aminopropionitrile, and γ-*N*-oxalyl-L-α,β-diaminopropionic acid (= ODAP), the principal agents responsible for osteolathyrism and neurolathyrism, respectively.

Vetches (*Vicia* sp., Fabaceae) may also cause cattle intoxication (neurolathyrism) due, in the case of *V. sativa* L., to a closely related substance, β-cyano-L-alanine.

B. *Leucaena* spp., *Mimosa* spp.: Mimosine

Biosynthetically, mimosine (= β-[*N*-(3-hydroxy-4-pyridyl)]-β-aminopropionic acid) and its metabolite are derived from lysine. These substances are responsible for the toxicity of certain Mimosaceae of southeast Asia and Australia, particularly *Leucaena.* Intoxication manifests itself by hair loss, followed by loss of appetite and weight, delayed growth, and perturbations of thyroid function. Mimosine inhibits the synthesis of proteins and nucleic acids.

C. Hypoglycins

This term designates the toxic principles of *Blighia sapida* König, a Sapindaceae from the Caribbean Islands and from Florida, originally native to Africa. This tree is cultivated for its fruits, the *ackees*: the arils that surround the base of the seeds are edible when ripe. Consuming the arils and the seeds of unripe fruits causes violent vomiting, convulsions, coma, and death. Severe hypoglycemia is observed, sometimes accompanied by hypokalemia. The toxic substances are methylene-cyclopropane-type amino acids, hypoglycin A (= 2-amino-4,5-methanohex-5-enoic acid) and its dipeptide form, hypoglycin B (conjugated with glutamic acid). During maturation, they disappear from the aril but not from the seed. These amino acids are metabolized to methylenecyclopropylacetic acid, which blocks the transport of fatty acids, acylCoA dehydrogenases, and neoglucogenesis: an energy deficit ensues, and is compensated by an intense acceleration of carbohydrate catabolism, hence the hypoglycemia which characterizes the intoxication.

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Amino Acid Derivatives

Cyanogenic Glycosides *

1. INTRODUCTION

Cyanogenesis is the ability of certain living organisms, plants in particular, to produce hydrocyanic acid. Except for the cyanolipids of Sapindaceae, cyanogenic substances are always glycosides of 2-hydroxynitriles commonly known as cyanogenic (or cyanogenetic*) glycosides.

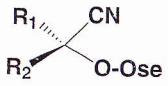
Although these are often referred to as cyanogenetic, the term cyanogenic seems more

Hydrolysis of these glycosides by endogenous glucosidases, then by hydroxynitrile lyases, generally follows tissue rupture induced by physical processes, such as crushing, chewing, or fungal infestation, which puts in contact the vacuolar glycosides and the cytoplasmic enzymes.

Cyanogenetic ability is common in the vegetable kingdom, in Filices, Gymnosperms, and Angiosperms; it is particularly pronounced in certain families: Rosaceae, Fabaceae, Poaceae, Araceae, Euphorbiaceae, Passifloraceae, and more. All organs of a plant may elaborate such compounds. In some cases, and this is probably to be related to a protective role, cyanogenesis is associated with a specific vegetative state, generally with young organs in an active growing phase (see sorghum).

2. STRUCTURE AND CHIEF TYPES OF CYANOGENIC GLYCOSIDES

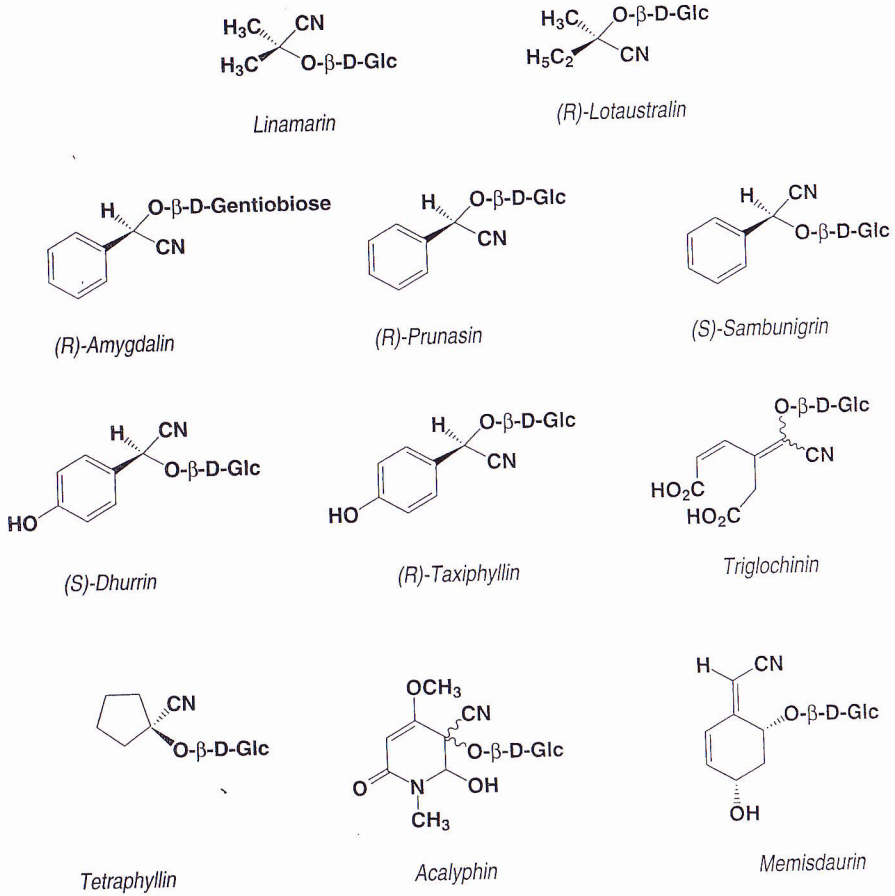
For convenience, the fifty or so known compounds may be classified as a function of the amino acid which is their biological precursor: phenylalanine, tyrosine (with an aromatic R1), leucine, isoleucine, valine (with an aliphatic R1 or aliphatic R1 and R2). In some cases R1 and R2 are part of a ring. Examples are cyclopentenoid derivatives from Violales (Passifloraceae, Flacourtiaceae), which probably arise from the metabolism of L-2-cyclopentene-1-glycine; another example is acalyphin, which is structurally close to the non-cyanogenic 3-cyanopyridones known in the Euphorbiaceae (see castor). One particular case is that of menisdaurin, found in the holly (*Ilex aquifolium* L.) berry: it does not release HCN by hydrolysis because of the cyanomethylene arrangement (moreover this type of compound itself is scarcely toxic).



The monosaccharide combined with the α-hydroxynitrile is almost always β-D-glucose, which may itself be linked to a second sugar (for example, amygdalin is the β-gentiobiosyl derivative of (*R*)-mandelonitrile); also known are glycosides with disaccharides as their sugar component, and in which a deoxyhexose is linked directly to the aglycone. Since the aglycone carbinol carbon is most often asymmetric (R1 ≠ R2), pairs of epimers arise. These are, as a general rule, produced by different plants (for example: (*S*)-dhurrin of sorghum and (*R*)-taxiphyllin of *Juniperus* sp. or *Phyllanthus* sp.). It is not rare for one compound to be produced by species pertaining to distant phyla (prunasin, linamarin, lotaustralin).

3. PROPERTIES, DETECTION, AND EXTRACTION

Glycosides of 2-hydroxynitriles are readily hydrolyzed at near neutral pHs by



Structure of cyanogenic glycosides

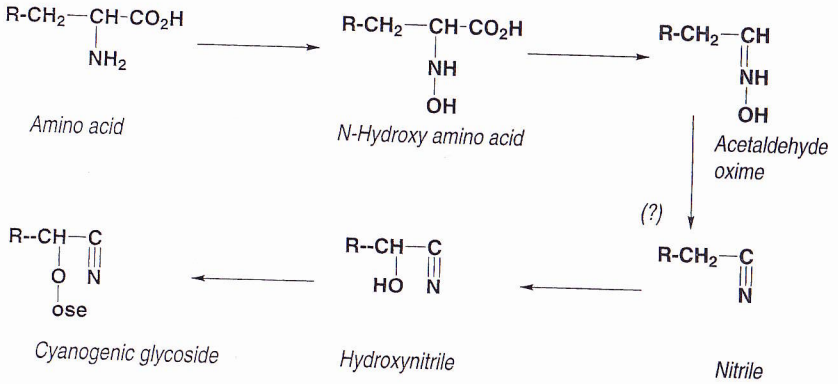
cyanohydrin. The latter is unstable and dissociates to hydrocyanic acid and a carbonyl compound, either an aldehyde or a ketone; this second reaction is catalyzed by a hydroxynitrile lyase. In mildly acidic medium and at elevated temperatures, the glycosides are hydrolyzed as they would be in the presence of glucosidases, and at near neutral pHs, the decomposition of the cyanohydrin is spontaneous and very rapid. The behavior in mildly alkaline medium varies with the structure: HCN is formed (as in acidic medium) or the nitrile group is transformed into a carboxylic acid without hydrolysis of the glycoside bond. If the structure includes an electron-withdrawing group, epimerization is easier and can take place at high temperature and at pH 7; it is enhanced in alkaline conditions.

This great fragility of cyanogenic glycosides makes their extraction and purification delicate. They require preliminary inhibition of the enzymes (by cooking in liquid nitrogen) and the use of alcohols and of chromatographic

Cyanogenic glycosides are easy to detect with a strip of filter paper impregnated with reagents able to give a color reaction with the hydrocyanic acid released upon crushing the plant material (e.g., picric acid/sodium carbonate or benzidine/cupric acetate). The impregnated strip of filter paper is placed at the opening of a test tube containing a small amount of the bruised drug. A classic quantitation method consists of suspending the drug in acidic water, then steam distilling, and titrating the hydrocyanic acid in the distillate with silver nitrate. GC analysis of trimethylsilyl derivatives is a more convenient means of identifying and simultaneously estimating the glycosides, even if their overall concentration is low.

4. BIOSYNTHETIC ORIGIN AND METABOLISM

These compounds arise from amino acids *via* the corresponding aldoximes, as shown by labeling experiments. The process is thought to involve two enzymatic complexes (or two multifunctional proteins), whereby it avoids immediate degradation of the intermediates. Normal catabolism of these glycosides frees hydrocyanic acid, which is immediately converted to asparagine, *via* the β-cyanoalanine formed by reaction of HCN with cysteine.



Principles of the biogenesis of cyanogenic compounds

5. TOXICITY OF HYDROCYANIC ACID AND OF CYANOGENIC PLANTS

Although hydrocyanic acid is a violent poison, it is important to remember that oral intake of cyanogenic drugs does not necessarily cause severe intoxication. This is because the range of dangerous concentrations (0.5-3.5 mg/kg) can only be achieved by rapid and massive ingestion of plant parts rich in cyanogenic glycosides: in the case of fruits, the pulp does not contain glycosides; in the case of leaves, the glycoside content is often high, but in general the leaves are not especially appetizing (e.g., cherry laurel leaves). In addition, the glycosides must be hydrolyzed in the digestive tract. Moreover, the human

rapidly detoxify cyanides to thiocyanates using a thiosulfate sulfurtransferase (= rhodanese); the resulting thiocyanates are eliminated in urine (30-60 mg/h).

Massive intoxication manifests itself by multiple symptoms that result from the cytotoxic anoxia caused by the combination of cyanide ions with cytochrome C oxidase; the reoxidation of cytochrome C is interrupted and molecular oxygen can no longer be used by the cell. A change in respiratory rhythm is frequently observed (acceleration and amplification), as well as headaches, dizziness, and inebriation. Next are consciousness disturbances, followed by a deep coma and respiratory depression. If the dose is small enough to not cause rapid death, an appropriate treatment must be applied expeditiously: stomach pumping, oxygen therapy, amyl nitrite, chelation of cyanide ions by hydroxycobalamin infusion, and stimulation of detoxification mechanisms (with sodium thiosulfate).

6. INTEREST IN CYANOGENIC PLANTS

Around 1970, a controversy developed regarding amygdalin and its hypothetical activity in cancer patients. Subsequently, rigorous trials demonstrated without ambiguity that this type of product (Laetrile) was totally devoid of activity, and that its use was irrational and dangerous. Only one species still finds use in pharmacy, namely the cherry laurel.

• CHERRY LAUREL, *Prunus laurocerasus* L., Rosaceae

Fresh cherry-laurel leaves are used to prepare cherry-laurel water. Titrated to contain 100 mg/100 g in total HCN (Fr. Ph., 10th Ed.), this water is used as an aromatizing agent, antispasmodic, and respiratory stimulant.

The Plant. This species, native to eastern Europe, is an evergreen shrub, with clusters of white flowers and with small ovoid drupes which are red at first, then turn black as they ripen; it is largely used for its ornamental qualities, especially in hedges. The leaf blades (12-15 x 5-7 cm) are entire, oblong, acuminate, coriaceous, shiny, and bear rounded nectaries near the junction to the petiole and on the underside. When crushed, they release a characteristic bitter almond odor. The prunasin (= (–)-(*R*)-mandelonitrile-β-D-glucoside) level ranges from 1.2 to 1.8 g per 100 g of fresh leaves.

Uses. The sole use of the drug is to obtain cherry-laurel water. Standardized to contain 100 (± 5) milligrams of total hydrocyanic acid per 100 grams, it must not contain more than 25 milligrams per 100 grams of the same acid in the free state; the minimum level of benzaldehyde is 300 milligrams per 100 grams. The preparation is identified by precipitation of CN^- ions as ferrocyanate, and by TLC detection of

benzaldehyde by gravimetry after precipitation as phenylhydrazone. The preparation must be kept in a tightly closed container away from light.

Traditionally, cherry-laurel water is used in the formulation of syrups for the treatment of broncho-pulmonary conditions, as a flavor and as a respiratory stimulant (some say it counterbalances, in opiate-containing syrups, the corresponding respiratory depressant effect).

Anglo-Saxon countries use the wild black cherry tree bark (*P. serotina* Ehrh.) or Virginian prune bark in the same type of preparation. The drug contains 0.2-0.3% prunasin, and is traditionally believed to have sedative and expectorant properties.

7. PLANTS WITH TOXIC POTENTIAL FOR HUMANS OR ANIMALS

A. Ornamental and Fruit Crop Rosaceae

A number of ornamental Rosaceae elaborate cyanogenic glycosides, including prunasin which predominates in vegetative organs, and amygdalin (= (–)-(*R*)-mandelo-nitrile-β-D-gentiobioside) which accumulates in the seeds. Thus these plants can release hydrocyanic acid.

• COTONEASTER, *Cotoneaster* spp., Pyracantha, *Pyracantha* spp.

Cotoneasters are thornless bushes, very ramified, with entire leaves and small fruits which are most often red. They are commonly used as ground cover or as borders in parks and gardens. The bark, leaves, flowers, and fruits are cyanogenic. The glycoside content of the fruit varies with the species and the degree of maturity; with the exception of *C. congestus* Baker and *C. praecox* Vilm., it is less than 50 mg/100 g (dry weight).

Pyracanthas are evergreen thorny shrubs with fruits that attract children just as often as those of cotoneaster. The vegetative organs are devoid of cyanogenic glycosides, and the fruits only contain a very small amount.

Young children frequently ingest the fruits of these common species. Poison control center statistics show that even in the most serious cases, only some gastrointestinal signs are observed. The real danger of Pyrancantha is probably its sharp thorns.

• MOUNTAIN ASH, *Sorbus aucuparia* L.

The fruits of this small tree with imparipinnate leaves and white flowers gathered in corymbs have a reputation for being antidiarrheal. Sometimes consumed as jam-

110 mg/100 g), and a glycoside whose lactone aglycone, parasorbic acid, may be irritating to the digestive tract mucosas. The seeds contain only traces of amygdalin.

• CHERRY LAUREL, *Prunus laurocerasus* L.

The leaves are rich in prunasin (see above), and the concentration of amygdalin in the seeds is substantial. In contrast, the pulp of the fruit, the only part of the plant that looks and tastes good enough to consume, has a very low level of cyanogenic glycosides. This uneven distribution of the toxic principles explains the contradictory literature on cherry-laurel intoxication: the seeds are most often spit out or swallowed whole, in which case intoxication is non-existent or almost unnoticed, or else they are chewed and the patient may present with some general symptoms (headaches, sleepiness, tachycardia in fewer than 2% of cases; when symptoms are observed, in four out of five cases they are digestive).

Cherry laurel clippings, like the leaves of North American *Prunus* (*P. serotina* Ehrh., *P. virginiana* L.), are toxic to herbivores, especially ruminants, and must not be left within their reach.

• APRICOT TREE, *Prunus armeniaca* L. and other *PRUNUS*

The pit or stone (the cotyledons of the seed) of the apricot, like the cotyledons of the seeds of various fruit crop Rosaceae (peach, plum, and especially bitter almonds [p. 138]) can cause more or less serious accidents because they contain amygdalin. Some cultivars of "bitter" apricot have a glycoside content equivalent to 240-350 mg HCN/100 g (apricot tree from the Balearic Islands) whereas the "sweet" cultivars contain almost none. Children are generally the poisoning victims. The intoxication can be severe (asthenia, vomiting, headaches, hypotension, tachycardia) and the literature contains case reports of death by respiratory arrest—even proper patient management (amyl nitrite, sodium thiosulfate, pure oxygen) cannot always prevent a fatal outcome.

B. Dietary Species: Manioc

• MANIOC, *Manihot esculenta* Crantz, Euphorbiaceae

Manioc is one of the dietary plants most anciently used by mankind. Use of its starch, cassava starch, was documented as early as 3000 B.C.; today it remains the chief starch source for several hundred million people in the tropical regions of the globe. In industrialized countries, cassava is better known as tapioca (see starch-

As all of the other species in the genus, *M. esculenta* originated in America and the various cultivars that emerged throughout its long history fall into two categories, improperly referred to as sweet or bitter. Both types contain a cyanogenic glycoside (linamarin). In the case of sweet manioc, this glycoside is preferentially located in the external parts of the tubercle and is therefore eliminated by traditional preparation procedures * (scraping and steeping then cooking), whereas in the case of bitter manioc it is spread in all starch-producing tissues.

Detoxification is rarely complete and the regular ingestion of the remaining cyanides is supposedly at the origin of the chronic symptoms observed in tropical regions. The prevalence of goiters in certain regions of Africa would be due to the antithyroid activity of the thiocyanates arising from cyanide metabolism. Moreover, several experimental studies support the hypothesis that the neuropathic ataxia that is relatively frequent in the same regions may be a symptom of the chronic toxicity of cyanides. This syndrome manifests itself, among other symptoms, by atrophy of the optic and auditory nerves, polyneuropathy, and an increase in blood thiocyanates. This neuropathy could be explained by the lack of sulfur-containing amino acids, subsequent to their utilization in the metabolism of cyanide ions.

C. Fodder

Some clovers (*Trifolium repens* L.) and other Fabaceae can very rarely cause incidents involving bovines, but it is mostly sorghum that causes severe intoxications. The problem may affect bovines and sheep who graze sorghum in the field, or who are fed sorghum as green fodder (*Sorghum vulgare* Pers. var. *sudanense* or "sudan-grass", Poaceae). It is at the start of the growth, when the plants are smaller than 10 cm, that the dhurrin level is maximal; it can reach 500 mg/100 g of green sorghum. The intoxication is rapid and often fatal.

D. Special Case: Cycadales and Cycasin

Cycadales are prespermaphytes that underwent considerable development in the Mesozoic era. Only a few dozen species survived that are distributed in nine genera, all of them in tropical and subtropical regions of the globe, as well as in a few more temperate regions: *Cycas* (from the Pacific to the Indian Ocean, and from the south of Japan to Australia), *Encephalartos* (Africa), *Macrozamia* (Australia), *Zamia, Ceratozamia* (America), and more. Plants of intermediate habit between those of tree ferns and palm trees, they have, for the most part, a medulla and fertilized

* For some authors, the rationale for the traditional preparation procedures is far from being that obvious; the same comment applies to the distinction between the bitter and sweet types and their distribution. See Nye, M.M. (1991). The Mis-measure of Manioc (*Manihot*

ovules rich in starch, hence their traditional use in human diet, particularly in Asia (*C. revoluta* Thunb., *C. circinalis* L.).

Cycadales toxicity is well known in Australia, where *Cycas* and *Macrozamia* are responsible for intoxications which result, in sheep, in hepatic cirrhosis with occlusion of the hepatic veins. In bovines, the observed symptoms are commonly ataxias linked to the neurotoxicity. Toxic symptoms (sclerosis, parkinsonism, dementia) have also been observed in humans following the ingestion of *Cycas*-based preparations. Experiments have shown a correlation between the acute toxicity and cycasin, a glycoside of methylazoxymethanol (MAM) found in all organs in the incriminated species, and sometimes referred to—erroneously—as "pseudo cyanogenic". It was also demonstrated that cycasin and MAM are carcinogens, when given by mouth to rats and other animals, and induce hepatic, intestinal, and renal tumors. The origin of the neurodegenerative manifestations observed in humans who consume *Cycas* remains disputed. They may be linked to an amino acid, β-methyl-amino-L-alanine—cycasin is eliminated by the food preparation procedure, therefore it is not at fault—or to a contamination of the flours.

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