Role of Carbohydrate Side Chains of Potato Glycoalkaloids in Developmental Toxicity

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As part of a program to improve the safety of plant-derived foods such as potatoes, the developmental toxicity of seven structurally related individual compounds was examined using the Frog Embryo Teratogenesis Assay-*Xenopus* (FETAX). The objective was to assess the role of the carbohydrate part of *Solanum* glycosides in influencing the developmental toxicity of these compounds. Comparative evaluations were carried out on the triglycosides α-chaconine and α-solanine, the diglycosides β_1- and β_2-chaconine and β_2-solanine, and the monoglycosides γ-chaconine and γ-solanine. The results show that biological activity is influenced by the chemical structure of the carbohydrate, i.e., galactose, glucose, or rhamnose; the number of carbohydrate groups making up the side chain attached to the 3-OH position of the aglycon solanidine; and the stereochemical orientation of the chaconine diglycosides. The developmental toxicity of these compounds in FETAX generally decreased following removal of the carbohydrates from the triglycosides.

Keywords: *Potatoes, glycoalkaloids, chaconines, solanines, relative potencies, food safety, frog embryos*

INTRODUCTION

Glycoalkaloids are potentially toxic secondary plant metabolites found in potatoes, tomatoes, and eggplants (Friedman et al., 1994; Jadhav et al., 1991; Vehora et al., 1984). They are consumed by animals and humans (Bushway and Ponnampalam, 1981; Friedman and Dao, 1992; Osman, 1983; Ponnampalam and Mondy, 1983; Sinden et al., 1984). Levels are especially high in green and damaged potatoes and in immature tomatoes (Dao and Friedman, 1994; Kozukue and Mizuno, 1990). Glycoalkaloids are produced during both the growth of the plant and postharvest storage.

Adverse effects following potato glycoalkaloid ingestion by animals and humans include anticholinesterase activity in the central nervous system, induction of hepatic ornithine decarboxylase (ODC), disruption of cell membranes, and possible teratogenicity (Caldwell et al., 1991; Keeler et al., 1991; Morris and Lee, 1984; Renwick, 1986; Renwick et al., 1984). We previously examined the relative embryotoxicities of structurally different *Solanum* alkaloids in the Frog Embryo Teratogenesis and frog membrane potential assays (Blankemeyer et al., 1992; Friedman et al., 1991, 1992). Our purpose was to better understand the structural features governing the developmental toxicity of these compounds. Our results show that glycoalkaloids are more toxic than corresponding aglycons lacking the carbohydrate groups. The two major potato glycoalkaloids, α-chaconine and α-solanine, are trisaccharides or triosides; i.e., they have three carbohydrate groups attached to the 3-position of the aglycon (Figure 1). Since one or more carbohydrate groups can, in principle, be hydrolytically cleaved by enzymatic hydrolysis in potatoes (Bushway et al., 1988, 1990) and, after consumption of the glycoalkaloids, by enzyme- and/or acid-catalyzed digestion, it was of interest to determine whether the hydrolysis products of these compounds, the so-called β- and γ-chaconines and solanines, with one or two carbohydrates each, behave similarly to the parent compounds in the embryo assays. The present study concerns the developmental toxicity of these glycoalkaloid hydrolysis products in FETAX.

MATERIALS AND METHODS

Test Materials. α-Chaconine and α-solanine were obtained from Sigma Chemical Co., St. Louis, MO. β_1-, β_2-, and γ-chaconines and β_2- and γ-solanines were isolated from incomplete hydrolysis mixtures of the parent compounds and characterized by high-pressure liquid chromatography and mass spectrometry (Friedman et al., 1993). Our efforts to isolate β_1-solanine were unsuccessful. All compounds produced a single peak on HPLC chromatograms.

FETAX Tests. The Frog Embryo Teratogenesis Assay-*Xenopus* (FETAX) was used to assess the developmental toxicity of the hydrolysis products of α-chaconine and α-solanine. The FETAX assay procedure followed the *ASTM Standard Guide for the Conduct of FETAX* (ASTM, 1991; Bantle et al., 1991). Two sets of 25 embryos each were placed into 60-mm covered glass Petri dishes with various concentrations of the test compounds dissolved in FETAX solution which contained 10.8 mM NaCl, 1.2 mM $NaHCO_3$, 0.58 mM $MgSO_4$, 0.44 mM $CaSO_4$, 0.4 mM KCl, and 0.14 mM $CaCl_2$.

The same four concentrations (milligrams per liter) of α-, β_1-, β_2-, and γ-chaconine were used with each clutch of embryos. With four dishes of controls and two replicates per concentration, each definitive experiment required 900 embryos. The same five concentrations (milligrams per liter) of α-, β_2-, and γ-solanine were employed with each clutch of embryos. With four dishes of controls and two replicates per concentration, each definitive experiment required 850 embryos. Embryos from the same clutch were used to reduce variation in each experiment.

Stock solutions of the chaconines were made by adding 5 mg of the compound to 500 mL of FETAX solution. The stock solutions of solanines were made by adding 10 mg of the compound to 500 mL of FETAX solution. Appropriate dilutions were made to achieve the final concentrations. No additional cosolvents were used to avoid the possibility of solvent–test material interactions. The pH of the solanine solutions was reduced from 8.1 to 6.9 to achieve solubility. The FETAX solution (including all controls) used for that experiment was also adjusted to pH 6.9, which is within limits set by the ASTM (1991). We have previously showed that α-chaconine and α-solanine are soluble

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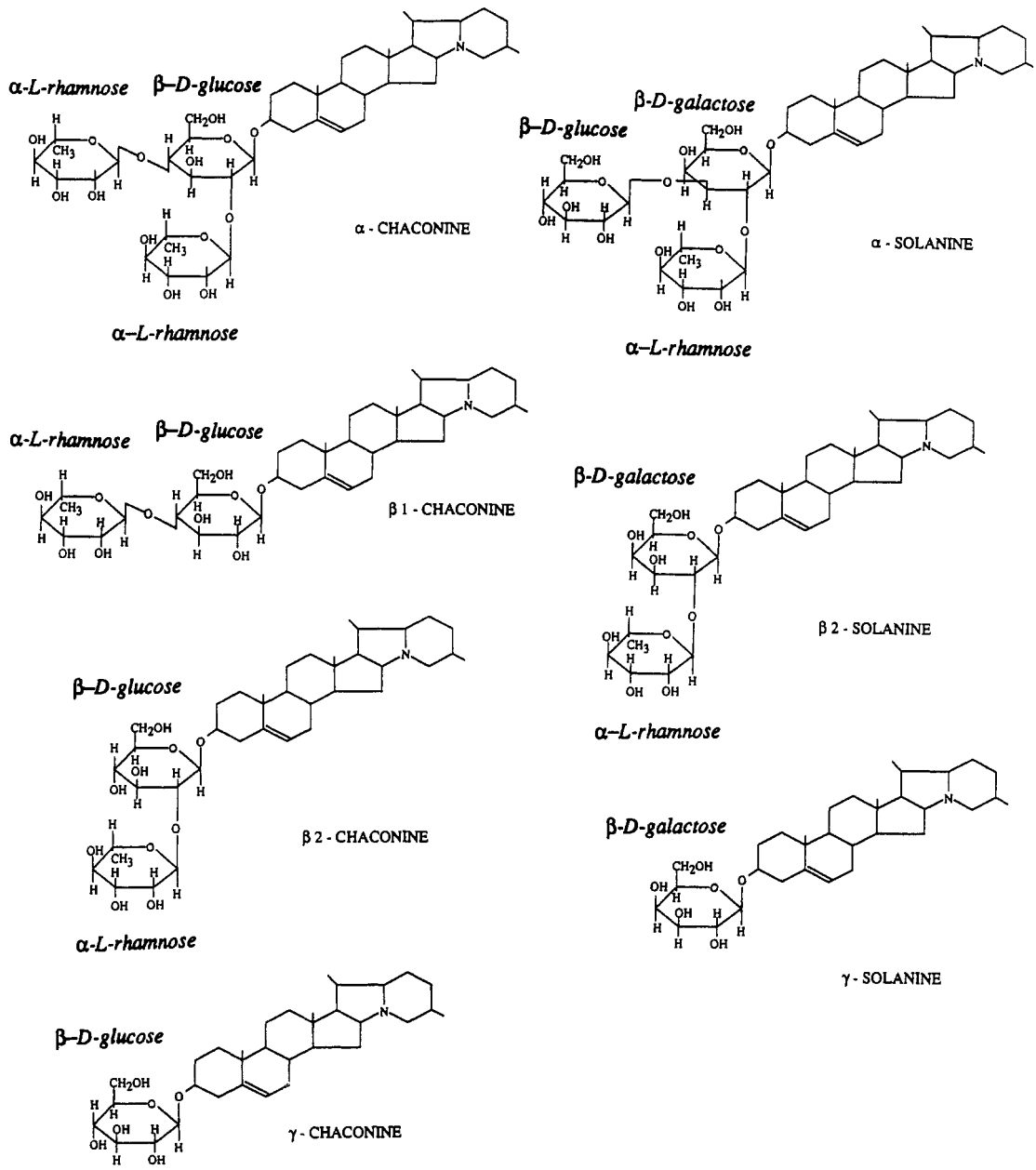

Figure 1. Structures of glycoalkaloids evaluated in FETAX.

in FETAX solution at these concentrations (Friedman et al., 1991; Rooke et al., 1943). Additional studies using HPLC to measure concentrations in solutions confirmed the solubilities of the hydrolysis products at the concentrations used.

The embryos were cultured at 24 ± 1 °C. Solutions were renewed every 24 h of the 4-day test and any dead embryos removed. The acidities of the stock and control dishes were measured each day to verify that pH was between 6.5 and 7.7. At 96 h, surviving (stage 46) embryos were fixed in 3% (w/v) formalin. Stage 46 embryos possess hind-limb buds and tightly coiled guts but do not yet feed. Malformed survivors, dead embryos, and the developmental stage were determined using a dissecting microscope (Nieuwkoop and Faber, 1975).

For each test, probit analysis (Tallarida and Murray, 1983) was used to determine the 96-h LC_{50} (median lethal concentration or the concentration causing 50% embryolethality), 96-h EC_{50} (malformation, or the concentration inducing gross terata in 50% of the surviving embryos), and a teratogenic index (TI) equal to LC_{50}/EC_{50}.

As a measure of growth, head–tail length was measured by following body contour using an IBM-compatible computer equipped with digitizing software (Jandell Scientific, Corte Madera, CA). For each test compound, the minimum concentration to inhibit growth (MCIG) was calculated using the t test for grouped observations. Values were considered significant at $P > 0.05$.

With α-chaconine controls, the number of dead embryos was 4% and the number of malformed embryos, 10.4%. The corresponding values for α-solanine were 8 and 5.5%, respectively. These limits are acceptable for FETAX assays (ASTM, 1991).

RESULTS AND DISCUSSION

In previous studies (Friedman et al., 1991, 1992) we showed that the developmental toxicity and embryotoxicity of the glycosides of the steroidal aglycon, solanidine, are strongly dependent on the carbohydrate residues attached to the steroidal secondary 3-OH groups. Both the nature and order of attachment of the carbohydrate residues appear to influence biological activity. Thus, the relative potency of α-solanine, a trioside with three sugars—glucose, galactose, and rhamnose—is significantly lower than that of α-chaconine, a trioside in which two rhamnose molecules and one glucose molecule are attached to the same aglycon.

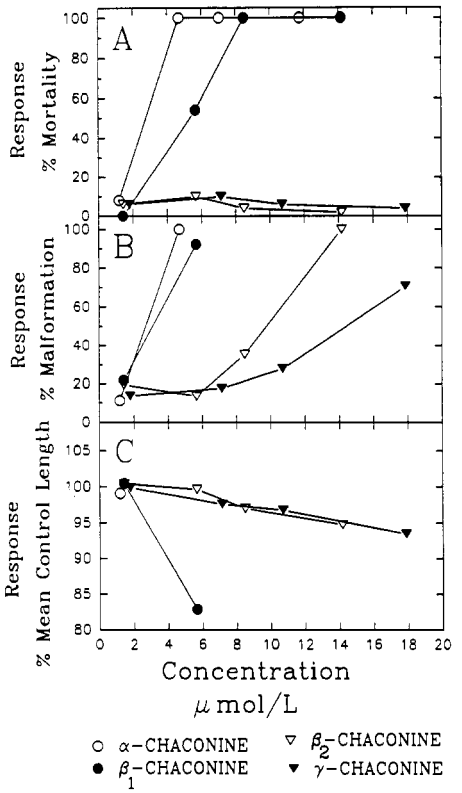

Figure 2. Comparison of the developmental toxicity of chaconines in FETAX.

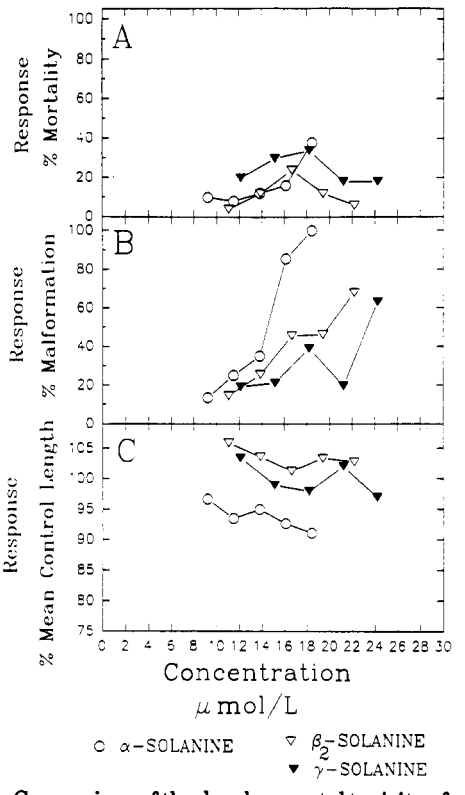

Figure 3. Comparison of the developmental toxicity of solanines in FETAX.

We suggested that the carbohydrate residues influence biological activity by participating in binding to sugar molecules associated with receptor sites of cell membranes. Since some of the hydrolysis products (metabolites) of α-chaconine and α-solanine shown in Figure 1 are found in plant tissues and could also be formed during normal digestion and metabolism of the parent compounds following ingestion, it was of interest to compare the relative potencies of potato glycoalkaloids with one, two, and three carbohydrate sugar residues attached to the 3-position of the aglycon solanidine.

FETAX Assay. In a previous study (Friedman et al., 1992) we showed that the developmental toxicity data we obtained for α-chaconine, α-solanine, solanidine, and tomatine in FETAX generally paralleled reported data on the teratogenicity induced by these compounds in pregnant hamsters. Related studies by Bantle et al. (1990) suggest that FETAX predicts the developmental toxicity of compounds previously tested in mammals with an accuracy of about 90%. These considerations and the additional advantages FETAX has over other short-term tests suggest that it merits wide use as a developmental screen for compounds that are sufficiently soluble in FETAX solution. According to Dresser et al. (1992), these advantages include the following: (a) *Xenopus* development is already well-known; (b) *Xenopus* embryos undergo fundamental developmental processes that are similar to those of mammals; (c) mating and ovulation can be induced at any time after sexual maturity; (d) embryos develop outside the frog, facilitating observation of development and malformations; and (e) developmental endpoints can be determined within a relatively short 96-h test period.

Most of the mortality seen with the α- and β_1-chaconines occurred within 24 and 48 h. This suggested that these compounds are interfering with very early development. After 48 h, there were very few deaths in the chaconine experiment. With the solanine compounds, embryos usually died at 96 h.

In Figures 2 and 3, the higher the percent response at a given concentration, the higher the developmental toxicity, as evidenced by higher mortality, malformation, and growth inhibition. Generally, the higher the mortality, the higher the developmental toxicity. In contrast, the lower the LC_{50} and EC_{50} values, the higher the developmental toxicity. Additional indications of teratogenic hazard are the severity of the malformation and whether growth of the embryos is inhibited at less than 30% of the MCIG value. Each endpoint must be considered separately when ranking developmental toxicity. Table 1 compares the developmental toxicities of all compounds evaluated in this study.

Chaconine Series. Figure 2 shows a plot of percent mortality versus concentration for the four compounds in the chaconine series. The results indicate that α-chaconine and β_1-chaconine were both quite lethal to embryos, with α-chaconine requiring somewhat less material to kill the same number of embryos. In contrast, β_2- and γ-chaconines induce very little mortality in the concentration range studied.

The data show that β_1-chaconine induced more malformations than β_2- and γ-chaconines. α-Chaconine also caused severe malformations that were very similar to those caused by β_1-chaconine. However, all of the malformed embryos died before 96 h. Previous experiments using lower concentrations of α-chaconine also showed these malformations (Friedman et al., 1991). The malformation curves for α- and β_1-chaconines are steep and have few points due to high mortality at the second and third concentrations tested. The embryos that died during the test frequently had anencephaly, as previously reported by Friedman et al. (1991). Microscopic inspection of surviving embryos revealed that the malformations induced by α- and β_1-chaconines were mostly slight to moderate facial malformations. However, gross malformations seem to be more common with α- and β_1-chaconines than with the β_2 and γ pair. Most of the

Table 1. Developmental Toxicity Ranking of Chaconines and Solanines Based on 96-h LC_{50}, EC_{50}, MCIG, and TI Values

compound	MW	clutch (embryos/clutch)	LC_{50}[a] (CI)[b]	EC_{50}[c] (CI)	MCIG[d]	TI[e]
α-chaconine	852.1	1 (200)	0.0022 (na)[f]	0.0020 (na)	<0.0047	1.1
β_1-chaconine	705.9	1 (200)	0.0043 (0.0038-0.0049)	0.0025 (0.0021-0.0029)	0.0057	1.72
β_2-chaconine	705.9	1 (200)	>0.014	0.0092 (0.0084-0.010)	0.0085	>1.5
γ-chaconine	559.7	1 (200)	>0.018	0.01397 (0.0125-0.0156)	0.0071	>1.2
α-solanine	868.0	2 (250)	0.0297 (0.025-0.035)	0.0131 (0.012-0.014)	<0.0092	2.26
β_2-solanine	721.2	2 (250)	>0.0222	0.0187 (0.017-0.021)	>0.0222	>1.2
γ-solanine	659.7	2 (250)	>0.024	0.0230 (0.022-0.024)	0.024	>1

[a] LC_{50}, concentration (μmol/L) causing 50% embryolethality in 96 h. [b] CI, 95% confidence interval. [c] EC_{50}, concentration (μmol/L) causing malformations in 50% of surviving animals in 96 h. [d] MCIG, minimum concentration (μmol/L) to inhibit growth. [e] TI, LC_{50}/EC_{50}(malformation). [f] na, not available.

malformations seen with β_2- and γ-chaconines were facial and gut malformations, mostly in the slight to moderate category. At the higher concentrations, the oral suckers were generally darkly pigmented.

Figure 2 and previous studies suggest (Friedman et al., 1991, 1992) a similar pattern of toxicity for growth. Thus, α- and β_1-chaconines have similar effects on the percent of mean control length and β_2- and γ-chaconines have similar effects on growth that are much less pronounced than those of α- and β_1-chaconines.

Solanine Series. Mortality of the embryos induced by α-solanine appear to be concentration-dependent (Figure 3). In contrast, mortality induced by β_2- and γ-solanines increased with concentrations up to 12 mg/L and then decreased. The mortalities for both compounds peaked at about the same concentration. These bell-shaped concentration–response curves were repeatable (data not shown).

Figure 3 shows that malformations induced by α-solanine increased nearly linearly with concentration in the range 8–16 mg/L. Solubility problems prevented higher concentrations of β_2- and γ-solanine from being tested. The number of malformations induced by β_2- and γ-solanines were similar to that from α-solanine up to a concentration of about 12 mg/L; at higher concentrations their malformation rates dropped below that of α-solanine. Malformations seen with α-solanine were mostly moderate loose or mid-gut coiling. Those observed with β_2- and γ-solanines were mostly slight to moderate gut coiling. However, they also included the same dark-pigmented oral suckers that β_2-chaconine and γ-chaconine induced.

The growth curves of β_2- and γ-solanine were again similar, while α-solanine inhibited growth markedly, significantly more than either β_2- or γ-solanine.

Role of Carbohydrates. Table 1 lists the calculated 96-h LC_{50}, EC_{50}, MCIG, and TI values for the compounds evaluated in this study. This allowed an analytical comparison of their developmental toxicities.

As already mentioned for the EC_{50}, LC_{50}, and MCIG values, the lower the number, the greater the potency. The results show that removal of a rhamnose residue from α-chaconine to form β_1-chaconine increases the LC_{50} value by a factor of 2, has less effect on the EC_{50} and MCIG values, and increases the TI from 1.1 to 1.72. Removal of the other rhamnose group from α-chaconine to form β_2-chaconine, which differs from the β_1-isomer only in stereochemistry since the compounds have identical compositions, increases the LC_{50} value by a factor of more than 6, the EC_{50} value by a factor of about 4.5, the MCIG value by a factor of 2, and the TI value from 1.1 to more than 1.5. Removal of both rhamnose residues from α-chaconine to produce the monoglycoside, glucosyl-solanidine (γ-chaconine), results in further decreases in overall toxicities as measured by the cited parameters.

Table 1 also shows that although the trends for the three solanines seem to be in the same directions as for the chaconine series, the absolute values for LC_{50}, EC_{50}, and MCIG are higher, for TI larger, and the differences among them smaller.

An earlier study (Friedman et al., 1991) found that the aglycon solanidine, formed on complete removal of the carbohydrate groups from either α-chaconine or α-solanine, had a low level of activity. This finding and the current results suggest that the carbohydrate side chains of the glycoalkaloids are paramount in influencing biological activity. Not only the number but also the type of carbohydrate, i.e., galactose, glucose, or rhamnose, as well as the order of attachment and the stereochemical orientation, e.g., β_1- and β_2-chaconines, affects the developmental toxicity of the glycoalkaloids. It is possible that removal of sugars may influence the transport of these compounds across cell membranes; i.e., toxicity differences may reflect transport phenomena as the side chain becomes smaller and less polar.

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