Kinetics of Acid-Catalyzed Hydrolysis of Carbohydrate Groups of Potato Glycoalkaloids α-Chaconine and α-Solanine†,‡

Mendel Friedman,* Gary McDonald, and William F. Haddon

Food Safety Research Unit, Western Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, 800 Buchanan Street, Albany, California 94710

As part of a broader plan designed to characterize *Solanum* glycoalkaloids and their hydrolysis products and biosynthetic intermediates, to identify plant enzymes in the biosynthetic pathways, and to develop a relative toxicity scale for glycoalkaloids, we examined conditions that favor the hydrolysis of carbohydrate portions of α-chaconine and α-solanine. These two triglycosides can each form two diglycosides, one monoglycoside, the so-called β_1-, β_2-, and γ-chaconines and -solanines, and a common aglycon, solanidine. An incomplete hydrolysis mixture should therefore contain nine compounds. Hydrolyses were carried out in 0.1, 0.2, and 0.5 N HCl–methanol at 38, 55, and 65 °C for various time periods. The individual carbohydrate residues in tri-, di-, and monosaccharides differed significantly in their susceptibilities to acid hydrolysis. Hydrolysis rates increased with HCl concentration and temperature. Hydrolytic stabilities of the carbohydrate groups attached to α-chaconine and α-solanine situated in a potato matrix appear to be similar to those of the pure compounds. By varying the hydrolysis conditions, it was possible to optimize the formation of specific compounds. Eight compounds were isolated and characterized with the aid of preparative chromatography on aluminum oxide columns, thin-layer chromatography, high-performance liquid chromatography, and mass spectrometry. Efforts to isolate β_1-solanine were unsuccessful. Our findings should facilitate characterization of biosynthetic intermediates in plants and of metabolites in animal tissues, as well as assessment of relative safety. Mechanistic aspects of the acid hydrolysis and the significance of the findings to food safety and plant molecular biology are discussed.

INTRODUCTION

According to Schreiber (1979), steroidal alkaloids have been isolated from nearly 300 species of Solanaceae, Illiaceae, and Lycopersicon plant families. There are approximately 60 steroidal alkaloids of pharmacological and toxicological interest. They generally occur as glycosides, in which the carbohydrate residues form a glycosidic linkage with one or more carbohydrate side chains at the 3-hydroxy position of either a solanidane, spirosolane, or spirostane which possess the C-27 steroidal skeleton of cholestane. The two major glycoalkaloids of commercial potatoes (*Solanum tuberosum* L.) and in several other *Solanum* and *Veratrum* species are α-solanine, with a branched α-L-rhamnopyranosyl-β-D-glucopyranosyl-β-galactopyranose (solatriose) side chain, and α-chaconine, with a bis(α-L-rhamnopyranosyl)-β-D-glucopyranose (chacotriose) side chain attached to a solanidine skeleton at the 3-hydroxy group (Figure 1).

Previous studies showed that the induction of liver enzymes (Caldwell *et al.*, 1991; Friedman, 1992), the disruption of cell membranes (Blankemeyer *et al.*, 1992, 1993), and the embryotoxicity and teratogenicity (Morris and Lee, 1984; Renwick *et al.*, 1984; Keeler *et al.*, 1991; Friedman *et al.*, 1991, 1992) of the glycosides are strongly dependent on the carbohydrate residues attached to the steroidal secondary 3-OH group. Both the nature and order of attachment of the carbohydrate residues appear to influence biological activity. We suggested that the carbohydrate residues affect biological and toxicological activity by interacting with receptor sites of cell membranes. Damage to DNA does not appear to be the cause of the cited biological effects (Friedman and Henika, 1992).

Potato plants are known to contain enzymes that catalyze the hydrolysis of α-chaconine and α-solanine (Kuhn and Low, 1955; Guseva and Paseschichenkko, 1957; Swain *et al.*, 1978; Filadelfi and Zitnak, 1982; Zitnak and Filadelfi, 1988; Bushway *et al.*, 1988, 1990; Stapleton *et al.*, 1991, 1992). Small amounts of the partial hydrolysis products are found in potato roots (Friedman and Dao, 1992; Friedman and Levin, 1992). The hydrolysis products could also be formed during normal digestion and metabolism of the parent compounds following ingestion. We therefore examined conditions that affect the acid-catalyzed hydrolysis of the carbohydrate residues of the glycosides. Our major objective was to maximize the partial hydrolysis of α-chaconine and α-solanine to permit isolation of all possible hydrolysis products for biological evaluation. To accomplish our objective, this study (a) defines the kinetic course of the hydrolysis of alkaloidal trisaccharides as a function of HCl concentration, time, and temperature; (b) compares susceptibilities to hydrolysis of tri-, di-, and monosaccharides; (c) describes the isolation of hydrolysis products by preparative chromatography; and (d) assigns structures to the isolated compounds with the aid of thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), and mass spectrometry.

* Author to whom correspondence should be addressed.

† Presented at the Division of Agricultural and Food Chemistry, 204th National Meeting of the American Chemical Society, Washington, DC, 1992; Abstract AGFD 88.

‡ M.F. dedicates this paper to the memory of his former major professor, Gerhard L. Closs. His untimely death during his tenure as the A. A. Michelson Distinguished Service Professor in the Department of Chemistry of the University of Chicago deprives us of a great scientific benefactor.

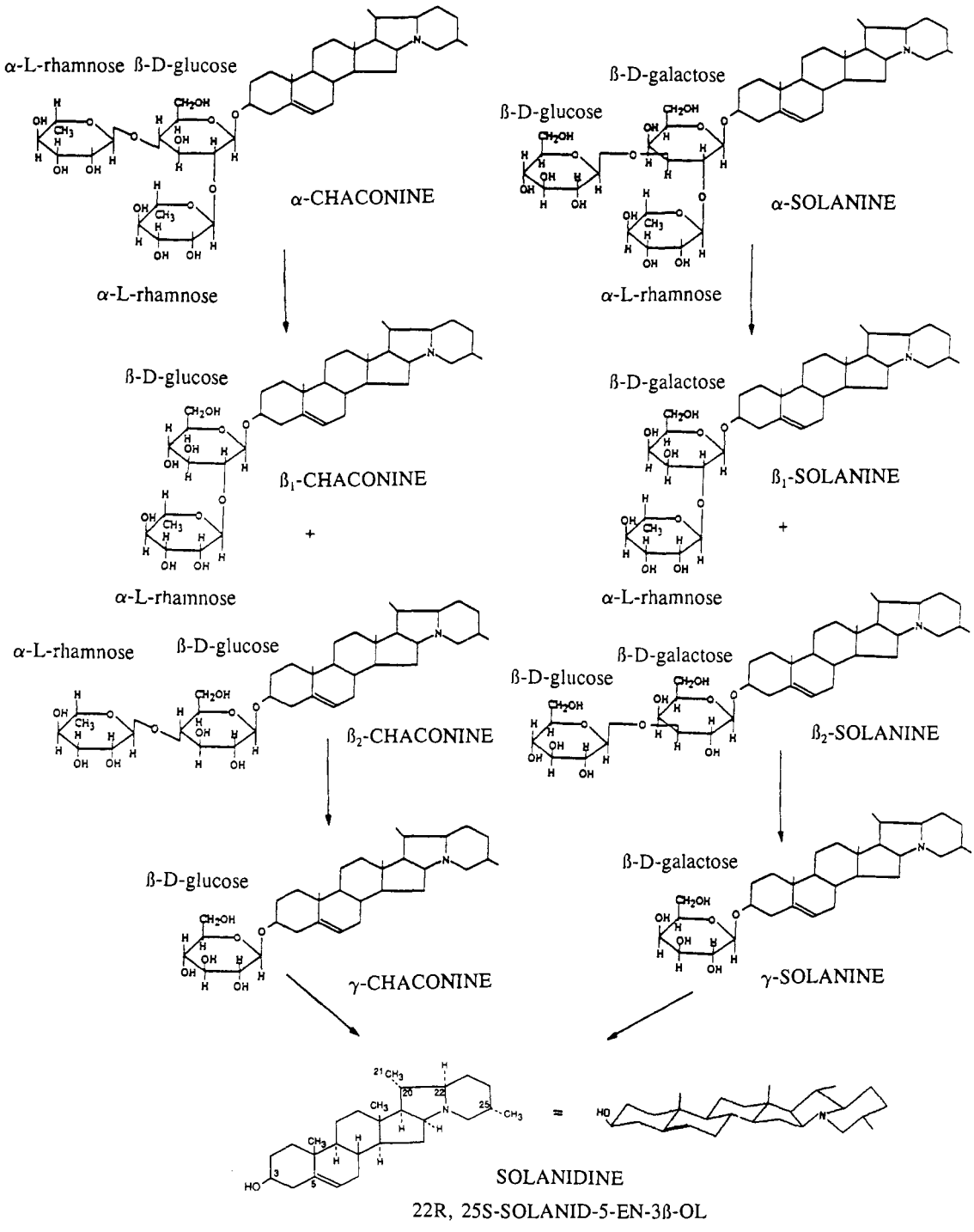

Figure 1. Structures of α-chaconine, α-solanine, and hydrolysis products.

MATERIALS AND METHODS

Materials. Anisaldehyde was obtained from Eastman Kodak, Rochester, NY. Silica-coated TLC plates were obtained from Merck, Darmstadt, Germany. The α-chaconine, α-solanine, solanidine, and aluminum oxide (activity grade 1) were obtained from Sigma Chemical Co., St. Louis, MO. Larger amounts of α-chaconine and α-solanine were isolated from sprouts of Russet potatoes purchased from a local store: fresh sprouts (1–3 cm) were washed and then macerated in a Waring blender with 1% acetic acid (300 mL/100 g) for 10 min. The slurry was filtered, and the solid cake was reextracted similarly. The combined filtrate was made basic with NH_4OH, heated to 70 °C, and cooled in a refrigerator overnight. The solution was then centrifuged (3500*g*) for 20 min and the supernatant discarded. The solid pellet was washed with cold 2% NH_4OH and recentrifuged. Next, the crude glycoalkaloid solid was transferred to a flask and heated to boiling with 250 mL of ethyl acetate–absolute ethanol (EtOH)–5% NH_4OH, 80:16:4 (Filadelfi and Zitnak, 1982). The solution was cooled and filtered. The solid residue was treated similarly twice more. The combined filtrate was evaporated, leaving crude α-chaconine (approximately 80%). The solid residue was heated with 250 mL of 95% EtOH and filtered. When evaporated, this gave crude α-solanine (approximately 85%). Pure compounds were obtained by chromatography, using an alumina column (20 × 2.7 cm) with water-saturated *n*-BuOH as the eluent (Kuhn and Low, 1955), and collecting 5-mL fractions. Pure compound fractions, as determined by TLC, were combined and recrystallized from 80% ethanol.

Melting Points. Uncorrected melting points were determined by placing the sample between two circular-slide cover glasses which were then placed on an electric heating block (Fisher-Johns apparatus). The rate of increase of heating was regulated to 1 °C/min, beginning at about 20 °C below the temperature at which browning first started, as noted in a preliminary run.

Acid Hydrolyses for Kinetic Studies. Hydrolyses were carried out in a flask fitted with a condenser in a circulating water bath heated to the appropriate temperature. Hydrolyses were all done at a concentration of 1 mg of substrate/mL of 0.1, 0.2, or 0.5 N HCl–methanol, typically 100 mg in 100 mL (exceptions are noted in the individual tables). The solvent was heated in the water bath and the test compound added. Aliquots (usually $^1/_5$ or $^1/_{10}$ of the total volume) were withdrawn at timed intervals. The aliquots were immediately neutralized with NH_4OH, evaporated to dryness, redissolved in 25 mL of H_2O, made basic with NH_4OH, and partitioned twice with 20 mL of 1-butanol (*n*-BuOH). The *n*-BuOH fractions were combined and washed with another 25 mL of 1% NH_4OH. The *n*-BuOH layer was separated and evaporated to dryness. The solid was redissolved in MeOH to give an approximate concentration of 1 mg/mL. One milliliter of this sample was then diluted with 9 mL of MeOH–acetonitrile–H_2O, 10:55:35. The diluent (20 μL) was then injected into the HPLC chromatograph for quantitation. Duplicate hydrolyses under the same conditions were ±2% for the first 30 min and then diverged to ±5%.

Preparative Acid Hydrolyses. Large-scale hydrolyses for preparation of the β and γ compounds were carried out similarly. Specifically, α-solanine (1 g) in 200 mL of 0.5 N HCl–methanol or α-chaconine (1 g) in 200 mL of 0.2 N HCl–methanol was heated at 65 °C for 60 min. The cooled solution was made basic with NH_4OH and then partitioned twice with 25-mL portions of *n*-BuOH. The combined *n*-BuOH layers were passed directly through an aluminum oxide column (25 × 3.7 cm). The collected fractions (10 mL) were examined by TLC for content of hydrolysis products. Fractions containing the same compound were combined and evaporated to dryness; the residue was then recrystallized from 80% EtOH.

Hydrolysis of Freeze-Dried Potato Powders. Washed potatoes (NDA 1725 high-glycoalkaloid-containing cultivar) were cut into small cubes, placed in a freeze-drying jar, and frozen by adding liquid nitrogen. The frozen product was quickly lyophilized. The dried sample was then ground in a Wiley mill to pass a 1-mm screen and stored at 0 °C. Hydrolyses were carried out by adding the powder (10 g) to 500 mL of 0.5 N HCl–methanol previously preheated to 65 °C. The flask was then placed into a 65 °C water bath. Samples (100 mL) were removed after 30, 60, and 90 min. These were treated as described earlier for the pure alkaloids, and the alkaloid composition was then determined by HPLC.

Thin-Layer Chromatography (TLC). TLC was performed on Merck precoated silica gel G plates, 0.25 mm thick. Development was in saturated chambers with chloroform–methanol–2% NH_4OH, 70:30:5. Spots were visualized either by placing the plates in a tank saturated with iodine vapor for 5 min or by spraying with anisaldehyde reagent (0.5 mL of anisaldehyde, 10 mL of MeOH, 0.5 mL of H_2SO_4, and 0.1 mL of acetic acid) and heating at 120 °C for 5 min (see Results and Discussion).

High-Performance Liquid Chromatography (HPLC). A Beckman Model 334 liquid chromatograph with a 427 integrator and a 165 UV–visible variable-wavelength detector was used (Friedman and Levin, 1992). Column was a Resolve C_{18} 3.9 × 300 mm HPLC column (Waters, Milford, MA). Flow rate was 1 mL/min, and detection was by UV at 200 nm. Eluent for glycoside determination was 100 mM ammonium phosphate, monobasic in 35% acetonitrile, adjusted to pH 3.5 with phosphoric acid. Eluent for aglycon determination was 10 mM ammonium phosphate in 60% acetonitrile, adjusted to pH 2.5.

Mass Spectrometry. In the absence of reference standards for the mono- and diglycosides of solanidine, mass spectrometry was used to characterize the material from TLC and HPLC peaks obtained from chromatography of the partial acid hydrolysates of α-chaconine and α-solanine. TLC spots were extracted with hot methanol prior to mass spectrometry. Samples were analyzed without derivatization by use of liquid secondary ion mass spectrometry (LSIMS) on a VG 70/70-HS mass spectrometer (Fisons, Inc., Manchester, U.K.) equipped with a cesium bombardment source (Antek Instruments, Palo Alto, CA) operated at 6-kV primary ion beam energy. Samples were applied as solutions to a glycerol matrix on a copper probe. Improved signals were achieved for some samples by addition of about 1 μL of 1 M acetic acid.

Table I. Melting Points of Alkaloids

compd	mp, °C
α-solanine	248 (browns), 256–258 (melts), 296–300 (decomposes)
β_2-solanine	250 (browns), 255–257 (melts), 295–298 (decomposes
γ-solanine	228 (browns), 240–242 (melts), 298–300 (decomposes)
α-chaconine	234–235 (melts), 296–299 (decomposes)
β_1-chaconine	199 (browns), 215–217 (melts), 294–298 (decomposes)
β_2-chaconine	231 (browns), 238–240 (melts), 296–299 (decomposes)
γ-chaconine	220 (browns), 232–234 (melts), 300–302 (decomposes)
solanidine	208 (browns), 215–217 (melts)

Table II. TLC Data for Alkaloids[a]

	solvent A		solvent B		solvent C		solvent D	
compd	R_f	$R_{\alpha\text{-s}}$[b]	R_f	$R_{\alpha\text{-s}}$	R_f	$R_{\alpha\text{-s}}$	R_f	$R_{\alpha\text{-s}}$
α-solanine	0.11	1.00	0.18	1.00	0.16	1.00	0.24	1.00
β_2-solanine	0.23	2.09	0.37	2.06	0.27	1.69	0.56	2.33
γ-solanine	0.46	4.18	0.59	3.28	0.48	3.00	0.65	2.71
α-chaconine	0.20	1.82	0.32	1.78	0.23	1.44	0.54	2.25
β_1-chaconine	0.27	2.45	0.40	2.22	0.30	1.88	0.56	2.33
β_2-chaconine	0.37	3.36	0.52	2.89	0.39	2.44	0.63	2.63
γ-chaconine	0.50	4.55	0.63	3.50	0.52	3.25	0.66	3.33
solanidine	0.84	7.64	0.87	4.83	0.80	5.00	0.80	3.33

[a] Solvent A: chloroform–methanol–2% NH_4OH, 70:30:5 (Jellema *et al.*, 1981). Solvent B: chloroform–methanol–1% NH_4OH, 65:35:5. Solvent C: chloroform–methanol–1% NH_4OH, 2:2:1, bottom layer (Jellema *et al.*, 1981; Filadelfi and Zitnak, 1983). Solvent D: chloroform–methanol–water, 5:5:1 (Jellema *et al.*, 1981). [b] $R_{\alpha\text{-s}}$: value relative to that of α-solanine.

RESULTS AND DISCUSSION

Structures of Hydrolysis Products. α-Chaconine can form two diglycosides (β_1, and β_2), one monoglycoside (γ-chaconine), and solanidine. α-Solanine can, in principle, also form two diglycosides (β_1, and β_2), one monoglycoside (γ-solanine), and solanidine (Figure 1).

Table I lists the melting points of all compounds.

Table II lists the R_f values from TLC of the partial hydrolysis products of α-solanine and α-chaconine. When analyzed by mass spectrometry, all samples yielded intense $(M + H)^+$ ions, in agreement with previous measurements on solanidine glycoalkaloids (Price *et al.*, 1985). LSIMS spectra, in glycerol matrix, for the aglycon solanidine and its parent glycosides, α-solanine and α-chaconine, are shown in Figure 2. Fragment ions at mass-to-charge ratios (m/z) 380, 204, and 150 in these spectra, and in those of the mono- and diglycosides of solanidine, arise from the steroidal portion of the structure (Budzikiewicz, 1964). Thus, the spectra characterize the compounds as solanidine glycoalkaloids.

For α-solanine, additional fragment ions at m/z 722 and 706 originate from cleavage of the glycosidic bonds accompanied by rearrangement of a single hydrogen. These fragment peaks correspond in mass to the possible protonated diglycoside acid hydrolysis products. In like manner, the m/z 560 peak has the mass of the protonated monoglycoside.

LSIMS spectra of the isolated diglycosides shown in Figure 3 correspond to β_1-chaconine and β_2-chaconine, both with $(M + H)^+$ at m/z 706 and β_2-solanine with $(M+H)^+$ at m/z 722. The underivatized β_1 and β_2 isomers of chaconine cannot be differentiated on the basis of mass spectra alone; differences in the recorded LSIMS spectra of these isomers, Figure 3A,B, apparently reflect differences in concentration in the LSIMS matrix rather than structural differences. For these compounds, R_f values from TLC and retention times from HPLC (Friedman and Levin, 1992) provide unequivocal identification when combined with gas–liquid chromatography (GLC) retention times and mass spectra of permethylated derivatives, which can differentiate between the 1→2 glycosidic linkage

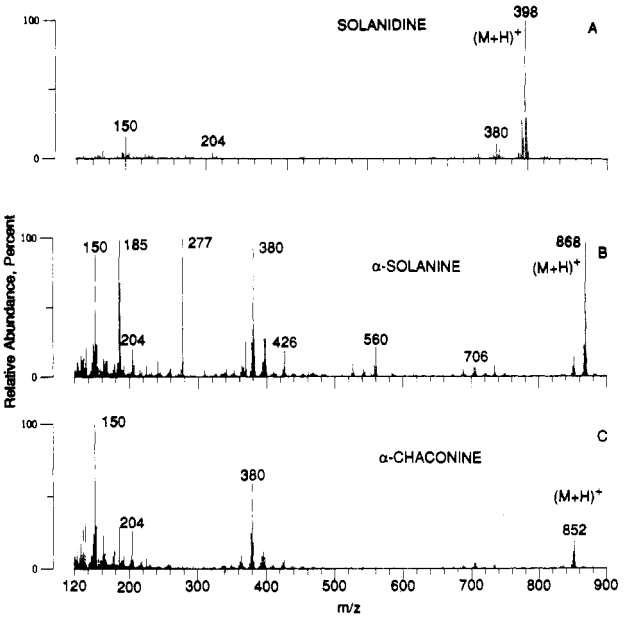

Figure 2. Positive ion LSIMS mass spectra of solanidine (A), α-solanine (B), and α-chaconine (C) in glycerol. Peaks at m/z 185 and 277 are derived from matrix.

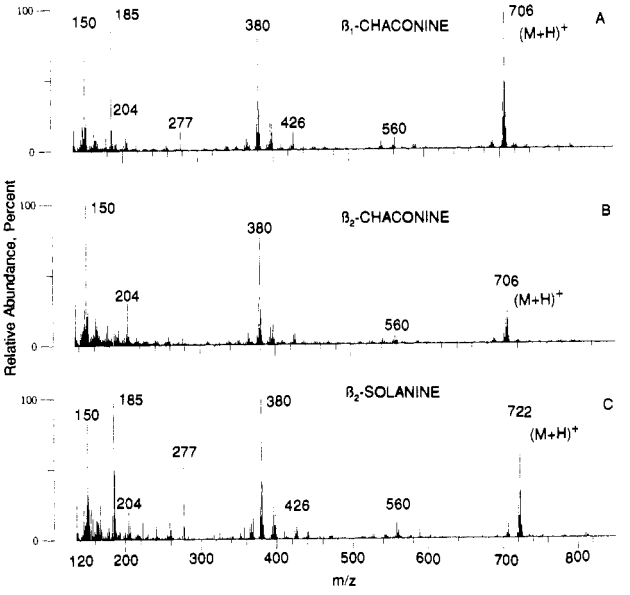

Figure 3. Positive ion LSIMS mass spectra of β_1-chaconine (A), β_2-chaconine (B), and β_2-solanine (C).

of β_1-chaconine and the 1→4 linkage of β_2-chaconine (Osman *et al.*, 1976; Swain *et al.*, 1978; Kitajima *et al.*, 1982).

The occurrence of the monoglycosides γ-solanine and γ-chaconine was also confirmed from their LSIMS spectra, which are shown in Figure 4. Their mass spectra have not been reported previously. As expected, these isomers give indistinguishable spectra, but identification by mass spectrometry is unequivocal because they originate from different parent glycosides.

Melting Points. Table I lists the melting points of the purified compounds. We noted that all compounds except α-chaconine continued to darken after melting, resembling the caramelization of sucrose. They also smelled of burnt sugar. Frothing occurred until the melt turned black and decomposed at around 300 °C for each compound. Some of the melting and/or decomposition points agreed with literature values and some did not. Wide ranges of values are listed for these compounds. As Porter (1972) has

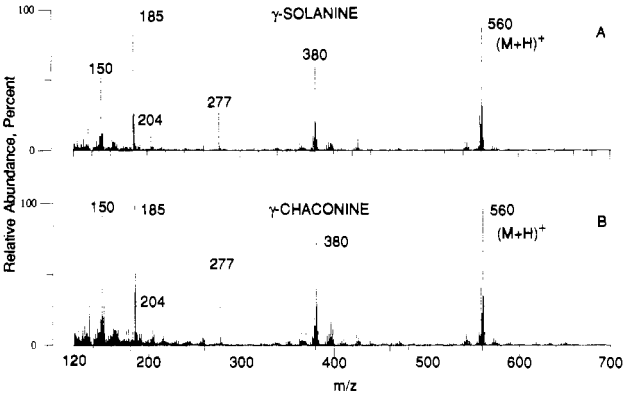

Figure 4. Positive ion LSIMS mass spectra of γ-solanine (A) and γ-chaconine (B).

pointed out, melting points of the various glycoalkaloids in and of themselves are unreliable as indicators of identity or purity. Different methods of heating may give widely varying values, as may the method of purification.

Thin-Layer Chromatography. TLC was used to monitor the column fractions after purification, to provide semiquantitative screens of hydrolysis mixtures, and to characterize the various compounds by R_f value. Table II lists the R_f and $R_{\alpha\text{-s}}$ values (R_f relative to that of α-solanine) for the hydrolysis compounds in several related solvent systems. For this study, the best results were obtained with solvent A, which gave adequate separation with very little spreading of the spots. Other solvent systems were tried, but the neutral or acidic systems or those with more than 10% water either gave poor separation or caused streaking.

Iodine vapor was used for visualizing spots in the column monitoring because it was fast and easy, involving no spraying or heating. Iodine vapor was also used for preparative TLC because the color is reversible, causing no chemical change in the compounds to be collected. The anisaldehyde spray was used to develop spots in the semiquantitative work because it is more sensitive and more specific than the iodine spray.

Factors Influencing Hydrolysis Rates. Tables III–XII present data from hydrolyses of the glycoalkaloids over time and under differing conditions. Tables III and IV cover α-chaconine; Tables V–VII, β- and γ-chaconines; Tables VIII and IX, α-solanine; Tables X and XI, β- and γ-solanines; and Table XII, mixture of α-chaconine and α-solanine. These data show that hydrolysis rates of starting materials and formation of hydrolysis products are accelerated by both HCl concentration in the range 0.1–0.5 N (Tables III and VIII) and temperature in the range 38–65 °C (Tables IV and IX).

Figure 5 compares the rates of disappearance of the different chaconines under the same hydrolytic conditions (0.2 N HCl–methanol, 65 °C). α-Chaconine seems to undergo stepwise hydrolysis, first to the β-chaconines, then to γ-chaconine, and finally to the aglycon. The first step can cleave either the 2-rhamnose or the 4-rhamnose, giving β_2- or β_1-chaconine, respectively. The data show that β_1-chaconine is produced twice as rapidly as the β_2 isomer. Once the diglycoside is formed, however, β_1-chaconine is more easily hydrolyzed than β_2-chaconine. This would seem to indicate that in the triglycoside the site of cleavage is more a factor of steric effects than bond strength. The γ-monoglycoside is relatively more difficult to hydrolyze to the aglycon, solanidine.

Figure 6 similarly compares the disappearance rates of the solanines (0.2 N HCl–methanol, 65 °C). The first step of hydrolysis is cleavage of the rhamnose residue from the

Table III. Effect of HCl Concentration on the Hydrolysis of α-Chaconine to β_1-, β_2-, and γ-Chaconines and Solanidine (Conditions: 0.1 and 0.2 N HCl–Methanol, 65 °C)

time, min	α-chaconine, μM %		β_1-chaconine, μM %		β_2-chaconine, μM %		γ-chaconine, μM %		solanidine, μM %	
	0.1 N	0.2 N	0.1 N	0.2 N	0.1 N	0.2 N	0.1 N	0.2 N	0.1 N	0.2 N
0	100[a]	100[b]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	84.0	83.8	5.8	8.2	9.4	7.2	0.8	0.6	0.0	0.0
20	74.5	68.2	11.1	14.7	11.8	14.0	2.6	3.0	0.0	0.0
30	65.5	55.4	14.7	20.9	14.5	16.8	5.2	6.9	0.0	0.0
40	51.4	45.9	21.3	24.2	17.7	18.0	9.5	11.9	0.0	0.0
50	46.8	40.2	20.6	25.8	17.2	18.2	14.9	15.5	0.4	0.2
60	45.8	31.5	22.7	27.5	15.9	18.2	14.9	22.0	0.7	0.8
70	39.3	27.5	24.1	27.5	18.3	17.1	17.2	26.7	1.1	1.3[c]

[a] Initial concentration of α-chaconine = 11.9 μM. [b] Initial concentration of α-chaconine = 10.3 μM. [c] After 80 min, μM % of solanidine is 2.0; after 90 min, 3.4.

Table IV. Effect of Temperature on the Hydrolysis of α-Chaconine to β_1-, β_2-, and γ-Chaconines and Solanidine (Conditions: 0.2 N HCl–Methanol at 38, 55, and 65 °C)

time, min	α-chaconine, μM %			β_1-chaconine, μM %			β_2-chaconine, μM %			γ-chaconine, μM %			solanidine, μM %		
	38 °C	55 °C	65 °C	38 °C	55 °C	65 °C	38 °C	55 °C	65 °C	38 °C	55 °C	65 °C	38 °C	55 °C	65 °C
0	100[a]	100[b]	100[c]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
15			77.9			11.0			10.7			0.4			0.0
30	97.9	76.9	55.4	1.8	15.6	20.9	0.3	6.5	16.8	0.0	0.9	6.9	0.0	0.0	0.0
40			45.9			24.2			18.2			11.9			0.0
50			40.2			25.8			18.2			15.5			0.2
60	95.1	49.0	31.5	3.5	24.9	27.5	1.5	14.8	18.2	0.0	11.4	22.0	0.0	0.0	0.8
70			27.5			27.5			17.1			26.7			1.3
80			23.0			27.5			15.9			31.6			2.0
90	90.4	31.2	20.8	6.5	32.6	27.5	3.1	15.0	13.3	0.0	20.3	34.9	0.0	0.9	3.4

[a] Initial concentration of α-chaconine = 11.7 μM. [b] Initial concentration of α-chaconine = 11.9 μM. [c] Initial concentration of α-chaconine = 10.3 μM.

Table V. Hydrolysis of β_1-Chaconine to γ-Chaconine and Solanidine (Conditions: 0.2 N HCl–Methanol, 65 °C)

time, min	β_1-chaconine		γ-chaconine, %	solanidine, %
	μM	%		
0	7.80	100.0	0.0	0.0
10	5.84	73.8	25.5	0.6
20	4.07	50.5	47.0	2.5
30	3.24	40.3	54.6	5.1
40	2.59	31.7	59.8	8.6
50	1.42	17.3	68.8	13.9
60	0.69	8.3	72.1	19.6

Table VI. Hydrolysis of β_2-Chaconine to γ-Chaconine and Solanidine (Conditions: 0.2 N HCl–Methanol, 65 °C)

time, min	β_2-chaconine		γ-chaconine, %	solanidine, %
	μM	%		
0	12.04	100.0	0.0	0.0
10	10.17	86.0	14.0	0.0
20	8.74	72.8	27.2	0.0
30	7.18	59.4	40.2	0.4
40	5.84	48.9	50.0	1.1
50	5.31	43.7	54.1	2.1
60	4.36	35.7	61.7	2.5
70	3.54	28.9	67.0	4.2

triglycoside, forming β_2-solanine exclusively. A very small peak was detected that could be due to β_1-solanine; however, we have been unable to confirm this (Swain *et al.*, 1978; Filadelfi and Zitnak, 1982). In any case, it would amount to no more than 0.1% of the total glycoalkaloid content of the mixture. β_2-Solanine is readily hydrolyzed, as measured by its disappearance, but an equivalent amount of γ-compound is not produced. Solanidine is formed much faster than γ-solanine. This could be caused either by the rapid hydrolysis of the γ compound as it is formed or by direct cleavage of the diglycoside. Since the data show that the hydrolysis of γ-solanine is slow, we conclude that β_2-solanine preferentially hydrolyzes to the aglycon.

Unknown Compounds. Hydrolysis of the glycoalkaloids formed small amounts of compounds of unknown

Table VII. Hydrolysis of γ-Chaconine to Solanidine (Conditions: 0.2 N HCl–Methanol, 65 °C)

time, min	γ-chaconine		solanidine, %
	μM	%	
0	1.43	100.0	0.0
10	1.41	98.6	1.4
20	1.34	97.1	3.6
30	1.43	93.5	6.5
40	1.35	88.2	11.8
50	1.42	85.0	15.0
60	1.27	77.9	22.1

structure in addition to the compounds listed in the figures and tables. Although these compounds were formed consistently and reproducibly, they represented less than 1% of the total parent glycoalkaloid content. Figure 7 is a typical HPLC chromatogram of the hydrolysis products of α-chaconine. Unknown peaks 1, 3, and 6 were isolated by TLC. Mass spectral data showed that peaks 1 and 3 have molecular weights of 851.5, the same as α-chaconine, and that peak 6 has a molecular weight of 705.4, the same as both the β-chaconines. This suggests that the peaks may be rearrangement products; for example, the cleaved rhamnose may reattach itself at a different site or by a different linkage. However, hydrolysis carried out in a 5% rhamnose solution, which might be expected to increase these peaks, had no effect on the results. Other possibilities include the following: (a) the new compounds are formed by acid-catalyzed isomerization of optically active centers in the carbohydrate residues to diastereomeric (anomeric) isomers; and (b) the double bond of the aglycon migrates to new positions, forming new isomers.

The chromatogram of an α-solanine hydrolysate (Figure 8) shows only one anomalous peak (peak 3). This seems to be a mixture of two or more closely related compounds. An attempt to characterize these compounds by mass spectrometry gave inconclusive results.

Hydrolysis of Potatoes. Dehydrated potato powders were hydrolyzed in 0.5 N HCl–methanol at 65 °C for 30, 60, and 90 min. Preliminary results indicate the suscep-

Table VIII. Effect of HCl Concentration on the Hydrolysis of α-Solanine to β_2- and γ-Solanines and Solanidine (Conditions: 0.1, 0.2, and 0.5 N HCl–Methanol, 65 °C)

time, min	α-solanine, μM %			β_2-solanine, μM %			γ-solanine, μM %			solanidine, μM %		
	0.1 N	0.2 N	0.5 N	0.1 N	0.2 N	0.5 N	0.1 N	0.2 N	0.5 N	0.1 N	0.2 N	0.5 N
0	100[a]	100[b]	100[c]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	94.3	97.4	84.0	5.7	2.6	16.0	0.0	0.0	0.0	0.0	0.0	0.0
20	90.6	90.0	61.6	9.4	10.0	31.1	0.0	0.0	3.2	0.0	0.0	4.1
30	86.3	83.8	48.4	13.7	15.7	38.0	0.0	0.5	5.7	0.0	0.0	7.9
40	82.4	78.4	37.5	17.6	20.6	40.9	0.0	0.9	8.1	0.0	0.0	13.5
50	78.0	72.7	30.2	22.0	25.1	42.4	0.0	1.7	10.4	0.0	0.5	16.9
60	73.2	68.0	23.6	24.6	28.7	42.3	0.9	2.1	12.0	1.1	1.2	22.2
70	68.3	63.5	18.7	27.8	31.5	41.0	1.6	2.6	13.4	2.3	2.4	26.9
80	64.9	59.1	15.1	30.5	32.0	38.9	1.6	3.2	14.0	3.0	5.7	32.0
90	62.0	54.0	12.9	32.3	34.4	37.2	1.6	4.8	15.2	4.1	6.7	34.7

[a] Initial concentration of α-solanine = 4.35 μM. [b] Initial concentration of α-solanine = 4.23 μM. [c] Initial concentration of α-solanine = 4.34 μM.

Table IX. Effect of Temperature on the Hydrolysis of α-Solanine to β_2- and γ-Solanines and Solanidine (Conditions: 0.2 N HCl–Methanol at 38, 55, and 65 °C)

time, min	α-solanine, μM %			β_2-solanine, μM %			γ-solanine, μM %			solanidine, μM %		
	38 °C	55 °C	65 °C	38 °C	55 °C	65 °C	38 °C	55 °C	65 °C	38 °C	55 °C	65 °C
0	100[a]	100[b]	100[c]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10			97.4			2.6			0.0			0.0
20			90.0			10.0			0.0			0.0
30	100	91.9	83.8	0.0	8.1	15.7	0.0	0.0	0.5	0.0	0.0	0.0
40			78.4			20.6			0.9			0.0
50			72.7			25.1			1.7			0.5
60	98.8	80.4	68.0	0.0	18.4	28.7	0.0	1.2	2.1	0.0	1.2	1.2
70			63.5			31.5			2.6			2.4
80			59.1			32.0			3.2			5.7
90	97.8	69.4	54.0	2.2	26.7	34.4	0.0	3.4	4.8	0.0	3.4	6.7

[a] Initial concentration of α-solanine = 11.7 μM. [b] Initial concentration of α-solanine = 12.0 μM. [c] Initial concentration of α-solanine = 4.3 μM.

Table X. Hydrolysis of β_2-Solanine to γ-Solanine and Solanidine (Conditions: 0.2 N HCl–Methanol, 65 °C)

time, min	β_2-solanine		γ-solanine, %	solanidine, %
	μM	%		
0	12.29	100.0	0.0	0.0
10	11.07	91.0	3.0	3.0
20	9.89	79.8	5.2	14.9
30	8.19	66.3	10.4	23.2
40	6.61	53.0	15.7	31.3
50	5.39	42.7	14.9	42.3
60	4.45	35.1	13.5	51.4

Table XI. Hydrolysis of γ-Solanine to Solanidine (Conditions: 0.2 N HCl–Methanol, 65 °C)

time, min	γ-solanine		solanidine, %
	μM	%	
0	2.61	100.0	0.0
10	2.58	98.1	1.9
20	2.53	97.1	2.9
30	2.49	94.5	5.5
40	2.42	91.3	8.7
50	2.33	86.8	13.2
60	2.18	80.8	19.2

tibilities of α-chaconine and α-solanine to hydrolysis in a potato matrix appear similar to those of the pure compounds. The extent of hydrolysis of the major glycoalkaloids in potatoes, α-chaconine and α-solanine, generally paralleled the hydrolysis of an artificial mixture of these two glycoalkaloids made up in a 52:48 ratio, similar to that found in potatoes (Table XII; Figure 9). Although the formation of the hydrolysis products was about the same for the two cases, the absolute amounts in potatoes were lower than in the artificial mixture, presumably because much of the acid in the potato experiment was used up in hydrolyzing starch to glucose rather than acting on the glycoalkaloids.

The chromatograms of the potato hydrolysates (Figure 10) contained several unknown peaks, presumably derived from the action of the acid on other potato constituents. These interfere with the quantitation of the alkaloids at low levels (less than 10 μg/mL). This means that actual potato samples would need a more extensive cleanup procedure than those used in this study if true quantitative results are to be achieved.

Mechanisms of Hydrolysis. Tables III–X show that the sum of micromoles of hydrolysis products is nearly equivalent to the amount of starting materials used. The quantitative recovery of hydrolysis products implies that side reactions of the carbohydrate side chains such as dehydration and/or cyclization to furan derivatives do not take place under the hydrolysis conditions used. Such side reactions are reported to occur during exposure of mono- and polysaccharides to strong acids at high temperature (BeMiller, 1967).

Acid and enzyme catalyses of carbohydrate hydrolysis have been studied extensively (BeMiller, 1967; Capon, 1969; Legler, 1990). The acid-catalyzed hydrolysis appears to be an S_N1-type reaction in which fast protonation of the glycosidic oxygen to form the conjugate acid is followed by cleavage of the exocyclic oxygen atom to produce a carbonium–oxonium ion intermediate. This intermediate then reacts with water to form the hydrolysis products. According to BeMiller (1967), both electronic and steric factors influence hydrolysis rates. These factors include ring size, configuration, conformation, and polarity of the sugar and size and polarity of the aglycon. BeMiller also notes that it is difficult to assign large differences in hydrolysis rates of disaccharides, polysaccharides, and related glycosides to a single factor.

Since both α-chaconine and α-solanine have the same aglycon, the observed differences in hydrolysis rates should be due only to differences in the composition of the trisaccharide side chain attached to the 3–OH group of

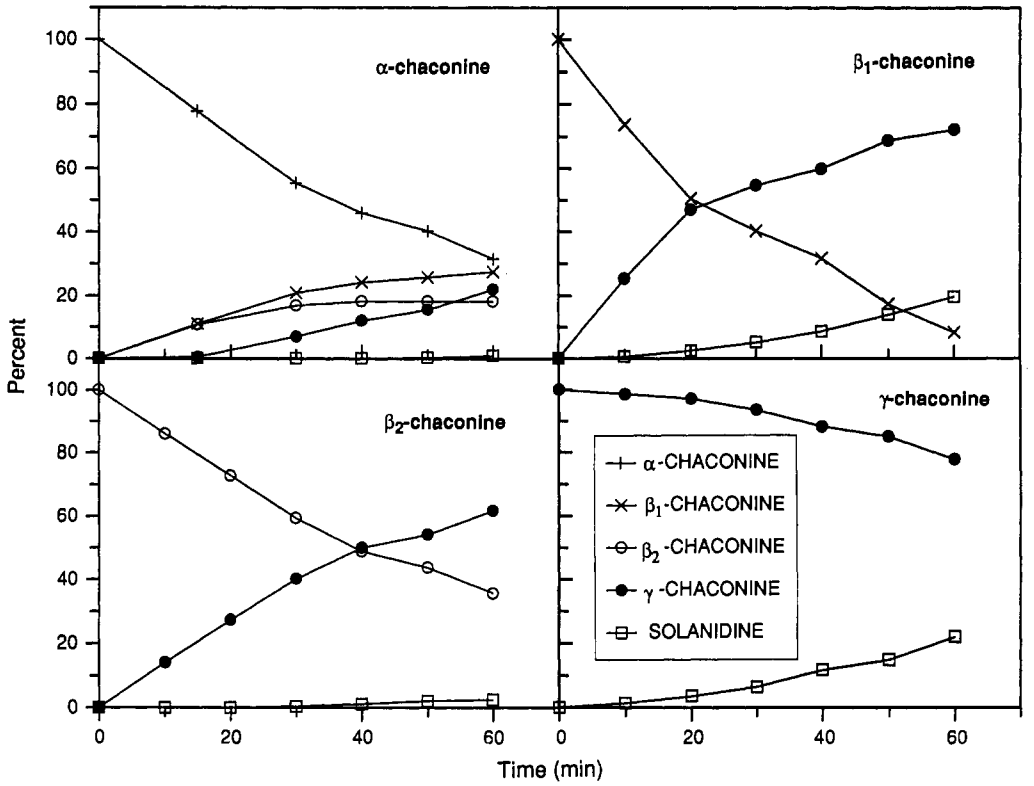

Figure 5. Comparison of hydrolysis rates of α-, β_1-, β_2-, and γ-chaconines in 0.2 N HCl–methanol at 65 °C.

Table XII. Hydrolysis of an α-Chaconine and α-Solanine Mixture (in a 52:48 Ratio Approximating That Present in Potatoes) to β_1-, β_2-, and γ-Chaconines, β_2- and γ-Solanines, and Solanidine (Conditions: 0.2 N HCl–Methanol, 65 °C)

time,	α-chaconine		β_1-chaconine		β_2-chaconine		γ-chaconine		α-solanine		β_2-solanine		γ-solanine		solanidine	
min	μM	%	μM	%	μM	%	μM	%	μM	%	μM	%	μM	%	μM	%
0	10.7	51.9	0.0	0.0	0.0	0.0	0.0	0.0	9.9	48.1	0.0	0.0	0.0	0.0	0.0	0.0
10	8.7	43.4	0.7	3.7	0.9	4.6	0.0	0.0	9.2	46.3	0.4	1.9	0.0	0.0	0.0	0.0
20	7.0	36.2	1.4	7.2	1.4	7.2	0.1	0.5	8.6	44.4	0.9	4.5	0.0	0.0	0.0	0.0
30	6.2	31.0	1.9	9.5	1.5	7.6	0.4	2.1	8.3	41.6	1.4	7.2	0.0	0.0	0.1	0.8
40	4.9	23.8	2.6	12.8	1.8	9.0	0.9	4.2	8.2	40.0	1.8	8.5	0.0	0.0	0.3	1.7
50	4.4	20.0	3.1	14.0	1.5	7.0	2.6	11.9	7.6	34.4	2.3	10.7	0.0	0.0	0.4	2.0
60	3.3	15.6	3.0	14.0	1.3	6.2	3.0	14.4	6.8	32.4	2.9	13.8	0.0	0.0	0.7	3.6
70	2.6	11.4	3.3	14.8	2.0	8.8	3.2	16.6	6.4	28.8	3.4	15.3	0.0	0.0	0.9	4.2
80	2.3	8.4	3.8	13.9	2.1	7.8	5.4	19.8	6.9	25.5	4.5	16.6	0.5	1.8	1.7	6.2

solanidine. This study shows that these differences are considerable when measured in terms of the observed hydrolysis products. Detailed kinetic studies on the hydrolysis of the carbohydrate side chain in glycoalkaloids apparently have not been previously reported. As far as we know, this is the first detailed kinetic study of the acid-catalyzed hydrolysis of the carbohydrate groups in glycoalkaloids. Our findings should stimulate interest in mechanistic studies to further define the parameters which govern hydrolysis rates of both acid- and enzyme-catalyzed reactions. Specifically, since the mechanism of acid hydrolysis postulates the formation of positively charged transition states, expectations are that both hydrolysis rates and distribution of hydrolysis products will be strongly influenced by the polarity of the solvent (Friedman, 1967). Preliminary studies show that this is indeed the case.

The situation is even more complicated because the tertiary nitrogen of the aglycon exists as the protonated (NH^+) form in strong acid solution. This implies that the transition state may have two positive charges. How solvent polarity would affect such an intermediate is not known.

Another mechanistic aspect of steroidal and alkaloidal solvolysis is relevant to hydrolyses of glycoalkaloids. Kupchan *et al.* (1966a,b) showed that the methanolysis of strophanthidin acetate and veratrum alkaloid acetates is enhanced as much as 4000-fold by bifunctional intramolecular general acid catalysis, as compared to steroid esters without nitrogen. In the case of alkaloidal esters, the authors postulate the involvement of the unshared electron pair of the ring nitrogen atoms in the catalysis. Although such facilitated solvolysis, the so-called Henbest–Kupchan effect (Kupchan *et al.*, 1962), of glycosidic bonds of the glycoalkaloids could not occur under the conditions of the present study, where the nitrogens are protonated, it could take place at higher pH values. Therefore, under basic conditions, the extent of intramolecular catalysis of hydrolysis of carbohydrate groups of the glycoalkaloids should be directly related to the nucleophilicities of the steroidal ring nitrogen atoms, as measured by basicities or pK values.

FUTURE STUDIES

Although this study covers only acid–methanol systems, preliminary studies show that about 1% of α-chaconine and 1% α-solanine are hydrolyzed in aqueous systems in 1 h under the acid conditions present in the digestive tracts of animals (1 N HCl, 38 °C). This value increases to 5% after 3 h. Hydrolysis could also occur under basic conditions after the glycoalkaloids pass from the stomach to the duodenum. Therefore, it is likely that the alkaloids

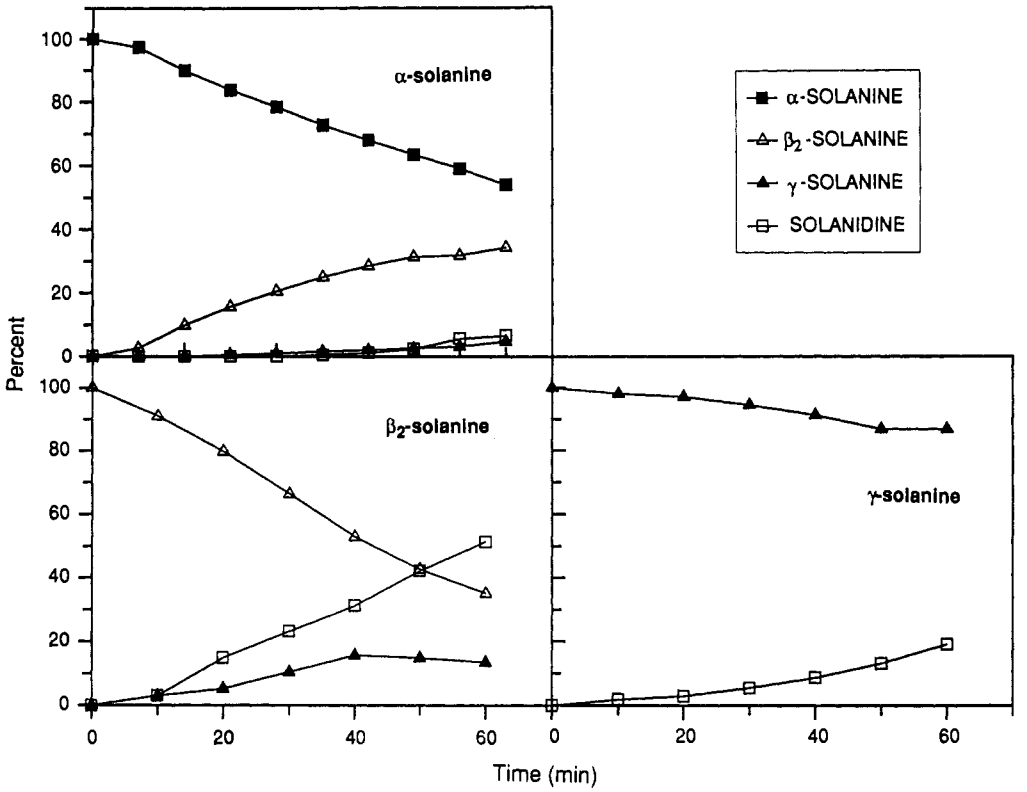

Figure 6. Comparison of the hydrolysis rates of α-, β-, and γ-solanines in 0.2 N HCl–methanol at 65 °C.

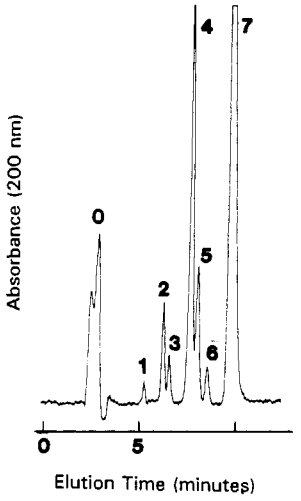

Figure 7. HPLC chromatogram of a partial acid hydrolysate of α-chaconine. Conditions: 0.2 N HCl–methanol, 65 °C, 1 h. 0, Solvent front; 1, unknown (MW 852); 2, α-chaconine; 3, unknown (MW 852); 4, β_1-chaconine; 5, β_2-chaconine; 6, unknown (MW 706); 7, γ-chaconine.

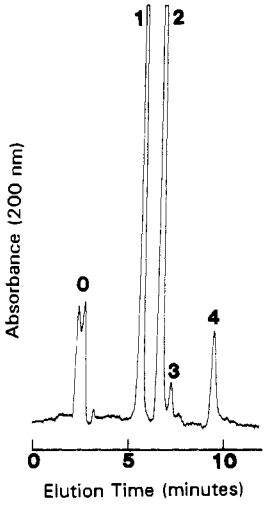

Figure 8. HPLC chromatogram of a partial acid hydrolysate of α-solanine. Conditions: 0.2 N HCl–methanol, 65 °C, 1 h. 0, Solvent front; 1, α-solanine; 2, β_2-solanine; 3, unknown; 4, γ-solanine.

are partly hydrolyzed following ingestion. However, these possibilities need to be confirmed with animal feeding studies.

The glycoalkaloids may also be susceptible to hydrolysis by enzymes (Legler, 1990). We do not know whether such glycosidases exist in the digestive tract or other organs such as the liver and kidneys. If they do, then the real target of metabolic, nutritional, and safety studies should be the resulting hydrolysis products.

Since both the number and nature of the carbohydrate residues attached to the 3–OH group of solanidine appear to be paramount in influencing relative safety, the described kinetic and synthetic studies should facilitate preparation of structurally different glycosides for biological evaluation. Our studies should also make it easier to measure the formation of the carbohydrate-containing intermediates during the biosynthesis of the glycoalkaloids in plants (Stapleton *et al.*, 1992) and to assess possible biological functions of carbohydrate groups in tomato alkaloids (Friedman *et al.*, 1992), anticarcinogenic quercetin glycosides (Leighton *et al.*, 1993), and saponins (Kitajima *et al.*, 1982; Wolf and Thomas, 1971).

Since the hydrolysis products may be less toxic than the parent compounds, exposure of potatoes to acid conditions may enhance food safety.

A final aspect that may be relevant to food safety and nutrition is possible heat-induced reactions of carbohydrate residues of glycoalkaloids with amino acids and proteins to form Maillard browning products (Friedman, 1991; Friedman and Molnar-Perl, 1990). If such browning reactions occur during food processing, they may lead to

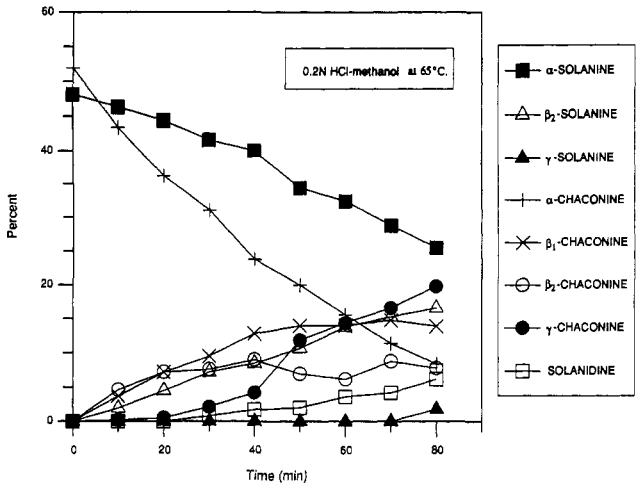

Figure 9. Kinetic course of the hydrolysis of a mixture of α-chaconine and α-solanine in a ratio approximating that in potatoes to β_1-, β_2-, and γ-chaconines, β_2- and γ-solanines, and solanidine. Conditions: 0.2 N HCl–methanol, 65 °C.

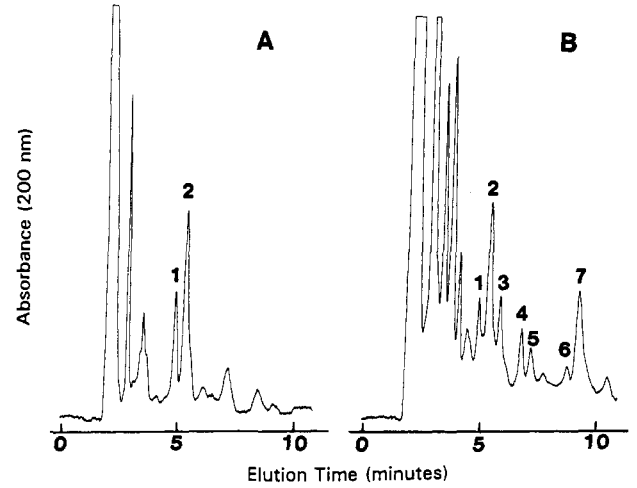

Figure 10. HPLC chromatograms of a partial acid hydrolysis of dried potato tubers. Conditions: 0.5 N HCl–methanol, 65 °C. (A) 0 min; 1, α-solanine; 2, α-chaconine. (B) 60 min; 1, α-solanine; 2, unknown hydrolysis product and α-chaconine (approximate ratio 9:1); 3, β_2-solanine; 4, β_1-chaconine; 5, β_2-chaconine; 6, γ-solanine; 7, γ-chaconine. Unlabeled peaks are solvent fronts and unknowns.

the removal of the carbohydrate groups of α-chaconine and α-solanine, forming less toxic compounds. As noted earlier in the discussion of melting point determination, the glycosides behave like carbohydrates under the influence of heat. Possible beneficial effects of acid treatment and browning reactions of glycoalkaloids in potatoes on food safety merit further study.

ACKNOWLEDGMENT

We are grateful to S. F. Osman and M. A. Filadelfi-Keszi for helpful comments.

LITERATURE CITED

BeMiller, J. N. Acid-catalyzed hydrolysis of glycosides. *Adv. Carbohydr. Chem.* **1967**, 22, 25–108.

Blankemeyer, J. T.; Stringer, B. K.; Rayburn, J. R.; Bantle, J. A.; Friedman, M. Effect of potato alkaloids, α-chaconine and α-solanine on membrane potential of frog embryos. *J. Agric. Food Chem.* **1992**, *40*, 2022–2025.

Blankemeyer, J. T.; Stringer, B. K.; Bantle, J. A.; Friedman, M. Correlation of the cell health assay of water quality (CHAWQ) with the frog embryo teratogenesis assay (FETAX). In *Environmental Toxicology and Risk Assessment*; Gorsuch, J. W., Dwyer, F. J., Ingersoll, C. G., La Point, T. W., Eds.; American Society for Testing Materials: Philadelphia, 1993; Vol. 2 (in press).

Budzikiewicz, H. Mass spectral fragmentation of steroidal alkaloids. *Tetrahedron* **1964**, *20*, 2029–2034.

Bushway, A. A.; Bushway, R. J.; Oh, C. H. Isolation, partial purification and characterization of a potato peel glycoalkaloid glycosidase. *Am. Potato J.* **1988**, *65*, 621–631.

Bushway, A. A.; Bushway, R. J.; Oh, C. H. Isolation, partial purification and characterization of a potato peel α-solanine cleaving glycosidase. *Am. Potato J.* **1990**, *67*, 233–238.

Caldwell, K. A.; Grosjean, O. K.; Henika, P. R.; Friedman, M. Hepatic ornithine decarboxylase induction by potato glycoalkaloids in rats. *Food Chem. Toxicol.* **1991**, *29*, 531–535.

Capon, B. Mechanism in carbohydrate chemistry. *Chem. Rev.* **1969**, *69*, 407–498.

Filadelfi, M. A.; Zitnak, A. Preparation of chaconines by enzymatic hydrolysis of potato berry alkaloids. *Phytochemistry* **1982**, *21*, 250–251.

Filadelfi, M. A.; Zitnak, A. A simple TLC standard for identification of potato glycoalkaloids. *Can. Inst. Food Sci. Technol. J.* **1983**, *16*, 151–153.

Friedman, M. Solvent effects in reactions of amino groups in amino acids, peptides, and proteins with α,β-unsaturated compounds. *J. Am. Chem. Soc.* **1967**, *89*, 4709–4713.

Friedman, M. Prevention of adverse effects of food browning. *Adv. Exp. Med. Biol.* **1991**, *289*, 171–215.

Friedman, M. Composition and safety evaluation of potato berries, potato and tomato seeds, potatoes, and potato alkaloids. In *Food Safety Assessment*; Finley, J. W., Robinson, S. F., Armstrong, D. J., Eds.; ACS Symposium Series 484; American Chemical Society: Washington, DC, 1992; pp 429–462.

Friedman, M.; Dao, L. Distribution of glycoalkaloids in potato plants and commercial potato products. *J. Agric. Food Chem.* **1992**, *40*, 419–423.

Friedman, M.; Henika, P. R. Absence of genotoxicity of potato alkaloids α-chaconine, α-solanine, and solanidine in the Ames *Salmonella* and adult and foetal erythrocyte micronucleus assays. *Food Chem. Toxicol.* **1992**, *30*, 689–694.

Friedman, M.; Levin, C. E. Reversed-phase high-performance chromatographic separation of potato glycoalkaloids and hydrolysis products on acidic columns. *J. Agric. Food Chem.* **1992**, *40*, 2157–2163.

Friedman, M.; Molnar-Perl, I. Inhibition of food browning by sulfur amino acids. 1. Heated amino acid-glucose systems. *J. Agric. Food Chem.* **1990**, *38*, 1642–1647.

Friedman, M.; Rayburn, J. R.; Bantle, J. A. Developmental toxicology of potato alkaloids in the frog embryo teratogenesis assay-Xenopus (FETAX). *Food Chem. Toxicol.* **1991**, *29*, 537–547.

Friedman, M.; Rayburn, J. R.; Bantle, J. A. Structural relationships and developmental toxicity of *Solanum* alkaloids in the frog embryo teratogenesis assay. *J. Agric. Food Chem.* **1992**, *40*, 1617–1624.

Guseva, A. R.; Paseshichenko, V. A. Enzymatic degradation of potato glycoalkaloids. *Biochemistry (Engl. Transl.)* Russian, **1957**, *22*, 792–799.

Jellema, R.; Elema, E. T.; Malingre, T. M. Fluorodensitometric determination of potato glycoalkaloids on thin-layer chromatograms. *J. Chromatogr.* **1981**, *210*, 121–129.

Keeler, R. F.; Baker, D. C.; Gaffield, W. Teratogenic *Solanum* species and the responsible teratogens. In *Handbook of Natural Toxins*; Keeler, R. F., Tu, A. T., Eds.; Dekker: New York, 1991; Vol. 6, pp 83–97.

Kitajima, J.; Komori, T.; Kawasaki, T.; Schulten, H. R. Basic steroid saponins from aerial parts of *Fritillaria thunberghii*. *Phytochemistry* **1982**, *21*, 187–192.

Kuhn, R.; Low, I. Chaconine. *Ann. Acad. Sci. Fenn., Ser. A2* **1955**, *60*, 488–495.

Kupchan, S. M.; Eriksen, S. P.; Friedman, M. General base-general acid catalysis of ester solvolysis. *J. Am. Chem. Soc.* **1962**, *84*, 4159–4160.

Kupchan, S. M.; Eriksen, S. P.; Friedman, M. Intramolecular catalysis. VIII. General base-general acid catalysis of ester solvolysis. *J. Am. Chem. Soc.* **1966a**, *88*, 343–346.

Kupchan, S. M.; Eriksen, S. P.; Liang, Y.-T. S. Intramolecular catalysis. IX. Intramolecular general base-general acid catalysis of ester solvolysis. *J. Am. Chem. Soc.* **1966b**, *88*, 347–350.

Legler, G. Glycoside hydrolases: mechanistic information from studies with reversible and irreversible inhibitors. *Adv. Carbohydr. Chem. Biochem.* **1990**, *48*, 319–384.

Leighton, T.; Ginther, C.; Fluss, L. The distribution of quercetin and quercetin glycosides in vegetable components of the human diet. In *Food and Cancer Prevention: Chemical and Biological Aspects*; Waldron, K. W., Johnson, I. T., Fenwick, G. R., Eds.; The Royal Society of Chemistry: Cambridge, England, 1993; pp 223–232.

Morris, S. C.; Lee, T. H. The toxicity and teratogenicity of *Solanaceae* glycoalkaloids, particularly those of the potato (*Solanum tuberosum*). *Food Technol. Aust.* **1984**, *36*, 118–124.

Osman, S. F.; Herb, S. F.; Fitzpatrick, T. H.; Sinden, S. Commersonine, a new glycoalkaloid from two *Solanum* species. *Phytochemistry* **1976**, *15*, 1065–1067.

Porter, W. L. A note on the melting point of α-solanine. *Am. Potato J.* **1972**, *49*, 403–406.

Price, K. R.; Mellon, F. A.; Self, R.; Fenwick, G. R.; Osman, S. F. Fast bombardment mass spectrometry of *Solanum* glycoalkaloids and its potential for mixture analysis. *Biomed. Mass Spectrom.* **1985**, *12*, 79–85.

Renwick, J. H.; Claringbold, W. D. B.; Earthy, M. E.; Few, J. D.; McLean, C. S. Neural-tube defects produced in Syrian hamsters by potato glycoalkaloids. *Teratology* **1984**, *30*, 371–378.

Schreiber, K. The steroid alkaloids of *Solanum*. In *The Biology and Taxonomy of the Solanaceae*; Hawkes, J. G., Ed.; Linnean Society Symposium Series 7; Academic Press: New York, 1979; pp 193–202.

Stapleton, A.; Allen, P. V.; Friedman, M.; Belknap, W. R. Isolation and characterization of solanidine glucosyltransferase from potato sprouts. *J. Agric. Food Chem.* **1991**, *39*, 1187–1293.

Stapleton, A.; Allen, P. V.; Tao, H. P.; Belknap, W. R.; Friedman, M. Partial amino acid sequence of potato solanidine UDP-glucose glucosyltransferase purified by new anion exchange and size exclusion media. *Protein Expression Purif.* **1992**, *3*, 85–92.

Swain, A. P.; Fitzpatrick, T. H.; Talley, E.; Herb, S. F.; Osman, S. F. Enzymatic hydrolysis of α-chaconine and α-solanine. *Phytochemistry* **1978**, *17*, 800–801.

Wolf, W. J.; Thomas, B. W. Ion-exchange chromatography of soybean saponins. *J. Chromatogr.* **1971**, *56*, 281–293.

Zitnak, A.; Filadelfi-Keszi, M. A. Isolation of β_2-chaconine, a potato bitterness factor. *J. Food Biochem.* **1988**, *12*, 183–190.

Received for review December 28, 1992. Revised manuscript received April 6, 1993. Accepted May 20, 1993.