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Review

Phytochemicals: The good, the bad and the ugly?

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Abstract

Phytochemicals are constitutive metabolites that enable plants to overcome temporary or continuous threats integral to their environment, while also controlling essential functions of growth and reproduction. All of these roles are generally advantageous to the producing organisms but the inherent biological activity of such constituents often causes dramatic adverse consequences in other organisms that may be exposed to them. Nevertheless, such effects may be the essential indicator of desirable properties, such as therapeutic potential, especially when the mechanism of bioactivity can be delineated. Careful observation of cause and effect, followed by a coordinated approach to identify the responsible entities, has proved extremely fruitful in discovering roles for phytochemical constituents. The process is illustrated by selected examples of plants poisonous to animals and include the steroidal alkaloid toxin of *Veratrum californicum* (Western false hellebore), piperidine alkaloids of *Lupinus* species (lupines), and polyhydroxy indolizidine, pyrrolizidine and *nor*tropane alkaloids of *Astragalus* and *Oxytropis* species (locoweeds), *Castanospermum australe* (Moreton Bay chestnut) and *Ipomoea* species (morning glories).
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Contents

1. Introduction

Phytochemical constituents are essential for the survival and proper functioning of plants. They provide protection

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against herbivores, microorganisms, and competitors; regulate growth (e.g. by delaying seed germination until an appropriate time); and control pollination, fertilization and the rhizosphere environment. In this respect phytochemicals are "good", at least with respect to the producing plant. However, their inherent biological activities can have decidedly adverse effects on other organisms and from that perspective the results may be "bad" or at the very least "ugly" since such entities may be killed or serious injured. Human nature is such that the "bad" is inherently more interesting than the "good" and the more striking the deleterious effect of any particular phytochemical, the more likely it is to be investigated. On the other hand, with some rare exceptions such as allelopathy, the complexities involved in unraveling the specific roles of phytochemicals in plant protection mitigate against research providing rewarding insights. Speculation as to the function of phytochemicals *in planta* is commonplace but concrete answers are infrequent.

In William Shakespeare's eponymous tragedy, Hamlet states: "...for there is nothing either good or bad, but thinking makes it so". This concept can be equally well applied to chemical and biological properties of plant constituents. Hypotheses and research approaches are governed by the perspective adopted as to whether the phytochemicals are "good" or "bad". Conversely, the "bad" properties observed for a phytochemical may prove to be essential leads to understanding biological function and consideration of the way in which these attributes can be employed, or the effects of concentration on activity, may elucidate "good" properties.

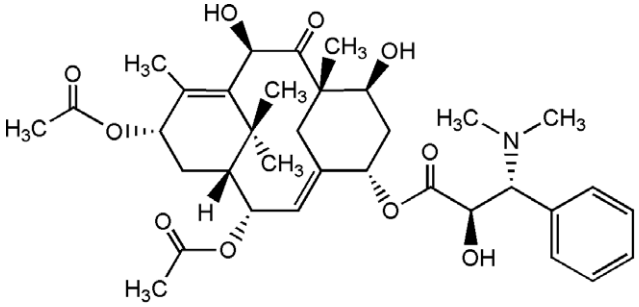
This situation is best exemplified by studying episodic poisoning of large animals by plants. The effects are often dramatic and readily apparent. Careful observation can usually identify cause and effect with relative ease and a considerable range of biological effects may be produced, encompassing the most serious such as death, organ damage, reproductive failure, and birth defects, to more benign but nevertheless economically important factors such as habituation/addiction, malnutrition, or failure to thrive. The greatest challenge to the phytochemist lies in identifying the specific compound(s) responsible for the observed effect, defining their mode of action and finally delineating a useful function for the phytochemical or its derivatives as, for example, a therapeutic drug.

2. Bioactive phytochemicals from plants poisonous to livestock

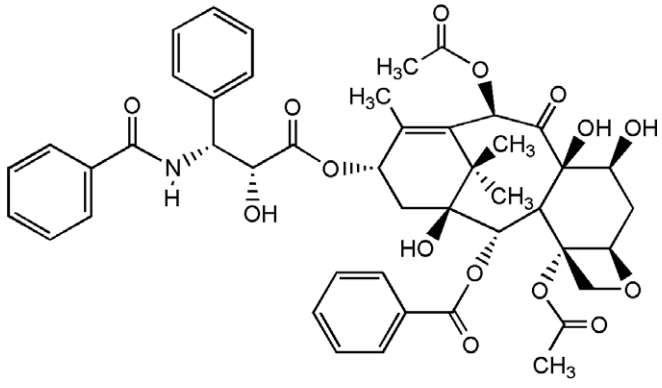
2.1. Taxus baccata and other Taxus species

English yew (*Taxus baccata* L., Taxaceae) has a long and storied history in Europe as a poisonous plant, causing sudden death in animals that consume the leaves and occasionally in humans, especially children, who may be attracted to the colorful fruits containing the poisonous seeds. In Elizabethan times and even earlier the tree was known not only for its toxicity but also for the superior nature of its wood for construction of the famous English longbows. Shakespeare writes: "The very beadsmen learn to bend their bows of double-fatal yew against thy state" in Richard II; the yew was "double-fatal" because of its poisonous nature and its use as a weapon of war. In the United States, *T. baccata* has been introduced for horticultural purposes and livestock poisonings are the result of animals gaining inadvertent access to the trees or from discarded clippings in pastures. In 1992, 35 out of a herd of 43 cattle died due to exposure in this manner (Panter et al., 1993). The plant is extremely toxic, with the minimal lethal dose of the leaves estimated to range from 50 to 100 g in humans to 500 g in cows (Wilson et al., 2001). Death is very sudden, due to cardiac arrest, and toxicity has been attributed to alkaloids such as taxine A (**1**) (Fig. 1) and taxine B together with minor congeners, all of which have a diterpenoid taxane moiety in common (Wilson et al., 2001). Cardiovascular effects are focused on atrial–ventricular conduction caused by alteration of calcium and sodium channel conduction (Tekol and Kameyama, 1987).

It appeared that the acute toxicity of *T. baccata* alkaloids could yield little of value either for experimental studies or for medicinal use, until Wani et al. (1971) employed bioassay-directed fractionation to isolate a taxane alkaloid, taxol (**2**), subsequently renamed paclitaxel, from the bark



(**1**) Taxine A



(**2**) Paclitaxel (taxol)

Fig. 1. Representative structures of diterpenoid taxines and taxanes occurring in *Taxus* spp.

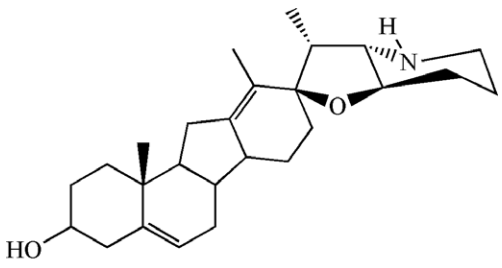
of Pacific yew (*Taxus brevifolia* Nutt.), a native species of the northwestern U.S. and Canada. In spite of its significant antileukemic and antitumor activity further investigation languished until it was shown to potently inhibit B16 melanoma and promote regression of mammary tumor xenografts. Discovery of its unique mechanism of action in stabilizing microtubules in tumor cells, leading to their overproduction with consequent mitotic arrest and cell death (Schiff and Horwitz, 1980), stimulated further research. This ultimately resulted in clinical application of the alkaloid for treatment of refractory ovarian cancer, breast, lung, and colon cancers, and AIDS-related Kaposi's sarcoma. Limited supplies of the natural product from Pacific yew have led to analysis for its presence in other more generally available *Taxus* species (Witherup et al., 1990) and production by semisynthesis from the precursor, baccatin III (Baloglu and Kingston, 1999), which can be obtained in high and sustainable yield from the needles of *T. baccata*. This has enabled comprehensive analysis of structure–activity relationships (Kingston, 2000). Inspection of the structures of the toxic alkaloids, the taxines, and the taxanes, paclitaxel and the baccatins, shows that they possess a diterpenoid moiety with many features in common, but their biological effects derive primarily from the substituents "decorating" this core.

As a result of these studies the English yew has been transformed in a few decades from a plant regarded for centuries with dread, because of its toxicity, to one that is exceptionally beneficial to mankind. Furthermore, the role of the Pacific yew has been acknowledged by placement of a plaque in the Gifford Pinchot National Forest, Washington State, near the site of the first collection of bark from which taxol was isolated (http://www.phcog.org/Taxus/Plaque.html).

2.2. *Veratrum californicum*

One of the most spectacular examples of birth defects caused by maternal consumption of poisonous plants is cephalic malformations, especially cyclopia, in lambs born to sheep ingesting western false hellebore or corn lily (*Veratrum californicum* Durand, Liliaceae) (Binns et al., 1963). The period of insult is confined to the 14th day of gestation and the syndrome is characterized primarily by a single or double globe with a single optic nerve and displaced or absent proboscis. The problem is known colloquially as "monkey-faced lamb" and although relatively common in some herds, proved difficult to elucidate because of the very specific time-frame involved. At later stages of development, different effects are produced, notably limb defects, tracheal stenosis, and embryonic death. Malformations are often so severe that the embryo is resorbed or all indications of parturition disappear, inducing prolonged gestation and resulting in large, stillborn lambs (James, 1977).

V. californicum contains a number of steroidal alkaloids of which cyclopamine (11-deoxojervine) (**3**) (Fig. 2) (Keeler, 1969) is predominant and has been incriminated as



(3) Cyclopamine

Fig. 2. The teratogenic steroidal alkaloid, cyclopamine, occurring in *Veratrum californicum*.

the primary teratogenic constituent (Keeler, 1978). Unsaturation at the 5,6-position is a requirement for teratogenesis, with the electrons of the basic nitrogen being alpha to the steroid plane also having an influence (Gaffield and Keeler, 1993). Despite the importance of this finding in relation to studies of plant-induced terata in sheep, for many years it appeared inconceivable that it could have wider application beyond that as an animal model of cyclopia and other birth defects in humans (Keeler, 1970, 1988) and investigation of structure–activity relationships. This situation changed dramatically in 1996 with the discovery of the role of the gene *Sonic hedgehog* (Shh) in the development of cyclopia in mice (Chiang et al., 1996), indicating that this could be the specific molecular target of cyclopamine and other steroidal alkaloids (Gaffield and Keeler, 1996). Signalling molecules secreted by Shh and its downstream genes, *Patched* (Ptc) and *Smoothened* (Smo) regulate embryonic patterning such as identity and position of organs. Cyclopamine blocks activation of the Shh response pathway by influencing the balance between active and inactive forms resulting from a conformational change in Smo, thus accounting for its teratogenic effects (Incardona et al., 1998). However, the gene also stimulates cell proliferation and is intimately involved in organs in which somatic stem cells persist, such as the skin, colon and connective tissue (including bone), controlling post-embryonic patterning and growth throughout life. Smo is a proto-oncogene, whereas Ptc is a tumor suppressor of basal cell carcinoma, medulloblastoma, fibrosarcoma and rhabdomyosarcoma. Cyclopamine has recently been shown to inhibit Shh signaling by binding directly to Smo and to override the consequences of oncogenic mutations in Smo and Ptc (Taipale et al., 2000; Chen et al., 2002). Such findings have created an explosion of interest in the potential for cyclopamine and its analogues as drugs against tumorigenesis and the alkaloid is currently under investigation as a treatment for diverse cancers. These include brain tumors (Sanchez and Ruiz i Altaba, 2005), prostate cancer (Mimeault et al., 2006), colorectal cancer (Qualtrough et al., 2004), breast (Katano, 2005) and ovarian (Chen et al., 2007) carcinomas. Cyclopamine may also find use for control of basal cell carcinoma (Athar et al., 2004) and as a topical treatment for psoriasis (Tas and Avci, 2004).

2.3. Lupinus and Nicotiana species

Maternal consumption of lupines (*Lupinus* spp., Fabaceae) and tobaccos (*Nicotiana* spp., Solanaceae), as well as hemlock (*Conium maculatum* L., Apiaceae), has long been associated with limb deformations (multiple congenital contractures), known as "crooked calf disease" and cleft palate in in cattle, goats and pigs (Shupe et al., 1967; Keeler et al., 1981; Panter et al., 1985). There is some overlap with regard to the type of terata produced in different animal species and the fact that the period of ingestion does not coincide with organogenesis indicated that a fundamental common mechanism of induction must exist.

Despite belonging to different plant families, the incriminated species all contain piperidine (Fig. 3) and/or quinolizidine alkaloids. Animal species susceptibility correlates with these different structural types, with piperidine alkaloid-containing lupines being teratogenic in both cattle and goats, whereas those containing quinolizidine alkaloids only produce terata in cattle. For this reason, most research has concentrated on species such as summer lupine (*Lupinus formosus* Greene) and longspur lupine (*Lupinus arbustus* Dougl. ex Lindl.), in which the piperidine alkaloids predominate (Panter et al., 1994, 1998), and tree tobacco (*Nicotiana glauca* Graham) (Keeler and Crowe, 1984) with attention focused primarily on ammodendrine (**4**) and the quinolizidine alkaloid, anagyrine, respectively.

Repeated collections of *L. formosus* from a site in Rio Vista, California gave extracts with high levels of ammodendrine (0.47% d.w.) and *N*-acetylhystrine (**5**). However, it was noted that the optical rotation of the ammodendrine varied with collection and that literature values ranged from $[\alpha]_D$ −20° to +15°. Furthermore, a sample collected in 2003 had $[\alpha]_D$ −2.9°, whereas a collection a few days later from a site in San Mateo, California, taxonomically established as identical, had 0.63% d.w. ammodendrine, with $[\alpha]_D$ +1.8°. Given the known propensity of both natural products and synthetic compounds to exhibit quite different biological effects, depending on their stereochemistry, it was apparent that the ammodendrine enantiomers needed to be individually isolated and tested.

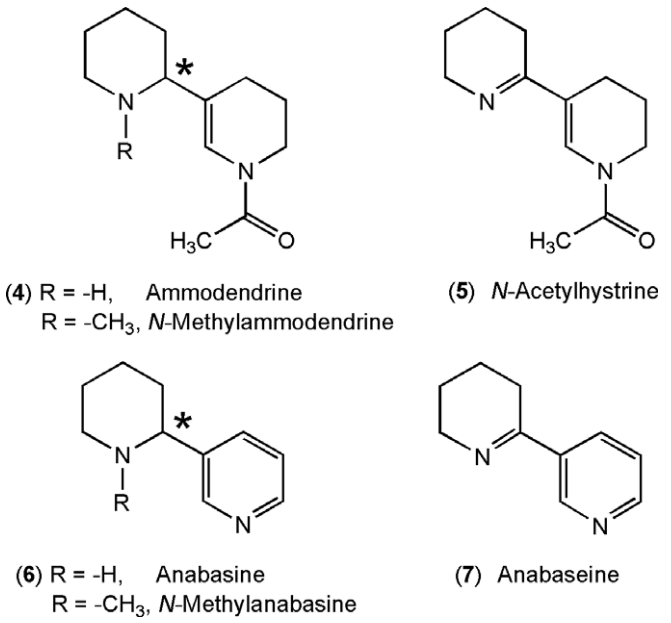


Fig. 3. Piperidine alkaoids occurring in *Lupinus* and *Nicotiana* spp.

Direct separation by HPLC on a chiral column proved not to be achievable but reaction of the enantiomeric mixture with 9-fluorenylmethoxycarbonyl-L-alanine (Fmoc-L-Ala-OH) gave a mixture of D- and L-Fmoc-L-Ala-ammodendrine diastereomers which were separable on a preparative reversed-phase C18 column. Hydrolysis of the Fmoc moiety from the separated diastereomers, followed by Edman degradation to remove the L-alanine group, gave the individual ammodendrine enantiomers (Fig. 4). Rederivatization of each with Fmoc-L-Ala-OH and analysis by HPLC showed an enantiomeric purity for D- and L-ammodendrine of 91% and 98%, respectively, with corresponding values for $[\alpha]_D^{24}$ of +5.4° and −5.7° (Lee et al., 2005). It was impossible to obtain sufficient quantities of the enantiomers by this method to test for teratogenic potential in livestock but measurement of toxicities in a mouse bioassay established the LD_{50} for L-ammodendrine as 115 ± 7.0 mg/kg and that of D-ammodendrine as 94.1 ± 7.8 mg/kg (Table 1). Although these values are statistically different, they are not as marked as might be expected. It is possible that metabolism occurs with loss of the asymmetric center, either through double-bond isomerization or by oxidation to introduce a double bond into the saturated piperidine ring. Adoption of a similar strategy for anabasine (**6**) (Fig. 3) from *N. glauca* enabled the separated enantiomers to be tested both in the mouse toxicity bioassay and for relative agonistic potency towards human fetal neuromuscular nicotinic receptor (nAChR). For both assays, *R*-(+)-anabasine was more potent than *S*-(−)-anabasine, although anabaseine (**7**), with no asymmetric center, was much more potent than either enantiomer (Table 1) (Lee et al., 2006).

Although the molecular mode of action of these alkaloids in producing limb defects and cleft palate is not known, the mechanical mechanism is now well-established. Radio-ultrasound experiments in pregnant animals infused with plant extracts or pure compounds via osmotic minipumps have shown that fetal movement is greatly reduced or completely suppressed (Panter et al., 1990). Using this technique, the specific periods of insult during which various animal species are susceptible to the teratogens have been established for different defects. For example, in goats cleft palate only is induced from days 35–41 of gestation but cleft palate and limb defects are induced from days 30–60 (Panter and Keeler, 1992). Cleft palate appears to be produced by immobilization of the tongue between the palatal shelves, interfering with palate closure. Such findings have important implications with respect to similar congenital defects in humans. Cleft palate can be consistently and reproducibly produced in goats by administration

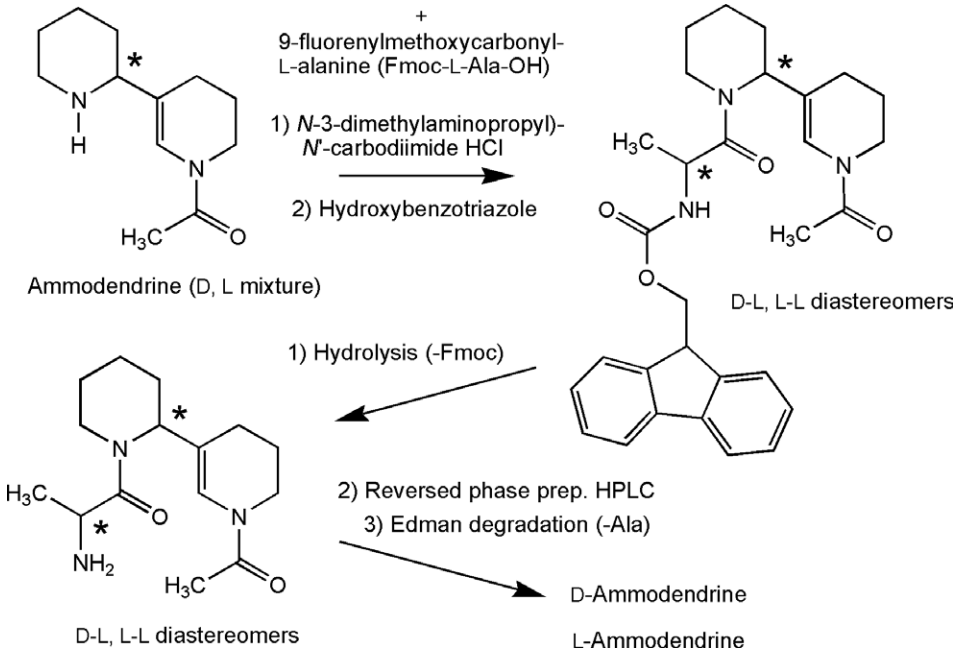


Fig. 4. Procedure for HPLC isolation of individual ammodendrine enantiomers.

Table 1
Comparison of toxicity and nicotinic agonist activity of D- and L-enantiomers of ammodendrine and anabasine

Alkaloid	Toxicity, LD_{50}, mg/kg (mouse)	Nicotinic agonist activity, μmol (human fetal muscle nAChR)
D-Ammodendrine	94.1 ± 7.8	n.d.
L-Ammodendrine	115.0 ± 7.0	n.d.
D-Anabasine	10.9 ± 0.9	2.6 ± 0.56
L-Anabasine	16.2 ± 1.0	7.1 ± 1.3

n.d. – Not determined.

of anabasine (Weinzweig et al., 1999a) and this animal model has been used to develop techniques for surgical repair *in utero* early in gestation, resulting in scarless healing (Weinzweig et al., 1999b). Surgery on children born with cleft palate involves a series of reconstructive procedures at an early age, first to correct the defect and then to rectify consequent problems, including formation of scar tissue. The successful development of an *in utero* operation would obviate some of these problems, due to the opportunity for scarless healing, and the availability of an animal model is an essential first step in this direction.

2.4. Astragalus and Oxytropis species

One of the earliest problems observed on North American rangelands caused by poisonous plants was that known as locoweed disease. Toxicity is due to consumption of certain species of the genera *Astragalus* and *Oxytropis* (Leguminosae) and encompasses a variety of syndromes, including neurological defects (from which the name "locoweed" derives) and depression, emaciation, abortion, birth defects and reproductive disturbances in both males and females. These problems and many of the causative species were clearly defined early in the 20th century by Marsh (1909). However, the identity of the toxin responsible for such diverse effects remained in doubt for another 70 years, until the discovery of the trihydroxyindolizidine alkaloid, swainsonine (**8**) (Fig. 5), in *Swainsona canescens* (Benth.) F. Muell., also a member of the Leguminosae (Colegate et al., 1979). This species and other members of the family, known as "poison peas", which are restricted to Australia, produce symptoms of poisoning remarkably similar to those of the locoweeds and all contain swainsonine. Subsequent research showed that the alkaloid was present in spotted locoweed (*Astragalus lentiginosus* Dougl. ex Hook) but co-occurring with the *N*-oxide (Molyneux and James, 1982) and two structurally related dihydroxyindolizine

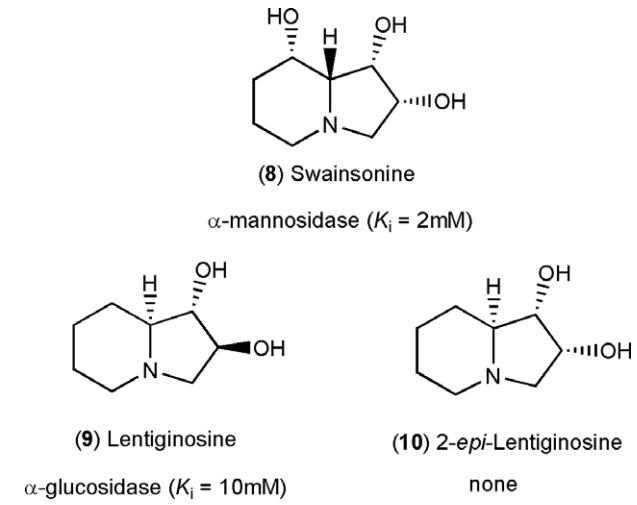


Fig. 5. Indolizidine alkaloid glycosidase inhibitors occurring in *Astragalus, Oxytropis* and *Swainsona* spp.

alkaloids, lentiginosine (**9**) and 2-*epi*-lentiginosine (**10**) (Pastuszak et al., 1990). Additional species of *Astragalus* and *Oxytropis*, historically associated with locoweed poisoning, were shown to contain swainsonine and the alkaloid has now been identified in these species from other parts of the world, such as China and South America, where similar poisoning episodes have been observed (Molyneux et al., 1991a, 1994). Recent research has indicated that swainsonine is produced, at least in some locoweed species, by an endophytic fungus, possibly an *Embellisia* species (Braun et al., 2003). This finding may account for the variability, and sometimes complete absence, of swainsonine in different populations of white locoweed (*Oxytropis sericea* Nutt.) and purple locoweed (*Oxytropis lambertii* Pursh.) (Gardner et al., 2001).

Swainsonine was initially isolated by bioactivity-directed fractionation, using inhibition of α-mannosidase as a biochemical probe (Colegate et al., 1979). This approach was a consequence of the observation that the symptoms of poisoning by *Swainsona* were clinically similar to those of genetic mannosidosis, caused by an absence or insufficiency of the enzyme α-mannosidase, and that the induced disease was phenotypical of the genetic disease (Dorling et al., 1978). The genetic disease is fairly common in Angus cattle and cats, and occasionally occurs in humans; it is classified as a lysosomal storage disease because processing of glycoproteins by α-mannosidase is incomplete and the aberrant products accumulate in the cell, causing vacuolation. Depending upon the organs affected, the clinical signs are manifested in a variety of ways, but neurological problems are commonplace and the most obvious. In contrast to swainsonine, lentiginosine inhibits not α-mannosidase but rather amyloglucosidase, although it does not inhibit other α-glucosidases (Pastuszak et al., 1990). On the other hand, 2-*epi*-lentiginosine did not exhibit any glycosidase-inhibitory activity. The levels of these dihydroxyindolizidine alkaloids in the plant are much lower than that of swainsonine and it is likely that any clinical signs would be minimal and completely masked by the α-mannosidase inhibition. However, the observation that differences in the number and stereochemistry of hydroxy groups distributed around the indolizidine nucleus can affect the specific enzyme that is inhibited was an important insight into structure–activity relationships, suggesting that potential inhibitors of each glycosidase might exist, either as new alkaloids or as synthetic analogues.

Glycoprotein processing is a fundamental metabolic function of all cells and the availability of specific inhibitors provides tools for either investigation or treatment of numerous disease states for which such mechanisms operate. The ability to induce an animal model of mannosidosis may provide benefits for the human patient, especially with regard to enzyme replacement therapy.

The rationale for treatment of cancer by inhibition of glycoprotein-processing pathways, using inhibitors such as swainsonine, has been reviewed by Goss et al. (1995). The alkaloid enhances natural killer cell activity, with important implications for anticancer and antimetastatic activity (Humphries et al., 1988; Bowlin et al., 1989). Administration of swainsonine to mice in the drinking water at 3 μg/mL for 24 h prior to injection of murine melanoma cells into the tail vein reduced pulmonary colonization of the lungs by 80%, and human melanoma xenografts were reduced by 50% at 10 μg/mL (Humphries et al., 1990; Dennis et al., 1990). A phase I study in humans with advanced malignancies, with swainsonine administered over 5 days by continuous infusion, showed remission of head and neck tumors in one patient and symptomatic improvement in two other patients. Oral administration in a phase IB clinical trial showed that the maximum tolerated dose was ca. 300 μg/kg/day and 150 μg/kg/day was recommended as the oral chronic intermittent dose (Goss et al., 1997). Swainsonine is extremely water soluble and pharmacokinetic studies in mice have shown that when administered in drinking water it is retained primarily in lymphoid tissue for at least 3 days (Bowen et al., 1997). The levels in the brain were low and it was concluded that at the levels studied, and for a short period of administration, exposure would be insufficient to produce neurological damage. This suggests that swainsonine could be administered intravenously in humans, prior to and following surgery, to prevent postoperative metastasis of tumor cells.

Swainsonine has also been shown to protect mice bearing melanoma-derived tumors from cytotoxicity of cyclophosphamide, an antitumor drug, without interfering with its chemotherapeutic activity. It also restored colony-forming cells in cultures of murine bone marrow treated with 3′-azido-3′-deoxythymidine (AZT), a myelosuppressive agent used in AIDS therapy, and human hematopoietic cells were protected from AZT toxicity *in vitro* (Klein et al., 1999). Swainsonine has important immunomodulatory effects, increasing colony forming unit (CFU) capacity of bone marrow cells in mice, indicating a potential to overcome the bone marrow suppressive effects of cancer chemotherapy and radiotherapy (Oredipe et al., 2003).

2.5. *Castanospermum australe*

The discovery of the polyhydroxy indolizidine alkaloids such as swainsonine in members of the family Leguminosae suggested that structurally analogous alkaloids would also have glycosidase inhibitory properties. One such alkaloid, already reported in the literature, was castanospermine (**11**) (Fig. 6) (Hohenschutz et al., 1981) which had been isolated from seeds of the monotypic Australian species *Castanospermum australe* A. Cunningham ex R. Mudie, also a member of the Leguminosae. This large, rain-forest tree is known as Black Bean or Moreton Bay Chestnut, the latter name due to its large chestnut-like seeds. These are known to be toxic to animals, especially horses, and have on occasion been responsible for human poisonings, although indigenous peoples ate them without ill-effects after they were ground and washed with water (Everist, 1974).

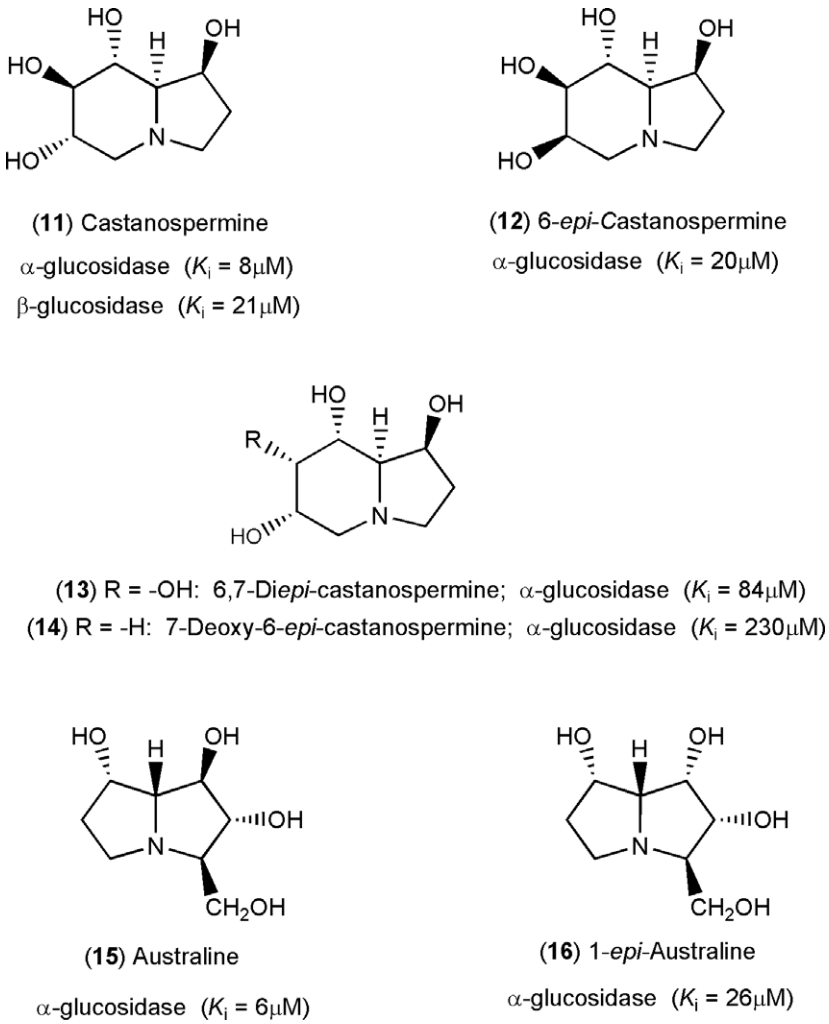


Fig. 6. Polyhydroxy indolizidine and pyrrolizidine alkaloid glucosidase inhibitors occurring in *Castanospermum australe*.

Testing of castanospermine for glycosidase-inhibitory activity showed that it was a potent inhibitor of α- and β-glucosidase (Saul et al., 1984). Subsequent fractionation of the residual extract remaining after crystallization of castanospermine led to the isolation of several indolizidine alkaloid epimers (**12**–**14**) (Molyneux et al., 1986, 1990, 1991b), together with analogous tetrahydroxy pyrrolizidine alkaloids, which were distinguished from castanospermines by the name australines (**15**, **16**) (Fig. 6) (Molyneux et al., 1988; Harris et al., 1989). The australines can be formally regarded as castanospermines that have undergone a ring-contraction with extrusion of a $-CH_2OH$ group; whether this actually occurs or whether the five-membered ring is formed by an alternate ring closure mechanism during the course of biosynthesis remains to be established. However, in spite of such differences in ring size and the number and stereochemistry of hydroxy groups, all of the castanospermines and australines isolated to date inhibit α-glucosidase to a greater or lesser extent. The most effective are castanospermine and australine (**15**), with K_i values of 8 and 6 μM, respectively (Saul et al., 1983, 1984; Tropea et al., 1989). It is also noteworthy that 6-*epi*-castanospermine (**12**), although having a mannose configuration of hydroxyl groups, does not inhibit either α- or β-mannosidase. Polyhydroxy alkaloids are absent or occur at very low levels in other parts of the plant and it seems likely that *C. australe* has a requirement for the seeds to sequester glucosidase inhibitors, possibly related to the ability of castanospermine to inhibit seed germination (Stevens and Molyneux, 1988).

Alexine, the 7a-epimer of australine, has been isolated from *Alexa* spp., South American legumes closely related to *C. australe*, and the stereochemistry of these epimers has been unequivocally established by NMR spectroscopy (Wormald et al., 1998). The scope of glycosidase inhibitory pyrrolizine alkaloids has been expanded by the isolation of tetra- and penta-hydroxyderivatives, namely casuarine and its 6-α-D-glucoside (Nash et al., 1994; Wormald et al., 1996) from *Casuarina equisetifolia* L. (Casuarinaceae) and *Eugenia jambolana* Lam. (Myrtaceae), and hyacinthines from *Hyacinthoides non-scripta* (L.) Chouard and *Scilla campanulata* Ait, both members of the Hyacinthaceae (Kato et al., 1999). *H. non-scripta* also contains monocyclic polyhydroxy pyrrolidines (Watson et al., 1997) and the presence

of glycosidase-inhibitory alkaloids may explain the toxicity of these plants to livestock. Such diversity in structures establishes the generality of polyhydroxy alkaloids as glycosidase inhibitors, many with specificity towards particular enzymes (Elbein and Molyneux, 1998).

As an inhibitor of acid α-glucosidase, castanospermine has potential use for the development of an animal model of Pompe's disease, a genetic disorder in humans characterized by absence or deficiency of the enzyme. The disease is classified as a lysosomal storage disease, characterized by accumulation of glycogen, especially in muscle tissue, and this effect has been reproduced in rats treated with castanospermine at 1.5–2.0 mg/g body weight (Saul et al., 1985).

Early experiments established that castanospermine might be effective in preventing the infectivity of influenza virus through alteration of the viral hemagglutinin (Pan et al., 1983). Suppression of the infectivity of a number of retroviruses has been a particularly noteworthy pharmacological effect reported for castanospermine. The most significant of these is the human immunodeficiency virus (HIV) responsible for AIDS, with the alkaloid preventing cellular recognition of the host and syncytium formation through changes in the structure of the glycoprotein coat of the virus (Gruters et al., 1987; Tyms et al., 1987; Walker et al., 1987). The prodrug, 6-*O*-butyryl-castanospermine, has been used in human clinical trials to overcome the water-solubility and consequent rapid excretion rate of the parent compound (Taylor et al., 1991). The same compound has been tested orally against herpes simplex virus type 1 (HSV-1) in a mouse model and found to produce a significant delay in lesion development and ~100-fold reduction of the virus load (Bridges et al., 1995). More recently, castanospermine has been shown to be effective *in vitro* against all four serotypes of dengue fever virus and to prevent mortality of mice infected with the virus at doses as low as 10 mg/kg bodyweight/day (Whitby et al., 2005; Schul et al., 2007). However, the alkaloid was ineffective against either yellow fever or West Nile virus.

Although antiviral activity appears to offer the most general therapeutic potential, castanospermine has also been shown to be immunosuppressive, promoting heart and renal allograft survival in rats (Grochowicz et al., 1996). In addition it demonstrates antiparasitic activity, preventing adhesion of *Plasmodium falciparum* to infected erythrocytes, thus providing protection against cerebral malaria through alteration of the cellular recognition processes (Wright et al., 1991). As with swainsonine, inhibition of glycoprotein processing by castanospermine inhibits metastasis of B16-F10 murine melanoma cells (Humphries et al., 1986) and induced tumor growth in nude mice (Ostrander et al., 1988). The ready availability of castanospermine from *C. australe* seeds, in which it occurs at levels of 0.3% fresh weight or higher (Saul et al., 1983), makes it a potentially attractive starting material for therapeutic application through synthetic modification to improve pharmacokinetics and overcome gastrointestinal upset.

2.6. Calystegia and Ipomoea species

The discovery of polyhydroxy alkaloids, otherwise known as imino-sugars (Stütz, 1999), raised an awareness among phytochemists that compounds with structural similarities should have analogous glycosidase-inhibitory properties. The observation by Tepfer et al. (1988) of two compounds exuded by the roots of the bindweed, *Calystegia sepium* (L.) R. Br. (Convolvulaceae), that influence the rhizosphere ecology of the plant as a nutritional resource for the specific microorganism *Rhizobium meliloti* strain 41, led to their identification as *nor*tropane polyhydroxy alkaloids (Goldmann et al., 1990). Recognition of their structural affinities to castanospermine, with a pair of five- and six-membered rings, albeit fused in a different manner (Fig. 7), led to their characterization as potent inhibitors of β-glucosidase and α- and β-galactosidase (Molyneux et al., 1993). Although bindweeds are generally regarded as nuisance weeds, rather than poisonous plants, the *nor*tropane alkaloids or calystegines were subsequently identified in *Ipomoea* species (Convolvulaceae) poisonous to livestock, namely *Ipomoea* sp. aff. *calobra* in Australia (Molyneux et al., 1995) and *Ipomoea carnea* Jacq. in Mozambique (de Balogh et al., 1999), in which they co-occur with swainsonine. Similar co-occurrence was found in *I. carnea* growing in Brazil (Haraguchi et al., 2003). This led to the analysis of additional *Ipomoea* species associated with a tremorgenic syndrome in Brazil, with *Ipomoea sericophylla* Meisn. and *Ipomoea riedelii* Meisn. being found to contain swainsonine at levels comparable to North American locoweeds. However, only *I. riedelii* contained calystegines, and they were absent from a third species, *Ipomoea asarifolia* Roem. & Schult., which had only trace amounts of swainsonine (Barbosa et al., 2006; Medeiros et al., 2003).

Fourteen different calystegines have been identified in the plant families Convolvulaceae, Moraceae, Solanaceae and Brassicaceae, with structures falling into three classes, calystegines A (**17**), B (**18**) and C (**19**) (Fig. 8), having three, four or five hydroxyl groups, respectively. Diversity within these three classes arises from the positional and stereochemical configuration of these hydroxyl groups

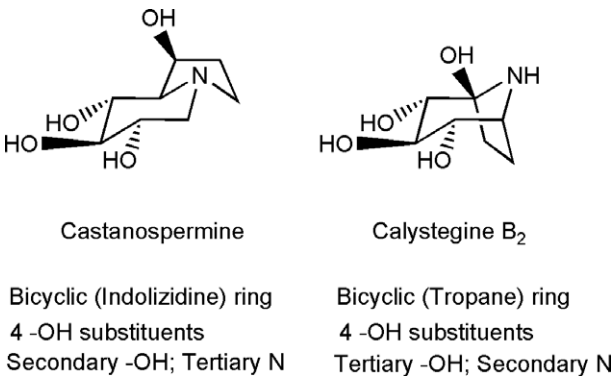


Fig. 7. Structural comparison of the polyhydroxy alkaloids castanospermine and calystegine.

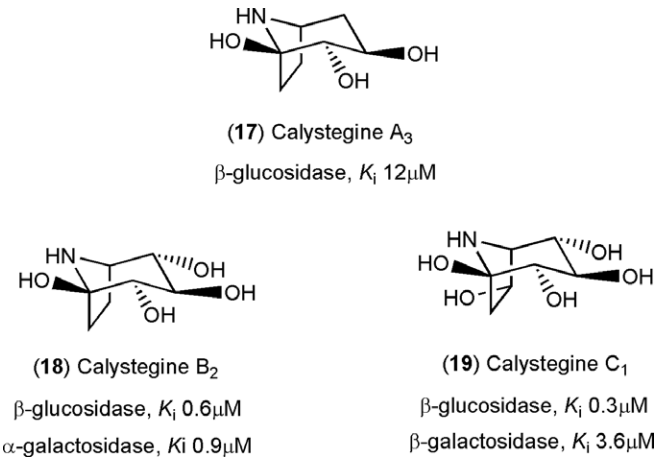


Fig. 8. Classes of calystegine alkaloids occurring in the plant families Convolvulaceae, Moraceae, Solanaceae and Brassicaceae.

(Molyneux et al., 1996). In contrast to swainsonine, castanospermine, and australines, and their congeners, the calystegines have been found in numerous edible fruits and vegetables, especially members of the Solanaceae such as potatoes, tomatoes and eggplants, with the latter containing up to 73 μg/g fresh weight (Asano et al., 1997). Keiner and Dräger (2000) have reported a total calystegine level of as much as 3.3 mg/g in fresh potato sprouts. Consumption of these has been associated with gastrointestinal upset and it is reasonable to suppose that the calystegines may be responsible, given their propensity to inhibit digestive and hepatic enzymes (Asano et al., 1997). The recent discovery of calystegines in cabbage varieties and other members of the Brassicaceae (Brock et al., 2006) raises concerns about the tolerable overall levels of these alkaloids in the human diet.

The relatively recent discovery of calystegines and their limited availability has provided little opportunity for evaluation for therapeutic properties. As inhibitors of α-galactosidase and α-glucosidase they offer potential for the development of animal models of the human lysosomal storage defects, Fabry's and Gauchers's diseases. Fabry's is caused by a deficiency of α-galactosidase, allowing the glycolipid, globotriaosylceramide, to accumulate in various organs, ultimately leading to renal insufficiency and cardiac complications. The disease can be treated by extremely expensive, recurrent enzyme replacement therapy. In contrast, Gaucher's occurs most frequently among Ashkenazi Jews and symptoms, depending on the disease type, are characterized by hepatomegaly, splenomegaly and neurological problems, the most serious of which are convulsions, mental retardation and dementia. The disease is caused by a genetic defect that catalyzes breakdown of lysosomal glucocerebrosidase, an α-glucosidase, resulting in an overall deficiency of the enzyme. Interestingly, *in vitro* experiments with human fibroblasts and lymphoblasts have shown that calystegines B_2 and C_1, as well as some polyhydroxy piperidine glycosidase inhibitors, may act as chemical chaperones, enhancing correct folding of residual enzyme and enabling improved trafficking to the lysosome, with consequent several-fold increase in enzyme activity (Ikeda et al., 2003; Chang et al., 2006).

3. Conclusions and perspective

The examples presented show how phytochemicals, investigated initially for their "bad" properties in poisoning animals may eventually prove to have "good" qualities of benefit to humans as therapeutic drugs. Unfortunately, the time-scale involved in such a transformation is extremely long, on the order of 40–50 years for compounds such as taxol and (possibly) cyclopamine since their first isolation and characterization. At this rate, compounds that have shown experimental promise as anticancer and antiviral agents, such as swainsonine and castanospermine, will not enter the clinic for another 15–25 years. Every effort should therefore be made to diminish this period, perhaps by consciously eliminating the perception that "bad" and "good" properties are mutually exclusive and identifying modes of action and commencing research on beneficial effects earlier in the process. At the same time, it may be worthwhile to take a fresh look at compounds such as the hepatotoxic pyrrolizidine alkaloids, for which no useful properties have yet been discovered. These alkaloids are very widespread in nature, comprising 370 structures from at least 560 plant species and often occur in high yield. For example, riddelliine (**20**) (Fig. 9) has been found in *Senecio riddellii* Torr. & Gray (Asteraceae) at up to 18% of the dry weight of the flowering plant (Molyneux and Johnson, 1984). Although some pyrrolizidine alkaloids have been shown to serve an ecological function in plant-insect herbivore relationships (Hartman and Witte, 1995) their occurrence across so many plant families argues for a more fundamental role, possibly maintenance of homeostasis of water content of plants.

Although the examples presented in this review consist entirely of various alkaloids, there is no reason to assume that therapeutic potential should be restricted to these structural types alone. Many other classes of plant toxins have been identified, such as sesquiterpene lactones, steroids and phenolics, and these also need to be investigated

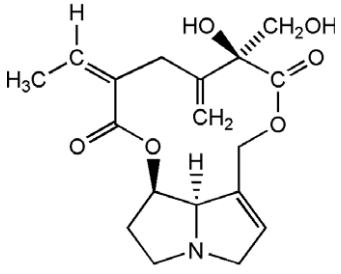


Fig. 9. The macrocyclic diester pyrrolizine alkaloid, riddelliine, occurring in *Senecio riddellii*.

for their biological properties. Discovery of new compounds of use to society, whether as medicines or for other purposes, will fulfill the words of Friar Laurence in Shakespeare's tragedy *Romeo and Juliet*: "For nought so vile that on the earth doth live, but to the earth some special good doth give... Within the infant rind of this small flower, poison hath residence and medicine power..."

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