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Journal of Chromatography B, 777 (2002) 289–309

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Review

Mammalian phytoestrogens: enterodiol and enterolactone

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Abstract

The mammalian phytoestrogens enterodiol (END) and enterolactone (ENL) are produced in the colon by the action of bacteria on the plant precursors matairesinol (MAT), secoisolariciresinol (SECO), their glycosides, and other precursors in the diet. Both END and ENL have been shown to possess weakly estrogenic and antiestrogenic activities, and it has been suggested that the high production of these antiestrogenic mammalian lignans in the gut may serve to protect against breast cancer in women and prostate cancer in men. Various in vitro experiments suggested END and ENL significantly inhibited the growth of human colon tumor cells, and the E2-induced proliferation of MCF-7 breast cancer cells was inhibited by ENL. The protective effects of mammalian lignans may be due to their ability to compete with E2 for the type II estrogen receptor, to induce sex hormone binding globulin (SHBG), to inhibit placental aromatase, and to act as antioxidants. This review mainly deals with the chemistry, quantitative analysis, biological properties and health effects of END and ENL.

Keywords: Reviews; Phytoestrogens, mammalian; Enterodiol; Enterolactone

Contents

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1570-0232/02/$ – see front matter
PII: S1570-0232(02)00281-7

1. Introduction

Phytoestrogens are a diverse group of compounds found in many edible plants that have, as their common denominator, a phenolic group that they share with estrogenic steroids. This phenolic group appears to play an important role in determining the estrogenic agonist/antagonistic properties of these compounds. Phytoestrogens have been categorized according to their chemical structures as isoflavones, lignans and coumestans [1].

The two most important lignan type phytoestrogens were identified as *trans*-2,3-bis(3-hydroxybenzyl)-γ-butyrolactone (enterolactone, ENL, **1**) and 2,3-bis(3-hydroxybenzyl)butane-1,4-diol (enterodiol, END, **2**) (Fig. 1) [2,3], which have been described as the major lignans present in serum, urine, bile and seminal fluids of humans and animals [3–7]. Because these two compounds are produced in animals as opposed to plants they are usually termed the mammalian lignans to distinguish them from lignans from plants. The mammalian-derived lignans differ from plant-derived lignans in possessing phenolic hydroxy groups only in the *meta*-position of the aromatic rings.

END and ENL are formed by bacteria in the intestinal tract [6,8] from the plant lignans matairesinol (MAT) and secoisolariciresinol (SECO) [9], which exist in various whole-grain cereals (barley, rye and wheat), seeds, nuts, legumes and vegetables. The evidence for dietary precursors was first obtained by changing the diet given to adult rats from commercial pellets to a semisynthetic diet, D7, which resulted in a marked and rapid decrease in the excretion of mammalian lignan in urine [8]. When the diet was reverted to commercial pellets, lignans reappeared in the urine.

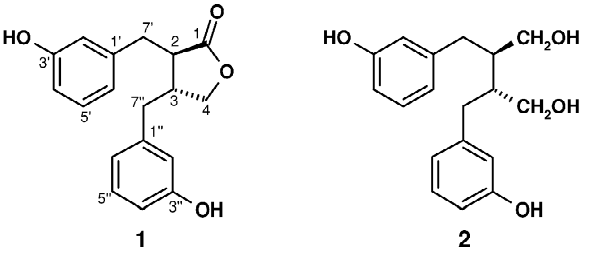

Fig. 1. Structures of lignan-type phytoestrogens ENL (**1**) and END (**2**).

Since identification of these two mammalian lignans after 1980, they received increasing attention because of their estrogen-like biological properties and pharmacological interaction with various enzymes and proteins. These activities may result in protection against some Western diseases. The highest phytoestrogen consumption and concentrations in urine and plasma are found in subjects living in countries with low cancer and coronary heart disease incidence, and the lowest values were found in breast cancer patients or in women at high risk for breast cancer [10].

This review mainly deals with the chemistry, analytical methods, biological properties and health effects of END and ENL.

2. Precursors and food sources

Two of the plant precursors known to form END and ENL upon bacterial fermentation are MAT (**3**) and SECO (**4**) (Fig. 2), respectively, although END can also form ENL [9]. In addition, glycosides of **3** and **4** are possibly the plant precursors of END and ENL, such as matairesinoside (**5**), secoisolariciresinol diglucoside (SDG, **6**), secoisolariciresinol 4-*O*-β-D-glucopyranoside (**7**) and so on (Fig. 2), since they are easily hydrolyzed to their aglycones by the β-glycosidase which is widespread in intestinal microorganisms [11]. SDG is the main component of flaxseed, its content varied between 11.7 and 24.1 mg/g in defatted flour and between 6.1 and 13.3 mg/g in whole flaxseeds [12].

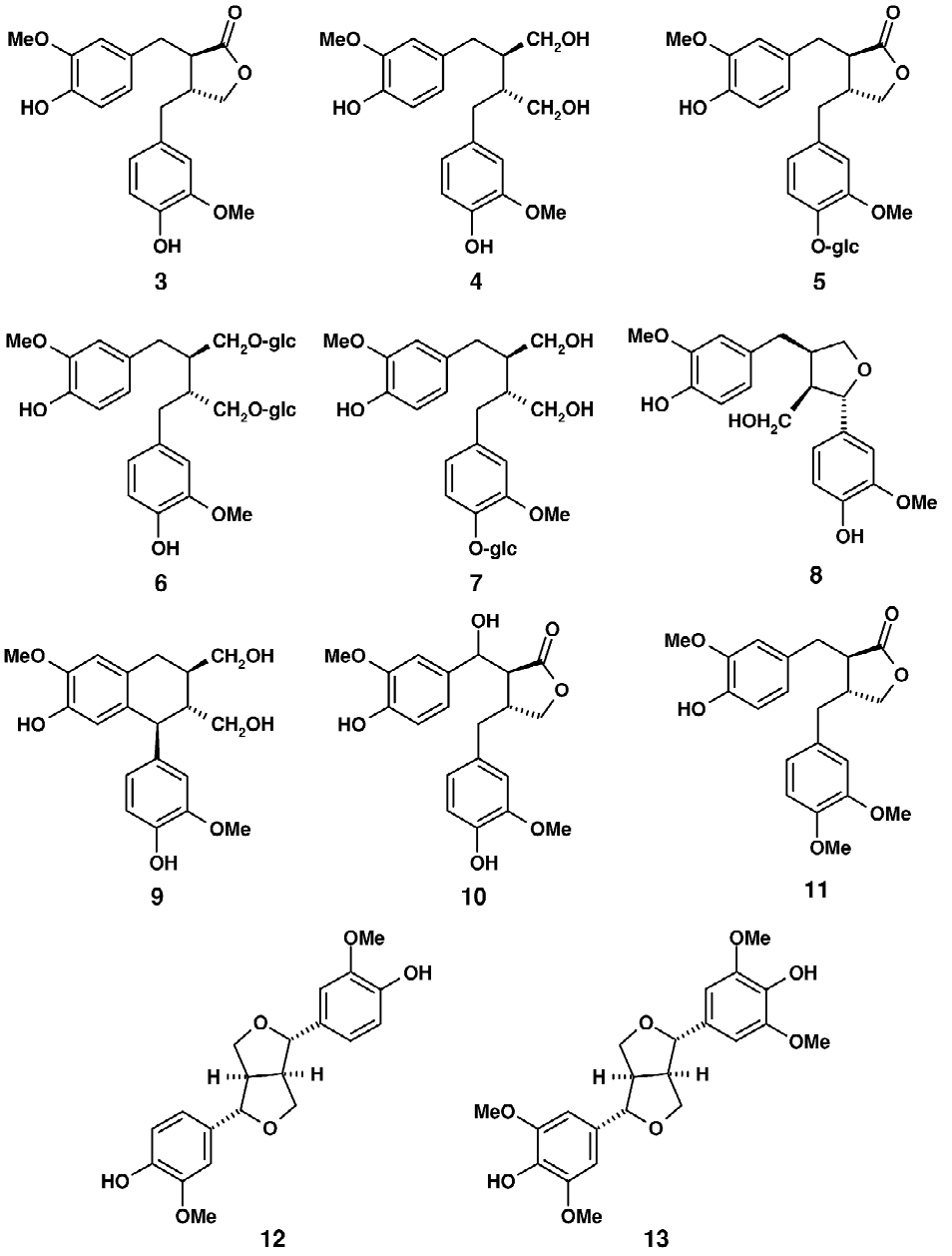

Fig. 2. Structures of plant precursors (**3**–**13**) of END and ENL.

Apart from the above precursors, lariciresinol (**8**), isolariciresinol (**9**) [13,14] and hydroxymatairesinol (**10**) [15] are also considered as precursors of END and/or ENL. Although the skeleton of lariciresinol (**8**) is different completely from END and ENL, the biosynthetic pathway from lariciresinol (**8**) to SECO (**4**) has been disclosed [16]. Hydroxymatairesinol (**10**), the most abundant single component of spruce lignans, was metabolized to ENL as the major metabolite in rat after oral administration, and the amounts of urinary ENL increased with the dose of hydroxymatairesinol (**10**) from 3 to 50 mg/kg [15].

More recently, pinoresinol (**12**) and syringaresinol (**13**) found in rye were also found as the precursors of END and ENL [17]. The glycosides of arctigenin (**11**) were not known to be transformed to ENL using rat gastric juice and intestinal microflora before [18,19], however Adlercreutz et al. [17] recently found that 4% of the arctigenin was metabolized to ENL during a 24-h fecal incubation.

In all, the plant precursors of END and ENL found up to now are SECO (**4**) and its glycosides (**6**, **7**), MAT (**3**) and its glycoside (**5**), lariciresinol (**8**), isolariciresinol (**9**), hydroxymatairesinol (**10**), arctigenin (**11**), pinoresinol (**12**) and syringaresinol (**13**), as shown in Fig. 2.

The precursors of END and ENL exist in various foodstuffs. Adlercreutz and Mazur [10] found high levels of SECO in garlic, carrots and broccoli. Horn-Ross et al. [20] found asparagus, as well as dried apricots and prunes, were the richest sources of SECO; garlic, along with carrots, cauliflower/brussels sprouts, and peaches were a moderate source, and sweet potatoes were an important source of MAT. Thompson et al. [21] developed an in vitro fermentation method using human fecal microbiota to simulate colonic fermentation. Using the method, the mammalian lignan END and ENL production from 68 different food products ranged from 21 to 67 541 μg/100 g sample. On a wet (as-is) basis as shown in Table 1, as a group, the oilseeds produced the highest mean concentration of total lignans (20 461±12 685), followed by the dried seaweeds (900±247), whole legumes (562±211), cereal brans (486±90), legume hulls (371±52), whole grain cereals (359±81), vegetables (144±23), and fruits (84±22). On a dry basis, oilseeds remained the highest producer of total lignans (23 123±14 414), followed by vegetables (1553±265), dried seaweeds (998±269), whole legumes (624±231), fruits (548±124), cereal brans (542±99), legume hulls (413±58), and whole cereals (407±93).

It was found that the lignan production from the flaxseed meal was 75 times higher than that from the seaweeds (second highest lignan-producing group) and 804 times higher than that from the fruits (lowest lignan-producing group) [21]. Lentils (1787), mekuba seaweed (1147), rapeseed (1130), triticale (924), soybean (863), hijiki seaweed (653), oat bran (651), corn bran (648), wheat bran (567), kidney beans (561), navy bean hull (535), alfalfa seed (498), whole wheat (490), purple rice (420), and garlic (407) were the other good producers of lignan, but still they were magnitudes lower than flaxseed.

Lignan production with the in vitro method related well to the urinary lignan excretion observed in rats and humans [8,22,23]. When the regular diet of humans was supplemented with flaxseed flour at a 10% level (based on dry matter), the urinary lignan excretion increased 21 times, about the same increase seen when the basal diet of rats was supplemented with flaxseed flour at a 10% level [23]. These strong relationships suggested the usefulness of the in vitro system in estimating the in vivo production of lignan from foods.

3. Identification

The structures of END and ENL were deduced from mass spectrometric, IR, UV and NMR data and were further confirmed by chemical synthesis.

The symmetry of END results in simple ^{1}H- and ^{13}C-NMR spectra, which were readily interpreted by inspection and by reference to published data for the related lignan dihydrocubebin [24]. The lack of symmetry in ENL, however, leads to more complex spectra. The ^{1}H-NMR spectrum showed a striking resemblance in the high-field region to those of MAT and its demethylether [25]. Nowadays, with various 2D NMR techniques, their NMR data were easily and unambiguously assigned as shown in Table 2.

In addition, Cooley et al. [26] proposed the conformational preference of ENL in solution (Fig. 3), derived from NOE data, which was found to correspond with that in solid state, obtained from an X-ray crystallographic study.

It should be pointed out that the natural ENL found in urine was racemic [3,5,27], while the optical rotation of synthetic (−)-ENL is $[\alpha]_D$ −38.4° [28]. Most lignans found in plants are optically active, having the absolute configuration as 3*R*,4*R*. We therefore present all formulae in the (3*R*,4*R*) configuration in this paper. The racemic was possibly due to isomerization by exchange of a hydrogen vicinal to the carbonyl group of the lactone [8].

Table 1
Mammalian lignan production from different foods[a] [21]

Foods	As-is (wet) basis		Foods	As-is (wet) basis	
	ENL	END		ENL	END
Oilseeds			Vegetables		
Flaxseed meal	8517	59 024	Garlic	81	326
Flaxseed flour	11 818	40 861	Squash	271	110
Rapeseed	975	155	Asparagus	136	238
Soybean	693	170	Carrot	284	62
Sunflower	201	195	Sweet potato	240	55
Peanuts	105	56	Broccoli	161	65
Cereal brans			Leek	24	174
Oat bran	265	386	Green pepper	162	33
Corn bran	168	480	Turnip	78	78
Wheat bran	269	298	Cauliflower	68	77
Barley bran	243	140	Beet	109	26
Rice bran	134	47	Snow pea	60	62
Whole cereals			Iceberg lettuce	58	63
Triticale	519	405	Onion	11	101
Wheat	411	79	String bean	40	56
Purple rice	340	80	Potato	33	50
Oats	251	89	Brussels sprout	57	18
Brown rice	169	128	Boston lettuce	27	47
Sorghum	199	56	Cabbage	30	34
Corn	199	31	Bok choy	44	14
Rye	69	91	Mushroom	43	13
Barley	41	74	Watercress	20	28
Dried whole legumes			Radish	25	10
Lentil	789	998	Celery	17	14
Kidney bean	329	232	Cucumber	18	11
Alfalfa seed	328	170	Tomato	11	10
Navy bean	352	108	Fiddle head	14	7
Faba bean	129	88			
Yellow pea	169	44	Legume hulls		
Pinto bean	154	47	Navy bean	370	165
Fruits			Lentil	278	119
Pear	112	69	Kidney bean	299	91
Plum	47	98	Field pea bean	262	49
Strawberry	41	38	Faba bean	143	79
Banana	55	14			
Orange	27	12	Dried seaweeds		
Cantaloupe	21	16	Mekuba	167	980
Apple	34	1	Hijiki	266	387

[a] Values expressed as μg/100 g sample.

4. Synthesis

Because END and ENL are produced in animals, the total synthesis is of great importance to confirm their structures in an early stage, and to get a sufficient amount for further study on the bioactivity, metabolism, pharmacokinetics, and fate of these compounds at target tissues in animals.

Groen et al. [28] reported the synthesis of (±)-ENL, together with (−)-ENL by recrystallization. Cooley et al. [29] utilize an adaptation of the traditional route to lignans of the 2,3-dibenzylbutane type to synthesize END and ENL. A stobbe condensation of 3-benzyloxybenzaldehyde with diethyl succinate gave bis-(3-benzyloxybenzylidene)-succinic acid. Catalytic hydrogenation, with simulta-

Table 2
NMR data of END and ENL

No.	END		ENL	
	^{1}H[a]	^{13}C [b]	^{1}H [c]	^{13}C [d]
1	3.53 (dd, 11.1, 5.3)			
	3.63 (dd, 11.1, 3.8)	61.7		179.7
2	1.98 (m)	44.4	2.59 (m)	46.5
3	1.98 (m)	44.4	2.50 (m)	41.2
4	3.53 (dd, 11.1, 5.3)			
	3.63 (dd, 11.1, 3.8)	61.7	3.87 (dd, 9.1, 7.6)	
		71.7	4.14 (dd, 9.1, 7.0)	
1′		143.8		139.2
2′	6.62 (ddd, 7.8, 2.2, 1.2)	116.9	6.59 (t, 1.6)	116.3
3′		158.2		157.2
4′	6.60 (ddd, 7.8, 2.2, 1.2)	113.7	6.70 (m)	113.9
5′	7.05 (t, 7.8)	130.1	7.18 (t, 7.9)	129.9
6′	6.62 (ddd, 7.8, 2.2, 1.2)	121.4	6.73 (ddd, 7.9, 1.6, 1.6)	120.9
7′	2.64 (m)	36.2	2.91 (m), 3.00 (m)	35.0
1″		143.8		139.7
2″	6.62 (ddd, 7.8, 2.2, 1.2)	116.9	6.47 (t, 1.6)	115.6
3″		158.2		157.2
4″	6.60 (ddd, 7.8, 2.2, 1.2)	113.7	6.74 (m)	113.9
5″	7.05 (t, 7.8)	130.1	7.15 (t, 7.9)	139.2
6″	6.62 (ddd, 7.8, 2.2, 1.2)	121.4	6.60 (ddd, 7.9, 1.6, 1.6)	120.1
7″	2.64 (m)	36.2	2.50 (m), 2.61 (m)	38.5

[a] In CD_3OD, 400 MHz (Ref. [25]).
[b] In CD_3OD, 100 MHz (unpublished data).
[c] In $CDCl_3$, 400 MHz (Ref. [25]).
[d] In $CDCl_3$, 100 MHz (unpublished data).

neous hydrogenolysis of the protecting benzyloxy groups, followed by reduction of the resulting 2,3-bis-(3′-hydroxybenzyl)-succinic acid with lithium aluminum hydride, and acidification, gave a mixture of END and ENL.

The conjugate addition of a thioacetal carbanion to butenolide followed by trapping of the enolate anion generated with a suitable electrophile provides a short, efficient approach to the construction of the basic lignan skeleton. Pelter et al. [30] have utilized a similar scheme to provide a general route to dibenzylbutyrolactones, including ENL.

However, the above methods were not enantioselective. Oeveren et al. [31] developed the enantioselective synthesis of natural dibenzylbutyrolactone lignans including (−)-ENL and (−)-END via tandem conjugate addition to γ-alkoxylbutenolides. The chiral butenolides (5*R*) (**14a**) and 5*S*(menthyloxy)-2(5*H*)-furanone (**14b**) (Fig. 4) are the key synthons in the methodology. Butenolides **14a** and **14b** have proven to be extremely valuable as

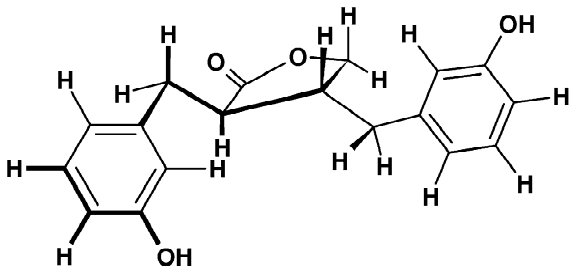

Fig. 3. Preferred conformation of ENL in solution, as determined by NOE (from Ref. [26]).

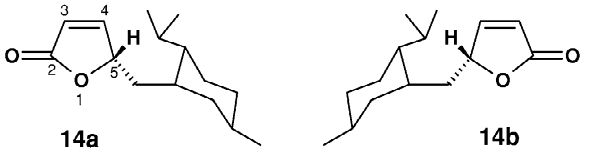

Fig. 4. Structures of the chiral butenolides (5*R*)- (**14a**) and (5*S*)-(menthyloxy)-2(5H)-furanone (**14b**).

a chiral dienophile and Michael acceptor, generally providing products with enantiomeric excess (ee) exceeding 99%, after removal of the auxiliary group D- or L-menthol.

As shown in Fig. 5, the dithiane **16** and the benzyl bromides **18** were prepared from the corresponding aromatic aldehydes **15**. The anions of dithioacetal **16** was generated by treatment of a solution of the dithioacetal in THF with *n*-butyllithium at −20 °C. The conjugate addition of lithiated dithiane **16** to **14a** at −80 °C was followed by quenching of the resulting lactone enolate anion **19** with benzy bromide **18** at −80 to −30 °C to yield the dibenzylbutyrolactone, **20**, with a complete lignan skeleton. A one-pot procedure for the conversion of lactone **20** to lactone **21** was designed, which proved to be highly efficient. The benzyl-protected lactone **21** was readily deprotected with H_2 on Pd/C to provide the enantiomerically pure lignan (−)-ENL. Furthermore, reduction of ENL with $LiAlH_4$ gave the enantiomerically pure lignan (−)-END. Similarly, (+)-END and (+)-ENL were obtained starting from the enantiomer **14b**.

5. Metabolism in vitro and in vivo

Earlier studies in germ-free rats and humans administered antibiotics had established that mammalian lignan production depends on the presence of

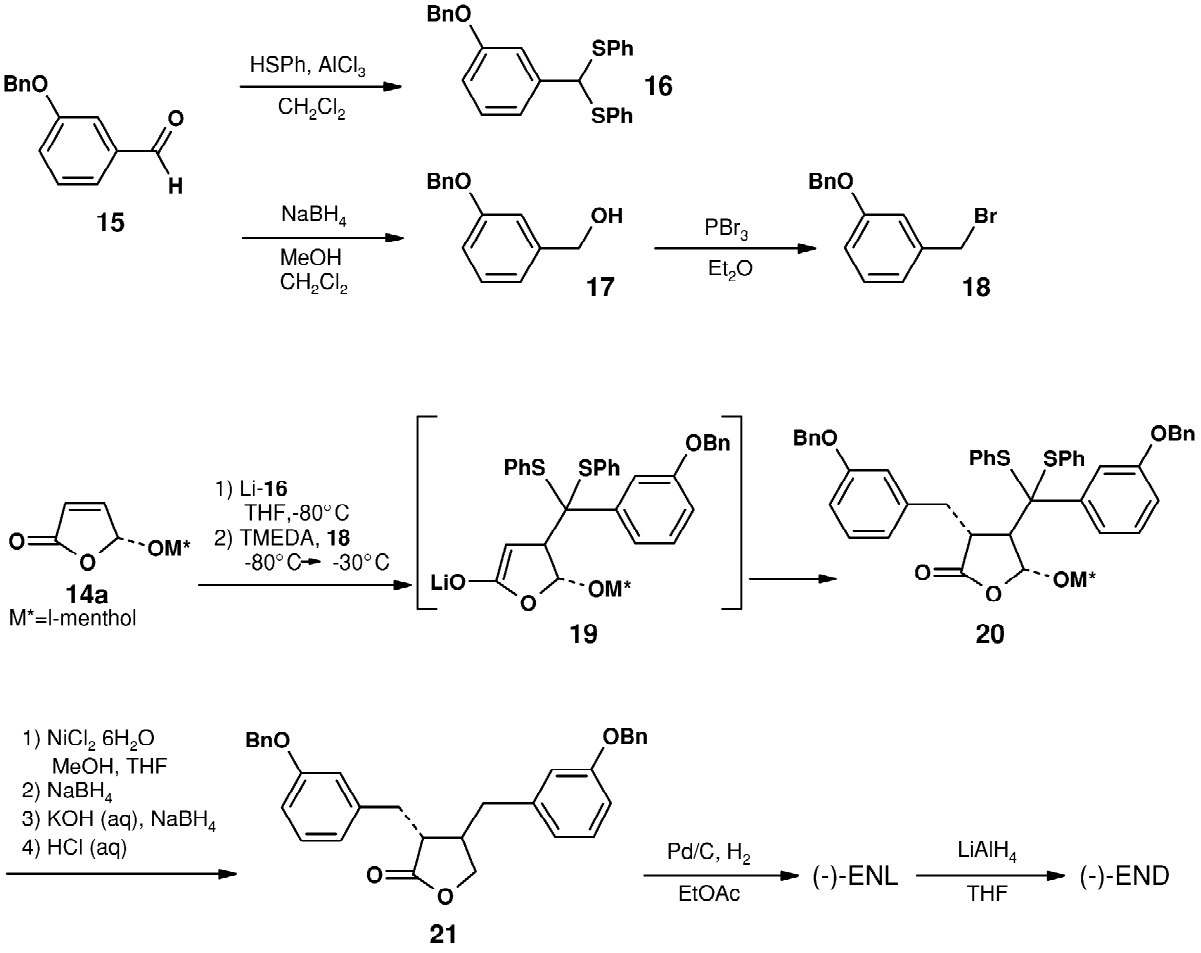

Fig. 5. Total synthesis of enantiomerically pure lignans (−)-END and (−)-ENL using chiral butenolide (**14a**) (from Ref. [31]).

bacteria in the intestinal tract [2]. In vitro studies have also demonstrated the efficient production of mammalian lignans from the dietary precursors by human fecal flora [9]. These suggest that the primary site of their production is the cecum and colon.

After removal of methyl and hydroxyl groups in precursors by intestinal bacteria, END and ENL are absorbed from the gut to appear in plasma and are then excreted in the urine and bile, predominantly as glucuronide or sulfate conjugates. Axelson and Setchell [32] reported that END and ENL in urine occur mainly as glucuronides. Likewise, Adlercreutz et al. [33] found 92% of END and 98% of ENL exist in the glucuronide conjugates. In addition to their glucuronide conjugates, there exists a small proportion of sulfoglucuronide, monosulfate and disulfate conjugates in urine.

Setchell et al. [9] first proposed the production and metabolism of END and ENL by human fecal flora in vitro. The authors speculated that SDG was metabolized to END, probably through hydrolysis, dehydroxylation and then demethylation by facultative bacteria, and then ENL was produced from END through oxidation by faculatative bacteria. Similarly they proposed the formation of ENL from MAT by dehydroxylation followed by demethylation by facultative aerobes.

Since the metabolic pathway of END and ENL was not clearly explained, Wang et al. [34] carried out an in vitro biotransformation of SDG, one of the precursors, to mammalian lignans END and ENL by human intestinal bacteria. Seven metabolites (**1**, **2**, **4**, **22–25**) (Fig. 6) were isolated after anaerobic incubation with a human fecal suspension. Furthermore, two bacterial strains, *Peptostreptococcus* sp. Strain SDG-1 and *Eubacterium* sp. Strain SDG-2, capable of demethylation and dehydroxylation, respectively, were isolated from a human fecal suspension. With the seven metabolites and the two bacterial strains, the possible metabolic pathway for the formation of ENL from SDG was proposed in Fig. 6. It seems reasonable that demethylation occurs prior to dehydroxylation since demethylated metabolites **22** and **24** were formed from SDG after incubation for a short period of time (20–30 h), whereas dehydroxylated ones **1**, **2**, **23** and **25** were obtained later than 48 h. In addition, no transformation of **4** or **23** was observed by a newly isolated strain of *E.* sp. strain SDG-2 having dehydroxylation ability.

In addition, transformation of **2**, **4** or SDG was effectively achieved by the human fecal suspension under anaerobic conditions, while similar results were not obtained under aerobic conditions or when a sterilized fecal suspension was used. These findings further suggest that the formation of END and ENL is not the result of spontaneous chemical reactions, but due to the metabolic reaction of viable intestinal bacteria under anaerobic conditions.

Using HPLC and GC–MS, Rickard and Thompson [35] conducted in vivo experiment and found the major urinary lignan metabolites of rats fed ^{3}H-SDG were END, ENL and its aglycon. However, there may be at least four other urinary metabolite derivatives of END or ENL which remained unidentified. None of the above in vitro metabolites **22**–**25** of SDG was identical to the four in vivo metabolites of ^{3}H-SDG, comparing with their mass data. The mass spectra obtained for the four unknown compounds did not also match that of the hydroxylated END or ENL metabolites detected in the urine of humans fed flaxseed [36] or produced from liver microsomes in vitro [37]. They also determined changes in postprandial blood levels of ^{3}H-SDG metabolites over a 24-h period with acute or chronic SDG treatment. Regardless of treatment, END, ENL and SECO accounted for 75–80% of urine radioactivity. The type of treatment had no effect on the levels of individual urinary metabolites of ^{3}H-SDG. Furthermore, in both the acute and chronic treatment groups, blood radioactivity levels peaked at 9 h, which remained steady until 24 h in the chronic SDG group. In the acute group, blood radioactivity started to drop at 24 h but still was not significantly different compared to the 12-h time point in another study [38]. Similar profiles were observed in premenopausal women given 25 g dose of flaxseed where plasma lignan levels peaked at 9 h postconsumption and remained at high level until 24 h postconsumption [39]. This suggests that the blood lignan kinetics are similar to flaxseed or SDG consumption and comparable between humans and rats.

To study the intestinal metabolism of lignans, the concentrations of plant and mammalian lignans (END and ENL) in intestinal digesta sampled along the intestinal tract of pigs, fed with rye-bread diets, were determined [40]. In the ileum, the lignans were mainly present as conjugated plant lignans, which were determined only when the analytical procedure

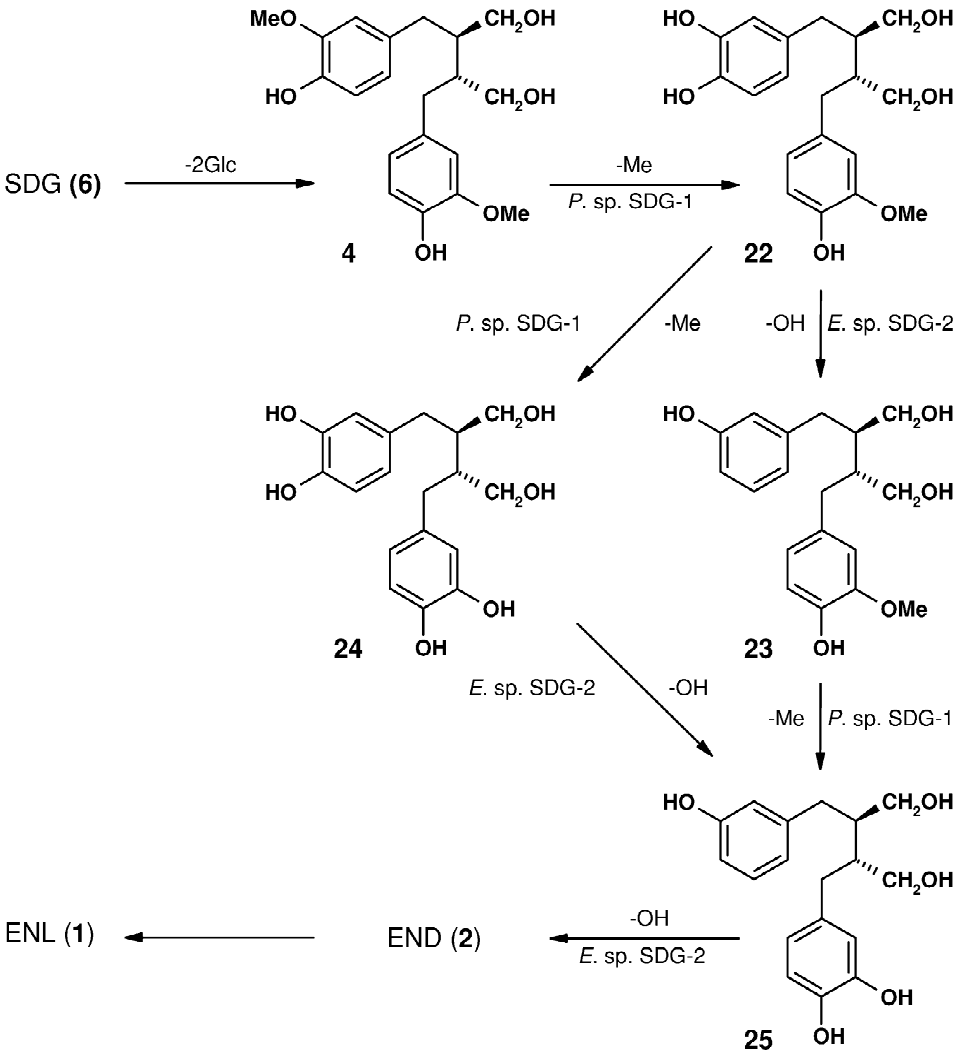

Fig. 6. Possible pathway for the transformation of ENL by *P.* Sp. strain SDG-1, *E.* sp. strain SDG-2 and human intestinal bacteria (from Ref. [34]).

included a hydrolysis step. Most of the plant lignans disappeared from the intestinal tract between the terminal ileum and the caecum.

After formation of the less polar END and ENL, they may be absorbed from gastrointestinal tract and undergo enterohepatic circulation, where they may be metabolized by liver and other tissue enzymes. Metzler et al. [37] recently disclosed that hepatic microsomes from rats and humans are able to hydroxylate END and ENL both at aliphatic and aromatic positions. Hepatic microsomes from aroclor-treated male Wistar rats biotransform ENL to twelve metabolites, six of which carry one additional hydroxy group at the aromatic and six at the aliphatic moiety according to HPLC–MS and GC–MS analysis. The aromatic hydroxylation products were identified with the help of synthesized reference compounds as ENL monohydroxylated in the *para*-position and in both *ortho*-positions of the original phenolic hydroxy group of either aromatic ring. Similarly, END is metabolized to three aromatic and four aliphatic monohydroxylated metabolites. Most of the metabolites of END and ENL were also formed with microsomes from uninduced rat, pig,

and human liver, suggesting that oxidative metabolism is a common feature in the disposing of these lignans in the mammalian organism.

Using the isolated microsomal metabolites as reference compounds, Metzler et al. [36] detected the nine aromatically hydroxylated metabolites (**25–33**) (Fig. 7) of END and ENL among the twelve in vitro metabolites in the urine of both female and male humans. Metabolites **30** and **31** may be formed through *ortho*-hydroxylation of ENL or may be intermediates in the bacterial conversion of SECO and/or MAT to ENL. Likewise, metabolite **25** may also represent an intermediate of the bacterial biotransformation of SECO to END. This point was also confirmed by the in vitro studies of SDG transformed by human intestinal bacteria [34].

The metabolites carrying one additional hydroxy group at the aliphatic moiety were not detected in human urine [41]. However, these metabolites were detected in the 6 h bile of the bile duct-catheterized female Wistar rats after administration intraduodenally at a dose of 10 mg/kg of END and ENL analyzed by HPLC and GC–MS. With END-dosed rats, three products of aromatic and two of aliphatic monohydroxylation were found, whereas six aromatic and five aliphatic monohydroxylated biliary metabolites were detected after administration of ENL. The structures of the in vivo metabolites arising from aliphatic hydroxylation could not be completely elucidated, however, they were identical to some of the formerly reported microsomal products according to GC retention times and mass spectra.

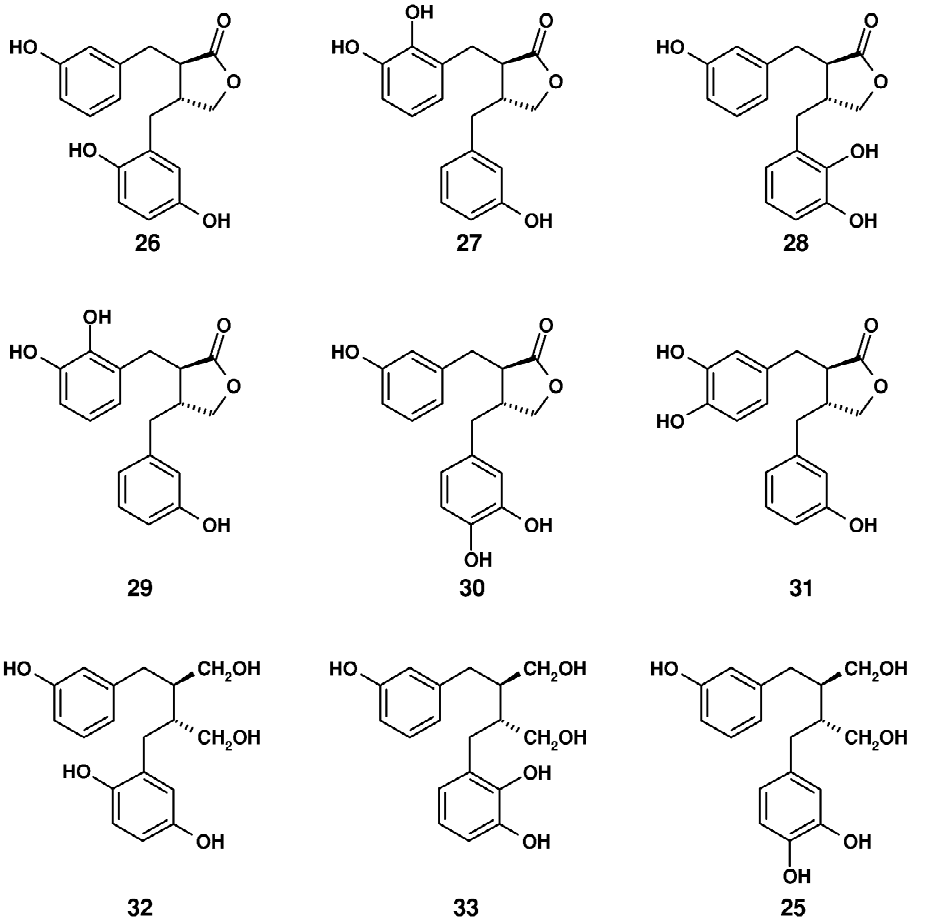

Fig. 7. Aromatically hydroxylated microsomal metabolites (**22**–**30**) of END and ENL (from Ref. [36]).

6. Analytical methods

Various analytical methods for detection and quantification of these two lignans, but especially ENL, in human biological fluids have been developed. These methods include gas chromatography (GC) or high-performance liquid chromatography (HPLC) alone or in combination with mass spectrometry (MS) and a time-resolved fluoroimmunoassay (TR-FIA) method.

6.1. GC–MS

GC or GC–MS methods are slightly complicated. Once the compounds are extracted, they need to be derivatized in order to be run through the gas chromatograph, a process that can be tedious and complicated. The first quantitative method for the determination of END and ENL in plasma was developed using isotope dilution GC–MS (ID-GC–MS) [42]. Using a similar technique, the quantitative determination of END and ENL in urine [43] and feces [44] was also performed.

Another novel approach to mammalian lignan quantification was developed by Shultz et al. [45], involving the application of GC coupled with ion mobility spectrometry (IMS) detection. IMS is a gas phase, atmospheric pressure detection technique in which ions are propelled by an electric field through a counter current gas flow [46–49]. The ions are separated based on their charge, mass, and collisional cross-sectional area and can be distinguished by their arrival time at a collector electrode [50]. IMS is inherently a low-resolution technique. Therefore, complex samples such as biological fluids require chromatographic separation before IMS detection. The use of GC preceding IMS reduces analyte interferences and avoids detector overload and matrix effects. The tunable selectivity of IMS allows the elimination of interfering chromatographic peaks, assuming that near-eluting compounds have different ion mobilities.

Using GC–IMS technique, both END and ENL concentrations in human plasma and urine, before and after whole wheat/flaxseed supplemented dietary intervention, were reported [45]. Fig. 8 shows the effect of nonselective and selective mobility monitoring on derivatized urine extract chromatograms, respectively.

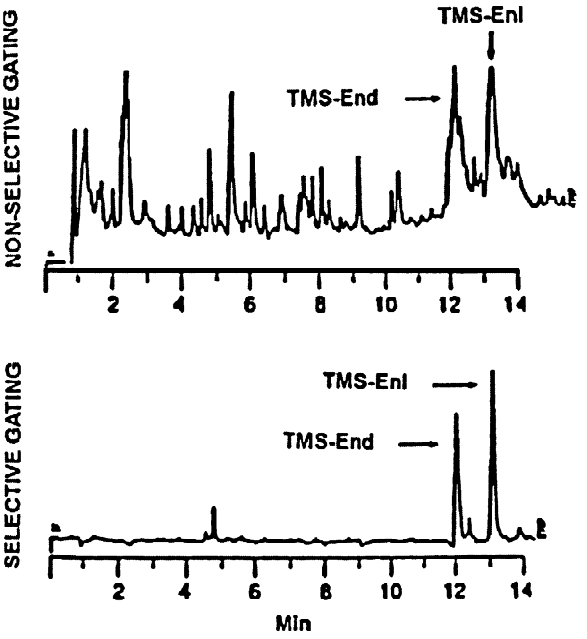

Fig. 8. Effect of selective gating on urinary extract chromatograms (from Ref. [45]).

The use of GC–IMS allows a sensitive and selective means of determining amounts of derivatized lignans in biological fluids. The unique IMS product ion spectra of lignans allow a high degree of selectivity, which is essential when dealing with extremely complex matrices such as urine and plasma. IMS sensitivity and selectivity facilitates the measurement of END levels in plasma, which was not detected successfully [43].

6.2. HPLC method

An HPLC method for profiling 13 phytoestrogens including END and ENL using coulometric electrode array detection was developed [51]. The sensitivity of the method was slightly less than that of the GC–MS method, but significantly higher compared to the HPLC methods using diode-array or UV detection [51]. This technique is based on the use of multiple electrochemical detectors placed in series after the analytical column and maintained at different potentials. This technique has significant advan-

tages in the detection of phenolic phytochemicals due to the inherent sensitivity, selectivity, and linear response range of electrochemical detectors. Also, because of the unique properties of the coulometric electrode, resolution of co-eluting solutes can be obtained based on small differences in their oxidation–reduction behaviors [52]. This expands the analytical capabilities for multicomponent analysis and can minimize sample prepurification steps when dealing with complex matrices such as urine. The utility of this approach for the analysis of plasma and urinary tea polyphenols has been successfully performed [53].

This method is useful for monitoring plasma phytoestrogen profiles in nonsupplemented samples. The higher the concentration of the compounds, the better the precision of the method; even if the concentration is close to the detection limit, determinations are satisfactory when compared to other methods with similar sensitivity. Through quantitative determination of END and ENL using GC methods in human plasma [42], urine [43] and feces [44] in omnivorous and vegetarian women, the excretion of ENL is at significantly higher levels than that of END, which is almost below the electrode array detection limit. Therefore, the HPLC method using coulometric electrode array detection is useful for quantitatively detecting ENL in different matrices such as plasma, urine and feces.

Urinary lignans including END and ENL have been analyzed by HPLC–MS [54]. Phytoestrogens were extracted from urine with Sep-Pak C_{18} cartridges. After hydrolysis with with glucuronidase/sulfatase aglycones were recovered by solid-phase extraction and then subjected to HPLC–APCI-MS analysis. A C_8 RP column was used, eluting with 0–50% acetonitrile in aqueous ammonium acetate over 15 min. After chromagraphic separation, the eluate stream was diluted with ammonium hydroxide and multiple reaction monitoring was carried out by selecting parent molecular ions and specific daughter ions formed by collision with argon–10% nitrogen.

6.3. TR-FIA method

The GC and HPLC methods for the quantitative determination of ENL and END are expensive, laborious, time-consuming, and not suitable for screening purposes in large populations. In addition, these methods are not sensitive enough for the assay of unconjugated phytoestrogens in plasma. Therefore there is a need for more convenient procedures and more sensitive methods for measuring unconjugated phytoestrogens in plasma and tissues.

Since antibodies to some phytoestrogens have been raised in rabbits as early as 1969, radioimmunoassay (RIA) methods for quantitative measurements of unconjugated formononetin in plasma [55], total and unconjugated daidzein and genistein in human biological fluids [56,57] were developed. As a continuous development of immunoassays, a time-resolved fluoroimmunoassay of plasma ENL was developed [58,59]. It combines the advantages of other nonradioisotopic assays (stability of the reagent and lack of radiation) with a 10- to 100-fold increase in sensitivity and assay range in comparison with conventional enzyme immunoassay (EIA) and fluoroimmunoassay (FIA) methods. It also shows low background interference and a wide dynamic range.

The excellence of time-resolved fluorometry in routine immunodiagnostic work, as employed in the dissociation-enhanced lanthanide fluroimmunoassay (DELFIA) system by Wallac Oy (Turku, Finland), is already well-established [60]. In the DELFIA system, a nonflurorescent chelate is employed to bind europium to the analyte. After the bioaffinity reaction is completed, the europium ions are dissociated from the chelates by means of an enhancement solution in which lanthanide ions form highly fluorescent complexes with components of the enhancement solution. The enhanced fluorescences are measured at a fixed time after excitation of the fluorophore. By this time, the background fluorescence has died away.

The TR-FIA for ENL is based on the fact that the europium-labeled and sample ENL compete in binding to a limited amount of highly specific antibody. When no unlabeled ENL exists, the tracer occupies all the antibody-binding sites, while if unlabeled ENL is present, it will compete with the tracer.

The TR-FIA method provided a new alternative procedure for the assay of ENL for large screening studies. The method is reliable, relatively inexpensive, sufficient, convenient and specific compared to the GC or HPLC methods.

Using a similar approach, Uehara et al. [61] also developed a rapid quantitative analysis of urinary ENL in human urine.

In conclusion, various methods such as GC, HPLC, and TR-FIA methods have been developed which are suitable for mammalian phytoestrogen ENL determination. However, due to the lower levels of END present in plasma, urine and feces, only the ID-GC–MS and GC–IMS methods show relatively higher sensitivity and selectivity for its quantitative measurement.

7. Biological activities

7.1. Estrogenic and antiestrogenic activity

The biological effects of estrogens are known to be extremely complex. Estrogens, including phytoestrogens, exert 'estrogenic' effects through binding to the estrogen receptor (ER). Ligands for ERα and ERβ can act as pure agonists, as antagonists, or have partial or selective agonist/antagonist activity. Whether an ER ligand acts as an agonist or antagonist in different tissues is determined by many factors [62–64]. Although the phytoestrogens bind less strongly to the estrogen receptor than estradiol (E2), their interaction with this receptor can produce both estrogenic and antiestrogenic responses in various cancer cell lines [65].

The estrogenic effects of phytoestrogens were first observed in Western Australian in 1946 as reproductive dysfunction in sheep, later known as 'clover' disease [66]. Since the detection of ENL in urine of humans, baboons, vervet monkey and rats, considerable interest was stimulated by the demonstration of a cyclic pattern in their excretion in women during the menstrual cycle, with maximum urinary levels occurring in the luteal phase, and increased excretion during early pregnancy [5,24,27], which suggested a physiological and biological role. The highly aromatic structure of these lignans and the similarity of their physicochemical properties to those of estrogens suggested that they might have estrogenic or antiestrogenic activity, especially since they have a phenyl ring which is common to all compounds with such activity.

Many studies have suggested the biphasic effects of phytoestrogens [65]. At relatively low doses, certain phytoestrogens express an estrogenic activity and stimulate cell growth, while at higher doses the same phytoestrogens appear to be antiestrogenic and suppress cell growth. At these higher doses, the phytoestrogens may enter this 'biphasic' mode and serve as protein kinase inhibitors or show topoisomerase activity.

The estrogenic activity of synthetic ENL was investigated by an assay of its systemic action on mouse uterine weight [7]. The control, E2, produced a graded uterine response but there was no significant increase in uterine weight with ENL, which showed that ENL had no estrogenic activity. However, later Jordan [67] demonstrated weak estrogenic activity in the stimulation of prolaction secretion and synthesis of progesterone receptor of ENL in vitro. The possibility that ENL may have antiestrogenic activity was disclosed by Waters and Knowler [68], who reported ENL has been shown to depress estrogen-stimulated rat uterine RNA synthesis.

It was well known that E2 has proliferative effects on estrogen dependent cancer cells and that antiestrogens inhibit this effect. To elucidate the estrogenic and antiestrogenic properties of ENL, Mousavi and Adlercreutz [69] studied, using MCF-7 breast cancer cells in culture, the in vitro effect of relatively low concentrations of ENL added both alone and in combination with E2. E2 (1 n*M*) and ENL (0.5–2 μ*M*) separately stimulated the proliferative of MCF-7 cells, but the combined compounds had no stimulatory effect at all compared to the controls. The phenomenon that the presence of E2 and ENL together inhibits each other's growth-stimulatory effect is possibly due to the fact that ENL prevents binding of E2 to the type II nuclear estrogen receptor. It was found that the control cells with no addition of ENL and E2 did not grow properly, most likely due to the complete absence of estrogens. While at a concentration above 10 μ*M* ENL in cells, a definite growth inhibitory effect could be observed. The above phenomenon agrees with the characteristic biphasic effect. The lignan ENL was also found to have a biphasic effect on DNA synthesis in MCF-7 cells, showing induction at 10–50 μ*M* (with a peak value of 210% at 10 μ*M*) and inhibition at high concentrations, with an IC_{50} of 82.0 μ*M* [70].

The estrogen-like activity of ENL at a concen-

tration of 1 μ*M* was consequently revealed by stimulating of pS2 mRNA expression in MCF-7 cells, which correlates positively with cellular proliferation [71].

The stimulatory and inhibitory concentrations of ENL differed according to different results. Mousavi and Adlercreutz [69] showed an inhibition of growth at >10 μ*M* ENL in MCF-7 cells, while Wang and Kurzer [70] showed inhibition at >50 μ*M* on DNA synthesis in MCF-7 cells. Similarly, Wang and Kurzer found that ENL at 10 μ*M* significantly increased DNA synthesis in MCF-7 cells induced by 0.01 n*M* E2, however, no effects were observed at other concentrations. These results differ from those of Mousavi and Aldercreutz [69], who reported that 1 μ*M* ENL decreased E2-induced growth in MCF-7 cells.

The phytoestrogen concentrations found to inhibit breast cancer cell growth have generally been >10 μ*M* in vitro. However, the serum concentration of ENL in humans is normally <10 μ*M* and most often <1 μ*M* [42–44,58,59]. The results of in vitro studies suggest that, at these concentrations, stimulation rather than inhibition, may be the primary effect of phytoestrogen on cell growth. It is therefore extremely important to broaden our understanding of the effects of phytoestrogens on breast cancer cell growth at low and high concentrations with particular attention to the effects at the probable concentrations in humans.

7.2. Binding to some proteins (SHBG, SBP, AFP)

Sex hormone binding globulin (SHBG) is the major plasma sex hormone transport protein with a high affinity towards endogenous estrogens [72]. It is produced in the liver and any manipulation of its synthesis or capacity to bind estrogen would have a dramatic effect on the estrogen levels that would be available to hormone-dependent cells [73]. It has been shown that some phytoestrogens are apparently effective stimulators of SHBG production in human liver cancer cells and subsequently suppress the growth of these cells in culture [73]. This suppression may be a result of the phytoestrogen's ability to enhance the level of SHBG, which could then bind a larger proportion of free estrogen, lowering the estrogen level available to the cancer cell and modulating its growth.

Using an in vitro assay, Schöttner et al. [74] tested the affinity of (±)-END and (±)-ENL to human SHBG in the presence of ^{3}H-labeled 5α-dihydrotestosterone as the ligand of SHBG. It was shown that (±)-END developed only a weak affinity (16±6%), (±)-ENL was of a comparable moderate intensity (55±3%). In addition, Adlercreutz et al. [75] found that ENL stimulated the synthesis of SHBG by HepG2 liver cancer cells in culture acting synergistically with E2.

A positive correlation between dietary intake of total fiber and urinary excretion of ENL has been shown previously. Adlercreutz et al. [76] found, in preliminary statistical studies, a highly significant positive correlation between intake of dietary total fiber/kg weight and plasma SHBG ($P<0.001$). These results suggested that fiber-rich food containing lignan precursors may, via production of mammalian lignans in the intestinal tract, stimulate SHBG synthesis in the liver and may in this way reduce the concentration of free hormones in plasma. In a subsequent study, significant positive correlation between urinary ENL, total lignan, total phytoestrogens and total diphenol excretion, and plasma SHBG, were found in a group of 23 Finnish women [75].

Sex steroid binding protein (SBP) has a specific high affinity for the sex steroids. About half of the circulating testosterone in men and 88% of the total estrogens in pregnant women are bound to SBP [77]. Thus any change in the concentration and/or binding properties of this protein will alter steroid metabolism by inducing large changes in the clearance rates of androgens and estrogens and in the availability of these hormones to target cells. Phytoestrogens could accelerate estrogen metabolic clearance because they inhibit steroid binding to circulating SBP, and SBP could carry phytoestrogens into target cells, where they may compete with endogenous estrogens for receptor sites and thus interfere with estrogen-mediated process [78,79]. It has been suggested that antiestrogenic activity of phytoestrogen was related to SBP [80]. The interaction between human SBP, and the lignans, nordihydrogaiaretic acid (NDGA), ENL, END and the isoflavonoids equol, genistein were studied by Martin et al. [80]. The phytoes-

trogens had different dose-dependent inhibitory effects on steroid binding by SBP. Their relative efficiencies were: ENL≥NDGA=equol>genistein for displacing E2 and equol>ENL>NDGA> genistein for displacing testosterone. END was much less active.

α-Fetoprotein (AFP) is believed to modulate the activity of estrogens [81–84] or fatty acids [84], to be an immunomodulator [85,86] and to regulate the growth of estrogen-sensitive cells [84–87]. Therefore, some effects of phytoestrogens appear to resemble those of AFP. Garreau et al. [88] examined the interaction of END and ENL with purified rat and human AFPs, to clarify the actions of these compounds in general and the function of AFP in particular. The competitive binding of its more usual ligands, estrogen for rat AFP and arachidonic acid for human AFP. They had differential inhibitory effects on the binding of estrone and E2 to rat AFP and the binding of arachidonic acid to both rat and human AFP. The inhibition was dose-dependent. The apparent dissociation constants (K_d) for ENL and END binding to AFP were: $1.7\pm0.4\cdot10^{-5}$ *M* and $1.7\pm0.4\cdot10^{-5}$ *M*, respectively. These finding indicate that END and ENL could play a role in AFP-dependent normal and pathological growth and development.

7.3. Inhibition of some enzymes

The two mammalian lignans, especially ENL, are inhibitors of several steroid metabolizing enzymes, such as aromatase, 5α-reductase, 7α-hydroxylase and 17β-hydroxysteroid dehydrogenase.

Earlier studies have shown that some flavones, flavanones, isoflavones, isoflavanones and α-naphthoflavones inhibit human estrogen synthetase (aromatase) [89–91], a cytochrome P-450 enzyme that catalyzes the conversion of androgens to estrogens in many tissues [92]. The aromatase in breast tissue and breast cancer may play some role in the development of the disease and in the response to treatment. Adlercreutz et al. [93] demonstrated that ENL is a moderate inhibitor of placental aromatase, while END is a somewhat weaker aromatase inhibitor, and that ENL has the same effect on a human choriocarcinoma cell line JEG-3. Consequently, Wang et al. [94] reported END (weak) and ENL (moderate) are inhibitors of aromatase enzyme activity in a preadipose cell culture system, which agreed with the observation of Adlercreutz et al. [93].

Evans et al. [95] described the inhibition of 5α-reductase by two lignans and six isoflavonoids in human genital skin fibroblast monolayers, and in benign prostatic hyperplasia tissue homogenates. In genital skin fibroblast monolayers, ENL was the most potent inhibitor. When prostate tissue homogenates were used, the compounds tested were better inhibitors of 5α-reductase isozymes 1 than 2. In addition, all of the compounds tested inhibited 17β-hydroxysteroid dehydrogenase activity in genital skin fibroblast monolayers.

Cholesterol 7α-hydroxylase is the rate-limiting enzyme in the formation of primary bile acids. Sanghvi et al. [96] demonstrated that END and ENL exhibit significant inhibitory properties against cholesterol 7α-hydroxylase activity in vitro. The authors speculated that lignans may provide some protection against colonic cancer, based on the correlation between colorectal cancer risk and fecal levels of the secondary bile acid, deoxycholic acid. Thus, inhibition of cholesterol 7α-hydroxylase by lignans would decrease primary bile acids and in turn, prevent accumulation of deoxycholic acid in the colon.

7.4. Antioxidant activity

Phytoestrogens have received considerable attention as potential cancer-preventing agents via their antiestrogenic activity [97,98]. However, there are also indications that several representatives of these groups may act via antioxidant mechanisms, especially in cells not expressing estrogen receptors [99,100]. The antioxidant activities of the flaxseed lignan SDG and its mammalian lignan metabolites, END and ENL, were evaluated in both lipid and aqueous in vitro model systems [101]. All three lignans significantly ($P\leq0.05$) inhibited the linoleic acid peroxidation at both 10 and 100 μ*M* over a 24–48 h period of incubation at 40 °C. In a deoxyribose assay, which evaluates the non site-specific and site-specific Fenton reactant-induced ·OH scavenging activity, SDG demonstrated the weakest activity compared to END and ENL at both 10 and 100 μ*M*; the greatest ·OH scavenging for END and

ENL was observed at 100 μ*M* in both assays. In addition, the incubation of pBR322 plasmid DNA with Fenton reagents together with SDG, ENL or END showed that they inhibited the DNA scissions. The efficacy of SDG and particularly the mammalian lignans END and ENL in acting as antioxidants in lipid and aqueous in vitro model systems, at relatively low concentrations, potentially achievable in vivo, is evidence of a potential anticarcinogenic mechanism of these compounds. Using chemiluminescence of zymosan-activated polymorphonuclear leukocytes, Prasad [102] investigated the antioxidant activity of SECO, END and ENL, utilizing two other antioxidants, SDG and vitamin E, for comparison. The antioxidant potencies of END, ENL and SDG were 4.86, 5.02, 4.35 and 1.27, respectively, as compared to vitamin E. SECO, END and ENL are, respectively, 3.82, 3.95 and 3.43 times more potent than SDG. Recently, Pool-Zobel et al. [103] pointed out that END and ENL were not effective in preventing H_2O_2-induced DNA damage in HT 29 cells and ENL did not reduce H_2O-induced intracellular oxidative stress determined by confocal laser scanning microscopy. However, they reduced the endogenous generation of oxidized DNA bases, indicating that these compounds do have some protective potential in colon cells.

7.5. Endogenous digitalis-like activity

Fagoo et al. [104] demonstrated that ENL can displace ^{3}H-ouabain from its binding sites on cardiac digitalis receptor, and inhibit dose dependently, the Na^+, K^+-ATPase activity of human and guinea-pig heart. These authors speculated that the γ-butyrolactone of ENL is common to ascorbic acid and cardiac glycosides, agents which also inhibit Na^+, K^+-ATPase activity. Furthermore, they suggested that the existence of lignans in mammals may account for the putative digitalis-like activity found in their tissues and fluids. In addition, Braquet and co-workers [105,106] found ENL inhibited Na^+, K^+-pump activity in human red cells. The inhibition of the Na^+, K^+-pump was obtained at doses 2–3 orders of magnitude higher than those required for ouabain. However, the authors cannot exclude that a glycosyl-(and/or butenolide)-derivative of ENL could be one endogenous ouabain-like factor. Later, Hirano et al. [107] further clarified the endogenous digitalis-like properties of END and ENL as determined by cross-reactivity with antidigoxin antibodies, Na^+, K^+-ATPase inhibition, and erythrocyte–ouabain binding inhibition.

8. Health effects

END and ENL are excreted in a cyclical pattern during the menstrual cycle and in higher amounts in urine during early pregnancy [5,24,27]. Phipps et al. [108] reported that daily flaxseed supplementation (10 g/day) could increase the luteal phase duration but had no effect on the follicular phase length. The effects of flaxseed ingestion on the menstrual cycle demonstrated a role for lignans in the relationship between diet and sex steroid action and possibly between diet and hormonally dependent cancer.

In postmenopausal women, Hutchins et al. [109] also found that consumption of flaxseed, in addition to their habitual diet increased excretion of END and ENL in a dose-dependent manner. Dietary studies and assays of urinary lignans in postmenopausal women showed that lignan excretion is significantly lower in urine of women with breast cancer than in normal omnivorous and vegetarian women [110]. Postmenopausal Japanese women, who eat large quantities of phytoestrogens as part of their normal diet, reportedly have fewer complaints of hot flushes, night sweats and other menopausal symptoms compared with women in other countries [65].

There is considerable evidence from epidemiological studies correlating high concentrations of lignans in body fluids with a low incidence of hormone-dependent tumors, in particular breast cancer [76,97,111]. The high concentration of phytoestrogens in the urine of Japanese men could be protective with regard to prostate cancer. Mammalian lignans have estrogenic effects in numerous biological systems and may, because of this property, inhibit development of prostatic cancer. Metzler et al. [112] recently investigated the genotoxicity of END and ENL at various endpoints in vitro. The results suggest that END and ENL are devoid of any genotoxicity under the experimental conditions.

8.1. Breast cancer

Breast cancer is the commonest cancer of women living in Western populations, and the incidence is rising in the UK [113]. The risk of breast cancer increases markedly with age, but its development is highly dependent on the hormones associated with ovarian function [114]. Although the rate of increase begins to slow after the menopause, events that occur premenopausally, and perhaps during adolescence, set the scene for later postmenopausal breast cancer. The evidence that diet has a role in the development of breast cancer initially came from population and migration studies [115,116], the subsequent cohort and case–control studies in human beings [117,118], and from animal experiments [119–121]. Early studies by Adlercreutz et al. [110] have shown that ENL excretion is lower in women who have breast cancer than in omnivorous and vegetarian women with no history or breast cancer. The authors speculated that the low rate of breast cancer in some women may be in part due to the presence of lignan precursors in their fibre-rich diets. It is possible that the lignans can serve in some antiestrogenic or antiproliferative capacity to suppress the growth of human malignancies, which has been confirmed by its antiproliferative effects on human breast cancer cell lines in vitro [69,70,122].

Ingram et al. [118] measured urinary isoflavone and lignan excretion in women newly diagnosed with breast cancer, and community controls. After adjustment for age at menarche, parity, alcohol intake, and total fat intake, a high excretion of both equol and ENL was associated with a substantial reduction in breast cancer risk, with significant trends through the quartiles. This is the first properly controlled prospective study to demonstrate that high excretion of some phytoestrogens is associated with a substantial reduction in breast cancer risk.

The high-lignan-producing diet i.e. flaxseed, is considered to be able to reduce the risk of breast cancer, although clear evidence cannot be obtained because other components in flaxseed may also exert some effect on tumorigenesis. A flaxseed-supplemented diet was considered appropriate to investigate the cancer-protective effect of a high-mammalian lignan-producing diet because of the exceptionally high level of plant lignans in flaxseed. A short term study by Serraino and Thompson [123] showed that flaxseed supplementation in a basal high-fat diet reduced the epithelial cell proliferation and nuclear aberration in the female rat mammary gland, suggestive of a protective effect for flaxseed at the initial stage of carcinogenesis. Subsequently, a long-term mammary tumorigenesis study using the dimethylbenzanthracene rat model, one of the most widely used models to study mammary tumorigenesis, confirmed the above findings [124]. Likewise, dietary treatment with 5% flaxseed or 1.5 mg/day SDG during early [124–126] and late [127] promotion stages of mammary carcinogenesis can inhibit tumor growth (size, number or multiplicity) in rats.

Insulin-like growth factor-I (IGF-I, 70 amino acids, $M_w = 7649$ g/mol) is a polypeptide synthesized mainly in the liver under the control of growth hormone [128] and is important for the development of terminal end buds in the mammary gland [129]. Many studies have shown an increased breast cancer risk in women with high levels of IGF-I [130–132]. Recently, the effect of flaxseed (5%) or SDG (1.5 mg/day) supplementation on plasma IGF-I levels was examined in rats treated with or without the carcinogen, *N*-methyl-*N*-nitrosourea (MNU) [133]. In MNU-free rats, flaxseed and SDG reduced plasma IGF-I levels, which were inversely related to urinary lignan excretion of END, ENL and SECO. In MNU-treated rats, only flaxseed significantly reduced plasma IGF-I concentrations. Similar to observations in MNU-free animals, there was a strong trend towards an inverse relationship between urinary lignan levels and plasma IGF-I in MNU-treatment rats. The similar effects seen with flaxseed and its equivalent dose of SDG on plasma IGF-I in MNU-free and MNU-treatment experiments, suggesting that the urinary lignans are largely responsible for the effects observed.

It is still early, however, to make firm recommendations regarding phytoestrogen intake and breast cancer prevention. The inclusion of one serving of soy and/or flaxseed per day in an otherwise diverse diet that has a high content of vegetable, fruit and grains would appear to have multiple positive heath benefits for younger women. In contrast, increasing circulation isoflavone or lignan levels in postmenopausal women, who are estrogen deficient, to levels known to stimulate

breast cancer cells in vitro may be potentially dangerous. Till now, no evidence is available that phytoestrogen supplementation in tablet form is protective against breast cancer or even safe, however, women with breast cancer are strongly advised to optimize their nutrient intake, nonsaturated fat protein sources such as fish and soy should be encouraged.

8.2. Prostate cancer

Prostate cancer is the most common hormone-related cancer in men, and an increasing incidence has been rising rapidly in western countries. A high consumption of beans, lentils and peas, tomatoes, and dried fruits is associated with a significantly decreased prostate cancer risk in Seventh Day Adventist men [134]. A prospective study of Japanese men living in Hawaii has shown a decreased prostate cancer risk in those who consume rice and tofu [135]. This is supportive of the hypothesis that environmental change, including diet, can impact cancer risk, even in later life [117].

Lignans (especially ENL), isoflavonoids and flavonoids are natural estrogenic compounds derived from soya, tea, fruits and vegetables and they have been proposed as chemopreventive agents in Asian men, in whom the incidence of prostate cancer is much lower than in men from the West. Of the population groups studied, Portuguese men have higher levels of ENL (162 ng/ml) in prostatic fluid compared with British men (20.3 ng/ml) and Hong Kong men (31.0 ng/ml) [136]. Even in some of the men from Portugal, very high levels of ENL ($>$600 ng/ml) were observed in the prostatic fluid. However, the mean plasma concentrations of ENL from the three centers were similar, at 6.2, 3.9 and 3.9 ng/ml in samples from Hong Kong, Portugal and Britain, respectively. The incidence of prostate cancer in Portugal is higher than that in Hong Kong and China, but about half that of British. Whether the high levels of ENL in prostatic fluid are associated with the high incidence of prostate cancer or not, is another interesting problem needing to be clarified. Recently, twenty-five patients with prostate cancer who were awaiting prostatectomy were instructed on a low-fat (20% of kilocalories or less), flaxseed-supplemented (30 g/day) diet [137]. Significantly lower proliferation rates and significantly higher rates of apotoric cell death were observed in the pilot study with a short-term (average duration: 34 days) dietary intervention, which suggest that a flaxseed-supplemented, low-fat diet may have an effect on prostate cancer biology possibly due to the effect of ENL.

8.3. Coronary heart disease (CHD)

CHD is a multifactorial disease, for which the main established risk factors are raised serum cholesterol, raised blood pressure and smoking. CHD accounts for 23% of deaths in women in the UK, and 30% of deaths in men, although rates have been falling since the late 1970s in the UK. Rates are low in Far East countries, such as Japan, and are also declining [138].

Lignans including ENL have been postulated to have a role in protection against CHD [10]. Although ENL may be simply a biomarker of healthy fibre-rich food, there are mechanisms through which ENL itself could actively protect against cardiovascular disease. Vanharanta et al. [139] reported a lower risk of acute coronary events in middle-aged men with high serum concentrations of the lignan ENL, when compared with those with lower serum ENL concentrations. This reduced risk observed in univariate analysis was also noted in multivariate analysis, after adjustment for nine predictive risk factors for CHD.

9. Conclusion

The phytoestrogens, END and especially ENL, would seem potentially to have wide-ranging effects on hormonal-related conditions such as breast and prostate cancer, cardiovascular disease and conditions associated with the menopause. Since they are transformed by the intestinal microorganisms in human body, the apparent health effects are attributed to digesting foods containing the precursors of the mammalian lignans. Nevertheless, there are still many unanswered questions about their potency, possible toxicity, and the role or mechanism of action of these compounds in the modulation of various diseases. Further studies are needed to determine the bioavailability of these compounds,

their metabolism and relative stability in vivo, as well as the dosage needed to achieve the desired biological effects.

10. Nomenclature

AFP	α-fetoprotein
CHD	coronary heart disease
DELFIA	dissociation-enhanced lanthanide fluoroimmunoassay
E2	estradiol
EIA	enzyme immunoassay
END	enterodiol
ENL	enterolactone
ER	estrogen receptor
FIA	fluoroimmunoassay
GC	gas chromatography
GC–IMS	gas chromatography–ion mobility spectrometry
GC–MS	gas chromatography–mass spectrometry
HPLC	high-performance liquid chromatography
HPLC–MS	high-performance liquid chromatography–mass spectrometry
ID-GC–MS	isotope dilution-gas chromatography–mass spectrometry
IGF	insulin-like growth factor
IMS	ion mobility spectrometry
MAT	matairesinol
MNU	*N*-methyl-*N*-nitrosourea
NDGA	nordihydrogaiaretic acid
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
RIA	radioimmunoassay
SBP	sex steroid binding protein
SDG	secoisolariciresinol diglucoside
SECO	secoisolariciresinol
SHBG	Sex hormone binding globulin
TR-FIA	time-resolved fluoroimmunoassay.

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