Structural Relationships and Developmental Toxicity of *Solanum* Alkaloids in the Frog Embryo Teratogenesis Assay-*Xenopus*

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Solanum plants produce potentially toxic alkaloids. As part of a program to improve safety of plant-derived foods such as potatoes, we examined the relative embryotoxicities of 13 structurally different compounds using the frog embryo teratogenesis assay-*Xenopus* (FETAX). Our purpose was to better understand structural features governing the developmental toxicology of these compounds. We measured the minimum concentrations needed to inhibit growth of the embryos, the median lethal concentration of 96-h exposure (96-h LC50), and the concentration inducing gross terata in 50% of the surviving animals [96-h EC50 (malformation)]. The following glycoalkaloids produced concentration–response curves: α-chaconine, α-solanine, solasonine, and α-tomatine. All compounds were tested at equimolar (0.005 and 0.015 mM) concentrations in order to develop a relative potency scale. The data showed that (a) glycoalkaloids are more toxic than corresponding aglycons lacking the carbohydrate groups, (b) for glycoalkaloids, the nature of the carbohydrate strongly influences potency, (c) the nitrogen of the steroid is required for teratogenicity, (d) the orientation of the unshared electron pair associated with the nitrogen atom does not affect potency, and (e) the presence of nitrogen in rings of non-steroidal alkaloids such as atropine, scopolamine, and ergonovine does not impart teratogenicity. The observed structural effects should facilitate predicting developmental toxicities of compounds of dietary interest without the use of live animals and provide information to guide selection of potato plants with a low content of specific toxic alkaloids. The possible significance of the results to food safety is discussed.

INTRODUCTION

Alkaloids and glycoalkaloids are nitrogen-containing secondary plant metabolites found in numerous plant species. More than 20 structurally different alkaloids have been recognized in potatoes and tomatoes and about 300 in other Solanaceae plants (Schreiber, 1979). The Solanaceae or "nightshade" family contains many plants important to animals and man, including potatoes (*Solanum tuberosum*), tomatoes (*Lycopersicon esculentum*), capsicum (*Capsicum frutescens*), eggplant (*Solanum melongena*), tobacco (*Nicotiana tabacum*), black nightshade (*Solanum nigrum*), and jimsonweed (*Datura stramonium*). These plants produce antinutritional and toxic compounds including glycoalkaloids, both during growth and after harvest.

Relatively high concentrations of glycoalkaloids have been found in Solanaceae plants consumed by man, such as potatoes (Friedman and Dao, 1992). Levels are especially high in green and damaged potatoes and immature green tomatoes. Glycoalkaloids are far more toxic to man than to other animals studied, although there appears to be considerable individual variation in the susceptibility of animals and humans (Morris and Lee, 1984; Keeler et al., 1991; Caldwell et al., 1991; Friedman, 1992; Friedman and Henika, 1992). The toxicity may be due to adverse effects on the central nervous system and to disruption of cell membranes, adversely affecting the digestive system and general body metabolism. The possible human toxicity of the Solanum glycoalkaloids has led to the establishment of guidelines limiting the glycoalkaloid content of new potato cultivars (Morris and Lee, 1984).

In order to decrease the biosynthesis of the most toxic compound(s) in the plant, it is necessary to define a relative toxicity scale to facilitate the design of suitable molecular biology experiments. In a previous study, we evaluated the embryotoxicity of several potato alkaloids in the frog embryo teratogenesis assay-*Xenopus* (FETAX) as well as the minimum concentration needed to inhibit the growth of the embryos (Friedman et al., 1991). In terms of these parameters, our results indicated that, overall, α-chaconine was about 3 times more toxic than α-solanine and that solanidine was not very toxic. This study (a) defines the relative potencies at equimolar concentrations of a series of structurally different Solanum alkaloids in the in vitro frog embryo assay and (b) relates the results to reported findings with hamsters and to the chemical structures of the test compounds.

MATERIALS AND METHODS

Test Materials. Test compounds were obtained from Biosynth AG, Switzerland; Roth AG, Germany; and Sigma Chemical Co., St. Louis, MO: atropine (Sigma), α-chaconine (Sigma lot 97F-7045), demissidine (Sigma lot 77F-0308), digoxigenin (Roth 7958), ergonovine (Sigma lot 94F-01451), scopolamine (Sigma), α-solanine (Roth 5414), solanidine (Roth 5329), solasodine (Sigma Lot 15F-40251), solasonine (Biosynth 2091), tomatidine (Sigma), and tomatine (Sigma lot 76F-5031).

FETAX Tests. Sets of 25 embryos were placed in 60-mm glass petri dishes with varying concentrations of the appropriate test compound dissolved in FETAX solution. For each test material, 6–10 concentrations were tested in duplicate. Four control dishes of 25 embryos each were exposed to FETAX solution alone, as described previously (Friedman et al., 1991).

For each test, probit analysis (Tallarida and Murray, 1980) was used to determine the 96-h LC50 (median lethal concentration

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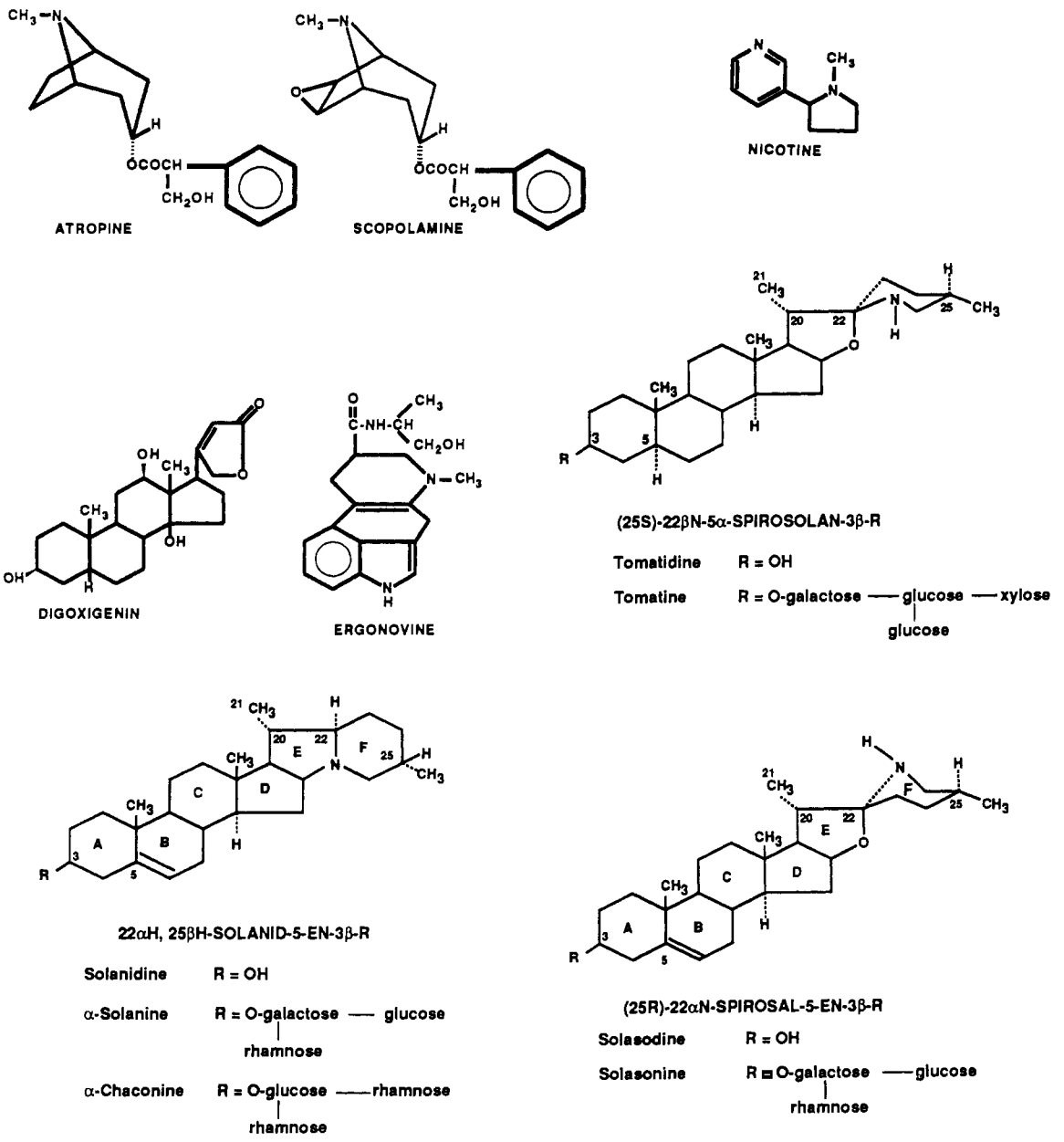

Figure 1. Structures of compounds evaluated in the FETAX test.

or concentration causing 50% embryolethality), 96-h EC50 (malformation) (concentration inducing gross terata in 50% of the surviving embryos), and 95% confidence intervals. These assays are used to define a teratogenic index (TI) of a test compound equal to (LC50/EC50). The TI is useful in assessing teratogenic potential (Bantle et al., 1990; Fort and Bantle, 1990). Head–tail length was measured by following body contour. For each test, the minimum concentration to inhibit growth (MCIG) was calculated using the t test for grouped observations ($P < 0.05$).

Experiments were conducted to compare developmental toxicity at the same molar concentration of alkaloids. Compounds were tested at 0.005 and 0.015 mM concentrations. The 0.005 mM concentration was selected because it was the highest concentration at which all the alkaloids were soluble. The 0.015 mM concentration was selected because it was within the developmental toxicity range of the most soluble of the alkaloids. Four dishes of 25 embryos each were used at each concentration. All other test conditions were the same as the concentration–response tests described above.

RESULTS

Rankings Based on 96-h LC50 and EC50 Values. Figure 1 depicts the structures of the compounds investigated in this study. Four glycoalkaloids were water soluble enough to produce concentration–response curves. These were α-solanine, α-solasonine, α-chaconine, and α-tomatine. Their millimole LC50 and EC50 data are compared from least to most developmentally toxic in Table I and Figure 2. Molarity rather than concentration (in mg/L) was used as a basis for comparison to account for differences in molecular weight.

Table I. Developmental Toxicity Ranking of Five Plant Alkaloids Based on 96-h LC50 and EC50 Values

compound	EC50,[a] mM	LC50,[a] mM	TI
α-solanine	0.010 (0.007–0.0132)	0.013 (0.012–0.014)	1.3[b]
solasonine	0.0057 (0.005–0.0067)	0.00633 (0.006–0.0067)	1.11
α-chaconine	0.0036 (0.0033–0.004)	0.004 (0.0034–0.0041)	1.11[b]
tomatine	0.0018 (0.0017–0.002)	0.0019 (0.0018–0.002)	1.05
nicotine	0.0028 (0.0025–0.0030)	0.869 (0.844–0.875)	310[c]

[a] 95% confidence intervals in parentheses. [b] Data by Friedman et al. (1991). [c] Data by Dawson et al. (1988).

Rankings Based on Exposure at Highest Soluble Concentrations. Figures 3–7 show rankings according to mortality, malformation, and growth, respectively. For each compound, 10% control mortality and 2.1% control

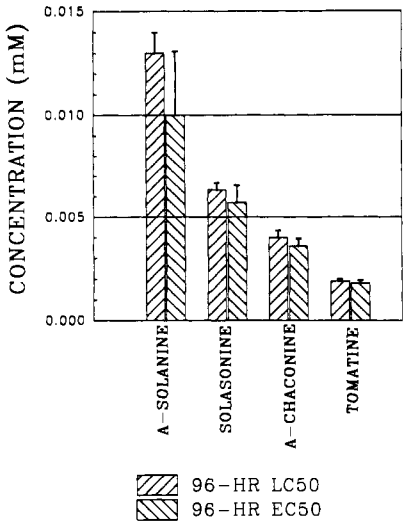

Figure 2. Ranking of four plant alkaloids based on relative 96-h LC50 and EC50 (malformation). The ranking is from lowest developmental toxicity (highest LC50 and EC50) to highest developmental toxicity (lowest LC50 and EC50). Bars represent 95% confidence intervals.

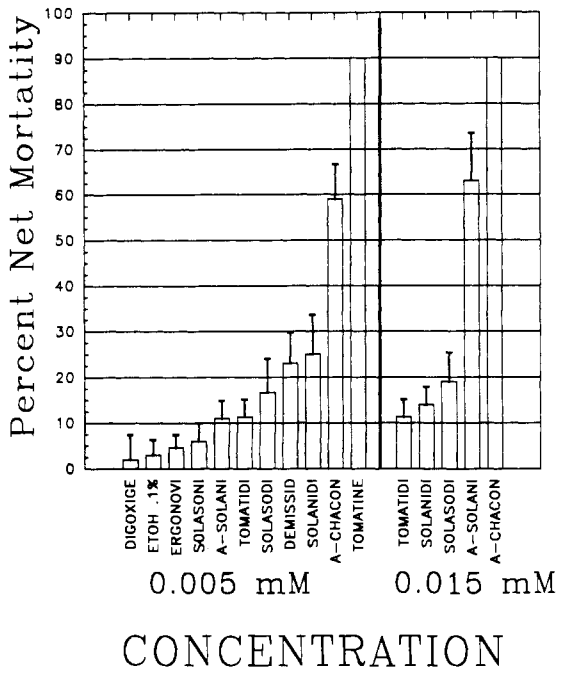

Figure 3. Ranking of plant alkaloids based on mortality. Rankings are based on experimental minus controls and are arranged from low mortality on the left to high mortality on the right. Both tomatine (0.005 mM) and α-chaconine (0.015 mM) caused 100% mortality and therefore do not appear on malformation and growth figures. ETOH refers to 0.1% v/v ethanol solvent controls. Bars are standard error of the mean of four replicates.

malformation were deducted. The plots show net results corrected for control values.

At 0.005 mM α-tomatine caused 100% mortality (90% net mortality shown in Figure 3). α-Chaconine also caused >50% mortality and malformation. Solasonine, however, only caused 29% malformation and little mortality. α-Solanine caused 11% net mortality and 17% net malformation.

Digoxigenin and ergonovine both caused less than 5% net mortality and less than 10% net malformation. Digoxigenin and ergonovine actually increased embryo growth slightly compared to control's. This is the reason for their negative reduction of growth (Figure 5).

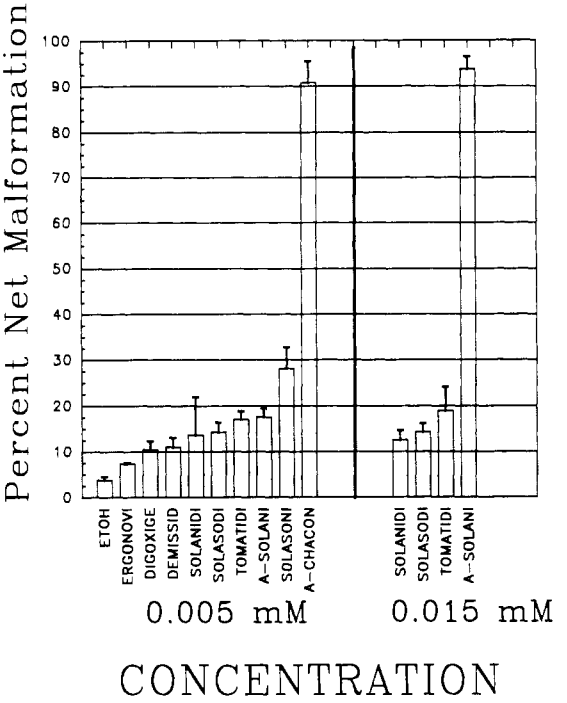

Figure 4. Rankings of plant alkaloids based on malformation. Rankings are based on experimental minus controls and are arranged from low mortality on the left to high mortality on the right. ETOH refers to 0.1% v/v ethanol solvent controls. Bars are standard error of the mean of four replicates.

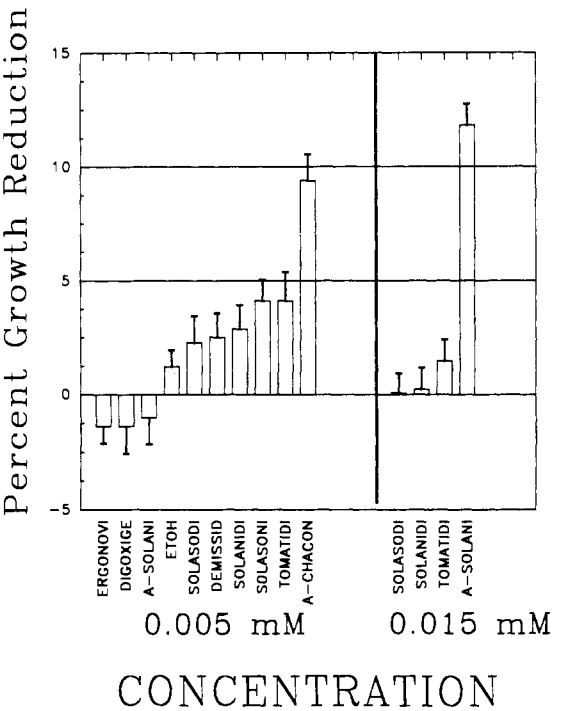

Figure 5. Ranking of plant alkaloids based on growth inhibition. Rankings are based on percent reduction of growth. In some cases, embryos were longer than controls resulting in negative growth reduction. However, these were not significantly different from controls. ETOH refers to 0.1% v/v ethanol solvent controls. Bars are standard error of the mean of four replicates.

The aglycons tomatidine, solasodine, demissidine, and solanidine all had between 10 and 30% mortality at 0.005 mM. Although they also showed a slight growth reduction, they were not significantly different from control's.

α-Solanine, tomatidine, solanidine, and solasodine were also tested at 0.015 mM. Tomatidine, solanidine, and solasodine showed no noticeable increase in effect, and in

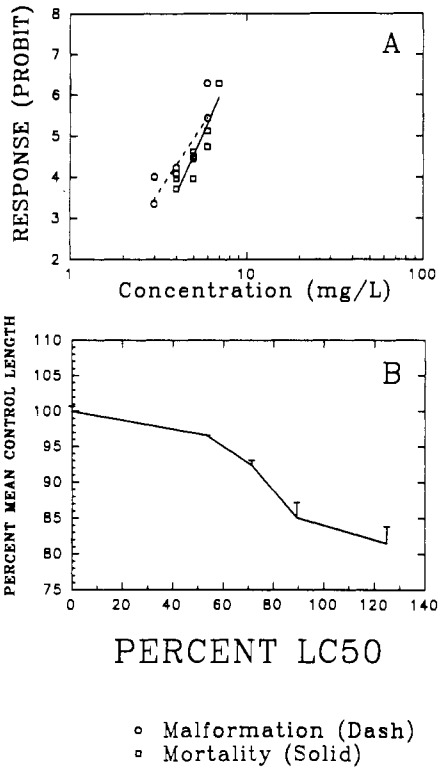

Figure 6. Solasonine concentration–response curves for malformation, mortality, and growth. Increasing concentrations of solasonine were tested in a solution of FETAX containing 0.1–0.3% v/v ethanol. Solvent-only controls were also included. A: □, mortality; ○, malformation. B: —, growth. Bars are for standard error of the mean.

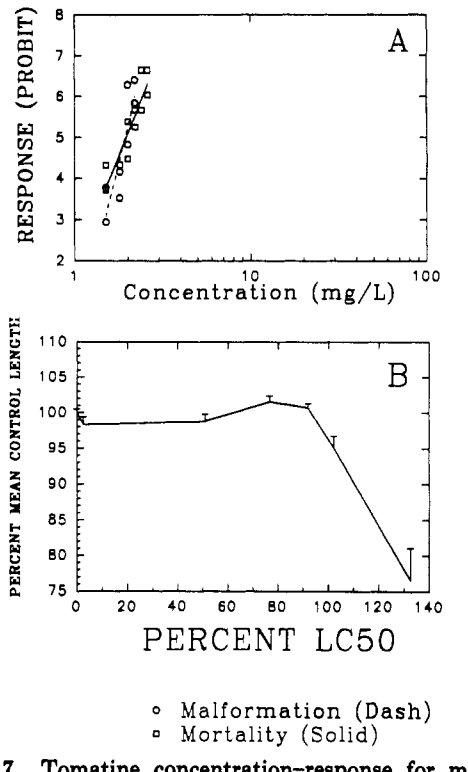

Figure 7. Tomatine concentration–response for mortality, malformation, and growth. Increasing concentrations of tomatine were tested in a FETAX solution in the presence of 0.1–0.3% v/v ethanol. Solvent-only controls were also included. The diluent was FETAX solution. A: □, mortality; ○, malformation. B: —, growth. Bars are for standard error of the mean.

one case a slight decrease was seen. Also no effect was seen on growth at 0.015 mM. α-Solanine caused 63% net mortality, 94% net malformation, and significantly reduced growth.

It must be remembered that the lower the LC50 and EC50, the higher the developmental toxicity (Figure 2). For Figures 3–5, the higher the percent response at a given concentration, the higher the toxicity. Each endpoint must be considered separately when ranking toxicity.

Atropine and scopolamine did not significantly affect mortality or malformation at the concentrations we tested. Three different range tests were performed, each testing progressively higher concentrations. Atropine and scopolamine were not graphed in Figures 3–5 because their toxicities were low.

Atropine has been tested up to 2.5 mg (8.63 mM), the limit of solubility in FETAX. No test produced greater than control mortality or malformation. Thus, all that we can say is it has a higher solubility than the potato alkaloids and its effects, as measured, are not different from ergonovine and digoxigenin.

Scopolamine was tested up to 4 mg/mL (10 mM) without reaching its limit of solubility. Mortality was the same as the control's, and malformations were not significantly different.

DISCUSSION

The frog embryo teratogenesis Assay-*Xenopus* (FETAX) was used in this study because it allowed rapid evaluation of the developmental toxicity. Compared to other short-term assays such as fish embryos, FETAX is faster because primary organogenesis is completed in only 4 days. Unlike fish assays, FETAX has undergone extensive validation using compounds of known mammalian teratogenicity and has attained a predictive accuracy of nearly 90% (Bantle et al., 1989). The in vitro frog embryo assays is, therefore, both time and cost effective and correlates with extensive evaluations in live mammals.

Early tests (Friedman et al., 1991) suggested that microsomes used for metabolic activation in FETAX had no effect on the developmental toxicity of potato alkaloids, so microsomes were not employed in this study. Compounds such as plant alkaloids can be ranked solely on the basis of the percent malformation caused at the same molar concentration (Figure 4).

Developmental Toxicity of Plant Alkaloids and Glycoalkaloids. Glycoalkaloids have the ability to induce spina bifida (the defective closure of the vertebral column), anencephaly (absence of part of the brain and skull), and embryotoxicity (Sharma et al., 1978; Morris and Lee, 1984; Keeler et al., 1991). Structural, stereochemical, and electronic configurations of the alkaloid seem to be paramount in influencing the teratogenic response. For example, Brown and Keeler (1978) suggested that 22*S*,25*R* epimers with a basic nitrogen atom in the terminal, non-steroidal F-ring, shared or unshared with ring E, and with bonding capabilities α to the steroid plane, may be teratogens (e.g., solasodine, jervine). In contrast, 22*R*,25*S* epimers with the unshared electron pair on nitrogen having a β orientation (e.g., demissidine) should not be teratogenic. Since the potato glycoalkaloids α-chaconine and α-solanine have the 22*R*,25*S* stereochemical configuration with the unshared electron pair projecting above the plane of the steroid ring system (β projection), this hypothesis predicts that these compounds should not be teratogenic. Limited studies summarized by Keeler et al. (1991) demonstrated that this prediction is not always realized, since both compounds turned out to be teratogenic in hamsters and other species (see below).

Keeler et al. (1991) and Friedman et al. (1991) attempted to explain the unexpected behavior of the two potato glycoalkaloids by suggesting that other factors could contribute to the teratogenic potencies. These include (a) enzyme-catalyzed inversion of the unshared electron from the β to an α orientation, (b) the presence of small amounts of the teratogenic 22*S*,25*R* epimers in potatoes and in the glycoside preparations evaluated for teratogenicity, (c) the presence of saponins, which may enhance gastrointestinal absorption by their action as emulsifying agents, (d) modification of teratogenicities by parallel toxicities in the gastrointestinal tract and in the liver (Dalvi and Jones, 1986; Caldwell et al., 1991), and (e) the possibility that the true teratogens are not the native alkaloids but metabolic transformation products. We will evaluate the embryotoxicity–teratogenicity effects of the structurally different alkaloids and the non-alkaloidal steroid digoxigenin in light of these comments.

Digoxigenin. Digoxigenin is the aglycon of the cardiac glycoside digoxin, produced by the plant *Digitalis lanata*. Such glycosides all have a lactone ring attached to the pentanoperhydrophenanthrene steroidal nucleus (Fieser and Fieser, 1959). Digoxigenin is used therapeutically in congestive heart diseases.

This steroidal compound was included in our comparative evaluation because, although structurally it resembles the steroidal alkaloids, it lacks a nitrogen-containing six-membered ring. Evidently, such a ring is required for teratogenicity since digoxigenin was not teratogenic in frog embryos.

Atropine and Scopolamine. Atropine and scopolamine are produced by Solanaceae plants including *Atropa belladonna* and *Datura stramonium* (Friedman and Levin, 1989). They both stimulate and depress the central nervous system. Structurally, the tropane ring of both consists of a piperidine ring fused to a tropane ring. Scopolamine has an additional epoxide group built into the tropane ring system (Figure 1).

Neither compound was toxic nor teratogenic to cultured frog embryos. These results suggest that nitrogen-containing ring systems of atropine-type compounds do not have the ability to induce the formation of terata.

Ergonovine. Ergonovine, produced by the parasitic fungus *Claviceps purpurea*, is the hydroxyisopropyl amide derivative of lysergic acid. Ergot alkaloids are also produced by morning glory (*Ipomoea violaceae*) plants (Friedman and Dao, 1990).

Although not derived from a Solanum plant, lysergic acid derivatives are widely distributed in nature, have numerous medical uses, and are also abused as drugs (Lewis and Elvin-Lewis, 1977). Our studies revealed that they do not induce a teratogenic response in embryos, even though structurally they have a nitrogen-containing six-membered ring as part of a polycyclic ring system (Figure 1). Evidently, such a structure does not have the ability to elicit a teratogenic response, at least not in frog embryos.

α-Chaconine, α-Solanine, Solanidine. The glycoalkaloids α-chaconine and α-solanine are found in potatoes (*S. tuberosum*). They each have three carbohydrate residues attached to the 3-OH group of the same aglycon solanidine (Figure 1). Solanidine contains 27 carbon atoms and one atom of tertiary nitrogen, which is part of the bicyclic ring E and F.

Data from Renwick et al. (1984) tabulated by Keeler et al. (1991) indicate that at an α-chaconine dose of 0.173 g/kg of body weight, 26% of pregnant hamsters died by day 8 of gestation and 66% of the surviving dams had deformed litters. The corresponding values for α-solanine at a dose of 0.200 g/kg were 8 and 57%, respectively. Solanidine was not evaluated in hamsters. α-Chaconine appears to be more toxic in terms of mortality and more teratogenic than α-solanine. Combining mortality plus teratogenicity gives a value of 92% for α-chaconine and 65% for α-solanine. Analyzed differently, these numbers show that of 100 litters, 25 were unaffected by either mortality or teratogenicity when gavaged with α-chaconine, versus 40 with α-solanine.

It should also be noted that high rates of malformation were seen in the hamster study only when there was significant maternal toxicity. The low TIs observed in this study and in our previous work (Friedman et al., 1991) suggest that malformations due to plant alkaloid toxicity occur near the cytotoxic concentration.

In the present work, we compared the relative potencies of several compounds at equimolar concentrations. Table I and Figures 2–5 show that at 0.005 mM concentrations, α-chaconine was about three times more toxic than α-solanine in terms of mortality, malformation, and growth inhibition and that solanidine was much less toxic than the two glycosides. Since α-chaconine and α-solanine differ only in the nature of the carbohydrate side chain attached to the corresponding 3-OH group of solanidine, the nature and possibly the number of the carbohydrate side chain residues appears to be paramount in influencing teratogenicity. This hypothesis is further reinforced by our observation (unpublished results) that hydrolytic removal of one or two carbohydrate residues results in a progressive decrease in embryotoxicity.

Solasonine and Solasodine. Solasonine is produced by a large number of Solanum plants including eggplants (*S. melongena*) and a number of potato cultivars other than *S. tuberosum* (Schreiber, 1979). Solasodine, the aglycon of solasonine, contains one more oxygen atom than solanidine. It has a spiroketal structure with a methyl group in the α-position in ring F and a double bond in ring B (Figure 1). Solasonine belongs to the 25*R* series. The molecule contains the same three carbohydrate residues found in α-solanine. The death rate of pregnant hamsters gavaged with 0.47 g/kg of solasonine for 8 days was 38%; the percent of formed litters from surviving dams was 0 (Keeler et al., 1991). The respective values for solasodine gavaged with 0.200 g/kg of body weight was 0 and 10, and for solasodine gavaged with 1.4 g/kg of body weight, 4 and 37.

Table I and Figures 2–6 show that solasonine's frog embryotoxicity and teratogenicity overall falls between those of α-solanine and α-chaconine. Thus, solasonine affected malformations, mortality, and growth at molar concentrations that were only about one-half those required for α-solanine to show the same effects. Since solasonine has the same carbohydrate side chains attached to the 3-OH group as α-solanine, differences in structural features of the respective nitrogen-containing ring systems may account for the observed differences in toxicities.

Solasodine was much less embryotoxic than the glycoside. Its toxicity is comparable to that of the other aglycons, solanidine and demissidine. The structure of demissidine is identical to that of solanidine except that the double bond of ring B is reduced.

Tomatine and Tomatidine. The steroidal glycoalkaloid tomatine has been isolated from tomatoes (*L. esculentum*). It has four carbohydrate residues attached to the 3-OH group of the aglycon tomatidine (Figure 1). Structurally, it is a spirosolane belonging to the 25*S* series, with a reverse *S* configuration at the spiroatom 22 compared to the spirosolane alkaloid, solasonine. Both tomatine and

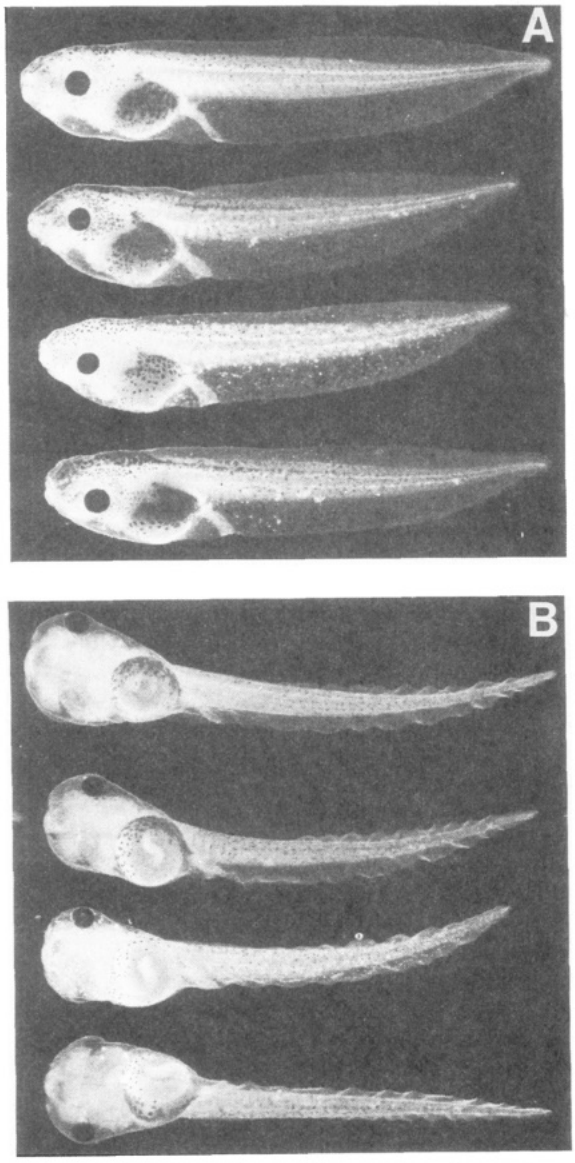

Figure 8. Effect of tomatine on *Xenopus* development, as seen from top to bottom: control and larvae exposed to 2, 2.4, and 2.6 mg/L, respectively. Note that pigmentation increases with increasing concentration. Gut coiling becomes progressively loose and embryo length is reduced as concentration increases. More severely malformaed larvae died by 48 h: A, side view of stage 46 (96-h) larvae: B, ventral view of stage 46 (96-h larvae).

solasonine serve as precursors for synthetic hormones such as progesterone (Fieser and Fieser, 1959).

Keeler et al. (1991) reported that the aglycon tomatidine was nontoxic and nonteratogenic when given as a gavage to pregnant hamsters. The glycoside tomatine, by contrast, was highly toxic though it induced no terata at a dose of one-eighth the molar equivalent of tomatidine.

Table I and Figures 2–5 and 7 show that tomatine is about 5 times more embryotoxic than α-solanine and caused the greatest (100%) mortality compared to all other compounds tested. Tomatine caused severe malformations similar to those induced by α-chaconine. These embryos typically died by 48 h. Among the survivors, malformations were generally scored as moderate (Figure 8). Abnormal pigmentation and loose gut coiling were the most frequent abnormalities encountered. The aglycon tomatidine strongly inhibited growth but mortality and teratogenicity (malformation) were of the same order as those resulting from the other aglycons.

Structure–Activity Relationships. Digoxigenin has the structural features of a steroid (Figure 1) but is not a steroidal alkaloid, since it lacks a nitrogen atom. It showed no effect on frog embryos, suggesting that nitrogen-containing steroids are potential teratogens. This suggestion is reinforced by the negative results with the Solanum alkaloids atropine and scopolamine, which contain a nitrogen atom as part of their bicyclic ring systems, and the lysergic acid derivative ergonovine, which has a six-membered piperidine ring attached to a polycyclic, but not steroidal ring system (Figure 1).

Our studies also show that the teratogenicity/embryotoxicity of the steroidal aglycon is strongly increased by the carbohydrate residues attached to the secondary 3-OH groups of the corresponding glycosides. This increase is not uniform, but depends on the nature and order of attachment of the carbohydrate residues. 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 the sugars rhamnose, glucose, and galactose are attached to the same aglycon. Other structural features also seem to influence relative toxicities. For example, the observed activity of solasonine in the frog embryo assay is intermediate between that of α-solanine and α-chaconine, even though solasonine has the same three carbohydrates as α-solanine. Evidently, structural features in the steroidal part of the glycoside also influence biological activity since both α-solanine and α-chaconine have a tertiary nitrogen atom as part of a bicyclic ring system, whereas solasonine contains a spiroketal structure with both oxygen and secondary nitrogen atoms. It is striking that tomatine, which has four sugar residues (Figure 1) attached to a spiroketal steroid, appears not to be teratogenic, although it is highly toxic to the frog embryos. It is possible that most embryos die before malformations can occur.

We can only speculate about possible biological mechanisms by which the carbohydrate side chains and the nitrogen-containing rings of the aglycons influence toxicity. One possibility is that the carbohydrate residues influence biological activity by participating in binding to sugar molecules associated with receptor sites of cell membranes. Possibly, the behavior of glycoalkaloids is similar to that of the glycoproteins called lectins (hemagglutinins), which also exhibit a high degree of "sugar specificity" in their ability to agglutinate red blood cells and other biological manifestations (Liener, 1989). The toxic effects of these molecules appear to be due to the ability of the carbohydrate part to bind to specific receptor sites on the surface of intestinal epithelial cells, resulting in cell damage and interfering with the absorption of nutrients across the intestinal wall. Incidentally, unlike potato glycoalkaloids, heat-sensitive potato lectins are largely destroyed during cooking.

In a previous study, Dawson et al. (1988) demonstrated a high TI for nicotine (Table I). However, unlike potato alkaloids, the potent teratogenicity of nicotine was largely deactivated by liver microsomes. Figure 1 shows that the structure of nicotine contains a tertiary nitrogen as part of a five-membered ring, in analogy with solanidine. The nicotine structure also contains a heterocyclic six-membered pyridine ring in analogy with ergonovine. Whether the mechanisms of teratogenicity induced by nicotine differs from that of the potato alkaloids and whether deactivation of nicotine also takes place in vivo awaits further studies.

The variable influence of the nitrogen-containing rings of the steroidal part of the molecules on toxicity could

arise from differences in stereochemical features associated with the structurally different rings and/or relative basicities of the secondary and tertiary nitrogen atoms. Although the stereochemistry of the unshared electron pairs on these nitrogens appears not to influence teratogenicity, basicity (pK) could, if the nitrogens are involved in binding to cell membrane receptor sites by charge transfer and/or hydrogen bonding interactions. Further studies are needed to explore these possibilities.

The justification for our attempt to relate structural features of naturally occurring Solanum alkaloids to observed biological activities deserves further comment. With natural compounds, we are limited by the structures provided by nature. Therefore, we may not be able to show the rigorous structure–activity correlations common with synthetic agricultural and medicinal compounds, where a specific structural feature can be artificially and systematically varied. Our attempt is similar to that of Rosenkrantz and Klopman (1990), who related common structural features of natural compounds associated with biological activities to carcinogenicities.

FUTURE STUDIES

An unanswered question is whether the glycoalkaloids would induce teratogenicity in pregnant mammals when, as part of a normal diet, they are subject to interaction with dietary nutrients and non-nutrients, digestion, absorption, transport, metabolism, and elimination. For example, since we do not know whether the carbohydrate residues associated with the steroid moieties are cleaved by hydrochloric acid in the stomach or by digestive enzymes, we do not know whether the glycosides or hydrolysis products are the actual toxicants. Only toxicity associated with oral ingestion of the pure glycoalkaloids, hydrolysis products, or alkaloid-containing potato extracts added to standard diets would give a realistic indication of potential health hazards to animals and humans. Since many of the reported animal studies were carried out by administering the alkaloids or plant extracts by gavage and/or injection, additional studies are needed to ascertain whether the reported findings can be confirmed by oral studies.

Another unanswered question is whether heat-labile lectins and heat-resistant inhibitors of digestive enzymes such as carboxypeptidase, chymotrypsin, and trypsin which are present in potatoes (Friedman, 1992; Lisinska and Leszczynski, 1989) modulate the biological effects of the glycoalkaloids when consumed as part of a potato-containing diet rather than individually. A related possibility is that the alkaloids themselves could act synergistically in vivo since in vitro studies on the disruption of cell membranes revealed strong synergism between α-chaconine and α-solanine (Roddick et al., 1988). Therefore, these considerations suggest that, although the embryotoxicity/teratogenicity data we obtained with FETAX generally parallel the reported data obtained with pregnant hamsters, additional studies are needed to relate the described in vitro frog embryo studies to teratogenicities of the structurally different compounds in higher animals when fed as part of a normal diet and to the disruption of membrane potentials of frog embryos (Blankemeyer et al., 1992). We expect that the in vitro FETAX assay will be a helpful guide for such studies and will turn out to be of value both as a developmental toxicity screen and as a useful model to study the mechanism of teratogenesis and its prevention without the need of live mammals.

Finally, since our data show that the aglycons lacking carbohydrate side chains are much less toxic than the glycosides, blocking the glycosylation step in the biosynthetic pathway should result in potatoes with significantly lowered toxicity (Stapleton et al., 1991, 1992).

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Registry No. Atropine, 51-55-8; scopalamine, 51-34-3; nicotine, 54-11-5; digoxigenin, 1672-46-4; ergonovine, 60-79-7; tomatidine, 77-59-8; tomatine, 17406-45-0; solanidine, 80-78-4; α-solanine, 20562-02-1; α-chaconine, 20562-03-2; solasodine, 126-17-0; solasonine, 19121-58-5.