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BIOLOGICAL ACTIVITIES OF LIGNANS

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Key Word Index—Biological activities; lignans, structure–activity relationships; mechanisms of actions.

Abstract—Lignans are widely distributed in angiosperms and gymnosperms. The range of their structures and biological activities is broad. Various lignans are known to have anti-tumour, antimitotic and antiviral activity and to specifically inhibit certain enzymes. Toxicity to fungi, insects and vertebrates is observed for some lignans and a variety of physiological activities have been documented. This review summarizes what is presently known about the biological activities of lignans.

INTRODUCTION

Lignans, long known as natural products, are distributed widely in the plant kingdom. More than 200 compounds in this general class have been identified and a great diversity in the chemical assembly of the two characteristic phenylpropanoid units, as well as the degree of oxidation, and types of substituents is apparent. A number of chemical reviews of lignan structures are available [1–6], the most recent dealing with compounds known as of 1976 [7]. Novel lignans continue to be described by natural products chemists at a steady rate and knowledge of their variety, as well as their range of occurrence in the plant kingdom, is continually expanding.

The breadth of the biological activities of these compounds has come to be appreciated relatively recently. Much interest has been focussed on their effectiveness as antineoplastic agents and research in this area has revealed several modes of action by which they can regulate the growth of mammalian cells. It is clear that lignans possess a variety of pharmacological actions in man, though the most interesting of these are subtle and not easily studied. The recent identification of lignans in human urine and blood suggests a need to pursue an understanding of their possible roles in human physiology.

At the ecological level, there is evidence that lignans play a role in plant–fungus, plant–plant and plant–insect interactions. At the molecular level, on the other hand, some are known to bind to the tubulin of microtubules, to interrupt nucleotide transport and DNA synthesis and to be specific inhibitors of certain enzymes.

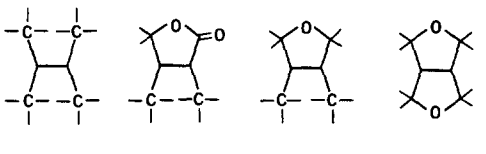
It appears that the biological role of lignans is just beginning to be understood. We feel that this is an appropriate time for information of this sort to be brought together in the form of a review

DEFINITION, BIOSYNTHESIS AND DISTRIBUTION

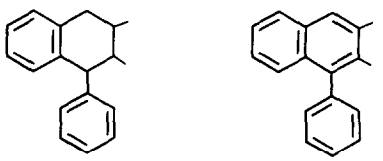
By definition, lignans are dimers of phenylpropanoid (C_6–C_3) units linked by the central carbons of their side chains [8]. Naturally occurring dimers that exhibit linkages other than this C_8–$C_{8'}$ type linkage are known as neolignans [9] and are more limited in number and phylogenetic distribution.

According to the way in which oxygen is incorporated into the skeleton, four structural groups of linear lignans can be recognized: lignans, or derivatives of butane (**A**); lignanolides, or derivatives of butanolide (**B**); monoepoxylignans, or derivatives of tetrahydrofuran (**C**) and bisepoxylignans or derivatives of 3,7-dioxabicyclo(3.3.0)-octane (**D**).

Further cyclization resulting from the introduction of a C-7/C-6″ linkage allows the existence of a large class of compounds collectively known as cyclolignans [10]. These occur either as tetrahydronaphthalene (**E**) or naphthalene (**F**) derivatives. This brief outline is to illustrate the basis for the variety of structures found in this class of compound. A comprehensive treatment of the



A B C D



E F

lignans known from each of these categories is available in the review edited by Rao [7].

It is assumed that lignan biosynthesis involves the combination of two phenylpropanoid units by oxidative coupling [11]. It would be desirable to cite some experimental evidence to support this widely held belief which forms the very basis for defining this class of compound. At the moment of writing, however, there is no definitive experimental work, existing considerations of biosynthesis being speculative in nature [12].

The distribution of lignans in the plant kingdom has been reviewed previously [12]. Table 1 presents a list of the plant families, arranged systematically, from which lignans have been isolated. The phylogenetic arrangement is that of Cronquist [13]. Fifty-five families of vascular plants are included. Gymnosperms are especially well represented, while the class Liliopsida, on the other hand, is represented by only a single grass, *Aegilops ovata* [14] from which one lignan has been isolated. Lignan-containing plants are distributed throughout the six subclasses of the Class Magnoliopsida in an apparently random fashion. Although certain families include many lignan containing taxa (e.g. Pinaceae, Rutaceae and Asteraceae), it is not clear whether this reflects the natural situation or a bias in selection by phytochemists. The widespread distribution of lignans, moreover, suggests that they play (or have played) an important role in plant evolution. The question of whether they are as widely distributed as are other plant phenolics awaits the accumulation of more phytochemical data.

Lignans have been isolated from all parts of plants. In many of the gymnosperms in which they occur they are important constituents of the wood [15]. They are also known from the wood of angiosperm trees [16, 17]. Some are present in tree bark [18, 19], while resin is an especially good source in some cases [20, 21]. They have been isolated from roots [22, 23], leaves [24, 25], flowers [26], fruit [27, 28] and even seeds [29, 30]. In several cases, long term explants, maintained in tissue culture, have been shown to produce lignans [31–33]. Synthesis of the compound, (+)-(1*R*,2*S*,5*R*,6*S*)-2,6-di(4-hydroxyphenyl)-3',7-dioxabicyclo(3.3.0)octane, has recently been elicited in suspension culture of red bean (*Vigna angularis*) by treatment with actinomycin D [34].

Little attention has been directed at the question of the cellular or intracellular production or distribution of lignans. Only the lignans of gymnosperm wood have been examined from this point of view. The heartwood of *Tsugsa heterophylla* contains patches of tracheids consisting of several types of cellular inclusions composed primarily of the lignans, matairesinol, hydroxymatairesinol and conidendrin [15]. These lignans also line tracheid cell walls and are deposited as pit encrustations. In the wood of *Picea abies* infected by the Basidiomycete, *Fomes annosus*, lignans accumulated in the 'reaction zone' which separates sound wood from infected wood [35].

BIOLOGICAL ACTIVITY OF LIGNANS

Antitumour activity

Lignans have aroused considerable interest because some of them display antitumour activities. This is particularly true of the podophyllotoxin group of lignans, constituents of the medicinal resin extracted from *Podophyllum* species [36–38]. These lignans have subsequently been identified in numerous plants as a result of screening for cytostatic activity [39–41]. Lignans from other classes, however, are also known to have antitumour activity.

Table 2 includes all the lignans that have been demonstrated, by either *in vitro* or *in vivo* methods, to possess cytostatic or antitumour activity. With the exception of three, SP-1, VP-16-213 and VM-26, all are naturally occurring and have been isolated from higher plants. The publications referred to [refs 20, 42–56] consist of reports on the biological testing and occurrence of the compounds listed.

In looking at these 33 compounds, it is difficult to identify a common structural characteristic which might explain their activity as antitumour agents. The task is complicated since it involves a comparison of data collected at different times and by researchers using different techniques. With these shortcomings in mind, we can, however, make several generalizations. Many of the compounds of Table 2 share the following features: (1) a five membered lactone ring; (2) a 3,4,5-trimethoxyphenyl group; (3) a methylenedioxy group and (4) two substituted phenyl groups separated by a four carbon chain.

Although a lactone ring is a common feature in the biological activity of other classes of compounds, its importance to the antitumour activity of lignans is not understood. Four of the 33 active lignans of Table 2 do not possess a lactone ring: compounds **1**, **2**, **3** and **8**. Moreover, compound **3** (burseran) differs from compound **4a** only in the presence of a furan ring rather than a lactone ring but both compounds possess antitumour activity [20]. Picropodophyllin (**6**) and picropodophyllic acid (**8**) differ only in that, in the latter compound, the lactone ring is opened. Although it has been reported that both compounds are active [43], the existing data do not indicate whether the activity of the acid is significantly less than that of the lactone [56]. Approximately half of the compounds of Table 2 possess a 3,4,5-trimethoxyphenyl moiety (17 of 33), indicating that this is not an absolute requirement. It is interesting to note, however, that 31 of the 33 compounds possess a methylenedioxyphenyl group, suggesting its importance in eliciting this response. The importance of the C_6–C_4–C_6 skeleton is difficult to assess. Although it may play a role in the case of the podophyllotoxins whose stereochemistries are quite similar, it does not appear to be a factor of general importance. The positioning of the two phenyl groups of the *Stegnacia* compounds (**12a–d**) is different from that of the podophyllotoxins, as well as the arylnaphthalene derivatives (**10a–f**, **11**). Those compounds that have not undergone cyclization to form a B ring (**1**, **3**, **4a** and **4b**) could exist in a number of possible configurations, most of which would not resemble very closely the compounds having a B ring (cyclolignans).

From Kelleher's study on the structure–activity relationship of podophyllotoxin analogues [56], it is possible to conclude that the configuration of the hydroxyl group of C_4 of the B ring is of some importance to antitumour activity, but the situation is complex. Epipodophyllotoxin (**7**), which differs from podophyllotoxin (**6a**) only in the stereochemistry of C-4, is an order of magnitude less effective as a cytotoxic agent. Positioning the hydroxyl group elsewhere on the molecule, however, does not have such a marked effect. β-Peltatin (**5g**), with a hydroxyl group on C-5 of the A ring is an even more potent antitumour agent and deoxypodophyllotoxin (**5b**),

Table 1. Distribution of lignans in the plant kingdom

		Order	Family
Division: Pterophyta		Filicales	Polypodiaceae
Division: Coniferophyta		Coniferales	Pinaceae
			Cupressaceae
			Taxodiaceae
			Podocarpaceae
			Araucariaceae
			Taxaceae
Division. Magnoliophyta			
Class	Subclass	Order	Family
Magnoliopsida	Magnoliidae	Magnoliales	Himantandraceae
			Magnoliaceae
			Winteraceae
			Austrobaileyaceae
			Eupomatiaceae
			Myristicaceae
		Laurales	Hernandiaceae
			Lauraceae
		Piperales	Piperaceae
		Aristolochiales	Aristolochiaceae
		Illiciales	Schizandraceae
		Ranunculales	Berberidaceae
	Hamamelidae	Urticales	Ulmaceae
			Urticaceae
		Fagales	Fagaceae
			Betulaceae
	Caryophyllidae	Caryophyllales	Phytolaccaceae
	Dilleniidae	Violales	Flacourtiaceae
			Cucurbitaceae
		Salicales	Salicaceae
		Ericales	Ericaceae
		Ebenales	Styracaceae
			Symplocaceae
	Rosidae	Rosales	Rosaceae
		Fabales	Fabaceae
		Myrtales	Thymelaeaceae
			Myrtaceae
			Combretaceae
		Santalales	Loranthaceae
			Balanophoraceae
		Euphorbiales	Euphorbiaceae
		Linales	Linaceae
		Polygalales	Polygalaceae
		Sapindales	Burseraceae
			Anacardiaceae
			Rutaceae
			Zygophyllaceae
		Apiales	Araliaceae
			Apiaceae
	Asteridae	Gentianales	Apocynaceae
		Solanales	Solanaceae
		Lamiales	Verbenaceae
			Lamiaceae
			Phrymaceae
		Scrophulariales	Oleaceae
			Scrophulariaceae
			Globulariaceae
			Acanthaceae
			Pedaliaceae
		Asterales	Asteraceae
Liliopsida	Commelinidae	Cyperales	Poaceae

Table 2. Lignans with known antitumour activity

Compound	Structure number	Reference
Nordihydroguaiaretic acid	1	42
(+)-Dimethylisolariciresinol-2α-xyloside	2	43
Burseran	3	20
(−)-*trans*-2-(3″,4″,5″-Trimethoxybenzyl)-3-(3′,4′-methylenedioxybenzyl) butyrolactone	4a	44
(−)-*trans*-2-(3″,4″-Dimethoxybenzyl)-3-(3′,4′-methylenedioxybenzyl) butyrolactone	b	44
Podophyllotoxin	5a	41, 45–48
Deoxypodophyllotoxin	b	40, 41, 48
3′-Demethylpodophyllotoxin	c	41
4′-Demethylpodophyllotoxin	d	41, 45–48
5′-Desmethoxypodophyllotoxin (morelsin)	e	43
α-Peltatin	f	41
β-Peltatin	g	41, 47, 48
β-Peltatin-A-methyl ether	h	48
5′-Desmethoxy-β-peltatin-A-methyl ether	i	43, 49
Podophyllotoxin glucoside	j	47, 48
Picropodophyllotoxin	6	47, 48
Epipodophyllotoxin	7	47, 48
Picropodophyllic acid	8	47
Austrobailignan-1	9	50
Diphyllin	10a	51
Justicidin-A	b	”
Diphyllin acetate	c	”
Diphyllinin	d	”
Diphyllinin monoacetate	e	”
Diphyllinin crotonate	f	”
Dehydroanhydropicropodophyllotoxin	11	43
Stegnacin	12a	52
Stegnangin	b	”
Stegnanol	c	”
Stegnalone	d	”
Podophyllic acid ethylhydrazide (SP-1)	13	53
4′-Demethyl epipodophyllotoxin ethylidene-β-D-glucoside (VP-16-213)	14a	54
VM-26	b	55, 56

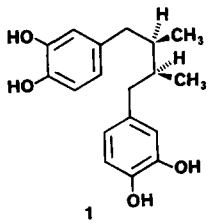
with no hydroxyl group, is almost equally effective.

Replacing the hydroxyl group of β-peltatin with the more bulky methoxyl group, to give β-peltatin-A-methyl ether (**5h**) or with glucose, to give β-peltatin β-D-glucopyranoside, however, results in significant reduction in cytotoxic activity [56]. Although glucosylation of the hydroxyl group of podophyllotoxin to give podophyllotoxin β-D-glucopyranoside (**5j**) results in marked reduction in activity, it does not abolish it completely [43, 48]. Moreover, the synthetic lignan derivatives used in cancer chemotherapy have been modified in just this way and are effective antitumour agents [53–55]. Decreasing the polarity of the C-4 glucose moiety by adding a phenyl group to it, as in the case of podophyllotoxin-benzylidene glucoside, results in a marked increase in cytotoxic potency [56]. This indicates that it is the polarity of the C-4 substituent rather than its size that is the more important factor in relation to antitumour activity.

The configuration about C-2 seems to play a significant role in determining antitumour action. Picropodophyllotoxin (**6**) differs only with respect to stereochemistry at this carbon and shows a markedly attenuated potency as a cytostatic agent. It is interesting to note, however, that this sort of modification is not sufficient to completely abolish activity [43, 48]. Substituting a furan ring for the lactone ring of podophyllotoxin severely reduces antitumour activity. The resulting compound, anhydropodophyllol, possesses in *in vitro* toxicity three orders of magnitude less than that of podophyllotoxin [56]. Finally, the 3,4,5-trimethoxy group does not appear to be a required feature in determining antitumour activity. The compound, 4′-demethylpodophyllotoxin (**5d**), displays an activity almost equivalent to that of podophyllotoxin [56].

Antimitotic activity

Many podophyllotoxins have been shown to possess antimitotic activity. This is illustrated by their ability to



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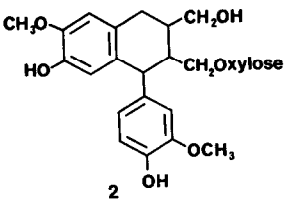

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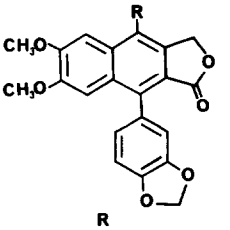
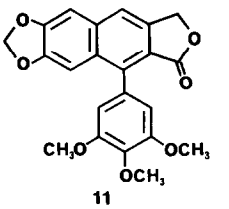


R

10 a OH
b OCH_3
c $OCOCH_3$
d $OC_4H_4O(OH)_2CH_2OH$
e $OC_4H_4O(OH)_2CH_2OCOCH_2$
f $OC_4H_4O(OH)_2CH_2OCOCHCHCH_3$



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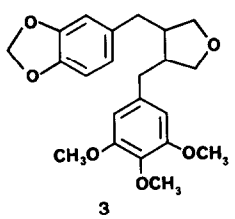

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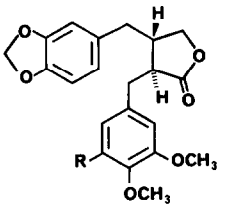
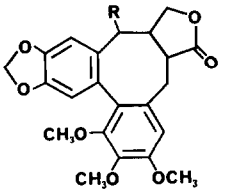
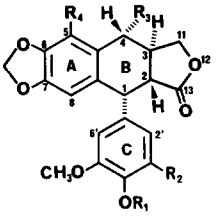


4 a R = OCH_3
b R = H

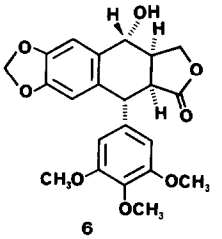


R

12 a OAc
b OCO-C(=CH_2)-CH_3
c OH
d =O



	R_1	R_2	R_3	R_4
5 a	CH_3	OCH_3	OH	H
b	CH_3	OCH_3	H	H
c	CH_3	OH	OH	H
d	H	OCH_3	OH	H
e	CH_3	H	H	H
f	H	OCH_3	H	OH
g	CH_3	OCH_3	H	OH
h	CH_3	OCH_3	H	OCH_3
i	CH_3	H	H	OCH_3
j	CH_3	OCH_3	OGlu	H



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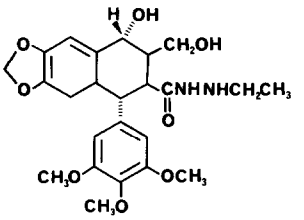

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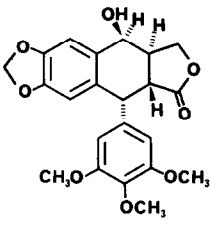

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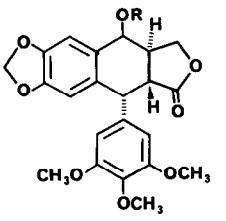

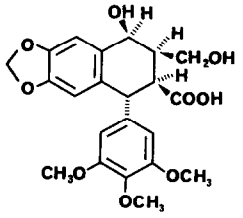

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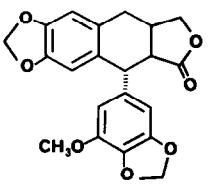
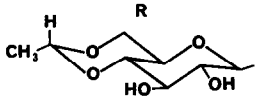
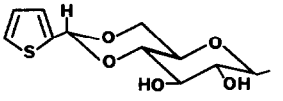


9

14 a



b



arrest cultured cells at metaphase [56–59] and to bind to purified tubulin preparations [56, 57, 60–64]. There is evidence that podophyllotoxin competes for the same binding site on tubulin as colchicine [57]. It has approximately twice the affinity for tubulin as that of colchicine, however, and appears to bind more rapidly [61]. Both compounds have two sites of attachment, only one of which is similar, this being specific for the 3,4,5-trimethoxyphenyl group [61].

Several structure–activity studies have been carried out on the antimitotic activity of podophyllotoxin type lignans and a number of conclusions can be drawn. There is apparently no difference in the tubulin binding activity of podophyllotoxin (**5a**) and deoxypodophyllotoxin (**5b**) [57, 63] which suggests that the C-4 hydroxyl is of little consequence. Epipodophyllotoxin (**7**), the C-4 stereoisomer of podophyllotoxin (**5a**) [57, 63] and 4′-demethylepipodophyllotoxin [61] are more than an order of magnitude less effective at binding tubulin than podophyllotoxin. This illustrates the importance of the stereochemical configuration of the C-4 proton in contributing to tubulin binding. As in the case of antitumour activity, a glucose moiety at the C-4 position of podophyllotoxin markedly reduces tubulin binding capacity [63]. It is especially interesting that the synthetic epipodophyllotoxin derivative containing a bulky substituent at C-4, VP-16-213 (**14a**), has no detectable effect upon microtubule assembly [62]. This is in contrast to its antitumour activity which is quite appreciable [53]. The stereochemistry about C-2/C-3 appears also to be of importance. Picropodophyllin (**6**) displays a much reduced ability to bind tubulin [57, 63, 64] and picropodophyllic acid (**8**) has no detectable activity [63].

The importance of the lactone ring of podophyllotoxin to tubulin assembly has been well documented. Replacing it with a furan ring results in a compound with several-fold diminished activity [57, 62, 63. 65]. Replacing the oxygen of the cyclic ether with carbon, sulfur or a sulfone group reduces the activity ten-fold, twenty-fold and completely, respectively [57].

As in the case of antitumour activity, shifting the hydroxyl group of podophyllotoxin to C-5 (for β-peltatin: **5g**) results in a moderate enhancement of inhibition of tubulin aggregation [63]. This activity, however, like antitumour activity, is reduced in compounds having either a methoxyl or glucose moiety at this position [63]. The guaiacyl compound, 4′-demethylpodophyllotoxin (**5d**), has approximately the same effect on tubulin aggregation as podophyllotoxin [63]. This is especially interesting in view of the belief that the site of attachment on the tubulin molecule is specific for the trimethoxy moiety [61].

Kelleher [56] has emphasized the correlation between antitumour activity and antimitotic activity of derivatives of podophyllotoxin. Although correlations exist between these two activities for some podophyllotoxin derivatives, suggesting a common mode of action, this is true for only a limited number of compounds. Noteworthy exceptions are podophyllotoxin glucoside (**5j**) and picropodophyllic acid (**8**); they have little effect upon tubulin polymerization but do have a demonstrable effect on some types of tumour cells. It seems unlikely, too, that all of the antitumour compounds from the other classes of lignans (Table 2) will be shown to affect microtubules. Further experimental evidence is required before any firm conclusions concerning the question of the relationship between the antitumour and antimitotic activity of lignans can be reached.

Effects on nucleic acids

The ability of podophyllotoxin and several of its derivatives to influence nucleic acid metabolism has been studied. Both podophyllotoxin and the derivative, VP-16-213 (**14a**), inhibit the synthesis of DNA, RNA and protein in HeLa cells at a concentration of 100 μM [65]. The effect was shown to be due to the ability of these compounds to inhibit facilitated diffusion of thymidine, uridine, adenosine and guanosine into the cell [66]. It was observed to be rapidly and completely reversible. Unlike podophyllotoxin, which inhibits cells at mitosis, VP-16-213 causes arrest in the G_1 phase of the cell cycle [53, 67]. This is probably due to the inhibitory influence of this agent upon nucleoside uptake.

The ability of lignanolides to damage DNA has also been established. Chromosomal damage in cells treated with an epipodophyllotoxin derivative has been observed [68]. Moreover, it was found that some podophyllotoxin analogues cause the appearance of a fraction of low molecular weight DNA, detectable after ultracentrifugation in an alkaline sucrose gradient [69]. The compounds, 4′-demethylpodophyllotoxin (**5d**), 4′-demethyldeoxypodophyllotoxin, 4′-demethylepipodophyllotoxin, α-peltatin (**5f**), VP-16-213 (**14a**) and VM-26 (**14b**) all cause DNA fragmentation while podophyllotoxin, deoxypodophyllotoxin, epipodophyllotoxin and β-peltatin were all inactive [69]. Note that all of the active compounds possess a 4′-hydroxyl group, while none of the inactive ones do. Also noteworthy is the greater activity of the epiderivatives, epipodophyllotoxin (**7**) and 4′-demethylepipodophyllotoxin. Both differ with respect to stereochemistry at C-4, which is in the *S* configuration.

When purified DNA, isolated from HeLa cells or from viruses, was treated with these compounds no fragmentation could be detected [69]. The effect therefore appears to be mediated by some cellular process.

Either of these effects of podophyllotoxin analogues on nucleic acid metabolism would likely have direct bearing upon their antitumour effects. There is evidence to suggest that the effects on nucleoside uptake and DNA structure occur via different mechanisms. Analogues possessing a 4′-methoxyl group appear to be more active in inhibiting nucleoside uptake than causing DNA fragmentation and, among the compounds tested, there is a poor correlation between these two activities [69].

Antiviral activity

It has been known for many years that the resin of several species of *Podophyllum* is effective in the treatment of condyloma acuminata, or venereal warts [36, 47]. In view of the long standing inclusion of this substance in the Pharmacopoeias of western industrial, Asian and native American civilizations, it is surprising that its use has only recently been placed on a sound experimental foundation. A crude extract of *Podophyllum peltatum* was observed to reduce the cytopathic effect of herpes simplex type II, influenza A and vaccinia viruses [70]. Podophyllotoxin (**5a**), β-peltatin (**5g**), deoxypodophyllotoxin (**5b**), picropodophyllotoxin (**6**) and α-peltatin (**5f**) were subsequently found to be active against measles and herpes simplex type I [71]. It was discovered independently that the

antiherpetic activity of the fruit of *Juniperus communis* was attributable to the presence of deoxypodophyllotoxin [72]. A variety of lignans were tested by Markkanen *et al.* [73] and a number of general observations concerning structural requirements for activity may be made. Podophyllotoxin, deoxypodophyllotoxin and β-peltatin are most potent and approximately equal in effectiveness, indicating that the C-4 hydroxyl is not an essential requirement for activity. Replacing the lactone ring of podophyllotoxin with a furan ring (anhydropodophyllol) results in a ten-fold reduction in activity. The stereoisomers, picropodophyllotoxin (**6**) and epipodophyllotoxin (**7**) are less active by yet another order of magnitude, while glycosides of podophyllotoxin, α- and β-peltatin are one thousand times less active than their respective aglycones. Dihydroanhydropodorhizol (**15a**) and its glucoside (**15b**) show a much reduced activity (1/1000th) when compared to the corresponding cyclic analogue, deoxypodophyllotoxin (**5b**). This observation points towards the importance of maintaining the two phenyl groups in a specific configuration relative to one another.

A less polar substituent than glucose at the C-4 position, as in the case of VP-16-213, does not alter the antiviral activity. This situation more closely resembles the case of inhibition of microtubule polymerization than antitumour activity, where derivatives like VP-16-213 possess marked activity.

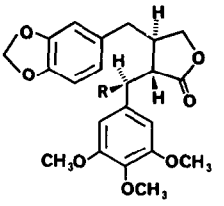
A second effect of podophyllotoxin derivatives on a virus mediated phenomenon has been reported. In this instance, however, the result of infection (by polyoma virus) is cell transformation. It is promoted by podophyllotoxin (**5a**), as well as other mitotic inhibitors that are unrelated structurally to lignans [74].

It is reasonable to hypothesize that these two effects on infection by viruses are a result of the inhibition of microtubule formation by lignans. Colchicine and the *Vinca* alkaloids (well known inhibitors of microtubule aggregation) also reduce the production of viruses by infected cells [75] and this has been shown to be at least partly the result of the inhibition of viral release [76]. Certain podophyllotoxin derivatives interfere with nucleic acid metabolism, an activity which may also play a role in the inhibition of viral replication. The question is an interesting one and amenable to experimentation.

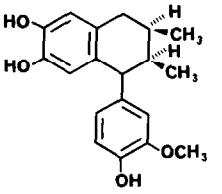
Inhibition of enzyme activity

Enzymes affecting respiration. The antitumour activity of podophyllotoxin has stimulated an interest into its possible effects on certain enzyme-catalysed processes of malignant tissues. When the tumours of rats injected with podophyllotoxin were analysed, a decrease in cytochrome oxidase, cytochrome *c* and succinoxidase activities, as well as a reduction in respiration, was observed [77–79]. Similar results were obtained with the derivatives β-peltatin (**5g**) [80] and a synthetic derivative, acetylpodophyllotoxin-ω-pyridinium chloride [81].

The structurally simpler lignan, nordihydroguaiaretic acid (NDGA) (**1**) inhibited glycolysis as a result of its ability to maintain pyridine nucleotides in their reduced state [42]. It was subsequently found to inhibit mitochondrial electron transport [82]. This is a result of the ability of NDGA to inhibit specifically both NADH-oxidase and succinoxidase enzyme systems (but not cytochrome oxidase or the coenzyme Q–cytochrome *c* reductase system) [82]. It was also active in inhibiting energy



15 a H
b glucose



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transfer reactions associated with site 1 phosphorylation [83]. More recently, nor-isoguaiacin, a related lignan (**16**), has also been found to inhibit succinoxidase, NADH-oxidase and mitochondrial energy transfer [84].

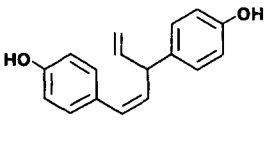
These results raise the question of the role of inhibition of respiration by lignans in relation to their antitumour activity [77]. Further evidence suggesting that the two activities are interdependent is provided by the observation that tumour damage induced by α-peltatin (**5f**), β-peltatin (**5g**) and podophyllotoxin (**5a**) is related to inhibition of respiration [85–89] and closely correlated with their ability to inhibit cytochrome oxidase activity in tumour cells [77]. Furthermore, the synthetic lignan derivative, podophyllotoxin-ω-pyridinium chloride was active in inhibiting aerobic metabolism resulting from the oxidation of malate, isocitrate and succinate [78, 90].

cAMP phosphodiesterase inhibition. In the screening of Chinese medicinal plants for their effects on cyclic AMP phosphodiesterase activity, six nor-lignans were identified as inhibitors [91]. *cis*-Hinokinoresinol (**17**) has been included as an example of one of the active compounds from this chemical class. Following this, a variety of lignans were tested and an informative structure–activity relationship study was published [28].

Three classes of lignans were represented in this study: lignanolides, monoepoxylignans and bisepoxylignans. Each showed varying degrees of cAMP phosphodiesterase inhibition. The common wood constituent, matairesinol (**18a**), showed a high degree of inhibitory activity. The guaiacyl ring seems to be the optimum substituent in eliciting the response. Arctigenin (**18b**), with one veratryl ring shows a slightly reduced activity and a compound with both aromatic substituents of the veratryl type (**18c**) is only one third as active as matairesinol. The importance of the 4′-substituent is unclear, however, since glucosylation of one position eliminates inhibitory activity (**18d, e, j**), whereas glucosylation of two positions (**18f, l**) results in a level of activity equivalent to that of the aglycone.

The stereochemistry of matairesinol at C-2 appears to have very little effect on cAMP phosphodiesterase inhibition; i.e. **18b** and **19a** and **b** have similar activities. Furthermore, hydroxylation of C-2 has no appreciable effect on enzyme inhibition as indicated by the activity of derivatives **18g, h** and **i**. This is in contrast to the case of the C-5 position. Hydroxylation of matairesinol at C-5 produces an inactive compound. Similarly, ring closure involving C-5 results in the formation of inactive derivatives: **20a** and **b** and **21**.

A wide variety of bisepoxylignans are also effective inhibitors of cAMP phosphodiesterase. As in the case of the lignanolides tested, the guaiacyl group appears to be most effective in contributing to the inhibitory activity.



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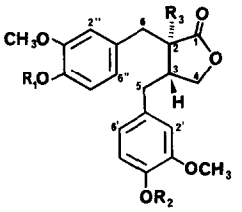
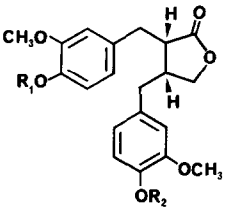
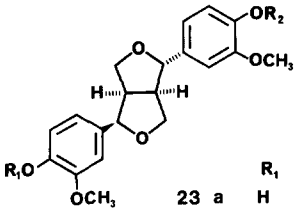


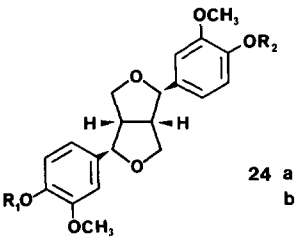
	R_1	R_2	R_3
18 a	H	H	H
b	H	CH_3	H
c	CH_3	CH_3	H
d	Glu	H	H
e	Glu	CH_3	H
f	Glu	Glu	H
g	H	H	OH
h	H	CH_3	OH
i	CH_3	CH_3	OH
j	Glu	H	OH
k	Glu	CH_3	OH
l	Glu	Glu	OH



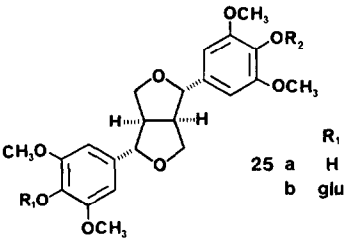
	R_1	R_2
19 a	H	CH_3
b	CH_3	CH_3



	R_1	R_2
23 a	H	H
b	CH_3	H
c	CH_3	CH_3
d	glu	H
e	CH_3	glu

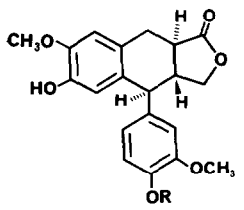


	R_1	R_2
24 a	H	H
b	glu	H

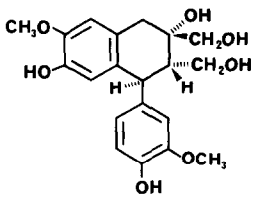


	R_1	R_2
25 a	H	H
b	glu	glu

Compound **22a** is more active than **22b** which is, in turn, more active than **22c**. Similarly, **23a** is more active than **23b** which is more active than **23c**. The syringyl group, however, is more effective than the veratryl group in conferring inhibitory activity, as seen from the activity of syringaresinol (**25a**). Remarkably, the peculiar relationship of the glucosides which was seen with the lignanolides is evident with the bisepoxylignans as well. A glucose moiety at the 4′-position of either aromatic group causes a marked reduction in inhibitory activity: this is a 2-fold decrease in the case of pinoresinol β-D-glucoside (**22d**) and **22e**. The presence of a glucose substituent at the 4′-positions of each ring, however, results in an activity equal to that of the aglycone (e.g. **22f** and **25b**). Derivatives of the C-1 position of the bisepoxy ring have also been prepared. The acetate (**22g**) has been found to be equally inhibitory to the parent compound and the alcohol (**22h**) slightly less so



	R
20 a	H
b	OCH_3



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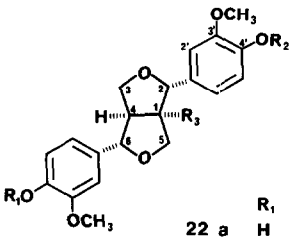
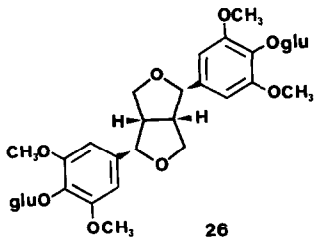


	R_1	R_2	R_3
22 a	H	H	H
b	CH_3	H	H
c	CH_3	CH_3	H
d	Glu	H	H
e	Glu	CH_3	H
f	Glu	Glu	H
g	H	H	OAc
h	H	H	OH

The stereochemistry of the furan–phenyl bond is evidently important to the inhibitory activity. (+)-Pinoresinol (**22a**), (+)-pinoresinol monomethyl ether (**22b**), (+)-pinoresinol dimethyl ether (**22c**) and (+)-pinoresinol β-D-glucoside (**22d**), in all of which both C-2 and C-6 are in the *S* configuration, are more effective in inhibiting cAMP phosphodiesterase than their corresponding (+)-epipinoresinol analogues (**23a–d**, respectively) in which C-2 is in the *S* and C-6 is in the *R*-configuration. (−)-Pinoresinol (**24a**) and (−)-pinoresinol β-D-glucoside (**24b**), in which both C-2 and C-6 are in the *R*-configuration, display cAMP phosphodiesterase inhibitory activities more than an order of magnitude less than (+)-pinoresinol (**22a**) or its β-D-glucoside (**22d**). Similarly, (+)-syringaresinol di-β-D-glucoside (**25b**) is much more active than (−)-syringaresinol di-β-D-glucoside (**26**).



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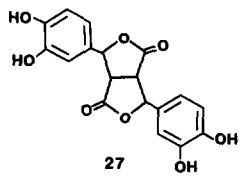

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The fungal metabolite, dehydrocaffeic acid dilactone (**27**), while not strictly speaking a lignan, since it is most likely of polyketide origin, is of interest. It has also been found to be an inhibitor of cAMP phosphodiesterase [92]. In the case of this dilactone, substitution of a catechol ring by a guaiacyl ring has no effect upon its activity but the analogue bearing two guaiacyl rings is inactive.

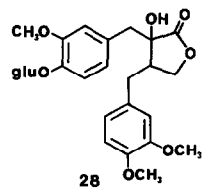
Other enzymes. Dehydrocaffeic acid dilactone has also been observed to be an inhibitor of the enzyme catechol-*O*-methyltransferase (COMT) [93]. As for cAMP phosphodiesterase inhibition, a single guaiacyl ring results in little activity while two guaiacyl rings abolish the activity completely.

Miscellaneous physiological effects

Cathartic activity. Extracts of *Podophyllum* species have long been known to produce catharsis. Podophyllotoxin was originally considered to be the active constituent [94], although α-peltatin, β-peltatin and 4′-demethylpodophyllotoxin were later shown to also produce the effect [95]. Podophyllin resin causes a decrease in the initial rate and amplitude of rhythmic contractions of isolated gut preparations followed by an increase in the force of contraction and a loss of tone [96]. Podophyllotoxin and picropodophyllotoxin are inactive in isolated gut preparations but produce diarrhea when administered to the living animal [97]. Similarly, intravenous injection of podophyllotoxin causes an increased peristalsis and intestinal tone, a response which is not altered by sectioning the vagus or splanchnic nerves [98]. Nor is the effect blocked by atropine [99].

Cathartic activity is not restricted to podophyllotoxin analogues. The compound, 2-hydroxyarctiin (**28**), has been found to be responsible for the cathartic activity of safflower meal [100].

Cardiovascular effects. Several isolated reports exist concerning the action of lignans on the cardiovascular system. Podophyllotoxin and picropodophyllin have a complex action on isolated heart preparations and in the whole animal causing a transitory rise [97] followed by a decrease in heart rate [95]. Particularly interesting is the



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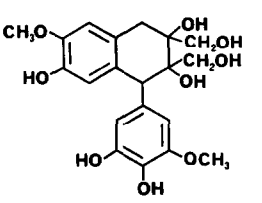
diglucoside of pinoresinol (**22f**) which has been isolated from the Chinese medicinal plant, *Eucommia ulmoides*, and found to be the active constituent in this antihypertensive preparation [101].

Allergenicity. The sawdust of red cedar (*Thuja plicata*) causes asthma and rhinitis in certain exposed individuals. The compound responsible has been found to be the lignan, plicatic acid (**29**) [102]. Although skin sensitivity or precipitating antibodies are not observed, it elicits immediate, late and dual reactions. Since the reaction takes place only in conditioned individuals, it is not thought to be the result of an irritant action, but a true allergic response [103].

Activity towards insects. A number of podophyllotoxin analogues are effective in inhibiting insect larval growth, especially β-peltatin-A-methyl ether, deoxypodophyllotoxin and deoxypicropodophyllin [104]. The bisepoxylignans, kobusin (**30**) and sesamin (**31**) were shown to inhibit the growth of silkworm (*Bombyx mori*) larvae [105], as were a number of neolignans [106, 107]. Sesamin (**31**) and sesamolin (in which one methylenedioxyphenyl group is linked to the bisepoxyfuran group via an ether bond) display weak juvenile hormone activity in the milkweed bug (*Oncopeltus fasciatus*) [108]. *p*-Benzolactone (**32**) is an insect feeding inhibitor [109].

Sesamin (**31**) and asarinin (**33**) are effective in enhancing the toxicity of a wide variety of insecticides [110, 111]. These lignans display the same potency as commercially used synthetic insecticide synergists. The related compounds, pinoresinol (**22a**) and its dimethyl ether (**22c**) are inactive. These observations point towards the importance of the methylenedioxy substituent in synergistic activity. Other studies have shown clearly that the meth-



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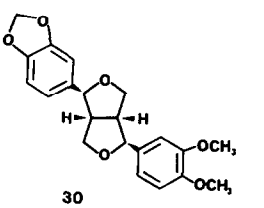

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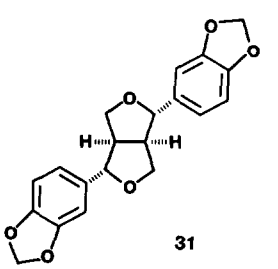

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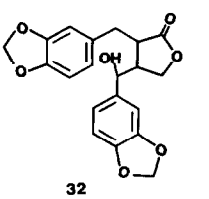

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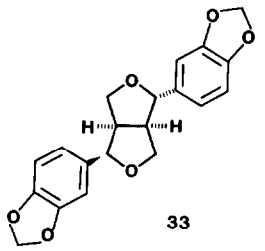
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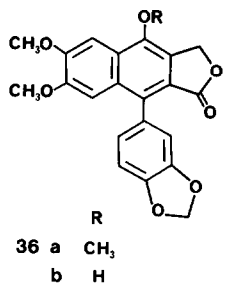
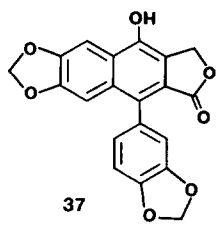

36 a CH_3
b H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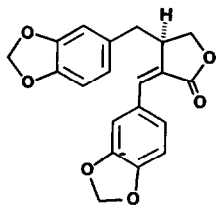
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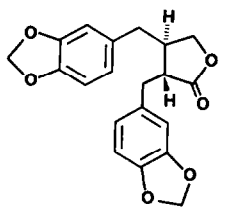
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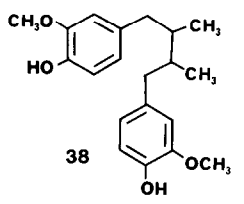
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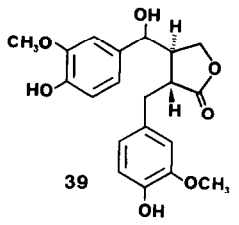
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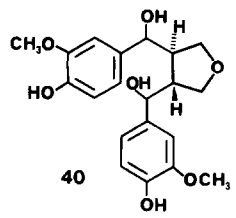
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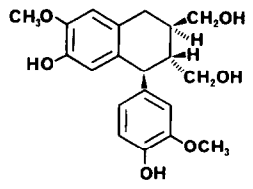
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ylenedioxy group is responsible for the inhibition of mixed function oxidase, the enzyme system that is responsible for the oxidation and inactivation of most toxins [112]. Other methylenedioxy containing lignans such as savinin (**34**) and hinokinin (**35**) are known also to be insecticide synergists [113]. It is possible that these compounds share this common biological activity as a result of the presence of the methylenedioxy group. Matairesinol (**18a**) and its dimethyl ether (**18c**), however, are both insecticide synergists [114] and neither possesses such a substituent. Thus, the chemistry underlying this biological phenomenon may not be quite so straightforward.

Piscicidal activity. The arylnaphthalene derivatives justicidin A (**36a**) and B (**36b**) and diphyllin (**37**) have been identified as piscicidal constituents of *Justicia hayatai* [25, 115]. Their toxicity is approximately equal in potency to that of rotenone. Nothing is known of the mechanism of action of these compounds.

Toxicity to mammals. The LD_{50} for mice of a range of podophyllotoxin derivatives has been determined [116]. The degree of toxicity was observed to correlate with the *in vivo* antimitotic effect.

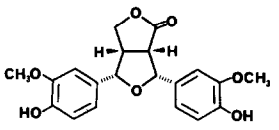
Antimicrobial activity. Nor-isoguaiacin (**16**) and dihydroguaiaretic acid (**38**) have been reported to be inhibitory to the growth of *Streptococcus* species, *Staphylococcus aureus*, *Bacillus subtilis* and, in the case of nor-isoguaiacin, to *Pseudomonas aeruginosa* as well [117]. Antifungal activity against *Rhizoctonia solani*, *R. oxisporum*, *Pythium* spp. and *Rhizopus nigrans* has also been reported for nordihydroguaiaretic acid and some of its derivatives [118].

Fungistatic activity. Evidence has been presented which suggests that lignans are produced in wood in response to fungal attack and that they play some role in preventing the subsequent degradation of wood. Spruce wood infected with the fungus, *Fomes annosus*, is known to contain highly elevated levels of matairesinol (**28a**), conidendrin (**20a**), hydroxymatairesinol (**39**) and liovil (**40**). Only hydroxymatairesinol and matairesinol, however, were inhibitory to the growth of the fungus [35]. Isoolivil (**41**) accumulates in the wood of *Prunus* sp. after fungal attack [119] and the wood of *Liriodendron tulipifera* produces syringaresinol (**24a**) and its dimethyl ether, as well as other compounds, upon injury [120]. Matairesinol (**18a**) is responsible for controlling infections due to *Lentinus lepideus* Fr. but not other fungi [121] and the wood of *Picea abies* infected with *Fomes annosus* produces a number of lignans (none of which were fully characterized) with fungistatic activity [122]. Some of the fungistatic activity of lignans is attributable to their inhibition of the extracellular fungal enzymes, cellulase, polygalacturonase, aryl-β-glucosidase and laccase [23].

Germination inhibitory activity. Several pieces of evidence are available which suggest that some lignans may play a role in the regulation of the physiological processes of plants. A monoepoxylignanolide (**42**) has been isolated from *Aegilops ovata* which is an inhibitor of germination. Its activity is strongly dependent upon radiation of specific wavelengths [14, 123]. Germination inhibition has also been observed for arctiin (**18e**) [124] and for a monoepoxylignan (**43**) isolated from potato root [125]. This compound is interesting because it is synthesized in response to infection by a nematode [125].

Miscellaneous physiological actions in man

Some of the most intriguing of the biological activities of lignans are particularly difficult to define. Although a distinct physiological effect is observed in response to lignan treatment, it is difficult to present a general model for their mechanism of action. Three categories have been constructed to accommodate these diverse activities:



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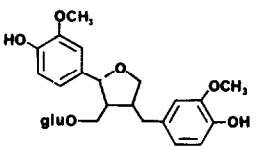

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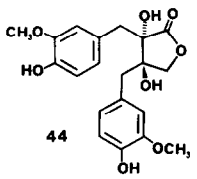

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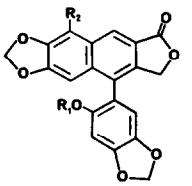
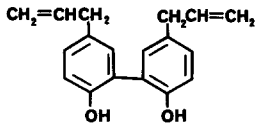


	R_1	R_2
45 a	H	OCH_3
b	CH_3	OCH_3
c	H	H



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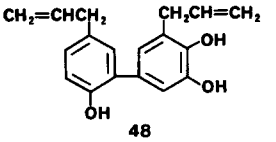

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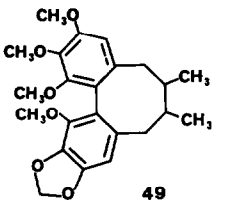

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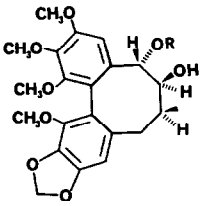
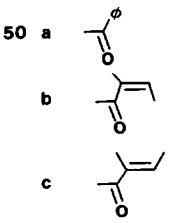
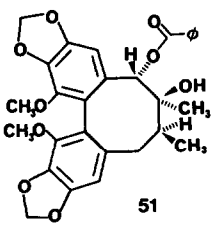


R



50 a b c



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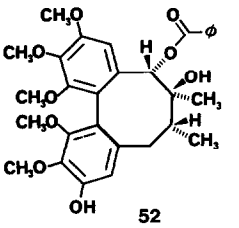

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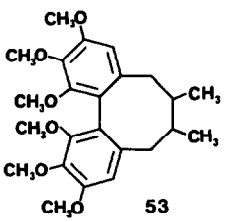

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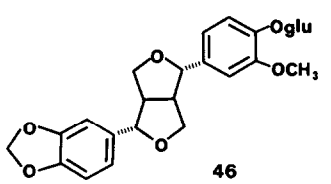

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activity on the central nervous system, protective activity against toxins and general stress-reducing activity.

Activity on the central nervous system. Lignans are capable of acting both as depressants and antidepressants. (+)-Nortrachelogenin (**44**) causes depression in rabbits [126] and prostalidins A, B and C (**45a, b** and **c**) produce a mild depression in rats and mice [127]. The bisepoxylignan glycoside, simplexoside (**46**) has CNS depressant activity in mice and rats while the aglycone is a stimulant [128, 129]. A number of the *Schizandra* lignans, discussed below, have also been reported to have activity on the central nervous system [130]. Finally, an ether extract of *Magnolia obovata* has been reported to have sedative and muscle relaxant activity [131]. The neolignans magnolol (**47**) and honokiol (**48**) are known to be principal constituents of this extract and likely account for at least a portion of its activity [132].

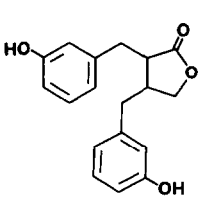
Protective activity against hepatotoxins. Some of the lignans of *Schizandra* fruit, a Chinese medicinal material, have been found to ameliorate the harmful effects of toxic drugs and to facilitate liver function and regeneration [133, 134]. Schizandrin B (**49**) is effective in protecting the liver in such a way and increases resistance to the toxic effects of digitoxin and indomethacin [133]. The schisantherin compounds A, B, C and D (**50a, b** and **c** and **51**), respectively, are also effective in protecting the liver from injury and lowering serum GPT levels [27]. This activity has been demonstrated in mice as well as human viral hepatitis patients [134]. Schisantherin E and deoxyschisantherin (**52**), however, are ineffective [27]. Although it is by no means clear how these compounds act, it is interesting to note that the five active compounds all contain at least one methylenedioxy group while neither of the inactive lignans possesses this moiety. Since the methylenedioxy group is responsible for mixed function oxidase inhibition and since the liver is the primary location of this enzyme, it is reasonable to expect that this is the mechanism of action of these compounds.

Stress reducing activity. A number of Asian medicinal plants of the Araliaceae which have long been used as tonics have been analysed and found to contain certain bisepoxylignans. One of these, (−)-syringaresinol diglucoside (**26**), reduces the weight of the adrenals, thymus and spleen in stressed animals; i.e. it reduces the normal physiological responses of the mammalian body to stress [135–137]. As a result, it allows for the elimination of

some of the harmful effects of stress and appears to facilitate increased physical, and perhaps also mental performance under stressful conditions [137]. Intriguing though this biological activity is, no explanation of its mode of action is available [138]. It is interesting to note that (+)-syringaresinol di-β-glucoside is an inhibitor of cAMP phosphodiesterase [28]. (−)-Syringaresinol di-β-D-glucoside is known to be much less effective than the (+)-stereoisomer, although precise data on its potency have not been published. It appears that (+)-syringaresinol di-β-D-glucoside, has not been examined for anti-stress activity.

Presence of lignans in mammals

The matter of the biological role of lignans has been given another dimension recently by the detection of several lignans in man and primates. Compounds **54** (enterolactone) and **55** (enterodiol) have been found, as glucuronides, in the urine of humans, baboons, vervet monkeys and rats [139–141]. Derivatives of both **54** and **55**, methoxylated in either one or both rings, have also been detected in vervet monkey urine [142]. Particularly interesting are the observations that the levels of compound **54** in human urine, plasma and bile is comparable to that of steroid metabolites [140] and that its urinary concentration in females shows a cyclical variation with time. Peak levels were detected during the luteal phase of the menstrual cycle [129, 130] and also during pregnancy [139]. The exciting possibility that these lignans possess hormonal activity in man should be treated with caution as there is evidence which suggests that they are probably metabolic products of the microflora of the gut [143]. Both compounds **54** and **55** are hydroxylated only in the *meta* position, a substitution pattern that is not observed in plant products. The biosynthesis of these compounds from dietary secolariciresinol glycoside has been proposed [143]. An enterohepatic route of circulation for these lignans has been shown to exist [142], suggesting and they play a role of some importance in the chemistry of the body and are possibly more than 'chemical noise.' Obviously much remains to be done to clarify the role of these compounds in mammalian physiology.



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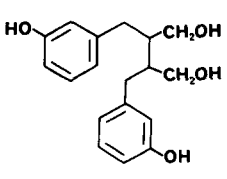

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SUMMARY

The variety of biological activities displayed by lignans is impressive. It is now pertinent to ask what are the biological targets or receptors for these physiologically active compounds, what are their stereochemical specificities for activation and whether commonalities exist among these diverse biological responses. More systematic investigations of their biological activities, utilizing larger numbers of compounds, should further our understanding of the structural prerequisites and determinants of biological activity and help us to discern whether a common mode of action underlies any of these apparently different biological activities. Studies of this type will not only uncover new uses for lignans, but will also provide physiological and biochemical information on the organisms and biological systems affected by them.

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