# Chemistry and Biological Activity of Steroidal Glycosides from the Lilium Genus


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REVIEW

# Chemistry and Biological Activity of Steroidal Glycosides from the *Lilium* Genus

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Plants from the *Lilium* genus are a rich source of chemical diversity and have been the focus of natural products chemistry research for over twenty years. This manuscript provides a background on the chemistry and nomenclature of steroidal glycosides, as well as a chronological account of the progress between the years of 1989 to 2014 with respect to their isolation and characterization from the genus. This review highlights the traditional use of lilies, as both food and medicine, and brings attention to the fact that the genus contains 110 accepted species of which the chemistry and biological activity of the steroidal glycosides from the majority of these species have not been investigated to date, making the genus a relatively untapped resource that contains a potential treasure trove of chemical diversity waiting to be discovered.



## 1. Introduction

The *Lilium* genus, Family Liliaceae, is comprised of 110 accepted species of flowering plants with wide geographical distribution.[1] Many *Lilium* species including ornamental cultivars and hybrids are cultivated for their esthetic value, and both the flowers and bulbs are regularly consumed in many parts of the world, particularly in Asia, as both food and medicine. The bulbs of several *Lilium* species, including *L. longiflorum* Thunb., *L. brownii* F. E. Br. ex Miellez, *L. pensylvanicum* Ker Gawl., and *L. pumilum* DC. have been used traditionally in China and Japan as a sedative, anti-inflammatory, antitussive and as a general tonic.[2, 3] The crude drug "Bai-he," used in traditional Chinese medicine, is prepared from the bulbs of *Lilium sp*. and is regularly used for lung ailments in China today. Although the medicinal use of *Lilium* is well documented, the compounds responsible for the reported properties are not known.

The Liliaceae family is a rich source of natural products displaying a vast range of structural diversity. A multitude of natural products have been isolated and characterized from Liliaceae including, dimeric *ent*-kaurane diterpenes,[4, 5] flavonoid glycosides, [6, 7] anthocyanins, [8, 9] stilbenes,[10] phenolics, [11] phenolic glucosides,[12] phenolic amides, [13] carotenoids,[14] sterols,[15] alkaloids,[16] and sulfur-containing compounds.[17] Most notably, there has been extensive work done on the isolation and characterization of steroidal alkaloids, particularly in the genus *Fritillaria*,[18] and steroidal glycosides including steroidal saponins[19-21] and steroidal glycoalkaloids [22, 23] from within the Liliaceae family.

Steroidal glycosides have been reported to exhibit a wide range of biological activities including antifungal,[24, 25] platelet aggregation inhibition,[26, 27] anti-cholinergic,[28] anti-diabetic,[29] anti-hypertensive,[30] cholesterol lowering,[20] anti-inflammatory,[31] antiviral,[32] and anticancer.[33-36] Additionally, steroidal glycosides have a wide variety of commercial uses including surfactants,[37] foaming agents,[38] vaccine adjuvants,[39] and serve as precursors for the industrial production of pharmaceutical steroids.[40]

Steroidal saponins have been found in over 100 plant families and in some marine organisms such as starfish and sea cucumber.[41] They are characterized by a steroid type skeleton glycosidically linked to carbohydrate moieties. Steroidal glycoalkaloids are characterized by a nitrogen containing steroid type skeleton glycosidically linked to carbohydrate moieties. In contrast to steroidal saponins, the occurrences of steroidal glycoalkaloids are, thus far, limited to the members of the plant families Solanaceae and Liliaceae.[18, 42]

Many natural products found in plants occur in trace quantities. As a result, the isolation and purification of these molecules in a sufficient enough yield for structural characterization and biological analysis remains to be a challenge. This is not the case for steroidal glycosides in the *Lilium* genus. *Lilium* plants are an exceptionally rich source of steroidal glycosides, making them an excellent starting material for the discovery of novel steroidal glycosides. For example, the levels of some steroidal glycosides in lily bulbs have been reported to

be as high as 8.84 mg/g and 4.59 mg/g in *L. longiflorum* and *L. pensylvanicum,* respectively.[43, 44]

The objective of this review article is to: 1) provide an account of the progress that has been made in the isolation, purification, and characterization of steroidal glycosides from the *Lilium* genus over the past twenty years, 2) highlight the ethnobotanical uses of *Lilium* species and the reported biological activities of the steroidal glycosides isolated from the genus, and 3) serve as a tool to aid future investigators in identifying known steroidal glycosides, and discovering new steroidal glycosides in the *Lilium* genus and other plants. Moreover, the aim of this review is to bring attention to the fact that the genus contains 110 accepted species of which the chemistry and biological activity of the steroidal glycosides from the majority of the species have not been investigated to date. This review was written with the intent of inspiring future plant biologists and chemists to continue to conduct phytochemical and biological investigations on the natural products contained within the *Lilium* genus and to revisit the chemistry of the lily species that have already been studied.

## 2. Classification of Saponins

Saponins are a structurally diverse class of natural products that are characterized by a non-polar sapogenin moiety glycosidically linked to one or more polar carbohydrate moieties. Based on the composition of sapogenin skeleton, they are generally classified into two major categories, triterpenoid saponins and steroidal saponins.[45] Triterpenoid saponins contain a thirty carbon aglycone and steroidal saponins contain a twenty-seven carbon aglycone. Both classes are derived from the thirty carbon precursor 2,3-oxidosqualene.[46] **(Figure 1)**. Isopentenyl pyrophosphate synthesized via the mevalonate pathway is the five carbon donor for the biosynthesis of terpenes in plants. Triterpenoid and steroidal sapogenins are synthesized from the thirty carbon hydrocarbon squalene, which is subsequently oxidized to squalene 2,3-epoxide, and then converted to tetra- or pentacyclic triterpenes by a family of 2,3-oxidosqualene cyclases. Following cyclization, the sapogenin moiety is subsequently mono- or poly- glycosylated by a wide variety of glycosyltransferase enzymes. In addition to the structure of the sapogenin, saponins are also classified according to the number of carbohydrate moieties that are glycosidically linked to the aglycone. Accordingly, they are referred to as mono-, bi-, or tridesmosidic, based on the number of carbohydrate moieties linked to the sapogenin skeleton **(Figure 2).**

Figure 1. Structures of (A) 2,3-oxidosqualene, (B) a triterpenoid sapogenin (quillaic acid), and (C) a steroidal sapogenin (diosgenin).[46-48]

Figure 2. Representative examples of (A) monodesmosidic and (B) bidesmosidic saponins isolated from *Lilium pumilum* Redouté and *L. pardalinum* Kellogg, respectively.[49, 50]

## 3. Steroidal Saponins

Steroidal saponins are widely found throughout the plant kingdom and have been reported in a broad range of orders including Solanales,[51] Ranunculales,[52] Sapindales, [53] Fabales,[54] Cyperales,[55] Liliales,[56] Dioscoreales,[57] Asparagales,[58] and Zingiberales.[59] In addition, steroidal saponins have been documented in over 100 plant families and in many marine organisms.[41] Steroidal saponins are divided into two main classes, spirostanols and furostanols, based on structural differences in the aglycone (**Figure 3**). Spirostanols have a six-ring structure (A – F rings), referred to as a spirostane skeleton, and are monodesmosidic, typically having a carbohydrate moiety β-glycosodically attached by an ether linkage to the C-3 carbon of the aglycone. Furostanols have a pentacyclic aglycone (A – E rings), referred to as a furostane skeleton, and are bidesmosidic with one carbohydrate moiety attached through an ether linkage at C-3 carbon and a second carbohydrate moiety attached by an ether linkage at the C-26 carbon. The most common furostanols have a single glucose linked at the C-26 position; however, multiple sugars can be attached, but this is less common. The sugar composition of the carbohydrate moiety of steroidal saponins most commonly include, D-glucose, D-galactose, D-glucuronic acid, D-galacturonic acid, L-rhamnose, L-arabinose, D-xylose, and D-fucose. All dextrorotatory form sugars are linked via a β-glycosidic linkage and all levorotatory form sugars are linked via a α-glycosidic linkage. The composition of the carbohydrate moiety can range from one sugar to multiple sugars and can be linked in a linear or branched arrangement **(Figure 4).** In addition, sugars can be attached by different interglycosidic linkages resulting in a vast number of possible structural arrangements. Structural differences in the carbohydrate moiety have been shown to play a role in the biological activity of the molecules and differential biological activity of steroidal saponins containing the same aglycone but differing only in carbohydrate composition has been previously reported.[35]

Figure 3. Structures of (A) a basic steroidal backbone (A-D rings), (B) a spirostane backbone, and (C) a furostane backbone.

Figure 4. Examples of different carbohydrate linkages: (A) linear arrangement (β-D-glu-(1→4)-β-D-glu), (B) branched arrangement (α-L-rha-(1→2)-α-L-xyl-(1→3)-β-D-glu), and (C) branched arrangement (α-L-rha-(1→2)-β-D-glu-(1→4)-β-D-glu).

## 4. Steroidal Alkaloids

Steroidal alkaloids are a structurally diverse class of natural products that have been isolated and characterized in a wide range of organisms including plants, marine animals, and terrestrial animals.[60] Structurally, steroidal alkaloids contain a steroid-type backbone and with a nitrogen atom incorporated into the molecule. The biosynthesis of steroidal alkaloids in plants differs from other plant alkaloids due to the fact that the carbon atoms in the molecule are derived from the mevalonic acid pathway, whereas the carbon backbones of other alkaloids are derived from amino acids.[60] Steroidal alkaloids are most commonly found in the plant families of Apocynaceae, Buxaceae, Liliaceae and Solanaceae. Interestingly, highly cytotoxic steroidal alkaloids have also been isolated and characterized from marine organisms such as the truncate *Ritterea tokiokal*,[61] and amphibians such as *Salamandra* species and *Phyllobates* species.[62]

## 5. Steroidal Alkaloids in Liliaceae

The occurrence of steroidal alkaloids in the Liliaceae family is well documented.[18] *Fritillaria,* a genus in the Liliaceae family, and *Veratrum*, a genus in the closely related Melanthiaceae family, have been recognized for centuries for

 

their pharmacological activities and have a long history of use in traditional medicine. In fact, *V. viride* has been documented to be used by Native Americans for the treatment of catarrh, the treatment of rheumatism, and as an insecticide against lice.[60] Steroidal alkaloids isolated from the Liliaceae family have been of great interest in pharmacology and have been documented to exhibit various putative biological activities including antihypertensive,[30] anticholinergic,[28] antifungal,[25] and anticancer.[36] In particular, the genera *Veratrum* and *Fritillaria*, have been the subject of extensive chemical characterization and pharmacological investigations. Hundreds of new steroidal alkaloids have been isolated from these plants and over 100 steroidal alkaloids have been isolated between the years of 1980 and 2005.[18] The steroidal alkaloids isolated from the Liliaceae have been classified into two main groups, isosteroidal alkaloids and steroidal alkaloids, on the basis of connectivity of the carbon skeleton.

### 6. Classification of Isosteroidal Alkaloids of Liliaceae

Isosteroidal alkaloids, also referred to as *Veratrum* steroidal alkaloids, are characterized by a C-nor-D-homo-[14(13→12)-*abeo*] ring system[18] (**Figure 5**). Isosteroidal alkaloids are further sub-divided into three groups according to the linkage patterns between rings E and F into cevanine type, veratramine type, and jervine type. The cevanine type, structurally characterized by the hexacyclic benzo [7,8] fluoreno [2,1-*b*] quinolizine nucleus, constitutes the largest class. The veratramine type is characterized by the absence of ring E and the presence of an aromatic ring D. Analogues with an unaromatized ring D are also placed in this class. The jervine type contains hexacyclic compounds that have the furan ring E fused onto a piperidine ring system forming an ether bridge between carbon atoms at C17 and C23.

Figure 5. Isosteroidal alkaloids of Liliaceae: representative examples of cevanine type (**A**), veratramine type (**B**), and jervine type (**C**) isosteroidal alkaloids, isolated from *F. imperialis, F. ningguoensis,* and *F. camtschatcensis,* respectively.[18]

### 7. Classification of Steroidal Alkaloids of Liliaceae

The steroidal alkaloids of Liliaceae, also referred to as *Solanum* steroidal alkaloids, are characterized by a six membered C-ring and a five-membered D-ring. The steroidal alkaloids are further sub-divided to two groups on the basis of the position of the nitrogen atom. Steroidal alkaloids with the nitrogen atom incorporated into an indolizidine ring are referred to as solanidine type. The solanidine type is derived from epiminocholestanes with the amino group incorporated into an indolizidine ring, resulting in a hexacyclic carbon framework. If the nitrogen atom is incorporated into a piperidine ring, they are of verazine type. The verazine type is based on the 22/23,26-epiminocholestane heterocyclic skeleton **(Figure 6).**

Figure 6. Steroidal alkaloids of Liliaceae: representative examples of solanidine type (A) and verazine type (B) steroidal alkaloids, isolated from *F. delavayi* and *F. ebeiensis* var. *purpurea,* respectively.[18]

### 8. Steroidal Alkaloids in *Lilium*

Although extensive work has been done on the characterization of steroidal alkaloids in the Liliaceae family, less is known of the steroidal alkaloids in the *Lilium* genus. In 1996 Noor-e-Ain reported the identification of two steroidal alkaloids from *L. candidum*, commonly known as the Madonna lily.[63] Two 22, 26-epiminocholestane-type steroidal alkaloids, named etioline and teinemine, which were previously found in several *Solanum* and *Veratrum* species were isolated from *L. candidum* bulbs.[60, 64] **(Figure 7)** In 2001, Erdoğan et al. also reported the isolation of etioline from *L. candidum* bulbs.[65] Although these compounds were isolated as free form steroidal alkaloids, the isolation procedure was performed over several weeks under acidic conditions, and it is unclear whether the conditions caused glycosidic cleavage, resulting in the formation of artifacts during the isolation process. Thus far, the glycosylated forms of these two steroidal alkaloids have not been identified in *L. candidum*. Nevertheless, these compounds are the only free form steroidal alkaloids that have been reported from the *Lilium* genus.

Figure 7. Structures of the steroidal alkaloids (A) etioline and (B) teiemine isolated from the bulbs of L. candidum.[60, 64]

### 9. Steroidal Glycoalkaloids

In contrast to the widespread distribution of steroidal alkaloids in plants and animals, steroidal glycoalkaloids, thus far, have only been identified in the Solanaceae and Liliaceae families.[42] Steroidal glycoalkaloids are characterized by a steroidal alkaloid type aglycone glycosidically linked to carbohydrate moieties. The most common aglycones of the steroidal glycoalkaloids can be classified based upon their structural features into six major groups **(Figure 8).** The first group is referred to as (**1**) spirosolanes and are characterized by an oxazaspirodecane ring system. The second group, (**2**) 22, 26-epiminocholestanes, are characterized by a 22/23, 26-epiminocholestane heterocyclic skeleton. The third group, (**3**) solanidanes, are characterized by a fused indolizidine ring system. The fourth group, (**4**) α–epiminocyclohemiketals, are characterized the presence of an α–epiminocyclohemiketal functionality. The fifth group, (**5**) 3-aminospirostanes, is characterized by an amino group at the C3-position. The sixth group, (**6**) leptines, are characterized by a fused indolizidine ring system with 23-hydroxy or 23-acetoxy moieties. The most common steroidal glycoalkaloids belong to the solanidane and the spirosolane groups.[42] The carbohydrate moiety of steroidal glycoalkaloids is β-glycosidically linked at the C-3 hydroxy position of the steroidal alkaloid backbone. The most common sugars are D-glucose, D-galactose, D-xylose, L-rhamnose, and L-arabinose. Similar to the steroidal saponins, all dextrorotatory form sugars are linked via a β-glycosidic linkage and all levorotatory form sugars are linked via a α-glycosidic linkage. The composition of the carbohydrate moiety can range from one sugar to multiple sugars, linked in a linear or branched arrangement.

Figure 8. Structures of the most common aglycones of the steroidal glycoalkaloids: (A) spirosolanes, (B) 22, 26-epiminocholestanes, (C) solanidanes, (D) α-epiminocyclohemiketals, (E) 3-aminospirostanes, and (F) leptines

### 10. Steroidal Glycosides from the *Lilium* genus (1989 through 2014)

Table 1 lists 110 accepted *Lilium* species and one hybrid (*L. speciosum x L. nobilissimum).* The table indicates which species have been documented to have a history of ethnobotanical use and lists the steroidal glycosides that have been isolated and

characterized from each species. In addition, the reported biological activities of the isolated compounds are indicated (**Table 1**). It is clear from this compilation that most of the lily species have not been evaluated for steroidal glycosides. Of the 32 species with historical medicinal uses, in only six (*L. pumilum*, *L. pardalinum*, *L. martagon*, *L. longiflorum*, *L. candidum*, and *L. brownii*) have there been steroidal glycoside analyses. Although there are certainly other classes of compounds that may contribute to the medicinal effects of *Lilium* species, it is likely that yet unknown steroidal glycosides may contribute to their biological activity.

Table 1 – List of accepted Lilium species: historical use, species name, steroidal glycosides, and biological activities.

***** indicates documented historical use as food or medicine.[66] Abbrev: **a**; inhibition of $Na^+/K^+$ ATPase. **b**; inhibition of cyclic AMP phosphodiesterase. **c**; inhibitory activity on 12-O-tetradecanoylphorobol-13-acetate (TPA) stimulated $^{32}P$-incorporation into phospholipids of HeLa cells. **d**; antifungal. **e**; bitter.

*Lilium* species are listed alphabetically, but steroidal glycosides are numbered chronologically by date of first report

### *L. pardalinum* Kellogg

Figure 9. Image of a *L. pardalinum* specimen taken in a private garden in Mercer, Pennsylvania, United States.

In 1989, Shimomura and co-workers reported on the isolation and structural elucidation of seven steroidal saponins **1 – 7**, trivially named pardarinosides A-G, from the fresh bulbs of *L. pardalinum* Kellogg, also referred to as the leopard lily or the panther lily (**Figure 10**).[50] It has been documented that the Atsugewi, Karok, and Yana Native American Indian tribes consumed the cooked bulbs of *L. pardalinum* as a food source.[67-69] The authors believed that the compounds from this study were the first report of steroidal saponins isolated from a *Lilium* species. The fresh lily bulbs (2.5 Kg) were extracted with hot methanol and the resulting bulb extract was partitioned between chloroform and water. The chloroform soluble fraction was then subjected to repeated silica gel column chromatography (CC) and gel permeation chromatography (GPC) (Sephadex LH-20®) to afford the pure compounds. Compound **1** was a spirostanol with a hydroxyl group at the C-21 position, and **2 – 6** were bidesmodic furostanols bearing an acetyl moiety at the C-26 position instead of a glucose and a methoxy moiety at the C-22 position. It is unclear whether the methoxy moiety was an artifact generated during extraction in hot methanol. No biological activity was reported for the compounds except for a comment on their bitter organoleptic properties. The bitter organoleptic proprieties were not quantified in the study.

Figure 10. Structures of compounds 1-7 isolated from *L. pardalinum*

### *Lilium pumilum* Delile (synonym: *L. tenuifolium* Fisch. Ex Schrank)

Figure 11. Image of a *L. pumilum* specimen taken in a private garden in Clinton, Canada.

In 1989, Mimaki and co-workers reported on the isolation and structural elucidation of two steroidal saponins **8** and **9**, trivially named tenuifolioside A and B, from the fresh bulbs of *Lilium pumilum* Delile (synonym: *L. tenuifolium*) (**Figure 12**).[49] Also known as the coral lily or shan dan in China, *L. pumilum* is listed as both a food and medicine.[70, 71] The fresh lily bulbs (4.7 Kg) were extracted with hot methanol under reflux and the resulting bulb extract was dissolved in water and sequentially partitioned between chloroform and 1-butanol. The 1-butanol soluble fraction was then subjected to repeated silica gel CC and GPC (Sephadex LH-20®) to afford the pure compounds. Through extensive structural elucidation experiments, **8** was determined to be a cholestane glucoside bearing two secondary hydroxyl groups, two tertiary hydroxyl groups and a carbonyl group. The structure of **8** was determined to be (20R, 22R)-3β,14α-20,22-tetrahydroxy 5α-cholestan-6-one 3-*O*-β-D-glucopyranoside, namely tenuifolioside A. Interestingly, **9** was determined to share the same sapogenin as **8**, but contained a β-D-allose saccharide moiety at the C-3 position of the aglycone instead of a β-D-glucose as in **8**. Accordingly, the structure of **9** was determined to be (20R, 22R)-3β,14α-20,22-tetrahydroxy 5α-cholestan-6-one 3-*O*-β-D-allopyranoside, namely tenuifolioside B. The authors comment about the potential chemotaxonomic significance of the compounds and that they have a slightly bitter taste, but report no other biological activities.

Figure 12. Structures of compounds 8 and 9 isolated from *L. pumilum*

### *Lilium philippinense Baker*

Figure 13. Image of a *L. philippinense* specimen taken at the Kyoto Botanical Garden, Japan.

In 1990, Espeso et al. reported the isolation of a steroidal glycoalkaloid **10** from the aerial parts of *L. philippinense,* commonly known as the Philippine lily, the banquet lily or the kanyon lily (**Figure 14**).[72] *L. philippinense* grows in the coniferous forests of the northern highlands of the Philippines. Interestingly, the compound was a veratramine type isosteroidal alkaloid glucoside, containing an unaromatized D ring. Prior to this work, the only steroidal glycoalkaloid previously isolated from the *Lilium* genus was from *L. cordatum*; however, this plant was taxonomically moved into the closely related genus *Cardiocrinum* (Endl.) Lindl. Nevertheless, in 1987, Nakano et al. reported the isolation and structural elucidation of (25*R*)- and (25*S*)-22,26-epimino-5α-cholest-22(*N*)-en-3β,6β-diol *O*(3)-β-D-glucopyranoside from *C. cordatum* (Nakano et al., 1987).

The plant material that was used in the study was collected in the Sagada Mountain Province during June through August and a voucher specimen was deposited at the University of Santo Tomas Research Center for Natural Sciences and the National Museum. In this study, the fresh aerial parts (1.3 Kg) were extracted with cold methanol. The resulting methanolic extract was concentrated and partitioned between diethyl ether and a 1% sulfuric acid solution. The aqueous layer was adjusted to pH 9 – 10 with aqueous ammonia and then extracted with chloroform. The resulting extract was repetitively chromatographed by preparative silica gel thin layer chromatography (TLC) and GPC (Sephadex LH-20®) to afford two steroidal alkaloids. Unfortunately, the authors only report on the structural elucidation of one of the alkaloids and no biological activities were reported. Based on the harsh extraction conditions,

under both acidic and basic conditions, it is unknown if the steroidal glycoalkaloid reported in this study was an artifact of the isolation process. For example, steroidal glycoalkaloids bearing additional saccharides units may be present in *L. philippinense* but glycosidic cleavage may have occurred during isolation, resulting in only the monoglycoside being isolated. The fact that two steroidal glycoalkaloids were isolated, but only one was characterized merits further investigation of the chemistry of this species.

Figure 14. Structure of steroidal glycoalkaloid isolated from *L. philippinense*

***Lilium brownii* F. E. Br. ex Miellez**

Figure 15. Image of a *L. brownii* specimen taken at Victoria Park, Hong Kong.

In 1990, Mimaki et al. reported on the isolation and structural elucidation of a 27-acyloxyspirostanol saponin **11**, trivially named brownioside, and a furospirostanol **14**, from the fresh bulbs of *Lilium brownii* F. E. Br. ex Miellez (synonym: *L. brownii* var. *brownii)* (**Figure 16**).[3] Some common names for *L. brownii* include Brown's lily and the Hong Kong lily, and it is one of the lily species commonly used in the traditional Chinese medicine "Bai-he," and as a result, the bulbs are sometimes themselves referred to as Bai-he. Bulbs from *L. brownii* have a long history of use in traditional medicine and are regularly used as both food and medicine predominately by Asian cultures today. Some documented uses include the treatment of various skin conditions including bruises, eczema, swelling, and wounds.[73, 74] Additionally, *L. brownii* bulbs are documented to have been used internally as a carminative, diuretic, and sedative and most notably for the treatment of coughs and other lung ailments, and as a general tonic.[75, 76]

In this study, the fresh lily bulbs (3.4 Kg) were extracted with methanol at 60 ºC and similar to the previous isolations by the same group, the resulting bulb extract was dissolved in water and sequentially partitioned between chloroform and 1-butanol. The 1-butanol soluble fraction was then subjected to repeated silica gel CC and GPC (Sephadex LH-20®) to afford the pure compounds. The $^{1}$H NMR and $^{13}$C NMR spectra for **11** were similar to that of diosgenin glycoside except that the C-27 methyl group was replaced by an oxymethyl group. The $^{1}$H NMR and $^{13}$C NMR data supported the presence of a 3-hydroxy-methylglutaric acid substituent that was confirmed upon alkaline hydrolysis, yielding 27-hydroxydiosgenin, 3-hydroxy-methylglutaric acid, and a disaccharide. Upon further spectroscopic analysis and chemical transformations, the structure of **11** was confirmed. The $^{1}$H NMR and $^{13}$C NMR spectra for compound **14** were consistent with that of a furospirostanol saponin, similar to that of aculeatiside A and B isolated from the roots of *Solanum aculeatissimum*. Upon further spectroscopic analysis and chemical transformations, the structure of **14** was fully confirmed. No biological activities were reported for either of the two compounds.

Later in 1990, Mimaki et al. reported on the isolation and structural elucidation of three additional saponins **12, 13** and **15** and two steroidal glycoalkaloids **16** and **17** from *L. brownii* (synonym: *L. brownii var. colchesteri).*[22] The fresh lily bulbs (4.5 Kg) were extracted with hot methanol and similar to the previous study, the resulting bulb extract was dissolved in water and sequentially partitioned between chloroform and 1-butanol. In this report, the 1-butanol soluble fraction was subjected to repeated silica gel CC, GPC (Sephadex LH-20®), followed by Diaion HP-20® CC, and finally preparative reverse phase high performance liquid chromatography (RP-HPLC) to afford the pure compounds. The authors comment that the *L. brownii var. colchesteri* used in the study is of Chinese origin and is commercially available in Japan for medicinal use. Nevertheless, they only report on the isolation and structural elucidation of the compounds and do not report on biological activities.

In 1998, on the basis of extensive spectroscopic analysis (IR, FAB-MS, $^{1}$H NMR, $^{13}$C NMR, DEPT, HMQC, and HMBC) and physical chemical properties, Ho and Chen reported on the isolation and structural elucidation of two furostanols **18** and **19** from the bulbs of *L. brownii.*[77] The fresh lily bulbs (15 Kg) were extracted with ethanol and subjected to solvent partitioning and CC to afford the purified compounds. **18** and **19** both contained two carbonyl functions in the aglycone and only differed in the level of saturation between the C-5 and the C-6 position. **18** was Δ-5 between the C-5 and the C-6 position and **19** was 5-α-H.

In 2012, Hong and et al. reported on the isolation and structural elucidation of fifteen steroidal glycosides from dried bulbs of *L. brownii.*[78] Eight of the compounds have been previously reported in several lily species. Seven of the compounds identified, **20** – **26**, were new steroidal glycosides that were not previously reported. Dried lily bulbs (6 Kg) were pulverized and extracted with ethanol under reflux and subjected to column chromatography and preparative HPLC to afford the purified compounds. Unfortunately, no biological assays were performed on the newly reported new compounds.

Figure 16. Structures of steroidal glycosides from *L. brownii.*

***Lilium mackliniae* Sealy**

Figure 17. Image of *L. mackliniae* taken at the Garden Cottage Nursery in the Highlands, United Kingdom.

In 1991, Sashida et al. reported on the isolation and identification of **11** and **12** previously identified in *L. brownii,* from the bulbs of *L. mackliniae* (**Figure 18**) .[23] In addition, a solanidine-based steroidal glycoalkaloid, solanidine *O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside **27**, was isolated and characterized. Interestingly, this steroidal glycoalkaloid was isolated previously from two *Fritillaria* species, *F. thunbergii* and *F. camtschatcensis.*[79, 80] *L. mackliniae* is native to northeast India and Burma, and it has been previously included taxonomically in the genus *Nomocharis.* In this study, the fresh lily bulbs (1.75 Kg) were extracted with hot methanol and the resulting bulb extract was dissolved in water and sequentially partitioned between chloroform and 1-butanol. The 1-butanol soluble fraction was then subjected to repeated silica gel CC and GPC (Sephadex LH-20®) and finally preparative RP-HPLC to afford the pure compounds. The bulbs are reported to have a significantly bitter taste but unfortunately the organoleptic properties of the steroidal glycosides **12, 13,** and **27** were not quantified in this study.

Figure 18. Structure of steroidal glycoalkaloid isolated from *L. mackliniae.*

***Lilium speciosum* Thunb.**

Figure 19. Image of a *L. speciosum* specimen taken at the Kyoto Botanical Garden, Japan.

In 1991, Sashida et al. reported on the isolation and identification of **11** and **13** previously identified in *L. brownii,* and a C-26 methoxy spirostanol **28** from the fresh bulbs of *L. speciosum,* also commonly referred to as the Japanese lily, the showy Japanese lily, or the speciosum lily (**Figure 20**).[81] The fresh lily bulbs (900 g) were extracted with hot methanol and the resulting bulb extract was dissolved in water and sequentially partitioned between chloroform and 1-butanol. The 1-butanol soluble fraction was then subjected to repeated silica gel CC, Diaion HP-20® CC and GPC (Sephadex LH-20®) to yield the purified compounds. According to the authors, **28** is the first report of a C-26 methoxylated spirostanol isolated from a natural source. Due to the fact that **28** was extracted in boiling methanol, it raises the possibility that methoxylation of the C-26 position occurred during isolation and **28** is an artifact of the isolation process. Further studies need to be conducted to clarify this possibility. No biological activities were reported for the compounds.

Figure 20. Structure of spirostanol saponin isolated from *L. speciosum.*

***Lilium pensylvanicum* Ker Gawl. (synonym: *L. dauricum*)**

Figure 21. Image of a naturalized *L. pensylvanicum* specimen growing in a seaside woodland grove in Helsinki, Finland.

In 1992, Mimaki et al. reported on the isolation of **28**, previously identified in *L. speciosum,* and the structural elucidation of six new steroidal glycosides **29 - 34** from the bulbs of *L. pensylvanicum* (synonym: *L. dauricum*), as known as the candlestick lily (**Figure 22**).[2] The authors comment that *L. pensylvanicum* along with three other *Lilium* species, namely, *L. brownii, L. pumilum, and Lilium longiflorum,* were documented to be used medicinally in Japan and China and that *L. pensylvanicum* was chosen for the study as part of their continued contribution to the phytochemical screening of the *Lilium* genus. Fresh *L. pensylvanicum* bulbs (1 Kg) were extracted with hot methanol and the resulting bulb extract was concentrated under reduced pressure. The extract was partitioned between water and 1-butanol and the 1-butanol soluble fraction was then subjected to silica gel CC, Diaion HP-20® CC and then GPC (Sephadex LH-20®) CC. The resulting steroidal glycoside fraction was subjected to octadecyl-silica (ODS) CC and then finally preparative RP-HPLC to afford compounds **28 - 34**. The $^{1}$H NMR and $^{13}$C NMR spectra for the aglycone of **29** and **30**, were in good agreement with the aglycone of **28**, (25R,26R)-26-methoxyspirost-5-en-3β-ol. On the basis of $^{1}$H NMR, $^{13}$C NMR, and partial acid hydrolysis and analysis of the resulting products by TLC and HPLC, **29** and **30** shared the same aglycone and only differed in the composition of the carbohydrate moiety. Compound **29** possessed an α-L-rhamnopyranosyl-(1→2)-O-[α-L-arabinopyranosyl-(1→3)]-β-D-glucopyranose moiety and compound **30** possessed an α-L-rhamnopyranosyl-(1→2)-O β-D-glucopyranosyl-(1→4)]-β-D-glucopyranose moiety. Acid hydrolysis of **31** yielded D-glucose, L-rhamnose, L-arabinose and (25R)-spirost-5-en-3β-ol, diosgenin, and the $^{1}$H NMR and $^{13}$C NMR spectra for the carbohydrate moiety matched that of **29.** Accordingly, **31** was deduced to be (25R)-spirost-5-en-3β-ol α-L-rhamnopyranosyl-(1→2)-O-[α-L-arabinopyranosyl-(1→3)]-β-D-glucopyranose. Based on the spectroscopic analysis, compounds **32** and **33** both shared a diosgenin based aglycone that contained a carbonyl group and a tertiary hydroxyl group and differed only in the carbohydrate moiety. Compound **32** was determined to be a diglycoside, and **33** was a triglycoside. Compound **34** was shown to be a triglycoside of tenuifoliol and shared the same aglycone as **8** and **9**, tenuifolioside A and B. The absolute configurations (20R, 22R) of the of the C-20 and C-22 positions were confirmed by converting tenuifoliol, liberated from tenuifolioside A via enzymatic hydrolysis to the corresponding isopropylidene derivative, and performing a one dimensional nuclear Overhauser (1D-NOE) experiment. Although the chemical structures for the six new steroidal saponins were described in this study, unfortunately, no organoleptic or biological properties were reported for the compounds.

Figure 22. Structures of steroidal glycosides isolated from *L. pensylvanicum.*

***Lilium hansonii* Leichtlin ex D. D. T. Moore**

Figure 23. Image of a *L. hansonii* specimen growing in private garden near Rome, Italy.

In 1992, Ori et al. reported on the isolation and structural elucidation of three new saponins **35**, **36**, and **38** and the isolation and identification of the known saponin **37**, previously identified in subterranean parts of the Chinese medicinal plant *Ophiopogon planiscapus*, from the bulbs of *L. hansonii* (**Figure 24**).[82, 83] Also known as Hanson's lily or Japanese Turk's cap lily, *L. hansonii* is native to the island of Dagelet located off of the coast of Korea. The authors comment that *L. hansonii* is noted for its resistance to viral diseases that are problematic with other *Lilium* species. In addition to the chemical characterization of the saponins, both the naturally occurring compounds, and chemically or enzymatically generated derivatives, were assayed for inhibition of cyclic AMP phosphodiesterase.

In this study, fresh lily bulbs (3.5 Kg) were extracted with hot methanol, sequentially partitioned between chloroform and 1-butanol, and the 1-butanol fraction was then subjected to silica gel CC, GPC (Sephadex LH-20®), and finally preparative RP-HPLC. On the basis of $^{1}$H NMR, $^{13}$C NMR, acid and enzymatic hydrolysis and analysis of the resulting products, **37** and **38** were determined to be 22-hydroxyfurostanol derivatives sharing the same carbohydrate moiety, and only differed in the level of saturation between the C-5 and the C-6 position. **37** was Δ-5 between the C-5 and the C-6 position and **38** was 5-α-H. In addition to **37** and **38**, two tigogenin based spirostanols **35** and **36** were also isolated and purified from the bulbs and the structures elucidated. On the basis of the $^{1}$H NMR and $^{13}$C NMR spectra, the aglycone of **35** and **36** were tigogenin derivatives both containing an axial 12-α-hydroxyl group. Similar to **37** and **38**, compounds **35** and **36** shared the same carbohydrate moiety and only differed in the level of saturation between the C-5 and the C-6 position. **35** was Δ-5 between the C-5 and the C-6 position and **36** was 5-α-H.

All four of the compounds **35 - 38** showed inhibition of cyclic AMP phosphodiesterase activity. The inhibitory activity for **35, 36, 37,** and **38**, were 103, 48.4, 177, and 34.5 $IC_{50}$ (x $10^{-5}$M), respectively. Interestingly, when the corresponding spirostanol of **35** was enzymatically generated and assayed, the

inhibitory activity increased approximately 150-fold from 103 to 0.7 $IC_{50}$ (x $10^{-5}$M). In contrast, when the rhamnose moiety of **9** was cleaved, inhibition activity of the resulting diglycoside decreased to > 500 x $10^{-5}$M. This study was the first report of the activity of steroidal saponins and analogs isolated from the *Lilium* genus on the inhibition of cyclic AMP phosphodiesterase.

Figure 24. Structures of steroidal glycosides isolated from *L. hansonii.*

***Lilium henryi* Baker**

Figure 25. Image of a *L. henryi* specimen growing in private garden near Rome, Italy.

In 1993, Mimaki et al. reported on the isolation and identification of **11** and **12** from the bulbs of *L. henryi,* commonly known as Henry's lily.[84] Compound **11** was previously isolated from *L .brownii, L. speciosum,* and *L. mackliniae,* and isolated from *L. regale* in the same study. Compound **12** was previously isolated from *L .brownii,* and *L. mackliniae.*

Compounds **11** and **12** and their derivatives, along with several other compounds isolated from *L. regale*, were assayed for inhibition of cyclic AMP phosphodiesterase activity in this study. The inhibitory activity for **11** and **12**, was 2.9 and 3.1 $IC_{50}$ (x $10^{-5}$M), respectively. The acid catalyzed cleavage and methyl esterification of the 3-hydroxy-3-methylglutarate group in both **11** and **12** reduced the inhibition of cyclic AMP phosphodiesterase activity, suggesting that this moiety plays a role in the activity.

***Lilium regale* E. H. Wilson**

Figure 26. Image of a *L. regale* specimen growing in the garden of a winery on the Naramata Bench, Okanagan, British Columbia, Canada.

In 1993, Mimaki et al. reported on the isolation and identification of three known saponins, **11, 39,** and **40** and two new saponins **41** and **42** from the bulbs of *L. regale,* commonly known as the regal lily or the royal lily (**Figure 27**).[84] Similar to *L. hansonii* and *L. henryii,* the authors note that the *L. regale* species exhibits an increased resistance to viral infections and that the resistance may be due to the phytochemical composition of the plant. Compound **11** was previously isolated from *L .brownii, L. speciosum,* and *L. mackliniae.* Compound **39**, gracillin, and **40**, trigofolenoside D, were previously isolated from *Costus speciosus* and *Trigonella foenum-graecum*, respectively. Compound **41** was an isomer of compound **12** only differing in the interglycosidic linkage of the terminal glucose. In **41**, the terminal glucose is linked via the C-3′ carbon of the inner glucose, whereas in **12,** the terminal glucose is linked via the C-4′ carbon of the inner glucose. Compound **42** was determined through a series of 2D NMR experiments to be a tetraglycoside with two hydroxyl moieties linked to the C17 and C27 carbons of the aglycone.

All five of the compounds **11**, **39 – 42** and their derivatives were assayed for inhibition of cyclic AMP phosphodiesterase activity. The inhibitory activity for **41**, **11**, and **39**, was 2.2, 2.9, and 6.1 $IC_{50}$ (x $10^{-5}$M), respectively. The furostanol saponin **40** and the spirostanol tetraglycoside **42** showed no inhibitory activity. Consistent with **12**, isolated from *L. henryii,* the acid catalyzed cleavage and methyl esterification of the 3-hydroxy-3-methylglutarate group in both **11** and **41** reduced the inhibition activity of the compounds.

In 1996, Gur'eva et al. reported on the isolation and identification of the previously known steroidal saponins **39, 43 – 47**,[82, 85-89] designated as lilioglycosides A, D, G, K, N, and R, respectively, from the bulbs of *L. regale* (**Figure 27**).[90] The authors noted that fifteen steroidal glycosides were separated by TLC and visualized using Sannié reagent, but only six were physically isolated by repeated CC. The three spirostanols **39**, **43** and **44** were identified by comparison of the $^{13}$C NMR spectra with that of the literature.[85-87] The three furostanols **45 - 47** were identified by stepwise acid hydrolysis and by methylation followed by the methanolysis of the permethylates. The structures were confirmed by gas-liquid chromatography (GLC) of the products along with the comparison of the IR spectra, UV spectra, melting points, and specific rotations with that of the literature. Although known compounds, this was the first report of these compounds from *L. regale*.

In 1997, Kintya et al. reported on the isolation and identification of two known saponins **11** and **13** designated as lilioglycosides E and F and four new saponins **48 - 51**, designated as lilioglycosides B, H, C, and I, respectively, from the bulbs of *L. regale* (**Figure 27**) .[91] The lily bulbs used in the study were collected in the territory of the Republic of Moldova. Compound **48** was determined to be a β-D-glucopyranoside of 27-hydroxydiosgenin, narthogenin, and had a (S) configuration at the C-25 position. Alkaline hydrolysis of **49** yielded 3-hydroxy-methylglutaric acid and narthogenin monoglucoside based on comparison by TLC with authentic standards. MS, IR, $^{1}$H NMR, and $^{13}$C NMR experiments identified the structure of **49** as (25R)-spirost-5-ene-3β,27-diol 3-O-β-D-glucopyranoside 27-(3-hydroxy-3-methylglutarate). Compound **50** was determined to be the triglycoside of **48**, and **51** was determined to be (25S)-spirost-5-ene-3β,27-diol 3-O-[O-a-L-rhamnopyranosyl-(1→2) ,[O-β-D-glucopyranosyl -(1→3)]-O-β-D-glucopyranoside 27-[-(S)-3-hydroxy-3-methylglutarate].

Figure 27. Structures of steroidal glycosides isolated from *L. regale.*

***Lilium speciosum x Lilium nobilissimum* (Makino) Makino**

Figure 28. Image of a *L. speciosum x L. nobilissimum* hybrid, cultivar 'Stargazer,' growing in a private garden in the United States.

In 1994, Nakamura et al. reported on the identification of four steroidal saponins from bulbs of the hybrid lily, *L. speciosum x L. nobilissimum* 'Stargazer', that were earlier isolated and characterized from several other *Lilium* species.[92] Compound **11**, previously identified from the bulbs of *L. regale, L. speciosum*, *L .brownii* and *L. mackliniae*, **12** previously identified from *L. regale, L. speciosum*, and *L. mackliniae,* **28** previously identified from *L. speciosum* and *L. pensylvanicum* and **30** previously identified from *L. pensylvanicum,* were isolated and identified from bulbs of the *L. speciosum x L. nobilissimum* for the first time. In addition to the known compounds, a new acetylated saponin **52** was isolated, purified, and the structure was elucidated (**Figure 29**).

*Lilium* species are readily capable of producing fertile interspecific hybrids with other members of the genus.[93, 94]

Cytological analysis of forty-six *Lilium* species revealed very little variation in the karyotypes within the genus; thus, interspecific hybridization is widely employed for the development of new ornamental *Lilium* cultivars.[95] This is the first report of the isolation of a new saponin from an interspecific *Lilium* hybrid. It is unclear whether the presence of the **52** was the result of the interspecific hybridization, or if the compound is present in the parental species, *L. speciosum* or *L. nobilissimum.* Novel steroidal glycosides have been reported from interspecific hybrids of solanaceous plants.[96] For example, a novel steroidal glycoside, not present in either parental species, was identified in the sexual hybrids of *S. acaule* and *Solanum* x *ajanhuiri.*[97] Similar to solanaceous plants, interspecific hybridization in members of the Liliaceae family may result in novel steroidal glycosides. This opens the possibility of a novel means to generate biologically active steroidal glycosides, increasing the potential chemical diversity available from within the genus. Surprisingly, of the over 13,000 lily cultivars registered in the fourth edition of *The International Lily Register* (*ILR*), the chemistry of 'Stargazer' is the only cultivar that has been investigated to date.[98]

In this study, lily bulbs were cultivated from bulbs purchased from Shunkouen Japan. Freshly harvested bulbs (5.0 Kg) were extracted using the standard procedure of the group. The bulbs were cut into pieces and extracted with hot methanol and the resulting extract was concentrated under vacuum and then partitioned between water and 1-butanol. The 1-butanol fraction was then subjected repeated to silica gel CC, Diaion HP-20 CC and ODS CC until pure compounds were obtained. The known compounds were identified by spectroscopic data and direct comparison by TLC to authentic standards. The new compound **52** was characterized by FAB-MS, IR, $^{1}$H NMR, $^{13}$C NMR, and chemical transformations. The acetyl group was deduced from a peak indicative of a carbonyl group in the IR spectrum (1724 cm$^{-1}$), $^{1}$H NMR (δ2.06, 3H s) and $^{13}$C NMR (δ170.9 and 20.8). The acetyl group was further substantiated by the comparison of both $^{1}$H NMR and $^{13}$C NMR spectra of the alkaline hydrolysis product of **52** with that of **30**.

All five of the saponins and their corresponding hydrolyzed and esterified derivatives were assayed for their inhibitory activity on 12-O-tetradecanoylphorobol-13-acetate (TPA) stimulated $^{32}$P-incorporation into phospholipids of HeLa cells. This *in vitro* assay has been shown to be in good agreement with anti-tumor activity *in vivo*. Of the naturally occurring compounds, only **52** had slight inhibitory activity (1.4% inhibition at a concentration of 5 μg ml$^{-1}$). The methyl ester derivative of **12** showed the most potent inhibitory activity of $^{32}$P-incorporation (40.3 % inhibition at 5 μg ml$^{-1}$), which is 10 times more potent than the steroidal sapogenin laxogenin, isolated from *Allium bakeri* tubers. The authors comment that this study is the first report of the anti-tumor activity of steroidal saponins.

Figure 29. Structure of spirostanol saponin isolated from hybrid lily, *L. speciosum* x *Lilium nobilissimum* 'Star Gazer.'

***Lilium longiflorum Thunb.***

Figure 30. Image of a commercial *L. longiflorum* specimen taken in New Jersey, United Sates.

In 1994, Mimaki et al. reported on the identification of five previously identified saponins **12**, **30**, **31**, **53**, and **58**, and the isolation and structural elucidation of two new spirostanol saponins **56** and **57** and two new furostanol saponins **54** and **55** from the bulbs of *L. longiflorum* (**Figure 31**).[99] *L. longiflorum,* commonly known as the Easter lily, the Bermuda lily, and the white trumpet lily, is native to the Ryukyu archipelago of Japan and the islands of eastern Taiwan.[93, 100] *L. longiflorum* has been listed along with *L. brownii* and *L. pumilum* as a traditional medicine in China.[101] Compound **12** was previously identified by the same group in *L. brownii,*[22] *L. mackliniae,* [23] and *L. henryi.*[84] Compound **30** and **31** was previously isolated from *L. pensylvanicum*[2] and **53** was previously identified in *Ophiopogon planiscapus.*[82] According to the authors, compound **58** was previously identified in *L. brownii.* Compound **58,** isolated from *L. longiflorum,* shares the same structure as the acid hydrolysis product of **12** isolated from *L. brownii.* It is unclear if this compound was identified as a natural product in *L. brownii* or solely as the acid catalyzed derivative of **12.**

In this study, fresh lily bulbs (5.0 Kg) were purchased from a local market in Heiwaen, Japan, and following the typical procedure; the bulbs were extracted with hot methanol and partitioned between water and 1-butanol. The 1-butanol fraction was then subjected to silica gel CC, Diaion HP-20 CC and then a second purification by silica gel CC. The final purification was performed by preparative RP-HPLC.

All nine compounds **12, 30, 31, 53 – 58**, and their derivatives were assayed for their inhibitory activity on 12-O-tetradecanoylphorobol-13-acetate (TPA) stimulated $^{32}$P-incorporation into phospholipids of HeLa cells. The spirostanol saponin **31** and the corresponding spirostanol saponins derived by enzymatic hydrolysis of the C-26 glucose from the furostanols **53** and **55** were cytotoxic towards the HeLa cells at 50 μg ml$^{-1.}$ The spirostanol derived from the corresponding furostanol **53** showed 25.2% inhibitions at 5 μg ml$^{-1}$. Compounds **56** – **58** showed 18.4, 1.5, and 12.6 inhibitory activities at 50 μg ml$^{-1}$ concentration, respectively, with no activity at the lower concentration of 5 μg ml$^{-1}$. None of the furostanol saponins assayed had inhibitory activity.

In 2010, Munafo et al. reported on the identification of several steroidal glycosides from the bulbs of *L. longiflorum* (**Figure 31**).[102, 103] The steroidal glycoalkaloid **17**, previously identified from the bulbs of *L. brownii* and the furostanol saponin **37** previously identified in the bulbs of *L. hansonii*[83] were isolated from the bulbs of *L. longiflorum* for the first time. In addition, a new acetylated steroidal glycoalkaloid **59** and two new furostanol saponins **60** and **61** were isolated and purified from *L. longiflorum* bulbs and the structures were elucidated by a combination of spectroscopic data ($^{1}$H NMR, $^{13}$C NMR, HMBC, HMQC, MS, IR), chromatographic data, and chemical analysis.

In this study, the isolation procedure was aimed at minimizing the formation of artifacts during the isolation process. Bulbs grown from tissue culture were used to avoid microbial modification, fresh lily bulbs were lyophilized prior to extraction, and methanol and elevated temperatures were avoided during the extraction procedure. The extraction consisted of grinding lyophilized lily bulbs (100 g) into a fine powder and washing the bulb powder with pentanes to remove lipids. The residual defatted material was then extracted with a mixture of ethanol and water (7:3, v/v) and the supernatant was collected and the residue discarded. The supernatant was then evaporated under reduced pressure and lyophilized. The lyophilized crude bulb extract was then dissolved in deionized water and washed with ethyl acetate and then extracted with 1-butanol. The 1-butanol

extract was then subjected to GPC (Sephadex LH-20®) and repeated preparative RP-HPLC to afford the pure compounds.

In 2011, a liquid chromatography – tandem mass spectrometry (LC-MS/MS) method performed in multiple reaction monitoring (MRM) mode was used for the quantitative analysis of the two steroidal glycoalkaloids **17** and **59** and the three furostanol saponins **37**, **60**, and **61**, in the different organs of *L. longiflorum*.[44] This was the first report describing the distribution of steroidal glycosides within the different organs of a *Lilium* species. Additionally, histological staining of bulb scales was performed to visualize the distribution of furostanols in these tissues. The results revealed differential furostanol accumulation in the basal plate, bulb scale epidermal cells, and vascular bundles, with little or no staining in the mesophyll of the bulb scale.

In another study in 2011, the five steroidal glycosides, **17**, **59**, **37**, **60**, and **61**, isolated and purified from *L. longiflorum* bulbs were evaluated for fungal growth inhibition activity against the plant pathogenic fungus, *Botrytis cinerea,* using an *in vitro* plate assay.[103] This was the first systematic investigation of the antifungal activity of steroidal glycosides isolated from a *Lilium* species. All of the compounds assayed showed fungal growth inhibition activity; however, the natural acetylation of C-6‴ of the terminal glucose in the steroidal alkaloid **59** increased antifungal activity by inhibiting the rate of metabolism of the compound by the *B. cinerea*. Acetylation of the glycoalkaloid may be a plant defense response to the evolution of detoxifying mechanisms by the pathogen. The biotransformation of the steroidal glycoalkaloids by *B. cinerea* led to the isolation and characterization of several fungal metabolites including the steroidal glycoalkaloid **16**, previously identified as a natural product from the bulbs of *L. brownii.* **16** were subsequently identified as not only a fungal metabolite of **17** and **59**, but also as a natural product constitutively present in *L. longiflorum* bulbs for the first time.

Figure 31. Structures of steroidal glycosides isolated from *L. longiflorum*

***Lilium martagon L.***

Figure 32. Image of a *L. martagon* specimen growing on the northern side of "Eggerkreuz" mountain in Ötscherland, Austria.

In 1996, Satou et al. reported on the isolation and characterization of two new saponins **62** and **63** from the bulbs of *L. martagon,* also referred to as martagon lily or the turban lily (**Figure 33**).[104] *L. martagon* is widely distributed geographically in Europe and Russia and is adaptable to many growing conditions including both sun and shade and various soil types. *L. martagon* is listed as a plant that has been used to treat cancer.[105] The author comments that although a few natural products have been isolated from this species, there has not been a systematic phytochemical investigation of the species, thus warranting the present study.

The bulbs used for this study were obtained from the Hokkaido Experiment Station of Medicinal Plants in Japan. Fresh lily bulbs (3.5 Kg) were extracted with hot methanol and partitioned between water and 1-butanol. The 1-butanol fraction was then subjected to repeated silica gel CC, Diaion HP-20 CC, silica gel CC, ODS CC and final purification was performed by preparative RP-HPLC and characterized by a combination of 1D and 2D NMR techniques and chemical transformations. The authors note that *L. martagon* readily hybridizes with *L. hansonii* to produce an ornamental hybrid garden lily, and that both *L. martagon* and *L .hansonii* contain the same spirost-5-ene derivatives and the corresponding α5-spirostan derivatives. They speculate that there is a good correlation between the secondary metabolites present in both species and their cross-compatibility; however, there is no data supporting their hypothesis. No biological activities of the compounds were reported in this study.

Figure 33. Structure of spirostanol saponin isolated from *L. martagon.*

***Lilium candidum L.***

Figure 34. Image of a *L. candidum* specimen growing in a private garden in Suceava County, Romania.

*L. candidum,* commonly known as the 'Madonna Lily,' has a long history of medicinal uses most notably for the external application for the treatment of burns and swellings.[106] In addition, *L. candidum* is listed as a treatment for cancer and inflammation. Due to its rich history as a medicinal plant, *L. candidum* has been the subject of several phytochemical investigations, resulting in the isolation and characterization of new several natural products. In 1998, Mimaki and co-workers reported on the identification of 10 steroidal saponins from bulbs of the *L. candidum.* Compound **13**, previously identified from the bulbs of *L. regale, L. longiflorum, L. hansonii, L. speciosum,* and *L. speciosum x L. nobilissimum*, **28** previously identified from *L. pensylvanicum, L. speciosum*, and *L. speciosum x L. nobilissimum,* **30** previously identified from *L. pensylvanicum* and *L. speciosum x L. nobilissimum,* **44** previously identified from *L. regale,* **53** previously identified from *L. longiflorum* and in the leaves of *Sabal causiarum* (O.F.COOK) BECC,[99, 107] and **68** previously identified from the seeds of *Costus speciosus*[108] were isolated and identified from bulbs of *L. candidum* for the first time.[106] In addition to the known compounds, four new saponins **64 – 67** were isolated and characterized (**Figure 35)**. The bulbs used for this study were obtained from the Hokkaido Experiment Station of Medicinal Plants in Japan. Fresh lily bulbs (2.0 Kg) were extracted with hot methanol and partitioned between water and 1-butanol. The 1-butanol-soluble portion was subjected to successive chromatographic separations by silica gel CC and ODS CC to afford the target compounds.

The inhibitory activity of the isolated steroidal glycosides on $Na^+/K^+$ ATPase was evaluated. Compounds **30**, **44**, and **28** were found to inhibit ouabain sensitive $Na^+/K^+$ ATPase with $IC_{50}$ values of 2.3 x $10^{-5}$ M, 1.4 x $10^{-5}$ M and 1.8 x $10^{-5}$ M, respectively. The remaining compounds were inactive, suggesting that the introduction of the hydroxyl group in the aglycone moiety resulted in the reduced inhibitory activity. The authors note that the results warrant the screening of the compounds for leukemia cell differentiation associated with $Na^+/K^+$ ATPase inhibition.

In 1998, Haladová et al reported on the isolation and identification of two known steroidal glycosides **30** and **58** from the bulbs of *L. candidum*.[109] These compounds were previously isolated from the bulbs of *L. pensylvanicum* and *L. longiflorum*, respectively. In 1999, Haladová and co-workers reported on the isolation and structural elucidation of a new steroidal glycoside

**69** together with the known steroidal glycoside **30** from the petals of *L. candidum* flowers. [110] There were no biological assays reported in either of the two studies.

In another study in 1999, Mimaki and co-workers reported on the characterization of six new steroidal glycosides including five new spirostanol saponins **70 – 74** and a new furostanol saponin **75**, from the bulbs of *L. candidum* (**Figure 35**).[111] The inhibitory activity of the new steroidal glycosides **70 – 75** on $Na^+/K^+$ ATPase was evaluated. Of all six compounds evaluated, only **70** and **73** showed inhibitory activity on $Na^+/K^+$ ATPase with $IC_{50}$ values of 2.2 x $10^{-5}$ M and 4.7 x $10^{-5}$ M, respectively. Consistent with the previous study, the presence of the hydroxyl group in the aglycone moiety of **71**, **72**, **74**, and **75** resulted in reduced inhibitory activity.

In a study conducted in 2000, Eisenreichova et al reported on the isolation and structural elucidation of a new steroidal glycoside **76** isolated from the fresh bulbs of *L. candidum.* In this study, fresh lily bulbs (1.7 Kg) were extracted with ethanol at room temperature. The extract was concentrated under reduced pressure and then partitioned between 1-butanol and water. The resulting organic fraction was successively chromatographed over silica gel to afford the purified compound.[112]

Figure 35. Structures of steroidal glycosides isolated from *L. candidum.*

## 11. Concluding remarks

Between the years of 1989 to 2014, over 75 steroidal glycosides have been isolated and characterized from members of the *Lilium* genus. Many of these steroidal glycosides have been reported to have biological activities. To date, phytochemical investigations focused on steroidal glycosides have only been conducted on 13 *Lilium* species, namely, *L. pardalinum, L. pumilum, L. philippinense, L. brownii, L. mackliniae, L. speciosum, L. pensylvanicum, L. hansonii, L. henryi, L. regale, L. longiflorum, L. martagon,* and *L. candidum,* despite the fact that over 30 species have been documented to have historical ethnobotanical uses. The genus contains 110 accepted species of which the chemistry and biological activity of the steroidal glycosides from the majority of the species have not been investigated thus far. Of the 13,000 registered lily cultivars, *L. speciosum x L. nobilissimum* cultivar 'Stargazer,' is the only cultivar of which the steroidal glycosides have been investigated. Hopefully this manuscript will inspire future plant biologists and natural product chemists to revisit the chemistry of the species that have already been studied and to conduct new phytochemical and biological investigations on the natural products chemistry of the steroidal glycosides contained within the *Lilium* genus.

## 12. Acknowledgements

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## References

1. *USDA, ARS, National Genetic Resources Program. Germplasm Resources Information Network - (GRIN) [Online Database]. National Germplasm Resources Laboratory, Beltsville, Maryland. URL: http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl?22119 (19 August 2012).*
2. Y. Mimaki, N. Ishibashi, K. Ori and Y. Sashida, *Phytochemistry*, 1992, **31**, 1753-1758.
3. Y. Mimaki and Y. Sashida, *Phytochemistry*, 1990, **29**, 2267-2271.
4. J. Z. Wu, C. Morizane, A. Iida, S. Ueda, Z. L. Zhou, M. Xu, M. Zhang, R. M. Li and T. Fujita, *Chemical and pharmaceutical bulletin*, 1995, **43**, 1448-1453.
5. J. Kitajima, T. Komori and T. Kawasaki, *Chem Pharm Bull*, 1982, **30**, 3912-3921.
6. E. Fattorusso, M. Iorizzi, V. Lanzotti and O. Taglialatela-Scafati, *Journal of agricultural and food chemistry*, 2002, **50**, 5686-5690.
7. J. A. Francis, W. Rumbeiha and M. G. Nair, *Life sciences*, 2004, **76**, 671-683.
8. K. Takeda, J. B. Harborne and R. Self, *Phytochemistry*, 1986, **25**, 2191-2192.
9. R. Nørbæk and T. Kondo, *Phytochemistry*, 1999, **50**, 1181-1184.
10. C. X. Zhou, J. Tanaka, C. H. Cheng, T. Higa and R. X. Tan, *Planta medica*, 1999, **65**, 480-482.
11. H. Shimomura, Y. Sashida and Y. Mimaki, *Phytochemistry*, 1986, **25**, 2897-2899.
12. Y. Shoyama, K. Hatano, I. Nishioka and T. Yamagishi, *Phytochemistry*, 1987, **26**, 2965-2968.
13. J. B. Park, *Journal of agricultural and food chemistry*, 2009, **57**, 8868-8872.
14. K. Tsukida and K. Ikeuchi, *VIII. Pollen carotenoids of Lilium longiflorum and of its cultivated hybrid, Bitamin*, 1965, **32**, 222-226.
15. T. Itoh, T. Tamura, T. Mitsuhashi and T. Matsumoto, *Phytochemistry*, 1977, **16**, 140-141.
16. H. Shimomura, Y. Sashida, Y. Mimaki and Y. Minegishi, *Phytochemistry*, 1987, **26**, 582-583.
17. V. Lanzotti, *Journal of chromatography. A*, 2006, **1112**, 3-22.
18. H. J. Li, Y. Jiang and P. Li, *Natural product reports*, 2006, **23**, 735-752.
19. J. Harmatha, B. Mauchamp, C. Arnault and K. Sláma, *Biochemical systematics and ecology*, 1987, **15**, 113-116.
20. H. Matsuura, *The Journal of nutrition*, 2001, **131**, 1000S-1005S.
21. C. X. Yang, S. S. Huang, X. P. Yang and Z. J. Jia, *Planta medica*, 2004, **70**, 446-451.
22. Y. Mimaki and Y. Sashida, *Chemical & pharmaceutical bulletin*, 1990, **38**, 3055-3059.
23. Y. Sashida, K. Ori and Y. Mimaki, *Chemical & pharmaceutical bulletin*, 1991, **39**, 2362-2368.
24. M. Sautour, T. Miyamoto and M. A. Lacaille-Dubois, *Journal of natural products*, 2005, **68**, 1489-1493.
25. C.-X. Zhou, J.-Y. Liu, W.-C. Ye, C.-H. Liu and R.-X. Tan, *Tetrahedron*, 2003, **59**, 5743-5747.

 

26. J. Zhang, Z. Meng, M. Zhang, D. Ma, S. Xu and H. Kodama, *Clinica chimica acta; international journal of clinical chemistry*, 1999, **289**, 79-88.
27. H. C. Huang, W. J. Tsai, S. L. Morris-Natschke, H. Tokuda, K. H. Lee, Y. C. Wu and Y. H. Kuo, *Journal of natural products*, 2006, **69**, 763-767.
28. A. H. Gilani, F. Shaheen, A. Christopoulos and F. Mitchelson, *Life sciences*, 1997, **60**, 535-544.
29. N. Nakashima, I. Kimura, M. Kimura and H. Matsuura, *Journal of natural products*, 1993, **56**, 345-350.
30. H. Oh, D. G. Kang, S. Lee, Y. Lee and H. S. Lee, *Planta medica*, 2003, **69**, 564-565.
31. B. Shao, H. Guo, Y. Cui, M. Ye, J. Han and D. Guo, *Phytochemistry*, 2007, **68**, 623-630.
32. B. Gosse, J. Gnabre, R. B. Bates, C. W. Dicus, P. Nakkiew and R. C. Huang, *Journal of natural products*, 2002, **65**, 1942-1944.
33. D. Acharya, A. C. Mitaine-Offer, N. Kaushik, T. Miyamoto, T. Paululat, J. F. Mirjolet, O. Duchamp and M. A. Lacaille-Dubois, *Journal of natural products*, 2009, **72**, 177-181.
34. G. R. Pettit, Q. Zhang, V. Pinilla, H. Hoffmann, J. C. Knight, D. L. Doubek, J. C. Chapuis, R. K. Pettit and J. M. Schmidt, *Journal of natural products*, 2005, **68**, 729-733.
35. Y. Mimaki, A. Yokosuka, M. Kuroda and Y. Sashida, *Biological & pharmaceutical bulletin*, 2001, **24**, 1286-1289.
36. Y. Jiang, H. Li, P. Li, Z. Cai and W. Ye, *Journal of natural products*, 2005, **68**, 264-267.
37. T. Yamanaka, J. P. Vincken, P. de Waard, M. Sanders, N. Takada and H. Gruppen, *Journal of agricultural and food chemistry*, 2008, **56**, 11432-11440.
38. B. Singh, T. K. Bhat and B. Singh, *Journal of agricultural and food chemistry*, 2003, **51**, 5579-5597.
39. Z. I. Rajput, S. H. Hu, C. W. Xiao and A. G. Arijo, *Journal of Zhejiang University. Science. B*, 2007, **8**, 153-161.
40. J. R. Hanson, *Natural product reports*, 2010, **27**, 887-899.
41. O. Guclu-Ustundag and G. Mazza, *Critical reviews in food science and nutrition*, 2007, **47**, 231-258.
42. E. Ghisalberti, *Nat. Prod. Commun.*, 2006, **10**, 859-884.
43. H. Wu, S. Zheng, Y. Bi and Y. Zhu, *Acta Horticulturae*, 2010, 295-298
44. J. P. Munafo, Jr. and T. J. Gianfagna, *Journal of agricultural and food chemistry*, 2011, **59**, 995-1004.
45. I. Abe, M. Rohmer and G. D. Prestwich, *Chemical Reviews*, 1993, **93**, 2189-2206.
46. K. Haralampidis, M. Trojanowska and A. E. Osbourn, *Advances in biochemical engineering/biotechnology*, 2002, **75**, 31-49.
47. T. Kuljanabhagavad, P. Thongphasuk, W. Chamulitrat and M. Wink, *Phytochemistry*, 2008, **69**, 1919-1926.
48. O. Espejo, J. C. Llavot, H. Jung and F. Giral, *Phytochemistry*, 1982, **21**, 413-416.
49. Y. Mimaki, Y. Sashida and H. Shimomura, *Phytochemistry*, 1989, **28**, 3453-3458.
50. H. Shimomura, Y. Sashida and Y. Mimaki, *Phytochemistry*, 1989, **28**, 3163-3170.
51. E. A. Ferro, N. L. Alvarenga, D. A. Ibarrola, M. C. Hellion-Ibarrola and A. G. Ravelo, *Fitoterapia*, 2005, **76**, 577-579.
52. A. Braca, J. M. Prieto, N. De Tommasi, F. Tome and I. Morelli, *Phytochemistry*, 2004, **65**, 2921-2928.
53. H. Achenbach, H. Hubner, W. Brandt and M. Reiter, *Phytochemistry*, 1994, **35**, 1527-1543.
54. T. Murakami, A. Kishi, H. Matsuda and M. Yoshikawa, *Chemical & pharmaceutical bulletin*, 2000, **48**, 994-1000.
55. A. E. Osbourn, *Phytochemistry*, 2003, **62**, 1-4.
56. A. Debella, E. Haslinger, O. Kunert, G. Michl and D. Abebe, *Phytochemistry*, 1999, **51**, 1069-1075.
57. M. Haraguchi, A. P. Zaccarias Dos Santos, M. Claudia Marx Young and E. Paulo Chui, *Phytochemistry*, 1994, **36**, 1005-1008.
58. M. Sautour, A.-C. Mitaine-Offer and M.-A. Lacaille-Dubois, *J Nat Med*, 2007, **61**, 91-101.
59. R. C. Lin, B. Hanquet and M. A. Lacaille-Dubois, *Phytochemistry*, 1996, **43**, 665-668.
60. A.-u. Rahman and M. I. Choudhary, in *The Alkaloids: Chemistry and Biology*, ed. A. C. Geoffrey, Academic Press, 1998, vol. Volume 50, pp. 61-108.
61. M. Lacaille-Dubois, *La Phytothe 'rapie Europe' enne*, 2002, **7**, 9-16.
62. J. W. Daly, T. F. Spande and H. M. Garraffo, *Journal of natural products*, 2005, **68**, 1556-1575.
63. F. Noor-e-Ain, University of Karachi, Karachi, 1996.
64. C.-N. Lin, M.-I. Chung and S.-Y. Lin, *Phytochemistry*, 1986, **26**, 305-307.
65. I. Erdogan, T. z, B. ener and R. Atta ur, *Biochemical systematics and ecology*, 2001, **29**, 535-536.
66. , United States Department of Agriculture - Agricultural Research Service 2011.
67. T. Garth, *Anthropological Records* 1953, **14**, 138.
68. S. Schenck and E. Gifford, *Anthropological Records*, 1952, **13**, 381.
69. E. Sapir and L. Spier, *Anthropological Records*, 1943, **3**, 251.
70. M. McGuffin, J. Kartesz, A. Leung and A. Tucker, *Herbs of commerce, ed. 2*, 2 edn., American Herbal Products Association, Silver Springs, MD, 2000.
71. Z. Wu and P. Raven, *Liliaceae*, Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis, 2000.
72. E. Espeso and B. Guevara, *Acta Manilana*, 1990, **38**, 83-87.
73. J. Uphof, *Dictionary of economic plants 2nd Ed.* , 2 edn., Verlag Von J. Cramer, Stuttgart, Germany, 1968.
74. L. Shih-chen, *Chinese medinal herbs.* , Georgetown Press San Francisco, 1973.
75. Anonomous, *A barefoot doctor's manual. DHEW Publication No. (NIH): 75-695.*, , 1974.
76. NAS, *Lost Crops of the Incas: Little-Known Plants of the Andes with Promise for Worldwide Cultivation.*, Ad Hoc Panel of the Advisory Committee on Technology Innovation, Board on Science and Technology for International Development, National Research Council, National Academies of Science. , 1989.
77. X. Hou and F. Chen, *Yao xue xue bao = Acta pharmaceutica Sinica*, 1998, **33**, 923-926.
78. X.-X. Hong, J.-G. Luo, C. Guo and L.-Y. Kong, *Carbohydrate research*, 2012, **361**, 19-26.
79. Y. Mimaki and Y. Sashida, *Chem Pharm Bull*, 1990, **38**, 1090-1095.
80. J. Kitajima, T. Komori, T. Kawasaki and H. Schulten, *Phytochemistry*, 1982, **21**, 187-192.

81. Y. Mimaki and Y. Sashida, *Phytochemistry*, 1991, **30**, 937-940.
82. Y. Watanabe, S. Sanada, Y. Ida and J. Shoji, *Chem Pharm Bull*, 1983, **31**, 3486-3495.
83. K. Ori, Y. Mimaki, K. Mito, Y. Sashida, T. Nikaido, T. Ohmoto and A. Masuko, *Phytochemistry*, 1992, **31**, 2767-2775.
84. Y. Mimaki, Y. Sashida, O. Nakamura, T. Nikaido and T. Ohmoto, *Phytochemistry*, 1993, **33**, 675-682.
85. R. Marker and J. Krueger, *Journal of the American Chemical Society*, 1940, **62**, 3349-3350.
86. Y. Watanabe, S. Sanada, A. Tada and J. Shoji, *Chem Pharm Bull*, 1977, **25**, 3049-3055.
87. T. Tsukamoto and T. Kawasaki, *Pharm Bull*, 1956, **4**, 104-108.
88. P. Kintya, N. Mashenko, N. Kononova and G. Lazur'evskii, *Khim. Prir. Soedin*, 1976, 267.
89. T. Kawasaki, T. Komori, K. Miyahara, T. Nohara, I. Hosokawa and K. Mihashi, *Chem Pharm Bull*, 1974, **22**, 2164-2175.
90. A. S. Gur'eva, P. K. Kintya, N. E. Mashchenko and A. S. Shashkov, *Chemistry of Natural Compounds*, 1996, **32**, 384-385.
91. P. Kintya, A. Gur'eva, N. Mashchenko and A. Shashkov, *Chemistry of Natural Compounds*, 1997, **33**, 658-662.
92. O. Nakamura, Y. Mimaki, H. Nishino and Y. Sashida, *Phytochemistry*, 1994, **36**, 463-467.
93. M. Hiramatsu, K. Ii, H. Okubo, K. L. Huang and C. W. Huang, *American journal of botany*, 2001, **88**, 1230-1239.
94. I. Preston, *Roy. Hort. Soc. Lily Year-Book*, 1933, 173-190.
95. R. N. Stewart, *American journal of botany*, 1947, **34**, 9-26.
96. V. Grassert and H. Lellbach, *Biochem. Biophys. Pflanzen*, 1987, **182**, 473-479.
97. S. F. Osman, T. A. Johns and K. R. Price, *Phytochemistry*, 1986, **25**, 967-968.
98. A. C. Leslie and R. H. Society, *International Lily Register and Checklist (1982)*, Royal Horticultural Society, 2002.
99. Y. Mimaki, O. Nakamura, Y. Sashida, Y. Satomi, A. Nishino and H. Nishino, *Phytochemistry*, 1994, **37**, 227-232.
100. E. H. Wilson, *The lilies of eastern Asia: a monograph*, Theophrastus, 1978.
101. *Jiangsu New Medical College (ed) The Dictionary Traditional Chinese Crude Drugs*, Shanghai Science and Technology Press, Shanghai, China, 1977.
102. J. P. Munafo, Jr., A. Ramanathan, L. S. Jimenez and T. J. Gianfagna, *Journal of agricultural and food chemistry*, 2010, **58**, 8806-8813.
103. J. P. Munafo, Jr. and T. J. Gianfagna, *Journal of agricultural and food chemistry*, 2011, **59**, 5945-5954.
104. T. Satou, Y. Mimaki, M. Kuroda, Y. Sashida and Y. Hatakeyama, *Phytochemistry*, 1996, **41**, 1225-1230.
105. J. Hartwell, *Plants used against cancer. A survey.*, Lloydia 1967-71:30-34.
106. Y. Mimaki, T. Satou, M. Kuroda, Y. Sashida and Y. Hatakeyama, *Chemical & pharmaceutical bulletin*, 1998, **46**, 1829-1832.
107. K. Idaka, Y. Hirai and J. Shoji, *Chem Pharm Bull*, 1988, **36**, 1783-1970.
108. S. Singh and R. Thakur, *Journal of natural products*, 1982, **45**, 667-671.
109. M. Haladová, E. Eisenreichová, P. Mučaji, M. Buděšínský and K. Ubik, *Collection of Czechoslovak chemical communications*, 1998, **63**, 205-210.
110. M. Haladová, E. Eisenreichová, P. Mučaji, M. Buděšínský and K. Ubik, *Die Pharmazie*, 1999, **54**, 159.
111. Y. Mimaki, T. Satou, M. Kuroda, Y. Sashida and Y. Hatakeyama, *Phytochemistry*, 1999, **51**, 567-573.
112. E. Eisenreichova, M. Haladova, P. Mucaji, M. Budesinsky and K. Ubik, *Die Pharmazie*, 2000, **55**, 549-550.