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Journal of Chromatography B, 777 (2002) 321–327

JOURNAL OF CHROMATOGRAPHY B

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Oxidative metabolites and genotoxic potential of mammalian and plant lignans in vitro

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Abstract

Certain plant lignans, e.g. secoisolariciresinol and matairesinol, are converted by the intestinal microflora to the mammalian lignans enterodiol and enterolactone, which are associated with beneficial health effects in humans. The metabolism of both mammalian and plant lignans in animals and humans is poorly understood, and most studies so far have focused on the conjugation of these diphenolic compounds. However, recent studies have demonstrated that mammalian and plant lignans are good substrates for cytochrome P450-mediated reactions, leading to numerous products of aliphatic and aromatic hydroxylation with microsomes in vitro. The current knowledge of the oxidative metabolism of food-related lignans is briefly reviewed in this paper, including published as well as unpublished data from our laboratory. Moreover, data on the genotoxic potential of the mammalian and plant lignans, determined at various endpoints in cultured mammalian cells, are included in this review.

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Keywords: Lignans

1. Introduction

The mammalian lignans enterodiol (END, Fig. 1) and enterolactone (ENL) are believed to protect against certain diseases common in Western industrialized societies, e.g. breast and prostate cancer as well as coronary heart disease [1–3]. END and ENL are known to be formed from various plant lignans (Fig. 1), e.g. secoisolariciresinol (SEC), matairesinol (MAT), pinoresinol (PIN) and lariciresinol (LAR) by human fecal microflora [4]. Such plant lignans occur in a variety of food items, i.e. fruits, legumes, and oilseeds [5]. When various foods were incubated with human fecal microbiota and the production of END and ENL measured, the highest yields of mammalian lignans were obtained with flaxseed [6]. Flaxseed has been shown to contain high concentrations of SEC, together with lesser amounts of PIN and MAT [7]. Accordingly, END and ENL together with their precursors SEC and MAT have been observed in the plasma of human subjects after the ingestion of flaxseed [8].

Once absorbed from the intestine, the mammalian and plant lignans are conjugated with glucuronic acid and sulfate which facilitates their clearance in the kidney and liver [9]. However, information on the metabolic fate of these important food-related lignans is poor. In particular, the question of oxidative metabolism has only recently been addressed [10–12], Hydroxylated metabolites, if formed, may be of endocrinological or toxicological importance. Jacobs and Metzler [10] first demonstrated that END and ENL are good substrates for cytochrome P450, as

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PII: S1570-0232(02)00276-3

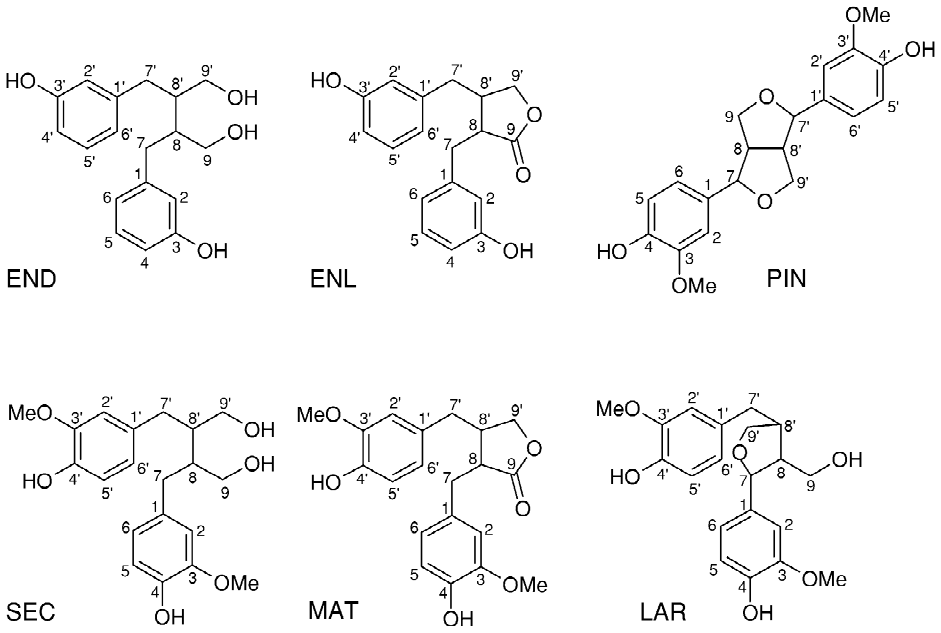

Fig. 1. Chemical structures of mammalian lignans (END, ENL) and plant lignans (SEC, MAT, LAR, PIN).

hepatic microsomes from various species including humans gave rise to a variety of aromatic and aliphatic hydroxylation products of END and ENL in vitro. Small amounts of oxidative metabolites of END and ENL were subsequently also demonstrated in the urine of humans kept on a flaxseed diet [11] and in the bile and urine of rats dosed with the mammalian lignans or fed flaxseed [12]. The present paper briefly reviews the oxidative metabolism of END and ENL and presents yet unpublished results on the microsomal metabolism of the plant lignans SEC and MAT. Moreover, in vitro studies on the potential of these four lignans to cause genetic damage are reviewed.

2. Methodology

The methods used for the generation, separation, and identification of lignan metabolites were described in detail in recent publications [10–12]. Briefly, lignans were incubated with microsomes prepared from the livers of aroclor 1254-treated male Wistar rats or from untreated rats, pigs or human liver. The microsomal metabolites were extracted with ethylacetate and analyzed by HPLC with a diode array detector (DAD) and by HPLC–MS using a TSQ-700 triple mass spectrometer equipped with an atmospheric pressure ionization-electrospray ionization chamber. GC–MS was carried out on a Finnigan GCQ system connected to an ion trap mass detector. Urine samples of volunteers kept on a flaxseed diet were solid-phase-extracted and the extracts hydrolyzed with β-glucuronidase/aryl sulfatase. Deconjugated lignans and their oxidative metabolites were purified by solid-phase extraction and analyzed as described above for microsomal metabolites. The same methodology was used to analyze the biliary metabolites of lignans present in the bile of female Wistar rats after intraduodenal administration of END, ENL, SEC and MAT [12]. For genotoxicity testing in our laboratory, V79 Chinese hamster lung fibroblasts were cultured and various endpoints studied as reported in detail previously [13].

3. Metabolism of mammalian and plant lignans

3.1. Oxidative metabolism of enterodiol and enterolactone

Incubation of END with hepatic microsomes from aroclor 1254-induced male Wistar rats yielded seven

metabolites, which were identified by GC–MS and HPLC–MS as monohydroxylation products of END [10]. Two major and two minor END metabolites were formed through aliphatic hydroxylation; their exact structures, i.e. the position of the hydroxyl group, could not yet be determined due to the lack of authentic reference compounds. Another three minor metabolites of END were formed by aromatic hydroxylation; comparison by GC–MS with synthetic reference compounds led to the unambiguous identification of the structures depicted in Fig. 2. Hepatic microsomes from untreated rats, pigs and humans gave rise to the same two major metabolites as observed with induced rat liver microsomes, although at lower yields. None of the minor metabolites was detected with microsomes from uninduced animals [10].

When ENL was incubated with aroclor-induced rat liver microsomes, six major and six minor metabolites were separated by GC–MS and HPLC–MS and identified as products of aliphatic and aromatic monohydroxylation. Three of the six major metabolites represented hydroquinones and catechols, arising from aromatic hydroxylation and unequivocally identified by means of synthetic reference compounds (Fig. 3), whereas the other three major metabolites were products of aliphatic hydroxylation and could not be definitively identified. Likewise, the minor metabolites comprised three aliphatic and three aromatic hydroxylation products. With microsomes from uninduced rat, pig, and human liver, aliphatic hydroxylation of ENL was more pronounced than aromatic hydroxylation [10].

When the urine of female and male volunteers who had ingested flaxseed for 5 days was analyzed for the presence of oxidative metabolites of END and ENL, only trace amounts of the three aromatic hydroxylation products of END and six of ENL were found [11]. Interestingly, none of the aliphatic hydroxylation products of END and ENL, which constitute major microsomal metabolites, could be detected in the human urine. In contrast, when END or ENL or flaxseed was administered to intact rats, small amounts of both aromatic and aliphatic hydroxylation products of END and ENL were detected in their urine [12]. Likewise, both types of hydroxylation products of the mammalian lignans could be found in the bile of female Wistar rats dosed with END or ENL [12].

3.2. Oxidative metabolism of secoisolariciresinol and matairesinol

When SEC was incubated with hepatic microsomes from aroclor-induced and non-induced male Wistar rats, the formation of seven oxidative metabo-

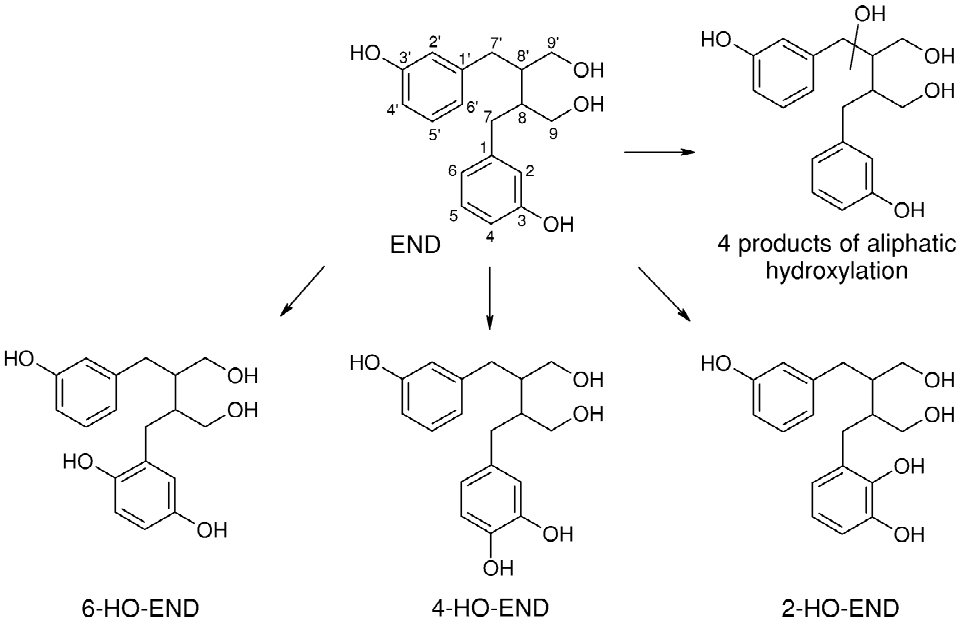

Fig. 2. Oxidative pathways in the metabolism of END.

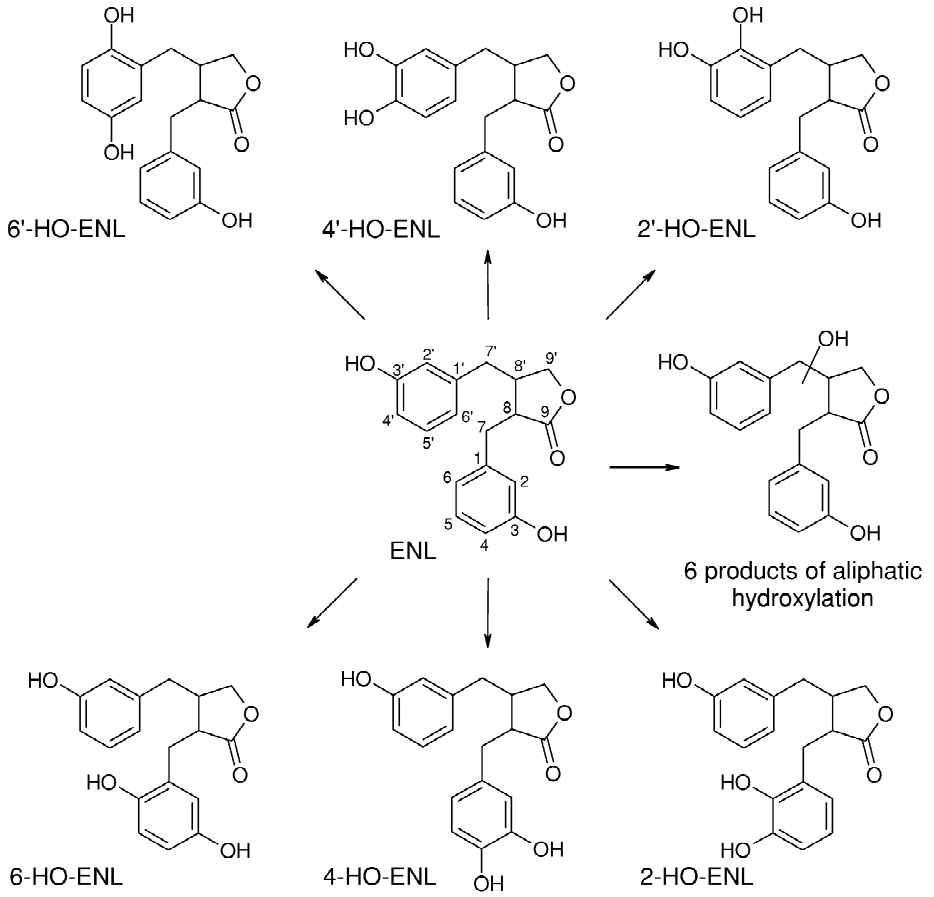

Fig. 3. Oxidative pathways in the metabolism of ENL.

lites was demonstrated by GC–MS analysis. Major products were LAR together with a metabolite tentatively identified as 7-hydroxy-SEC (Niemeyer et al., unpublished). Small amounts of three aromatic hydroxylation products and isolariciresinol (ISL) were also detected (Fig. 4). Human microsomes were able to metabolize SEC to LAR and traces of ISL.

Incubation of MAT with rat liver microsomes gave rise to ten metabolites according to GC–MS analysis (Niemeyer et al., unpublished). Major products were 7′-hydroxy-MAT, a metabolite with opened lactone ring, and demethylated MAT (Fig. 5). Very small amounts of several aromatic hydroxylation products were also detected. Surprisingly, incubation of MAT with human hepatic microsomes did not yield oxidative metabolites to any appreciable extent.

4. Genotoxic potential of mammalian and plant lignans

Because several other phytoestrogens, e.g. genistein and coumestrol, have recently been reported to display genotoxic activity in vitro [14–18], we have studied the effects of END, ENL, SEC and MAT at certain endpoints for genotoxicity in cultured Chinese hamster V79 cells [13]. The results are summarized in Table 1. Neither the mammalian nor the plant lignans induced micronuclei or hprt mutations, or affected the microtubule system in cultured cells

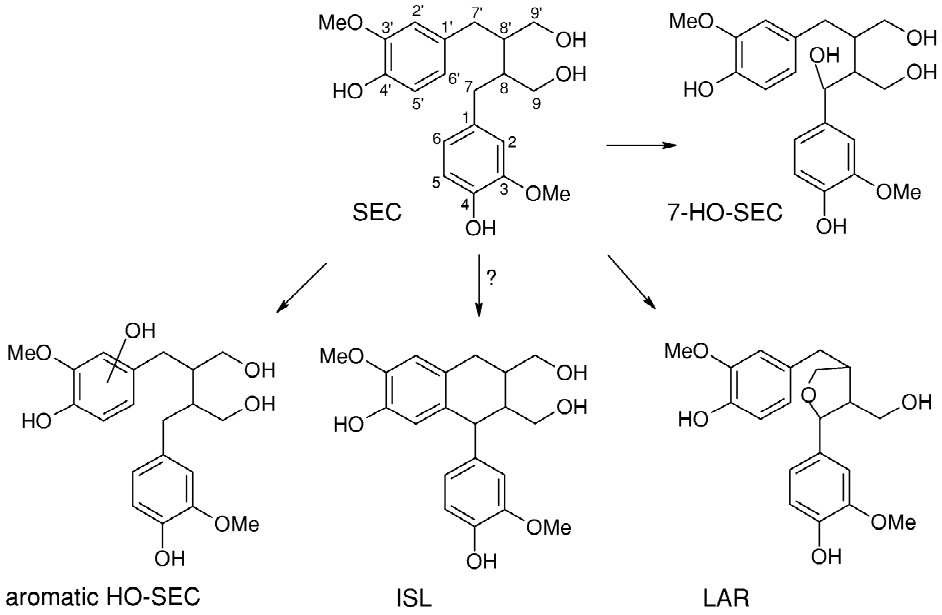

Fig. 4. Oxidative pathways in the metabolism of SEC.

or under cell-free conditions. In this respect, the lignans resembled the isoflavone daidzein and differed from genistein and coumestrol, which exhibited clastogenic and gene mutagenic activity in V79 cells [14,15].

5. Conclusion

The studies reviewed here clearly demonstrate that END, ENL, SEC and MAT are good substrates for cytochrome P450-mediated hydroxylation reactions when incubated with microsomes in vitro. Hydroxylation takes place at aliphatic and aromatic positions of the parent lignan molecule. For END and ENL, microsomes from non-induced rat, pig and human liver favored aliphatic over aromatic hydroxylation, whereas induction of rat liver microsomes with aroclor lead to an increased formation of aromatic hydroxylation products [10]. SEC and MAT were preferentially hydroxylated at the aliphatic moiety by both induced and non-induced rat liver microsomes.

Several questions must now be asked concerning the biological significance of oxidative lignan metabolism. Firstly, it is important to know whether hydroxylation products of lignans are formed under in vivo conditions. Lignans already contain two hydroxy groups and can therefore become conjugated without further hydroxylation. Searches for hydroxylated END and ENL in the urine of humans fed a flaxseed diet have revealed the excretion of small amounts of aromatic but not aliphatic hydroxylation products [11], whereas 7-hydroxy-ENL and 7-hydroxy-MAT were tentatively identified in the urine of humans after ingestion of flaxseed [1]. The reason for this discrepancy is presently unclear. Both types of oxidative metabolites were detected in the bile and urine of rats dosed with END and ENL [12]. It was estimated that the total amount of oxidative metabolites was less than 3% of the dosed lignans. Further studies are needed to better assess the role of oxidative lignan metabolites in vivo.

Another interesting aspect of the oxidative metabolism of lignans is the formation of biologically active products. The addition of a hydroxyl group to the diphenolic structure of the parent lignan may decrease or increase the estrogenic activity or confer genotoxic potential. Whereas the parent lignans END, ENL, SEC and MAT were devoid of genotoxicity in vitro (Table 1), nothing is known to date about the genotoxic potential of hydroxylated lignans. In particular, the products of aromatic hydroxy-

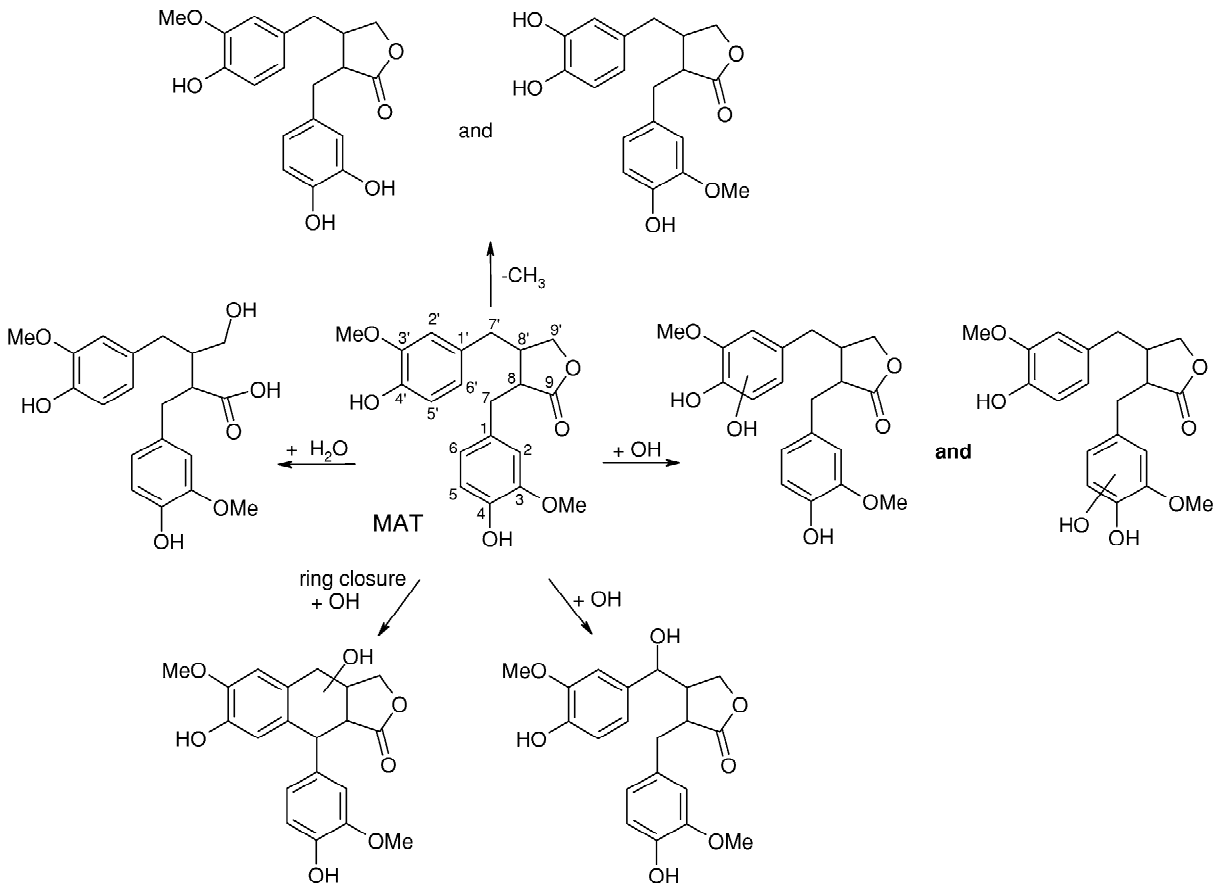

Fig. 5. Oxidative pathways in the metabolism of MAT.

Table 1
Summary of the genotoxic effects of various isoflavone, coumestane and lignan phytoestrogens in cultured V79 cells at different endpoints in vitro

Endpoint	GEN	DAI	COM	END	ENL	SEC	MAT
Induction of micronuclei							
CREST-positive	–	–	–	–	–	–	–
CREST-negative	+++	–	++	–	–	–	–
Interference with MT							
Disruption of CMTC	–	–	–	–	–	–	–
Mitotic spindle	–	–	–	–	–	–	–
Cell-free MT assembly	–	–	–	–	–	–	–
Induction of mitotic arrest	–	–	–	–	–	–	–
Mutations at the hprt locus	(+)	–	++	–	–	–	–

GEN, genistein; DAI, daidzein; COM, coumestrol; MT, microtubule; CMTC, cytoplasmic microtubule complex;. END, ENL, SEC and MAT were tested up to their limit of solubility (100 μM).

lation, which are hydroquinones and catechols, may be genotoxic, but hydroxylation at the benzylic position may also provide a metabolite, e.g. 7-hydroxy-ENL, for further activation to a genotoxin, e.g. by conjugation with sulfate in analogy to the situation with tamoxifen [19]. These possibilities should be explored, as should be the enzymology of the oxidative biotransformation, i.e. which forms of cytochrome P450 are involved in the formation of the various metabolites. Only if such data are known is it possible to obtain a full appreciation of the fate and possible health effects of these important phytoestrogens in humans.

6. Nomenclature

DAD	diode array detector
END	enterodiol
ENL	enterolactone
GC	gas chromatography
HPLC	high-performance liquid chromatography
HO	hydroxy
hprt	hypoxanthin phosphoribosyl transferase
ISL	isolariciresinol
LAR	lariciresinol
MAT	matairesinol
MS	mass spectrometry
PIN	pinoresinol

Acknowledgements

Studies conducted in our laboratory were supported by the Deutsche Forschungsgemeinschaft Me 574/9-2). The skillful and dedicated work of Cornelia Hodapp, Doris Honig, Renate Loske and Sybille Mayer is gratefully acknowledged.

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