Part III: Combined Glycoside Hydrolysis and Solasodiene Formation— Guidelines for Commercial Hydrolysis

Kinetic models for glycoside hydrolysis and solasodiene formation are combined, and using previous experimental data the effect of changes in reaction conditions on hydrolysis times and the proportion of solasodiene in the final hydrolysed product are established. These are used to give a set of guidelines for commercial hydrolysis. The use of the models for new commercial situations is explained.

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SCOPE

Mathematical models have been proposed to describe the hydrolysis of pure α -solasonine and α -solamargine (Part I), and the further reaction of pure solasodine to produce solasodiene (Part II). The proposed models have been shown to describe adequately these three independent reactions. Methods have been presented for determination of the model parameters from experimental data. Any commercial hydrolysis will be more complex, involving all of these three reactions. The individual

kinetic models need to be combined to describe such systems. The description of the hydrolysis reaction must be extended to incorporate solasodiene formation from the released solasodiene so that the time for hydrolysis and the proportion of solasodiene in the final product can be predicted. Consideration of the predictions of this combined model, using parameter values obtained in the studies of the individual reactions, should provide guidance on the optimization of the hydrolysis process.

CONCLUSIONS AND SIGNIFICANCE

Previous models are easily extended to more complex hydrolysis situations; a model is established for the most general hydrolysis system, that of the hydrolysis of a mixed glycoside substrate with allowance for solasodiene formation from the released solasodine. Using the experimental rate data for α -solasonine hydrolysis (from Part I) and that for the solasodine to solasodiene conversion (from Part II), estimates of the reaction times (for specified conversions) and of solasodiene percentages in the final product have been obtained. For the conditions

studied, solasodiene percentages in the final product are low (generally less than 1%); the problem of solasodiene formation is not as great as previously reported, provided that the reaction is stopped once hydrolysis is complete. Reaction rates increase with acid concentration and temperature, but decrease sharply with the addition of water to an alcohol solvent. The glycoside concentration has no effect unless solubility limits of the intermediates are exceeded.

INTRODUCTION

The triglycosides solasonine and solamargine, extracted from *Solanum* plants, are hydrolyzed using mineral acid in aqueous alcohol solution to yield the aglycone solasodine. A mathematical description of the hydrolysis reactions has been developed and experimentally tested (Part I). Part II presents a study of the conversion of solasodine to the impurity solasodiene, which occurs slowly at the normal hydrolysis conditions.

The overall aim of this study of glycoside hydrolysis is to provide a mathematical description of a system such as those used in commercial processing. In such systems, hydrolysis of both triglycosides and the undesired conversion of solasodine product to solasodiene occur simultaneously. Furthermore, the original glycoside mixture presented for hydrolysis may already contain the di- and mono-glycosides which are intermediates in the triglycoside hydrolysis. Thus it is necessary to combine the hydrolysis models and the solasodiene formation model to describe these more complex systems. The following combinations of the individual models are considered below:

- (a) Hydrolysis of a single triglycoside substrate combined with solasodiene formation from the solasodine product.
- (b) Simultaneous hydrolysis of the triglycosides α -solasonine and α -solamargine with concurrent solasodiene formation.

(c) Hydrolysis of a mixed glycoside substrate with concurrent solasodiene formation.

GLYCOSIDE HYDROLYSIS AND SOLASODIENE FORMATION

The modelling of the α -solasonine hydrolysis process considered previously (Part I) assumed that the solasodine once formed did not further react. Obviously this is not true since a slow conversion of solasodine to solasodiene is known to occur. If this latter conversion is incorporated into the hydrolysis modelling, the net rate of production of solasodine (S) is given by the simplified hydrolysis model:

$$\frac{d[S]}{dt} = k_3([\alpha S_n] + [\beta_1 S_n] + [\beta_2 S_n] + [\gamma S_n]) - k_s[S] \quad (1)$$

(cf. Eq. 6 in Part I; Eqs. 2, 3, 4 and 5 in Part I are unchanged) or by the modified hydrolysis model:

$$\frac{d[S]}{dt} = k_{31}([\beta_2 S_n] + [\gamma S_n]) + k_{32}(\beta_1 S_n] + [\alpha S_n]) - k_s[S]$$
(2)

(cf. Eq. 22 in Part I; Eqs. 18, 19, 20 and 21 in Part I are unchanged). In both cases, the rate of formation of solasodiene at any specified acid concentration is taken as

$$\frac{d[S_d]}{dt} = k_s[S] \tag{3}$$

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(cf. Eq. 1 in Part II).

For the reaction of initially pure α -solasonine, the solutions are, for the simplified model:

$$[S] = \frac{k_3[\alpha S_n]_t}{k_s - k_3} \left(e^{-k_3 t} - e^{-k_s t} \right) \tag{4}$$

(cf. Eq. 11 in Part I)

$$[S_d] = \frac{[\alpha S_n]_i}{k_s - k_3} \{ k_s (1 - e^{-k_3 t}) - k_3 (1 - e^{-k_s t}) \}$$
 (5)

and for the modified model:

$$\begin{split} [S] = & \frac{(k + K'k_{31})[\alpha S_n]_i}{k_s - k_{21} - k_{32}} \{ e^{-(k_{21} + k_{32})t} - e^{-k_s t} \} \\ & - \frac{K'k_{31}[\alpha S_n]_i}{k_s - k_{31}} (e^{-k_{31}t} - e^{-k_s t}) \end{split} \tag{6}$$

(cf. Eq. 26 in Part I)

 $[S_d] =$

$$\frac{(k_{31}-k_{32})[\alpha S_n]_i \{k_s (1-e^{-(k_{21}+k_{32})t})-(k_{21}+k_{32})(1-e^{-k_s t})\}}{(k_{31}-k_{21}-k_{32})(k_s-k_{21}-k_{32})} - \frac{K'[\alpha S_n]_i}{k_s-k_{31}} \{k_s (1-e^{-k_{31}t})-k_{31}(1-e^{-k_s t})\}$$
(7)

where $K'=k_{21}(k_{31}-k_{21}-k_{32})^{-1}$. The fractional yield of solasodine $[S]/[\alpha S_n]_t$ and the solasodine to solasodine ratio $[S_d]/[S]$ may now be predicted at any time tfrom Eqs. 4 and 5 for the simplified model or from Eqs. 6 and 7 for the modified model.

SIMULTANEOUS HYDROLYSIS OF α -SOLASONINE AND α -SOLAMARGINE

Figure 2 in Part I shows that the hydrolysis of solasonine and solamargine proceed independently of each other. The result of hydrolysis of a mixture of known proportions of the two glycosides may be determined by linear combination of the individual results from α -solasonine and α -solamargine. If f is the mole fraction of solasonine in the initial triglycoside mixture, $[\alpha S]_i$ the initial total triglycoside concentration and k_6 the rate constant for solamargine hydrolysis, corresponding to k_3 in the solasonine hydrolysis model, then (using the simplified model without solasodiene formation)

$$\frac{[S]}{[\alpha S]_1} = f(1 - e^{-k_3 t}) + (1 - f)(1 - e^{-k_6 t})$$
 (8)

(cf. Eq. 11 in Part I)

If solasodiene formation is included, Eqs. 4 and 5 become respec-

$$\frac{[S]}{[\alpha S]_t} = \frac{k_3 f}{k_s - k_3} (e^{-k_3 t} - e^{-k_s t}) + \frac{(1 - f)k_6}{k_s - k_6} (e^{-k_6 t} - e^{-k_s t})$$
(9)

$$\begin{split} \frac{[S_d]}{[\alpha S]_i} &= \frac{f}{k_s - k_3} \{ k_s (1 - e^{-k_3 t}) - k_3 (1 - e^{-k_s t}) \} \\ &+ \frac{1 - f}{k_s - k_6} \{ k_s (1 - e^{-k_6 t}) - k_6 (1 - e^{-k_6 t}) \} \end{split} \tag{10}$$

Similar equations may be generated for the modified model.

HYDROLYSIS OF A MIXED GLYCOSIDE SUBSTRATE

The analysis presented above assumes that the substrate presented for hydrolysis consists of pure triglycosides. However, the glycoside mixture presented for commercial hydrolysis may already be partially hydrolyzed (for example, by natural degradation processes or by recycle operation) and consist of a mixture of α -, β - and γ -glycosides as well as the solasodine aglycone itself. To accommodate this possibility the original rate equations (Eqs. 2 to 6 or Eqs. 18 to 22 in Part I) must be solved allowing for nonzero initial concentrations of all species involved. For the hydrolysis of mixed solasonine and solamargine glycosides, the equations for the solasodine and solasodiene concentration at any time t become, for the simplified model:

$$[S] = [S]_{i}e^{-k_{s}t} + \frac{fk_{3}[G]_{i}}{k_{s} - k_{3}}(e^{-k_{3}t} - e^{-k_{s}t})$$

$$+ \frac{(1 - f)k_{6}[G]_{i}}{k_{s} - k_{6}}(e^{-k_{6}t} - e^{-k_{s}t})$$

$$[S_{d}] = [S_{d}]_{i} + [S]_{i}(1 - e^{-k_{s}t})$$

$$+ \frac{f[G]_{i}}{k_{s} - k_{3}}\{k_{s}(1 - e^{-k_{3}t}) - k_{3}(1 - e^{-k_{s}t})\}$$

$$+ \frac{(1 - f)[G]_{i}}{k_{s} - k_{6}}\{k_{s}(1 - e^{-k_{6}t}) - k_{6}(1 - e^{-k_{s}t})\}$$

$$(12)$$

where $[G]_i = ([\alpha S_n]_i + [\beta S_n]_i + [\gamma S_n]_i + [\alpha S_m]_i + [\beta S_m]_i + [\gamma S_m]_i)$ and represents the total initial glycoside concentration. fin this case represents the mole fraction of all solasonine-derived glycosidic species in the initial glycoside mixture. Similar solutions may be produced for the modified model, although the form of the final equations is more complex.

GUIDELINES FOR COMMERCIAL HYDROLYSIS

Experimental results expressed in terms of rate constants for the two proposed models for hydrolysis of α -solasonine have been tabulated in Part I and the complementary results for the solasodine to solasodiene conversion in Part II. These results may be used with the combined models developed above to predict the characteristics of solasonine hydrolysis; variation of the reaction conditions would be expected to effect solamargine hydrolysis in an analogous fashion.

The reaction time necessary to reach a specified conversion of glycoside to aglycone from a single triglycoside (for example, $[S]/[\alpha S_n]_i = 0.95$ or 0.99) may be calculated from Eq. 4 (simplified model) or Eq. 6 (modified model). The expected solasodiene levels after these hydrolysis times may be then obtained from Eqs. 4 and 5 (simplified model) or Eqs. 6 and 7 (modified model). The results are summarized in Tables 1 and 2 for the simplified and modified hydrolysis models respectively.

Tables 1 and 2 can be used to give a set of guidelines for commercial-scale hydrolysis. These are based on the two primary objectives for the hydrolysis-minimization of both the hydrolysis time and the solasodiene formation. The first observation to be made from the results in Tables 1 and 2 is that solasodiene formation is not as great a problem as past workers report. This indicates that the main problem with commercial hydrolysis in the past has been lack of knowledge of the end point of hydrolysis reaction. To illustrate this point consider alteration of $[S]/[\alpha S_n]_i = 0.99$ to $[S]/[\alpha S_n]_i = 0.95$ as the end point of the hydrolysis. This gives a reduction in the hydrolysis time of 30 to 35% and a reduction in the fraction of solasodiene formed of 35 to 40% (Tables 1 and 2). Thus there is a great advantage in limiting the degree of completion of the reaction. Two alternatives are possible: 1. the solasodine produced may be transferred into a nonpolar solvent and the remaining glycosides rehydrolyzed; or 2. the hydrolysis can be carried out in an aqueous-alcohol solvent chosen so that precipitation of the final solasodine (as the hydrochloride salt) can occur. Filtration then yields the final product leaving the remaining glycosides in solution for continued hydrolysis.

The effects of the reaction variables studied are easily seen from Tables 1 and 2 and may be listed as:

(a) Increased acid concentration gives a reduction in the hydrolysis time but an increase in the proportion of solasodiene formed. This is a consequence of the different responses of the acidity functions for the two types of reaction to a change in acid concentration—as acid concentration is increased, the acidity function for solasodiene formation increases more rapidly than the

Table 1. Characteristics of α -Solasonine Hydrolysis Based on Predictions from Simplified Model

	Reaction Conditions					Predictions for $[S]/[\alpha S]_i = 0.95$		Predictions for $[S]/[\alpha S]_i = 0.99$	
		Solvent		$\{G\}_i$	Hydrolysis	$100 [S_d]/[S]$	Hydrolysis	$100 [S_d]/[S]$	
Substrate	[HCl] _f (mol/L)	(mol % H ₂ O/ isopropanol)	Temp.	(10 ³ mol/L)	Time (min) (from Eq. 4)	(from Eqs. 4 and 5)	Time (min) (from Eq. 4)	(from Eqs. 4 and 5)	
αS_m	1.0	40	70	2	697	1.3	1,070	2.2	
αS_n	1.2	40	70	2	321	0.59	494	1.0	
αS_n	2.0	40	70	2	70	1.2	107	2.0	
αS_n	0.5	40	70	2	1,110	0.38	1,710	0.65	
αS_n	1.0	40	75	2	151	0.54	233	0.91	
αS_n	1.0	40	60	2	1,540	0.50	2,370	0.85	
αS_n	1.0	0	70	2	20	0.16	30	0.27	
αS_n	1.0	20	70	2	123	0.34	189	0.58	
αS_n	1.0	60	70	2	651	1.4	1,000	2.4	
αS_n	1.0	40	70	1	333	0.61	512	1.0	
αS_n	1.0	40	70	5	344	0.63	529	1.1	

Table 2. Characteristics of α -Solasonine Hydrolysis Based on Predictions from Modified Model

Reaction Conditions					Predictions for $[S]/[\alpha S]_i = 0.95$		Predictions for $[S]/[\alpha S]_i = 0.99$	
Substrate	(HCl); (mol/L)	Solvent (mol % H ₂ O/ isopropanol)	Temp. (°C)	[G] _i (10 ³ mol/L)	Hydrolysis Time (min) (from Eq. 6)	100 S _d /[S] (from Eqs. 6 and 7)	Hydrolysis Time (min) (from Eq. 6)	100 $[S_d]/[S]$ (from Eqs. 6 and 7)
αS_m	1.0	40	70	2	609	1.1	907	1.8
αS_n	1.0	40	70	2	312	0.55	452	0.89
αS_n	2.0	40	70	2	70	1.1	103	1.8
αS_n	0.5	40	70	2	968	0.31	1,400	0.50
αS_n	1.0	40	75	2	162	0.54	233	0.86
αS_n	1.0	40	60	2	1,400	0.43	2,040	0.70
αS_n	1.0	0	70	2	19	0.15	27	0.23
αS_n	1.0	20	70	2	120	0.31	174	0.51
αS_n	1.0	60	70	2	571	1.2	824	1.9
αS_n	1.0	40	70	1	341	0.61	508	1.0
αS_n	1.0	40	70	5	315	0.55	461	0.90

acidity function for glycoside hydrolysis.

(b) As expected from the form of the proposed models for glycoside hydrolysis and solasodiene formation, a change in glycoside concentration does not significantly affect the hydrolysis time or the extent of acid loss. However, there is a limit to this generalization in that, if the solubilities of the intermediate species are exceeded, slower reaction rates will result.

(c) Increased reaction temperature gives a reduction in hydrolysis time, but there is little effect on the proportion of solaso-diene formed. This is a consequence of the reactions having similar dependence on temperature. [Arrhenius activation energies are calculated to be 33.7 (\pm 2.7%) kcal/mol (simplified model) or 34.6 (\pm 2.6%) kcal/mol (modified model) for glycoside hydrolysis (based on k_T values) and 33.5 (\pm 5.8%) kcal/mol for solasodiene formation.]

(d) Increased proportion of water in the solvent (in the range 0 to 60 mol %) gives an increase in both the hydrolysis time and the proportion of solasodiene formed. This is due to the decrease in acidity function on addition of water to an aqueous-alcohol mixture, the effect being much larger for glycoside hydrolysis than for solasodiene formation. The variation of the Hammett acidity function in aqueous-isopropanol solvents at 25°C has been measured (Tourky et al., 1972) and shows a similar variation to that of k_T and k_T .

Of all the reaction variables studied, the proportion of water in the solvent is shown to have the greatest effect on both hydrolysis time and solasodiene formed. The results indicate that it would be best to use pure alcohol solvents, since this gives both decreased hydrolysis times and decreased solasodiene formation. However, commercial operation with anhydrous sytems could cause difficulties. The presence of only trace amounts of water would decrease the acidity of the hydrolysis solution markedly, increasing hydrolysis times and thus making prediction of the end point of the reaction difficult. As mentioned previously, prediction of the end point of the reaction is critical if the proportion of solasodiene

formed is to be minimized. As an example, Table 2 shows that the addition of only 5.5% by volume water to pure isopropanol (giving 20 mol % $\rm H_2O$) increases the hydrolysis time by a factor of 6.3 (530% increase). Operation in 20 to 40 mol % $\rm H_2O$ /isopropanol solvent will give more consistent results (although longer hydrolysis times) and would be recommended for commercial operation.

A further problem with the use of acidified anhydrous isopropanol is the increased acid loss reaction. While this increase is not important for the hydrolysis reaction itself (since the hydrolysis reaction is accelerated to a similar extent), it results in an increased consumption of acid and makes storage of acidified solutions more difficult.

The acid concentration should be kept low. As well as reducing the proportion of solasodiene formed this reduces both the amount of acid required and the corrosion of the equipment, but it does give increased hydrolysis time. Such increased hydrolysis times may be reduced again by raising the reaction temperature, since this has been shown not to affect significantly the proportion of solasodiene produced. A probable reaction temperature would be the normal boiling point of the reaction mixture so that the reaction could be carried out under atmospheric reflux conditions (82.4°C for pure isopropanol). At such temperatures an acidity of 0.5N HCl would give a suitable reaction time (approximately 20 min for 40 mol % H₂O/isopropanol).

The concentration of glycoalkaloids in the reaction mixture should be close to the solubility limit of the least soluble intermediate of the reaction (either γS_n or γS_m) to inimize both solvent and acid consumption. This upper limit is of the order of 5 g/L for 20 mol % $\rm H_2O/isopropanol$ at 20°C.

Choice of acid and alcohol should be restricted to HCl and propanol to minimize acid loss prior to and during the reaction. Use of propanol has the added benefit that any propylchloride formed is liquid at room temperature and so does not escape through water-cooled condensers nor give problems of pressure build-up in stored acidified solutions.

A procedure has been developed which can be applied to the commercial hydrolysis of the glycosides from Solanum aviculare and Solanum laciniatum.

Choice between models depends on accuracy required and analytical capability for quantitative determination of the solasodine species. The modified model gives a better prediction of $[S]/[\alpha S]_{\ell}$ as a function of time, but the simplified model has the advantage of being easier to use and requiring less expensive analytical equipment. The simplified model slightly underestimates the values of $[S]/[\alpha S]_i$ towards the end of the reaction. This results in the prediction of longer hydrolysis times by the simplified model with consequent overprediction of the degree of solasodiene formation. (Compare Tables 1 and 2.)

Obtaining design data for any particular set of reaction conditions involves experimental determination of the rate constants from pure substrates. For a commercial substrate of known composition, the design equations then provide predictions of conversion and solasodiene formation. The procedure is as follows.

Using Simplified Model

Analytical Requirements

- Ability to determine quantitatively total glycosides, solasodine and solasodiene
- Ability to distinguish quantitatively solasonine-derived glycosides from solamargine-derived glycosides (to estimate f) Substrate Requirements
 - Pure α -solasonine, α -solamargine and solasodine
 - Pure solasodiene for calibration of analysis **Procedure**

Laboratory-scale hydrolysis is carried out under the required reaction conditions on pure samples of α -solasonine and α -solamargine. By measuring $[S]/[\alpha S]_t$ after various reaction times values of k_3 and k_6 can be obtained as outlined previously (Part I). Accurate evaluation of k_s requires knowledge of the variation of k_s with acid concentration; reaction of a pure sample of solasodine under the required reaction conditions and at two different acid strengths is sufficient to do this. Acid loss under the same reaction conditions is required. The iterative procedure specified in Part II is used. With values of k_3 , k_6 and k_s known, Eqs. 11 and 12 may be used to evaluate $[S]/[G]_i$ and an estimate of f.

To simplify this procedure solasodiene formation may be ignored. In this case evaluation of k_s is not required and $k_s = 0$ is substituted into Eq. 11. This would be adequate if the reaction conditions chosen are similar to those discussed above, where estimates of solasodiene contents in the final products have already been made. It may also be satisfactory to set $\{S_d\}_{i \in \mathcal{S}_d}$ equal to zero. With all of these simplifications, Eq. 11 becomes the simple Eq. 8.

Using Modified Model

Analytical Requirements

- Ability to distinguish quantitatively each of the glycosidic species as well as solasodine and solasodiene
- High-pressure liquid chromatography, the most suitable analytical method (Crabbe and Fryer, 1980)

Substrate Requirements

- Pure α -solasonine, α -solamargine and solasodine
- Pure solasodiene for calibration of analysis Procedure

As for simplified model, but now $[S]/[\alpha S]_t$ and $[\alpha S]/[\alpha S]_t$ need to be monitored in the trial reaction. Values of the rate constants can be obtained as previously outlined (Part I) and once known can be used to evaluate $[S]/[G]_t$ and $[S_d]/[S]$ at any time t using equations similar to Eqs. 11 and 12 but for the modified model. In this case, however, it is necessary to know the proportion of each species in the starting material.

CONCLUSIONS

Models for hydrolysis of Solanum glycosides to solasodine and for the simultaneous conversion of solasodine to solasodiene have been combined. Using values of model parameters obtained from separate experimental studies, it is now possible to predict the reaction time required for a specified conversion and the concentration of solasodiene in the solasodine product.

Study of model predictions indicates that in commercial operation of 6 hydrolysis it is important to limit reaction time to that needed for 95 to 99% conversion of glycosides to the aglycone; in this way the solasodiene content of the product can be kept well below 1%. Satisfactory operation would be achieved with up to 5 g/L glycosides in 20 to 40 mol % water-isopropanol mixtures with 0.5N HCl, operated at refluxing temperature. Attempts to reduce reaction time by using higher acid concentrations will lead to higher levels of solasodiene in the product. Lower water concentration in the solvent would reduce both reaction time and solasodiene contamination, but reaction time for specified conversion becomes extremely sensitive to water concentration, so that prediction of the end point of the reaction is very difficult. At lower reaction temperatures, the required reaction time is increased but there is no change in solasodiene content. Use of alternative alcohols and acids may result in unacceptable loss of acid.

NOTATION

= fraction of solasonine-derived glycoside species in initial mixture [G] = concentration of total glycosides = first-order rate constant for solasodiene formation = rate constant for solasodine formation from solasonine-related species (simplified model) = rate constant for solasodine formation from solamargine-related species (simplified model) $k_{21}, k_{31}, k_{-} =$ rate constants in modified model k_T $= k_1 + k_2 + k_3$ (simplified model) $\frac{k_T}{k'}$ $= k_{11} + k_{21} + k_{31} + k_{32}$ (modified model) $=k_{21}(k_{31}-k_{21}-k_{32})^{-1}$ [**S**] = concentration of solasodine $[S_d]$ = concentration of solasodiene $[\alpha S]$ = sum of concentrations of α -solasonine and α -solamargine $[\alpha S_n]$ = concentration of α -solasonine $[\beta_1 S_n]$ = concentration of β_1 -solasonine $[\beta_2 S_n]$ = concentration of β_2 -solasonine

Subscript

 $[\beta S_n]$

 $[\gamma S_n]$

= initial concentration

 $= [\beta_1 S_n] + [\beta_2 S_n]$

= retention time

= concentration of γ -solasonine

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