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

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Chemistry and Biological Activity of Steroidal Glycosides from the Lilium Genus

John P. Munafo Jr and Thomas J. Gianfagna*

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. 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. punilum DC. have been used traditionally in China and Japan as a sedative, anti-inflammatory, antitussive and as a general tonic. 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 enkaurane diterpenes, flavonoid glycosides, anthocyanins, stilbenes, phenolics, phenolic glucosides, phenolic amides, carotenoids, sterols, alkaldoids, and sulfur-containing compounds. Most notably, there has been extensive work done on the isolation and characterization of steroidal alkaloids, particularly in the genus Fritillaria, and steroidal glycosides including steroidal saponins and steroidal glycoalkaloids from within the Liliaceae family.

Steroidal glycosides have been reported to exhibit a wide range of biological activities including antifungal, platelet aggregation inhibition, anti-cholinergic, anti-diabetic, anti-hypertensive, cholesterol lowering, anti-inflammatory, antiviral, and anticancer. Additionally, steroidal glycosides have a wide variety of commercial uses including surfactants, foaming agents, vaccine adjuvants, and serve as precursors for the industrial production of pharmaceutical steroids.

Steroidal saponins have been found in over 100 plant families and in some marine organisms such as starfish and sea cucumber. 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. 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 precursor 2,3-epoxide, and then converted to tetra- or pentacyclic triterpenes by a family of 2,3Eoxidosqualene 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 (guillai 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. pardinum* 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 Solanaceae,\(^{51}\) Ranunculaceae,\(^{52}\) 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.\(^{60}\) 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-glycosidically 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-galacturonic acid, D-galacturonic acid, L-ramnose, L-arabinose, D-xyllose, 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.\(^{85}\)

Figure 4. Examples of different carbohydrate linkages: (A) linear arrangement (β-D-glucopyranosyl(1–4)β-D-glucopyranosyl), (B) branched arrangement (α-L-rhamnopyranosyl(1–2)α-L-xylpyranosyl(1–3)β-D-glucopyranosyl), and (C) branched arrangement (α-L-rhamnopyranosyl(1–2)β-D-glucopyranosyl(1–4)β-D-glucopyranosyl).\(^{95}\)

### 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 tokiokai*,\(^{62}\) and amphipods 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.\textsuperscript{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,\textsuperscript{30} anticholinergic,\textsuperscript{28} antifungal,\textsuperscript{25} and antitumor.\textsuperscript{36} In particular, the genera Veratrum and Fritillaria,\textsuperscript{32} 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.\textsuperscript{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\textsuperscript{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,\textsuperscript{24} veratramine type, and jervine type. The cevanine type,\textsuperscript{25} structurally characterized by the hexacyclic benzo [7,8] fluoreno[2,1-b] quinolizidine 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. camtschatica, respectively.\textsuperscript{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 epininocholestanes 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-epininocholestane 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.\textsuperscript{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.\textsuperscript{65} Two 22, 26-epininocholestane-type steroidal alkaloids, named etioline and teiemine, which were previously found in several Solanum and Veratrum species were isolated from L. candidum bulbs.\textsuperscript{60,64} (Figure 7) In 2001, Erdoğan et al. also reported the isolation of etioline from L. candidum bulbs.\textsuperscript{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.\textsuperscript{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.\textsuperscript{62} 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 oxazaspirodecanine ring system. The second group, (2) 22, 26-epininocholestanes, are characterized by a 22/23, 26-epininocholestane 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 splisoalane groups.\textsuperscript{65} 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-xylene, 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-epininocholestanes, (C) solanidanes, (D) α-epiminocyclohemiketals, (E) 3-aminoSpirostanes, and (F) leptines.

Table 1 lists 110 accepted Lilium species and one hybrid (L. speciosum × 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. longiforum, 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. Abbrev: a; inhibition of Na+/K+ ATPase. b; inhibition of cyclic AMP phosphodiesterase. c; inhibitory activity on 12-O-tetradecanoylphorobol-13-acetate (TPA) stimulated 32P-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). 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. 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 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 cholestanone 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-tetradecanoylphorbol-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-aloce saccarhide 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-tetradecanoylphorbol-22-tetrahydroxy 5α-cholestan-6-one 3-O-β-D-allylopyranoside, 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.

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). L. philippinense grows in the coniferous forests of the northern highlands of the Philippines. Interestingly, the compound was a veratrane 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 (25R)- and (25S)-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

In 1990, Mimaki et al. reported on the isolation and structural elucidation of a 27-acloxySpirostanol 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 15). 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 ailments, and most notably for the treatment of coughs and other lung ailments. 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.

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

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 subjected to solvent partitioning and CC to afford the purified compounds. The authors comment that 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

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 17). In addition, a solanidine-based steroidal glycoalkaloid, solanidine-O-α-L-rhamnopyranosyl(1→2)-α-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*. *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 subjected to repeated silica gel CC, GPC (Sephadex LH-20®), followed by Dionex 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, 1H NMR, 13C NMR, DEPT, HMOC, 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*. 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 A-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*. 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 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). 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*. *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 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.
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). 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 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*)

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). The authors comment that *L. pensylvanicum* along with three other *Lilium* species, namely, *L. brownii*, *L. pumilum*, and *L. 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 LHE20®) to yield the purified compounds. The resulting steroidal glycoside fraction was subjected to octadecyl-silica (ODS) CC and then finally preparative RP-HPLC to afford compounds 28 - 34. The 1H NMR and 13C 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 1H NMR, 13C 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 1H NMR and 13C 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, tenuifoliolide 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 tenuifoliolide 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

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). Also known as Hanson’s lily or Japanese Turk’s cap lily, *L. hansonii* is native to the island of Kagoshima, 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 LHE-20®), and finally preparative RP-HPLC. On the basis of 1H NMR, 13C 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 5α-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 1H NMR and 13C NMR spectra, the aglycone of 35 and 36 were tigogenin derivatives both containing an axial 12α-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 5α-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 IC50 (x 10-5M), 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 IC₅₀ (x 10⁻³ M). In contrast, when the rhamno moiety of 9 was cleaved, inhibition activity of the resulting diglycoside decreased to > 500 x 10⁻³ 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*.

51 *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.⁴⁴ 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 IC₅₀ (x 10⁻³ 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.

53 *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).⁴⁴ 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, graccilin, and 40, trigolenoside 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 IC₅₀ (x 10⁻³ 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 steroid saponins 39, 43 – 47, 82, 85-89 designated as liliglycosides A, D, G, K, N, and R, respectively, from the bulbs of *L. regale* (Figure 27).⁵⁰ The authors noted that fifteen steroidal glycosides were separated by TLC and visualized using Sanné reagent, but only six were physically isolated by repeated CC. The three spirostanols 39, 43 and 44 were identified by comparison of the ¹³C 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 liliglycosides E and F and four new saponins 48 - 51, designated as liliglycosides B, H, C, and I, respectively, from the bulbs of *L. regale* (Figure 27).⁶⁵ 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-hydroxyspirostanone, 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 with TLC with authentic standards. MS, IR, ¹H NMR, and ¹³C NMR experiments identified the structure of 49 as (2RS)-spirost-5-ene-3β,27-diol 3-β-D-glucopyranoside 27-(3-hydroxy-3-methylglutarate). Compound 50 was determined to be the triglycoside of 48, and 51 was determined to be (2S)-spirost-5-ene-3β,27-diol 3-O-[α-L-rhamnopyranosyl-(1→2), [O-β-D-glucopyranosyl-(1→3)]O-β-D-glucopyranosyl]-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.⁵⁵,⁹⁴
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. 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. For example, a novel steroidal glycoside, not present in either parental species, was identified in the sexual hybrids of S. acaule and Solanum x ajahnui. 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.

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 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, 1H NMR, 13C NMR, and chemical transformations. The aglycon particle was deduced from a peak indicative of a carboxyl group in the IR spectrum (1724 cm⁻¹), 1H NMR (δ 62.06, 3H s) and 13C NMR (δ 170.9 and 20.8). The aglycon particle was further substantiated by the comparison of both 1H NMR and 13C 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-tetradecanoylphorbol-13-acetate (TPA) stimulated 3H-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% inhibition 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, Munafa et al. reported on the identification of several steroidal glycosides from the bulbs of L. longiflorum (Figure 31). 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 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 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*. 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. 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-6α 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.**

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). *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. 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, and 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 α,β-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.**

It was the first systematic investigation of the 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.

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 IC50 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*. 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 IC50 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 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 IC50 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.

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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 of the species has not been investigated thus far. Of the 13,000 registered lily cultivars, L. candidum 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.

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