Acid-Catalyzed Partial Hydrolysis of Carbohydrate Groups of the Potato Glycoalkaloid α-Chaconine in Alcoholic Solutions

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As part of an effort to improve the safety of plant-derived foods, the role of the carbohydrate side chain has been explored in biological effects of potato glycoalkaloids such as α-chaconine. This steroid glycoalkaloid has a trisaccharide attached to the 3-hydroxy position of the steroidal aglycon solanidine. This study attempts to define the effect of structurally different alcohols on the partial hydrolysis of α-chaconine to β_1-chaconine, β_2-chaconine, γ-chaconine, and solanidine. Partial hydrolyses were carried out in 97.5% alcohol–0.25 N HCl at 60 °C. HPLC was used to measure the distribution of hydrolysis products as a function of time. The rate of hydrolysis of α-chaconine in the straight-chain alcohol solutions was as follows: methanol > ethanol = 1-butanol > propanol > pentanol ≫ water. The longer the chain, the slower the rate of hydrolysis except for the anomalous result that the extent of hydrolysis in 1-butanol was equal to that in ethanol. However, hydrolysis in 2-butanol was slower than in 1-butanol. Surprisingly, hydrolysis in *tert*-butyl alcohol was slowest, proceeding more slowly than even in 1-pentanol. The formation of γ-chaconine was also greatly reduced in *tert*-butyl alcohol. Mechanistic rationalizations are offered to explain the observed trends in terms of the hydrophobic–hydrophilic nature of the glycoalkaloids and the solvation properties of the alcohols. The results should be generally useful for optimizing or minimizing the formation of specific hydrolysis products.

Keywords: *Carbohydrate hydrolysis; α-chaconine; β_1-chaconine; β_2-chaconine; γ-chaconine; food safety; glycoalkaloids; potatoes; solanidine; α-solanine*

INTRODUCTION

Potatoes are known to contain enzymes that hydrolyze the glycosidic part of the naturally occurring glycoalkaloids α-chaconine and α-solanine (Bushway et al., 1988, 1990; Filadelfi and Zitnak, 1982; Swain et al., 1978; Zitnak and Filadelfi, 1988). While these hydrolysis products are normally only a small part of the total glycoalkaloids present in potatoes, we have noted that after prolonged storage of sprouted potatoes, all of the α-chaconine is converted to β_2-chaconine while the α-solanine is unaffected (unpublished results). What triggers this change is not known. Also, the fate of the glycoalkaloids in the digestive tract is, as yet, unknown. Acid and/or enzymatic hydrolysis may occur. Finally, attempts to genetically suppress the enzymes involved in glycosylation of the aglycon solanidine (Stapleton et al., 1991, 1992, 1994) could result in increased accumulation of the hydrolyzed forms. Thus, it is important to determine the biochemistry of all forms of chaconine and solanine as well as solanidine.

As part of this objective, we are currently attempting to develop a chemical structure–biological activity relationship for the glycoalkaloids of potatoes, tomatoes, and eggplants. Previously, we examined the role of the carbohydrate side chain of α-chaconine and α-solanine in developmental toxicity (Rayburn et al., 1994). This involved testing the partial hydrolysis products, i.e. the β disaccharides and the γ monosaccharide, as well as the parent α trisaccharide, and the aglycon, solanidine (Figure 1). The results showed that the biological activity was influenced by both the nature and the number of sugars making up the carbohydrate moiety attached to the 3-OH position of the aglycon. The developmental toxicity generally decreased with stepwise removal of sugar units from the chacotriose and solatriose side chains.

To obtain sufficient quantities of the hydrolysis products for further biological studies, we felt it would be beneficial to examine and optimize conditions that would favor the formation of the different hydrolysis products. In Friedman et al. (1993), we reported that hydrolysis rates in methanol increased with HCl concentration and temperature and decreased with the amount of water in methanol–water solutions. In the course of that study, it also became apparent that the rate of hydrolysis and composition of the mixture was considerably altered by substituting ethanol for methanol. This study extends our earlier observations by comparing the extent of hydrolysis of α-chaconine and the concurrent formation of the four hydrolysis products (see Figure 1) under otherwise identical conditions (0.25 N HCl, 60 °C) in a series of alcohol solutions of increasing number of methyl and methylene groups, both straight chain and branched. Included are methanol, ethanol, propanol (1-propanol), 2-propanol, 1-butanol, isobutyl alcohol (2-methyl-1-propanol), 2-butanol, *tert*-butyl alcohol (2-methyl-2-propanol), and pentanol (1-pentanol). We also studied the effect of adding water to the ethanol solutions.

The main objective of this study was to elucidate the role of the solvent in the hydrolysis of glycoalkaloids in order to facilitate optimizing or minimizing conditions for the formation of specific hydrolysis products.

MATERIALS AND METHODS

Materials. α-Chaconine was prepared from Russet potato sprouts as described in Friedman et al. (1993). Solvents purchased commercially, methanol (99.9%), propanol (99.9%), 2-propanol (99.9%), and pentanol (BP 137.7–138.0 °C) were Baker Analyzed reagents (J. T. Baker Chemical Co., Phillipsburg, NJ); 1-butanol (99.9%), isobutyl alcohol (>99%), and *tert*-butyl alcohol (>99%) were obtained from EM Science (Cherry

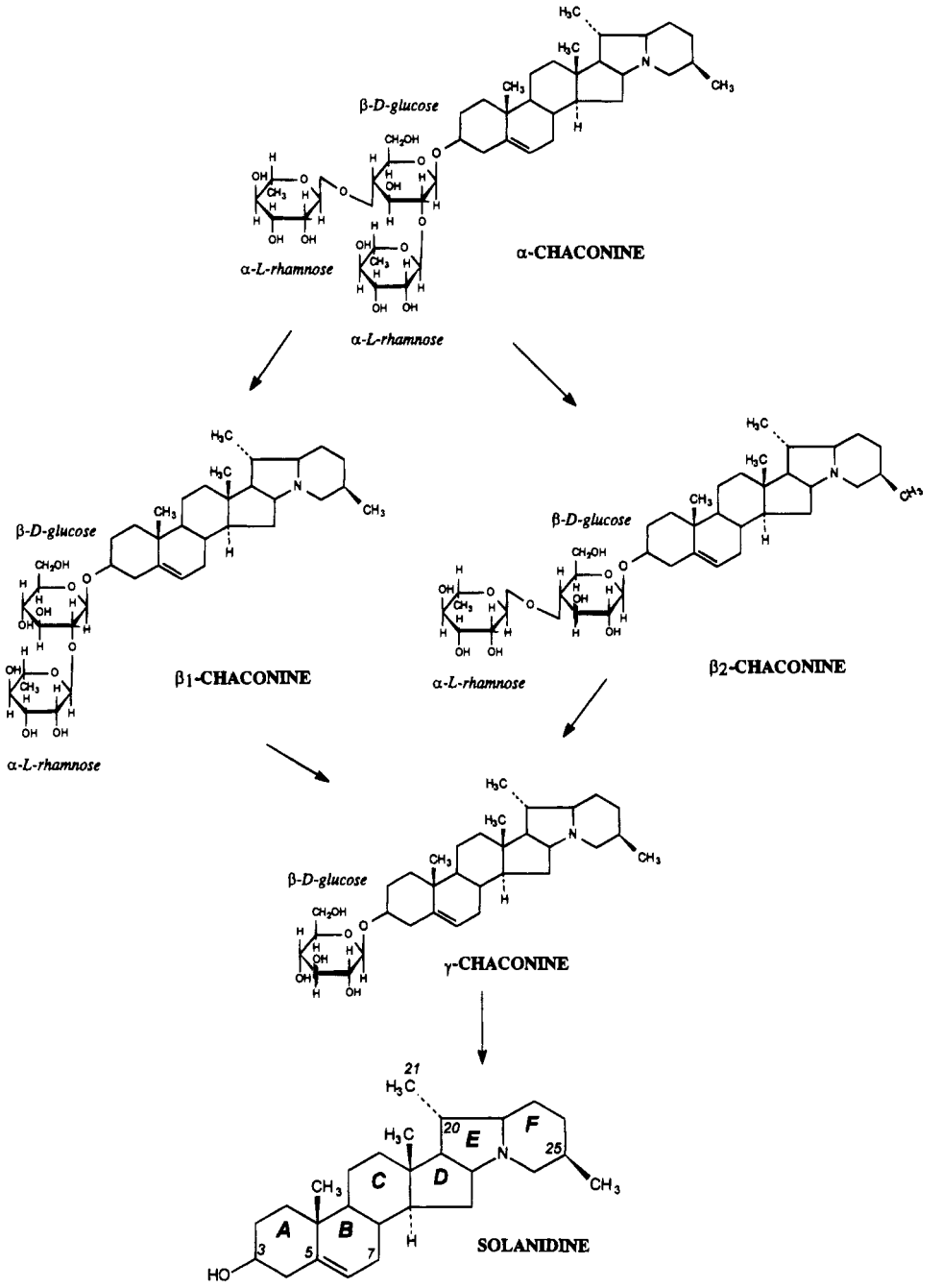


Figure 1. Structures of α-chaconine and hydrolysis products.

Hills, NJ); 2-butanol (>99%) was Fisher Certified (Fisher Scientific, Fair Lawn, NJ); and ethanol (>99%) was from Quantum Chemical Corp. (Anaheim, CA).

Acid Hydrolysis. Hydrolyses were carried out in flasks fitted with condensers placed in a circulating water bath set at 60 °C. The 97.5% 0.25 N HCl hydrolysis solutions were prepared by taking 487.5 mL of alcohol and adding 10.4 mL of 12 N HCl and 2.1 mL of H_2O to a final volume of 500 mL. The 95, 90, 80, and 50% ethanol solutions were prepared similarly using 10.4 mL of 12 N HCl and appropriate amounts of alcohol and water to a final volume of 500 mL. The solution (120 mL) was heated in the water bath for 1 h. α-Chaconine (24 mg) was added, and 20 mL aliquots were taken at 0, 20, 40, 60, 80, and 100 min intervals. The aliquots were immediately neutralized with NH_4OH. They were then evaporated to dryness, resuspended in 25 mL of H_2O, made basic with NH_4OH, and partitioned twice with 20 mL portions of water-saturated butanol. The butanol fractions were combined and evaporated to dryness. The residues were dissolved in 1 mL of methanol and diluted to 10 mL with methanol–acetonitrile–water (10:55:35). These solutions (20 μL) were then analyzed directly by HPLC.

Composition of Partial Acid Hydrolysates. Authentic standards of β_1-, β_2-, and γ-chaconines and solanidine were isolated from a partial hydrolysis mixture and characterized with the aid of mass spectrometry and other techniques as described previously (Friedman et al., 1993). The quantitative composition of each hydrolysis mixture was calculated with the aid of linear concentration plots (standard curves) obtained with α-chaconine and these hydrolysis products.

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). Glycoalkaloids were analyzed on a Resolve C_{18}, 90A, 5 μm, 3.9 × 300 mm column (Waters Chromatography, Milford, MA) at a flow rate of 1 mL/min. Eluent was 100 mM ammonium phosphate, monobasic, in 35% acetonitrile adjusted to pH 3.5 with phosphoric acid.

Solanidine was analyzed on a Supelcosil LC-18 DB column (Supelco, Bellefonte, PA) at a flow rate of 0.5 mL/min. Eluent was 10 mM ammonium phosphate, monobasic, in 60% acetonitrile adjusted to pH 3.0 with phosphoric acid. Figure 2 shows typical chromatograms of a partial hydrolysate analyzed for glycoalkaloids (A) and aglycons (B).

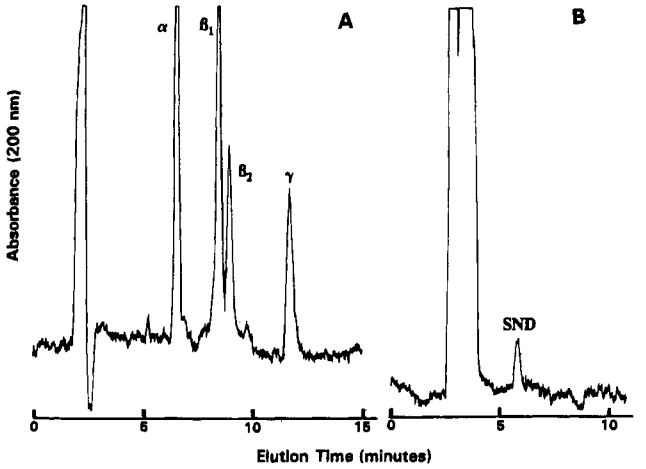


Figure 2. HPLC chromatogram of a partial hydrolysate of α-chaconine. Conditions: 97.5% 1-butanol; 0.25 N HCl; 60 °C; 60 min. (A) Glycoalkaloids (Resolve C_{18} column; flow rate, 1 mL/min of 100 mM ammonium phosphate, monobasic, in 35% acetonitrile adjusted to pH 3.5 with phosphoric acid): α = α-chaconine, β_1 = β_1-chaconine, β_2 = β_2-chaconine, γ = γ-chaconine. (B) Aglycons (Supelcosil LC-18 DB column; flow rate, 0.5 mL/min of 10 mM ammonium phosphate, monobasic, in 60% acetonitrile adjusted to pH 3.0 with phosphoric acid): SND = solanidine.

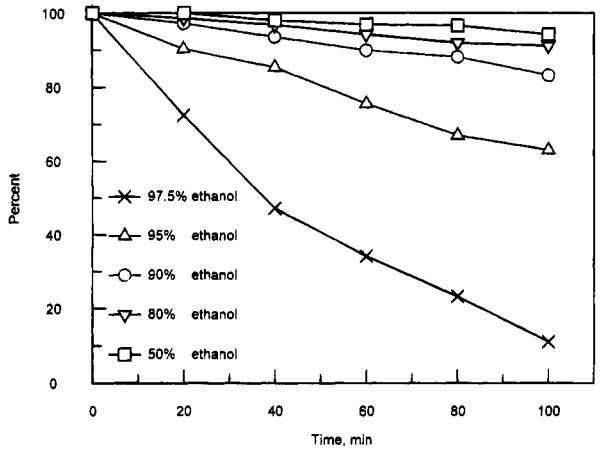


Figure 3. Effect of water in ethanol–water mixtures on the extent of hydrolysis of α-chaconine. Conditions: 0.25 N HCl; 60 °C.

RESULTS AND DISCUSSION

Hydrolysis Conditions. Of the two main potato glycoalkaloids α-chaconine was selected for this study because it undergoes acid-catalyzed hydrolysis under milder conditions than α-solanine. In addition, the hydrolysis produces both isomeric diosides (β_1- and β_2-chaconine), the monoside (γ-chaconine), and the aglycon (solanidine), all in reasonable quantities. α-Solanine produces only one dioside, β_2-solanine, and the yield of γ-solanine is quite small (Friedman et al., 1993). We selected 60 °C because this temperature gives a reasonable rate of hydrolysis and is below the boiling point of methanol. We used 0.25 N HCl because this concentration permits the hydrolysis to proceed with minimal side reactions.

Effect of Water on the Extent of Hydrolysis. Since water is required for hydrolysis and since the extent of hydrolysis of α-chaconine in pure water is low, our first objective was to establish the effect of water in the hydrolysis solutions. Figure 3 shows the hydrolysis of α-chaconine in five mixtures of ethanol–water ranging from 50 to 97.5% ethanol. Ethanol was chosen

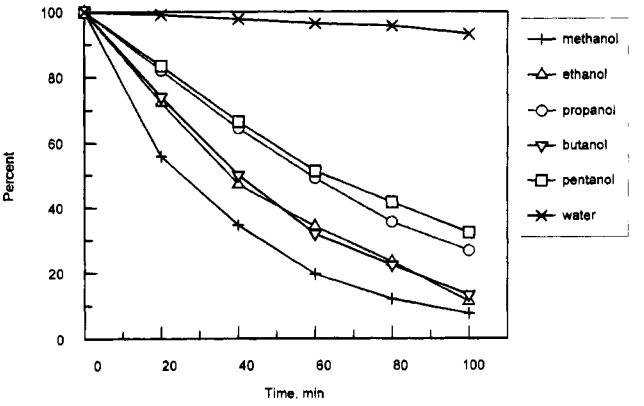


Figure 4. Comparison of the hydrolysis (disappearance) of α-chaconine in various alcohols and water. Conditions: 97.5% alcohol or water; 0.25 N HCl; 60 °C.

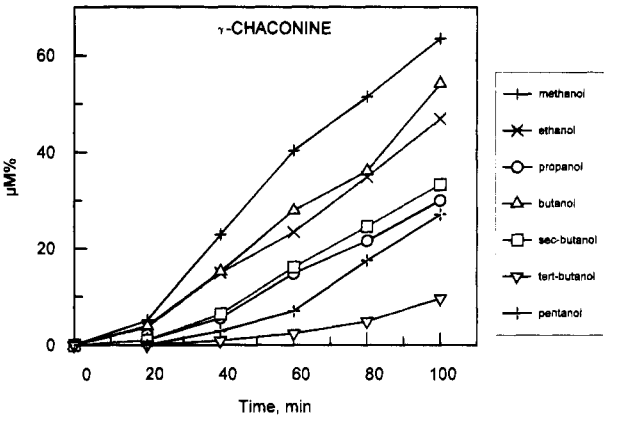


Figure 5. Comparison of rates of formation of γ-chaconine in various alcohols. Conditions: 97.5% alcohol; 0.25 N HCl; 60 °C.

for the extended study so as to determine if there would be any change in glycoalkaloid content of potatoes if cooked in or consumed with alcoholic beverages. Preliminary experiments with methanol and propanol showed similar trends. Comparing the results with the pure water curve (Figure 4) shows that 80% ethanol is little different from pure water and that 90% ethanol is only slightly better. The difference between 95 and 97.5% is, however, striking. At this level, small changes in the amount of water can dramatically affect the rate of hydrolysis. For this reason we used 97.5% alcohol solutions for all further studies.

Generally, solvents such as water facilitate hydrolysis through hydrogen-bonding interactions with the substrate. With the glycoalkaloids, however, solvation of the carbohydrate side chain by water molecules appears to have little or even a negative effect on the hydrolysis mechanism.

We have no obvious explanation for the described effects of water in the hydrolysis. Below, we offer some mechanistic rationalizations.

Effect of Alcohol on Hydrolysis and Product Distribution. Figure 4 compares the disappearance of the starting material, α-chaconine, and Figure 5 the formation of γ-chaconine in the same alcoholic solutions. Figures 6 and 7 and Table 1 give the composition of hydrolysis mixtures, as determined by HPLC, over time for a series of alcoholic solutions. The results in these figures strikingly demonstrate the influence of the structure of the alcohol on hydrolysis rates. The results show that the order of the rate of hydrolysis for α-chaconine in the straight-chain alcohols was as fol-

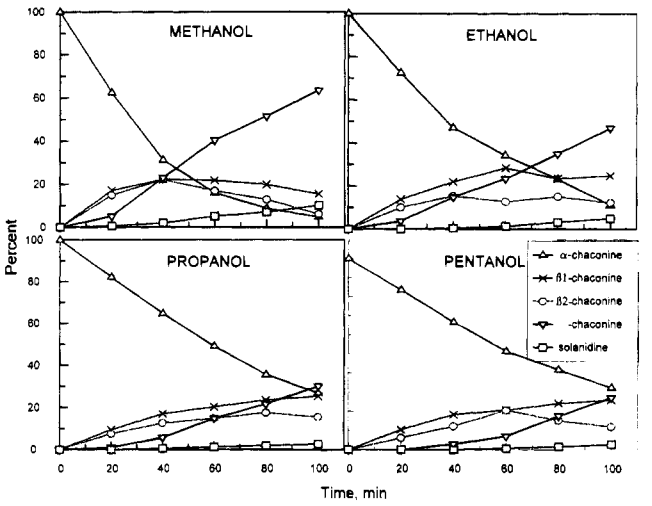


Figure 6. Comparison of hydrolysis rates of α-chaconine to β_1-, β_2-, and γ-chaconines and solanidine in methanol, ethanol, propanol, and pentanol. Conditions: 97.5% alcohol; 0.25 N HCl; 60 °C.

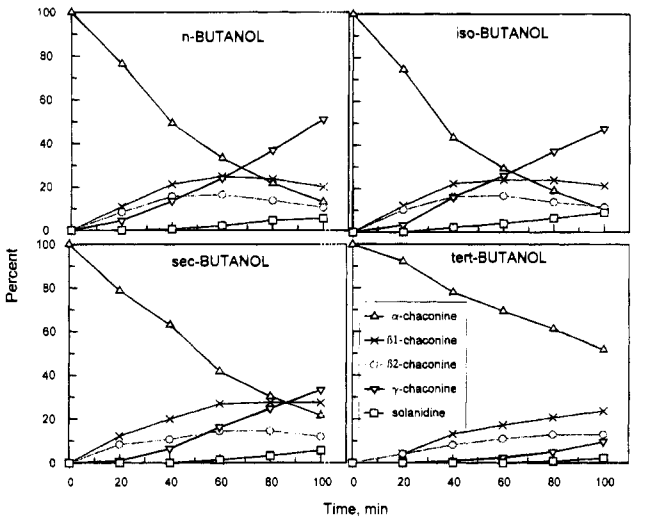


Figure 7. Comparison of hydrolysis rates of α-chaconine to β_1-, β_2-, and γ-chaconines and solanidine in 1-butanol, isobutyl alcohol, 2-butanol, and *tert*-butyl alcohol. Conditions: 97.5% alcohol; 0.25 N HCl; 60 °C.

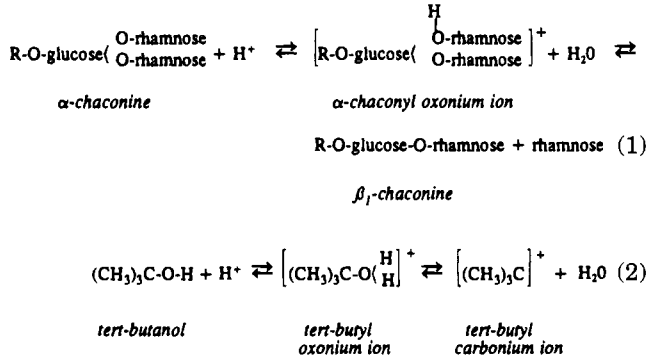
lows: methanol > ethanol = butanol > propanol > pentanol ≫ water.

A more detailed examination of the trends in hydrolysis rates revealed that the longer the chain, the slower the rate of hydrolysis except for the unexpected result that the rate in 1-butanol, a four-carbon-atom alcohol, equaled the rate in ethanol, a two-carbon-atom alcohol. Among the branched-chain alcohols, rates in 2-propanol equaled those in propanol; those in isobutyl alcohol were similar to hydrolysis rates in 1-butanol. However, reactions in 2-butanol were somewhat slower than in 1-butanol; those in *tert*-butyl alcohol were slowest compared to all other alcohols, even slower than in pentanol. These results imply that the use of different alcohols makes it possible to generate mixtures with different distributions of hydrolysis products.

The formation of γ-chaconine and solanidine in *tert*-butyl alcohol was also greatly reduced compared to that in other alcohols evaluated. Possible reasons for these differences are also not immediately apparent. Below, we speculate that differences in charge distribution in reactants and solvents and steric factors may be responsible for the observed effects.

Mechanisms of Solvent Effects. Table 1 shows that the sum of micromoles of hydrolysis products is equivalent within experimental error to the micromoles of the starting material α-chaconine. This result suggests that side reactions of the carbohydrate side chains of α-chaconine, such as cyclization, dehydration, and furan formation, reported to occur during the acid hydrolysis of carbohydrates under different conditions (BeMiller, 1967; Capon, 1969; Legler, 1990) did not occur under the hydrolysis conditions selected for this study. In this connection it is worth noting that we were unsuccessful in attempts to carry out the hydrolysis in the dipolar solvent dimethyl sulfoxide (DMSO), a solvent known to facilitate reactions with charged transition states (Friedman, 1967; Friedman and Koenig, 1971). Although α-chaconine disappeared rapidly, we could not find any corresponding hydrolysis products, presumably because some other reaction such as ring cleavage occurred (results not shown).

According to BeMiller (1967) and Capon (1969), the mechanism of acid-catalyzed hydrolysis of oligo- and polysaccharides is an S_N1-type reaction in which fast protonation of glycosidic oxygen to form the conjugate acid is followed by cleavage of the exocyclic oxygen atom to produce the hydrolysis product(s) (eq 1). Both elec-



tronic and steric factors including ring size, configuration, conformation, and polarities of the sugar and aglycon influence hydrolysis rates.

Examination of the structure of α-chaconine (Figure 1) shows that it consists of a polar, hydrophilic trisaccharide side chain attached to a hydrophobic tertiary nitrogen-containing steroid moiety. Since this tertiary nitrogen atom is protonated in the acid solution used for hydrolysis, the transition state may have two positive charges, one on the glycosidic oxygen linking the carbohydrate groups and the other on the tertiary nitrogen of the steroid ring. As stepwise hydrolysis proceeds and the number of sugars in the side chain is consecutively reduced, the hydrolysis products presumably become successively less polar.

The situation with respect to the intermediate hydrolysis products is quite complex since it reflects both the appearance of products derived from α-chaconine and their partial disappearance when they are themselves concurrently hydrolyzed. The overall hydrolysis scheme is a complex, dynamic series of competitive simultaneous and consecutive reactions. Crabbe and Fryer (1983a–c) devised and used such a scheme to optimize the hydrolysis of the glycoalkaloid solasonine to the aglycon solasodine. They found that reaction rates in 2-propanol–water increased with acid concentration and temperature and decreased with the addition of water to the alcohol solvent. They ascribed differences in hydrolysis rates in aqueous–alcoholic solutions to differences associated with the acidity

Table 1. Hydrolysis of α-Chaconine to β_1-, β_2-, and γ-Chaconines and Solanidine in Different Solvents (97.5%, 0.25 N HCl, 60 °C)

solvent	μM at 0 min[a]	total μM at 60 min[b]	μM % after 60 min				
			α-chaconine	β_1-chaconine	β_2-chaconine	γ-chaconine	solanidine
water	4.64	4.49	96.4	1.8	1.8	0.0	0.0
methanol	4.41	4.43	19.6	21.0	14.4	41.5	3.4
ethanol	4.51	4.48	34.2	28.4	12.8	23.4	1.3
propanol	4.14	4.00	49.0	20.2	14.8	14.8	1.2
2-propanol	4.26	3.97	52.1	21.7	13.6	12.1	0.5
1-butanol	4.30	4.23	31.9	23.9	15.1	27.9	1.2
isobutyl alcohol	4.35	4.54	29.3	24.0	16.7	26.0	4.0
2-butanol	4.21	4.07	41.6	26.7	14.4	16.1	1.3
tert-butyl alcohol	4.68	4.54	69.4	17.2	11.0	2.4	0.0
pentanol	4.58	4.28	51.2	20.6	20.6	7.0	0.7

[a] α-Chaconine found in 20 mL aliquot. [b] Sum of α-, β_1-, β_2-, and γ-chaconines and solanidine in 20 mL aliquot.

function, a measure of the proton-donating ability of the solvent. Devising an analogous model that optimizes the intermediate products rather than the final aglycon is a challenging problem.

The observed decreasing trend in the hydrolysis rates with increasing chain length of the series of straight-chain alcohols consisting of methanol, ethanol, propanol, and pentanol appears to parallel the reported decreases of dielectric constants and dipole moments in the same series (Streitwieser and Heathcock, 1985). However, the hydrolysis rate in 1-butanol did not follow the decreasing trend but was similar to that of ethanol in several experiments. We have no explanation for this observation.

Several branched-chain alcohols were also examined including the butanol series. Butanol and isobutyl alcohol gave similar results. This is not surprising as they are both primary alcohols with the branching occurring at the secondary carbon. The secondary alcohols, 2-propanol and 2-butanol, showed either no effect or a slight decrease in rates, respectively. However, the tertiary alcohol, *tert*-butyl alcohol, showed a dramatic decrease in the rate of hydrolysis of α-chaconine and the resulting β-chaconines. One possible explanation for this trend is that the mechanism of hydrolysis also involves protonation of the solvent as illustrated in eq 2. Stabilization of the positive charge on the tertiary butyl oxonium and carbonium ions is facilitated by hyperconjugation with methyl group hydrogens (Streitwieser and Heathcock, 1985). The OH group of *tert*-butyl alcohol competes with the glycosidic oxygen of the glycoalkaloids for protons. This could result in a decreased hydrolysis rate since the effective concentration of protons (H^+) available for hydrolyzing the glycoside is reduced. Analogous protonation would be less in secondary and least in primary alcohols. Steric hindrance of the surrounding methyl groups of *tert*-butyl alcohol could also be a significant factor in slowing the reaction but does not seem to affect the hydrolysis in secondary alcohols.

Preliminary experiments (not shown) with the hydrolysis of α- and β_2-solanines in *tert*-butyl alcohol seem to confirm that there is little if any direct hydrolysis of the β_2-solanine to solanidine—bypassing γ-solanine—as was observed in the previous study with methanol (Friedman et al., 1993). This finding implies that the observed sequential hydrolysis of α-chaconine in *tert*-butyl alcohol might also occur with other glycoalkaloids. These considerations do not account for the anomalous behavior of 1-butanol and isobutyl alcohol, however.

One other factor could influence hydrolysis rates. It is well-known that glycoalkaloids and aglycons possess surfactant properties (Roddick, 1974). Formation of surfactants between alcohols and alkaloids could alter rates by controlling diffusion of H^+ from HCl to the glycosidic oxygen atoms of the carbohydrate moieties which, as mentioned, need to be protonated before they can be cleaved. In fact, the mechanism may be analogous to enzyme catalysis in nonaqueous solvents. An enzyme "sees" only the tightly bound water layer, the so called "essential layer" (Gupta, 1992; Zaks and Klibanov, 1988). The increase in hydrolytic activity with increasing percent ethanol may be due to an effective concentration of H^+ in the immediate layer surrounding the glycoalkaloid molecules.

Evidently, the role of alcoholic solvents in the hydrolysis of amphilic-type molecules is not a clear-cut proposition. One portion of the molecule may prefer dissolution in a hydroxylic solvent, and the other portion may favor a hydrocarbon solvent (Streitwieser and Heathcock, 1985). The amphilic nature of the glycoalkaloids is also supported by their ability to disrupt cell membranes, presumably by interaction with the hydrophilic–hydrophobic bilayers of the membranes (Blankemeyer et al., 1992, 1995). Solvents such as methanol with the right balance of hydrophilic–hydrophobic character would then be the most efficient hydrolysis media for glycoalkaloids.

Applications. This and a previous study (Friedman et al., 1993) permit making the following recommendations for maximizing the formation of specific hydrolysis products:

(*1*) γ-Chaconine was produced in quantity by hydrolyzing α-chaconine in 0.25 N HCl in methanol at 65 °C for 30–40 min. This would be the preferred method for a product that is readily formed and not easily hydrolyzed.

(*2*) If the compound is readily formed but is easily hydrolyzed, e.g. β_1- or β_2-chaconine, *tert*-butyl alcohol at 60–70 °C for several hours will slow the secondary reaction and should give a good yield.

(*3*) When total conversion to the aglycon is desired, the biphasic solvent method of van Gelder (1984) is recommended. If, however, the use of carbon tetrachloride is not practical, 97.5% ethanol or other alcohol at 75 °C for several hours in 0.5–1.0 N HCl is also useful. The increased temperature accelerates the hydrolysis compared to methanol, and the still relatively mild conditions keep side reactions to a minimum.

(*4*) When the desired product is not formed because the carbohydrate preferentially cleaves off two or more sugars at a time, the use of *tert*-butyl alcohol for 12 h or more could induce the hydrolysis to proceed in a stepwise manner. We have used 0.25 N HCl in *tert*-butyl alcohol to reduce the direct hydrolysis of β_2-solanine to solanidine, which significantly increases the yield of γ-solanine.

The applicability of the described solvent effects to the controlled hydrolysis of carbohydrate groups in other glycoalkaloids (Friedman et al., 1994), nonsteroidal glycosides such as digoxin and rutin (Kuhlmann et al., 1973; Leighton et al., 1993), glycoproteins such as soybean hemagglutinin (Liener, 1989), oligo- and polysaccharides such as sucrose, cellulose, and starch (BeMiller, 1967; Capon, 1969; Legler, 1990), and Maillard reaction products such as maltotriulose glycine (Schumacher and Kroh, 1994) merits further study.

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