REVIEWS

Biomedical Applications of Poisonous Plant Research

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Research designed to isolate and identify the bioactive compounds responsible for the toxicity of plants to livestock that graze them has been extremely successful. The knowledge gained has been used to design management techniques to prevent economic losses, predict potential outbreaks of poisoning, and treat affected animals. The availability of these compounds in pure form has now provided scientists with tools to develop animal models for human diseases, study modes of action at the molecular level, and apply such knowledge to the development of potential drug candidates for the treatment of a number of genetic and infectious conditions. These advances are illustrated by specific examples of biomedical applications of the toxins of *Veratrum californicum* (western false hellebore), *Lupinus* species (lupines), and *Astragalus* and *Oxytropis* species (locoweeds).

INTRODUCTION

With the westward migration of the pioneers during the 1800s and early 1900s and the subsequent development of livestock industries in the western United States, poisonous plants were soon recognized as an important cause of economic loss to the livestock industry. Because of these losses and consequent requests for assistance by ranchers and related groups, research was initiated by the U.S. Department of Agriculture on the effects of poisonous plants on livestock. The earlier investigations involved the identification of specific poisonous plants and description of the intoxications, with limited efforts to identify plant toxins. Following World War II and the availability of more sophisticated scientific equipment, efforts were focused more on the identification of plant constituents responsible for toxicity and the mechanisms of action of such compounds (*1*, *2*).

At this time, members of the ranching communities asked that research be initiated on a number of problems causing great economic loss to producers. These included a cyclopian-type birth defect, with a single- or double-globe centrally located eye, that had occurred in lambs throughout central Idaho for many years. The malformation was determined to be the result

of ewes grazing the plant *Veratrum californicum* on the 14th day of gestation, and the toxins were shown to be steroidal alkaloids, primarily cyclopamine. With this knowledge a grazing strategy was initiated that has effectively prevented these malformations from occurring. At about the same time, it became apparent that cows grazing certain species of the *Lupinus* genera gave birth to calves with serious skeletal deformities and cleft palates. As many as 40% of the pregnant cows grazing lupine-infested ranges delivered deformed calves that had to be euthanized, resulting in the loss of millions of dollars. Research was initiated that demonstrated that the primary fetal insult occurred during days 40–70 of gestation and that the lupine alkaloids principally responsible for these defects were ammodendrine and anagyrine. Grazing regimens have now been developed to ease the economic burden of these losses. During the same period research was initiated on toxicological effects of certain species in the genera *Astragalus* and *Oxytropis* (locoweeds). This group of plants is one of the most devastating of the poisonous plant problems, not only in the United States but also worldwide. When consumed over 3–4 weeks by livestock and many species of wildlife, locoweed causes permanent neurological damage, wasting, congestive heart failure when grazed at high elevations, reproductive failure, abortions, and skeletal birth defects. The toxin has been established as swainsonine, an indolizidine alkaloid. Research is continuing to develop methods to prevent such losses, treat

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affected animals, and ensure the safety to humans of meat and milk from animals grazing in locoweed-endemic areas.

More recently, it has been observed that Ponderosa pine needles, when grazed by late-term pregnant cows, cause abortions. Incidence can be as high as 100% in a herd, and because it generally occurs in the last trimester of gestation, some calves are born alive (premature birth) but usually do not survive. Mortality in the cows can be excessive, with high economic losses not only because of animal death but in long-term reproductive effects such as retained placentas and delayed estrus. This condition occurs in most of the western states and in several foreign countries. The abortifacient compound in pine needles has been identified as the labdane diterpene, isocupressic acid, but the mechanism of action is not yet fully understood and predictive models that would lead to strategies to prevent losses remain to be developed.

The above plants, and many others that are less widely distributed or produce episodic poisoning incidents, are toxic because they biosynthesize and accumulate compounds that provide a protective function against predation or a competitive advantage relative to other plants and microorganisms. Such bioactive compounds have adverse effects on various biological systems when consumed by animals, and natural products chemistry has been remarkably successful over the past few decades in isolating and identifying these constituents, providing livestock producers with significant relief from the economic threat from poisonous plants. Although the latter task is by no means completed, and indeed may be increasing because of the current high costs and low returns, the knowledge gained from such research may have broader implications for society as a whole. It is a common paradigm in the search for new drugs in human medicine to investigate constituents of plants that have an ethnopharmacological basis for their use. Plants that are poisonous have been much less commonly studied, perhaps because of a perception that toxic compounds will always exhibit adverse effects. Nevertheless, it is not unreasonable to suppose that by changing the dose the outcome may be altered and that bioactive constituents could be manipulated to yield more positive or beneficial results. This review provides an overview of the advances in understanding human disease states that have resulted from recent investigations of the toxic principles derived from *Veratrum*, *Lupinus*, *Astragalus*, and *Oxytropis* species that have been commonly responsible for livestock poisoning.

VERATRUM CALIFORNICUM

V. californicum (Liliaceae) (**Figure 1**), commonly known as western false hellebore, grows throughout the mountains of the western United States in areas that are readily grazed by sheep. During the mid-20th century, up to 25% of pregnant ewes that grazed in the mountains of central Idaho gave birth to lambs suffering from serious craniofacial defects (*3*). These anomalies varied from the extreme malformation, cyclopia (**Figure 2**), to mildly deformed upper jaws. Basque sheepherders referred to the most common affliction as "chatto', which was also known as "monkey-face" lamb disease (*1*). Ewes carrying twins, or single malformed lambs, had a prolonged gestation during which the lambs continued to grow in utero. Although *Veratrum* is relatively unimportant as a plant poisonous to adult sheep, it is extremely toxic to a developing embryo or fetus. Ingestion of *Veratrum* by sheep on day 14 of gestation was shown to induce grotesque birth defects in offspring, dramatically highlighted by cyclopia (*4*). The birth of deformed lambs was eventually prevented by implementing appropriate range management strategies (*5*). R. F. Keeler of the Poisonous Plant Research

Figure 1. *V. californicum* (western false hellebore; veratrum), the plant responsible for craniofacial birth defects in lambs.

Figure 2. Cyclopian-type birth defect in a lamb induced by maternal consumption of *V. californicum*.

Laboratory (PPRL) in Logan, UT, found that the primary *Veratrum* alkaloid responsible for terata induction was 11-deoxojervine, which he aptly named cyclopamine, **1** (**Figure 3**) (*6, 7*). He further discovered that cyclopamine induced not only craniofacial malformations on day 14 but also limb defects on days 28−31 (*8*) and tracheal stenosis on days 31−33 (*9*). Experiments with ewes given *Veratrum* showed that the number not pregnant was equal to or greater than the number having malformed lambs, indicating that the damage to the developing embryo was severe enough to stop embryonic development (*10*). In addition to sheep, cyclopamine induced cyclopia also in rabbits and chick embryos (*11*). The application of *Veratrum* alkaloids as tools to study embryonic development has been reviewed (*12*).

Molecular Targets of *Veratrum* Teratogens. Recent advances in molecular biology and genetics have provided insight into the mechanisms underlying the induction of teratogenic

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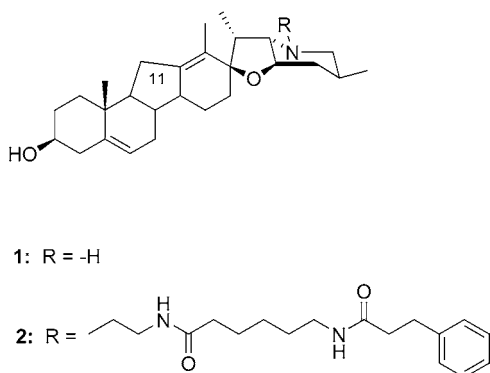*J. Agric. Food Chem.,* Vol. 52, No. 11, 2004 **3213**

Figure 3. Structures of cyclopamine, **1** (11-deoxojervine), the primary *Veratrum* alkaloid responsible for terata induction in lambs, and 3-keto-*N*-aminoethyl-aminocaproyl dihydrocinnamoylcyclopamine, **2** (KAAD-cyclopamine), a synthetic analogue of cyclopamine exhibiting 10–20-fold higher potency than the parent alkaloid.

expressions by cyclopamine. Hedgehog proteins, so-called because mutated larvae appear similar to a crew-cut hedgehog with a back covered by chitinous spines, are intimately involved in diverse processes such as the development of the limbs, skin, eye, lung, teeth, and nervous system and the differentiation of sperm and cartilage (*13*). Two key research discoveries revealed the crucial role of the *Sonic hedgehog* gene (the video game icon possesses synophthalmia with a noselike structure below the eyes) in the developmental patterning of the mammalian forebrain. First, mouse embryos that lacked functional copies of Sonic hedgehog displayed severe holoprosencephaly (failure of division of the embryonic forebrain) that included cyclopia (*14*); second, loss-of-function mutation at the human Sonic hedgehog locus was correlated with holoprosencephaly, for example, hypotelorism, cebocephaly, midline cleft lip, and a single central upper incisor (*15*). Because administration of either cyclopamine, **1**, or jervine (11-ketocyclopamine) to gastrulation-stage embryos induced cyclopia at high incidence in several experimental animals, the *Veratrum* alkaloids were considered to be likely to either inhibit or disrupt Sonic hedgehog-mediated patterning of the neural tube (*16*).

Investigation of a variety of Sonic hedgehog-dependent cell types derived from the neural tube and somites of chick embryo explants with cyclopamine-induced malformations has shown clearly that virtually all aspects of Sonic hedgehog signaling are interrupted in these tissues upon exposure to cyclopamine (*17*, *18*). These studies concluded that the jerveratrum alkaloids cyclopamine and jervine exert their primary teratogenic effect on developing embryos by potently and selectively blocking Sonic hedgehog signal transduction (*17*, *18*). A review on the control of Sonic hedgehog activity and signaling in the neural tube has been published (*19*).

An early hypothesis proposed that defects in intracellular cholesterol transport and homeostasis formed a common basis for the induction of holoprosencephaly by cyclopamine and the 7-dehydrocholesterol reductase inhibitor AY-9944 (*17*). However, this hypothesis was discounted for several reasons: of several compounds evaluated, only cyclopamine completely inhibited Sonic hedgehog signaling in the absence of cytotoxicity, and teratogenic concentrations did not block cholesterol transport; cyclopamine and other steroidal alkaloids that blocked Sonic hedgehog signaling only weakly showed similar activity in cholesterol transport assays; and, steroids such as U18666A and progesterone that are weak inhibitors of Sonic hedgehog markedly disrupted cholesterol homeostasis by affecting the vesicular trafficking of the Niemann-Pick C1 protein (*20*).

Rather than supporting a correlation between cholesterol homeostasis and Sonic hedgehog signaling, an alternative proposal suggested that the functions of both the Niemann-Pick C1 protein and the structurally similar Sonic hedgehog receptor, Patched, involve a common vesicular pathway (*20*). Furthermore, the structural features of steroidal alkaloids previously shown to be responsible for the induction of holoprosencephaly in whole-body animals were shown to be essential in blocking Sonic hedgehog signaling in vitro (*20*). The role of cholesterol in Sonic hedgehog signaling and teratogen-induced holoprosencephaly has been reviewed (*21*, *22*).

The Hedgehog receptor, Patched, functions as a molecular brake to inhibit downstream signaling initiated by Smoothened, an associated membrane protein (*23*, *24*). Binding of Sonic hedgehog to Patched relieves the inhibition of Smoothened and allows downstream signaling that eventually leads to activation of the *Gli* gene family of transcription factors (*25*). In studies with mouse embryonic fibroblasts transfected with Smoothened complementary DNA, cyclopamine treatment suppressed Sonic hedgehog-independent activation of the response pathway (*26*). This observation indicates a target of cyclopamine downstream of Patched that involves a mechanism not requiring direct interference with Sonic hedgehog binding (*26*). Thus, cyclopamine was suggested to inhibit the Sonic hedgehog pathway by antagonizing Smoothened, possibly by influencing the balance between active and inactive forms of this protein (*26*). Further research clearly demonstrated that cyclopamine antagonized Hedgehog signaling by binding directly to the Smoothened heptahelical domain (*27*). Cells transfected with a labeled cyclopamine derivative showed specific cross-linking of Smoothened and also bound specifically to a fluorescent analogue of cyclopamine (*27*). All of these observations demonstrate that cyclopamine inhibition of Hedgehog signal transduction is derived from binding to Smoothened.

Because Smoothened could be inhibited by cyclopamine, other small molecules that modulated Smoothened function were sought (*28*, *29*). Whereas cyclopamine was discovered by a natural accident, systematic screening of chemical libraries has identified a class of chlorobenzothiophenes known as leiosamines that serve as agonists or activators of Hedgehog signaling (*28*, *29*). Combinatorial chemical techniques have also provided several synthetic arylamino Hedgehog pathway inhibitors known either as SANTs (*28*) or Cur61414 (*29*, *30*). The latter compound blocks elevated Hedgehog signaling activity that results from oncogenic mutations in Patched-1 (*30*). Thus, Hedgehog signaling may potentially be either up-regulated or down-regulated by low molecular weight synthetic molecules (*31*).

Cyclopamine as a Biological Probe. Although neutralization of Sonic hedgehog function may be accomplished with either the polyclonal antibody Ab 80 or the anti-Sonic hedgehog antibody 5E1, more stringent inhibition of Sonic hedgehog signaling has been performed with cyclopamine. An example of the exploitation of cyclopamine as a probe in understanding biological development is provided by research on Hedgehog-dependent oligodendrocyte specification in the telencephalon (*32*). Cyclopamine inhibited oligodendrocyte progenitor development in cultures of mouse basal forebrain, indicating that Hedgehog signaling is required for oligodendrogenesis in the ventral telencephalon. Previous research had established that, in the caudal neural tube, oligodendrocyte precursors originate in the ventral neuroepithelium under the influence of Sonic hedgehog before migrating throughout the spinal cord and brainstem and subsequently differentiating into myelin-forming cells. Another example involved the use of cyclopamine

inhibition of Indian hedgehog signaling, which resulted in the promotion of chondrocyte maturation in limb explants of mouse and chick embryos (*33*). Cyclopamine inhibition of Indian hedgehog showed that bone morphogenetic protein signaling does not act as a secondary signal of Indian hedgehog but instead signals independently in the regulation of hypertrophic differentiation of chondrocytes (*33*).

Although extensive redundancy exists between Sonic hedgehog and other hedgehogs in zebrafish midline signaling, cyclopamine treatment showed that Sonic hedgehog appears to be the only hedgehog operative in the early bud, an observation that is important in the interpretation of the Sonic hedgehog null fin phenotype (*34*). Further research in zebrafish showed that cyclopamine treatment blocked the spread of retinal neurogenesis, indicating that several hedgehog genes cooperate to drive the wave of neurogenesis in the zebrafish retina (*35*). This study demonstrates that the role played by hedgehog signaling in retinal differentiation is conserved between flies and fish and therefore supports a common evolutionary origin of the animal eye (*35*).

Other applications of cyclopamine provided information on prostate ductal bud formation (*36*) and gut formation in leech (*37*). In the former research, a nearly total loss of *Gli1* expression, and lesser inhibition of *Gli2* and *Gli3* in urogenital explants, accompanied the dramatic inhibition of prostate bud formation by cyclopamine. In the latter study, cyclopamine induced malformation of the foregut, anterior midgut, and coelomic mesenchyme in *Helobdella* (leech) but showed no apparent effect on the segmental patterning of mesoderm and ectoderm. These results indicate an ancestral role of *hedgehog* family genes in bilaterian animals that was associated with gut formation and/or neural differentiation, rather than segmentation (*37*).

Altered Expression of Organ Tissue. The inhibition of mammalian Sonic hedgehog signal transduction has been correlated with several terata (brain defects, shortened limbs, and malformed jaws) that were induced in lambs whose mothers had ingested *Veratrum*. For example, the rapid, extensive expansion of the developing chick midbrain and forebrain has been retarded, and overall head size was reduced following treatment with cyclopamine (*38*). Second, treatment of regenerating fins in zebrafish with cyclopamine initially reduced and then inhibited fin outgrowth (*39*). These results implicate Sonic hedgehog and bone morphogenetic protein signaling in the proliferation or differentiation of specialized bone-secreting cells in the blastema. They further suggest that Sonic hedgehog expression might be controlled by regulatory feedback mechanisms that define the region of bone secretion in the outgrowing fin (*39*). Finally, mouse embryos exposed to the Hedgehog-pathway inhibitor jervine partially phenocopied the lower jaw defects of *Prx1* and *Prx2* null mutants and further suffered loss of the mandibular incisors (*40*). In the latter study, a novel molecular pathway has been proposed that identifies a mechanism that links the *Prx* genes to Hedgehog signaling in order to shape the developing mandibular arch (*40*).

Expression of organ tissue of numerous mammalian organs can be either enhanced or inhibited upon administration of cyclopamine. For example, exposure of embryonic chicks to cyclopamine promotes pancreatic development (*41*). Cyclopamine inhibition of Sonic hedgehog signaling apparently permits expansion of portions of the endodermal region of the foregut where Sonic hedgehog signaling does not occur, resulting in pancreatic differentiation in a larger area of the foregut endoderm (*41*). Understanding the mode of action of drugs such

Table 1. Human Developmental Malformations and Tumors Due to Mutations in Sonic Hedgehog Signaling Network Genes

gene	syndrome or disease (*ref*)
Sonic hedgehog (*Shh*)	holoprosencephaly (*15*)
	basal cell carcinoma (*45*)
	medulloblastoma (*45*)
	breast carcinoma (*45*)
Patched (*Ptc*)	basal cell nevous syndrome (*50, 51*)
	sporadic basal cell carcinomas (*52*)
	medulloblastoma (*53, 54*)
	trichoepithelioma (*55*)
	breast carcinoma (*56*)
	meningioma (*56*)
	esophageal carcinoma (*57*)
Smoothened (*Smo*)	basal cell carcinoma (*58, 59*)
Gli3	polydactyly, syndactyly, hypertelorism, hypothalamic hamartoma, and anal anomalies (*48*)

as cyclopamine may further the development of cell-replacement therapies for pancreatic diseases such as diabetes mellitus.

Another instance of cyclopamine-induced expansion of organ tissue is represented by the development of an overabundance of fungiform papillae on anterior embryonic rat tongue, with a density that eliminates interpapilla spacing (*42*). This research demonstrated a prominent role for Sonic hedgehog in fungiform papilla induction and patterning and indicated differences in the morphogenetic control of fungiform and circumvallate papilla development and their numbers. A previously unknown, broad competence of dorsal lingual epithelium to form fungiform papillae on both anterior and posterior oral tongue was also revealed (*42*).

An example of inhibition of organ tissue formation is provided by cyclopamine-treated mice that showed striking inhibition of hair follicle morphogenesis (*43*). The hair follicle is a source of stem cells and the site of origin for several epithelial skin cancers (*44*). Because basal cell carcinoma appears to be caused by mutations in genes involved in the Sonic hedgehog signaling pathway, future research may reveal how constitutive activation of the pathway in keratinocytes contributes to the formation of basal cell carcinomas (*45*). However, cyclopamine treatment did not disrupt skin development beyond its effect on follicle morphogenesis (*46*).

Sonic hedgehog is dynamically expressed during murine external genitalia development. Recent results suggest a dual mode of Sonic hedgehog function, first, by regulation of initiating genital tubercule outgrowth and, second, by subsequent genital tubercle differentiation (*47*). Inhibition of Sonic hedgehog signaling by 5,6-dihydrojervine was shown to induce abnormal genital tubercle development (*47*).

Potential Biomedical Applications of Cyclopamine. Secreted signaling molecules of the hedgehog family exert many essential patterning roles during the development of organisms as diverse as humans and *Drosophila*. Although hedgehog proteins most commonly affect cell fate, they can also stimulate cell proliferation. A variety of diseases and clinical disorders result from mutations in the human *Sonic hedgehog* gene and in additional downstream genes such as *Patched* or *Smoothened* that comprise its intracellular signaling pathway (*48, 49*). Included among these diseases are not only holoprosencephaly and various tumors but also several forms of polydactyly that are derived from genetic defects in network genes (**Table 1**) (*15, 45, 48, 50−59*).

Holoprosencephaly can occur in families as part of rare but inherited disorders that encompass a spectrum of malformations

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from mild cognitive effects to severe physical impairment (*60*). The syndrome is relatively common in early embryogenesis, occurring in 1 of 250 spontaneous abortions. The ability of cyclopamine to both induce holoprosencephaly in experimental animals and strongly inhibit Sonic hedgehog signal transduction offers the potential to enhance understanding of the development of the human brain and spinal cord, in particular, at the cellular and molecular level (*61*).

Patched has been implicated as the gene involved in basal cell nevous syndrome, with mutations observed in ∼30−40% of afflicted patients (*62*). Basal cell nevous syndrome, an autosomal dominant condition known also as Gorlin's syndrome, displays a wide spectrum of phenotypes including general overgrowth, polydactyly, and fused or bifed ribs (*63, 64*). Basal cell nevous syndrome is characterized by a large number of basal cell carcinomas, and patients are at further risk for the muscle tumor rhabdomyosarcoma and a greatly increased incidence of the brain tumor medulloblastoma (*64, 65*). Several mutations in the human ortholog of Patched have been detected both in sporadic medulloblastomas and in primitive neuroecto-dermal tumors (*56*). In addition, Patched mutations have been identified in breast carcinoma, a meningioma (*56*), esophageal squamous carcinoma (*57*), and trichoepithelioma (*55*), another type of skin cancer. Activating mutations in Smoothened are also found in appreciable instances of sporadic basal cell carcinomas and primitive neuroectodermal tumors (*59*). Upon inactivation of Patched, Smoothened may become active and independent of Sonic hedgehog control, leading in turn to activation of Sonic hedgehog target genes that might play a role in tumor development.

Cyclopamine treatment caused regression of murine tumor allografts in vivo and induced rapid death of cells from freshly resected human medulloblastomas, but not from other brain tumors. These results established a specific role for hedgehog pathway activity in medulloblastoma growth and showed that cyclopamine can induce tumor regression by specific effects on the hedgehog pathway (*66*). An investigation by the same researchers showed that cyclopamine suppressed hedgehog pathway activity in a wide range of digestive tract tumors, including most of those originating in the esophagus, stomach, bilary tract, and pancreas (*67*). Another study reported that inhibition of hedgehog signaling by cyclopamine induced apoptosis and blocked proliferation in a subset of pancreatic cell lines both in vivo and in vitro (*68*). Finally, the effect of cyclopamine was investigated on small-cell lung cancer (SCLC), an aggressive, highly lethal malignancy with primitive neuro-endocrine features (*69*). Treatment of SCLC cells with either cyclopamine or 3-keto-*N*-aminoethyl-aminocaproyl dihydrocin-namoylcyclopamine, **2** (KAAD-cyclopamine), an analogue of cyclopamine exhibiting 10−20-fold higher potency than the parent alkaloid, silenced hedgehog pathway activation in vitro (*69*). The vulnerability of SCLC to hedgehog pathway blockade might provide a new pharmacologic approach to this disease.

Studies in mouse fibroblast cell lines using KAAD-cyclop-amine, **2**, show that activation is blocked of both the hedgehog response pathway and abnormal cell growth associated with oncogenic mutations in both Patched and Smoothened (*26*). Thus, cyclopamine or its derivatives are proposed as potential "mechanism-based" therapeutic agents for the treatment of tumors arising from disruption of components of the hedgehog pathway (*26*).

Because of its propensity to interfere with cholesterol metabolism that results in a decrease in cholesterol synthesis and an accumulation of late biosynthetic intermediates, cyclop-

amine was evaluated as an inhibitor of multidrug resistance in tumor cells. Intrinsic or acquired resistance of tumor cells to cytotoxic drugs is a major cause of failure of cancer chemo-therapy. Both cyclopamine and the spirosolane alkaloid toma-tidine were observed to act as potent and effective chemosen-sitizers in multidrug-resistant cells (*70*). Both of these steroidal alkaloids are comparable in potency and efficacy to verapamil, a commonly used reversal agent used in multidrug research. Thus, plant steroidal alkaloids such as cyclopamine and toma-tidine, or their analogues, might serve as chemosensitizers in combination chemotherapy with conventional cytotoxic drugs for treating multidrug-resistant cancer (*70*).

Three clinically distinct human polydactyly syndromes are associated with mutations in another family of transcription factors that aid the regulation of target genes of Sonic hedgehog (*48*). These syndromes include Greig cephalopolysyndactyly, Pallister−Hall syndrome, and postaxial polydactyly type A1. Because of Sonic hedgehog pathway involvement in various types of cancer, questions arise concerning the mechanism by which the pathway affects cell division and whether different types of tumor cells are transformed by common events. Hedgehog inhibitors such as cyclopamine might be effective in controlling the onset or progression of certain lesions or disease states in nonpregnant adults because of the low toxicity of drug levels that are effective in blocking Sonic hedgehog (*26*). The use of cyclopamine or its analogues as templates in the development of medicinal agents or drugs for treatment of these afflictions could then be envisioned.

Future Research. Further molecular biological research should reveal both the role of Patched and Smoothened in Sonic hedgehog signal transduction and the cellular conditions that permit induction of holoprosencephaly and various other disease states described above. The potent and selective inhibition by cyclopamine of Sonic hedgehog signaling may serve as an important tool in obtaining this information by offering a chemical probe that will supplement genetic and antibody probes (*71*). Studies on the effects of cyclopamine on craniofacial development will further supplement research on other terato-gens such as the effects of retinoids on the development of facial primordia. The research obtained from these combined ap-proaches should permit an elaboration of the normal develop-mental processes that generate and pattern the human face.

Cyclopamine is considered to be the primary prototype of a small molecule that reveals the logic and timing of vertebrate development (*72*). Models derived from research using cyclop-amine as a probe or tool could provide insight into understanding the etiologic role of environmental agents in various human birth defects. Dissection of the Sonic hedgehog network might further reveal how this network interacts with other signal transduction pathways, for example, those of the transforming growth factor-β (TGF-β) and Wnt families. A pathological role for the hedgehog and Wnt pathways has emerged from research demonstrating a high number of certain human tumors associ-ated with mutations in these pathways (*73*). It has been proposed that tumorigenesis associated with hedgehog and Wnt pathway activation may occur due to misspecification of cells toward stem cell or stem cell-like fates (*73*).

LUPINUS/CONIUM/NICOTIANA

The establishment of appropriate animal models is essential if new techniques and procedures are to be applied to human conditions. *Lupinus*, *Conium*, and *Nicotiana*, even though belonging to different families (Fabaceae, Apiaceae, and Solan-aceae, respectively), are classified together in this discussion

because of common features in their toxicity and potential biomedical application. These include the syndrome of plant-induced skeletal malformation (multiple congenital contractures, MCC) and cleft palate in livestock; the mode of action of these plants in the induction of fetotoxicity and teratogenesis, especially cleft palate; the established use of *Nicotiana glauca* (tree tobacco) and anabasine therefrom as a standard to describe the mechanism of action; and the characterization of a goat model to develop fetal surgical techniques and procedures for in utero cleft palate repair in humans during a privileged (midgestation) period of fetal scarless healing.

Briefly, the syndrome of plant-induced cleft palate and contracture skeletal malformations in livestock is the same whether it is induced by *Lupinus, Conium,* or *Nicotiana* species. However, the piperidine toxins from each plant inducing these malformations possess different toxic potencies because of different chemical structural entities (*74*). In general, the teratogenic piperidines meet specific structural criteria for teratogenesis (*75*). These differences in structural characteristics are important in the manifestation of the mechanisms of action, such as reduction of fetal movement and fetal malpositioning (*76*). The chemical structural differences and mechanism of action will be discussed with reference to the evolution and establishment of the goat model for human biomedical applications.

The cleft palates induced by toxic plants in the goat model closely mimic the human cleft palate condition (*77, 78*). This model is also useful for histological comparison of the prenatal- and postnatal-repaired cleft palate and comparison of craniofacial growth and development. Therefore, this model provides an ideal congenital model to study the etiology of cleft palate in humans, develop fetal surgical techniques in utero, and compare palate histology after prenatal or postnatal repair. The impetus for the biomedical application using these plants and the specific animal model selected has evolved over time and occurred because of the discovery of certain specific biological effects in the goat and their relationship with similar conditions in humans (*76−80*).

Description of the Syndrome. Research at the PPRL on plant-induced skeletal malformations and cleft palate began in the late 1950s when musculoskeletal defects in newborn calves known as "crooked calf disease" (**Figure 4A**) were attributed to maternal ingestion of *Lupinus* spp. (*81−85*). A high incidence of cleft palate was also associated with lupine-induced crooked calf disease (*86, 87*) (**Figure 4B**). Other significant craniofacial deviations such as asymmetry of the skull, maxillary hypoplasia, brachygnathia, or malocclusion often accompany cleft palate. Lupine-induced cleft palate and skeletal malformations continue to cause heavy losses to the cattle industry in the western United States (*88*; personal communications, Dr. Clive Gay, WSU, 1997; Gary Walker, Lewisville, ID, 2001).

In the late 1960s and early 1970s in the midwestern and southern states, epidemic proportions of skeletal malformations from *Conium maculatum* and *Nicotiana tabacum* in pigs were recorded (*89, 90*). It was determined that poison-hemlock (*C. maculatum*) (**Figure 5**) and burley tobacco (*N. tabacum*) were responsible. The induced malformations in newborn pigs appeared similar to and were eventually described as those in lupine-induced crooked calf disease, that is, contracture-type skeletal malformations (arthrogryposis, scoliosis, kyphosis, and torticollis) and cleft palate. In the early 1970s, similar malformations were reported in a herd of cattle in northern Utah, but no lupine was found in the pastures. *C. maculatum* was implicated and later confirmed to be the cause (*91*).
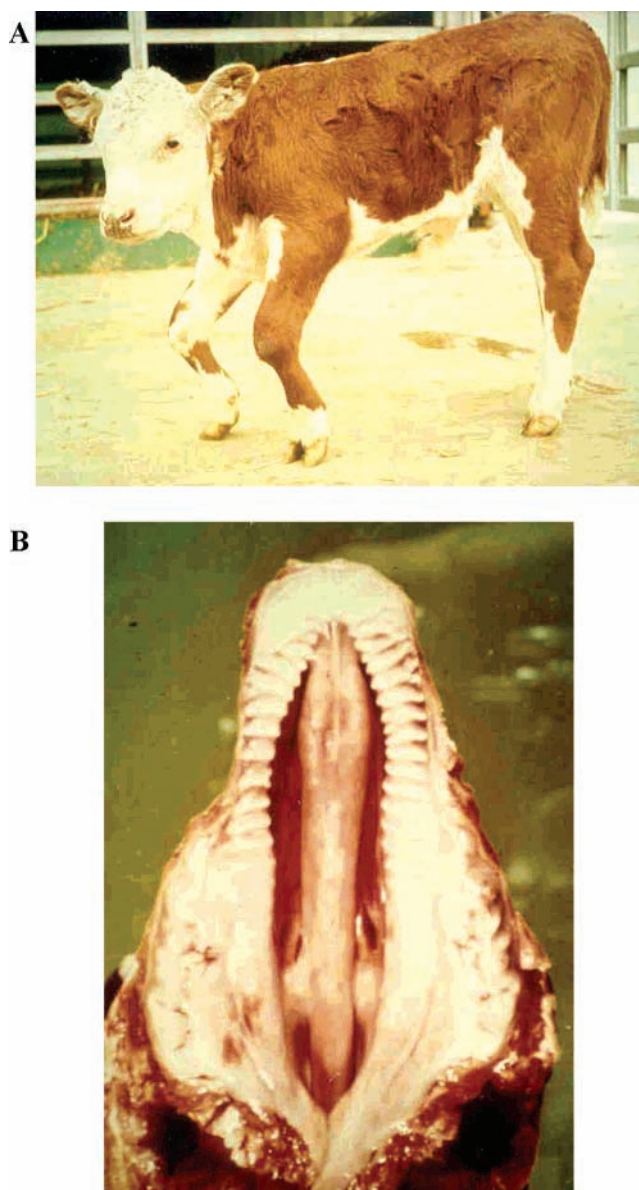

Figure 4. (**A**) Teratogenic "crooked calf disease" resulting from maternal ingestion of *Lupinus* spp.; (**B**) cleft palate associated with lupine-induced crooked calf disease.

Clinical signs of poisoning in all livestock species are similar when this group of piperidine alkaloid-containing plants is involved and begins with nervousness, depression, grinding of the teeth, frothing around the mouth, relaxation of the nictitating membrane of the eye, frequent urination and defecation, and lethargy. These initial signs progress to muscular weakness, tremors and fasciculations, ataxia, collapse, sternal recumbency leading to lateral recumbency, respiratory failure, and death. Signs may appear as early as 1 h after ingestion and progressively get worse over the course of 24−48 h even if further ingestion does not occur. Generally, if death does not occur within this time frame, the animal recovers completely. Extensive experimental feeding trials confirmed the teratogenic effects of *C. maculatum* and *N. tabacum*. The simple piperidine alkaloids (**Figure 6**) coniine, **3**, and γ-coniceine, **4**, from *Conium,* and anabasine, **5**, from *N. tabacum,* were the primary teratogenic piperidines identified. Later research confirmed that these alkaloids caused identical birth defects in cattle, pigs, sheep, and goats (*75, 76, 91−95*). Thus, research on *Conium, Nicotiana,* and *Lupinus,* as a group, over the past 40 years at

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Figure 5. *C. maculatum* (poison hemlock), responsible for contracture-type skeletal malformations in cattle.

Figure 7. *L. formosus* (summer lupine), a teratogenic lupine species containing the piperidine alkaloids ammodendrine, *N*-methylammodendrine, and *N*-acetylhystrine.
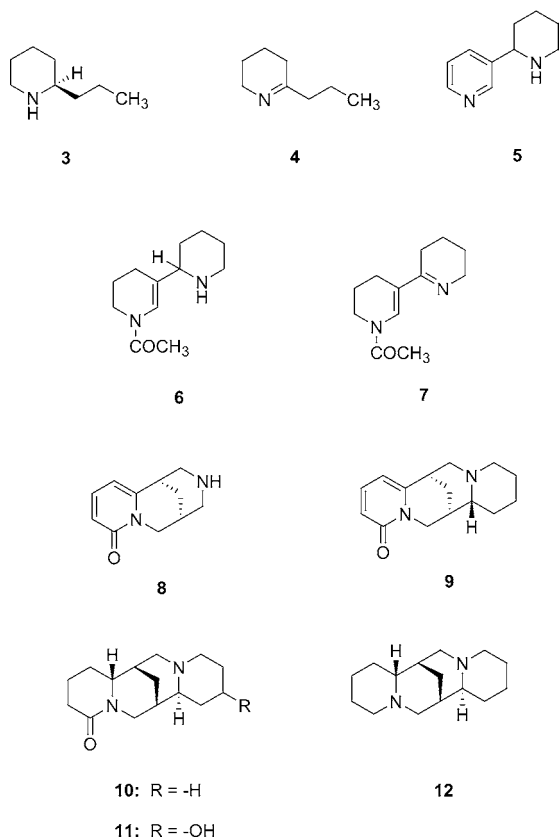

Figure 6. Structures of piperidine and quinolizidine alkaloids from teratogenic *Lupinus, Conium,* and *Nicotiana* species.

the PPRL has focused on the identification of the plant species responsible, isolation and characterization of teratogenic alkaloids, comparison of toxicoses among livestock species, definition of the susceptible stages of pregnancy, and description of the mechanism of action. This research has demonstrated that the birth defects induced by lupines, *Conium* and *Nicotiana* spp.,

are the same and their biological activities occur by similar, if not the same, mechanisms of action (*76*).

Although the toxic and teratogenic syndrome in livestock is the same for *Conium*, *Nicotiana*, and *Lupinus*, there is a difference in manifestation of the teratogenic effects in cattle versus sheep and goats depending upon which lupine species is ingested, that is, those lupine species containing quinolizidine alkaloids versus those containing piperidine alkaloids. The reasons behind this difference are not yet known. The lupine species containing piperidine alkaloids (ammodendrine, **6**, and *N*′-methylammodendrine) are teratogenic in cattle and goats; however, lupine species containing quinolizidine alkaloids are teratogenic only in cattle. This difference between cattle and goats was believed to be metabolic (*96*), but later feeding trials in sheep, goats, and cows failed to support a metabolic theory as the only difference (*97*). The exact reason for this difference between cows and small ruminants is still unknown.

Of the three genera, lupines have been the most economically important to the livestock industry and continue to cause large losses (*88*, *98*). Even though the economic losses to the livestock industry are significant and often devastating, the potential benefits to human medicine through spin-off research and discovery of biomedical tools that may provide medical breakthroughs in the treatment of certain diseases may somewhat overshadow the adverse effects. Thus, as the research in this area evolved, two key factors have surfaced in the study of the mechanism of action and advancement of research on the human condition: the goat as a preferred animal model and *N. glauca* and anabasine as a preferred test plant/alkaloid. With these two factors in hand, advancements in studying the human cleft palate condition are rapidly moving forward (*77*, *78*, *80*).

Mechanism of Action and Insult Periods. More than 170 quinolizidine alkaloids have been structurally identified from the Fabaceae family, and of these only one, anagyrine, is believed to be teratogenic in cattle (*96*, *99*). Eighteen western American lupine species have been shown to contain anagyrine, and 14 of these contain teratogenic levels (*100*). A limited number of lupines contain piperidine alkaloids and include *Lupinus formosus* (summer lupine) (**Figure 7**), *Lupinus arbustus* (longspur lupine), and some varieties of *Lupinus argenteus* (silvery lupine) and *Lupinus sulphureus* (yellow lupine). The piperidine alkaloids from lupines believed to exhibit teratogenic activity include ammodendrine, **6**, *N*′-methylammodendrine, and *N*′-acetylhystrine, **7**.

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Little is known about individual quinolizidine alkaloid toxicity or the biochemical mechanism of action; however, 14 quinolizidine alkaloids isolated from *Lupinus albus*, *Lupinus mutabilis*, and *Anagyris foetida* were analyzed for their affinity to nicotinic and/or muscarinic acetylcholine receptors (*101*). Of the 14 compounds tested, the α-pyridones (cytisine, **8**, and *N*-methylcytisine) showed the highest affinities at the nicotinic receptor (0.14 and 0.05 μM, respectively), as measured by displacement of the radiolabeled ligand [^{3}H]nicotine, whereas several quinolizidine alkaloid types, including the teratogen anagyrine, **9** (IC$_{50}$ = 132 μM), were active at the muscarinic receptor. Lupanine, **10**, which is widely distributed in legumes as a major alkaloid, displayed an IC$_{50}$ of 5 μM at the nicotinic receptor and is 100 times more active than hydroxylated lupanines or alkaloids of the multiflorine series. Thus, 14 alkaloids have been tested from strongest to weakest binding affinity for nicotinic and muscarinic receptors (*101*). If one compares binding affinities of the teratogen, anagyrine, in nicotinic versus muscarinic receptors, there is a 16 times greater binding affinity to muscarinic receptors. Perhaps information about the maternal or fetal mechanism of toxicity or teratogenicity of anagyrine can be inferred from this comparison.

In other experiments, lupanine, **10**, 13-hydroxylupanine, **11**, and sparteine, **12**, were shown to block ganglionic transmission, decrease cardiac contractility, and increase contractility of uterine smooth muscle (*102*). Yovo et al. (*103*) confirmed the nicotinic cholinergic receptor blocking activity of lupanine and sparteine, but both compounds were weak antagonists at the muscarinic cholinergic receptor in their assay. This is somewhat inconsistent with information reported by Schmeller et al. (*101*) as they reported that sparteine was the 2nd most potent muscarinic antagonist of the 14 quinolizidine alkaloids tested, whereas lupanine was 12th. The toxicity of lupanine administered by intraperitoneal injection to mice was less than that of sparteine, with LD$_{50}$ values of 175 versus 36 mg/kg, respectively (*103*, *104*). Although this information is important, there remains the question as to why anagyrine, **9**, is teratogenic in the cow and other quinolizidine alkaloids that are very similar structurally including the stereoisomer of anagyrine, thermopsine, are not (*96*, *105*).

Eight piperidine alkaloids have been identified from *C. maculatum*, and three of those have been demonstrated to possess toxic and teratogenic activities (*74*, *106*). The toxicities of the three predominant alkaloids have been compared, and γ-coniceine, **4**, was found to be ∼7 times more toxic than coniine, **3**, and ∼13 times more toxic than *N*-methylconiine (*74*, *107*). Toxicological and pharmacological studies have been done in cats, mice, and chicks with coniine, γ-coniceine, and *N*-methylconiine. Oral LD$_{50}$ values in mice for γ-coniceine, coniine, and *N*-methylconiine were reported to be 12, 100, and 204.5 mg/kg, respectively (*107*). Similarly, the LD$_{50}$ values via the intravenous route were 2.5, 11.4, and 20.5 mg/kg, respectively (*74*). A similar relationship occurs with three of the main active piperidine alkaloids in tobaccos, that is, anabasine, **5**, anabaseine, and *N*-methylanabasine from *N. glauca* and ammodendrine, **6**, *N*-acetylhystrine, **7**, and *N*′-methylammodendrine in *L. formosus* (*74*).

The predominant action of piperidine alkaloids from *Conium* is nicotinic in nature, consisting of initial transient stimulation followed by a more persistent depression of the autonomic ganglia, neuromuscular junctions, and medulla (*107*, *108*). Pharmacological investigations with coniine have shown it acts as a neuromuscular blocking agent at the neuromuscular junction either on the motor end-plates or at the fine nerve terminals (*107*). The neuromuscular effects are complex and involve multiple sites but resemble nicotine in its central and peripheral effects. However, coniine, **3**, and γ-coniceine, **4**, cause contracture defects and cleft palate, whereas nicotine does not (*90*, *93*, *94*). Peripheral actions on smooth muscle are caused by initial ganglionic stimulation and subsequent blockage in cats, mice, guinea pigs, and rats (*108*). Coniine produces blockade of spinal reflexes, which has been attributed to the increased permeability of membranes to potassium ions. The pharmacological properties of γ-coniceine and *N*-methylconiine are very similar to those of coniine except that γ-coniciene is more stimulatory to autonomic ganglia and *N*-methylconiine has a greater blocking effect (*108*). We believe that other piperidine toxins from *Lupinus* and *Nicotiana* (*74*) are also neuromuscular blockers that inhibit fetal movement during critical stages of gestation, resulting in contractures and cleft palate (*76*, *79*).

Pharmacologic information about the *Conium* alkaloids adds further biochemical evidence upon which a mechanism of action may be hypothesized. It has been suggested that coniine and γ-coniceine possess some curare-like effects (*107*). However, curare does not cause the initial stimulation the *Conium* alkaloids do, but induces a highly selective paralysis of motor end-plates in skeletal muscle and also paralyzes autonomic ganglion cells. The clinical effects of curare are very similar to the depressing effect of the *Conium* alkaloids, coniine and γ-coniceine, but less so for *N*-methylconiine. Could the teratogenicity of *Conium* be attributed to a mechanism similar to that of curare? It is a matter of conjecture whether the teratogenic effects, which are believed to be due to reduced fetal movement during critical stages of gestation, might be attributed to the same mechanism of action, namely, the blockade of effector cells inervated by preganglionic cholinergic nerves in the fetus. If this is the case, then curare should cause arthrogrypotic-type malformations in livestock similar to those induced by *Conium*, assuming there were no metabolic, pharmacokinetic, or excretionary differences. Interestingly, curare and D-tubocurarine are reported to cause arthrogryposis in chicks when administered in eggs and cleft palate in rats (*109*). Furthermore, it has been demonstrated in preliminary studies with goats that curare infused into the embryonic vesicle during early gestation caused severe contracture skeletal defects and cleft palate similar to that induced by *Conium*, *Nicotiana*, and *Lupinus* (K.E.P., unpublished data).

Keeler and Balls (*106*) fed commercially available structural analogues of coniine to pregnant cows to compare structural relationships to teratogenic effects. Results suggested that the piperidine alkaloids must meet certain chemical structural criteria to be teratogenic. On the basis of these data, it was speculated that the piperidine alkaloids with either a saturated ring or a single double bond in the ring, with a side chain of at least three carbon atoms in length adjacent to the nitrogen atom, were potential teratogens (*106*). The piperidine alkaloids described in this section meet these criteria. Additionally, those alkaloids with a double bond adjacent to the nitrogen atom are more toxic than either the fully saturated or *N*-methyl derivatives (*74*).

The proposed mechanism of action for *Lupinus*-induced malformations and cleft palate involves a chemically induced reduction in fetal movement much as one would expect with a sedative, neuromuscular blocking agent, or anesthetic (*76*). The mechanism of action of the teratogenic effects of *Lupinus*, *Conium*, and *Nicotiana* spp. is believed to be similar if not identical (*110*). This mechanism of action was supported by experiments using radio-ultrasound where a direct relationship between reduced fetal activity and severity of contracture-type skeletal defects and cleft palate in sheep and goats was recorded.

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Further research suggested that this inhibition of fetal movement must be over a protracted period of time during specific stages of gestation. This has been supported using a goat model and feeding trials with piperidine alkaloid-containing plants at various stages of gestation. Thus, the mechanism of action of the lupine-induced malformations is apparently an alkaloid-induced reduction in fetal movement similar to the neuromuscular blocking effect of curare or succinyl choline (*76*; K.E.P., unpublished data). This effect has been described and is believed to be responsible for the skeletal contracture malformations and cleft palate (*76, 110*). The skeletal malformations are theoretically caused by abnormal alignment, bowing, or twisting as a result of the in utero positioning and/or abnormal tendon and ligament development resulting from the lack of movement (*76*). The rib cage abnormalities and wedging of the vertebrae of the spinal column and asymmetry of the head are apparently a direct result of malpositioning in utero. The cleft palate is believed to result from mechanical interference by the tongue between the palatal shelves at the programmed time of closure day 38 in goats and between days 40 and 50 in cows (*79, 111*).

Ultrasonographic studies demonstrated that strong fetal movement normally becomes evident in the untreated goat at about day 35 of gestation and that the first movements are extension-type of the fetal head and neck. The heads of fetuses under the influence of anabasine through days 35−41 of gestation remained tightly flexed against the sternum, and no movement was seen. Subsequently, the newborn goats had cleft palate but no other defects. Panter and Keeler (*79*) suggested that these cleft palates were caused by mechanical interference by the tongue between palate shelves during programmed palate closure time [day 38 in goats (*76*); between days 40 and 50 in cows (*111*)]. This inhibition of fetal movement specifically decreased head extension during that critical time and resulted in cleft palate.

In addition to the ultrasonographic studies, which provided direct evidence of reduced fetal movement, the nature of some of the defects in calves from cows gavaged with *L. formosus* and in goats gavaged with *C. maculatum* seed or *N. glauca* plant offers other evidence of the importance of lack of fetal movement in normal development (*96*). These defects included depressions in the rib cage, legs, or spinal column, suggesting a mechanical impact from malpositioning, that is, pressure of a sibling or the head turned back on the rib cage. On the basis of ultrasonographic studies, the action of teratogens appears to be directly on the fetus through inhibited fetal movement rather than via maternal toxicity. Fetal movement inhibition persists between doses for a much greater duration than do signs of overt toxicity in the dam (*76*). Furthermore, manual manipulation of the fetus at the time of ultrasound examination during feeding trials revealed that there was adequate space in the lumen of the uterus for normal body movement, yet the fetus remained totally immobile.

In addition to the malpositioning induced by teratogenic alkaloids, we also suspect that the length of time of continuous exposure of the fetus to the teratogen and the sustained effects over the specific stages of gestation are important in the induction of cleft palate and MCC. This is evident in an experiment in which high incidence of cleft palate was induced during gestation days 32−41 and 35−41, but no clefts were induced during days 36−40, 37−39, or 38 (*80*). The major difference in the incidence of induced cleft palate between goats and sheep may be the length of time of sustained reduced fetal movement over the palate closure period. Fetal goats are continuously inhibited between doses, whereas we believe fetal

sheep experience brief intermittent periods of recovery (i.e., fetal movement) between doses (*76, 79, 80, 95*).

Whereas the mechanical mechanism can be a major factor of cleft palate formation in goats, the molecular mechanism is yet unknown. A reasonable hypothesis, however, is that it may be due to a unique fetal pharmacologic neuromuscular blockade. This is supported by preliminary experiments in which a curare extract and succinylcholine with known pharmacologic activity was infused via osmotic minipumps into the amniotic sac of fetal goats during susceptible periods of gestation (K.E.P., unpublished data). Cleft palate and severe MCC-type skeletal defects were induced. This evidence is preliminary, and further research is needed to verify the biochemical mechanisms of action.

Recent research has confirmed that the goat is a good congenital model for cleft palate research because of the high incidence and predictability of induction (*80*), and a congenital model for the study of the fetal cleft palate in humans was recently characterized (*77, 78*). The sheep was previously established as a preferred model for surgically created cleft palates and subsequent fetal repair (*112*). The goat model has the potential to complement the surgical sheep model for the study of cleft palate and is far superior. Although cleft palates do occur and can be induced in sheep, the incidence has been too low and unpredictable for routine study of cleft palate (*80*).

The periods of gestation when the fetus is susceptible to these plant teratogens have been defined in cattle, sheep, pigs, and goats. The specific insult periods are as follows: swine, 30−41 days of gestation for cleft palate only, 40−53 days of gestation for forelimb, spine, and neck defects, and 50−63 days of gestation for pelvic limb involvement; cattle, 40−100 days of gestation for cleft palate, limb, spine, and neck and 40−50 days of gestation for cleft palate only; and sheep and goats, 35−41 days of gestation for cleft palate only and 30−60 days of gestation for cleft palate and limb defects (*74, 76, 79, 88, 93, 94*). Research using radio-ultrasound to observe fetal movement confirmed early speculation and demonstrated that fetal movement was reduced by maternal ingestion of the teratogens and, in fact, was completely suppressed during the feeding periods when defects were induced (*76*).

As mentioned earlier, the diurnal duration of time during which fetal movements are inhibited is important. For example, fresh *Conium* plant was fed to pregnant sheep and goats during gestation days 30−60 and fetal movement was monitored at 45, 50, and 60 days of gestation over a 12 h period after dosing (*76*). *Conium* plant inhibited fetal movement for 5−9 h after gavage, but by 12 h fetal movement had returned to that similar of controls. The lambs and kids had no cleft palates and only slight to moderate carpal flexure (buck knees), which spontaneously resolved a few weeks after birth. On the other hand, *Conium* seed (with higher teratogen concentration) or *N. glauca* inhibited fetal movement during the entire 12 h period between dosages (twice daily) over the treatment period of 30−60 days. Severe limb, spine, and neck defects and cleft palate occurred.

Even though research at the PPRL has been limited to the three genera mentioned above, there are many other plant species that contain piperidine and quinolizidine alkaloids structurally similar to those known to be toxic and teratogenic. These include species of the genera *Genista*, *Prosopis*, *Lobelia*, *Cytisus*, *Sophora*, *Pinus*, *Punica*, *Duboisia*, *Sedum*, *Withania*, *Carica*, *Hydrangea*, *Dichroa*, *Cassia*, *Ammondendron*, *Liparia*, *Colidium*, and others. Many plant species or varieties from these genera may be included in animal and human diets; however, toxicity and teratogenicity are a matter of dose, rate of ingestion,

time of insult, and alkaloid level and composition in the plant, and toxicity may not always be readily correlated with ingestion.

The research already described for *Lupinus*, *Conium*, and *Nicotiana* has application for the livestock industry but also has important implications in human health. Research by Panter et al. (*76*) suggests that fetal movement and inhibition thereof by these plants is the cause of the described congenital defects and that inhibition of fetal movement over a protracted period by any agent may be a common cause for induction of congenital defects in mammals, including humans. In fact, there is circumstantial evidence that the alkaloids in *L. latifolius* may have been the cause of arm and hand deformities in a baby boy, via maternal ingestion of goat's milk (*113*).

Use of Anabasine To Induce Cleft Palate in Goats. Although the mechanisms of action of the piperidine alkaloid-induced contractures and cleft palate are the same, the potencies of the alkaloids differ, based on structural characteristics (*74*). The structural differences among these piperidine alkaloids have led to a focus on anabasine, **5** (**Figure 6**), the piperidine teratogen from *N. glauca*, because of its potency and consistent activity among different livestock species, that is, cattle, pigs, sheep, and goats. Therefore, *N. glauca*, extracts therefrom, and the pure alkaloid anabasine have been selected to advance investigations about the mode of action of this group of plants to induce contracture malformations and cleft palate. Similarly, goats have been selected as the animal model of choice to study the mechanism of action because of their susceptibility to the teratogenic effects, especially cleft palate (*80*), and their small size, ease of handling, consistent response, and availability (*79*, *80*). Most recently, goats have been demonstrated to be the ideal model for the study of the etiology of cleft palate induction and development of fetal biomedical procedures for human application (*77*, *78*, *80*).

Biomedical Application. Although the development of a small ruminant model, the goat, was primarily to study the mechanism of action of crooked calf disease in cattle, this model, using *N. glauca* and anabasine-rich extracts therefrom to induce fetal cleft palate, has become an important tool in the study of the mechanism of cleft palate induction in humans and fetal biomedical research (*77*, *78*). This research is currently focused on the privileged period of fetal scarless healing and development of in utero surgical procedures to repair human cleft palates early in gestation. To emphasize the significance of this research, Jeff Weinzweig, M.D., a plastic surgeon at Brown University, Providence, RI, has stated: **"Children born with cleft palate often undergo a series of operations to correct the ensuing deformities, only the first of which is the actual palate repair at the age of 6−12 months. For many children, speech remains a major problem as well as craniofacial development. Our goal, of course, is to eliminate the need for any of these reconstructive procedures by performing the cleft palate repair in utero. Never, more than now, in the age of fetal surgery has this been a real possibility. Despite this, what is truly exciting is that we now have a congenital goat model of cleft palate as well as the model of in utero cleft repair."** The biomedical value of this goat model using *N. glauca* plant or anabasine-rich extracts therefrom to induce cleft palates has been demonstrated.

Whereas fetal surgical intervention in life-threatening circumstances has been established (*114*), the role of fetal intervention (i.e., surgery) in the treatment of non-life-threatening congenital anomalies remains a source of much debate (*115*). In 1999 a surgical team at Vanderbilt University operated on a human fetus in utero at 21 weeks of gestation to repair a spinal defect, spina bifida (*116*). Even though the fetal surgery was successful and was later highlighted in *Life* magazine (*117*), the debate continues. Regardless of the debates over fetal surgical intervention, procedures and techniques must be established in animal models that closely resemble the human anomaly to demonstrate the utility and safety of the in utero procedures.

Recently, a congenital model for cleft palate in the goat was described and characterized (*77*, *78*). The methodology and techniques used to successfully repair congenital cleft plates in utero was also presented, and successful scarless palatal healing and development after repair demonstrated (*78*). This model closely simulates the etiopathogenesis of the human anomaly. Thus, in utero cleft palate repair early in gestation, on or before day 85 in the goat, is feasible and results in scarless healing of the mucoperiosteum and velum. Furthermore, this congenital cleft palate goat model is highly reproducible with little variation, representing an ideal animal model (*80*).

Previous cleft palate work had focused on the development of surgically created models in sheep (*112*). Other models to study fetal wound repair were developed in the mouse, rat, rabbit, guinea pig, pig, opossum, and monkey (*118*). The smaller, short-gestation models, such as the mouse, rat, guinea pig, and rabbit, allowed fetal manipulation only later in gestation, when the postoperative intrauterine period is short and after the privileged period of fetal scarless healing had passed. In contrast, larger, long-gestation animals, such as the monkey, sheep, and goat, provide for easier and more extensive manipulation during the period of early gestation (< day 100). Early surgical intervention has proven to be far superior to postnatal repair as fetal repair is often scarless and there is a longer postoperative intrauterine period for monitoring healing and development. This is especially true of craniofacial defects, because early surgical repair maximizes the advantage of the privileged fetal environment for scarless healing and minimizes neonatal disfiguring lip and palate scarring (*119*).

N. glauca-induced cleft palate and musculoskeletal contracture malformations in the goat have proven to be excellent models to study the mechanism of action of similar lupine-induced malformations in cattle (*76*, *79*). The model has shown that alkaloid-induced reduction in fetal movement over specific periods of gestation was directly responsible for cleft palate and musculoskeletal malformations. Similar models have established the specific cleft palate induction periods in pigs (gestation days 30−45) (*94*), goats (35−41 days of gestation) (*79*), and cattle (40−50 days of gestation) (*111*). Consequently, *N. glauca* and anabasine-rich extracts therefrom are now used almost exclusively in conjunction with the goat model to study in general the mechanism of action of plant-induced cleft palate and MCC, to further define susceptible periods of gestation, and, most recently, to study the etiopathogenesis of cleft palate development and the potential for fetal intervention and in utero repair in humans.

The sheep had been previously established as a model to study methods of in utero scarless repair of cleft palates and cleft lips, but these clefts were surgically created (iatrogenic) (*112*). Although the surgical sheep model has been successfully used for developing early techniques, there are certain clinical limitations when using a surgical model versus a model in which the malformation is induced or congenital or more "natural". The limitation of the surgical model is expressed in the following statement by Hedrick et al. (*112*): **"Ideally, a large animal model that intrinsically forms clefts would provide the best model with which to evaluate the efficacy of fetal cleft**

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repair...no such practical intrinsic model exists...the fetal lamb is not known to form clefts intrinsically in response to teratogens...clefts in this model must be created surgically." Whereas the fetal lamb does intrinsically form cleft palates, as demonstrated in a recent study (*80*), the incidence is so low and the occurrence so unpredictable that using the sheep model has been impractical. The goat congenital model is more efficient and closely mimics human cleft palates (*77*, *78*).

As stated previously, the primary mechanism responsible for cleft palate induction in this model is believed to result from an alkaloid-induced reduction in fetal movement during the palate closure period (*76*, *79*). Ultrasound imaging of fetuses gavaged with *N. glauca* during the normal programmed palate closure period of 35−41 days showed a tight flexure of the head and neck, with negligible space between the chin and sternum. These observations are in contrast to the intermittent head and neck extension movements in untreated fetal goats that normally begin about days 35−38 of gestation (*120*). Therefore, inhibited movement of the fetal tongue with prolonged maintenance between palatal shelves, combined with the hyperflexed position of the head and neck, is believed to be the mechanical mechanism of alkaloid-induced cleft palate formation in goats (*79*). This proposed mechanism is supported by similar theories offered for the pathogenesis of cleft palate in the Pierre Robin Syndrome (PRS), the etiology of which is heterogenic, resulting from fetal malposition due to multiple causes (*121*). Like the alkaloid-induced condition, this malpositioning prevents the tongue from descending from the nasal cavity and interposition of the tongue between the palatal shelves will prevent or delay the fusion of the palate at the midline.

In conclusion, this research has significant implications in the management of fetal cleft palates in humans in addition to its important applications in agricultural research. Understanding periods of fetal susceptibility, identifying teratogenic plants and specific toxins therefrom, and understanding mechanisms of action will provide information for livestock managers whereby losses might be reduced.

ASTRAGALUS AND *OXYTROPIS* AND OTHER PLANTS WITH RELATED TOXINS

Locoweed poisoning epitomizes livestock poisoning in general and has probably stimulated more research activity than any other poisonous plant problem. The earliest settlers of the American West were plagued by losses caused by animals consuming the plants responsible. The latter were not clearly defined, and some of the earliest research undertaken by the U.S. Department of Agriculture to prevent widespread livestock losses was focused specifically on the locoweed problem. In 1909, Marsh (*122*) published a monograph that clearly established certain species of the genera *Astragalus* and *Oxytropis* (Fabaceae) as the causative agents and defined the animals affected, symptoms of poisoning, pathological lesions, conditions under which poisoning occurs, and potential prevention techniques and remedies. It should be emphasized that *Astragalus* and *Oxytropis* species are commonly known as "milkvetches", but only those species established as producing the symptoms of locoism in animals should be properly named as "locoweeds".

Consumption of locoweeds by animals produces a multitude of syndromes, including, but not limited to, neurological problems, depression, emaciation (**Figure 8**), reproductive disturbances, birth defects, and abortion. However, lambs fed locoweed have been shown to gain weight more quickly during the first week to 10 days of consumption than control lambs.

Figure 8. Cow exhibiting emaciation as a consequence of locoweed consumption.

Figure 9. *A. lentiginosus* (spotted locoweed), a typical locoweed species, containing the glycosidase-inhibitory alkaloids swainsonine, lentiginosine, and 2-epilentiginosine.

Specific problems observed are a consequence of numerous factors, including animal species, nutritional status, prior exposure to the plant, pregnancy, and environmental conditions (*123*). For example, cattle grazing the plants at high altitudes exhibit congestive right-heart enlargement and edema of the brisket (*124*, *125*), with nursing calves exhibiting the symptoms prior to their mothers, indicating that the toxin is transferred in the milk and either that it is concentrated in lactation or that young animals are more susceptible.

Despite numerous attempts to identify the toxin, in the course of which it was variously attributed to inorganic components such as selenium or barium, it was not until 1982 that the primary toxic constituent of spotted locoweed (*Astragalus lentiginosus*) (**Figure 9**) was unequivocally established as the indolizidine alkaloid swainsonine (**13**) (**Figure 10**) (*126*). Swainsonine was first isolated and identified as the toxic constituent of *Swainsona* species, known as "poison peas", a genus of legumes restricted to Australia but closely related to the locoweeds both in physical appearance and also in their effects on livestock (*127*). Although many species of *Astragalus* and *Oxytropis* do not contain swainsonine, it has been detected in all of those that are generally regarded as locoweeds, including widely distributed species such as woolly loco (*A. mollissimus*) and white loco (*O. sericea*) (*128*). The toxin has also been found in a number of *Astragalus* and *Oxytropis* species in other parts of the world, such as South America and areas of China (*129*). It has recently been shown to occur in members of the morning glory family (*Ipomoea* species) that are poisonous to sheep in Australia and to goats in Mozambique and Brazil, respectively (*130−133*). In addition to swainsonine, *A.*

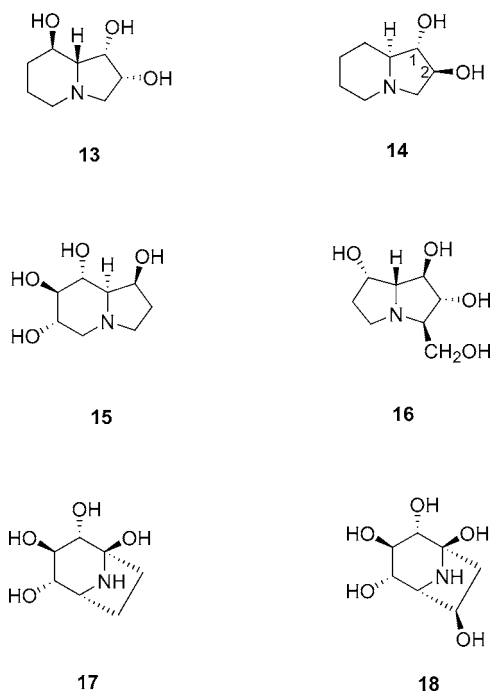**3222** *J. Agric. Food Chem.,* Vol. 52, No. 11, 2004

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Figure 10. Representative structures of glycosidase-inhibitory alkaloids of the indolizidine (swainsonine, **13**; lentiginosine, **14**; and castanospermine, **15**), pyrrolizidine (australine, **16**), and tropane (calystegines B2, **17**, and C1, **18**) classes.

Figure 11. *C. australe* (Moreton Bay chestnut or black bean), showing the seeds and pods that contain the glycosidase inhibitors castanospermine and australine.

lentiginosus also contains minor amounts of its *N*-oxide derivative and lentiginosine (**14**) and 2-epilentiginosine (**Figure 10**), two structurally related dihydroxy alkaloids (*134*).

Although the toxin content of locoweeds is not very high, generally not more than 0.2% of the dry weight of the plant, it is found in all above-ground parts of the plant and is more concentrated in the flowers and seeds. However, because of its exceptional potency, it has been calculated that levels >0.001% can cause poisoning if the plant is consumed over a sufficient period of time (*129*). The alkaloid appears to be remarkably stable, and even plants dead for as long as two years retain enough of the toxin to cause locoism (*135*). The potential hazard of any particular species to livestock can be assessed by analysis of a representative plant sample for the presence of swainsonine.

The initial isolation of swainsonine from *Swainsona* species by Colegate et al. (*127*) resulted from the recognition that the poisoning induced in livestock by these plants was biochemically, morphologically, and clinically similar to the genetic disease mannosidosis, which results from an insufficiency of the enzyme α-mannosidase. It was therefore hypothesized that the toxin was an inhibitor of α-mannosidase, and this enzyme was then used as a probe for such bioactivity and ultimately the separation and purification of the active compound. The chemical structure of swainsonine is not complex and has many similarities to the simple sugar mannose, which it appears to mimic. As a result of this emulation, and the presence of a nitrogen atom in the molecule, it suppresses the action of the enzyme α-mannosidase, which is essential for the proper functioning of all animal cells. This enzyme trims sugar molecules from complex molecules known as glycoproteins (sugar-proteins) within the cell. Once the correct number of sugars has been trimmed off, the smaller molecules can be targeted for either retention, incorporation into the cell-wall structure, or release from the cell to serve the specific functions for which they have been synthesized (*136*). Failure of the trimming process results in an accumulation of complex

molecules within the cell until it can no longer contain them and vacuolation results. After a sufficient number of cells have been damaged in this way, the signs of poisoning appear in the animal. Because all cells depend on the proper functioning of α-mannosidase, many different organs can be damaged, including the brain, heart, reproductive system, and digestive system. The particular organs affected and signs of poisoning depend on the amount of swainsonine consumed and the period of exposure, as well as external factors such as nutritional status, grazing altitude, and pregnancy. Like sugars, swainsonine is very water-soluble and therefore distributed to many parts of the body. It is also rapidly excreted, primarily in the urine, but in lactating animals a portion of it is transferred to the milk, so that nursing calves or lambs may become locoed (*137, 138*). This fast excretion rate suggests that occasional consumption of locoweeds for short periods is unlikely to have serious adverse effects but that continuous consumption, even at relatively low levels, generally results in typical signs of locoweed poisoning. It is therefore important to remove animals from locoweed-infested land as soon as grazing of the plant is observed.

The recognition that swainsonine was an inhibitor of α-mannosidase as a consequence of its ability to interact with receptor sites for mannose substrates on the enzyme suggested that structurally similar alkaloids might have similar properties. One such alkaloid, castanospermine (**15**) (**Figure 10**) (*139*), had been isolated from seeds of the black bean or Moreton Bay chestnut (*Castanospermum australe*) (**Figure 11**), an Australian rainforest tree, although the bioactivity of this compound had not been evaluated. When tested, castanospermine was found to be a potent inhibitor of α- and β-glucosidases, enzymes that are also essential for glycoprotein processing (*140*). The large leguminous seeds, which litter the ground beneath the trees, had been known to be toxic to livestock, especially cattle and horses, and even, on occasion, humans. The discovery of the biochemical activity of this alkaloid therefore suggested a close analogy to locoweed poisoning, although the signs of poisoning are significantly different with pronounced gastrointestinal disturbances, as might be expected from inhibition of digestive glucosidases, but no discernible neurological damage.

An extensive chemical examination of *C. australe* seeds resulted in the identification of three structurally related indolizidine alkaloids, differing from castanospermine only in the orientation of specific hydroxyl groups around the ring system.

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Table 2. Induced Phenocopies of Lysosomal Storage Diseases: Plant Sources, Alkaloids Reponsible, and Enzymes Inhibited

plant sources	alkaloids present	enzymes inhibited	lysosomal disease	primary symptoms
locoweeds (*Astragalus* and *Oxytropis* spp.)	swainsonine	α-mannosidase	mannosidosis	depression staggering gait
poison peas (*Swainsona* spp.)				neurological problems emaciation
Moreton Bay chestnut/black bean (*Castanospermum australe*)	castanospermine	α-glucosidase	Pompe's	gastric disturbances glycogen in muscle
morning glories (*Ipomoea calobra, I. carnea,* and *I. polpha*)	calystegines B_1 and C_2 swainsonine	β-glucosidase α-galactosidase α-mannosidase	Gaucher's Fabry's mannosidosis	epileptiform seizures enlarged spleen neurological problems

Three additional alkaloids based on the pyrrolizidine ring system were also isolated, of which australine (**16**) is typical (**Figure 10**). All of these new alkaloids inhibited α-and β-glucosidases to a greater or lesser extent but were present at significantly lower levels than castanospermine (*136, 141*). However, the identification of swainsonine, castanospermine, and australine, together with isomers differing only in their stereochemistry, suggested that many similar compounds capable of mimicking sugars might exist in nature as glycosidase inhibitors.

More recently this structural reasoning has led to the identification of hydroxylated alkaloids belonging to the tropane class, known as calystegines, which inhibit α- and β-galactosidases and β-glucosidase (*142*). At present this group consists of 14 alkaloids differing in the number, disposition, and stereochemistry of the hydroxyl group substituents, with calystegines B_2 (**17**) and C_1 (**18**) (**Figure 10**) being the most commonly found (*143*). Various combinations of these alkaloids have been discovered in several genera belonging to the plant families Convolvulaceae, Solanaceae, and Moraceae. The most interesting livestock toxicity situation occurs with respect to certain *Ipomoea* species, which have been found to poison sheep in Australia and goats in Africa. Analysis of *I. calobra* and *I. polpha* from Queensland (*130*) showed that the plants contained not only calystegines B_2 and C_1 but also the locoweed toxin, swainsonine, with an analogous pattern of alkaloids subsequently being detected in *I. carnea* from Mozambique (*131*) and Brazil (*135*). The toxicity of these plants therefore results from the effect of the toxins on at least three different enzymes, and many of the symptoms correlate with those observed in locoism induced by α-mannosidase inhibition, exacerbated by inhibition of α-galactosidase and β-glucosidase.

Glycosidase-inhibitory alkaloids, which were initially represented by swainsonine alone, have now expanded to encompass a number of structural classes, including indolizidines, pyrrolizidines, tropanes, and simpler pyrrolidine and piperidine analogues. Their bioactivity has stimulated the synthesis of counterparts that have not been found to occur naturally, and these now outnumber those isolated from natural sources. To include so many disparate structural types under a single classification, the generic name of "polyhydroxy alkaloids" has been applied, because the presence of multiple hydroxyl groups is a consistent feature, and their chemistry, biochemistry, and biological activity have been extensively reviewed (*144*).

Biomedical Applications. The fundamental cellular function of glycoprotein processing primarily affects *N*-linked glycoproteins, which are involved in numerous essential physiological functions, especially cell−cell recognition reactions critical to pathogenesis, inflammation, parasitism, development, cell adhesion, and symbiosis. Consequently, as might be expected from a class of compounds that inhibits glycosidases, the polyhydroxy alkaloids exhibit an exceptional diversity of biological effects,

including insecticidal, herbicidal, antimicrobial, and therapeutic activities. Discovery and isolation of many of the alkaloids has been a result of observations of the ultimate clinical effects that result from the consumption by animals of plants containing these bioactive compounds. The capability of polyhydroxy alkaloids to disrupt the general cellular function of glycoprotein processing leads to the expectation that these compounds should have therapeutic potential for the treatment of various disease states even though their mammalian toxicity is an obvious concern with regard to their medicinal application. However, this is not a unique problem as many, if not most, drug candidates have significant toxicity and it is well-recognized that an appropriate dose−response relationship which minimizes harmful side effects can often be achieved. Moreover, adverse effects due to glycosidase inhibition, such as the neurological damage caused by swainsonine, often develop quite slowly and appear to be reversible if ingestion of the alkaloid is terminated relatively early, as would be the situation with most drug regimens. However, when ingestion is continued, signs of poisoning persist.

Investigation of the alkaloids for therapeutic potential has so far concentrated on three major disease states, namely, the treatment of cancer and inhibition of metastasis, as antiviral agents, and as antiparasitics. Structurally related compounds have also been used as antidiabetic drugs (*145*). Recent comprehensive reviews have described the therapeutic potential for all structural classes of naturally occurring glycosidase inhibitors, together with their synthetic analogues (*143, 144*). Awareness of these alkaloids first arose because of their effects as the toxic components of poisonous plants, but many are now being identified in plants not established as toxic and even in common food plants for humans (*146*).

Animal Models for Lysosomal Storage Diseases. Collectively, genetic diseases in animals occurring as a consequence of an insufficiency of glycoprotein processing enzymes are known as lysosomal storage diseases and have counterparts in humans. These are mannosidosis, Pompe's disease, Gaucher's disease, and Fabry's disease, which arise from a deficiency or, in severe cases, absence of the enzymes α-mannosidase, α-glucosidase, β-glucosidase, and α-galactosidase, respectively. The availability of specific inhibitors of these enzymes provides a mechanism for the induction of phenocopies of these genetic diseases in animal models. For example, feeding experiments with castanospermine in rats resulted in vacuolation of hepatocytes and skeletal myocytes and glycogen accumulation, consistent with Pompe's disease or type II glycogenesis (*147*). Similarly, young rats treated with swainsonine developed axonal dystrophy in the central nervous system as a consequence of lysosomal storage of incompletely processed mannosides, which has a parallel in genetic mannosidosis (*148*). The specific alkaloids known to induce phenocopies of lysosomal storage

diseases are shown in **Table 2**, together with their plant sources and signs of poisoning. Additional experiments using these and other alkaloids should provide useful information for early diagnosis and possible means of intervention to interrupt the progression of such diseases.

Antimetastatic and Antiproliferative Activity. Swainsonine has received particular attention as an antimetastatic agent, and this effect has been shown to be due to enhancement of natural killer T-cells and increased susceptibility of cancerous cells to their effect (*149−151*), as well as an antiproliferative effect that is independent of but also additive to that of interferon (*152*). The targeting of carbohydrate-processing pathways in the Golgi through glycoprotein-processing inhibitors represents a novel approach to development of anticancer agents (*153*).

Lymphoid (MDAY-D2) and melanoma (B16-F10) cells were less metastatic when grown in 0.3 μg/mL swainsonine for 48 h prior to injection into the tail vein of mice, and addition of 2.5 μg/mL to the drinking water further reduced lung colonization by the melanoma cells (*154*). This effect of swainsonine is not limited to a single tumor type, because spontaneous metastasis of murine B16-BL6 melanoma and M5076 reticulum sarcoma cells to the lung and liver, respectively, was reduced as a function of the amount in the drinking water at levels up to 3 μg/mL (*155*).

Comparison experiments with human colon carcinoma (HT29) cell xenografts in athymic nude mice showed that swainsonine in the drinking water or systemic administration of human interferon-α2 reduced tumor growth rates by 49 and 53%, respectively; in combination these treatments reduced tumor growth by 78% (*152*). In vivo experiments with mice have shown that in animals provided with drinking water containing 3 μg/mL of swainsonine for 24 h prior to injection with B16-F10 murine melanoma cells, pulmonary colonization was reduced by >80% (*156*) and growth rates of human melanoma xenografts in mice were reduced by 50% by administration either at 10 μg/mL in the drinking water or at 0.5 mg/kg/day by osmotic minipump (*157*). Similarly, mice implanted with a gastric wall orthotopic carcinoma showed inhibition of the tumor volume of 74% and reductions in liver and peritoneal metastasis of 75 and 67%, respectively (*158*).

In 16 human patients with advanced malignancies, a phase IB clinical trial consisting of biweekly oral swainsonine administered in escalating doses of 50−600 μg/kg/day showed the maximum tolerated dose to be ~300 μg/kg/day (*159*). In the preceding phase I study, with swainsonine administered by continuous infusion over 5 days, one patient had objective remission of >50% in tumors of the head and neck and two other patients showed symptomatic improvement. Limiting adverse events were primarily elevation in serum AST and dyspnea, together with fatigue, anorexia, and abdominal pain; it was concluded that 150 μg/kg/day by oral chronic intermittent administration could be tolerated. Pharmacokinetic studies in mice have indicated that the levels of alkaloid and period of administration would be insufficient to produce neurological damage, with highest tissue levels appearing in the bladder, kidney, and thymus and lowest in the brain (*160*). Additional experiments showed that swainsonine administered in the drinking water was predominately retained in lymphoid tissue and retained therein for at least 3 days (*161*). It has been suggested that postoperative metastasis of tumor cells in humans could be suppressed by intravenous administration of the alkaloid prior to and following the surgery. Clinical trials in humans with very advanced malignancies showed that lysosomal α-mannosidases and Golgi mannosidase II were inhibited and

some improvement in clinical status occurred (*159*). An analogue of swainsonine has been reported to be in phase II clinical trials for treatment of renal cell carcinoma (*162*). Castanospermine has also been reported to suppress metastasis in mice (*163*), but studies with this alkaloid have been only preliminary in comparison with those using swainsonine.

In addition to its antimetastatic and antiproliferative effects, it has been shown that swainsonine may also be useful in alleviating the effects of chemotherapeutic toxicity. The alkaloid protected C57BL/6 mice bearing melanoma-derived tumors from toxicity induced by cyclophosphamide without altering the efficacy of the drug to inhibit tumor growth. Swainsonine also increased bone marrow cellularity and the number of circulating white blood cells in mice treated with the AIDS drug 3′-azido-3′-deoxythymidine (AZT), at levels typically causing severe myelosuppression. Human myeloid progenitor cells were similarly protected from AZT toxicity in vitro (*164*). Survival and bone marrow proliferation were increased in various strains of mice treated with the cytotoxic agents, methotrexate, fluorouracil, cyclophosphamide, and doxorubin (*165*). Analogous effects were observed in mice subjected to radiation (*166*).

It is likely that enhanced anticancer activity will derive from the use of semisynthetic derivatives of swainsonine that enhance the pharmacokinetics of the alkaloid through reduction in the water solubility of the alkaloid and consequent rapid excretion. Dennis et al. (*167*) have shown that certain carbonoyloxy derivatives of the 2- or 8-hydroxy substituents of swainsonine can be prepared that, although poor inhibitors of α-mannosidases in vitro, enter cells at a rate similar to that of the parent compound and are hydrolyzed to swainsonine by cellular esterases. However, the more lipophilic derivatives were ~10 times less effective in inhibiting Golgi oligosaccharide processing, probably due to a reduced ability to enter the tumor cells. Swainsonine and its derivatives had comparable activities as stimulators of bone marrow cell proliferation when administered intraperitoneally to mice.

Antiviral Activity. Castanospermine has been shown to be capable of suppressing the infectivity of a number of retro viruses, including the human immunodeficiency virus (HIV) responsible for AIDS (*168−170*). This effect is a direct consequence of glycoprotein-processing inhibition, resulting in changes in the structure of the glycoprotein coat of the virus. Cellular recognition of the host is therefore prevented, and syncytium formation is suppressed. Despite this significant effect, the alkaloid suffers from the disadvantage that it is highly water-soluble and therefore rapidly excreted. This problem has been overcome by derivatization to give 6-*O*-butanoylcastanospermine (*171, 172*), and this compound has undergone clinical trials against AIDS in humans, either alone or in combination with AZT, with the only significant side effect being gastrointestinal disturbances, as might be predicted. 6-*O*-Butanoylcastanospermine (Celgosivir; MBI-3253) has recently been reported to be in phase I/II trials as an oral prodrug of castanospermine for treatment of chronic hepatitis C infections and has been administered to >600 subjects in clinical trials to date (*173*).

Immunosuppressive and Antiparasitic Activities. The ability of polyhydroxy alkaloid glycosidase inhibitors to prevent cellular recognition has resulted in their use in studies of clinical situations when suppression of an immune response would be desirable or for use against parasitic diseases. Thus, in vivo experiments have shown that castanospermine can be used as an immunosuppressive drug, promoting heart and renal allograft survival in rats (*174*). Similarly, parasitic diseases may also be

controlled by altering cellular recognition processes. Swainsonine has been demonstrated to inhibit the association of *Trypanosoma cruzi*, the causative agent of Chagas' disease, with host cells by formation of defective mannose-rich oligosaccharides on the cell surface (*175*), whereas castanospermine provides protection against cerebral malaria by preventing adhesion of *Plasmodium falciparum* to infected erythrocytes (*176*).

There is no doubt that the polyhydroxy alkaloids have considerable potential for treatment of a variety of disease states in humans and animals. The primary challenge in introducing them as commercial drugs is to minimize any potential for toxicity and enhance the specificity of their beneficial effects. Improvement of their pharmacokinetic properties should result in much lower dose rates being necessary so that undesirable side effects are limited. Increased specificity of action may also be achieved by preparation of appropriate synthetic derivatives and additional research to provide a comprehensive understanding of structure−activity relationships.

Future Outlook. The examples given above of poisonous plants that have yielded toxins that have already been adopted as tools for biomedical investigations were first investigated for the problems inflicted on the livestock industry several decades earlier. It is probable that some of the poisonous plant problems only now being investigated will prove to have similar applications. The situation with regard to abortions caused by Ponderosa pine is an example of such a problem in which the potential benefits can be visualized, but the knowledge obtained has not developed sufficiently. Cows grazing pine needles near term often give birth to a calf that may survive with extra care, indicative of a premature birth rather than abortion. This and other evidence suggests that the mechanism of pine needle abortion mimics a normal birth process. Although the toxin has been identified as isocupressic acid (*177*), its metabolic conversions have been only partially elucidated, and its mode of action is not known. Nevertheless, it is obvious that the compound could be a valuable tool to investigate the mechanisms of parturition, and it could even be used in the livestock industry to initiate delivery at an opportune time for the producer. Similarly, horses fed white snakeroot (*Eupatorium rugosum*) develop subendocardial fibrosis and degeneration of the heart. The toxin in white snakeroot is normally excreted in the milk in lactating cows and has been historically associated with the poisoning of humans known as "milk sickness" (*178*). This suggests that the above lesions could be found in the heart of a nursing colt, or possibly fetal foals, whose mother was grazing the plant. Information of this type may provide valuable insight into the mechanisms of cardiac damage in utero or through ingestion of harmful substances via lactation.

Investigation of poisoning of animals and humans by plants may sometimes only indirectly lead to discovery of potential therapeutic drugs. For example, the acute toxicity of *Taxus* species, especially the English yew (*T. baccata*) (*179, 180*), led to the identification of taxines A and B as the toxins, but it was taxol, an accompanying constituent present in amounts too small to be responsible for the toxicity, that was isolated from the bark of *T. brevifolia*, structurally characterized (*181*), and eventually developed as a remarkably successful agent against ovarian tumors, breast cancer, Kaposi's sarcoma, and non-small-cell lung cancer. It is doubtful whether any *Taxus* species would ever have been subjected to such comprehensive investigation of its constituents had it not been for the serious livestock losses caused by *T. baccata* and its relatives. In other cases, such as the hepatotoxic pyrrolizidine alkaloids that occur in many plant genera, often at very high levels, there is a plethora of knowledge regarding their physiological effects, but no useful application has been discovered so far. It is likely that the only biomedical application for this knowledge will be to prevent transfer of pyrrolizidine alkaloids into the human food chain through use of inappropriate herbal remedies such as comfrey or contamination of grains and honey (*182, 183*).

All of the plants discussed in this review, and many others, are serious economic problems to the livestock industry. It is possible that those livestock producers who have spent most of their lives combating those plants may eventually benefit not only from a reduction in losses but also from medical treatments resulting from research on the toxins of these very plants. As illustrated by the above examples, it has taken several decades after the identification of the specific toxins for their potential in biomedical research or as therapeutic agents to be recognized. The archetypes discussed suggest that such potential could be developed much more rapidly if it is taken into consideration at the earliest stages of investigations of the chemistry of poisonous plants and sufficient effort devoted to elucidating the biological mode of action of individual constituents.

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