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Medicinal History of North American *Veratrum*

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Abstract

Plants belonging to the genus *Veratrum* have been used throughout history for their medicinal properties. During the 19th and 20th centuries, phytochemical investigations revealed a host of steroidal alkaloids in *Veratrum* species, some of which are potent bioactives. This review discusses *Veratrum* species that grow in North America with a focus on the medicinal history of these plants and the steroidal alkaloids they contain. While significant reviews have been devoted to singularly describing the plant species within the genus *Veratrum* (botany), the staggering breadth of alkaloids isolated from these and related plants (phytochemistry), and the intricacies of how the various alkaloids act on their biological targets (physiology and biochemistry), this review will straddle the margins of the aforementioned disciplines in an attempt to provide a unified, coherent picture of the *Veratrum* plants of North America and the medicinal uses of their bioactive steroidal alkaloids.

Keywords: steroidal alkaloids; Bezold-Jarisch reflex; hedgehog signaling pathway; hypertension; cancer

Abbreviations:

USDA	United States Department of Agriculture
NRCS	National Resources Conservation Service, formerly the U.S. Soil Conservation Service
IUPAC	International Union of Pure and Applied Chemistry
HPE	Holoprosencephalopathy, a developmental abnormality in which the prosencephalon of the embryo fails to divide into separate hemispheres causing defects in facial and brain structures
Hh	Hedgehog, used in reference to the complete series of biochemical events and structures that make up the embryonic hedgehog signaling pathway
hh	Invertebrate hedgehog gene, originally discovered in *Drosophila*
shh	Vertebrate Sonic hedgehog gene
ihh	Vertebrate Indian hedgehog gene
dhh	Vertebrate Desert hedgehog gene
Shh	Sonic hedgehog protein, potent morphogen coded for by *shh*
Shh-N_p	Active Sonic hedgehog protein, 19 kDa N-terminal fragment produced by autolytic cleavage of Shh and subsequent palmitoylation of the N-terminus and addition of cholesterol to the C-terminus
Ptch	Patched, a twelve pass transmembrane transport protein integral to the hedgehog signaling pathway which serves as the binding site for the active Sonic hedgehog protein and regulates the activity of Smoothened
Smo	Smoothened, a seven transmembrane G-protein like receptor integral to the hedgehog signaling pathway; initiates the signaling cascade regulating the activity of Gli transcription factors

NBCCS	Nevoid basal cell carcinoma syndrome, an autosomal-dominant inherited condition characterized by craniofacial malformations, spina bifida, and polydactylism in addition to high rates of basal cell carcinoma and cancers of the heart, ovaries, skin, and central nervous system
BCC	Basal cell carcinoma, most common non-melanoma skin cancer; characterized by uncontrolled growth of cells in the *stratum basale*, the skin's lowest layer
PNET	Primitive neuroectodermal tumor, tumors originating in cells derived from the neuroectoderm such as medulloblastomas
KAAD-cyclopamine	3-keto N-aminoethyl aminocaproyl dihydrocin-namoyl cyclopamine, a potent hedgehog pathway antagonist derived from the natural product cyclopamine
CSC	Cancer stem cell, highly proliferative undifferentiated tumor cells, which give rise to rapidly dividing "bulk" tumor cells

Introduction: A Botanical Overview of the Genus Veratrum

Veratrum is a genus of perennial flowering herbs of the Liliacea or Melanthiacea family, depending on taxonomic treatment, that are typically found in woodland or alpine areas and confined to the Northern hemisphere (Zomlefer et al. 2003). Plants of the genus *Veratrum* are widespread, with 17-45 distinct species, the bulk of which are native to Eastern Asia, the genus's likely origin (Liao et al. 2007; Trier 2011). Eleven *Veratrum* species are endemic to North America (Liao et al. 2007; USDA NRCS 2013). *Veratrum* plants have a long and storied history of medicinal use, the foundation of which rests on a diverse spectrum of steroidal alkaloids produced by members of this genus.

Over the past century investigators have uncovered more than 100 distinct steroidal alkaloids in *Veratrum* plants (see reviews by Kupchan 1961; Tomko and Votický 1973; Atta-Ur-Rahman and Choudhary 1998; and Li et al. 2006), some of which are potent bioactives. This review focuses only on the species of *Veratrum* found in North America with an emphasis on the various bioactive steroidal alkaloids they contain. First, the distinct species of *Veratrum* plants in North America will be covered, including their range, growing conditions, and alkaloids isolated from each. Second, the use of *Veratrum* alkaloids as therapeutic agents for hypertension in the mid-1900s will be surveyed. Lastly, the discovery of teratogenic *Veratrum* alkaloids and recent interest in the jervanine alkaloid cyclopamine as a model antagonist of the embryonic Hedgehog signaling pathway will be discussed.

North American Veratrum Species

Liao and colleagues encapsulate the complex taxonomy of *Veratrum* species very well in their 2007 study of flower color in the genus, stating, "The number of species depends largely on taxonomic treatment of four widely spread species complexes, viz *V. album* L., *V. nigrum* L, *V. maackii* Regal from Eurasia and *V. viride* Aiton from North America" (pp 177). Indeed, it is quite difficult to categorize *Veratrum* species purely on morphological features as these can exhibit distinct local variation, with different species grading into one another geographically (for example, *V. oxysepalum* and *V. grandiflorum*, members of the genetically assigned *V. album* complex, have been traditionally defined along an arbitrary north/south division in Japan and Korea, see Kupchan 1961 and Zomlefer et al. 2003). In deciding which North American species to include and discuss, we have relied on Dr. Wendy Zomlefer's 2003 phylogenetic analysis of *Veratrum* in addition to the United States Department of Agriculture's (USDA) PLANTS database (Zomlefer 2003; USDA NRCS 2013). The overarching sections assigned by Dr. Zomlefer (2003) are used for organization. A graphic depiction of the distribution of *Veratrum* species in North America has been created (*Figure 1*). Details regarding each North American *Veratrum* species, including morphology, habitat, range and steroidal alkaloids have been included as a table in the appendix.

Zomlefer's Section *Veratrum* [Clade B]

Veratrum plants typically have a long, thick rhizome featuring many smaller roots and capped by a bulb. Plants are tall (1-2 m) and feature a pseudostem formed by overlapping sheaths at the sessile base of broad oval to elliptical leaves, which extend up the stem to the inflorescences. Plants of this section have 32 chromosomes (Zomlefer et al. 2003). North American species within section *Veratrum* are the primary focus of this review, as they have been investigated extensively for steroidal alkaloid content.

Veratrum viride

Veratrum viride (green false hellebore) has a discontinuous geographical distribution, growing throughout eastern North America, from Quebec to Georgia, and is also found across the western margin of the continent from Alaska to California and inward to the mountainous regions of Montana, Idaho, and Alberta (Kupchan 1961; Zomlefer et al. 2003; USDA NRCS 2013). The species has been divided into two subspecies based on the east/west distribution (the western variety of *V. viride* was formerly considered the separate species *V. eschoscholtzii*; any data specific to this species will be grouped with *V. viride* for the purposes of this review) and may or may not be a variant that should be grouped in the *V. album* complex (Youngken 1952; Youngken 1953; Kupchan 1961; Zomlefer et al. 2003; USDA NRCS 2013). *V. viride* spans a large variety of habitats from wet woods and moist coastal regions in Alaska and Quebec to mountain meadows in northern California, Idaho, Montana, and North Carolina. As is to be expected with such a widespread species, local morphological variations exist, but *V. viride* generally feature green perianths (Kupchan 1961).

Veratrum tenuipetalum

Veratrum tenuipetalum (Colorado false hellebore) is a species found primarily in Colorado and in the southern reaches of Carbon County, Wyoming, but is disputed taxonomically (Johnston 2002; USDA NRCS 2013). Unfortunately, this species has not been sampled for phylogenetic study. As a result, *V. tenuipetalum* A. Heller is often considered the same species as *V. californicum* var. *californicum*, which grows outside of Colorado, based on morphology and ecological factors (Kupchan 1961; Johnston 2002; Iler and Inouye 2013). *V. tenuipetalum* is commonly found in subalpine meadows and aspen forests and can feature very large inflorescences (greater than 1.5 m) which are studded by small white perianths (Iler and Inouye 2013).

Veratrum fimbriatum

Veratrum fimbriatum (fringed false hellebore) is confined to a narrow coastal strip of northern California between Fort Bragg and Fort Ross. *V. fimbriatum* is distinct from other North American members of the genus due to its narrow, low-elevation habitat (reported to extend only 5 km inward from the coastline and up a few hundred meters in elevation), heavily fringed tepals, and large wingless seeds (Taylor 1956a; Kupchan 1961; Zomlefer et al. 2003; USDA NRCS 2013).

Veratrum insolitum

Veratrum insolitum (Siskiyou false hellebore) is found primarily in northern California and southwestern Oregon with a small isolated population documented in southwestern Washington. *V. insolitum* features wooly, grey ovaries and inflorescences covered in small, white and fringed tepals. Notably, the species is often found growing in serpentine and diorite soils on mountain slopes, which stands in contrast to the stagnant wet conditions preferred by many other members of the genus (Kupchan 1961; Washington Department of Natural Resources 2003; Zomlefer et al. 2003; USDA NRCS 2013).

Veratrum album

Veratrum album (white false hellebore) is a primary *Veratrum* species of Europe which "forms a complex of broad geographic clines to which there are many striking local exceptions in all regions" (Kupchan 1961, pp 8). *V. album* is often treated as a complex of species consisting of *V. lobelianum*, *V. grandiflorum*, and *V. oxysepalum* due to its widespread, variable nature. Phylogenetic analyses of *V. album* populations in Europe strongly suggest the species entered Europe from the east, consistent with an ancient East Asian origin of the species and genus (Liao et al. 2007; Treier and Müller-Schärer 2011). *V. album* also expanded eastward, likely across the ancient Bering Land Bridge, and modern populations exist in Alaska (Treier and Müller-Schärer

2011; USDA NRCS 2013). Members of the *V. album* complex are generally identified by their large white tepals but local variations can include small green, white or even yellow tepals (Kupchan 1961; Zomlefer et al. 2003; USDA NRCS 2013).

Veratrum californicum

Veratrum californicum var. *californicum* (Californica false hellebore) is found in the American west, particularly in high meadows of Idaho, Oregon, Utah, California, and New Mexico (Kupchan 1961; USDA NRCS 2013). The species prefers moist, subalpine mountain environments, typically in areas that receive significant snowpack during the winter months which contribute to the chilling required to break dormancy as well as early season moisture (Sun et al. 2012; Doniger and Shock 2011). *V. californicum* has unique soil requirements, growing naturally in acidic (pH 6.3) sandy loams that are high in calcium and iron (Shock and Shock, 2011). *V. californicum* has large, heavily flowered inflorescences that feature broad white tepals (Kupchan 1961).

Veratrum californicum var. *caudatum* (Cascade false hellebore) can be found at lower elevations from north central Idaho to the coastal mountains of Washington and Oregon (Kupchan 1961; USDA NRCS 2013). *V. californicum* var. *caudatum* features a notable unbranched extension of the main stem which is studded with large, green to white perianths during flowering (Kupchan 1961).

Zomlefer's Section *Fuscoveratrum* [Clade C]

Fuscoveratrum plants tend to feature slender leaves that are located at the base and a very small to non-existent rhizome. Tepals are often conspicuously clawed. Plants of section *Fuscoveratrum* have 16 chromosomes (Zomlefer et al. 2003).

Veratrum latifolium, virginicum, woodii, hybridum and *parviflorum* are circumscribed within the genus *Veratrum* and can be found in the south and east of North America (Zomlefer et al. 2003). These plants have been categorized as a separate genus *Melanthium* at different points in time based on their distinct morphological characteristics, such as narrow petiloate leaves and clawed tepals which stand in contrast to the broad sessile leaves and unclawed tepals of other North American *Veratrum* species, such as *V. viride* or *V. californicum* (Zomlefer et al. 2001; Zomlefer 2003). Yet, phylogenetic analyses of the internal transcribed spacer region of nuclear ribosomal DNA of different *Veratrum* plants placed these four species as a subsect of the genus *Veratrum*, albeit in the distinct *Fuscoveratrum* section (Zomlefer et al. 2001; Zomlefer et al. 2003). *Veratrum* species of section *Fuscoveratrum* have not been the subject of phytochemical investigation focused on steroidal alkaloids, thus there will not be further discussion of these species in this review.

Veratrum Alkaloid Overview and Biosynthesis

IUPAC formally defines alkaloids as, "Basic nitrogen compounds (mostly heterocyclic) occurring mostly in the plant kingdom (but not excluding those of animal origin)," excluding amino acids, peptides, proteins, nucleotides, nucleic acids, amino sugars and antibiotics (IUPAC 2006). However, a more serviceable definition comes courtesy of Pelletier (1983), in which he states, "An alkaloid is a cyclic organic compound containing nitrogen in a negative oxidation state which is of limited distribution among living organisms" (pp 26). Of this wide reaching class of chemical compounds, steroidal alkaloids are a subset defined by the presence of a steroidal (cyclopentanophenanthrene or derivative) skeleton with a nitrogen atom either as an integral part of the molecule or as a side chain. Notably, steroidal alkaloids are not derived from amino acids but instead find their roots in steroids or triterpenoids. Due to similarities with endogenous steroids in the body (corticosteroids, hormones, anabolic steroids), steroidal alkaloids have been a major subject of pharmacological investigation (Atta-ur-Rahman and Choudhary 1998).

Among the many different steroidal alkaloids that exist in nature, two broad groups have been isolated from *Veratrum* species: those featuring the typical cyclopentanophenanthrene skeleton of cholesterol (sometimes referred to as the *Solanum* alkaloids) and those which have a C-nor-D-homosteroidal skeleton featuring a five-membered C-

ring formed through bond migration from C-12 to C-13 via a Wagner-Meerwan rearrangement mechanism (*Figure 2*) (Hirschman et al. 1954; Mitsuhashi and Shimizu 1963). Alkaloids of this latter group are commonly referred to as *Veratrum* alkaloids and were some of the first steroidal alkaloids known to science (Li et al. 2006; Meissner 1819). In fact, the term "alkaloid" was coined in 1819 by German pharmacist Wilhelm Meissner in his description of what is now known to have been a mixture of *Veratrum* alkaloids isolated from the related plant *Schoenocaulon officiale* (sabadilla or cevadilla), which is native to Mexico and was brought back to Europe in the late 1500s (Meissner 1819; also Wood 1856, Pelletier 1983; Zomlefer et al. 2006).

Within the *Solanum* alkaloids, two further classes are evident, those of the verazine type featuring a distinct imine containing ring and those of the solanidine type in which the nitrogen containing ring has become fused to the rest of the cyclic system. This has been hypothesized to happen through the nucleophilic attack of the lone pair of electrons on the nitrogen atom to a suitable electophilic atom containing a good leaving group on the D-ring, to form a tertiary amine (Kaneko et al. 1977). The *Veratrum* alkaloids can also be further divided into distinct structural groups: the veratranine, jervanine, and cevanine types. The veratranine group is defined by the presence of an aromatic D-ring, whereas alkaloids of the jervanine type feature a tetrahydrofuran E-ring linking the amine containing F-ring to the D-ring through a spiroconnection at C-17. Cevanine alkaloids are distinct from the other two classes of *Veratrum* alkaloids in that they have a six-membered E-ring hypothesized to form through nucleophilic attack of the nitrogen in the piperidine ring on a neighboring methyl that has a suitable leaving group (such as a phosphate group) (Kaneko et al. 1978; Kaneko et al. 1979). Cevanine alkaloids are highly hydroxylated in *Veratrum* plants, with 7 to 9 atoms of oxygen, and feature α-ketol and hemiketal linkages between C-4 and C-9 of the A and B rings respectively (Krayer and Meilman 1977; Kaneko et al. 1970a). While many genera in Liliaceae produce steroidal alkaloids, some chemotaxanomic distinctions can be drawn. Cevanine alkaloids of the 4,9-olide and ester types are common in *Veratrum, Zigadenus*, and *Schoenocaulon* but in *Fritillaria*, 5α-cevanine steroidal alkaloids are common (Li et al. 2006). Of the *Veratrum* alkaloids, those of the cevanine and jervanine types have garnered the most medical attention as therapeutics for their action as hypotensive and chemotherapeutic agents respectively.

As may be surmised from the structural similarities between the *Solanum* and *Veratrum* alkaloids, the two classes share a biosynthetic pathway, which has been partially investigated (*Figure 3*). What is known about the biosynthesis of *Veratrum* alkaloids comes from work using radiolabeled carbon isotopes in *V. grandiflorium* (part of the *V. album* complex) by Kaneko and colleagues in the 1970s (Kaneko et al. 1970a, b; 1972; 1975-1979). By administering acetate-1-^{14}C and cholesterol-4-^{14}C to *V. album* and measuring the incorporation of these radioisotopes into the alkaloids jervine and veratramine, Kaneko and colleagues discovered that the synthesis of steroidal alkaloids in *Veratrum* likely begins with acetate which is transformed into cholesterol through the mevalonate pathway (Kaneko et al. 1970a, b). Cholesterol then undergoes a series of oxidations, reductions, and rearrangements that build on the cyclopentanophenanthrene steroidal scaffold to produce the different classes of steroidal alkaloids.

Kaneko and colleagues elucidated four important parts of the biosynthetic pathway for *Veratrum* alkaloids. First, the source of nitrogen for the piperidine ring (this appears in the biosynthetic pathway as verazine) is likely L-arginine (discovered using the ^{15}N-labelled amino acid), which accumulates in the dormant rhizome prior to budding (*Figure 3a*) (Kaneko et al. 1976). Second, the conversion of solanidine to the *Veratrum* type alkaloids, which feature a C-nor-D-homosteroidal skeleton, is light dependent and indicates that the Wagner-Meerwan type rearrangement of epirubijervine likely requires the input of ATP (*Figure 3b*) (Kaneko et al. 1972; 1979; Heretsch et al. 2010). Third, the isolation of the intermediate compound procevine suggests that cevanine-type alkaloids are formed by nucleophilic attack from the nitrogen lone pair electrons of the piperidine ring to a suitable electrophilic atom containing a good leaving group attached to C-18 of isorubijervine, prior to rearrangement to the C-nor-D-homosteroidal skeleton (*Figure 3c*) (Kaneko et al. 1978; 1979). Lastly, Kaneko et al. discovered a common precursor to both the veratranine and jervanine type alkaloids when administration of jervine to plants being fed radioactive acetate led to the production of radioactive veratramine at the expense of radioactive jervine. This result suggested an allosteric model of inhibition at the site of synthesis for the two compounds (Kaneko et al. 1970a). Kaneko et al. found cyclopamine (11-deoxojervine) to be a possible precursor after radioactive cyclopamine fed to cultivated *V. grandiflorum* plants produced both radioactive jervine and veratramine, 26.1% and 12.1% respectively (*Figure 3d*). The low rate of veratramine synthesis from cyclopamine suggests the possibility of additional synthetic routes for this alkaloid that precede the formation of cyclopamine and jervine, but no published work has addressed what these may be (Kaneko et al. 1970b).

Early Medicinal Use of Veratrum Plants

The powerful physiological effects of *Veratrum* did not go unnoticed by early peoples and the plants were variously regarded as medicine or poison. Considering that the epicenter of the genus's diversity is in eastern Asia, it is not surprising that rhizome and root material of *Veratrum* plants are prominent in Chinese medicine, referred to as "Li-lu," and has been used for centuries (Li et al. 2006). Similarly, certain western Native American tribes, such as the Shoshone, used *Veratrum* medicinally, applying the crushed rhizome of *V. viride* to snake bite wounds, using a tea to treat veneareal diseases, and chewing the raw root to help with sore throats and colds (Sweet 1976). The rhizome of *V. viride* was also used in a qualification test by some Native American tribes in northeast North America; the man who could best withstand its emetic properties upon ingestion was judged to have the strength to be the group's leader (Osgood 1835). *V. album*, a common *Veratrum* species in Europe, has a long history of medicinal use, dating back as far as Hippocrates in which dilutions of the plant are prescribed to produce upward purging (Adams 1886). Gerard wrote of *V. album's* (white hellebore) emetic effects with particular poignancy in his influential 1633 treatise *The Herbal or General History of Plants*, reporting that "root of white Hellebor procureth vomite mightly, wherein consisteth his chiefe virtue, and by that means voideth all superfluous slime and naughtie humours" (pp 441). Through the 1700s, preparations of *V. album* root and rhizomes were used medicinally in Europe primarily for their emetic properties. It wasn't until the discovery of the first pure alkaloids (e.g. morphine in 1805 and quinine in 1817) and the pursuit of "vegetable chemistry" came into being that chemical investigation of *Veratrum* plants was first attempted (Mathew 1989; Pelletier 1983).

19th Century Use of Veratrum plants and Early Phytochemistry

The first isolation of *Veratrum* (and quite possibly steroidal) alkaloids was accomplished by the German pharmacist Wilhelm Meissner in 1819 from the seeds of *S. officiale* (as mentioned above). Concurrently, French researchers Pelletier and Caventou were also investigating the extracts of *S. officiale* seeds and obtained a substance, to which they gave the name veratrine, much like the dirty-white colored solid isolated by Meissner. While stymied in their attempts to characterize the substance as either acid or base (possibly due to the resulting hydrolysis of the esterified carboxylic acid moieties from the steroidal backbone which masked the alkaline nature of the parent molecule) they found it to act similarly to morphine and strychnine when subjected to concentrated nitric acid, producing a blood red color. Alongside their experiments with veratrine, Pelletier and Caventou published the first chemical investigation of steroidal alkaloids isolated from the rhizome *V. album*, noting that the resulting substance differed little from that obtained from *S. officiale* seeds (Pelletier and Caventou 1820). It is now known that the likely compounds in the veratrine isolated by Pelletier and Caventou were cevadine and veratridine, C-3 esters of veracevine, while those from *V. album* were likely polyallylic esters of germine, protoverine, and zygadenine (Krayer and Meilman 1977).

At the same time Pelletier and Caventou were investigating the extracts of *V. album*, their contemporaries in America had noted the similarities in appearance and medicinal qualities between the white hellebore (*V. album*) common in Europe and their own American or green hellebore (*V. viride*). The first account of *V. viride's* use in the medical literature is from 1835, in which Dr. Charles Osgood described the plant's physiological effects upon ingestion as well as a chemical investigation of the rhizome resulting in a product "thrown down" by ammonia that was of "a clear white colour, pulverulent, inodorous, and very acrid, producing a peculiar stinging sensation when taken upon the tongue" (Osgood 1835, pp 295). Dr. Osgood's description of his product is similar to veratrine and he mentions that it was commonly believed that the active principle of *V. viride* was identical to that of veratrine (Osgood 1835; Wormley 1876). Dr. Osgood recommended that *V. viride* be used as a treatment for inflammatory diseases and rheumatism, as it reduced the frequency and strength of the pulse (Osgood 1835).

While Dr. Osgood brought *V. viride* to the attention of the medical community for the first time, it wasn't until Dr. Wesley C. Norwood of Cokesbury, South Carolina, began producing and bottling a tincture of the rhizome in the 1850s that this crude extract of mixed steroidal alkaloids came into wider use ("Norwood's Tincture" was made by macerating eight ounces of the dried rhizome in a pint of officinal alcohol, 90-95% ethanol, for two weeks) (Wood 1856). Eventually, production of this tincture was taken over by a branch of the religious group the Shakers, and it was distributed widely in the central and western parts of the United States (Rhame 1957; Estes 1992). With its growing medical footprint, the extract won both loyal supporters and detractors (Rhame 1957). The latter considered the extract to a be a dangerous remedy due to its ability to induce striking bradycardia and apnea, forceful vomiting,

and sedation to the point of collapse when administered as directed on Norwood's vials (Baker 1859). Those who adjusted Norwood's dosage to more manageable levels, such as Dr. Paul Baker of Augusta, Georgia, became advocates of its use in just about every situation from epilepsy to pneumonia (Baker 1859; Rhame 1957).

> …for, always let it be remembered and never forgotten, that *Veratrum viride* controls the circulation by its sedative influence upon those nerves whose 'abberated action' forces the heart and arteries into such an unnatural and dangerous turmoil (Baker 1859, pp 580)

Though early medical practitioners were quite perceptive in their analysis of the gross affects *V. viride* extracts had on the nervous and circulatory system, it wasn't until a decade later that insight into the underlying mechanism of action of cevanine ester-alkaloids in the body started to come to light.

Physiology of the Hypotensive Response Caused by Cevanine Ester-Alkaloids

In 1867, German physiologists Albert von Bezold and Ludwig Hirt were investigating the action of veratrine on the heart when they observed that the sudden hypotension and bradycardia after administration of this mixed alkaloid extract could be relieved by severing the vagus nerve. von Bezold and Hirt assumed this effect resulted from stimulation of sensory nerves on the inner surface of the heart which then inhibited the vasomotor center leading to peripheral vasodilation (von Bezold and Hirt 1867). This conclusion did not go unchallenged and the mechanism behind the vasodepressive response caused by veratrine was a source of controversy in the field of cardiac physiology for the following sixty years (see the review by Krayer and Meilman 1977 for an excellent early history). Finally, in 1939, the Austrian team of Jarisch and Richter (1939a, b) unequivocally confirmed von Bezold and Hirt's hypothesis that the bradycardia and hypotension caused by veratrine were the result of reflex action originating in the ventricles of the heart and transmitted via the afferent and efferent pathway of the vagus nerves (Krayer 1961; Aviado and Aviado 2001). The triad of bradycardia, hypotension, and apnea observed upon administration of cevanine ester-alkaloids is now known as the Bezold-Jarisch reflex (Aviado and Aviado 2001).

Subsequent investigations revealed that reflex vasodepression and cardiodecelerator effects caused by certain cevanine-ester alkaloids do not depend solely on receptors in the heart, but are the result of nonspecific stimulation of mechanoreceptors located throughout the circulatory system, including the aorta, carotid sinus, and pulmonary vascular bed (Krayer 1961; Krayer and Meilman 1977; Aviado and Aviado 2001). The specific mechanism of this stimulation lies in the ability of hypotensive cevanine ester alkaloids, such as veratridine, cevadine and protoveratrines A and B, to bind to voltage-gated sodium channels in cells which feature excitable membranes such as nerves, skeletal muscles, and cardiac cells (Krayer and Mendez 1942; Honerjäger et al. 1982; Ulbricht 1998). Upon alkaloid binding, the sodium channel remains in an open state, creating a positive inotropic effect which delays repolarization of the cellular membrane and causes multiple discharges from each neuronal stimulus (Nánási et al. 1990; Wang and Wang 2003). The hypotensive cevanine-ester alkaloids bind to the sixth transmembrane segment of the second homologous domain (D2-S6) within an α-subunit in the voltage-gated sodium channel (type 2 receptor site); this binding is readily reversible, which explains the relatively short duration of the bradycardic and hypotensive effects (Meilman 1956; Meilman and Krayer 1977; Wang and Wang 2003). While all electrical cells are subject to the positive inotropic effects of hypotensive cevanine-ester alkaloids, the cardiac cells and mechanoreceptors in the left-ventricle of the heart play an important role in the Bezold-Jarisch reflex, which is a specific manifestation of the left-ventricle mechanoreceptor reflex. Under normal physiological conditions, the left-ventricle mechanoreceptor reflex continually coordinates peripheral resistance with myocardial contractility so as to keep arterial pressure constant, but hypotensive cevanine ester alkaloids cause myocardial contractility to increase due to their positive inotropic effects, thus causing peripheral resistance and blood pressure to decrease (Estrin et al. 1979).

Modern Phytochemistry and Use of Veratrum Alkaloids in the Treatment of Hypertension

Phytochemical understanding of *Veratrum* alkaloids expanded rapidly in the early 20th century due to advances in separation techniques. Though late 19th century researchers Mitchell (Wormley 1876), Wright and Luff (1879; Wright 1879), and Salzberger (1890) obtained amorphous alkaloid resins from *V. album* and *V. viride*, the German investigator W. Poethke was the first modern chemist to successfully purify *Veratrum* alkaloids of the cevanine type[1,2], procuring the ester alkaloids protoveratrine and germerine in addition to the alkamines germine and protoverine from the rhizomes of *V. album* in 1937 (Poethke 1937a, b). Poethke's work caught the attention of

researchers Lyman Craig and Walter Jacobs at the Rockefeller Institute for Medical Research in New York City, New York, who had been working on the degradation of the related alkaloid veracevine (the alkamine base of the ester alkaloids found in veratrine obtained from the seeds of *S. officiale*). Craig and Jacobs subsequently shifted their focus to the alkaloids of the native *V. viride*, the American or green false hellebore.

During the course of their investigations on *Veratrum* alkaloids (of which there were many, the reader is directed to the full series "The Veratrine Alkaloids" beginning in 1937 and ending in 1956), Craig, Jacobs, and colleagues found Poethke's germine to be an isomeric form of the compound veracevine, the esters of which were utilized for their hypotensive properties by Jarisch and Richter in their investigations of cardiac reflexes (see above) (Craig and Jacobs 1943a; Jarisch and Richter 1939a, b). Similarly, protoverine (the alkamine of protoveratrine) was found to be a 27-carbon base that behaved similar to veracevine and germine (Jacobs and Craig 1943; Craig and Jacobs 1943b). These correlations were some of the first chemical evidence hinting at the hypotensive underpinnings of *Veratrum* extracts. Though not identical to the principle components of veratrine (as hypothesized over 100 years before, see Osgood 1835), the ester alkaloids in *Veratrum* species were conspicuously close; trials of veratrine to treat hypertension had already begun and testing pure *Veratrum* alkaloids in a similar manner was a logical next step (Krayer and Mendez 1942; Krayer et al. 1944).

The renewed interest in *Veratrum* alkaloids in the 1940s came at a lull in their medicinal use; *Veratrum* extracts had largely fallen out of favor by the early 20th century due to difficulties with dosing, a result of the variable composition of the mixed alkaloid extracts (Meilman and Krayer 1950). One use for *Veratrum* extracts that persisted was in the treatment of eclampsia (Bryant and Flemming 1940). A mixed alkaloid extract of *V. viride*, Veratrone, was marketed to physicians in the early 20th century for use in obstetric emergencies (Hawes 1917). However, when chemists such as Poethke, Craig, Jacobs, and others developed methods to obtain pure alkaloids from *Veratrum* plants, medical researchers seized the opportunity to test these alkaloids as hypotensive agents in isolation rather than as variable mixtures.

One of the first alkaloids evaluated in this manner was protoveratrine, a principle alkaloid from *V. album* (first isolated by Salzberger in 1890 and later purified by Poethke, Craig and Jacobs). Krayer and colleagues demonstrated that protoveratrine could be used as a hypotensive agent, first in animal models and then in human trials, and in dosages that limited toxic effects (Krayer et al. 1944; Meilman and Krayer 1950). Subsequent phytochemical analysis by numerous groups revealed that protoveratrine was actually a mixture of two tetraacyl esters of protoverine differing only at C-3 (Nash and Brooker 1953; Kupchan and Ayres 1960). Clinical studies with the two compounds advanced protoveratrine B as the preferred drug candidate as it had similar potency to protoveratrine A but fewer emetic side-effects (Winer 1960).

Whereas the protoveratrines were easily isolated from *V. album*, it was not until 1952 that this group of compounds, along with another ester, termed neoprotoveratrine, was identified in *V. viride* (Klohs et al. 1952). Rather, investigations by Fried and colleagues (1949; 1950) found that the principal alkaloids responsible for the hypotensive effects of *V. viride* were esters of germine, coined germidine and germitrine. Continuing purification of amorphous fractions from *V. viride* expanded the list of germine esters to include neogermitrine, germbudine and isogermbudine (Fried et al. 1952; Myers et al. 1952). As the search for *Veratrum* alkaloids expanded into species beyond *V. viride* and *V. album*, the last group of hypotensive ester alkaloids, those based on the alkamine zygadenine, were found in *V. fimbriatum* (Klohs et al. 1953). Vetroylzygadenine, the zygadenine ester isolated by Klohs et al. from *V. fimbriatum*, had previously only been isolated from *Zigadenus venenosus* (death camas) a related plant within the *Liliacea* family, blurring the chemotaxonomic lines separating these genera[3] (see *Figure 5* for an overview of cevanine ester-alkaloid bases) (Kupchan and Deliwala 1953).

The isolation of new hypotensive *Veratrum* alkaloids[4] proceeded rapidly in the early 1950s, and researchers needed to assess the relative ability of these compounds to lower blood pressure before widespread clinical was feasible. As assessed in anesthetized dogs, the 3, 7, 15 triesters of germine (e.g. germitrine and neogermitrine) were found to be most potent, followed by the 3, 6, 7, 15 tetraesters of protoverine (e.g. protoveratrine A and B), then 3, 15 diesters of germine (germbudine and neogerbudine), and lastly vetroylzygadenine, a C-3 ester of zygadenine (reviewed in Kupchan 1961). With these data in hand, numerous pharmaceutical companies developed formulations of cevanine ester alkaloids, isolated from *V. viride* and *V. album*, for clinical use. Principle among these pharmaceutical products were a purified but crude mixture of polyacyl germine esters from *V. viride* that went by the trade names Veriloid (Riker Labs, now part of 3M), Vergitryl (E.R. Squibb & Sons), and Vertavis (Irwin, Neisler & Company) and a

mixture of protoveratrines A and B that were marketed under the names Provell maleate (Eli Lilly & Co.) and Veralba (Pittman Moore Company)(Grimson 1955). Demand was deemed high enough that initial investigations began into the cultivation of wild *Veratrum* plants, which proved to be a challenging endeavor due to their slow rate of growth, chilling requirements, and low germination rates (Taylor 1956b).

Compounding the difficulties encountered in obtaining a reliable supply of *Veratrum* alkaloids due to the fickle growing demands of the wild plants, the alkaloids had clinical limitations as well. For one, the reflex action (Bezold-Jarisch reflex) responsible for the hypotensive effects of cevanine ester alkaloids was rapid in onset (minutes) but short lived in duration (less than three hours). This made *Veratrum* alkloids only suitable for control of hypertensive emergencies and limited their use in chronic hypertension (Fries and Stanton 1948; Meilman and Krayer 1950; Meilman 1956; Winer 1960; Page and Sidd 1972). Also, the narrow range between toxic and therapeutic dosages (approximately 30%) made titrating dosages in patients problematic, though the toxic effects could be rapidly reversed through the administration of atropine (Meilman 1956; Page and Sidd 1972). The emetic side effects once thought to be the result of impurities in the crude alkaloid extracts being used were found to be the result of stimulation of the nodose-ganglion and therefore intrinsic to the hypotensive alkaloids themselves. Toxic doses also produced feelings of epigastric and substernal oppression in addition to nausea, sweating, marked hypotension, bradycardia, heart block and even extrasystoles (Meilman 1956). Despite these limitations, hypotensive *Veratrum* alkaloids made their way into mainstream clinical use, and by 1973, more than one million prescriptions for extracts of *V. viride* had been written (Page and Sidd 1972; Farnsworth and Morris 1976). However, the use of *V. viride* alkaloids was eventually abandoned as better-tolerated and more effective pharmaceutical agents such as methyldopa and diazoxide were developed to treat hypertensive emergencies (Page and Sidd 1972).

The Discovery of Teratogenic Veratrum Alkaloids

In the late 1950s, at the height of interest in hypotensive *Veratrum* alkaloids, sheep herders in the highlands of Idaho began to notice an unusually high rate of lambs being born with cyclopean-type malformations (a form of holoprosencephalopathy, HPE) and recruited investigators from the United States Department of Agriculture's (USDA) Agricultural Research Service-Poisonous Plant Research Laboratory in Logan, Utah, to help solve the problem (Binns et al. 1962; James 1999). The investigators noted that all the affected ewes were newly pregnant and had been grazing in moist seepage meadows studded with stands of *V. californicum*. After ruling out a possible genetic link, researchers recreated the cyclopean malformations through feeding trials with *V. californicum*, cementing the plant as the causal agent. Only ewes that fed on *V. californicum* between the second and third weeks of pregnancy, approximately the 14th day of gestation, were susceptible to the teratogen, suggesting interference with the development of the neural plate (Binns et al. 1962; Keeler and Binns 1964; Binns et al. 1965; James 1999; Gaffield 2000).

Consequently, the search for the causal agent turned toward *Veratrum* alkaloids. Researchers Richard Keeler and Wayne Binns proceeded to systematically test crude alkaloid fractions obtained from ethanol and benzene soaks of *V. californicum* biomass for their teratogenic potential in sheep. They ruled out the hydrophilic cevanine type ester alkaloids as both commercial preparations and fractions containing these compounds failed to produce teratogenic responses (Keeler and Binns 1966c). Keeler and Binns narrowed their search to fractions containing an unidentified glycoside (alkaloid X), an unidentified alkamine (alkaloid V), jervine, and veratrosine (Keeler and Binns 1964; 1966b). Notably, Keeler and Binns also isolated veratramine from *V. californicum* and found it (along with veratrosine) to cause teratogenic malformations in sheep, but these malformations were distinct from the cyclopia they set out to investigate, being characterized instead by hypermobility of the knee joints leading to bow-legged lambs unable to stand (Keeler and Binns 1964; 1966a; 1967).

Further chemical investigations revealed alkaloid V to be identical to 11-deoxojervine, first isolated by Masamune and colleagues from *V. grandiflorum* in 1965, and alkaloid X to be its C-3 glycoside (Keeler 1969a, b). Keeler assigned the trivial names cyclopamine and cycloposine, respectively, to these compounds in recognition of their teratogenic effects (Keeler 1968; Keeler 1969b). In the same time period, Keeler and Binns carried out definitive feeding trials in both ruminant (sheep) and non-ruminant animal models (rabbits) with purified steroidal alkaloids that established cyclopamine, cycloposine, and jervine as the compounds in *V. californicum* responsible for the cyclopean-malformations first observed over a decade earlier (Keeler and Binns 1968; Keeler 1970b; 1971a; James 1999).

In the course of their phytochemical search for the teratogenic agents in *V. californicum*, Keeler and Binns advanced knowledge of the steroidal alkaloids in North American *Veratrum* species greatly. Prior to their investigations, scientific work on *Veratrum* had focused on the hypotensive cevanine-type alkaloids at the expense of the free alkamine bases. Keeler and Binns not only identified a number of new compounds never before isolated from North American *Veratrum* species (cyclopamine, cycloposine, and muldamine), they identified an otherwise unknown physiological role of jervanine-type steroidal alkaloid compounds, that of vertebrate teratogen (Keeler 1978). Following the initial identification and structural elucidation of the jervanine-type steroidal alkaloids in *V. californicum*, Keeler and colleagues turned to the more difficult question of how these compounds interfere with proper fetal development.

Using derivative compounds and structurally related, non-teratogenic species, such as tomatidine, (*Figure* 5) in a hamster model Brown and Keeler were able to deduce a number of chemical features that affected teratogenicity of the jervanine steroidal alkaloids. Initial work suggested that only alkaloids with a rigid terminal furanopiperidine ring system, such as jervine and cyclopamine, produced teratogenic effects, possibly due to the positioning of the nitrogen's lone pair of electrons on the α-side of the ring system (Keeler 1970a; 1971a; 1978; Keeler et al. 1976; Brown and Keeler 1978c). Additionally, large N-alkyl groups significantly reduced the teratogenicity of jervine derivatives whereas N-methyl and N-formyl derivatives remained relatively teratogenic, suggesting that both the steric and electronic state of the nitrogen atom were important for bonding at the active site (Brown and Keeler 1978b, c). Another hypothesis, based on the potent cyclopamine derivative 11-deoxojervine-4-en-3-one, was that the unsaturation of the A-B ring system in the jervanine alkaloids, which is similar to endogenous steroids, might cause them to impair hormone function during embryogenesis (Keeler 1970a; Brown and Keeler 1978a). Later work substantiated this early observation, revealing that the relative unsaturation at C-5 and C-6 in steroidal alkaloids was more closely linked to incidence of cyclopean birth defects than the position or basicity of the nitrogen (Gaffield and Keeler 1993).

The structure-activity studies described above, though interesting basic science, were irrelevant to human health as jervanine alkaloids are not found at high levels in any food crops and the related solanidane and spirosolane alkaloids, which occur in potatoes, are considerably less teratogenic (Gaffield 2000). The information was also of little practical use to the veterinary community as the incidence of cyclopia in grazing animals dropped to near zero once livestock holders knew to keep newly pregnant animals away from fields containing *V. californicum* (Gaffield 2000). Yet, the story of *V. californicum* and one-eyed sheep did not go unnoticed among researchers studying activation of the Hedgehog (Hh) signaling pathway during embryogenesis; a scientific happenstance with far-reaching consequences for modern cancer treatment.

Of One-Eyed Sheep and Hedgehogs

In the late 1970s researchers Christiane Nüsslein-Volhard and Eric Wieschaus were studying embryonic pattern formation in *Drosophilia melanogaster* when they discovered a genetic locus they named hedgehog (*hh*), as the loss of function mutant produced a larvae covered in pointy denticles reminiscent of a hedgehog (the two would go on to win a Nobel prize in medicine in 1995 with Edward B. Lewis for their foundational work on the genetics of early development) (Nüsslein-Volhard and Wieschaus 1980). Research in the early 1990's uncovered three homologous hedgehog genes in vertebrates: Sonic hedgehog (*shh*), Indian hedgehog (*ihh*), and Desert hedgehog (*dhh*). The proteins encoded by the aforementioned genes form concentration gradients in the embryo that direct aspects of cellular growth and differentiation during embryogenesis. Each hedgehog protein controls distinct developmental processes but the Sonic hedgehog protein (Shh) is the best studied, most widely expressed in the developing embryo, most potent, and directs the widest range of events. Additionally, all three hedgehog proteins undergo the same modifications and interact with the same cellular response elements (discussed below) and as such, the term Hh signaling will be used throughout the rest of this review although the majority of research in this area has dealt with Shh (Pathi et al. 2001; Varjosalo and Taipale 2008).

Shh protein is a morphogen requisite for the development of proper bilateral symmetry and dorsal ventral patterning; it also guides the formation of hands and feet, the central nervous system, and most epithelial tissues (Echelard et al. 1993; Krauss et al. 1993; Riddle et al. 1993; Varjosalo and Taipale 2008). Sonic hedgehog loss of function mutants (*shh*$^{-/-}$) develop holoprosencephaly (HPE) as the neural vesicle that gives rise to the optic nerves, optic chiasm, eyes, and pituitary gland fails to divide bilaterally (Cooper et al. 1998; Gaffield 2000). Notably, Shh is secreted as a 47-49 kDa pro-peptide that undergoes autolytic cleavage followed by palmitoylation at the N-terminus and covalent

addition of a molecule of cholesterol to the C-terminus of the resulting 19 kDa fragment to form the active peptide Shh-N_p (Ihh and Dhh are similarly modified) (Porter et al. 1996; Pathi et al. 2001). Disorders of cholesterol biosynthesis impact *shh* signaling by impeding the formation of the active peptide and are often characterized by HPE (Cooper et al. 1998).

The mechanism of the Hh signaling begins with the stoichiometric binding of Shh-N_p to the transmembrane transporter protein Patched (Ptch) (Marigo et al. 1996). Ptch, when not bound to Shh-N_p, inhibits the seven-transmembrane protein Smoothened (Smo), a G protein-coupled-like receptor. Upon binding of Shh-N_p, this inhibition is lifted, activating a signaling cascade originating at Smo and culminating in the activation of the Gli family of zinc-finger transcription factors in vertebrates. The Gli transcription factors (particularly Gli-1 and Gli-2) up-regulate the transcription of Hh target genes, which enhance cellular proliferation and the epithelial-mesenchymal transition (Ingham and McMahon 2001; Lum and Beachy 2004; Kasper et al. 2006; Kiesslich et al. 2012).

Informed by the observation that jervine interfered with cholesterol metabolism, researchers at Johns Hopkins University School of Medicine demonstrated that jervine and cyclopamine, two jervanine alkaloids naturally occurring in North American *Veratrum* species, interfere with Hh signaling but do so without modifying the processing of Shh to Shh-N_p (Beachy et al. 1997; Cooper et al. 1998). Furthermore, addition of exogenous Shh-N_p does not save Hh signaling from alkaloid-mediated inhibition (Cooper et al. 1998). Within a month, another group of researchers at the University of Washington published near identical results indicating that cyclopamine inhibited Hh signaling without disrupting the conversion of Shh to Shh- N_p and did not interfere with cholesterol metabolism (Incardona et al. 1998). Both groups, noting that the transmembrane protein Ptch features a sterol-sensing domain, hypothesized that cyclopamine and jervine inhibited Hh signal transduction by interfering with Ptch's ability to participate in intracellular cholesterol trafficking (Cooper et al. 1998; Incardona et al. 1998). However, later investigations revealed that cyclopamine antagonizes the Shh pathway by binding directly to Smo, Ptch's downstream response element and does not interfere with cholesterol metabolism (Incardona et al. 2000; Taipale et al. 2000; Chen et al. 2002). Jervine has been found to bind in the same location, although with less affinity (EC_{50} of 500 nM vs 300 nM for cyclopamine), and there is evidence that the structurally similar alkaloids tomatidine, solasodine, and solanidine may act on Smo but are very weak inhibitors (Taipale et al. 2000; Lipinski et al. 2007; Stanton and Peng 2010). The discovery that cyclopamine inhibited Hh signal transduction provided developmental biologists examining early embryogenesis with a novel molecular probe that circumvented the need for gene knockout, but, more importantly, it identified a therapeutic target for diseases characterized by aberrant Hh signaling (Gaffield 2000).

Use of Veratrum Alkaloids in the Treatment of Aberrant Activity of the Hedgehog Signaling Pathway

The first major human disease linked to malfunctions in the Hh pathway was nevoid basal cell carcinoma syndrome (NBCCS, also Gorlin syndrome and basal cell nevus syndrome), an autosomal-dominant inherited condition. People with NBCCS suffer from myriad developmental irregularities including craniofacial malformations, spina bifida, and polydactylism and are highly susceptible to a range of tumors, especially those originating in the heart, ovaries, skin, and central nervous system. Yet, as the name suggests, the most common NBCCS tumor type is basal cell carcinoma (BCC). Individuals with NBCCS develop BCC lesions with increasing frequency in the second decade of life and the incidence of BCCs remains high throughout adulthood, requiring frequent surgical removal (Lo Muzio 2008). This stands in contrast to BCC lesions that develop in fair skinned people later in life from cumulative exposure to UV light. While NBCCS is rare (prevalence of 1/57,000 to 1/256,000), BCC is the most common form of skin cancer (representing approximately 80% of non-melanoma skin cancers, lifetime incidence risk of 30% for people with light skin) and the incidence rate of this malignancy is rapidly increasing (Wong and Lear 2003; Rubin et al. 2005; Lo Muzio 2008; Rogers et al. 2010).

Research in the mid-1990s linked both inherited and *de novo* mutations in the gene coding for Ptch, a principal protein in the Hh signaling pathway, to NBCCS as well as sporadic BCC lesions (Gailani et al. 1996; Hahn et al. 1996; Johnson et al. 1996; Wolter et al. 1997). These mutations caused inactive Ptch proteins, a result that explained many of the developmental malformations seen in NBCCS; Ptch genes are expressed by the sclerotome, spinal cord, and limbs during fetal development and are actively expressed in human skin during adulthood (Goodrich et al. 1996; Marigo et al. 1996; Johnson et al. 1996). Building on this discovery, researchers also found activating mutations in genes coding for Smo in samples taken from sporadic BCC lesions (Xie et al. 1998). Similarly, genetic

investigations of primitive neuroectodermal tumors (PNETs), such as medulloblastoma, revealed high incidences of mutations in both Ptch and Smo (Raffel et al. 1997; Wolter et al. 1997; Reifenberger et al. 1998; Hahn et al. 1999; Zurawel et al. 2000). By the end of the decade, some estimated that up to 40% of sporadic BCCs and 25% of PNETs could have either loss of function mutations in Ptch or activating mutations in Smo, both of which cause the Hh pathway to be pathogenically over-expressed (Taipale et al. 2000). The growing link between mutations in the Hh signaling pathway and the development of certain cancers paved the way for targeted inhibition of the Hh pathway with small molecules, the first of which to be tested was the *Veratrum* alkaloid cyclopamine (Taipale et al. 2000).

Using a novel cell line sensitive to Shh-N_p created from NIH 3T3 fibroblast cells transfected with Gli-Luc and TK-Renilla reporters (Shh Light II cells), investigators Johns Hopkins University School of Medicine demonstrated that cyclopamine could inhibit activating oncogenic mutations in Ptch and Smo, preventing the spread of tumorigenic cells *in vitro*. These researchers also tested synthetic derivatives of cyclopamine, one of which, 3-keto N-aminoethyl aminocaproyl dihydrocin-namoyl cyclopamine (KAAD-cyclopamine), was 10-20 times more potent. The action of these small molecules indicated the target receptor was downstream of Ptch, possibly at the level of Smo (Taipale et al. 2000). Later work demonstrated definitively that cyclopamine binds directly to Smo, specifically to the transmembrane heptahelical bundle, and suggested that Ptch may regulate Smo through the controlled pumping of small, endogenous inhibitors (Chen et al. 2002; Incardona et al. 2002). The net result of the successful inhibition of the Hh signaling pathway with small, synthetic compounds was the instigation of pre-clinical trials assessing the utility of these molecules as therapeutic agents.

One of the first successful demonstrations of Hh antagonists as therapy came from a murine model of medulloblastoma, in which the administration of cyclopamine and KAAD-cyclopamine prevented growth of medulloblastoma cells *in vitro* and also caused shrinkage of tumor allografts *in vivo*. Additionally, cyclopamine and KAAD-cyclopamine caused rapid cell death in cultured human medulloblastoma cells *in vitro* (Berman et al. 2002). Subsequent studies using cyclopamine as a molecular probe demonstrated Hh pathway over-expression to be a factor in a murine model of BCC (Athar et al. 2004), pancreatic adenocarcinoma (Thayer et al. 2003), prostate cancer (Khardakar et al. 2004), small cell lung cancer (Watkins et al. 2003), cancers of the digestive tract (Berman et al. 2003), psoriasis (Taş and Avci 2004), and breast cancer (Kasper et al. 2009; Zhang et al. 2009). To date, Hh signaling has been implicated in the pathogenesis of over twenty types of cancer (see Kar et al. 2012 for a current review). A full account of the intricacies of Hh signaling in cancer is outside the scope of this review but the emerging consensus is that Hh and other early embryonic signaling pathways (such as Notch and Wnt) are fundamental to the maintenance of so-called cancer stem cell (CSC) populations. CSCs are undifferentiated, self-renewing cells within a tumor mass that are resistant to traditional chemotherapeutics (which target the rapidly dividing bulk tumor cells) and tend to be highly proliferative, likely leading to recurrence and metastasis of existing tumors (the reader is directed to the current review on the subject by Kiesslich et al. 2012).

As the list of tumor types sensitive to blockade of the Hh signaling pathway steadily grew, targeting the pathway emerged as an increasingly viable means of treating cancer (Rubin and de Sauvage 2006). The discovery and development of a wide range of synthetic and semi-synthetic Hh pathway antagonists followed, one of which, Genentech's GDC-0449 (vismodegib) has passed through clinical trials and is now approved for use in the treatment of advanced metastatic BCC (Robarge et al. 2009; Sekulic et al. 2012; Tang et al. 2012; Pazdur 2012). For more information on current small molecule antagonists and agonists of the Hh pathway, see the 2012 review by Yun and colleagues.

With the tremendous growth of research on the Hh pathway and cancer, it may be somewhat surprising that cyclopamine, the *Veratrum* alkaloid fundamental to early pre-clinical trials that established the field, is not being pursued as a pharmaceutical agent. However, the utility of cyclopamine as a therapeutic agent for cancer treatment is impeded by a number of molecular characteristics. First, cyclopamine is sparingly soluble (5 μg/mL) in aqueous solutions (Yun et al. 2012). Second, the ether bridge formed by the spiro-connection at C-17 is acid labile, rendering the molecule biologically inactive if it passes through the stomach (Keeler 1970b; 1978; Wilson et al. 2010). Lastly, many synthetic Hh pathway antagonists display greater potency than cyclopamine; cyclopamine has an EC_{50} of 300 nM, KAAD-cyclopamine has an IC_{50} of 20 nM, and GDC-0049's has an IC_{50} of 13 nM (Rominger et al. 2009; Stanton and Peng 2010; Yun et al. 2012). Consequently, medicinal chemists have sought to modify and build on cyclopamine's structure to improve its pharmacological attributes.

Modifications to cyclopamine's structure have included the addition of carbohydrate moieties to the piperidine ring nitrogen to improve aqueous solubility as well as the addition of prostate specific antigen and glucuronide at the same location to create prodrugs for the treatment of prostate cancer and glioblastoma respectively (Zhang et al. 2008; Kumar et al. 2008; Hamon et al. 2010). A tartrate of cyclopamine has also been developed and this salt is readily soluble in aqueous solutions (Xie and Garrossian 2011). KAAD-cyclopamine is another example of substituent addition at the piperidine nitrogen and also features increased unsaturation in the A/B ring system. These modifications resulted in higher potency and aqueous solubility in comparison to cyclopamine, albeit the structure retains cyclopamine's susceptibility to acidic conditions (Taipale et al. 2000; Tremblay et al. 2009). Perhaps the most notable cyclopamine derivative is the compound IPI-926 (saridegib) developed by Tremblay and colleagues at Infinity Pharmaceuticals (2008; 2009). This compound was created by expanding the D-ring of cyclopamine to create a heptacyclic configuration that minimizes the allylic effects of the oxygen atom in the E-ring, thus making it less acid labile, and adding a sulfonamide moiety to C-3 to improve aqueous solubility and potency (Tremblay et al. 2009). IPI-926 demonstrated early promise; this drug advanced to a Phase 2 clinical trial in combination with gemcitabine for metastatic adenocarcinoma but the trial was stopped early when preliminary analysis revealed that median survival of patients in the treatment arm was less than that of the control arm (Infinity Pharmaceuticals 2012a). Additionally, Infinity stopped Phase 2 trials of IPI-926 for the treatment of chondrosarcoma and myelofibrosis when the treatment arms failed to outperform placebo (Infinity Pharmaceuticals 2012b). A Phase 1 trial of IPI-926 in combination with cetuximab for recurrent advanced head and neck cancers is in process (University of Colorado, Denver 2013).

Appendix:

Table 1: *Veratrum* species of North America: Morphological features, range, habitat, and alkaloids

Species and Common Name	Morphological Features	Range	Habitat	Alkaloids				References
Veratrum viride Green false hellebore	Up to 2 m tall when flowering; broad, pointed lower leaves; crowded paniculate inflorescences; generally features green perianths, lanceolate tepals; moderately hairy ovaries	Eastern North America from Quebec to Georgia; western North America from Alaska to California, inland to the mountainous regions of Montana, Idaho, and Alberta	Wet woods and moist coastal regions in Alaska and Quebec to mountain meadows in northern California, Idaho, Montana, and North Carolina	isorubijervine germine germitrine germerine veratramine	neogermitrine rubivirine jervine isojervine rubijervine	germidine protoveratrine A&B protoveratridine neogermbudine neogermidine veramivirine	pseudojervine veratrosine isorubijervosine escholerine desacetyl-protoveratrine veratroylzygadenine	Kupchan 1961, Mathew 1989, Li et al. 2006, USDA NRCS 2013
Veratrum californicum var *californicum* California false hellebore	1-2 m in height, broad lower leaves which continue up pseudostem; dark brown to black rhizome; crowded large inflorescences; creamy white tepals with glabrous ovaries	Washington, Oregon, Idaho, Montana, Utah, California, Nevada, New Mexico	Moist, sandy loam soils in subalpine meadows and forests, generally prefers north facing slopes which retain snow	cyclopamine cycloposine dipotassium cevine veratrosine	germine isorubijervine jervine veratramine	rubijervine muldamine protoveratrine A & B	protoverine pseudojervine veracevine	Mathew 1989, Kupchan 1961, Keeler and Binns 1966a, Keeler 1974*, USDA NRCS 2013
Veratrum album (includes ssp: *V. lobelianum, V. oxysepalum, V. Grandiflorum* White false hellebore	Up to 2 m tall when flowering; paniculate inflorescences with very short perdicles (2-3 mm); often large, broad white tepals but local variations can include small green to white tepals, and even yellow	Primarily Eurasia; Alaska	Damp mountain meadows	jervine rubijervine geralbine germerine germitrine germitetrine veratroylzygadenine veralomidine veralomine veracintine 3-acetyl-15-veratroylgermine verdinine	veralobine veralkamine veralinine veratrine veratrobasine veralbidine veramanine isojervine verdine veramine germinaline germinine	15-veratroylgermine rhamnoveracintine neojerminalanine verussurine methyljervine-*N*-3'-propanoate veramarine verazine 3,15-*O,O'*-(2-methylbutyroyl)germine veralodine veralosine protoveratrine A & B	germbudine 15,-*O*-(2-methylbutyroyl)germine pseudojervine *O*-acetyljervine isorubijervine isorubijervosine diacetylveralkamine tetrahydroveralkamine 1-hydroxy-5,6-dihydrojervine jervinone	Kupchan 1961, Mathew 1989 Li et al. 2006, USDA NRCS 2013,
Veratrum fimbriatum Fringed false hellebore	Heavily fringed green to white tepals; large nectaries; wingless, large seeds	Coastal northern California between Fort Bragg and Fort Ross	Coastal; sea level to several hundred meters in elevation, 5 km inland	jervine pseudojervine neogermitrine				Taylor 1956a, Mathew 1989, USDA NRCS 2013
Veratrum californicum var *caudatum* Cascade false hellebore	Stem terminates in long, heavily flowered tail; large green-to-white perianths; glabrous ovaries	Western Oregon and Washington; north-central Idaho	Lower elevation swampy meadows and coastal areas	NA, likely similar to var *californicum*				Kupchan 1961, Mathew 1989, USDA NRCS 2013
Veratrum tenuipetalum Colorado false hellebore	Over 2 m tall when flowering; heavily flowered inflorescences; small white perianths	Colorado, Wyoming	Moist openings and forest canopies, depleted riparian areas	NA, likely similar to *V. californicum*				Kupchan 1961, Mathew 1989, Johnston 2002, USDA NRCS 2013
Veratrum insolitum Siskiyou false hellebore	1 to 1.5 m tall; hairy upper pseudostem; very white-wooly, paniculate inflorescences, heavily flowered with small yellow-white perianths; densely wooly ovaries	Northern California, southwestern Oregon, Washington	Serpentine and diorite soils, wet openings in mixed-evergreen forests	NA				Kupchan 1961, Mathew 1989, Washington Dept of Natural Resources 2003, USDA NRCS 2013

*Keeler R (1974) Isolation of rubijervine from *Veratrum californicum*. Phytochemistry 13:2336-2337

Notes

[1] Near the same time, researchers Saito and colleagues in Japan isolated the *Veratrum* alklaoids jervine and veratramine from *V. grandiflorum*, a member of the *V. album* complex (Saito and Suginome 1936, Saito 1940). Though not hypotensive *Veratrum* alkaloids, these compounds would be the subject of greater medical investigation in the 1960s and 1970s. See the section on teratogenic *Veratrum* alkaloids.

[2] Seiferle et al. produced the first modern isolation of jervine, pseudojervine, rubijervine, protoveratridine, and germine from *V. viride* (Seiferle et al. 1942).

[3] Notably, Kupchan and colleagues also isolated the alkamine germine from *Zigadenus venenosus*, further cementing the phytochemical relationship between the species of *Veratrum* and *Zigadenus* (Kupchan and Deliwala 1953).

[4] The numerous permutations of cevanine ester-alkaloids with hypotensive qualities are substantial and the reader is directed to see the outstanding reviews by Kupchan (1961) and Krayer and Meilman (1977) for a more complete discussion of the phytochemistry and medical applications of these compounds respectively.

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Figure Captions

Figure 1: Geographic distribution of *Veratrum* species in North America. Emphasis is placed on species within Zomlefer et al.'s (2003) section *Veratrum* as these plants have been heavily investigated for the bioactive steroidal alkaloids they contain. Illustration adapted from distribution ranges in (Utech 2002) in Flora of North America, volume 26.

Figure 2: Steroidal alkaloids have been isolated from a wide range of plant and animal sources. Species of the genus *Veratrum* produce two broad classes of steroidal alkaloids: the *Solanum* alkaloids, which feature the classic cyclopentanophenanthrene ring structure and the *Veratrum* alkaloids, which feature a rearranged C-nor-D-homosteroidal ring structure in the C-ring is five membered and the D-ring is six membered.

Figure 3: Biosynthetic pathway for steroidal alkaloids in *Veratrum* plants as elucidated by Kaneko and colleagues in *V. grandiflorum*. 3a: The nitrogen source for *Veratrum* alkaloids is likely L-arginine (Kaneko et al. 1976). 3b: Based on the observation that solanidine accumulates in etiolated plants and is then rapidly turned into *Veratrum* alkaloids featuring a C-nor-D-homosteroidal skeleton upon illumination, Kaneko et al. (1972, 1979) hypothesized that this conversion is light and energy dependent. 3c: Isolation of procevine suggests attack of the nitrogen atom on a suitable leaving group on C-18 precedes ring rearrangement in the formation of cevanine type alkaloids (Kaneko et al. 1978, 1979). 3d: Cyclopamine was found to be an intermediate compound from which veratramine and jervine can be formed (Kaneko et al. 1970a, b).

Figure 4: Cevanine alkamine bases. Esters of these bases reversibly bind to fast-sodium channels and lead to the Bezold-Jarisch reflex (hypotension, bradycardia, apnea) when injected into circulation.

Figure 5: Cyclopamine, cycloposine and jervine produce teratogenic effects in vertebrates by interfering with Hh signaling whereas the structurally similar and related alkaloids veratramine and tomatidine do not.

Figure 1

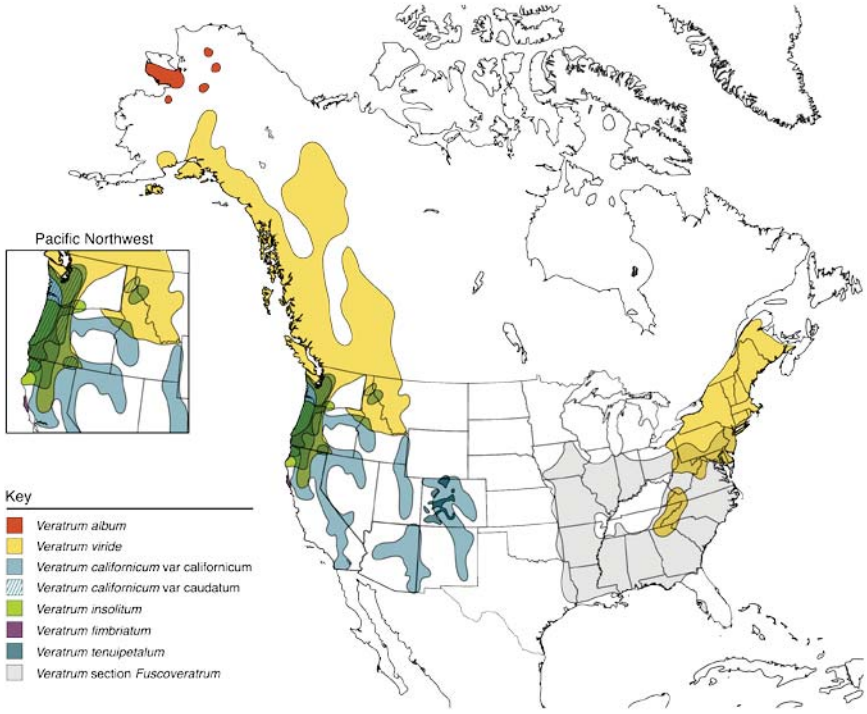

Figure 2

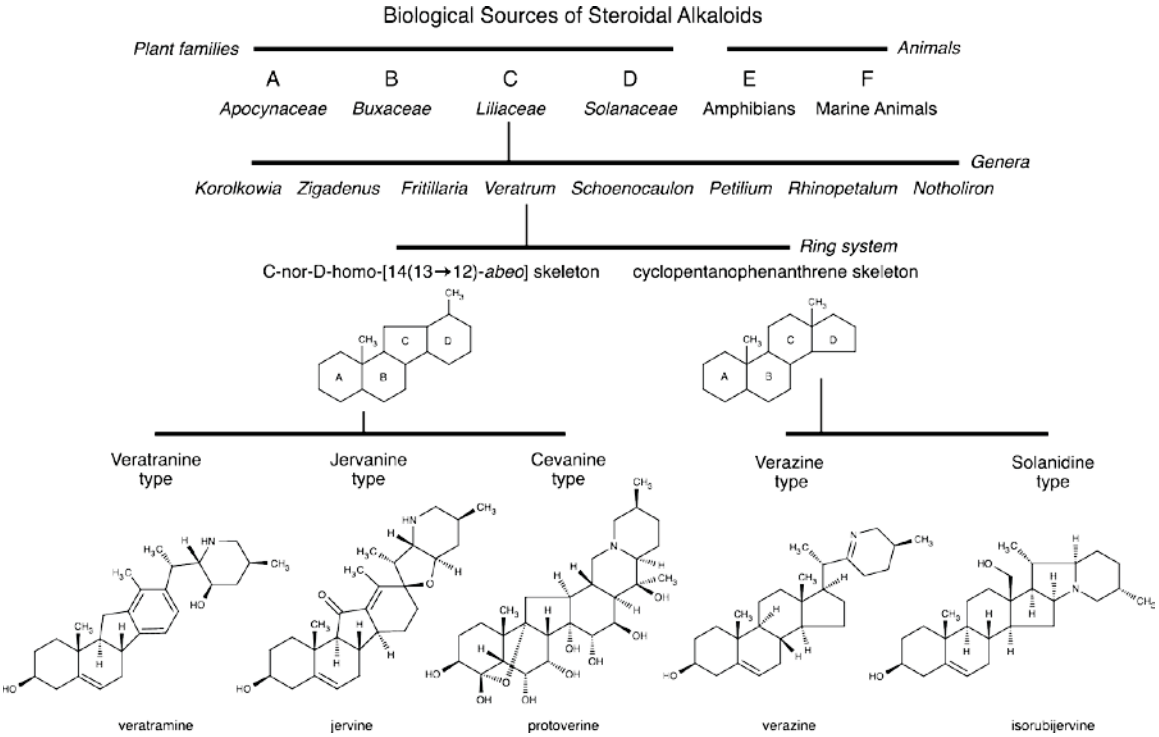

Figure 3

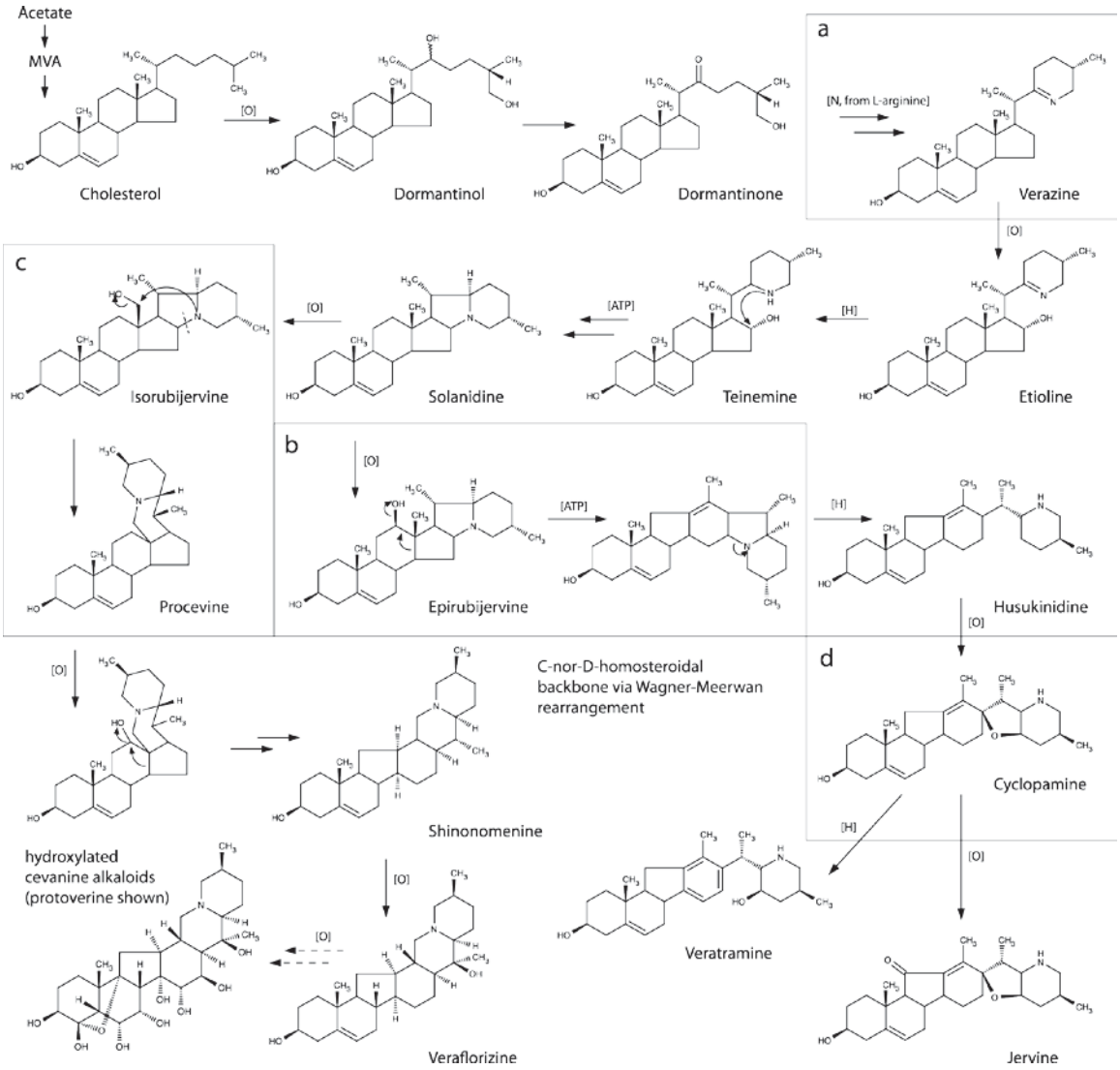

Figure 4

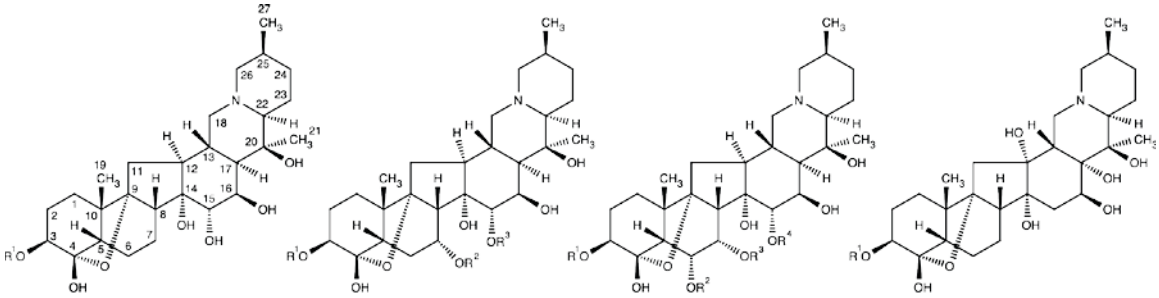

Figure 5