

**Isopentenoids in Plants**  
**Biochemistry and Function**

Edited by

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STEROIDAL GLYCOALKALOID BIOSYNTHESIS AND FUNCTION IN SOLANUM SPP.

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1. INTRODUCTION

Tuber bearing species of the Solanum group contain a class of secondary metabolites commonly referred to as glycoalkaloids. These compounds are, in fact, nitrogen derivatives of steroidal glycosides. Although more accurate designations of these compounds have been suggested such as "alkaloid imperfecta" [1] the term glycoalkaloid remains their most common designation and we will use it in this review. These compounds (as glycosides or the free aglycone) are not restricted to Solanum species; they are common to other Solanaceae such as Lycopersicon and are also found in Liliaceae family. Although this review is primarily concerned with glycoalkaloid biosynthesis in Solanum many of the biosynthetic studies that will be described are based on experiments using Veratrum plants.

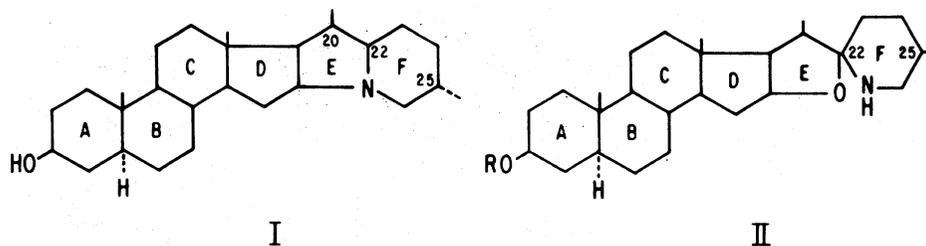
Many excellent reviews of glycoalkaloid chemistry and biochemistry are available [2,2a,3-5]. This review will emphasize the more recent investigations of glycoalkaloid biosynthesis and function.

## 2. BIOSYNTHESIS

The basic ring structures of Solanum alkaloids (Fig. 1) are solanidane (I) and spirosolane (II). The similarity in structure of spirosolanes to sapogenins (which are also found in Solanum) is readily apparent (replace N with O). A partial list of spirosolanes and solanidanes that have been identified in Solanum species is given in Table 1.

Labeling experiments have demonstrated that the common steroid precursors are incorporated into both solanidanes and spirosolanes. Cholesterol [6], cycloartenol and lanosterol [7] are all efficient precursors to these compounds. Biosynthesis leading to these and other steroids in plants has been reviewed by Heftmann [8].

The bioconversion of cholesterol to spirosolanes or solanidanes has recently been studied. The E and F rings result from derivatization and cyclization of the cholesterol side chain. Tschesche [9] has proposed an hypothetical pathway for this process which is based on labeling data and the isolation of intermediates (Fig. 2, scheme B). A similar pathway, with minor variations, has been offered by Kaneko [10] (Fig. 2, Scheme A). The sequence of steps, specifically when the nitrogen function is introduced, is the major difference in these two proposals. There is general agreement that cholesterol is initially converted to the C-26 hydroxy derivative [11-14]. Kaneko [10] has proposed that the steps subsequent to C-26 hydroxy synthesis are the formation of dormantinol (IIIa) and dormantinone (IVa) since these compounds have been isolated from Veratrum grandiflorum, a very active solanidine producing species. However, Tschesche [15], in labeling studies, has found that (25-R)-26-aminocholesterol (IIIb) was incorporated into solasodine in S. dulcumara which would suggest that derivatization at C-22 occurs after the introduction of the amino group. These results are not mutually exclusive and further research is necessary to determine which mechanism is operative (it may be possible that different mechanisms are operative in different species).



I a  $\Delta^5$  UNSAT.  
 I b  $\Delta^5$  UNSAT., 23 O-C-CH<sub>3</sub>

II a  $\Delta^5$  UNSAT.

Figure 1. Solanum aglycone ring structure.

Neither 22-oxo, 16,26-dihydroxycholesterol, 16,22,26-trihydroxycholesterol, or 16-hydroxy, 26-aminocholesterol are precursors to solasodine, soladulcidine, tomatidine, or tomatidenol [16]. This, as well as the fact that the epimino compounds, verazine (VI) and etioline (VII) have been found in plants that also contain solanidanes as spirosoLANES [16], indicates that C-16 hydroxylation occurs subsequent to ring F formation. The

Table 1. Aglycones of Solanum Glycoalkaloids

Aglycone	Structure*
Solanidine	Ia (20S,22R,25S)
Demissidine	I (20S,22R,25S)
Acetylleptinidine	Ib (20S,22R,25S)
Tomatidine	II (22S,25S)
Soladulcidine	II (22R,25R)
Solasodine	IIa (22R,25R)
Tomatidenol	IIa (22S,25S)

\*See Figure 1

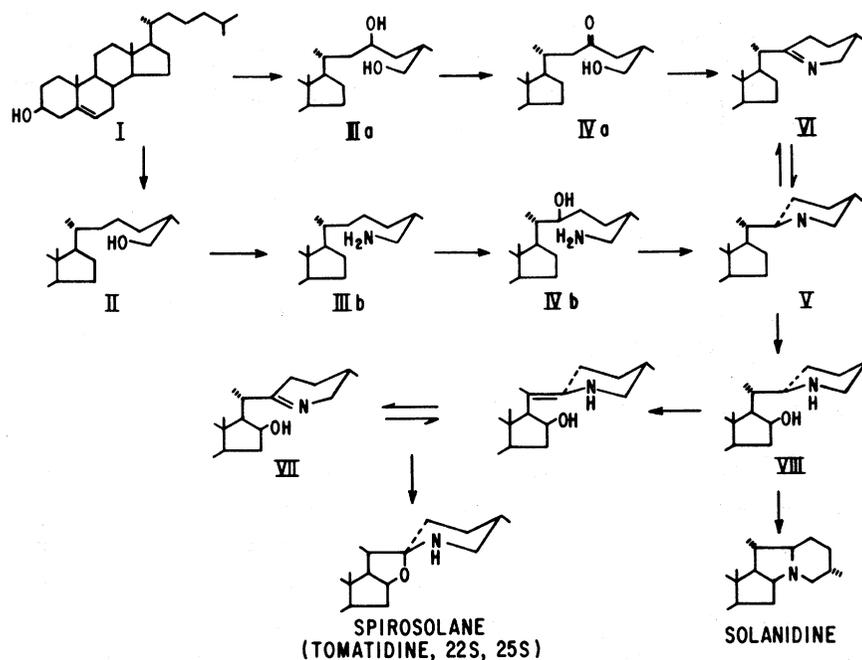


Figure 2. Biosynthetic pathway for E and F rings of aglycones.

sequence of steps from verazine and etioline to either spiroso-  
lanes or solanidananes is hypothetical and has not been confirmed  
by either the isolation of intermediates or incorporation of  
labeled precursors. In the cyclization step to form the D ring  
in tomatidine from labeled cholesterol, the  $16\beta$ -hydrogen of choles-  
terol is lost specifically [17].

Arginine has been postulated as the apparent source of nitro-  
gen in verazine [18]. Arginine content in the rhizomes of *V.*  
*grandiflorum* maximized at a level about four times that of other  
amino acids or ammonia before verazine formation then declines  
just prior to verazine synthesis (Fig. 3). Labeled Arginine ( $^{15}\text{N}$ )  
is incorporated at levels twenty times that of  $\text{NH}_4\text{Cl}$ .

Other aglycone structures that occur in *Solanum* species result  
from minor modification of the solanidane or spirosolane skeleton.  
Solanaviol ( $12\beta$ -hydroxysolasodine) has been isolated from *S.*

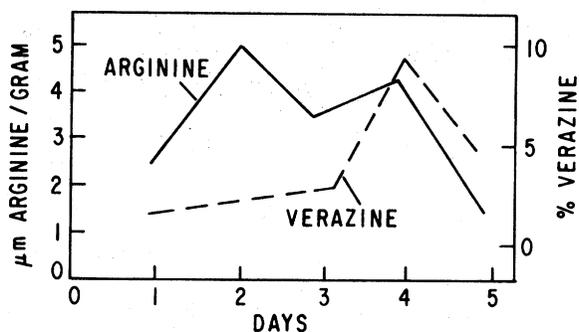
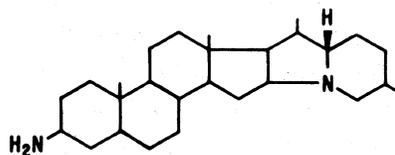


Figure 3. Arginine vs verazine contest. From Kaneko *et. al.* [18].

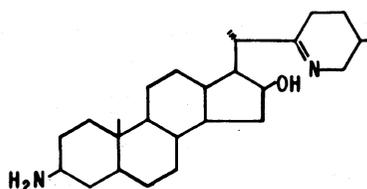
aviculare [19] and leptinidine (23-acetylsolanidine) and leptinine (23-hydroxysolanidine) and leptinine (23-hydroxysolanidine) [2] has been isolated from S. chacoense. The saturated analogues of solanidine (i.e., demissidine), solasodine (soladulcidine) and tomatidenol (tomatidine) are probably synthesized from the unsaturated aglycone via the conjugated ketone as has been demonstrated for steroids [20].

Aglycones that contain 3 $\beta$ -amino groups have recently been reported. Solanogantine (IX), which also contains the unusual  $\beta$ -configuration for the C-22 proton has been isolated from S. giganteum [21] and solacallinidine (X) has been isolated from S. callium [22]. The latter compound has been found in the presence of the analogous 3 $\beta$ -hydroxy compound. A postulated pathway for the formation of the 3-amino compounds from the 3-hydroxy compound is shown in Fig. 4.

The 3-O-glycosides of the Solanum alkaloids, which are more prevalent than the free aglycones in many Solanum species, can con-



IX



X

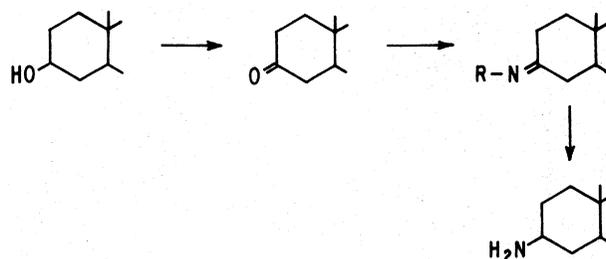


Figure 4. Hypothetical pathway for introduction of amino group at C-3.

tain from one to four sugar units. Rhamnose, glucose, galactose, and xylose are the only sugars that have been reported to be present in the carbohydrate. The biosynthesis of the glycoside is thought to proceed in a stepwise addition of the sugar. This pathway would be similar to flavone glycoside biosynthesis [23] although this has not been substantiated experimentally. The glycosylation of solanidine by potato tuber [24], cell free preparations of potato tuber tissue [25], and crude enzyme preparations of potatoes [26] has been demonstrated. In experiments in our laboratory we have been able to demonstrate the stepwise addition of glucose to solanidine in cell free systems [25] (Fig. 5). Solanidine is rapidly converted to 3-O-glucosyl solanidine and this in turn is converted to a diglucosyl solanidine of undetermined linkage. In solutions containing tuber slices and supplemented with rhamnose, a product tentatively identified as a rhamnosylglucose derivative of solanidine has been isolated.

### 3. REGULATION OF GLYCOALKALOID BIOSYNTHESIS

Environmental factors that affect glycoalkaloid synthesis in harvested potato tubers have been reviewed by Jadhav and Salunkhe [5]. Sinden and Webb [27] have determined the total glycoalkaloid content (TGA) of potato varieties grown in different locations. Glycoalkaloid levels in tubers increase under conditions that also cause chlorophyll levels to rise which probably accounts for the folklore prohibition against eating green potatoes. During plant develop-

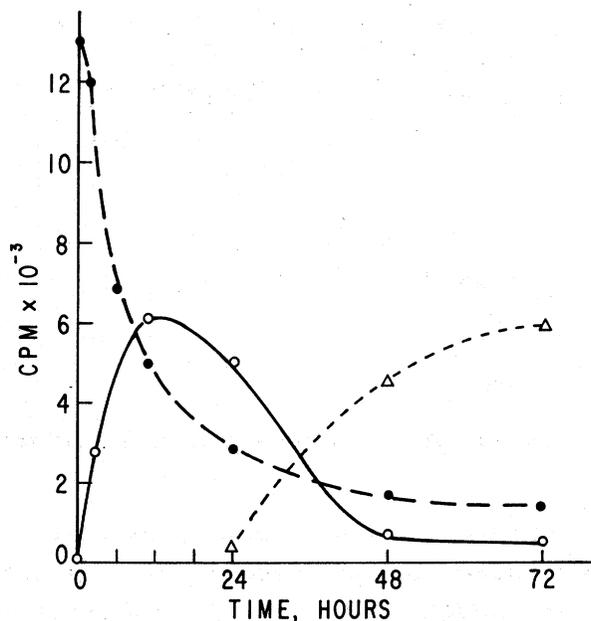


Figure 5. Glycosylation of solanidine in vitro.  
 O = solanidine, O = glucosyl solanidine, Δ = diglucosyl solanidine.

ment, glycoalkaloid concentration increases as the plant matures and then decreases after full maturity [28]. The decrease observed may result from turnover or from a divergence of the biosynthetic pathway to the closely related saponins. Bennett [29] has shown that cholesterol-4-<sup>14</sup>C is converted to neotigogenin (XI), Δ<sup>16</sup>-5α-pregnenolone (XII) and tomatine in Lycopersicon pimpinellifolium. Although this suggests a pathway such as shown in Fig. 6 to account for lower TGA there is no evidence, to date, to support the conversion of tomatidine to XIII or that saponin synthesis, at maturity, is at the expense of glycoalkaloid synthesis.

Partial or complete hydrolysis of the glycosidic moiety of α-solanine and α-chaconine by potato glycosidases has been demonstrated [30,31]. This may be a mechanism of lowering measured glycoalkaloid levels (free aglycones will generally not be determined in standard TGA analysis).

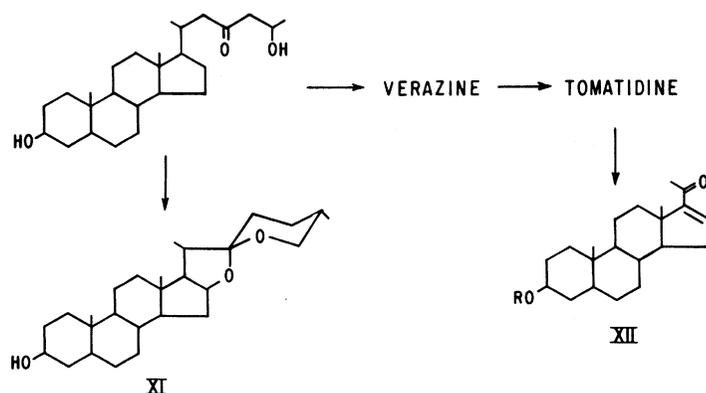


Figure 6. Proposed mechanisms for decreased glycoalkaloid levels in mature tissue.

Under conditions of stress, the glycoalkaloid content of potato tuber tissue changes quantitatively and qualitatively. Frizpatrick *et. al.* [32] have determined the TGA increases in potato slices over prolonged storage times; five-fold increases were not uncommon. Kennebec tuber slices synthesize the glycoalkaloids  $\alpha$ - and  $\beta$ -solamarine which contain the tomatidenol aglycone not normally found in Kennebec tubers [33].

In our examination of cultured potato tissue we detected glycoalkaloids only in some callus tissue cultures that had formed roots [34]; however, we were unable to detect any glycoalkaloids in unrooted cultures.

The genetics of glycoalkaloid inheritance has been investigated. Ross [35] originally suggested that suppression of glycoalkaloid synthesis is inherited in a dominant manner, however, other studies [36,37] do not support this hypothesis. Sanford and Sinden [38] found no reliable correlation between glycoalkaloid levels in parents and offspring although TGA values in many cases were an average value of those found in the individual parents. In  $F_2$  progeny from clones of *S. Chacoense* that contains different glycoalkaloids, McCollum and Sinden [39] found new glycoalkaloids resulting from the combination of the sugars from one glycoalkaloid

in one parent with the aglycone from another glycoalkaloid in the other parent. These workers hypothesized that the biosynthesis of glycoalkaloids commersonine and solanine was controlled by alternative codominant alleles at a single gene locus and a major gene for chaconine synthesis segregated independently of this locus.

#### 4. FUNCTION OF GLYCOALKALOIDS IN SOLANUM

Are glycoalkaloids necessary for the survival of Solanum species? This question takes on added significance since these compounds are undesirable constituents in edible plants because of their toxicity. It has been the object of potato breeders to produce cultivars that are as low as possible in glycoalkaloid concentration, however, it is doubtful that many commercial varieties which are low in TGA could survive in certain environments without the use of insecticides and fungicides. Because there is a major concern about limiting the use of pesticides, plant breeders are exploring the concept of natural host resistance in the context of an integrated pest management program. The function of glycoalkaloids thus becomes more than academic for potato breeders. There is considerable data correlating pest resistance with glycoalkaloid content. The species S. chacoense has a significant resistance to the Colorado potato beetle (CPB) [40]. This species generally contains a high level of glycoalkaloids; some clones contain the leptines which are deterrents to CPB in in vitro tests [41]. S. chacoense is a parent of an experimental cultivar, Lenape, which was never released because of apparent toxicity problems.

An attempt has been made to correlate glycoalkaloid structure to CPB resistance [42], however, leptines which seem to be high on the list of repellency do not contain the requisite structural features as determined in this study. The relationship of TGA to field resistance has been investigated by Tingey et. al. [42]. In measuring the TGA of ten wild, tuber-bearing species of Solanum that differed significantly in resistance to potato leaf hopper, an extremely good correlation was observed (Fig. 7). Although other

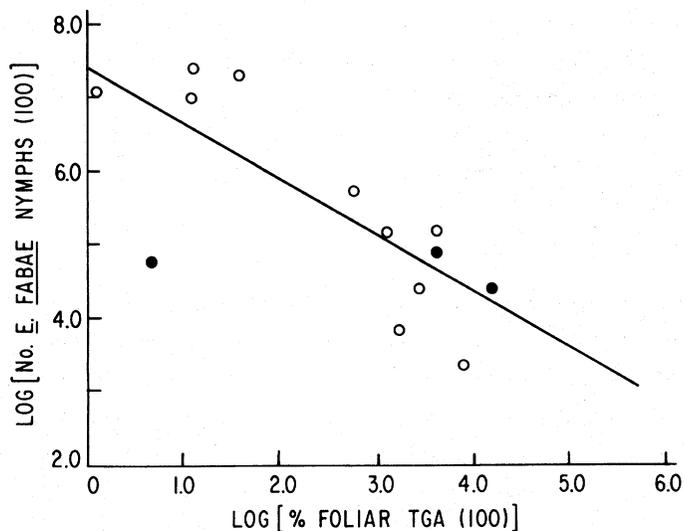


Figure 7. TGA foliar content vs. leaf hopper (*Epoasea Fabae* (Harris)) infestation.  $\circ$  = Species with secretory trichomes. From Tingey et al. [43].

concomitant factors may be the actual resistance vectors, these studies suggest that lowering glycoalkaloid levels through breeding may also decrease natural resistance. By increasing our understanding of glycoalkaloid biosynthesis we may, someday, be able to control the level of these compounds for maximum pest resistance (in the aerial parts of the plant) and maintain safe levels in the in the edible portion of the plant.

The use of the *Solanum* alkaloids as synthetic precursors to steroidal hormones is a classic example of the beneficial role of plant secondary metabolites have had for mankind. Whether these compounds have significant benefits to the plant kingdom remains to be determined.

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