Bioactivities of Glycoalkaloids and Their Aglycones from *Solanum* Species

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ABSTRACT: Potatoes, tomatoes, and aubergines are all species of the *Solanum* genus and contain a vast array of secondary metabolites including calystegine alkaloids, phenolic compounds, lectins, and glycoalkaloids. Glycoalkaloids have been the subject of many literature papers, occur widely in the human diet, and are known to induce toxicity. Therefore, from a food safety perspective further information is required regarding their analysis, toxicity, and bioavailability. This is especially important in crop cultivars derived from wild species to prevent glycoalkaloid-induced toxicity. A comprehensive review of the bioactivity of glycoalkaloids and their aglycones of the *Solanum* species, particularly focused on comparison of their bioactivities including their anticancer, anticholesterol, antimicrobial, anti-inflammatory, antinociceptive, and antipyretic effects, toxicity, and synergism of action of the principal *Solanum* glycoalkaloids, correlated to differences of their individual molecular structures is presented.

KEYWORDS: glycoalkaloids, structure−activity relationship, anticancer, aglycones, synergism

INTRODUCTION

Glycoalkaloids, a class of nitrogen-containing steroidal glycosides, are biologically active secondary plant metabolites and are commonly found in plants of the *Solanum* genus. These include many common vital agricultural plants including potato (*Solanum tuberosum*), tomato (*Solanum lycopersicum*), and aubergine (*Solanum melongena*).

Glycoalkaloids are not required for plant growth and function. However, they have been associated with plant resistance to pests and pathogens and have been shown to exhibit a concentration-dependent toxicity to a wide range of organisms from fungi[1−12] to humans.[13−18] In 1826, α-solanine **1** was the first glycoalkaloid to be reported as a natural constituent of potatoes,[19] and it was considered to be the only compound of this class present until 1954, when α-chaconine **2** was found.[20−22] The tomato glycoalkaloid tomatine was not discovered until 1948;[7,8,23−25] however, it was later found that the reported "tomatine" was in fact a mixture of the glycoalkaloids tomatine **3** and dehydrotomatine **4**.[26] α-Solasonine **5** and α-solamargine **6**, the two major steroidal alkaloid glycosides found in aubergines, were discovered much later and in over 100 other species.

The toxicity of glyoalkaloids in humans is well documented, with "solanine" poisoning from blighted, green, or sprouted potatoes being reported as early as 1980.[14−16,27−29] Accordingly, current safety regulations limit their content in the edible tuber to 20 mg/100 g fresh weight (fw).[30,31] The mechanism of toxicity induced by glycoalkaloids is associated with their membrane-disruptive properties[32−34] and their inhibition of acetylcholinesterase activity.[35−39] Bioactivity of glycoalkaloids is not limited to their toxicity; they have been reported to possess anticancer, anticholesterol, and anti-inflammatory properties, for example, and some of these effects have been reviewed.[1,14,16,30,40−47] Although there have been multiple reviews of individual glycoalkaloids in the literature, including separate reviews of potato[45] or tomato[1] glycoalkaloids, this is the first review to examine and compare the bioactivities, toxicities, and synergisms of action of the principal *Solanum* glycoalkaloids. Furthermore, the mechanisms of action are discussed, and the relationship between molecular structure and bioactivity profile is presented.

STRUCTURES OF GLYCOALKALOIDS IN *SOLANUM* SPECIES

Glycoalkaloids consist of two structural components, which accounts for their amphiphilic nature. The aglycone unit consists of a hydrophobic 27-carbon skeleton of cholestane with nitrogen incorporated into the F ring. The second unit is a hydrophilic carbohydrate side chain attached at the 3-OH position (Figure 1). The aglycones of glycoalkaloids, also referred to as alkamines, are classified into five different categories depending on their structures:[48] solanidanes, which have fused indolizidine rings; spirosolanes, which have an oxa-azaspirodecane system; and the (22,26)-epiminocholestanes, α-epiminocyclohemiketals, and 3-aminospirostanes (Figure 2).[48,49] Most of the glycoalkaloids found in *Solanum* species belong to solanidane and spirosolane classes. At least 90 structurally unique steroidal alkaloids have been identified in over 350 *Solanum* species.[46]

The structural relationship and some common sources of the major *Solanum* glycoalkaloids are described in Figure 3.

The major glycoalkaloid components (comprising >95% of the glycoalkaloid content) found in commercial *S. tuberosum* (potato) cultivars, are α-solanine **1** and α-chaconine **2**. These can also be found in many other *Solanum* species.[50−52] Both glycoalkaloids α-solanine **1** and α-chaconine **2** contain the solanidine aglycone **16**. The glycoalkaloids dehydrocommersonine **21** and dehydrodemissine **35**, found in the wild species *S. commersonii* and *S. canasense*,[53−56] also possess the solanidine aglycone **16**. Commersonine **24**, isolated from *S. commersonii*[54,57] and *S. chacoense*,[50,57] and demissine **23**, from the wild potato species *S. demissum* and *S. chacoense*,[50,51,54,57] both possess the demissidine aglycone **22**. Demissidine **22** and solanidine **16** both possess solanidane structures, the only difference being the degree of saturation between C5 and C6 (Figure 4).

The major glycoalkaloid component of *S. lycopersicum* (tomato) α-tomatine **3** has a spirostane ring, specifically the aglycone tomatidine **25**. α-Tomatine **3** can also be found in at least 15 other *Solanum* species.[26,50,51,54,55,58] The glycoalkaloid sisunine **26**, which has been found in *S. acaule* and *S. ajanhuiri*,[59] also possesses the tomatidine aglycone **25**. In addition to α-tomatine **3**, the tomato also contains the glycoalkaloid dehydrotomatine **4**.[26] Isolates of *S. commersonii*[53] also contain dehydrotomatine **4**, which possesses the dehydrotomatidine **36** aglycone subunit. α-Solamarine **29** and β-solamarine **28** share the same aglycone (dehydrotomatine **4**), both of which can be found in
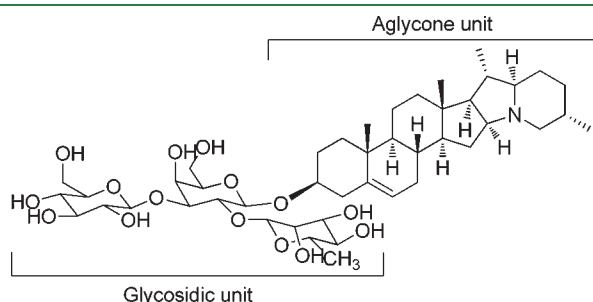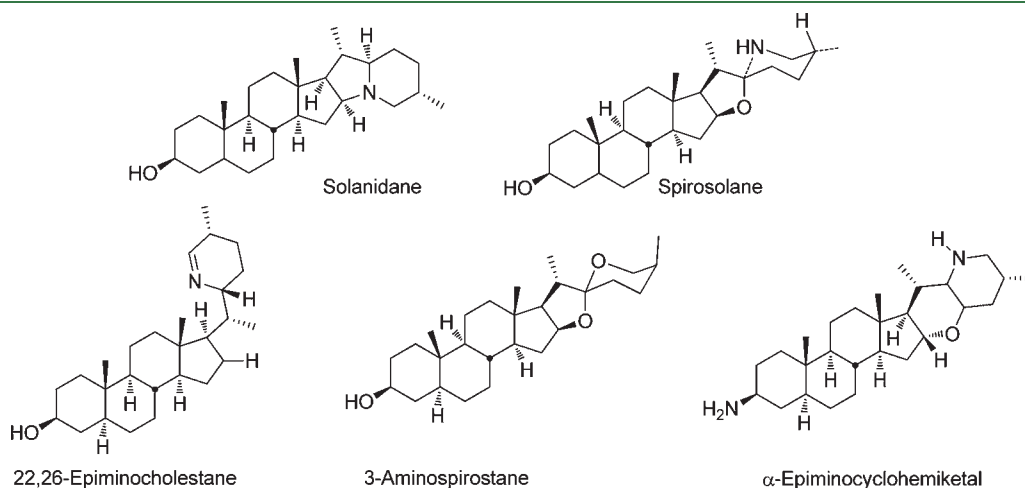
S. brachycarpum[55] and *S. phureja*.[60] Furthermore, α-solamarine **29** has been reported as a constituent of *S. × curtilobum*,[51] *S. phureja × juzepczukii*,[51,58] *S. candolleanum*, *S. medians*, and *S. multiinterruptum*,[50] whereas β-solamarine **28** has been isolated from *S. × juzepczukii*,[51,58] *S. trilobatum*,[61] *S. dulcamara*,[62] and *S. curtilobum*.[51]

In the case of the chief aubergine glycoalkaloids α-solasonine **5** and α-solamargine **6**, both possess the aglycone solasodine **34**, which also contains a spirostane ring in its structure. α-Solasonine **5** has been isolated from the following *Solanum* species: *S. berthaultii*, *S. platanifolium*, *S. ambosinum*, *S. multidissectum*, *S. spegazzinii*, *S. candolleanum*, *S. vernei*, *S. stoloniferum*, and *S. sodomaeum*.[50,55,63−65] Extracts of *S. berthaultii*, *S. paludosum*, *S. ambosinum*, *S. multidissectum*, *S. spegazzini*, *S. bukasovii*, *S. candolleanum*, *S. santolallae*, *S. stoloniferum*, *S. sodomaeum*, and *S. vernei* contain α-solamargine **6**.[50,55,63,65,66] Few records of *Solanum* species containing the glycoalkaloids soladulcine A **31**, soladulcine B **33**, and β-soladulcine **32** are documented in the literature. All three possess the soladulcidine aglycone **30**. Soladulcine B **33** was isolated from *S. lyratum*,[67] whereas soladulcine B **33** and β-soladulcine **32** were detected in *S. dulcamara*.[68,69]

The second structural unit of the glycoalkaloid is a glycosidic residue (Figure 5), which is attached at the 3-hydroxyl position on the A ring of the aglycone. The saccharides consist of different combinations of D-glucose, D-galactose, D-xylose, and L-rhamnose in the form of tri- or tetrasaccharides. Both α-chaconine **2** and α-solanine **1** have a trisaccharide attached to solanidine **16**. In α-chaconine **2** the trisaccharide is a branched bis-α-L-rhamnopyranosyl-β-D-glucopyranose **12** (chacotriose) unit, whereas α-solanine **1** possesses a branched α-L-rhamnopyranosyl-β-D-glucopyranose-β-galactopyranose **13** (solatriose) side chain. The tomatidenol glycosides β-solamarine **28** and α-solamarine **29** are both glycosides of chacotriose **12** and solatriose **13**, respectively. The solasodine glycoalkaloids α-solamargine **6** and α-solasonine **5** have the same side chains as α-chaconine **2** and α-solanine **1**, respectively. The demissidine glycoalkaloids demissine **23** and commersonine **24** both possess a tetrasaccharide. Demissine **23** and α-tomatine **3** are both glycosides of lycotetraose. Commersonine **24** and dehydrocommersonine **21** possess the tetraose commertetraose **15**.

It is worth noting that a group of glycoalkaloids called "leptines", which are principally isolated from potato leaves, consist of either
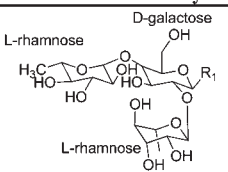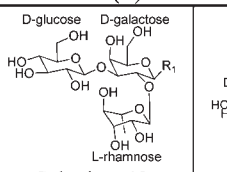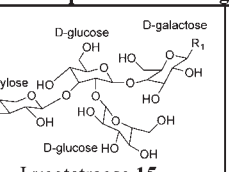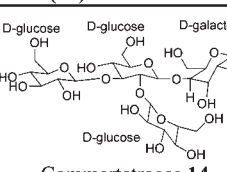

Figure 1. Structure of α-solanine **1**.

Figure 2. Structure of steroidal alkamines.
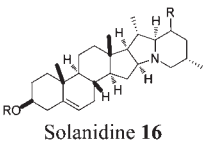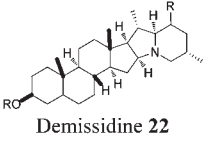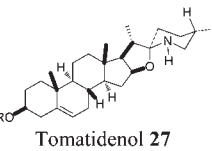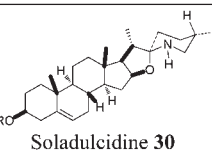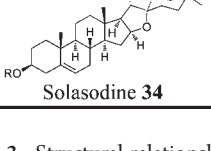
Aglycones R^1	Glycosidic Residues (R) attached at 3-OH position on the aglycone (R^1)			
	Chacotriose 12	Solatriose 13	Lycotetraose 15	Commertetraose 14
Solanidine 16	α-Chaconine 2 (R=H) **Leptine I 17** (R=OAc) **Leptinine I 18** (R=OH) *(potato)*	α-Solanine 1 (R=H) **Leptine II 19** (R=OAc) **Leptinine II 20** (R=OH) *(potato)*		Dehydrocommersonine 21 *(potato)*
Demissidine 22			Demissine 23 *(potato)*	Commersonine 24 *(potato)*
Tomatidine 25			α-Tomatine 3 *(tomato)*	Sisunine 26 *(potato hybrids)*
Tomatidenol 27	β-Solamarine 28 *(deadly nightshade)*	α-Solamarine 29 *(deadly nightshade)*	Dehydrotomatine 4 *(tomato)*	
Soladulcidine 30	Soladulcine A 31 *(deadly nightshade)*	β-Soladulcine 32	Soladulcine B 33 *(deadly nightshade)*	
Solasodine 34	α-Solamargine 6 *(aubergine)*	α-Solasonine 5 *(aubergine)*		

Figure 3. Structural relationships of the principal *Solanum* glycoalkaloids.

the 23-acetoxy or 23-hydroxy solanidine aglycone. The leptines possess the acetoxy moiety, and the leptinines possess a hydroxy moiety at position 23. Leptine I **17** and leptinine I **18** both possess the chacotriose **12** trisaccharide; leptine II **19** and leptinine II **20** are both attached to solatriose **13**. The leptines have been found in the leaves of *S. chacoense* but not in the leaves of *S. tuberosum*.[55,70−73]

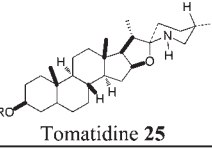In addition to the glycoalkaloids discussed, unusual spirosolane or solanidine type glycoalkaloids have been detected in *Solanum* plants,[74−77] for example, esculeoside A **37** (Figure 6), which was isolated from tomato.[78] Also, novel glycoalkaloids are reported due to the hybridization and or genetic modification of *Solanum* plants, for example, sisunine **26**.[59]

■ GLYCOALKALOID HYDROLYSIS AND BIOSYNTHESIS

Naturally occurring glycoalkaloids are called α-compounds. The sugar chains are subject to hydrolytic cleavage by chemical or enzymatic means. Stepwise cleavage of the glycoside side chain leads to β- and γ-compounds in the trisaccharides and β-, γ-, and δ-compounds in the case of tetrasaccharides. Figure 7 shows the hydrolysis products of the principal potato glycoalkaloids α-solanine **1** and α-chaconine **2**, the majority of which have been isolated from nature except β_1-solanine **38**.[45] The tomato glycoalkaloid α-tomatine **3** exhibits similar pathways of hydrolysis[1] as do the aubergine glycoalkaloids α-solasonine **5** and α-solamargine **6**.[79] Chemical hydrolysis has been performed on a wide range of glycoalkaloids to ascertain the importance of the glycosidic residues for bioactivity.

The mechanism of in vivo hydrolysis by the plant is not yet fully elucidated. However, in 1953 enzymes present in the sap from cultivated potato sprouts were shown to exhibit hydrolysis activity toward the sugar bonds.[80] The incomplete hydrolysis of α-chaconine **2** and direct hydrolysis of α-solanine **1** to solanidine **16** was first demonstrated by Swain et al.[81] The
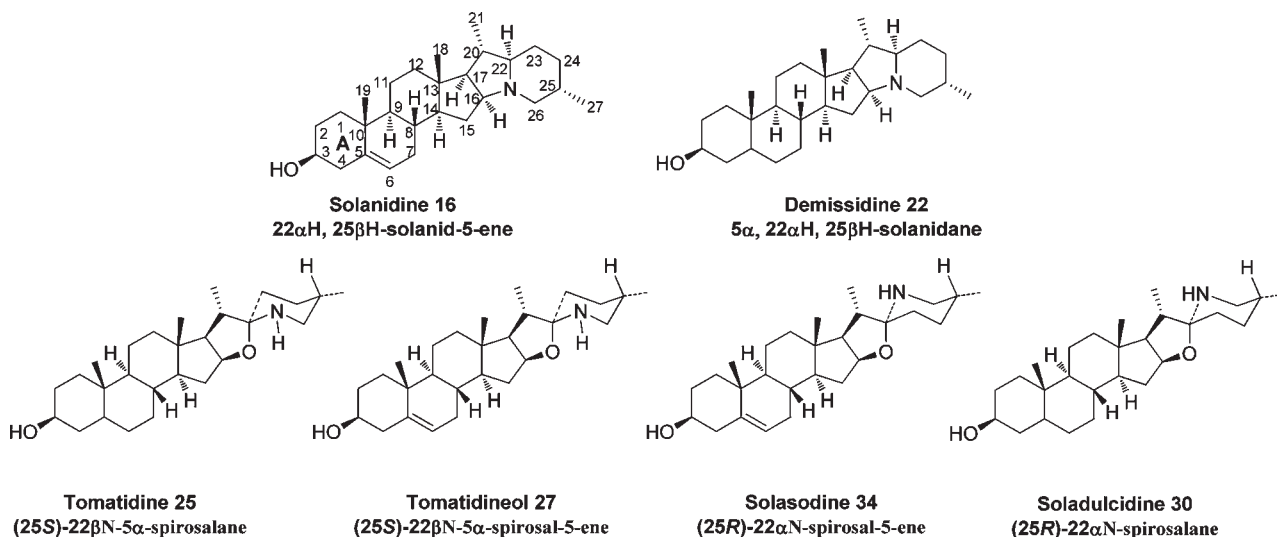
Figure 4. Structures of the aglycone units of *Solanum* glycoalkaloids.
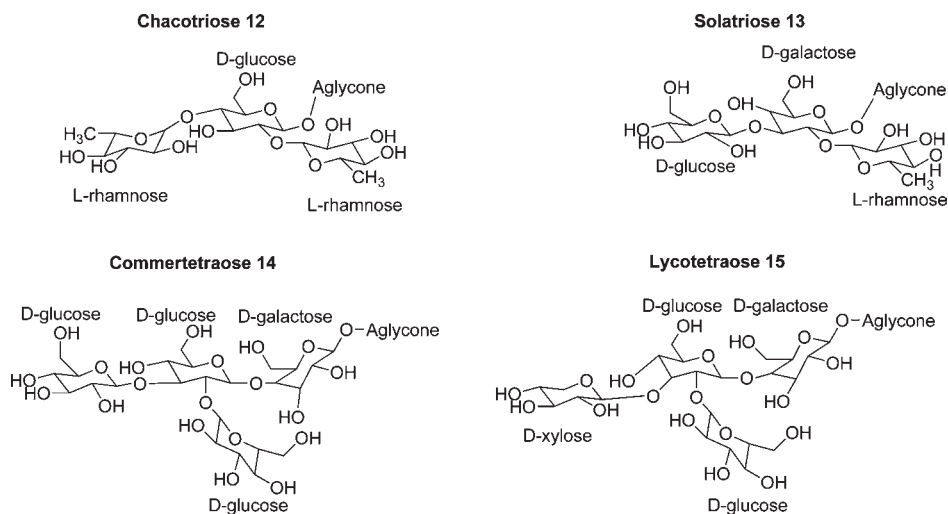
Figure 5. Structures of carbohydrate side chains.
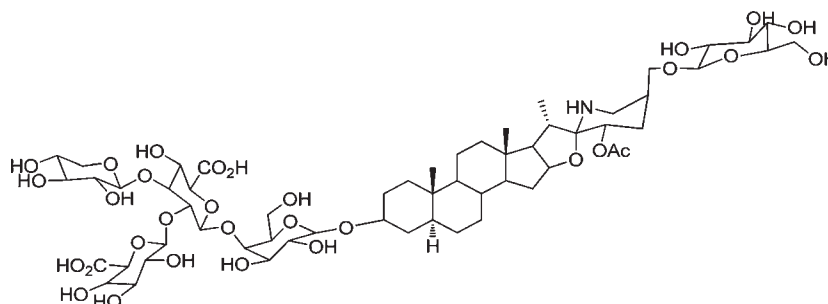
Figure 6. Structure of esculeoside A **37**.

kinetics of enzymatic hydrolysis has also been examined.[82] Rhamnosidase has been isolated and partially purified from different parts of the potato;[83] this enzyme is involved in the hydrolysis of the rhamnose moieties from α-chaconine **2**[84] and α-solanine **1**.[85]

Several glycosidases have been isolated from bacteria and fungi found on the *Solanum* spp. Glycosidases that hydrolyze α-tomatine to its hydrolysis products β_1-**42**, β_2-**43**, γ-**44**, and δ-tomatine **45** and its aglycone tomatidine **25** are produced by phytopathogenic fungi, which grow on rotting tomatoes. These
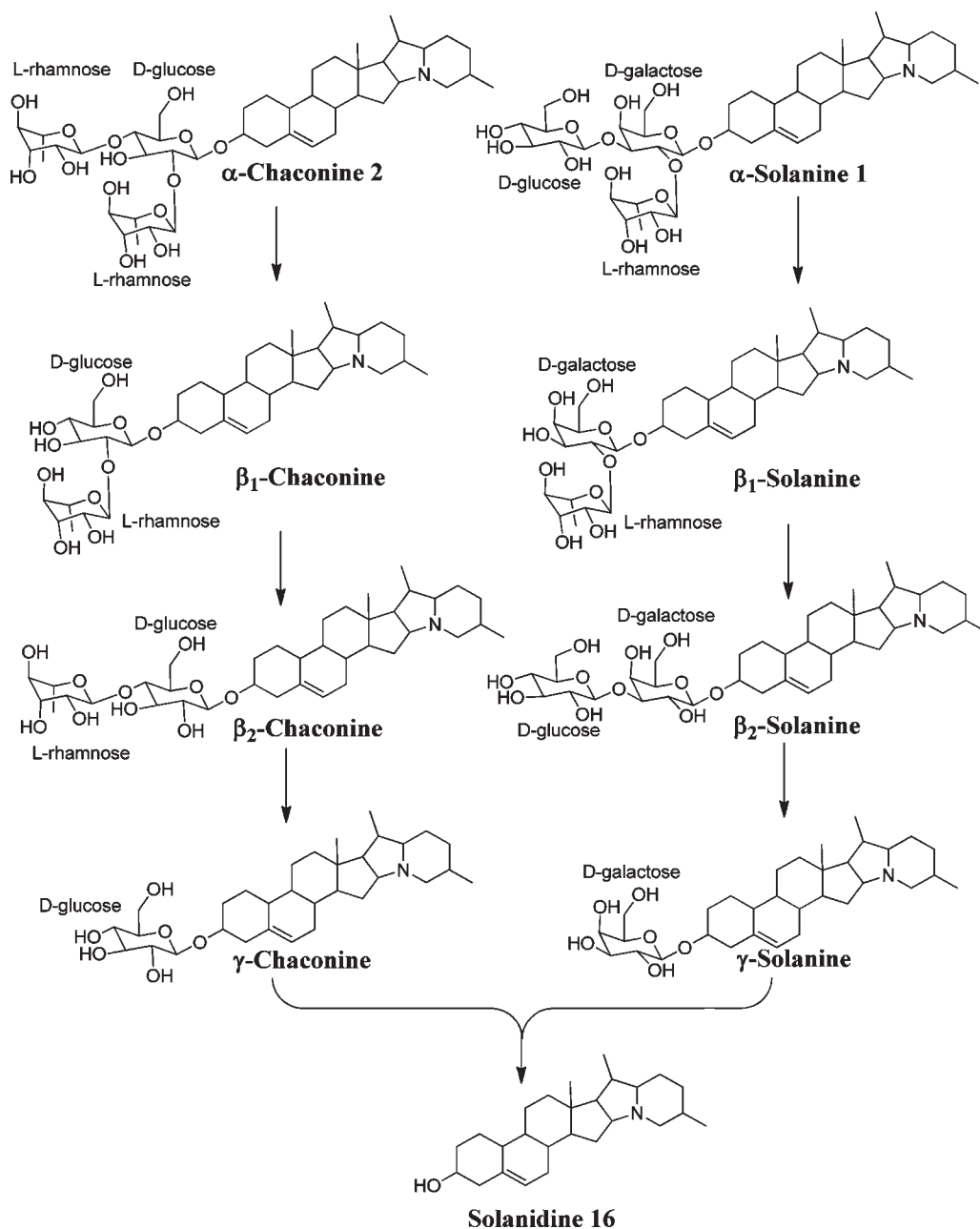
Figure 7. Structures of the hydrolysis products of α-chaconine **2** and α-solanine **1**.

fungi have been isolated and characterized.[86−90] Three strains of filamentous fungi have been isolated, which contain glycosidases that hydrolyze α-chaconine **2** but not α-solanine **1**.[91] The chemical hydrolysis of potato, tomato, and aubergine glycoalkaloids has been performed, and it has been highlighted that by careful selection of solvent, temperature, and alcoholic solution, access to the desired hydrolysis product is possible. Rates of acid hydrolysis were increased with higher acid concentrations and temperatures and decreased with increasing volumes of water in mixed alcohol−water solutions. The nature of the alcohol present in the aqueous and nonaqueous media strongly influenced the course of hydrolysis.[92−100]

The biosynthetic pathways of glycoalkaloid synthesis have not yet been fully elucidated. It is, however, generally accepted that the aglycone moiety of glycoalkaloids is derived from cholesterol (Figure 8).[1,46,101−106] The biogenesis of the principal glycoalkaloids has been presented and discussed in detail elsewhere, and a summary of the postulated pathways presented is depicted in Figure 8.[101]

DISTRIBUTION AND ANALYSIS OF GLYCOALKALOID CONTENT IN THE PLANT

Glycoalkaloid Content and Distribution in the Plant. More than 80 glycoalkaloids have been identified in various potato species; the principal glycoalkaloids are illustrated in Figure 9. The principal potato glycoalkaloids in commercial cultivars are α-solanine **1** and α-chaconine **2**. The ratios of these can vary between tissues and cultivar and are dependent on growing conditions. Glycoalkaloid levels in potatoes are increased by a

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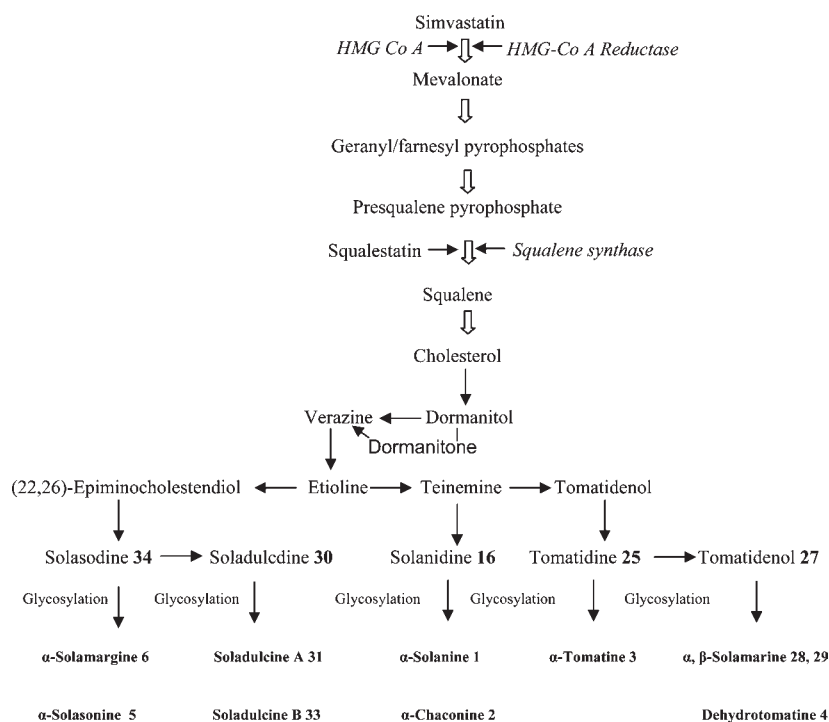
Figure 8. Biogenesis of principal *Solanum* glycoalkaloids.
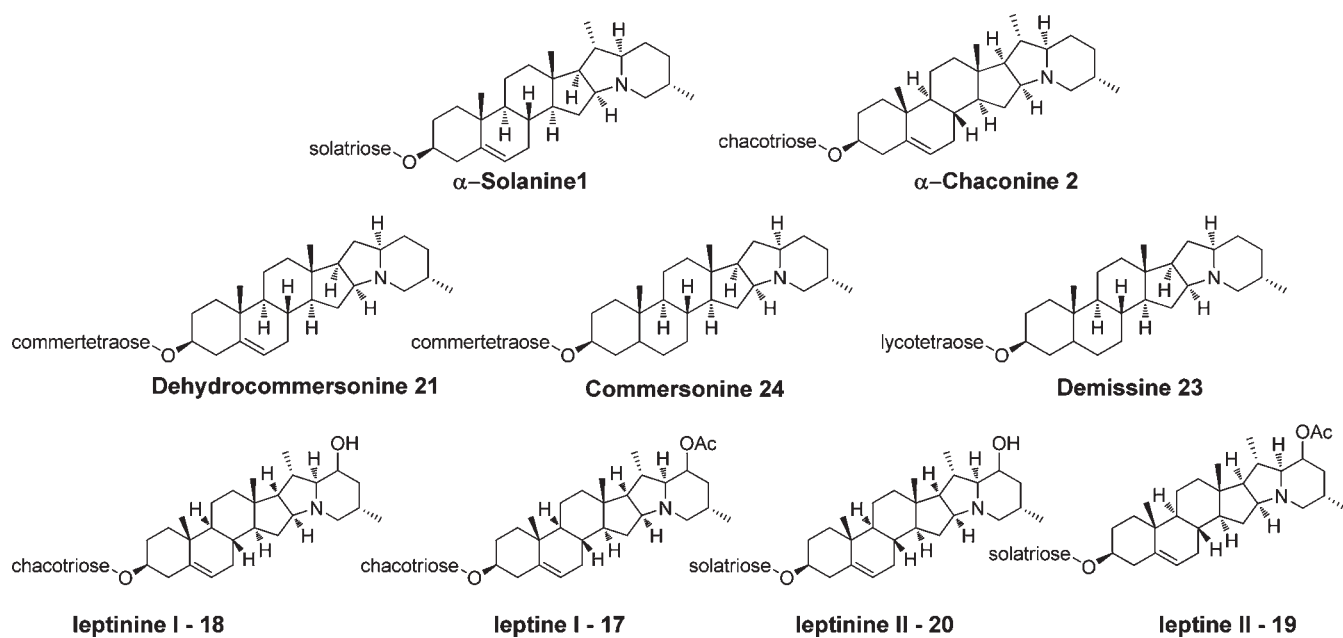
Figure 9. Structures of potato glycoalkaloids isolated to date.

number of factors; unfavorable climatic conditions such as extreme temperatures[107] or exposure to artificial light or sunlight[108−111] can cause a 300-fold increase in glycoalkaloid content; increases in glycoalkaloid content depend on the wavelength of light used;[112] and both mechanical[113] and insect damage, particularly by the Colorado potato beetle, can lead to high concentrations of glycoalkaloids.[114]

Moreover, postharvest conditions such as storage temperatures, light, and mechanical injury during processing can all dramatically increase the glycoalkaloid content in potatoes.[85,115−117] In many cases increased glycoalkaloid content is associated with greening of the potato.[118,119] Storage of potatoes for sale under conditions where light is excluded to prevent the synthesis of chlorophyll (opaque packing or red or blue light excluded-colored packing material) can minimize the glycoalkaloid levels. High glycoalkaloid levels are found in potato tuber sprouts (Table 1); both retailers and consumers should be encouraged to prevent sprouting by storing potatoes at low

Table 1. Concentration of Glycoalkaloid in Various Potato Plant Parts (Adapted from Reference 16)

plant part	GA concentration (mg/kg fresh weight)	refs
flowers	2150–5000	123, 124
leaves	230–1000	125
stems	23–33	128
roots	180–400	128
bitter-tasting tubers	250–800	126
whole tuber	10–150	127
skin (2–3% of tuber)	300–640	123, 124
peel (10–12% of tuber)	150–168	123, 124
flesh	12–100	123, 124
cortex	125	127
pith	undetectable	127
sprout	2000–7300	123, 124

temperatures. It is also worth noting that glycoalkaloid composition and content are unaffected by all modes of cooking;[120] however, a significant proportion of glycoalkaloids can be removed by peeling the potato.[121,122] The majority of the plant parts possess glycoalkaloids except the pith (Table 1).

Glycoalkaloid concentrations in commercially grown cultivars are generally in the range of 1.1–35 mg/kg fresh weight (fw); however, in wild cultivars a substantial difference is observed, with glycoalkaloid content ranging from 6 to 432 mg/100 g fw.[128] In 1970 a new American cultivar, 'Lenape' contained an average glycoalkaloid concentration of 27 mg/100 g; however, under environmental stress these levels rose to 65 mg/100 g. Because the acceptable level is 20 mg/100 g, the 'Lenape' cultivar was removed from the commercial market.[129] It is important to note that glycoalkaloid content is comparative only when the tubers compared are of similar dimensions.[130] Furthermore, when potatoes were examined for their palatability, tuber tissues from potatoes that had glycoalkaloid concentrations in excess of 14 mg/100 g were rated as bitter by a taste panel.[131] Tissues that possessed a glycoalkaloid content in excess of 22 mg/100 g also produced a mild to severe burning sensation in the mouth and throat.[131] Therefore, it was postulated that humans affected the domestication of the potato by selection of a cultivar with reduced quantities of glycoalkaloid, and this assumes an ability to recognize glycoalkaloid levels among potato varieties. However, when a taste panel test examined 14 cultivars including wild and genetically modified varieties with various levels of glycoalkaloid contents, no significant correlation was observed.[132] Later the same author examined a much wider range of species and found that selection for reduced toxicity did in fact occur as part of the domestication of the potato.[132]

Potatoes and potato products are constantly being modified to enhance pest and pathogen resistance, low glycoalkaloid levels, yield, and quality. The most common methods to perform this are genetic modification and interspecific hybridization[133] at an experimental level but not at a commercial level; both methods have been employed to successfully incorporate resistance to biotic and abiotic factors. The content of glycoalkaloids is a genetically controlled property, and their concentration varies significantly among potato varieties.[107,134–137] Choice of cultivar is an extremely important factor as wild species such as *S. chacoense, S. brachycarpum, S. hjertingii, S. hougasii, S. kurtzianum, S. medians, S. pinnatisectum,* and *S. polyadenium* possess 5–10

times the total glycoalkaloid concentration when compared with *S. tuberosum*. Hybridization with these species could lead to unacceptable glycoalkaloid content in the progeny;[55] therefore, it is imperative that the glycoalkaloid composition of all species being produced in breeding programs be defined. However, it has been demonstrated that it should be possible to reduce the concentration of glycoalkaloids by backcrossing with the cultivated genotypes or selection of a clone with normal glycoalkaloid content.[138] Furthermore, somatic hybrids are employed to introduce desirable characteristics to new cultivars. Hybrids between frost-tolerant *S. commersonii* and frost-sensitive *S. tuberosum* resulted in a commercial cultivar with increased frost tolerance.[139–141] Somatic hybridization also led to resistance to *Ralstonia solanacearum,*[142] late blight,[143–145] potato virus Y and potato leaf roll virus,[144–149] potato virus X,[146,150] *Erwinia carotovora,*[151] and the potato cyst nematode.[152]

In comparison with potato glycoalkaloids, the tomato glycoalkaloids are relatively nontoxic; for example, a bitter-tasting Peruvian cultivar (*Lycopersicon esculentum* var. *cerasiforme*), with an α-tomatine **3** content in the range of 500–5000 mg/kg is widely consumed without any acute adverse effects.[153] As mentioned previously the principal tomato glycoalkaloid tomatine is a mixture of α-tomatine **3** and dehydrotomatine **4** and is found in all parts of the plant. High glycoalkaloid content in tomatoes is generally associated with immature plants (Table 2). α-Tomatine **3** content generally is reduced upon maturation of the tomato to 5 mg/kg; similar to the potato glycoalkaloids, cooking did not affect glycoalkaloid content in foods.[40,154]

Other tomato glycoalkaloids include the bitter principal factor tomatoside A isolated from tomato seeds,[155,156] esculeoside A **37** and esculeoside B, dehydrotomatoside from tomato fruits,[156,157] filotamine,[158] and lycoperosides A–H from leaves and fruits of the tomato plants.[78,159,160] Genetic modification has been utilized to increase the tolerance of tomato leaf and fruit tissues to heat and UV-B stress,[161] resistance to the root infecting pathogen *Ralstonia solanacearum,*[162] and bacterial wilt and rot[163] and to improve processing properties.[164] Transgenic tomatoes have also been analyzed for their glycoalkaloid content, and negligible differences were observed between the parent and the somatic hybrid.[26,154]

α-Solasonine **5** and α-solamargine **6** are the principal glycoalkaloids of the aubergine species; the total glycoalkaloid content in the aubergine is far less than in other plants of the *Solanum* species. Furthermore, the highest concentration of glycoalkaloids is usually in the peel; in the aubergine, the highest concentration is found in the flesh. The mesocarp with seeds was found to contain 7–38 mg/100 g and the mesocarp without seeds 1–4 mg/100 g; the peel possessed negligible quantities of glycoalkaloids.[165] Aubergine glycoalkaloids and their concentrations in wild and cultivated species have received little attention in the literature. Although there have been reviews in the literature on aubergine biotechnology,[166] in the majority of cases no analysis for glycoalkaloid content has been performed. Transgenic aubergine has been employed to combat abiotic stresses such as osmotic stress induced by salt, drought, and chilling stress[167] and biotic stresses such as spider mites,[168] the fruit and shoot borer,[169] and the Colorado potato beetle[170,171]; however, no analysis of glycoalkaloid content has been performed. When compared with the potato glycoalkaloid, the aubergine glycoalkaloids are relatively nontoxic; however, they have exhibited embryotoxicity and teratogenic effects,[172] and therefore determination of the glycoalkaloid content is warranted.

Table 2. Glycoalkaloid Content of Parts of the Tomato Plant (Adapted from Reference 44)

tomato plant part	dehydrotomatine 4 (mg/kg fresh weight)	α-tomatine 3 (mg/kg fresh weight)
large immature green fruit	14	144
small immature green fruit	54	465
roots	33	118
calyxes	62	795
leaves	71	975
small stems	138	896
large stems	142	465
flowers	190	1100
senescent leaves	330	4900

When three aubergine species and their accessions were examined for their glycoalkaloid content, the common aubergine *S. melongena* and three accessions had 1.1−2.0 mg/100 g, *S. aethiopicum* had 1.95 mg/100 g, and the variants possessed 1.1−5.4 mg/100 g. *S. macrocarpon*, another cultivated species, contained 221 mg/100 g, and the variants of this species contained 140−148 mg/100 g. The *S. macrocarpon* cultivar contained a significantly higher glycoalkaloid content and has been associated with a bitter taste. Also, a difference in glycoalkaloid content between cultivar and transgenic species was observed in all cases, and significant differences in glycoalkaloid ratios were observed between cultivars.[173]

Glycoalkaloid Analysis. The extraction protocols of amphilic glycoalkaloids and their hydrolysis products have been thoroughly evaluated[35,96,98−100,174−177] and reviewed.[46,178] A wide range of analytical techniques have been employed to identify and quantitate glycoalkaloids, in particular, to ascertain their concentration in plants and to examine the wide range of bioactivities associated with these molecules. Analyses of potato,[46,179] aubergine,[173] and tomato[1] glycoalkaloids have been compared and reviewed in the literature. The principal method used to quantify glycoalkaloids is high-performance liquid chromatography (HPLC); one disadvantage, however, is that the UV chromophore for glycoalkaloids is at short wavelengths (200−210 nm), where it can be subject to interference due to the presence of other compounds.[180−183] This has been circumvented by the use of pulsed amperometric detection[26,154,184] and sample purification.[185] However, to perform a structural determination of novel glycoalkaloids, the main methods employed are nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry (MS).

Due to the advancement of two-dimensional 2D NMR experiments such as COSY, NOESY, ROESY, TOCSY, DQF-COSY HMQC, and HMQBC and the availability of high magnetic fields, the qualification of glycoalkaloids employing NMR is fast becoming routine. However, it should be highlighted that NMR usually requires relatively large amounts of material in comparison to mass spectroscopy and is inefficient in the structural assignment of glycoalkaloid mixtures. Initial analysis of a novel glycoalkaloid routinely involves acid hydrolysis, yielding an aglycone and a glycosidic unit. The absolute structural assignment of the aglycone and saccharides is usually performed with a combination of correlation spectroscopy (COSY) and total correlation spectroscopy (TOCSY) for ^{1}H NMR and heteronuclear multiple quantum coherence (HMQC) for assignment of the ^{13}C NMR spectrum.[64] Rotating-frame Overhauser

effect spectroscopy (ROESY) is the NMR experiment employed to decipher the structure of the glycosidic linkage of the tri- and tetrasaccharides of glycoalkaloids.[64,186,187] Full NMR spectral assignment has been performed on the following: glycoalkaloids [α-solasonine 5,[64,188,189] α-solamargine 6 and khasianine 47,[64,188] α-tomatine 3,[186,190,191] and dehydrotomatine 4 [192]], aglycones solanidine 16,[193,194] leptidine and acetylleptidine,[193] solasodine 34,[64,189,195] and tomatidine 25,[186,191] and saccharides solatriose 13 and chacotriose 12 [64,188] lycotetraose,[190] and the khasianine oligosaccharide.[64,188] Some novel *Solanum* glycoalkaloids (ravifoline[64]), aglycones (7α-hydroxytomatidine and 7α-hydroxytomatidenole[196] and solancardinol[197]), and oligosaccharides (solashabanine, solaradixine, and solaradinine[189] and the ravifoline oligosaccharide[64]) have also been assigned by NMR analysis.

Mass spectrometry is one solution to the identification of the individual components in glycoalkaloid mixtures. Gas chromatography coupled with mass spectrometry (GC-MS) has been employed by a number of researchers, but this method is only useful for the individual structural determination of aglycones or sugar residues. Therefore, a prehydrolysis step by chemical or enzymatic means is required. Furthermore, a derivitization step of the aglycone or the saccharide is also required in most cases. Preparation of partially methylated alditol acetate of the sugar moieties,[57,97,198] or preparation of the trimethylsilyl,[177,199] pentafluoropropionyl,[200] or acyl[201] products of the aglycone is required to convert the aforementioned compounds into more volatile and thermally stable derivatives. Underivatized aglycones can also be analyzed.[202−205] Both electron spray ionization and chemical ionization measurements have been employed in conjunction with high-resolution mass spectroscopy to determine the empirical formulas of molecular and fragment ions of both aglycones and the tri- and tetrasaccharides of glycoalkaloids. This method allows the identification of novel glycoalkaloids; however, full structural determination of novel moieties is not possible.[114,175,204,206] Liquid chromatography−mass spectroscopy (LC-MS) is analogous to GC-MS in that it is a useful, rapid method for the quantitative detection of glycoalkaloids, aglycones, and oligosaccharides. However, limited structural information is obtained from this method of analysis. Unlike GC-MS, derivatization or hydrolysis is not required for analysis. In the majority of cases purification of the crude material is required before analysis can be performed. Online solid phase extraction with LC-MS is one solution to this and allows rapid screening of glycoalkaloids in plant extracts.[185]

Fast atom bombardment mass spectroscopy (FABMS), also known as liquid secondary ion mass spectrometry (LSIMS), is a useful technique for the analysis of nonvolatile and/or thermally unstable compounds. FABMS has been a useful technique in association with NMR spectroscopy for the identification and structural assignment of novel glycoalkaloids;[160,207] no derivatization or preliminary purification is necessary with this technique. FABMS spectra yield molecular weight information, and significant fragment ions are achieved with individual cleavage of carbohydrate groups possible.[94,154,198] FABMS has allowed direct analysis of juice from etiolated sprouts, and the fragmentation patterns of ten glycoalkaloids were reported.[208] The method employed was not quantitative; it did, however, provide a very efficient qualitative protocol for glycoalkaloid analysis in crude mixtures.[208] A major disadvantage of FABMS for the characterization of the molecular weight distribution is the fragmentation of parent ions during ionization. The study of single components

Table 3. Analytical Techniques Employed To Detect Glycoalkaloids

analytical method	glycoalkaloid source	refs
high-performance liquid chromatography	potato	45, 135, 180, 181, 213−218
	tomato	1, 26, 154, 219−222
	aubergine	223
medium-pressure liquid chromatography	potato	224
thin layer chromatography	potato	55, 96, 225, 226
	tomato	97
	aubergine	55
immunoassays and enzyme-linked immunosorbent assay	potato	227−229
	tomato	184, 230−232
biosensors	potato	233−236
calorimetric detection	potato	237
	bitter nightshade	238
	aubergine	55
	tomato	239
radioligand assay	potato	240, 241
	tomato	242

from the molecular weight distribution with FABMS is possible only by using tandem mass spectroscopy(MS/MS).[209]

The employment of MS/MS as an analytical method for the structural determination of glycoalkaloids has gained momentum in the past decade. The identification of glycoalkaloids is usually made first by their molecular weight and further confirmed by comparing the resultant (MS/MS) fragmentation pattern with literature standards. Tandem MS with scanning array detection is one of the state-of-the-art methods for glycoalkaloid analysis as it provides information on the nature of the aglycone unit and the nature of the glycosidic linkages. α-Tomatine **3** has been structurally assigned with as little as 200 fmol by four-sector MS/MS with scanning array detection in a single scan.[210] Furthermore, positions and linkages of the tri- and tetrasaccharides were obtained by diagnostic fragmentation of the $[M + H]^+$ ion. This method of analysis is rapid and requires small quantities of sample. It is an efficient protocol for the analysis of glycoalkaloid mixtures. The problems associated with the low intensity of collision-induced dissociation fragment ions was overcome by the use of scanning array detectors.[210]

Positive and negative ion tandem mass spectrometry methods have been compared. The positive ion method proved advantageous as it had a lower detection limit, proved to be more structurally informative due to the increased amount of fragment ions, and increased capabilities for the analysis of crude mixtures.[210] High- and low-energy collision-induced disassociation methods were also compared. Charge-driven reactions dominated the low-energy fragmentations, and more complex fragmentation patterns and multiple bond cleavages were associated with the high-energy collision-induced disassociation.[211] The interpretation of fragmentation patterns of the aubergine, potato, and tomato glycoalkaloids has been performed; therefore, this provides a useful reference for glycoalkaloid analysis by

MS/MS.[158,210−212] Moreover, the employment of MS/MS has led to the discovery of a previously undiscovered tomato glycoalkaloid (filotomatine) in tomato leaves.[158] Liquid chromatography−electrospray ionization−tandem mass spectrometry has also been investigated and has been proved to be an accurate, rapid, high-throughput quantification and qualification protocol for the examination of crude glycoalkaloid mixtures.[212]

In conclusion, it should be highlighted that to ascertain the complete structure of a novel glycoalkaloid, MS/MS methods alone are insufficient. FABMS or LSIMS is a useful method for molecular weight determination and provides some access to some structural information. However, MS/MS is a useful aid for both molecular weight and structure determination of both purified and glycoalkaloid mixtures. The information based on these studies needs to be combined with advanced 2D NMR spectroscopy and chemical experimentation to determine the stereochemistry, identity, and attachment of the carbohydrate residues and aglycone moiety. A summary of the other analytical methods employed for glycoalkaloid analysis is outlined in Table 3. These methods often involve hydrolysis of the carbohydrate moiety and individual analysis of the aglycone and saccharide units.

ADVERSE PHYSIOLOGICAL EFFECTS OF GLYCOALKALOIDS AND THEIR AGLYCONES

Pests and Pathogens of *Solanum* Species. The deterrent properties of glycoalkaloids against pests and pathogens were first reported in the 1950s. A positive correlation between total leaf glycoalkaloid content of wild potato species and resistance to Colorado potato beetle (*Leptinotarsa decemlineata*) was obtained; this in turn led to the discovery of a number of novel glycoalkaloids.[243,244] Adverse effects of these secondary

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metabolites on insect behavioral and developmental biology have been reviewed as well as considerations in manipulation of foliar glycoalkaloids in breeding for varietal resistance to insects.[245] High glycoalkaloid levels impart host plant resistance; however, they are associated with a bitter taste and, at high concentration, toxic effects are observed.

The Colorado potato beetle is a major predator of many plants of the *Solanum* spp., especially the potato. The potato cultivar *Solanum chacoense* 'Bitter' is resistant to the Colorado potato beetle, and this has been attributed to the presence of large amounts of leptine glycoalkaloids, which are found only in the foliage of the plant. Crosses between *S. tuberosum* and *S. chacoense* were shown to reduce feeding and increase mortality of the Colorado potato beetle.[72,246−248] It has also been demonstrated that increasing the leptine concentration by light exposure led to an increased mortality to the larvae of the Colorado potato beetle.[249] The tomato glycoalkaloid α-tomatine **3** exhibited field resistance,[118] caused retarded growth, and delayed development of the Colorado potato beetle. Tomatidine **25** showed no activity against the Colorado potato beetle, which highlights the necessity of the lycotetraose carbohydrate unit **14** for activity.[250] To provide elevated resistance to the Colorado potato beetle, Cooper et al.[251] combined genetic engineering and traditional breeding to increase the amount of leptines and *Bacillus thuringiensis* toxins in *S. chacoense*, which led to complete inhibition of feeding and growth; mortality was near 100% in the Colorado potato beetle. Also, levels of glycoalkaloids in potatoes stressed by the Colorado potato beetle were much higher than those concentrations in undamaged plants. This result indicates that a food crop not protected from common pests may produce elevated levels of natural toxins, possibly affecting the degree of toxicity of the final product.[252] As discussed later, one of the modes of action of glycoalkaloids is the inhibition of acetylcholinesterase. When homogenates from several insect species were assayed for inhibition of acetylcholinesterase by α-chaconine **2**, the acetylcholinesterase of the Colorado potato beetle was up to 150-fold less sensitive when compared with the other species tested. It was postulated that the Colorado potato beetle has developed a level of resistance to glycoalkaloid-induced toxicity.[37] This resistance to glycoalkaloid effects was also observed when extracts of potato leaves were tested on the semi-isolated heart of three beetle species, *Z. atratus*, *T. molitor*, and *L. decemlineata*. Cardio-inhibition by glycoalkaloid was observed in *Z. atratus*, and a negligible effect was observed in *T. molitor* and *L. decemlineata*; this has been attributed to evolutionary adaptation to potato glycoalkaloids.

Another significant potato pathogen is the potato cyst nematode. This parasite can cause major losses in crop yield.[253,254] Potato root leachate contains hatching factors, which stimulate the hatch of second-stage juveniles, from eggs, within cysts of both potato cyst nematode species, the golden potato cyst nematode (*Globodera rostochiensis* Woll.) and the white potato cyst nematode (*Globodera pallida*). Jones et al.[255−258] have examined the effect of potato glycoalkaloids α-solanine **1** and α-chaconine **2** present in potato root leachate as minor hatching chemicals; the major hatching factors in potato root leachate were sesquiterpenes. The two potato cyst nematode species exhibited different hatching sensitivities to the potato glycoalkaloids. In the absence of the host plant, these hatching factors could be applied to infested soil, which would induce a "suicide hatch". This suicide hatch could be an environmentally friendly method of potato cyst nematode control.

Increased foliar concentration of glycoalkaloids has also been associated with increased mortality to the potato leafhopper (*Empoasca fabae*).[259] Furthermore, when a range of glycoalkaloids were examined against the potato leafhopper, tomatine **3** proved to have the highest mortality (95%) at the lowest concentration.[260] The potato aphid (*Macrosiphum euphorbiae*) and green peach aphid (*Myzus persicae*) are significant potato pests and can cause substantial damage and losses in crop yield.[261] In addition, they are responsible for the transmission for a number of potato viral diseases, including potato virus X, potato virus Y, and potato leafroll virus.[262] The effects of *Solanum* glycoalkaloids and aglycones were examined on the potato aphid; the aglycones were found to be relatively inactive when compared with their glycoalkaloid counterparts. α-Chaconine **2** and α-solamargine **6** decreased reproduction; in contrast, their paired glycoalkaloids α-solanine **1** and α-solasonine **5**, respectively, increased reproduction rates. Furthermore, Gunter et al.[263,264] also demonstrated that lycotetraose-bearing glycoalkaloids were responsible for the strongest deterrent and toxic effects as well as decreased reproduction rates. Larval growth of the red flour beetle (*Tribolium castenum*) was inhibited in the presence α-solamargine **6**, α-solasonine **5**, and α-tomatine **3**. In the case of the tobacco hornworm (*Manduca sexta*), α-tomatine **3** was the only glycoalkaloid tested that showed marked inhibitory activity.[265]

A more general pest of the potato is the snail *Helix aspersa*; α-solanine **1** and α-chaconine **2** have both been reported to deter snail feeding, α-chaconine **2** being the more potent inhibitor. Moreover, a synergistic feeding deterrent activity was reported between the paired potato glycoalkaloids α-solanine **1** and α-chaconine **2**.[266] When tested, the α-solasonine **5** and α-solamargine **6** extracts of *Solanum mammosum* were found to be fatally toxic to *Lymnea cubensis* snails at 10 mg/L and to *Biomphalaria glabrata* snails at 25 mg/L; the aglycones solasodine **34** and tomatidine **25** were found to be completely inactive,[267] and α-tomatine **3** showed molluscicidal activity at concentrations as low as 4−10 mg/L against *B. glabrata* snails.[268] Extracts of glycoalkaloids from Moroccan *Solanum* species *S. sodomaeum* seeds and leaves and the berries of *S. elaeagnifolium* were also shown to have molluscicidal activity.[269] The molluscicidal activity of glycoalkaloids is an important property, as one of the main methods to control schistosomiasis is to use molluscides to reduce the numbers of snails and therefore the transmission of the disease to man.[270]

Animals. To improve the safety of plant-derived foods for the human diet, much research has focused on the structurally different glycoalkaloids and metabolites in the *Solanum* spp. To ascertain the safety of glycoalkaloids, the biological potencies and mechanisms of action need to be determined. Animal models are employed to determine their respective toxicities and to elucidate the mechanism of glycoalkaloid-induced toxicity.

The embryotoxicity of glycoalkaloids is well established. Frog, rat, mouse, hamster, and bovine embryos have been employed as test models. Bell et al.[271] demonstrated that α-solanine **1**, when administered parenterally into mice, induced embryotoxicity and that aspirin potentiated the toxic effects. The relative toxicities of steroidal glycoalkaloids and aglycones were also examined in mice. α-Solamargine **6** is more potent in disrupting cell membranes than α-solasonine **5** by a factor of 2 or 3;[172] both glycoalkaloids possess the same aglycone (solasodine **34**) but have different glycoside chains. α-Chaconine **2** was shown to be
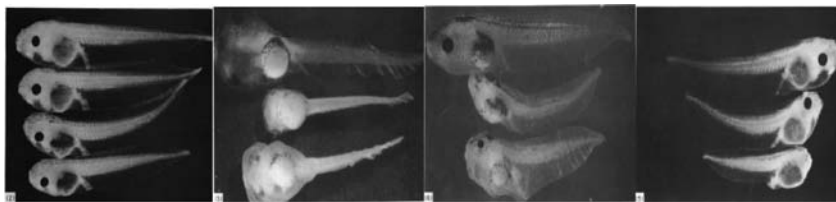
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Figure 10. Results of FETAX assay.[172]

4 times more potent than α-solanine **1**.[272] α-Tomatine **3** has also been compared with tomatidine **25** by the same research group, and the glycosidic residue was required for membrane-disrupting activity. It was concluded that these results further reinforce the importance of the carbohydrate side chain for bioactivity. The intraperitoneal LD_{50} values for α-solanine **1**, α-chaconine **2**, and α-tomatine **3** in mice were 27, 30, and 34 mg/kg body weight (bw) respectively, and for most animals, the intraperitoneal LD_{50} values of the various glycoalkaloids were around 30–60 mg/kg bw. Toxicological studies revealed that the solanidanes **7** seem to be more toxic than their corresponding spirosolanes **8**.[273,274]

The frog embryo teratogenesis assay-*Xenopus* (FETAX) is a useful method for the evaluation of the relative potencies of steroidal glycoalkaloid toxicity. Dessar et al.[275] outlined the advantages. *Xenopus* embryos undergo fundamental developmental processes that are similar to those of mammals. Also, *Xenopus* development has been studied extensively, mating and ovulation can be induced at any time after sexual maturity, and development is external, which facilitates the observation of the developmental process and any malformations that may occur. Finally, the rapidity of *Xenopus* development allows for the determination of developmental end points within a 96 h test period. It has been used to predict the teratogenic potential of structurally different glycoalkaloids and biosynthetic intermediates. α-Chaconine **2** was shown to be more toxic than α-solanine **1** by a factor of 3; severe head and facial malformations were observed[276] (Figure 10).

The aglycones solanidine **16**, solasodine **34**, and demissidine **22** were found to be much less toxic to frog embryos.[276] The role of the carbohydrate chain was also evaluated by the FETAX assay. The hydrolysis products of α-chaconine **2** and α-solanine **1** were examined (see Figure 7). Again, the carbohydrate side chain of the glycoalkaloids was found to be paramount to activity; the order of attachment, the number of glycosides, the type of glycoside, and the stereochemistry of attachment influenced activity. In most cases the hydrolyzed products were less active than the parent steroidal glycoalkaloid. It was postulated that the removal of sugars may influence the transport of these compounds across cell membranes.[95]

The potato, aubergine, and tomato glycoalkaloids have been shown to alter the membrane potential of *Xenopus laevis* frog embryos as well as the active transport of sodium by frog skin,[172,272,273] and this change in membrane potential has been used to explain the embryotoxic and teratogenic effects seen in the presence of glycoalkaloids. Interestingly, a number of agents have been employed to protect against glycoalkaloid-induced embryotoxicity. Glucose-6-phosphate and nicotine adenosine dinucleotide phosphate (NADP) have been shown to protect against glycoalkaloids, although the mechanism of this is not yet understood.[277] Folic acid and the folic acid analogue methotrexate have also protected against α-chaconine **2**-induced toxicity, by a reduction in the depolarization of the membrane potential

caused by α-chaconine **2**, in a concentration-dependent manner. Also, the relatively high concentrations of folic acid needed to achieve a protective effect in both cases suggested that protection required a "pharmacological" rather than a much lower "nutritional" concentration of folic acid.[278,279] Embryotoxicity was also observed in early chick embryos upon exposure to α-solanine **1**.[280] α-Chaconine **2**, α-solanine **1**, and solanidine-*N*-oxide **48** were shown to inhibit preimplantation upon in vitro exposure of bovine oocytes, therefore preventing in vitro bovine embryo development.[281]

Humans. There have been no reports of the ill effects of glycoalkaloids with the aubergine or the tomato, but a number of reports have correlated potato glycoalkaloids with toxic effects in humans. Consumption of potatoes containing high levels of glycoalkaloids, which impart a bitter taste, are reported to cause severe illness and even death. In cases of mild glycoalkaloid poisoning symptoms include headache, vomiting, and diarrhea. Neurological symptoms were also reported, including apathy, restlessness, drowsiness, mental confusion, rambling, incoherence, stupor, hallucinations, dizziness, trembling, and visual disturbances. Fatality has been attributed to the ingestion of potatoes and potato leaves and berries with high levels of glycoalkaloids.[14–16,27–29] The reports of the susceptibility of humans to glycoalkaloid poisoning are varied. Cases of lethal poisoning have been reported at estimated oral doses of >3–6 mg total glycoalkaloid/kg bw, and mild poisoning occurs in the range of 1–5 mg/kg bw.[216,282] It is suspected that a higher percentage of mild glycoalkaloid poisoning occurs; however, the symptoms are similar to those of other gastrointestinal disturbances and remain under-reported. Another source of high glycoalkaloid content could arise from the feeding of potato peel waste from the manufacturing industry to livestock as a feed supplement.[283] Given the high content of glycoalkaloid in peel, this process should be monitored and restrictions put in place.

Due to human toxicity, 200 mg (total glycoalkaloid/kg fresh weight) in potatoes is accepted as the upper safety limit.[16] The joint FAO/WHO Expert Committee on Food Additives (JECFA) reported a total glycoalkaloid content of <100 mg/kg potatoes (fw) as being of no concern.[284] High glycoalkaloid levels in potatoes are associated with a bitter taste, and very high levels cause a burning sensation in the throat and mouth.[15] To date, little is known about the bioavailability, metabolism, and pharmacokinetics of glycoalkaloids in humans. The potato glycoalkaloids have received some attention in this area as described below. However, little or no investigation into the bioavailability, metabolism, and pharmacokinetics of the other *Solanum* glycoalkaloids has been undertaken, which needs to be rectified to fully develop a safety profile for these compounds.

Hellenäs[216] and Mensinga et al.[285] investigated the in vivo metabolism of potato glycoalkaloids α-solanine **1** and α-chaconine **2** after the consumption of mashed potatoes. Both groups reported an accumulation of potato glycoalkaloids in the body,

and clearances of glycoalkaloids required >24 h. Also, the biological half-life of α-chaconine **2** was much longer than that of α-solanine **1**, and substantial interpatient differences were observed in both studies. Gastrointestinal effects were observed at doses of <2 mg/kg bw, which disagreed with previous reports. The fact that solanidine **16** has been detected in the blood after oral consumption of potatoes suggested that hydrolysis must occur at some stage;[216] however, it could also be an artifact of the analytical procedure. Glycoalkaloids have been demonstrated to disrupt epithelial barriers in a dose-dependent fashion in both cell culture models and in sheets of mammalian intestine. Furthermore, IL-10 gene-deficient mice, animals with the genetic predisposition to develop inflammatory bowel disease, demonstrated a greater degree of small intestinal epithelial barrier disruption and inflammation when their epithelium was exposed to potato glycoalkaloids.[286]

BENEFICIAL EFFECTS OF GLYCOALKALOIDS AND THEIR AGLYCONES

Although glycoalkaloids are perceived as potentially toxic, studies during the past 10 years suggest that they may also possess beneficial effects depending on dose and conditions of use. These potential uses include anticancer, anti-inflammatory, antinociceptive, antipyretic, anticholesterol, antifungal, and antibacterial effects.

Anticancer Properties of Glycoalkaloids and Aglycones. Most, perhaps all, human tumors possess six essential alterations in cell physiology that collectively dictate malignant growth: self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, evasion of programmed cell death (apoptosis), limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis.[287,288] Glycoalkaloids have exhibited apoptotic activity and chemopreventative effects against known carcinogens, which are discussed in the following sections.

Aubergine Glycoalkaloids and Aglycones. α-Solamargine **6** has been shown to inhibit growth in a number of cancer cell lines, for example, colon, prostate, breast, human hepatoma, JT-26 cells,[289] and human lung cancer cells (H441, H520, H66, H69).[290] It has been demonstrated to exhibit a greater cytotoxic effect than cisplatin, methotrexate, 5-fluorouracil, epirubicin, and cyclophosphamide against human breast cancer cell lines.[291] The accepted mechanism of action is generally described as apoptotic. The rhamnose moiety of the trisaccharide of α-solamargine **6** is essential for anticancer activity; this was determined by comparing the activity of α-solamargine **6** with that of the structurally similar glycoalkaloid khasianine **47** (Figure 14). The only difference in structure between α-solamargine **6** and khasianine **47** is the absence of L-rhamnopyranosyl moiety in the attached saccharide. A dramatic decrease in anticancer activity was observed with khasianine **47** due to the absence of this monosaccharide.[292] Shiu et al.[291] outlined the mechanism of α-solamargine **6** apoptosis as follows: α-solamargine **6** up-regulates the expressions of external death receptors and also leads to an increased intrinsic ratio of Bax to Bcl-2, which results in the release of mitochondrial cytochrome *c* and activation of caspase-8, caspase-9, and caspase-3 in the cells, which indicates that α-solamargine **6** triggered extrinsic and intrinsic apoptotic pathways of breast cancer cells.[291,293]

Cisplatin causes cancer cell apoptosis through caspase-8/caspase-3 and Bax/cytochrome *c* pathways, but the resistance to cisplatin is correlated with Bcl-2 and Bcl-xL overexpression.

However, the overexpression of Bcl-2 and Bcl-xL can be broken through by α-solamargine **6**. The combined treatment of α-solamargine **6** with cisplatin significantly reduced Bcl-2 and Bcl-xL expressions and enhanced Bax, cytochrome *c*, and caspase-9 and -3 expressions in breast cancer cells. Thus, the combined use of α-solamargine **6** and cisplatin may be effective in cisplatin-resistant breast cancer.[291,294]

In addition to the mechanism of action described above, α-solamargine **6** also has been employed as a mechanism to overcome drug resistance in human breast cancer cell lines. Overexpression of HER2 [human EGFR (epidermal growth factor receptor)2]/neu is associated with drug resistance, promotion of angiogenesis, lymph node metastasis, and a poor prognosis in breast cancer.[295] On activation, HER2/neu promotes cell proliferation in a manner similar to that of a growth factor. In normal cells, the HER2/neu receptor triggers signal transduction pathways that control normal cell growth, differentiation, motility, and adhesion in several cell lines.[296] In tumor cells, HER2/neu gene expression is uncontrolled. Amplification of the HER2/neu gene and protein overexpression have been demonstrated in a variety of human carcinomas, including ovarian and breast cancers.[294] α-Solamargine **6** induced downregulation of the HER2/neu expression and enhanced cisplatin, methotrexate, and 5-fluorouracil;[297] trastuzamab and epirubicin mediated toxicity.[298,299]

There are very few reports of cytotoxic effects of α-solasonine **5**; however, it has been reported to act synergistically with α-solamargine **6** against cancer cell lines.[65] Curaderm^BEC5, a proprietary plant preparation containing α-solasonine **5** and α-solamargine **6** (both being solasodine glycoalkaloids), is now available for the treatment of skin cancers, and an intravenous preparation has entered a phase II clinical trial for the treatment of internal cancers. Furthermore, when these glycoalkaloids were administered by intralesion injection into solid tumors, a rapid regression of the tumor was observed.[300−307]

Solasodine **34**, the aglycone of α-solamargine **6** and α-solasonine **5**, has exhibited potent antitumor activity; furthermore, the hydrochloride salt of solasodine **34** is in preclinical trials.[308,309] Trouillas et al.[310] observed DNA fragmentation when human1547 osteosarcoma cells were treated with α-solasodine **34** for 24 h, suggesting apoptosis induction. Interestingly, when the anticancer activity of solasodine **34** is compared with the glycoalkaloids α-solamargine **6** and α-solasonine **5**, the activity is comparable; therefore, the trisaccharide side chain does not appear to be essential for activity, which is an unexpected result when compared with the potato glycoalkaloids.[44,292] Synthetic modification of the solasodine aglycone at the 3-hydroxyl position has led to a dramatic increase in in vitro anticancer activity in PC-3 cell lines (Figure 11).[311]

Li et al.[79] examined α-solamargine **6** and α-solasonine **5** and their hydrolysis products for their antiproliferative activities. They were examined against HCT-8 tumor cells and via a MTT assay. The results show that α-solamargine and α-solasonine exhibit strong cytotoxic activities with IC_{50} values of 10.63 and 11.97 μmol/L, respectively. 6-O-Sulfation was performed on the sugar chain of the glycoalkaloids and the products examined for their anticancer activity, but no increase in activity was observed. To ascertain the importance of the heterocyclic nitrogen in the F ring, Kim et al.[312] acetylated at this position, and a complete loss of anticancer activity resulted.

A number of plant extracts containing the spirosolane glycoalkaloids α-solamargine **6** and α-solasonine **5** and the aglycone

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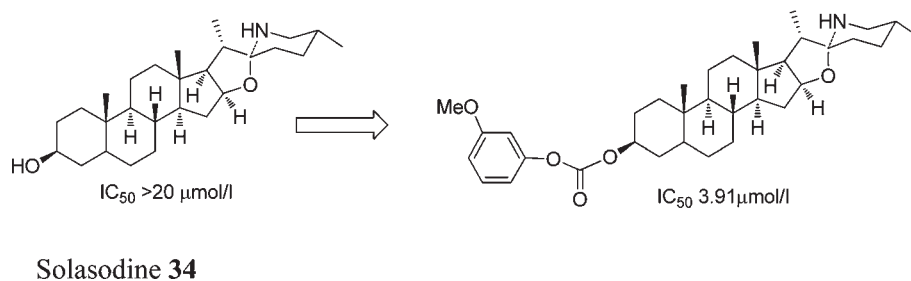
Figure 11. Synthetic modification of solasodine **34** results in increased anticancer activity.

solasodine **34** have been examined for their anticancer activity. Extracts from *Solanum xanthocarpum*, which contained the glycoalkaloids α-solamargine **6** and α-solasonine **5**, the hydrolysis product β_2-solamargine **49**, and the aglycone solasodine **34** were examined for activity. Once again, the rhamnose sugar was found to be essential for activity as β_2-solamargine **49** was inactive against colon carcinoma cells. Solasodine **34** was only weakly toxic, which contrasts previous reports.[313] Cytotoxic activity of extracts from *S. crinitum* and *S. jabrense* were examined against Ehrlich carcinoma and human K562 leukemia cells; solasodine **34** displayed negligible activity, and the acetylated form of α-solasonine **5** displayed the highest cytotoxic effect in this study.[314]

Solanum aculeastrum Dunal is a medicinal plant that has long been used to treat various cancers and many other conditions in the Eastern Cape Province of South Africa.[315] *S. aculeastrum* caused significant inhibition of growth in HeLa MCF7 and HT29 cells by induction of cell cycle arrest and apoptosis.[316] However, it is imperative to note that these extracts not only inhibited the growth of cancer cell lines but were also cytotoxic to noncancerous cells. Therefore, there are significant safety considerations due to the lack of differentiation between cells. Aboyade et al.,[317−319] who examined the toxicity of these extracts in the male Wistar rats for 28 days, observed negligible toxic effects, but further studies are required to examine efficacy.

Potato Glycoalkaloids. The cytotoxic effects of a range of concentrations (0.016, 0.08, 0.4, 2, 10 μg/mL) of α-solanine **1** were investigated on several tumor cell lines from the digestive system and were screened using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The apoptotic potential of α-solanine **1** in tumor cells was evaluated by laser confocal microscopy; this examined any morphological changes, apoptosis, rate, and cell cycle of the tumor cells. Human hepatocarcinoma (Hep G2) cells are relatively sensitive to the cytotoxic effect of α-solanine **1**, with an IC$_{50}$ of 14.47 μg/mL. The other cell lines investigated, SGC-7901 and LS-174, were found to be less sensitive, with IC$_{50}$ values of >50 μg/mL. The rate of apoptosis in Hep G2 cells was found to have a dose-dependent response, with the highest apoptotic rate of 32.2% achieved for 10 μg/mL of α-solanine **1**. Cell cycle observations revealed that α-solanine **1** suppressed the cells of the G$_2$/M phase, and the S phase cells increased dramatically for treated groups. Western blot revealed that α-solanine **1** induced apoptosis in Hep G2 cells by inhibiting the expression of Bcl-2 protein. α-Solanine **1** can inhibit the proliferation of human liver cancer cells Hep G2 in vitro, but it has a potent toxicity (LD$_{50}$ = 45 mg/kg) intraperitoneally, so safety experiments should be performed prior to preventive or therapeutic treatments against carcinomas in vivo.[320]

The antiproliferative activity and cytotoxicity of 15 *Solanum jamesii* accessions at two concentrations (5, 10 μg/mL) were examined for their inhibition of human colon (HT-29) and (LNCaP) prostate cancer cell lines. Inhibition of proliferation was observed in both HT-29 and LNCaP, although proliferation of HT-29 was significantly reduced with 5 μg/mL of all *S. jamesii* tuber extracts; however, 10 μg/mL was required to inhibit proliferation of LNCaP cancer cell lines. No cytotoxic effects were observed when compared with the controls. It should be noted that the tuber extract also contained a variety of other compounds, for example, chlorogenic acid, caffeic acid, sinapic acid, rutin hydrate, and myricetin. The inhibition of proliferation may require the presence of these other phytochemicals.[321] Lee et al.[44] examined the potato glycoalkaloids α-chaconine **2** and α-solanine **1** and their hydrolysis products at four concentrations (0.1, 1, 10, 100 μg/mL) against human colon (HT-29) and liver (Hep G2) cell lines over 4 h to assess the role of the glycosides in the anticancer activity of glycoalkaloids. The parent glycoalkaloids were the most potent compounds in all test systems once again; the liver cells displayed higher sensitivity to the glycoalkaloids examined: 39.5% inhibition of liver cells was observed with 0.1 μg/mL of α-chaconine **2**. Although the inhibition of both cell lines increased with concentration, the increase was not a linear function of concentration. At concentrations of 100 μg/mL glycoalkaloid, a minor reduction in inhibition is observed when compared with inhibition by the hydrolysis products at the same concentration; however, this is more likely due to a cytotoxicity at high concentrations. At 10 μg/mL a comparison of the inhibition of cancer cells between glycoalkaloid and hydrolysis products shows a significant reduction in activity. The potencies reported for α-tomatine **3** and α-chaconine **2** (1 μg/mL) against the liver (Hep G2) cells were higher than the anticancer drugs doxorubicin and camptothecin. Different combinations of α-chaconine **2** and α-solanine **1** were examined for synergistic activity, but a comparable reduction in MTT activity was observed in both AGS and Hep G2 cell lines. The selectivity of glycoalkaloids for cancer cells was ascertained by examining α-tomatine **3**, α-chaconine **2**, and α-solanine **1** on normal human liver Chang cell lines. Comparable growth inhibition of normal Chang cell lines was observed with cancer cell lines.[44]

Different concentrations (25, 50, 100 μg/mL) of α-chaconine **2** and α-solanine **1** from five varieties of potatoes (Dejima, Jowon, Sumi, Toya, Vora valley) were used to examine the effect of altering the concentration and ratios of these compounds on the growth of cancer cell lines. HeLa cervical, Hep G2 liver, U937 lymphoma, AGS and KATI II stomach human cancer cells, and Chang normal human liver cells were examined.[43] Both individual glycoalkaloids and glycoalkaloid mixtures were in most cases more active in the Hep G2 cancer cell lines than in the Chang normal human liver cell lines. Furthermore, three concentrations (0.1, 1, 10 μg/mL) of α-chaconine **2** and α-solanine **1** were

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initially examined individually with both the MTT assay and microscopy. In both α-chaconine **2** and α-solanine **1** the inhibition increased with dose and converged at higher concentrations, with α-chaconine **2** showing increased inhibitory activity. It had been suggested that this may be due to a toxicity mechanism. However, a significant disappearance of cancer cells was observed in the photographs, which is consistent with an anticarcinogenic mechanism. It was also shown that certain combinations of glycoalkaloids that acted synergistically may improve the therapeutic outcome of these compounds.[43]

In conclusion, the undifferentiating destruction of both cancer and noncancerous cell lines in the aforementioned studies,[43,44] leads to questions of therapeutic uses of glycoalkaloids due to safety considerations. However, it is difficult to translate the results of an in vivo trial in vitro. Therefore, both animal and human experiments are essential to confirm or disprove the in vivo data observed in these studies. Moreover, this acute toxicity is in contradiction with a recent study in which concentrations of ~200 mg/kg (via mashed potato) were fed to human volunteers and no adverse systemic effects were observed,[285] which further adds to the safety debate concerning glycoalkaloids.

α-Chaconine **2** has been reported to possess antimetastatic activity;[322] this was tested in a dose- and time-dependent manner by examining its activity against the highly metastatic A549 cells, which were treated with various concentrations of α-chaconine **2** (1, 1.25, 1.5, 1.75, 2 μg/mL) in the wound-healing assay and the Boyden chamber assay over 24 and 48 h. When examined at concentrations between 0 and 1.5 μg/mL, compared to the untreated control, α-chaconine **2** did not significantly affect the A549 cells, which indicates that α-chaconine **2** is not cytotoxic to the A549 cells at these doses. Cell viability significantly decreased at higher doses (1.5–2 μg/mL of α-chaconine **2**) for 24 and 48 h as a result of a dose- and time-dependent loss in A549 cell viability. Therefore, doses below 1.5 μg/mL of α-chaconine **2** were employed to examine antimetastatic effects of α-chaconine **2** on A549 cells. α-Chaconine **2** caused a dose-dependent inhibition on the migration and invasion of the metastatic A549 cells. An optimum concentration of 1.5 μg/mL of α-chaconine **2** led to an inhibition of migration of A549 cells of 75% after a 48 h incubation period. The mechanism of action was described as a reduction of matrix metalloproteinase-2 and matrix metalloproteinase-9 activities, which involved the suppression of the phosphoinositide 3-kinase/AKT/NF-κB signaling pathway.[322]
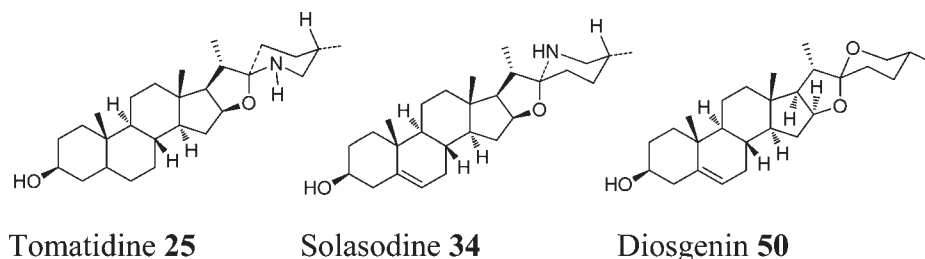
Tomato Glycoalkaloids. A mixture of α-tomatine **3** and dehydrotomatine **4** (10:1 respectively) produced a strong inhibitory effect to the growth of liver cancer and human colon cancer cell lines employing the in vitro MTT assay. A dose-dependent inhibition of HT29 colon cancer cells at levels ranging from 38.0 to 81.5% and of human HepG2 cancer cells at levels from 46.3 to 89.2% was reported. To further expand this study and elucidate the mechanism of action, Friedman et al.[44] examined different concentrations of α-tomatine **3**.[44] Six green and three red tomato extracts, from different varieties and various levels of maturity, were investigated for their ability to induce cell death in human cancer and normal cells using a MTT assay. Pure samples of α-tomatine **3**, dehydrotomatine **4**, tomatidine **25**, and tomatidenol **27** were also examined for anticarcinogenic effects. Compared to untreated controls, the high-tomatine green tomato extracts strongly inhibited the growth of the following human cancer cell lines: breast (MCF-7), colon (HT-29), gastric (AGS),

and hepatoma (liver) (Hep G2), as well as normal human liver cells. There was little inhibition of the cells by the three red tomato extracts, which contained a low tomatine concentration. α-Tomatine **3** was highly effective in inhibiting all of the cell lines. Dehydrotomatine **36**, tomatidenol **27**, and tomatidine **25** had little, if any, effect on cell inhibition. The results show that the susceptibility to destruction varies with the nature of the alkaloid and plant extract and the type of cancer cell. The mechanism of action is not yet fully understood; however, it was suggested that it may be the result of multiple molecular events including the formation of complexes with cholesterol, potentiation of the immune system, and direct destruction of cancer cells via disruption of cell membranes. This latter process is initiated by binding (intercalation) of tomatine to cholesterol located within cell membranes. It was concluded that the bioavailability and in vitro binding of carcinogens by both tomatine and dehydrotomatine require further study.[323]

The cytotoxicity of esculeoside A **37**, a relatively new glycoalkaloid, was ascertained by Fujiwara et al.[78] and was found to be less cytotoxic to MCF-7 human breast cancer cell lines and less active than α-tomatine **3**. When Lee et al.[44] examined a range of glycoalkaloids and metabolites against colon and liver cancer cells, the potencies of α-tomatine **3** and α-chaconine **2** inhibition at a concentration of 1 μg/mL were found to be higher than those of the anticancer drugs doxorubicin and camptothecin. The hydrolysis products β_1-**42**, γ-**44**, δ-tomatine **45**, and tomatidine **25** were examined for their inhibitory activity, and unlike the potato and aubergine glycoalkaloids, a dramatic reduction in activity was observed upon removal of each glycoside.

α-Tomatine **3** has also been reported to have antimetastatic properties in human lung adenocarcinoma. α-Tomatine **3** can inhibit the invasion and migration of A549 human adenocarcinoma cells in an in vitro model. α-Tomatine **3** can suppress cancer cell invasion, and the inhibition of migration possibly may occur through inactivation of PI3K/Akt or ERK signaling pathways, exerting inhibitory effects on NF-jB, c-Fos, and c-Jun transcriptional factors, inhibiting NF-kB and AP-1 DNA binding activities, decreasing MMP-2, MMP-9, and u-PA activities, and then having an antimetastatic effect.[324] In vivo studies in rainbow trout have shown α-tomatine **3** to possess chemopreventive properties. Dibenzo[*a,l*]pyrene (DBP) is a well-known carcinogen. Upon cofeeding α-tomatine **3** with DBP, a reduction of 48.7% in the incidence of liver tumors was observed as was a 36.6% reduction in stomach tumor formation compared with DBP alone. The tomatine-containing diet did not induce changes in mortality, and no adverse pathological effects were observed.[323]

Anti-inflammatory, Antinociceptive, and Antipyretic Effects. The anti-inflammatory effects of tomatoes were first observed in the 1960s; the isolation of two antihistamine-like substances from crown gall-bearing tomato stems was reported, and one of the active constituents was identified as α-tomatine.[325−328] When α-tomatine **3** was tested on isolated organ preparations, it was found to exert a nonspecific effect in antagonizing the contractions induced by histamine, bradykinin, SRS-A, acetylcholine, and 5-hydroxytryptamine.[328] When injected into guinea pigs, α-tomatine **3** was found to be highly active in preventing the effects of intradermally injected histamine and bradykinin on capillary permeability, and it exerted some protection against the effects of a lethal histamine aerosol.[328] Filderman et al.[329] examined the anti-inflammatory activity of α-tomatine **3** in rats using the carrageenan-induced rat

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Tomatidine **25**   Solasodine **34**   Diosgenin **50**

Figure 12. Chemical structures of the aglycones **25** and **34** and the glucocorticoid **50**.

paw edema test as most anti-inflammatory drugs seem to possess anti-carrageenan activity. It was demonstrated that α-tomatine **3** exhibited a fairly potent anti-inflammatory activity; however, the corresponding aglycone tomatidine **25** was ineffective at dose levels of 10−20 mg/kg in all three tests. However, it was subsequently reported that the potent anti-inflammatory effects of tomatidine **25** caused a decrease in nitric oxide production by 66%.[330] The structure of steroidal alkaloids is similar to that of glucocorticoids (Figure 12). Steroidal alkaloids are essentially nitrogen analogues of steroid saponins such as diosgenin, which is a precursor of steroidal hormones and anti-inflammatory steroids. In addition, corticoids mainly inhibit inflammatory responses requiring iNOS-mediated NO production and COX-2 expression. Tomatidine **25** suppressed the COX-2 protein level in a dose-dependent manner, and PGE2 production was decreased by tomatidine **25**. These results indicated that tomatidine **25** could modulate inflammatory effects through inhibition of the COX pathway.[330]

The ethanol extract of potato tubers (*S. tuberosum* L.) has been evaluated for antinociceptive and anti-inflammatory activities in mice. The acute treatment of mice with an ethanolic extract from the potato tuber at doses of 100 and 200 mg/kg, by oral administration, produced a significant antinociceptive effect in the acetic acid-induced writhing, formal induced pain licking, and hot-plate induced pain. Also, the ethanolic extract of potato tubers significantly inhibited both carrageenan- and formalin-induced inflammation in mice as well as arachidonic acid-induced ear edema in mice.[331] The methanol extract of *Solanum ligustrinum*, a native Chilean plant, contained steroidal glycoalkaloids and exhibited a significant anti-inflammatory effect in guinea pigs, and an antipyretic effect was observed in rabbits.[332] The chloroform and aqueous extract of *Solanum nigrum* displayed antinociceptive, anti-inflammatory, and antipyretic effects.[333,334]

Anticholesterol. One of the first reports of anticholesterol action of glycoalkaloids was in the 1950s; Schulz et al.[335] had shown tomatine **3** binds cholesterol in vitro in a 1:1 ratio. α-Solanine **1** and α-chaconine **2** also possess the cholesterol-complexing properties but with lowered activity in comparison.[336] Moreover, α-tomatine **3** cholesterol complex precipitation was quantified in vitro by utilization of radioactive cholesterol.[337] The aglycone of α-tomatine **3**, tomatidine **25**, did not possess cholesterol-binding properties,[240] which reinforces the requirement of the carbohydrate side chain for activity. Cayen tested complexation in vivo and reported that dietary α-tomatine **3** formed an insoluble complex with cholesterol in the rat and increased sterol excretion.[338] α-Tomatine **3** possessed the least toxic effects of all the glycoalkaloids in the rat. α-Tomatine **3** also increased hepatic and intestinal cholesterol synthesis, which was explained by the reduction of the amount of cholesterol reaching the liver by enterohepatic circulation.

Overall, no significant reductions of plasma levels of cholesterol, phospholipids, and triglycerides were observed.[338]

Sterol synthesis varies among species. The liver of the rat has an exceptionally high rate of sterol synthesis and makes an important contribution to the newly synthesized pool of sterol in the extrahepatic issue. In most other species, for example, the hamster, the liver has a negligible contribution to sterol synthesis.[339] Disruption of the cholesterol balance is regulated by the modulation of LDL transport in hamsters,[340−342] as it is in humans.[343] Therefore, the hamster is a better animal model to examine the anticholesterol effects of glycoalkaloid as it has analogous sterol synthesis pathways to the human.[344] Friedman et al.[42] demonstrated the low toxicity of α-tomatine **3** by feeding hamsters about 20 mg/day, and they grew at the same rate as the control hamsters. The diet that included tomatine decreased LDL cholesterol by 41%, did not change HDL cholesterol, and decreased the LDL/HDL cholesterol ratio by 45%. The same group also examined semipurified green or red freeze-dried tomato powders. In comparison to the control diet without tomatoes, 44 and 59% reductions in LDL cholesterol were observed for red and green tomatoes, respectively.[41]

Antimicrobial Activity. The antifungal, antibacterial, and antiviral activities of glycoalkaloids are well documented; such activity has been attributed to the fact glycoalkaloids are part of the plant's chemical defenses against various pathogens, namely, fungi, bacteria, and viruses. The potato glycoalkaloids α-solanine **1** and α-chaconine **2** have been demonstrated to inhibit spore generation and hyphal growth on agar of *Alternaria brassicicola* and *Phoma medicaginis* and on growth in liquid culture of these species and *Ascobolus crenulatus* and *Rhizoctonia solani*.[3,6] Administration of α-solanine **1** alone caused minor inhibition; however, a marked enhancement in inhibition was observed upon coadministration with α-chaconine **2**,[3,6] once again reiterating the synergistic effects of glycoalkaloids. A significant reduction in activity of the glycoalkaloid solely was observed at pH 6; however, the mixture of glycoalkaloids was not as susceptible to changes in pH. *R. solani* generally exhibited reduced sensitivity, and this was attributed to the fact that it is a major potato pathogen,[3,6] which supports the earlier thesis that specialist pests and pathogens are less affected by host-specific glycoalkaloids. Fewell et al.[4] also suggested that the germination of spores is a better test parameter than measurement of hyphal extension. In *P. medicaginis* a dramatic reduction in hyphal extension was observed with solamargine **6** and solasonine **5**, but no significant inhibition of spore germination was observed at relatively high concentrations. In contrast, α-solanine **1** induced an increase in spore production and a reduction in hyphal extension when examined on *Fusarium caeruleum*.[5] A crude extract of tomatoes was shown to inhibit the in vitro growth of *Fusarium oxysporum* f. sp. *lycopersici*.[7] *Penicillium notatum* growth has also been inhibited

by crude α-tomatine **3**.[8] Later, α-tomatine **3** was shown to exhibit a reduction in vegetative development but an increase in sporulation in *F. oxysporum* f. sp. *lycopersici* species.[9] Therefore, due care is advised in the interpretation of results regarding the inhibition of fungal growth by glycoalkaloids; preferably both sporulation and hyphal growth should be examined simultaneously.

Solamargine **6** and solasonine **5** have been shown to inhibit the development of mycelium in *P. medicaginis* and *R. solani*. A pH effect was also observed with these glycoalkaloid; generally, a greater inhibition was observed with increasing pH. Solamargine **6** exhibited the highest potency, and *P. medicaginis* proved to be the more susceptible species. Solasonine was inactive against *R. solani* over the pH range of 5−8. Synergism was also observed between these glycoalkaloids, especially against *R. solani*, which otherwise was immune to glycoalkaloid inhibition. A mixture (1:1) of the two glycoalkaloids produced a marked inhibition. The synergistic inhibition had reduced sensitivity to pH changes, and spore germination in *Alternaria brassicicola* was significantly reduced by solamargine **6** but unaffected by solasonine **5**. In *P. medicaginis*, neither glycoalkaloid caused inhibition. In combination, the two compounds caused synergistic inhibitory effects in both species, but to a much greater extent in *A. brassicicola*. The radial growth of *Alternaria solani*, the fungus responsible for early potato blight, was inhibited by the glycoalkaloids α-solanine **1** (33% inhibition) and α-chaconine **2** (56%), whereas the aglycone solanidine showed the highest inhibitory activity (72%); this is a rare example of the aglycone moiety exhibiting an enhanced activity over the parent glycoalkaloid. An increase in the occurrence of *A. solani* was observed with a decrease of glycoalkaloid content in leaves due to plant age.[10]

A range of glycoalkaloids have exhibited antiviral activity. When tested against herpes simplex virus type 1, each of the glycoalkaloids examined exhibited activity (β-solamarine **29**, solamargine **6**, α-chaconine **2** > α-tomatine **3** > α-solasonine **5**);[345] the aglycones (solanidine **16**, solasodine **34**, and tomatidine **25**) proved inactive against the virus.[346] It would appear, therefore, that the sugar moiety is essential for activity. Moreover, α-solanine **1** showed negligible antiviral activity; as it shares the same trisaccharide as α-chaconine **2**, the identity of the aglycone structure determines the degree of activity. *Herpes simplex*, *Herpes genitalis*, and *Herpes zoster* are also reported to be inactivated by the glycoalkaloids solamargine **6** and solasonine **5**.[347] The inactivation of the virus has been suggested to result from insertion of the glycoalkaloid into the viral envelope.[346] Extracts from *Solanum torvum* inhibited herpes simplex virus type 1 activity.[348] The antiviral activity of glycoalkaloids is pH dependent.

Ethanol, acetone, and water extracts from the fruits and leaves of *S. aculeastrum*, which is used in traditional medicine to treat various human and animal diseases, were examined for activity against a range of bacteria: *Bacillus cereus*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Micrococcus kristinae*, *Streptococcus pyogenes*, *Escherichia coli*, *Salmonella pooni*, *Serratia marcescens*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. The leaf extracts of the two solvents showed moderate activity against *Micrococcus kristinae*. The acetone extract of the leaves showed activity against Gram-positive bacteria except *Staphylococcus epidermidis*, whereas there was no activity against the Gram-negative bacteria at the highest concentration tested with the exception of *Salmonella pooni*. In a similar experiment, moderate activity by *Solanum aculeastrum* was reported against *E. coli*,

S. aureus, *P. aeruginosa*, *K. pneumoniae*, *S. faecalis*, and *B. subtilis*.[349,350] α-Solanine **1** and α-chaconine **2** showed a concentration-dependent inhibition of *Corynebacterium sepedonicum*.[351] Extracts of *Solanum nigrescens* were confirmed as useful herbal treatment of *C. albicans* vaginitis.[352] Antidermatophytoses activity was observed with extracts of *Solanum americanum* and *Solanum nigrescens*.[353] Mice treated with low levels of α-solanine **1** and α-chaconine **2** were resistant to lethal doses of *Salmonella typhimurium*.[354]

The antimalarial activity of glycoalkaloids has been examined in mice; α-chaconine **2** displayed the highest degree of suppression (76% suppression of parasitemia) of malarial infection in a dose-dependent fashion. α-Tomatine **3**, α-solamargine **6**, α-solasonine **5**, and α-solanine **1** displayed 65, 64, 57, and 41% suppression of parasitemia, respectively. Furthermore, to examine the importance of the carbohydrate moiety, the 6-O-sulfated chaconine was examined, but this led to a reduction of antimalarial activity (42%), indicating the importance of the free hydroxyl group on the chacotriose **12** side chain of α-chaconine **2**.[355]

■ MECHANISM OF ACTION: MEMBRANE DISRUPTION

The biological activity of glycoalkaloids of the *Solanum* species derives mainly from two properties: (a) inhibition of acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholinesterase (BuChE, EC 3.1.1.8) (AChE is responsible for terminating cholinergic transmission at the neuromuscular junction and the central nervous system); and (b) complexation with membrane 3β-hydroxy sterols, thereby causing membrane disruption and loss of integrity of the membrane.

The inhibition of acetylcholinesterase by *Solanum* glycoalkaloids was first reported in 1953 by Pokrovskii,[356] who demonstrated that the water extracts of the sprouting potato tubers suppressed the activity of the nonspecific blood serum cholinesterase. Later, Orgell et al.[37] discovered that a wide variety of aqueous extracts of a number of solanaceous plants possessed human acetylcholinesterase inhibitory activity.[357] α-Chaconine **2** is a potent inhibitor of a number of insect AChE, except for the Colorado potato beetle, a specialist pest; this been attributed to insect adaptation due to feeding on potato foliage. The inhibition of AChE and BuChE is an important effect, but there have been varying reports of activities, which has partially been attributed to the fact that enzymes from a variety of sources (plasma vs serum) have been examined (Table 4). For example, AChE from fetal bovine serum is less sensitive to AChE inhibition by α-tomatine **3** than human AChE.[358] Another source of discrepancy could be differences in the purity of the glycoalkaloids and aglycones being tested. Roddick et al.[36] and Nigg et al.[38] purchased high-purity (<95%) glycoalkaloids and aglycones, whereas Orgell et al.[357] and Harris and Whittaker[359] examined purified extracts from solanaceous plants.

It is clear from Table 4 that the aglycone unit alone is largely inactive against the cholinesterase enzymes. However, the marked reduction in inhibitory activity of the solasodine-based glycoalkaloids α-solasonine **5** and α-solamargine **6**, which possess the same trisaccharide units as the solanidine-based glycoalkaloids, indicates that, even though the sugar unit is required for activity, it is the structure of the aglycone unit which determines the extent of inhibition. Furthermore, the inaction of steroids that do not possess a nitrogen atom in the aglycone emphasizes the requirement of a heterocyclic nitrogen for activity.[360]

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Table 4. Reported Inhibition by Glycoalkaloids of AChE and BuChE in the Literature

glycoalkaloid/aglycone	concentration (μM)	cholinesterase	% inhibition (max)	ref
chaconine 2	2.88	human BuChE	67	38
chaconine 2	10	bovine AChE	54	36
chaconine 2	40	insect AChEs	>50	37
chaconine 2	40	bovine AChE	45	39
chaconine 2	40	eel AChE	26	39
solamargine 6	100	bovine AChE	13	36
solanine 1	potato peel extract	human AChE	80	359
solanine 1	40	bovine AChE	45	39
solanine 1	40	eel AChE	26	39
solanine 1	2.88	human BuChE	47	38
solanine 1	2.88	human BuChE	50	38
solanine 1	10	bovine AChE	44	36
solanine 1	5	human AChE	50	357
solanine 1 + chaconine 2	5 (1) + 5 (2)	bovine AChE	37	36
solasonine 5	100	bovine AChE	16	36
solasonine 5 + solamargine 6	100	bovine AChE	0.5	36
tomatine 3	33	bovine and eel AChE	negligible	39
tomatine 3	100	human AChE	57	36
tomatine 3	100	human AChE	∼50	357
solanidine 16	100	bovine AChE	11	36
tomatidine 25	100	bovine AChE	15	36
solasodine 34	100	bovine AChE	9	36
solanidine 16	100	human AChE	14	36
tomatidine 25	100	human AChE	−10	36
solasodine 34	100	human AChE	0.6	36

Unlike membrane lysis, the inhibition of AChE and BuChE has been shown to be pH independent.[36] No evidence of synergism was observed in the inhibition of these enzymes by the glycoalkaloids.[36] The inhibition of BuChE by glycoalkaloids is reversible[38] as it meets the criteria of reversibility outlined by Dawson;[361] for example, inhibition is reversed by dilution, independent of time, decreases with increasing substrate concentration, and reversed by dialysis. However, Alozie et al.[362] classified α-chaconine 2 as a noncompetitive mixed-type inhibitor, and rat BuChE was inhibited by 49% in vivo when α-chaconine 2 was administered 10 mg/kg intraperitoneally.

The second principal mechanism of action of glycoalkaloids is the disruption of biological membranes. Individual animal cells, fungal tissues, and plant organelles have been utilized as test systems, but liposomes are good models for membrane-disruptive properties of glycoalkaloids as they mimic the phospholipid bilayer in vivo, a fact that aids mechanism elucidation. α-Tomatine 3 has displayed a pH dependence on the leakage of peroxidase from liposomes; the optimum pH for disruption was found at pH 7.2,[363] which is consistent with findings of disruption of animal and plant cells.[363,364] Concentrations between 10 and 100 μM are required for disruptive activity. The extent of loss of membrane integrity was correlated with the concentration of sterols in the membrane.[365] Unlike acetylcholinesterase inhibition, a marked synergistic effect has been reported between the potato glycoalkaloids and the destabilization of cell membranes. α-Solanine 1 has been shown to have negligible membrane disruption at concentrations up to 1 mM. α-Chaconine 2, on the other hand, led to extensive membrane destabilization at ≤100 μM.[5,11,366−368] However, upon coadministration of chaconine

and solanine (50:50 ratio), the maximum leakage of peroxidase into the supernatant, that is, liposome membrane lysis, was observed (70%).[369]

Segal et al.[364,370,371] proposed that the aglycone liberated by surface glycosidases was the active form in membrane studies. Roddick et al.[365] reported that the importance of the carbohydrate moiety was also important as β_2-chaconine 51, which differs only in one rhamnose moiety from α-chaconine 2, was also examined, and a dramatic decrease in lysis was observed when compared to α-chaconine 2. All of the activity was attributed to the binding of 3β-hydroxy sterols in the membrane.[369] Synergism between α-solanine 1 and α-chaconine 2 has also been documented in living systems such as animal, plant, and viral cells. Roddick et al.[372] examined rabbit erythrocytes, red beet cells, and *Penicillium notatum*. Once again, α-chaconine 2 displayed higher membrane disruptive activity than α-solanine 1, with maximum activity toward erythrocytes and fungal protoplasts being observed with a 70% α-chaconine 2/30% α-solanine 1 mixture, whereas a 40% α-chaconine 2/60% α-solanine 1 mixture produced maximal effects with beet cells.

Keukens et al.[32−34] carried out a number of systematic studies on model membranes to ascertain the mechanism of action of glycoalkaloid-induced membrane disruption. Lipid vesicles were employed as test systems, and sterols present in the membrane were found again to be required for glycoalkaloid activity. Molecular modeling between solanidine aglycone and cholesterol was also performed to predict the orientation of the molecules in the lipid bilayer. Another determining factor of activity was the requirement of a planar-structured sterol, especially in the case of α-chaconine 2; α-tomatine 3 was found to be far less susceptible

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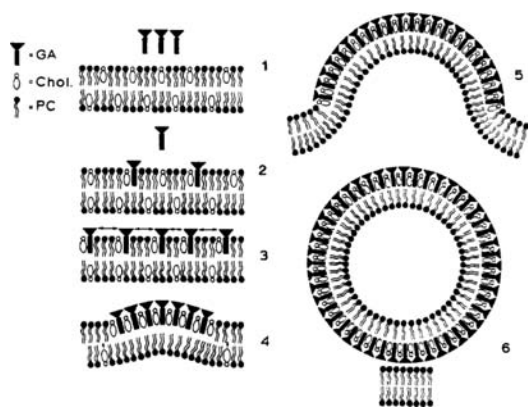dx.doi.org/10.1021/jf200439q |*J. Agric. Food Chem.* 2011, 59, 3454–3484

Figure 13. Mechanism of glycoalkaloid-induced membrane disruption [32].

to the planarity of the sterol. The hydrolyzed sugars of α-chaconine **2** and α-tomatine **3** were examined for their membrane lytic properties, and, in all cases, the tri- and tetrasaccharide sugars of α-chaconine **2** and α-tomatine **3**, respectively, were required for activity. Once again, synergism was observed with α-solanine **1** and α-chaconine **2**; although when α-chaconine **2** and α-tomatine **3** were combined, no synergistic effect was observed.

Therefore, a stepwise mechanism of membrane disruption was proposed for α-chaconine **2** as follows (Figure 13): With insertion of the glycoalkaloids into the membrane, the aglycone moiety reversibly binds to sterols in the membrane in a 50:50 ratio (step 2). When the glycoalkaloid—sterol complexes reach a certain density in the phospholipid bilayer, intermolecular electrostatic interactions between the glycosidic residues of the glycoalkaloids catalyze the development of an irreversible matrix of glycoalkaloid—sterol complexes (steps 3 and 4). In turn, the sterols in the external membrane become immobilized due to complexation; therefore, Keukens proposed that the sterols of the inner leaflet will probably invert and substitute the immobilized sterol glycoalkaloid layer. Membrane budding will arise due to the fact that the glycoalkaloid—sterol complex, which possesses a relatively large polar headgroup, does not have a cylindrical shape (steps 5 and 6). The phospholipids of the interior leaflet of the membrane become encapsulated in the final structure during separation from the membrane and form a monolayer (step 6). Tubular structures are formed due to the three-dimensional structure of the sugar moiety, causing faster growth of the matrix in one direction compared to the other. Keukens then examined his theories derived from model membranes on biomembranes, and his mechanism of action of glycoalkaloids on membranes translated effectively to the biological membranes.[33]

Walker et al.[373] compared the interaction of α-tomatine **3** with mixed monolayers containing sterols, the phospholipids, and egg sphingomyelin. Once again, a pH dependence was observed for disruption of the bilayer. α-Tomatine **3** interacted strongly with sitosterol **52** and cholesterol **53**, although no interaction was observed with sitosterol glycoside **54**. Glycoalkaloids were also shown to adversely affect intestinal permeability in both cell culture models and in sheets of mammalian intestine. Furthermore, IL-10 gene-deficient mice and animals with the genetic predisposition to develop irritable bowel syndrome were more susceptible to glycoalkaloid-induced membrane disruption and inflammation.[286] Increased expression of cholesterol biosynthesis genes in Caco-2 cells, in a concentration-dependent response, was observed. It was suggested that this could be utilized

as a biomarker for glycoalkaloid-induced toxicity. Other signaling pathways that have been altered upon exposure of intestinal epithelial cells to glycoalkaloids include growth factor signaling pathways mediated by EFG, HGF, VEGF, IFG-R1, and EGFR and the P13K/AKT, JNK and ERK signaling pathways. These pathways are essential for a diverse array of cellular processes and are possibly involved in glycoalkaloid-induced toxicity.[374−376]

To further elucidate the membrane activity of glycoalkaloids, modified chacotriose-containing glycoalkaloids were examined. Rings E and F were modified; all were attached to the chacotriose sugar and tested for activity (Figure 14).[35] The membrane disruption activity of the glycoalkaloids was lost with the opening of ring E of the aglycone, which alters the rigidity and enhances the basicity of the glycoalkaloid. Dihydrosolasodine A chacotrioside **55** exhibited no lysis at 500 μM; no acetylcholinesterase inhibition was observed either. N-Nitrosolamargine **56** possessed no anticholinesterase activity or lytic properties, which indicates the nitrogen lone pair is required for activity. The nitrogen lone pair has been proposed to be involved in the formation of a bioactive iminium ion species.[312] β-Solamarine **28** displayed marked erythrocyte- and liposome-disruptive properties. Overall, it was concluded with respect to the aglycone subunit that an intact E ring, the unshared pair of electrons on the nitrogen of the F ring, and the solanidane and spirosalane rings are essential for membrane lytic activity.[35]

A final mechanistic aspect of the membrane-disruptive activity is the influence of glycoalkaloids on the membrane potential and sodium transport of biomembranes. FETAX was employed to examine the effects of potato glycoalkaloids on frog membranes. The glycoalkaloids were shown to alter the membrane potential; in addition, a reduction in sodium active transport was reported.[272,273] α-Tomatine **3** exhibited the highest potency toward membrane depolarization. Enhanced permeability of the brush border was reported, which could in turn lead to an increase in uptake of macromolecules such as allergens. A synergistic effect with α-solanine **1** and α-chaconine **2** was also demonstrated.[377] Overall, a number of mechanistic pathways have been reported for the bioactivity of glycoalkaloids. It is clear, however, that anticholinesterase activity and membrane disruptive activity is involved. However, further mechanistic investigation is warranted.

■ SYNERGISTIC ACTIVITY OF GLYCOALKALOIDS

Isolates from Solanaceae typically possess naturally "paired" glycoalkaloids, for example, α-solanine **1** and α-chaconine **2** in the potato, α-solasonine **5** and α-solamargine **6** in the aubergine, and α-tomatine **3** and dehydrotomatine **4** in the tomato. The presence of paired glycoalkaloids has been principally attributed to plant evolution.[1] Glycoalkaloids have been tested for their bioactivity; however, a marked increase in their biological activities was observed when glycoalkaloids were administered as mixtures. Moreover, the biological potency is dependent on the ratio of the glycoalkaloid mixture employed.

The ratio of α-chaconine **2** to α-solanine **1** present in the potato, depending on the cultivar, ranges from 1.2 to 2.6:1. The ratio for peel is generally in the range of about 2:1, which is much higher than for flesh, with values near ∼1.5:1.[40,214] α-Chaconine **2** and α-solanine **1** have been examined on a vast array of biological systems, and α-chaconine **2** has always exhibited higher potency. However, a marked synergistic effect has been observed when α-chaconine **2** and α-solanine **1** are used in

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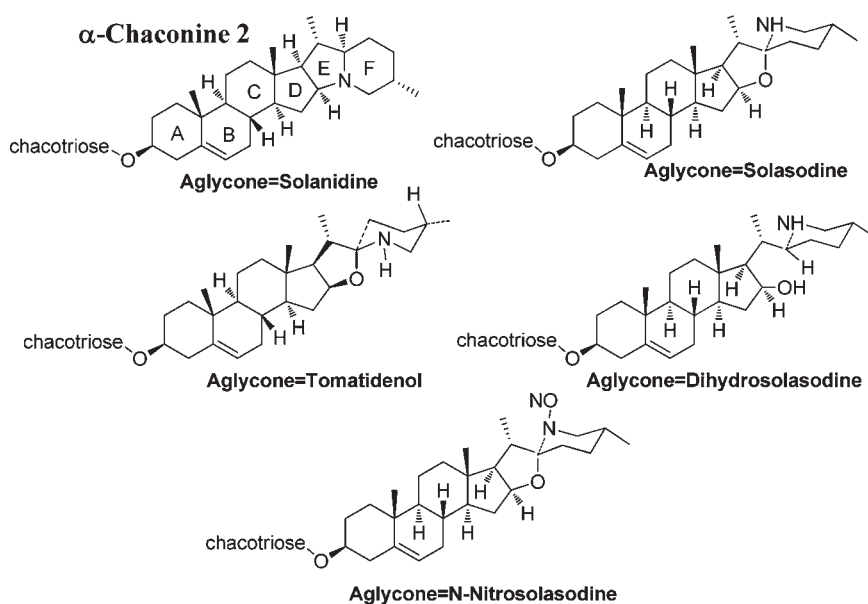dx.doi.org/10.1021/jf200439q |J. Agric. Food Chem. 2011, 59, 3454–3484

Figure 14. Structures of ring-modified chacotriose **12**-containing glycoalkaloids.[35]

combination. Roddick et al.[369,378] were some of the first to report the synergistic effects of glycoalkaloids; they found a 50% mixture of α-chaconine **2** and α-solanine **1** led to a significant increase in membrane-disruptive activity. It was also found that when one of the glycoalkaloids was replaced with α-tomatine **3**, digitonin **57**, or β_2-chaconine **39**, the synergistic activity was lost. The fact that β_2-chaconine **39** differs from α-chaconine **2** by only one rhamnose sugar indicates the specificity of the synergism and reiterates the importance of the carbohydrate residue for activity.

The individual and synergistic activities of α-solanine **1** and α-chaconine **2** were also examined in mammalian erythrocytes, red beet cells, and *Penicillium notatum*. Once again, α-chaconine **2** was found to have the highest lytic action in all three test systems. A 50% mixture of α-solanine **1** and α-chaconine **2** produced synergistic effects in all three test systems. In beet cells, the mixture had a greater toxicity than α-chaconine **2** alone. With erythrocytes and fungal protoplasts, the maximum synergistic activity was noted with mixtures containing approximately 70% α-chaconine **2**. With beet cells a 40% chaconine mixture possessed the highest disruptive activity. Cholesterol binding in vitro was also subject to synergistic effects, and the maximum response was observed to the 50% mixture.[372] Different potato cultivars were examined against human cervical, liver, lymphoma, and stomach cancer cells to examine the ratios of α-solanine **1** and α-chaconine **2**; synergism was observed, and it was concluded that further examination of different ratios was warranted.[43] Ratio-dependent synergistic activity was observed in inhibition of snail feeding[266] and development toxicity of *Xenopus* frog embryos.[379] The antifungal activity of α-solanine **1** and α-chaconine **2** was also examined against *Ascobolus crenulatus*, *Alternaria brassicicola*, *Phoma medicaginis*, and *Rhizoctonia solani*. Although α-chaconine **2** has higher antifungal activity, a 1:1 mixture of α-solanine **1** and α-chaconine **2** was shown to possess synergistic effects. A wide range of ratios showed enhanced activity in comparison to α-chaconine **2** alone.[3]

The synergistic effects of a range of glycoalkaloids were examined for their antifungal activity; as expected, a pronounced synergistic effect was observed with α-solanine **1** and α-chaconine **2**. The aubergine glycoalkaloids α-solasonine **5** and α-solamargine **6**, on the other hand, have received contrasting reports. When examined against fungal cultures isolated from *Solanum americanum* and *S. carolinense*[2] and two strains of *Trypanosoma cruzi*,[380] no evidence of synergism was documented. Contrastingly, when tested against *Phoma medicaginis* and *Rhizoctonia solani*, combinations of 50 μM of each glycoalkaloid produced synergistic effects against both fungi.[4] With regard to the membrane-disrupting properties of these glycoalkaloids, synergistic activity has also been reported. Different glycoalkaloid combinations were examined; with α-solamargine **6** and α-chaconine **2** the effect was additive, whereas α-solasonine **5** and α-solanine **1** together caused no lytic activity.[381] The synergistic activity of the tomato glycoalkaloids α-tomatine **3** and dehydrotomatine **4** remains unreported; this is primarily due to the fact that dehydrotomatine **4** was not discovered until 1994[26,382] and α-tomatine **3** was in fact a mixture of glycoalkaloids. Therefore, further research is warranted in this area. The various combinations and ratios of glycoalkaloids merit further investigation. Purified α-tomatine **3** and dehydrotomatine **4** need to be examined both individually and as a mixture.

■ STRUCTURE–ACTIVITY RELATIONSHIP OF GLYCOALKALOIDS AND AGLYCONES

Glycoalkaloids are divided into two main subunits, the aglycone unit and the carbohydrate unit. Investigation into the activity of glycoalkaloids is principally performed by either modification or removal of the carbohydrate unit or modification of the aglycone unit. The composition of the carbohydrate side chain of glycoalkaloids is of vital importance in defining their biological activities, and this has been investigated using a number of techniques: the sugars of the tri- and tetrasaccharides can be removed individually by chemical hydrolysis. A number of glycoalkaloids possess the same aglycone unit but different glycosidic residues; therefore, their activities can be compared and chemical modification of the carbohydrate chain could allow systematic investigation into the structure–activity
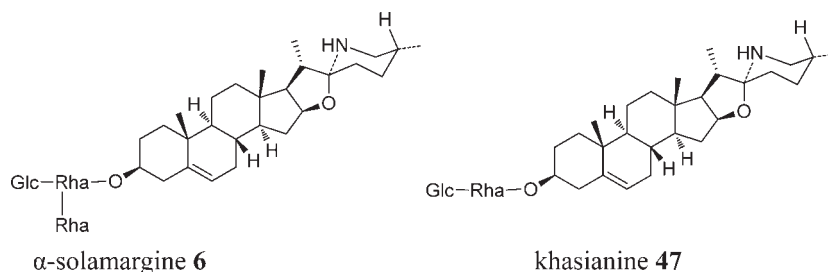
α-solamargine **6**

khasianine **47**

Figure 15. Structures of the glycoalkaloids **6** and **47**.
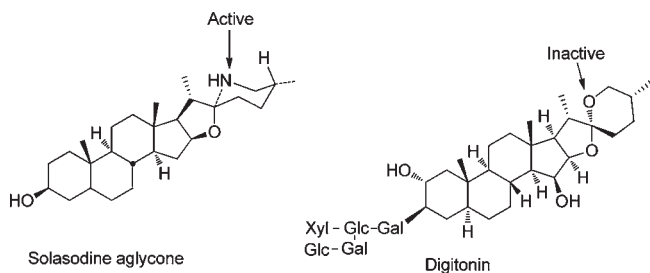

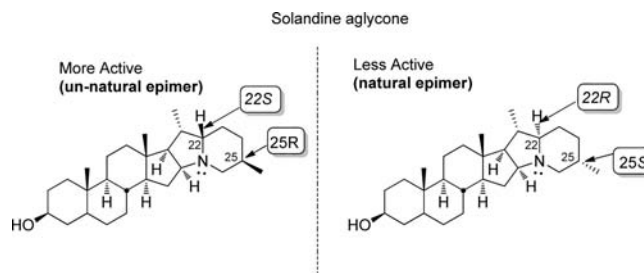**Figure 16.** Structures of digitonin **57**.

Figure 17. Configuration at C22 and C25 and aglycone activity.

relationship of the glycosides. The nature of the aglycone moiety is also of vital importance for bioactivity; synthetic modification of the aglycone has helped to outline a structure—activity relationship for these compounds. The significance of the aglycone moiety can also be examined by comparing glycoalkaloids with the same aglycone but differing carbohydrate moieties.

The hydrolysis products of glycoalkaloids have been examined in a number of biological systems and compared with the bioactivity of the parent glycoalkaloid. The majority of cases report a reduction in biological activity. Rayburn et al.[95] have reported that systematic removal of sugars from potato glycoalkaloids led to a reduction in teratogenicity; α-**2** > β_1-**59** > β_2-**39** > γ-**40** chaconine and α-**1** > β_2-**60** > γ-**41** solanine. A marked reduction in anticancer activity of the aubergine glycoalkaloids α-solasonine **5** and α-solamargine **6** was also observed upon saccharide removal.[383] Moreover, both β_1-**59** and β_2-**39** chaconine were shown to have no effect on membrane integrity when compared with the activity of the parent glycoalkaloid α-chaconine **2**.[32] In contrast, β_2-chaconine **39** resulted in in vitro bovine and human acetylcholinesterase inhibitions comparable to those of α-chaconine **2**.[36] The fact that β_2-chaconine **39** differs from α-chaconine **2** only by one rhamnose sugar indicates the specificity of the bioactivity of these glycoalkaloids and reiterates the importance of the carbohydrate residue for activity.[369,378] Chang et al.[292] investigated the significance of the rhamnose moiety in α-solamargine **6** by comparing it to khasianine **47**; a ~4-fold reduction in apoptotic activity was reported at 20 μg/mL, and it was suggested that the 2′ rhamnose moiety affects the dihedral angle of the glycosidic bond, which in turn increases activity (Figure 15). These results concur with the mechanism of action outlined by Keukens et al.[32−34] that carbohydrate residues influence biological activity by participating in binding to sugar molecules associated with receptor sites of cell membranes. Therefore, they are essential for biological activity. In contrast to this, when the effects of glycoalkaloids
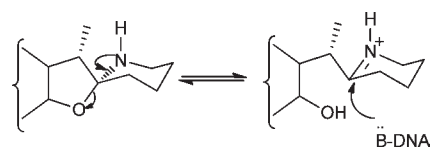
Figure 18. Mechanism of action of DNA disruptive activity of solasodine aglycone **34**.[381]

were examined in vitro on the growth of colon and liver carcinoma cell lines, comparable inhibition was observed between the parent α glycoalkaloid and the β hydrolysis products. In addition, the aglycone moieties solanidine **16** and solasodine **34** were found to possess growth inhibitions similar to those of the parent glycoalkaloids α-chaconine **2** and α-solanine **1** and α-solasonine **5** and α-solamargine **6**, respectively. However, this was not found to be the case with the hydrolysis products of α-tomatine **3**; a dramatic reduction in activity was observed with removal of any glycosidic residue. Furthermore, the aglycone tomatidine was inactive in comparison to the parent glycoalkaloid.[44] However, the anti-inflammatory activity of tomatidine **25** was found to exceed that of the parent glycoalkaloid α-tomatine **3**.[384] Therefore, there are contrasting reports on the importance of the tri- and tetrasaccharide moieties, and it is clear that this is dependent on the biological system being examined and the mode of examination, for example, in vivo versus in vitro.

The composition of the trisaccharide is easily examined as some glycoalkaloids share a common aglycone and different sugars; for example, the paired potato glycoalkaloids α-chaconine **2** and α-solanine **1** both possess the solanidine aglycone **16** but different glycosidic linkages, namely, chacotriose **12** and solatriose **13**, respectively. Higher potency has been demonstrated by α-chaconine **2** in a wide range of test systems when compared with α-solanine **1**, for example, inhibition of acetylcholinesterase,[35−37] toxicity in mice,[385] and sodium active
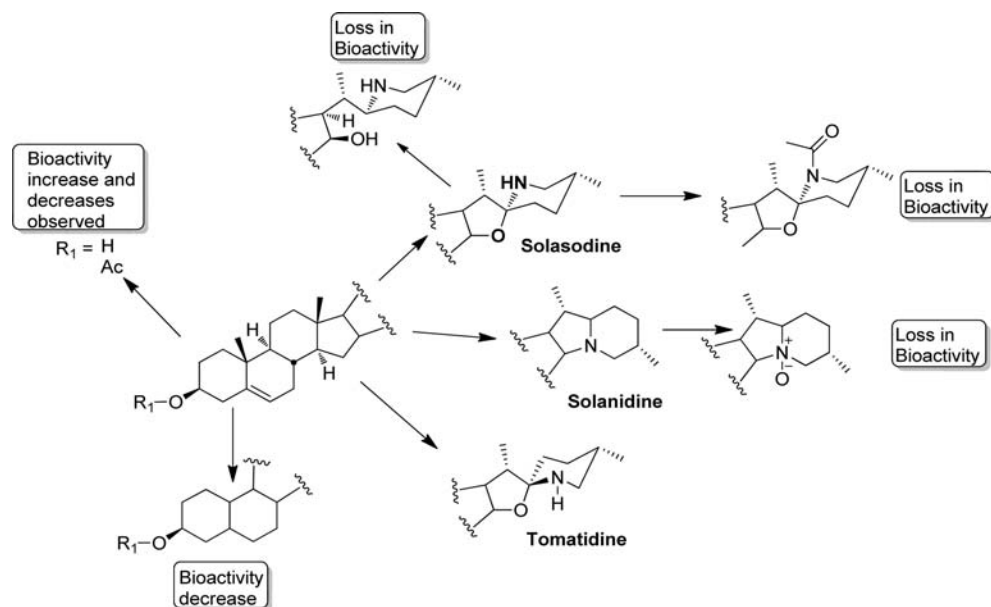
Figure 19. Influence of synthetic modification of the aglycone moiety on bioactivity.

transport in frog skin.[272,273] A similar trend is observed with the paired aubergine glycoalkaloids α-solamargine **6** and α-solasonine **5**. α-Solamargine **6** consistently displays higher activities in biological systems than α-solasonine **5**, for example, anticancer,[79] development toxicity in frog embryos,[172,276] and membrane-disrupting properties.[381]

In the case of membrane lytic activity, the aglycones tested display negligible activity; the chacotriose-containing glycoalkaloids (α-chaconine **2** and α-solamargine **6**) exhibited increased membrane lytic activity over solatriose **13**-containing glycoalkaloids (α-solanine **1** and α-solasonine **5**). α-Tomatine **3** possessed the highest membrane lytic activity, as it contains distinct aglycone and sugar moieties.[33] A structural comparison cannot be derived due to the fact that the lycotetraose sugar **14** or the tomatidine aglycone **25** is not common to any of the other glycoalkaloids tested. Chemical modification of the glycosidic unit is relatively unreported; Zhao et al.[79,383] performed sulfation of the carbohydrate residue of solanine **1**, chaconine **2**, and α-solamargine **6**, and a reduction in anticancer activity was observed for the sulfated analogues. Overall, there is scope for further investigation into the area of carbohydrate modification to enhance the beneficial bioactivity or suppress the toxicity of glycoalkaloids. For example, to examine the activity of the tomatidine aglycone **25**, it would be interesting to attach the chacotriose sugar **12** and compare the results with those documented in the literature. Although the lycotetraose **14** and chacotriose **12** sugars have been synthesized,[386–389] they have not been attached to alternate aglycones and tested for bioactivity, which would help further elucidate the importance of the carbohydrate attachments.

In the case of the acetylcholinesterase inhibition, whereas the potato glycoalkaloids α-solanine **1** and α-chaconine **2** displayed marked inhibition, α-solasonine **5** and α-solamargine **6**, the aubergine glycoalkaloids, exhibited little inhibition. This indicates the importance of the solanidine aglycone **16** for activity and highlights the significance of the nature of the aglycone moiety in acetylcholinesterase activity. However, it should be noted that the sugars are required for activity, as when the

individual aglycone units were tested, negligible inhibition was observed.[36] The heterocyclic nitrogen of the aglycone unit is also paramount for activity as when compared with spirostane steroids, for example, digitonin **57**, no depression of acetylcholinesterase activity is observed[360] (Figure 16). The configuration of the nitrogen of solanidine was investigated by Brown et al.,[390] and it was found that the unnatural 22S,25R solanidine diastereomer possessed the most teratogenic activity when examined in the hamster. It was postulated that the configuration with the basic nitrogen accessible to the α steroid face is necessary for activity (Figure 17). Furthermore, the free electron pair on the nitrogen is also a requirement for activity; Roddick et al.[35] performed a nitration at this position, which led to a loss of lytic activity. It has been postulated that the involvement of the nitrogen lone pair leads to an electrophilic iminium ion species, which results in DNA disruption (Figure 18). Substitution at nitrogen would prevent the formation of the bioactive iminium ion species.[381]

The double bond between C5 and C6 on the solanidine **16** ring of the glycoalkaloid of chaconine **2** is required for lytic activity; in the case of α-tomatine **3**, loss of the double bond is less detrimental when compared to α-chaconine **2**.[32] Figure 19 summarizes the overall synthetic modification made to investigate the structure–activity profile of glycoalkaloids. It is clear that in most cases the modification of the F ring of any of the aglycone moieties led to a loss of activity. The saturation between C5 and C6 is more important for the solanidane glycoalkaloids than the tomatidine glycoalkaloids. A range of modifications have been made at the 3-hydroxyl group, and biological activity has varied, both increasing and decreasing depending on the biological system assessed.

In conclusion, a substantial number of investigations have demonstrated that structural modification of both the carbohydrate and aglycone moieties has a significant impact on the bioactivity of the glycoalkaloids. However, systematic comparison and variation of structure within an individual biological system are required to enable construction of a comprehensive structure–activity relationship.

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