 Hz, H, H-7′), 2.49 (t, *J* = 12.8 Hz, 2H, H-7′), 3.57 (q, *J* = 6.1 Hz, 2H, H-9), 3.04 (t, *J* = 9.8 Hz, 2H, H-9), 3.40 (d, *J* = 4.3 Hz, 2H, H-9′), 3.38 (d, *J* = 4.0 Hz, 2H, H-9′), 3.34 (d, *J* = 5.4 Hz, 2H, H-9′), 3.32 (d, *J* = 5.6 Hz, 2H, H-9′), 4.36 (d, *J* = 4.8 Hz, 1H, H-7), 5.81 (s, 2H, −O−CH_2−O−), 5.92 (s, 1H, H-6), 6.12 (d, *J* = 10.5 Hz, 1H, H-5′), 6.30 (d, *J* = 8.4 Hz, 1H, H-6′), 6.43 (s, 1H, H-3), 6.49 (s, 1H, H-2′); ^{13}C (125 MHz, *d*-DMSO) δ 121.8 (C-1), 149.5 (C-2), 98.0 (C-3), 145.4 (C-4), 139.8 (C-5), 110.7 (C-6), 37.3 (C-7), 42.9 (C-8), 61.5 (C-9), 129.9 (C-1′), 114.9 (C-2′), 143.8 (C-3′), 143.4 (C-4′), 116.0 (C-5′), 126.7 (C-6′), 31.4 (C-7′), 33.5 (C-8′), 63.2 (C-9′), 100.53 (O−CH_2−O).

ST-4. *[(Z)-6-(4-(3,4-dihydroxybenzylidene)-3-(hydroxymethyl)tetrahydrofuran-2-yl)benzo[d][1,3]dioxol-5(7aH)-one]:* UV λ_{max}^{MeOH} = 279; MW = 358; ESI$^-$-MS, *m*/*z* 357 [M − H]$^-$; ^{1}H (500 MHz, *d*-DMSO) δ 2.47 (m, H, H-8), 3.44 (d, *J* = 4.2 Hz, 2H, H-9), 3.46 (d, *J* = 4.3 Hz, 2H, H-9), 4.07 (t, *J* = 8.4 Hz, 2H, H-9′), 3.91 (d, *J* = 5.2 Hz, 2H, H-9′) 3.93 (d, *J* = 5.1 Hz, 2H, H-9′), 4.12 (m, H, H-7), 4.43 (d, *J* = 8.5 Hz, H, H-5), 6.13 (s, 2H, −O−CH_2−O−), 6.60 (d, *J* = 6.8 Hz, H, H-6), 6.66 (d, *J* = 8 Hz, H, H-7′), 6.78 (d, *J* = 1.7 Hz, H, H-2′), 7.04 (d, *J* = 8.2 Hz, H, H-5′), 7.47 (d, *J* = 1.3 Hz, H, H-3), 7.65 (d, *J* = 1.5 Hz, H, H-6′); ^{13}C (125 MHz, *d*-DMSO) δ 132.3 (C-1), 197.6 (C-2), 107.8 (C-3), 151.6 (C-4), 82.9 (C-5), 117.7 (C-6), 49.1 (C-7), 53.1 (C-8), 59.6 (C-9), 131.2 (C-1′), 113.9 (C-2′), 144.8 (C-3′), 148.0 (C-4′), 108.1 (C-5′), 124.9 (C-6′), 115.2 (C-7′), 145.1 (C-8′), 70.0-(C-9′), 102.1 (O−CH_2−O).

ST-5. *[(Z)-2-(4-(3,4-dihydroxybenzylidene)-3-(hydroxymethyl)tetrahydrofuran-2-yl)-4,5-dihydroxycyclohexa-2,5-dienone]:* UV λ_{max}^{MeOH} = 280; MW = 346; ESI$^-$-MS, *m*/*z* 345 [M−H]$^-$; ^{1}H (500 MHz, *d*-CD_3OD) δ 2.67 (m, H, H-8), 3.59 (dq, *J* = 3.6, 11.4, 19.3 Hz, 2H, H-9), 4.06 (dt, *J* = 4.1, 5.7 Hz, H, H-9′), 4.19 (m, H, H-9′), 4.19 (m, H, H-7), 4.58 (d, *J* = 9.0 Hz, H, H-5), 6.73 (s, H, H-2′), 6.73 (d, *J* = 8.5 Hz, H, H-6), 6.85 (d, *J* = 8.2 Hz, H, H-5′), 6.89 (s, H, H-3), 7.46 (q, *J* = 2.1 Hz, H, H-7′), 7.48 (d, *J* = 2.1 Hz, H, H-6′); ^{13}C (125 MHz, *d*-CD_3OD) δ 133.5 (C-1), 200.3 (C-2), 115.1 (C-3), 152.5 (C-4), 85.2 (C-5), 119.8 (C-6), 50.2 (C-7), 54.2 (C-8), 60.9 (C-9), 146.6 (C-1′), 130.3 (C-2′), 116.1 (C-3′), 146.3 (C-4′), 115.9 (C-5′), 123.6 (C-6′), 116.4 (C-7′), 146.4 (C-8′), 71.9 (C-9′).

ST-6. *[2,3-bis(3,4-dihydroxybenzyl)-4-hydroxybutanal]:* UV λ_{max}^{MeOH} = 273; MW = 332; ESI$^-$-MS, *m*/*z* 329 [M−H]$^-$; ^{1}H (500 MHz, *d*-CD_3OD) δ 2.11 (m, H, H-8), 2.57 (d, *J* = 5.8 Hz, H, H-7), 2.59 (s, H, H-7), 2.62 (q, *J* = 5.2, 8.4 Hz, H, H-7), 2.67 (d, *J* = 5.2 Hz, H, H-7′), 3.20 (q, *J* = 5.0, 7.5 Hz, H, H-8′), 3.34 (m, H, H-8′), 3.49 (d, *J* = 1.9 Hz, H, H-9), 6.24 (dd, *J* = 1.2, 7.0 Hz, H, H-2), 6.39 (d, *J* = 5.2 Hz, H, H-3), 6.44 (s, H, H-6), 6.79 (d, *J* = 8.1 Hz, H, H-5′), 7.38 (dd, *J* = 8.3, 1.8 Hz, H, H-6′), 7.36 (d, *J* = 1.8 Hz, H, H-2′), 9.38 (s, H, H-9′); ^{13}C (125 MHz, *d*-CD_3OD) δ 128.8 (C-1), 125.4 (C-2), 115.0 (C-3), 143.2 (C-4), 143.4 (C-5), 115.4 (C-6), 32.1 (C-7), 39.6 (C-8), 63.3 (C-9), 126.0 (C-1′), 115.2 (C-2′), 145.3 (C-3′), 150.8 (C-4′), 115.2 (C-5′), 121.5 (C-6′), 30.6 (C-7′), 42.1 (C-8′), 201.6 (C-9′).

ST-7. *[2,3-bis(3-hydroxybenzyl)butane-1,4-diol]:* UV λ_{max}^{MeOH} = 274; MW = 302; ESI$^-$-MS, *m*/*z* 301 [M−H]$^-$; ^{1}H (500 MHz, *d*-DMSO) δ 1.89 (m, 2H, H-8,8′), 2.59 (m, 4H, H-7′,7″), 3.37 (dd, *J* = 10.6, 3.1 Hz, 2H, Hb-9,9′), 3.49 (dd, *J* = 10.5, 4.7 Hz, 2H, Ha-9,9′), 6.56 (m, m, 6H, H-2′,-4′,6′,2″,4″,6″), 7.01 (t, *J* = 7.7 Hz, 2H, H-5′,5″); ^{13}C (125 MHz, *d*-DMSO) δ 143.0 (C-1), 115.9 (C-2), 157.1 (C-3), 112.5 (C-4), 128.9 (C-5), 119.6 (C-6), 34.0 (C-7), 42.6 (C-8), 60.2 (C-9), 143.0 (C-1′), 115.9 (C-2′), 157.1 (C-3′), 112.5 (C-4′), 128.9 (C-5′), 119.6 (C-6′), 34.0 (C-7′), 42.6 (C-8′), 60.2 (C-9′).

ST-8. *[3,4-bis(3-hydroxybenzyl)dihydrofuran-2(3H)-one]:* UV λ_{max}^{MeOH} = 280; MW = 298; ESI$^-$-MS, *m*/*z* 297 [M − H]$^-$; ^{1}H (500 MHz, *d*-DMSO) δ 2.49 (m, 1H, Hb-7″), 2.35 (m, 1H, Ha-7″), 2.63 (m, 1H, H-8′), 3.16 (dd, *J* = 13.8, 7.0 Hz, 1H, Ha-7′), 3.34 (dd, *J* = 13.8, 5.3 Hz, 1H, Hb-7′), 3.16 (m, 1H, H-8), 6.91 (d, *J* = 2.2 Hz, 1H, H-2″), 6.99 (d, *J* = 7.9 Hz, 1H, H-6″), 7.15 (m, 3H, H-2′,6′), 7.42 (ddd, *J* = 8.0, 2.2, 1.0 Hz, 1H, H-4″,4′), 3.50 (d, *J* = 8.0 Hz, 2H, H-9′), 7.65 (t, *J* = 8.0 Hz, 1H, H-5″,5′); ^{13}C (125 MHz, *d*-DMSO) δ 136.6 (C-1), 117.9 (C-2), 156.8 (C-3), 111.4 (C-4), 126.7 (C-5), 120.6 (C-6), 39.6 (C-7), 40.1 (C-8), 177.4 (C-9), 136.6 (C-1′), 117.9 (C-2′), 156.8 (C-3′), 111.4 (C-4′), 123.2 (C-5′), 118.3 (C-6′), 39.8 (C-7′), 39.9 (C-8′), 72.4 (C-9′).

RESULTS

Transformation of STG Metabolites by a HIB Mixture. After anaerobic incubation of STG with a bacterial mixture of human feces, the culture was extracted with *n*-BuOH and the extract was subjected to Diaion HP-20, Sephadex LH-20, and preparative RP-18 column chromatography. Eight metabolites (**ST-1**−**ST-8**) were isolated and identified by ESI-MS and one-dimensional (1D) and 2D-NMR. The ^{1}H and ^{13}C NMR spectra of **ST-1** were in good agreement with those reported for sesaminol (*18*, *19*).

Compound **ST-2** was detected as a major metabolite after 24 h of incubation of STG in a human fecal suspension. Compound **ST-3** has the same molecular weight as **ST-2**. The molecular weight of the isolated metabolites was determined by LC-MS. Both **ST-2** and **ST-3** had a [M − H]$^-$ ion at *m*/*z* 359 with electrospray ionization (ESI). The molecular ions of **ST-2** and **ST-3** were 10 mass units less than that of **ST-1**; therefore, **ST-2** and **ST-3** could be reduced and demethylated products of ST-1. The full assignment of each proton and carbon signal was confirmed by ^{1}H-detected HMQC and HMBC experiments (**Figure 1**).

The structure of STG metabolite (**ST-2**) isolated from human feces can be clearly established by ^{1}H and ^{13}C NMR spectral data. H-8 appeared as a multiplet due to coupling with H-8′, H-7, and H-9, whereas H-8′ appeared as a multiplet due to coupling with H-9′ and H-8, as confirmed by COSY. HMBC showed coupling between H-6 (δ 6.31)/H-3 (δ 6.41) and C-5 (δ 142.3). The position of C-2 (δ 151.0) was assigned from couplings with H-6 (δ 6.31) and H-7 (δ 4.19), and the positions of C-2 (δ 151.0) and C-4 (δ 147.4) were assigned from coupling with H-6 (δ 6.31) in HMBC. Moreover, HMBC showed the coupling between methylenedioxyphenyl protons (δ 5.76) and C-5 (δ 142.3), confirming the presence of the methylenedioxyphenyl group on this aromatic ring. On the other aromatic ring of the molecule, HMBC showed the coupling of H-2′ (δ 6.53) and C-7′ (δ 33.3). The position of C-4′ (δ 144.0) was assigned from couplings with H-2′ (δ 6.53) and H-5′ (δ 6.20), and the positions of C-3′ (δ144.1) and C-1′ (δ 132.3) were assigned from couplings with H-5′ (δ 6.20) in HMBC. The presence of hydroxyl groups on this aromatic ring caused upfield shifts in positions C-3 and C-4 and downfield shifts in positions C-3′ and C-4′ compared with the corresponding positions on the aromatic ring possessing the methylenedioxyphenyl group. The aromatic carbons were assigned from couplings to attached hydrogens in HSQC. Thus, the structure of the STG metabolites (**ST-2**) was established as 4-[((3*R*,4*R*)-5-(6-hydroxybenzo[*d*][1,3]dioxol-5-yl)-4-(hydroxymethyl)tetrahydrofuran-3-yl)methyl]benzene-1,2-diol. The proton signals (including chemical shifts and coupling constants) of **ST-3** due to the furan ring in the ^{1}H NMR spectrum were similar to those of **ST-2**, suggesting a similar stereochemistry at C-7, C-8, C-7′, and C-8′. Compared to **ST-2**, the furan carbons of **ST-3** had upfield shifts in positions C-7 (δ 37.3), C-8 (δ 42.9), C-7′ (δ 31.4), and C-8′ (δ 33.5) and downfield shifts in positions C-7 (δ 40.8), C-8 (δ 47.6), C-7′ (δ 33.3), and C-8′ (δ 40.6). The structure of **ST-3** was consequently determined to be a stereoisomer of **ST-2**, 4-[((3*S*,4*R*)-5-(6-hydroxybenzo[*d*][1,3]dioxol-5-yl)-4-(hydroxymethyl)tetrahydrofuran-3-yl)-methyl]benzene-1,2-diol by NMR and MS (**Figure 1**).

The molecular ion of compound **ST-4** at *m*/*z* 357 [M − H]$^-$ in the ESI-MS spectrum was 2 mass units less than that of **ST-2**, indicating that **ST-4** is an oxidation product of **ST-2** and **ST-3**. In the NMR spectra of **ST-4**, H-8 (δ 2.47) appeared as a multiplet due to coupling with H-7 and H-9, whereas H-9′ (δ 4.07) appeared as a multiplet due to coupling with H-7′ and H-8, as confirmed by COSY and NOESY. HMBC showed coupling between H-7 (δ 4.12)/H-3 (δ 7.47) and C-1 (δ 132.3). ^{1}H NMR showed meta-coupling between H-6′ and H-2′ (*J* = 1.7 Hz) and ortho-coupling between H-6′ and H-5′ (*J* = 8.2 Hz). The position of C-2 (δ 197.6) was assigned from couplings with H-3 (δ 7.47), and the positions of C-2 (δ 197.6) and C-1 (δ 132.3) were assigned from coupling with H-3 (δ 7.47) in HMBC. Moreover, HMBC showed coupling between the methylenedioxyphenone protons (δ 102.1) and C-4

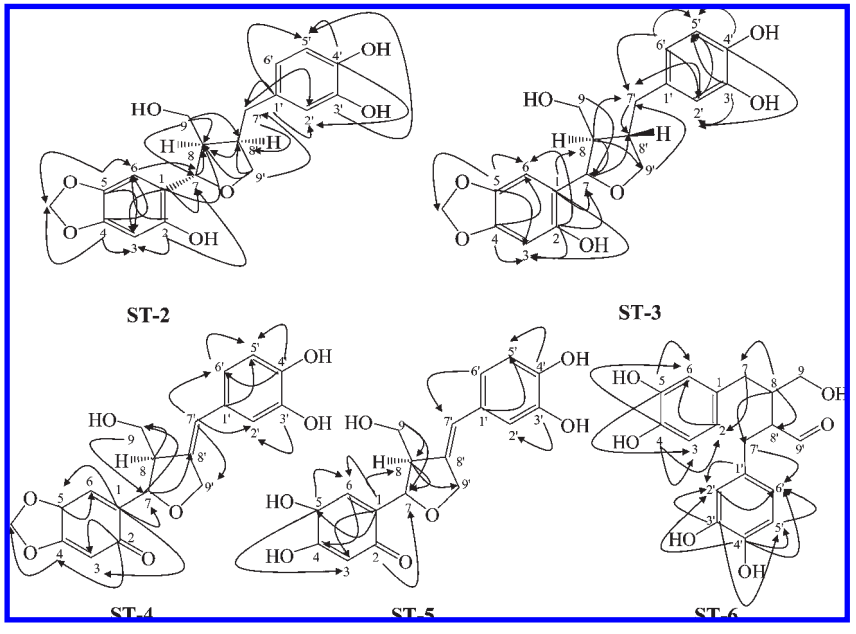

Figure 1. HMBC correlations of compounds **ST-2**, **ST-3**, **ST-4**, **ST-5**, and **ST-6**.

(δ 151.6), confirming the presence of the methylenedioxyphenone group on this aromatic ring. On the other aromatic ring of the molecule, HMBC showed the couplings between H-2′ (δ 6.78) and C-7′ (δ 115.2). The positions of C-4′ (δ 148.0) and C-3′ (δ 144.8) were assigned from couplings with H-5′ (δ 7.04) and H-2′ (δ 6.78), respectively, and the positions of C-4′ (δ 148.0) and C-1′ (δ 131.2) were assigned from couplings with H-5′ (δ 7.04) in HMBC (**Figure 1**). The structure of **ST-4** was assigned as (*Z*)-6-[4-(3,4-dihydroxybenzylidene)-3-(hydroxymethyl)tetrahydrofuran-2-yl]benzo[*d*][1,3]dioxol-5(7a*H*)-one.

The molecular ion of compound **ST-5** at *m*/*z* 345 $[M - H]^-$ in the ESI-MS spectrum was 12 mass units less than that of **ST-4**, indicating that **ST-5** is a demethylenation product of **ST-4**. This was supported by its ^1H and ^{13}C NMR. In contrast to one methylene group in **ST-4**, no signal of a methylene group was observed in **ST-5**. Furthermore, a signal of H-5 was shifted upfield by 4.58 ppm, whereas that of H-6 was shifted downfield by 6.73 ppm, indicating that demethylenation had taken place at C-5. This was further proved by an HMBC experiment (**Figure 1**). The position of C-2 (δ 200.3) was assigned from coupling with H-7 (δ 4.19), and the positions of C-1 (δ 133.5) and C-5 (δ 85.2) were assigned from coupling with H-3 (δ 6.89) in HMBC. HMBC showed coupling between H-6 (δ 6.73)/H-3 (δ 6.89) and C-1 (δ 133.5). On the other aromatic ring of the molecule, HMBC showed coupling between H-6′ (δ 7.48) and C-7′ (δ 116.4). The positions of C-4′ (δ 146.3) and C-1′ (δ 146.6) were assigned from couplings with H-5′ (δ 6.85) in HMBC. H-8 (δ 2.67) appeared as a multiplet due to coupling with H-7 and H-9, whereas H-9 (δ 3.59) appeared as a multiplet due to coupling with H-7, as confirmed by COSY and NOESY. The structure of **ST-5** was assigned as (*Z*)-2-[4-(3,4-dihydroxybenzylidene)-3-(hydroxymethyl)tetrahydrofuran-2-yl]-4,5-dihydroxycyclohexa-2,5-dienone (**Figure 1**).

The molecular ion of compound **ST-6** at *m*/*z* 331 $[M - H]^-$ in the ESI-MS spectrum was 16 mass units less than that of **ST-5**, indicating that **ST-6** is a reduction and dehydroxylation product of **ST-5**. This was supported by its ^1H and ^{13}C NMR spectral data. H-8 (δ 2.11) appeared as a multiplet due to coupling with H-7, whereas H-7 (δ 2.57, 2.62) appeared as a multiplet due to coupling with H-9, as confirmed by COSY and NOESY. ^1H NMR showed meta-coupling between H-6′ and H-2′ (J = 1.75 Hz) and ortho-coupling between H-6′ and H-5′ (J = 8.1 Hz). The position of C-4 (δ 143.2) was assigned from couplings with H-3 (δ 6.39) and H-6 (δ 6.44), and the positions of C-4 (δ 143.2) and C-5 (δ 143.4) were assigned from coupling with H-3 (δ 6.39) in HMBC. The position of C-5 (δ 143.4) was assigned from couplings with H-3 (δ 6.39) and H-6 (δ 6.44), and the positions of C-4 (δ 143.2) and C-5 (δ 143.4) were assigned from coupling with H-6 (δ 6.44) in HMBC. On the other aromatic ring of the molecule, HMBC showed couplings between H-6′ (δ 7.38) and C-7′ (δ 30.6). The position of C-4′ (δ 150.8) was assigned from couplings with H-6′ (δ 7.38), and the positions of C-4′ (δ 150.8) and C-3′ (δ 145.3) were assigned from couplings with H-2′ (δ 7.36) in HMBC. The structure of ST-6 was established as 2,3-bis(3,4-dihydroxybenzyl)-4-hydroxybutanal (**Figure 1**).

The ^1H and ^{13}C NMR data of compounds **ST-7** and **ST-8** agreed well with those reported for END and ENL, respectively (*9*, *20*). After a week of incubation with human intestinal microbiota, compound **ST-8** was identified as ENL, a final metabolite of STG. Therefore, compound **ST-8** was determined to be ENL [3,4-bis(3-hydroxybenzyl)dihydrofuran-2(3*H*)-one]. A compound having the same structure was obtained as the final metabolite of arctigenin, pinoresinol diglucoside, and sesamin (*21*).

Transformation of STG Metabolites by Intestinal Microbiota. These metabolic processes are further supported by a time course

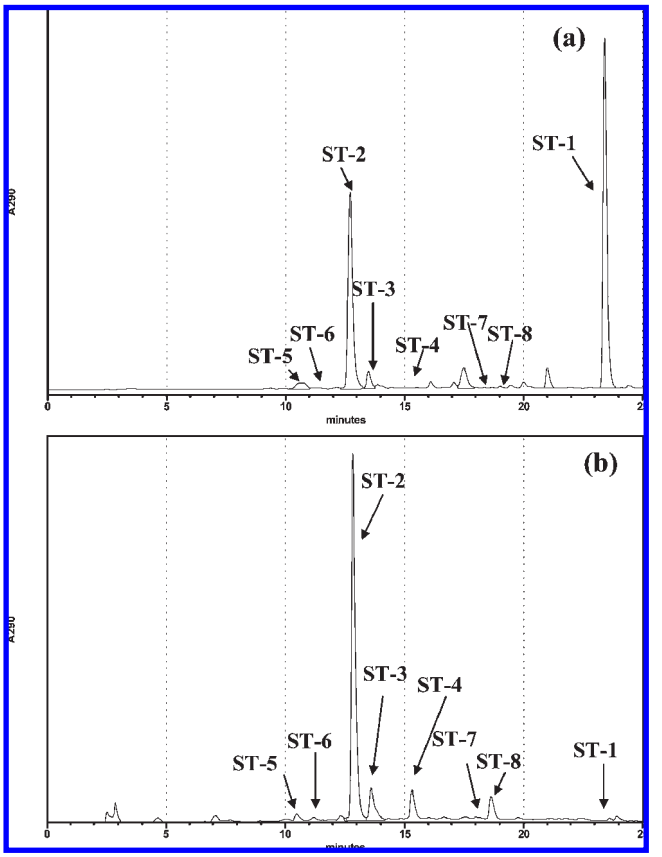

Figure 2. HPLC chromatograms of sesaminol triglucoside metabolites by microflora of rats and humans: (**a**) cultivated sesaminol triglucoside with rat microflora; (**b**) cultivated sesaminol triglucoside with human microflora.

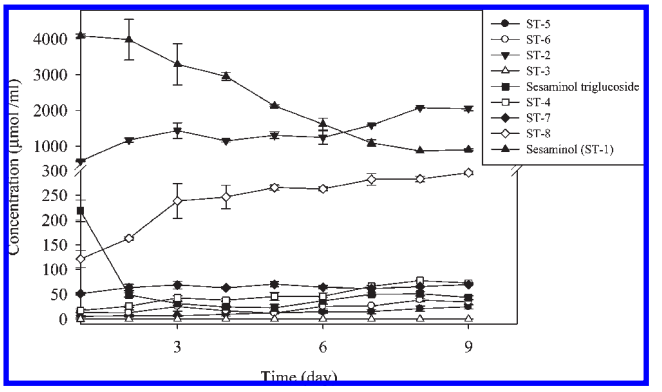

Figure 3. Time course of the transformation of sesaminol triglucoside to major enterolignans by microbiota of rats.

experiment, monitored by HPLC. The major STG metabolites were **ST-1** and **ST-2** by rat intestinal microbiota, but **ST-2** was the main metabolite by human intestinal microbiota (**Figure 2**). **Figure 3** shows the time course for the formation of **ST-2** and the consumption of sesaminol (**ST-1**). A stepwise conversion from STG to **ST-2**, **-3**, **-4**, **-5**, **-6**, **-7**, and **ST-8** (ENL) occurs, whereas **ST-8** appears to be stable even after prolonged incubation. The isolated rat intestinal microbiota showed potent ability to transform **ST-1** to **ST-8**. Compound **ST-2** formed after 24 h of incubation and reached a maximum concentration after 1 week. The time course experiment confirmed that the **ST-1** is responsible for the transformation to **ST-2** and **ST-8**.

Possible Transformation Pathway of STG by Intestinal Microbiota. On the basis of the structures of metabolites of STG and the

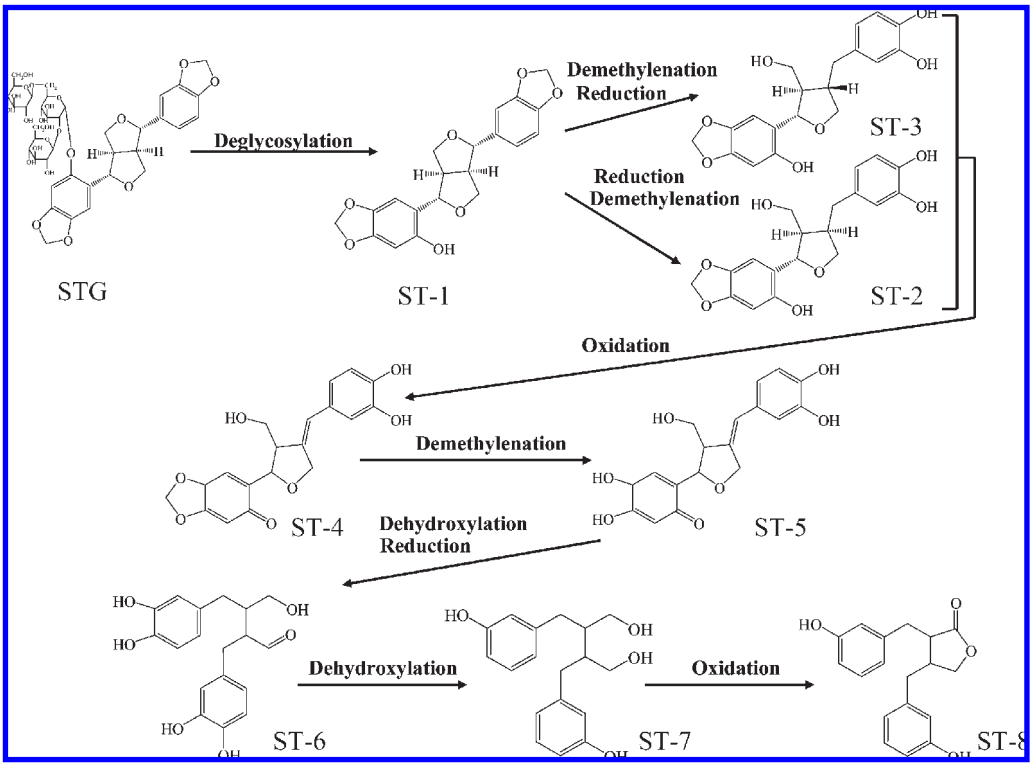

Figure 4. Possible pathway for the biotransformation of sesaminol triglucoside by rat.

time course experiments monitored by HPLC, a possible metabolic pathway was proposed (**Figure 4**). The transformation of STG by human intestinal microbiota includes four types of reactions: hydrolysis of glucoside, demethylenation of a methylene group, reduction of a tetrahydrofuran moiety, and oxidation of methylenedioxyphenyl to methylenedioxyphenone or of dibenzylbutanediol to dibenzylbutyrolactone. The present study (**Figure 4**) gives us a much clearer understanding of the bacterial biotransformation of plant lignans to mammalian lignans, because the conversion passes through all three other types of precursors (compounds **ST-2**, **-3**, **-4**, and **-5** are furano lignans, compounds **ST-6** and **ST-7** are dibenzylbutanes, and compound **ST-8** is a dibenzylbutyrolactone). The reductive cleavage of the furofuran rings, **ST-1** to **ST-4** via **ST-2** and **ST-3** (furofurans to dibenzylbutanes via furans), proceeds with the retention of stereochemistry at C-8 and C-8′ in **ST-2** and **ST-3**.

DISCUSSION

At least seven plant lignans are now thought to be precursors of the mammalian lignans, END and ENL (*22*). These precursors may be divided into four types: dibenzylbutanes (secoisolariciresinol), dibenzylbutyrolactones (matairesinol, 7-hydroxymatairesinol, and arctigenin), furano lignans (lariciresinol), and furofuran lignans (pinoresinol and syringaresinol). Of these plant lignans, the transformation pathways of secoisolariciresinol diglucoside (SDG), pinoresinol diglucoside (PDG), and arctiin (the glucoside of arctigenin) by human intestinal microbiota have been extensively studied (*16*, *20*, *21*). It has also been shown that sesamin could be converted to ENL and END in humans (*22*, *23*).

The metabolism of arctiin and tracheloside has been studied; the glycosides of arctigenin and trachelogenin were incubated by rat intestinal microbiota. During the 24 h reaction time the sugar moieties were cleaved after 5 h, and some demethylation of the substituted phenolic ring was observed (*24*, *25*). In our study, STG was first deglycosylated to **ST-1** by intestinal microbiota; **ST-1**, like other lipophilic lignans (sesamin) possessing a methylenedioxyphenyl moiety, undergoes oxidative biotransformation and demethylenation of its methylenedioxyphenyl group to form a hydroxylated catechol metabolite (*26*). **ST-1** may be demethylenated into **ST-2** and **ST-3** and then further oxidized to **ST-4** in its methylenedioxyphenyl to methylenedioxyphenone moiety. **ST-4** can be further demethylenated to **ST-5**. **ST-5** could further undergo reductive cleavage of its furofuran rings to **ST-6**, similar to PDG (*16*). Further metabolism of the furan ring, demethylation, and dehydroxylation of intermediate metabolites can be carried out by intestinal microbiota in the same way as for furofuran lignans such as pinoresinol and common lignans such as lariciresinol and SDG.

Enterolactone production was a steadily proceeding slow process (*27*, *28*). This result is supported by the findings of this study and others (*29*−*31*). A previous study of ours shows that a portion of dietary STG is absorbed and metabolized and is then transported to other tissues, but is removed from the body after consumption. In this study, we observed only limited conversion of STG to END and ENL by in vitro fermentation with human and rat fecal microbiota. Therefore, it is assumed that STG is mainly metabolized in the feces to **ST-2**; they are then metabolized by the intestinal microbiota to hydroxylated metabolites. Hence, the primary site of STG metabolism can be the same as that of the more hydrophilic lignan precursors such as SDG, which is metabolized by intestinal microbiota to **ST-2** and **ST-3**.

In our study, the possible metabolism pathway of sesaminol triglucoside was metabolized via intestinal microbiota. The biotransformation of lignans by microbiota has been reported. Transit time of material through the large intestine is an important factor affecting the availability of dietary components to the host, primarily because colonic bacterial fermentation can influence circulating concentrations of compounds produced by colonic bacteria (*9*, *32*). Our results demonstrate that sesame seed is one of the richest dietary sources of mammalian lignan precursors, and sesaminol triglucoside is one of them.

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