JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Studies on the Metabolism of the Plant Lignans Secoisolariciresinol and Matairesinol

HEIKE B. NIEMEYER,[†] DORIS M. HONIG, SABINE E. KULLING,[‡] AND MANFRED METZLER*

Institute of Food Chemistry and Toxicology, Department of Chemistry and Biosciences, University of Karlsruhe, P.O. Box 6980, 76128 Karlsruhe, Germany

The plant lignans secoisolariciresinol and matairesinol occur in numerous foods such as oilseeds, whole grains, vegetables, and fruits. We have studied the hitherto unknown oxidative metabolism of secoisolariciresinol and matairesinol in hepatic microsomes from untreated and Aroclor 1254-induced Wistar rats and from humans. Five oxidative metabolites of secoisolariciresinol and 10 oxidative metabolites of matairesinol were detected in rat liver microsomes, and their chemical structures were elucidated. The pathways in the metabolism of both secoisolariciresinol and matairesinol included aliphatic and aromatic hydroxylation, whereas oxidative demethylation was only observed for matairesinol. Human hepatic microsomes were able to metabolize secoisolariciresinol whereas matairesinol was only poorly metabolized. This study clearly shows that secoisolariciresinol and matairesinol are substrates of cytochrome P450-mediated metabolism. However, from preliminary experiments with rats dosed orally with secoisolariciresinol and matairesinol, it appears that the intestinal absorption and subsequent oxidative metabolism of these plant lignans occur only to a very small extent due to the highly efficient conversion of secoisolariciresinol and matairesinol to the mammalian lignans enterodiol and enterolactone by the gut microflora.

KEYWORDS: Plant lignans; microsomal metabolites; secoisolariciresinol; matairesinol; lariciresinol; mass spectrometry

INTRODUCTION

Secoisolariciresinol diglycoside and matairesinol are plant lignans occurring in a variety of different foods, e.g., oilseeds, whole grains, vegetables, and fruits (*1*, *2*). Lignans are of increasing interest due to their potential anticarcinogenic, estrogenic, and antiestrogenic activities (*3*−*6*) and antioxidative effects (*7*−*10*). They are discussed as protective agents against hormone-dependent cancers, especially breast and prostate cancer (*4*, *11*−*13*). High levels of lignans are found in flaxseed, with secoisolariciresinol diglycoside as the major and matairesinol as the minor lignan constituents (*14*). Recently, isolariciresinol, pinoresinol, and lariciresinol were identified in flaxseed as additional lignan compounds (*15*, *16*). After ingestion, secoisolariciresinol diglycoside and matairesinol are converted by intestinal bacteria to the mammalian lignans enterodiol and enterolactone (**Figure 1**), which were identified in the urine of rats and humans (*17*−*19*). The extent of conversion depends on the activity of the gut microflora and the use of antimicrobials and varies intervidually (*20*−*23*). An intermediate bacterial metabolite of secoisolariciresinol diglycoside is its aglycone, secoisolariciresinol. Furthermore, enterodiol can be metabolized to enterolactone in the intestine (*24*).

The mammalian metabolism of the plant lignans secoisolariciresinol diglycoside and matairesinol is still unknown. The proportion of secoisolariciresinol and matairesinol escaping bacterial metabolism in the intestine will undergo absorption, and oxidative metabolites could possibly be formed in the mammalian organism, which may be of biological and toxicological relevance. For example, chemopreventive and antioxidative effects were recently reported for 7′-hydroxy-matairesinol, a further plant lignan (*25*, *26*) that is also a putative hydroxylation product in the oxidative metabolism of matairesinol.

In the present study, we have investigated the oxidative in vitro metabolism of secoisolariciresinol and matairesinol in rat and human hepatic microsomes. Using gas chromatography−mass spectrometry (GC-MS) analysis and reference substances, the structures of three oxidative metabolites were definitely elucidated. For several other oxidative metabolites of the two plant lignans, no reference compounds were available, and their tentative identification is presented. In a preliminary experiment, secoisolariciresinol and matairesinol were orally administered to Wistar rats, and metabolites of the plant lignans were searched for in the rat urine.

* To whom correspondence should be addressed. Tel: +49-721-608-2132. Fax: +49-721-608-7255. E-mail: Manfred.Metzler@chemie.uni-karlsruhe.de.

[†] Present address: Chemisches und Veterinäruntersuchungsamt Karlsruhe, Weissenburger Strasse 3, 76187 Karlsruhe, Germany.

[‡] Present address: Institute of Nutritional Physiology, Federal Research Centre for Nutrition, Haid-und-Neu-Strasse 9, 76131 Karlsruhe, Germany.

10.1021/jf030263n CCC: $25.00 © 2003 American Chemical Society
Published on Web 09/03/2003

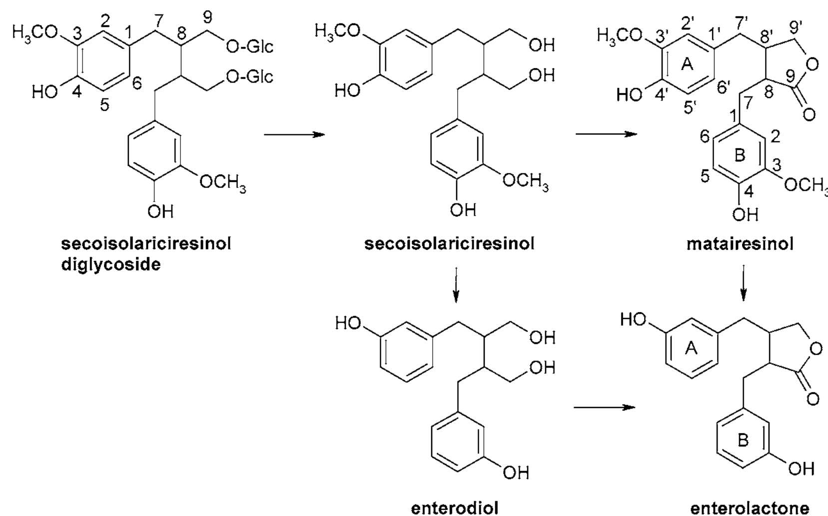

Figure 1. Bacterial transformation of the plant lignans secoisolariciresinol diglycoside and matairesinol to the mammalian lignans enterodiol and enterolactone.

MATERIALS AND METHODS

Chemicals and Materials. (±)-Secoisolariciresinol was kindly provided by M. Schöttner (University of Bayreuth, Germany). (±)-Matairesinol was synthesized in our laboratory by E. Jacobs according to a modification of the method of Pelter et al. (*27*). (±)-Lariciresinol was a gift from Dr. L. B. Davin (Washington State University, U.S.A.). 7′-Hydroxy-matairesinol was kindly provided by Dr. R. Santti (University of Turku, Finland) and consisted of two diastereomers. The purity of secoisolariciresinol and matairesinol was ≥99 and ≥92% according to GC-MS analysis. Deuterated d9-BSA was purchased from C/D/N Isotopes Inc. (Quebec, Canada). All other chemicals were purchased from major suppliers and were of the highest purity available.

Preparations and Incubations of Liver Microsomes. Microsomes were prepared from the liver of untreated and Aroclor-treated male Wistar rats and from human liver as described by Lake (*28*). To induce cytochrome P450 enzymes, animals were treated with a single intraperitoneal dose of 500 mg Aroclor 1254/kg body weight, dissolved in sesame oil at 100 mg/mL, and sacrificed on day 6 after treatment. Protein concentrations were measured using the Pierce bicinchoninic reagent. Cytochrome P450 concentrations were determined as described by Omura and Sato (*29*). Hepatic microsomes from untreated and Aroclor-treated rats were found to contain 0.59 and 1.69 nmol cytochrome P450 per mg protein, respectively. Human liver microsomes contained 0.32 nmol cytochrome P450 per mg protein.

Incubations of secoisolariciresinol and matairesinol with liver microsomes were carried out as previously reported (*30*). A standard incubation mixture of 4 mL total volume contained 100 nmol of secoisolariciresinol or matairesinol dissolved in 100 μL of dimethyl sulfoxide (DMSO) or methanol, 4 mg of microsomal protein (4.5 mg for untreated rat liver microsomes), and a NADPH-generating system (1.21 mM $NADP^+$, 4.3 mM $MgCl_2$, 9.4 mM D,L-isocitrate, and 1.8 U isocitrate dehydrogenase) in 0.1 M potassium phosphate buffer, pH 7.4. Incubations (37 °C, 60 min) were stopped by the addition of 4 mL of ethyl acetate. Lignans and metabolites were extracted with 3 × 4 mL of ethyl acetate, the extract was evaporated to dryness under reduced pressure at 40 °C, and the residue was dissolved in 200 μL of methanol or a methanol/water mixture. For further purification, the methanolic solution was diluted with water and applied to a 9 mm × 10 mm, short body RP-18 cartridge (Waters) preconditioned with 10 mL of water, 5 mL of methanol, and 10 mL of water. After they were washed with 5 mL of water, the lignans and their metabolites were eluted with 5 mL of methanol. The eluate was evaporated to dryness under reduced pressure or a stream of nitrogen. The residue was either redissolved in 200 μL of methanol or methanol/water for high-performance liquid chromatography (HPLC)−UV/diode array detection (DAD) analysis with fractionation of the metabolites or directly treated with BSTFA, BSA, or d9-BSA overnight at room temperature for GC-MS analysis. Control incubations were carried out with heat-inactivated microsomes, without microsomes, without substrate, or without the NADPH-generating system.

HPLC−UV/DAD Analysis and Fractionation of in Vitro Metabolites. HPLC analysis was carried out on a 250 mm × 4.6 mm i.d., 5 μm Prodigy ODS column (Phenomenex) using a linear solvent gradient from 25 B to 29% B in 55 min (solvent A: water/methanol 84:16 v/v, adjusted to pH 2.8 with formic acid; solvent B: methanol) with a flow rate of 1 mL/min. Aliquots of 20−50 μL were injected for the analysis of metabolites. Detection was carried out at 283 nm with both a Lamda-Max model 480 UV detector (Waters) and a Series 1100 DAD detector (Hewlett-Packard) to obtain UV spectra (wavelength range 200−400 nm) of the metabolites. For GC-MS analysis, secoisolariciresinol metabolites were separated by HPLC into two fractions: fraction 1 was collected from 5.0 to 21.5 min elution time; in fraction 2, the major metabolite was collected from 21.5 to 25.0 min. Metabolites of matairesinol were collected from 5.0 to 37.0 min elution time, prior to the matairesinol peak. After they were evaporated to dryness under reduced pressure at 40 °C or by freeze-drying, the metabolites were trimethylsilylated as described above.

GC-MS Analysis. GC-MS analysis was carried out using a GCQ ion trap mass detector (Finnigan MAT) with the following conditions: 28.7 m × 0.25 mm i.d.; 0.25 μm MDN-5S fused silica capillary column (Supelco, Taufkirchen, Germany); flow of He, run pressure from 10 to 20 psi; column temperature program, 1 min isothermic at 60 °C, then from 60 to 250 °C at 30 °C/min, 10 min isothermic at 250 °C, from 250 to 275 °C at 1 °C/min; injector temperature, from 50 to 275 °C at 8 °C/s; transfer line temperature, 275 °C; ion source temperature, 200/250 °C; ionization energy (EI), 70 eV; injection volume, 0.5−2 μL. Full scan mass spectra (m/z 50−850) were recorded at a rate of two spectra per second. Detection of secoisolariciresinol, matairesinol, and their metabolites was achieved by selecting up to three characteristic ions from the full scan data using the Finnigan GQ 2.31 data analysis software.

RESULTS

Microsomal Metabolites of Secoisolariciresinol. In the symmetric secoisolariciresinol molecule, three aromatic and two aliphatic sites are available for hydroxylation (**Figure 1**). Furthermore, demethylation of the methoxy groups as well as combinations of hydroxylation and demethylation reactions may occur in the oxidative metabolism of secoisolariciresinol.

HPLC−UV analysis of the extracts obtained from the incubations of secoisolariciresinol with microsomes from untreated and Aroclor-treated rats and from humans gave rise to the same qualitative result. In addition to parent secoisolariciresinol, which had a retention time of 25.8 min, only one peak eluting at 22.8 min was detected, which was not present in control incubations. Its absorption maxima were 283 and 229 nm as determined by HPLC−DAD analysis. The extent of conversion of secoisolariciresinol to this metabolite peak

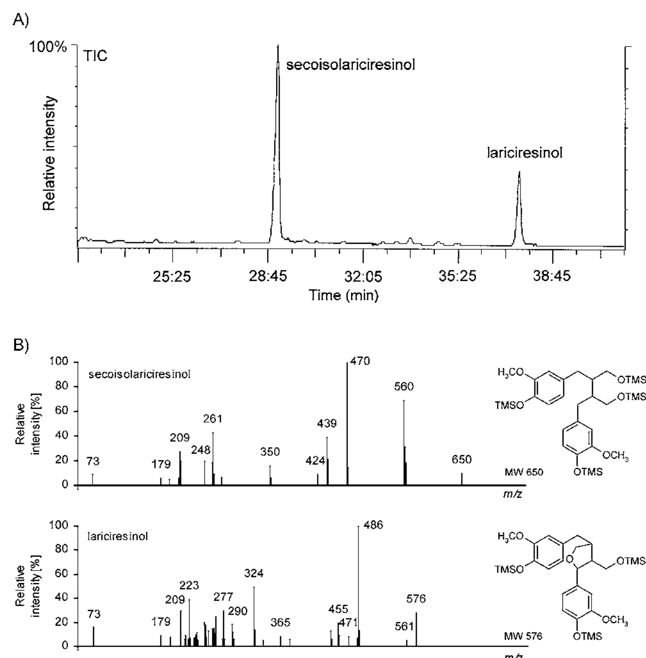

Figure 2. (**A**) GC-MS chromatogram of secoisolariciresinol metabolites from Aroclor-treated rat liver microsomes (TMS derivatives). (**B**) Mass spectra and chemical structures of secoisolariciresinol and lariciresinol (TMS derivatives).

(calculated as peak area of metabolite/∑ (peak area of metabolite + remaining secoisolariciresinol)) depended on the microsomes used and was approximately 30% for induced rat, 21% for untreated rat, and 16% for human liver microsomes, assuming identical absorption coefficients at the detector wavelength of 283 nm.

GC-MS analysis of the incubation mixtures again revealed only one major peak other than secoisolariciresinol in the total ion current chromatogram (**Figure 2A**). This metabolite had a molecular ion of *m*/*z* 576 (**Figure 2B**) and was unequivocally identified as lariciresinol by comparison of its GC retention time and mass spectrum with that of authentic (±)-lariciresinol and with mass spectrometric data described in the literature (*31*−*33*). Derivatization with d9-BSA was used to determine the number of hydroxyl groups, because each hydroxyl gives rise to a shift of nine mass units when d9-BSA is used instead of nonlabeled BSA. The molecular ion of the major secoisolariciresinol metabolite shifted to *m*/*z* 603 (**Table 1**), in accordance with the three hydroxyl groups in the lariciresinol molecule.

To identify further secoisolariciresinol metabolites present in smaller amounts, the incubation mixture with rat liver microsomes was fractionated by HPLC, and the fraction collected between 5.0 min and the retention time of lariciresinol was analyzed by GC-MS. Because hydroxylation and/or demethylation products of secoisolariciresinol may be expected as metabolites, characteristic fragment ions for these compounds were selected from the full scan spectra. The characteristic ions for the trimethylsilylated derivatives were *m*/*z* 648, 558, and 297 for monohydroxylation products and *m*/*z* 708, 618, and 528 for demethylation products. The loss of 90 mass units due to a TMSOH group is a typical fragmentation of lignans, e.g., secoisolariciresinol (**Figure 2B**). The search for the calculated ions led to the detection of five monohydroxylated metabolites of secoisolariciresinol (peaks I, II, IV, V, VI; **Figure 3**), whereas demethylation products, combinations of hydroxylation and demethylation (*m*/*z* 706, 616, and 267), or dihydroxylation products (*m*/*z* 736, 646, and 556) were not observed. Metabolites I, IV, and V were obtained after incubation of secoisolariciresinol with microsomes from both Aroclor- and untreated rats. Metabolites II and VI were formed from untreated rat liver microsomes but were not always clearly detectable in the incubations with Aroclor-treated rat liver microsomes. The mass spectra of the five compounds are depicted in **Figure 4**.

Monohydroxylation products of secoisolariciresinol have a molecular weight of 738 after trimethylsilylation. However, compounds I and II exhibit virtually identical mass spectra without a molecular ion of *m*/*z* 738 (**Figure 4**). As observed previously for metabolites of enterodiol and enterolactone, aliphatic hydroxylation products easily undergo elimination of the newly introduced hydroxyl group, resulting in the lack of a molecular ion in their mass spectra (*30*). It is therefore assumed that compounds I and II are aliphatic hydroxylation products of secoisolariciresinol. The base peak at *m*/*z* 297 probably arises from a benzylic cleavage of 7-hydroxylated secoisolariciresinol (**Figure 5**), as the same type of fragmentation was observed in the mass spectrum of authentic 7′-hydroxy-matairesinol. Com-

Table 1. Mass Spectra of Secoisolariciresinol and Matairesinol Metabolites from Rat Liver Microsomes after Derivatization with d9-BSA (in Parentheses, Corresponding TMS Derivatives)

compound	*m*/*z*
secoisolariciresinol	686 (650), 587 (560), 488 (470), 457 (439), 368 (350), 261 (270), 257 (248), 218 (209)
lariciresinol (peak VII)[a]	603 (576), 504 (486), 473 (455), 342 (324), 286 (277), 232 (223), 218 (209)
hydroxysecoisolariciresinol (peak I)[a]	684 (648), 585 (558), 554 (527), 486 (468), 473 (455), 342 (324), 315 (297), 218 (209)
hydroxysecoisolariciresinol (peak II)[a]	585 (558), 554 (527), 486 (468), 342 (324), 315 (297), 218 (209)
isolariciresinol (peak III)[a]	684 (648), 585 (558), 554 (527), 486 (468), 473 (455), 455 (437), 388 (379), 218 (209)
hydroxysecoisolariciresinol (peak IV)[a]	684 (648), 585 (558), 367 (349), 315 (297), 270 (261), 218 (209)
hydroxysecoisolariciresinol (peak V)[a]	783 (738), 684 (648), 585 (558), 554 (527), 367 (349), 315 (297), 270 (261), 218 (209)
hydroxysecoisolariciresinol (peak VI)[a]	783 (738), 684 (648), 585 (558), 367 (349), 316 (298), 218 (209)
matairesinol	520 (502), 502 (487), 302 (293), 218 (209)
demethylated matairesinol (peak A)[b]	587 (560), 369 (351), 311 (293), 285 (267), 218/219 (209/210)
ring-opened matairesinol (peak B)[b]	700 (664), 682 (649), 601 (574), 583 (559), 474 (456), 382 (364), 256 (247), 219 (210)
hydroxymatairesinol (peak C)[b]	617 (590), 518 (500), 399 (381), 315 (297), 218 (209)
hydroxymatairesinol (peak D)[b]	617 (590), 518 (500), 315 (297), 218 (209)
demethylated matairesinol (peak E)[b]	587 (560), 569 (545), 470 (455), 302 (293), 285 (268), 218 (209)
hydroxymatairesinol + ring closure (peak F)[b]	615 (588), 516 (498), 471 (453), 410 (392), 397 (379), 311 (302), 283 (274), 218 (209)
hydroxymatairesinol (peak G)[b]	617 (590), 383 (365), 315 (297), 283 (267), 218 (209)
hydroxymatairesinol (peak H)[b]	617 (590), 383 (365), 342 (324), 315 (297), 218 (209)
hydroxymatairesinol (peak I)[b]	617 (590), 316 (298), 285 (267)
hydroxymatairesinol (peak J)[b]	617 (590), 316 (298), 285 (267), 218 (209)

[a] Peak number according to **Figure 3**. [b] Peak number according to **Figure 7**.

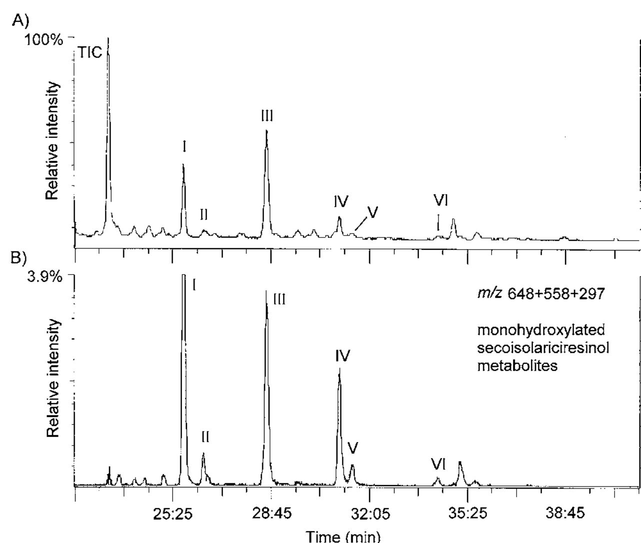

Figure 3. GC-MS chromatogram of secoisolariciresinol metabolites present in the HPLC fraction 1 of the incubation with untreated rat liver microsomes (TMS derivatives). (**A**) Total ion current (TIC) and (**B**) monohydroxylated secoisolariciresinol metabolites reconstructed from full scan spectra by selection of characteristic ions.

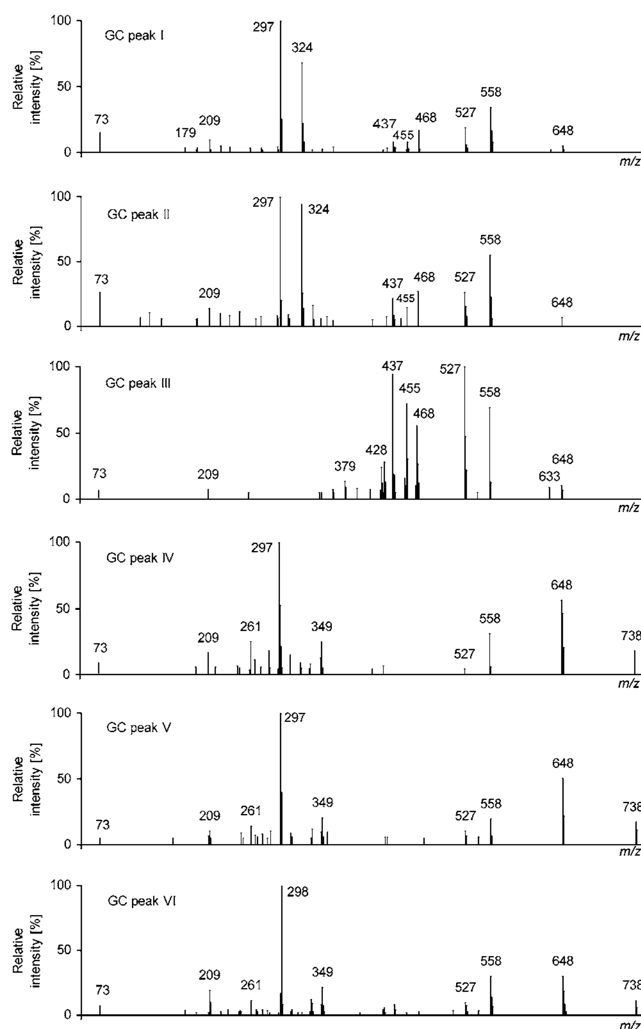

Figure 4. Mass spectra of secoisolariciresinol metabolites (TMS derivatives). Peak numbers are as shown in **Figure 3**.

pounds I and II are therefore proposed to represent diastereomers of 7-hydroxy-secoisolariciresinol. The mass spectrometric fragmentation proposed in **Figure 5** is consistent with the mass spectra obtained after trimethylsilylation with d9-BSA, e.g., showing that the fragment ion *m/z* 648 is shifted to *m/z* 684 and must therefore contain four hydroxyl groups (**Table 1**).

The mass spectrum of peak III resembles that of metabolite I in the higher mass range but lacks the fragment ions at *m/z* 297 and 324 (**Figure 4**). The same mass spectrum as well as the corresponding fragmentation pattern was described earlier for isolariciresinol, occurring in flaxseed, in human urine, and in Norway spruce (*15*, *31*, *32*). The assignment of the fragment ions was corroborated by derivatization with d9-BSA (**Table 1**). Peak III was thus identified as isolariciresinol with *m/z* 648 as molecular ion. However, it is not clear at this time whether isolariciresinol is a true metabolite of secoisolariciresinol, because Haworth and Kelly (*34*) have reported that lariciresinol, which is the major microsomal metabolite of secoisolariciresinol, is chemically unstable and may generate isolariciresinol. Moreover, traces of isolariciresinol were present in the secoisolariciresinol used for the microsomal incubations.

In the mass spectra of metabolites IV, V, and VI, the molecular ion at *m/z* 738 was quite prominent (**Figure 4**). This suggests aromatic hydroxylation products of secoisolariciresinol, which are more stable against fragmentation than aliphatic ones according to our previous study on the metabolites of enterodiol and enterolactone (*30*). Elimination of TMSOH (90 mass units) from the molecular ion can be observed twice, resulting in the fragment ions *m/z* 648 and 558. The base peak at *m/z* 297 probably arises from benzylic cleavage at the hydroxylated ring, as observed earlier for aromatic hydroxylation products of enterodiol and enterolactone (*30*). Derivatization with d9-BSA corroborates these postulated fragmentations (**Table 1**). It is therefore concluded from the mass spectra that metabolites IV, V, and VI are aromatic hydroxylation products of secoisolariciresinol. The position of the newly introduced hydroxyl groups cannot be definitively assigned without reference substances.

When the complete extract from incubations of secoisolariciresinol with microsomes from human liver was analyzed by GC-MS, the major product other than parent secoisolariciresinol was lariciresinol. Trace amounts of isolariciresinol, but none of the aliphatic or aromatic hydroxylation products found with rat liver microsomes, could be detected.

Microsomal Metabolites of Matairesinol. As the matairesinol molecule is not symmetrical (**Figure 1**), six aromatic and five aliphatic sites are theoretically available for hydroxylation. In addition, demethylation is a conceivable metabolic reaction.

HPLC−UV analysis of the extract of the incubations of matairesinol with liver microsomes from Aroclor-treated and untreated rats revealed seven products (peaks 1−7; **Figure 6**) that were not present in control incubations. Assuming identical absorption coefficients for matairesinol and its metabolites, the overall metabolic conversion of matairesinol was estimated as 43 and 37% with microsomes from treated and untreated rats, respectively (calculated as $\sum$ peak areas of metabolites/$\sum$ (peak areas of metabolites plus remaining matairesinol)). With human liver microsomes, no metabolites could be detected by HPLC−UV analysis.

The extract from the incubation with untreated rat liver microsomes was purified by HPLC, and the fraction containing the metabolites, collected from 5.0 to 37.0 min, was subjected to GC-MS analysis (**Figure 7A**). When the mass spectra were searched for ions characteristic for products of monohydroxylation (*m/z* 590, 500, and 297) and demethylation (*m/z* 560, 268, and 267) of matairesinol, six hydroxylated (peaks C, D, G, H, I, J; **Figure 7B**) and two demethylated matairesinol metabolites

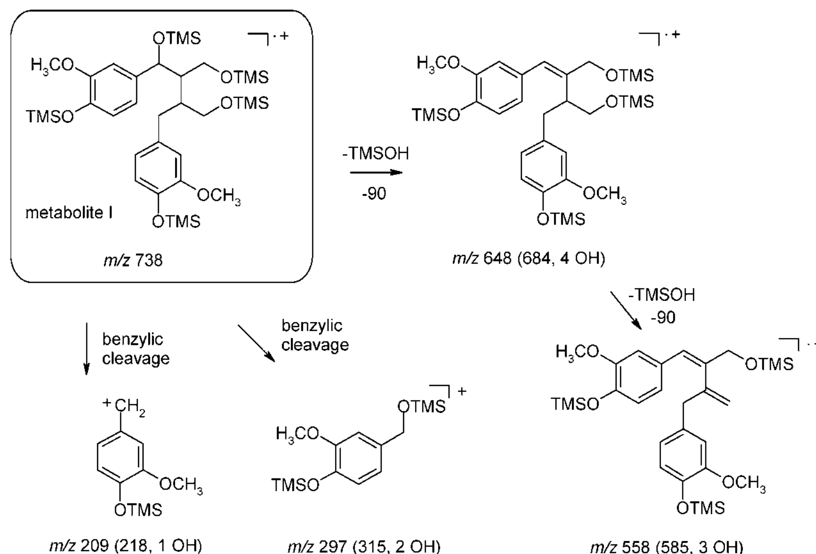

Figure 5. Proposed mass fragmentation scheme for secoisolariciresinol metabolite I. Numbers in parentheses refer to ions obtained after derivatization with d9-BSA and the deduced number of hydroxyl groups.

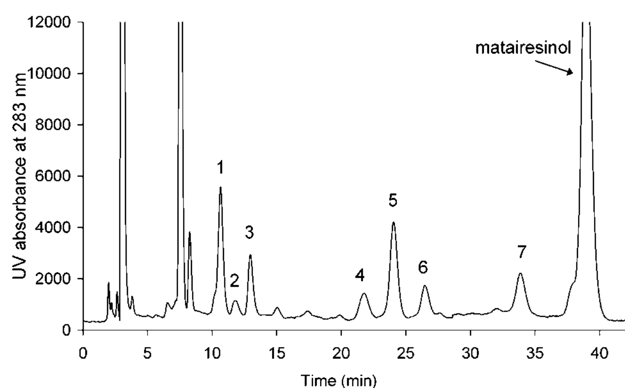

Figure 6. HPLC profile of matairesinol metabolites from untreated rat liver microsomes.

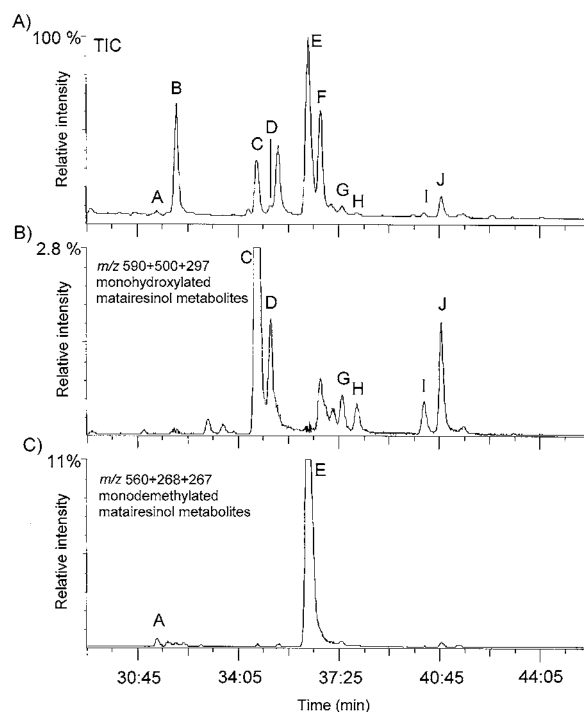

Figure 7. GC-MS chromatogram of matairesinol metabolites formed in from untreated rat liver microsomes (TMS derivatives). (**A**) TIC, (**B**) monohydroxylated matairesinol metabolites reconstructed from full scan spectra by selection of characteristic ions, and (**C**) monodemethylated matairesinol metabolites reconstructed from full scan spectra by selection of characteristic ions.

(peaks A and E; **Figure 7C**) were detected. Their mass spectra are depicted in **Figure 8**. No products of dihydroxylation (expected *m/z* 678, 588), double demethylation (*m/z* 618, 268), or a combination of monohydroxylation and monodemethylation (*m/z* 648, 558, and 268) were observed. However, two further major metabolites (GC peaks B and F; **Figure 7A**) displayed the typical loss of 90 mass units from their molecular ions of *m/z* 664 and *m/z* 588, respectively (**Figure 8**).

Metabolites A und E are proposed to represent monodemethylation products of matairesinol. Demethylation appears to occur at either ring although to a different extent, because the amounts of metabolites A and E differ widely (**Figure 7A**). The proposed structures were corroborated by derivatization with d9-BSA (**Table 1**). No reference compounds were available to completely identify the structures of metabolites A and E.

Metabolite B exhibited a molecular ion of *m/z* 664 (**Figure 8**), shifted to *m/z* 700 with d9-BSA (**Table 1**) indicative of the presence of four hydroxyl groups. The molecular weight of metabolite B should thus be 376, which differs from that of matairesinol by 18 mass units. Because two additional hydroxyl groups are present, a likely structure is that of matairesinol with an opened lactone ring, exposing alcoholic and carboxylic acid functions. The ring opening may be the result of an esterase-catalyzed hydrolysis or of a spontaneous hydrolysis after metabolic formation of an unstable hydroxylation product at C-9′. The proposed structure is supported by other ions of the mass spectrum, e.g., the pronounced elimination of TMSOH yielding *m/z* 574, followed by elimination of the benzylic moiety (*m/z* 210) to yield *m/z* 364. The ions at *m/z* 574 and 364 still contain the trimethylsilylated carboxyl group and give rise to *m/z* 456 and *m/z* 247, respectively, upon decarboxylation.

Metabolite F exhibited a molecular ion of *m/z* 588, and derivatization with d9-BSA revealed the presence of three hydroxyl groups (**Figure 8** and **Table 1**). The molecular ion differs from that of monohydroxylated matairesinol by two mass units. This implies a ring closure, in analogy to that leading from secoisolariciresinol to isolariciresinol (see above), which

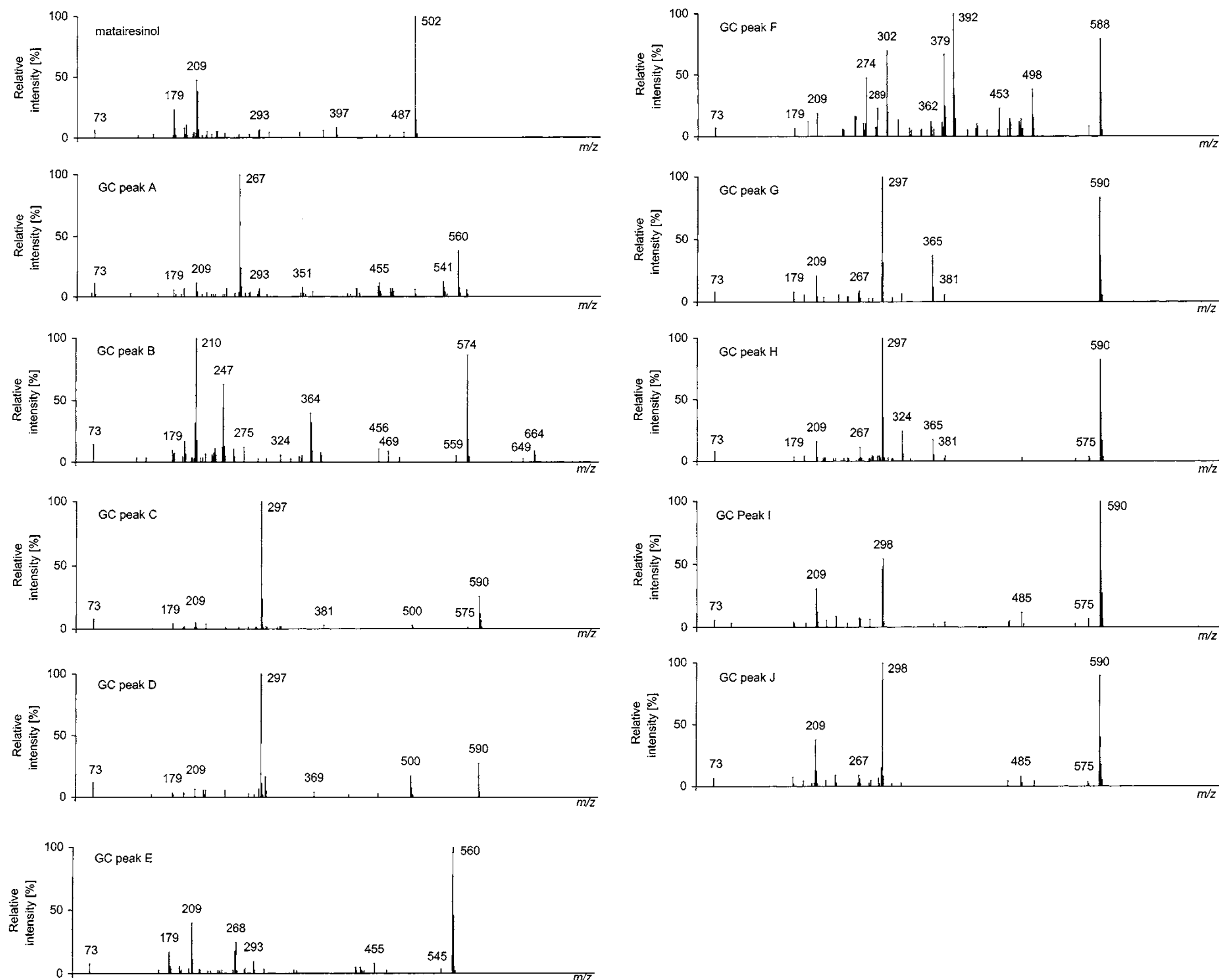

Figure 8. Mass spectra of matairesinol metabolites (TMS derivatives). Peak numbers are as shown in **Figure 7**.

is consistent with the other mass spectrometric data. The fragment of [M − 90] suggests that the hydroxyl group is located in the aliphatic moiety of the molecule.

The remaining six matairesinol metabolites all share a molecular ion of *m*/*z* 590 and represent monohydroxylated matairesinol metabolites, as mentioned above. The mass spectra of metabolites C and D exhibited the base peak at *m*/*z* 297 and rather low intensities of both the molecular ions and the [M − 90] fragments (**Figure 8**). The same type of mass spectrum was reported by Ekmann (*32*) for 7′-hydroxy-matairesinol isolated from Norway spruce and obtained in our laboratory upon GC-MS analysis of authentic 7′-hydroxy-matairesinol, which consists of two stereoisomers. The intense fragment ion at *m*/*z* 297 arises from benzylic cleavage between C-7′ and C-8′. Metabolites C and D were unambiguously identified as isomers of 7′-hydroxy-matairesinol by cochromatography with the reference substance.

Metabolites G, H, I, and J exhibited very intense molecular ions at *m*/*z* 590 but no fragment ions at *m*/*z* 500 in their mass spectra (**Figure 8**), indicating more stable aromatic hydroxylation products of matairesinol. Again, strong fragment ions of *m*/*z* 297 were observed for all metabolites, resulting from benzylic cleavage of the hydroxylated ring. Although there are differences in the intensity of minor fragment ions, it is presently not possible to determine the site of hydroxylation in ring A or B from the mass spectra without reference compounds.

When the extract obtained from the incubation of matairesinol with human liver microsomes was analyzed by HPLC−UV, no metabolites were detectable. GC-MS analysis revealed the presence of only traces of one aromatic hydroxylation (corresponding to peak I; **Figure 8**) and one demethylation product (corresponding to peak E; **Figure 8**).

In Vivo Metabolites of Secoisolariciresinol and Matairesinol in Rat Urine. In a preliminary experiment, single doses of 10 mg/kg body weight of secoisolariciresinol and matairesinol dissolved in corn oil containing 2.5% DMSO were orally administered to female Wistar rats and the urine was collected for 24 h. Following cleanup and conjugate hydrolysis according to the method described by Jacobs et al. (*35*), the extract was analyzed by GC-MS after trimethylsilylation both without and with prior fractionation by HPLC. The 24 h urine of the same animals prior to administration of the plant lignans was used as control. The major compounds observed in the urine of the dosed animals were the mammalian lignans enterodiol and enterolactone, which were excreted in higher amounts than the administered plant lignans secoisolariciresinol and matairesinol. This demonstrates the extensive conversion of the plant lignans to the mammalian lignans by intestinal bacteria. No oxidative metabolites of secoisolariciresinol and matairesinol were detected when their characteristic fragment ions were searched in the full scan mass spectra. These findings suggest that oxidative

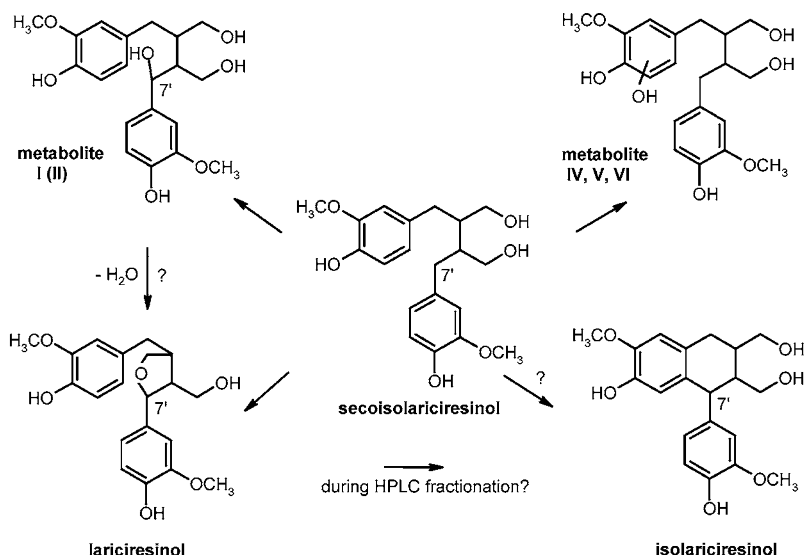

Figure 9. Oxidative metabolism of secoisolariciresinol in rat liver microsomes.

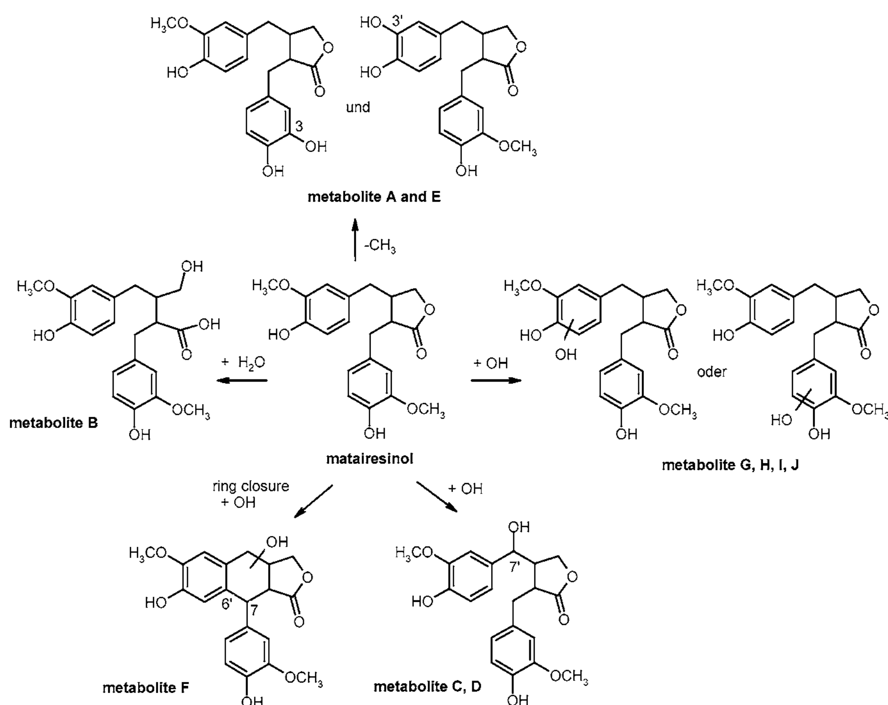

Figure 10. Oxidative metabolism of matairesinol in rat liver microsomes.

metabolites of the plant lignans may not play a significant role in intact organisms in vivo, probably due to the rapid bacterial metabolism to enterodiol and enterolactone prior to absorption in the intestine. Traces of products of aromatic hydroxylation, but not of aliphatic hydroxylation, of enterodiol and enterolactone were detected in the rat urine. However, because recent observations in our laboratory have disclosed that aromatic hydroxylation products of mammalian lignans can be formed as artifacts under the conditions of urine collection and conjugate hydrolysis, in particular in the presence of ascorbic acid (Niemeyer et al., unpublished), we conclude that oxidative metabolites of enterodiol and enterolactone were not excreted in the rat urine to any appreciable extent.

DISCUSSION

The objective of this study was to elucidate the chemical structures of the major oxidative metabolites of the plant lignans secoisolariciresinol and matairesinol formed in vitro. In rat liver microsomes, secoisolariciresinol gave rise to at least five novel products, which are represented as a metabolic scheme in **Figure 9**. According to their mass spectra, three products of aromatic monohydroxylation (metabolites IV, V, and VI) and two products of aliphatic hydroxylation at the benzylic C-7 position (metabolites I and II) were detected. The major metabolite of secoisolariciresinol with rat and human liver microsomes was identified as lariciresinol and is presumably formed from 7-hydroxy-secoisolariciresinol by further reaction of the newly introduced hydroxyl group with one of the methylenehydroxyl groups. No products of dihydroxylation, oxidative demethylation, or combinations thereof were detected. Isolariciresinol was also identified among the microsomal products but may arise as an artifact from lariciresinol.

Incubation of matairesinol with rat liver microsomes gave rise to 10 metabolites depicted in **Figure 10**. In addition to

products of monohydroxylation at the aromatic rings (metabolites G, H, I, and J) and at the benzylic positions (metabolites C and D), two metabolites (A and E) arising from oxidative demethylation were detected. Metabolite B is most likely formed by hydrolysis of the lactone ring of matairesinol, whereas metabolite F probably results from ring closure between C7 and C6′ but carrying an additional hydroxyl group in the aliphatic moiety of the molecule. Surprisingly, virtually no metabolites of matairesinol were generated by human liver microsomes. This suggests that the oxidative metabolism of matairesinol is mediated by specific isoforms of cytochrome P450 present in rat liver but not in human liver.

When secoisolariciresinol and matairesinol were administered to female Wistar rats by gavage, no oxidative in vivo metabolites of the two plant lignans were detected in the 24 h urine. Most of the administered dose of secoisolariciresinol and matairesinol was found as the bacterial conversion products enterodiol and enterolactone.

Our study shows that secoisolariciresinol and matairesinol are substrates of the cytochrome P450 system in rat and human liver, although matairesinol appears to be only very poorly oxidized by human liver. In intact rats, however, both plant lignans are very efficiently converted to their corresponding mammalian lignans enterodiol and enterolactone, leaving only little secoisolariciresinol and matairesinol for direct absorption. No oxidative in vivo metabolites of secoisolariciresinol or matairesinol were detected. It is well-known that the conversion of plant lignans to mammalian lignans in humans depends on the activity of the gut microflora and can vary considerably between individuals and under certain medical conditions, e.g., after application of antibiotic drugs (*23*, *36*). Little is known about the bacterial strains capable of carrying out the conversion (*37*). In the case of a reduced activity of the gut microflora, the oxidative metabolism of secoisolariciresinol and matairesinol might take place to a larger extent.

ABBREVIATIONS USED

BSA, *N*,*O*-bis(trimethylsilyl)acetamide; d9-BSA, BSA with deuterated TMS groups; BSTFA, *N*,*O*-bis(trimethylsilyl)trifluoroacetamide; TMS, trimethylsilyl.

ACKNOWLEDGMENT

We thank Drs. L. B. Davin, M. Schoettner, and R. Santti for providing reference compounds. We greatly appreciate the help of Dr. R. Loske with the GC-MS analyses.

LITERATURE CITED

(1) Mazur, W. M. Phytoestrogen content in foods. *Baillière's Clin. Endocrinol. Metab.* **1998**, *12*, 729−742.

(2) Mazur, W. M.; Uehara, M.; Wähälä, K.; Adlercreutz, H. Phytoestrogen content of berries, and plasma concentrations and urinary excretion of enterolactone after a single strawberry-meal in human subjects. *Br. J. Nutr.* **2000**, *83*, 381−387.

(3) Murkies, A. L.; Wilcox, G.; Davis, S. R. Phytoestrogens. *Endocrinol. Metab*. **1998**, *83*, 297−303.

(4) Thompson, L. U. Experimental studies on lignans and cancer. *Baillière's Clin. Endocrinol. Metab.* **1998**, *12*, 691−705.

(5) Rickard, S. E.; Thompson, L. U. Phytoestrogens and lignans: effects on reproduction and chronic disease. In *Antinutrients and Phytochemicals in Food*; Shahidi, F., Ed.; American Chemical Society: Washington, DC, 1997; pp 273−296.

(6) Thompson, L. U.; Seidl, M. M.; Rickard, S. E.; Orcheson, L. J.; Fong, H. H. S. Antitumorigenic effect of a mammalian lignan precursor from flaxseed. *Nutr. Cancer* **1996**, *26*, 159−165.

(7) Kitts, D. D.; Yuan, Y. V.; Wijewickreme, A. N.; Thompson, L. U. Antioxidant activity of the flaxseed lignan secoisolariciresinol diglycoside and its mammalian lignan metabolites enterodiol and enterolactone. *Mol. Cell. Biochem.* **1999**, *202*, 91−100.

(8) Prasad, K. Hydroxyl radical-scavenging property of secoisolariciresinol diglycoside (SDG) isolated from flax-seed. *Mol. Cell. Biochem.* **1997**, *168*, 117−123.

(9) Harper, A.; Kerr, D. J.; Gescher, A.; Chipman, K. J. Antioxidant effects of isoflavones and lignans, and protection against DNA oxidation. *Free Radical Res.* **1999**, *131*, 149−160.

(10) Niemeyer, H. B.; Metzler, M. Antioxidant activities of lignans in the frap assay (ferric reducing antioxidant power assay). In *Biologically Active Phytochemical in Food—Analysis*, *Metabolism*, *Bioavailability and Function*; Pfannhauser, W., Fenwick, G. R., Khokar, S., Eds.; Royal Society of Chemistry: Cambridge, U.K., 2001; pp 394−395.

(11) Adlercreutz, H.; Mazur, W.; Bartels, P.; Elomaa, V.-V.; Watanabe, S.; Wähälä, K.; Landström, M.; Lundin, E.; Bergh, A.; Damber, J.-E.; Åman, P.; Widmark, A.; Johansson, A.; Zhang, J.-X.; Hallmans, G. Phytoestrogens and prostate disease. *J. Nutr*. **2000**, *130*, 658−659.

(12) Rose, D. P. Dietary fiber, phytoestrogens, and breast cancer. *Nutrition* **1992**, *8*, 47−51.

(13) Adlercreutz, H. Phytoestrogens: epidemilogy and a possible role in cancer protection. *Environ. Health Perspect.* **1995**, *103* (Suppl. 7), 103−112.

(14) Mazur, W.; Fotsis, T.; Wähälä, K.; Ojala, S.; Salakka, A.; Adlercreutz, H. Isotope dilution gas chromatographic-mass spectrometric method for the determination of isoflavonoids, coumestrol, and lignans in food samples. *Anal. Biochem.* **1996**, *233*, 169−180.

(15) Meagher, L. P.; Beecher, G. R.; Flanagan, V. P.; Li, B. W. Isolation and characterization of the lignans, isolariciresinol and pinoresinol, in flaxseed meal. *J. Agric. Food Chem.* **1999**, *47*, 3173−3180.

(16) Sicilia, T.; Niemeyer, H. B.; Honig, D. M.; Metzler, M. Identification and stereochemical characterization of lignans in flaxseed and pumpkin seeds. *J. Agric. Food Chem.* **2003**, *51*, 1181−1188.

(17) Stitch, S. R.; Toumba, J. K.; Groen, M. B.; Funke, C. W.; Leemhuis, J.; Vink, J.; Woods, G. F. Excretion, isolation and structure of a new phenolic constituent of female urine. *Nature* **1980**, *287*, 738−740.

(18) Setchell, K. D. R.; Lawson, A. M.; Mitchell, F. L.; Adlercreutz, H.; Kirk, D. N.; Axelson, M. Lignans in man and animal species. *Nature* **1980**, *287*, 740−742.

(19) Setchell, K. D. R.; Lawson, A. M.; Conway, E.; Taylor, N. F.; Kirk, D. N.; Cooley, G.; Farrant, R. D.; Wynn, S.; Axelson, M. The definitive identification of the lignans *trans*-2,3-bis(3-hydroxybenzyl)-γ-butyrolactone and 2,3-bis(3-hydroxybenzyl)-butane-1,4-diol in human and animal urine. *Biochem. J.* **1981**, *197*, 447−458.

(20) Axelson, M.; Setchell, K. D. R. The excretion of lignans in rats—evidence for an intestinal bacterial source for this new group of compounds. *FEBS Lett.* **1981**, *123* (2), 337−342.

(21) Adlercreutz, H.; Fotsis, T.; Heikkinen, R.; Dwyer, J. T.; Goldin, B. R.; Gorbach, S. L.; Lawson, A. M.; Setchell, K. D. R. Diet and urinary excretion of lignans in female subjects. *Med. Biol.* **1981**, *59*, 259−261.

(22) Rowland, I. R.; Wiseman, H.; Sanders, T. A. B.; Adlercreutz, H.; Bowey, E. A. Interindividual variation in metabolism of soy isoflavones and lignans: influence of habitual diet of equol production by the gut microflora. *Nutr. Cancer* **2000**, *36*, 27−32.

(23) Kilkkinen, K.; Pietinen, P.; Klaukka, T.; Virtamo, J.; Korhonen, P.; Adlercreutz, H. Use of oral antimicrobials decreases serum enterolactone concentration. *Am. J. Epidemiol.* **2002**, *155*, 427−477.

(24) Axelson, M.; Sjövall, J.; Gustafsson, B. E.; Setchell, K. D. R. Origin of lignans in mammals and identification of a precursor from plants. *Nature* **1982**, *298*, 659−660.

(25) Saarinen, N. M.; Wärri, A.; Mäkelä, S. I.; Eckermann, C.; Reunanen, M.; Ahotupa, M.; Salmi, S. M.; Franke, A. A.; Kangas, L.; Santti, R. Hydroxymatairesinol, a novel enterolactone precursor with antitumor properties from coniferous tree (*Picea abies*). *Nutr. Cancer* **2000**, *36*, 207−216.
(26) Oikarinen, S. I.; Pajari, A.; Mutanen, M. Chemopreventive activity of hydroxymatairesinol in adenomatous polyposis coli-multiple intestinal neoplasia (apc) (min) mice. *Cancer Lett.* **2000**, *159*, 183−187.
(27) Pelter, A.; Ward, R. S.; Satyanarayana, P.; Collins, P. Synthesis of lignan lactones by conjugation addition of thioacetal carbanions to butenolide. *J. Chem. Soc. Perkin Trans. 1* **1983**, 643−647.
(28) Lake, B. G. Preparation and characterisation of microsomal fractions for studies on xenobiotic metabolism. In *Biochemical Toxicology*; Snell, K., Mullock, B., Eds.; IRL Press: Oxford, 1987; pp 183−215.
(29) Omura, T.; Sato, R. The carbon monooxide-binding pigment of liver microsomes: I. Evidence for its hemoprotein nature. *J. Biol. Chem.* **1964**, *239*, 2370−2377.
(30) Jacobs, E.; Metzler, M. Oxidative metabolism of the mammalian lignans enterolactone and enterodiol by rat, pig and human liver microsomes. *J. Agric. Food Chem.* **1999**, *47*, 1071−1077.
(31) Bannwart, C.; Adlercreutz, H.; Wähälä, K.; Brunow, G.; Hase, T. Detection and identification of the plant lignans lariciresinol, isolariciresinol and secoisolariciresinol in human urine. *Clin. Chim. Acta* **1989**, *180*, 293−302.
(32) Ekman, R. Analysis of lignans in Norway spruce by combined gas chromatography−mass spectrometry. *Holzforschung* **1976**, *30*, 79−85.
(33) Miki, K.; Ito, K.; Sasaya, T. Lignans from heartwood of *Larix leptolepis* Gord. *Mokuzai Gakkaishi* **1979**, *25*, 665−670.
(34) Haworth, R. D., Kelly, W. Constituents of natural phenolic resins. VIII. Lariciresinol, cubebin and some stereochemical relationships. *J. Chem. Soc.* **1937**, 384−391.
(35) Jacobs, E.; Kulling, S. E.; Metzler, M. Novel metabolites of the mammalian lignans enterolactone and enterodiol in human urine. *J. Steroid Biochem. Mol. Biol.* **1999**, *68*, 211−218.
(36) Setchell, K. D. R.; Lawson, A. M.; Boriello, S. P.; Harkness, R.; Gordon, H.; Morgan, D. M. L. Lignan formation in man−microbial involvement and possible roles in relation to cancer. *Lancet* **1981**, *2* (8236), 4−7.
(37) Wang, L. Q.; Meselhy, M. R.; Li, Y.; Qin, G. W.; Hattori, M. Human intestinal bacteria capable of transforming secoisolariciresinol diglucoside to mammalian lignans, enterodiol and enterolactone. *Chem. Pharm. Bull. (Tokyo)* **2000**, *48*, 1606−1610.

Received for review April 8, 2003. Revised manuscript received July 21, 2003. Accepted July 21, 2003. This study has been supported by the Deutsche Forschungsgemeinschaft (Grant Me 574/9-2).

JF030263N