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# Toxic Chemical Compounds of the Solanaceae

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***This paper is dedicated to Professor P. Joseph-Nathan for his 65th birthday.***

The Solanaceae is comprised of some 2500 species of cosmopolitan plants, especially native to the American continent. They have great value as food, like the well-known potato, tomato and eggplants, and medicines, like species of *Atropa*, *Withania* and *Physalis*, but many plants of this family are toxic, and sometimes lethal to mammals, in particular to man. Some of them also produce hallucinations and perceptual changes. The toxic species of this family are characterized by the occurrence of a variety of chemical compounds, some of which are responsible for the toxicity and lethality observed after ingestion, while others are suspected to be toxic. In this review, the following toxic compounds belonging to different members of the Solanaceae family are described: Tropane alkaloids (*Atropa, Datura, Hyoscyamus, Mandragora*); pyrrolidine and pyrrolic alkaloids (*Nierembergia*, *Physalis*, *Solanum*); protoalkaloids (*Nierembergia*); glycoalkaloids (*Lycopersicon*, *Solanum*); nicotine (*Nicotiana*); cardenolides (*Cestrum*, *Nierembergia*); capsaicinoids (*Capsicum*); kaurene-type tetracyclic diterpenes (*Cestrum*); steroidal glycosides (*Cestrum*, *Solanum*); 1,25-dihydroxyvitamin $D_3$ and vitamin $D_3$ (*Cestrum, Solanum, Nierembergia*); and withasteroids, withanolides (*Withania*), and physalins (*Physalis*). Other bioactive chemical constituents of members of this family are sugar esters and lectins. Phenylpropanoids are not included in this paper.

**Keywords:** Solanaceae, toxic compounds.

The Solanaceae contains some 2500 species of cosmopolitan plants, which are shrubs, vines, trees and bushes, especially native to the American continent. Many plants of this family have great value for man as food (for example potatoes, tomatoes, eggplants, and peppers), some of which were introduced into Europe after the conquest of America [1]. Many others are ornamental plants, while a great number are toxic, and sometimes lethal to mammals, in particular to man [2-4]. Furthermore, a group of Solanaceae is known to produce hallucinations and perceptual changes.

This family is characterized by the occurrence of a variety of chemical compounds [5-7]. Some of them are responsible for the toxicity and lethality in mammals after ingestion of solanaceous plants, while others are suspected to be toxic. There are also compounds that have been earlier reported as showing some extent of toxicity, but their beneficial biological activities encouraged research in this latter sense.

The toxic solanaceous compounds are:
(**1**) **Tropane alkaloids:** Genera: *Atropa*; *Datura*; *Hyoscyamus*; *Mandragora*.
(**2**) **Pyrrolidine and pyrrolic alkaloids:** Genera: *Nierembergia* (example, *N. hippomanica*); *Physalis* spp; *Solanum* (example, *S. sturtianum*).
(**3**) **Protoalkaloids: Phenethylamines:** Genus: *Nierembergia*.
(**4**) **Glycoalkaloids:** Genera: *Lycopersicon*; *Solanum.*
(**5**) **Nicotine:** Genus: *Nicotiana* (example, *N. tabacum*).
(**6**) **Cardenolides:** Genera *Cestrum* (example, *C. parqui*); *Nierembergia* (example, *N. aristata*).

(**7**) **Capsaicinoids:** Genus: *Capsicum*.
(**8**) **Kaurene-type tetracyclic diterpenes:** Genus *Cestrum* (example, *C. parqui*).
(**9**) **Steroidal glycosides (neutral saponins):** Genera: *Cestrum* (example, *C. parqui*); *Solanum* (example, *S. nigrum*).
(**10**) **1,25-Dihydroxyvitamin $D_3$ and vitamin $D_3$:** Genera: *Cestrum* (example, *C. diurnum*); *Solanum* (example, *S. glaucophylum*, also called *S. malacoxylum*); *Nierembergia* (example, *N. veitchii*).
(**11**) **Withasteroids:** (**a**) **Withanolides:** Genus: *Withania*. (**b**) **Physalins:** Genus: *Physalis*.
(**12**) **Other bioactive chemical constituents:**
(**a**) **Sugar esters**. (**b**) **Lectins**.

(**1**) **Tropane alkaloids:** Tropane alkaloids are distributed within the families Solanaceae, Erythroxylaceae, Proteaceae, Euphorbiaceae, Rhizophoraceae, Convolvulaceae and Cruciferae. These alkaloids are characteristic of the genera *Datura*, *Brugmansia* (tree datura) and *Duboisia* of the Solanaceae. However, the distribution is more widespread, with novel tropane derivatives in families not traditionally associated with these bases. The chemotaxonomy and geographical distribution of tropane alkaloids have been reviewed [8].

The starting compound for the biosynthesis of the tropane alkaloids is ornithine and methylornithine is the first intermediate. Plants containing these alkaloids have been used throughout recorded history as poisons, but many of the alkaloids do have valuable pharmaceutical properties. The biotechnological production of natural products of pharmaceutical value, based on genomic tools has been discussed [9].

Several recipes for preparing ointments and beverages from medieval times used to include psychoactive Solanaceae plants [10]. In fact, these plants are drugs with a lot of tradition in the history of witchcraft [11], for example, the European plants known as '*the trio of the delirium'*: deadly nightshade (*Atropa belladonna*) [12], henbane (*Hyoscyamus niger*) [13], and mandrake (*Mandragora officinarum*) [14]; also some *Datura* species, for example, *D. metel* ('floripondio'), *D. ferox* ('chamico'), *D. stramonium* (‘stramon’; ‘devil's fig’), which is related to the Mexican 'toloache' (*D. innoxia*) that was used by the famous sorcerers of Catemaco in Veracruz and in other regions of Mexico, and *Datura* (*Brugmansia*) *insignis*, *D. suaveolens*, *D. aurea* and *D. arborea* (the last three species known as 'floripondios' in Mexico, 'yas' or 'borrachero' in some regions of Central America, and 'estramonios' in Spain). All these *Datura* species are biologically complex, and have been used as hallucinogens from very ancient times, mainly in the Andes and in the Amazon region, where they are called 'toá' [15,16]. These species contain atropine, scopolamine and hyoscyamine as active principles. Different analytical methods have been reported for the analysis and separation of these toxic alkaloids. Recently, high performance capillary electrophoresis was successfully used [17].

Unlike other hallucinogens, these tropane alkaloids do not increase the sensorial perception, although their effects take place at very diverse levels: mouth dryness, tachycardia, body temperature increase, pupil enlargement, mental confusion, conscience obnubilation, and loss of recent memory. Drowsiness, delirium and coma are shown with high doses [18].

When the Spaniards arrived in America, they found that the aborigines not only had surprising herbalist knowledge, but also they used plants for transforming their consciousness to other realities. In spite of the American Inquisition, the traditions and secrets of the herbs have survived in this continent, blended with tradition and European religion, leading to the phenomenon of witchcraft-chamanism that is still present in many towns of the American continent [19].

*D. innoxia* ('toloache'), native to America, has been used for therapeutical and ritual purposes since before the arrival of the Spaniards to the continent. The ‘yaquis’ and the ‘zuni’ ethnic groups attributed to the drug the power of flying or transporting the soul toward the infinite. The ‘navajos’ used it to induce visions, to diagnose illnesses and to heal. Some North American tribes also used this *Datura* species for some adolescence initiation rites, in which the symbolic transit between death and rebirth justified the potent preparations. In Mexico, the use of this drug has not diminished either in the religious magic ceremonies or as a therapeutic agent.

The main alkaloid of *D. innoxia* is scopolamine (Figure 1), and in lesser proportion, atropine. Atropine is the racemic form of hyoscyamine (Figure 2), and is used to dilate the pupils of the eye. Atropine is also a central nervous system (CNS) stimulant, and is used for the treatment of nerve gas poisoning. It is known that scopolamine is an authentic hallucinogen.

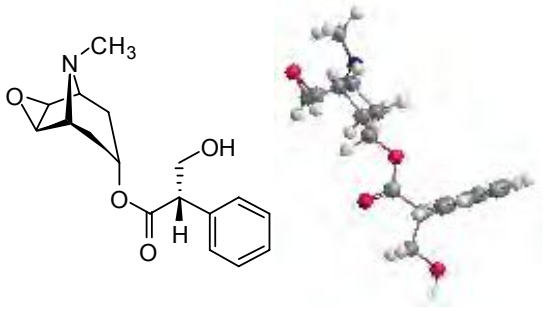
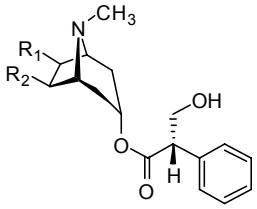


**Figure 1**: (-)-Scopolamine (hyoscine).

Hallucinations are not only visual, as with LSD or mescalin, but also auditory and even tactile. Often, contact with reality is completely lost, and an external observer can see the intoxicated subject sustaining incoherent talk with nonexistent people or behaving out of context [19,20].

*D. stramonium*, also referred to as jimsonweed, thorn apple, stramon, and Jamestown weed, is a hallucinogenic plant that causes severe anticholinergic toxicity after accidental, recreational or intentional consumption of any part of the plant [21]. All parts of the plant, especially the seeds, contain hyoscyamine and scopolamine as toxic principles. Clinical symptoms are those of atropinic poisoning, particularly dryness of mouth, mydriasis, flushing, tachycardia, and agitation, as well as visual and auditory hallucinations [22,23]. Severe and even fatal complications (coma, respiratory distress, and death in more than 5% of cases) are not rare since the lethal concentration of hyoscyamine and scopolamine is close to the level at which delirium occurs [24]. The presence of tropane alkaloids in urine was demonstrated by gas chromatography-mass spectrometry (GC-MS) [22]. Blood samples taken twelve hours after *D. stramonium* ingestion were analyzed by liquid chromatography and mass spectrometry (LC-MS/MS) [24]. *D. stramonium* poisoning of horses has also been reported [25,26]. The outbreak was characterized by protracted and repeated colic attacks due to impaction of the large colon and/or caecum without any other anti-muscarinic signs. *D. stramonium* seed extract has a rapid onset of effects and was shown to be useful for treatment of organophosphate poisoning [27].

*D. ferox* ('chamico') is also a very toxic plant, which causes important cattle losses. We have studied the poisoning of farm animals in Argentina due to the accidental ingestion of chamico seeds mixed with the animals' food, and further isolated the lethal tropane alkaloids [28].



| | |
|---|---|
| (-)-Hyoscyamine | $R_1$=R2=H |
| 6β-Hydroxyhyoscyamine (Anisodamine) | R1=OH, R2=H |
| 7β-Hydroxyhyoscyamine | R1=H, R2=OH |
| 6β,7β-Dihydroxyhyoscyamine | R1=R2=OH |

**Figure 2**: Hyoscyamine derivatives.

Recently, the genes involved in tropane alkaloid biosynthesis have been reported and an unusual cytochrome $P_{450}$ ($CYP_{80}F_1$) has been identified using a combined EST and virus-induced gene silencing (VIGS) approach [29].

(-)-Scopolamine is a (-)-(1*S*,3*S*,5*R*,6*R*,7*S*,8*S*)-6,7-epoxy-3-[(*S*)-tropoiloxy]tropane, also known as hyoscine (Figure 1). The difference from atropine (racemic form of hyoscyamine) (Figure 2) is an oxygen bridge between C-6 and C-7.

Atropine, hyoscyamine, scopolamine and hyoscine are parasympatholytic compounds. Atropine and scopolamine cause a central and peripheral anticholinergic blockade of the muscarine receptors located in the CNS, heart, intestine and other tissues. Psychiatric symptoms include restlessness, excitement, hallucinations, euphoria, and disorientation, but also stupor, coma and respiratory depression [30].

As result of the parasympathic system inhibition, salivary and stomach secretions diminish, thus giving rise to mouth dryness [31]. The symptoms also include mydriasis with slow reaction to light, blurred vision for near objects with occasional transitory blindness [31]; tachycardia, sometimes accompanied by hypertension, skin blushing due to skin vasodilation, sweat decrease, and hyperthermia that can reach 42°C [12].

Like atropine, and hyoscyamine, scopolamine is an anticholinergic agent that in low doses blocks the cholinergic receptors of the brain, depressing the impulses of the nervous terminals, while in high doses, these compounds cause stimulation before depression [32,33]. In doses of more than 10 mg in children and more than 100 mg in adults, scopolamine causes convulsions, severe depression,

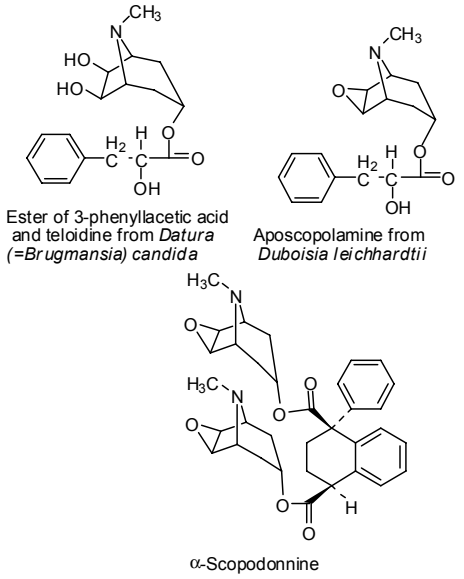



**Figure 3**

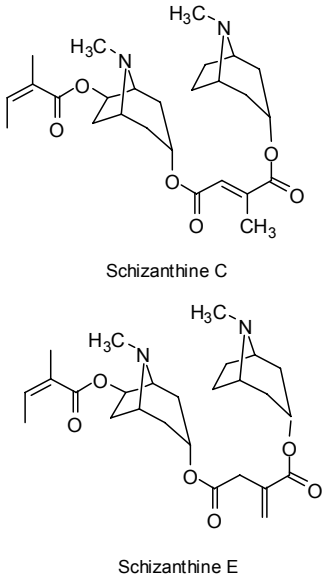



**Figure 4**

heart arrhythmias, severe tachycardia, fibrillation, breathing disturbances, vascular collapse, and death [12,34]. The maximum effect is reached after 1 to 2 hours and then it diminishes very slowly. Scopolamine has a mean life of two and a half hours, and it is metabolized in the liver, giving tropic acid and scopine. Only 10% is excreted by the kidneys without being metabolized. Traces appear in the sweat and maternal milk. It crosses the placental barrier and acts on the fetus [12].

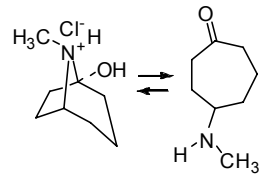
Scopolamine is still widely used in anaesthetic practice and in other medical fields [35], such as treatment of motion sickness, depression [36], abdominal pain associated with cramps induced by gastrointestinal spasms [37], and for the prevention of brain damage and cognitive dysfunction induced by toxic organophosphate nerve agents, which cause severe adverse effects and long term changes in the peripheral and central nervous systems [38-40]. The antiemetic efficacy of transdermal scopolamine has also been reported [31].

Psychopharmacological studies in humans and animals have shown that a systemic cholinergic blockade may induce deficits in learning and memory. Tan *et al.* [41] examined the effects of scopolamine on morphine-induced conditioned place preference (CPP), thus showing that the effects of the systemic cholinergic blockade on morphine-induced CPP depended on the morphine exposure time. Furthermore, scopolamine induced disruption of latent inhibition that was prevented by antipsychotic drugs and an acetylcholinesterase inhibitor [42].

Tropane alkaloids are chemical plant defenses. Scopolamine, the most abundant tropane alkaloid of *Brugmansia suaveolens*, in an artificial diet, increased mortality and prolonged the developmental time of the larvae of the generalist noctuid moth *Spodoptera frugiperda* [43].

*D.* (*Brugmansia*) *candida* also contains the ester of 3-phenyllacetic acid and teloidine (Figure 3) [44]. Aposcopolamine, and dimeric aposcopolamine, which contains two scopine rests as substituents, were isolated from *Duboisia leichhardtii* [45,46]. α- and β-Scopodonnine were obtained by dimerization of aposcopolamine [47], and were also isolated from *Datura innoxia* seeds [48] (Figure 3). Several quaternary alkyl halides were synthesized from these scopodonnines and were shown to be myorelaxants [49]. Two stereospecific oxidoreductases constitute a branch point in tropane alkaloid metabolism [50].

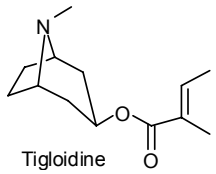
Aerial parts and roots of *Schizanthus grahamii*, *S. hookeri, S. porrigens* and *S. pinnatus* contain tropane alkaloids and in particular the ditropinesters called schizanthines A to M, and X to Z [51-53] (Figure 4). The separation of isomeric tropane alkaloids from *S. grahamii* was performed by non-aqueous capillary electrophoresis [54]. These alkaloids were further analysed by very fast gas chromatography [55], as well as being isolated and identified by two fully automated HPLC-NMR methods [56]. Recently, a rapid *in vitro* propagation system leading to formation of shoots from callus, roots, and plantlets was developed for *S. hookeri* [57].



(+)-Physoperuvine
= 5-Hydroxytropane chlorhydrate

**Figure 5**

Physoperuvine, which is a quaternary 5-hydroxy-tropanium chloride, was obtained from leaves and roots of *Physalis peruviana* (Figure 5) [44,58,59], which inhibited growth and induced apoptosis of human Hep G2 cells in culture [60].

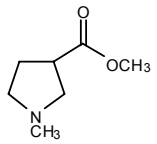


Tigloidine

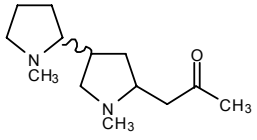
**Figure 6**

Scopolamine and tigloidine (3-β-tigloyloxytropane; Figure 6) were present in most *Physalis* and *Nierembergia* species [61,62].

(**2**) **Pyrrolidine and pyrrolic alkaloids:** Pyrrolidine alkaloids are also found in solanaceous plants, for example, hygrine and anaferine, and others in most *Physalis* species, the methyl ester of homohygrinic acid in *Solanum sturtiatum*, and *N*-methylpyrrolidinylhygrines A and B (both epimers) in *Datura innoxia* (Figure 7).

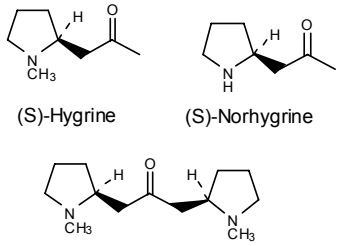


Methylester of the
homohygrinic acid
from *Solanum sturtiatum*



*N*-methylpyrrolidinylhygrine A and B
(both epimers from *Datura innoxia*)

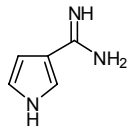
**Figure 7**



(S)-Hygrine (S)-Norhygrine

Cuscohygrine

**Figure 8**



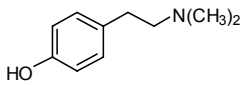
Pyrrol 3-carbamidine toxic principle of
*Nierembergia hippomanica*

**Figure 9**

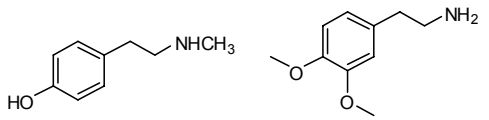
Hygrine and norhygrine (Figure 8), together with other alkaloids, steroids, and the dihydroecdysteroid 7,8-dihydroajugasterone C were isolated from *Nierembergia hippomanica* in our laboratories [61,62]. Hygrine and derivatives are also ornithine-derivatives.

The toxic principle of *N. hippomanica* was isolated and identified as pyrrole 3-carbamidine in our laboratories [63] (Figure 9). It is worth mentioning that a variety of non-toxic, acylated *O*-glycosides of flavonoids have also been isolated from *N. hippomanica* [64-66].

(**3**) **Protoalkaloids (phenethylamines):** Phenethyl-amines are not usual compounds of the Solanaceae, and they are found in other families, such as Poaceae and Cactaceae. β-Phenylethylamine, *N*-methyltyramine, tyramine, and hordenine have been isolated by us from *N. hippomanica* [61,62] (Figure 10).



Hordenine



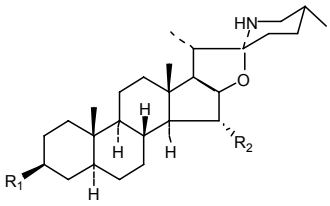
*N*-Methyltyramine 3,4-Dimethoxyphenethylamine

**Figure 10**

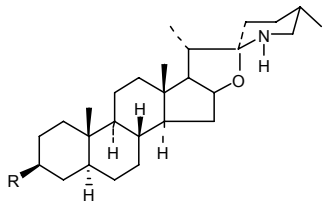
These protoalkaloids contributed to the toxicity of this solanaceous plant with peripheric nervous signs, because of being sympathomimetic agents. Phenethylamines also showed central nervous signs due to crossing the blood brain barrier, as we have demonstrated by Tc-99m labeling [67].

(**4**) **Glycoalkaloids**: The Solanaceae is characterized by the occurrence of glycoalkaloids, which are basic saponins. Since these glycoalkaloids are widely distributed in *Solanum* species, they used to be called *Solanum*-alkaloids, and also alkaloid glycosides, in which the aglycone is a steroid alkaloid, and the sugar moiety is a branched trisaccharide (triose) or tetrasaccharide (tetraose) bonded to the C-3 hydroxy group of the steroid. According to the chemical skeleton of the aglycone ($C_{27}$-steroid alkaloids = alkamines), the steroid alkaloids of the Solanaceae can have the basic structure of spirosolanols (Figure 11), solanidanes (Figure 12), 22,26-iminocholestanes (Figure 12), 3-amino-spirostanes (Figure 13), and solanocapsine/ solanopubamines (Figure 13). The last group has an amino group at C-3, and a nitrogenous function in the E/F-rings.

Spirosolanols



Solasodane skeleton $R_1$=$R_2$=H, (22 α, 25 *R*)
Soladulcine $R_1$=OH, $R_2$=H
Solasodine $\Delta^{5,6}$; $R_1$=OH, $R_2$=H.
15α-Hydroxysolasodine $\Delta^{5,6}$ ;$R_1$=$R_2$=OH,

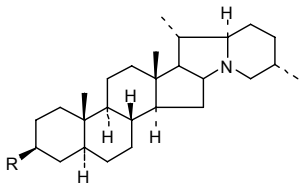


Tomatidane skeleton R=H, (22 β,25 *S*)
Tomatidine R= OH
Tomatidenol $\Delta^{5,6}$; R=OH

**Figure 11**: Spirosolanols (aglycones of the glycoalkaloids).

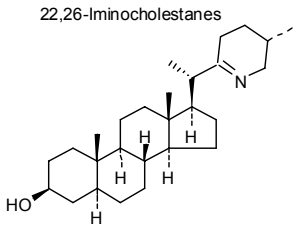
*Solanum*, *Lycopersicon* and *Physalis* species contain these toxic glycoalkaloids. Solanidine is the steroidal aglycone of some potato glycoalkaloids and a very important precursor for the synthesis of hormones and some pharmacologically active compounds [68-71].

Solidanes



Solidane skeleton R=H
Demissidine R=OH, Solanidine $\Delta^{5,6}$; R=OH
Leptinidine $\Delta^{5,6}$; HO-23; R=OH

22,26-Iminocholestanes



Solacongestidine
Solafloridine HO-16, Dihydrotomatillidine oxo-24
Tomatillidine $\Delta^{5,6}$; oxo-24

**Figure 12**: Solanidanes and 22,26-iminocholestanes.

Recently, a new chemistry model within Density Functional Theory, called CHIH-DFT, was used to calculate the molecular structure of the aglycone solanidine, as well as to predict its IR and UV spectra [72]. Likewise, the molecular structure of the glycoside γ-solanine (Figure 12) was calculated and its IR and UV-vis spectra, and some other electronic parameters were predicted [73].

Glycoalkaloids are involved in plant defenses against insects and other pests, and have a variety of adverse as well as beneficial effects in cells, animals, and humans [74,75]. The two major glycoalkaloids present in tubers of *S. tuberosum* are α-solanine and α-chaconine, which together account for 95% or more of the glycoalkaloid content. The pharmacology and toxicology of the potato glycoalkaloids, as well as the anticarcinogenic and other beneficial effects have been recently reviewed [76].

Both solanidine glycosides, α-solanine and α-chaconine, induced craniofacial malformations, whereas the spirosolanes tomatidine and tomatine were non-teratogenic, even at high dosage. Solasodine (Figure 11) was teratogenic, although at a dosage nearly ten-fold that required for terata induction by cyclopamine. Conformational analysis was used to relate induced teratogenicity to a negatively charged center accessible to the α-side of the steroidal plane. This correlation was based

upon two comparisons, that of the spirosolanes solasodine *vs* tomatidine and of 22*S*,25*R*-solanidine *vs* 22*R*,25*S*-dihydrosolanidine (demissidine) [77].

The steroidal glycoalkaloids are degradated once inside the organism to the aglycone, for example, solasodine, which may penetrate, by simple diffusion, the placental barrier and/or the brain blood barrier and impact the fetuses. *S. lycocarpum* fruit may act as a phytohormone, promoting perhaps some neural alterations that at the adult age may impair the sexual behavior of the experimental female without impairing the fertility and sexual hormone synthesis. These observed changes can be the direct consequence of the toxic actions of the steroidal alkaloid on the female offspring during fetal development [78].

Recently, the efficacy and mechanisms of *α*-solasonine and α-solamargine-induced cytolysis on two strains of *T. cruzi* were reported [79].

The ethanol extract of potato has shown antinociceptive and anti-inflammatory activities in mice [80]. Solasodine, and the methanol extract of *S. trilobatum* also showed significant antiinflammatory activity [81].

The antiproliferative activities against human colon (HT29) and liver (HepG2) cancer cells of a series of structurally related compounds were examined. Evaluations were carried out with the potato trisaccharide glycoalkaloids α-chaconine and α-solanine; the disaccharides $\beta_1$-chaconine, $\beta_2$-chaconine, and $\beta_2$-solanine; the monosaccharide γ-chaconine and their common aglycone solanidine; the tetrasaccharide potato glycoalkaloid dehydro-commersonine; the potato aglycone demissidine; the tetrasaccharide tomato glycoalkaloid α-tomatine, the trisaccharide $\beta_1$-tomatine, the disaccharide γ-tomatine, the monosaccharide δ-tomatine, and their common aglycone tomatidine; the eggplant glycoalkaloids solamargine and solasonine and their common aglycone solasodine; and the nonsteroidal alkaloid jervine [82]. All compounds were active in the assay, with the glycoalkaloids being more active than their hydrolysis products. The effectiveness against liver cells was greater than that against the colon cells. Potencies of α-tomatine and α-chaconine at a concentration of 1 μg/mL against the liver carcinoma cells were higher than those observed with the anticancer drugs doxorubicin and camptothecin. Since α-chaconine, α-solanine, and α-tomatine also inhibited normal human liver HeLa (Chang) cells, these compounds were proposed for either preventative or therapeutic treatments against carcinomas [82].

Five saponins, two steroidal alkaloids (solasodine, solanidine), and one sterol (stigmasterol) have been tested for their biological activities on human 1547 osteosarcoma cells. Differences in activity were studied in terms of proliferation rate, cell cycle distribution and apoptosis induction. By using molecular modeling, spatial conformation and electron transfer capacity were calculated. The second property has been investigated by the HOMO repartition and the corresponding energy. Correlation between the experimental and the theoretical data showed the importance of the hetero-sugar moiety and the 5,6-double bond in the biological activity (apoptosis and cell cycle arrest) on the human 1547 cell line. The importance of conformation at C-5 and C-25 carbon atoms was also discussed [83].

Glycoalkaloids have been reported to inactivate the *Herpes simplex*, *H. zoster* and *H. genitalis* viruses in humans, while the aglycones, including solasodine, might protect against skin cancer. Extracts of glycoalkaloids or solanidine were used to obtain a potential skin cancer preparation for clinical research [71]. Dried potato sprouts were used to obtain glycoalkaloids and solanidine. The yield of solanidine in a liquid-liquid system for the hydrolysis of glycoalkaloids was higher than that obtained using solid-liquid-liquid systems for glycoalkaloid hydrolysis from potato vines [71].

A cream formulation containing purified glycoalkaloids from the fruits of *Solanum sodomaeum* was effective in the treatment of malignant human skin tumors, basal cell carcinomas, squamous cell carcinomas and benign tumors, keratoses and keratoacanthomas. Steroidal glycosides were isolated from the underground parts of *S. sodomaeum*, and their antiproliferative activity against human promyelocytic leukemia (HL-60) cells was reported. Five compounds exhibited stronger activity than cisplatin [84].

The mixture of solasodine glycosides, called BEC, of the fruits of *S. linnaeanum* (devil's apple), which consisted of the triglycosides solasonine, solamargine and di- and mono-glycosides, retarded the progress of ocular squamous cell carcinoma in Hereford cattle. BEC had antineoplastic properties against a wide

variety of human cancers in cell culture, tissue culture, and was very effective against terminal tumors in animals [85].

Toxic ornamental *Solanum* species used in gardening are: *S. jazminoides* or *S. aviculare* ('solano'), *S. crispum* ('tomatillo' or 'natre'; South American plant with white fruits), *S. pseudocapsicum* ('Jerusalem cherry' bush, with small star-shaped white flowers, and red berries with many white seeds when ripening); *S. pyracanthum* (African species with violet flowers and thorny leaves), *S. seaforthianum* ('tears of San Pedro' or 'hiedro'; South American perennial vine, which can reach 6 m high, with beautiful scarlet-colored fruits), *S. woodlands* (native to Costa Rica; a climbing bush that can reach 6 m high, with very showy hanging flowers).

The invasive *Solanum* species that are toxic are: *S. dulcamara* ('European bittersweet', 'dulcamara'), a shrub with red fruits when ripening that contain solanine, thus being very dangerous, even lethal if ingested by small children; *S. sodomeum* ('devil's tomatera'), a South African bush with thorny stems and leaves that can reach 3 m high and which shows a 2 cm yellow fruit. This plant is widely distributed in the Mediterranean area; *S. viarium* ('apple of tropical soda', native to Brazil and Argentina), an invasive species in many countries of America, characterized by its white-spotted clear green fruits, which become greenish-yellow when ripening; *S. eleagnifolium* ('silverleaf nightshade'); *S. carolinense* ('horsenettle'), a perennial plant with thorny stems and leaves, clear violet to white flowers, and a green fruit; *S. nigrum* ('black nightshade' or 'devil's tomatillo'), which is very similar to dulcamara, but with greenish-yellow fruits that become black when ripening, and not red as those of dulcamara. Because of its higher content of glycoalkaloids, for example solanine, chaconine, and solasodine, this species is even more dangerous than the sweet nightshade. The other nightshades have as their toxic principle solanine, usually in leaves, sprouts, and unripe berries. The ingestion results in acute hemorrhagic gastroenteritis, weakness, excessive salivation, dyspnea, tremors, progressive paralysis, prostration, and death [86]. Treatment is based on pilocarpine, physostigmine, and gastrointestinal protectors. The seeds can contaminate grain, and recently, the toxicity of *S. bonariense* ("Naranjillo") to cattle has been reported in western Uruguay [87].

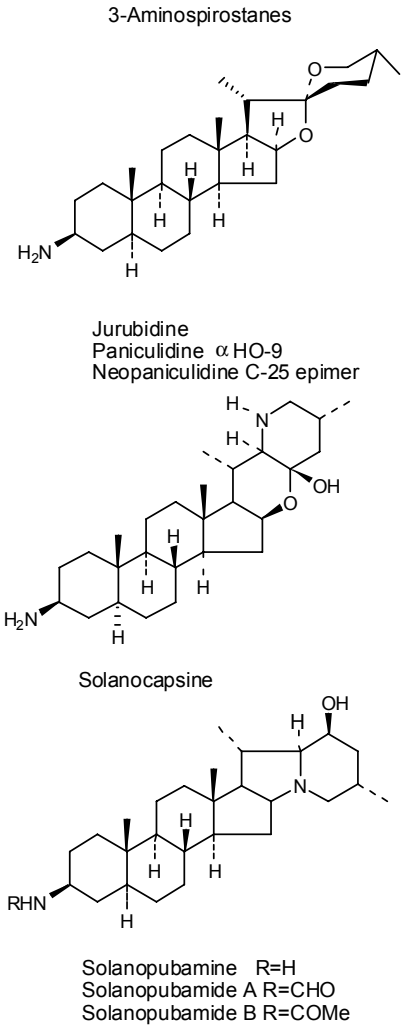
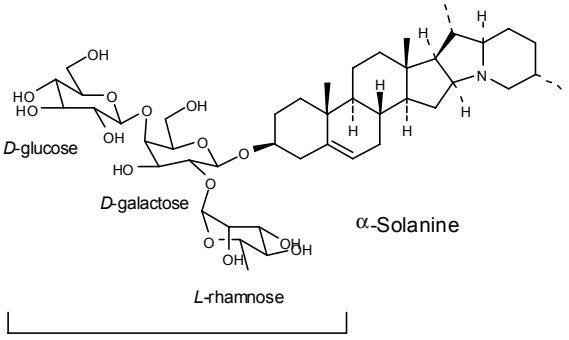



**Figure 13**: 3-Aminospirostanes, solanocapsine/solanopubamines.

Of the edible *Solanum* species, the eggplant (*S. melongea*) contains solanine, even in the fruits, but once cooked, it loses the toxicity, and is edible. The potato plant (*S. tuberosum*) and the new buds are toxic because they contain *α*-solanine (Figure 14) and α-chaconine (Figure 15), concentrated mainly in the flowers and sprouts (200 to 500 mg/100 g) [88]. These glycoalkaloids have the same aglycone, solanidine, but a different sugar moiety.

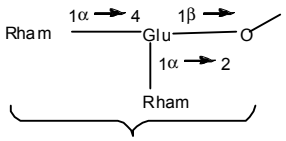
Immuno-capillary electrophoresis with laser-induced fluorescence (CE-LIF) detection was used for the determination of total glycoalkaloids in potatoes [89,90] and was shown to be a rapid alternative to traditional ELISA and HPLC methods. Nonaqueous capillary electrophoresis coupled with electrospray ionization-ion trap mass spectrometry (MS and MS/MS) detection was used for the identification and quantification of potato glycoalkaloids and their




Solatriose
Other Glycoalkaloids

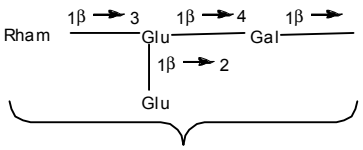
α-Solamarine= Solatrioside of Tomatidenol
α-Solasonine= Solatrioside of Solasodine

**Figure 14**




Chacotriose

β-Solamarine=chacotrioside of Tomatidenol
α-Chaconine=chacotrioside of Solanidine
α-Solamargine=chacotrioside of Solasodine




Lycotetraose

Tomatine, Demissine
Glycosides of lycotetraose

**Figure 15**

relative aglycones [91]. Also, *α*-solanine and *α*-chaconine have been recently determined by HPLC, based on the chemiluminescent reaction of *tris*(2,2'-bipyridine)ruthenium(III) [92]. The detection limits of α-solanine and α-chaconine were 1.2 and 1.3 ng/mL, respectively. The solanidine hydrolytic extraction in different solid-liquid-liquid systems was reported [69], which combined three different processes: extraction of glycoalkaloids from potato vines, their hydrolysis to solanidine, and the extraction of solanidine, in a single step. The IR and MS spectra of isolated solanidine were recorded.

The occurrence of these glycoalkaloids in potatoes gives a bitter taste above 14 mg/100g, and a burning sensation to the mouth and throat above 20 mg/100g [93]. At these concentrations solanine and other potato glycoalkaloids are toxic [1]. They are not destroyed during normal cooking because the decomposition temperature of solanine is about 243°C.

Potatoes also contain proteinase inhibitors, which act as effective defenses against insects and micro-organisms, but are not toxic to humans because of being destroyed by heat. Lectins or hemagglutinins are also present in potatoes, but are destroyed by heat, and potatoes are usually cooked before being eaten (see section 12.b. of this paper).

Other toxic genera are *Lycopersicon* ('tomato'), for example *L. esculentum*, and *Physalis* ('ground cherry'), for example, *P. viscosa*.

Tomatoes accumulate a variety of secondary metabolites, including phytoalexins, protease inhibitors, and glycoalkaloids as protection against adverse effects of hosts of predators, such as fungi, bacteria, viruses, and insects. Tomato glycoalkaloids are mainly *α*-tomatine and dehydrotomatine [94]. A new α-tomatine isomer, named filotomatine (MW 1033), has been reported, which shares a common tetrasaccharide structure (i.e., lycotetraose) (Figure 15) with α-tomatine and dehydrotomatine, and with soladulcidine as aglycone [95]. RP-HPLC with electrospray ionization (ESI) and ion trap mass spectrometry (ITMS) were used.

Many of the glycoalkaloids found in nightshades have pharmacological and toxicological effects on humans due to their significant anticholinesterase activity and disruption of cell membranes [91]. When the activity of cholinesterase is strongly inhibited, the nervous system control of muscle movement is disrupted, resulting in muscle twitching, tremors, paralyzed breathing, and convulsions [96]. Upon ingestion of glycoalkaloids, it is considered that joints are damaged due to inflammation and altered mineral status, although this is not clear enough. However, Solanaceae glycoalkaloids can contribute to excessive loss of calcium from bone and excessive deposits of calcium in soft tissues.

***Mechanism of action:*** The glucoalkaloid, solanine is a direct irritant of the esophagic and gastric mucosa. Solanine inhibits acetylcholinesterase (AchE), as do most glycoalkaloids. Some of them, in addition, are atropine-type anticholinergic agents [88].

Generally, leaves (especially withered) and green fruits are the toxic parts of *Solanum* plants, except for *S. eleagnifolium*, whose ripe fruits are more toxic

than the green ones. The toxic components are usually labile to heat, except for *S. tuberosum* (potato) [86].

Poisoning due to ingestion of glycoalkaloids has variable indications. However, in most cases, gastrointestinal problems (abdominal pain, vomiting, hemorrhagic diarrhea), and CNS signs (apathy, drowsiness, salivation, progressive weakness or paralysis, dyspnea, bradycardia, circulatory collapse, dilated pupils, tremors, incoordination and convulsions) can be distinguished. All these symptoms lead to paralysis, loss of consciousness, shock, coma, breathing depression, and death. Usual injuries include erythema and ulceration of the esophagic and gastric mucosa [88].

(**5**) **Nicotine:** The pyridine alkaloids nicotine, nornicotine, anabasine and anatabine are characteristic of *Nicotiana* spp. (Figure 16), which include cultivated (*N. tabacum*), wild (*N. attenuata* and *N. trigonophylla*), and tree tobacco (*N. glauca*). Ornithine is the biosynthetic precursor of the pyrrolidine that occurs in the alkaloids of tobacco (nicotine, nornicotine), and other Solanaceae. Nicotine is a starting compound of numerous further tobacco alkaloids. Anabasine is a teratogenic agent, but nicotine is not. Wild and cultivated tobaccos contain some anabasine. Anabasine is the main alkaloid of the leaves of *N. debneyi* (*ca*. 50%) However, 85-99% of the total alkaloid content of tree tobacco, *N. glauca*, is anabasine. Anatabine is the main alkaloid of *N. otophora* (60%) [6].

Nicotine is a pyridine alkaloid with an asymmetric carbon, which occurs in high concentration in the leaves of the tobacco plant (*N. tabacum*). It accounts for nearly 5% of the weight of the plant. Of both isomers, *L*-nicotine [(*S*)-3-(1-methylpyrrolidin-2-yl) pyridine] is the active form, and it is found in tobacco (Figure 16).

Poisoning due to consumption of tobacco leaves and stems has been reported in cattle, horses, sheep, and swine, as well as dogs and even humans (after consuming the leaves as boiled greens).

Livestock progress from: excitement, shaking and twitching, rapid breathing, staggering, weakness and prostration, coma, descending paralysis of the central nervous system, to death by respiratory failure. Recently, nicotine exposure and bronchial epithelial cell nicotinic acetylcholine receptor expression in the pathogenesis of lung cancer have been reported [97].

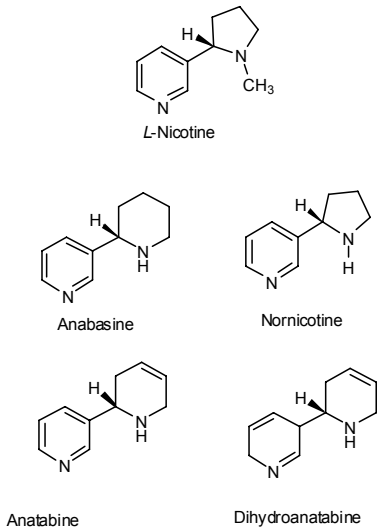



**Figure 16**

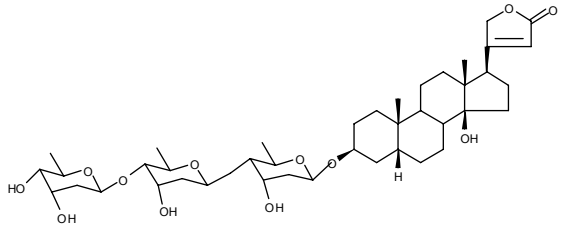
Deformed offspring due to ingestion of anabasine in tobacco has been documented in cattle, sheep, and swine. These deformities are clinically the same as those caused by maternal consumption of either lupine or poison hemlock: carpal flexure, cleft palates, arthrogryposis of the forelimbs and curvature of the spine [98,99].

Smoking exerts complex central and peripheral nervous system, behavioral, cardiovascular, and endocrine effects in humans and is a primary risk factor for various cancers. Nicotine, a major constituent of tobacco, is the compound that is responsible for the development and maintenance of tobacco dependence. The balance between nicotine neuroprotection and toxicity depends on dose, developmental stage and regimen of administration [100]. The absorbed nicotine is rapidly metabolized to inactive cotinine by $CYP_2A_6$ in the human liver [101]. Genetic variation in the $CYP_2A_6$ gene can increase or decrease enzyme activity through altering either the protein's expression level or its structure and function. $CYP_2A_6$ genetic variation has been recently reviewed [102] taking into account its impact on *in vivo* nicotine kinetics, different phenotyping approaches for assessing *in vivo* $CYP_2A_6$ activity, and other sources of variation in nicotine metabolism, such as gender. There are large interindividual variations in the rate of nicotine metabolism within groups of individuals having the same $CYP_2A_6$ genotype. $CYP_2B_6$ genetic variation is associated with the metabolism of nicotine and cotinine among individuals with decreased $CYP_2A_6$ activity [103]. The relationships between smoking behavior and the risk of cancer have been reported [101].

NO-synthase (NOS) is a heme-containing enzyme that catalyzes the oxidation of *l*-arginine to nitric oxide, an important cellular signaling molecule. Recently, it was found that aqueous extracts of tobacco cigarettes caused the inactivation of the neuronal isoform of NOS (nNOS) and that this might explain some of the toxicological effects of smoking. The exact identity of the chemical inactivator(s) is not known. Lowe *et al*. [104] recently analyzed extracts prepared from a variety of plants looking for nNOS inactivation. They found 18 plants that contained a chemical inactivator(s) of nNOS, 6 of which were members of the Solanaceae. Probably, the same or related chemical inactivators are involved.

(**6**) **Cardenolides**: Cardenolides are characteristic 3-*O*-glycosides (triosides or tetraosides) with an α,β-unsaturated γ-lactone at C-17, and a β-HO-14, which are responsible for the toxicity and lethality of some solanaceous plants. They are well-known components of the heart drug digitalis, for example, digitoxin (Figure 17), and which in Solanaceae produce similar heart effects, besides gastrointestinal disturbances.

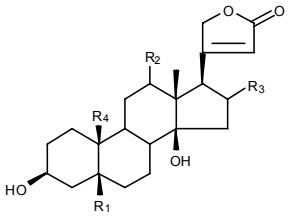
The mechanism of action of the cardiac glycosides consists of inhibition of the Na/K-ATPase enzyme system, which results in increased intracellular Na and decreased K. The poisoning due to cardiac glycosides is acute and characterized by gastrointestinal (abdominal pain, vomiting, diarrhea) and cardiovascular signs (bradycardia, arrhythmias, heart block, asystole). The lesions account for severe gastroenteritis, and agonal hemorrhages in the heart, and serous and mucous membranes.




Digitoxin

**Figure 17**: The cardiac glycoside, digitoxin.

Few cardenolides have been isolated from the Solanaceae. The toxic *Cestrum parqui* contains gitoxigenin glycosides (Figure 18) as cardenolides, which were isolated and identified in early studies. Later studies were performed on the toxic tetracyclic diterpenes [105-107].




Digitoxigenin $R_1$=$R_2$=$R_3$=H, $R_4$=$CH_3$
Gitoxigenin $R_1$=$R_2$=H, $R_3$=OH, $R_4$=$CH_3$
Digoxigenin $R_1$=$R_2$=OH, $R_3$=H, $R_4$=$CH_3$
6,7-Dehydrostrophantidin $R_1$=OH, $R_2$=$R_3$=H
$R_4$=CHO, $\Delta^{6,7}$

**Figure 18**: Aglycones of cardiac glycosides.

The toxic *Nierembergia aristata* contains three cardenolides, 17-*epi*-11α-hydroxy-6,7-dehydrostrophanthidin 3-*O*-β-boivinopyranoside, 6,7-dehydrostrophanthidin 3-*O*-β-boivinopyranoside, and 6,7-dehydrostrophanthidin 3-*O*-β-oleandro- pyranoside, of which the last one showed a significant cytotoxicity towards eleven cancer cell lines [108].

(**7**) **Capsaicinoids:** *Capsicum* includes many species of sweet and hot peppers, and chili peppers, such as Tabasco peppers and 'habaneros'. In the spice category, paprika is derived from *C. annum*, the common red pepper, and cayenne pepper from *C. frutescens*. Tabasco sauce contains high amounts of *C. annuum*, and is also considered a nightshade food. According to Dewit and Bosland [109], there are five species of *Capsicum* peppers native to the American continent: *C. pubescens*, C. *baccatum*, *C. annuum*, *C. frutescens* and *C. chinense*. The hottest chili peppers belong to the *C. chinense* group, including the notorious 'habanero'. This species is native to the Amazon Basin of South America.

*Capsicum* species contain members of the vanilloid family, currently known as capsaicinoids [110]. The extracts contain at least five capsaicinoids of well-known pungency, of which three are predominant: capsaicin (8-methyl-*N*-vanillil-6-nonenamide), dihydrocapsaicin and nordihydrocapsaicin (Figure 19). Capsaicinoids are produced in the plant probably as a defense against herbivore animals. Birds in general are not sensitive to capsaicinoids, and thus serve to disperse the seeds. *Capsicum* extract and capsaicin modulate T cell-immune responses, and their immunomodulatory effects on murine Peyer's patch (PP) cells are partly due to both transient receptor potential vanilloid 1 (TRPV1)-dependent and -independent pathways [111].

Recently, two glucosides, capsaicin-β-D-glucopyranoside and dihydrocapsaicin-β-D-glucopyranoside have been isolated from the fruit of *C. annuum* cultivar '*High Heat*' [112]. They were sequentially purified by acetone, *n*-hexane, and acetonitrile extractions, followed by medium-pressure liquid chromatography and RP-HPLC. Their chemical structures were elucidated by $^{1}$H- and $^{13}$C NMR spectroscopy, and hydrolysis with *α*- and β-glucosidases. The glucosides were also detected in various pungent cultivars of *C. annuum*, *C. frutescens*, and *C. chinense* by liquid chromatography-mass spectrometry (LC-MS) [112]. However, these glucosides were not detected in nonpungent cultivars of *C. annuum*. Furthermore, a positive correlation was observed between the quantity of the capsaicinoids, capsaicin, and dihydrocapsaicin, and their glucosides.

Capsaicin is also used as medication and as tear gas. In high quantities it can be very toxic. The poisoning symptoms are difficulty to breathe, blue skin and convulsions. However, accidental poisoning due to chili consumption is rare.

In fact, capsaicin is a neurotoxin [113], which has been studied in diverse pain anomalies because, after causing an initial irritation, it produces analgesic effects, and substantial relief to the pain associated with osteoarthritis, rheumatic arthritis, *Herpes* and diabetic neuropathy. The effects on the nervous system of chili peppers have been recently reported [113]; nerve regeneration and a clinical trial design are included.

Capsaicin is used in urology for the treatment of dysfunctions, like vesical hyperactivity, detrusor hyperreflexia and vesical pain. Capsaicin activates the sensorial nervous fibers by binding to the vanilloid subtype 1-receptor (vanilloid-1 receptor), a non-selective cationic channel, present in the peripheral terminals of the nociceptive neurons [114,115]. After activation of the vanilloid-1 receptor, capsaicin and other vanilloids desensitize the sensorial neurons, turning them refractory to subsequent stimuli that cause pain [116]. Besides desensitizing the type-C afferent neurons, capsaicin also alters the release, from the peripheral terminals, of substance P, neurocinine A, a peptide related to the gene of calcitonin and other neurotransmittors/neuropeptides, which act in the inflammation response.

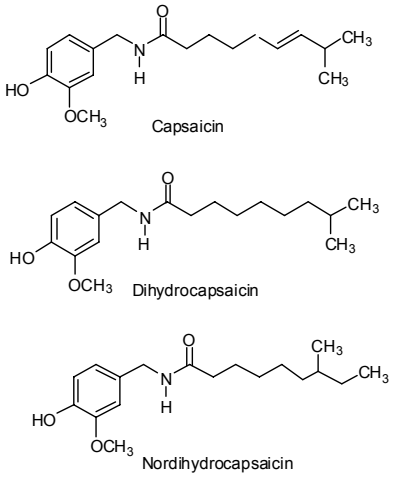



**Figure 19**

It is thought that the effect of capsaicin on pain is mediated by its action on the sensory neurons with amielinic type-C axons, which take part in the transmission of nociceptive information toward the CNS. They also release different proinflammatory mediators that participate in fibers of the bladder, which transmit nociceptive signs to the CNS, and are those that seem to play a role in the sign transmission that gives rise to detrusor hyperactivity [117,118]. It is probable that an identical or similar mechanism accounts for the effects of capsaicin in the bladder.

Recently, Soontrapa *et al*. [119] evaluated the effectiveness of capsaicin in 25 patients with either hyperactive or hypersensitive bladder with primary detrusor instability. These investigators graded as very high the effectiveness of capsaicin for hyperactive and hypersensitive bladder and for primary detrusor instability.

In most studies there were no complications due to the treatment with capsaicin. However, the initial disturbance (suprapubic ardour sensation or pain), associated with capsaicin instillation is an important dissuasive factor for the most widespread use of treatment with capsaicin [117].

Recently, Takahashi *et al*. [120] reported that capsaicin inhibited catecholamine secretion and synthesis *via* suppression of $Na^{+}$ and $Ca^{2+}$ influx through a vanilloid receptor-independent pathway. These authors studied the effects of capsaicin on catecholamine secretion and synthesis in cultured bovine adrenal medullary cells.

Capsaicinoids showed antitumor activity. These compounds adhered to the proteins in the

mitochondria of the cancerous cells giving rise to apoptosis, without damaging the surrounding healthy cells, because the mitochondrial biochemistry of the cancerous cells is very different from that of the normal cells. However, capsaicin also exhibited a carcinogenic potential. To clarify the mechanism for expression of the potential carcinogenicity of capsaicin, Oikawa *et al*. [121] examined DNA damage induced by capsaicin in the presence of a metal ion and several kinds of cytochrome $P_{450}$ (CYP) using $^{32}$P-5'-end-labeled DNA fragments. Capsaicin induced Cu(II)-mediated DNA damage efficiently in the presence of $CYP_1A_2$ and partially in the presence of 2D6. DNA damage was inhibited by catalase and bathocuproine, a Cu(I) chelator, suggesting that reactive species derived from the reaction of $H_2O_2$ with Cu(I) participate in DNA damage. Formation of 8-oxo-7,8-dihydro-2'-deoxyguanosine was significantly increased by $CYP_1A_2$-treated capsaicin in the presence of Cu(II). Therefore, Cu(II)-mediated oxidative DNA damage by CYP-treated capsaicin seemed to be relevant for the expression of its carcinogenicity [121].

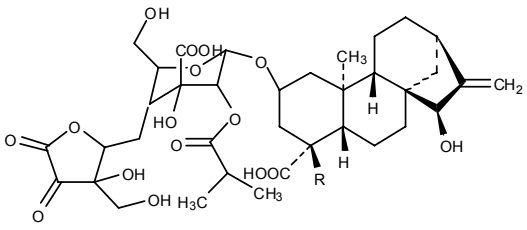
Various procedures for the extraction of capsaicinoids have been assessed as an alternative to conventional Soxhlet and sonication methods. Analysis of the capsaicinoids was also performed in order to improve the obtention and quantitation of these compounds. Recently, capsaicinoids were extracted from peppers by pressurized liquid extraction (PLE) [122]. These compounds were determined by RP-HPLC, with detection by fluorescence spectrophotometry and MS. The stability of capsaicin and dihydrocapsaicin has been studied at different temperatures (50-200$^o$C), and several extraction variables have been assayed. Finally, the PLE method developed has been applied to quantify the capsaicinoids present in three varieties of hot peppers cultivated in Spain, quantifying five capsaicinoids: nordihydrocapsaicin, capsaicin, dihydrocapsaicin, an isomer of dihydrocapsaicin, and homodihydrocapsaicin [122].

A microwave-assisted extraction (MAE) procedure combined with $^1$H-NMR spectroscopy was reported [123], and optimized for the extraction and quantitative determination of capsaicin in *C. frutescens*. The optimized MAE method provided extracts that were analyzed quantitatively using $^1$H NMR spectroscopy without either any preliminary clean-up or derivatization steps. In the $^1$H NMR spectrum of the crude extracts, the doublet at δ 4.349-4.360 was well separated from other resonances in deuterated chloroform. The quantity of the compound was calculated from the relative ratio of the integral value of the target peak to that of a known amount of dimethylformamide as internal standard.

The morphology, productive yield, main quality parameters and genetic variability of eight landraces of hot pepper (*C. annuum* ssp.) from Southern Italy have been recently reported [124]. Chemical and genetic investigations were performed by HPLC and random amplified polymorphic DNA (RAPD)-PCR, respectively. In particular, carotenoid and capsaicinoid (pungency) contents were considered as the main quality parameters of hot pepper. For the eight selected samples, genetic similarity values were calculated from the generated RAPD fragments and a dendrogram of genetic similarity was constructed. All eight landraces exhibited characteristic RAPD patterns that allowed their characterization. The results led to the identification of three noteworthy populations, suitable for processing, which fitted into different clusters of the dendrogram [124].

A liquid chromatography-electrospray ionization/ time-of-flight mass spectrometry (LC-ESI/TOF-MS) method has been developed for the direct and simultaneous determination of capsaicin and dihydrocapsaicin in fruit extracts from different *Capsicum* genotypes [125]. Chromatographic separation of capsaicin and dihydrocapsaicin was achieved with a RP chromatography column, using a gradient of methanol and water. Quantification was achieved using as internal standard (4,5-dimethoxy-benzyl)-4-methyloctamide, a synthetic capsaicin analogue not found in nature. Analytes were base-peak resolved in less than 16 min, and limits of detection were 20 pmol for capsaicin and 4 pmol for dihydrocapsaicin [125].

Capsaicinoids were determined in habanero pepper extracts by a chemometric analysis of UV spectral data [126]. The method consisted of partial-least-squares (PLS-1) multivariate regression modeling techniques in conjunction with UV spectral data obtained on alcoholic extracts of habanero peppers (*C. chinense*). The PLS-1 regression models were developed by correlating the known total concentration of the two major capsaicinoids (capsaicin and dihydrocapsaicin) in the extracts, as determined by HPLC, with spectral data. The regression models were subsequently validated with laboratory-prepared test sets [126].



Parquin R=H
Carboxyparquin R=COOH

**Figure 20**

(**8**) **Kaurene-type tetracyclic diterpenes:** *Cestrum parqui* L'Hérit. (common name, 'green cestrum', 'duraznillo negro', 'palqui') is a perennial plant, 1 to 2 m high, native to the south of South America, especially Argentina and Chile. It is also found in Australia where it was introduced as an ornamental plant [127] because of the clusters of yellow flowers, and the violet to black berries. The bush has a characteristic, unpleasant scent, which is rejected by livestock. However, the plant can be consumed by animals recently arrived to the sector or when it is the only green forage, thus causing accidental poisonings [128,129].

The compounds responsible for the toxicity to cattle are diterpenoid (kaurene) glycosides, which are mitochondrial poisons [130]. Parquin and carboxyparquin (Figure 20) are toxic kaurenoid glycosides very similar to the diterpenoid atractyloside, which has been isolated from *Wedelia glauca* (Asteraceae) in our laboratories [131], together with other tetracyclic diterpenes [132]. In particular, carboxyparquin, fed to mice at doses of approximately 4 mg/kg, led to severe liver damage and signs of kidney damage. Carboxyparquin is also present in *Cestrum elegans* and *C. tomentosum*. *ent*-Kaurene tetracyclic diterpenes have shown antimicrobial activity [133,134], and *ent*-kaur-16-en-19-oic acid cytotoxic activity against KB cells [135].

Injuries were found first in the liver. Together with this liver damage, hemorrhages were observed as petechias and ecchymosis in the heart (epi, peri and endocardium); hemorrhages were also seen in the gall bladder and in the bowel serous. The hemorrhagic lesions would be due to a decrease of clotting factors due to hepatic dysfunction [136]. The hepatic damage resulted in an increase of aspartate-aminotransferase and the prothrombine time. These values were used for diagnosis and prognosis in the non-fatal intoxications. A microhistological technique was used to confirm the diagnosis of ruminant intoxication by *C. parqui* [137].

The clinical signs in the intoxicated animals appeared abruptly as prevailing excitement, incoordination, muscular tremors, sweat, abdominal pain, anorexy and dyspnea. Death occurred 4 to 8 hours after the beginning of the clinical signs, although longer evolution is known [107,127,128]. The mortality was about 80 to 100%. The lethal dose for bovines was 10 g dry matter/kg body weight [128], with variations throughout the year.

(**9**) **Steroidal saponins:** Saponins can be divided into steroidal saponins, triterpene saponins and glycoalkaloids (basic saponins). Steroidal saponins (steroidal glycosides) are widespread in the Solanaceae. The steroidal sapogenins of this family are usually spirostanes, but a few spirofuranes are known (Figure 21).

The properties of the saponins are the formation of stable foams when agitated in water, the formation of oil-in-water emulsions, hemolytic activity, hormonal modulation, antiinflammatory, hypocholesterolemic, diuretic, antimicrobial and inhibition of cancer cell growth, stimulation of the immune system, and protection against bone loss. Sapogenins have a direct antioxidant activity, which may results in other benefits, such as reduced risk of cancer and heart diseases [138]. The compounds have a variety of applications in the pharmaceutical, cosmetics, food, detergents and mining industries, for example ore separation in industrial and mining operations, and products such as photographic emulsions and films, creams, shampoos, and adjuvants in animal vaccines [138]. Not only their detergent properties have led to their cosmetic use, but also the antifungal, antibacterial, antiinflammatory and vasoprotecting properties, in addition to their emollient effects, for example occlusion, humectant and lubrication [139,140]. Saponins are used in cosmetics and for the manufacture of pharmaceutical compositions for treating the skin in order to increase the amount of collagen IV in the dermo-epidermal junction [141].

The antimycotic activity of *Solanum chrysotrichum* is useful for the treatment of fungal foot infections, extracts being applied externally in the treatment of the superficial mycosis due to *Tinea pedis*, without producing secondary effects [142]. The extract of *S. chrysotrichum* showed biological activity against

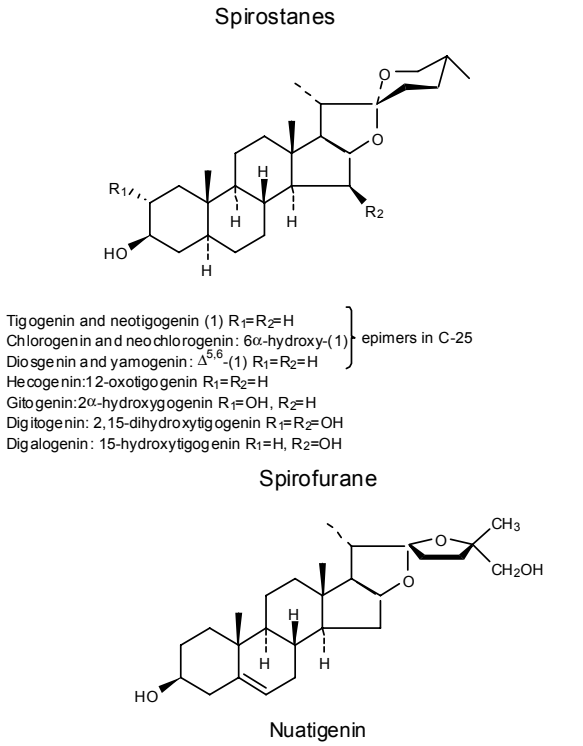



**Figure 21**

dermatophytes and yeasts. A shampoo containing a standardized extract of *S. chrysotrichum* showed therapeutic effectiveness for the topical treatment of *Pityriasis capitis* (dandruff), which is a seborrhoeic dermatitis of the scalp, associated with the yeast genus *Malassezia* [143].

An example of a saponin is dioscine (Figure 22). The sugar moiety, bonded to HO-3 of the sapogenin, is usually a branched trisaccharide or tetrasaccharide. In this example, it is a branched trisaccharide called chacotriose.

Saponins occur in plants as a multi-component mixture of compounds of very similar polarities. Therefore, the separation of each component requires a combination of different chromatographic techniques, for example, first separation of the mixture on $C_{18}$ columns, followed by further purification on a normal phase silica gel column. Especially difficult is the determination of saponins in plant material, as these compounds do not possess chromophores and their profiles cannot be registered by UV. The techniques for quantification of saponins in plant material have been recently reviewed [144]. These include the application of evaporative Light scattering detection (ELSD) for saponin profiling and quantification; liquid chromatography-electrospray mass spectrometry (LC/ESI/MS) for saponin

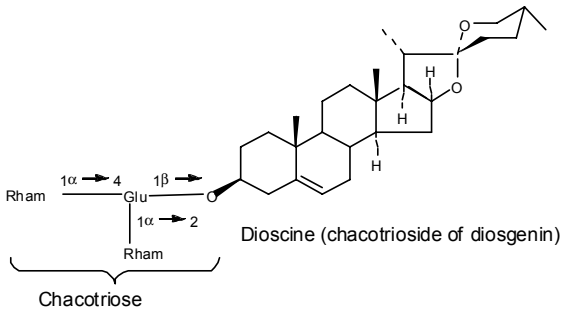



**Figure 22**

determination; and sensitive and compound specific ELISA tests for some saponins.

Saponins, if swallowed, may be poisonous, when they are called sapotoxins, and may cause urticaria (skin rash) in humans. *Cestrum parqui,* which is a toxic species of Argentina and Chile, is one of the examples of a solanaceous plant that contains toxic tetracyclic diterpenes, and also toxic saponins, which have the spirostanols gitogenin, digitogenin, and tigogenin as sapogenins (Figure 21) [105-107,145]. The saponins showed a strong irritating action on the digestive mucous membrane, and also produced degeneration of the hepatic tissues [106]. Saponins were considered responsible for the liver necrosis. The occurrence of cardiotonic glycosides caused stimulus of the vagus action on the heart, leading to bradycardia, atrioventricular blockade and death by heart failure [106].

Two steroidal glycosides, parquisoside A and B were also isolated from the aerial parts of *C. parqui*. The common aglycone was a steroid of the spirostane series, named parquigenin [146], which had the structure (3β,24*S*,25*S*)-spirost-5-ene-3,24-diol, *i.e.* a (24*S*,25*S*)-24-hydroxydiosgenin. On the basis of detailed spectroscopic studies and chemical analysis, the structures of parquisosides A and B were elucidated as (3β,24*S*,25*S*)-spirost-5-ene-3,24-diol 3-*O*-{[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)}-β-D-glucopyranoside and (3β,24*S*,25*S*)-spirost-5-ene-3,24-diol 3-*O*-{[α-L-rhamnopyranosyl)-(1→4)-α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)}-β-D-glucopyranoside, respectively [146].

Recently, two steroidal saponins were purified from cayenne pepper (*Capsicum frutescens*) [147]. Both contained the same aglycone, but differed in the number of glucose moieties: the first saponin had four (MW 1081) and the second had three (MW 919).

The larger saponin was slightly fungicidal against the nongerminated and germinating conidia of *Aspergillus flavus*, *A. niger*, *A. parasiticus*, *A. fumigatus*, *Fusarium oxysporum*, *F. moniliforme* and *F. graminearum*, whereas, the second saponin (MW 919) was inactive against these fungi. The results indicated that the absence of one glucose molecule affected the fungicidal and aqueous solubility properties of these similar molecules [147].

Six steroidal saponins, solanigrosides C-H, and the saponin degalactotigonin were recently isolated from the toxic plant *Solanum nigrum* [148]. Their chemical structures were elucidated by spectroscopic analysis, chemical degradation, and derivatization. All seven compounds were tested for their cytotoxicity using four human tumor cell lines (HepG2, NCI-H460, MCF-7, SF-268). Only degalactotigonin was cytotoxic, with $IC_{50}$ values of 0.25-4.49 μM [148].

**(10) 1,25-Dihydroxyvitamin $D_3$ and Vitamin $D_3$:** Cholecalciferol (vitamin $D_3$) and its metabolites are responsible, together with other conduction regulators, of the complex homeostasis pathways of calcium and phosphorus in vertebrates. These minerals are used in many biological processes, such as the regulation of muscular and nervous functions, and the development and maintenance of bone structure.

Vitamin $D_3$, previtamin $D_3$, and other metabolites, previously known to be synthesized only in mammals and birds, have been detected also in the so-called calcinogenic plants. The intermediate is formed by a photoreaction at a wavelength of 250-310 nm from the 7-dehydro derivative (Figure 23).

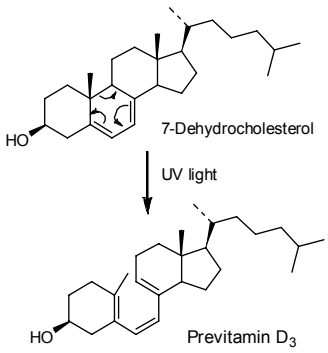



**Figure 23**

Secosteroids, such as previtamin $D_3$, show *cis-trans* isomerism in an equilibrium shifted toward the *S-trans*, *S-cis* contribution (Figure 24).

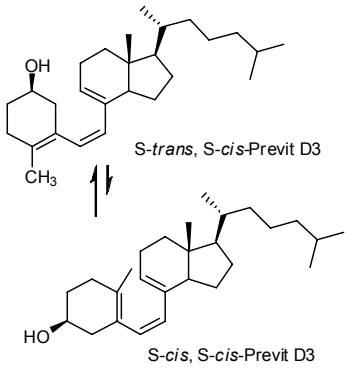



**Figure 24**

The intermediates and enzymes of the vitamin $D_3$ pathway have been detected in plant, cell and tissue cultures [149]. Studies with *in vitro* plant systems revealed a functional role of vitamin $D_3$ compounds [150].

On the basis of cladistic analysis the presence of these compounds was predicted to be a characteristic of Angiosperms [149]. Both plants and animals seemed to possess a similar synthetic route to 1α,25-$(OH)_2D_3$. The 1α,25-$(OH)_2D_3$ was shown to behave in plant systems in the same way as in animal systems through binding to a specific receptor (vitamin D receptor, VDR) [151]. Recently, competition assays allowed the detection of binding sites for [$^3$H]-1α,25-$(OH)_2D_3$ in cultured *S. glaucophyllum* cells [151]. The data demonstrated that *S. glaucophyllum* cells, which produced 1α,25-$(OH)_2D_3$, also expressed a cognate binding protein(s) and were able to respond to 1α,25-$(OH)_2D_3$ [151].

Vitamin $D_3$, applied exogenously in solution, stimulated $^{45}Ca^{2+}$ uptake into the shoots of potato plantlets of micropropagated potato (*S. tuberosum*) cultivars [152,153]. Therefore, the vitamin D group apparently played a similar role in both plants and animals by affecting Ca absorption and Ca-mediated cellular functions.

1α,25-$(OH)_2D_3$, the hormonally active form of vitamin $D_3$, has been shown to be a potent negative growth regulator of breast cancer cells both *in vitro* and *in vivo*. In addition to regulating gene transcription via the specific intracellular VDR, 1α,25-$(OH)_2D_3$ induced rapid, non-transcriptional responses involving activation of transmembrane signal transduction pathways, like growth factors and peptide hormones [154].

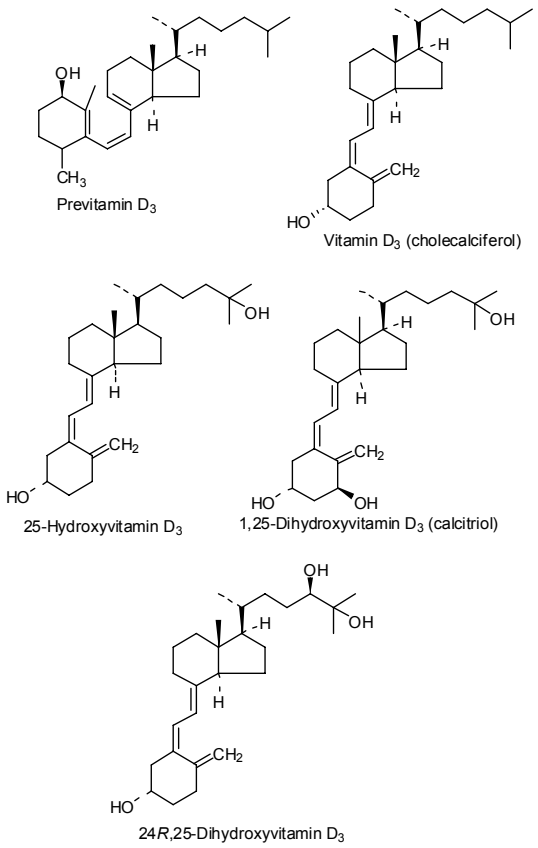



**Figure 25**

1α,25-$(OH)_2D_3$ was shown to inhibit serum induced activation of ERK-1 and ERK-2 mitogen-activated protein kinases (MAPKs) [155]. The non-receptor tyrosine kinase *Src* was involved in the pathway leading to activation of ERK 1/2 by serum. Capiati *et al*. [154] showed that 1α,25-$(OH)_2D_3$ inhibited the MAPK cascade by inactivating *Src* tyrosine kinase through a mechanism mediated by the VDR and tyrosine phosphatases.

Enzootic calcinosis or metastasic calcification is produced in mammals by ingestion of the following solanaceous calcinogenic plants, *Solanum glaucophyllum* (native to South America), *Nierembergia veitchii* (native to South America), and *Cestrum diurnum* (native to southern USA, particularly Florida). Another non-solanaceous calcinogenic plant is *Trisetum flavescens*, which is native to southern Germany. Spontaneous calcinoses of animals are chronic debilitating diseases characterized by mineralization of the soft tissues, especially in the cardiovascular system and lungs. Most of these diseases are associated to 1,25-$(OH)_2D_3$ (Figure 25) present in the calcinogenic plants [156].

*Solanum glaucophyllum* Desf. (syn., *S. malacoxylum* Sendtn.) (c.n., 'duraznillo blanco') is responsible for an enzootic calcinosis in cattle, horses, and sheep in Argentina, Brazil, Paraguay and Uruguay [157].

Toxicity has also been induced in rabbits, mice and rats. This disease, called 'enteque seco', is characterized by the calcification of the soft tissues, especially aorta, heart, lungs and kidneys [158,159]. Although excess ingestion of *S. malacoxylon* can cause hypercalcemia, hyperphosphatemia, sometimes hypomagnesemia and soft tissue calcification, controlled administration has useful therapeutic applications [160].

*S. glaucophyllum* contains high levels of the previtamin $D_3$, cholecalciferol (1,25-dihydroxy-vitamin $D_3$; 1,25-$(OH)_2D_3$), as a glycoside [161], and other metabolites, such as vitamin $D_3$ and 25-$(OH)D_3$ [162] (Figure 25), and diosgenin, as well as enzymes (cholecalciferol-25-hydroxylase and 25-hydroxy-cholecalciferol-hydroxylase) that are able to hydroxylate cholecalciferol to active metabolites. Glycoalkaloids are also present, for example α-solanine and α-solamargine.

The occurrence of 7-dehydrocholesterol, vitamin $D_3$, 25-$(OH)D_3$ and/or 1α,25-$(OH)_2D_3$ in *S. glaucophyllum* [163,164], *Lycopersicon esculentum* (tomato), *S. tuberosum* (potato), *Cucurbita pepo* (zucchini) [165], *Nicotiana glauca* [166] and other species has been demonstrated, involving a light-dependent pathway in the formation of vitamin $D_3$ in plants similar to that of vertebrates. However, other evidence supported the operation of a non-photolytic reaction for vitamin $D_3$ synthesis [150].

*Solanum glaucophyllum* callus cultures were found to contain 17β-estradiol, estrone, and also abundant estrogen binding sites [167]. The results provided the first evidence for the existence of estrogen binding proteins structurally related to the mammalian ER α subtype in a higher plant system. The action of estrogen might be mediated by the classical nuclear estrogen receptor or through putative receptors with non-classical localization. There was evidence showing antiapoptotic effects of estradiol in various cell types. Recently, Vasconsuelo *et al*. [168] showed that 17β-estradiol, at physiological concentrations, abrogated DNA damage, PARP cleavage and mitochondrial cytochrome c release induced by $H_2O_2$ or etoposide in mouse skeletal muscle C2C12 cells. This protective action, which involved PI3K/Akt

activation and BAD phosphorylation, was inhibited by antibodies against the estrogen receptor (ER) α or β isoforms, or transfecting siRNA specific for each isoform.

The influence of UV light (6 h radiation with 254 nm) in the calcinogenic activity of *T. flavescens* and *N. veitchii* has been tested [169]. Mello and Habermehl [170] also reported the incubation effects of rumen fluid on aqueous extracts of *T. flavescens*, *S. glaucophyllum*, *N. veitchii*, and *Cestrum laevigatum*. The rachitic chicken test was performed, and the serum levels of Ca, P, and alkaline phosphatase were determined. Extracts of *S. glaucophyllum*, and *C. diurnum*, as well as 1,25-$(OH)_2$vit$D_3$-25-*O*-glucoside, gave (without incubation) an increased activity, while with incubation a small additional effect was observed. Comparable effects were obtained with 1,25-$(OH)_2$vit$D_3$-1-*O*-glucoside, as well as 1,25-$(OH)_2$vit$D_3$-3-*O*-glucoside [170].

Cell differentiation damage induced by calcinogenic plants has been analyzed in skin, aorta and lungs [171]. Aguirre *et al*. [172] reported the bone and growth cartilage changes after subacute poisoning of rabbits with *S. glaucophyllum*. Recently, Gil *et al*. [173] validated a method to estimate the amounts of 1,25-$(OH)_2D_3$ in *S. glaucophyllum* leaves, based on a purification using $C_{18}$ minicolumns, and RIA assays with an antibody raised in rabbits by injection of the acid-$C_{22}$, 1*α*-(OH)vitamin $D_3$. Data were expressed as glycoside equivalent to 1,25-$(OH)_2D_3$ in ng/g of dry leaves, and further compared with those of 1,25-$(OH)_2D_3$ levels [μg of 1,25-$(OH)_2D_3$/g dry leaves] measured, in the same samples, by HPLC with UV detection, after enzyme cleavage. Then, *S. glaucophyllum* leaves were first incubated with rumen fluid, followed by $C_{18}$-OH solid phase extraction, and HPLC analysis.

The calcinogenic annual plant *Nierembergia veitchii* is widely distributed in Rio Grande do Sul, Brazil [174], and in northern Argentina [175]. Arterial lesions consisted of diffuse calcification of the media with areas of cartilage and bone metaplasia and infiltration of macrophages and occasionally multinucleated giant cells. Diffuse intimal thickening (DIT) was very prominent and, in some areas, reached several times the thickness of the media [174].

Oliveira Vasconcelos *et al*. [176] described the cells and extracellular matrix of DIT in spontaneous calcinosis of sheep induced by *N. veitchii*. Morphometric, immunohistochemical and ultra-structural studies were carried out on the DIT in arteries of seven sheep with clinical signs of naturally occurring enzootic calcinosis due to the ingestion of this plant. By immunohistochemistry *α*-actin was detected in cells of the media and in cells forming the intimal thickening. Receptors for 1,25-$(OH)_2$vitamin $D_3$ were detected in nuclei of intimal, medial and endothelial cells [176].

Stimulation of these receptors induced cell proliferation and differentiation in several tissues [177,178], and there was an increase in DNA synthesis and a decrease in heparan sulfate, a glycosaminoglycan responsible for the inhibition of the proliferation of these cells, allowing multiplication of the smooth muscle cells [179].

DIT is a consistent component of arteriosclerotic lesions in *N. veitchii* induced calcinosis of sheep, and the predominant cells in this process are the smooth muscle cells originating from the predecessors of the media. It is suggested that the inducing factor for the arterial changes is 1,25-$(OH)_2D_3$ present in *N. veitchii* [176].

Similar observations were reported in experimental poisoning by vitamin D in rabbits, and in enzootic calcinosis in sheep [174]. DIT was described in calcinosis of cattle [180] and of sheep [174] as an increase of elastic and collagen fibers or subendothelial cell proliferation without a definitive identification of the cell types involved.

Most experiments indicated that the active component of calcinogenic plants most probably should be a glycoside of the metabolite 1,25-$(OH)_2D_3$. The vitamin D-like activity of the calcinogenic plants *S. glaucophyllum*, *C. diurnum*, *T. flavescens* and *N. veitchii* was evaluated by testing different extracts by oral application to rachitic chicks [181]. The serum was analyzed to determine the level of calcium, phosphorus and alkaline phosphatase. The results demonstrated the presence of substances with vitamin D-like activity in the four plants. Only *S. glaucophyllum* and *C. diurnum* contained hydrosoluble substances with a high vitamin D-like activity, which was indicated by the significant high levels of calcium and phosphorus combined with a reduced activity of the alkaline

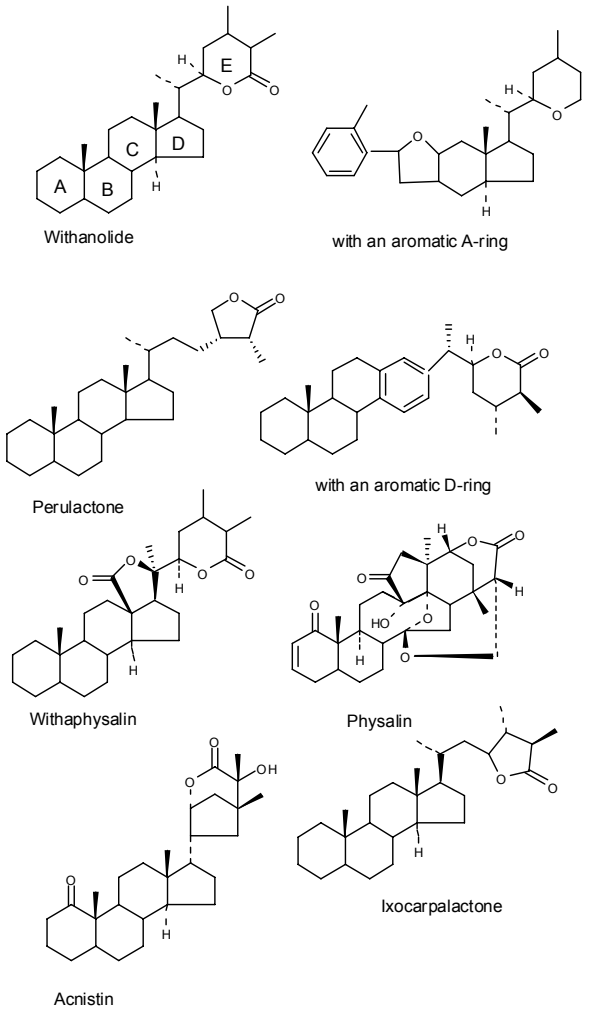



**Figure 26**

phosphatase. The hydrosoluble activity of the active substance in both plants is most probably due to the presence of a glycoside of 1,25-$(OH)_2D_3$ in O-25. *N. veitchii* and *T. flavescens* contained only minor concentrations of these hydrosoluble substances. The four plants were evaluated quantitatively: *S. glaucophyllum* 82,800 IU of vit D/kg, *C. diurnum* 63,200 IU of vit D/kg, *N. veitchii* 16,400 IU/kg and *T. flavescens* 12,000 vit D IU/kg. All concentrations were calcinogenic [181].

(**11**) **Withasteroids:** Withasteroids are polyoxygenated ergostane derivatives, which are mainly found in the Solanaceae. Withasteroids have a lactone group at C-26, and a variety of structures, which can be accordingly classified into eight groups: withanolides, two types of modified withanolides (aromatic A- and D-rings), withaphysalins, acnistins, ixocarpalactones, perulactones and physalins [182]. The skeletons of each group of compounds are shown in Figure 26. These eight structural groups are found

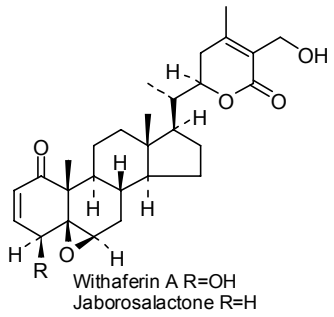



**Figure 27**: Withaferin A and jaborosa-lactone A.

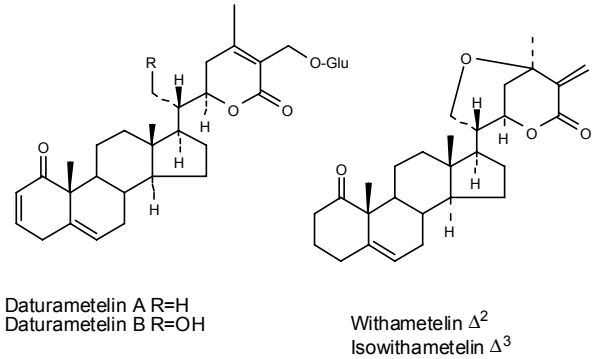
in the following solanaceous genera: *Acnistus, Datura, Deprea, Dunalia, Iochroma, Jaborosa, Lycium, Nicandra, Physalis, Salpichroa, Trechonates, Tubocapsicum, Withania,* and *Witheringia.* A great diversification in ergostane structure is observed in two genera, *Withania* and *Physalis*, which mainly produce withanolides and physalins, respectively.

**(a) Withanolides:** Withanolides (steroidal lactones) are the most abundant withasteroids. These compounds are considered to be precursors of the withaphysalins and acnistins. Withanolides are subdivided into two main classes according to the orientation (α or β) of the lateral chain at C-17. The β-withanolides are the most frequent.

*Withania somnifera*, whose leaves are used for the treatment of tumors, contains withanolides with antibiotic and mytosis inhibitory activity; these compounds are also found in species of *Acnistus*, *Datura*, *Jaborosa*, *Physalis* and *Tubocapsicum*. Examples of this group are withaferin A and jaborosa-lactone A (Figure 27).

Withaferin A was the first withanolide isolated from *W. somnifera*. Thereafter, many other structures of this group of steroids were obtained from the Solanaceae. The antimicrobial activity of withaferin A was described in 1956, before the total identification of the chemical structure [6].

Withanolides, such as daturalactone, were isolated from *Datura ferox*, *D. quercifolia* and *D. stramonium*. Withametelin, isowithametelin (both $C_{28}$-withanolides), and the glucosides daturametelin A and B ($C_{34}$-withanolides) were obtained from *D. metel* (Figure 28). (+)-Jaborol, which has modified A/B rings with further aromatization in ring A, was obtained from *Jaborosa magellanica* (Figure 28 [6].



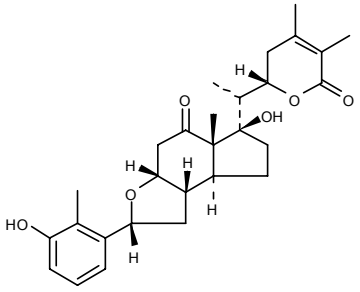




**Figure 28**.

Some withanolides are chlorinated, such as physalolactone and 4-deoxyphysalolactone (both $C_{28}$-withanolides) from *Physalis peruviana* (Figure 29). Others contain sulphur, such as withaperuvin H ($C_{30}$-withanolides) (Figure 29).

Withanolides and related ergostane-type steroids have been reviewed [183]. Furthermore, the chemistry and bioactivity of the withanolides from South American Solanaceae have been recently reviewed [184].

Withanolides are not toxic and/or lethal to mammals, but some of them have shown insecticidal activity. Several ring D aromatic withanolides, for example salpichrolides, showed an antifeedant effect on larvae of *Musca domestica* [185] and *Tribolium castaneum* [186], delayed the development of *Ceratitis capitata* larvae to puparia, and were lethal to *C. capitata* adults [187]. The naturally occurring compounds, salpichrolide A [(20*S*,22*R*,24*S*,25*S*,26*R*)-5α,6α:22,26:24,25-triepoxy-26-hydroxy-17(13→18)-*abeo*-ergosta-2,13,15,17-tetraen-1-one], salpichrolide B [(20*S*,22*R*,24*S*,25*S*,26*R*)- 5α,6α:22,26:24,25-triepoxy-1,26-dihydroxy-17(13→18)-*abeo*-ergosta-2,13,15,17-tetraene], salpichrolide C [(20*S*,22*R*,24*S*,25*S*,26*R*)-22,26:24,25-diepoxy-5α,6β, 26-trihydroxy-17(13→18)-*abeo*-ergosta-2,13,15,17-tetraen-1-one], and

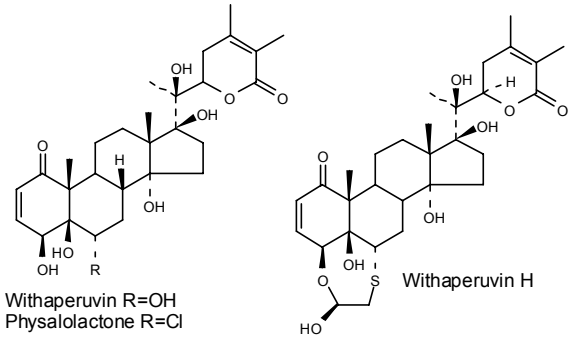



**Figure 29**

salpichrolide G [(20*S*,22*R*, 24*S*,25*S*,26*R*)-5α,6α:22,26:24,25-triepoxy-15,26-dihydroxy-17(13→18)-*abeo*-ergosta-2,13,15,17-tetraen-1-one], previously isolated from *Salpichroa origanifolia*, and other new synthetic analogues were tested [185-187].

Some withanolides exhibited selective phytotoxicity. 4,7,20-Oxowithanolides from *Iochroma australe* showed selective herbicidal activity against weed species. In fact, the extract and the major constituent (17*S*,20*R*,22*R*)-4β,7β,20α-trihydroxy-1-oxowitha-2,5,24-trienolide reduced growth of the radical of *Sorghum halfpence* (monocotyledon) and *Chenopod album* (dicotyledonous), but had no significant effect on either germination or radical length of the commercial crop species *Lactic sativa* [188]. Recently, four withanolides of the twelve new withanolides isolated from *Jaborosa rotate* [189] and three withanolides from *J. calescent* var. *calescent* and *J. calescent* var. *bipinnatifida* [190] showed selective phytotoxicity toward monocotyledonous and dicotyledonous species. The new withanolide, jaborosalactone 43, with a spiranoid δ-lactone at C-22, was isolated from *J. kurtzii*, together with jaborosalactone 44, a 12-oxowithanolide, and showed selective phytotoxicity toward the dicotyledon species, *Lactuca sativa* (lettuce) [191].

Active secondary metabolites are usually biosynthesized in small quantities in the plant. An alternative technology could be the *in vitro* culture of desirable medicinal plants to increase the yield of specific drug components. The biotechnological approaches, such as shooty teratomas, tissue culture, and transformed cultures, were reported for the production of bioactive secondary plant metabolites, for example withanolides [192]. Successful micro-propagation protocols for cloning of some medicinal plants have been developed. Regeneration occurred via organogenesis and embryogenesis in response to auxins and cytokinins [193].

Besides other techniques, genetic engineering of medicinal plants using *Agrobacterium*-mediated transformation has many advantages that include fast growth and high levels of stable production of secondary metabolites, making them commercially and economically feasible. Genetic fidelity of tissue culture raised plants can be ascertained by using molecular markers [194].

*Withania somnifera* has been used in traditional Indian medicine as a tonic and antistress supplement. Pharmacological activities of *W. somnifera* include physiologic and metabolic restoration, antiarthritic, antiaging and immunomodulatory activities, use as a nerve tonic, for cognitive function improvement in geriatric states, and recovery from neurodegenerative disorders, such as convulsions and tardive dyskinesia, [195-198]. *In vitro* and *in vivo* molecular pharmacological investigations have elucidated the association of these activities with the withanolides present in the plant [199].

Biogenesis of withanolides appeared to be highly restricted to a few genera [200], and *W. somnifera* produced the largest number of withanolides with diversified functional groups and regio/stereo-forms, some of which possess specific therapeutic significance [198,201]. Withaferin A and withanone were the major withanolides of the plant, whereas the amount of withanolide A (5α,20α-dihydroxy-6α,7α-epoxy-1-oxowitha-2,24-dienolide) was usually very low [202]. Withanolide A induced nerve development and improved nervous system function, for example it showed strong neuropharmacological properties of promoting neurite outgrowth and synaptic reconstruction [203,204]. Therefore, it could be potentially useful in neurological disorders like Alzheimer's and Parkinson's diseases, convulsions, and cognitive function impairment. However, its usual very low levels in the plant and its occurrence mainly in the roots of *W. somnifera* led to exploration of its production in *in vitro* cultures.

Withanolides like withanone, withaferin A, and withanolide D have been shown to be present in several organogenic cultures, including hairy roots [205,206]. Methanolic extracts of leaves from plantlets growing in tissue culture and those transferred to the greenhouse were evaluated for immunomodulatory activity. While the extract from greenhouse samples showed potent immuno-suppressive activity, those from tissue cultured samples did not. Fractionation and characterization of withanolides revealed the presence of withaferin A in the greenhouse samples. Therefore, *Withania* species may require a longer time and better differentiation, and also a natural environment for the production of withaferin A [205]. Withanolide D-containing fractions were studied for their anti-metastatic activity using B16F-10 melanoma cells in C57BL/6 mice.

Because of the root specific production of withanolide A, root cultures, particularly hairy roots, that can grow rapidly in simple media and can be easily upscaled in a bioreactor were the first choice for production *in vitro*. However, despite several reports on withanolide profiles (withaferin A, withanolide D, *etc*.) of *Agrobacterium rhizogene*-transformed hairy roots of *W. somnifera*, the presence of withanolide A has not been detected in those cultures [207,208]. Withanolides reported in these hairy root cultures were those mainly produced by the aerial parts.

The production of withanolide A was recently reported for the first time in *in vitro* shoot cultures of two experimental lines of *W. somnifera* - *RS Selection-1* (*RS-Sel-1*) and *RS Selection-2* (*RS-Sel-2*) in the presence of different plant growth regulators on MS medium. *RS-Sel-1* showed greater biogenesis/accumulation of withanolide A than *RS-Sel-2* [198]. The presence of other withanolides (like withaferin A) observed in these cultures was similar to that previously reported [205,206].

The enhanced *de novo* biogenesis of withanolide A in shoot cultures was corroborated by radiolabel incorporation studies using [2-$^{14}$C] acetate as a precursor. The *in vitro* shoot cultures also produced withaferin A, the main withanolide of the aerial parts of *W. somnifera* [198], as previously reported [206]. This previous report suggested that its biogenesis in the cultures was strongly modulated by the hormonal balance/ratio in the media.

Developmental variability was introduced into *W. somnifera* using genetic transformation by *Agrobacterium rhizogenes*, with the aim of changing withasteroid production. Inoculation of *W. somnifera* with *A. rhizogenes* strains LBA 9402 and A4 produced typical transformed root lines, transformed callus lines, and rooty callus lines with simultaneous root dedifferentiation and redifferentiation [209]. These morphologically distinct transformed lines varied in T-DNA content, growth rates, and

withasteroid accumulation. Accumulation of withaferin A was greatest in the transformed root line WSKHRL-1, thus being the first detection of withaferin A in the roots of *W. somnifera*. All the rooty callus lines accumulated both withaferin A and withanolide D. Four of these callus lines produced both withaferin A and withanolide D, and they grew faster than the transformed root lines. The presence of withasteroids in undifferentiated callus cultures of *W. somnifera* was reported for the first time [209].

Lee *et al.* [210] studied whether the quantity and quality of light affected growth and development of *W. somnifera* plantlets. Growth and histophysiological parameters [stomatal characteristics, chloroplast pigment concentrations, photosynthesis, and transpiration] of *W. somnifera* plantlets regenerated under either various light intensities, or monochromatic light, or under a mixture of two colors of light in tissue culture conditions were analyzed. Chlorophylls and carotenoids, stomatal number, rate of photosynthesis and transpiration, stomatal conductance, and water use efficiency increased with increasing photon flux density up to 60 μmol/ $m^2$ s. Light quality also affected plantlet growth and physiology. Highest growth was observed under fluorescent and in a mixture of blue and red light. Thus, both the quality and the quantity of light affected the growth of plantlets, development of stomata and physiological responses differently depending on the intensity and the wavelength of the light [210].

Microemulsion electrokinetic chromatography (MEEKC) with a diode-array detector was developed for the simultaneous analysis of natural withanolides, including withaferin A, withacnistin and iochromolide [211]. A capillary electrochromatographic (CEC) method was developed to separate these withanolides, including the critical pair, withacnistin and iochromolide, which was achieved in less than 5 min. Further, CEC was interfaced with ESI-MS. Finally, the described methods were applied to the qualitative analysis of withanolides in *Iochroma gesnerioides* plant extract [211].

A variety of withanolides has also been isolated from *W. coagulans*, for example coagulin F and coagulin G [212], two withanolides, and a withacoagulin [213]. Four withanolides of *W. somnifera* were butyrylcholinesterase inhibitors, but only three compounds were active against acetylcholinesterase [214]. A dimeric withanolide, ashwagandhanolide, has recently been isolated from *W. somnifera* [215]. A detailed spectroscopic evaluation revealed its identity as a dimer with an unusual thioether linkage. This compound displayed growth inhibition against human gastric (AGS), breast (MCF-7), central nervous system (SF-268), colon (HCT-116), and lung (NCI H460) cancer cell lines. In addition, it inhibited lipid peroxidation and the activity of the enzyme cyclooxygenase-2 *in vitro* [215].

Physagulins L, M and N, together with physagulin D, were the withanolides isolated from *Physalis angulata* [216], all of which showed weak trypanocidal activity against trypomastigotes, an infectious form of *Trypanosoma cruzi*, the etiologic agent for Chagas' disease [216].

Fifteen new withanolides, and the known withanolide D and 17$\alpha$-hydroxywithanolide D, were isolated from *Tubocapsicum anomalum* using bioassay-directed fractionation, eight of which showed significant cytotoxic activity against Hep G2, Hep 3B, A-549, MDA-MB-231, MCF-7, and MRC-5 cell lines [217]. The structures were determined by spectroscopic and chemical methods, and the absolute configurations were established by circular dichroism analysis and by the Mosher ester method. Two structures were further confirmed by X-ray crystallographic analysis [217].

Withanolide glycosides named daturametelins H-J, together with daturataturin A and 7,27-dihydroxy-1-oxowitha-2,5,24-trienolide, were recentlly isolated from *Datura metel*. The latter nonglycosidic compound showed the highest antiproliferative activity towards the human colorectal carcinoma (HCT-116) cell line [218]. The structures were determined mainly by spectroscopic techniques, including 2D-NMR (HMBC, HMQC, $^1$H,$^1$H-COSY, NOESY), and MS [218]. Recently, from the flowers of *D. metel*, ten new withanolides were isolated: withametelins I-P, 1,10-*seco*-withametelin B, and 12β-hydroxy-1,10-*seco*-withametelin B, together with seven known withanolides [219]. The structures were elucidated by spectral data, and the absolute stereochemistry was confirmed by single-crystal X-ray analysis. Withametelins I, K L, and N exhibited cytotoxic activities against A549 (lung), BGC-823 (gastric), and K562 (leukemia) cancer cell lines [219].

(**b**) **Physalins:** The genus *Physalis* comprises herbaceous and perennial plants, distributed mainly in the temperate zones of Central and South America. Considering the level of biogenetic oxidation, the genus is the most evolved of the Solanaceae family. This position is evidenced by the presence of polyoxygenated ergostane-derivatives, withasteroids, having lactones, epoxides, and enone functions. The enzymatic system of *Physalis* species has the ability to oxidise the carbons of the steroidal nucleus and the lateral chain, except for C-8, C-9 and C-11, giving rise to a variety of chemical structures, for example, physalins, withaphysalins, ixocarpalactones, and acnistins (Figure 26).

Physalins are $C_{28}$-secosteroid, lactone-type constituents of *Physalis* spp. (steroid derivatives of the 13,14-*seco*-16,24-cycloergostane-type, with a keto function at C-15). The chemistry and spectral data of some withasteroids obtained from *Physalis* species have been recently reviewed [182].

Physalins A to V have been obtained from *Physalis* species, for example *P. alkekengi, P. alkekengi* var. *francheti, P. angulata, P. ixocarpa, P. lanifolia, P. minima, P. peruviana, P. phyladelphia, P. pubescens,* and *P. viscosa.* More than twenty physalins have been isolated from these species, and further structures are being obtained [220]. Moreover, a chlorinated physalin was recently isolated from *P. minima*, which consisted of eight fused rings involving three lactones [221]. The structures were elucidated by 1D ($^1$H NMR, $^{13}$C NMR, DEPT-$^{13}$C NMR) and 2D (COSY, HMQC, HMBC) NMR spectroscopic analysis, and the relative stereochemical assignments were based on 1D NOESY correlations and analysis of coupling constants.

Physalins have a carbonyl group at C-1, and differ in the oxygenated pattern of rings A and B, giving rise to nearly 9 types [182]; for example physalins K and O have a peroxide in the A-ring. There are also distinctions in other parts of each molecule.

Physalin S is characterized by the absence of olefinic signals in the A-ring, but shows methyne proton signals at high field in the $^1$H-NMR spectrum, thus indicating the presence of a cyclopropane ring between C-3 and C-5.

Withasteroids have shown antimicrobial, anti-inflammatory, immunomodulatory**,** antitumor, and

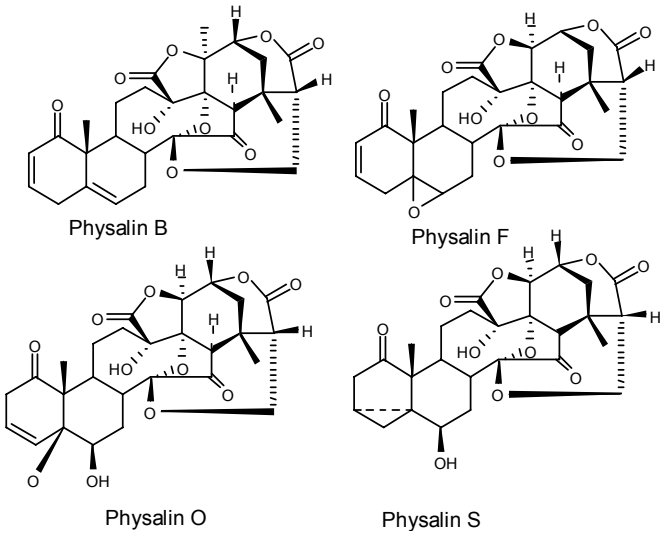



**Figure 30**

trypanocidal activities. Physalins have shown biological activities in humans against immunodeficiency, inflammatory processes, and tropical endemic diseases. Withaphysalins from *Acnistus arborescens* also showed potent cytotoxic activity against a panel of human cancer cell lines [222].

Some physalins have been described as having potent antimycobacterial and antitumor effects [223,224], and those obtained from *Physalis minima,* and *P. minima* var. *indica* showed a potent leishmanicidal activity against the promastigotes of *Leishmania major* [225,226]. Physalins from *P. angulata* also showed antiinflammatory activities [227]. The mechanisms of the anti-inflammatory effects of physalins B and F from *P. angulata* were studied in a model of intestinal ischaemia and reperfusion injury, showing similar effects to those of dexamethasone. Furthermore, the *in vivo* antiinflammatory actions of physalins B and F were mostly due to the activation of glucocorticoid receptors [228].

Physalin F from *P. angulata* exhibited *in vitro* and *in vivo* antitumor effects, whereas physalin B was inactive both *in vitro* and *in vivo* [223]. Further reports on other physalins from *P. angulata* showed strong cytotoxicity against multiple tumor cell lines, including KB, A431, HCT-8, PC-3, and ZR751, with $EC_{50}$ values less than 4 μg/mL [229]. Magalhães *et al*. [230] reported considerable *in vitro* cytotoxicity against several cancer cell lines of physalin B and physalin D isolated from *P. angulata*, thus explaining the ethnopharmacological use.

Recently, the biological evaluation of withanolides, withangulatins, and a group of physalins from *P. angulata* against a panel of human cancer cell lines showed that withanolides and physalins with 4β-hydroxy-2-en-1-one and 5β,6β-epoxy moieties are potential cytotoxic agents [231].

(**12**) **Other bioactive chemical constituents:** The Solanaceae family contains other type of compounds that have shown a variety of bioactivities. Although they are not toxic to mammals, they have shown pesticide activity. These compounds are sugar esters and lectins. Phenylpropanoids are also present in the Solanaceae, but these compounds are not considered in this paper.

(**a**) **Sugar esters:** Trichomes on the leaf surface of many Solanaceae produce and secrete exudates affecting insects, microbes, and herbivores. A variety of sugar esters, also known as acyl sugars or polyol esters, are contained in the exudates and natural wax of the leaves of tobacco [232,233], wild tobacco, *Nicotiana gossei* [234], and other *Nicotiana* species [235], wild tomato (*Lycopersicon pennellii*) [236], tomato [237] and potato species [238,239]. The primary insecticidal compounds within these glandular trichomes are sucrose esters, which were shown in several *Nicotiana* spp. to produce resistance to infestation by green peach aphids, two-spotted spider mites, tobacco hornworm, and greenhouse whitefly [240,241]. Acyl glucose esters isolated from *Datura wrightii* glandular trichomes showed bioactivity against three native insect herbivores [242].

Sugar esters were capable of controlling arthropod plant pests, such as greenhouse whiteflies, sweet potato whiteflies, aphids, mites and psyllids [243,244]. Sucrose octanoate is now registered by the U.S. Environmental Protection Agency (EPA) for a wide variety of insect and mite pests for use on agricultural crops, such as apples, citrus, cotton, grapes, and pears, and to control pests in greenhouses, and of ornamental crops, household and garden plants [244-246]. These compounds are also used by the food industry.

The sugar esters disrupt the physiological functions of the pest, but not the host. Although the mode of action is unknown, it has been suggested that the compounds, like sucrose laurate and sucrose octanoate, act as contact insecticides and behave as surfactants to dewax the insect's outer protective coating causing death by rapid dehydration and/or attack by microbes [245]. The residual sugar esters, after application as insecticides, are biodegradable, and rapidly hydrolyze to harmless sugar and fatty acids.

Metabolic engineering of gland exudation showed potential for improving pest/disease resistance, and for facilitating molecular farming. A cytochrome $P_{450}$ hydroxylase gene specific to the trichome gland was identified [247] and both antisense and sense co-suppression strategies were used to investigate its function.

The acetone fraction of the tobacco leaf surface lipid containing glucose esters and sucrose esters inhibited both tumor necrosis factor-*α* (TNF-*α*) release from BALB/3T3 and KATO III cells induced by okadaic acid and tumor promotion by okadaic acid on mouse skin initiated with 7,12-dimethylbenz(*a*)anthracene (DMBA) [248]. The inhibition of TNF-α release with synthetic disaccharide esters was also reported, such as 4,4'- and 6,6'-diester-trehaloses, and 6,6'-diamide-trehaloses. Since TNF-α is a proinflammatory cytokine playing a role in various pathological states, these non-toxic sugar esters, for example diester-trehaloses of $C_8$ to $C_{12}$ fatty acids, and mimics of disaccharide monoesters, such as *n*-dodecyl-β-D-maltoside, which are inhibitors of TNF-*α* release, appear to be promising cancer-preventive agents of a new type [248].

A novel acylated sucrose ester physaloside A was obtained from *Physalis viscosa* [249], and recently, three 2,3,1',3'-tetraacyl- and two 2,3,3'-triacylsucroses, nicandroses A-E, were isolated from the fruits of *P. nicandroides* var. *attenuata* [250].

Tobacco synthesizes unique proteins, called T-phylloplanins, only in the glands of a particular procumbent trichome type (short glandular trichome) that apparently does not secrete the well known diterpenes and sugar esters produced and secreted by tall glandular trichomes of tobacco [251,252]. T-phylloplanins are defensive proteins for tobacco plants.

(**b**) **Lectins:** Plant lectins are considered a heterogeneous group of proteins with a variety of biological roles. The functions of plant lectins were divided into external activities, such as harmful effects on aggressors, and internal roles, for example in the transport and assembly of appropriate ligands, or in the targeting of enzymatic activities. Structural

and functional aspects of plant and animal lectins, as well as their application to crop protection and in tumor therapy by immunomodulation have been analyzed [253]. Most identified plant lectins can be classified into seven families of structurally and evolutionarily related proteins, each of which is characterized by its own typical sugar-binding motif [254].

Solanaceae lectins comprise a group of unique chimeric plant proteins. Several species of the Solanaceae accumulate lectins in seeds and/or vegetative tissues [254]. These lectins include *Solanum tuberosum* agglutinin (STA) from potato, *Lycopersicon esculentum* agglutinin (LEA) from tomato and *Datura stramonium* agglutinin (DSA) from *Datura*. The molecular structure of the Solanaceae lectins can only be resolved by either determining the complete amino acid sequence of the proteins or cloning of the corresponding genes.

Tubers of *Solanum tuberosum* contain a number of chitin-binding proteins, which have possible functions in defense against pathogens [255]. A major protein of the tuber is the chitin-binding hydroxyproline-rich lectin, which may be involved in the defense mechanism of the plant. This potato lectin (STA) is an unusual glycoprotein containing approximately 50% carbohydrate by weight. Of the total carbohydrates, 92% is contributed by *L*-arabinose, which is *O*-linked to hydroxyproline residues. The ferric chloride-orcinol colorimetric assay (Bial's test), which is specific for pentoses, was developed for monitoring the presence and the purification of potato lectin [256].

Van Damme *et al*. [257] reported a re-investigation of the potato tuber lectin and the molecular cloning of the corresponding gene. Evidence was presented that the classical potato lectin comprised two homologous modules of twin hevein domains, interspersed by an extensin-like domain of approximately 60 amino acid residues. The hevein domain was named after hevein, a small 43 amino acid residue, chitin-binding protein found in the latex of the rubber tree (*Hevea brasiliensis*). The revised structure of the potato lectin confirmed the canonical chimeric nature of the Solanaceae lectins, but also indicated that the physiological role of the chimeric lectin should be revised.

The overall structure of the potato lectin was, to a certain extent, reminiscent of that of some animal collectins (collagen-like lectins), which play an important role in the recognition and binding of microorganisms [258]. Although the potato lectin differs from the collectins due to a double-headed rod-like structure, the extensin-like domain might similarly act as the collagen domain of the collectins. It might imply why the potato lectin specifically interacts with some strains of *Pseudomonas solanacearum* [259] and the *Datura stramonium* lectin interferes with bacterial motility [260].

As potato lectin activated and degranulated both mast cells and basophils by interacting with the chitobiose core of immunoglobulin E (IgE) glycans, a high intake of potato might increase the clinical symptoms as a result of non-allergic food hypersensitivity in atopic subjects [261].

The pattern of lectin binding in the cerebellum of calves poisoned with *Solanum fastigiatum* var. *fastigiatum* was studied [262]. The lectin-binding pattern was compatible with a glycolipid storage disease.

Peumans *et al.* [263] reported the cDNA cloning of a putative tomato leaf lectin. This lectin consisted of two similar chitin-binding modules, each comprising two contiguous hevein domains, interspersed by a short proline-rich domain containing a single Ser[Pro]n repetitive motif. The elucidation of this structure confirmed the chimeric nature of the Solanaceae lectins, but indicated that the previously proposed model of the molecular structure of the tomato lectin needed to be revised. Oguri *et al*. [264] found a 2S storage albumin from the seed of tomato (*Lycopersicon esculentum* L. cv. *Cherry*) that cross-reacted with antiserum to the fruit lectin, and named it Lec2SA. A sequence similarity was found between the large subunit of Lec2SA and the peptide sequence from tomato lectin. However, Lec2SA lacked the carbohydrate-binding domain. Thus, tomato lectin is a chimeric lectin sharing the seed storage protein-like domain that is incorporated into the gene encoding tomato lectin through gene fusion [264]. Sugar chain-binding specificity of tomato lectin was analyzed using lectin blot, and high mannose-type *N*-glycans produced by plants and yeast were recognized [265].

A lectin, with a molecular mass of 79 kDa, and with specificity toward rhamnose and *O*-nitrophenyl-β-D-galactopyranoside, was isolated from samta tomato fruits. The lectin stimulated the mitogenic response in mouse splenocytes and inhibited human

immunodeficiency virus-1 reverse transcriptase with an $IC_{50}$ of 6.2 μM [266].

Tobacco (*N. tabacum* L. cv *Samsun NN*) leaves accumulated a cytoplasmic/nuclear lectin, called Nictaba, in response to methyl jasmonate [267]. To check whether, and if so to what extent, the specific induction of this lectin applied to related species, a collection of 19 *Nicotiana* species, covering 12 *Nicotiana* sections and eight *N. tabacum* cultivars, was screened for their capability to synthesize the jasmonate-inducible lectin. Protein analyses confirmed that only nine out of the 19 species examined synthesized lectin after jasmonate treatment [268].

Jasmonic acid (JA) and its derivatives, commonly designated as jasmonates, are an important group of plant signalling molecules that play an important role in some reproductive processes, as well as in the regulation of plant metabolism and defense against pathogen and insect herbivores [269]. Although the effects of jasmonates on developmental and defense-related processes are well understood, the exact mechanism(s) of the signalling cascades that relate the synthesis of jasmonates and the activation of target genes still remain to be fully elucidated. A mutant was isolated from tomato (called the *jasmonate-insensitive-1* or *jai1 mutant*) [270] as in other plants, indicating that the mode of action of jasmonates might be the same in all plants. The heterogeneity in jasmonate-induced responses applies not only to the synthesis of species-specific secondary metabolites, but also to the accumulation of proteins. Numerous JA-responsive genes have been identified that are clearly upregulated and lead to the synthesis of jasmonate-inducible proteins (JIPs). Recently, the jasmonate-induced expression of the *N. tabacum* leaf lectin (Nictaba) was reported [271].

Sasaki *et al.* [272] found that a lectin, *Datura stramonium* agglutinin (DSA), induced irreversible differentiation in C6 glioma cells. Proliferation of four human glial tumour cells was inhibited by DSA [272]. Further, these differentiated human glial tumor cells had long processes and a high content of glial fibrillary acidic protein similar to differentiated C6 glioma cells. These observations suggested that DSA might be useful as a new therapy for treating glioma without side effects.

A monomeric mannose/glucose-binding lectin, with a molecular mass of 29.5 kDa and an *N*-terminal sequence GQRELKL showing resemblance to that of the lectin-like, oxidized, low-density, lipoprotein receptor from the rabbit, were isolated from the seeds of red cluster pepper *Capsicum frutescens* var. *fasciculatum* [273]. The lectin showed strong mitogenic activity toward spleen cells isolated from Balb/c mice. The lectin also showed antifungal activity, thus inhibiting the germination of *Aspergillus flavus* and *Fusarium moniliforme* spores and hyphal growth in the two fungi [273].

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