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Diterpenes

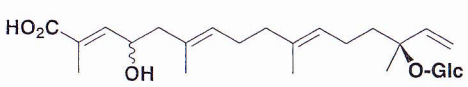
1. INTRODUCTION

Diterpenes constitute a vast group of C_{20} compounds arising from the metabolism of *2E,6E,10E-geranylgeranyl pyrophosphate* (= GGPP). Found in some insects and in various animal organisms, they are especially widespread in plants. Some are ubiquitous (e.g., gibberellins), whereas others have a more limited distribution. They are particularly abundant in the Lamiales and Asterales—over 1,200 compounds representing about one hundred skeletons have been reported in the Asteraceae alone!—and they are more scattered in the Gentianales, Geraniales, and Fabales.

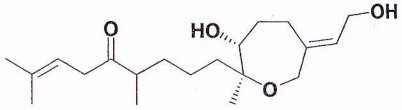
2. CHIEF STRUCTURAL TYPES. BIOGENETIC ORIGIN

The structure of diterpenes is highly variable and strictly dependent on their biogenesis. A classification based on biogenesis is therefore logical.

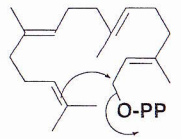
Acyclic Compounds. Acyclic compounds are not the most commonly occurring diterpenes. They may be linear like capsianoside, or comprise a lactone or an oxygen-containing ring arising from the opening of oxiranes (zoapatanol).



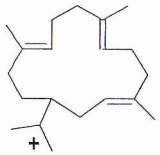
Capsianoside



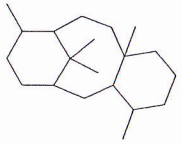
Zoapatanol



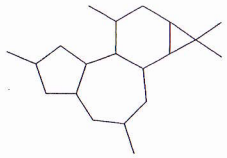
Geranylgeranylpyrophosphate



Cembrane type



Taxane



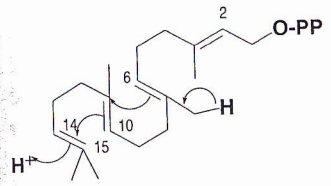
Tigliane

Cyclic Compounds. Cyclic compounds result from the two modes of cyclization of the precursor GGPP:

1. A cyclization due to pyrophosphate acting as a leaving group, and the resulting carbocation alkylating a double bond. Most often this is the terminal isopropylidene, and the mechanism leads to the formation of a macrocycle. The macrocyclic polyunsaturated cation is very reactive. It can be stabilized (e.g., by cembrenoid formation in the gummy exudate of tobacco leaf) or, frequently, lead to a polycyclic structure by intramolecular nucleophilic substitution, leading for example to taxane, tigliane, daphnane, or ingenane.

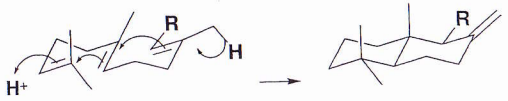
2. A cyclization catalyzed by an acid, like the one which leads to triterpenes, but without preliminary epoxidation: the result is a substituted decahydronaphthalene. This type of cyclization leads to two enantiomeric series, which differ by opposite configurations at C-5, C-9, and C-10. The series is known as "normal" when the A and B rings are fused like in the steroids, and "*ent*" (enantio-) for the mirror image: examples include labdane and *ent*-labdane, and kaurane and *ent*-kaurane (in fact the prefix "normal" is generally omitted).

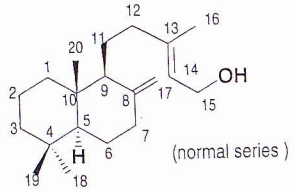
- ***Bicyclic Diterpenes***. The biosynthesis of bicyclic diterpenes is directed toward one or the other series by the conformation of the linear precursor (GGPP) on the surface of the enzyme which catalyzes the cyclization. The reaction sequence consists of the protonation of the C-14 double bond of the precursor, followed by the 1,2-antiparallel additions of the C-6 and C-10 double bonds to form, in both cases, a *trans*-decalin. The (hypothetical) carbocation resulting from this cyclization can, like in the other terpenoid series, be stabilized: 1. by the elimination of a proton and formation of a double bond; 2. by hydration; or 3. by a rearrangement.



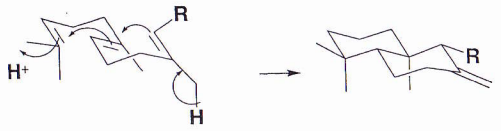
2*E*, 6*E*, 10*E*-GGPP

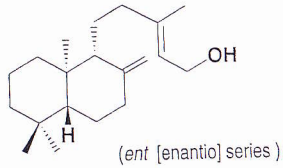
Cyclisation of geranyl-geranyl-PP : formation of labdanes and ent-labdanes. Examples of labdane structures



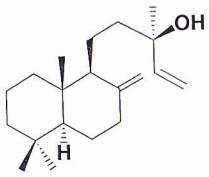


(normal series)

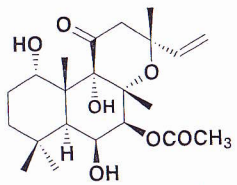




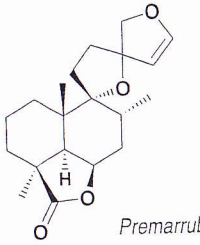
(*ent* [enantio] series)



Manool



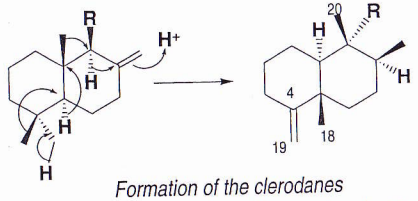
Forskolin



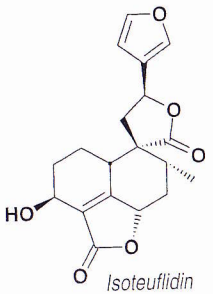
Premarrubiin

Potential oxidation at various stages (e.g., manool, labdanolic acid) makes possible the formation of cyclic ethers or lactones (e.g., forskolin, premarrubiin).

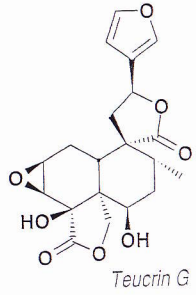
The most common rearrangement includes both proton migrations and methyl group migrations, and leads to clerodanes ("*friedo*" rearrangement). Both enantiomeric series are known, the substituents at C-8 and C-9 may be *cis* or *trans* relative to one another, and the products may be substantially functionalized (for an example, see the germanders).



Formation of the clerodanes and examples of the neoclerodanes characteristic of Teucrium *spp.*



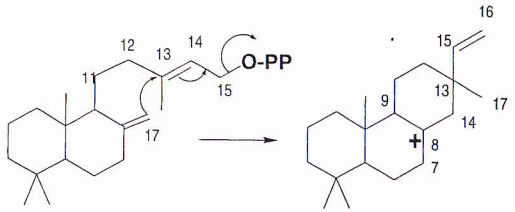
Isoteuflidin



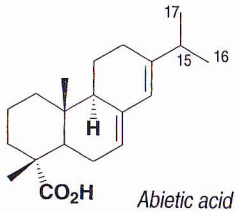
Teucrin G

(*neoclerodanes*)

• ***Tri- and Tetracyclic Diterpenes***. The hypothetical carbocation arising from labdadienyl pyrophosphate can cyclize in several ways: attack on C-13 by the exocyclic double bond, migration of the C-13 double bond, stabilization of the cation by elimination of a proton from C-7 or C-14 (leading to isopimaranes or pimaranes), or further rearrangement (leading to abietanes, cassanes, or rosanes).

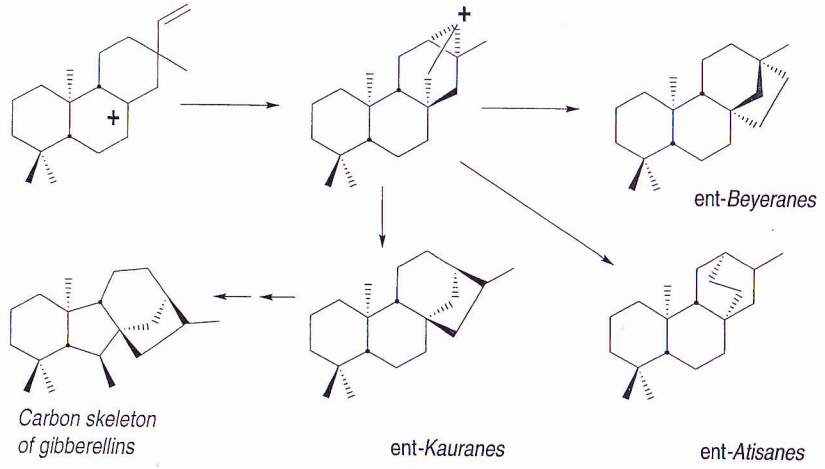


Formation of the pimaranes



Abietic acid (abietanes)

The tetracyclic structures arise, in theory, from the cyclization of the *ent*-pimarenyl cation. The figure below illustrates some of its cyclization possibilities.



Carbon skeleton of gibberellins

ent-*Kauranes*

ent-*Atisanes*

ent-*Beyeranes*

3. INTEREST IN DITERPENES

Diterpenes have limited therapeutic applications. Except for the tricyclic terpenes from yews, which are undergoing clinical trials, no pure diterpene is currently produced by the pharmaceutical industry.

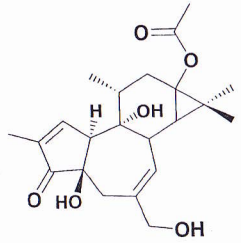
Several diterpene-containing drugs are ingredients of phytotherapeutic products or, as simple galenicals, ingredients of allopathic proprietary drugs. Examples are the gum plants, which are Lamiaceae containing marrubiin, or the germanders, which will be covered below despite a (nearly) complete lack of evidence that the diterpenes that they contain have any role in the activity attributed to them.

Some diterpenes, however, do have some therapeutic potential, for example the following: forskolin from *Plectranthus barbatus* Andr. (= *Coleus forskohlii*, Lamiaceae) with its antihypertensive properties; prostratin from *Homalanthus nutans* (Forster) Pax (Euphorbiaceae) with its antiretroviral properties; diterpenoid quinones from *Salvia miltiorrhiza* Bunge, of interest in the treatment of various heart conditions; tetracyclic diterpenes (e.g., oridonin, lasiokaurin) from *Isodon* (= *Rabdosia,* Lamiaceae), some of which (*R. rubescens* and other species, China) have undergone clinical trials; structures isolated from marine organisms, but also from Lamiaceae (borjatriol and other diterpenes from *Sideritis mugronensis* Borja and closely related species) with anti-inflammatory and analgesic properties; and diterpenoid amides from *Ryania speciosa* Vahl. (Flacourtiaceae), which are toxic to insects and act by paralyzing muscles.

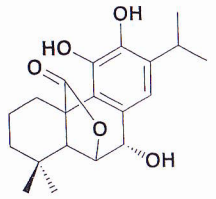
The co-carcinogenic activity of the diterpenoid esters of the Euphorbiaceae and Thymelæaceae, and paradoxically, the cytotoxic activity of some of them suggest interesting research directions.

In addition to the therapeutic potential, note the following:

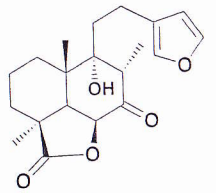
- the antioxidant properties of the phenolic diterpenes of some Lamiaceae (see rosemary, p. 539 and sage, p. 540);
- the sweetening properties of stevioside from *Stevia rebaudiana* (p. 653)



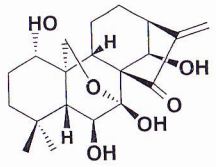
Prostratin



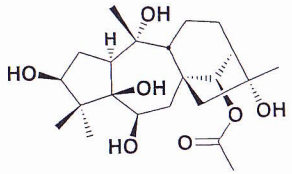
Rosmanol



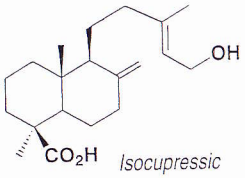
Ballotinone



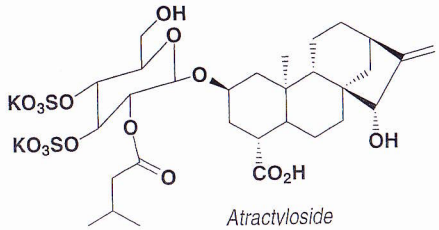
Oridonin



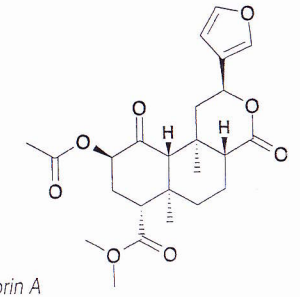
Grayanotoxin 1



Isocupressic acid



Atractyloside



Salvinorin A

- the intense hallucinogenic properties of salvinorin A, an active diterpene from the leaves of *Salvia divinorum* Epling & Jativa, a Lamiaceae masticated fresh (or smoked) by the Mazatec Indians of northeastern Oaxaca, Mexico, for its psychoactive effects, which aid in ritual divination, and to treat various disorders: 200 µg of salvinorin A, vaporized and inhaled, trigger effects close to those of most other known hallucinogens;

– the action on the uterus of kaurenoic acid and its derivatives: it is believed to be the explanation for the activity of a traditional nahua drug, *zoapatle* (*Montanoa tomentosa* Cerv., Asteraceae). *Zoapatle* is used in Mexico for birth control and to induce labor (*cihuapahtli*, which gave *zoapatle,* means "women's remedy"). This is reminiscent of the African use of some *Aspilia* (Asteraceae) as galactagogues and to relieve menstrual cramps. The leaves of these species contain diterpenic acids (kaurenoic, grandiflorenic) which, *in vitro*, stimulate the contractions of guinea pig uterus previously treated with estrogens. Certain *Aspilia* are also consumed by chimpanzees*. Finally, isocupressic acid—a labdane-type diterpene—is responsible for the premature calvings induced in cows by the ingestion of the needles of a North American pine, *Pinus ponderosa* Laws (Pinaceae) or of *Cupressus macrocarpa* Laws. Isocuppressic acid is thought to alter hormonal regulation, causing a decrease in blood flow to the fetus.

Several diterpenes are violent poisons. The species that elaborate them are dangerous to humans and animals, and include Thymelæaceae, Euphorbiaceae, and Ericaceae that contain phorbol esters and related compounds, as well as Asteraceae that contain carboxyatractyloside, such as the Mediterranean thistle (*Atractylis gummifera* L.) or cockleburs (*Xanthium* spp). We shall cover below the toxicity of diterpenoid alkaloids, some of which are used in therapeutics (e.g., aconitine).

The physiological role of diterpenes, like that of many other secondary metabolites, is not well understood. Except for the gibberellins (which are known to be growth hormones), some are postulated to have a protective role against predators, and others are constituents of coatings on foliage and might limit water losses.

* "Zoopharmacognosy" experts find evidence consistent with the hypothesis that chimpanzees have a medicinal use for *Aspilia*. Initially, it was postulated that these leaves were consumed for the antibacterial properties of thiarubrine A (p. 172). However, the finding of this polyalkyne was not confirmed, but that of kauranes that stimulate uterine contractions was confirmed. In addition, female chimpanzees consume three times more *Aspilia* leaves than males do. Page, J.E., Balza, F., Nishida, T. et Towers, G.H.N. (1992). Biologically Active Diterpenes from *Aspilia mossambicensis*, a Chimpanzee Medicinal Plant, *Phytochemistry*, **31**, 3437-3439.

On "zoopharmacognosy", see mainly: Rodriguez, E. and Wrangham, R. (1993). Zoopharmacognosy: The Use of Medicinal Plants by Animals, *in* "Phytochemical Potential of Tropical Plants", (Downum, K.R., Romeo, J. and Stafford, H.A., Eds.), p. 89-106, Plenum Press, New York.

4. DITERPENE-CONTAINING DRUGS USED IN THERAPEUTICS

A. Yews, *Taxus* sp., Taxaceae

Yews have been known since remote antiquity for their toxicity to humans and domesticated animals, and have been making medical headlines for a few years: directly or indirectly, they provide two diterpenoid anticancer compounds with a novel mechanism of action, namely paclitaxel and docetaxel.

The Plant. The eight species in this genus, which is the only one in the Taxaceae family, are all found in the northern hemisphere. They are characterized by leaves that are flattened limp needles, almost in a single plane, with two yellowish-green stomata bands on the underside. The male flowers have 6-14 anthers shaped like shields and the female apparatus is reduced to an ovule surrounded by scales. The fecundated ovule is wrapped in a red aril which becomes fleshy upon ripening. A microscopic examination of the leaf cut shows an epidermis with heavily cutinized cells, as well as the absence of hypoderm and resin ducts.

The European yew is *T. baccata* L., a large dioecious tree which grows slowly and lives for an exceptionally long time. Outside of Europe, common species are *T. brevifolia* Nutt. and *T. canadensis* Marshall in North America, and the Japanese yew, *T. cuspidata* Siebold & Zucc., and the Himalayan yew, *T. wallichiana* Zucc, in Asia. These species are all very similar and difficult to distinguish. They can sometimes produce hybrids. For example, the hybrid of *T. baccata* and *T. cuspidata* is *T.* x *media* Rehder, whose numerous cultivars (e.g., *'hicksii', 'densiformis', 'fastigiata'*) are prized as ornamental plants.

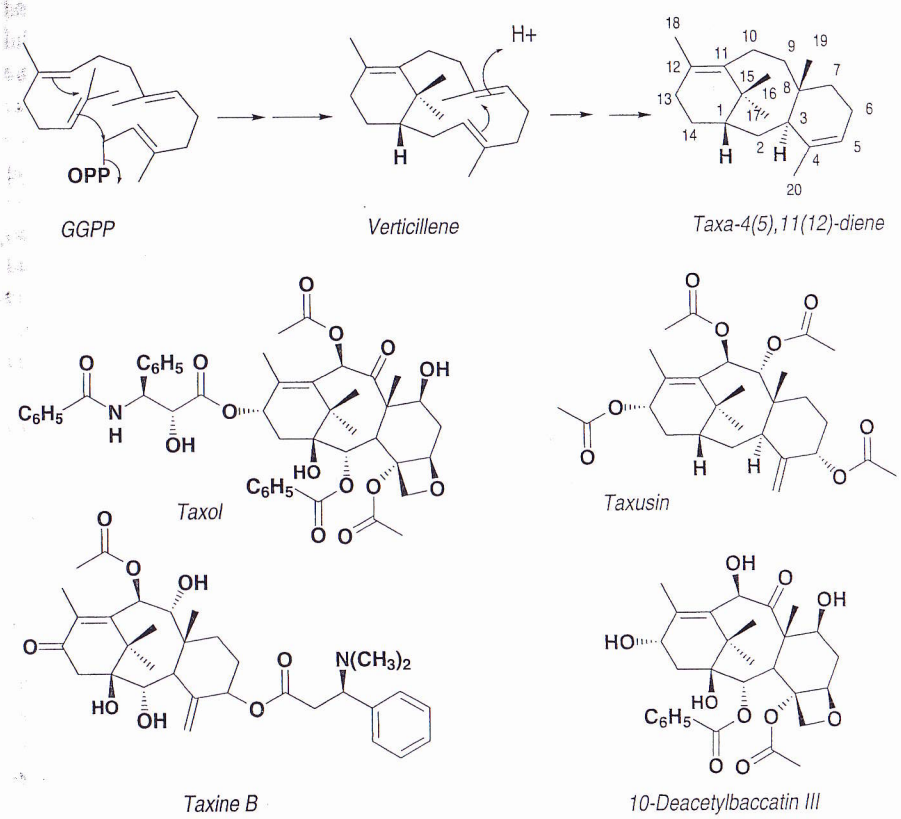
Once abundant, the European yew became heavily exploited to manufacture bows, then for woodwork and marquetry, because of the remarkable qualities of its wood (hardness, homogeneity, grain). Growing in the shade as well as in the sun, adapted to most kinds of soils, and able to withstand bitter cold, yews are frequently planted in parks and gardens for ornamental purposes, especially as hedges: they lend themselves to clipping.

Chemical Composition. Different categories of metabolites are represented in the leaves and stems, including saccharides, polysaccharides and cyclitols, fatty acids, sterols, bisflavonoids (sciadopitysin, kayaflavone), proanthocyanidins, lignans, and cyanogenic glycosides.

The most interesting constituents are tricyclic diterpenes with a taxane nucleus, including taxusines, taxagifin, baccatin III and derivatives, taxine (a complex mixture of taxine A, B, and their derivatives), taxol, cephalomannine, taxicins and derivatives. Some are strictly diterpenoids (e.g., baccatins), whereas others have an amide function (e.g., taxol), or are esters of 3-dimethylamino-3-phenylpropionic acid (e.g., taxines), so that they are sometimes considered to be pseudoalkaloids.

TAXUS BACCATA L.

diphosphate * to bicyclic verticillene, then to taxa-4(5),11(12)-diene. The latter becomes functionalized in various ways.

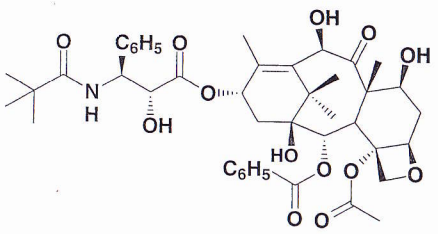


Taxol Sources. Taxol (paclitaxel [INN], marketed in France as Taxol® since 1994) was initially isolated from the bark of the trunk of Pacific yew (*T. brevifolia* Nutt.), but was found only in traces (0.01%): at best, a one hundred year-old tree would produce about 3 kg of bark, in other words 300 mg of taxol. Even with optimized extraction methods, about 7 t of dried bark would be required to yield 1 kg of taxol. Thus the compound cannot be produced on an industrial scale without eventually destroying the species! Systematically screening the *Taxus* genus resulted in the selection and cultivation of cultivars whose leaves constitute an exploitable (and sustainable) source of taxol: in the case of *T.* x *media*, the taxane concentration

* German researchers recently questioned the fact that mevalonate is the precursor of taxol. On the basis of labeled precursor experiments conducted on *T. chinensis* cell cultures, they are inclined to propose an origin similar to that which was described in a bacterium by Rohmer *et al.* in 1993 (see p. 469). Eisenreich, W., Menhard, B., Hylands, P.J., Zenk, M.H. and Bacher, A. (1996). Studies on the Biosynthesis of Taxol: the Taxane Carbon Skeleton is

of the leaves exceeds 0.1% and the taxol concentration can reach 0.06%, particularly in the "*hicksii*" cultivar. The yield of taxol is closely dependent on the drying process (40-50°C). It is also quite feasible to prepare taxol by semisynthesis from structural analogs such as 10-desacetylbaccatin III. This is a diterpene found in substantial quantities (0.02-0.1%) in an easily sustainable starting material: the leaves of the European yew, *T. baccata*, or of various cultivars of other yews (*wallichiana, cuspidata*, x *media*).

Synthetic work has also led from the same 10-desacetylbaccatin III to esters at C-13, in which *N*-benzoyl-3-phenylisoserine is replaced by a structural analog. One of these analogs, *N*-debenzoyl-*N*-tert-butoxycarbonyl-10-deacetyltaxol (= docetaxel, INN), was marketed in France in 1996 under the name of Taxotère®. Docetaxel and paclitaxel have also been approved by the FDA.



Docetaxel

Other synthetic approaches to taxol and its derivatives. Two total syntheses of taxol were published in 1993. Until now, their interest has been purely academic. Research efforts focused on tissue culture have not produced any results of note. On the other hand, endophytic fungi discovered on yews are capable of producing taxanes. These organisms (*Taxomyces andreanae*, *Pestalotiopsis microspora*) produce only small quantities of taxanes (60-70 µg/L at best).

Pharmacological Properties and Uses of Paclitaxel and Docetaxel

• ***Paclitaxel.*** Paclitaxel, like some other natural substances, is a mitotic spindle poison, but its mode of action is very specific: it promotes the assembly of tubulin dimers into microtubules, which it stabilizes by inhibiting their depolymerization *.

The first indication of paclitaxel was the treatment of advanced ovarian tumors, including those that no longer respond to cis-platinum. Other indications were

* Substances that possess the same mechanism of action have been isolated in 1996 from bacteria: *Sorangium cellulosum*. These substances, epothilone A and B, are macrolides for which several total syntheses have already been published. *Cf.*: Höfle, G., Bedorf, N., Steinmetz, H., Schomburg, D., Gerth, K. and Reichenbach, H. (1996). Epothilone A and B – Novel 16-Membered Macrolides with Cytotoxic Activity: Isolation, Crystal Structure, and Conformation in Solution, *Angew. Chem. Int. Ed. Engl.*, **35**, 1567-1569. More recently, a diterpenoid glycoside, eleutherobin, was found to have identical properties (Lindel, T., Jensen, P.R., Fenical, W., Long, B.H., Casazza, A.M., Carboni, J. and Fairchild, C.R. (1997). Eleutherobin, a New Cytotoxin that Mimics Paclitaxel (Taxol) by stabilizing Microtubules, *J. Am. Chem. Soc.*, 119, 8744-8745.

recognized more recently: 1. treatment of metastatic breast cancer unresponsive to treatment that includes an anthracycline; 2. advanced ovarian tumors in combination with cis-platinum. Posology: 135-175 mg/m², depending on the indication; by infusion every 3 weeks, after appropriate pre-medication. The toxicity is non-trivial, and includes neutropenia, peripheral neuropathy, cardiovascular problems, alopecia, nausea, vomiting, and hypersensitivity reactions to the solvent, polyethoxylated castor oil.

• ***Docetaxel.*** The indication of docetaxel is the single-drug therapy of breast cancer, locally advanced, or metastatic and resistant, or in relapse, after chemotherapy that has included an anthracycline (posology: 100 mg/m² by infusion every 3 weeks, after appropriate pre-medication). Like its natural homolog, docetaxel is highly toxic: adverse effects include severe neutropenia, hypersensitiviy reactions, cutaneous reactions, and water retention.

A critical 1996 review of the first clinical results ever obtained emphasized that although an encouraging number of tumors responded to these very costly compounds, to date, the two compounds do not radically improve the bleak prognosis of the conditions for which they are indicated. Other clinical trials are in progress to explore the benefit of combinaion chemotherapy (e.g., taxoids and adriamycin, or fluorouracil and folinic acid, or epirubicin) and evaluate other indications, particularly non small cell lung cancer. In addition, synthetic patents for novel structural analogs are being applied for on a regular basis.

Yew: a Toxic Plant. The toxicity of yew has been exploited for war (arrow poison), to commit murders, and even, in rare cases, for suicide attempts (leaves as such or in infusion). This toxicity is also well known to veterinarians and farmers. It results, almost always, in the instant death of the intoxicated animals (bovines, horses). Currently, poison centers only report intoxications linked to the ingestion of the pseudofruits by young children. Very frequently, the seed (which contains the toxic principles) is ingested without having been chewed, or else spit out (its contents are very bitter), which explains why serious poisoning is rare. In case of suspected ingestion of "berries", virtually all practitioners recommend induced vomiting, and if the vomit does contain "berries", hospitalization.

The intoxication by the leaf (or the seed) begins with digestive symptoms (nausea, vomiting, abdominal pains, diarrhea) and neurological symptoms (sleepiness, lethargy), and is characterized by hypotension and perturbations of the cardiac rhythm: bradycardia at 25-30 beats/min and ventricular arrhythmia, generally fatal in the absence of emergency care. There is no specific treatment.

B. Gum Plants, *Grindelia* spp., Asteraceae

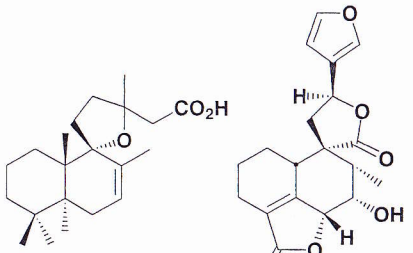
"The part of *Grindelia* that is used as a drug consists the dried flowering tops of *G. robusta* Nutt., *G. squarrosa* (Pursh) Dunal., *G. humilis* Hook. & Arn., or *G. camporum* Greene" (Fr. Ph., 10th Ed.).

The official gum plants are Californian species which have semi-amplexicaul, rigid, dentate, and shiny leaves, with a characteristic underside displaying a network formed by the secondary veins. The inflorescences are heterogamous capitulums of orangy-yellow color, surrounded by an involucre of imbricate and coriaceous bracts; these are armed with curved thorns which are shiny and have scattered small lentils of brown resin.

Gum plant resin chiefly contains diterpenoid acids. Grindelic acid and about twenty other compounds with a labdane skeleton have been characterized in the various species, which also produce sesquiterpenes. Note also the presence of phenolic acids, polyalkynes, flavonols, and saponins with an aglycone of the polyhydroxylated oleanolide type.

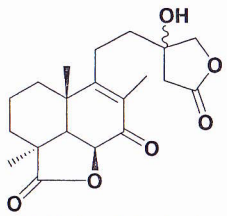
The identification of the drug entails a microscopic examination to show the presence of massive and globulous glandular trichomes, multicellular covering trichomes, rigid elements with sharp teeth on the edges, and echinulate pollen grains. Next is showing the presence of flavonoids (by the cyanidin reaction) and diterpenes (by reacting a petroleum ether extract with acetic anhydride and sulfuric acid). The assay includes a TLC analysis of the flavonoids (with visualization under UV) and the diterpenes (with visualization by vanillin in the presence of sulfuric acid), as well as the quantitation of the residue insoluble in hexane: it must be not less than 6%.

The pharmacology of the diterpenes of gum plants is not well known. The extract (80% ethanol) partially prevents the carrageenan-induced rat paw edema (*per os*, 100-200 mg/kg). The antibacterial and anti-inflammatory activities of the fluid extract of *G. squarrosa* are thought to be due to the phenolics. The drug has a reputation for being an antitussive and a spasmolytic, and is traditionally used to treat cough [French Expl. Note, 1998]. The tincture and the fluid extract are ingredients of different syrups combining white horehound, hedge mustard, niaouli, ipecac, balsam of Tolu, sundew, ethyl-morphine, or codeine, to name only a few.

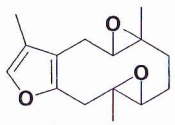


Grindelic acid

Teucrin A



13-Hydroxyballonigrolide



Glechomafuran

C. Diterpene-containing Lamiaceae

Here we shall group various Lamiaceae which are seldom used. They are known to contain diterpenes, but their activity has not been demonstrated. Specifically, there are no data to link the presumed activities to the presence of diterpenes.

• GERMANDER, *Teucrium* spp., Lamiaceae

Several species in this genus used to be folk remedies. One example is the wall germander, *T. chamædrys* L., an herbaceous plant common in France on dry soils, except if they are siliceous. The flowering aerial parts contain triterpenes, flavonoids, an essential oil, and most of all, lactonic diterpenes from the neoclerodane series, e.g., teufline, teucrins A to G, teucvin, teuflidin, and isoteuflidin. A review and interpretation of the published phytochemical data showed that the diterpene composition depends closely on the geographical origin.

The pharmacology of this drug has not been studied. Thus, it is based on tradition alone that the wall germander claims the following indications: to treat the symptoms of mild diarrhea, as an adjunct in weight loss programs, and locally, as a mouthwash for oral hygiene. At the beginning of 1992, French poison control centers reported several cases of acute hepatitis * resulting from the administration of phytopharmaceuticals containing wall germander: the responsible proprietary products have been removed from the market, and compounding or dispensing wall germander preparations is proscribed in France.

The other germander species remained on the list of plants eligible for the abridged application dossier for a French government marketing authorization or *dossier abrégé d'AMM* until the end of 1997, despite the fact that they are not better known than the wall germander **. These species, which no longer appear in the 1998 French Explanatory Note, may claim the following indications:

- *T. polium* L., aerial part: traditionally used to treat the symptoms of digestive ailments and of neurotonic disorders in adults and children, especially for minor sleeplessness.

- cat thyme (*T. marum* L., flowering tops): same indications as for the white horehound (see below).

Comment. It has been demonstrated experimentally that hepatic necrosis can be induced by a germander extract enriched in diterpenes, and that the preliminary administration of cytochrome P450 activators or inhibitors increases or decreases the toxicity: thus, the activation of the furano-neoclerodanes appears to be a prerequisite for the toxic activity. The furan nucleus is directly responsible for the toxicity: teucrin A is hepatotoxic, but the mixture of tetrahydroteucrins (13*R* and 13*S*) is devoid of toxicity. The diterpenes cause rapid and massive cellular death by apoptosis by increasing intracellular calcium levels and by stimulating various

* Similar case reports have been recorded in Canada: Laliberté, L. and Villeneuve, J.-P. (1996). Hepatitis after the Use of Germander, a Herbal Remedy, *Can. Med. Assoc. J.*, **154**, 1689-1692.

** And despite the fact that *T. polium* infusions have been incriminated in one extreme case of liver damage more recently; Mattei, A., Rucay, P., Samuel, D., Feray, C., Reynes, M. and Bismuth, H. (1995). Liver Transplantation for Severe Acute Liver Failure after Herbal Medicine (*Teucrium polium*) Administration. *J. Hepatol.* **22**, 597.

calcium-dependent enzymes. This involvement of the furan nucleus is no surprise, because it is well known to be hepatotoxic, like menthofuran, a product of pulegone metabolism (see p. 547). Similarly, perilla-ketone from *Perilla frutescens* L. (Lamiaceae) is well known for its pulmonary toxicity: it is a 1-[3-furyl]-4-methylpentan-1-one that gets oxidized by cytochrome P450 to a butene dialdehyde which reacts rapidly with biological macromolecules.

• White Horehound, *Marrubium vulgare* L., Lamiaceae

The white horehound is a perennial herb which grows wild in neglected areas. It was official at the end of the nineteenth century, and reappeared in the French Pharmacopoeia in January 1989 (dried flowering tops). It is also described in the latest edition (1990) of the BHP.

The identification of the drug includes a morphological examination (downy stems, leaves irregularly crenate with reticulated veins, compact whorled flowers spaced far apart, calyx with teeth shaped like fish hooks), a microscopic examination (covering trichomes shaped like candelabras), and showing the presence of tannins (positive reaction with $FeCl_3$). TLC analysis shows the presence of different constituents, which can be visualized by vanillin in the presence of sulfuric acid.

The chemical composition of the white horehound is not well known: traces of essential oil, many flavonoids (flavone *O*-glycosides, *C*-glycosides, glucosylated flavone lactate), and diterpenes (marrubiin, the chief labdane-type lactone [0.3-1%], probably occurs as premarrubiin, its furan-type precursor).

The white horehound is not well known pharmacologically. It is thought to be an expectorant and a choleretic. The ethanolic extract is weakly anti-inflammatory in rats and marrubiic acid, in other words the product resulting from the opening of the lactone of marrubiin, is a choleretic, also in rats.

In the absence of objective clinical data, the phytopharmaceuticals based on the white horehound are traditionally used orally, in France, for the symptomatic treatment of cough in case of acute benign bronchial disease [French Expl. Note, 1998]. The German Commission E monograph mentions the choleretic effect of marrubiic acid and lists two indications for this drug: lack of appetite and dyspepsia, and catarrh of the respiratory tract. There are no toxicological data and the occurrence of furan-type diterpenes invites caution in the use of this plant whose health benefits are unclear. In the United States, the absence of demonstrated activity has led the FDA to ban its use in over-the-counter antitussives.

• Black Horehound, *Ballota nigra* L., Lamiaceae

The black horehound is an herb, very common in piles of rubble and vacant lots, and particularly polymorphic: the Flora Europaea lists six subspecies in Europe, two of which are frequently encountered in western Europe: subsp. *nigra* and subsp. *fœtida* (Lam.) Havek. The leaves are limp, tomentose, and smell moldy. They contain

flavonoids, phenylpropanoid glycosides (e.g., verbascoside [= acteoside], forsythoside), and furanoid labdane-type derivatives: the main constituent in commercial samples is 13-hydroxyballoniginolide. Other analyses have characterized, in specimens from various sources, ballotenol, 7-oxomarrubiin (= ballotinone), 7α-acetoxy-marrubiin, and a prefuranoid structure, preleosibirin.

The drug (flowering tops) has a reputation for being an antispasmodic, a calming agent for coughing fits, a sedative, and an anxiolytic. Animal experiments tend to confirm all this and compounds such as verbascoside are known to exert a sedative activity in mice.

According to the 1998 French Explanatory Note, the black horehound may claim the following indications orally: traditionally used to treat the symptoms of neurotonic disorders in adults and children, especially for minor sleeplessness, and traditionally used for the symptomatic treatment of cough. There are no data on toxicity and because black horehound can contain furan-type labdanes, it must be used with caution. In any event, its health benefits are unclear. In the rare cases where side effects were reported, they were described as fatigue. (See note *, p. 659).

• Ground Ivy, *Glechoma hederacea* L., Lamiaceae

Like the above Lamiaceae, ground ivy is official (Fr. Ph., 10th Ed.) and listed in the 1998 French Explanatory Note. The chemistry of this common species with creeping stems and small cordate leaves is not well known. Does the drug contain marrubiin? The assay required by the French Pharmacopoeia implies it, and it also specifies that the drug gives a positive reaction with $FeCl_3$ (tannins). In contrast, the occurrence of flavonoids, sesquiterpenes (glechomafuran or glechomanolide, depending on the chemotype), ursolic acid, and essential oil containing monoterpenoid ketones—in the odoriferous varieties—is well established. A hydroxylated octadecadienoic acid, which activates adenylate cyclase in platelets and inhibits cell migration, has also been isolated. Ground ivy, whose ethanolic extract is weakly anti-inflammatory on the carragenate-induced edema of the rat paw, is seldom used. In France, it may claim (traditional) indications identical to those of the white horehound.

There are no clinical data to confirm the reputation of ground ivy. Nothing is known of its potential toxicity to humans, but there have been case reports of fatal intoxication in animals (e.g., pulmonary emphysema in horses).

• Scutellaria, *Scutellaria* spp., Lamiaceae

There are no scutellaria listed in the French Pharmacopoeia or in the Annex of the French Herbal Remedies, Notice to Applicants for Marketing Authorization (= *avis aux fabricants*) of 1990 (or in the French Explanatory Note of 1998). In Europe, scutellaria are used mostly in the United Kingdom. The dried aerial parts of *S. lateriflora* L., which used to be official in France and in the United States at the

beginning of the twentieth century, are described in the 1983 BHP as sedative and anticonvulsant. In China, the root of *S. baicalensis* Georgi is official (*huangqin*), and is one of the drugs most often used to treat bacterial infections of the respiratory and gastrointestinal tract. It is also used in Japan (*wogon*), often in mixtures. It contains over thirty flavonoids in a wide range of concentrations; many are not substituted on the B ring, and many are substituted at C-2' or C-2',C-6'. The main compound (12-17%) is a flavone, baicalin, the derivative of baicalein (= trihydroxy-5,6,7-flavone) 7-*O*-glucuronide. The chemistry of the other scutellaria species is not well known.

Scutellaria are mentioned here only because they are suspected of hepatotoxicity after several recent case reports—in England, Norway, Australia, and Japan. The genus *Scutellaria* is known to elaborate furan-type diterpenes, but in most of the published case reports, the suspected remedy was a complex mixture, and most often, the plant at fault was not identified with certainty—*S. baicalensis* may be adulterated with several species in the genus. *Teucrium* were even being sold under the name of scutellaria, in the United Kingdom and in the 1980s.

Although these accidents are absolutely exceptional, particularly in Asia, recall that in the case of *S. lateriflora,* there are no experimental data to substantiate the properties that are claimed or the therapeutic use.

5. DITERPENE-CONTAINING DRUGS OF POTENTIAL INTEREST

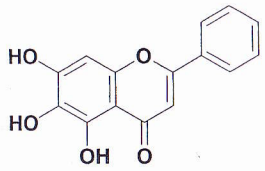
• COLEUS, *Plectranthus barbatus* Andr. (*Coleus forskohlii*), Lamiaceae

This *Plectranthus* species grows wild in the warm and subtropical temperate areas of India, Burma, and Thailand. A perennial herb with fleshy fibrous roots, it is cultivated in several states of India for use as a condiment. It contains diterpenes whose basic skeleton is 11-oxo-manoyl oxide (8,13-epoxy-labd-14-en-11-one). The chief constituent is forskolin, initially isolated under the name coleonol. This compound has a positive inotropic action on the myocardium, and by decreasing peripheral vascular resistance, it exerts an antihypertensive activity. The inotropic action results from the stimulation of adenylate cyclase, an increase in cAMP, the activation of cytoplasmic protein kinases, the decrease of the Na-K-ATPase activity at the membrane level, and from the activation of the slow calcium channels. Preliminary studies in humans have shown that forskolin indeed increases the contractility of the myocardium without increasing oxygen consumption, and that it is a vasodilator (it decreases peripheral resistance). Furthermore, forskolin possesses bronchodilating properties which could be of interest to treat asthma. It also causes a substantial and lasting decrease in intra-ocular pressure. Structural analogs have been synthesized and are undergoing pharmacological and even clinical studies (e.g., NKH 477). While awaiting complementary studies, forskolin remains a useful tool for the biochemical study of adenylate cyclase and of the

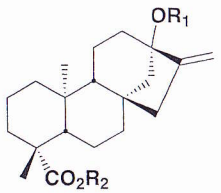
• *Stevia rebaudiana* Bertoni, Asteraceae

This perennial herb has oblong and crenulate leaves, tastes sweet, and is indigenous to the high altitude regions of Brazil and Paraguay; it is cultivated in many areas of the world, including Brazil, Israel, and Asia (Japan, Korea, China).

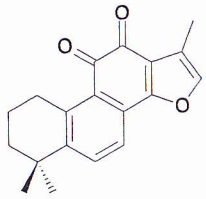
The leaves contain a series of glycosides formed from an *ent*-kaurenoid alcohol: steviol (consider stevioside *, rebaudiosides, and dulcoside). The glycoside can represent up to 10% of the weight of the leaf. It can be extracted with water, re-extracted with butanol, and it is generally purified by filtration (charcoal) and crystallization. Stevioside as a sweetener is approximately 200 times more potent than sucrose; it seems devoid of toxicity. A weak antihormonal activity had been reported, but could not be found again in later experiments. Some questions remain: there are indications of a mutagenic activity of certain metabolites of steviol formed *in vitro* by human hepatic microsomes, therefore metabolic studies on this compound must be pursued. Currently used in Japan, stevioside is also marketed in Brazil, Paraguay, and other countries. It can be combined with glycyrrhizin. In the United States, it does not have the "Generally Recognized As Safe" status and has not been approved by the FDA (as an additive, although it has been approved as a dietary supplement). It is not on the list of additives authorized by the European Union either.



Baicalein



R_1 = *glc(1–>2) glc,*
R_2 = *glc : Stevioside*



Tanshinone II

• *Salvia miltiorrhiza* Bunge, Dan shen (China), Tan-jin (Japan), Lamiaceae

The roots of this herbaceous plant, which bears purple or violet flowers grouped into spikes, are a traditional remedy in eastern medicine. They have a reputation for

* Interestingly, this type of structure—which has only been isolated from two out of about one hundred species of *Stevia* investigated—has been found in a species of *Rubus* (*R. suavissimus* S. Lee = *R. chingii* Hu, Rosaceae). This plant, whose leaves contain over 5% of a steviol glycoside, is traditionally used in China (Guangxi) to prepare "sweet" beverages; see Ohtani, K., Aikawa, Y., Kasai, R., Chou, W.-H., Yamasaki, K. and Tanaka, O. (1992). Minor Diterpene Glycosides from Sweet Leaves of *Rubus suavissimus*. *Phytochemistry*, 31, 1553-1559.

being a sedative, bactericide, cardiac stimulant (stasis, edema), and are used, among other things, to treat certain cardiac disorders.

Their composition is well known, or at least that of the pigments which impart their reddish-brown color to the subterranean parts. These pigments are diterpenoid lactones with an abietane skeleton. Orthoquinones (tanshinones I and II-A, B, V, VI and derivatives, cryptotanshinone, miltirone, miltionones) and paraquinones (isotanshinones, danshenxinkun A-C) occur alongside lactone derivatives. Phenolics have also been isolated: magnesium and either ammonium or potassium lithospermates B (a rosmarinic acid dimer), salvianolic and rosmarinic acids, and a benzofuranoid derivative.

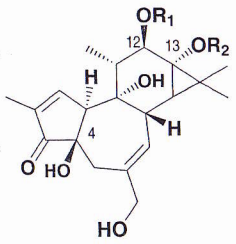
Dan-shen quinones are antioxidants, and many of them are bacteriostatics and active against various dermatophytes. Tanshinones I and VI and cryptotanshinone prevent the complications of myocardial ischemia (on the isolated rat's heart). In China, tanshinone II-A, solubilized by sulfonation, has undergone successful clinical trials for the treatment of angina pectoris. Experiments in normal and uremic animals indicate that the drug extracts may improve renal function (decrease in uremia, and increase in the speed of glomerular filtration, and of excretion of urea and creatinine). This activity appears to be due to lithospermate B and to involve prostaglandins. The same pure compound, tested on the rabbit heart after ischemia-reperfusion, is efficacious in reducing tissue damage.

6. DITERPENE-CONTAINING TOXIC PLANTS: ESTERS OF PHORBOL AND OF INGENOL AND RELATED COMPOUNDS

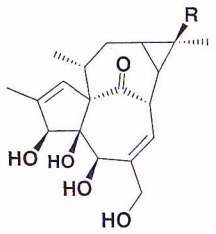
Several plant species owe their toxicity to diterpenoid esters of complex structure, of the tigliane, ingenane, or daphnane type. The distribution of these compounds is restricted to two families, the Euphorbiaceae and Thymelæaceae.

The concentrations are generally low and the composition is always very complex: the seeds of croton contain 25 phorbol esters and some 4-deoxy-4α-phorbol (R_1, R_2 = acetate, tiglate, butyrate, octanoate, decanoate, dodecanoate, and so on, see formulas p. 655). The same complexity is observed with the derivatives based on other skeletons (e.g., mono-, di-, and triesters of ingenol; orthoesters of fatty acids and of polyhydroxylated daphnanes [resiniferonol and derivatives: huratoxin and related compounds]).

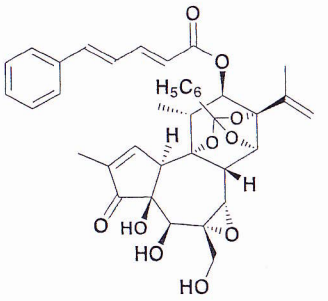
Almost all of these compounds are toxic: they are drastic cathartics, and induce, by contact with the skin or with the mucosal membranes, an intense inflammatory reaction; they are also co-carcinogens. The compound most studied, and with the strongest activity, is 12-*O*-tetradecanoylphorbol-13-acetate (= TPA), one of the most potent known inducers of skin tumors in mice. In addition, these esters have a large number of cellular and biochemical effects which make them interesting investigational tools in biology and pharmacology: among other activities, TPA activates protein kinase C by taking the place of the endogenous diacylglycerol.



Phorbol esters
R_1 = *tetradecanoate,*
R_2 = *acetate* : TPA



Ingenol and 17-hydroxy-ingenol



Mezerein

• THYMELÆACEAE

This small heterogeneous family of about fifty genera is represented in western Europe by a dozen species from two genera: *Thymelæa* and *Daphne*.

The *Daphne* indigenous to western Europe are shrubs or subshrubs, and some are cultivated for their ornamental qualities. The seeds and bark of the various species contain toxic diterpenes.

• *D. laureola* L. Known as spurge laurel, this species is a subshrub with flexible stems, common in the woods. The leaves are indeciduous and coriaceous, and have a blade which is shiny on the upper side and dull on the underside. The greenish flowers are grouped into small racemes. The fruits - green drupes which turn black - are inserted in the middle of the rosette of leaves.

• *D. mezereum* L. A bushy subshrub of 0.5 to 1.5 m, the February daphne or *mezereon* becomes covered with very fragrant pink flowers at the beginning of the spring. The leaves, which appear later on, are lanceolate, entire, limp, deciduous, and grouped at the end of the branches. The fruits are scarlet drupes with a green seed which turns brown, and they are inserted under the rosette of leaves. This species grows in the woods and is common in the east of France and in the nearby mountains.

• Other species, of more limited distribution, are also toxic: *D. gnidium* L. (the spurge flax or in French *garou*) is a bush indigenous to the French Atlantic coast and to the Mediterranean area, with erect stems and white flowers; *D. cneorum* L. has prostrate stems and fragrant pink flowers, and is specific to mountain wood skirts.

The toxic substances are daphnetoxin (bark) and mezerein (seeds). The barks, upon contact with the skin or mucosal membranes, cause substantial irritation *. The

* Some authors report that in former times, beggars resorted to these plants to induce redness and pustules to invite pity! *Daphne* intoxications were common back when spurge flax was used for medicinal purposes [as a revulsant (!) or to induce fixation abscesses], but have now become exceptional, at least in the medical and toxicological literature: apparently they are not uncommon in children of the French region of Franche-Comté (J. Vaquette, personal

ingestion of the fruits (the part most often at fault) precipitates an ulceration of the mucosas of the digestive tract. The symptoms include violent digestive spasms, hypersalivation, vomiting, hoarseness, swallowing difficulties, diarrheas, headaches, nausea, and neurological symptoms (convulsions).

The treatment is essentially symptomatic. The toxin is removed, the inflammation of the mucosas is treated (with astringents), and the neurological symptoms are treated (with barbiturates).

• EUPHORBIACEAE

Toxic diterpenes are found in at least 14 of the 300 genera in this family, including *Aleurites, Croton* * (Crotonoideae), *Excoecaria, Euphorbia, Hippomane, Hura, Jatropha*, and *Sapium* (Euphorbioideae), to name only the best known species.

The concentrations of toxic compounds are normally very low (0.05-0.1%). All of them are irritants for the skin and the mucosal membranes, and have substantial toxicity when taken orally by animals, and by humans (tung oil, an industrial oil from *Aleurites* **, is very harmful). Some authors hold these diterpenes responsible for the high frequency of certain cancers in the Caribbean populations who consume infusions of the leaves of *C. flavens* L. on a regular basis. Their ability to activate viruses involved in carcinogenesis has also been shown (Epstein-Barr virus).

Toxic spurges

In western Europe, spurges are herbs with leaves reduced to little or nothing, and with pseudoflowers (cyathus). They secrete an irritating milky latex and are known for their drastic purgative properties (e.g., mole plant, *Euphorbia lathyris* L.), their toxicity to fish (e.g., *E. helioscopia* L.), their aggressive action on the skin (wart weed), and their ability to induce serious conjunctivitis (e.g., *E. esula* L.). In fact, the toxic diterpenoid esters are found in most of the *Euphorbia* that are indigenous to western Europe, including the most common species, such as *E. peplus* L.

Accidents due to contact with the skin are the most frequent, especially for gardeners: redness, swelling, and the formation of vesicles are the main symptoms.

* "Croton Oil" was formerly used. It was obtained from *Croton tiglium* L., not to be confused with the house plant, *Codiaeum variegatum* (L.) Blume, var. pictum Muell. Arg., which is known to cause occupational contact eczema on rare occasions.

** *Aleurites* are Asian species whose seeds produce drying oils: tung oil or Chinese wood oil (*A. fordii* Hemsl., *A. montana* Lour.). An accident due to mistaking Aleurites fruits for edible chestnuts was recently described. See Lin, T.J., Hsu, C.I., Lee, K.H., Shiu, L.L. and Deng J.F. (1996). Two Outbreaks of Acute Tung Nut (*Aleurites fordii*) Poisoning, *J. Toxicol. – Clin.*

If the latex ever gets into an eye, immediate and profuse rinsing is necessary, and medical attention must be sought (topical antibiotic therapy, anti-inflammatory), even though, as a general rule, the symptoms resolve over a few days.

Ornamental cactiform spurges are also a serious hazard (e.g., candelabra cactus, *E. lactea* Haw.). In contrast, it is clearly established that the colorful bracts of poinsettia (*Euphorbia pulcherrima* Willd. ex Klotzsch) present no particular danger. In the Caribbean Islands, the manchineel tree poses a problem (*Hippomane mancinella* L.): skin contact with all parts of the tree causes severe vesicular dermatitis, in some cases purpuric; fruit ingestion induces hemorrhagic labial erosion and edema of the pharynx, sometimes to the point of making tracheotomy necessary.

• SPECIAL CASE: TOXIC HONEYS

Several case reports of intoxication have been recorded in Turkish hospitals following the ingestion of honey made by bees gone honey-gathering on *Rhododendron*. The toxicity of *Rhododendron*—like that of other Ericaceae (*Pieris, Kalmia*)—can also, under exceptional circumstances, affect animals (e.g., lack of food during the snowy season in the mountains, zoo animals), or, very rarely, children. The toxic principle in these species is grayanotoxin I (= acetyl-andromedol), which causes nausea, vomiting, hypotension (50-60 mm Hg), bradycardia (25-50 beats/min) and perturbations of the cardiac rhythm, tingling in the extremities, extreme fatigue, dizziness, and loss of consciousness.

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