1.25
Lignans: Biosynthesis and Function

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1.25.1 INTRODUCTION

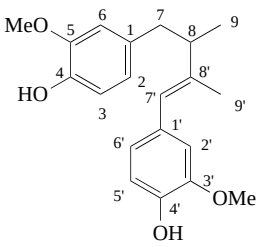
The lignans are a very common, structurally diverse, group of plant natural products of phenylpropanoid origin.[1] They display important physiological functions *in planta*, particularly in plant defense,[2–5] and are most efficacious in human nutrition and medicine, given their extensive health protective and curative properties.[6–9]

This chapter primarily describes the intricate biochemical pathways established in lignan biosynthesis, particularly as regards phenylpropanoid coupling. It must be emphasized at the outset, however, that the biochemical outcome of enzymatic coupling was previously widely held to result only in formation of randomly linked racemic products. This hypothesis, originally adopted for lignin biopolymer formation (but see Chapter 3.18), could not explain the observed optical activities of the vast majority of naturally occurring lignans (discussed in Section 1.25.5). In contrast, research has established that most, if not all, phenylpropanoid coupling reactions catalyzed by purified plant proteins and enzymes *in vitro* can be either regio- or stereoselectively controlled, as are their subsequent stereospecific metabolic conversions. This contribution therefore attempts to summarize the progress made in this area, as well as in providing an assessment of the rapidly growing importance of lignans in plant growth, development, and survival, and in human usage.

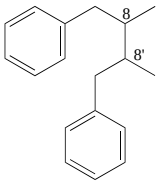
1.25.2 DEFINITION AND NOMENCLATURE

Around the turn of the nineteenth century, a number of plant phenolic substances from various species were isolated and given trivial names, prior to their chemical structures being determined. One of these, guaiaretic acid, from guaiacum resin obtained from *Guaiacum officinale* heartwood, was later shown to contain the skeletal formula (**1**) by Schroeter *et al.*[10] It was subsequently proposed[11] that it and related compounds represented a unique class of dimeric phenylpropanoid substances linked exclusively through 8—8′ bonds, e.g., (**2**). In order to provide a system for their classification, Haworth[11] introduced the term "lignane" (later shortened to lignan) to define these substances; they were considered to result from regiospecifically linking two "cinnamyl" (C_6C_3) molecules to give compounds such as guaiaretic acid (**1**) and pinoresinols (**3a,b**). This initial classification, unfortunately, failed to account for either other dimeric lignan skeletal types that were

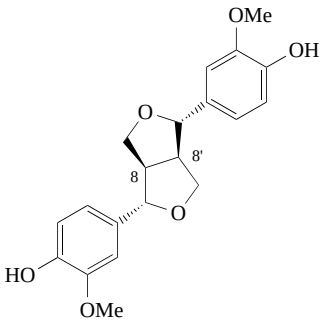
present in many plant species and tissues or much larger molecules (oligomeric lignans) that could also exist. A derivative term, neolignan, was introduced to account for the other coupling modes, e.g., megaphone (**4**) (8—1′ linked), dehydrodiconiferyl alcohols (**5a,b**) (8—5′ linked) and *erythro*/*threo* guaiacylglycerol 8—*O*—4′ coniferyl alcohol ethers (**6a,b**) (8—*O*—4′ linked),[12–14] but this was later modified to encompass only presumed allylphenol-derived coupling products, such as the lignans



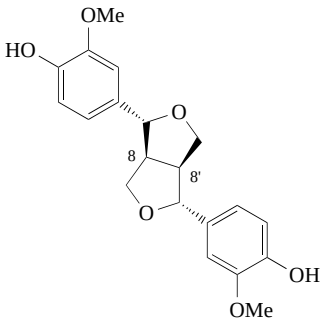
(1) Guaiaretic acid
$[\alpha]_D = -94^\circ$
(Guaiacum officinale)



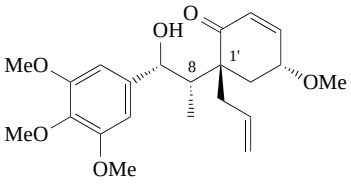
(2)



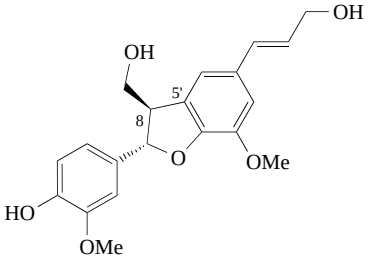
(3a) (+)-Pinoresinol
$[\alpha]_D^{22} = +61.6^\circ$ ($CHCl_3$)
(Forsythia europaea)



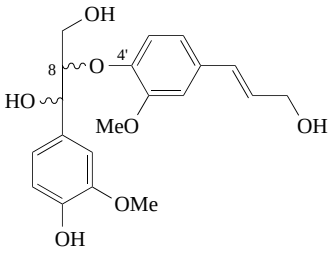
(3b) (–)-Pinoresinol
$[\alpha]_D^{25} = -34.7^\circ$ ($CHCl_3$, *c* = 0.91)
(Daphne tangutica)



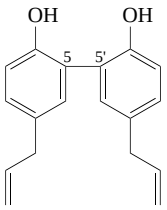
(4) (–)-Megaphone
$[\alpha]_D^{27} = -23.0^\circ$ (EtOH, *c* = 0.15)
(Aniba megaphylla)



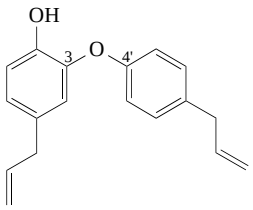
(5a,b) (±)-Dehydrodiconiferyl alcohols



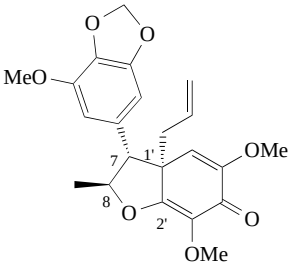
(**6a**,**b**) (±)-*erythro*/*threo* Guaiacylglycerol 8–*O*–4′ coniferyl alcohol ethers



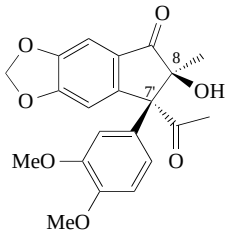
(7) Magnolol
(*Magnolia virginiana)*



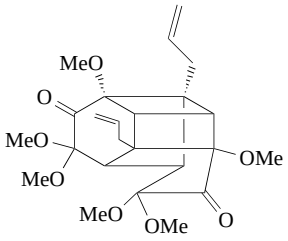
(8) Isomagnolol
(Sassafras randaiense)



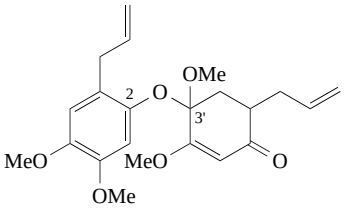
(9) Chrysophyllon 1A
(Licaria chrysophylla)



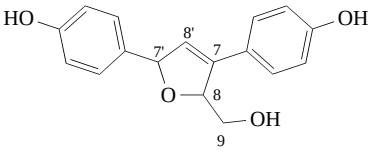
(10)
(Virola sebifera)



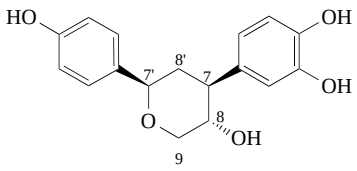
(11) Isoasatone
$[\alpha]_D^{20} = 0^\circ$
(Asarum taitonense)



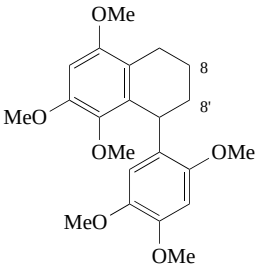
(12) Lancilin
(Aniba lancifolia)



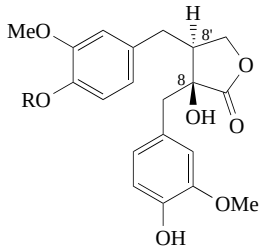
(13) (–)-Cryptoresinol
$[\alpha]_D^{25} = -170.4^\circ$ (MeOH, *c* = 1.25)
(Cryptomeria japonica)



(14) (–)-Hydroxysugiresinol
$[\alpha]_D^{25} = -19^\circ$ (EtOH, c 1)
(Sequoia sempervirens)



(15) Pachypostaudin A
$[\alpha]_D^{24} = 0^\circ$ ($CHCl_3$, $c = 0.8$)
(Pachypodanthium staudtii)



(16) (–)-Nortrachelogenin, R = H
$[\alpha]_D^{17} = -16.8^\circ$ (EtOH, $c = 0.178$)
(17) (–)-Trachelogenin, R = Me
$[\alpha]_D^{23} = -43.3^\circ$ (EtOH, $c = 0.25$)
(Trachelospermum asiaticum)

(**4**) and (**7**)–(**12**).[15] As a further complication, the term norlignan[16] was adopted to depict lignan-like metabolites which lacked either a carbon at the C-9 and/or C-9′ positions, e.g., cryptoresinol (**13**),[17] hydroxysugiresinol (**14**),[18] and pachypostaudin A (**15**),[19] or a methyl group on the aromatic methoxyl, e.g., nortrachelogenin (**16**)[20,21] versus trachelogenin (**17**).[20,22]

It is now well established that the products of phenylpropanoid coupling have a range of structural motifs[8,14,15,23,24] and molecular sizes,[25–30] rather than being restricted to 8—8′ linked moieties as previously contemplated. Moreover, since there appear to be only a relatively small number of distinct skeletal forms, the term lignan can be conveniently used to encompass all skeletal types, provided that the precise linkage type is stipulated, e.g., 8—8′, 8—1′, 8—5′, 8—*O*—4′, 5—5′, 3—*O*—4′, 7—1′, 8—7′, 1—5′, and 2—*O*—3′.

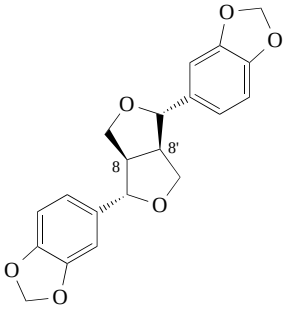
Of the various lignan types known, however, those that are 8—8′ linked appear to be the most widespread in nature, based on current chemotaxonomic data;[23] they can also be further subdivided into the substituted furofurans, tetrahydrofurans, dibenzylbutanes, dibenzylbutyrolactones, aryltetrahydronaphthalenes, arylnaphthalenes, dibenzocyclooctadienes, etc. (e.g., Structures (**18**)–(**25**)). Although not lignans proper, there are also related natural products of mixed metabolic origin, e.g., so-called flavonolignans[31–33] which are described in Section 1.25.11.

Finally, given that lignans can also exist in oligomeric form,[25–29] it is necessary at this point to introduce terminology to distinguish them from the corresponding lignin biopolymers. In this regard, since upper limits of molecular size have not been established for the oligomeric lignans, perhaps the easiest distinction lies with their physiological roles/functions: the lignin biopolymers have cell wall structural roles, whereas oligomeric lignans are nonstructural components (discussed in Section 1.25.13.6).

1.25.3 EVOLUTION OF THE LIGNAN PATHWAY

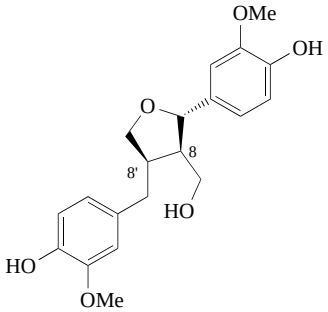
The evolutionary adaptation of plants to land was accompanied by massive elaboration of the phenylpropanoid pathway.[23] The salient features are summarized in Figure 1, and the reader is referred to Chapter 3.18 for a comprehensive description of each enzymatic step in the pathway. Nevertheless, assimilated carbon is directed to provide a variety of plant phenolics, such as the lignans and lignins, suberins and flavonoids, with the latter two metabolic classes being formed via merging both phenylpropanoid and acetate pathways. Accordingly, the transition of plants from an aquatic to a terrestrial environment was greatly facilitated via formation of lignans (protective/defense functions), lignins (structural support), suberins (formation of protective barriers preventing excessive water loss), and flavonoids (flower petal pigments, signaling molecules, UV-B screening, and so forth).[23] Together, these metabolites account for more than 30% of the carbon in vascular plants; however, note that lignans and lignins have never been convincingly demonstrated as present in aquatic plants, such as algae.

Furofuran

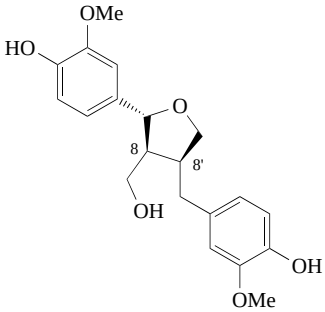


(18) (+)-Sesamin
$[\alpha]_D^{20}$= +68.36° ($CHCl_3$, c = 24.45)
(*Sesamum indicum*)

Tetrahydrofuran

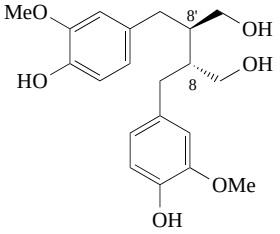


(19a) (+)-Lariciresinol
$[\alpha]_D^{25}$= +18° (Me_2CO, c = 1.0)
(*Araucaria angustifolia*)

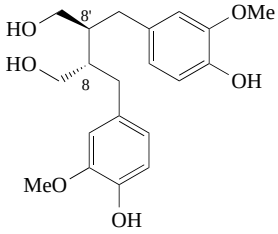


(19b) (–)-Lariciresinol
$[\alpha]_D^{25}$= –12.3° (Me_2CO, c = 0.94)
(*Daphne tangutica*)

Dibenzylbutane

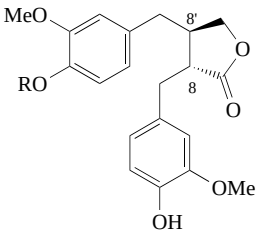


(20a) (–)-Secoisolariciresinol
$[\alpha]_D^{25}$= –35.6° (Me_2CO, c = 1.07)
(*Podocarpus spicatus*)

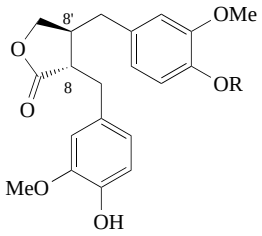


(20b) (+)-Secoisolariciresinol
(*Linum usitatissimum*)

Dibenzylbutyrolactone



(21a) (–)-Matairesinol, R = H
$[\alpha]_D^{18}$= –48.6° (Me_2CO, c = 2.409)
(*Podocarpus spicatus*)

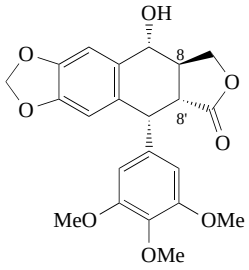


(21b) (+)-Matairesinol, R = H
$[\alpha]_D^{20}$= +37.0° (MeOH, c = 1.0)
(*Selaginella doederleinii*)

(22a) (–)-Arctigenin, R = Me
$[\alpha]_D^{23}$= –34.6° (EtOH)
(*Forsythia intermedia*)

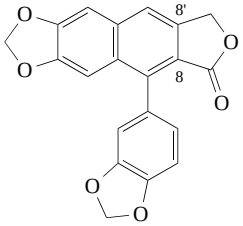
(22b) (+)-Arctigenin, R = Me
$[\alpha]_D^{23}$= +28.05° (EtOH, c = 1.23)
(*Wikstroemia indica*)

Aryltetrahydronaphthalene



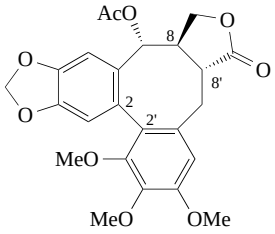
(23) (–)-Podophyllotoxin
$[\alpha]_D$ = –109° (EtOH)
(*Podophyllum hexandrum*)

Arylnaphthalene



(24) Justicidin E
(*Justicia procumbens*)

Dibenzocyclooctadiene



(25) (–)-Steganacin
$[\alpha]_D$ = –122.6° ($CHCl_3$, c = 1.02)
(*Steganotaenia araliacea*)

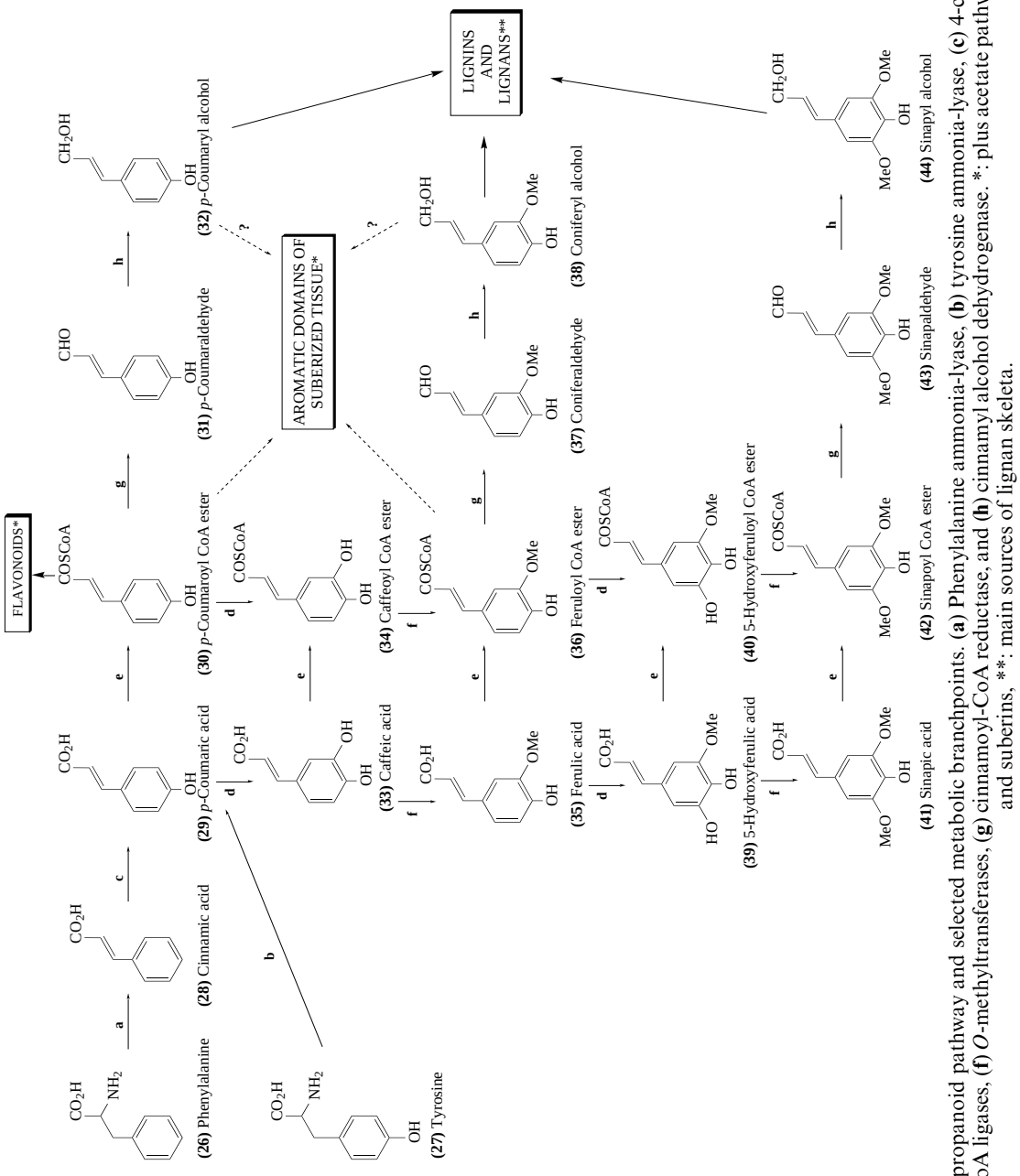


Figure 1 The phenylpropanoid pathway and selected metabolic branchpoints. (**a**) Phenylalanine ammonia-lyase, (**b**) tyrosine ammonia-lyase, (**c**) 4-cinnamate hydroxylase, (**d**) hydroxylases, (**e**) CoA ligases, (**f**) *O*-methyltransferases, (**g**) cinnamoyl-CoA reductase, and (**h**) cinnamyl alcohol dehydrogenase. *: plus acetate pathway for both flavonoids and suberins, **: main sources of lignan skeleta.

1.25.4 OCCURRENCE

Lignans are present in a large number of vascular plant species, ranging from ''primitive'' hornworts, liverworts, and ferns to the woody gymnosperms and woody/herbaceous angiosperms. They have been found in all plant parts including (woody) stems, rhizomes, roots, seeds, oils, exuded resins, flowers, fruits, leaves, and bark tissues.[8,23,24,34] However, their amounts can vary extensively between tissues and species, and in some instances their deposition can be massive. For example, in western red cedar (*Thuja plicata*) heartwood, dimeric lignans and higher oligomers can constitute up to 20% of the dry weight of the plant tissue,[35] where both (oligomeric) lignans and lignin biopolymers coexist in the same (heartwood) tissue.[36] In terms of localization of lignans *in planta*, evidence points to different sites depending upon the tissue/species involved. For example, in western red cedar heartwood, they appear to be mainly deposited as nonstructural infusions (secreted from specialized cells such as the ray parenchyma) into the prelignified sapwood,[28] whereas in flax (*Linum usitatissimum*) they seem to be covalently bound to carbohydrate moieties in the seed. On the other hand, in *Linum flavum*, lignans apparently accumulate in vacuoles (discussed in Section 1.25.13.6).[37]

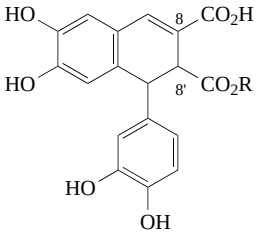
The lignans are a structurally very diverse class of natural products, with more than several thousand distinct structures known at the dimer level alone.[8,14–16,38–42] Interestingly, they are typically found in optically active form, although the particular antipode can vary with plant species. For example, (+)-pinoresinol (**3a**) is present in *Forsythia europaea*,[43] whereas its (−)-antipode (**3b**) occurs in *Daphne tangutica*,[44] and (−)-arctigenin (**22a**) has been isolated from *Forsythia intermedia*,[45] with the (+)-form (**22b**) being found in *Wikstroemia indica*.[46] A discussion of optical activity in lignans and radical coupling mechanisms is given in Section 1.25.5.

1.25.4.1 Lignans in ''Early'' Land Plants

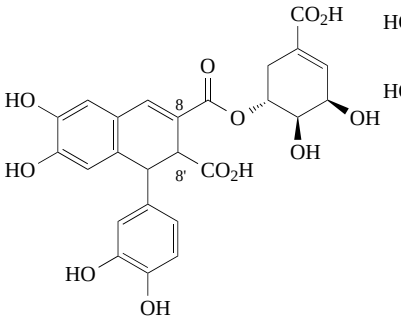
Based on DNA sequence analyses and neontological/paleontological data,[47–49] it is considered that bryophyte-like plants, which include the liverworts (Hepaticae), hornworts (Anthocerotae), and mosses (Musci), were part of the terrestrial flora that emerged some 20–40 million years or so prior to the ''pretracheophytes'' and vascular plants. The earliest documented examples of presumed bryophyte-like fossilized remains come from both lower Devonian (~408 million years ago) and early Silurian (~438 million years ago) records: the early Silurian spore fossils possess cell wall ultrastructures reminiscent of those present in extant liverworts,[50] whereas lower Devonian fossils show some similarities to modern thaloid hepatics (liverworts).[51] Moreover, gene sequence analyses of the large subunit of ribulose 1,5-bisphosphate carboxylase/oxygenase (rbcL) in the chloroplast, has suggested that liverworts are at the base of the embryophyte (embryo-containing plants) lineage and that the hornworts represent the closest lineage to vascular plants,[52] i.e., the liverworts emerged first, then the mosses, and finally the hornworts, before the first tracheophytes.

Lignans are found in both liverworts and hornworts, although none have been reported in mosses. Consideration of their structures is of interest, as it may give useful insight into how the lignan pathway evolved. Thus, lignans present in liverworts (*Pellia epiphylla* (**45**)–(**48**),[53,54] *Jamesoniella autumnalis* (**49**)–(**54**),[55] and *Scapania undulata* (**54**)[56]) contain optically active 8—8′ linked lignans which provisionally appear to be caffeic acid (**33**) derived. They can also be ligated to shikimic acid moieties (**55**) as shown in (**47**),[53] or contain pendant aryl groups that have undergone fission and recyclization to afford lactones as in (**49**)–(**52**) and (**54**).[54] On the other hand, egonol-2-methylbutanoate (**56**) and the optically active lignan, (−)-licarin A (**57**), present in the liverworts, *Riccardia multifida* subsp. *decrescens*[57,58] and *Jackiella javanica*,[59,60] respectively, appear to be derived from 8—5′ coupling of allylphenols, eugenol (**58**)/isoeugenol (**59**) and *p*-hydroxyarylpropene (**60**). Of these, egonol-2-methylbutanoate (**56**) in *R. multifida* has two noteworthy features: namely, the apparent absence of a C-9 carbon and the introduction of a methylenedioxy group. Hitherto, methylenedioxy bridge formation was thought to occur in the lignan pathway with the advent of the gymnosperms.[23]

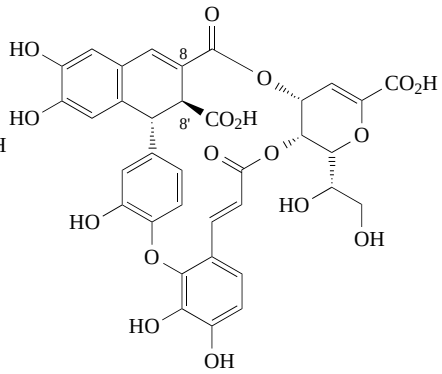
The only report of a lignan present in hornworts (*Megaceros flagellaris*, *Notothylas temperata*, and *Phaeoceros laevis*)[61,62] is that of (+)-megacerotonic acid. Two different structures (**61**) and (**62**) were reported by the same authors for this metabolite in two different publications in the same year, but without any cross-referencing or explanation given to account for the different skeletal representations.[61,62] In a subsequent total chemical synthesis study by Brown *et al.*,[63] the structure of megacerotonic acid was unequivocally established to be compound (**61**).



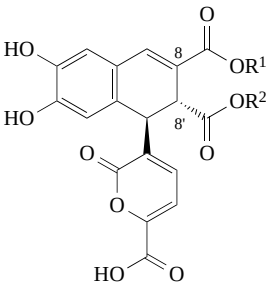
(45) R = H, $[\alpha]_D^{20}$ = –130.77˚ (MeOH)
(46) R = Me, $[\alpha]_D^{20}$= –112.11˚ (MeOH)
(Pellia epiphylla)



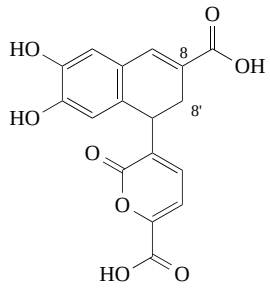
(47) $[\alpha]_D^{20}$= –122.19˚ (MeOH)
(Pellia epiphylla)



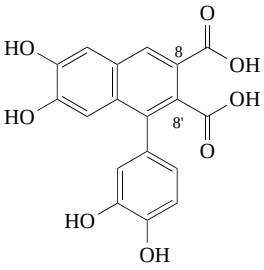
(48) Pelliatin $[\alpha]_D^{20}$= –106.68˚ (MeOH)
(Pellia epiphylla)



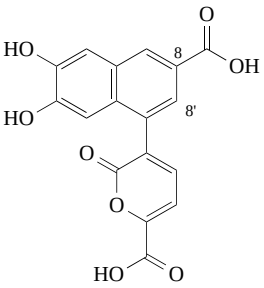
(49) $R^1 = R^2$ = H, $[\alpha]_D^{20}$= +54.88˚ (MeOH, *c* = 0.30)
(50) R^1 = Me, R^2 = H, $[\alpha]_D^{20}$= +33.49˚ (MeOH, *c* = 1.70)
(51) R^1 = H, R^2 = Me, $[\alpha]_D^{20}$= +50.48˚ (MeOH, *c* = 0.95)
(Jamesoniella autumnalis)



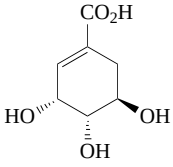
(52) $[\alpha]_D^{20}$= +156.52˚ (MeOH, *c* = 0.08)
(Jamesoniella autumnalis)



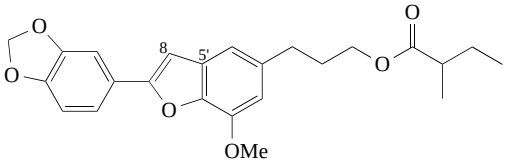
(53)
(Jamesoniella autumnalis)



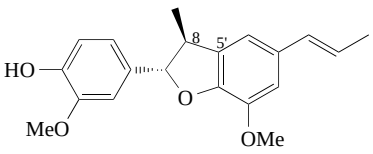
(54) Scapaniapyrone A
(Jamesoniella autumnalis)
(Scapania undulata)



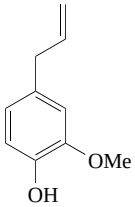
(55) Shikimic acid



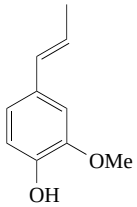
(56) Egonol 2-methylbutanoate
(Riccardia multifida, subsp. *decrescens)*



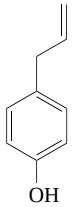
(57) (–)-Licarin A
$[\alpha]_D$ = –43˚ (c 9.0)
(Jackiella javanica)



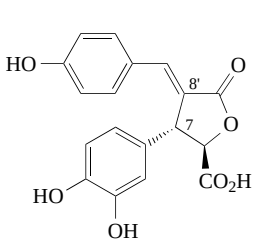
(58) Eugenol



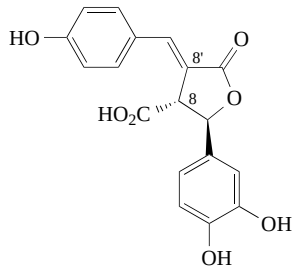
(59) Isoeugenol



(60) *p*-Hydroxyarylpropene



(61)



(62)

(+)-Megacerotonic acid
$[\alpha]_D$ = +233.0° (5% AcOH, *c* = 1.66)
(Megaceros flagellaris)

Lignans are also found in the ferns (Pteridophytes), and include the presumed caffeic acid (**33**) derived 8—2′ linked lignans, (−)-blechnic acid (**63**) and its shikimate derivative, (−)-brainic acid (**64**) from *Blechnum orientale*, *Struthiopteris amabilis*, *Struthiopteris niponica*, *Woodwardia orientalis*, *Woodwardia prolifera*, and *Brainea insignis* (Blechnaceae).[64] Additionally, (−)-lirioresinol A [=(−)-epi-syringaresinol] (**65**), (−)-lirioresinol B [=(−)-syringaresinol] (**66b**), (+)-wikstromol [=(+)-nortrachelogenin] (**67**), (−)-nortracheloside (**68**), and (+)-matairesinol (**21b**) are present in *Selaginella doederleinii* Hieron. (Selaginellaceae), a small perennial pteridophyte found in south and southwestern China at low altitude.[65]

The remaining lignans known to be present in the fern *Pteris vittata* (Pteridaceae), are the 8—5′ and 8—8′ linked glucosides of (−)-dihydrodehydrodiconiferyl alcohol (**69**) and (+)-lariciresinol (**70**).[66] Initially, lignan (**69**) was claimed to have the *cis* configuration at positions 7 and 8; however, subsequent studies by Wallis and co-workers established it to be *trans* (**71**).[67] Other than the lignan dimers, no higher oligomers have been reported in either liverworts, hornworts, or ferns.

1.25.4.2 Lignans in Gymnosperms and Angiosperms (General Features)

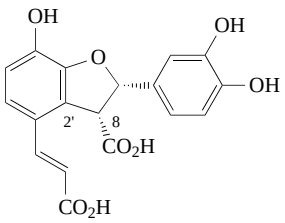
In contrast to the relatively few examples of lignans in early land plants, the gymnosperms were accompanied by a massive increase in lignan structures, particularly representatives of the optically active 8—8′ linked tetrahydrofurans, dibenzylbutanes, dibenzylbutyrolactones, aryltetrahydronaphthalenes, and arylnaphthalenes.[23] The vast majority reported thus far are *E*-coniferyl alcohol (**38**)-derived dimers; however, they can also exist in trimeric and higher oligomeric forms.[25–29,36] Other lignan types, e.g., 8—5′ and 8—*O*—4′ linked, are present in varying amounts in the gymnosperms.

The evolutionary transition to the angiosperms also witnessed a massive increase in formation of (oligomeric) lignan structural types. While the most widespread are again the optically active 8—8′ linked, other coupling modes, affording 8—1′, 8—5′, 5—5′, 7—1′, 8—7′, 1—5′, 8—*O*—4′, 2—*O*—3′, and 3—*O*—4′ coupled products also appeared—particularly in the Magnoliiflorae.[23] By contrast, relatively few lignans of any type have been reported in the monocotyledons. The few described primarily consist of cyclobutane dimers, such as the dihydroxytruxillic acids (**72**) and (**73**) from *Setaria anceps* cv *Nandi* (Poaceae, Commeliniflorae)[68,69] and acoradin (**74**) found in *Acorus calamus* (Araceae, Ariflorae).[70]

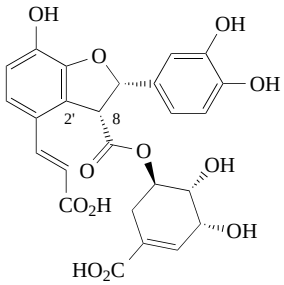
However, rather than attempting to distinguish between the various lignans on the basis of plant family or origin, it is perhaps more instructive to discuss the distinct structural types in terms of known or perceived biosynthetic pathways, as understood at the enzyme, protein, and molecular levels. A discussion of optical activity is, however, first required.

1.25.5 OPTICAL ACTIVITY OF LIGNAN SKELETAL TYPES AND LIMITATIONS TO THE FREE RADICAL RANDOM COUPLING HYPOTHESIS

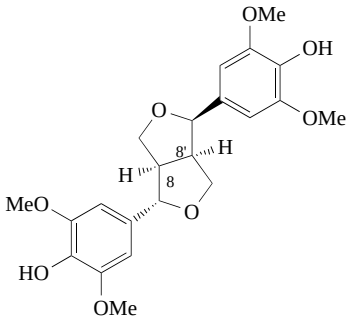
No description of lignan structure would be complete without first discussing the changing paradigm for control of enzymatic free-radical coupling of phenylpropanoids. Initially, based on the study of presumed lignin biopolymer formation, a random free-radical coupling process was favored as being the only operative mechanism.[71,72] However, this earlier concept has been dramatically revised,[73,74] and it is important to discuss the basis for this changing view.



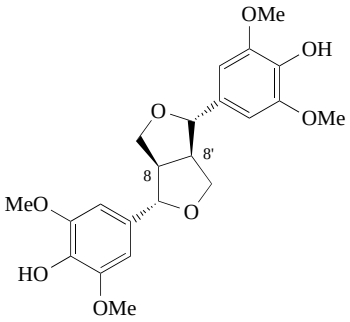
(63) (–)-Blechnic acid
$[\alpha]_D^{23}$ = –28° (MeOH, *c* = 1.0)
(Blechnum orientale)



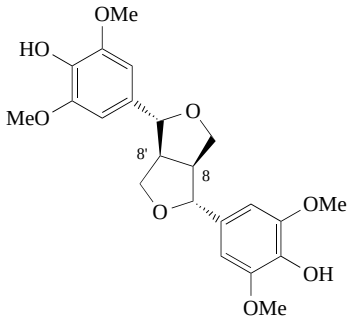
(64) (–)-Brainic acid
$[\alpha]_D^{24}$ = –42° (MeOH, *c* = 1.0)
(Blechnum orientale)



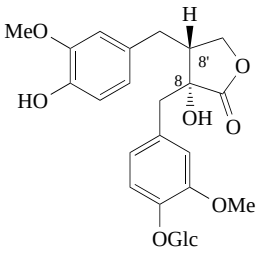
(65) (–)-Lirioresinol
= (–)-Episyringaresinol
(Selaginella doederleinii)



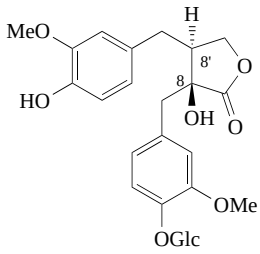
(66a) (+)-Lirioresinol B
= (+)-Syringaresinol



(66b) (–)-Lirioresinol B
= (–)-Syringaresinol
(Selaginella doederleinii)



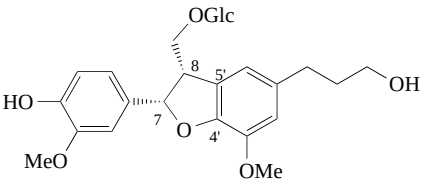
(67) (+)-Wikstromol
= (+)-Nortrachelogenin
(Selaginella doederleinii)



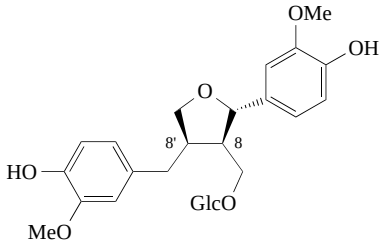
(68) (–)-Nortracheloside
(Selaginella doederleinii)

In the original random free-radical coupling hypothesis, substrate monomers, such as the monolignols, *p*-coumaryl (**32**), coniferyl (**38**), and sinapyl (**44**) alcohols, were envisaged to undergo single-electron oxidations (oxidase-catalyzed) to afford the corresponding free-radical species, where the only enzymatic requirement was generation of the free-radical intermediates. Under such conditions, at least *in vitro*, random coupling can then occur at several sites on the molecule to initially afford racemic lignan dimers, and Figure 2 illustrates this random coupling hypothesis using *E*-coniferyl alcohol (**38**) as an example: the major coupling products formed are the three racemic 8—5′, 8—8′, and 8—*O*—4′ linked lignan dimers (**5**), (**3**), and (**6**), with the 8—5′ dimer predominating in coupling frequency *in vitro*.

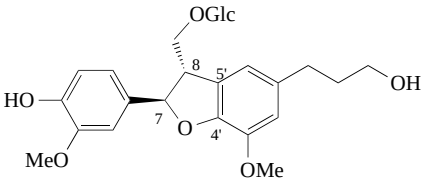
According to this view, there was believed to be no further requirement for additional enzyme/protein involvement in determining the outcome of lignin biopolymer assembly, other than reoxidation of the dimeric species. Therefore, unlike any other biopolymer, lignin formation was thought to be satisfactorily duplicated by the random encounter of its free-radical precursors *in vitro*. However, this random assembly mechanism, in fact, never gave a biopolymer duplicating lignin structure (see Chapter 3.18); nor could it explain the observed optical activity of numerous lignans



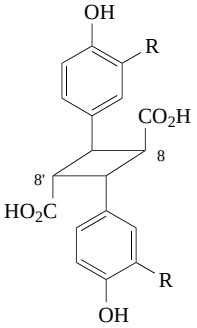
(69) *cis*-Dihydrodehydrodiconiferyl alcohol-9–*O*–β-D-glucoside (incorrectly assigned)



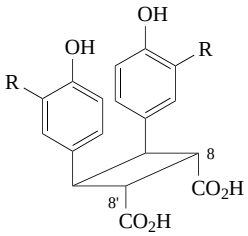
(70) Lariciresinol-9–*O*–β-D-glucoside
$[\alpha]_D^{27}$= –39.77° (Me_2CO, *c* = 0.88)
$[\alpha]_D^{20}$of aglycone = +15.7° (Me_2CO, c 0.35)
(Pteris vittata)



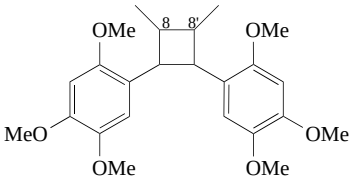
(71) *trans*-Dihydrodehydrodiconiferyl alcohol-9–*O*–β-D-glucoside
$[\alpha]_D^{22}$= –23.6° (MeOH, c = 0.98)
$[\alpha]_D^{22}$ of aglycone = +8.5° (Me_2CO, *c* = 0.96)
(Pteris vittata)



(72) Dihydroxytruxillic acid
R = H or OMe
(Setaria anceps)



(73) Dihydroxytruxinic acid
R = H or OMe
(Setaria anceps)



(74) Acoradin
$[\alpha]_D$ = 0° (*c* = 1.1)
(Acorus calamus)

occurring in a wide variety of plant species. Indeed, lignan optical activity could presumably only result via one of three ways: (i) where stereoselective phenylpropanoid coupling occurs, thereby explicitly controlling both the regio- and stereochemistries; (ii) where racemic coupling occurs, but where one of the enantiomeric forms is selectively metabolized; and/or (iii) where both stereoselective and random coupling can occur, but to different extents in different tissues and cell types.

Each of these three possibilities had to be considered since, in many cases, the optical rotation, $[\alpha]_D$, value for a particular lignan can differ substantially between plant species: For example, Table 1 shows the variation in reported optical rotations for syringaresinols (**66**) obtained from different plants.[44,75–85] As can be seen, these range from being optically active [(+)- or (−)-], to being nearly racemic. These observations, however, only serve to remind us that an $[\alpha]_D$ determination does not reveal the enantiomeric purity of isolated lignans, or, indeed, provide any insight as to whether the enantiomer(s) originate from the same subcellular compartment, cell type, or tissue.

The application of chiral HPLC methodologies, suitable for the facile separation of various lignan optical antipodes, has significantly helped in the study of this class of natural products, i.e., whether for examination of enantiomeric purity and/or outcome of phenylpropanoid coupling.[86–88] The two major chiral stationary phases currently employed for separation of (+)- and (−)-lignan antipodes are either cellulose carbamate coated on silica gel (Chiralcel columns, Daicel), or a macrocyclic

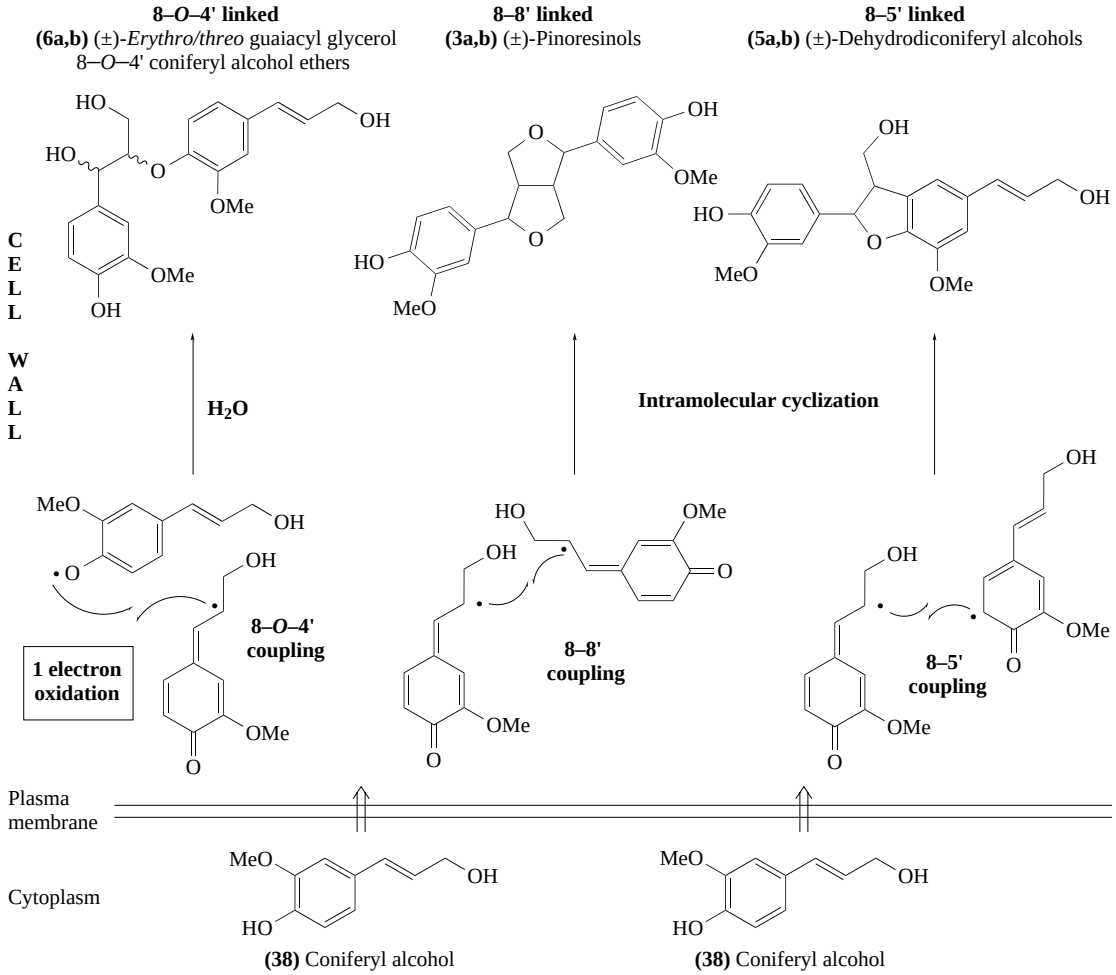


Figure 2 Illustration of the monolignol random coupling hypothesis leading to lignin as according to Freudenberg.[71,72]

Table 1 Specific rotations and enantiomeric compositions of syringaresinol (**66**) isolated from different species.[76] (Enantiomeric composition was determined following chiral column chromatography.)

			Syringaresinol (%)	
Species	$[\alpha]_D$ *reported*	*Ref.*	(+)-(**66a**)	(−)-(**66b**)
Aspidosperma marcgravianum	−34.8	77		
Holocantha emoryi	−32.5	78		
Xanthoxylum ailanthoides	−9.6	79	38	62
Daphne tangutica	−2.1	44	48	52
Xanthoxylum inerme	0	80	48	52
Stellera chamaejasme	+3.0	81		
Liriodendron tulipifera	+19.0	82		
Hedyotis lawsoniae	+23.0	83		
Eucommia ulmoides	+44.0	84	88	12
Liriodendron tulipifera	+48.9	85		

glycopeptide covalently bound to silica (5 μm), such as Vancomycin (Chirobiotic V column, Advanced Separation Technologies). The basis for their separations is the reversible formation of transient diastereomeric complexes formed between each enantiomer and the chiral stationary phase.[76]

Conditions have thus been developed for separation of (+)- and (−)-enantiomers of various lignans obtained, for example, by chemical syntheses, such as the pinoresinols (**3a,b**), seco-isolariciresinols (**20a,b**), syringaresinols (**66a,b**), and dehydrodiconiferyl alcohols (**5a,b**) (see Figures 3(a) to 3(d)). Further, chiral HPLC analysis of certain lignans from selected plant species, e.g., *F. intermedia*, has revealed that, in many instances, lignans, such as pinoresinol (**3**) and seco-isolariciresinol (**20**), occur in optically pure form (Figures 3(e) and 3(f)). On the other hand, with the syringaresinols (**66a,b**) isolated from different sources, it was observed (Figure 4 and Table 1) that both antipodes could either occur in enantiomeric excess (e.g., in *Xanthoxylum ailanthoides* and *Eucommia ulmoides*; Figures 4(b) and 4(a)) or were in nearly racemic amount (e.g., *D. tangutica* and *Xanthoxylum inerme*; Figures 4(c) and 4(d)). Some convincing scientific explanation was thus required for the noted, but often differing, optical activities in the lignans.

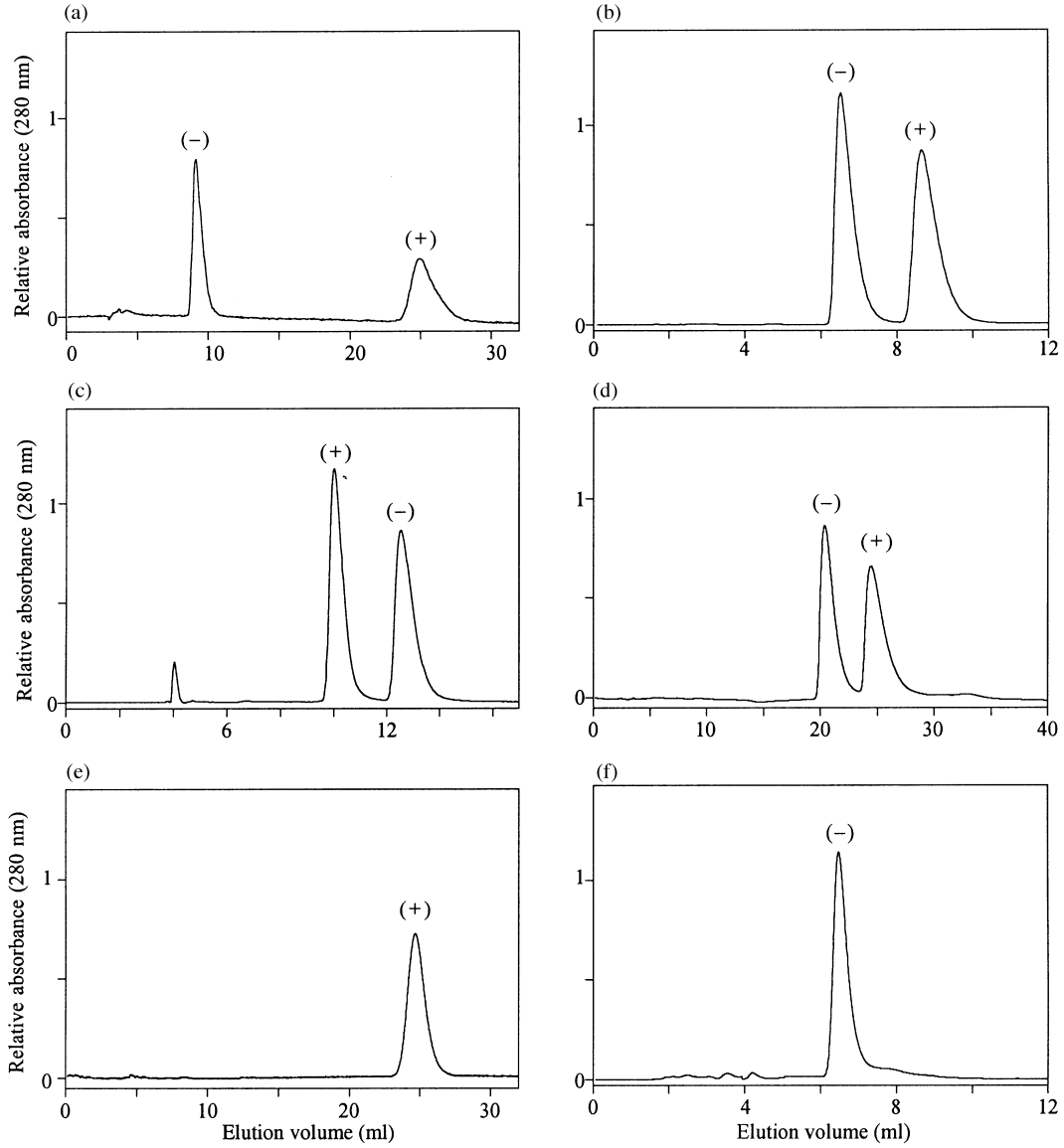


Figure 3 Chiral HPLC separation of selected lignans. (**a**) (±)-Pinoresinols (**3a,b**), (**b**) (±)-secoisolariciresinols (**20a,b**), (**c**) (±)-syringaresinols (**66a,b**), (**d**) (±)-dehydrodiconiferyl alcohols (**5a,b**) as well as (**e**) (+)-pinoresinol (**3a**), and (**f**) (−)-secoisolariciresinol (**20a**) isolated from *Forsythia intermedia*. (±)-Pinoresinols (**3a,b**), (±)-syringaresinols (**66a,b**), and (±)-secoisolariciresinols (**20a,b**) were individually resolved using a Chiralcel OD column (Daicel),[86,88] whereas resolution of (±)-dehydrodiconiferyl alcohols (**5a,b**) employed a Chirobiotic V column (Advanced Separation Technologies) with MeOH-NH_4NO_3 (1:9) as solvent system (flow rate: 1 ml min^{-1}).

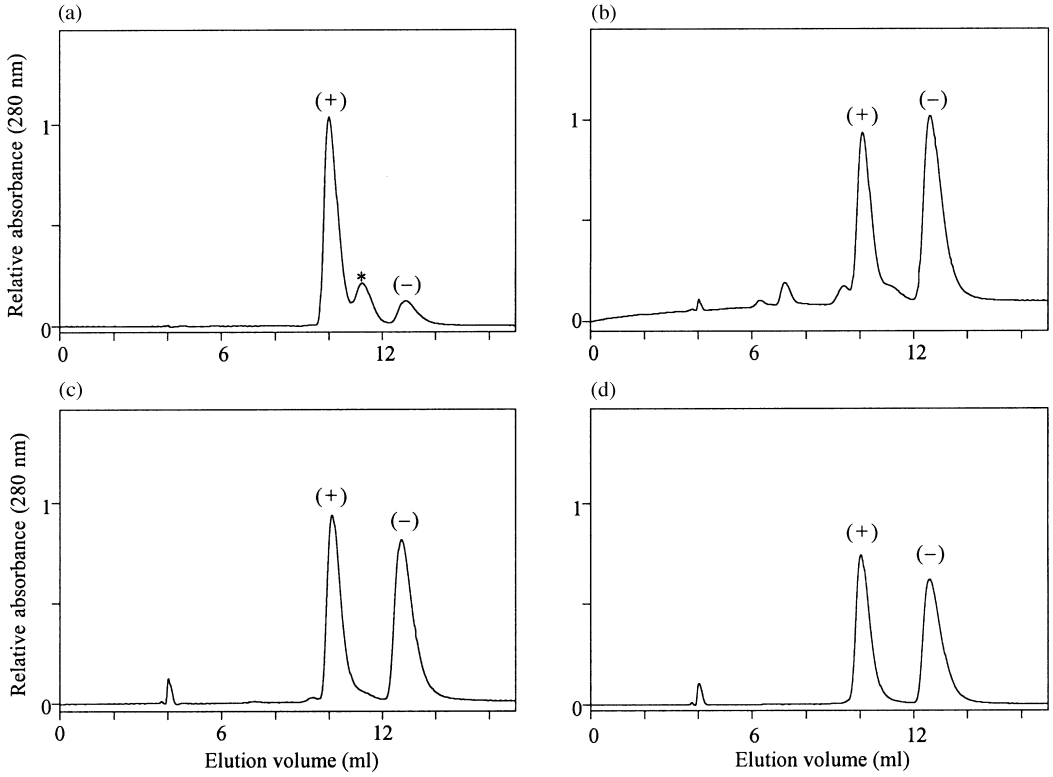


Figure 4 Chiral HPLC separation of syringaresinols (**66a,b**) isolated from different plant species. (**a**) *Eucommia ulmoides*, (**b**) *Xanthoxylum ailanthoides*, (**c**) *Daphne tangutica*, and (**d**) *Xanthoxylum inerme*.[76] For elution conditions, see Figure 3 legend (* = contaminant).

1.25.6 8—8′ STEREOSELECTIVE COUPLING: DIRIGENT PROTEINS AND *E*-CONIFERYL ALCOHOL RADICALS

1.25.6.1 Dirigent Proteins Stipulate Stereoselective Outcome of *E*-Coniferyl Alcohol Radical Coupling in Pinoresinol Formation

Since lignan optical activity cannot readily be explained by random coupling, it was essential to delineate how monolignol coupling control might be achieved *in planta*. Initial investigations used *Forsythia* species, given that it is an abundant source of the optically pure 8—8′ linked lignans, (+)-pinoresinol (**3a**) and (−)-matairesinol (**21a**): it was discovered that *Forsythia* stem residues, following removal of readily soluble proteins, were capable of preferentially stereoselectively converting *E*-coniferyl alcohol (**38**) into the 8—8′ linked (+)-pinoresinol (**3a**) in ~60% enantiomeric excess.[86] In contrast, all previous bimolecular phenoxy radical coupling processes, whether engendered biochemically[89] or chemically,[71] only afforded the racemic lignans, (±)-dehydrodiconiferyl alcohols (**5a,b**), (±)-pinoresinols (**3a,b**), and (±)-*erythro*/*threo* guaiacylglycerol 8—*O*—4′ coniferyl alcohol ethers (**6a,b**) as previously discussed.

(+)-Pinoresinol (**3a**) was ultimately demonstrated to be formed *in vitro* when two distinct proteins were added together; this resulted in conferring the requisite stereoselectivity to the coupling of two *E*-coniferyl alcohol (**38**) molecules. The first protein was of circa 78 kDa molecular size, as determined by both gel permeation chromatography (Sepharose S200, Pharmacia) and analytical ultracentrifugation. SDS-PAGE analysis, on the other hand, gave a single band of 26–27 kDa suggesting that the native protein existed as a trimer. This 78 kDa protein lacked any (oxidative) catalytic capacity by itself, and was unable to directly engender formation of (+)-pinoresinol (**3a**) from *E*-coniferyl alcohol (**38**). The second protein exhibited a typical plant laccase EPR spectrum, but did not catalyze stereoselective coupling. It did, however, oxidatively convert *E*-coniferyl alcohol (**38**) into the well-known racemic lignan products *in vitro*, i.e. (**5a,b**), (**3a,b**), and (**6a,b**) in a ratio of circa 4:2:1 (Figure 5(a)).

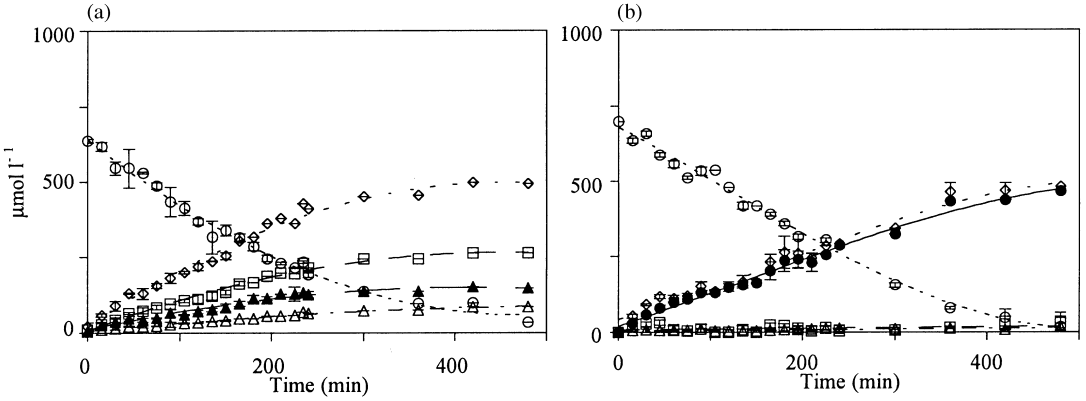


Figure 5 Time courses for *E*-coniferyl alcohol (**38**) depletion and formation of corresponding lignans during incubation in the presence of (**a**) an oxidase, and (**b**) dirigent protein and an oxidase.[87] ○, Coniferyl alcohol (calculated as dimer equivalents); □, (±)-dehydrodiconiferyl alcohols (**5a,b**); ●, (+)-pinoresinol (**3a**); ▲, (±)-pinoresinols (**3a,b**); △, (±)-*erythro*/*threo* guaiacylglycerol 8—*O*—4′ coniferyl alcohols (**6a,b**); and ◇, total of all lignans (after Lewis *et al.*[87]).

When both proteins were combined together in judicious amounts, however, essentially only conversion of *E*-coniferyl alcohol (**38**) into (+)-pinoresinol (**3a**) was observed. This is illustrated in Figure 5(b) which shows the effect of both proteins on stereoselective coupling, and the term dirigent protein[87] (Latin, *dirigere*: to guide or align) was introduced to describe the perceived unique function of the 78 kDa protein (discussed in Section 1.25.6.5). Significantly, stereoselective coupling occurred regardless of whether a one-electron oxidase (e.g., laccase) or a one-electron oxidant (such as FMN) was used in conjunction with the dirigent protein (see Figures 6(b) and 6(d)). In both cases, dirigent

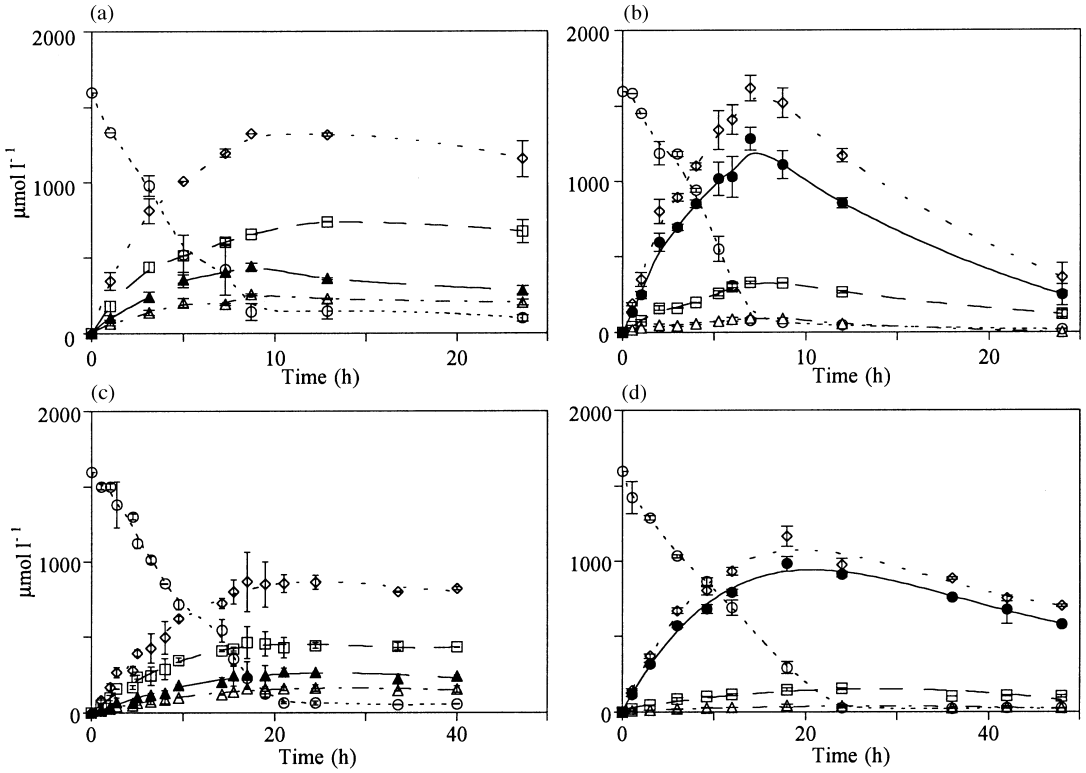


Figure 6 Time courses for *E*-coniferyl alcohol (**38**) depletion and formation of corresponding lignans during incubation in the presence of (**a**) laccase, (**b**) dirigent protein and laccase, (**c**) FMN, and (**d**) FMN and dirigent protein. See Figure 5 for key (after Lewis *et al.*[87]).

protein addition had little, if any, effect on the rate of *E*-coniferyl alcohol (**38**) depletion (see Figures 6(a) to (d)). Moreover, stereoselectivity was only observed with *E*-coniferyl alcohol (**38**) as a substrate, but not when either *E*-*p*-coumaryl (**32**) or *E*-sinapyl (**44**) alcohol was used.

1.25.6.2 Cloning of the Gene Encoding the Dirigent Protein and Recombinant Protein Expression in Heterologous Systems

The cDNA of two *Forsythia* dirigent protein genes (psd-Fi1 and psd-Fi2; Figures 7(a) and 7(b)) were obtained by a polymerase chain reaction guided strategy, and sequence analyses revealed the presence of secretory signal peptides, potential *N*-glycosylation and serine/tyrosine phosphorylation sites.[74,90] Each cDNA encoded a protein subunit of only circa 18 kDa, after taking into account the secretory signaling sequences, indicating that the presumed 26 kDa subunit was extensively glycosylated.

The authenticity of the dirigent protein clone(s) was proven by obtaining recombinant dirigent proteins: these were heterologously expressed in both *Spodoptera frugiperda* (fall army worm) Sf9 cells[74] using a baculovirus based expression system,[91] and in *Drosophila melanogaster* cells cotransfected with the DES (drosophila expression system) vector (Invitrogen);[92] both expression systems were chosen over *Escherichia coli* since they carry out glycosylation/post-translational modifications. As illustrated in Figure 8, the purified recombinant proteins were functionally capable of conferring the requisite stereoselectivity to [9-^{3}H]coniferyl alcohol (**38**) coupling, provided that single-electron oxidative capacity (e.g., laccase) was supplied, thereby establishing the gene sequence(s) to be correct.[74,90] These findings were also confirmed using deuterated [9-$^{2}H_2$, $OC^{2}H_3$]coniferyl alcohol (**38**) as substrate.

1.25.6.3 Sequence Homology Comparisons

The *Forsythia* dirigent protein sequence has no significant level of homology to any other protein of known function when using the BLAST/BLAST-Beauty database search tool.[93,94] It did, however, show significant identity ($\sim$64%) with a ''pea disease resistance response gene 206-d'' of unknown function, which is induced in conjunction with isoflavone reductase.[95] Low sequence homology levels were also noted with portions of three other plant genes, of no known function, i.e., *Arabidopsis thaliana*, wheat (*Triticum aestivum*), and barley (*Hordeum vulgare*). The *Arabidopsis* gene possessing limited sequence homology was the ''hypothetical protein'' random BAC clone (40% similarity over 82% of the sequence, Genbank accession number SF000657), whereas that from wheat (47% similarity over 56% of the sequence, Genbank accession number U32427) is associated with systemic acquired resistance and is induced by benzothiadiazole.[96] Finally, the barley gene encoded a ''putative 32.7 kDa jasmonate-induced'' gene (Genbank accession number U43497), with some 50% similarity over 35% of the sequence of the dirigent protein. No meaningful comparisons could be made with other gene sequences outside of the plant kingdom, and it can, therefore, be concluded that the dirigent protein genes are unique. Current information does not, however, give any incisive insight into how these gene(s) may have evolved, or as to what its progenitor function may have been.

1.25.6.4 Comparable Systems

Since various 8—8′ linked lignans are present in many plant species, isolation and characterization of dirigent protein analogues and homologues was of interest. Accordingly, dirigent protein involve-

Figure 7 Complete sequence of *Forsythia intermedia* dirigent protein cDNA (**a**) psd-Fi1, and (**b**) psd-Fi2. The signal peptide cleavage sites are indicated by an arrow (before Arg-25 and His-25 in psd-Fi1 and psd-Fi2, respectively, in the native proteins, and before Thr-22 in the recombinant psd-Fi1 proteins). Potential *N*-glycosylation sites (Asn-52, Asn-65, Asn-122, and Asn-140 in psd-Fi1; Asn-51, Asn-64, Asn-121, and Asn-139 in psd-Fi2) and serine (Ser-123 in psd-Fi1, Ser-28 in psd-Fi2) and tyrosine phosphorylation (Tyr-183 in psd-Fi1, Tyr-182 in psd-Fi2) sites are indicated by underlining. The stop codons are indicated by an asterisk (after Lewis *et al.*[74]).

(a)

```
     ATTTCGGCACGAGATTAAACCAAACATGGTTTCTAAAACACAAATTGTAGCTCTTTTCCT
                                M  V  S  K  T  Q  I  V  A  L  F  L

  36 TTGCTTCCTCACTTCCACCTCTTCCGCCACCTACGGCCGCAAGCCACGCCCTCGCCGGCC
  13  C  F  L  T  S  T  S  S  A  T  Y  G  R  K  P  R  P  R  R  P
                                   ⇑           ⇑
  96 CTGCAAAGAATTGGTGTTCTATTTCCACGACGTACTTTTCAAAGGAAATAATTACCACAA
  33  C  K  E  L  V  F  Y  F  H  D  V  L  F  K  G  N  N  Y  H  N

 156 TGCCACTTCCGCCATAGTCGGGTCCCCCCAATGGGGCAACAAGACTGCCATGGCCGTGCC
  53  A  T  S  A  I  V  G  S  P  Q  W  G  N  K  T  A  M  A  V  P

 216 ATTCAATTATGGTGACCTAGTTGTGTTCGACGATCCCATTACCTTAGACAACAATCTGCA
  73  F  N  Y  G  D  L  V  V  F  D  D  P  I  T  L  D  N  N  L  H

 276 TTCACCCCCAGTGGGTCGGGCGCAAGGGATGTACTTCTATGATCAAAAAAATACATACAA
  93  S  P  P  V  G  R  A  Q  G  M  Y  F  Y  D  Q  K  N  T  Y  N

 336 TGCTTGGCTAGGGTTCTCATTTTTGTTCAATTCAACTAAGTATGTTGGAACCTTGAACTT
 113  A  W  L  G  F  S  F  L  F  N  S  T  K  Y  V  G  T  L  N  F

 396 TGCTGGGGCTGATCCATTGTTGAACAAGACTAGAGACATATCAGTCATTGGTGGAACTGG
 133  A  G  A  D  P  L  L  N  K  T  R  D  I  S  V  I  G  G  T  G

 456 TGACTTTTTCATGGCGAGAGGGGTTGCCACTTTGATGACCGATGCCTTTGAAGGGGATGT
 153  D  F  F  M  A  R  G  V  A  T  L  M  T  D  A  F  E  G  D  V

 516 GTATTTCCGCCTTCGTGTCGATATTAATTTGTATGAATGTTGGTAAACAATTTAGCCGTA
 173  Y  F  R  L  R  V  D  I  N  L  Y  E  C  W  *

 576 TATATATATATATATGGCTATACATATTTCATAGAATCCAGATTTGCTGTTTCAAATGTG
 636 TGTTTCTTTAGTTGTGCCACCAATAAAAAAATGTACACATTATTTAATAAATATAATTAT
 696 TTAATGTGTTCATTTTTGAAGTTAAATTTAAGTTGTATTTATTTGATTATGTATAAATTC
 756 TCTATTAGTAAAATAGTCAAAGTGACACATATTCAAGACGACATATGTAACTTTATTTCA
 816 TATCTTCAACAAGTTCAATAATGTCATATATATTGTACTATTGAAAAAAAAAAAAAAAAA
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(b)

```
     AATTCGGCACGAGGAAAAATGGCAGCTAAAACACAAACCACAGCCCTTTTCCTCTGCCTC
                       M  A  A  K  T  Q  T  T  A  L  F  L  C  L

  43 CTCATCTGCATCTCCGCCGTGTACGGCCACAAAACCAGGTCTCGACGCCCCTGTAAAGAG
  15  L  I  C  I  S  A  V  Y  G  H  K  T  R  S  R  R  P  C  K  E
                                  ⇑
 103 CTCGTTTTCTTCTTCCACGACATCCTCTACCTAGGATACAATAGAAACAATGCCACCGCT
  35  L  V  F  F  F  H  D  I  L  Y  L  G  Y  N  R  N  N  A  T  A

 163 GTCATAGTAGCCTCTCCTCAATGGGGAAACAAGACTGCCATGGCTAAACCTTTCAATTTT
  55  V  I  V  A  S  P  Q  W  G  N  K  T  A  M  A  K  P  F  N  F

 223 GGTGATTTGGTTGTGTTTGATGATCCCATTACCTTAGACAACAACCTGCATTCTCCTCCG
  75  G  D  L  V  V  F  D  D  P  I  T  L  D  N  N  L  H  S  P  P

 283 GTCGGCCGGGCTCAGGGAACTTATTTCTACGATCAATGGAGTATTTATGGTGCATGGCTT
  95  V  G  R  A  Q  G  T  Y  F  Y  D  Q  W  S  I  Y  G  A  W  L

 343 GGATTTTCATTTTTGTTCAATTCTACTGATTATGTTGGAACTCTAAATTTTGCTGGAGCT
 115  G  F  S  F  L  F  N  S  T  D  Y  V  G  T  L  N  F  A  G  A

 403 GATCCATTGATTAACAAAACTAGGGACATTTCAGTAATTGGAGGAACTGGTGATTTTTTC
 135  D  P  L  I  N  K  T  R  D  I  S  V  I  G  G  T  G  D  F  F

 463 ATGGCTAGAGGGGTAGCCACTGTGTCGACCGATGCTTTTGAAGGGGATGTTTATTTCAGG
 155  M  A  R  G  V  A  T  V  S  T  Ð  A  F  E  G  D  V  Y  F  R

 523 CTTCGTGTTGATATTAGGTTGTATGAGTGTTGGTAAATTTACCTTATTTTTCCATTTTCT
 175  L  R  V  D  I  R  L  Y  E  C  W  *

 583 TGAGTTTGACTCGGATTTGACTAATAATGTCTTCTGTAATCCTTGTTTTTGATCAATTTG
 643 TGGCGATTTTATCAATTAGTGATTGTTTGGTTCATATTTTAATCTGTTAAAAAAAATTGT
 703 GGTCAAAAGCCAATAACCACAACCGTAGGGAGTTTTTTCCGTTAAGGGGAAAAAAAAGTA
 763 TGTCCATGTGTTACTACGTTTTCAATTTCATTCAAAATTTGCTTTTCAATCATCTTCTTC
 823 AAAAAAAAAAAAAAAAA
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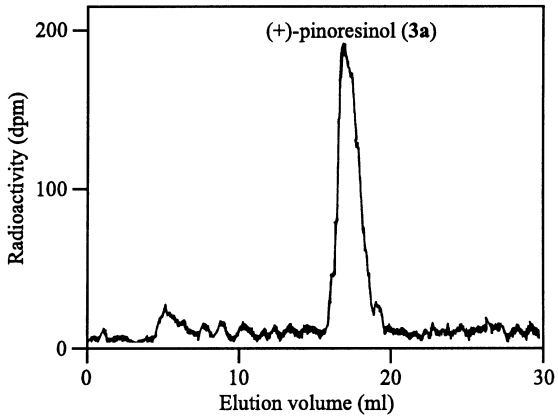


Figure 8 Chiral HPLC analysis of [9,9′-^{3}H]-pinoresinol (**3**) formed by incubation of recombinant dirigent protein with [9-^{3}H]coniferyl alcohol (**38**) in the presence of laccase as oxidant.[74] For elution conditions and column employed see Figure 3 legend.

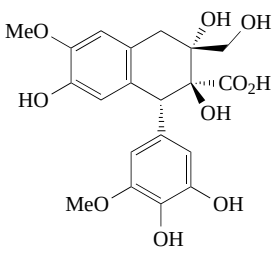
ment in (+)-pinoresinol formation was detected in sesame (*Sesamum indicum*), whereas in flax (*Linum usitatissimum*) the corresponding dirigent protein stipulated formation of the (−)-antipode[97] i.e., the proteins from different plant sources can have different stereoselectivities to give (+)- and (−)-pinoresinols (**3a**) and (**3b**), respectively.

The *Forsythia* dirigent protein cDNA, psd-Fi1, was used to probe the cDNA libraries of two gymnosperms, western red cedar (*T. plicata*) and western hemlock (*Tsuga heterophylla*), which accumulate 8—8′ linked lignans, plicatic acid (**75**) and (α)-conidendrin (**76**),[30] respectively, in their heartwood. Two dirigent protein-like cDNAs were isolated from *T. plicata* (psd-Th1 and psd-Th2) and eight from *T. heterophylla* (psd-Tp1 to psd-Tp8).[74,90] Using a PCR-guided approach on DNA extracted from different plant species, dirigent protein genes were also identified in Manchurian ash (*Fraxinus mandschurica*, Salicaceae: psd-Fm1 and psd-Fm2) and aspen (*Populus tremuloides*, Oleaceae: psd-Pop1 and psd-Pop2). These have a very high degree of similarity/identity[74] (from 91.9% and 85.4% for psd-Fm1 and psd-Fm2 versus psd-Fi1 to 66.1% and 61.2% for psd-Tp1 versus psd-Fi1), as shown in Table 2.

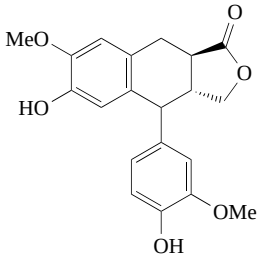
Table 2 Similarity/identity between the different dirigent protein-like clones isolated and the dirigent protein clone psd-Fi1 from *Forsythia intermedia*.

		With signal peptide	
Plant species	*cDNA*	*Similarity* (%)	*Identity* (%)
Forsythia intermedia	psd-Fi2	87.6	81.6
Thuja plicata	psd-Tp1	66.1	61.2
	psd-Tp2	77.4	61.8
	psd-Tp3	78.5	61.8
	psd-Tp4	83.3	67.4
	psd-Tp5	78.4	60.0
	psd-Tp6	76.3	57.5
	psd-Tp7	79.0	59.1
	psd-Tp8	79.0	59.1
Tsuga heterophylla	psd-Th1	69.9	60.8
	psd-Th2	68.5	61.4
Fraxinus mandschurica	psd-Fm1	91.9	85.4
	psd-Fm2	91.9	85.4
Populus tremuloides	psd-Pop1	77.2	66.7
	psd-Pop2	77.2	66.7

The high similarity/identity between different dirigent protein clones isolated and detected indicates that the dirigent proteins may be ubiquitous throughout the plant kingdom, although it



(75) Plicatic acid



(76) (–)-α-Conidendrin

remains to be established whether they stipulate only 8—8′ coupling or other modes (e.g., 8—1′, etc.) as well (see Sections 1.25.8 and 1.25.9).

1.25.6.5 Perceived Biochemical Mechanism of Action

Free-radical coupling reactions, as catalyzed by nonspecific (per)oxidases, cannot control either the regio- or stereochemistries of product formation, when more than one potential coupling site is on the substrate molecule. On the other hand, nature extensively utilizes free-radical coupling processes, with circa 30–40% of all organic carbon being linked together in this way, e.g., lignans, lignins, suberins, melanins, insect cuticles, etc. Given the extensive deployment of such coupling reactions *in vivo*, it could therefore be anticipated that nature had some means to control or stipulate the outcome of phenoxy free-radical coupling.

The discovery of the *Forsythia* dirigent protein, stipulating both the regio- and stereochemical fate of *E*-coniferyl alcohol (**38**) coupling during (+)-pinoresinol (**3a**) formation, thus gave a new perspective as to how control of free-radical reactions occurred *in vivo*; this was further supported by analysis of its unique 18 kDa gene sequence which revealed no counterpart elsewhere. It is believed that the dirigent protein effectuating (+)-pinoresinol (**3a**) formation requires glycosylation of the 18 kDa subunit to give the 26 kDa glycoprotein which then forms the functional ~78 kDa dirigent protein trimer.

There are three distinct biochemical mechanisms[87] that can be envisaged as operative, with each discussed in terms of relative likelihood based on data interpretation. The first is generation of free-radicals from *E*-coniferyl alcohol (**38**), by action of nonspecific oxidase(s)/oxidant(s), with the free radicals then binding to the dirigent protein prior to stereoselective coupling. In this case, the free-radical species are orientated on the 78 kDa protein prior to coupling, hence the term dirigent (Latin, *dirigere*: to guide or align). Alternative possibilities are that *E*-coniferyl alcohol (**38**) molecules are themselves bound to the dirigent protein, and appropriately orientated to give (+)-pinoresinol (**3a**) following one-electron oxidation. It is anticipated that this could only occur when either the substrate phenolic groups were exposed so that they could readily be oxidized by an oxidase or oxidant, or via an electron transfer mechanism between the oxidase/oxidant and an electron acceptor site or sites on the dirigent protein.

Lines of evidence, however, suggest "capture" of phenoxy radical intermediates by the dirigent protein. This is because both the rates of substrate depletion and product formation were largely unaffected by the presence of the dirigent protein. This is consistent with a free-radical capture mechanism which would only affect coupling specificity when single-electron oxidation of coniferyl alcohol (**38**) is rate-determining. The electron transfer mechanism is ruled out, on the basis that no new ultraviolet-visible chromophores were observed in either the presence or absence of an auxiliary oxidase or oxidant, under oxidizing conditions. Preliminary kinetic data were also in agreement with free-radical capture based on the formal Michaelis constant (K_m) and maximum velocity (V_{max}) values characterizing the conversion of *E*-coniferyl alcohol (**38**) into (+)-pinoresinol (**3a**), with the dirigent protein alone and in the presence of the various oxidases or oxidants.[87] With a free-radical capture process, the Michaelis–Menten parameters obtained will only represent formal rather than true values, given that the highest free-energy intermediate state during the conversion of *E*-coniferyl alcohol (**38**) into (+)-pinoresinol (**3a**) is unknown and the relation between the concentration of substrate and that of the corresponding intermediate free-radical in open solution has not been delineated.

With these qualifications in mind, formal K_m and V_{max} values for the dirigent protein preparation were estimated in the presence and absence of the oxidase, laccase and the oxidant, FMN. The preliminary kinetic parameters so obtained were in harmony with the finding that the dirigent protein does not substantially affect the rate of *E*-coniferyl alcohol (**38**) depletion in the presence of the oxidase/oxidant, and are thus in accord with the working hypothesis that the dirigent protein functions by capturing free-radical intermediates which then undergo stereoselective coupling. Accordingly, it is of considerable importance to establish the site(s) on the dirigent protein that involve substrate binding, together with the number of monomeric forms binding to the 18 kDa subunit and to the preformed 78 kDa protein.

1.25.7 PINORESINOL METABOLISM AND ASSOCIATED METABOLIC PROCESSES

The 8—8′ linked lignan, pinoresinol (**3**), is a central intermediate in lignan metabolism, which, depending upon the plant species, can be converted into a rather large number of natural products of important plant physiological and pharmacological functions (see Sections 1.25.13 and 1.25.14). Delineation of the various biochemical pathways associated with its metabolism has been carried out using sesame (*S. indicum*), *Magnolia kobus*, *Forsythia* species, flax (*L. usitatissimum*), western red cedar (*T. plicata*), western hemlock (*T. heterophylla*), *Linum flavum*, and *Podophyllum* species. Together, these plants contain various furanofuran, furano, dibenzylbutane, dibenzylbutyrolactone, and aryltetrahydronaphthalene lignans, and, therefore, provide the opportunity to study the formation of distinct 8—8′ linked lignan skeletal forms.

1.25.7.1 *Sesamum indicum*: (+)-Piperitol, (+)-Sesamin, and (+)-Sesamolinol Synthases

Sesame (*S. indicum*) seeds are rich in furanofuran lignans, of which (+)-sesamin (**18**) and the unusual oxygenated derivative, (+)-sesamolin (**77**) are the most abundant (Figure 9). They differ from (+)-pinoresinol (**3a**) by methylenedioxy bridge formation and, in the latter case, by an oxygen insertion between the furanofuran group and the aryl moiety. Their physiological roles appear to be as protective antioxidants, thereby helping stabilize sesame seed oil from the rapid onset of rancidity.[4,98,99]

Sesame seed pod development is a gradual maturation process, being initiated some 14 days or so after flowering in the oldest tissues nearest the stem base. New seed pods are then continually formed over a six-week period, and Figure 10 shows a maturing *S. indicum* plant with its seed pods at different maturation stages. Plants at this stage were used for tracer experiments, employing both radio- and stable isotopically labeled lignan precursors, where it was found that sesamin (**18**) and sesamolin (**77**) were both formed *de novo* in maturing seed.[100,101] The possible biochemical routes to both lignans are shown in Figure 9.

When racemic (±)-[3,3′-O^{14}CH$_3$]pinoresinols (**3a,b**) were administered to intact sesame seeds taken from pods from eight-week-old plants, it was found that only the (+)-antipode (**3a**) was metabolized into (+)-piperitol (**78**), (+)-sesamin (**18**), and (+)-sesamolin (**77**); the corresponding (−)-enantiomer (**3b**) was not utilized.[101] It was also found that the relative efficacy of metabolism of (+)-[3,3′-O^{14}CH$_3$] pinoresinol (**3a**) into each of these lignans varied with the stage of maturation of the developing seed pod. Incorporation into sesamolin (**77**) was highest at the early stages of seed pod maturation, whereas conversion into sesamin (**18**) and piperitol (**78**) was greatest at the later stages.

Confirmation of these radiochemical observations was attained by administering (±)-[9,9′-^{2}H$_2$, 3,3′-OC^{2}H$_3$]pinoresinols (**3a,b**) to sesame seed. The enzymatically synthesized (+)-[9,9′-^{2}H$_2$, 3-OC^{2}H$_2$, 3′-OC^{2}H$_3$]piperitol (**78**) so obtained displayed a molecular ion [M$^+$ +9] at *m*/*z* 365, corresponding to the presence of nine deuterium atoms.[101] This established intact incorporation of the precursor during methylenedioxy bridge formation, in a conversion catalyzed by (+)-piperitol synthase.

Microsomal preparations from this seed pod maturation stage were examined as a potential source of (+)-piperitol synthase. These were incubated with (±)-[3,3′-O^{14}CH$_3$]pinoresinols (**3a,b**) but only gave radiolabeled [3-O^{14}CH$_2$, 3′-O^{14}CH$_3$]piperitol (**78**) when NADPH (1 mM) was added.[101] The conversion was completely enantiospecific, since only the radiolabeled (+)-antipode was formed (Figure 11). Confirmation of the radiochemical findings was achieved using isotopically labeled

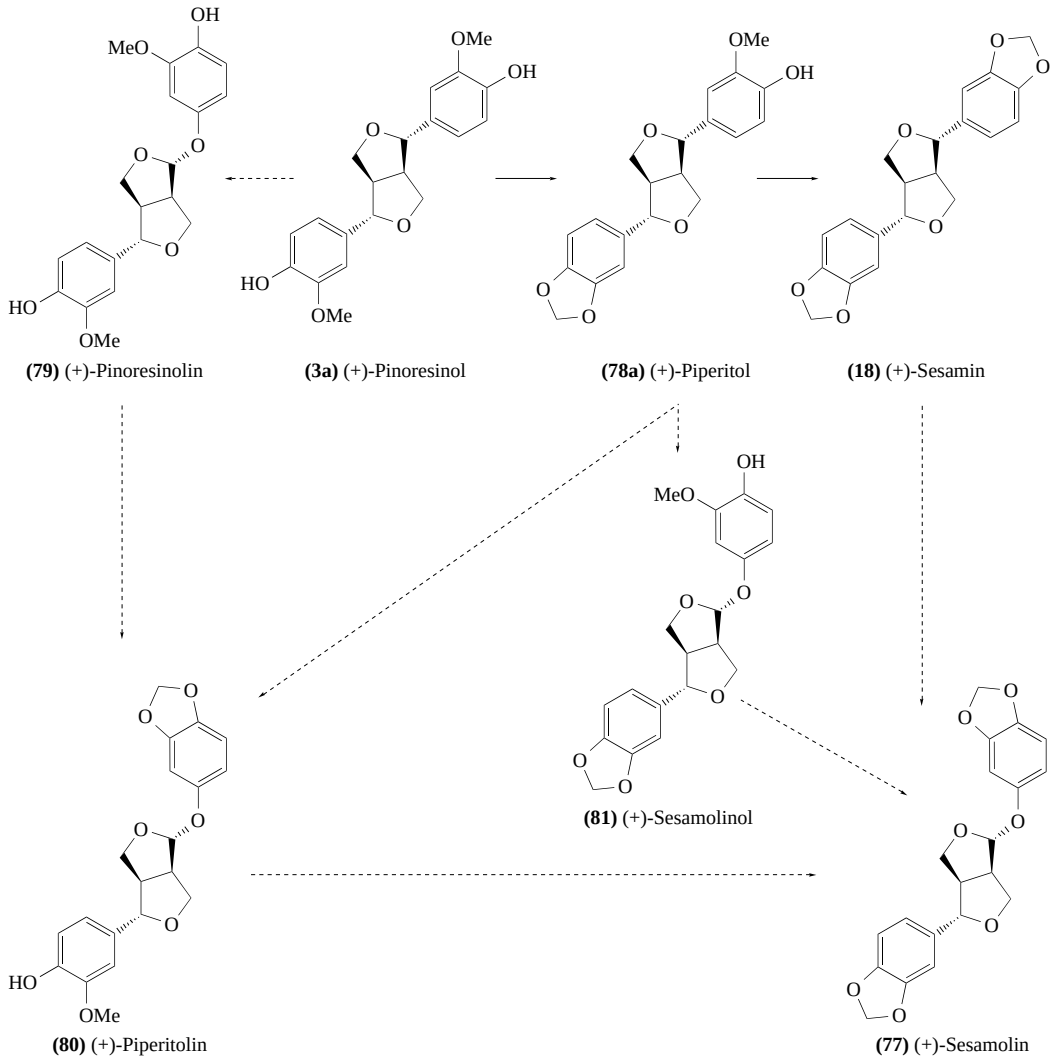


Figure 9 Possible biosynthetic pathways to (+)-sesamolin (**77**) from (+)-pinoresinol (**3a**) in *S. indicum*. (**78b** would be the opposite enantiomer.)

(±)-[9,9′-$^2H_2$, 3,3′-OC^2H_3]pinoresinols (**3a,b**) as substrates, where the enzymatically generated (+)-piperitol (**78**) displayed the expected molecular ion [M^+ +9] at *m/z* 365 as before.[101]

(+)-Piperitol synthase is an O_2-requiring, NADPH-dependent, cytochrome P450 enzyme, with temperature and pH maxima of 40 °C and 7.5, respectively. Cytochrome P450 inhibitors, clotrimeizol (300 μM), miconazol (300 μM), and cytochrome c (170 μM), completely inhibited the enzyme, with tropolone and metyrapone being less efficient (Table 3). It is also strongly inhibited by carbon monoxide (90% inhibition in a CO/O_2 (9:1) atmosphere).[101] These results are consistent with previous studies of benzophenanthridine alkaloid[102,103] and isoflavonoid[104] biosynthetic pathways, where methylenedioxy bridge generation during the formation of such natural products was catalyzed by NADPH-dependent, cytochrome P450 monooxygenase(s).

Interestingly, the *S. indicum* microsomal preparation only effectively converted (+)-pinoresinol (**3a**) into (+)-piperitol (**78**), but not into (+)-sesamin (**18**). Incubation with (+)-piperitol (**78**) did, however, give (+)-sesamin (**18**).[105] Accordingly, there may be two distinct cytochrome P450s involved in (+)-piperitol (**78**) and (+)-sesamin (**18**) formation, respectively. The enzymology associated with oxygen insertion between the aryl group and furanofuran to give sesamolin (**77**) also awaits delineation. It is perhaps significant, however, that ketone (**82**) was isolated from *S. indicum* seeds,[106] and studies will establish if it is a pathway intermediate to (+)-sesamolin (**77**) via rearrangement.

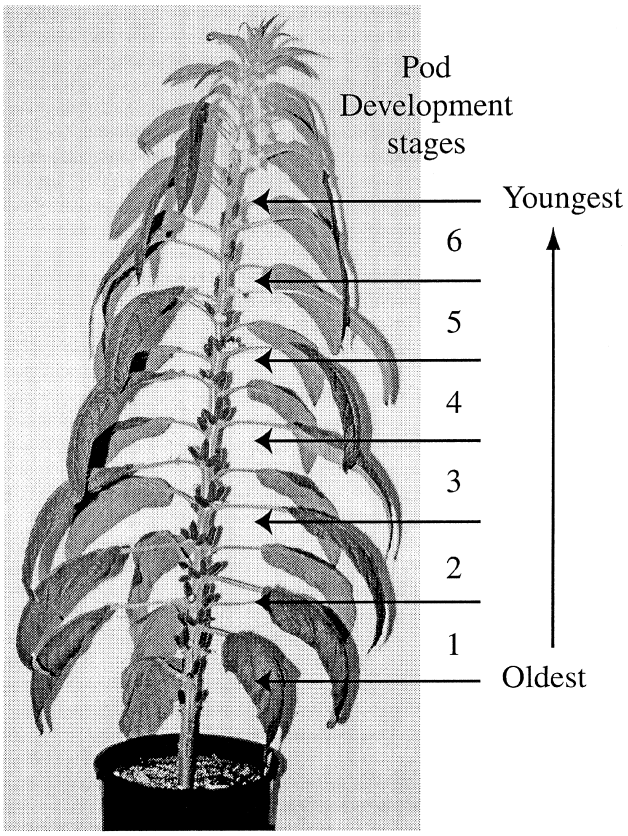


Figure 10 Sesame (*S. indicum*) plant showing pods at different maturation stages.

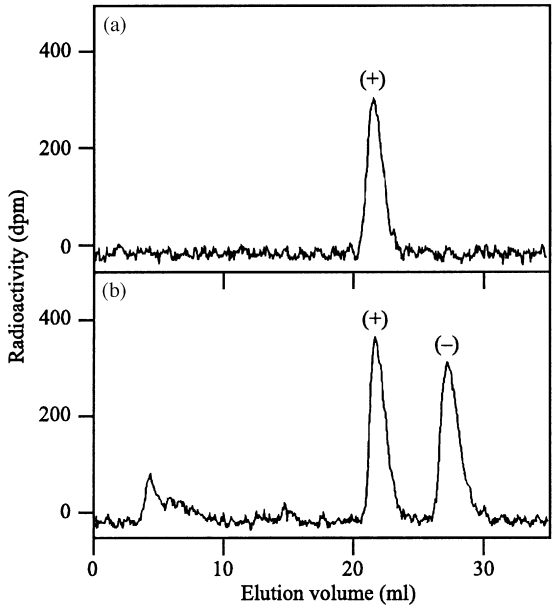
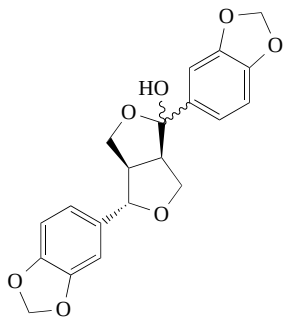


Figure 11 Chiral HPLC separations of (+)- and (−)-piperitols (**78a,b**): (a) (+)-[3-O^{14}CH$_2$, 3′-O^{14}CH$_3$]-Piperitol (**78a**) obtained after incubation of (±)-[3,3′-O^{14}CH$_3$]pinoresinols (**3a,b**) with a *S. indicum* microsomal preparation and (b) (+)- and (−)-antipodes of synthetic piperitols (**78a**) and (**78b**) (after Jiao *et al.*[101]).

Table 3 Effect of various cytochrome P450 enzyme inhibitors on the activity of microsomal bound (+)-piperitol synthase from *S. indicum*. (Standard assay conditions were employed, where 100% activity = 1.46 pkat for (+)-piperitol synthase.)[101]

Cytochrome P450 inhibitor	*Inhibitor concentration* (μM)	*Inhibition* (%)
Clotrimeizol	300	100.0
	35	100.0
Miconazol	300	100.0
	35	71.2
Cytochrome c	170	100.0
	17	52.4
Tropolone	300	65.5
	35	54.4
Metyrapone	300	46.4
	35	26.8

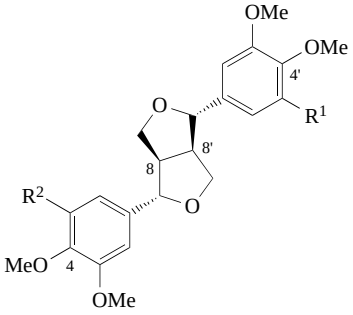


(82) Ketone

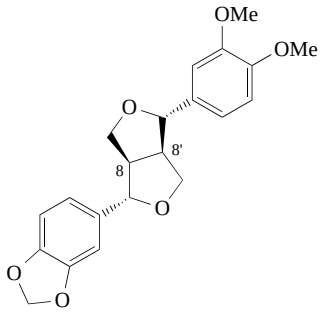
1.25.7.2 *Magnolia kobus*: Pinoresinol and Pinoresinol Monomethyl Ether *O*-Methyltransferase(s)

Magnolia kobus var. *borealis* is a member of the Magnoliaceae, which accumulates furanofuran and tetrahydrofuran lignans in its leaves, e.g., (+)-eudesmin (**83**), (+)-magnolin (**84**), (+)-yangambin (**85**), (+)-kobusin (**86**), and (+)-kobusinols A (**87**) and B (**88**).[107] Its lignans primarily differ from pinoresinol (**3**) by the degree of methoxylation and/or methylenedioxy bridge formation on the aromatic rings, as well as reductive modifications (discussed in Section 1.25.7.3.1). Thus, the methylation patterns (i.e., at C-4/C-4′) for these lignans have a very different regiospecificity to that observed for monolignol formation, which only occurs at the *meta* (C-3) position (see Figure 1 for comparison). Miyauchi and Ozawa have carried out studies to begin to characterize the *M. kobus* lignan *O*-methyltransferases using crude cell-free extracts from leaves (Scheme 1).[108] These were individually incubated with both racemic (±)-pinoresinols (**3a,b**) and (±)-pinoresinol monomethyl ethers (**89**), in the presence of *S*-adenosyl-L-[methyl-^{14}C]methionine, in order to ascertain whether the *O*-methyltransferases were enantiospecific or not. With (±)-pinoresinols (**3a,b**) as substrates, both (+)- and (−)-[4-O^{14}CH$_3$]pinoresinol monomethyl ethers (**89**) were formed, where the (+)-enantiomer predominated (3:1) over the (−)-form; dimethylation affording (+)-eudesmin (**83**), however, was not observed. On the other hand, with (±)-pinoresinol monomethyl ethers (**89**) as substrates, both (+)- and (−)-eudesmins (**83**) were obtained with formation of the (+)-enantiomer again being favored (~2:1).

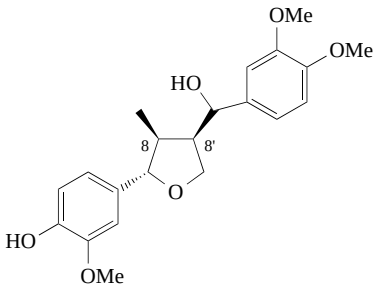
Thus, the crude *O*-methyltransferase preparation catalyzing formation of eudesmin (**83**) *in vitro* from pinoresinol (**3**) is either not stereospecific, or more likely contains various *O*-methyltransferases which differ in their specificities towards each enantiomeric form. These possibilities will only be distinguished when the purified *O*-methyltransferases are obtained. As discussed below (in Section 1.25.7.3.3), this lack of being fully enantiospecific is consistent with previous observations made, using *Forsythia* crude cell-free preparations, in the study of *O*-methylation of matairesinol (**21**) to give arctigenin (**22**).[109]



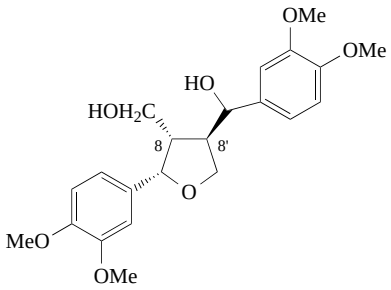
(83) $R^1 = R^2 = H$, Eudesmin, $[\alpha]_D^{25} = +64.0°$ (MeOH, *c* = 0.01)
(84) $R^1 = H$, $R^2 = OMe$, Magnolin, $[\alpha]_D^{25} = +55.9°$ (MeOH, *c* = 0.35)
(85) $R^1 = R^2 = OMe$, Yangambin, $[\alpha]_D^{25} = +63.49°$ (MeOH, *c* = 0.03)



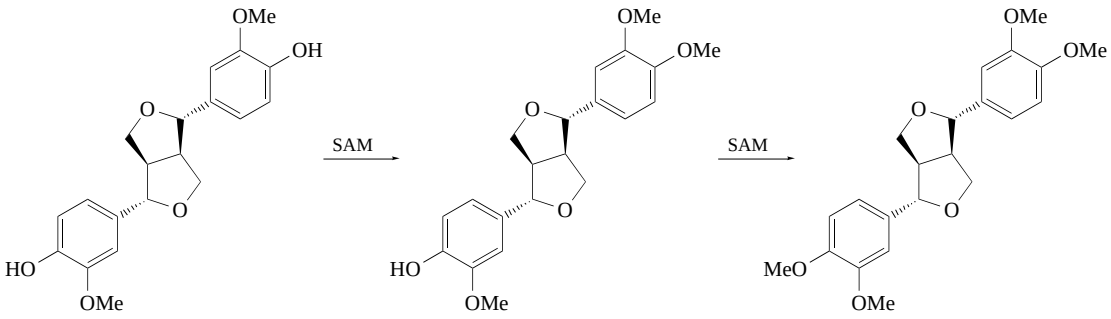
(86) Kobusin, $[\alpha]_D^{25} = +48.0°$ (MeOH, *c* = 0.31)



(87) Kobusinol A, $[\alpha]_D^{25} = +98.2°$ (MeOH, *c* = 0.28)



(88) Kobusinol B, $[\alpha]_D^{25} = +16.4°$ (MeOH, *c* = 0.22)



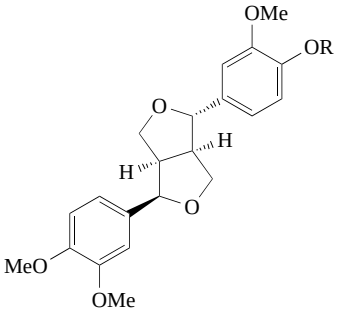
(3) Pinoresinol **(89)** Pinoresinol monomethyl ether **(83)** Eudesmin

Scheme 1

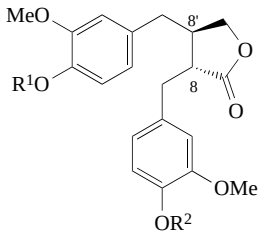
1.25.7.3 *Forsythia intermedia* and *Forsythia suspensa*

Forsythia species accumulate various 8—8′ linked lignans in differing amounts: (+)-pinoresinol (**3a**), (+)-phillygenin (**90**), and (+)-phyllirin (**91**) are present in *F. suspensa*, whereas *F. viridissima* accumulates (−)-matairesinol (**21a**), (−)-arctigenin (**22a**), and (−)-arctiin (**92**). The hybrid, *F. intermedia* (*F. suspensa* × *F. viridissima*), on the other hand, contains all of these lignans.[45]

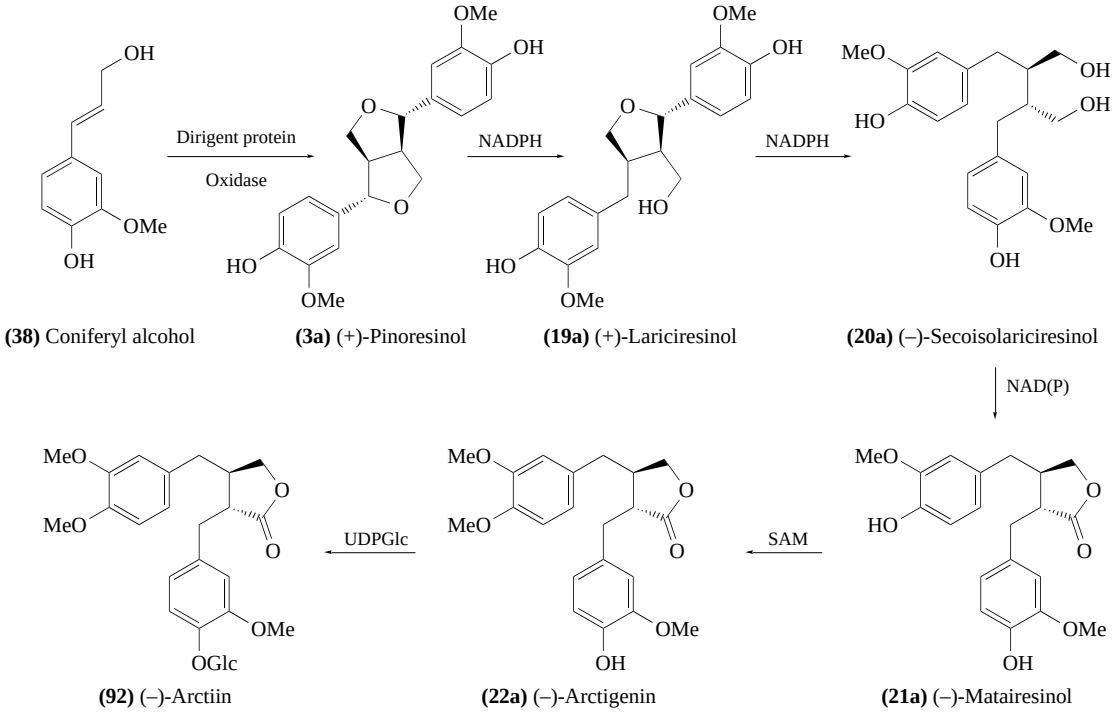
The lignan biosynthetic pathway defined in the *Forsythia* plant family is summarized in Scheme 2, being initiated with the dirigent protein mediated stereoselective coupling of *E*-coniferyl alcohol (**38**) to give (+)-pinoresinol (**3a**) (Section 1.25.6). This is sequentially reduced to afford (+)-lariciresinol (**19a**) and (−)-secoisolariciresinol (**20a**), respectively, with stereospecific dehydrogenation of the latter giving (−)-matairesinol (**21a**), which is subsequently converted into (−)-arctigenin (**22a**) and (−)-arctiin (**92**). The enzymology associated with (+)-pinoresinol (**3a**) metabolism in *F. intermedia* is described below.



(90) R = H, (+)-Phillygenin
(91) R = Glc, (+)-Phillyrin



(21a) $R^1 = R^2$ = H, (–)-Matairesinol
(22a) R^1 = Me, R^2 = H, (–)-Arctigenin
(92) R^1 = Me, R^2 = Glc, (–)-Arctiin



(38) Coniferyl alcohol **(3a)** (+)-Pinoresinol **(19a)** (+)-Lariciresinol **(20a)** (–)-Secoisolariciresinol

(92) (–)-Arctiin **(22a)** (–)-Arctigenin **(21a)** (–)-Matairesinol

Scheme 2

1.25.7.3.1 (+)-Pinoresinol/(+)-lariciresinol reductase

The first enzyme identified in the lignan pathway was the bifunctional, NADPH-dependent, (+)-pinoresinol/(+)-lariciresinol reductase.[88,110,111] This circa 35 kDa enzyme was purified (>3000-fold) to apparent homogeneity from *F. intermedia*,[111] and shown to catalyze the enantiospecific conversion of (+)-pinoresinol (**3a**) into (+)-lariciresinol (**19a**), and (+)-lariciresinol (**19a**) into (−)-secoisolariciresinol (**20a**); the corresponding antipodes (**3b**) and (**19b**) did not serve as substrates.

It is a type A reductase, as established using [4*R*-^{3}H] and [4*S*-^{3}H]NADPH as cofactors, since only the pro-*R* hydrogen on the nicotinamide ring of NADPH was abstracted and transferred to the lignan product.[88] As shown in Scheme 3, this hydride transfer from NADPH during furanofuran lignan reduction could occur in any one of three ways, i.e., via direct hydride attack onto the furano ring or the regenerated quinone methide, resulting in either retention or inversion of apparent configuration at C-7/C-7′. Alternatively, hydride attack could occur at either face and result in racemization. That an ‘‘inversion’’ of configuration mechanism occurred was established by exam-

ination of the products obtained following individual incubation of (±)-[7,7′-$^2H_2$]pinoresinols (**3a,b**) and [7,7′-$^2H_3$]lariciresinols (**19a,b**) with (+)-pinoresinol/(+)-lariciresinol reductase, in the presence of NADPH (1.6 mM): ^{1}H NMR spectroscopic analyses (Figure 12) of the enzymatically generated lariciresinol (**19**) and secoisolariciresinol (**20**) revealed that hydride (deuteride) transfer occurred in

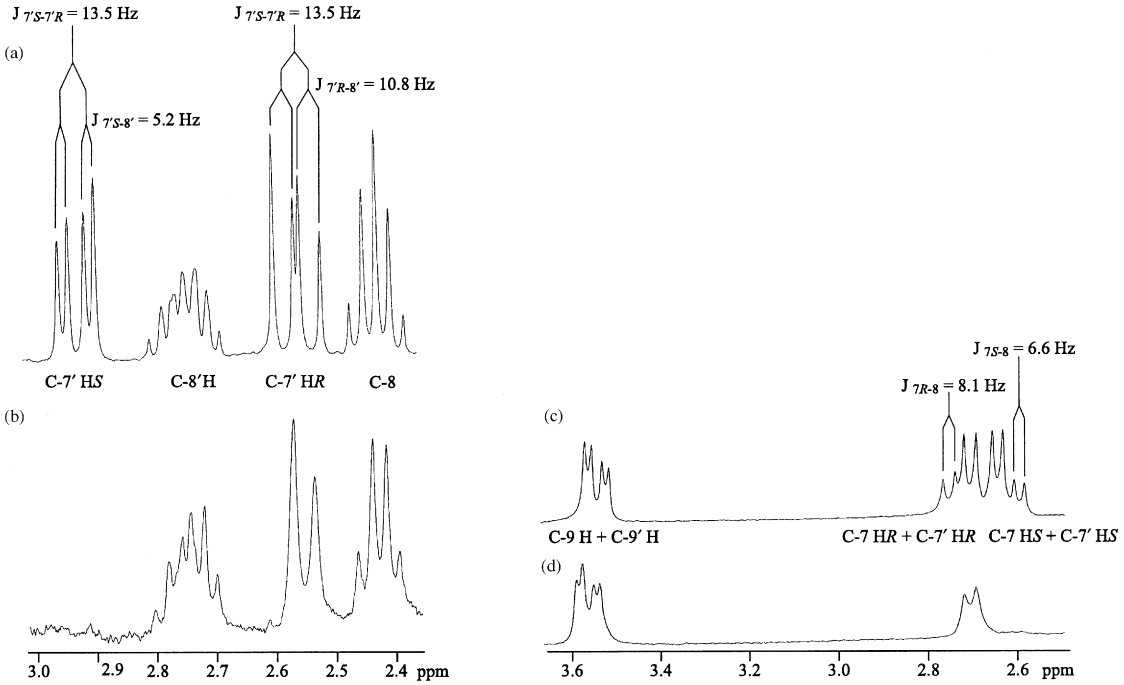
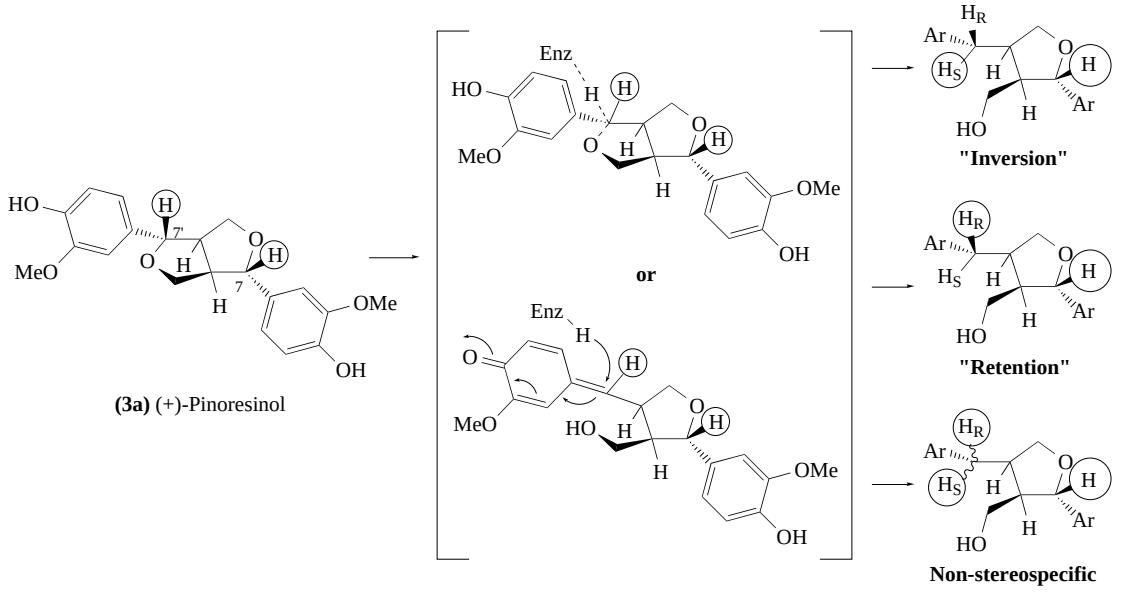


Figure 12 Partial ^{1}H NMR spectra of lariciresinol (**19**) showing spectral regions for C-7′, C-8′, and C-8 proton resonances ((a) and (b)) and secoisolariciresinol (**20**) showing spectral regions for C-7 and C-9 proton resonances ((c) and (d)). (a) Synthetic (±)-lariciresinols (**19a,b**), (b) enzymatically synthesized (+)-[7,7′*S*-$^2H_2$]lariciresinol (**19a**) obtained following incubation with (±)-[7,7′-$^2H_2$]pinoresinols (**3a,b**), (c) synthetic (±)-secoisolariciresinols (**20a,b**), and (d) enzymatically synthesized (−)-[7,7′-$^2H_3$]secoisolariciresinol (**20a**) obtained following incubation with (±)-[7,7′*S*-$^2H_3$]lariciresinols (**19a,b**).[88]



Scheme 3

a completely stereospecific manner, with the incoming hydride (deuteride) taking up the pro-*R* position to give (+)-[7,7′*S*-$^2H_2$]lariciresinol (**19a**) and (−)-[7,7′*S*-$^2H_3$]secoisolariciresinol (**20a**), respectively, i.e., with "inversion" of configuration at C-7 and C-7′.

The nucleotide sequence for the gene encoding (+)-pinoresinol/(+)-lariciresinol reductase has a single open reading frame encoding a polypeptide of 312 amino acids (Figure 13), giving a calculated molecular mass of 34.9 kDa.[111] The authenticity of its sequence was established via assay of functionally recombinant (+)-pinoresinol/(+)-lariciresinol reductase, as either its β-galactosidase fusion protein[111] or the "native" protein in a pSBETa[112] vector system expressed in *E. coli*: both recombinant proteins catalyzed the enantiospecific conversion of (+)-pinoresinol (**3a**) into (+)-lariciresinol (**19a**), and the latter into (−)-secoisolariciresinol (**20a**): the corresponding antipodes were not used as substrates (Figure 14). Sequence analysis of the cDNA also revealed that the NADPH-binding domain was situated close to the N-terminus, consisting of three conserved glycine (as GXGXXG, with X representing any residue) and four conserved hydrophobic residues (underlined in Figure 13); these are required for correct packaging for domain formation.[113] The sequence also revealed considerable homology (63.5% to 61.6% similarity and 44.4% to 41.3% identity) to isoflavone reductases (see Table 4), which catalyze the analogous reduction of α,β unsaturated ketones during isoflavonoid formation.[111] For example, *Pisum sativum* isoflavone reductase converts 2′-hydroxypseudobaptigenin (**93**) into (3*R*)-sophorol (**94**), a precursor of the phytoalexin, (+)-pisatin (**95**) (Scheme 4). Moreover, given that pinoresinol-derived lignans appear to be found in even the most primitive extant plants (e.g., ferns, gymnosperms), whereas the isoflavonoids may be more restricted (e.g., to the Leguminosae), it is tempting to speculate that pinoresinol/lariciresinol reductase is the evolutionary forerunner of the isoflavonoid reductases.[34] (+)-Pinoresinol/(+)-lariciresinol reductase also contains five conserved possible phosphorylation sites, including Thr-302 (casein kinase II-type protein phosphorylation site) suggesting that its activity might be regulated by protein kinase cascades;[34,111] a somewhat analogous situation also holds for isoflavonoid reductase(s).

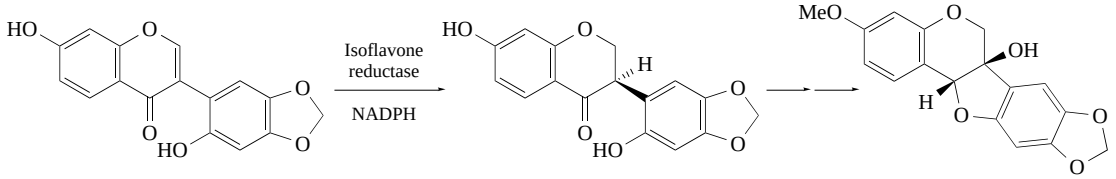
In agreement with these findings, (+)-pinoresinol/(+)-lariciresinol reductase has also been detected in cell-free extracts from *Forsythia koreana* by Umezawa *et al.*[114]

Table 4 Homology between (+)-pinoresinol/(+)-lariciresinol reductase from *Forsythia intermedia* and isoflavone reductases from three legumes.[111]

Plant species	*Similarity* (%)	*Identity* (%)
Cicer arietinum	63.5	44.4
Medicago sativa	62.6	42.0
Pisum sativum	61.6	41.3

1.25.7.3.2 (−)-Secoisolariciresinol dehydrogenase

This $NAD(P)^+$-requiring enzyme catalyzes the enantiospecific conversion of (−)-secoisolariciresinol (**20a**) into (−)-matairesinol (**21a**).[115,116] It has been purified (> 6000-fold) to apparent homogeneity from *F. intermedia* stem tissue, and is an ~32 kDa protein, as estimated by SDS-PAGE analysis.[117,118] Interestingly, the presumed lactol (**96**) intermediate was not detected in any of



(93) 2-Hydroxypseudobaptigenin **(94)** (3*R*)-Sophorol **(95)** (+)-Pisatin

Scheme 4

```
     AATTCGGCACGAGAAAAACAGAGAGAGATGGGAAAAAGCAAAGTTTTGATCATTGGGGGT
                                  M  G  K  S  K  V  L  I  I  G  G

  34 ACAGGGTACTTAGGGAGGAGATTGGTTAAGGCAAGTTTAGCTCAAGGTCATGAAACATAC
  12 T  G  Y  L  G  R  R  L  V  K  A  S  L  A  Q  G  H  E  T  Y

  94 ATTCTGCATAGGCCTGAAATTGGTGTTGATATTGATAAAGTTGAAATGCTAATATCATTT
  32 I  L  H  R  P  E  I  G  V  D  I  D  K  V  E  M  L  I  S  F

 154 AAAATGCAAGGAGCTCATCTTGTATCTGGTTCTTTCAAGGATTTCAACAGTCTGGTCGAG
  52 K  M  Q  G  A  H  L  V  S  G  S  F  K  D  F  N  S  L  V  E

 214 GCTGTCAAGCTCGTAGACGTAGTAATCAGCGCCATTTCTGGTGTTCATATTCGAAGCCAT
  72 A  V  K  L  V  D  V  V  I  S  A  I  S  G  V  H  I  R  S  H

 274 CAAATTCTTCTTCAACTCAAGCTTGTTGAAGCTATTAAAGAGGCTGGAAATGTCAAGAGA
  92 Q  I  L  L  Q  L  K  L  V  E  A  I  K  E  A  G  N  V  K  R

 334 TTTTTACCATCTGAGTTTGGAATGGATCCTGCAAAATTTATGGATACGGCCATGGAACCC
 112 F  L  P  S  E  F  G  M  D  P  A  K  F  M  D  T  A  M  E  P

 394 GGAAAGGTAACACTTGATGAGAAGATGGTGGTAAGGAAAGCAATTGAAAAGGCTGGGATT
 132 G  K  V  T  L  D  E  K  M  V  V  R  K  A  I  E  K  A  G  I

 454 CCTTTCACATATGTCTCTGCAAATTGCTTTGCTGGTTATTTCTTGGGAGGTCTCTGTCAA
 152 P  F  T  Y  V  S  A  N  C  F  A  G  Y  F  L  G  G  L  C  Q

 514 TTTGGCAAAATTCTTCCTTCTAGAGATTTTGTCATTATACATGGAGATGGTAACAAAAAA
 172 F  G  K  I  L  P  S  R  D  F  V  I  I  H  G  D  G  N  K  K

 574 GCAATATATAACAATGAAGATGATATAGCAACTTATGCCATCAAAACAATTAATGATCCA
 192 A  I  Y  N  N  E  D  D  I  A  T  Y  A  I  K  T  I  N  D  P

 634 AGAACCCTCAACAAGACAATCTACATTAGTCCTCCAAAAAACATCCTTTCACAAAGAGAA
 212 R  T  L  N  K  T  I  Y  I  S  P  P  K  N  I  L  S  Q  R  E

 694 GTTGTTCAGACATGGGAGAAGCTTATTGGGAAAGAACTGCAGAAAATTACACTCTCGAAG
 232 V  V  Q  T  W  E  K  L  I  G  K  E  L  Q  K  I  T  L  S  K

 754 GAAGATTTTTTAGCCTCCGTGAAAGAGCTCGAGTATGCTCAGCAAGTGGGATTAAGCCAT
 252 E  D  F  L  A  S  V  K  E  L  E  Y  A  Q  Q  V  G  L  S  H

 814 TATCATGATGTCAACTATCAGGGATGCCTTACGAGTTTTGAGATAGGAGATGAAGAAGAG
 272 Y  H  D  V  N  Y  Q  G  C  L  T  S  F  E  I  G  D  E  E  E

 874 GCATCTAAACTTTATCCAGAGGTTAAGTATACCAGTGTGGAAGAGTACCTCAAGCGTTAC
 292 A  S  K  L  Y  P  E  V  K  Y  T  S  V  E  E  Y  L  K  R  Y

 934 GTGTAGTTGAAAGCTTTCCATTATTATTGTAATAATATTTAAATCAGTATGTAGTTTTAA
 312 V  *

 994 ATTTCGTTAAATAATATGTGTTGAATTTTGCTTCCAAAAA
```

Figure 13 Complete sequence of *F. intermedia* (+)-pinoresinol/(+)-lariciresinol reductase cDNA plr-Fi1. The NADPH binding domain conserved residues are underlined. The five possible phosphorylation sites that are conserved among (+)-pinoresinol/(+)-lariciresinol reductase and the characterized isoflavone reductases are double underlined. The stop codon is indicated by an asterisk.[111]

the enzyme assays using native protein (Scheme 5). The genes encoding (−)-secoisolariciresinol dehydrogenase were obtained using a PCR-guided strategy, coupled with the screening of the *F. intermedia* cDNA library. Two of the five cDNA clones obtained, SMDEHY631 and DEHY130, were subsequently expressed in *E. coli*, and both gave proteins capable of enantiospecifically converting (−)-secoisolariciresinol (**20a**) into (−)-matairesinol (**21a**). Both had single open-reading frames encoding polypeptides of 272 amino acids, giving calculated molecular masses of 29 kDa, and isoelectric points of ~6.1. However, in contrast to the native dehydrogenase, the corresponding lactol (**96**) in each instance was observed as an intermediate, during conversion of (−)-secoisolariciresinol (**20a**) into (−)-matairesinol (**21a**) (Scheme 5).

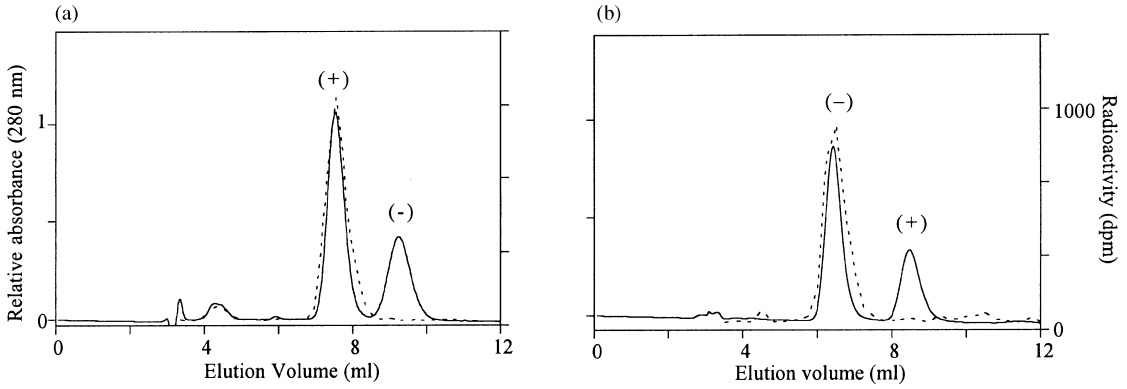
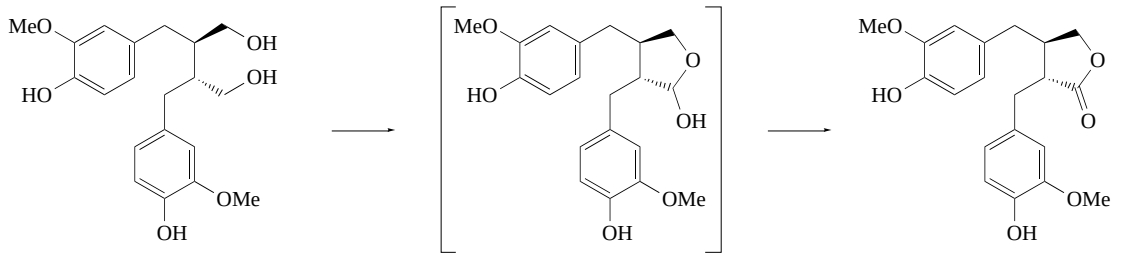


Figure 14 Chiral column HPLC analysis of lignans (a) lariciresinol (**19**) and (b) secoisolariciresinol (**20**). (±)-Pinoresinols (**3a,b**) were incubated with recombinant (+)-pinoresinol/(+)-lariciresinol reductase in the presence of [4*R*-^{3}H]NADPH, with unlabeled (±)-lariciresinols (**19a,b**) and (±)-secoisolariciresinols (**20a,b**) added as radiochemical carriers. The solid line represents the UV absorbance trace (280 nm), whereas the dashed line shows that radioactivity is only incorporated into (+)-lariciresinol (**19a**) and (−)-secoisolariciresinol (**20a**), respectively.[111] For elution conditions see Figure 3.

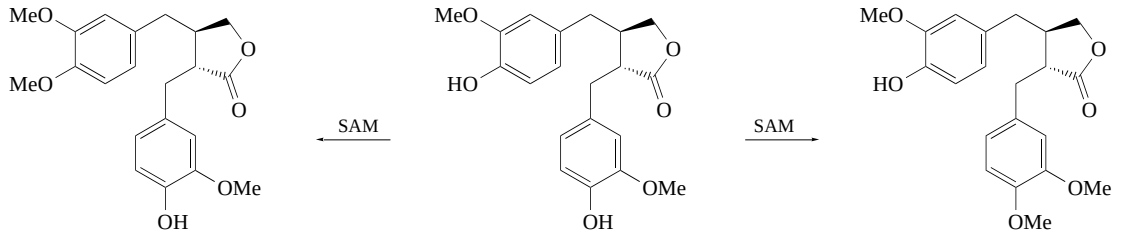


(20a) (–)-Secoisolariciresinol **(96)** (–)-Lactol **(21a)** (–)-Matairesinol

Scheme 5

1.25.7.3.3 Matairesinol O*-methyltransferase*

Cell-free extracts of *F. intermedia* were examined in order to attempt to establish whether *O*-methylation of matairesinol (**21**) giving arctigenin (**22**) occurred in an enantio- and regiospecific manner.[109] Incubation with (±)-[Ar-^{3}H]matairesinols (**21a,b**), in the presence of *S*-adenosyl-L-methionine, gave both radiolabeled arctigenin (**22**) and isoarctigenin (**97**) (Scheme 6), with the (−)-enantiomeric form being preferentially obtained in both cases (2:1 and 2.5:1, respectively), i.e., *O*-methylation was neither regio- nor stereospecific. As for eudesmin (**83**) biosynthesis, purification of the actual *O*-methyltransferase involved in catalyzing the formation of (−)-arctigenin (**22a**) is required in order to determine whether the *O*-methyltransferase is truly enantio- and regiospecific.



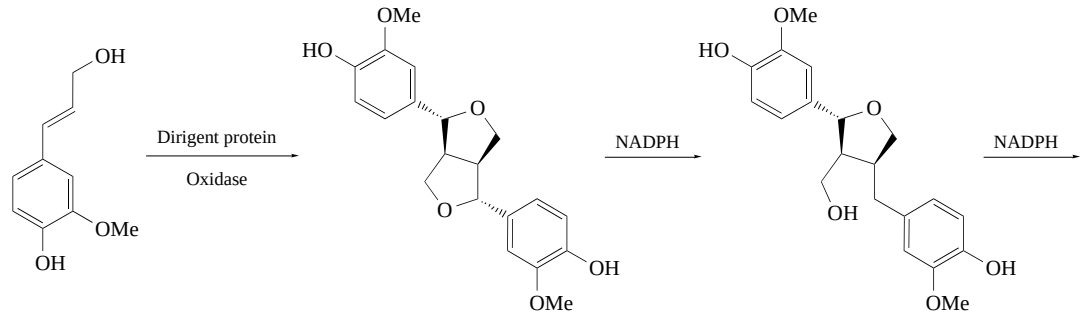
(22) Arctigenin **(21)** Matairesinol **(97)** Isoarctigenin

Scheme 6

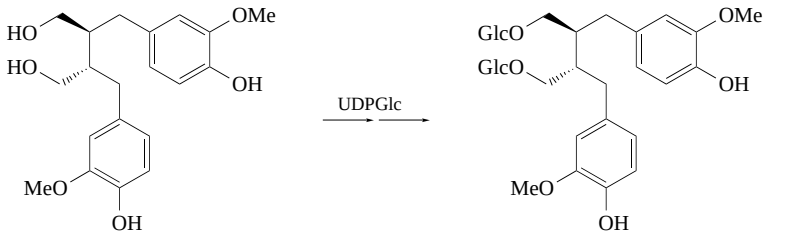
1.25.7.4 *Linum usitatissimum*: (−)-Pinoresinol/(−)-Lariciresinol Reductase and (+)-Secoisolariciresinol Glucosyltransferase(s)

Flaxseed (*L. usitatissimum*, Linaceae) has been used for several millennia for medicinal purposes. There is considerable renewed interest in it because of the high levels (3–3.5%) of secoisolariciresinol diglucoside (**98**), which has an important role in the diet due to its protection against onset of breast and prostate cancers[119,120] (see Section 1.25.14), and can be solubilized from flaxseed under very basic conditions (e.g., 0.3 M sodium methoxide).[121] Hydrolysis of secoisolariciresinol diglucoside (**98**) gives the aglycone (**20**), the chiral analysis (Chiralcel OD, Daicel) of which revealed that essentially only the (+)-antipode (**20b**) was present.[97]

The proposed biochemical pathway to secoisolariciresinol diglucoside (**98**) is shown in Scheme 7. Preliminary experiments[97] have indicated that stereoselective coupling of *E*-coniferyl alcohol (**38**) occurs to give (−)-pinoresinol (**3b**). Based on results from enzyme assays, using cell-free extracts of developing seeds, this is then reduced to first give (−)-lariciresinol (**19b**) and then (+)-secoisolariciresinol (**20b**). Screening procedures are under way to obtain the corresponding gene(s) for both the dirigent protein and reductase(s) from this source.



(38) Coniferyl alcohol **(3b)** (–)-Pinoresinol **(19b)** (–)-Lariciresinol



(20b) (+)-Secoisolariciresinol **(98)** (+)-Secoisolariciresinol diglucoside

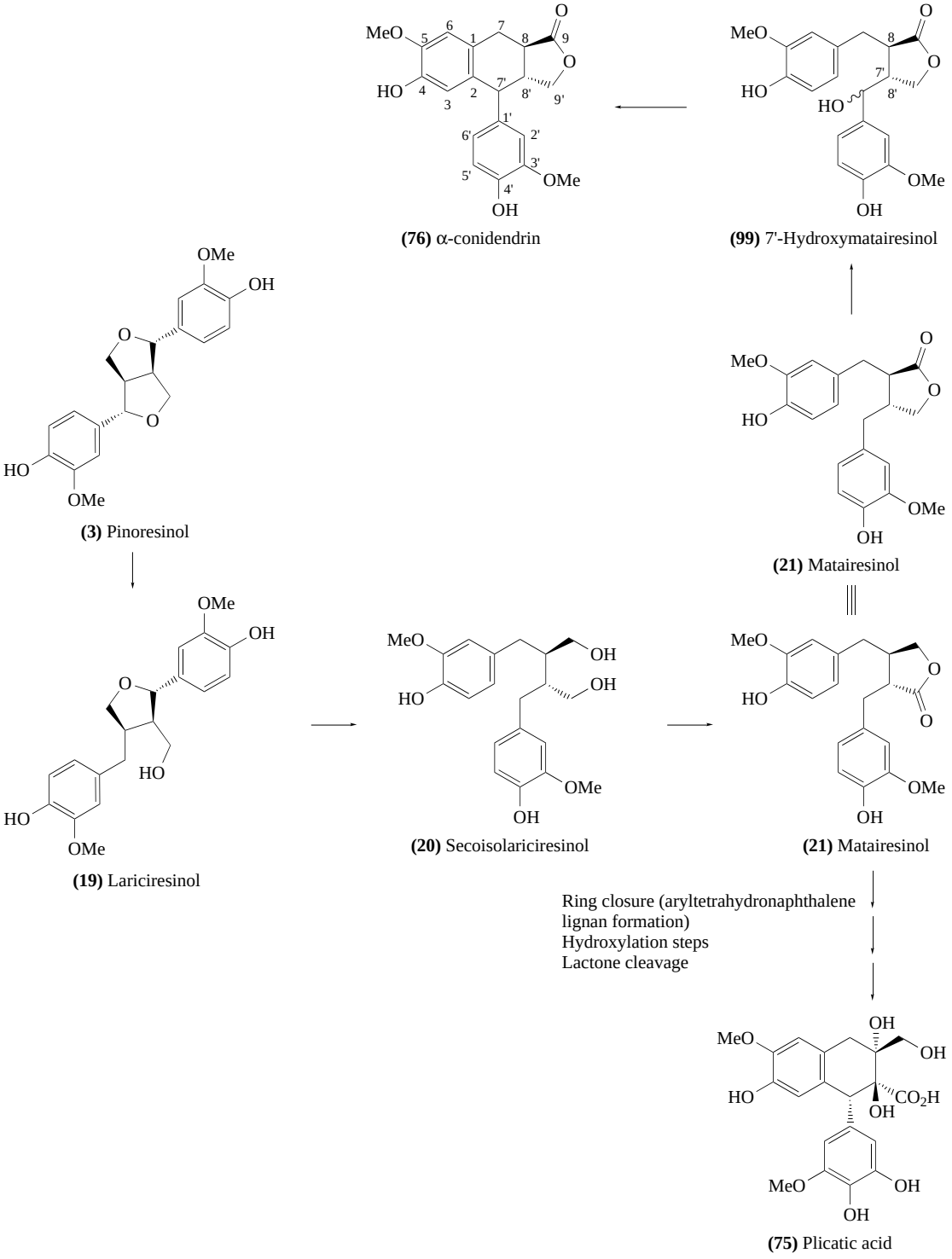
Scheme 7

Preliminary assays, with partially purified secoisolariciresinol glucosyltransferase, in the presence of UDP[1-^{3}H]glucose and (±)-secoisolariciresinols (**20a,b**), have revealed that maximum activity of the glucosyltransferase occurs during the second week of seed development.

1.25.7.5 *Thuja plicata* and *Tsuga heterophylla*: Pinoresinol/Lariciresinol Reductases and Other Enzymatic Conversions

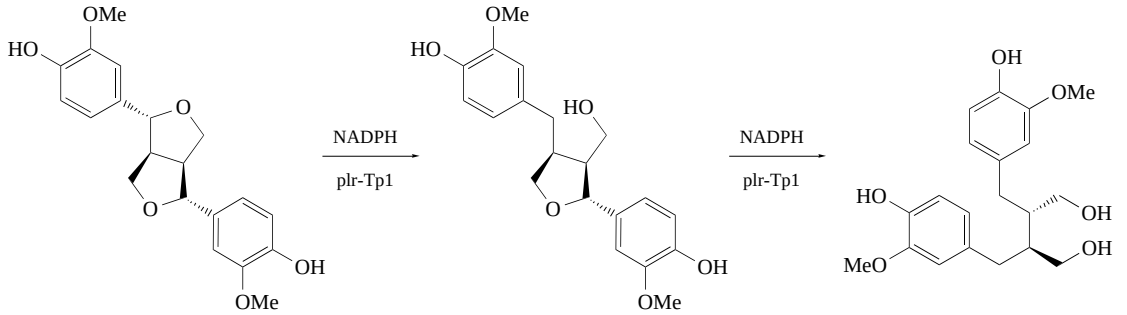
Western red cedar (*Thuja plicata*) and western hemlock (*Tsuga heterophylla*) are two long-living gymnosperms, with the former having life spans in excess of 3000 years. Of the two species, cedar heartwood is particularly valued for its color, durability, and texture, this in part being due to the massive deposition (~20% of dry weight) of heartwood (oligomeric) lignans, derived from plicatic acid (**75**) and its congeners.[35] In a somewhat analogous manner, western hemlock accumulates various lignans, such as 7′-hydroxymatairesinol (**99**) (0.3% in sapwood) and α-conidendrin (**76**) (0.05% in sapwood and 0.15 to 0.2% in heartwood).[122] Based on their respective chemical structures, and the results from studies in the authors' laboratory, it was rationalized that both plicatic acid (**75**) and α-conidendrin (**76**) were pinoresinol-derived (Scheme 8). Plicatic acid (**75**) could result from

matairesinol (**21**) via ring closure (aryltetrahydronaphthalene ring formation), hydroxylations (at both aromatic and aliphatic positions), and lactone ring cleavage, whereas α-conidendrin (**76**) might result from regiospecific hydroxylation at the 7′-position of matairesinol (**21**) to give 7′-hydroxymatairesinol (**99**), with subsequent ring closure. It was important to establish, therefore, whether the corresponding pinoresinol/lariciresinol reductases were present in these plant systems in addition to the dirigent proteins (see Section 1.25.6.4).



Scheme 8

Thus, screening of a western red cedar cDNA library, via a PCR-guided strategy, gave two pinoresinol/lariciresinol reductase cDNAs with ~58% identity and ~70% similarity to plr-Fi1. Their recombinant proteins, when expressed in *E. coli*, displayed distinct pinoresinol/lariciresinol reductase activities.[30,123,124] The first, plr-Tp1, catalyzed the NADPH-dependent conversion of (−)-pinoresinol (**3b**) into (−)-lariciresinol (**19b**), this being subsequently reduced to (+)-secoisolariciresinol (**20b**) (see Scheme 9), with the enantiospecificity of the conversion being determined by chiral HPLC analysis of the enzymatic products so obtained (Figure 15(a)). The second, plr-Tp2, isolated from the same plant source, converted (+)-pinoresinol (**3a**) into (+)-lariciresinol (**19a**), and then into (−)-secoisolariciresinol (**20a**) (see Scheme 10 and Figure 15(b)). That is, western red cedar has genes encoding two reductive pathways with differing enantiospecificities. Although the biological significance of both pathways needs to be established, this study reveals that the pathway to plicatic acid (**75**) indeed occurs via involvement of both dirigent proteins and pinoresinol/lariciresinol reductase(s).



(3b) (–)-Pinoresinol **(19b)** (–)-Lariciresinol **(20b)** (+)-Secoisolariciresinol

Scheme 9

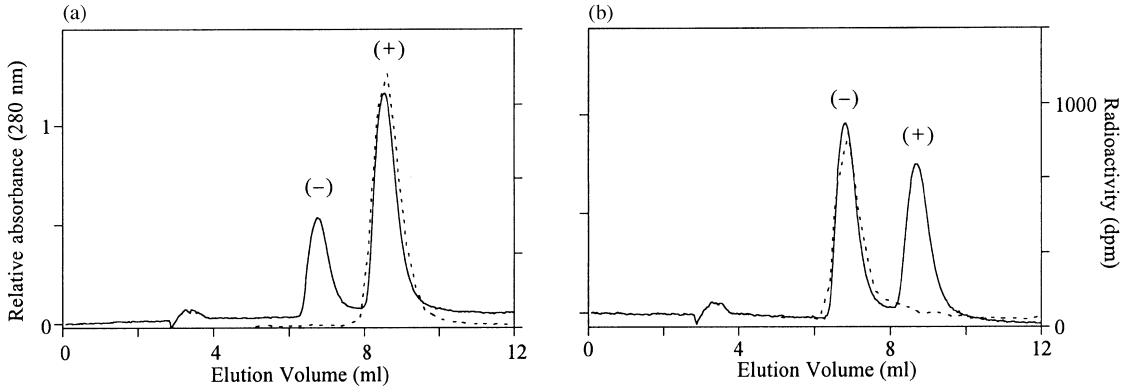
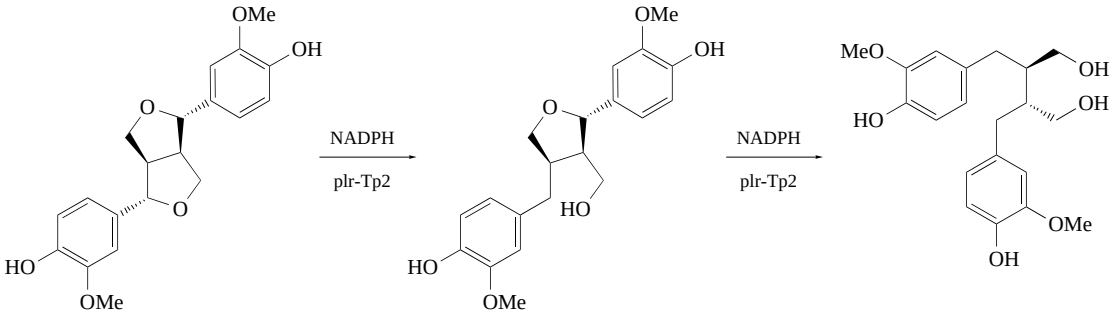


Figure 15 Chiral column HPLC analysis of the secoisolariciresinol (**20**) obtained after incubation of (±)-[3,3′-$O^{14}CH_3$]pinoresinols (**3a,b**) with recombinant pinoresinol/lariciresinol reductase, in the presence of NADPH. (a) plr-Tp1, and (b) plr-Tp2. Unlabeled (±)-secoisolariciresinols (**20a,b**) were added as radiochemical carriers. The solid line represents the absorbance trace at 280nm, whereas the dashed line shows the radioactivity incorporated into the corresponding secoisol ariciresinol (**20**) product.

Further characterization of the recombinant reductases, plr-Tp1 and plr-Tp2, resulted in the discovery that both differ substantially from their *Forsythia* counterpart: plr-Tp1 preferentially converts (−)-pinoresinol (**3b**) into (−)-lariciresinol (**19b**), but can also slowly reduce (+)-pinoresinol (**3a**) to give (+)-lariciresinol (**19a**) although the latter is not further metabolized. The converse situation was observed for plr-Tp2. It preferentially converts (+)-pinoresinol (**3a**) into (+)-lariciresinol (**19a**), but can also slowly reduce (−)-pinoresinol (**3b**) into (−)-lariciresinol (**19b**). As for plr-Tp1, plr-Tp2 is fully enantiospecific for lariciresinol (**19**) reduction, since it only utilizes (**19a**) as a substrate and not its (−)-enantiomer (**19b**).[30,124]

In a comparable manner, a *T. heterophylla* cDNA library was screened, this resulting in two



(3a) (+)-Pinoresinol **(19a)** (+)-Lariciresinol **(20a)** (–)-Secoisolariciresinol

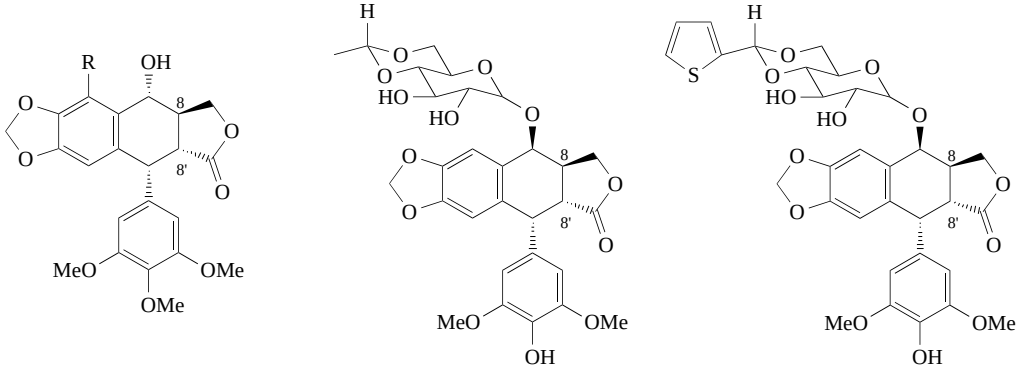
Scheme 10

putative cDNAs encoding pinoresinol/lariciresinol reductase with ~73% similarity and ~62% identity.

Taken together, these findings may help explain the results of Umezawa and Shimada where the formation of secoisolariciresinol (**20**), catalyzed by cell-free extracts from *Arctium lappa* petioles, was investigated.[125] In that study, coniferyl alcohol (**38**) was incubated with the cell-free preparation in the presence of H_2O_2 and NADPH. Under these conditions, nonspecific peroxidase catalyzed coupling occurs to give the racemic lignans, (±)-(**5a,b**), (±)-(**6a,b**), and (±)-(**3a,b**), with the latter then undergoing NAPDH-dependent reduction to afford secoisolariciresinol (**20**), whose (+)-enantiomer (**20b**) was in enantiomeric excess (e.e. 20%). Additionally, (+)-secoisolariciresinol (**20b**) was isolated from MeOH extracts of *A. lappa* petioles in 78% enantiomeric excess.[125] These data are consistent with both (+)- and (−)-pinoresinol/lariciresinol reductases being present in this species, as previously described for western red cedar.

1.25.7.6 *Linum flavum* and *Podophyllum hexandrum*: Podophyllotoxin and its Pinoresinol Precursor

(−)-Podophyllotoxin (**23**) accumulates up to 4% of the dry weight in *P. hexandrum* (Podophyllaceae) rhizomes[126] and (−)-5-methoxypodophyllotoxin (**100**) up to 3.5% of the dry weight in *L. flavum* (Linaceae) roots.[127] Podophyllotoxin (**23**), as its etoposide (**101**) and teniposide (**102**) derivatives, is used in treatment of various cancers (see Section 1.25.14.2). Because of the limited supply of *Podophyllum* rhizomes, due to their intensive collection in the wild, there is a growing interest in defining and exploiting the podophyllotoxin (**23**) biosynthetic pathway. This is in part because studies, using both cell suspension and root tissue cultures, have not been very successful in producing significantly elevated levels of either podophyllotoxin (**23**) or (−)-5-methoxypodophyllotoxin (**100**).[127–132] Accordingly, both defining the pathway and establishing what factors affect its induction seem to be worthwhile goals in preparation for future biotechnological manipulations.



(23) R = H, (–)-Podophyllotoxin
(100) R = OMe, (–)-5-Methoxypodophyllotoxin

(101) Etoposide **(102)** Teniposide

Preliminary investigations by Broomhead *et al.* suggested that (−)-matairesinol (**21a**) might be a precursor of podophyllotoxin (**23**).[133] Accordingly, it was important to establish whether the biochemical pathway to matairesinol (**21**) was also present in *L. flavum*. Thus, cell-free extracts from *L. flavum* roots were incubated with (±)-pinoresinols (**3a,b**) (20 mM) in the presence of NADPH (20 mM), where it was established that both (+)-lariciresinol (**19a**) and (−)-secoisolariciresinol (**20a**) were formed.[134] Furthermore, a partially purified secoisolariciresinol dehydrogenase preparation was also obtained which, when incubated with (±)-[Ar-^{2}H]secoisolariciresinols (**20a,b**) and NAD, only gave (−)-[Ar-^{2}H]matairesinol (**21a**). The enzymatically formed (−)-[Ar-^{2}H]matairesinol (**21a**) had a cluster of ions centered at *m/z* 360 (Figure 16(a)) relative to that of (±)-[Ar-^{2}H]secoisolariciresinol (**20a,b**) cluster of ions at *m/z* 364 (Figure 16(b)), thereby establishing the intact enzymatic conversion, and thus verification of the pathway to (−)-matairesinol (**21a**) in this species.

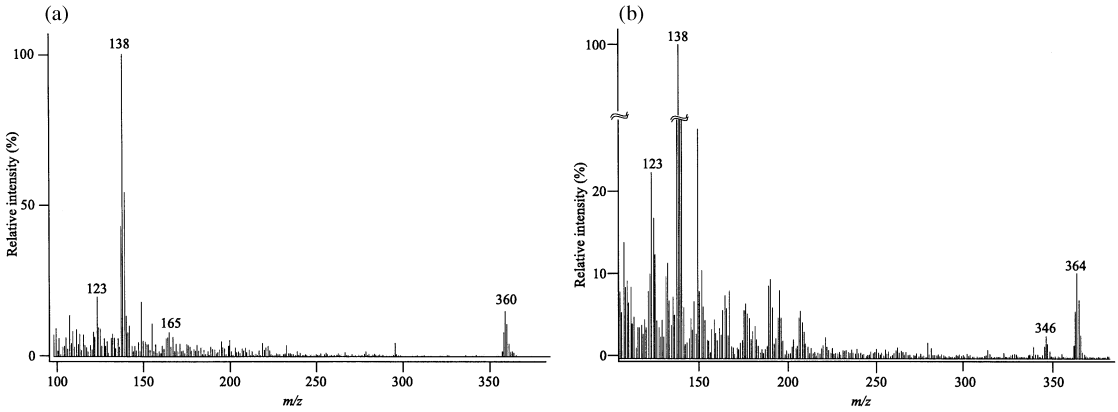


Figure 16 Mass spectra of (a) (−)-[Ar-^{2}H]matairesinol (**21a**) obtained following incubation with a partially purified secoisolariciresinol dehydrogenase preparation of *Linum flavum* and (±)-[Ar-^{2}H]secoisolariciresinols (**20a,b**) in the presence of NAD, (b) (±)-[Ar-^{2}H]secoisolariciresinol (**20a,b**) substrates.

Thus, in summary, pinoresinol (**3**) formation and metabolism have been shown to occur in *Forsythia* species, *Linum usitatissimum*, *Thuja plicata*, *Tsuga heterophylla*, *Linum flavum*, and *Podophyllum peltatum*, thereby establishing its pivotal role in 8—8′ linked lignan biosynthesis.

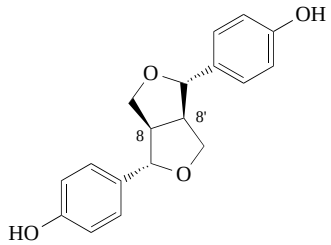
1.25.8 ARE DIRIGENT PROTEIN HOMOLOGUES INVOLVED IN OTHER 8—8′ PHENOXY RADICAL COUPLING PROCESSES?

In addition to coniferyl alcohol (**38**) derived 8—8′ linked lignans, there are optically active 8—8′ linked lignans which provisionally appear to result from coupling of other monomeric precursors, such as *E*-*p*-coumaryl alcohol (**32**), *E*-sinapyl alcohol (**44**), various hydroxycinnamic acids/allylphenols, and their derivatives. Although each of their biochemical pathways await delineation, the occurrence of these lignans suggests the presence of dirigent proteins with differing substrate specificities. This, in turn, is consistent with the existence of an entire new class of proteins, and this possibility is discussed below using just a few examples for illustrative purposes only.

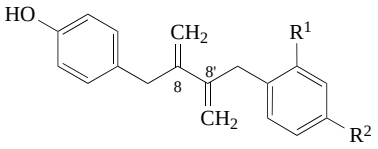
1.25.8.1 Ligballinol (*p*-Coumarylresinol) and Related Structures

Based on the chemical structures of ligballinol (= *p*-coumarylresinol) (**103**), termilignan (**104**), anolignan B (**105**), and thannilignan (**106**), it can be proposed that all are either *p*-coumaryl alcohol (**32**) or *p*-hydroxyarylpropene (**60**) derived. Of these, ligballinol (*p*-coumarylresinol) (**103**) was first isolated in optically active form from squirting cucumber (*Ecballium elaterium*, Cucurbitaceae) fruits,[135] but also accumulates in enantiomeric excess in red bean (*Vigna angularis*, Leguminosae) cell suspension cultures treated with actinomycin D.[136] Interestingly, although both isolates have very distinct $[\alpha]_D$ values suggesting different enantiomeric compositions, the chiral HPLC analyses of the ligballinol (*p*-coumarylresinol) (**103**) preparations have not been reported. Termilignan

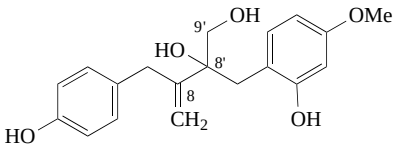
(**104**), anolignan B (**105**), and the optically active thannilignan (**106**), from *Terminalia bellerica* (Combretaceae),[137] can also be proposed to partly result from regiospecific/stereoselective *E*-*p*-coumaryl alcohol (**32**) coupling, although they might also derive from the corresponding allylphenol (**60**). Whether such coupling is under the control of dirigent-like proteins awaits delineation, as does identification of the nature of the precursors involved.



(103) Ligballinol = *p*-coumarylresinol
(*Ecballium elaterium*), $[\alpha]_D$ = –7.1° (MeOH, *c* = 1.61)
(*Vigna angularis*), $[\alpha]_D^{28}$ = +25.3° (MeOH, *c* = 0.06)



(104) R^1 = OH, R^2 = OMe, Termilignan
(105) R^1 = H, R^2 = OH, Anolignan B
(*Terminalia bellerica*)



(106) (–)-Thannilignan
$[\alpha]_D^{24}$ = –63.9° ($CHCl_3$, *c* = 0.3)
(*Terminalia bellerica*)

1.25.8.2 Syringaresinol and Medioresinol

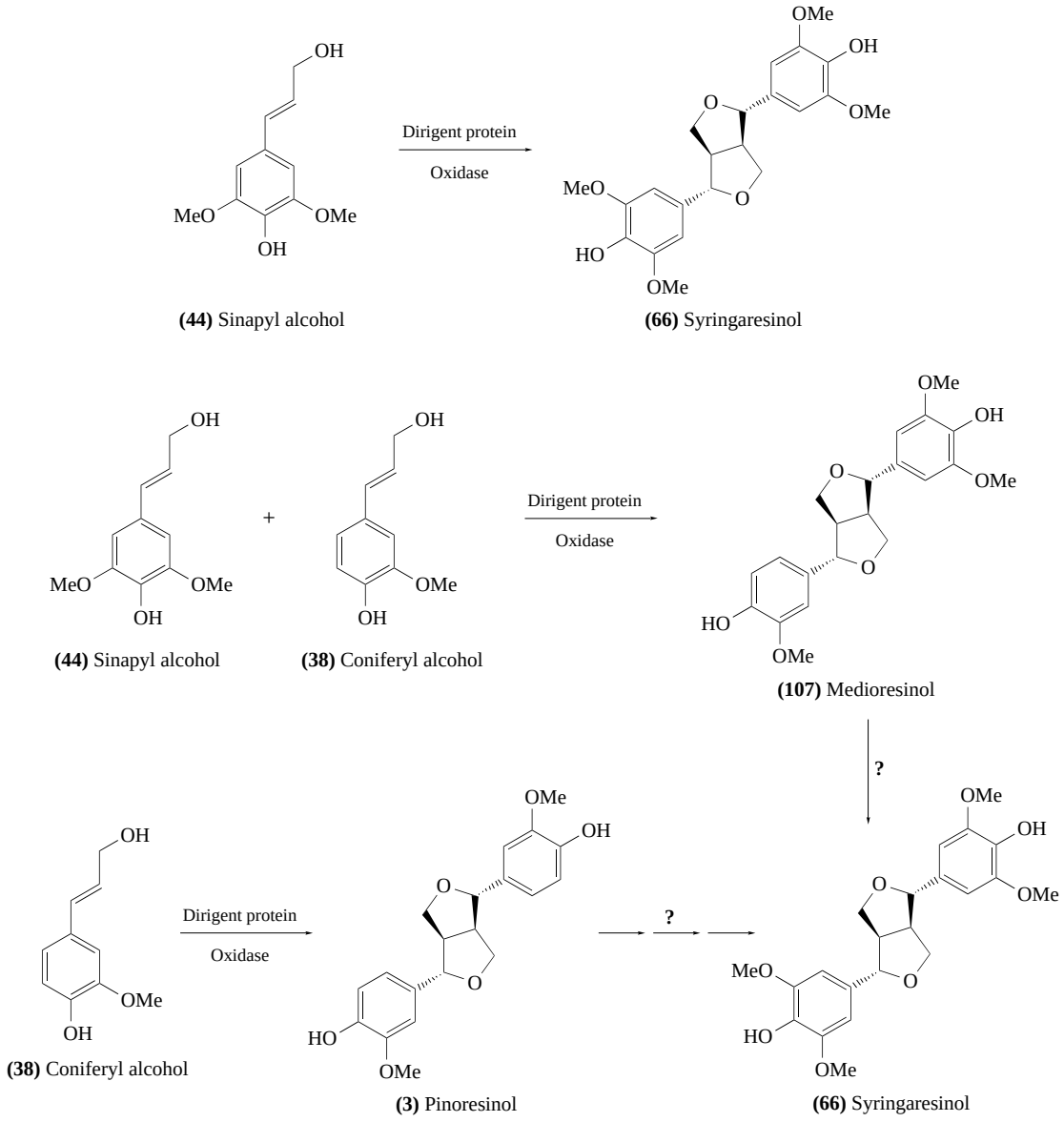
As summarized in Table 1, syringaresinol (**66**) is found in different species, with predictably different degrees of enantiomeric purity depending upon the plant species involved. Its formation could result from either dirigent protein mediated coupling of sinapyl alcohol (**44**), albeit with more than one mode of stereoselective coupling to afford both (+)- and (−)-enantiomers (**66a**) and (**66b**), respectively, or perhaps less likely, it could result from modification of preformed pinoresinol (**3**). As an added consideration, optically active medioresinol (**107**) present in *Eucommia ulmoides* (Eucommiaceae),[138] might result from either heterogeneous coupling of *E*-coniferyl (**38**) and sinapyl (**44**) alcohols, or be formed through pinoresinol (**3**) modification (Scheme 11). Accordingly, the precise enzymology involved in their formation awaits full clarification, including that of the role of dirigent proteins.

1.25.8.3 *Pellia* Liverwort Lignans

Lignans present in liverworts,[53–60] such as *Pellia epiphylla*, appear to be formed via stereoselective coupling of *E*-caffeic acid (**33**) molecules, as shown in Scheme 12. Thus, following stereoselective coupling, the regenerated diphenol on ring A can then participate in nucleophilic attack onto the quinone methide of ring B to generate the resulting aryldihydronaphthalene derivative (**45**). Although the enantiomeric composition of these lignans has yet to be described, their pronounced optical rotations suggest involvement of dirigent proteins.

1.25.8.4 Lignanamides

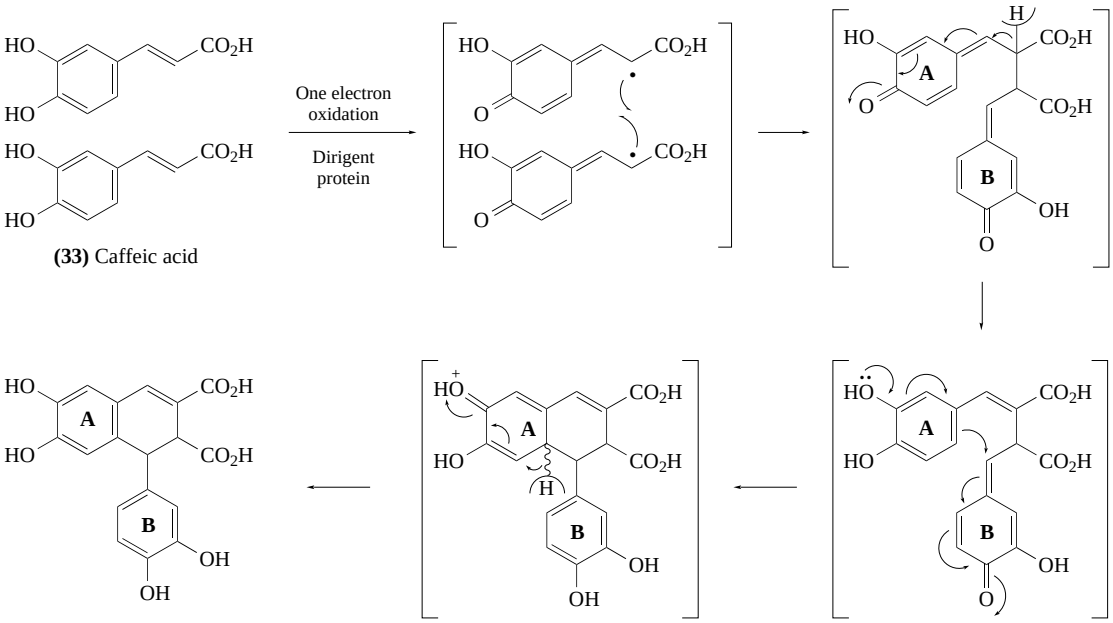
Various plant species, particularly in, but not restricted to, the Solanaceae accumulate *p*-coumaroyl and feruloyl tyramine derivatives (**108**) and (**109**), with these being considered to be incorporated (at least in part) into the aromatic component of the suberin biopolymer.[139–141] Feruloyl (**109**), sinapoyl (**110**), and caffeoyl (**111**) tyramine derivatives, and the optically active aryldihydronaphthalene lignanamides (**112**) and (**113**), have also been isolated from *Porcelia macrocarpa*



Scheme 11

(Annonaceae) branch tissue.[142] It is, accordingly, again tempting to speculate that the lignanamides result from dirigent protein mediated stereoselective coupling (cf. the liverwort lignans).

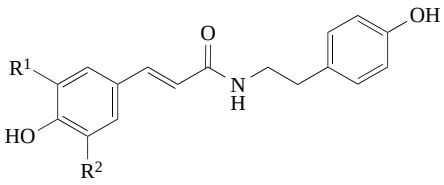
Fruits of *Xylopia aethiopica* (Annonaceae) also contain optically active 8—8′ linked (−)-cannabisin B (**114**) and (−)-cannabisin D (**115**), together with smaller amounts of the racemic lignans, (±)-grossamide (**116**) and (±)-demethylgrossamide (**117**).[143] Perhaps significantly, the small amounts of racemic products (**116**) and (**117**) co-occur with larger amounts of the corresponding "monomeric" tyramine derivatives (**108**), (**109**), and (**111**). Indeed, based on the methodologies employed for their isolation (plant tissue grinding over long periods, lengthy extractions, numerous chromatographic steps), formation of the racemic dimers could result from nonspecific coupling (artifact formation) during isolation. This is because during their isolation disruption of the plant material will result in the tyramine derivatives (**108**), (**109**), and (**111**) coming into contact with nonspecific oxidases/oxidants. Surprisingly, few studies seem to consider the possibility that isolated (racemic) phenolic dimers (and higher oligomers) may be artifacts, in spite of the readily oxidizable nature of such compounds.



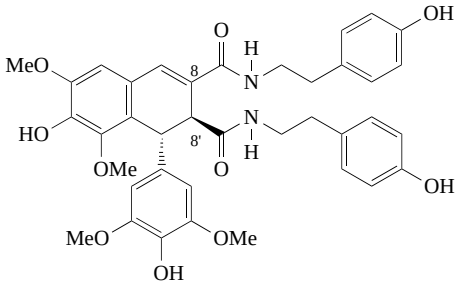
(33) Caffeic acid

(45)
$[\alpha]_D^{20}$= –130.77° (MeOH)
(*Pellia epiphylla*)

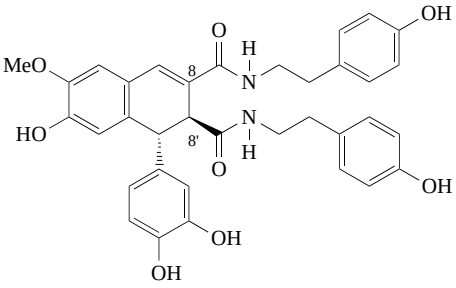
Scheme 12



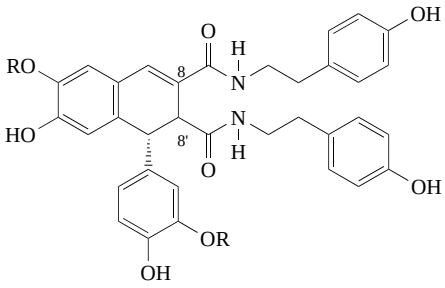
(108) $R^1 = R^2$ = H, *p*-Coumaroyl tyramine
(109) R^1 = OMe, R^2 = H, Feruloyl tyramine
(110) $R^1 = R^2$ = OMe, Sinapoyl tyramine
(111) R^1 = OH, R^2 = H, Caffeoyl tyramine



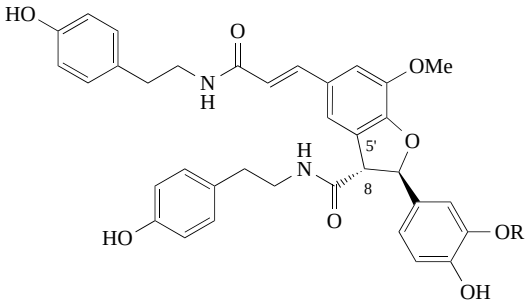
(112)
$[\alpha]_D^{25}$= –20° (MeOH, *c* = 0.062)
(*Porcelia macrocarpa*)



(113)
$[\alpha]_D^{25}$= –12° (MeOH, *c* = 0.085)
(*Porcelia macrocarpa*)

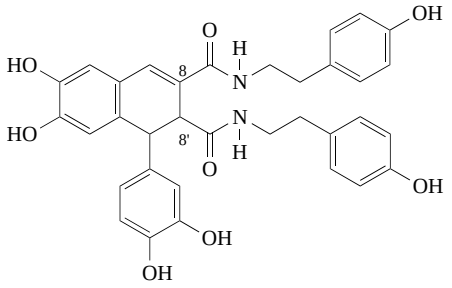


(114) R = H, Cannabisin B, $[\alpha]_D$ = –38° (MeOH, *c* = 0.25)
(115) R = Me, Cannabisin D, $[\alpha]_D$ = –46° (MeOH, *c* = 0.25)

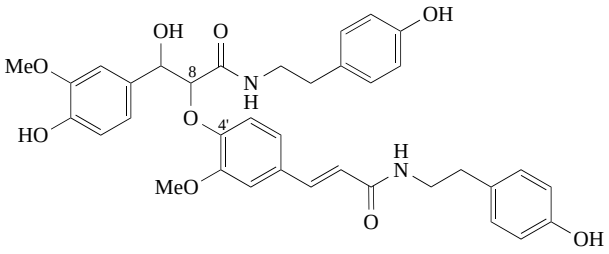


(116) R = Me, (±)-Grossamide
(117) R = H, (±)-Demethylgrossamide

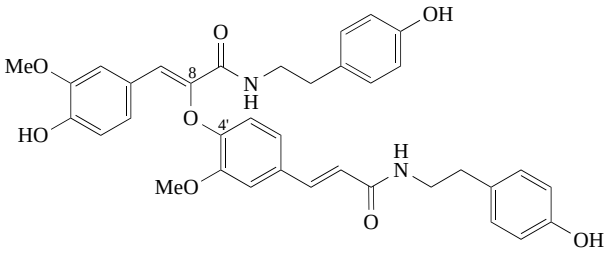
Cannabis sativa (Cannabidaceae) fruits[144–146] also reputedly contain racemic 8—8′ ((**114**), (**115**), (**118**)), 8—*O*—4′ ((**119**), (**120**)), and 8—5′ ((**116**), (**117**)) linked lignan amides, together with their "monomeric" precursors (**108**), (**109**), and (**111**). At a first glance, the occurrence of these dimers would appear to favor random coupling mechanisms *in vivo*. However, such reports of racemic products being present in plant tissues must be viewed with some caution, since once again the isolation conditions employed were very harsh, and it cannot be ruled out that these "randomly linked" dimers are instead just isolation artifacts.



(118) Cannabisin A



(119) Cannabisin E



(120) Cannabisin F

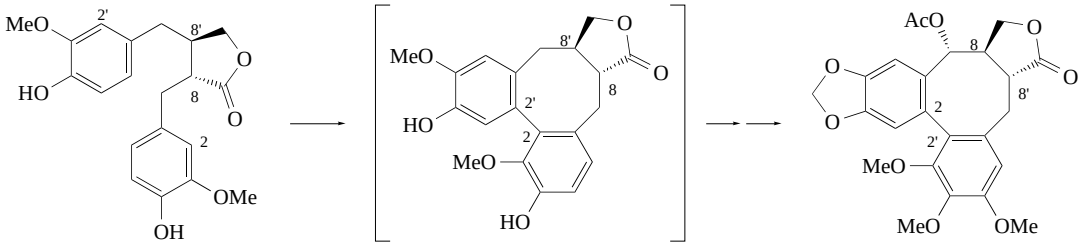
Artifact formation may also account for the occurrence of racemic grossamide (**116**) in bell pepper (*Capsicum annuum* var. *grossum*, Solanaceae) roots.[147] In this case, homogenized roots (21 kg) were extracted for 336 h with 70% EtOH (160 L), prior to any chromatographic purification, i.e., under conditions which would certainly favor nonspecific coupling and artifact formation from the monomeric precursors present in the tissue.

1.25.8.5 Guaiaretic Acid, Steganacin, and Gomisin A

Certain plant species contain relatively unusual types of optically active 8—8′, as well as 8—8′, 2—2′ linked lignans that appear to be derived via either allylphenol and/or monolignol coupling. Examples include the 8—8′ linked (−)-guaiaretic acid (**1**) from *Guaiacum officinale*,[10] (−)-steganacin (**25**) from *Steganotaenia araliacea* (Umbelliferae),[148] and (+)-gomisin A (**121**) from the fruits of *Schizandra chinensis* (Schizandraceae).[149,150] The formation of these optically active lignans is again of considerable interest: (−)-guaiaretic acid (**1**) can be envisaged to result via stereoselective coupling of (iso)eugenol (**58**/**59**) (cf. the mechanism shown in Scheme 12), in a process presumably mediated by the corresponding dirigent protein.

(−)-Steganacin (**25**), on the other hand, could result from dirigent protein mediated coupling to give (+)-pinoresinol (**3a**), this being metabolized into (−)-matairesinol (**21a**) or a substituted butyrolactone equivalent (Scheme 13). A most interesting biochemical conversion then follows which stipulates 2—2′ intramolecular coupling: this may involve a cytochrome P450 catalyzed process, in a manner comparable to diphenol intramolecular coupling processes leading to the alkaloids (*R*,*S*)-berbamunine, (*R*,*S*)-norberbamunine, and (*R*,*R*)-guattegaumerine from (*S*)-coclaurine, (*R*)-*N*-methylcoclaurine, and (*S*)-*N*-methylcoclaurine, respectively, in *Berberis stolonifera* plant cell cultures.[151]

(+)-Gomisin A (**121**) appears to be derived from dirigent protein mediated stereoselective coupling of (iso)eugenol (**58**/**59**) (Scheme 14). If correct, this would initially afford the bis-quinone methide which could then be acted upon by the corresponding reductase to generate the putative



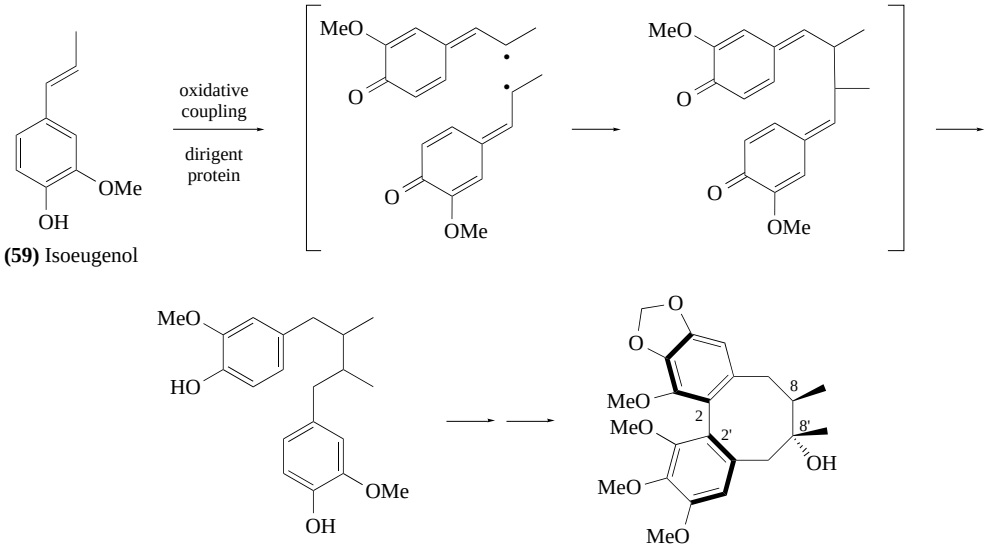
(21) Matairesinol or a substituted butyrolactone equivalent

2–2' intramolecular coupling

(25) (–)-Steganacin
$[\alpha]_D$ = –122.6° ($CHCl_3$, c 1.02)
(*Steganotaenia araliacea*)

Scheme 13

dibenzylbutane (**122**). The latter (or some equivalent) could then undergo 2—2′ coupling, as well as various hydroxylation, methylation, and methylenedioxy bridge formation steps, to ultimately afford (+)-gomisin A (**121**).



(59) Isoeugenol

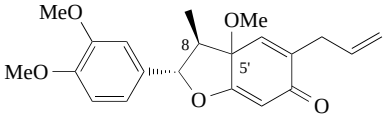
(122)

(121) (+)-Gomisin A
$[\alpha]_D$ = +67.9° ($CHCl_3$)
(*Schizandra chinensis*)

Scheme 14

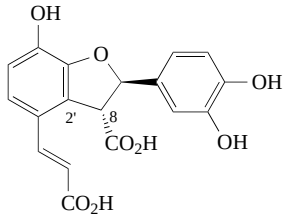
1.25.9 MISCELLANEOUS COUPLING MODES: ARE DIRIGENT PROTEINS ALSO INVOLVED?

Consideration of the chemical structures and optical rotations of many other isolated lignans strongly suggests involvement of dirigent proteins stipulating distinct coupling modes. This is illustrated with only three examples, namely the 8—2′ linked (−)-blechnic acid (**63**) from the fern *B. orientale*,[64] the 8—5′ linked (+)-denudatin B (**123**) from *Magnolia denudata* (Magnoliaceae)[152] and the 8—1′ linked (−)-megaphone (**4**) from *Aniba megaphylla* (Lauraceae).[153]

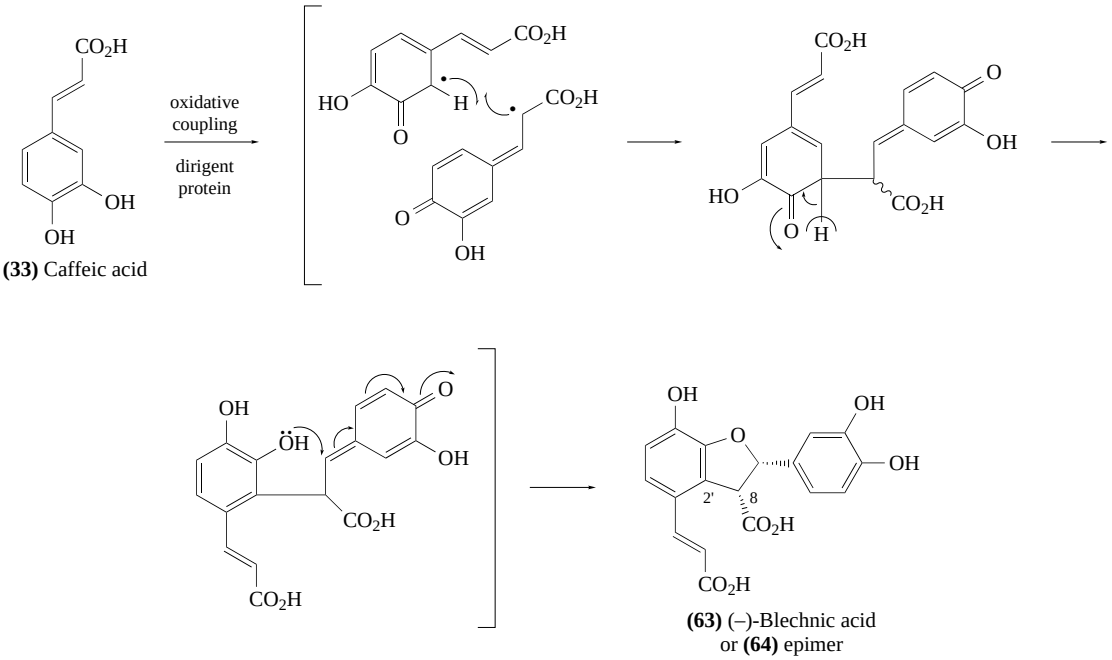


(123) (+)-Denudatin B
$[\alpha]_D$ = +82.7° (MeOH, *c* = 2.67)
(*Magnolia denudata*)

Blechnic acid (**63**), which reputedly co-occurs with its epimeric form, 7-epiblechnic acid (**124**), and other conjugates in *B. orientale*, provisionally appears to result from stereoselective coupling of two caffeic acid (**33**) molecules. Its formation can be envisaged to occur through the involvement of a dirigent protein-mediated process in a manner analogous to that for (+)-pinoresinol (**3a**) formation in *Forsythia* species, with the proposed biosynthetic scheme shown in Scheme 15. In a somewhat analogous manner, formation of both (+)-denudatin B (**123**) and (−)-megaphone (**4**) can be envisaged to occur via stereoselective 8—5′ and 8—1′ coupling of two (iso)eugenol (**58**/**59**) derived compounds, followed by skeletal modifications as needed. Accordingly, these and many other examples again strongly suggest that a class of dirigent proteins exists with each engendering distinct coupling modes.



(124) (–)-7-Epiblechnic acid
$[\alpha]_D^{23}$ = –145° (MeOH, *c* = 1.0)
(*Blechnum orientale*)



(33) Caffeic acid

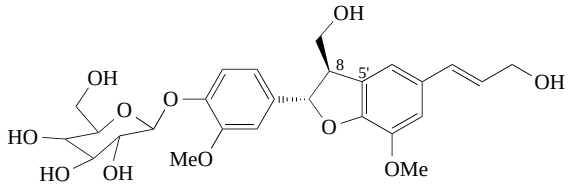
(63) (–)-Blechnic acid
or **(64)** epimer

Scheme 15

1.25.10 8—5′ AND 8—*O*—4′ COUPLING OF MONOLIGNOLS AND ALLYLPHENOLS AND THEIR ASSOCIATED METABOLIC PROCESSES

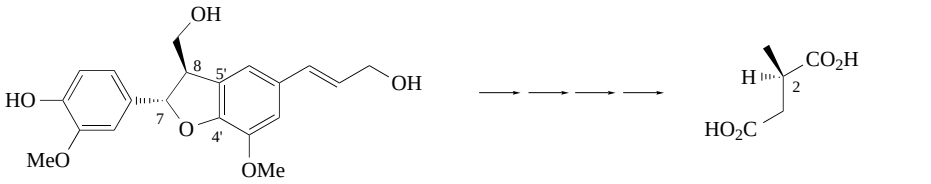
A number of plant species in the pteridophytes, gymnosperms, and angiosperms contain 8—5′ and 8—*O*—4′ linked lignans, in addition to their 8—8′ constituents. These lignans can, depending upon the plant species from which they are isolated, exist in either optically pure or near racemic form. Of these, the most frequently reported are the dehydrodiconiferyl alcohols (**5a**) and (**5b**), the guaiacylglycerol 8—*O*—4′ coniferyl alcohol ethers (**6**), and derivatives thereof. Another example of an 8—5′ linked lignan is that of the presumed allylphenol-derived lignan, licarin A (**57**).[59,154] The

proposed main metabolic role of lignans (**5**) and (**6**) *in vivo* are as intermediates in lignin formation[71] (but see Chapter 3.18), whereas other derivatives, such as dehydrodiconiferyl alcohol glucosides (**125a,b**), are reputed to be cell wall components functioning as cytokinins[155–157] (but see Section 1.25.13.5).



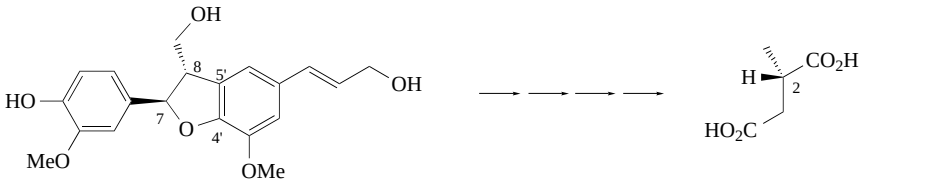
(125a,b)

Prior to discussing the biochemical processes known to involve the common 8—5′/8—*O*—4′ coupled products, a brief discussion of their relative configurations and optical activities is required. In a study by Hirai *et al.*,[158] each enantiomer of dehydrodiconiferyl alcohol (**5a,b**) was separated and subsequently degraded by chemical means to give methylsuccinic acid (**126**) via aromatic ring fission (Scheme 16). Following analysis of the configurations of the resulting methylsuccinic acids (**126a**) and (**126b**) at carbon 2 (corresponding to C-8 of lignan (**5**)), it was deduced that the (+)- and (−)-forms of dehydrodiconiferyl alcohol (**5a**) and (**5b**) had 7*S*, 8*R* and 7*R*, 8*S* configurations, respectively. Interestingly, the enantiomers of dehydrodiconiferyl alcohol (**5a**) and (**5b**) have $[\alpha]_D^{25}$ values of $+36.3^\circ$ (MeOH, $c = 0.168$) and -44.6° (MeOH, $c = 0.186$),[159] whereas the synthetic 7′—8′ dihydro derivatives (**127a**) and (**127b**) (Scheme 17) have lower $[\alpha]_D^{25}$ values, i.e., $+5.1^\circ$ (MeOH, $c = 0.196$) and -9.18° (MeOH, $c = 0.244$), respectively. On the other hand, phenols (**128a**) and (**128b**), which have only a single chiral center due to 7—*O*—4′ ether reductive cleavage, have $[\alpha]_D^{25}$ values of $+25.84^\circ$ (MeOH, $c = 0.209$) and -29.35° (MeOH, $c = 0.184$).



(5a) (7*S*, 8*R*)-(+)-Dehydrodiconiferyl alcohol

(126a) (*R*)-(+)-Methylsuccinic acid



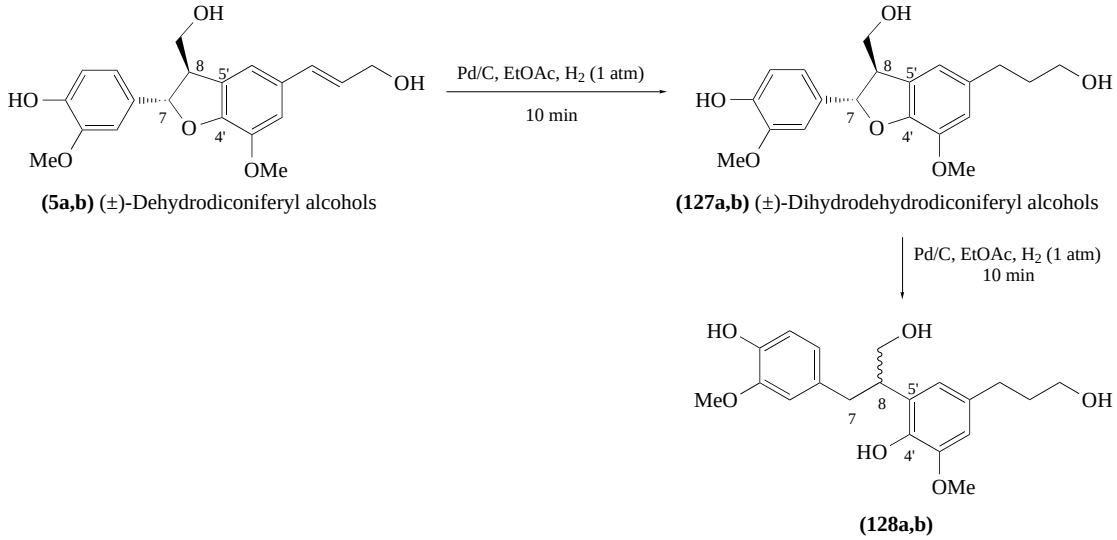
(5b) (7*R*, 8*S*)-(−)-Dehydrodiconiferyl alcohol

(126b) (*S*)-(−)-Methylsuccinic acid

Scheme 16

While most ^1H and ^{13}C NMR spectroscopic studies of 8—5′ linked lignans have established a *trans* configuration for the constituents attached to carbons 7 and 8,[160–162] there are a number of studies which concluded that various 8—5′ linked lignans were in a *cis* configuration.[66,163–165] Detailed analyses by Wallis and co-workers, however, established that only the *trans* isomers were natural products, and that the proposed *cis* configurations resulted from misinterpretation of spectroscopic data.[67]

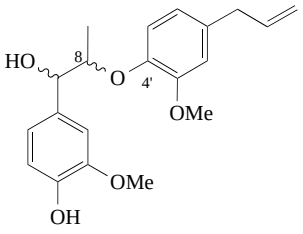
With this background regarding optical activities and configurations, naturally occurring 8—5′ linked lignans seem to be present in different plants with differing degrees of enantiomeric purity, based on their observed optical rotations, e.g., (−)-licarin A (**57**) from the liverwort *Jackiella javanica*,[59] which has an $[\alpha]_D = -43^\circ$ ($c = 9.0$), is presumed to be optically pure, whereas the same compound (**57**) in *Eupomatia laurina* is apparently racemic.[166] Additionally, the aglycone (**127**) of *trans*-dihydrodehydrodiconiferyl alcohol glucoside (**71**), $[\alpha]_D^{21} = -8.5^\circ$ ($c = 0.96$, acetone), from the fern, *P. vittata*,[66] is also apparently in significant enantiomeric excess, based on comparison with the optical rotation data obtained for the synthetic analogue (**127b**) described above. Optical



Scheme 17

activities for numerous other 8—5′ linked lignans have also been described in the gymnosperms and angiosperms. In general, however, these have a wide range of optical rotations.

Finally, the 8—*O*—4′ linked lignans (**6**), which exist *in vivo* in both *erythro* and *threo* form, have diastereomers that are readily separable by conventional chromatographic methodologies.[87] Although the chiral separation of each form has not been reported, enantiomeric separation was achieved with synthetic analogues, i.e., *erythro* and *threo* forms of lignan (**129**).[16]



(129) (±)-*threo/erythro*

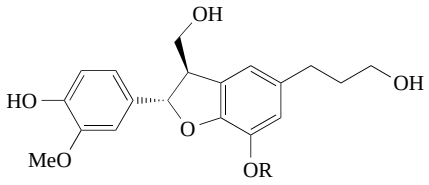
1.25.10.1 Formation and Metabolism of 8—5′ and 8—*O*—4′ Linked Lignans

As for the 8—8′ linked lignans, the fact that many 8—5′ and 8—*O*—4′ lignans are either enantiomerically pure or in enantiomeric excess requires an explicit biochemical explanation, in terms of whether the coupling steps are stereoselective, and/or if subsequent metabolic conversions are enantiospecific.

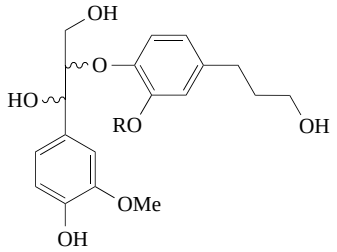
However, since no systematic study has been reported, it is premature to discuss in any detail how 8—*O*—4′ and 8—5′ coupling may be either regio- or stereoselectively controlled, and whether dirigent protein mediation is involved. On the other hand, the optical activities noted for both (−)-licarin A (**57**) from *J. javanica*[59] and the (−)-aglycone (**127b**) from deglycosylation of (−)-*trans*-dihydrodehydrodiconiferyl alcohol glucoside (**71**) found in *P. vittata*[66] suggest that stereoselective coupling of (iso)eugenol (**58**/**59**) and coniferyl alcohol (**38**), respectively, occurs—at least in these species—but this needs to be established at the protein/enzyme level.

Significant progress has been made though in delineating postcoupling metabolic conversions of dehydrodiconiferyl alcohol (**5**) and guaiacylglycerol 8—*O*—4′ coniferyl alcohol ether (**6**) in loblolly pine (*Pinus taeda*) cell suspension cultures.[159,168–170] Not unexpectedly, based on chemical characterization of previously isolated lignans in the Pinaceae,[168–170] the most common modifications are the regiospecific reduction of the 7′—8′ allylic double bond of (**5**) and (**6**), and demethylation at carbon 3′ (see compounds (**127**) and (**130**)–(**132**)). Additional regiospecific transformations also

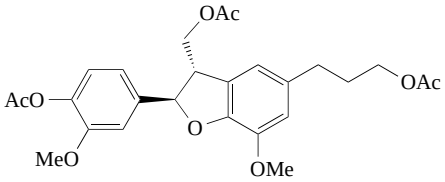
occur, which involve 7—*O*—4′ reduction (**128**), and in other species, acylation of the 9 and 9′ aliphatic hydroxyl groups with acetate, *p*-coumaroyl, and feruloyl moieties (**133**)–(**136**).



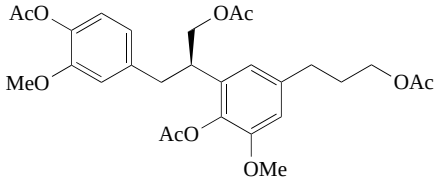
(127a) R = Me, Dihydrodehydrodiconiferyl alcohol
$[\alpha]_D^{25}$= +5.1° (MeOH, c = 0.6)
(130) R = H, Cedrusin, $[\alpha]_D^{25}$= +3.2° (MeOH, *c* = 1.0)
(*Pinus massonian*) *a*



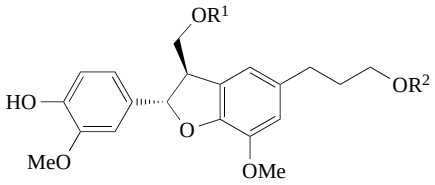
(131) R = H
(132) R = Me
(*Pinus massonian*) *a*



(133) *Tran s*Dihydrodehydrodiconiferyl alcohol triacetate
$[\alpha]_D^{25}$= –66° ($CHCl_3$, *c* = 0.8)
(*Cryptomeria japonic*) *a*



(134)
$[\alpha]_D^{25}$= –2.5° ($CHCl_3$ *c* = 2.0)
(*Cryptomeria japonic*) *a*



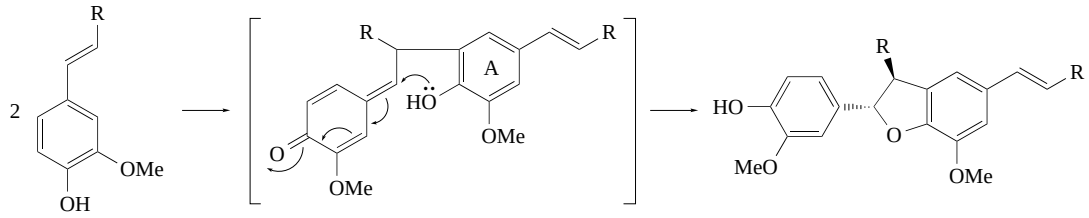
(135) R^1 = R^2 = *p*-coumaroyl, $[\alpha]_D^{28}$= +2.0° (MeOH, *c* = 0.5)
(136) R^1 = *p*-coumaroyl, R^2 = feruloyl, $[\alpha]_D^{28}$= +2.3° (MeOH, *c* = 0.5)
(*Corylus sieboldian*) *a*

1.25.10.1.1 Phenylcoumaran 7—O—4′ ring reduction

The 8—5′ linked lignans, such as dehydrodiconiferyl alcohol (**5**) and licarin A (**57**), both contain phenylcoumaran ring structures, which result from nucleophilic attack of the phenol group of ring A onto the quinone methide, as shown in Scheme 18. In certain gymnosperms, such as *Cryptomeria japonica*, there are other 8—5′ linked lignans whose structures might be considered to result from reduction of the 7—*O*—4′ benzylic ether of the phenylcoumaran ring. In *C. japonica*, these metabolites e.g., (**134**) have low optical rotations ($[\alpha]_D^{25} = -2.5^\circ$, $c = 2.0$, $CHCl_3$)[171] suggesting that reductive cleavage might only be regio- rather than enantiospecific. Such reductions are mechanistically analogous to those for pinoresinol/lariciresinol and isoflavonoid reductases.

In this regard, there have been a number of reports of so-called isoflavone reductase homologues, which show considerable sequence homology to both isoflavone and pinoresinol/lariciresinol reductases, e.g., from *A. thaliana*, *Nicotiana tabacum*, *Solanum tuberosum*, *Zea mays*, and *Lupinus albus* (summarized by Dinkova-Kostova *et al.*[111]). None of these homologues have, however, any known catalytic function.

During screening of a *P. taeda* cDNA library, one such homologue was obtained,[159] and Figure 17 shows its gene sequence. The corresponding recombinant protein was subsequently expressed in *E. coli*, but was unable to reduce (±)-pinoresinols (**3a,b**). On the other hand, when incubated with



(38) R = CH_2OH, Coniferyl alcohol
(59) R = Me, Isoeugenol

(5) R = CH_2OH, Dehydrodiconiferyl alcohol
(57) R = Me, Licarin A

Scheme 18

(±)-dehydrodiconiferyl alcohols (**5a,b**), it catalyzed 7—*O*—4′ reduction of both enantiomers to give the products (**128a,b**),[172] albeit at low specific activity compared with pinoresinol/lariciresinol reductase. As for pinoresinol/lariciresinol and isoflavone reductases, it is a type A reductase, since only the 4 pro-*R*, but not the 4 pro-*S*, hydride is abstracted from NADPH during reductive cleavage. In contrast to the enantiospecific *Forsythia* pinoresinol/lariciresinol reductase, the recombinant 7—*O*—4′ reductase was capable of effectively reducing both (+)- and (−)-antipodes (**5a**) and (**5b**).

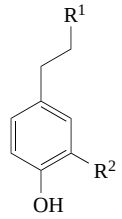
```
     GGCACGAGGTTTCAGGGCCGACATGGGAAGCAGGAGCAGGATACTCCTAATTGGCGCAACAGGATACATTGGTCGCCATGTTGCCAAGGC   68
   1                      M  G  S  R  S  R  I  L  L  I  G  A  T  G  Y  I  G  R  H  V  A  K  A   23
  69 TAGCCTTGATCTCGGCCATCCCACCTTCCTTCTGGTTAGAGAGTCCACTGCTTCTTCTAATTCTGAGAAAGCCCAGCTCCTGGAATCCTT  158
  24  S  L  D  L  G  H  P  T  F  L  L  V  R  E  S  T  A  S  S  N  S  E  K  A  Q  L  L  E  S  F   53
 159 CAAGGCCTCTGGTGCTAATATAGTCCATGGATCCATAGATGATCATGCAAGCCTTGTGGAGGCAGTGAAGAATGTGGATGTAGTAATCTC  248
  54  K  A  S  G  A  N  I  V  H  G  S  I  D  D  H  A  S  L  V  E  A  V  K  N  V  D  V  V  I  S   83
 249 CACAGTTGGATCACTACAGATAGAGAGCCAGGTCAATATTATCAAGGCTATTAAAGAAATTGGAACCGTCAAGAGGTTTTTTCCATCTGA  338
  84  T  V  G  S  L  Q  I  E  S  Q  V  N  I  I  K  A  I  K  E  I  G  T  V  K  R  F  F  P  S  E  113
 339 GTTCGGGAATGATGTTGATAACGTCCATGCAGTGGAGCCTGCAAAGAATGTGTTTGAGGTGAAAGCCAAAGTCCGTAGGGCAATCGAAGC  428
 114  F  G  N  D  V  D  N  V  H  A  V  E  P  A  K  N  V  F  E  V  K  A  K  V  R  R  A  I  E  A  143
 429 AGAGGGTATTCCTTATACATACGTCTCTAGCAACTGTTTTGCAGGGTATTTCCTGCGAAGCCTCGCACAGGCTGGCCTAACAGCTCCTCC  518
 144  E  G  I  P  Y  T  Y  V  S  S  N  C  F  A  G  Y  F  L  R  S  L  A  Q  A  G  L  T  A  P  P  173
 519 AAGAGATAAAGTTGTCATTCTTGGAGATGGAAATGCCAGAGTTGTCTTTGTAAAAGAGGAAGACATTGGAACATTTACAATCAAGGCAGT  608
 174  R  D  K  V  V  I  L  G  D  G  N  A  R  V  V  F  V  K  E  E  D  I  G  T  F  T  I  K  A  V  203
 609 GGACGACCCCAGAACGTTGAACAAGACCTTATACTTGAGGCTTCCTGCCAATACTCTGTCTTTAAATGAGCTTGTGGCTCTCTGGGAGAA  698
 204  D  D  P  R  T  L  N  K  T  L  Y  L  R  L  P  A  N  T  L  S  L  N  E  L  V  A  L  W  E  K  233
 699 GAAGATTGATAAGACTCTGGAGAAGGCCTACGTGCCCGAGGAGGAGGTTCTTAAATTAATCGCAGATACACCATTCCCAGCTAATATTAG  788
 234  K  I  D  K  T  L  E  K  A  Y  V  P  E  E  E  V  L  K  L  I  A  D  T  P  F  P  A  N  I  S  263
 789 CATAGCAATTAGTCATTCTATCTTCGTGAAAGGAGATCAAACAAATTTTGAAATTGGACCTGCTGGTGTAGAGGCTAGTCAGCTGTACCC  878
 264  I  A  I  S  H  S  I  F  V  K  G  D  Q  T  N  F  E  I  G  P  A  G  V  E  A  S  Q  L  Y  P  293
 879 TGATGTGAAATACACCACCGTCGACGAGTACCTGAGCAATTTTGTGTGAACTATGCAACATTCTTCAACGAACCTAATAGATTAAATAGT  968
 294  D  V  K  Y  T  T  V  D  E  Y  L  S  N  F  V  *                                            309
 969 GGTTTCTATGAAAGTTTATATAAGGCATTTTGCCAAATTGTTGGTTATCGCCTTTCATAGATATTCGACAAAATAGAACCTCCAAAATAG 1058
1059 AACCTCCTGTTTAGTATATTATGATAAGCCAGGCTAGGTAGATCCTTCACACATTTCTGTTCTGTAAGAAGCAAACATATAAAATATCCG 1148
1149 TTTTTTGTTTTTTGATTAAAAAAAAAAAAAAAAACTCGAGGGGGGCCCGTACCCAAT                                  1205
```

Figure 17 Complete sequence of *Pinus taeda* 7—*O*—4′ -reductase cDNA, plrh-Pt.

Thus, it appears there is a family of reductases in phenylpropanoid metabolism, which catalyze the reduction of isoflavonoids (e.g., hydroxypseudobaptigenin (**93**)), 8—8′ linked lignans (e.g., pinoresinol (**3**) and lariciresinol (**19**)), and 8—5′ linked phenylcoumarans (e.g., dehydrodiconiferyl alcohol (**5**)). The latter, however, differs from the others by the ability to effectively reduce both (+)- and (−)-antipodes (**5a**) and (**5b**) of the substrate, i.e., it is regio- rather than enantiospecific.

1.25.10.1.2 Allylic 7—8′ bond reduction

A common structural modification of 8—5′ and 8—*O*—4′ lignans *in planta*, including in the Pinaceae such as *Pinus taeda* cell suspension cultures, is that of the so-called "dihydro" derivatives, whose structures are based mainly on dihydrodehydrodiconiferyl alcohol (**127**)[160–162,164,165,168,171–185] and guaiacylglycerol 8—*O*—4′ dihydroconiferyl alcohol ethers (**132**).[165,172,178–180] However, in addition to these substances, other "dihydro" compounds frequently co-occur, including dihydro-*p*-coumaryl (**137**)[178] and dihydroconiferyl (**138**)[162,165,173,176,178] alcohols, as well as various dihydrocinnamic acids e.g., (**139**) and (**140**).[173,178]

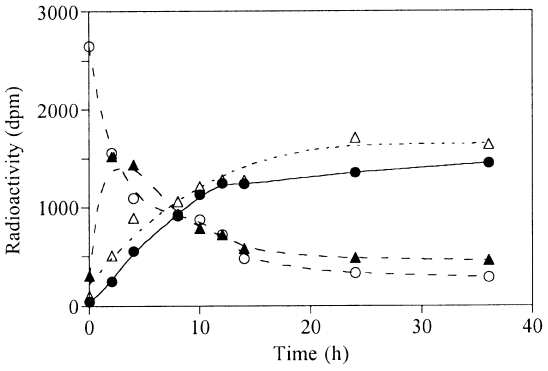


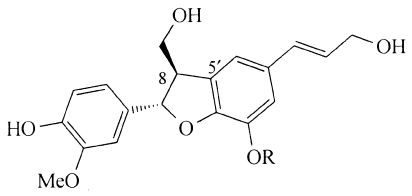
(137) $R^1 = CH_2OH$, R^2 = H, Dihydro *p*-coumaryl alcohol
(138) $R^1 = CH_2OH$, R^2 = OMe, Dihydroconiferyl alcohol
(139) $R^1 = CO_2H$, R^2 = H, Dihydro *p*-coumaric acid
(140) $R^1 = CO_2H$, R^2 = OMe, Dihydroferulic acid

P. taeda suspension cultures have been used to define the biochemical processes involved in allylic double bond reductions of dehydrodiconiferyl alcohols (**5a,b**), where using partially purified reductase preparations, it was established that NADPH (but not NADH) was required as a cofactor.[169] Individual incubation of (**5a,b**) with [4*R*-^{3}H] and [4*S*-^{3}H]NADPH, respectively, revealed that only the 4 pro-*R* hydride was transferred to the reduced product (**127**), with the 7′—8′ allylic bond reductase being capable of utilizing both (+)- and (−)-antipodes (**5a**) and (**5b**) as substrates. It needs to be determined, however, whether the reductase reduces the allylic double bond directly, or whether some (oxidized) intermediate is involved. The only other known allylic bond reductase is that of enoyl acyl carrier protein reductase in fatty acid biosynthesis,[186,187] which requires the double bond to be in conjugation with a carbonyl group. The purified dehydrodiconiferyl alcohol 7′—8′ double bond reductase is thus required, in order to mechanistically determine how this reduction is achieved. Additionally, it needs to be established as to whether the same enzyme catalyzes formation of all of the various "dihydro" natural products albeit with different substrate specificities, or if a family of these reductases exists with each catalyzing distinct transformations.

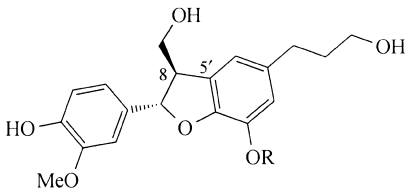
1.25.10.2 Regiospecific *O*-Demethylation at Carbon 3′ and Monosaccharide Functionalization

In *P. taeda* cell suspension cultures, (±)-dehydrodiconiferyl alcohols (**5**) can also undergo further metabolism involving regiospecific demethylation at C-3′. As shown in Figure 18, when (±)-[9,9′-$^3H_2$]dehydrodiconiferyl alcohols (**5**) were incubated with *P. taeda* cell suspension cultures for different time intervals, both enantiomers were either rapidly demethylated to give demethyldehydrodiconiferyl alcohol (**141**), or reduced to afford dihydrodehydrodiconiferyl alcohols





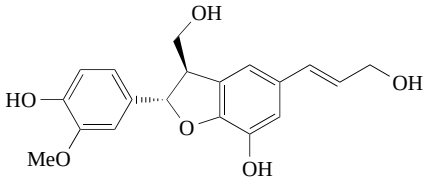
(**5a,b**) R=Me, (±)-Dehydrodiconiferyl alcohols
(**141a,b**) R=H, (±)-Demethyldehydrodiconiferyl alcohols



(**127a,b**) R=Me, (±)-Dihydrodehydrodiconiferyl alcohols
(**130a,b**) R=H, (±)-Cedrusins

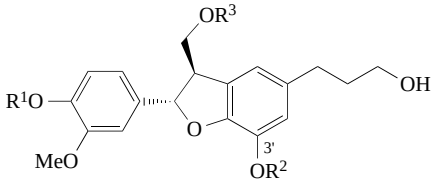
Figure 18 Time course of metabolism of (±)-dehydrodiconiferyl alcohols (**5a,b**) by *Pinus taeda* cell suspension cultures. ○, (±)-dehydrodiconiferyl alcohols (**5a,b**); ▲, (±)-dihydrodehydrodiconiferyl alcohols (**127a,b**); ●, (±)-demethyldehydrodiconiferyl alcohols (**141a,b**); △, (±)-cedrusins (**130a,b**).

(**127a,b**).[170] In turn, both metabolites (**127a,b**) were finally converted into (±)-demethyldihydrodehydrodiconiferyl alcohols = (±)-cedrusins (**130a,b**);[170] the enzymology involved in this demethylation awaits full characterization.



(141)
(*Pinus taeda*)

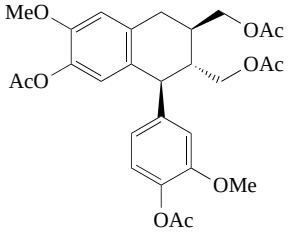
Additionally, these and related derivatives, whether from dihydrodehydrodiconiferyl alcohol (**127**), dehydrodiconiferyl alcohol (**5**), and guaiacylglycerol 8—*O*—4′ (dihydro)coniferyl alcohol ethers (**6**) and (**132**) are frequently found conjugated to monosaccharides. They are typically attached to the C-4 phenolic hydroxyl groups with either glucose or rhamnose, e.g., (**142**) and (**143**) in *Picea abies*[172] and *Pinus silvestris*,[165,182] and even if the demethylated 3′ hydroxyl group is in its free phenolic form. In other species, such as *Clematis stans*,[177] the glucose moiety is attached to the 3′ hydroxyl position of cedrusin (**130**) (see (**144**)), but not to the 4-hydroxy functionality, whereas in *Licaria chrysophylla* (Lauraceae),[162] it is attached to C-9 of dihydrodehydrodiconiferyl alcohol (**127**) (see (**145**)). The significance, if any, of these different monosaccharide attachments is unknown.



(142) R^1 = Glc, R^2 = R^3 = H
(143) R^1 = Rha, R^2 = R^3 = H
(*Pinus silvestris*)
(144) R^1 = H, R^2 = Glc, R^3 = H, Clemastanin A
(*Clematis stans*)
(145) R^1 = R^2 = H, R^3 = Glc
(*Licaria chrysophylla*)

1.25.10.3 Acylation

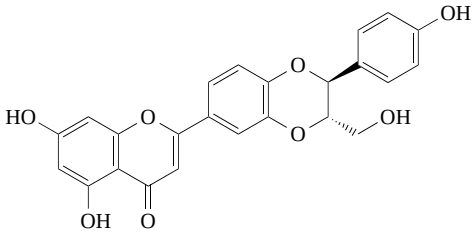
A few lignans have also been reported in species such as *C. japonica* and *Corylus sieboldiana* which contain acyl, *p*-coumaroyl, or feruloyl moieties conjugated to the lignan skeleton (**133**)–(**136**).[171,174] These have been noted for both 8—5′ (**133**)–(**136**) and 8—8′ (**146**)[171] linked lignans, but nothing has been established about how these *trans*-esterifications and coupling reactions occur.



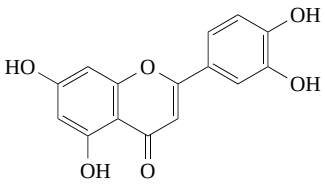
(146)
(*Cryptomeria japonica*)

1.25.11 MIXED DIMERS CONTAINING MONOLIGNOLS AND RELATED MONOMERS

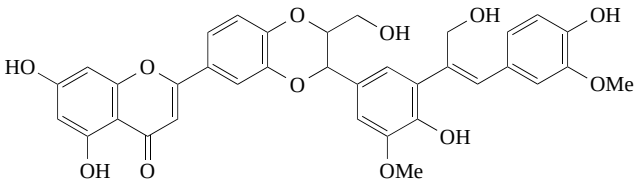
In addition to the *bona fide* lignan skeletal types described throughout this chapter, there are other natural products trivially described as being flavonolignans, coumarinolignans, stilbenolignans, etc. These appear to result either from coupling of monolignols, or in some cases, lignans, with other phenolic substances, such as flavonoids. These include, for example, the flavonolignan, sinaiticin (**147**) from *Verbascum sinaiticum* (Scrophulariaceae) leaves[188] which appears to result from regio/stereospecific coupling of luteolin (**148**) with *p*-coumaryl alcohol (**32**). Indeed, given that the only optical center present in the molecule results from monolignol attachment, how this coupling is controlled needs to be defined. Related substances such as the optically active hydnowightin (**149**) are also present in seeds of *Hydnocarpus wightiana* (Flacourtiaceae),[32] again suggesting enzymatic control of coupling in both a regio- and stereospecific manner. More complex flavonolignans also exist, such as pseudotsuganol (**150**), which consists of pinoresinol (**3**) linked to a dihydroquercetin (**151**) moiety,[31,189] this being found in the outer bark of Douglas fir (*Pseudotsuga menziesii*) in optically active form ($[\alpha]_D = +20.2^\circ$ ($c = 0.25$, MeOH)).



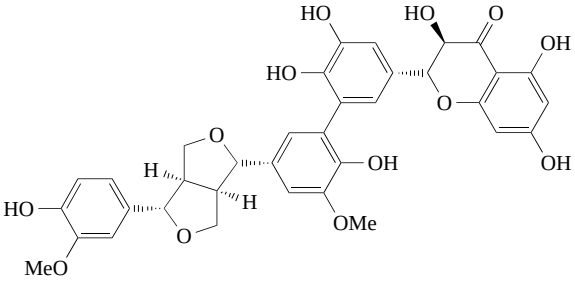
(147) Sinaiticin
$[\alpha]_D = -15.2°$ (MeOH, $c = 0.0033$)
(*Verbascum sinaiticum*)



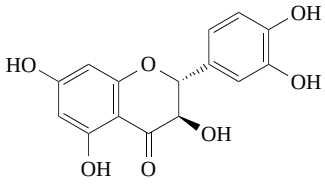
(148) Luteolin



(149) Hydnowightin
$[\alpha]_D = +40°$ (MeOH, $c = 0.55$)
(*Hydnocarpus wightiana*)

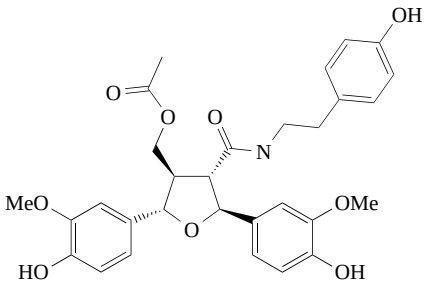


(150) Pseudotsuganol
$[\alpha]_D = +20.2°$ (MeOH, $c = 0.25$)
(*Pseudotsuga menziesii*)

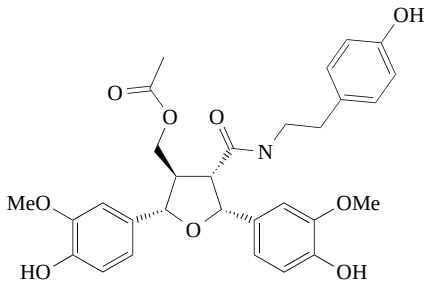


(151) Dihydroquercetin
(*Pseudotsuga menziesii*)

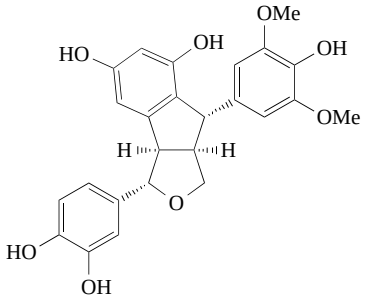
Another interesting group of mixed dimers are the lignanamides, the jacpaniculines (**152**) and (**153**) from the fruits of *Jacquemontia paniculata* (Convolvulaceae).[190] These presumably result from coupling of *E*-coniferyl alcohol (**38**) with feruloyl tyramine (**109**), although nothing can be concluded about how coupling might be carried out, since the enantiomeric purity of these metabolites has not been described. On the other hand, the stilbenolignans, such as maackoline (**154**) from *Maackia amurensis* (Leguminosae) heartwood,[191] are apparently racemic suggesting nonspecific coupling of the presumed sinapyl alcohol (**44**) and stilbene precursors.



(152) Jacpaniculine
(*Jacquemontia paniculata*)

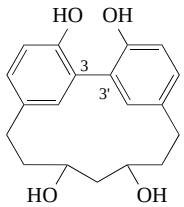


(153) Isojacpaniculine
(*Jacquemontia paniculata*)

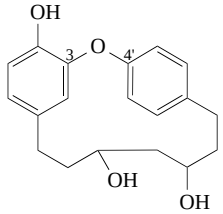


(154) Maackoline
$[\alpha]_D = 0$
(*Maackia amurensis*)

Other ''mixed dimers'' include (+)-megacerotonic acid (**61**) from *M. flagellaris*,[61–63] (−)-cryptoresinol (**13**) from *C. japonica* (Pinaceae),[17] and the diarylheptanoids, alnusdiol (**155**) and maximowicziol A (**156**) from *Betula maximowicziana* (Betulaceae) heartwood.[192] (+)-Megacerotonic acid (**61**) co-occurs with rosmarinic acid (**157**), and its formation (Scheme 19) may result from oxidative coupling as shown, i.e., where generation of the transient biradical species, derived from (**158**) gives, following ring closure and rearomatization, the 7—8′ linked lignan (**61**). A comparable mechanism may account for the formation of the lignan, (−)-cryptoresinol (**13**). The diarylheptanoids (**155**) and (**156**), by contrast, appear to result from oxidative coupling of two *p*-coumaryl alcohol (**32**) molecules via 3—3′ or 3—*O*—4′ bonds. Interestingly, these metabolites contain an additional carbon attached to the 9/9′ carbons. This suggests that the linear diarylheptanoid moiety is first formed, followed by phenoxy radical coupling; in the latter case, intramolecular coupling may result from action of a cytochrome P-450 type oxidase, such as previously noted for (*R*,*S*)-berbamunine, (*R*,*S*)-norberbamunine, and (*R*,*R*)-guattegaumerine.[151]



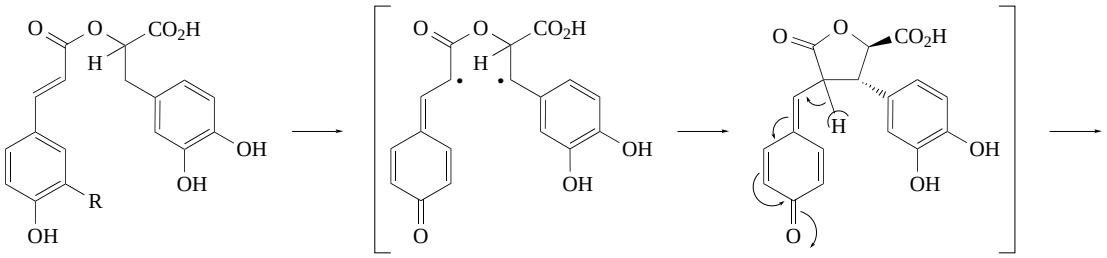
(155) (–)-(a*S*, 9*S*, 9'*S*)-Alnusdiol
$[\alpha]_D^{23} = -46.0°$ (MeOH, *c* = 0.85)
(*Betula maximowicziana*)



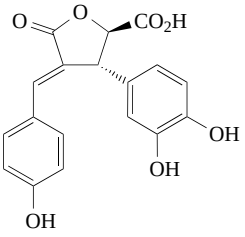
(156) (–)-rel-(*p*R, 9*S*, 9'*S*)-Maximowicziol A
$[\alpha]_D^{23} = -86.3°$ (MeOH, *c* = 0.66)
(*Betula maximowicziana*)

Finally, a quite abundant group of ''mixed'' dimers are the so-called cyclobutane lignans found in the Poaceae (Commeliniflorae) (**72**) and (**73**)[68,69] and the Ariflorae (**74**).[70] These are presumed to be primarily formed via photodimerization of either juxtaposed hydroxycinnamic acids (e.g., (**29**) and (**35**)), aldehydes (e.g., (**31**) and (**37**)), alcohols (e.g., (**32**) and (**38**)), or even allylphenols (e.g., (**58**)–(**60**)). These molecules are typically attached to the various cell wall fractions of grasses and

grains. They can apparently be formed by either head-to-head, head-to-tail, or tail-to-tail coupling; all are believed to be optically inactive.



(157) R = OH, Rosmarinic acid
(158) R = H, presumed precursor of (+)-megacerotonic acid



(61)

Scheme 19

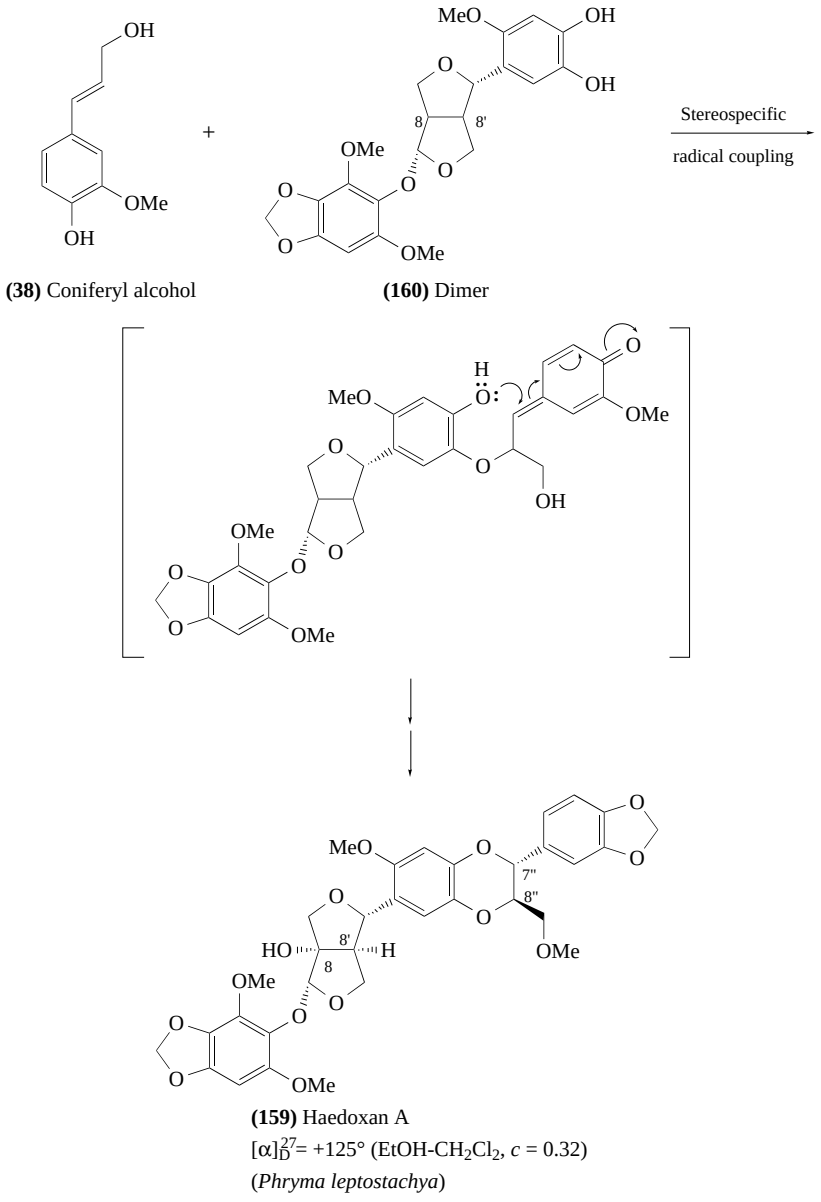
1.25.12 LIGNANS AND SESQUILIGNANS: WHAT IS THE RELATIONSHIP TO LIGNIN FORMATION?

The view has long been held,[72] without rigorous scientific proof, that monolignol (glycoside)s are transported from the cytoplasm into lignifying cell walls where they undergo sequential random coupling to give biopolymeric lignins via transient (oligomeric) lignan formation. However, this view must be tempered by the fact that ''random'' coupling of monolignols *in vitro* never gave an adequate representation of the natural lignin biopolymer(s), in terms of, for example, frequencies of inter-unit linkages[73] (see Chapter 3.18). In contrast, lignification proper has been proposed to occur via end-wise polymerization of the monolignols at discrete points in the cell wall, this being envisaged to occur along a template viewed to consist of arrays of dirigent protein sites[74] or some proteinaceous equivalent, with the initial lignin strand then replicating via a template mechanism[193–195] (see Chapter 3.18 and work by Lewis *et al.*[73]). Such a process would preclude the formation of transient dimeric and higher oligomeric forms undergoing random coupling, but would explain all the known features of lignin proper *in situ*.

On the other hand, the original random coupling hypothesis was supported in part from analyses of acetone-water extracts from homogenized sapwood and heartwood tissues. Such preparations were mainly obtained from the Pinaceae, and were thought at the time to contain ''native or Brauns lignins.''[196] Curiously, while they shared some structural similarities with lignin biopolymers (e.g., being coniferyl alcohol (**38**)-derived), they were never proven: (i) to be present in lignifying cell walls, (ii) to have any structural roles, and (iii) to be formed via direct monomer polymerization.

Today, these ''Brauns and native lignins'' appear only to be nonstructural, nonlignin oligomeric sesquilignans, which depending upon the plant system involved can apparently exist in a variety of different molecular sizes (see Section 1.25.13.6). Their roles appear to be primarily in defense. For example, the roots of the herbaceous plant *Phryma leptostachya* (Phrymaceae) contain a series of insecticidal oligolignans, such as haedoxan A (**159**) and its congeners,[197] whose formation is clearly under stereospecific enzymatic control (Scheme 20). It contains a modified 8—8′ furanofuran skeleton, with an additional C_6C_3 moiety linked through the 7″—8″ positions as noted for flavonolignans such as sinaiticin (**147**). Presumably it is formed via stereospecific radical–radical coupling of coniferyl alcohol (**38**) to the preformed dimer (**160**) or some equivalent thereof, which then undergoes further metabolism to give haedoxan A (**159**). Such substances clearly would be unable

to undergo further conversion to give lignin biopolymer(s) proper, or even to be incorporated into the same via a template-replication process.



(38) Coniferyl alcohol

(160) Dimer

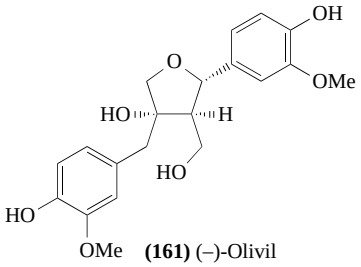
(159) Haedoxan A

$[\alpha]_D^{27}$= +125° (EtOH-CH_2Cl_2, *c* = 0.32)

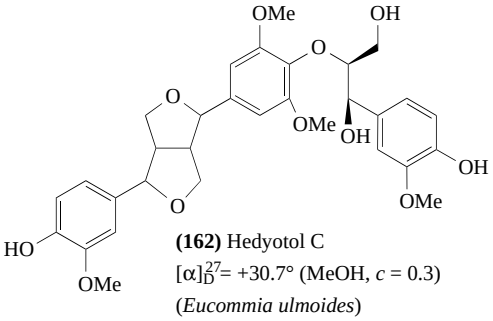
(*Phryma leptostachya*)

Scheme 20

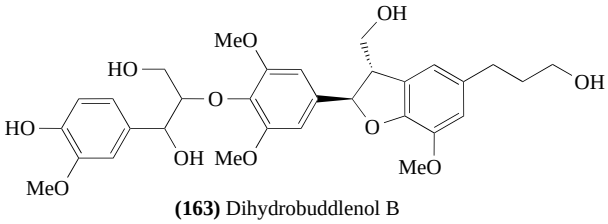
Other sesquilignans frequently consist of monolignols conjugated to optically active lignan dimers, such as medioresinol (**107**), secoisolariciresinol (**20**), matairesinol (**21**), dihydrodehydrodiconiferyl alcohol (**127**), and olivil (**161**). These include substances such as hedyotol C (**162**) from *E. ulmoides*[198] bark tissue and *Hedyotis lawsoniae* (Rubiaceae) leaves,[83] which can be viewed to result from coupling of medioresinol (**107**) with coniferyl alcohol (**38**). Another example is dihydrobuddlenol B (**163**) from *Prunus jamasakura* (Rosaceae) bark,[199] which consists of a 5-methoxydihydrodehydrodiconiferyl (**164**) moiety attached to a coniferyl alcohol (**38**) residue via an 8—*O*—4′ linkage. Others include 7′-hydroxylappaol E (**165**) from hemlock (*T. heterophylla*) sapwood,[200] consisting of 7′-hydroxymatairesinol (**99**) linked to coniferyl alcohol (**38**) via an 8—*O*—4′ bond, as well as the lappaols (**166**) and (**167**) from the roots of *Arctium lappa* (Compositae)[26,201] and the cerberalignans (**168**)–(**170**) from *Cerbera manghas* and *Cerbera odollam* (Apocynaceae) stem tissue.[202–204] The lappaols (**166**) and (**167**) are primarily matairesinol (**21**)-derived lignans linked via 8—5′ bonds to coniferyl alcohol (**38**) residues, whereas the cerberalignans (**168**)–(**170**) are mainly



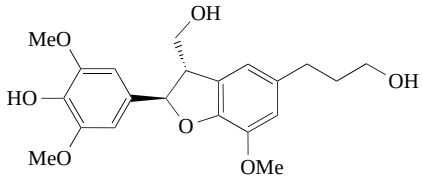
(161) (–)-Olivil
$[\alpha]_D^{26}$= –48.5° (MeOH, *c* = 1.5)
(*Cerbera manghas, C. odollam*)



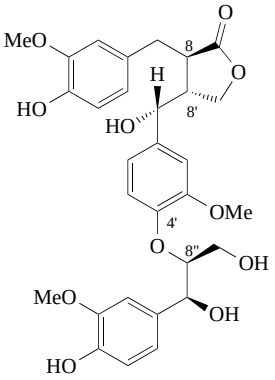
(162) Hedyotol C
$[\alpha]_D^{27}$= +30.7° (MeOH, *c* = 0.3)
(*Eucommia ulmoides*)
(*Hedyotis lawsoniae*)



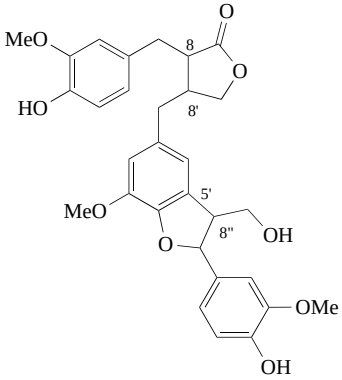
(163) Dihydrobuddlenol B
$[\alpha]_D^{29}$= –28.2° (MeOH, *c* = 0.43)
(*Prunus jamasakura*)



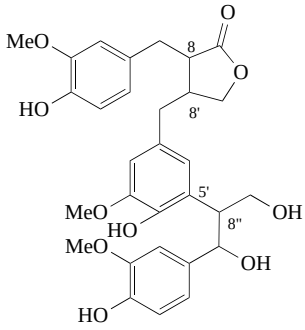
(164) 5-Methoxydihydrodehydrodiconiferyl alcohol



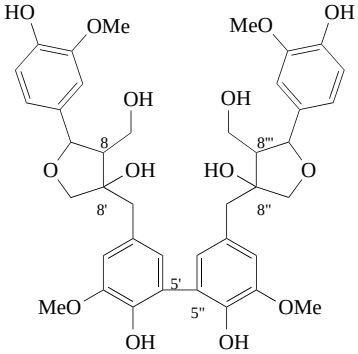
(165) (–)-7'-Hydroxylappaol E
$[\alpha]_D^{25}$= –3.7° (MeOH)
(*Tsuga heterophylla*)



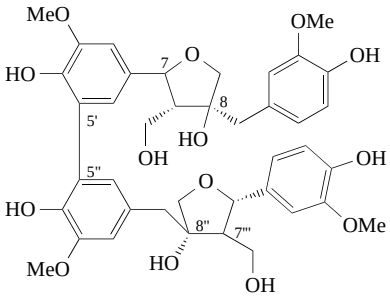
(166) Lappaol A
$[\alpha]_D^{20}$= –17.4° (MeOH, *c* = 1.0)
(*Arctium lappa*)



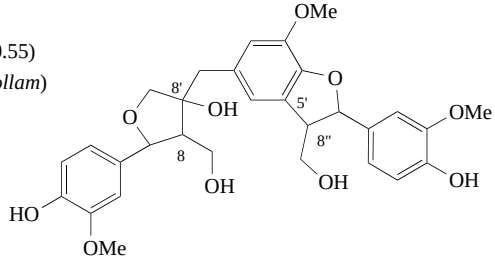
(167) Lappaol C
$[\alpha]_D^{20}$= –55° (MeOH, *c* = 1.0)
(*Arctium lappa*)



(168) Cerberalignan A
$[\alpha]_D^{20}$= –76° (MeOH, *c* = 0.55)
(*Cerbera manghas, C. odollam*)



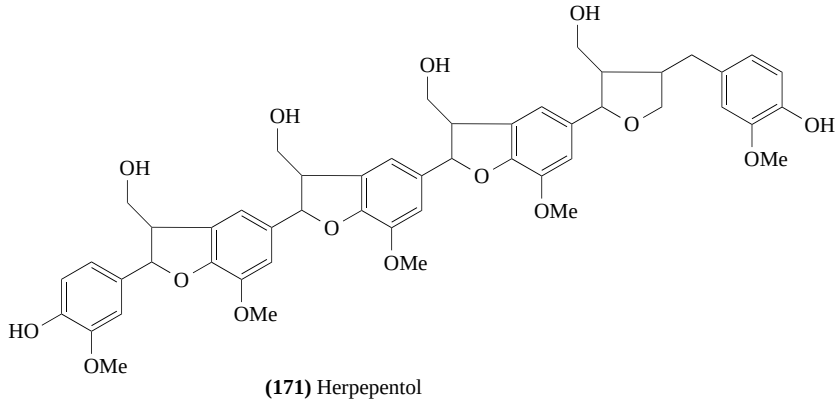
(169) Cerberalignan B
$[\alpha]_D^{20}$= –75.4° (MeOH, *c* = 0.35)
(*Cerbera manghas, C. odollam*)



(170) Cerberalignan D
$[\alpha]_D^{20}$= –50.6° (MeOH, *c* = 0.98)
(*Cerbera manghas, C. odollam*)

(−)-olivil (**161**)-derived molecules linked head-to-head, tail-to-tail, or head-to-tail. As before, however, isolation procedures, such as for the cerberalignans, were extremely lengthy and the various lignans isolated were present together with much larger amounts of the presumed precursors, e.g., olivil (**160**), again raising the possibility of artifact formation.

Perhaps one of the most unusual sesquilignan structures proposed is that of herpepentol (**171**), described as present in methanol extracts of grains from *Herpetospermum caudigerum* Wall. (Cucurbitaceae).[205] Based on its preliminary characterization by FAB MS and ^{1}H NMR spectroscopy, a coniferyl alcohol (**38**) derived 8—5′, 8—5′, 8—5′, 8—8′ linked pentamer was proposed. Although such a substance could only result from endwise coupling (cf. lignin), a more definitive study is, however, necessary to establish that its proposed structure is correct.



(171) Herpepentol
(*Herpetospermum caudigerum* Wall.)

Thus, the preponderance of available evidence reveals that (sesqui)lignans have structures which cannot be derived directly via simple free-radical coupling of monolignols, and therefore lead to lignin formation. Moreover, the structural modifications typically encountered (e.g., methylenedioxy bridge formation, allylic double bond reduction, etc.) give substances which (bio)chemically cannot readily undergo further coupling to form high molecular weight lignin biopolymers. Accordingly, there is no convincing evidence linking (sesqui)lignan formation to that of the structural lignin biopolymers, and both pathways must be viewed as being biochemically, configurationally, temporally, and spatially distinct (discussed in Section 1.25.13.6).

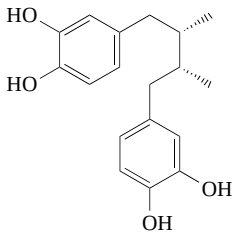
1.25.13 PHYSIOLOGICAL ROLES *IN PLANTA*

Although identification of the physiological roles of (oligomeric) lignans is still very much in its infancy, significant progress in defining some of their functions has been made. The best documented roles appear to be primarily defense related, namely, antioxidant, biocidal, feeding deterrent, and allelopathic properties. As for other systems, and although this aspect is seldom examined, a specific biological activity can be associated with a particular enantiomeric form. Using a pharmacological example to illustrate this point, (−)-trachelogenin (**17**) inhibits the replication of HIV-1 *in vitro*, whereas the corresponding (+)-antipode is much less effective.[7] Another significant role of lignans in certain species is in heartwood formation, since they affect color, durability, quality, and texture of the resulting wood: these substances have occasionally been erroneously described as ''abnormal or secondary lignins.'' Other putative roles proposed for the (oligomeric) lignans, include that of cytokinins[155–157] and as intermediates in lignification,[206] although there are significant counter-arguments to both proposed functions (see Sections 1.25.13.6 and 1.25.12).

1.25.13.1 Antioxidant Properties

Nordihydroguaiaretic acid (**172**), a major constituent of the resinous exudate of the creosote bush (*Larrea tridentata*),[207] is one of the most powerful antioxidants known,[208–210] whereas others such as sesamolin (**77**), sesamin (**18**), and sesamolinol (**81**) from sesame (*S. indicum*) seeds display less potent but still striking antioxidant properties.[4,98,99] Indeed, it is perhaps no coincidence that several oil

seed bearing plants, such as sesame and flax, contain high lignan levels, which accordingly help stabilize lipid (oil) components against oxidative degradation and onset of rancidity.



(172) Nordihydroguaiaretic acid

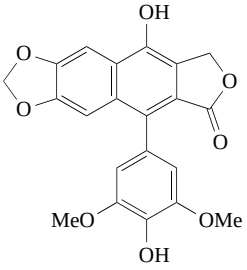
Because of these properties, several studies have been directed toward defining the precise modes of action of lignans *in planta* and in human applications. Thus, nordihydroguaiaretic acid (NDGA) (**172**) competitively inhibits soybean meal lipoxidase-catalyzed oxidation of sodium linoleate,[210,211] an important antioxidant property given that lipoxidase is directly involved in the autooxidation of unsaturated fatty acids during vegetable and seed oil manufacture. Indeed, NDGA (**172**) was a common antioxidant in various foodstuffs until 1972, when its use was discontinued following indications that it had toxic effects on the kidneys.[209] It is used instead in nonfood applications, such as in stabilizing polymers, rubber, perfumery oils, and photographic formulations.

1.25.13.2 Antifungal and Antimicrobial Effects

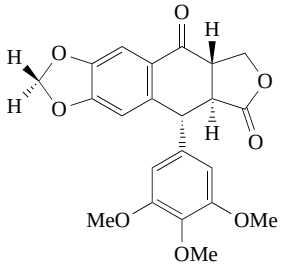
Many lignans have antifungal and antimicrobial properties. For example, termilignan (**104**) and (−)-thannilignan (**106**), from the popular Indian traditional medicinal plant, *T. bellerica* (Combretaceae), have potent activities against the fungus *Penicillium expansum*: the minimum amounts of each lignan required to inhibit fungal growth (1 and 2 μg respectively), compared favorably with conventional treatment levels with nystatin (0.5 μg).[137]

Additionally, formation of (−)-matairesinol (**21a**) and related metabolites, e.g., 7′-hydroxy-matairesinol (**99**) and α-conidendrin (**76**), is induced in *Picea abies* upon infection by *Fomes annosus*,[212,213] which, in turn, limits further fungal growth.[212–214] This observation may partly help explain the massive deposition of lignans in the heartwood of western red cedar (*T. plicata*)[35] which, in conjunction with tropolones, helps confer protection to this plant species thereby enabling life spans in excess of 3000 years to be reached.

Other lignans reputed to have antifungal properties include representatives from *Podophyllum hexandrum*[215] and the katsura tree (*Cercidiphyllum japonicum*, Cercidiphyllaceae):[216] 4′-*O*-demethyl-dehydropodophyllotoxin (**173**), and picropodophyllone (**174**) from *P. hexandrum* reportedly have antifungal activities against *Epidermophyton floccosum*, *Curvularia lunata*, *Nigrospora oryzae*, *Microsporum canis*, *Allescheria boydii*, and *Pleurotus ostreatus*, although no quantitative data were given,[215] and magnolol (**7**) from *C. japonicum* accumulates in twig cortical tissue in response to *Fusarium solani* f. sp. *mori* invasion.[216] Magnolol (**7**) is also effective against *Aspergillus niger* and *Tricophyton mentagrophytes* with minimum inhibitory concentrations (MIC) of 30 and 2.5 μg ml^{-1}, respectively, which compares well with the control using amphotericin B (MIC = 30 and 15 μg ml^{-1}).[217]



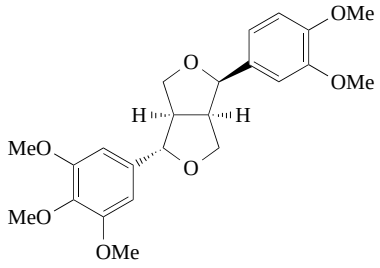
(173) 4'–*O*–Demethyldehydropodophyllotoxin



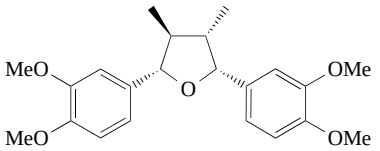
(174) Picropodophyllone

There have also been a limited number of studies examining both regio- and enantiospecific conversions of lignans in response to exposure to various fungi. For example, (±)-eudesmins (**83**), when incubated with *Aspergillus niger*, are converted into both pinoresinol monomethyl ether (**89**) ($[\alpha]_D^{29} = -12.8^\circ$, enantiomeric excess 39.3%) and pinoresinol (**3**) ($[\alpha]_D^{29} = -57.6^\circ$, enantiomeric excess 100%), where the (−)-antipode was more rapidly demethylated than its (+)-counterpart.[218] Why this organism preferentially metabolizes one particular enantiomer may be of significance in plant–fungus interactions. This may also be particularly important in lignin biodegradation studies, which most commonly use lignans rather than lignins for their assays. Unfortunately, none of the lignin-degradation studies have examined whether conversions with lignans are enantiospecific.

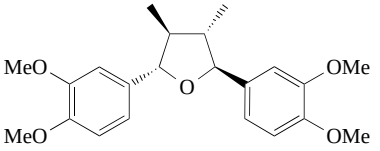
A number of other lignans are also regiospecifically demethylated at the *para* position by *A. niger*, notably (+)-magnolin (**84**), (+)-epimagnolin A (**175**), (+)-veraguensin (**176**), (+)-galbelgin (**177**), and galgravin (**178**), but not (+)-yangambin (**85**).[219–221] This fungus can also de-ethylate diethyl pinoresinol (**179**) and its monoethyl derivative (**180**) to give pinoresinol (**3**), but has no effect on dipropyl (**181**) and dibutyl (**182**) analogues.[222]



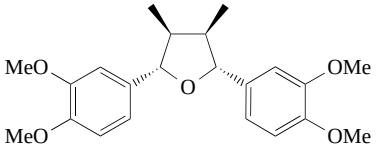
(175) (+)-Epimagnolin



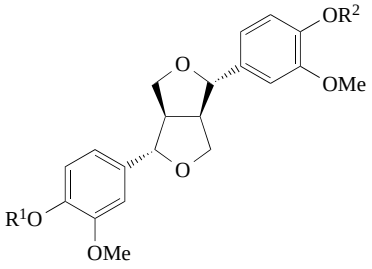
(176) (+)-Veraguensin



(177) (+)-Galbelgin



(178) Galgravin



(179) $R^1 = R^2 =$ Et, (+)-Diethyl pinoresinol

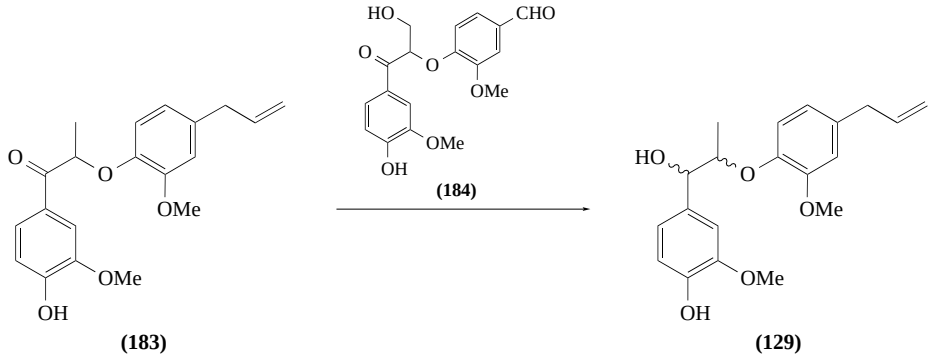
(180) $R^1 =$ Et, $R^2 =$ H, (+)-Monoethyl pinoresinol

(181) $R^1 = R^2 = n$Pr, (+)-Dipropyl pinoresinol

(182) $R^1 = R^2 = n$Bu, (+)-Dibutyl pinoresinol

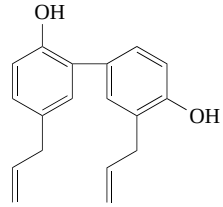
Interestingly, and while the significance is again unknown, *Fusarium solani* enantiospecifically converts Δ8′-hydroxy-3,3′dimethoxy-7-oxo-8-*O*-4′-lignan (**183**)—when incubated in the presence of the ketone (**184**)—into the *threo*/*erythro* lignans (**129**) in a ratio of 2:3 (Scheme 21). For both *threo* and *erythro* forms, only one enantiomer was formed, revealing that the reductive step was fully enantiospecific.[167]

Lignans also have antibacterial properties: magnolol (**7**) and honokiol (**185**) inhibit *Staphylococcus aureus*, *Bacillus subtilis*, and *Mycobacterium smegmatis* bacterial growth with a MIC of 5–10 μg ml^{-1}, which is comparable to or better than the activity of streptomycin sulfate (MIC = 10, 10, and 2.5 μg ml^{-1}, respectively)[217], and nordihydroguaiaretic acid (**172**) is effective against salmonella, penicillium, *M. pyrogenes*, and *Saccharomyces cerevisiae*.[209] Extracts from the aril tissue of *Myristica*

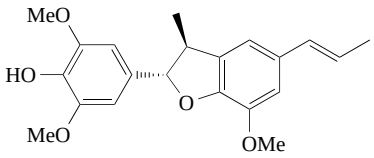


Scheme 21

fragrans (mace) are also used in Sri Lanka for dental caries prevention (antiplaque formation), with dehydrodiisoeugenol (licarin A) (**57**) and 5′-methoxydehydrodiisoeugenol (**186**) being characterized as the major antibacterial principles. Both inhibit the growth of *Streptococcus mutans* at concentrations of 12.5 μg ml^{-1}.[223]



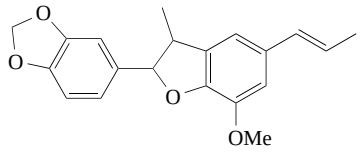
(185) Honokiol



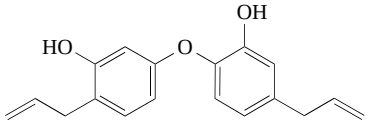
(186) 5-Methoxydehydrodiisoeugenol

1.25.13.3 Insecticides, Nematocides, Antifeedants, and Poisons

(+)-Haedoxan A (**159**), isolated from the roots of the herbaceous perennial plant, *Phryma leptostachya*, is perhaps the best known insecticidal lignan.[197,224–226] In combination with piperonyl butoxide (a synergist), it has excellent insecticidal activity, when administered orally to several lepidopterous insect larvae and houseflies, e.g., *Musca domestica*, $LD_{50} = 0.25$ ng per fly,[225] this being comparable to that of commercial synthetic pyrethroids. Its physiological effect results in muscle relaxation, feeding cessation, general paralysis, and death, thereby causing similar effects to the insect neurotoxins, nereistoxin, ryanodine, and reserpine.[197] Another insecticidal lignan is the 8,5′-linked, licarin B (**187**), isolated from *Myristica fragrans*, which is effective against silkworm (*Bombyx mori*) fourth instar larvae (at 300 ppm in the diet), with death occurring 3 days or so after treatment.[227] Additionally, the lignans (**188**), (**189**), and magnolol (**7**), isolated from *Magnolia virginiana* (Magnoliaceae), cause 100% mortality to mosquito (*Aedes aegypti*) larvae at concentrations of ~10 ppm within 2 hours, this being comparable to valinomicin treatment as a control.[228]



(187) Licarin B



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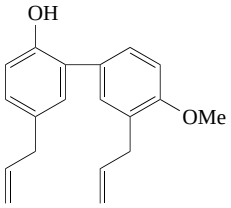
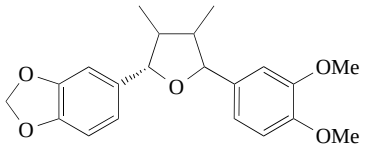


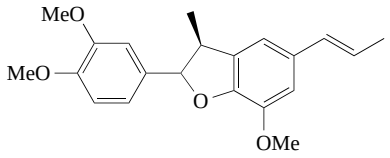
(189)

Growth inhibitory properties of lignans have also been described: (+)-epimagnolin A (**175**), isolated from the flower buds of *Magnolia fargesii*, inhibits the growth of *D. melanogaster* larvae[229] at concentrations greater than 1 mg ml^{-1}, whereas licarin A (**57**), (−)-machilusin (**190**), and lignans

(**191**) and (**192**) from the leaves of *Machilus japonica* function by inhibiting *Spodoptera litura* larval growth when added to their diets ($EC_{50} = 0.20$, 0.19, 0.13, and 0.24% w/w, respectively).[230] (+)-Sesamin (**18**) and (+)-sesamolin (**77**) from *S. indicum* also synergistically act with natural juvenile hormone to prevent metamorphosis in the milkweed bug (*Oncopeltus fasciatus*) at amounts of 10 and 1 μg, respectively.[231] Moreover, (+)-sesamin (**18**) and *epi*-sesamin (**193**) function with pyrethrum insecticides, by inhibiting oxidative degradation (cytochrome P450 oxygenase system) in the gut of the ingesting organism.[5,232]



(190) (–)-Machilusin



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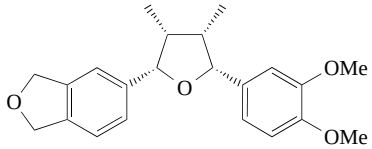

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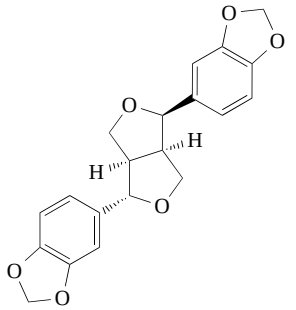
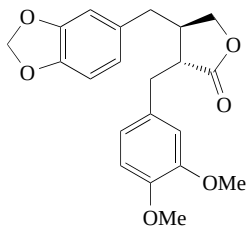


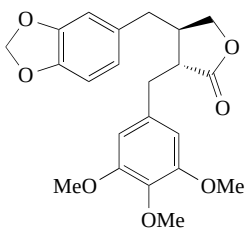
(193) *Epi*-sesamin

Lignans confer protection against nematodes.[233,234] For example, (−)-matairesinol (**21a**) and (−)-bursehernin (**194**), at concentrations of ~50 μg ml^{-1}, inhibit the hatching of potato cyst nematodes, *Globodera pallida* and *G. rostochiensis*, by 55% and 70%, respectively, when compared with controls using $ZnSO_4$ and ethanol: the hatching inhibitory dose (16.42 μg ml^{-1}) for bursehernin (**194**) reduced hatching by 50% over a 2-week period. The presence of the methylenedioxy bridge seems to play an important role, since when replaced with either a methoxyl, one hydroxyl, or two hydroxyl groups, the inhibitory activity was greatly reduced. Interestingly, lariciresinol 9-*O*-*β*-D-glucoside (**70**), isolated from the roots and stolons of potatoes, accumulates in response to infection with *G. rostochiensis*,[235] perhaps also suggesting a nematocidal role.

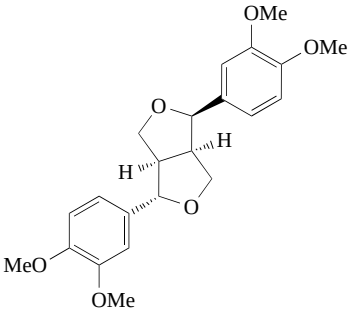


(194) Bursehernin

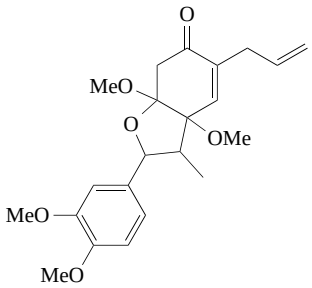
Several lignans display antifeedant and/or toxicity effects. (−)-Yatein (**195**) has antifeedant properties when added to the diets of adult granary weevil beetles (*Sitophilus granarius*) and confused flour beetle (*Tribolium confusum*) with an estimated total coefficient of deterrency of 189 and 158, respectively, where values > 200 indicate very good feeding-deterrent activity.[236] (+)-Eudesmin (**83**)/(+)-*epi*-eudesmin (**196**) from *Parabenzoin praecox*, and (−)-piperenone (**197**) from *Piper futokadsura*, have antifeedant activities (90–100%) against *S. litura* larvae when provided at concentrations of 0.05%, 1.0%, and 0.005% in the diet.[237,238] Justicidins A (**198**) and B (**199**) from *Justicia hayatai* var. *decumbens*, on the other hand, show strong toxicity against *Oryzias latipes* at levels comparable to that of rotenone and 10 times higher than that of pentachlorophenol. *J. hayatai* has been used for many centuries as a fish-poison by the natives of the Pescadores (Pung Fu islands) of Taiwan.[239]



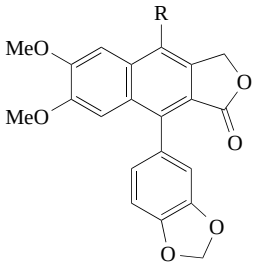
(195) Yatein



(196) *Epi*-eudesmin



(197) Piperenone



(198) R = OMe, Justicidin A
(199) R = H, Justicidin B

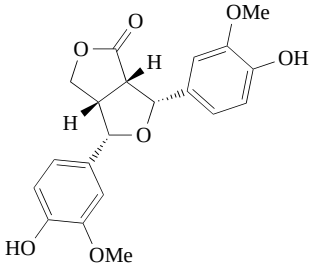
The lignanamides, (±)-grossamide (**116**), (±)-demethylgrossamide (**117**), (−)-cannabisin B (**114**), and (−)-cannabisin D (**115**), isolated from *X. aethiopica* also display antifeedant properties at 5000 ppm against subterranean termite (*Reticulitermes speratus*) workers: index values of 1.91, 29.49, 7.10, and 12.93, respectively, were obtained, where < 20 indicates significant feeding-deterrent activity.[143]

1.25.13.4 Allelopathy

Various lignans have powerful allelopathic properties. For example, nordihydroguaiaretic acid (**172**), when supplied at concentrations of $\sim 20\ \mu g\ l^{-1}$, is able to dramatically reduce the seedling root growth of barnyard grass, green foxtail, perennial ryegrass, annual ryegrass, red millet, lambsquarter, lettuce, and alfalfa, as well as the hypocotyl growth of lettuce and green foxtail.[240] Additionally, the monoepoxylignanolide (**200**) of *Aegilops ovata* is reputedly a unique germination inhibitor of *Lactuca sativa* (lettuce) achenes, this effect being greater in the light than under darkness.[241] Arctiin (**92**) from *Arctium lappa* inhibits the germination of 11 out of 12 different plant species tested at concentrations of $\sim 5\ \mu g\ \mu l^{-1}$, and the levels of arctiin (**92**) parallel that of the annual rhythm of germination.[242] The furofurans, fargesin (**201**) and sesamin (**18**), are germination inhibitors of peanut (*Arachis hypogaea*) seeds (lipid-storing seeds), but not of rice (*Oryza sativa*) seeds (carbohydrate-storing seeds),[243] where the investigators suggested that this effect might be on processes/enzymes controlling lipid metabolism. Interestingly, in the same studies, eudesmin (**83**), which lacks a methylenedioxy group, was much less active, suggesting that the methylenedioxy group is needed for biological activity.

1.25.13.5 Cytokinin-like Activities

Certain lignans have been implicated to function as cytokinins during plant growth and development, although they are only effective at very high concentrations. For example, a cytokinin-like



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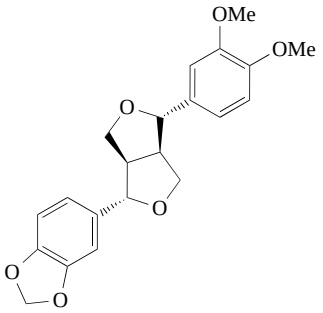


(201) Fargesin

function has been proposed for (±)-dehydrodiconiferyl alcohol 4-*O*-glucosides (**125a,b**), since they can stimulate cell division of tobacco (*N. tabacum*) cells and replace cytokinin in pith and callus cultures:[155–157] both (+)- and (−)-dehydrodiconiferyl alcohol 4-*O*-glucosides (**125a**) and (**125b**) stimulated pith growth, at concentrations of ~10 μM, in a manner comparable to that of the cytokinin, zeatin riboside (0.1 μM). On the other hand, they apparently did not stimulate shoot formation from leaf explants as normally observed for cytokinins.[155] These investigators also proposed that the glucosides were being mobilized from their roles as cell-wall components, even though this could not be the case since they were extracted from the callus cultures by methanol/water extraction. That is, although their subcellular origins were not determined, they could not have been cell-wall constituents based on this solubilization property. Moreover, these data must be viewed as only a tentative indication of any cytokinin role *in vivo*, and more extensive studies are required to prove that this is indeed a true function.

1.25.13.6 Constitutive and Inducible (Oligomeric) Lignan Deposition and Nonstructural Infusions, "Abnormal" and "Stress" Lignins

The physiological roles of lignans discussed thus far are those of antioxidant, biocidal, allelopathic, and antifeedant agents, as well as a putative role as cytokinins. These properties, in turn, lead to the question of what are the factors controlling their induction/constitutive formation *in planta*? However, with the appropriate gene[87,90,111] and promoter sequences (data not shown) in hand, together with their proteins and antibodies, meaningful experiments can be undertaken to define how their formation is regulated, and the temporal and spatial nature of expression of the biosynthetic pathways involved.

What is known about sites of lignan accumulation is both rudimentary and variable, with evidence from different studies perhaps pointing to different locations. One study suggests that 5-methoxypodophyllotoxin (**100**) is present in the vacuolar compartments of *Linum album* cell suspension cultures, this being based on crude subcellular (organelle) fractionation studies.[37] That it is supposedly present in the vacuoles might also suggest a similar location for the constitutively formed insecticidal lignan, haedoxan A (**159**), in the roots of *P. leptostachya*, and the cytotoxin, podophyllotoxin (**23**), in the rhizomes of *P. peltatum*/*P. hexandrum*. In seed tissues, such as sesame and flax, nothing is definitively known about their lignan subcellular locations, a situation which also holds for lignans in flowers, fruits, leaves, and bark.

Lignan deposition in sapwood and heartwood tissues of certain woody plants has been a matter of particular interest, given the long-standing confusion surrounding the nature of various heartwood metabolites and whether they are lignins, lignans, or oligomeric lignans (see Section 1.25.12); this, in turn, has led to the use of lax terminology to describe such constituents, e.g., as "abnormal and secondary" lignins.

Heartwood formation itself is initiated, at some undetermined point, in the center (pith) region of mature prelignified secondary xylem wood. What initiates or induces its formation is unknown, although the metabolic composition (lignans, flavonoids, alkaloids, etc.) can vary extensively with the species. Nevertheless, it is this phenomenon that provides the various woody types, with these differing in terms of color, quality, durability, and rot resistance, e.g., the black color of ebony wood and the reddish-brown color of western red cedar are due to their distinctive heartwood

metabolites, whereas the whitish-yellow of spruce results from the near absence of any heartwood metabolites.

Heartwood-forming substances are considered to be initially formed and released from specialized ray parenchyma cells, with these substances further infusing into neighboring tracheids and/or fibers/vessels (Figure 19). As proposed by Chattaway nearly 50 years ago,[244,245] they are exuded through parenchyma cells via pit apertures into the lumen of adjacent dead, prelignified, cells and then diffuse into neighboring, prelignified cells to ultimately afford the heartwood tissue. In agreement with this contention, Hergert's analysis of western red cedar and western hemlock constituents also led to the conclusion that heartwood-forming substances accumulated in ray parenchyma cells, prior to becoming insoluble infusions/deposits in tracheid cells.[29] Indeed, for these reasons, Hergert cautioned that analytical results obtained from the analysis of wood samples must specify the physiological conditions and/or tissue, e.g., whether sapwood, heartwood, compression wood, diseased wood, etc. Unfortunately, most investigations treat woody tissue as if it were homogeneous.

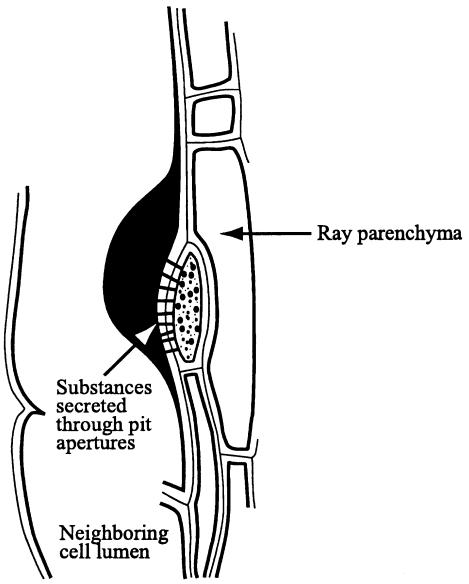
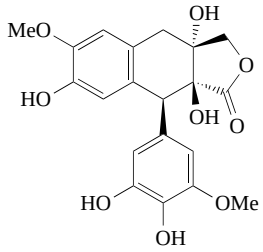


Figure 19 Secretion of heartwood constituents by ray parenchyma cells into the lumen of neighboring cells appears to occur through pit apertures. After Chattaway.[244]

Heartwood metabolites are frequently, but erroneously, also described as "extractives," based on the fact that a portion can be removed by aqueous/organic solvent treatment. It is seldom acknowledged, however, that only a proportion of these metabolites is solubilized, with the remaining often requiring harsher conditions, e.g., as commonly employed for lignin dissolution. To a lesser extent, comparable substances to those present in heartwood can also be formed in sapwood, in response to biological challenges, such as insect attack.

Unfortunately, imperfect characterization of such heartwood/sapwood metabolites, particularly when lignan derived, has led to their descriptions as "abnormal" lignins, "secondary" lignins, and "Brauns native" lignins. These substances are, however, not lignins, but instead constitute (see Section 1.25.12) a fundamentally distinct biochemical class of nonstructural, nonlignin metabolites which can be produced in a range of sizes and form insoluble deposits during heartwood formation, i.e., they differ from lignins in terms of temporal and spatial deposition, configurations of the molecules, postcoupling modifications, and physiological function. In western red cedar, for example, the lignan-derived components can range from monomers, such as plicatic acid (**75**) and plicatin (**202**) to oligomers (MW $\sim$10 000), with a portion only being removed under conditions required for lignin removal.[27–29,36] Another example is western hemlock heartwood, the lumen of whose tracheid cells can contain either 7′-hydroxymatairesinol (**99**) or α-conidendrin (**76**).[246] This heterogeneity is particularly interesting, since it suggests that certain parenchyma cells may be involved in formation of specific metabolites, regardless of the fact that complex mixtures may ultimately result in the developed heartwood via the infusion process previously discussed.[244,245]



(202) (–)-Plicatin

As described in Section 1.25.12, other nonlignin, nonstructural metabolites are that of the ''Brauns native'' lignins[196] and ''abnormal'' lignins[247] present in both Pinaceae heartwood and sapwood (although typically by 10-fold less in the latter). These substances, e.g., in loblolly pine (*Pinus taeda*), are primarily dehydrodiconiferyl alcohol (**5a,b**) derived, and result from lignan modifications via allylic double bond reduction, demethylation, and phenylcoumaran ring opening.[171–173] As before, they are released into the prelignified sapwood via specialized cells, and have chemical structures which preclude them from being able to undergo polymerization to give lignin biopolymers (see Section 1.25.12 and Chapter 3.18).

The term, ''stress'' lignins, has also been introduced to describe inducible ''lignin-like'' responses, but again with no explicit biochemical explanation as to what it actually meant. For example, *P. taeda* cell suspension cultures (and those of other plants in the Pinaceae) can be induced, at high sucrose concentrations, to form a lignin-like ''extracellular'' precipitate.[168] Detailed analysis of these substances revealed, however, that this inducible response resulted from lignan coupling as for the ''abnormal'' lignins, and as such they more closely resemble constituents formed during heartwood deposition which are formed by a distinct biochemical pathway to that of the lignins.

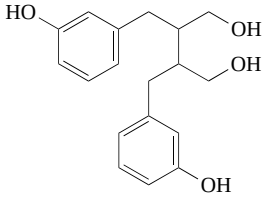
This, however, again underscores the necessity to use molecular probes to comprehensively distinguish, both temporally and spatially, between lignin and (oligomeric) lignan formation and deposition.

1.25.14 ROLES IN HUMAN NUTRITION/HEALTH PROTECTION AND DISEASE TREATMENT

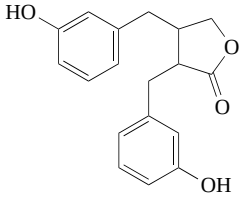
Lignans have long held considerable importance in medicine, human health, and nutrition, and a brief description of some of their most significant applications is summarized below.

1.25.14.1 Nutrition/Health and Protection against Onset of Breast and Prostate Cancers: Secoisolariciresinol, Matairesinol, and Sesamin

Dietary lignans, e.g., secoisolariciresinol (**20**) and matairesinol (**21**), have significant roles in conferring health protection, particularly against the onset of breast and prostate cancers. During digestion, they are metabolized into the ''mammalian'' lignans, enterodiol (**203**) and enterolactone (**204**), these being first detected in the urine of female rats and humans.[248,249] Interestingly, excretion of these lignans had a cyclic pattern during the menstrual cycle, which reached a maximum during the luteal phase. It has also been shown with rats that the levels of enterodiol (**203**) increase substantially when flaxseed was provided, due to metabolism of secoisolariciresinol diglucoside (**98**).[250]

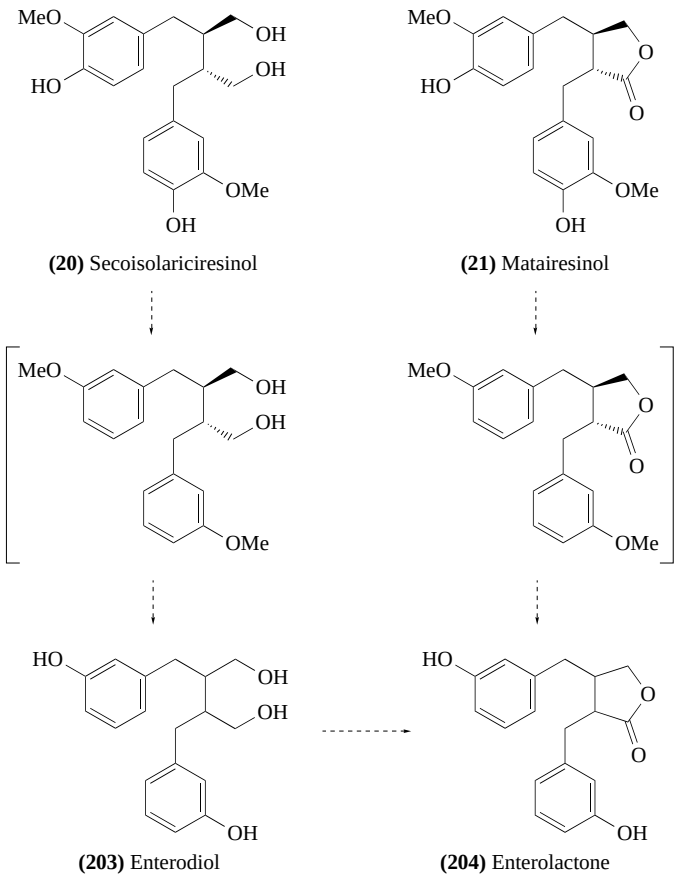


(203) Enterodiol



(204) Enterolactone

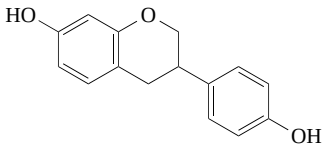
Recognition of the health-protection effects of dietary lignans began with observations of significant metabolic profile differences in the urinary excretions from individuals at low risk for breast/prostate cancers, and those at high risk or who had contracted these cancers. Low breast cancer risk Finnish women, for example, had high levels of mammalian lignan-derived metabolites, enterodiol (**203**) and enterolactone (**204**), in their body fluids (urine, plasma, and bile), whereas those at high risk did not (< 5% of total).[251–253] This was subsequently recognized to be a form of chemoprotection which was ultimately correlated with a vegetarian-like diet (grains, fibers, seeds, and berries) rich in the lignans, secoisolariciresinol (**20**) and matairesinol (**21**). Their subsequent conversion into the protective ''mammalian'' lignans, enterodiol (**203**) and enterolactone (**204**), occurs in the gut[254,255] via loss of the hydroxyl functionalities at C-4 and C-4′ and demethylation at C-3 and C-3′. Scheme 22 shows a possible biochemical pathway.



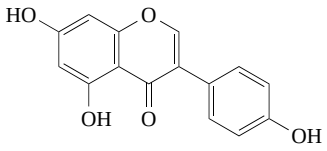
Scheme 22

Dietary lignans (as well as isoflavonoids) impart chemoprotective effects due to their antioxidant,[4,98,99] weak oestrogenic/antioestrogenic,[256–258] anti-aromatase,[259,260] and anticarcinogenic/antitumor[261–265] properties, thereby protecting against the initiation of various sex hormone-induced cancers. Their importance can be illustrated using as an example the sex hormone binding globulin (SHBG). This binds circa 50% of circulating testosterone in men, and 80% of the oestrogen in women[258] and thus the availability of sex hormone to target cells is greatly affected by changes in both its concentration and/or binding properties. Postmenopausal women excreting large amounts of mammalian lignans have higher levels of SHBG than omnivores or breast cancer patients,[265] and other studies have shown that mammalian lignans and isoflavonoids interact with SHBG in a dose-dependent manner, with enterolactone (**204**) > equol (**205**) > genistein (**206**) for displacing estradiol (**207**), and equol (**205**) > enterolactone (**204**) or enterodiol (**203**) > genistein (**206**) for testosterone (**208**) displacement.[258,266] These effects occur at levels (5–50 μM) which correspond well with the

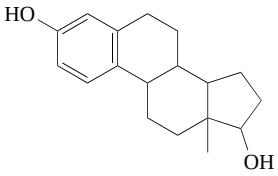
concentrations of plant-derived dietary diphenols in body fluids, following ingestion of vegetarian and soy diets, thereby suggesting that sex-hormone binding is modulated by their presence.



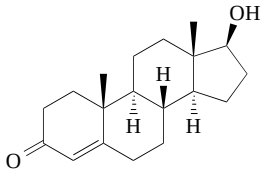
(205) Equol



(206) Genistein



(207) Estradiol



(208) Testosterone

There is also chemoprotection against the onset of tumors. Sprague–Dawley female rats, pretreated with the (pro)carcinogen dimethylbenzanthracene, had reduced incidence rates (37% reduction) and tumor masses (46% reduction in mammary tissues)[261] when rats were administered a diet containing secoisolariciresinol diglucoside (**98**), and the number of valid putative preneoplastic markers for colon carcinogenesis in male rats also declined.[263] Treatment with flaxseed or the flax lignan, secoisolariciresinol diglucoside (**98**), also significantly reduced epithelial cell proliferation, as well as the number of aberrant crypts and aberrant crypt foci, viewed to be early indicators of colon cancer risk.[263] In this regard, it is noteworthy that both Japanese and Caucasian men have comparable numbers of precancerous colonic aberrations; however, in Japanese men, somehow these do not become cancerous to the same extent, an observation which is viewed as a dietary consequence.[267] Such dietary differences may also help explain the difficulties in inducing carcinogenesis in primates, even when procarcinogens are added to the diet. For example, administering oestrogens with benzpyrene/dibenzanthracene to rhesus monkeys did not result in carcinogenesis, even after 8 years.[264] This effect is presumed to be due to the fact that primates have diets which result in the massive accumulation and excretion of lignan (as well as isoflavone) diphenolics.

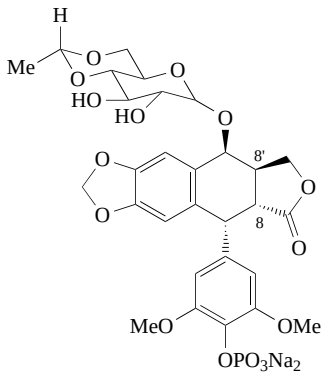
Flaxseed is the richest source of mammalian lignan precursors, containing levels 75–800 higher than any other plant food, and is being widely investigated for its cancer protective effects. Incubation of its most abundant lignan, (+)-secoisolariciresinol diglucoside (**98**), with cultured human fecal microflora *in vitro*, suggested the metabolic pathway (Scheme 22) for its conversion into the mammalian lignans: that is, intestinal bacteria hydrolyzed the sugar moiety to release secoisolariciresinol (**20**), this being presumably followed by dehydroxylation and demethylation to give the mammalian lignan enterodiol (**203**), which was oxidized to enterolactone (**204**). As indicated in Scheme 22, enterolactone (**204**) is also considered to result from catabolism of the plant lignan, matairesinol (**21**).[268] Once formed, both mammalian lignans undergo enterohepatic circulation,[269] where a good correlation exists with their presence and the reduced incidence rates of hormone-related cancers.

Chemoprotection can also be correlated with antioxidant abilities, e.g., of secoisolariciresinol diglucoside (**98**) as a radical scavenger.[270] For example, hydrogen peroxide, when subjected to photolysis under ultraviolet light in the presence of salicylic acid, is involved in the formation of the $OH^{\bullet}$ adduct products, 2,3-dihydroxybenzoic acid (DHBA) and 2,5-DHBA. When secoisolariciresinol diglucoside (**98**) was present, however, a concentration-dependent decrease in the formation of 2,3-DHBA and 2,5-DHBA occurred due to scavenging of $OH^{\bullet}$ radicals by secoisolariciresinol diglucoside (**98**). On a somewhat related topic, dietary sesamin (**18**) also has the effect *in vivo* of elevating levels of the antioxidant, γ-tocopherol, in rat plasma and liver, leading to the suggestion that a sesame-rich diet increases availability of antioxidants (vitamin E) in the body. This, in turn, decreases the risk of a number of diseases directly related to free-radical formation, such as more rapid onset of aging;[271] sesame lignans can also cause an increase in vitamin E activity in rats fed a low α-tocopherol diet.[272]

1.25.14.2 Antitumor Properties: Podophyllotoxin and other 8—8′ Lignans

There are essentially only a handful of plant natural products used in medicine today in cancer treatment, of which one is podophyllotoxin (**23**) from *Podophyllum peltatum* and *Podophyllum hexandrum*. Its pharmacological usage dates back many centuries, when may apple (*P. hexandrum*) alcoholic extracts, obtained from rhizomes and roots, were employed first as a poison and later, in smaller doses, for treatment of various pathological conditions.[273] The cytotoxic effect of these extracts was subsequently found to be due to podophyllotoxin (**23**), which led to its use as an antitumor agent against various malignancies. It was later shown that podophyllotoxin (**23**) was readily taken up by the cells due to its small size and hydrophobicity, with tubulin binding (at a different site to that occupied by the *Vinca rosea* alkaloids) and microtubular assembly inhibition arresting cells in mitosis;[274,275] this occurred in a manner more rapid and reversible than colchicine. Its action leads to cytoskeletal arrest of cell division and ultimately cell death.

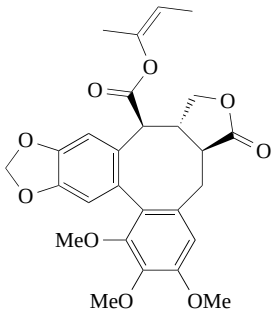
In spite of its antitumor promise, the clinical applications of direct administration of podophyllotoxin (**23**) were greatly compromised by severe (gastrointestinal) toxicity experienced by those under treatment. Accordingly, a significant effort was launched to identify means whereby the drug could be delivered with reduced cytotoxicity. This led to the development, and subsequent widespread application, of its semisynthetic derivatives, etoposide (**101**) and teniposide (**102**),[276,277] which are used (alone or in conjunction with other drugs, e.g., cisplatin) for treatment of Hodgkin's lymphomas, non-Hodgkin's lymphomas, small cell lung cancers, and acute leukemias.[278–280] The semisynthetic derivatives (**101**) and (**102**), however, were found to differ in their mechanism of action. The added sugar moieties prevented tubulin interactions from occurring, and thus microtubular assembly was not inhibited. The antitumor action was, instead, a consequence of an ability to form stable tertiary complexes with topoisomerase II and its substrate DNA leading to formation of numerous double-stranded DNA breaks. In turn, this results in large DNA fractures, thereby arresting cells in their life cycle at the G_2 phase, and ultimately causing cell death.[281] At present, etoposide phosphate (**209**), the phosphorylated form of etoposide (**101**), is undergoing clinical tests, since its application may be more convenient due to its increased water solubility.[282,283]



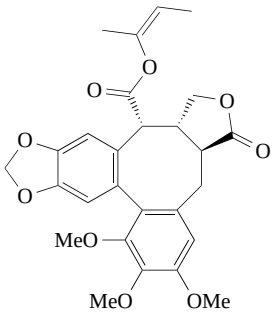
(209) Etoposide phosphate

Other lignans have promising anticancer properties: (−)-steganacin (**25**) and (−)-steganangin (**210**), isolated from *S. araliacea* stem bark and wood, exhibit antileukemia activities both against the *in vivo* murine P-388 lymphocytic leukemia test system and *in vitro* against cells derived from a human carcinoma of the nasopharynx cell culture.[148] It is thought that this antimitotic activity is through an effect on spindle microtubules, as for podophyllotoxin (**23**), with the chirality about the pivotal biphenyl bond and the orientation of the lactone carbonyl being essential for antitumor activity.[284] *Epi*-steganangin (**211**) and steganoate B (**212**) also have cytotoxic properties against 11 different human tumor cell lines,[285] and (−)-burseran (**213**) from *Bursera microphylla* (Burseraceae) displays antitumor properties against human epidermoid carcinoma of the nasopharynx cell culture.[286,287] (+)-Wikstromol (**67**), from *Wikstroemia foetida* var. *oahuensis* Gray (Thymeleaceae) is also active against the P-388 lymphocytic leukemia test system.[288]

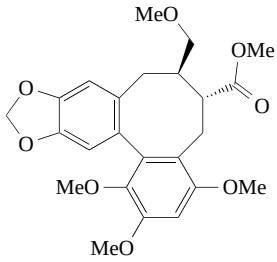
Finally, phyllanthin (**214**) and hypophyllanthin (**215**), from *Phyllanthus amarus* Schum. & Thonn. (Euphorbiaceae), enhance cytotoxic responses mediated by vinblastine in the multidrug-resistant KB cell line. However, alone they had no significant cytotoxic activity with a large number of mammalian cells.[289]



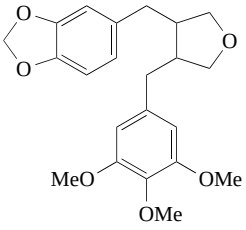
(210) (–)-Steganangin



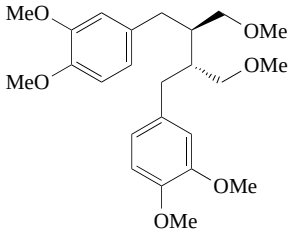
(211) (–)-Episteganangin



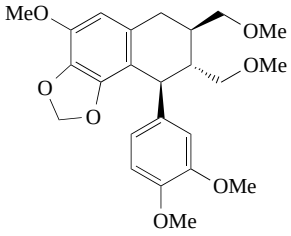
(212) (–)-Steganoate B



(213) Burseran



(214) Phyllanthin



(215) Hypophyllanthin

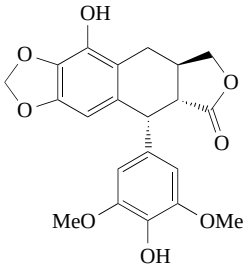
1.25.14.3 Hepatotoxic Preventive Effects

Schizandra chinensis fruit is an important component of various traditional Asian medicines. Its ''kita-gomisi'' extract is used as an antitusive and tonic,[149] whereas the ''Sheng Mai San'' formulation is employed in the treatment of coronary heart disease treatment.[290] The fruits have also been used in Japan and East Asia for the treatment of elevated serum aminotransferase activity in acute hepatitis.[291]

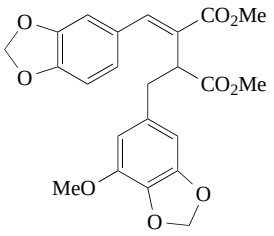
Schizandra fruit contains significant levels of the 8—8′,2-2′ linked lignans, such as gomisin A (**121**).[292] This lignan appears to have an excellent ability in protecting the liver from a variety of liver-damaging agents, such as the hepatotoxic compounds, CCl_4, galactosamine, and lipopolysaccharides.[291,293,294] This protection has been correlated with several enzymatic processes, e.g., inhibition of leukotriene biosynthesis preventing arachidonic acid release,[295] and prevention of acetaminophen-induced liver injury in rats by inhibiting lipid peroxidation.[291] Prevention or limitation of acetaminophen-caused intoxication by gomisin A (**121**) is considered to be due to the reduction of aminotransferase activity in serum and suppression of lipoperoxide accumulation in liver.[291] Liver regeneration, following partial hepatectomy, is also stimulated by gomisin A (**121**), and this is thought to occur via stimulation of ornithine decarboxylase activity leading to putrescine and spermidine accumulation (polyamines play an important biochemical role in liver regeneration)[296] as well as DNA and RNA biosynthesis.[297] Gomisin A (**121**) also has an ability to inhibit 3′-methyl-4-dimethylamino-azobenzene induced liver carcinogenesis,[298,299] as well as limiting muscular damage induced by excessive exercise.[300]

1.25.14.4 Antiviral Properties

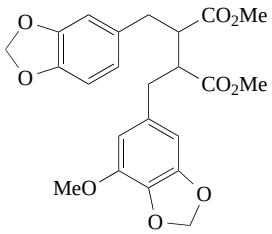
Many lignans have been demonstrated to exhibit quite potent antiviral properties: podophyllotoxin (**23**) and (−)-α-peltatin (**216**) prevent development of murine cytomegalovirus plaques in mouse 3T3-L1 cells, as demonstrated *in vitro* by reduction of plaque numbers by $\sim 50\%$ at a concentration of 10 ng ml^{-1}.[301] Rhinacanthin E (**217**) and rhinacanthin F (**218**), from the medicinal plant *Rhinacanthus nasutus* (Acanthaceae), also have antiviral activities against the influenza type A virus. Using the hemadsorption inhibition assay, these lignans have EC_{50} values of 1.7 and < 0.94 μg ml^{-1}, whereas with a cytopathic effect assay dosage levels were 7.4 and 3.1 μg ml^{-1}, respectively. For both assays, control values with amantadine and ribavirin were 0.054 and 3.7 μg ml^{-1}, respectively.[302]



(216) (–)-α-Peltatin

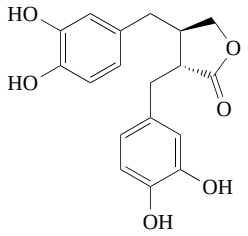


(217) Rhinacanthin E



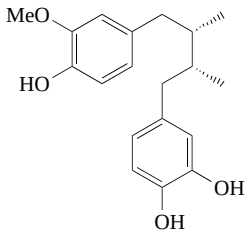
(218) Rhinacanthin F

A number of lignans inhibit replication of the human immunodeficiency virus (HIV), albeit with different modes of action. (−)-Arctigenin (**22a**) and (−)-trachelogenin (**17**) inhibit HIV-1 replication in infected human cell systems,[7] with (−)-arctigenin (**22a**) suppressing integration of proviral DNA into the cellular DNA genome,[303] whereas it was inactive with purified HIV-1 integrase.[304] 3,3′-Demethylarctigenin (**219**) (a catechol analogue), on the other hand, gave a strong inhibition of HIV-1 integrase.[304]

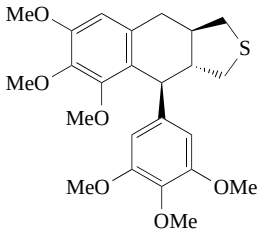


(219) 3,3'-Demethylarctigenin

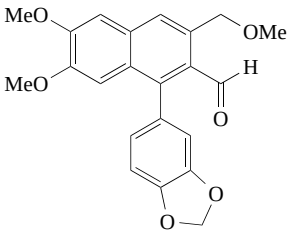
Antiviral effects have also been noted for constituents from the creosote bush,[6,305] a plant widely used in traditional medicine among the indigenous people of America for digestive disorders, rheumatism, venereal diseases, and sores. One of its lignans, 3′-*O*-methylnordihydroguaiaretic acid (**220**),[6] inhibits HIV Tat-regulated transactivation *in vivo* (EC_{50} = 25 μM), and it is thought that this occurs by interrupting not only the life cycle of wild-type HIV, but also of reverse transcriptase or protease mutant viruses.[305] Other tetrahydronaphthalene lignan analogues have been tested as potent inhibitors of HIV-1; of these, the most effective is compound (**221**), which has an ED_{50} of 0.8 μM with an IC_{50} of 58 μM. It functions as a noncompetitive inhibitor of HIV-1 reverse transcriptase with respect to both template-primer and substrate (dGTP),[306] as does phyllamycin B (**222**) and retrojusticidin B (**223**) from *Phyllanthus myrtifolius* (Euphorbiaceae; IC_{50} = 3.5 and 5.5 μM, respectively) with respect to template primer and triphosphate substrate.[307]



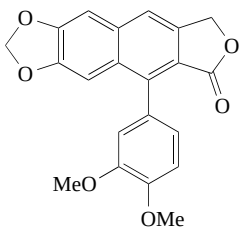
(220) 3'-*O*-Methylnordihydroguaiaretic acid



(221)



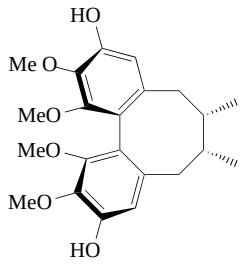
(222) Phyllamycin B



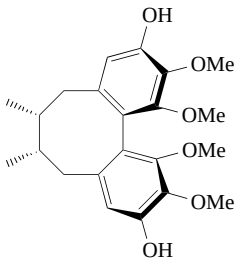
(223) Retrojusticidin B

Of the *Schizandra* lignans, examined for anti-HIV activity, (−)-gomisin J (**224a**) displays beneficial effects, whereas gomisins A (**121**), D (**225**), E (**226**), and N (**227**), deoxyschizandrin (**228**), and (+)-gomisin J (**224b**) do not.[308] Interestingly, the synthetic bromine analogue (**229**) of (−)-gomisin J was 33-fold more effective than (−)-gomisin J (**224a**) itself. Studies of its action suggested it to be both a noncompetitive inhibitor of HIV-1 reverse transcriptase, and a mixed (noncompetitive and uncompetitive) inhibitor with respect to the primer-template.[308] The bromo derivative (**229**) was also effective against 3′-azido-3′-deoxythymidine (AZT) resistant HIV-1, as well as synergistically

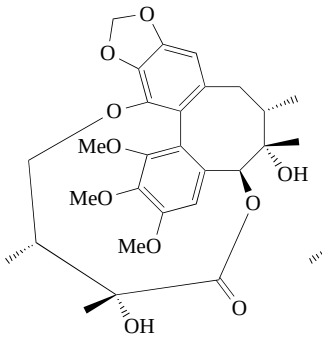
acting with AZT. Finally, anolignan A (**230**) and anolignan B (**105**), from *Anogeissus acuminata* (Combretaceae), synergistically inhibit HIV-1 reverse transcriptase,[309] whereas interiotherin A (**231**) and schisantherin D (**232**), isolated from *Kadsura interior* (Schizandraceae), inhibit HIV replication with EC_{50} values of 6.1 and 1.0 μM, respectively.[310]



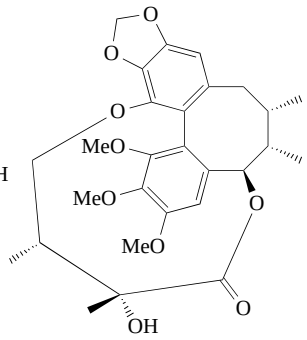
(224a) (–)-Gomisin-J



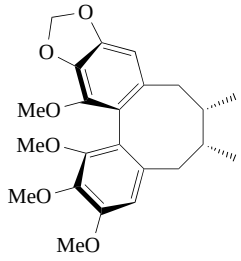
(224b) (+)-Gomisin-J



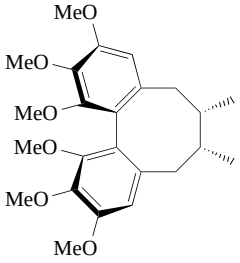
(225) Gomisin-D



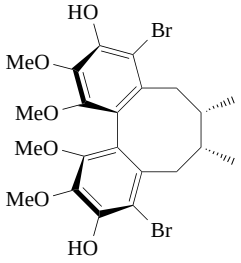
(226) Gomisin-E



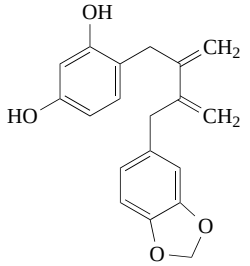
(227) Gomisin-N



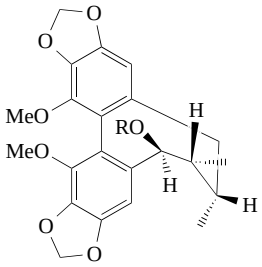
(228) Deoxyschizandrin



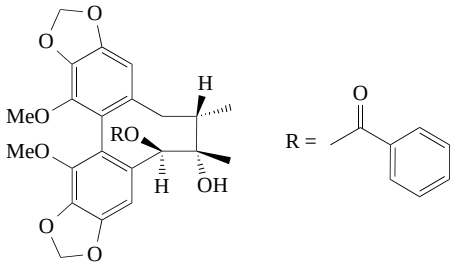
(229)



(230) Anolignan A



(231) Interiotherin A

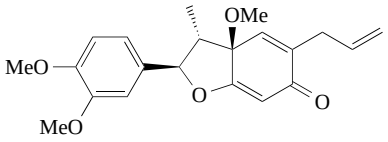


(232) Schisantherin D

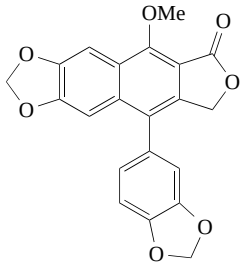
1.25.14.5 Miscellaneous Health Benefits: Anti-inflammatory, Antiasthmatic, and Antidepressant Effects

Kadsurenone (**233**), from *P. futokadsura* (Piperaceae), is a platelet-activating factor, a potent mediator of inflammation, and an asthma antagonist.[311] Such effects have also been ascribed to fargesin (**201**)/eudesmin (**83**) from *Magnolia biondii* (Magnoliaceae),[312] as well as yangambin A (**85**) from *Ocotea duckei* (Lauraceae)[313] and neojusticin A (**234**), justicidin B (**199**), taiwanin E (**235**), and its methyl ether (**236**) from *Justicia procumbens* (Acanthaceae).[314]

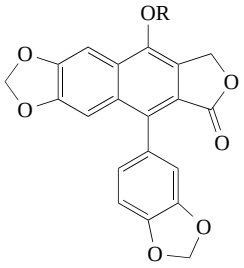
Potential antiasthmatic agents are evaluated on their abilities to inhibit cyclic nucleotide phosphodiesterase, this being responsible for hydrolyzing cAMP and cGMP into their respective 5′-mononucleotides. That is, increases in cellular levels of cAMP and cGMP have been implicated in relaxation of the airway smooth muscle, given that elevated levels of cAMP prevent the activation of pro-inflammatory cells. Lignans and norlignans with demonstrable cAMP phosphodiesterase



(233) Kadsurenone

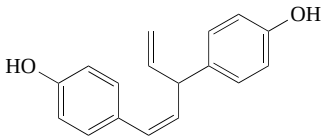


(234) Neojusticin A

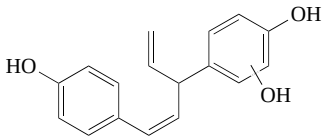


(235) R = H, Taiwanin E
(236) R = Me, Taiwanin E methyl ether

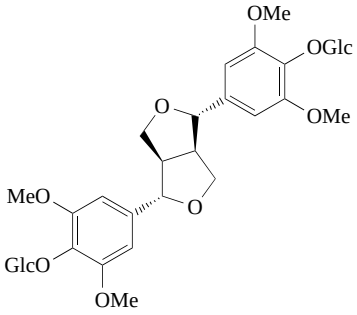
inhibitory properties include: (+)-pinoresinol (**3a**)/(−)-matairesinol (**21a**) from *Forsythia* species,[315] *cis*-hinokiresinol (**237**)/oxy-*cis*-hinokiresinol (**238**) from *Anemarrhena asphodeloides*,[316] and (+)-syringaresinol-di-*O*-*β*-D-glucopyranoside (**239**)/(+)-hydroxypinoresinol 4′,4″-di-*O*-*β*-D-glucopyranoside (**240**) from *Eucommia ulmoides* bark.[317] Additionally, arylnaphthalene analogues have been synthesized and tested as cyclic nucleotide phosphodiesterase IV inhibitors: compound (**241**) inhibits phosphodiesterase ($IC_{50} = 0.057$ μM) and displays antispasmodic activities; it is 8-fold more active than rolipram ($ED_{50} = 2.3$ mg kg^{-1} versus 19 mg kg^{-1} i.v.) in the guinea pig antigen-induced bronchoconstriction model. However, it was 8-fold less active than rolipran in a histamine-induced bronchospasmotic assay ($ED_{50} = 0.08$ mg kg^{-1} versus 0.01 mg kg^{-1} i.v.).[318]



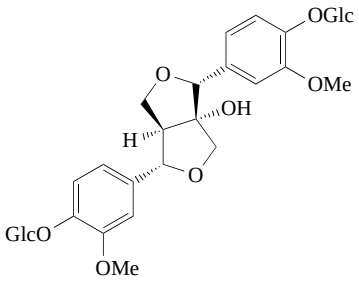
(237) *cis*-Hinokiresinol



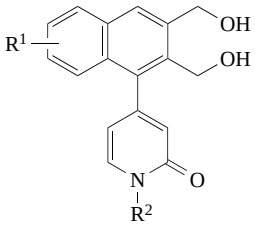
(238) Oxy-*cis*-hinokiresinol



(239) (+)-Syringaresinol di-*O*-β-D-glucopyranoside



(240) (+)-Hydroxypinoresinol 4,4'-di-*O*-β-D-glucopyranoside

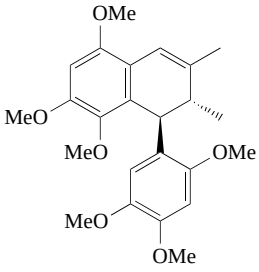


(241)
R^1 = 6,7-$(OEt)_2$
R^2 = *N*-(2-methoxyethyl)

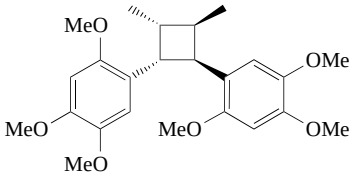
Magnoshinin (**242**) and magnosalin (**243**), isolated from *Magnolia salicifolia* buds, also display anti-inflammatory effects comparable to hydrocortisone acetate.[319,320] Diphyllin acetyl apioside (**244**) and tuberculatin (**245**) are active against inflammation induced by 12-*O*-tetradecanoylphorbol acetate (TPA): the 50% inhibitory doses for acute TPA inflammation were 0.27 and 1.23 μmol/ear for (**244**) and (**245**), respectively; the former is a more potent inhibitor than indomethacin.[321]

Lignans also exhibit antidepressant activities, e.g., prostalidins A, B, and C (**246**)–(**248**), from *Justicia prostata* (Acanthaceae), a plant native to the Western Himalayas.[322]

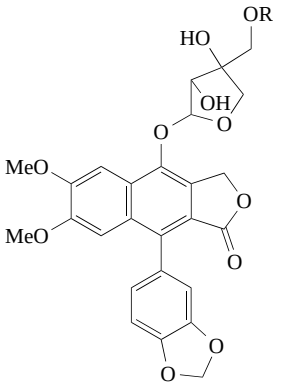
Finally, the cardiovascular effects of lignans are very significant: Siberian ginseng (*Acanthopomax senticosus*), which is widely used in Asia, has the effect of helping sustain cardiovascular activity during prolonged exercise;[323] this has been attributed to the lignan, (+)-syringaresinol di-*O*-*β*-D-glucoside (**239**).[324]



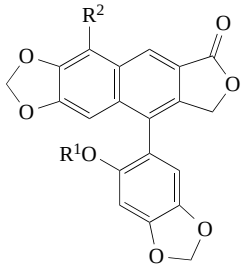
(242) Magnoshinin



(243) Magnosalin



(244) R = Ac, Diphyllin acetyl apioside
(245) R = H, Tuberculatin



(246) R^1 = H, R^2 = OMe, Prostalidin A
(247) R^1 = Me, R^2 = OMe, Prostalidin B
(248) R^1 = R^2 = H, Prostalidin C

1.25.15 CONCLUDING REMARKS

The foregoing discourse has described the knowledge gained in delineating how stereoselective and regiospecific control of coupling occurs *in planta*. The discovery of dirigent protein mediated phenolic coupling, leading to both (+)- and (−)-pinoresinols (**3a**) and (**3b**), depending upon the species, strongly suggests the involvement of related proteins which stipulate distinct coupling modes. Indeed, all research studies reveal that steps associated with both coupling and subsequent metabolic conversions are, as for all other natural products, under full biochemical control.

Re-examination of various claims for ''abnormal'' lignins, ''secondary'' lignins and related substances has revealed that they are nonlignin, nonstructural, oligomeric lignan infusions being secreted into neighboring prelignified cells (such as in heartwood) via specialized cells. Additionally, because of their susceptibility to further oxidation during isolation, it cannot be ruled out that some of the sesquilignans are not, in fact, artifacts of the isolation process.

Additional work will lead to further clarification of the (oligomeric) lignan and lignin forming processes, which must now be viewed as being fully distinct, in terms of biochemical processes involved in their formation, and their structural configurations, as well as in their temporal and spatial deposition *in vivo*.

Finally, the importance of the various lignan skeleta in both plant physiology (particularly defense) and in human nutrition and medicine continues to grow, as the properties of this massive class of natural products continue to be discovered.

ACKNOWLEDGMENTS

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1.25.16 REFERENCES

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