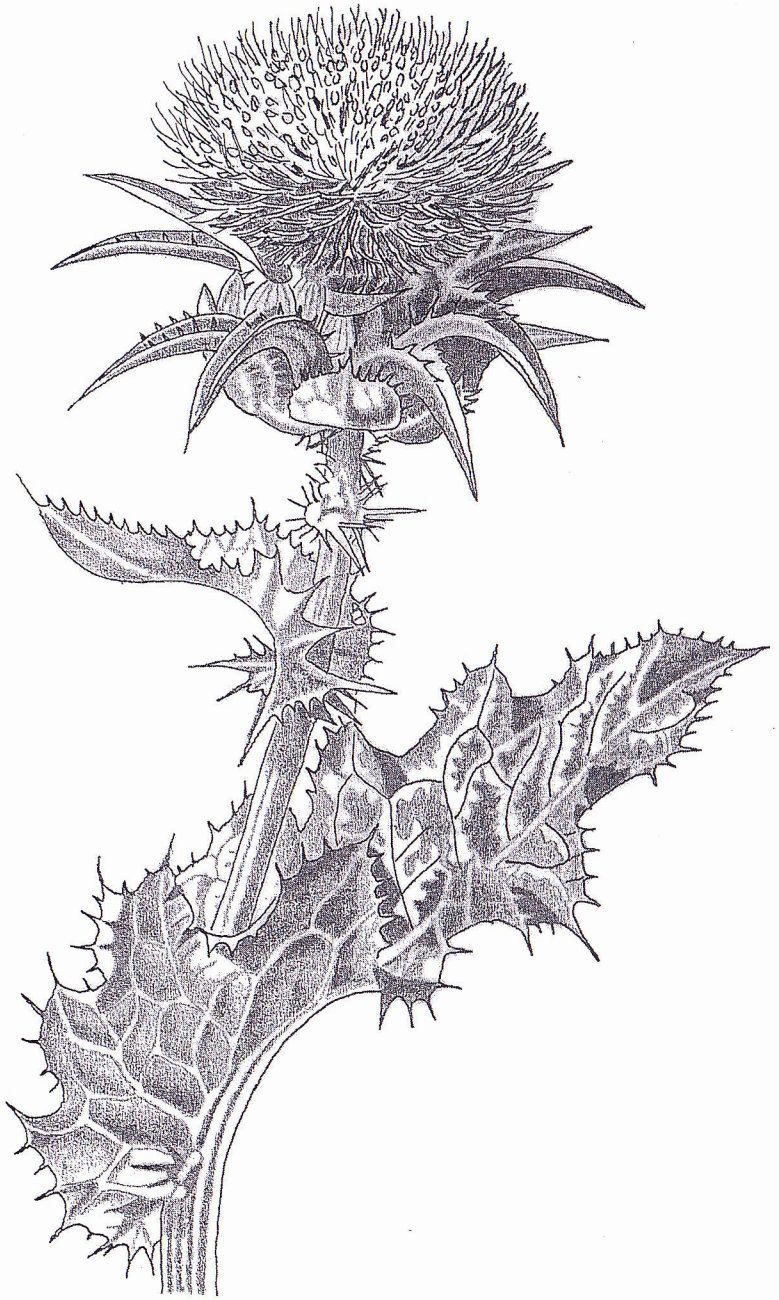
Lignans, Neolignans, and Related Compounds

1. GENERALITIES

In the current state of knowledge, we may distinguish four groups of compounds formed by condensation of phenylpropane units: lignans, neolignans, "oligomers", and norlignans. In addition, it is customary to add to these lignoids, or hybrid lignans.

The term lignan commonly designates compounds whose skeleton results from bonding between the β carbons of the side chains of two units derived from 1-phenylpropane (8-8' bond).

Neolignans are also condensation products of phenylpropanoid units, but the actual bond varies and involves no more than one β carbon (8-3', 8-1', 3-3', 8-*O*-4' for example).

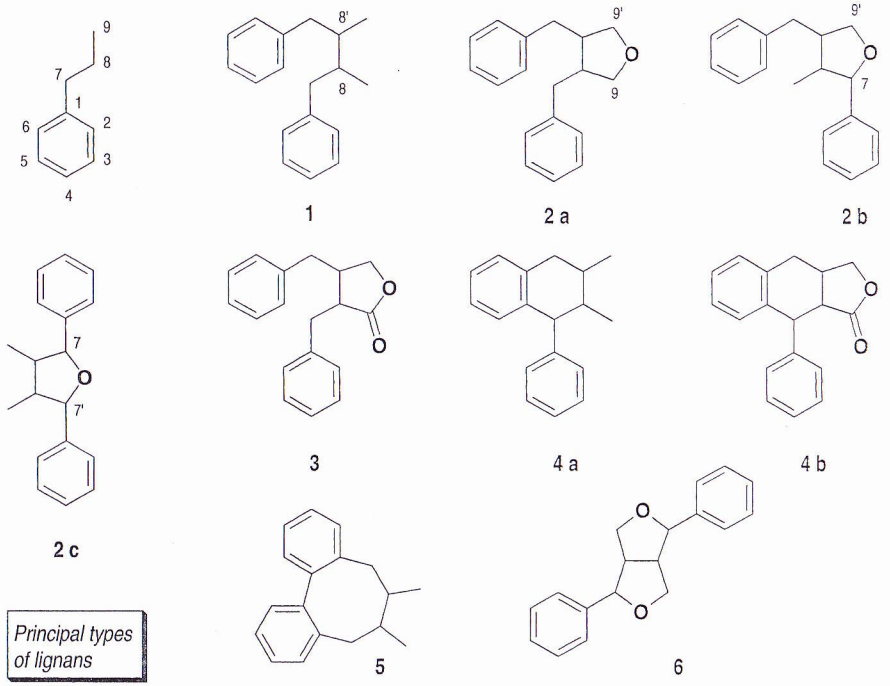


The term oligomers is improper and designates lignans or neolignans resulting from the condensation of two to five phenylpropanoid units (e.g., sesqui- and dilignans of burdock seeds, lithospermic acid).

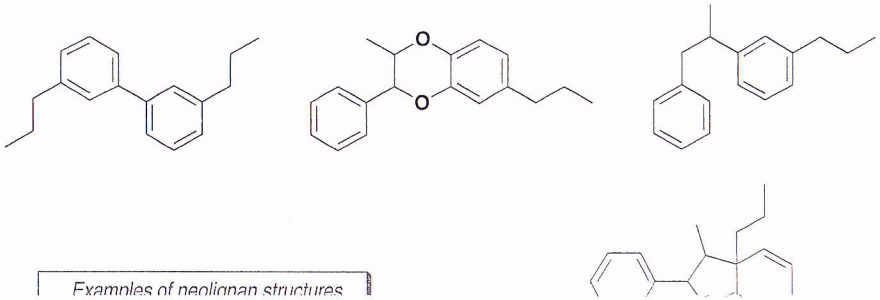
Norlignans are probably specific to Gymnosperms and have a C_{17} skeleton.

The last group consists of "lignoids", also called hybrid lignans, a name that emphasizes their mixed biosynthetic origin: flavonolignans of St. Mary thistle or of *Hydnocarpus* sp., coumarolignans of various Simaroubaceae, and xantholignanoids such as kielcorin of St. John's wort.

Among lignans *per se*, it is customary to distinguish six fundamental structural groups. The simplest are dibenzylbutanes (8-8' bond: see structure **1**) which, by cyclization, yield three types of monofuranoid lignans (cyclization in 9-*O*-9', 7-*O*-9',



Principal types of lignans

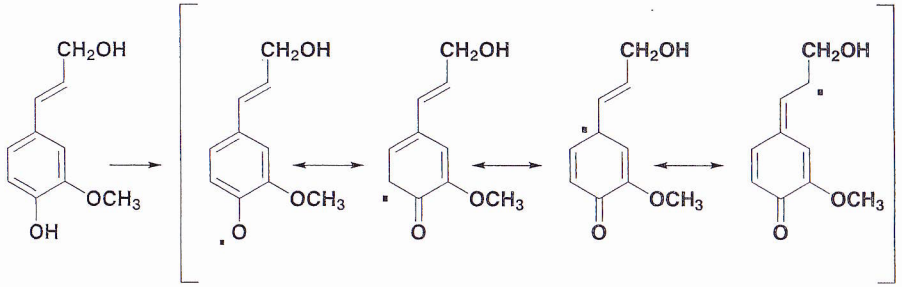


Examples of neolignan structures

7-*O*-7': **2 a-c**) and butyrolactones (**3**). The cyclization may involve one aromatic carbon atom (arylnaphthalenes: **4 a-b**) or two (dibenzocyclo-octanes: **5**). Double cyclization between 7-*O*-7' and 9-*O*-9' leads to furanofuranoid lignans: **6**.

Among neolignans, numerous coupling possibilities result in a greater structural diversity (see table on p. 281).

Chemotaxonomically, lignans are widely distributed; several hundred compounds have been isolated in about 70 families. In Gymnosperms they occur mainly in wood, whereas in Angiosperms they have been identified in all tissues. Neolignans appear to have a narrower distribution; they are especially common in Magniolales and Piperales, two orders characterized by the frequent occurrence of propenyl- and allylphenols.

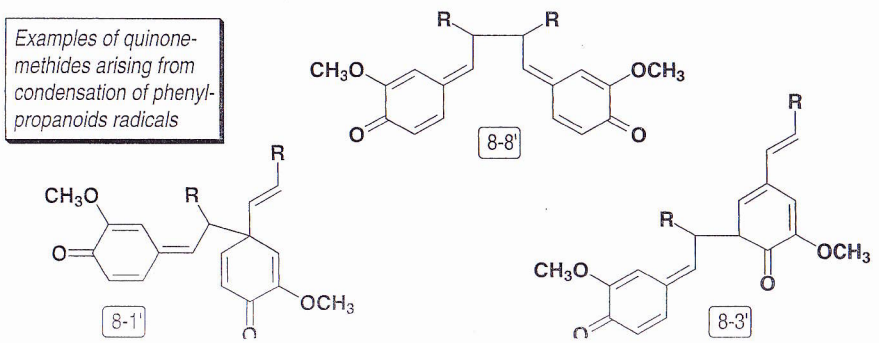


Coniferyl alcohol: oxidation and radical mesomerism

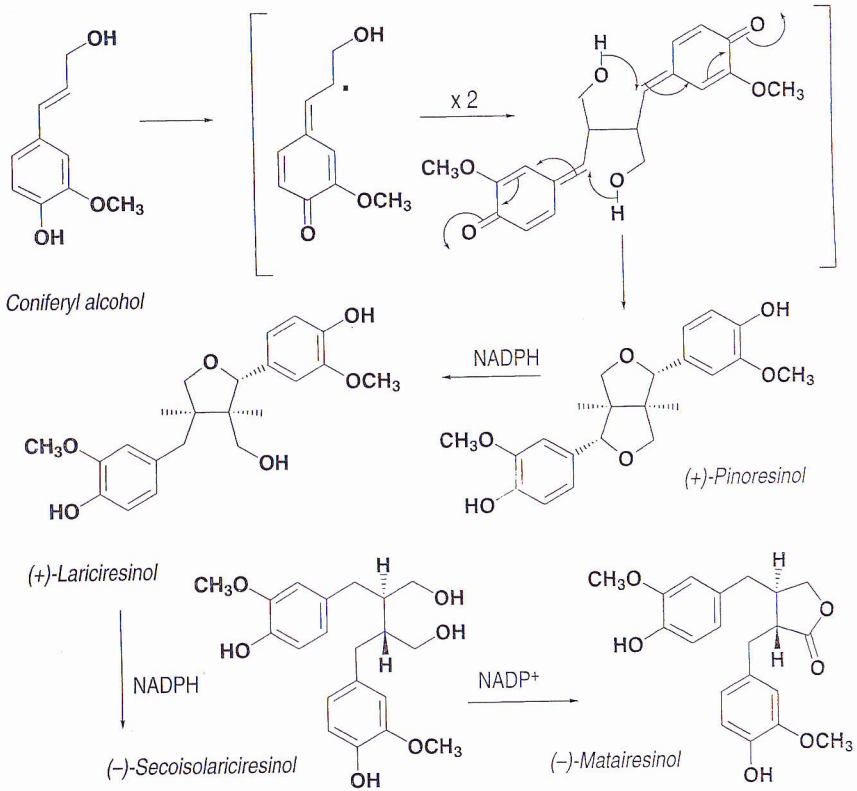
2. BIOSYNTHETIC ORIGIN

Experimental data are scarce, and in most cases we can only present partially confirmed hypotheses. Since lignans are optically active, they must result from stereospecific coupling catalyzed by an enzyme (as shown in *Forsythia* spp.). It is conceivable that the oxidation of a precursor (for example coniferyl alcohol, see figure) would lead to a radical which could exist as one of four mesomers. In theory, this would make possible a very large number of couplings, among which five would be common (8-8', 8-1', 8-3', 8-*O*-4', and 3-3'). In the case of neolignans, it is possible, although not proven, that the radicals involved in the coupling reactions

Examples of quinone-methides arising from condensation of phenyl-propanoids radicals



arise from allyl- and propenylphenols. Logically, the condensation of two radicals would lead to a methide-quinone, which could then become aromatic again and thereby induce cyclization (to form, for example, furanofurans or dihydro-benzofurans). In many cases the intermediate may be hydroxylated.



Biosynthesis of lignans : origin of matairesinol

3. BIOLOGICAL INTEREST IN LIGNANS

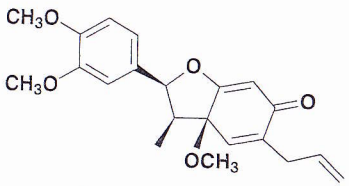
In plants, lignans and neolignans probably play an important defense role: antibacterial, antifungal, and antifeedant properties have been described for many compounds in this group.

Although many lignans, mostly arylnaphthalenes and dibenzocyclo-octanes, possess cytotoxic and antimitotic properties, only the semisynthetic derivatives of podophyllotoxin are exploited in therapeutics. Among lignan-related compounds, the flavanolignans of the akenes of St. Mary thistle are responsible for hepato-protective properties that have been demonstrated *in vitro* and in animals; for this reason they are included in the formulation of proprietary drugs available in Europe. In addition, several lignan-containing drugs are used in Chinese medicine, particularly the fruit of

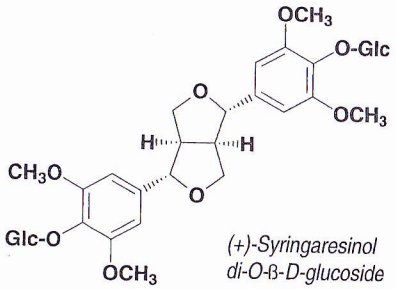
Other lignans and neolignans are of potential interest in various domains: enzyme inhibition, especially of cAMP phosphodiesterase by (+)-matairesinol, or 5-lipoxygenase, and leukotriene biosynthesis by justicidin E and its analogs; anti-platelet-aggregation activity of syringaresinol; calcium blocking activity of trachelogenin; antihypertensive action of the bis β-D-glucoside of (+)-pinoresinol; antiviral activity of nordihydroguaiaretic acid (NDGA) derivatives and of various naphthalene- or tetrahydronaphthalene-type cyclolignans; and potentiation of the insecticidal action of pyrethrins by furano-furans of the unsaponifiable matter of sesame oil.

In the group of neolignans, kadsurenone, isolated from the stems of a Chinese plant well known as antiallergic and antirheumatic, and used as such in the south of China (*haifengteng*, *Piper futokadsura* Sieb. & Zucc., Piperaceae), is a specific inhibitor of the *platelet activating factor* (= PAF), a mediator probably involved in anaphylactic shock, inflammation, or allergic reactions. It inhibits the binding of PAF to its receptors on the platelet membrane; it also inhibits the degranulation of neutrophils. An activity of the same type has been recognized for various other neolignans, as well as for furanofuranoid lignans (e.g., magnolol, aschantin).

Under the heading of miscellaneous activities, note that lignans and their metabolites in humans (enterodiol, enterolactone) are receiving increasing attention from nutritionists, because they may decrease the risk of carcinogenesis in the prostate, colon, and breast, among others. Their action may be linked to an interaction with estrogen receptors, to their antioxidant activity and/or to aromatase inhibition.



Kadsurenone



(+)-Syringaresinol di-O-ß-D-glucoside

4. DRUGS CONTAINING LIGNANS AND RELATED COMPOUNDS

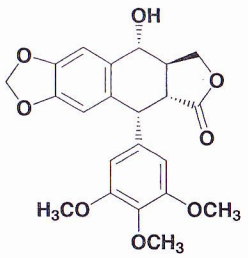
- **MAY APPLE OR MANDRAKE,**
  Podophyllum peltatum L., Berberidaceae

The resin of podophyllum rhizome, traditionally used as a contact cathartic, is a source of podophyllotoxin. This antimitotic lignan is extracted from various sources to be transformed into the semisynthetic antineoplastic derivatives teniposide (INN) and etoposide (INN). Proprietary drugs based on podophyllotoxin and its derivatives

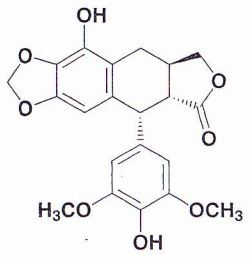
may not be renewed. Podophyllin, which no longer appears in the latest edition of the French Pharmacopoeia, is still listed in other pharmacopoeias (e.g., Helvetica VII).

The Plant, the Drug. This small plant, perennial by a rhizome, has an aerial stem of about 30 centimeters which ends with two opposite and palmatilobate leaves at the base of which is inserted a solitary, trimerous, white flower. The species is wild in the damp and shady forests of the eastern United States and Canada. The rhizome, cut into reddish-brown fragments (5-20 x 0.5 cm), has knots with insertion scars of aerial stems, and scars, smaller and more numerous, of roots. A microscopic examination reveals the presence of resin cells, calcium oxalate prisms, and starch grains. The morphology and especially the size of these various elements allows one to distinguish *P. peltatum* and *P. hexandrum* (see below).

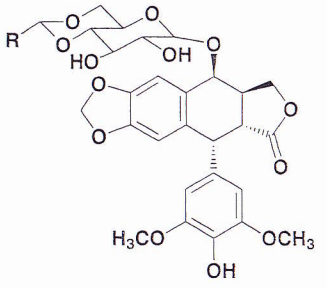
Chemical composition. The drug contains 3 to 6% resin. Known in the past as podophyllin, this resin can be obtained by diluting an alcoholic extract with water that is eventually acidified: it precipitates, is collected, then dried. The main constituents of the resin are 1-aryltetrahydronaphthalenes: podophyllotoxin (20%), α- and β-peltatins (5 and 10%, respectively), desoxypodophyllotoxin, and close derivatives. Some of these compounds occur as glycosides. All compounds in the series have a lactone which is *trans*-fused to the adjacent ring. The structure, very strained, is unstable: epimerization at carbon C-2 (*via* the enolate) is immediate in slightly alkaline medium. The resulting products, which are *cis*-fused, are stable, but practically inactive. Picropodophyllin is one example. Epi derivatives are also known, with an inverted configuration at carbon C-4 (4-*S*).



Podophyllotoxin



α-Peltatin



R = CH_3, *Etoposide*
R = thienyl, *Teniposide*

Pharmacological Activity. Podophyllotoxin and peltatins inhibit the growth of experimental tumors induced in the mouse. Their action takes place at the level of the microtubules. The competitive inhibition of colchicine binding to tubulin shows that the mechanism of action is similar: podophyllotoxin, a mitotic spindle poison, inhibits the polymerization of tubulin and stops cell division at the beginning of the metaphase. Picropodophyllin is practically inactive, and glycosides are less active than genins, but their side effects are less pronounced.

Synthetic work and the study of structure-activity relationships have made

relatively limited side effects. These products are demethylated at 4', belong to the epi series, and their hydroxyl in position C-4 is part of a glycoside linkage with a glucose, of which two of the hydroxyl groups (at C-4" and C-6") are blocked by acetalization as thienylidene (teniposide) or ethylidene (etoposide). These derivatives, in contrast with podophyllotoxin, are inactive on the assembly of the microtubules, but they stop the cell cycle at the end of the S phase or at the beginning of the G2 phase, by forming a linkage with topoisomerase II, an enzyme necessary to DNA replication.

Toxivcity. Podophyllotoxin is extremely toxic. Following ingestion (or skin contact), it causes gastrointestinal distress, and later on, encephalopathy and peripheral neuropathy with severe hematological manifestations. The intoxication is sometimes fatal and in most cases, it induces walking difficulties and other neurological sequelae that can last for months.

Uses. The resin was long used as a laxative and cholagogue: it used to be an ingredient of the French "*petites pilules Carter pour le foie*" or "little Carter pills for the liver". The resin is no longer used except for the extraction of podophyllotoxin, which is also commonly extracted from another species of the *Podophyllum* genus, namely *P. hexandrum* Royle (= *P. emodi* Wall.). The latter, of Himalayan origin, contains 6 to 12% resin, in which the concentration of podophyllotoxin is around 40%.

Uses of podophyllotoxin.

- In the treatment of external condylomas. Compounded preparations of podophyllin used to be very concentrated, and for a long time, they were the sole form used for this indication. Because they were particularly toxic and caused extremely severe intoxications, they were replaced by an alcoholic podophyllotoxin solution at 5%, packaged in a small vial (3.5 mL) with a child-proof cap. The efficacy of this product (French Liste I, i.e., a prescription drug which may not be renewed) was confirmed by clinical trials and justifies the following indication: external condyloma acuminatum not more than 4 cm^2 in area, as an alternative to other therapies (cryotherapy, surgery). The antimitotic properties of this lignan make pregnancy and breast-feeding absolute contraindications; it is never to be administered to children. In women of child-bearing age, an efficacious contraceptive method must be prescribed before the beginning and throughout the duration of the treatment.

- To obtain semisynthetic derivatives prescribed in the hospital and under strict medical observation:

- *Etoposide* is active when used alone in chemotherapy, but it is most commonly prescribed in combination in various multiple-drug chemotherapy protocols with the following indications: embryonic carcinoma of the testicles, small

duration of survival—and other bronchogenic carcinomas, placental choriocarcinoma, previously treated breast cancer, malignant lymphoma (Hodgkin's disease or other), and acute leukemia. The product is available as an injectable solution; there are also capsules for oral administration. Common doses: IV, 50-150 mg/sq m/day x 1-3 day, for dilution and slow infusion; *per os*, double doses for the same duration or small daily doses in a few particular cases (e.g., palliative treatment). The main side effects are proportional to the dose and are hematological: granulopenia and thrombopenia. Blood tests must be regular and must begin before the start of treatment. Pregnancy and breast feeding are contraindications.

- Common indications of ***teniposide*** are * the treatment of Hodgkin's disease, non-Hodgkin's lymphomas, and brain and bladder tumors. It can be used—in a hospital setting—in single-drug therapy for the induction of remission (30 mg/sq m/day in 5-day cycles; 4-5 cycles 10-21 days apart) or for maintenance (60 mg/sq m/day once weekly for several months), as well as in multiple-drug chemotherapy. The formulation is a solution in polyethoxylated castor oil to be diluted and administered exclusively by infusion. As before, toxicity is hematological; in addition, the excipient can induce a risk of immediate anaphylactic-type reaction with acute respiratory distress. In case of extravasation, teniposide and etoposide cause tissue necrosis.

The pharmaceutical industry recently made available a prodrug, etoposide phosphate. In contrast to etoposide, the phosphate is water soluble (injectable lyophilisate), which makes it possible to use concentrated solutions and short infusion times. Etoposide phosphate is rapidly hydrolyzed to etoposide by serum phosphatases.

The high interest in the therapeutic potential of podophyllotoxin-type lignans continues to be reflected by the synthesis and evaluation of structural analogs, especially C-4 substituted derivatives (esters, amine and arylamine derivatives, azides) and aminoglycosides. Some are currently undergoing clinical trials (e.g., GL331, a C-4 arylamine derivative of epipodophyllotoxin).

• St. Mary Thistle or Blessed Milk Thistle, *Silybum marianum* (L.) Gaertn., Asteraceae

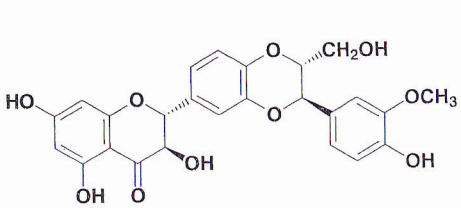
The fruit of St. Mary thistle, not official in France, is known for its hepato-protective properties due to flavonolignans.

The Plant, the Drug. The leaf blade of this biennial plant, marbled with white along the ribs, is bordered by thorny teeth. The purple florets, all tubular, are gathered into a capitulum tucked in an involucre with thorny external bracts. The plant is common to underdeveloped areas of southern Europe, northern Africa, and

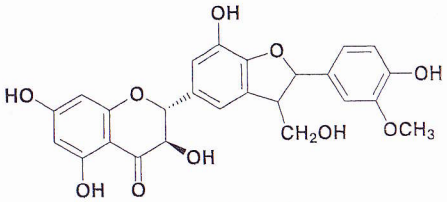
* ...or were, in France, where teniposide was taken off the market in June 1998.

western Asia. The drug consists of the rough black akenes with the remainder of a flower crown on their tops, which is a pale yellow cylindrical scale.

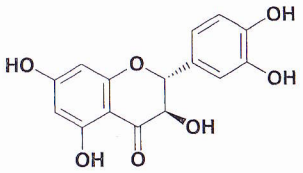
Chemical Composition. The drug contains 20 to 30% lipids, proteins, sugars, and flavonoids, including quercetin, taxifolin, eriodyctiol, and chrysoeriol. The constituents responsible for the activity are flavonolignans initially isolated as a mixture of addition products of a phenylpropanoid alcohol, coniferyl alcohol, onto a 2,3-dihydroflavonol, taxifolin. This mixture, commonly known as silymarin, represents 1.5 to 3% of the weight of the drug. Silybin, the major constituent of the mixture, is a benzodioxane, a 1:1 mixture of two diastereoisomers (7"*R*, 8"*R* and 7"*S*, 8"*S*). The other constituents of silymarin are silydianin, an oxatricyclodecene resulting from the cycloaddition of coniferyl alcohol on the *o*-quinone derived from taxifolin, and silychristin, a dihydro-benzofuranic structure. In other varieties (for example in a white-flower variety), these products occur alongside 3-deoxy derivatives of regio-isomers of silybin (silandrin) and silydianin (silymonin).



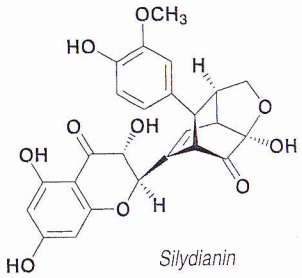
Silybin



Silychristin



Taxifoline



Silydianin

Tests. The drug can be identified by its microscopic characteristics, by TLC (visualization by spraying with aminoethanol diphenylborate), and it is possible to determine the flavonolignan content by spectrophotometry of the derivatives obtained by reaction with 2,4-dinitrophenylhydrazine; HPLC can also be used.

Pharmacological Activity. Multiple experimental studies tend to demonstrate the antihepatotoxic activity of silymarin and its constituents: prevention of the toxic effects of carbon tetrachloride, galactosamine, and other toxins at the level of the hepatic parenchyma, protection (in the mouse, IV) against the harmful effects of phalloidin administered parenterally (but the effect is only partial if the flavonolignans are administered after phalloidin). Silymarin inhibits membrane lipid

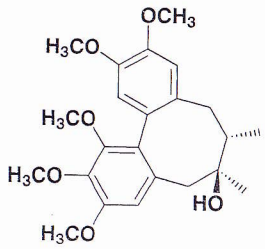
peroxidation, acts as a free radical scavenger, and inhibits the formation of leukotriene B4 at low doses (isolated Kupffer cells). It is thought to have a stabilizing effect on membranes, and in the case of the *Amanita* toxin, it may compete for binding sites. In addition, it stimulates RNA-polymerase: the stimulation of protein synthesis may increase the hepatic tissue regeneration capacity.

The clinical efficacy of silymarin is difficult to establish and varies with the nature of the aggression on the liver (chronic, viral). This activity is evaluated with hepatic function tests (transaminases and others), is rather modest, and only manifests itself in long-term treatments. Silymarin seems to improve the survival rate of alcoholic cirrhosis patients. It is devoid of acute or chronic toxicity and has practically no side effects (rare and mild digestive symptoms). Scarcely absorbed in the intestine, its bioavailability can be increased by complexation with phosphatidylcholine (silipide).

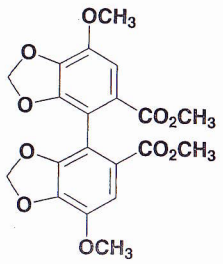
Uses. In Germany and in other European countries, silymarin or extracts titrated for silymarin are promoted as a treatment, *per os*, for liver damage from poisoning and as an adjunctive treatment for chronic liver disease and cirrhosis; an injectable form is used to treat *Amanita phalloides* poisoning. In France, phytomedicines containing St. Mary thistle akenes are traditionally used orally for the symptomatic treatment of functional digestive signs thought to have a hepatic origin [French Expl. Note, 1998]. The very low water solubility of the flavanolignans makes it unlikely that (the very rarely used) herbal tea forms have an antihepatotoxic activity.

• SCHIZANDRA, WUWEIZI, *Schizandra chinensis* (Turcz.) Baillon, Schizandraceae

Traditional Chinese medicine attributes to the fruits of this creeping plant of northern China tonic, antitussive, and CNS stimulating properties. The seeds contain about 30 lignoid-type compounds with a dibenzocyclo-octane skeleton: schisandrin, gomisines A, B, C..., S, T, deoxyschisandrin, and more. Experiments in animals show that the alcoholic extract obtained from the kernel of the seed is antihepatotoxic: it prevents the histopathological changes and the increase in *alanine aminotransferase* (ALAT) induced by carbon tetrachloride or galactosamine



Schizandrin



Biphenyl dimethyl dicarboxylate

intoxication. The principal lignans tested so far would act by inhibiting lipid peroxidation (indeed several of them are antioxidants *in vitro* and stimulate superoxide dismutase and catalase activity). The ethanolic extract of the seeds as well as a synthetic compound, *biphenyldimethyldicarboxylate* (= BDD), have undergone clinical tests and are used (in China) to treat hepatitis from various etiologies. According to Tyler, the efficacy of this drug has not been demonstrated and its use is not recommended.

• CHAPARRAL, *Larrea divaricata* Cav. subsp. *tridentata* (DC.) Felger & Lowe Zygophyllaceae

Chaparral or creosote bush is a shrub native to the arid areas of the United States Southwest and Mexico. Its stems and leaves are coated with a thick layer of resin. Chemically, the leaves are characterized by flavonoids, triterpenes, and lignans, particularly nordihydroguaiaretic acid (= NDGA *). This tetraphenolic diarylbutane can represent 40-50% of the resin that covers the leaf. NDGA is a good antioxidant. It was used as such until medium- and long-term toxicity studies in rodents showed a high frequency of liver damage. The anticancer reputation of this lignan—active *in vitro*—has not been confirmed by clinical observations.

Very popular in the United States, the leaf infusion is purported to have a beneficial effect on the common cold, the flu, diarrhea, and urinary infections; the drug is an antioxidant and some believe in its anti-aging effect. Far from providing the pharmacological basis for these claims, a review of the scientific literature shows that chaparral—infusions, capsules, or tablets consumed in large quantities over many weeks—was responsible for several cases of severe liver damage with jaundice, some of which evolved toward cirrhosis, and two of which required a liver transplant. The drug is also considered responsible for one case of kidney adenocarcinoma. Although these cases remain statistically exceptional, in the absence of proof of any kind of beneficial effect, it is best to not use chaparral. External contact with chaparral (or NDGA) can cause allergic dermatitis.

• *HYPOXIS ROOPERI* T. Moore, Hypoxidaceae

Hypoxis are herbaceous perennial plants with a bulb or tuberized rhizome (family close to Amaryllidaceae) found mostly in the southern part of Africa. Several species in the genus are used in traditional medicine, especially for urinary disorders (South Africa), prostatic hyperplasia (Malawi), and even cancer (Caribbean Islands).

* This lignan is a constituent of guaiacum resin from *Guaiacum officinale* L., a small tree native to central America. Guaiacum tincture is a traditional reagent used to detect oxidases and peroxidases. NDGA can be used in experimental pharmacology as a lipoxygenase

The *H. rooperi* bulb contains a glycoside which most authors agree to classify in the *nor*lignan group, hypoxoside [= (1*E*)-1,5-bis (3'-hydroxy-4'-*O*-β-D-glucopyranosylphenyl) pent-1-en-4-yne]. In the presence of β-glucosidase, hypoxoside is converted to rooperol, which is cytotoxic *in vitro*. Preliminary trials in humans indicate that the glycoside is not toxic.

H. rooperi is currently used in Germany as an extract promoted as a treatment for benign prostate hyperplasia. The activity that is attributed to this extract may be due to glycosides of sterols. The infusion of dried bulb is used in South Africa for the same indication.

SPECIAL CASES: Lithospermic acid derivatives

Structurally, lithospermic acid is a dihydrobenzofuran visibly formed by three molecules of caffeic acid. Its structure (and that of "tetramers" such as rabdosin) is close to that of another caffeic derivative, namely rosmarinic acid: we found it more judicious to not separate the coverage of these compounds, which in addition are found in the same families (Boraginaceae, Lamiaceae) (see phenolic acids, Java tea, p. 250).

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