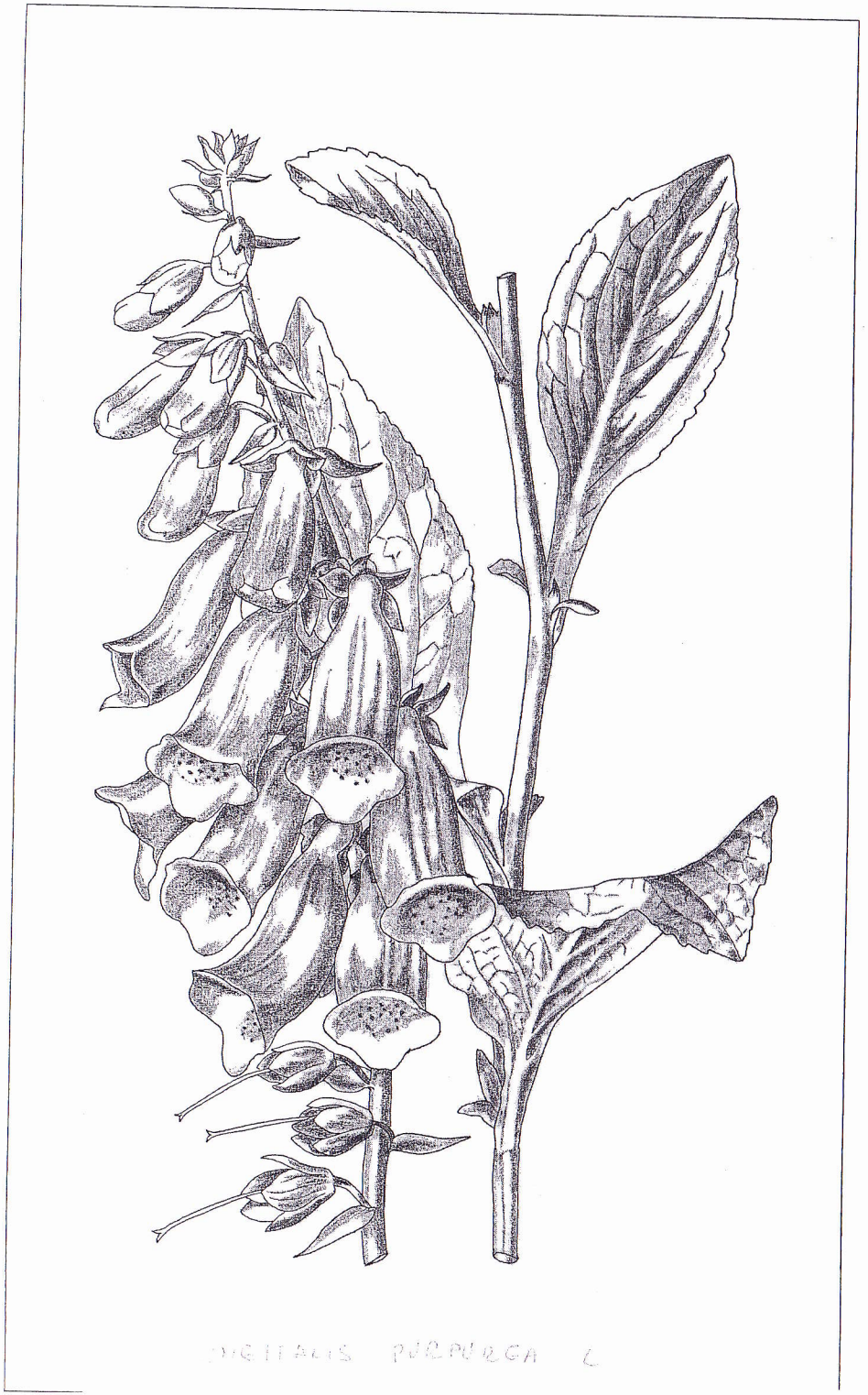


Cardiac Glycosides: Generalities

1. INTRODUCTION

Cardiac glycosides form a well-defined and highly homogeneous group from a structural, as well as pharmacological, standpoint. Despite a narrow margin of safety, these drugs, which are natural compounds of plant origin, are still useful in the long-

decrease functional limitations, and decrease the frequency of acute decompensation. Neither the new synthetic inotropic agents on the market, which turned out to be disappointing and even dangerous, nor ACE inhibitors—no matter what their advantages, particularly in terms of survival—have completely overshadowed the importance of cardiac glycosides, especially digoxin.

Except for squill (*Drimia maritima* [L.] Stearn), which physicians have known since antiquity for its diuretic properties, the drugs in this group remained unused for a very long time. The "heart tonic" activity of the lily of the valley was not recognized until the sixteenth century, the properties of the foxglove not until the end of the eighteenth century, and the *Strophanthus* did not become official until the beginning of the twentieth century, after Livingstone experienced their effects.

Despite this lack of awareness of the therapeutic potential of the drugs in this group, some of them had been known and exploited for their cardiac toxicity since remote times: in Africa, and also in Asia, they were—and still are occasionally *—ingredients of arrow poisons, which frequently combine cardiac toxins with irritating substances to facilitate the diffusion of the toxins through the tissues. Examples include some Apocynaceae: the seeds of *Strophanthus* spp., (see p. 745), the wood and root of *Acokanthera* spp. (e.g., *A. schimperi* [A. DC.] Schweinf, from Tanzania [= *A.ouabaio* Poisson]), and the latex of *Adenium* (e.g., *A. boehmianum* Schinz from Namibia). Another example from Africa is that of Asclepiadaceae such as *Parquetina nigrescens* (Afzel.) Bullock., the latex of which was used in Zaire. In Malaysia and in a large part of southeast Asia, it was mostly the latex of a Moraceae, *Antiaris toxicaria* (Pers.) Leschen., that was used, as well as the bark of a Celastraceae, *Lophopetalum javanicum* (Zoll.) Turcz. In South America, the main species used were from the genera *Naucleopsis* and *Maquira* (Moraceae). Other species also provided ordeal poisons (e.g., *Menabea venenata* Baillon, Asclepiadaceae).

2. DISTRIBUTION

Cardiac glycosides have a fairly limited distribution in several dozen genera unevenly scattered in about fifteen families. There are many such genera in the Asclepiadaceae (e.g., *Asclepias, Calotropis, Carissa, Cryptostegia, Gomphocarpus* [= *Pachycarpus*], *Menabea, Periploca, Xysmalobium*) or in the Apocynaceae (e.g., *Acokantera, Adenium, Apocynum, Cerbera, Nerium, Strophanthus, Thevetia*), but

* In 1996, the Kuala Lumpur hospital recorded a case report of death subsequent to the ingestion of *pokok ipoh*, an arrow poison consisting chiefly of the latex of *Antiaris toxicaria* (Pers.) Leschen. (seeHo, L.M., Cheong, I. and Jalil, H.A. (1996). Rhabdomyolysis and Acute Renal Failure following Blowpipe Dart Poisoning, *Nephron*, **72**, 676-678). In the late 1960s, multiple case reports of criminal use of arrow poisons were recorded in Kenya (see Maitai, C.K., Muraguri, N. and Patel, H.A. (1973). A Survey on the Use of Poisoned Arrows in Kenya during the Period 1964-1971. *East African Med. J.* **50**, 100-104).

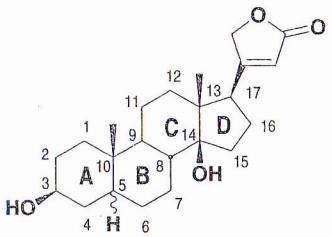
in most other families, the ability to elaborate these structures is only found in a very limited number of genera: *Cheiranthus, Erysimum* (Brassicaceae), *Euonymus, Lophopetalum* (Celastraceae), *Cotyledon, Kalanchoe, Tylecodon* (Crassulaceae), *Coronilla* (Fabaceae), *Homeria, Moraea* (Iridaceae), *Boweia, Convallaria, Urginea* (Liliaceae), *Antiaris, Antiaropsis, Castilla* (Moraceae), *Adonis, Helleborus* (Ranunculaceae), *Digitalis* (Scrophulariaceae), and *Corchorus* (Tiliaceae). All of the organs may contain cardiac glycosides, but except for a few rare cases, the concentrations are low (lower than 1%). These compounds are found in animals only in exceptional cases: bufadienolides occur in toads (*Bufo*) and cardenolides in Lepidoptera, but in this particular case, they originate in the food, in that the caterpillars feed on Asclepiadaceae, Apocynaceae, or Brassicaceae, and are thereby protected from their natural predators. This is not, however, a general rule: some Coleoptera synthesize their cardio-active aglycones from phytosterols (*Chrysolina* sp.).

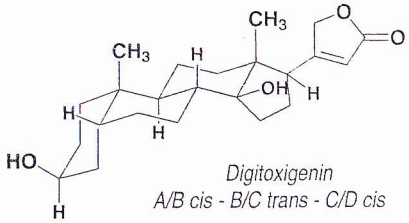
3. STRUCTURES

The structures are remarkably homogeneous, and comprise a steroidal aglycone of the (C_{23}) cardenolide type or of the (C_{24}) bufadienolide type, and a sugar moiety, most often an oligosaccharide.

A. Structure of the Aglycones

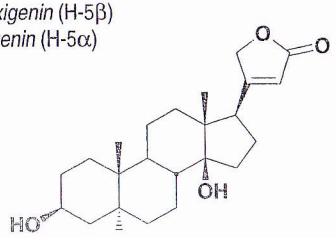
All of the aglycones have in common the classic, tetracyclic, steroidal nucleus. The A, B, C, and D rings normally have a *cis-trans-cis* configuration (digitoxigenin) or, less often, a *trans-trans-cis* configuration (uziragenin and Asclepiadaceae aglycones). In some cases (rare: scillarenin), a 4,5-double bond imparts some



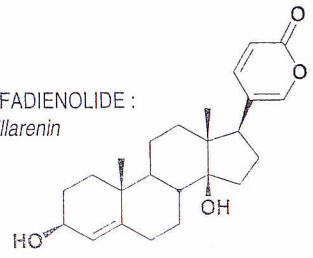


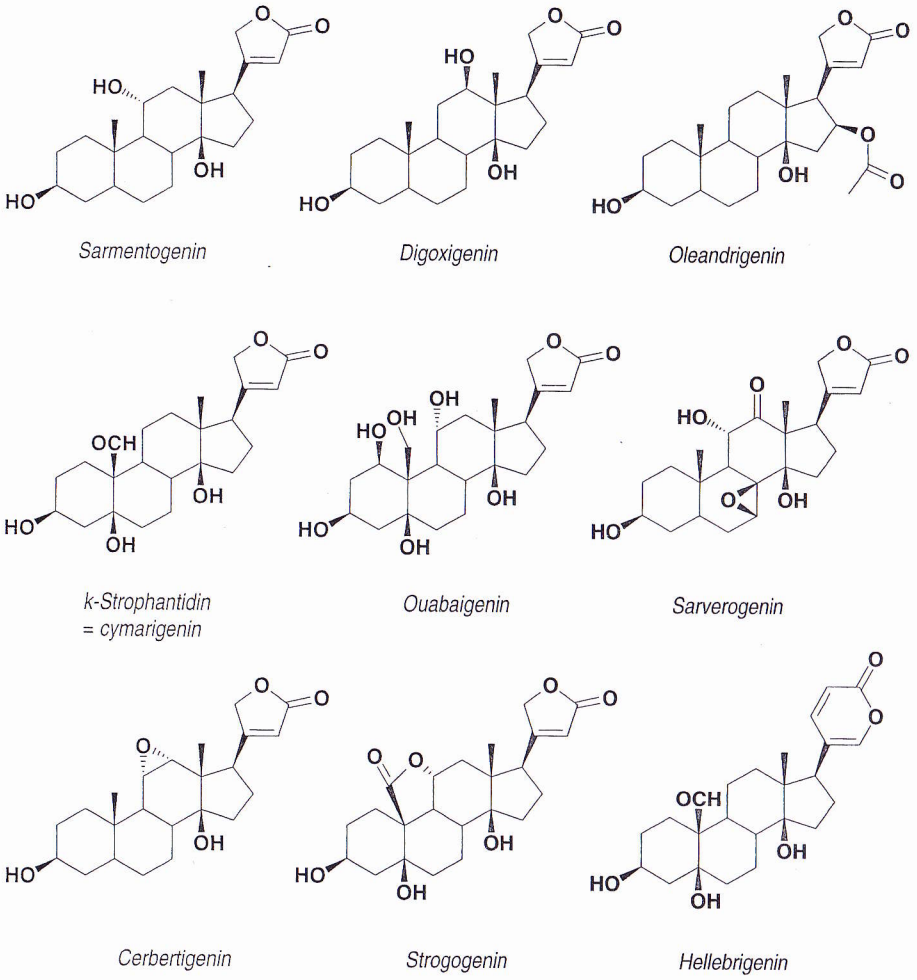
Digitoxigenin
A/B cis - B/C trans - C/D cis

CARDENOLIDES :
Digitoxigenin (H-5β)
Uzarigenin (H-5α)



BUFADIENOLIDE :
Scillarenin





planarity to the molecule. Also common to all of the aglycones is the presence of two hydroxyl groups: one is a 3β secondary alcohol, the other is a 14β tertiary alcohol. The final structural element common to all of the aglycones is the presence of a β substituent at C-17: an α,β-unsaturated lactone.

The size of the lactone ring distinguishes two groups of aglycones: the C23 cardenolides with an α,β-unsaturated γ-lactone (= butenolide) and the C24 bufadienolides with a di-unsaturated δ-lactone (= pentadienolide).

Structural Variations. They are rather limited and consist of additional hydroxyl groups: 11α (sarmentogenin), 12β (digoxigenin), 16β (gitoxigenin), 5β (k-strophantidin), 1 (ouabaigenin), or at several of these positions (ouabaigenin, diginatigenin). In rare cases, one of these hydroxyl groups can be esterified by formic acid (gitaloxigenin) or by acetic acid (oleandrigenin). The oxidation can also result in a ketone function at C-12 (sarverogenin) or in an epoxide (11,12 in

cerbertigenin; 7,8 in sarverogenin). In many compounds, the angular methyl group at C-10 is oxidized to a secondary alcohol (ouabaigenin) or to an aldehyde (k-strophantin, hellebrigenin); lactonization with the hydroxyl group at C-11 is then possible (strogogenin). In exceptional cases, the C ring can be unsaturated ($\Delta^{11,12}$), and *Thevetia* sp. are known to contain C-*nor*-D-*homo*-cardenolides (e.g., cannogenin homologs).

B. Structure of the Sugar Moiety

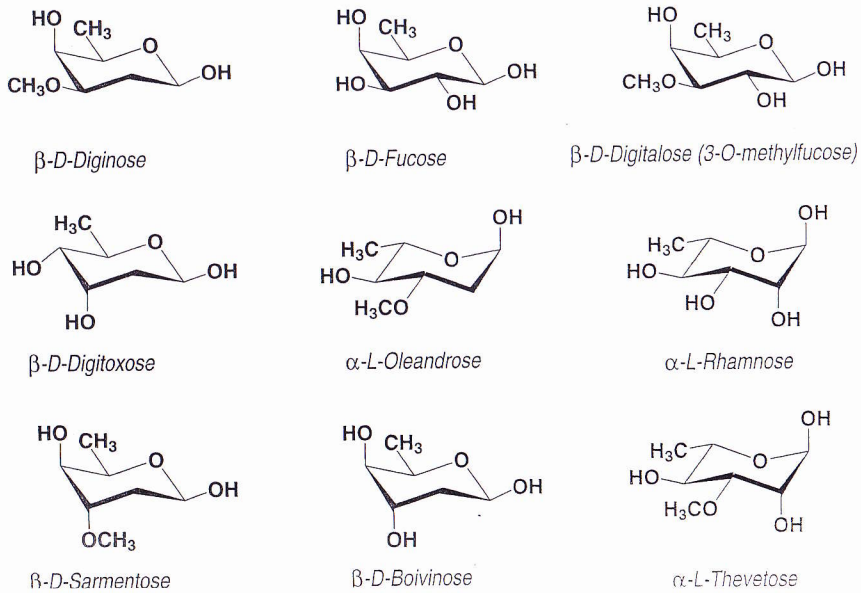
The majority of the saccharides found in cardiac glycosides are highly specific. They are 2,6-dideoxyhexoses, such as D-digitoxose (= 2,6-dideoxy-D-allose), and 2,6-dideoxy-3-methylhexoses, such as L-oleandrose (= 2,6-dideoxy-3-methyl-L-mannose) or D-diginose (= 2,6-dideoxy-3-methyl-D-galactose).

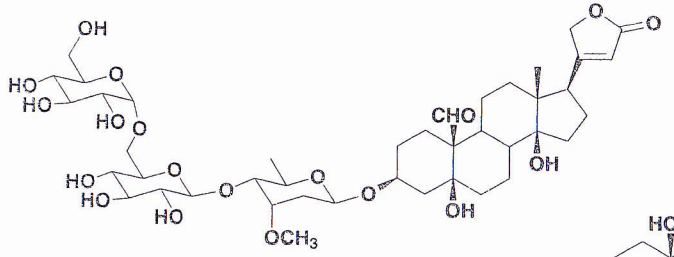
In addition to these specific saccharides, 6-deoxyhexoses also occur (L-rhamnose, D-fucose), as well as 6-deoxy-3-methylhexoses, such as L-thevetose (= 6-deoxy-3-methyl-L-glucose), and D-digitalose (= 6-deoxy-3-methyl-D-galactose).

Glucose can also be found in the glycosides, in which case it is found at the end of an oligosaccharide. Finally, note that one hydroxyl group on a sugar can be acetylated (see below, acetyldigoxin).

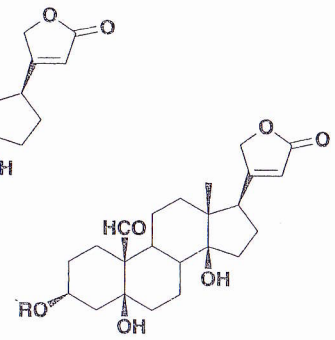
C. Structures of the Glycosides, Structure-activity Relationships

The sugar moiety is generally linked to the aglycone through the hydroxyl group at C-3, or, in the particular case of the Asclepiadaceae, at both C-3 and C-2 with a 1,4-dioxane ring as a result. This sugar moiety consists of a monosaccharide (for

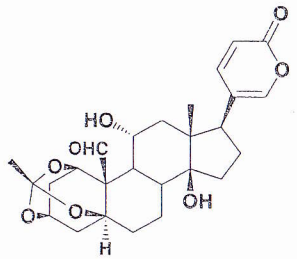




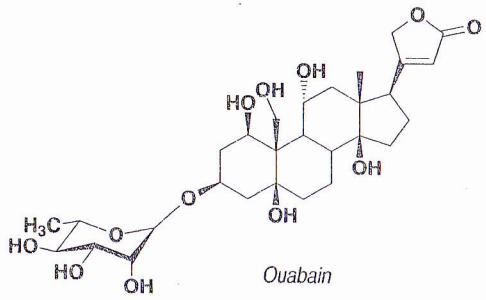
K-Strophantoside



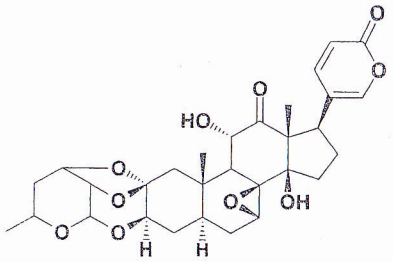
R = β-D-glc-(1—>4)-α-L-rha-(1—>) : *Convalloside*
R = α-L-rha-(1—>) : *Convallatoxin*



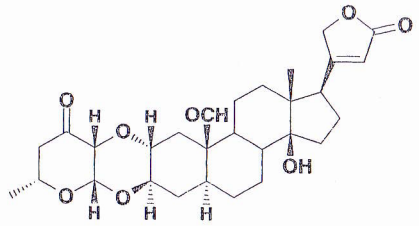
Bryophylline A (= bryotoxin C)



Ouabain



Orbicuside A



Usharidin

example, ouabain is ouabaigenin 3-rhamnoside), or, very frequently, of an oligosaccharide. The latter may comprise two to four units. When there is a glucose unit, it is always terminal. Generally, *primary* and *secondary* glycosides are distinguished. The former, found in the fresh plants, comprise a terminal glucose molecule which is readily eliminated (particularly upon drying) to yield the latter. An example is the convalloside-convallatoxin pair (see also, in the next chapter, the glycosides of the foxglove or of the squill).

The structures are a little different in Asclepiadaceae and Crassulaceae. In those families, the occurrence of aglycones hydroxylated at C-2 allows the formation of a ring (tyledoside A, uscharidin, calotropin). In the case of Crassulaceae, the linkage with the saccharide can even involve three bonds (orbicuside); glycosides are never

found: bryophilline A in *Kalanchoe* is a 1,3,5-orthoacetate and other bufadienolides are simples esters (acetate or gluconate).

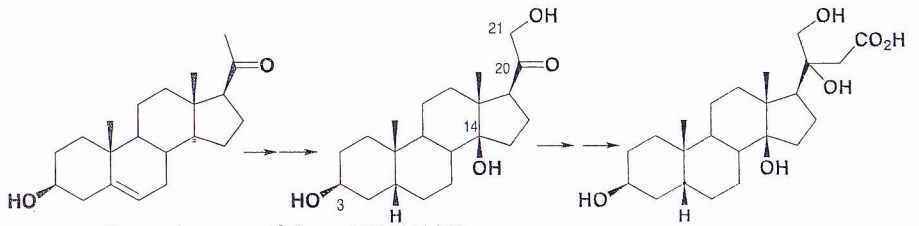
The **structure-activity relationships** are clear: the cardiac activity is linked to the aglycone. The sugar moiety does not participate directly in the activity, but its presence enhances the activity and modulates it by modifying the polarity of the compound. The presence of a certain number of structural elements is required for, or at least favorable, to the activity:

- The lactone at C-17. The presence of a X=C-C= function (where X is a heteroatom) is required, and it must be in the β configuration.
- The configuration of the rings. The activity is maximized when the A, B, and C rings are in the *cis, trans, cis* configuration. The activity is greatly diminished when the A and B rings are *trans* fused (e.g., uzarigenin), but is maintained when the A ring is partially unsaturated, as in the case of the squill glycosides, which have a 4,5-dehydro-aglycone. The C and D rings must be *cis* fused.
- The substituents. The inversion of the configuration at C-3 diminishes the activity, but 3-deoxy compounds are not completely inactive. In the case of the tertiary alcohol function at C-14, its presence is a very favorable element, but it is not so much its presence, as it is the configuration of C-14 that matters: 14-epi-digitoxigenin is inactive, whereas 14-deoxydigitoxigenin (with a 14β-hydrogen) is slightly active (although 8β,14β-epoxy derivatives are inactive).

None of the countless structural modifications attempted in this series has improved the performance of the natural glycosides: furthermore, none has provided a better therapeutic index.

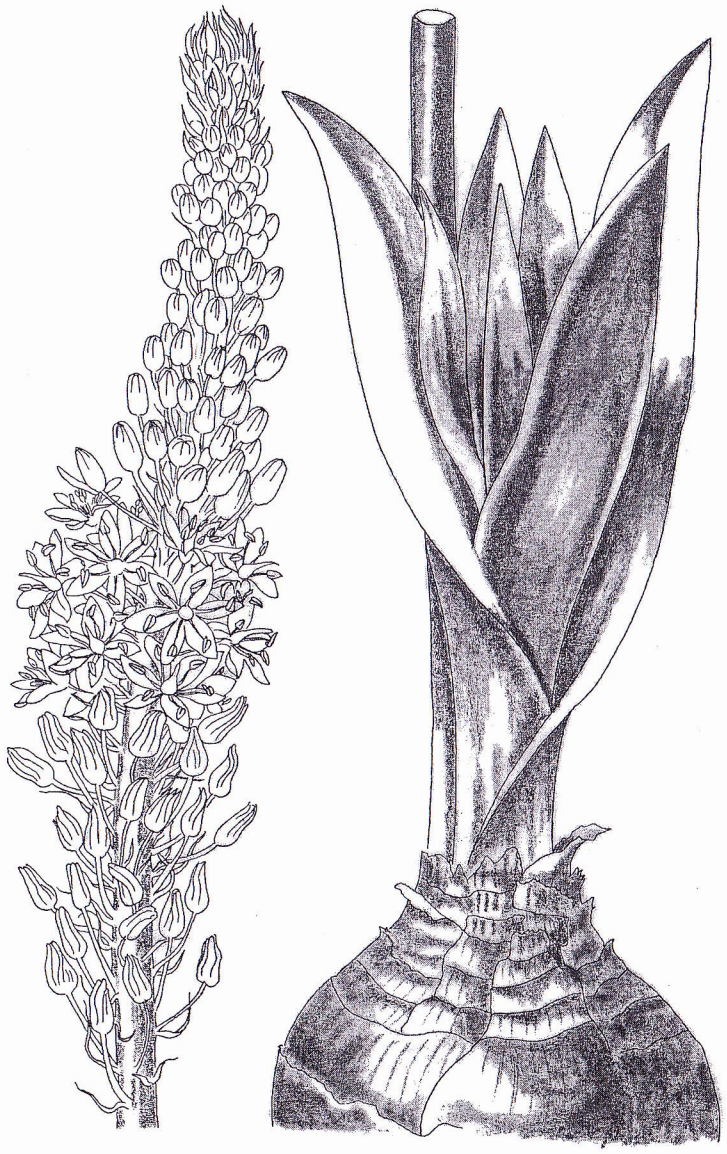
4. BIOSYNTHETIC ORIGIN

It is generally accepted that cardenolides arise from the condensation of a derivative from the pregnane series (a functionalized 20-ketopregnane, such as 5β-pregnan-3,14,21-triol-20-one) with a two-carbon unit (acetate). Also known is the fact that various (C_{23}) norcholanic acids are efficiently incorporated by *D. purpurea*. The introduction of the hydroxyl group at C-14, with simultaneous inversion of the configuration at that carbon is likely to occur early, although the exact reaction sequence has yet to be demonstrated.



Pregnenolone *5β-Pregna-3,14,21-triol-20-one*

Suggested intermediates in the biosynthesis of cardenolides



DRIMIA MARITIMA (L.) Stearn

5. PHYSICO-CHEMICAL PROPERTIES, CHARACTERIZATION, AND QUANTITATION

As a general rule, the glycosides are fairly soluble in water, and slightly soluble in ethanol and chloroform: digitoxin is far more soluble in chloroform than digoxin, which is fairly soluble in dilute ethanol and in ethanol-chloroform mixtures. Both are sparingly soluble in ethyl acetate. Primary glycosides such as lanatoside C are water-soluble, soluble in dioxane, sparingly soluble in chloroform (1 g in 2 L), and virtually insoluble in methanol. The presence of the lactone renders the molecule labile, and likely to open in an alkaline medium.

A. Characterization

The concentration of cardiac glycosides is often low, and precludes the direct application of the different characterization methods: preparing purified and concentrated extracts is necessary. The normal technique (which is not compatible with the presence of formylated derivatives) for the preparation of these extracts is a purification by precipitation with lead acetate: extraction of the pulverized drug by a mixture of 50% ethanol and lead acetate solution. After boiling, cooling, and elimination of the residue by centrifugation, the cardiac glycosides present in the supernatant are extracted with chloroform. It is this chloroform solution that undergoes the characterization reactions and chromatographic analyses.

Color Reactions. They can be due to the sugars or to the aglycones.

Color Reactions of the Sugars. The only color reactions of the sugars that are of interest are those specific to 2,6-dideoxyhexoses. The reaction most frequently carried out is that using xanthydrol, also known as the Pesez reaction: addition of xanthydrol to a glycoside solution in concentrated acetic acid, heating in a water bath, and development of a red color.

The Keller-Kiliani reaction may also be used: addition of concentrated sulfuric acid containing traces of ferric salts to a glycoside solution in concentrated acetic acid also containing ferric salts; a reddish-brown ring develops and the acetic acid solution slowly turns blue-green. Glycosides such as ouabain (whose sugar moiety is rhamnose, i.e., a 6-deoxyhexose) do not produce this reaction, but other glycosides, which comprise 2,6-dideoxyhexoses, but are not cardio-active, do produce a positive reaction: for example digitanol glycosides comprising D-diginose (i.e., 2,6-dideoxy-3-methyl-D-galactose), such as diginoside or digifolein.

Color Reactions of the Aglycones. The classic reactions of steroids may be used, but their lack of specificity limits their usefulness. In the case of the cardenolides, it is far more interesting to run specific reactions, linked to the presence of the α,β-unsaturated γ-lactone, such as the Kedde reaction or the Baljet

reaction. What these reactions have in common is the use of an aromatic nitro derivative which, in an alkaline medium (sodium hydroxide), will form a deeply colored adduct with the lactone: the resulting purplish-red color is fairly stable. The Baljet reaction uses picric acid and yields a stable orangy color. These reactions are negative with saponins, and either negative or much weaker with bufadienolides. The digitanol-glycosides sometimes give weakly positive reactions.

Fluorescence Reactions

Cardiac glycosides form, under acidic conditions, fluorescent dehydrated derivatives: 14-dehydro derivatives, and in the case of aglycones substituted at C-16, 14,16-didehydro derivatives. In the latter case, the fluorescence is much more intense, since the resulting trienone has three double bonds conjugated with the carbonyl group. These reactions are mostly useful to visualize chromatograms (TLC).

In practice, the Jensen reaction is used by spraying the plates with trichloroacetic acid in solution in ethanol. The simultaneous use of an oxidant (chloramine T) allows the observation of fluorescent spots of different colors, which facilitates the interpretation of the chromatograms. Phosphoric acid can also be used, alone or mixed with sulfuric acid and ferric chloride: after heating, a red color appears (Tattje reaction). Again, the reaction is much more sensitive with glycosides comprising an aglycone substituted at C-16 than with those of the digitoxin or digoxin type.

Digitanol-glycosides do not give these fluorescent reactions.

B. Quantitation

The quantitation of cardiac glycosides is frequently delicate, because of the complex composition of the drugs containing them and because of their low concentrations. Pharmacopoeias most often require a quantitation of the aglycones.

These quantitations, which are most often colorimetric, take advantage of the characteristic reactions of the lactone (cardenolides): to quantitate pure glycosides (digitoxin, digoxin), the French Pharmacopoeia uses the Baljet reaction (picric acid); to quantitate the aglycones of the foxglove, it uses the Kedde reaction (with 3,5-dinitrobenzoic acid).

To quantitate the aglycone, the glycosides present in the supernatant after purification by precipitation with lead acetate are hydrolyzed in the presence of hydrochloric acid, and the aglycones are extracted repeatedly with chloroform. The chloroform extracts are evaporated, the residue redissolved in ethanol, and 3,5-dinitrobenzoic acid is added; finally, the absorbance at 540 nm is measured. Simultaneously, a reference standard consisting of a pure glycoside is treated under strictly identical conditions.

The direct colorimetric quantitation of the glycosides begins with an extraction with an aqueous or hydroalcoholic solution and continues with a purification: purification by precipitation with lead acetate, or re-extraction of the liquid extract

with a non-miscible solvent, or both. Over the past few years, several methods have been developed for HPLC: they allow an estimate of the total glycoside concentration, and of the relative proportions of the different glycosides, which must be known to correctly estimate the value of the drug for extraction purposes.

6. PHARMACOLOGICAL PROPERTIES

Cardiac glycosides act on the heart at different levels: force and speed of contraction, rate, and electrophysiological properties. These effects result in electrocardiographic changes frequently observed during treatment.

• Increase in contractility. Cardiac glycosides increase the force and speed of contraction of the heart. In patients with cardiac insufficiency, this positive inotropic effect translates into an increase in cardiac output, an increase in cardiac work capacity without any increase in oxygen consumption, a decrease in heart rate, and, indirectly, a decrease in arterial resistance. The glycosides are thought to act at the membrane level, by inhibition of the Na-K ATPase, which would result in an increase of the intracellular calcium ion concentration. It is also postulated that they decrease the activation of the renin-angiotensin-aldosterone system, lower the level of circulating catecholamines, and restore baroreceptor sensitivity.

• In patients with cardiac insufficiency, cardiac glycosides decrease the heart rate (negative chronotropic effect) by affecting the autonomic nervous system (indirect parasympathomimetic effect).

• Electrophysiological changes. The negative dromotropic action—of cholinergic origin—manifests itself by a decrease in the conduction velocity at the *atrium-ventricle* (= A-V) junction, and an increase in the refractory period of the A-V node (hence their use to treat supraventricular rhythm abnormalities); there is no action on the intraventricular conductivity.

In patients with cardiac insufficiency, contractility is diminished, as is cardiac output (decrease in systolic ejection or stroke volume), whereas the post-systolic residual blood volume in the ventricles and oxygen consumption are increased; the decrease in contractility leads to a reflex sympathic vasoconstrictive activity. The renal output is diminished, resulting in sodium retention and edema. Cardiac glycosides, by improving the cardiac output, decrease the reflex sympathic vasoconstrictive activity; the resistance to ventricular ejection decreases, the venous return improves, and the heart rate tends to decrease.

The pharmacokinetics of cardiac glycosides are closely dependent on the polarity of the molecule, especially on the degree of hydroxylation of the aglycone. This aspect of cardiac glycosides, like their pharmacodynamic properties and

specialized texts. Here, we shall merely note that a compound which is not highly hydroxylated, such as digitoxin, is lipophilic: when administered orally, it is rapidly and completely resorbed; it binds strongly to plasma proteins and its elimination in the bile and urine is slow (half-life > 6 days with very high individual variability). Digoxin is less lipophilic and resorbed much less in the intestine (80%); it does not bind very much to plasma proteins and diffuses rapidly into the tissues; it is not extensively metabolized, and is eliminated chiefly and fairly rapidly by the kidneys (half-life = 36 hours). A very polar compound, such as ouabain, may only be administered intravenously (resorption in the digestive tract is very poor), and its renal excretion is very rapid. Nontoxic plasma concentrations range from 10 to 30 and 1 to 2 ng/mL for digitoxin and digoxin, respectively.

7. USES OF DRUGS CONTAINING CARDIAC GLYCOSIDES

These drugs are not used crude, and their galenicals, because of their irreproducible activity, have been abandoned in favor of pure glycosides produced by the extraction industry. In France, the different glycosides obtained by extracting the leaves of foxgloves (*D. lanata, D. purpurea*) are used in current medical practice. Ouabain from *Strophanthus* was long prized in emergency medicine, but is no longer marketed in France. In other European countries, proscillaridin A, extracted from squill bulbs, is also currently in use.

8. THERAPEUTIC INDICATIONS

The usefulness of cardiac glycosides for the treatment of chronic cardiac insufficiency has long been the topic of a controversy which multiple randomized trials were designed to resolve. The most recent study—which included 7,788 patients—showed that digoxin, if taken daily (0.25 mg for 70% of patients, mean duration of the monitoring: 37 months; patients simultaneously treated with an ACE inhibitor, or a diuretic, or both) decreased the mortality due to the aggravation of the chronic cardiac insufficiency but did not alter the overall mortality (whereas other inotropic agents increase it). The study also showed a marked decrease (>20%) of the number of hospitalizations for aggravation of the chronic cardiac insufficiency. Previous studies had demonstrated the efficacy of cardiac glycosides in improving the symptoms and quality of life of patients with cardiac insufficiency. This is also true of patients who received an ACE inhibitor and diuretics: a randomized trial showed that the discontinuation of cardiac glycosides led, in most cases, to a deterioration of the status of the patients.

The efficacy of cardiac glycosides must not overshadow the fact that that ACE inhibitors (and diuretics) are actually considered by the majority of prescribers as the basic treatment for cardiac insufficiency: they delay the advance of the disease and decrease mortality. In general, it is when the insufficiency becomes advanced that cardiac glycosides are added to the basic treatment.

Cardiac glycosides are currently indicated for:

1. cardiac insufficiency with low output (generally in combination with diuretics), particularly when there is atrial fibrillation;

2. supraventricular rhythm abnormalities: to slow down or decrease atrial fibrillation or flutter.

The prescriber must take into account the numerous contraindications (ventricular hyperexcitability [extrasystoles], A-V block with no pacemaker, IV calcium therapy). Physicians and pharmacists must also keep in mind the multiple potential drug interactions common to all of the cardiac glycosides, which impose as many warnings:

- IV calcium salt therapy, in which case serious, perhaps fatal, rhythm abnormalities may arise (this is in fact a contraindication);
- adsorbents (activated charcoal, cholestyramine, antacids) modify the digestive absorption of cardiac glycosides, and should be taken at a different time (at least two hours away);
- drugs that lower the blood potassium concentration (some diuretics, but also contact laxatives, corticoids, or amphotericin B) increase the toxicity of cardiac glycosides (because the lack of potassium enhances cardiac rhythm abnormalities).

Other interactions, specific to each cardiac glycoside, must also be considered: a few examples are with enzyme inducers (phenobarbital and rifampin are not to be taken with digitoxin or acetyldigitoxin), quinidine, sulfasalazine, verapamil (not to be taken with digoxin), amiodarone (not to be taken with any cardiac glycoside), midodrine, bretylium (digitoxin), and so on.

9. GUIDELINES FOR PRESCRIPTION

The choice of a cardiac glycoside (with rapid, slow, or intermediate speed of onset of action) depends on the type and stage of cardiac disease, and on the renal function and electrolyte balance of the patient. Commonly, the prescription—generally of an oral form—begins with a loading dose for optimal "digitalization" and continues with a weekly maintenance dose schedule spread over several days per week (or consisting of a daily dose, depending on the exact glycoside). Average doses must be adapted individually as a function of clinical data (return to a stable heart rate of 70) and electrocardiographic data.

The normal doses in the elderly are generally reduced. In patients with hepatic or renal insufficiency, it is best to regularly monitor diuresis, blood pressure, heart rate, and blood potassium: it all depends on the nature of the cardiac glycoside, and therefore on the fact that it is— is not, or is not very extensively—metabolized by the liver; its chief route of elimination is also to be considered. In some cases, it may be useful to monitor the drug plasma concentration to adjust the posology.

The first symptoms of a possible overdose are digestive (nausea, vomiting, diarrhea), then visual (yellow vision), neurological (confusion, headaches), and cardiac (e.g., rhythm abnormalities, bradycardia and perturbations of automaticity, ventricular extrasystoles, junctional tachycardia, A-V block). In case of overdose

the treatment must be discontinued, the blood potassium concentration brought back to normal (K+ administration), potential bradycardia is corrected by administering atropine, and potential hyperexcitability is corrected by administering phenytoin. In case of massive intoxication, the patient must be hospitalized in a specialized unit where the appropriate treatment can be applied and can include cholestyramine, phenytoin, and temporary ventricular pacing. The administration of digoxin specific antibodies (Fab fragments produced by sheep immunized against digoxin and now available in France) is an efficacious and expeditious treatment for serious cardiac glycoside poisoning, although it is expensive.

10. BIBLIOGRAPHY (see also p. 749)

Albrecht, H.P. (1999). Cardiac Glycosides, *in* "Naturally Occuring Glycosides", (Ikan, R., Ed.), p. 83-123, John Wiley & Sons, Chichester.

Bounhoure, J.-P. and Galinier, M. (1996). Le traitement de l'insuffisance cardiaque, *Presse Méd.*, **25**, 1076-1082.

Collectif (1996). Anticorps antidigitaliques, *La Revue Prescrire*, **16**, 842-845.

The Digitalis Investigation Group (1997). The Effect of Digoxin on Mortality and Morbidity in Patients with Heart Failure, *New Engl. J. Med.*, **336**, 525-533; comment: Packer, M. End of the Oldest Controversy in Medicine. Are We Ready to Conclude the Debate on Digitalis? *ibid.*, 575-576.

Kreis, W., Hensel, A. and Stuhlemmer, U. (1998). Cardenolide Biosynthesis in Foxglove, *Planta Med.*, **64**, 491-499.

Neuwinger, H.D. (1994). Afrikanische Arzneipflanzen und Jagdgifte – Chemie, Pharmakologie, Toxikologie, Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart.

Renwick, J.A.A. (1997). The Role of Cardenolides in a Crucifere-insect Relationship, *Adv. Exp. Med. Biol.*, **405**, 111-121.

Steyn, P.S. and van Heerden, F.R. (1998). Bufadienolides of Plant and Animal Origin, *Nat. Prod. Rep.*, **15**, 397-413.

Théry, C. (1994). La digoxine et les inhibiteurs de l'enzyme de conversion dans le traitement de l'insuffisance cardiaque chronique, *Thérapie*, **50**, 211-218.

Williamson K.M. (1996). Is There a Role for Digoxin in Patients With Systolic Dysfunction? *Pharmacotherapy*, **16**, 37S-42S.

Cardiac Glycosides: Chief Drugs

1. DRUGS USED FOR THE EXTRACTION OF GLYCOSIDES

• FOXGLOVES, *Digitalis* spp., Scrophulariaceae

The genus *Digitalis* comprises about twenty species, essentially all European and herbaceous. Although all of the species in the genus contain cardenolide glycosides, only two are used for the extraction of digitoxin, digoxin, and closely related derivatives: the Grecian foxglove, *Digitalis lanata* Ehrh. (digoxin and other compounds) and the purple foxglove, *Digitalis purpurea* L. (digitoxin). Only the purple foxglove is the subject of a monograph in the European Pharmacopoeia,

they are prescription drugs which may not be renewed). It is also listed in many other national pharmacopoeias.

The 10th edition of the French Pharmacopoeia specifies that the drug "consists of the dried leaves [... and] it must contain not less than 0.3% cardenolide glycosides expressed as digitoxin [...] and calculated relative to the dried drug".

• The purple foxglove, described under this name in 1542, recommended during the seventeenth century by Parkinson, and touted as an antiepileptic, was not really used until after 1785, when W. Withering described its beneficial effects in the treatment of "dropsy". It did not truly become a medication until the first pure compound was isolated: digitalin (C. Nativelle, 1868); it is a potential industrial source of digitoxin.

• The Grecian foxglove is widely cultivated. Industrially, it is the starting material for the extraction of the glycosides currently on the market: it allows the production of digoxin and digitoxin, as well as derivatives of its secondary glycosides (e.g., desacetyl-lanatoside C).

The Plants: Morphology, Distribution

• The purple foxglove is a biennial or perennial herb. In the first year, the plant only has a dense rosette of leaves, and the floral stalk (0.5-1.5 m) does not appear, at the earliest, until the second year. The leaves are petiolate at the base of the stalk and sessile at the top, and are alternate. The blade is decurrent on a great part of its length at the base, and roughly and irregularly crenate. A network of veins is very prominent on the underside, which is very pubescent. The inflorescence is a long unilateral raceme of pentamerous, gamopetalous, zygomorphous, and hypogynous flowers. The purple corolla is weakly bilabiate and has bright red spots with a white rim. The androecium is didynamous and the style filiform. The fruit is an ovoid bilocular capsule surrounded by a indeciduous calyx; it releases, by septicidal dehiscence, a multitude of tiny seeds. The species grows wild on the siliceous soils of western Europe where it colonizes semi-shady areas; it is not found in Mediterranean areas.

• The Grecian foxglove is perennial, likes calcareous soils, and has sessile, lanceolate, and glabrous leaves that are acuminate at the top and have an entire margin. The flowers are grouped in a long and dense raceme. The bracts of the inflorescence and the calyx are very pubescent; the corolla is creamy white with brown veins, and is deeply bilabiate, with the lower lip as long as the tube, whereas the upper lip is bifid and very short. The species is indigenous to the central and southern regions of Europe where it grows wild on sunny limestone slopes.

The Drugs: Sources

• The natural habitat of the purple foxglove has a dense enough population in certain areas to permit the collection of wild plants. The plant can also be cultivated

(Netherlands). Optimization has allowed the selection of varieties rich in digitoxin. The leaves are harvested at the end of the first year, when both the yield in leaves and the glycoside concentration are at a maximum.

• The Grecian foxglove used for extraction comes exclusively from cultivation. The species is not very demanding and is easy to grow, even on soils that are poor in limestone. It is cultivated in the Netherlands— the chief producer—and in France, where the optimization of producing varieties (by the *Institut Technique Interprofessionel des Plantes à Parfums, Médicinales et Aromatiques* = ITEIPMAI) has sparked a recent rebound in the production (several dozen hectares).

The drug must be dried rapidly at a temperature as low as possible, or even at ambient temperature, to avoid glycoside decomposition. The low temperature must be compensated by an intense ventilation. The drug must be stored under an anhydrous atmosphere and away from light, and its residual water must be approximately 5%. In any event, the storage time is limited.

The Drugs: Macroscopic and Microscopic Characteristics

• The purple foxglove leaf is large (10-40 x 4-15 cm). The blade is oval with a subacute apex, decurrent on much of the length of the petiole, grayish-green and almost glabrous on the upper side, whitish and very pubescent on the underside. The veins are pinnate and anastomosed on the edges of the blade, forming a prominent network on the underside. The dried leaf is brittle and often broken.

• The Grecian foxglove leaf is oval-lanceolate to lanceolate (10-30 x 1-4 cm). The blade is dark green, glabrous, and its margin entire. The secondary veins form a very acute angle with the midrib; they do not form a prominent network on the underside.

• The microscopic examination shows, in *D. purpurea*, highly prominent veins on the underside of the blade; a few glandular trichomes with a unicellular stalk and a head generally uni- or bicellular; and uniseriate covering trichomes with a blunt point, comprising three to five cells, one or several of which are often articulated, with finely verrucose or slightly striated walls. The epiderm cells have anticlinal walls that are either straight or slightly sinuate.

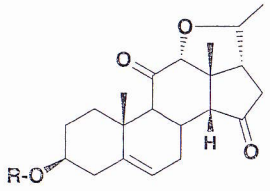
• The epidermis of the leaf of Grecian foxglove bears only glandular trichomes with a unicellular stalk and a bicellular head. The upper epidermis is characterized by cells with a beaded surface.

Chemical Composition. The *Digitalis* leaf contains numerous compounds.

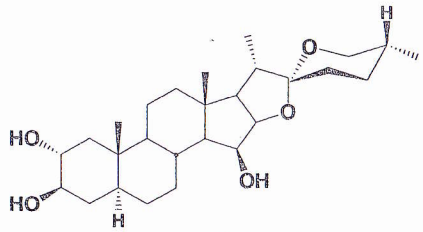
- Flavonoids (flavones and flavone glycosides).
- Anthraquinones (methoxylated derivatives of 2-methylanthraquinone and alizarin derivatives).

- Saponins: digitonin, tigonin, i.e., glycosides with a spirostane-type aglycone (digitogenin, tigogenin) and with a ramified oligoside. They are found in the leaves, and especially abundant in the seeds.

- Digitanol-glycosides: digitalonin, diginoside, digifolein. These glycosides include a C_{21} pregnane-type aglycone and a deoxy-sugar (D-digitalose, D-diginose, L-oleandrose). Diginigenin is characterized by the presence of a tetrahydrofuran ring (12,20-ether) and by two carbonyl functions (at C-11 and C-15); it is sometimes hydroxylated at C-2 (digifologenin —> digifolein). Sometimes, the aglycone also does not include an ether bridge (for example, purprogenin is 5-pregnen-3β,14β,15α-triol-15,20-dione). The concentration of these compounds is always very low.



R = H : *Diginigenin*
R = digitalose : *Digitalonin*
R = diginose : *Diginoside*



Digitogenin

- Cardiac glycosides: both species contain cardenolides, but their compositions differ markedly, quantitatively and qualitatively.

Purple foxglove. The cardenolide concentration in the leaves of wild foxgloves fluctuates between 0.1 and 0.4%. Nearly thirty glycosides have been characterized in the drug. The relative proportions of the different series vary (with the chemotype), but those varieties in which series A predominates (>50%) are used preferentially.

The *primary glycosides*—known as purpurea glycosides—occur in the fresh plant; they comprise a sugar moiety linked to the aglycone through the hydroxyl group at C-3 and consisting of four monosaccharides: one (terminal) molecule of D-glucose and three molecules of a 2,6-dideoxyhexose, namely D-digitoxose. If the drug is dried without any particular precautions, the primary glycosides are rapidly hydrolyzed by digipurpidase, a β-glucosidase that occurs in the leaves. The hydrolysis products have lost their terminal glucose: they are referred to as *secondary glycosides*. These are not vulnerable to the action of β-glucosidases, but they can be hydrolyzed in acidic conditions.

The chief cardenolides of the leaf of *D. purpurea* fall into three series defined by the structure of their aglycone.

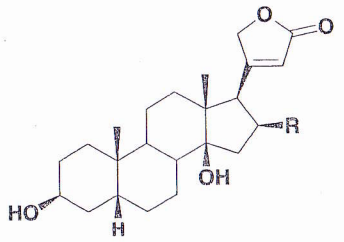
- The **A series**: the aglycone is digitoxigenin (3β,14β-dihydroxylated). The primary glycoside is purpurea glycoside A, and the secondary glycoside is digitoxin (or, in French, *digitoxoside* *), also known as digitalin.

* The glycosidic nature of these compounds naturally requires the suffix *-oside* in French; however, usage, as well as the French Pharmacopoeia, have chosen in French the suffix *-ine*

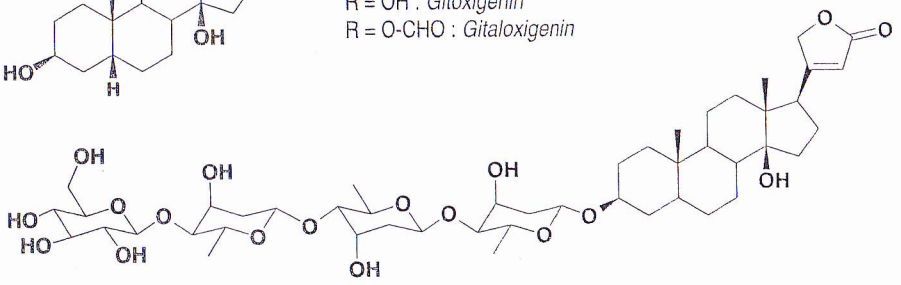
- The **B series**: the aglycone is gitoxigenin (3β,14β,16β-trihydroxylated). The primary glycoside is purpurea-glycoside B, and the secondary glycoside is gitoxin.

- The **E series**: the aglycone is gitaloxigenin (16-formylgitoxigenin). The primary glycoside is purpurea glycoside E or glucogitaloxin, and the secondary glycoside is gitaloxin.

Alongside these chief compounds, minor glycosides have been isolated from the drug, with aglycones identical to those of the above (A, B, or E), but with a sugar moiety consisting of a monosaccharide or disaccharide: strospeside (gitoxigenin D-digitaloside), digiproside (digitoxigenin D-fucoside), odoroside (digitoxigenin D-digitaloside), gluco-strospeside, and more.



R = H : *Digitoxigenin*
R = OH : *Gitoxigenin*
R = O-CHO : *Gitaloxigenin*



Purpureaglycoside A

Digitoxine (= digitoxoside = digitalin)

Grecian foxglove. Like the other species, it contains saponins (tigonin) and a small quantity of digitanol-glycosides, some of which are specific (digifologenin 3-oleandroside). The concentration of cardenolides can be greater than 1%. As in the previous case, a very large number of compounds has been identified (about sixty) and their structures are closely related. The chief constituents by far are glycosides from the A and C series. Selection favors clones in which series C predominates (ranging from over 50 to nearly 75%).

In this case, the *primary glycosides* are lanatosides. As in purpurea glycosides, the sugar moiety comprises four monosaccharides (three D-digitoxose and one D-glucose, always in the terminal position), but the digitoxose molecule next to the glucose is acetylated at C-3, and this is what characterizes lanatosides.

The hydrolysis of lanatosides by β-glucosidase yields acetylated secondary glycosides. For example, lanatoside A (a glycoside of digitoxigenin) affords acetyl-digitoxin (more precisely, a mixture of α- and β-acetyldigitoxin, the former acetylated at C-3 and the latter at C-4). The same lanatosides are readily hydrolyzed under alkaline conditions: lanatoside A yields purpurea glycoside A (= desacetyl-lanatoside A); during this reaction there is a risk of simultaneous opening of the

lactone ring. Experience shows that during the industrial extraction, part of the lanatoside A can be degraded to digitoxin.

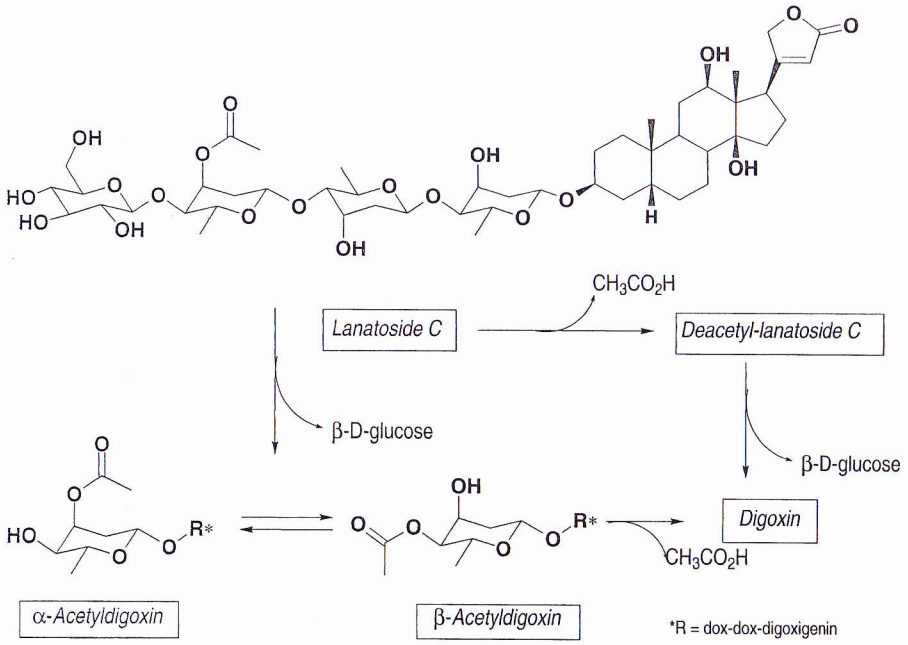
The chief cardenolides of the leaf of *D. lanata* fall into five series of unequal importance:

- The **A, B,** and **E series.** Alongside lanatoside A, B, and E, which occur in the fresh plant, the dried plant contains the corresponding secondary glycosides: acetyldigitoxin, acetylgitoxin, and acetylgitaloxin. Desacetylated products can be found in the extracts in variable proportions.

- The **C series.** The aglycone that characterizes this series is digoxigenin (3β,14β,12β-trihydroxylated). The primary glycoside is lanatoside C and the secondary glycoside is acetyldigoxin. By alkaline hydrolysis, the latter yields digoxin.

- The **D series.** This series, which is minor quantitatively, includes the glycosides (lanatoside D, acetyldiginatin) of diginatigenin (3β,14β,12β,16β-tetrahydroxylated).

Like the purple foxglove, the drug also contains glycosides with only one or two monosaccharides.



Pharmacologic Activity. Therapeutic Indications, Contraindications, Precautions. See Generalities above.

Tests for the Purple Foxglove. The French Pharmacopoeia provides a detailed description of the microscopic characteristics of the powdered leaf, which allow the

that *D. lanata* is in fact a falsification! The drug is identified by showing the presence of cardenolides (color reaction with dinitrobenzoic acid in alkaline conditions) and of the 2-deoxysugars (color reaction in the presence of xanthydrol). These color reactions are applied after extraction at high temperature of the glycosides with a mixture of 50% alcohol and a solution of lead acetate, followed by re-extraction of the supernatant hydroalcoholic phase by chloroform. It is on the same chloroform solution that a TLC analysis is conducted to show the presence of digitoxin and purpurea glycoside A and B (fluorescence at 365 nm after spraying trichloroacetic acid and chloramine T, followed by heating to 100-105 °C). The assay *per se* includes: 1. a verification of the absence of foreign elements (absence of leaves of *D. lanata*, i.e., absence of glabrous leaves with epidermic cells whose anticlinal walls look like a rosary when examined face on); 2. loss on drying (<6%), total ash (<12%), and ash insoluble in hydrochloric acid (<5%).

The quantitation is that of the aglycones: extraction of the glycosides (H_2O), purification (lead acetate), hydrolysis (HCl), extraction of the aglycones ($CHCl_3$), color reaction (dinitrobenzoic acid), and measurement of absorbance. A standard digitoxin solution must undergo the same procedure, including the measurement of absorbance, in parallel. The official drug must contain not less than 0.3% cardenolide glycosides calculated as digitoxin relative to the dried drug.

Uses of Digitalis. The purple foxglove has long been used in simple galenicals (powder, tincture); it can also be used, like the Grecian foxglove, for the industrial extraction of the glycosides.

At present, the following compounds are marketed in France: digitoxin (digitalin), digoxin, acetyldigitoxin, and desacetyl-lanatoside C (INN: deslanoside). Digitoxin and gitoxin are listed in the French Pharmacopoeia (as controlled substances on French *liste I*, i.e., prescription drugs which may not be renewed). Some countries use a semisynthetic derivative, namely β-methyldigoxin (medigoxin). In the United States, cardiac glycosides are not controlled substances, but they are prescription drugs.

Dosage

Digitoxin (in France, a controlled substance on *liste I*, i.e., a prescription drug which may not be renewed; not a controlled substance, but a prescription drug in the United States). In France, digitoxin (= digitalin) is available in tablets (0.1 mg). In adults, the initial digitalization is generally achieved by administering from 0.8 to 1.2 mg in two to four days. The maintenance dose ranges from 0.4 to 0.8 mg (4-8 tablets) to be taken over several days per week. The doses must be decreased for patients with renal insufficiency: maximum doses, 1 mg/single dose, 2 mg/day. In the United States, digitoxin is available in tablets (0.05 mg and 0.1 mg). In adults, the initial digitalization is achieved with 0.2 to 0.6 mg-doses over four days. The maintenance doses range from 0.05 to 0.3 mg daily, the most common dose being 0.15 mg daily.

Digoxin (in France, a controlled substance on *liste I*, i.e., a prescription drug

United States). In France, digoxin is available in tablets (0.25 or 0.125 mg), in solution for oral administration (0.05 mg = 1 mL), as well as in solution for IV injection (1 ampule = 0.5 mg) and in solution for pediatric IV injection (1 ampule = 0.05 mg). In adults, the loading dose ranges from 1 to 2 mg/day (to be divided into several portions), and the maintenance dose is 0.25 mg/day in one or two portions. Doses must be decreased as a function of creatininaemia in the patient with renal insufficiency (there is a good correlation between the renal clearance of creatinine and that of digoxin): maximum doses, 1 mg/single dose, 2 mg/day. In the United States, digoxin is available for adults in solution-filled capsules (0.05, 0.1, or 0.2 mg), in tablets (0.125, 0.25, and 0.5 mg), in ampules for injection (0.5 mg), and in closed injection systems (premeasured doses of 0.25 or 0.5 mg in cartridge-needle units). In adults, the initial digitalization is achieved with 0.4 to 1.25 mg-doses. The maintenance doses range from 0.05 to 0.5 mg daily.

Desacetyl-lanatoside C (in France, a controlled substance on *liste I*, i.e., a prescription drug which may not be renewed; not a controlled substance, but a prescription drug in the United States, known as deslanoside). In France, desacetyl-lanatoside C is available in solution for IV injection (1 ampule = 0.4 mg). Dosage is as follows: initial digitalization, 0.8 mg/day, emergency (acute pulmonary edema), 0.4-1.6 mg/day, maintenance, 0.4-0.6 mg/day (adults).

• SQUILL, *Drimia maritima* (L.) Stearn = *Urginea maritima* (L.) Baker, Liliaceae

Until recently (1991) the French pharmaceutical industry was marketing proscillaridin, a secondary glycoside prepared from the bulbs of various squills, *Drimia maritima*, but also *D.indica* (Roxb.) Jessop (= *U. indica* [Roxb.] Kunth). This compound remains in use in other countries of the European Union.

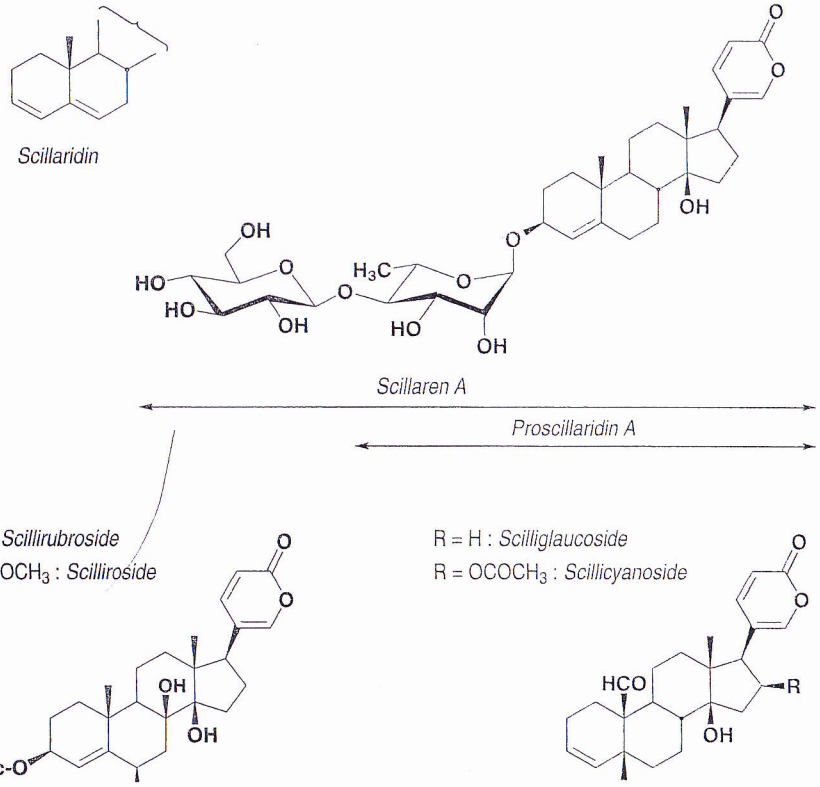
For a long time, the belief was that there were two varieties of squill, the white squill, containing scillarenin glycosides, and the red squill, containing scilliroside. In reality, *U. maritima* is an aggregate of at least six species. Some have a white bulb: examples are *U. maritima* (L.) Baker in the strict sense of the term (a hexaploid indigenous to the Iberian peninsula), *U. hesperia* Webb. and Berth. (a tetraploid from Tenerife), *U. pancration* (Steinh.) Philippe (a diploid from southern Italy), and a tetraploid species from Egypt. Others have a red bulb (*U. numidica* [Jord. & Fourr.] Grey, a Tunisian tetraploid), or a bulb ranging from white to red (*U. aphylla* [Forskal] Speta, a tetraploid from Greece and Turkey). We shall see below that these species have different compositions and that the traditional distinction between white and red squill is insufficient.

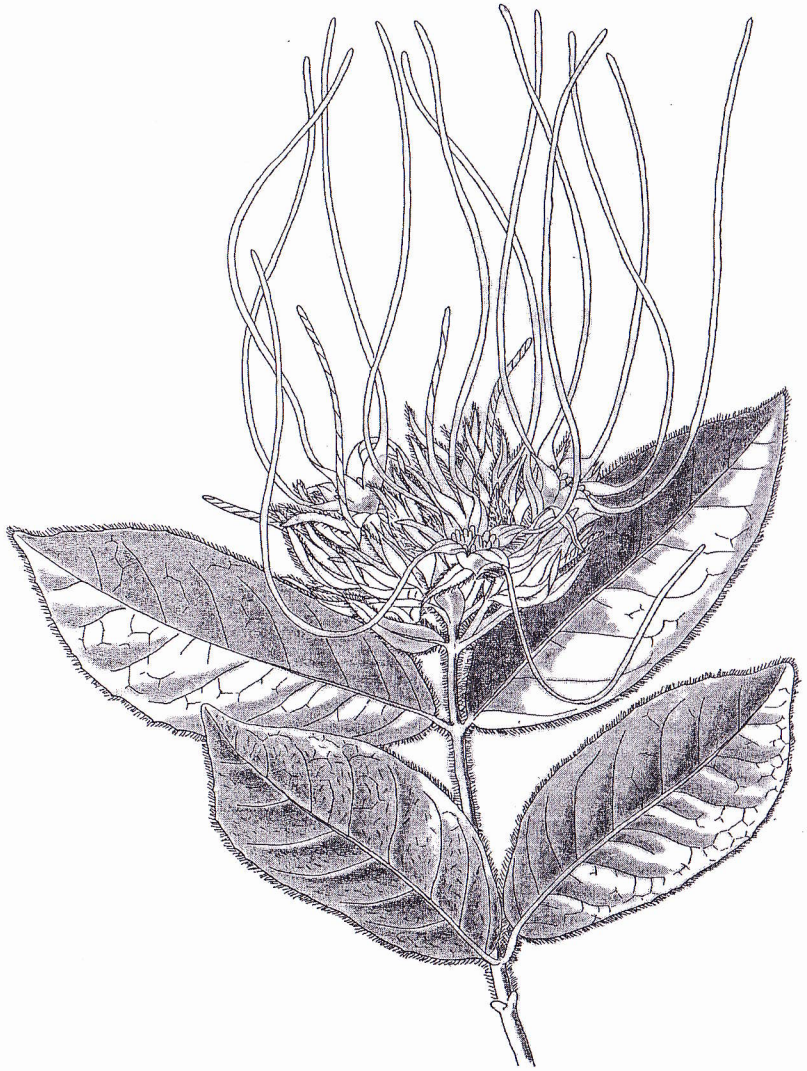
The Plant, the Drug. The squill is a perennial Mediterranean plant with a

kilograms. The leaves are entire and lanceolate; the flowers are trimerous with petaloid sepals, and are arranged in a long tight raceme on a floral stalk (1-2 m). The drug was the subject of a monograph in the 8th edition of the French Pharmacopoeia; it is still official in several countries (for example in DAB 10, and in the *British Pharmacopoeia* = BP 1988 [which also lists *D. indica*]). The inner scales, thick and fleshy, are cut in the transverse direction to facilitate drying. As a result, the drug consists of translucent strips of variable color; it is hygroscopic and difficult to store. The cut and the powder are characterized by the presence of large raphides of calcium oxalate (200-500 x 5-15 µm), which are either isolated or bundled.

Chemical Composition. Although the bulb is known to contain fructanes, condensed tannins, and flavonoids, the focus of attention is the cardiac glycosides.

The bulb of *D. maritima s. s.* contains up to 4% bufadienolides. The chief constituents are scillarenin glycosides or scillarigenin (glucoscillaren A and scillaren A), or its 11β-hydroxylated derivative, namely scilliphaeosidin (i.e., scillaphaeoside and glucoscillaphaeoside). In addition, scillicyanoside is found. Scillaren A loses one molecule of glucose on enzymatic hydrolysis to yield proscillaridin A. Note that the acidic hydrolysis of proscillaridin A does not give the aglycone (scillarenin), but rather its dehydration product, scillaridin.





STROPHANTHUS HISPIDUS DC.

The other species in the *maritima* group have a composition that differs qualitatively or quantitatively: *U. hesperia* has a similar composition, but lower concentrations (1-2% total bufadienolides), *U. pancration* does not contain scillaren A, but contains scillirubroside and scilliroside instead (even though it has a white bulb), and the latter constituent also occurs in the Egyptian tetraploids with white bulbs. In contrast, the red bulbs contain no (*U. aphylla*) or little (*U. numidica*) scilliroside.

The tetraploid form of *D. indica*—an Indian species that can be used for industrial extraction—has a composition very similar to that of *D. maritima s.s.*: it differs, however, by the absence of glucoscillaren A, and by the presence of scilliglaucosidin 3-rhamnoside; another difference is the red color which develops when a drop of iodine solution is added to the powder.

Pharmacological Properties and Uses. Proscillaridin is a heart tonic which is active orally, and is eliminated rapidly, therefore it is safe to use. It has positive inotropic, and weakly negative dromotropic and chronotropic effects, as well as specific diuretic effects superimposed on its heart tonic properties. Proscillaridin A used to be administered orally in France (1.5-2.5 mg/day for the loading dose; 1-2 mg/day for the maintenance dose). Traditionally, red squill is considered a rat poison. Anglo-saxon authors—and the British Pharmacopoeia, in agreement with them—recommend squill as an expectorant.

Scilliroside is an ingredient of granules, concentrates, pellets, and other products designed to exterminate rodents. These products are toxic and cause accidents in domesticated carnivores on a regular basis; dogs are particularly sensitive to this heart tonic. The symptoms of intoxication are those of cardiac glycoside poisoning (nausea, vomiting, rhythm alterations).

• STROPHANTHUS, *Strophanthus* spp., Apocynaceae

African *Strophanthus* are sarmentose bushes or large vines, and for a long time, they were an important source of arrow poisons. The fruits consist of 2 follicles that can exceed 30 cm in length and contain several hundred seeds. On top of the seed is a large (5-10 cm) fan-shaped pappus of silky hairs which can contain, depending on the species, 3-8% cardenolides.

Principal species: *S. hispidus* DC. and *S. gratus* (Wall. & Hook.) Baillon found from the Gulf of Guinea to Zaire; *S. kombe* Oliver, from Tanzania to Kenya; *S. sarmentosus* DC.; *S. eminii* Asch. & Pax; and *S. tholonii* Franchet.

Strophanthus has very limited uses—particularly *S. gratus*—but it remains a source of ouabain. This compound is very polar and is practically not absorbed orally. It is a heart tonic with a rapid onset and short duration of action (for IV use in emergencies). As of 1997, it is no longer marketed in France.

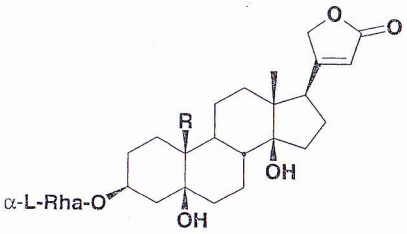
2. TOXIC PLANTS CONTAINING CARDIAC GLYCOSIDES

Several plants in our environment represent a potential danger because they synthesize cardiac glycosides. Although some of them have been used for criminal purposes, or in suicide attempts, they are rarely responsible for serious intoxications: most of the time, the marked bitterness of the glycosides and the early vomiting often induced by their absorption, prevent the ingestion of lethal quantities. Yet their danger should not be overlooked and these plants require great caution, especially when very young children are involved.

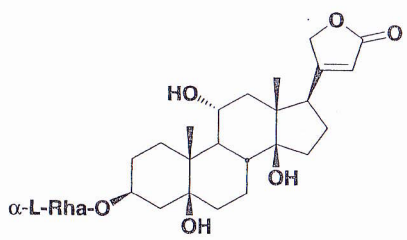
• Lily of the Valley, *Convallaria majalis* L., Liliaceae

The lily of the valley is a small herbaceous plant of the European, North American, and western Asian underwoods. Largely cultivated for its ornamental value, it is characterized by two radical cone-shaped leaves and a lateral raceme of bell-shaped flowers of delightful fragrance. The flowering tops contain saponins and 0.1-0.5% cardenolides (dried drug); these are absent from the pulp of the fruit, and mainly concentrated in the flowers and in the seeds.

The composition is complex (nearly 40 glycosides built upon about ten aglycones) and closely dependent on the geographical origin. The chief glycosides have an aglycone with a 5β-hydroxyl group and an angular methyl at C-10, which occurs either as an aldehyde (i.e., k-strophanthidin of convalloside, convallatoxin, desglucocheirotoxin), a hydroxymethyl (convallatoxol), or a methyl group (for example in locundjoside, which has an 11α-hydroxylated aglycone).



R = CHO : *Convallatoxin*
R = CH_2OH : *Convallatoxol*



Lokundjoside

Convallatoxin is a very active glycoside, but—fortunately—it is very poorly absorbed in the intestine (≤ 10%). Although the ingestion of lily of the valley is frequently at the origin of calls to poison centers, symptoms are observed in only 10-15% of cases. They are almost always gastrointestinal (nausea, vomiting). Serious intoxications seem exceptional (e.g., due to mistaking the subterranean parts with those of an edible species). The toxicity of the water from the flower vases having contained lily of the valley is often reported, but does not seem to be confirmed by

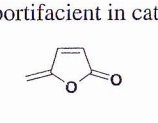
• Spring Adonis, *Adonis vernalis* L., Ranunculaceae

The aerial parts of this species were the subject of a monograph in the French Pharmacopoeia until 1972 (9th edition). This small perennial plant, with cauline leaves deeply pinnatisect and with large separate flowers with 12-18 yellow petals, is rare in France. The drug contains flavone *C*-glycosides and 0.2-0.5% cardenolides. The chief active constituent is cymarin, which can be hydrolyzed to k-strophantidin and D-cymarose. It occurs alongside adonitoxin, which yields L-rhamnose and adonitoxigenin upon hydrolysis: this aglycone differs from k-strophantidin by the absence of a 5β tertiary alcohol and the presence of a 16β secondary alcohol. There are no reports of intoxications due to this species.

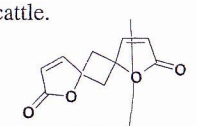
• Christmas Rose, *Helleborus niger* L., Ranunculaceae

Several glycosides with an aglycone of the bufadienolide type are thought to impart some toxicity to this species, also known as black hellebore, and cultivated for its ornamental qualities. This toxicity has been known since antiquity, and is

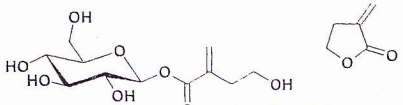
* Protoanemonin - a "hemiterpenoid" lactone - is responsible for the allergic contact dermatitis induced by various Ranunculaceae indigenous to western Europe (ranunculus [buttercup], anemone, clematis). The toxicity sometimes observed with fresh plants disappears in the dried fodder: upon drying, protoanemonin, which is the hydrolysis product of a glycoside, namely ranunculin, dimerizes to form anemonin (which is not toxic). Ranunculin is particularly abundant in *H. niger* and *R. bulbosus*. Protoanemonin imparts antibacterial properties to the pulsatilla and it is thought to be an antispasmodic. This species (*Pulsatilla vulgaris* Miller) is used in phytotherapy (fresh flowering aerial parts) and especially in homeopathy. Phytopharmaceuticals based on pulsatilla are traditionally used to relieve menstrual pain, to treat the symptoms of neurotonic disorders in adults and children, especially in case of minor sleeplessness, and for the treatment of cough [French Expl. Note, 1998]. The German Commission E monograph states that the known uses for this drug have not been substantiated. It warns that using the fresh plant can cause severe irritation of the skin and mucosal membranes, and that high oral doses can induce renal and urinary side effects. Therefore, Commission E does not authorize therapeutic use. In any event, the use of this plant must be proscribed in pregnant women; protoanemonin is cytotoxic, and it is thought to be teratogenic and abortifacient in cattle.



Protoanemonin



Anemonin



Tuliposide and Tulipalin

Products of similar structure (tulipalin A and B), which arise from the hydrolysis of glycosides (tuliposides A, B, D, E), are responsible for dermatitis and even for respiratory problems in some cases, all of which are induced, especially in horticulturists, by the handling of tulip bulbs (*Tulipa* sp.) or by *Alstroemeria aurea* Graham or *A. x ligtu* L.

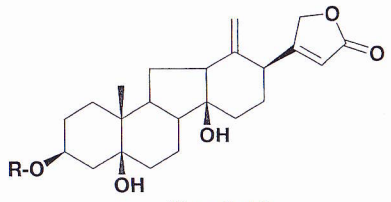
also (mostly?) due to the presence of saponins and of protoanemonin * (p. 747). The green hellebore is said to have the same properties. The intoxications are rare, and manifest themselves by tingling in the mouth and throat, vomiting, diarrhea, and mydriasis. These *hellebores* must not be confused with the white hellebore or veratrum (Liliaceae), whose toxicity is due to steroidal alkamines.

- **OLEANDER (ROSE LAUREL),**
  Nerium oleander L., Apocynaceae

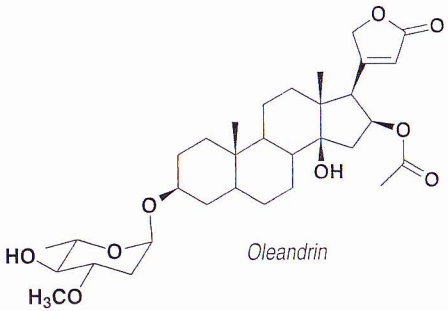
This species is a shrub or a small tree with indeciduous, coriaceous, and lanceolate leaves, which have numerous and tight pinnate secondary veins. The flowers are pink, regular, pentamerous, grouped in terminal corymbs, and they have an infundibuliform corolla. Oleander grows wild in the Mediterranean area and is also widely cultivated further north (where it must be moved into greenhouses in the winter). The leaves contain about 1.5% cardenolides: the chief constituent is oleandrin or 3-*O*-(α-L-oleandrosyl)-16-acetoxygitoxigenin. It occurs alongside related derivatives (e.g., the glycoside of gitoxigenin and 4-*O*-(β-D-glucosyl)-β-D-digitalose). Note also the presence of weakly active cardenolides (uzarigenin glycosides) or even inactive ones (glycosides of adynerigenin, of D-diginose, and D-digitalose). The drug has been used for the extraction of glycosides. The leaves and seeds are sometimes responsible for intoxications, which are caused by mistaken identification or suicide attempts. Nausea, vomiting, mental confusion, bradycardia, and hyperkalemia characterize oleander poisoning, which can cause death rapidly (ventricular fibrillation). Because the leaf is tough and bitter, the accidental poisoning of young children is typically without serious consequences. Oleander poisoning in companion animals, particularly in dogs, are not that rare, and in areas of the world where the species is abundant (e.g., Middle East, California), cattle intoxications are infamous (e.g., 142 bovines died in California between 1989 and 1995).

- **YELLOW OLEANDER,**
  Thevetia neriifolia Juss. = *T. peruviana* K. Schum., Apocynaceae

This shrub, indigenous to tropical America, has been introduced in most of the warm areas of the globe because of its striking ornamental qualities: leaves with secondary venation pinnate and dense, flowers with a spread out corolla, yellow, with overlapping lobes. The seeds contain cardenolides which have been used in therapeutics. Their composition is complex: thevetosides and gentiobiosyl-thevetosides of digitoxigenin (e.g., thevetin B), of uzarigenin, and of cannogenin. The leaves contain related compounds as well as C-*nor*-D-*homo*-cardenolides, which are thevetiosides. In Sri-Lanka, yellow oleander seeds are frequently used in suicide attempts (3-4 seeds and more can be fatal). The fruit is a globose drupe the size of a plum, sometimes tempting to young children. The symptoms observed after leaf, fruit, or seed ingestion are typical of cardiac glycoside poisoning



Thevetioside



Oleandrin

- **DIGITALIS**

Intoxications in humans are exceptional (the bitterness is highly deterrent). They can occur out of mistaken identification (with comfrey, borage [?], and others), or out of ignorance. Poisoning in animals is just as rare (contaminated fodder).

3. BIBLIOGRAPHY

Digitalis

Gheorghiade, M. (1997). Digoxin Therapy in Chronic Heart Failure, *Cardiovasc. Drugs Ther.*, **11**, 279-283.

Haïat, R. (1997). Substances inotropes positives (digitoxine, digoxine) - Principes et règles d'utilisation, *Revue Prat.*, **47**, 1723-1727.

Hauptman, P.J. and Kelly, R.A. (1999). Digitalis, *Circulation*, **99**, 1265-1270.

Hoelz, H., Kreiss, W., Haug, B. and Reinhard, E. (1992). Storage of Cardiac Glycosides in Vacuoles of *Digitalis lanata* Mesophyll Cells, *Phytochemistry*, **31**, 1167-1171.

Ikeda, Y., Fujii, Y., Nakaya, I. and Yamazaki, M. (1995). Quantitative HPLC Analysis of Cardiac Glycosides *Digitalis purpurea* Leaves, *J. Nat. Prod.*, **58**, 897-901.

Smith, N., Temple, W. and Woods, D. (1993). Acute Toxicity of Drugs. 2. Digoxin, *Pharm. J.*, 179-181.

Taboulet, P. (1993). L'intoxication digitalique. Encore trop de morts malgré l'efficacité des anticorps spécifiques, *Revue Prat. (Médecine Générale)*, **7**, (217), 35-41.

Wiegrebe, H., and Wichtl, M. (1993). High-performance Liquid Chromatographic Determination of Cardenolides in *Digitalis* Leaves after Solid-phase Extraction, *J. Chromatogr.*, (630), 402-407.

Toxic Plants

Abe, F., Yamauchi, T., Yahara, S. and Nohara, T. (1994). Glycosides of 19-Formyl-thevetiogenin and 5α-Thevetiogenin from *Thevetia neriifolia*, *Phytochemistry*, **37**, 1429-1432.

Ahlawat, S.K., Agarwal, A.K. and Wadhwa, S. (1994). Rare Poisoning with Cerebra Thevetia (yellow oleander): a Report of Three Cases, *Trop. Doct.*, **24**, 37-38.

Brustbauer, R. and Wenisch, C. (1997). Bradykardes Vorhofflimern nach Genuß von