Isolation and Characterization of the Lignans, Isolariciresinol and Pinoresinol, in Flaxseed Meal

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The isolation and characterization of the lignans, isolariciresinol, pinoresinol, secoisolariciresinol, and matairesinol, potent phytoestrogens, from flaxseed meal are described. This is the first report of isolariciresinol and pinoresinol being detected in a food. The extraction method selected combined the removal of the lignan glycosides from the plant matrix with an alcoholic solvent system, followed by acid hydrolysis to release the aglycons. A reversed-phase high-performance liquid chromatography with diode array detection system was used for initial separation and detection of the lignans at 280 nm in the acid-hydrolyzed methanolic extract. Lignan trimethylsilyl ether derivatives were characterized by gas chromatography/mass spectrometry. Secoisolariciresinol is the major lignan in flaxseed; isolariciresinol, pinoresinol, and matairesinol were identified as minor lignan components.

Keywords: *Phytoestrogens; lignans; isolariciresinol; pinoresinol; flaxseed; Linum usitatissimum; secoisolariciresinol; matairesinol*

INTRODUCTION

The lignans are phytoestrogens of continuing interest (Murkies et al., 1998) due to their demonstrated antitumorigenic (Jenab and Thompson, 1996; Thompson et al., 1996a,b), estrogenic and antiestrogenic (Collins et al., 1997; Kurzer et al., 1995b; Mousavi and Adlercrutz, 1992), and anti-aromatase (Wang et al., 1994) properties. The lignans secoisolariciresinol and matairesinol (Figure 1) are found in a variety of foods and are at their highest levels in flaxseed (*Linum usitatissimum*) (Mazur et al., 1996). They are believed to be the plant precursors of the lignan metabolites enterolactone and enterodiol (Figure 1) referred to as the mammalian lignans, first discovered in human urine by Setchell et al. (1983). The mammalian lignans are produced from plant lignans by in vitro human fecal flora metabolism (Borriello et al., 1985). Fecal inoculum has been utilized to analyze the mammalian lignan production from plant precursors in various foods (Thompson et al., 1991). This incubation has shown that flaxseed contains higher levels of total lignans (enterolactone and enterodiol) than other plant foods. There is increasing interest in flaxseed in human nutrition (Kurzer et al., 1995b; Thompson, 1995) as it gains popularity as a health food, a dietary supplement, and an ingredient in bread, muffins, and breakfast cereals (Jenkins, 1995; McCord and Rao, 1997).

Secoisolariciresinol ([*R*-(*R**′,*R**)]-2,3-bis[(4-hydroxy-3-methoxyphenyl)methyl]-1, 4-butanediol) and matairesinol ([3*R*-*trans*]-dihydro-3,4-bis[(4-hydroxy-3-methoxyphenyl)methyl]-2(3*H*)-furanone) are members of the oxydiarylbutane and diarylbutyrolactone classes of lignans, respectively. Lariciresinol ([2*S*-(2α,3*β*,4*β*)]-tetrahydro-2-(4-hydroxy-3-methoxyphenyl)-4-[(4-hydroxy-3-methoxyphenyl)methyl]-3-furanmethanol), a furanoid lignan (Figure 2), and isolariciresinol ([1*S*-(1α,2*β*,3α)]-1,2,3,4-tetrahydro-7-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-6-methoxy-2,3-naphthalenedimethanol), a tetrahydronaphthalene lignan, as well as secoisolariciresinol and matairesinol have previously been detected in urine (Bannwart et al., 1989, 1984). Arctigenin ((3*S*,4*S*)-3-[(3-methoxy-4-hydroxyphenyl)methyl]-4-[3,4-dimethoxyphenyl)methyl]dihydro-2(3*H*)-furanone), a diarylbutyrolactone lignan, pinoresinol ([1*S*-(1α,3α,4*β*,6α)]-4,4′-(tetrahydro-1*H*,3*H*-furo(3,4-)furan-1,4-diyl)bis(2-methoxyphenol), a furofuran lignan, and nordihydroguaiaretic acid (1,4-bis[3,4-dihydrooxyphenyl]-2,3-dimethylbutane) (NDGA) have not been reported in human urine, plasma, feces, or flaxseed. However, NDGA has been found in chaparral (Obermeyer et al., 1995), a desert shrub found in parts of the southwestern United States and Mexico,

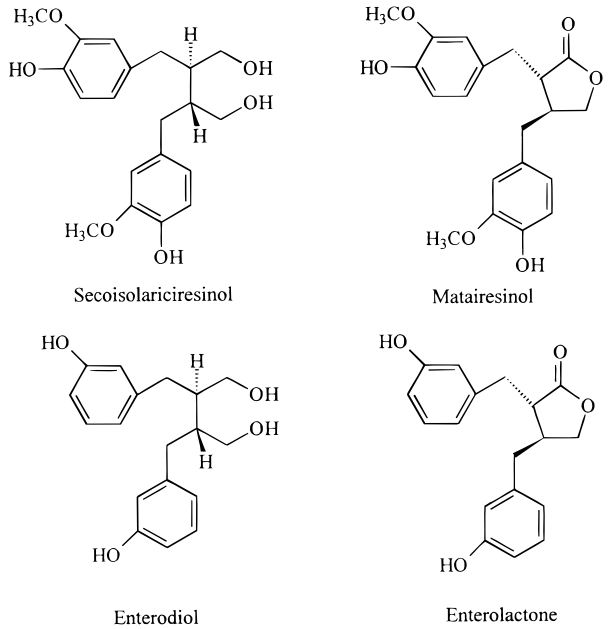

Figure 1. Structures of the lignans secoisolariciresinol, matairesinol, enterodiol, and enterolactone.

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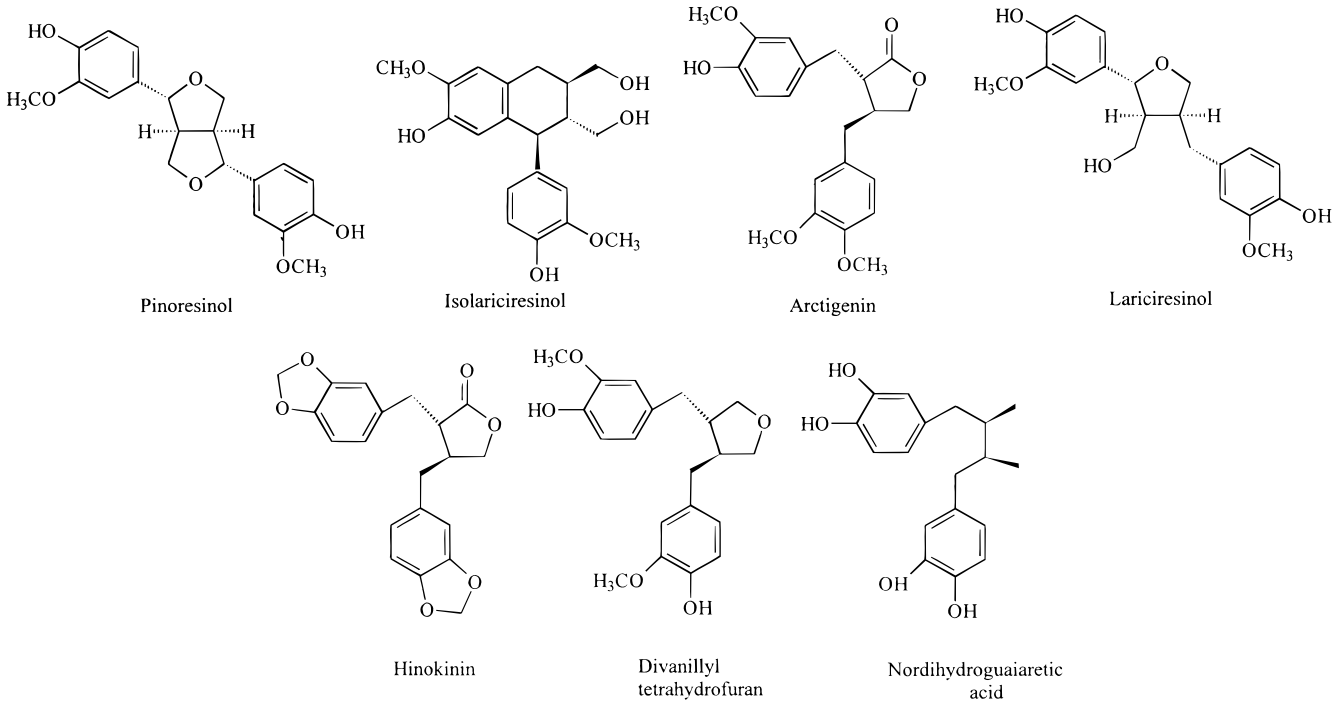

Figure 2. Structures of the lignans pinoresinol, isolariciresinol, lariciresinol, arctigenin, hinokinin, divanillyltetrahydrofuran, and nordihydroguaiaretic acid.

which is used as a herbal infusion and is not a common food. Hinokinin (3*S*,4*S*-3,4-bis(1,3-benzodioxol-5-ylmethyl)dihydro-2-(3*H*)-furanone), a diarylbutyrolactone lignan, was used as a mass spectral standard, due to differences in the fragmentation pattern when compared to the other lignans of interest.

Extraction of lignan glycosides from the plant matrix is the preliminary step in their isolation. The traditional enzymatic hydrolysis with β-glucuronidase/sulfatase (Setchell et al., 1983; Adlercrutz et al., 1991a) was first developed for the analysis of the urinary metabolites, enterolactone and enterodiol glucuronides. Enzymatic hydrolysis (Obermeyer et al., 1995) has been used to liberate secoisolariciresinol from flaxseed and chaparral (*Larrea tridentata*) with subsequent analysis by high-performance liquid chromatography with ultraviolet spectrometric detection (HPLC−UV) and by high-performance liquid chromatography mass spectrometry (HPLC−MS). No matairesinol could be detected in flaxseed by these UV and MS methodologies. The quantitative determination of the lignans secoisolariciresinol and matairesinol in plant-derived foods (Mazur et al., 1996) was achieved by stable isotope dilution gas chromatography mass spectrometry (ID-GC/MS) using deuterated analogues of the respective analytes. A five-step extraction and purification procedure consisted of rehydration with water, followed by enzymatic and then acid hydrolysis to convert the diphenolic glycosides into their respective aglycons. Purification by two ion-exchange chromatographic routines is followed by derivatization, a time-consuming, though quantitative, method.

An alternative approach to enzymatic extraction of the flaxseed meal by an aqueous buffer is to use an organic solvent. Secoisolariciresinol diglycoside has been extracted from flaxseed (Westcott and Muir, 1995) into an alcoholic solvent (either methanol or ethanol), followed by base-catalyzed hydrolysis to liberate lignan diglycosides. Extraction of defatted (removal of fats by extraction with hexane) flaxseed powder with methanol or dioxane/water (Harris and Haggerty, 1993) or with an ethanolic extraction (Amarowicz et al., 1994) has been utilized.

Our aim was to isolate and characterize several of the minor lignans from defatted flaxseed meal (DFM) using a simple extraction procedure. It is well-known that secoisolariciresinol diglycoside is the major lignan component of flaxseed (Bambagiotti-Alberti et al., 1994; Harris and Haggerty, 1993). After examining several of the literature methods (Obermeyer et al., 1995; Mazur et al., 1996) for analyzing lignans in foods, we chose an extraction method that combined the removal of the glycosides from the plant matrix with an aqueous organic solvent system (the meal had been defatted prior to this step), followed by acid hydrolysis to release the aglycon. Separation and purification of the lignan extract were achieved with high-performance liquid chromatography with a diode array detection system (HPLC−DAD) on a semipreparative C_{18} column. The identity of each lignan was then confirmed by GC/MS using retention times and mass spectra of their trimethylsilyl ether (TMS) derivatives.

MATERIALS AND METHODS

Chemicals. Acetonitrile, ethyl acetate, hexanes, and methanol of HPLC grade and acetic and hydrochloric acid were purchased from Fisher Scientific, Fair Lawn, NJ. Pyridine was purchased from Aldrich Chemical Co., Milwaukee, WI, and *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) was purchased from Pierce Chemical Co., Rockford, IL. Inorganic membrane filters Anotop 25, 0.2 μm, 25 mm, and Whatman No. 4 filter paper were purchased from Whatman International Ltd., Maidstone, U.K.

Standards. The lignan standards, secoisolariciresinol and matairesinol, were purchased from T. A. Hase, University of Helsinki, Helsinki, Finland. NDGA was purchased from Sigma Chemical Co., St. Louis, MO. The pinoresinol was a gift from N. G. Lewis, Washington State University, Pullman, WA. Arctigenin and hinokinin were gifts from M. P. Doyle, The University of Arizona, Tucson, AZ. Defatted flaxseed meal

(DFM) was purchased from Arthur D. Little, Cambridge, MA, product code *FLX050192H0092B, location *152.

HPLC. HPLC semipreparative separations were carried out on a Beckman 114M HPLC system with a Microsorb (Rainin Instrument, Woburn, MA) semipreparative C_{18} reverse-phase column (25 cm × 10 mm i.d.; 5 μm particles), protected with a Brownlee C_{18} reverse-phase guard cartridge. The HPLC system was equipped with an HP 1100 DAD, with detection set at 280 nm to monitor the lignans, and peaks were scanned between 200 and 400 nm for identification purposes. Elution was carried out with a flow rate of 0.6 mL/min using the following solvent systems: solvent A = water/glacial acetic acid (99.8:0.2 v/v) and solvent B = acetonitrile. An initial ratio of 70A:30B was followed by a linear gradient to 50A:50B, over 55 min, then back to 70A:30B, for equilibration of the system over 5 min.

GC/MS. The electron impact (EI) mass spectra were acquired on a Finnigan-MAT TSQ70 (San Jose, CA) triple-stage mass spectrometer operated in the positive ion mode interfaced to a Varian 3400 GC. The ion source was at 150 °C, ionization energy = 70 eV, and filament current = 200 μA. The GC column was a J&W Scientific (Folsom, CA) 15 m × 0.25 mm (i.d.), 0.25 μm, DB-1701 with helium as the carrier gas at 68 cm/s. The oven was maintained at an initial temperature of 100 °C for 1 min and then increased to 300 °C at a rate of 27 °C/min.

Extraction and Hydrolysis. Defatted flaxseed meal (72 g) was divided into 4 g portions and each extracted with 50 mL of methanol/water (80:20) for 4 h at 55 °C in a shaker water bath. The methanolic extract was filtered (Whatman No. 4) and concentrated by rotary evaporation (35 °C). The resulting aqueous extract (10 mL) was hydrolyzed with 0.8 mL of hydrochloric acid (final concentration of ~1 M) for 1 h at 100 °C in a Pierce Reacti-Therm heating block. The acid hydrolysate was diluted with water (10 mL) and extracted twice with (20 mL) ethyl acetate/hexanes (1:1). The dry flaxseed meal extract was redissolved in methanol, filtered through a 0.2 μm inorganic membrane filter, and applied to the HPLC (see HPLC paragraph for conditions). Multiple fractions of the DFM lignan extract were collected from the HPLC, as individual well-defined peaks and the broader areas between, then pooled. These pooled aqueous fractions were extracted with ethyl acetate to remove the lignans and dried, and a portion was subjected to derivatization for subsequent GC/MS analysis. Further HPLC−DAD separation of each fraction using the conditions described above gave the pure lignans secoisolariciresinol, matairesinol, isolariciresinol, divanillyltetrahydrofuran, and pinoresinol.

Derivatization. Trimethylsilyl (TMS) ether derivatives were formed with BSTFA and pyridine (1:1). A 10 μL aliquot was added to the dry lignan sample, kept at room temperature for 20 min, and then taken to dryness. Isooctane (20−40 μL) containing 1% BSTFA−pyridine was added to the sample prior to GC/MS analysis.

RESULTS

Lignans Detected in Flaxseed Meal. The identification of pinoresinol (Figure 2), isolated from a DFM extract, is carried out by a comparison of the HPLC retention time (Table 1) on a C_{18} reverse-phase column (Figure 3) and GC retention time on a nonpolar column (Figure 4) in combination with the mass spectral data (Figure 5a) from a synthetic standard. The mass spectrum of the isolated isolariciresinol (Figure 5b) is in good agreement with that reported in the literature (Bannwart et al.,1989). We were unable to obtain a sample of pure isolariciresinol to serve as a standard. The level of secoisolariciresinol isolated from the DFM extract was greater than the levels of other lignans, such as isolariciresinol, pinoresinol, or matairesinol. Isolariciresinol appears at higher levels than either matairesinol or pinoresinol, which are in comparable levels. Lariciresinol was not detected in the DFM analyzed. It was observed that, unlike other lignans, because of the presence of free hydroxyls, NDGA is readily oxidized to orthoquinone structures, which then polymerize. It was found that NDGA was not stable in methanol, and the concentration of a standard decreased rapidly within 4 days, even when kept in a −20 °C freezer.

Table 1. Comparison of Retention Times (Minutes) for Lignan Aglycons by HPLC−DAD with Those of the Lignan TMS Derivatives by GC/MS

	GC/MS		HPLC−UV	
lignan	isolated	standard	isolated	standard
NDGA[a]		8.01		44.3
isolariciresinol	8.32		*b*	
secoisolariciresinol	8.44	8.41	16.8	16.9
anhydroseco[a]	9.04		36.0	36.2
matairesinol	10.54	10.48	31.6	31.5
lariciresinol		10.01		18.6
hinokinin		10.55		17.9
arctigenin		11.13		40.5
pinoresinol	11.19	11.17	26.8	25.9

[a] NDGA, nordihydroguaiaretic acid; anhydroseco, divanillyltetrahydrofuran. [b] Isolariciresinol was not resolved on HPLC due to a coeluting sterol peak.

Acid Hydrolysis. It was observed that acid hydrolysis of the methanolic lignan extract led to the isolation of divanillyltetrahydrofuran (anhydrosecoisolariciresinol) (Figure 2), which has been reported previously (Mazur et al., 1996) as an artifact of acid hydrolysis resulting from the dehydration of secoisolariciresinol and not as a naturally occurring lignan. Acid hydrolysis (using hydrochloric acid with a final concentration of ~1 M for 1 h at 100 °C in a heating block) of a synthetic secoisolariciresinol sample led to the formation of only divanillyltetrahydrofuran, with no unreacted secoisolariciresinol detected by GC/MS or HPLC−DAD. In contrast, acid hydrolysis of the DFM extract afforded greater levels of secoisolariciresinol than divanillyltetrahydrofuran, suggesting that the plant matrix has a protective effect during acid hydrolysis of the methanolic extract. In addition, acid hydrolysis of secoisolariciresinol diglycoside (isolated from DFM) under identical conditions yielded a mixture of secoisolariciresinol and divanillyltetrahydrofuran, with no unreacted secoisolariciresinol diglycoside (SDG) detected by GC/MS or HPLC−DAD.

DISCUSSION

This is the first report of the lignans isolariciresinol and pinoresinol being detected in a plant food (flaxseed). Isolariciresinol has previously been detected in the urine of two different Finnish omnivorous women (Bannwart et al., 1989). In this study with GC/MS analysis, isolariciresinol eluted before secoisolariciresinol (Figure 4) on a nonpolar column. It was found in the present study that isolariciresinol had a shorter retention time on GC than the other lignans isolated from flaxseed (Table 1). Pinoresinol, a lignan usually associated with coniferous trees (Ayres and Loike, 1990), has not been reported in human urine, plasma, or feces or flaxseed or other foods. It has been reported previously that secoisolariciresinol (Obermeyer et al., 1995; Mazur et al., 1996) and matairesinol (Mazur et al., 1996) are found in flaxseed and flaxseed meal (Table 2). The level of secoisolariciresinol detected in flaxseed is much greater than that of matairesinol (Mazur et al., 1996), which is apparently below the detection level for some earlier methods employing HPLC−UV spectroscopy. However, others reported detection of secoisolaricires-

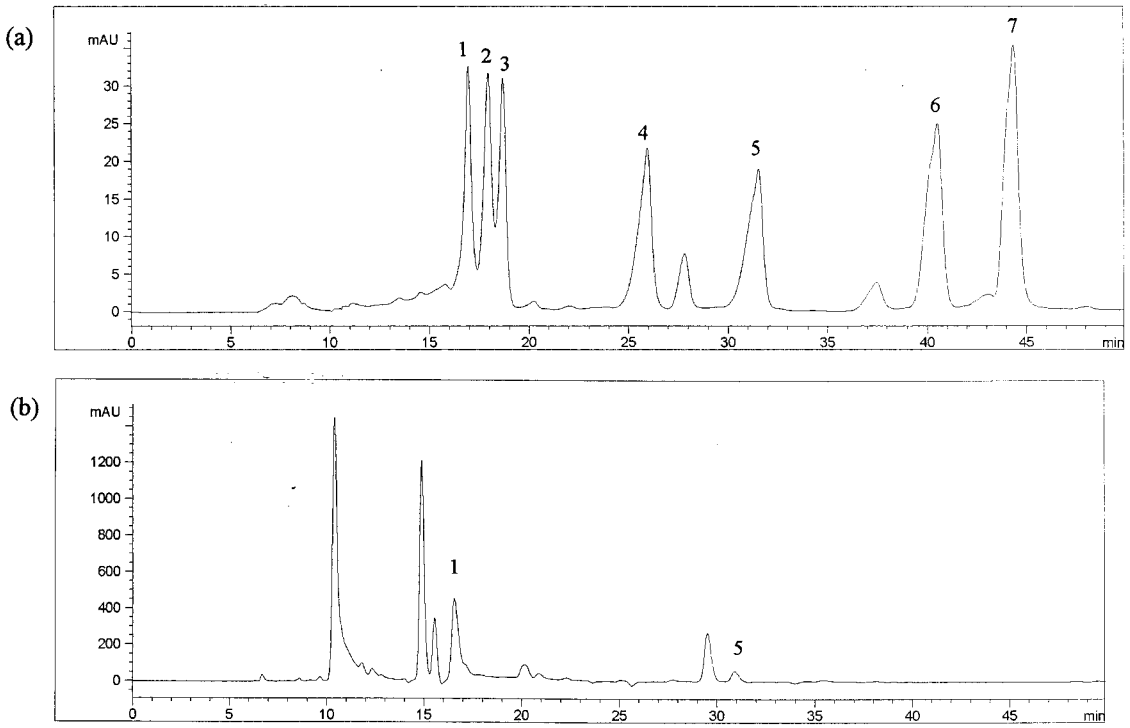

Figure 3. HPLC−DAD chromatogram (minutes) of (a) the synthetic standards and (b) an acid-hydrolyzed methanolic flaxseed meal extract ($\lambda = 280$ nm). Peak identification: 1, secoisolariciresinol (16.9); 2, hinokinin (17.9); 3, lariciresinol (18.6); 4, pinoresinol (25.9); 5, matairesinol (31.5); 6, arctigenin (40.5); 7, nordihydroguaiaretic acid (44.3).

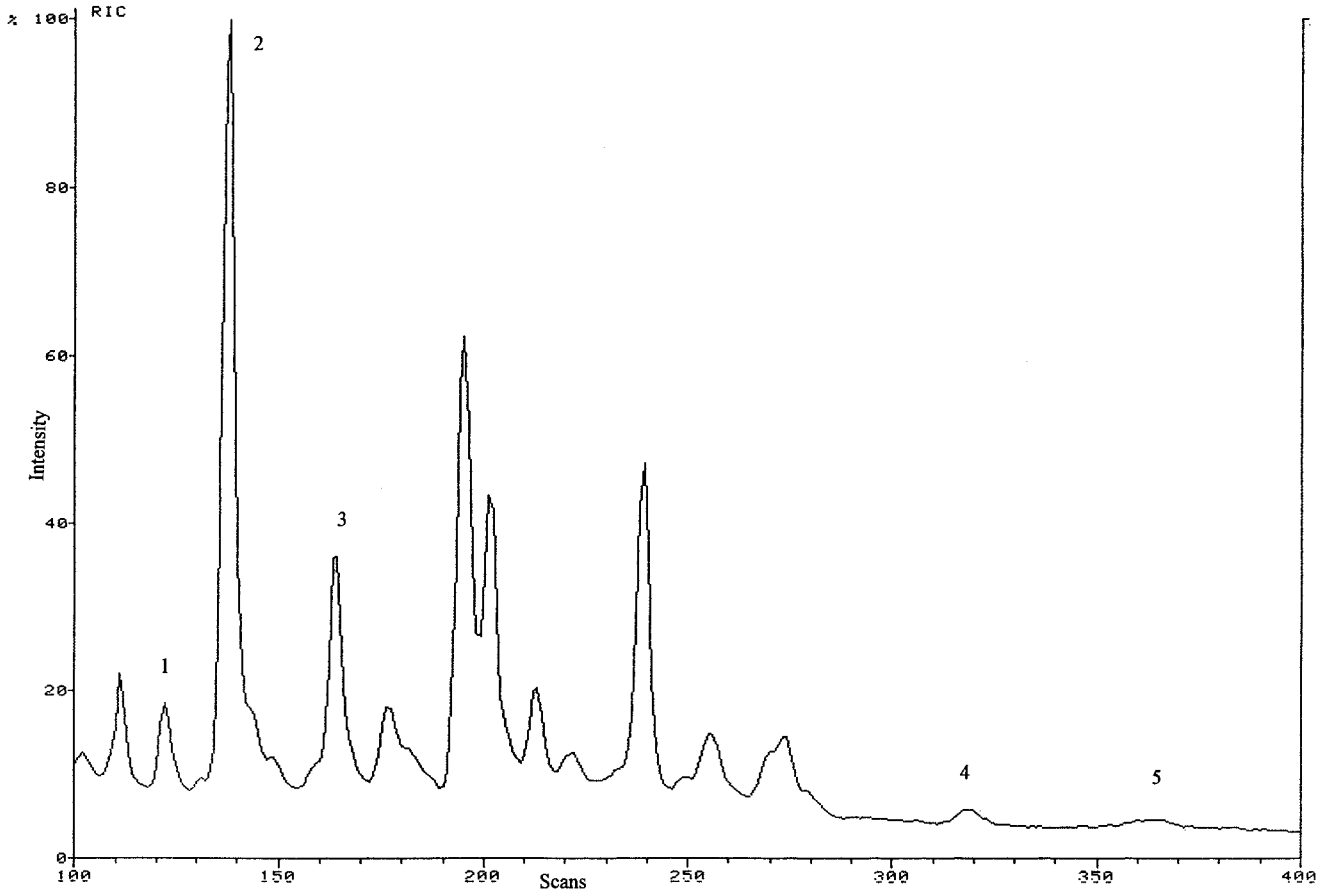

Figure 4. GC/MS chromatogram (minutes) of an acid-hydrolyzed methanolic flaxseed meal extract. Peak identification: 1, isolariciresinol (8.32); 2, secoisolariciresinol (8.44); 3, divanillyltetrahydrofuran (9.04); 4, matairesinol (10.54); 5, pinoresinol (11.19).

inol (Bannawart et al., 1989) and matairesinol (Bannwart et al., 1984) in urine samples (Table 2). No lariciresinol was observed in the DFM extract analyzed even though it has been detected in a urine sample from a Helsinki omnivore and two Boston macrobiotics (Bannwart et al., 1989). These people were not supple-

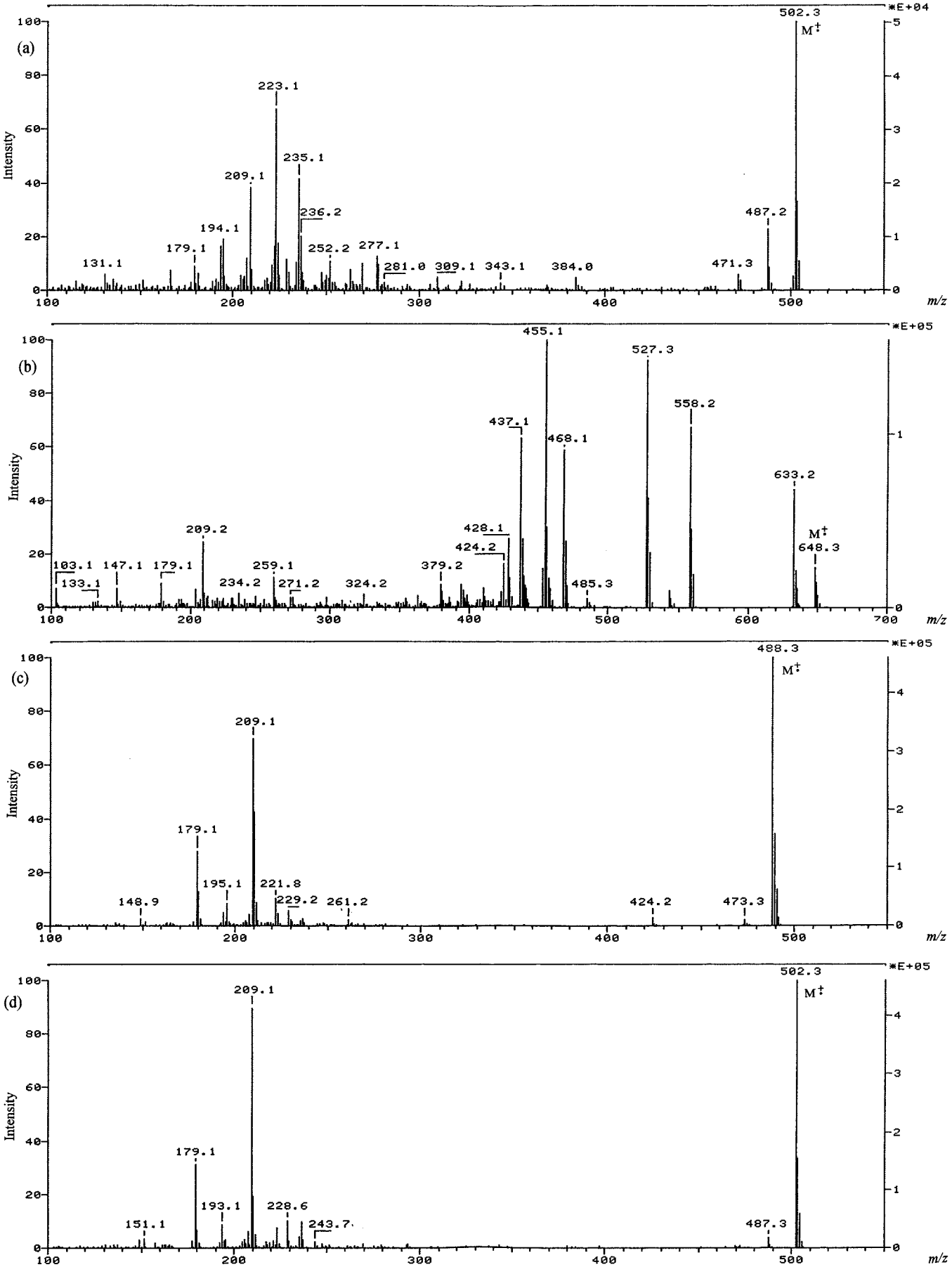

Figure 5. Mass spectra of TMS derivatives of isolated (a) pinoresinol, (b) isolariciresinol, (c) divanillyltetrahydrofuran, and (d) matairesinol.

Table 2. Comparison of Lignan Aglycons Isolated from Food, Urine, and Feces

		plant lignans						mammalian lignans		
source	food item	Seco[g]	Mati	Isol	Lari	Pino	NDGA	EL	ED	study
food	linseed[a,c]	−	−	−	−	−	−	+	+	Borriello et al. (1985)
	foods[a,c]	−	−	−	−	−	−	+	+	Thompson et al. (1991)
	flaxseed[b,d]	+	nd	−	−	−	nd	nd	nd	Obermeyer et al. (1995)
	foods[b,c]	+	+	−	−	−	−	−	−	Mazur et al. (1996)
	rye[b,c]	+	+	−	−	−	−	−	−	Nilsson et al. (1997)
	flaxseed[a,c,f]	+	−	−	−	−	−	+	+	Nesbitt and Thompson (1997)
	flaxseed[a,c]	+	−	−	−	−	−	+	+	Thompson et al. (1997)
	tea/coffee[b,c]	+	+	−	−	−	−	−	−	Mazur et al. (1998)
	beer[b,c]	+	+	−	−	−	−	−	−	Lapcik et al. (1998)
	flaxseed	+	+	+	nd	+	nd	−	−	current study
urine	N[b,c]	−	−	−	−	−	−	+	+	Setchell et al. (1983)
	N[b,c]	−	+	−	−	−	−	−	−	Bannwart et al. (1984)
	N[b,c]	+	−	+	+	−	−	−	−	Bannwart et al. (1989)
	N[b,c]	−	+	−	−	−	−	+	+	Adlercreutz et al. (1991a)
	N[b,c]	−	−	−	−	−	−	+	+	Adlercreutz et al. (1991b)
	F[b,c]	−	+	−	−	−	−	+	+	Lampe et al. (1994)
	S[b,c]	−	−	−	−	−	−	+	+	Hutchins et al. (1995)
	V[b,c]	−	−	−	−	−	−	+	+	Kirkman et al. (1995)
	N[b,d]	−	−	−	−	−	−	+	+	Horn-Ross et al. (1997)
	S[b,e]	−	−	−	−	−	−	+	+	Gamache and Acworth (1998)
feces	N[b,c]	−	+	−	−	−	−	+	+	Adlercreutz et al. (1995)
	F[b,c]	−	+	−	−	−	−	+	+	Kurzer et al. (1995a)

[a] Fecal digestion of sample prior to analysis. [b] Enzymatic hydrolysis (β-D-glucuronidase/sulfatase). [c] Analysis by GC/MS. [d] Analysis by HPLC−UV. [e] Analysis by HPLC with coulometric array detection. [f] Homemade and commercial products containing flaxseed. [g] Seco, secoisolariciresinol; Mati, matairesinol; Isol, isolariciresinol; Lari, lariciresinol; Pino, pinoresinol; NDGA, norodihydroguaiaretic acid; EL, enterolactone; ED, enterodiol; +, detected; −, not analyzed; nd, not detected; N, normal diet; F, fed flaxseed powder; S, fed soy products; V, carotenoid and cruciferous vegetable and soy diet.

mented with flaxseed and may be obtaining the lariciresinol from another plant food source in their diet. In addition, variations in the lignan content of flaxseed may be due to variety, seasonal changes, geographic location, and processing conditions (Thompson et al., 1997).

Extraction Methodology. Preliminary observations suggested that enzyme hydrolysis of DFM is not as efficient at removing lignans from the plant matrix as extraction with an organic solvent system. The new methodology combining aqueous methanolic extraction and subsequent acid hydrolysis renders a lignan extract that can be further purified by partitioning with ethyl acetate and hexanes. With HPLC−DAD (Figure 3b) there was the issue of interference from coeluting peaks with secoisolariciresinol, matairesinol, and isolariciresinol when monitoring was done at 280 nm. The secoisolariciresinol and matairesinol peaks were larger than the interfering compounds, but the retention time for isolariciresinol (which elutes before secoisolariciresinol on HPLC) was not determined because of the large coeluting sterol peak. Purification of the HPLC fractions prior to GC/MS analysis was not required. The crude lignan extract could be applied to GC/MS (Figure 4) without the need for a C_{18} preparative column or an ion-exchange column used in other reported methods (Setchell et al., 1983; Mazur et al., 1996) as the lignan peaks of interest were well resolved on the nonpolar column. Formation of the TMS derivatives also facilitated the separation of lignans that were soluble in isooctane from more polar compounds that were insoluble in this solvent.

Mass Spectral Identification of Lignans. As can be seen in Figure 5 there is structural diversity among the lignans that is also reflected in their mass spectra. The major lignan identified in the hydrolyzed methanol/water extract of DFM is secolariciresinol, which was previously found by Mazur (1996). The newly identified and other yet unidentified lignans constitute a small portion of the methanol/water extractable lignans of DFM. This is the first reported GC/MS total ion current profile (Figure 4) of lignan TMS ethers from DFM. The ion current profile is complex and contains other major peaks identified as TMS ethers of monohydroxylated C_{28} and C_{29} mono and diene sterols.

The literature on the mass spectral characteristics of lignans has been reviewed (Ayres and Loike, 1990). All of the lignans isolated have several characteristic low mass ions. The fragment ion at *m/z* 209 is attributable to the stable substituted tropylium ion structure. This ion is strong and is often the base peak in lignans containing two methoxytrimethylsilyl ether benzylic moieties with either the C-7 or C-7′ containing one or two hydrogens. However, the *m/z* 209 ion is not observed for hinokinin or NDGA. The ion *m/z* 209 shifts to 239 with the addition of a second methoxy group to the aromatic rings.

The aliphatic TMS ether groups are lost as TMSOH, whereas the phenolic TMS ethers do not readily lose TMSOH. The methoxyl groups appear to be preferentially lost from the phenyl ring. This does not appear to depend on the leaving group capability as much as the ring position. This is supported by the mass spectrum (not shown) of the NDGA TMS ether. NDGA does not contain a methoxyl group, but instead two adjacent hydroxyl groups. The loss of TMSOH is the third most abundant ion in the spectrum. The presence of an ion at *m/z* 209 does not imply the presence of a benzylic moiety in the molecule, as can be seen from the moderately abundant *m/z* 209 ion in the TMS ethers of pinoresinol (Figure 5a) and isolariciresinol, the mass spectrum of which has also been obtained on a quadrupole mass spectrometer (Bannwart et al., 1989). Isolariciresinol shows the characteristic loss of the two TMS groups (−90 u) due to the primary hydroxyl groups (Figure 5b) to give the fragment ions at *m/z* 558 and 468. The molecular ion is at *m/z* 648 with a base peak at *m/z* 455 (M − 193) due to loss of one of the aliphatic silyl groups as TMSOH (−90 u), plus the loss of a second aliphatic silyl group as TMSO−CH_2 (−103 u). The loss of 103 u is detected with and without retention of charge from the ions at *m/z* 103 and 545 (M − 103). Typical ions were observed from the loss of aliphatic hydroxyl, phenolic silyl ether groups, and phenolic methyl ethers or any combination thereof. In the mass spectrum of

isolariciresinol, these are found at *m/z* 633 (M − CH_3, from phenolic silyl ether group), *m/z* 558 and 468 (M − TMSOH and losses of two TMSOH, respectively), *m/z* 527 (M − TMSOH + OCH_3), and *m/z* 437 [M − (2(TMSOH) + OCH_3)]. Ions are also observed for the methoxyl hydroxyl substituted benzyl (*m/z* 209) moiety with concomitant loss of formaldehyde (30 u) at *m/z* 179. Finally, ions are found for multiple bond cleavages of the aliphatic ring portion of the molecule. Pinoresinol is unique among the lignans analyzed in that the molecular ion is also the base peak at *m/z* 502 with an envelope of ions centered around *m/z* 233. The absence of alcoholic hydroxyl groups usually yields an intense molecular ion.

If selected ion monitoring (SIM) for molecular ions or ions associated with the diarylbutane structure rather than total ion (TI) monitoring is utilized in GC/MS (Adlercreutz et al., 1991a,b; Bannwart et al., 1984, 1989; Lampe et al., 1994; Mazur et al., 1996) analysis (see Table 2), lignans with differing structures such as isolariciresinol and pinoresinol will not be detected.

Lignan Biosynthesis. Early work by Umezawa et al. (1990) showed that in vivo labeling experiments with *Forsythia intermedia* cell-free extracts result in the formation of the lignans (−)-secoisolariciresinol and (−)-matairesinol from two coniferyl alcohol molecules. Further research on the stereoselective and enantiospecific transformation mechanisms of lignan biogenesis with *F. intermedia* (Katayama et al., 1992, 1993; Chu et al., 1993) showed that the cell-free preparations catalyze the stepwise conversion of pinoresinol into secoisolariciresinol via lariciresinol. The pinoresinol/lariciresinol reductases found in *F. intermedia*, a deciduous shrub also known as Golden Bells, catalyze the unusual benzylic ether reduction in plants leading to benzylaryltetrahydrofuran, dibenzylbutane, dibenzylbutyrolactone, and aryltetrahydronaphthalene lignans. Although *F. intermedia* and flaxseed, *Linum usitatissimum*, are not related species, the isolation of pinoresinol and lariciresinol as key intermediates in the former suggests that lignan biosynthesis in the latter may occur by a similar mechanism. Our isolation and characterization of pinoresinol from flaxseed supports this theory. Although no lariciresinol was detected in the DFM analyzed, the isolariciresinol isolated may be an intermediate, the result of further reduction of lariciresinol in the flaxseed lignan biosynthetic pathway.

Lignan Metabolism. Secoisolariciresinol and matairesinol are believed to be the plant precursors of the mammalian lignans enterolactone and enterodiol (Boriello et al., 1985). These mammalian lignans, produced by metabolic conversions in the gastrointestinal tract by intestinal microflora, are detected in urine, feces (Table 2), plasma (Morton et al., 1994), and prostatic fluid (Morton et al., 1997a,b). Studies have been performed on the metabolism of lignans in animal models (Rickard and Thompson,1998; Thompson et al., 1996a,b). Feeding labeled secoisolariciresinol diglycoside (SDG) to rats resulted in >50% of the lignans being excreted in the feces and 30% in the urine, with low tissue retention 24 h after administration. The tissues with the greatest concentrations, intestines, liver, and kidney, are involved in SDG metabolism. With appreciable levels of metabolites found in estrogen-sensitive tissues such as the uterus (Rickard and Thompson, 1998), it should be possible to detect any lignans that are not metabolized by the microflora in the gastrointestinal tract in either feces or urine, if they are present in the ingested plant foods.

However, some food-derived lignans may not be detected in human fluids because they lack the ability to bind to certain active sites. Secoisolariciresinol, isolariciresinol, 3,4-divanillyltetrahydrofuran, enterolactone, and enterodiol, but not pinoresinol, have shown a binding affinity to human sex hormone binding globulin (SHBG) in an in vitro assay (Schoettner et al., 1997). This may explain why pinoresinol has not been detected in human studies as there is no method for transport from the gastrointestinal tract to the plasma or urine. It may well be present in the feces at very low levels that are below the detection limits of earlier methods.

In conclusion, the extraction of DFM with aqueous methanol followed by acid hydrolysis has led to the isolation and identification of isolariciresinol and pinoresinol as minor lignan components. What role these lignans may play in the potential health benefits of flaxseed remains to be determined. There are additional compounds with lignan-like characteristics in the DFM extract analyzed, which will be reported at a later date.

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