Coumarins

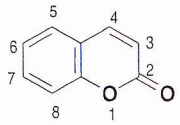
1. INTRODUCTION

Coumarins owe their class name to "coumarou", the vernacular name of the tonka bean (*Dipteryx odorata* Willd., Fabaceae), from which coumarin itself was isolated in

approximation, to be the lactones of the 2-hydroxy-*Z*-cinnamic acids. Over one thousand coumarins have been described, and the simplest among them are widely distributed in all of the vegetable kingdom. Certain families among Angiosperms produce a wide range of structures: Fabaceae, Asteraceae, and especially Apiaceae and Rutaceae, in which the most complex molecules occur.

2. CHEMICAL STRUCTURE AND CLASSIFICATION

Except for a few rare cases, including coumarin *per se*, all coumarins are substituted by a hydroxyl group in position 7. Umbelliferone, which is 7-hydroxy-coumarin itself, is the precursor of the 6,7-di- and 6,7,8-trihydroxylated coumarins. The hydroxyl groups of these simple coumarins are either methylated, or oftentimes one of them is engaged in a glycosidic linkage. Particularly common are skimmin (7-*O*-glucosyl-umbelliferone), aesculin and cichoriin (6- and 7-*O*-glucosyl-aesculetin, respectively), scopolin and fraxin (glycosides of scopoletin and fraxetin). One



Coumarin

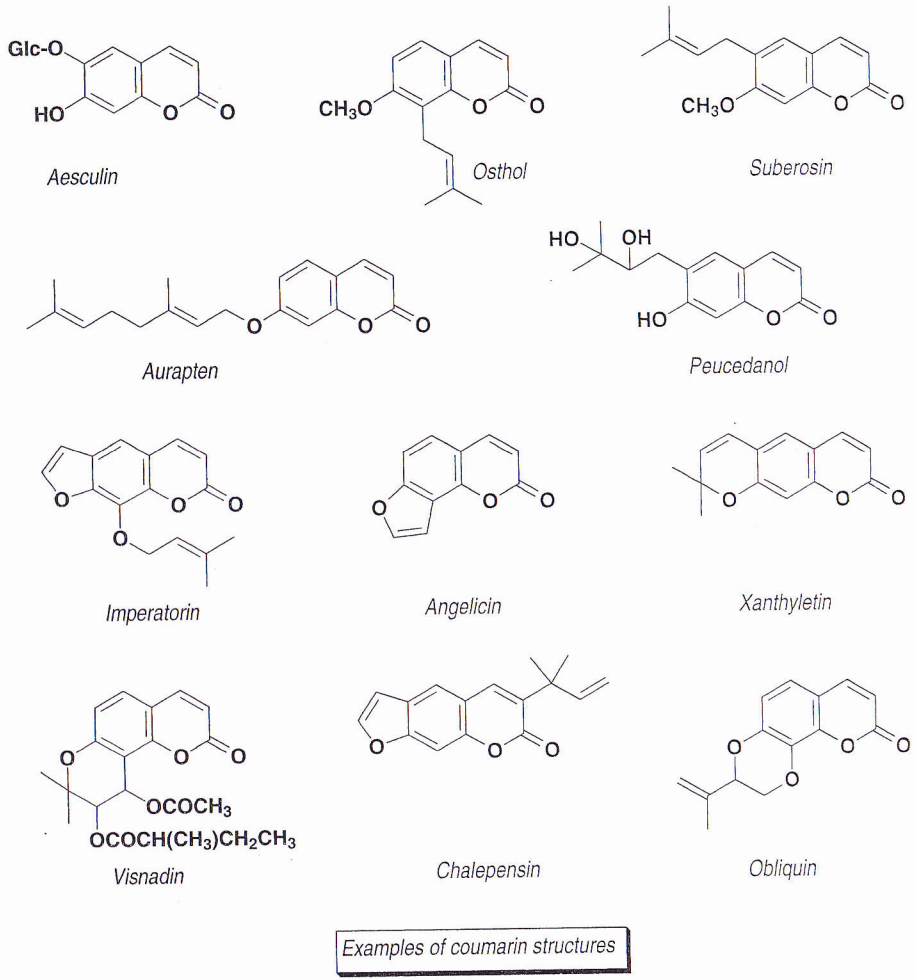
$R_1 = R_3 = H$, $R_2 = OH$; *Umbelliferone*
$R_1 = R_3 = H$, $R_2 = OCH_3$; *Herniarin*
$R_1 = R_2 = OH$, $R_3 = H$; *Aesculetin*
$R_1 = OCH_3$, $R_2 = OH$, $R_3 = H$; *Scopoletin*
$R_1 = OCH_3$, $R_2 = R_3 = OH$; *Fraxetin*

structural feature which is common to many coumarins is prenylation: *O*-prenylation or, more frequently, prenylation on the ring in the 6- or 8-position of umbelliferone or of herniarin (aurapten, suberosin, osthol). In more exceptional cases, there may be a five-carbon residue at C-3. The high reactivity of the isoprene chain (C_5, C_{10}, or less commonly, C_{15}) explains the large number of derived structures (epoxidized, mono- and dihydroxylated, cyclized, e.g., swietenol, peucedanin). Prenylation is also the origin of polycyclic coumarins, furano- and pyrano-coumarins, linear (e.g., psoralen, imperatorin, xanthyletin, chalepensin), and angular (e.g., angelicin, visnadin) coumarins. In a few cases, a benzodioxane cycle may be formed (e.g., obtusin; see also, in the next chapter, coumarinolignans).

3. BIOSYNTHESIS

A. Origin of Simple Coumarins

Like other phenylpropanoids, coumarins arise from the metabolism of phenylalanine *via* a cinnamic acid, *p*-coumaric acid. The specificity of the process resides in the 2-hydroxylation (i.e., in the *ortho* position relative to the 3-carbon chain); next comes the photocatalyzed isomerization of the double bond (*E* → *Z*)



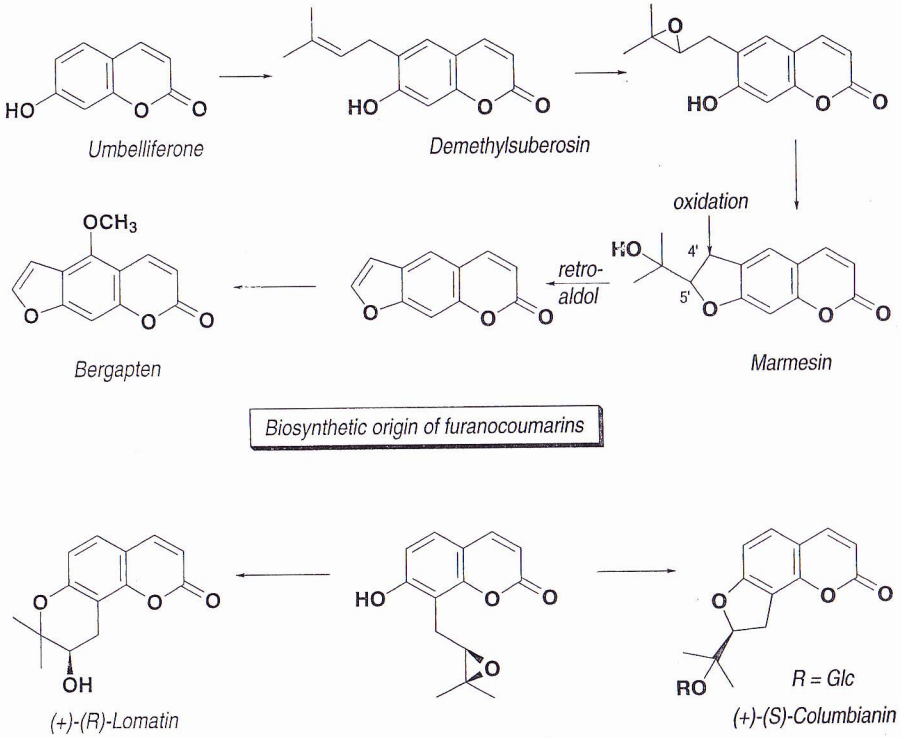
Examples of coumarin structures

acid occurs, precluding lactonization. In such cases, coumarin only arises after tissue injury and enzymatic hydrolysis (see below the example of melilotoside which yields coumarin upon hydrolysis). The formation of di- and trihydroxy-coumarins and of their ethers involves the hydroxylation of umbelliferone rather than the lactonization of the corresponding cinnamic acids (for example caffeic or sinapic acids).

B. Formation of Furano- and Pyranocoumarins

Systematic tracer experiments demonstrated that prenylation of the benzene ring by *dimethylallyl pyrophosphate* (= DMAPP) in the 6- or 8-position of a 7-hydroxy-coumarin is the origin of the extra ring which characterizes these molecules. Prenylation at C-6 yields the so-called "linear" furano- and pyranocoumarins; at C-8

The cyclization of 6- or 8-isoprenylcoumarin is probably due to nucleophilic attack by the hydroxyl group in the 7-position on the epoxide formed by oxidation of the double bond of the isopentenyl residue. The product of this reaction depends on the orientation of the nucleophilic attack: it is either a hydroxyisopropyl-dihydrofuranocoumarin, or, in the case of an attack on the tertiary carbon, a hydroxydimethyldihydropyranocoumarin. The occurrence, within one Apiaceae species, of (+)-(*R*)-lomatin and (+)-(*S*)-columbianin supports this hypothesis (see figure below).



The proposed mechanism for the formation of furanocoumarins includes two successive steps: stereospecific oxidation at C-4' and elimination of the hydroxyisopropyl residue at C-5' by retroaldol condensation.

Substitution at C-5 or C-8 or in both positions of furanocoumarins occurs later and is catalyzed by oxidases and *O*-methyltransferases.

4. PROPERTIES, EXTRACTION, AND CHARACTERIZATION

Coumarins in the free state are soluble in alcohols and in organic solvents such

to take advantage of the properties specific to the lactone: opening and solubilization in alkaline conditions, closing in acidic medium. It is also possible, in some cases, to use sublimation. However, the applicability of these two procedures is limited by the risk of inducing alterations of the original structure. The risk of artefact formation also exists with chromatographic techniques on classic stationary phases (silica gel), mainly for acylated coumarins; gel fractionation then becomes of interest for compounds in the free state as well as glycosides. Semipreparative HPLC is widely used (on normal and reverse phase).

Coumarins have a characteristic UV spectrum which is heavily influenced by the nature and the position of substituents, and by alkalinization (KOH, $NaOCH_3$). When examined under UV light, TLC spots from coumarin-containing drugs have colors which are enhanced in the presence of ammonia, and range from blue to yellow and purple. For a quantitative estimate of these compounds within a drug, it is possible to use a spectrofluorometric technique (after elution of the TLC spots), or, more simply, HPLC.

5. PHARMACOLOGICAL PROPERTIES AND USES

The pharmacological interest of coumarin-containing drugs is limited. Aesculin is said to be a venous tonic and a vascular protective agent. Sweet clover extract is used for the symptomatic treatment of venous and lymphatic vessel insufficiency. Some furanocoumarins are photosensitizers, therefore they are indicated for the therapy of psoriasis and vitiligo. Visnadin, a pyranocoumarin isolated from khella, has been extracted and marketed for its coronary vasodilator effect and promoted as having a favorable action on senile cerebral insufficiency. Coumarin is known for its antiedema properties and has undergone clinical trials in patients with advanced cancer: it is an immunostimulant with a cytotoxic activity. It is rapidly metabolized in the liver to 7-hydroxycoumarin, and it can, in rare cases, induce severe hepatonecrosis. A small number of drugs used in phytotherapy contain coumarins: mouse-ear, angelica, ash*, sweet woodruff... but that the relationship between the presence of coumarins in these drugs and the activity attributed to them by folk medicine has not always been established.

* We cannot exclude that the anti-inflammatory and analgesic properties attributed to the ash tree are due to coumarins (inhibition of lipoxygenase and cyclooxygenase metabolism?). The leaf also contains an iridoid, excelsioside. The dried leaf—from *Fraxinus excelsior* L. or *F. oxyphylla* M. Bieb., not less than 2.5% total hydroxycinnamic derivatives (Fr. Ph., 10th Ed.)—is traditionally used to enhance the urinary and digestive elimination functions, to facilitate the renal elimination of water, as an adjunct in weight loss programs, and for the symptomatic treatment of minor pains in the joints [French Expl. Note, 1998]. The German Commission E does not authorize the use of this drug because its usefulness has not been adequately substantiated; nevertheless, there is no known risk and its use in mixtures is not formally prohibited.

MELILOTUS OFFICINALIS L.

6. CHIEF COUMARIN-CONTAINING DRUGS

COUMARIN

Coumarin itself was marketed in France until late 1996. It was indicated to treat the lymphedema of the arm subsequent to breast cancer radiotherapy and surgery (90-135 mg/day). The large number of hepatitis cases attributable to coumarin (2-4 cases per 10,000 in France) led to the removal from the market of coumarin-containing pharmaceuticals. Identical cases (including one death) were reported in Switzerland and in Australia, always with high doses. Coumarin can still be found, but at low doses, in medicines containing sweet clover (see below).

The French government regulations, taking into account the 1988 European directive, specify that the residual coumarin content in foods and beverages must be not more than 2 mg/kg. In the particular case of taffy, caramel-containing confectionery products, and alcoholic beverages, the limit is raised to 10 mg/kg. It is 50 mg/kg for chewing gums.

• COMMON HORSE CHESTNUT, (bark); Aesculin, Aesculetin, 4-Methylaesculetin

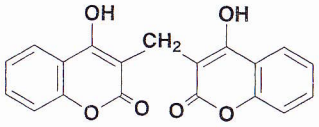
Aesculin is found in the bark of the common horse chestnut tree (*Æsculus hippocastanum* L., see saponin-containing drugs), but it can also be obtained from other plant species. Considered a vascular protective agent, this glycoside, as well as aesculetin and its methylated derivative, both synthetic, are ingredients of proprietary drugs which, depending on their formulation (combinations with flavonoids, ruscus extracts, local anesthetics, tocopherol, ascorbic acid) are promoted as a treatment for the symptoms of venous and lymphatic vessel insufficiency (functional symptoms, edema), or the functional symptoms of the acute attack of piles, or both. Phytotherapeutic products containing the bark of the stem of common horse chestnut tree are traditionally used, orally and topically, to treat the functional symptoms of cutaneous capillary fragility (ecchymosis, petechiae); for the subjective symptoms of venous insufficiency (fullness in the legs); and for hemorrhoidal symptoms [French Expl. Note, 1998].

• SWEET CLOVER, *Melilotus officinalis* (L.) Pallas, Fabaceae

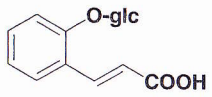
Sweet clover flowering tops are official (Fr. Ph., 10th Ed.), used in folk medicine, and used to prepare extracts prescribed for minor venous circulatory disorders.

The Drug: Origin and Composition. Sweet clover is a small plant with green grooved stems, trifoliolate leaves with 2 lanceolate stipules, flowers with yellow

and neglected fields in almost all of Europe, it is known to contain saponins with a pentacyclic triterpenoid aglycone, flavonoids, and about 15 phenolic acids. All species of *Melilotus* contain, especially in the young leaves, melilotoside, the glucoside of 2-hydroxycinnamic acid, which, following facile hydrolysis, lactonizes to coumarin. In the event of fungal contamination, 2-hydroxycinnamic acid may be metabolized to form an anticoagulant, dicoumarol.



Dicoumarol



Melilotoside

Tests. The French official drug is identified by its macro- and microscopic characteristics, particularly by the presence, in the cuts and in the powder, of bicellular echinulate covering trichomes with a right angle bend. Coumarin is identified by a color reaction (coupling with 4-diazoniobenzenesulfonate), and its presence shown alongside that of 2-hydroxycinnamic acid by TLC of a methanolic extract.

Properties and Uses. Animal experiments have shown the antiedemic properties of sweet clover extract, which also increases venous and lymphatic flow rates, and decreases capillary permeability. Coumarin, which is not an anticoagulant, stimulates the reticulo-endothelial system and the proteolytic power of macrophages.

Sweet clover extract, in combination with rutin (5 mg of coumarin for 1 g of fluid extract [or 30 mg of dry extract]), and based on placebo-controlled clinical trials, is indicated for the treatment of the symptoms of venous and lymphatic vessel insufficiency (fullness in the legs, pains, restless legs syndrome) and to treat the functional symptoms of the acute attack of piles. Other combinations (butcher's broom, hesperidin-methylchalcone) are also indicated for metrorrhagias due to contraception by minipills or linked to intra-uterine contraceptive devices, following suitable clinical and other tests. In France, phytopharmaceuticals based on sweet clover are traditionally used, orally and topically, to treat the symptoms of cutaneous capillary fragility (ecchymosis, petechiae); for the subjective symptoms of venous insufficiency (fullness in the legs); and for hemorrhoidal symptoms. They are used orally to treat gastrointestinal disturbances (epigastric bloating, impaired digestion, eructations, flatulence); as an adjunctive therapy for the painful component of functional gastrointestinal disturbances; and in the symptomatic treatment of neurotonic disorders in the adult and in the child, for example for minor sleeplessness. The 1998 French Explanatory Note specifies that it can be used topically for eye irritation or discomfort of various etiologies (for example eye strain, smoky atmospheres).

The German Commission E monograph lists internal uses limited to those that are justified by the activity on venous and lymphatic vessels, including itching, fullness in the legs, nocturnal cramps, edema, post-thrombotic [illegible]

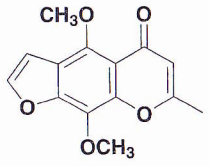
congestion, and hemorrhoids. The drug and its preparations are also used topically for contusions, sprains, or superficial bleeding (its wound-healing activity has been shown in animal experiments). Recall that the coumarin-type anticoagulants currently synthesized were designed with dicoumarol as a model—an anticoagulant arising from coumarin upon fungal contamination of sweet clover. Dicoumarol is responsible for cattle intoxications *.

• KHELLA, *Ammi visnaga* Lam., Apiaceae

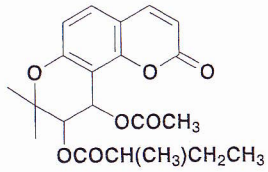
Khella is an annual Mediterranean herb which grows wild from Morocco to the Near East, and has bi- or tripinnatisect leaves with linear segments and white flowers grouped in large compound umbels. The drug (official in Germany, DAB 10) consists of the fruits which are tiny mericarps, flattened, generally separate, and very bitter.

The chief constituents of the drug that have been identified are furanochromones (2-4%), including khellin (0.3-1.2%), visnagin (0.05-0.3%), khellol, and khellinol, and angular pyrano-coumarins (0.2-0.5%), including visnadin, samidin, and dihydrosamidin. The drug also contains lipids (up to 18%), furanoacetophenones, flavonoids (flavonols and flavonol sulfates), and 0.2-0.3 mL/kg essential oil.

Khellin is a spasmolytic agent. Visnadin is a coronary vasodilator and a positive inotropic, bradycardic, and spasmolytic agent, probably as a result of its calcium blocking activity, shown *in vitro*. Khellin, like carbocromen (INN), a synthetic coumarin that remains available in several European countries, but not in France, has been used in the preventive therapy of *angina pectoris*. It continues to be used as a coronary vasodilator in Germany, where khella is seldom used in infusion, but where khellin and extracts titrated for khellin are frequently used in the composition of spasmolytic medicines.



Khellin



Visnadin

The fruits of another *Ammi* species, *A. majus* L., are an industrial source of furanocoumarins, mainly of xanthotoxin (= ammoidin = 8-*methoxypsoralen* = 8-

* All around the Mediterranean, sheep herds are often poisoned by another species rich in coumarins, the giant fennel (*Ferula communis* L., Apiaceae). The anticoagulant prenylated 4-hydroxycoumarins contained in this plant are responsible for the symptoms that are observed, including hemorrhagic diarrhea, hematuria, and internal hemorrhage. See Tligui, N. and Ruth, G.R. (1994). *Ferula communis* Variety *brevifolia* Intoxication of Sheep. *Am. J. Vet. Res.*, 55,

MOP = methoxsalen). The combination treatment regimen of psoralen (P) and UV radiation of 320-400 nm wavelength, commonly referred to as UVA, is known by the acronym PUVA (see below).

- **Mouse-Ear,**
  Hieracium pilosella L., Asteraceae

Mouse-ear, also called hawkweed, is a small ground cover-type plant seen in dry lawns, with a rosette of oval leaves (1-12 x 0.5-2 cm) covered with long silky white hairs, with a solitary hairy flowering stem, and a sulfur-yellow capitulum with an involucre covered with blackish glandular trichomes. The entire plant is used (Fr, Ph., 10th Ed.), and contains umbelliferone (in glycosidic form), ortho-dihydroxy-cinnamic derivatives (not less than 2.5%), and flavonoids, as well as inulin in the roots. Some rather old studies attribute to umbelliferone the bacteriostatic activity of the drug, which used to be applied to the treatment of brucellosis in veterinary medicine. In the absence of pharmacological or clinical data, the drug is traditionally used to enhance urinary and digestive elimination functions and to enhance the renal excretion of water [French Expl. Note, 1998].

- **Angelica,**
  Angelica archangelica L., Apiaceae

The drug (Fr. Ph., 10th Ed.) consists of the dried root stock of this biennial species characteristic of damp locales, and cultivated, among other things, for its petioles, used in confectionery, and its fruits, a liquor starting material. The drug contains up to 6 mL/kg of an essential oil rich in hydrocarbons (β- and α-phellandrenes, a-pinene, Δ^3-carene, sesquiterpenoid hydrocarbons) and is characterized by macrocyclic lactones (15-pentadecanolide, 13-tridecanolide) (Fr. Ph.: >2 mL/kg; lateral roots 2.5 times richer than main root). It also contains numerous coumarins: simple, furanoid and hydroxyisopropylfuranoid, linear and angular (e.g., osthol, bergapten, xanthotoxin, angelicin, archangelicin). Apparently there have been no studies on the pharmacology of the drug; the essential oil has a spasmolytic effect on isolated organs. Based on tradition [French Expl. Note, 1998], angelica root stock and fruits are used orally to treat the symptoms of gastrointestinal disturbances (bloating, impaired digestion, eructations, flatulence), and as an adjunct in the treatment of the painful component of gastrointestinal distress. The German Commission E monograph lists the lack of appetite in addition to the same types of indications as in France, but only for the root (the fruit activity is considered to be insufficiently demonstrated). Patients are advised to avoid prolonged exposure to sunlight or to UV irradiation during the treatment (furanocoumarins can induce photodermatitis).

Angelicas are widely used in Asia. In China, the dried root of *A. dahurica* (Fisch. ex Hoffm.) Benth. & Hook. f. (*baizi*) is reputed to [illegible]

analgesic (headaches, toothaches) and *A. sinensis* (Oliv.) Diels (*danggui*) has similar uses. In Japan, it is *A. acutiloba* (Sieb. & Zucc.) Kitag. that is mostly used.

- **Sweet Woodruff,**
  Galium odoratum (L.) Scop., Rubiaceae

Sweet woodruff (also called *Asperula odorata* L.) has characteristic greenish quadrangular stems bearing, at each node, two acuminate and shiny leaves (2-4 x 0,5-1 cm), with edges rough to the touch, and with four to six stipules identical to the leaves. The flowers (0.3 cm) are yellowish-white and have a bell-shaped corolla with four lobes. The dried drug contains approximately 1% coumarin and an iridoid: asperuloside. The French Pharmacopoeia identifies the drug by its microscopic characteristics and by TLC analysis of a methanolic extract (coumarin identification). The aerial parts of sweet woodruff are traditionally used to treat neurotonic symptoms (minor sleep disturbances), and also to treat gastrointestinal disorders (like angelica, see French Expl. Note, 1998). The German Commission E does not authorize the use of this drug because its usefulness has not been adequately demonstrated; however, there is no known risk.

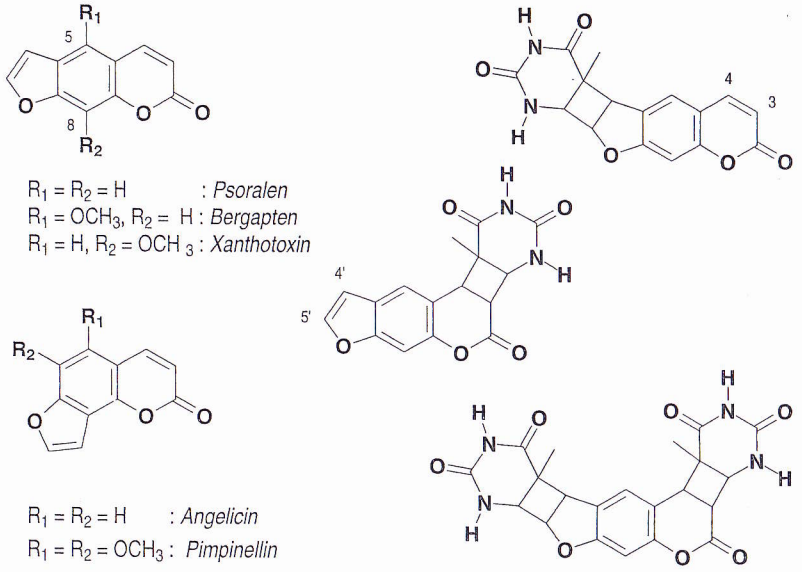
- **Tonka Bean,**
  Dipteryx odorata (Aublet) Willd., Fabaceae

Cultivated in Venezuela, this tree is used for its seed or bean which contains 1 to 3% coumarin. These seeds, as well as those of another *Dipteryx* species of Brazilian origin, are used to flavor tobacco. The coumarin used in perfumery is in large part synthetic. Coumarin is also found in substantial quantities in various Poaceae (for example in vernal grass, *Anthoxanthum odoratum* L.).

7. FURANOCOUMARINS AND PHOTOTOXICITY

It has long been known that various plant species from different parts of the world are capable of causing a transient cutaneous hyperpigmentation. It is said that the juice of *Ammi visnaga* L. (Apiaceae) was used by the Egyptians to delete the signs of vitiligo and that ayurvedic medicine used, to the same end, the seeds of a *Psoralea* (Fabaceae). It is also known that these and other species can cause acute dermatitis, sometimes with vesicles coalescing into large blisters. In many cases, a hyperpigmentation follows which may last a long time (berlock dermatitis). In the absence of specific treatment, relieving the symptoms is in order (cold compresses, possible corticosteroids). These cutaneous accidents reflect phototoxicity: they always occur after contact with the plant or product followed by exposure to sunlight, and they are enhanced by humidity.

The phototoxic constituents, common to all these species, are linear furanocoumarins: psoralen, bergapten (= 5-*methoxypsoralen* = 5-MOP), and xanthotoxin (= xanthotoxol = 8-MOP); angular furanocoumarins are only weakly toxic (pimpinellin, angelicin). It has been shown that these furanocoumarins can undergo cycloadditions at C-3, C-4 or at C-4', C-5', or both with the pyrimidine bases of DNA. Furanocoumarins may each be involved in one or two cycloadditions, and in the latter case, they can form cross-links between the base pairs of the nucleic acids and induce lesions to the genome. It is possible that these properties have something to do with phototoxicity, the mechanism of which remains to be elucidated: they mainly explain the mutagenic and carcinogenic properties.



A. Chief Species Incriminated

The plants involved all belong, as far as European species are concerned, and except for the fig tree, *Ficus carica* L., Moraceae, either to the Apiaceae family, or to the Rutaceae family.

Most often, they are cultivated species, and the reported dermatitis is observed in farmers or in employees of the industries processing the plants. The following are considered phototoxic by contact: angelica, celery, parsley, lovage, and the numerous *Citrus* species. Other species are at times at fault, either because they are found in our natural environment (such is the case for hogweed, *Heracleum sphondylium* L., for the garden parsnip, *Pastinaca sativa* L., or for the rue plant, *Ruta graveolens* L.), or because they are cultivated as ornamental plants, such as the horticultural varieties of fraxinella (the biblical burning-bush, *Dictamnus albus* L.) or the giant hogweed (*Heracleum mantegazzianum* Sommier & Levier). Of course, similar risks of phototoxicity exist with dermatology, perfumery, or cosmetic products whose formulation includes furanocoumarins or essential oils of *Citrus*

(particularly bergamot). The risks of phototoxicity after ingestion are very limited: although such accidents are rare, celery can cause phototoxic manifestations, particularly in PUVA treatment patients.

B. Other Toxic Coumarins

Some coumarins synthesized by lower Fungi are toxic; for example aflatoxins are carcinogenic. These polycyclic toxins, which arise biosynthetically from a deca-acetate, are produced by various strains of *Aspergillus* which develop, under the right conditions of temperature and humidity, during storage of plant materials, especially peanuts. Their absence must be carefully verified in animal feed (cattle cake) and human food (oil, milk, butter).

Since this topic exceeds the scope of this text, the interested reader may refer to the numerous articles and monographs published on mycotoxins.

8. FURANOCOUMARIN APPLICATIONS

A. Medical Applications

The photodynamic sensitizing properties of bergapten and methoxsalen are applied during PUVA treatment, or photochemotherapy of psoriasis and other dermatological disorders. The technique consists of the administration, generally by the oral route, of the furanocoumarin (0.6 mg/kg of 8-MOP or 1.2 mg/kg of 5-MOP), followed, 2 or 3 hours later, by exposure to UV radiation of long wavelength (320-380 nm or UVA). UVA exposures must be brief initially (1-3 J/cm^2) and are lengthened progressively to 6-8 J/cm^2, generally in three weekly sessions; results are generally obtained, for severe psoriasis, in about 20 sessions. Localized treatment is possible (e.g., in case of hepatic insufficiency), but must be conducted with the greatest caution. PUVA treatment is contraindicated for pregnant women and children, in case of cutaneous disorders aggravated by sunlight, and in case of renal or cardiac insufficiency. This therapy is not without risks: gastrointestinal disorders (8-MOP), dry skin, photosensitization (pruritus, burns, hence the need to avoid overexposure with protective clothing, sunscreens), and later on, accelerated aging of the crystalline lens of the eye (sunglasses during treatment and in the hours that follow), aging of the skin, and pigmentation problems. Long-term PUVA treatment increases the risk of cancer: studies in the late 1990s confirm the possible induction of squamous-cell carcinoma and melanoma, many years after the treatment begins. However, experts feel that as long as the number of indications is limited, young and fair-skinned patients are excluded (with possible exceptions), prior treatments are taken into account, and the number of sessions and the doses delivered (cumulative maximum 1,500 J/cm^2) are tightly controlled, PUVA treatment remains useful, especially for extensive psoriaris, because it improves the patients quality of life.

B. Other Applications, Cosmetic Products

Natural products such as bergamot oil are authorized as photodynamic sensitizers in sun lotions. They increase the number of melanocytes, as well as their melanin production; this is how they provide extra protection against UV radiation. However, their use in tanning and cosmetic products is not without risk: the occurrence of symptoms linked to phototoxicity is not exceptional. The phototoxic reaction is influenced by many factors: type of skin, skin hydration, time elapsed between the application of furanocoumarin-containing products and irradiation, duration and frequency of irradiation, and so forth. A determining factor in the occurrence of dermatitis is the vehicle being used: for equal doses, oily solutions (or oil/water emulsions) induce no reactions, whereas alcoholic solutions facilitate penetration and lead to phototoxicity.

The demonstrated role of furanocoumarins in the genesis of skin cancers has led to questioning the wisdom of their use* in sun lotions and other products. For some years, manufacturers have voluntarily limited the furanocoumarin content of their products to 15 ppm, and in 1995, CAPT (the *Comité européen pour l'adaptation au progrès technique* or European committee for adaptation to technical progress) proposed a directive that would require that all sun-related products containing more than 1 ppm of psoralen be taken off the market.

A comment is in order about 3-aryl and 4-arylcoumarins. Biosynthetically, 3-arylcoumarins are isoflavonoids (see p. 347). The biosynthetic origin of 4-arylcoumarins remains hypothetical: they are neither derivatives of a 2'-hydroxylated cinnamic acid, nor flavonoid derivatives. Most likely they are formed, as neoflavenes, by the condensation of a 1-phenylpropane onto an arene that arose from the condensation of a polyacetate. Often referred to as "neoflavonoids", they will be covered later in order to reflect their mixed biosynthetic origin.

9. BIBLIOGRAPHY

Generalities

Casley-Smith, J.R., Morgan, R.G. and Piller, N.B. (1993). Treatment of Lymphedema of the Arms and Legs with 5,6-Benzo-[α]-pyrone, *New Engl. J. Med.*, **329**, 1158-1163.

Estévez-Braun, A. and González, A.G. (1997). Coumarins, *Nat. Prod. Rep.*, **14**, 465-475.

* Some manufacturers seek refuge in the concept of photochemoprotection—the notion that using a 5-MOP-containing, melanostimulating sunscreen allows the duration of exposure to sunlight to be reduced —to justify promoting their products. See Chadwick, C.A., Potten, C.S., Cohen, A.J. and Young, A.R. (1994). The Time of Onset and Duration of 5-Methoxypsoralen Photochemoprotection from UVR-induced DNA Damage in Human Skin, *Br. J. Dermatol.*, 131, 483-494.

Ghosh, P., Markin, R.S. and Sorrell, M.F. (1997). Coumarin-induced Hepatic Necrosis, *Am. J. Gastroenterol.*, **92**, 348-349.

Hoult, J.R.S. and Payá, M. (1996). Pharmacological and Biochemical Actions of Simple Coumarins: Natural Products with Therapeutic Potential, *Gen. Pharmac.*, **27**, 713-722.

O'Kennedy, R. and Thornes, R.D. (1997). Coumarins–Biology, Applications and Mode of Action, Wiley, Chichester.

Sweet Clover

Puschner, B., Galey, F.D., Holstege, D.M. and Palazoglu, M. (1998). Sweet Clover Poisoning in Dairy Cattle in California, *J. Am. Vet. Med. Assoc.*, **212**, 857-859.

Khella

Rauwald, H.W., Brehm, O. and Odenthal, K.-P. (1994). The Involvment of a Ca^{2+} Channel Blocking Mode of Action in the Pharmacology of *Ammi visnaga* Fruits, *Planta Med.*, **60**, 101-105.

Durate, J., Vallejo, I., Perez-Vizcaino, F., Jimenez, R., Zarzuelo, A. and Tamargo, J. (1997). Effects of Visnadine on Rat Isolated Vascular Smooth Muscles, *Planta Med.*, **63**, 233-236.

Furanocoumarins

Béani, J.C. and Leccia, M.T. (1996). Puvathérapie. Accidents, surveillance, indications d'emploi, *Concours Med.*, **118**, 1191-1199.

Bethea, D., Fullmer, B., Syed, S., Seltzer, G., Tiano, J., Rischko, C., Gillespie, L., Brown, D, and Gasparro, F.P. (1999). Psoralen Photobiology and Photochemotherapy: 50 Years of Science and Medicine, *J. Dermatol. Sci.*, **19**, 78-88.

Diawara, M.M. and Trumble, J.T. (1997). Linear Furanocoumarins, *in* "Handbook of Plant and Fungal Toxicants", Felix D'Mello, J.P., Ed., p. 175-189, CRC Press, Boca Raton.

Lindelöf, B., Sigurgeirsson, B., Tegner, E., Larkö, O., Johannesson, A., Berne, B., Christensen, O.B., Andersson, T., Törngren, M., Molin, L., Nylander-Lundqvist, E. and Emtestam L. (1991). PUVA and Cancer: a Large-scale Epidemiological Study, *Lancet*, **338**, 91-93.

McNeely, W. and Goa, K.L. (1998). 5-Methoxypsoralen. A Review of its Effects in Psoraisis and Vitiligo, *Drugs*, **56**, 667-690.

Stern, R.S., Nichols, K.T. and Väkevä, L.H. (1997). Malignant Melanoma in Patients Treated for Psoriasis with Methoxsalen (Psoralen) and Ultraviolet A Radiation (PUVA), *New Engl. J. Med.*, **336**, 1041-1045; comment: Wolff, K., Should PUVA Be Abandonned? *ibid.*, 1090-1091.