# Part II: Formation of Solasodiene

The undesirable conversion of solasodine product to solasodiene which occurs during the acid hydrolysis of solasodine glycosides has been mathematically modelled. The model incorporates acid loss which results from reaction of acid with alcohol solvents. The model has been used to obtain kinetic data for the reaction at a number of different reaction conditions.

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## SCOPE

During hydrolysis of solasodine glycosides to produce solasodine, the solasodine formed may react further to give solasodiene. The proportion of solasodiene in the final hydrolysed product is reportedly dependent on reaction conditions such as reaction temperature, but published information on this dependence is inadequate and conflicting. No attempt has been made to obtain kinetic data for the reaction of solasodine to solasodiene.

Analysis of the compounds involved by high pressure liquid chromatography enables the hydrolysis reaction to be followed and the process modelled mathematically as described in part I of this paper. If the solasodine to solasodiene reaction were also modelled, the expected solasodiene contents of hydrolysed products could be easily and accurately predicted.

## CONCLUSIONS AND SIGNIFICANCE

The conversion of solasodine to solasodiene is acid-catalyzed. As with glycoside hydrolysis, reaction rates are not first order with respect to acid concentration but can be described in terms of an acidity function. The acidity function for the solasodiene formation reaction differs from that for glycoside hydrolysis. The solasodiene formation reaction is slow; acid loss during the period of the reaction becomes significant and must be incorporated into the model if it is to describe the process accurately. An approximate method of doing this is proposed; solution of the equations to obtain rate constants for the combined solasodiene formation/acid loss process is necessarily an iterative procedure and involves determination of the variation of the acidity function for solasodiene formation with acid concentration. The proposed model is shown to give a good description of both the solasodiene formation and the acid loss reaction and provides kinetic data which may be combined with that from glycoside hydrolysis to give prediction of the solasodiene content in the final hydrolyzed product.

## INTRODUCTION

Solasodine, a potential raw material for steroid manufacture, occurs in *Solanum* plants as the triglycosides solasonine and solamargine. After extraction from the plant material, these glycosides must be hydrolyzed to release the aglycone solasodine. The hydrolysis is usually carried out with mineral acid in aqueous alcohol solution. In Part I, the reaction steps involved in the cleavage of sugars from the glycosides have been modelled mathematically, so that with the appropriate kinetic data, the rate of conversion to solasodine can be predicted.

Under acid hydrolysis conditions, the solasodine product undergoes a slow reaction to solasodiene, which then constitutes an impurity which is difficult to remove. It is important to establish reaction conditions which will minimize the solasodiene content of the product while giving an acceptable rate of hydrolysis. Of major interest are the effects of acid concentration, glycoside concentration, temperature and solvent composition.

In studies of glycoside hydrolysis, Labenskii and Koretskaya (1961) and Labenskii and Kuzovkova (1965) reported an increase in the formation of solasodiene relative to solasodine as acid concentration was increased. Crabbe (1980) has pointed out, however, that the sampling times which were the same in each case, all exceeded the time required for complete hydrolysis. Since an increase in acid concentration will cause more rapid hydrolysis and solasodiene forms only from the solasodine aglycone, the reported

effect may be due simply to the longer time for which the solasodine aglycone product was exposed to the acid conditions. The data of Cionga et al. (1967) support this criticism and suggest that the solasodiene formation rate is not increased relative to the hydrolysis rate as the acid concentration is increased.

Labenskii and Kuzovkova (1965) reported less solasodiene formation with higher glycoside concentrations, but only when using hydrochloric acid. This effect may be due to a greater proportion of the solasodine-hydrochloride salt precipitating at the higher concentrations. Labenskii and Koretskaya (1961) found higher proportions of solasodiene at higher temperatures; because the sampling time exceeded the time for complete hydrolysis in each case, this may again be a misleading result due simply to faster hydrolysis at the higher temperatures. Increased temperatures will lead to higher rates of hydrolysis and of solasodiene formation, but it is not clear what the effect is on the relative rate.

Weston (1976), Cionga et al. (1967), and Labenskii and Koretskaya (1961) all reported higher proportions of solasodiene with increasing water content. If the claim by Weston (1976) that the reaction rates for hydrolysis increase as the proportion of water is increased is valid, these reports may also be misleading. It is unfortunate indeed that, in so many of these experimental studies, the test mixtures were sampled and analyzed only at a fixed time, usually after the hydrolysis reaction was complete.

The work reported in this paper is a study of the solasodine to solasodiene reaction, separated from the hydrolysis reaction which produces the solasodine. Only with this approach is it possible to provide reliable kinetic data. The experimental results can be used to confirm a mathematical model of the reaction system, and this model used then, in conjunction with the model for glycoside hydrolysis, to provide a full description of these two concurrent reactions.

## MODELLING SOLASODINE TO SOLASODIENE REACTION

A number of reactions are possible for solasodine under acidic conditions once the $3\beta$ oxygen of solasodine is protonated. If sugars are still attached to the $3\beta$ position, the favored reaction is cleavage of the sugar (i.e., glycoside hydrolysis). Once the sugars are all removed and the solasodine aglycone formed, the preferred product is solasodiene via an El route. At high acid concentrations, solasodine may react to give different products and the solasodiene formed may further react (Crabbe, 1980).

Based on the El route the rate equation for the conversion of solasodine $(S)$ to solasodiene $(S_d)$ may be written

$$\frac{-d[S]}{dt} = k'_s h[S] = \frac{d[S_d]}{dt} \tag{1}$$

where $h$ is an acidity function giving a measure of the ability of the solvent to protonate the $3\beta$ oxygen of solasodine. The variation of $h$ with acid concentration in this case will be different from that of the acidity function for glycoside hydrolysis (Eq. 1, Part I). This involvement of acidity functions in the rate equation has been discussed in much greater detail by Crabbe (1980).

### ACID LOSS

In acidified aqueous alcohol solvents, acid is lost as a result of substitution or elimination reactions with the alcohol. The rate of acid loss can be neglected in studies of the rate of glycoside hydrolysis, as long as the correct choice of acid and alcohol is made. (See Part I.) However, the rate of diene formation is relatively slow; in considering this reaction the acid loss cannot be neglected, no matter what acid or alcohol is chosen. It is necessary to allow mathematically for the change in acidity of the solution in Eq. 1.

The alternate mechanisms for acid loss are either SN1 or SN2 nucleophilic substitution. For a secondary alcohol such as isopropanol, the probabilities of SN1 and SN2 reactions are both low (Roberts and Caserio, 1964) making choice of mechanism for the reaction difficult. A generalized rate equation for acid loss by either SN1 or SN2 substitution without back reaction is (Crabbe, 1980):

$$\frac{-d[H^+]}{dt} = k'_a[H^+]^p \tag{2}$$

which gives

$$\frac{[HA]_i}{[H^+]} = (1 + (p - 1)[HA]_i^{p-1}k'_a t)^{1/(p-1)} \tag{3}$$

where $[HA]_i$ is the initial acid concentration, and $k'_a$ and $p$ are constants.

As written, Eq. 3 is not suitable for incorporation into Eq. 1. Instead a variation of $h$ with time is required. If it is assumed that for these reactions $h \simeq c[H^+]^n$ where $c$ and $n$ are constants, Eq. 3 yields:

$$h_i/h = (1 + (p - 1)[HA]_i^{p-1}k'_a t)^{n/(p-1)} \tag{4}$$

This may be written

$$h_i/h = e^{n k'_a [HA]_i^{p-1} t} \tag{5}$$

if

$$k'_a[HA]_i^{p-1}t < 0.1$$

Figure 1, based on data given by Arnett and Mach (1966), shows two typical acidity functions and their measured values for various acid concentrations. The range of acidities used in our studies was 0.5N to 4.0N; over this range $c[H^+]^n$ adequately describes these acidity functions and would be expected to apply to the solasodiene formation acidity function. The errors introduced by using Eq. 5 instead of Eq. 4 are less than 3% for $k'_a[HA]_i^{p-1}t = 0.1$. If $k'_a[HA]_i^{p-1}t > 0.1$, the assumptions leading to Eq. 3 (i.e., back reaction ignored) will be violated, causing errors larger than those introduced by this simplification.
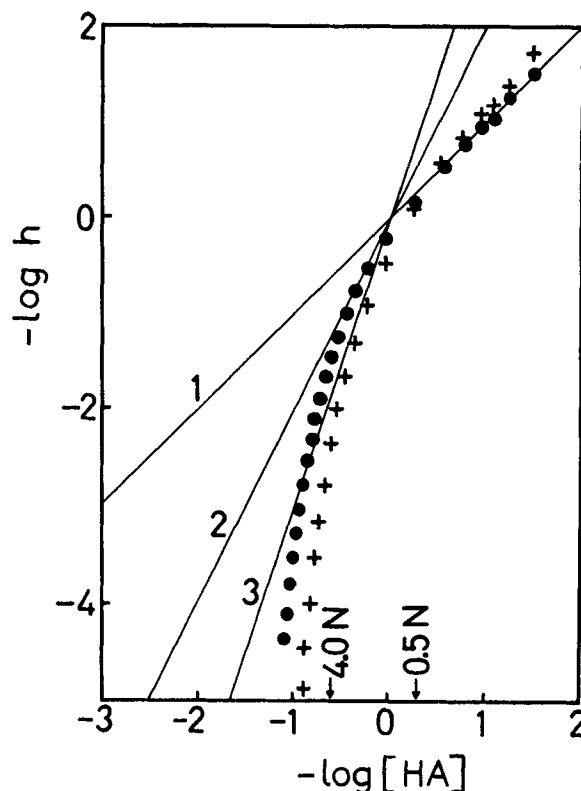


Figure 1. Variation of the acidity functions $h_o$ and $h'_R$ with acid concentration [HA]. (Experimental points from Arnett and Mach (1966) for aqueous solution of HCl at 25°C). ● $h \equiv h_o$, Hammett acidity function; + $h \equiv h'_R$ Modified Deno acidity function; 1: $h = [HA]$; 2: $h = [HA]^2$; d: $h = [HA]^3$.

### SOLASODIENE FORMATION IN PRESENCE OF ACID LOSS

To model the solasodine to solasodiene reaction in a system in which acid loss is significant, Eq. 5 may be introduced into Eq. 1:

$$\frac{d[S]}{dt} = k'_s[S]h_i e^{-n k'_a[HA]_i^{p-1}t}$$

For any specific initial acid concentration we may replace $k'_s h_i$ by $k_{si}$ and $n k'_a[HA]_i^{p-1}$ by $k_a$, yielding

$$ln \frac{[S]}{[S]_i} = \frac{-k_{si}}{k_a}(1 - e^{-k_a t}) \tag{6}$$

### EVALUATION OF RATE CONSTANTS

Use of Eq. 6 to calculate values for $k_{si}$ and $k_a$ from experimental data is necessarily an iterative procedure. Evaluation of $k_{si}$ may be made from a plot of $ln [S]/[S]_i$ vs. $1 - e^{-k_a t}/k_a$ only if $k_a$ is known. To determine $k_a$ a plot of $ln h/h_i$ vs. $t$ must be made (Eq. 5). This requires knowledge of the variation of $h$ with $[H^+]$ (or equivalently the variation of $k_s$ $(= k'_s h)$ with $[H^+]$). A series of experiments at different initial acid concentrations is required to provide this information. The proposed iterative procedure is as follows:

(a) Equation 1 may be solved assuming no acid loss, giving

$$ln \frac{[S]}{[S]_i} = -k_{si}t \tag{7}$$

An estimate of $k_{si}$ at each initial acid concentration and a plot of $k_{si}$ vs. $[HA]_i$ may be made.

(b) From Eq. 5

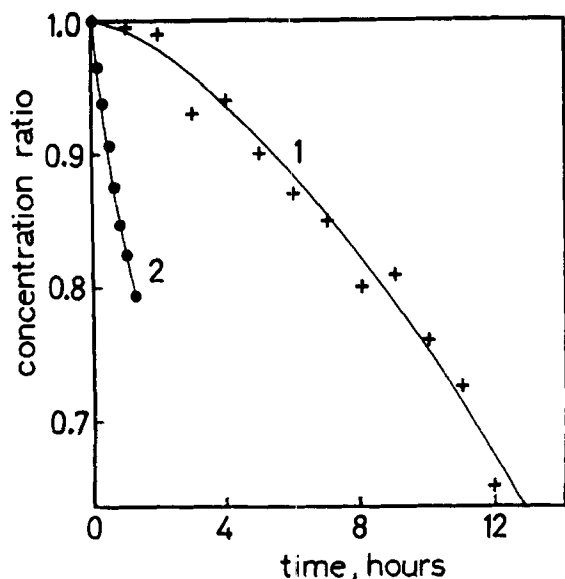$$ln \frac{h}{h_i} = -k_a t = ln \frac{k_s}{k_{si}} \tag{8}$$

Figure 2. Change in $([S] + [S_d])/[S]_i$ and $[S]/([S] + [S_d])$ during reaction of $10^{-3}$ M solasodine with 4N HCl in 20 mole % $H_2O$/isopropanol at 70°C. 1: $([S] + [S_d])/[S]_i$; 2: $[S]/([S] + [S_d])$.
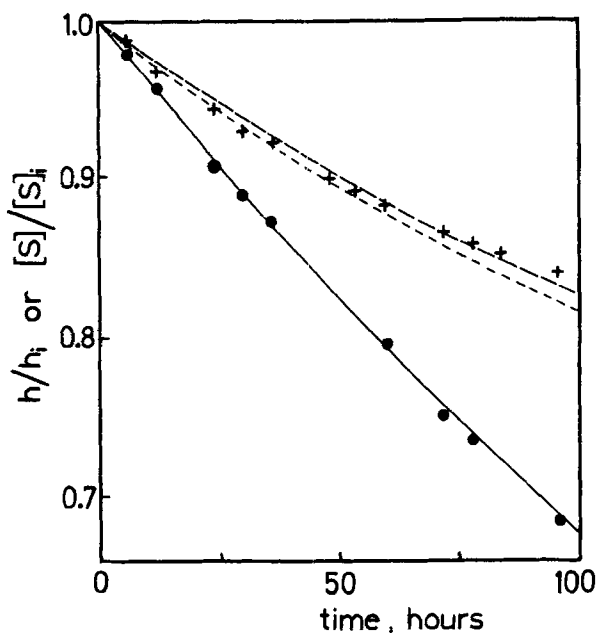


Figure 3. Acid loss and solasodine conversion for the reaction of $2 \times 10^{-3}$ M solasodine with 1N HCl in 20 mol % $H_2O$/isopropanol at 70°C. Acid Loss: ● experimental $h/h_i$; —— prediction, Eq. 8. Solasodine Conversion: + experimental $[S]/[S]_i$; --- Eq. 6; - - - - - - Eq. 12.



Figure 4. Plot of $k_s$ and $k_a$ against initial acid concentration $[HCl]_i$. Solasodine Conversion: ● experimental $k_s$; —— Eq. 10; Acid Loss: + experimental $k_a$; - - - - Eq. 11.

experiments reported in Part I. Solasodine was prepared by partially hydrolyzing a mixture of glycosides and separating the aglycone from the unhydrolyzed glycosides by extraction into dimethyl benzene. Under these conditions only trace amounts of solasodiene (<0.2%) appeared in the final product. Analysis of the reaction products was made using high pressure liquid chromatography (Crabbe and Fryer, 1979, 1980) Because of the slow rate of formation of solasodiene and the possibility of extraneous reactions, the solasodine to solasodiene reaction was followed to only 10 to 20% conversion. To be able to predict solasodiene levels in glycoside hydrolysis products, it is not necessary to follow the reaction further. Figure 2 shows results for the reaction of solasodine in 4N HCl followed to greater than 20% conversion. The extent of extraneous reactions is indicated by the departure of the value of $([S] + [S_d])/[S]_i$ from unity. However, the fitted curves in Figure 2 suggest that for $[S]/[S]_i > 0.8$, $([S] + [S_d])/[S]_i > 0.99$.

Reaction of solasodine was carried out at a number of different reaction conditions so that, as well as establishing the suitability of the proposed model, the effect of reaction variables (temperature, solvent, acid concentration, glycoside concentration) could be evaluated. To enable the proportion of solasodiene in glycoside hydrolysis products to be predicted, the solasodine to solasodiene reactions were carried out at reaction conditions identical to those reported in Part I for the hydrolysis reactions.

## ACID LOSS EXPERIMENTS

Quantitative measurement of the acid loss in the reaction solution was carried out independently of the solasodine to solasodiene conversion reaction, but the two sets of reactions were followed simultaneously using the same acidified solvent and the same reaction temperature to avoid any systematic error in the reaction variables.

## RESULTS AND MODEL PREDICTIONS

Figure 3 shows the concentration-time profile for the solasodiene formation and acid loss (for the reaction of $2 \times 10^{-3}$ M solasodine with 1N HCl in 20 mol % $H_2O$/isopropanol at 70°C). The experimental points are compared with predictions of the fitted model,

since $k_s = k'_s h$ and $k_{st} = k'_s h_t$ where $k'_s$ is a constant independent of $h$. Using the plot of $k_{st}$ vs. $[HA]_t$, the experimental values of $[H^+]/[HA]_t$ may be converted to values of $k_s/k_{st}$ and Eq. 8 used to evaluate $k_a$.

(c) Since values of $k_a$ are known, Eq. 6 may be used to give new estimates of $k_{st}$ and a new plot of $k_{st}$ vs. $[HA]_t$ made.

(d) Steps (b) and (c) are repeated until the values of $k_{st}$ converge.

## SOLASODIENE FORMATION EXPERIMENTS

The reaction of pure solasodine in acidified aqueous isopropanol solvents was carried out in an analogous fashion to that for the glycoside hydrolysis
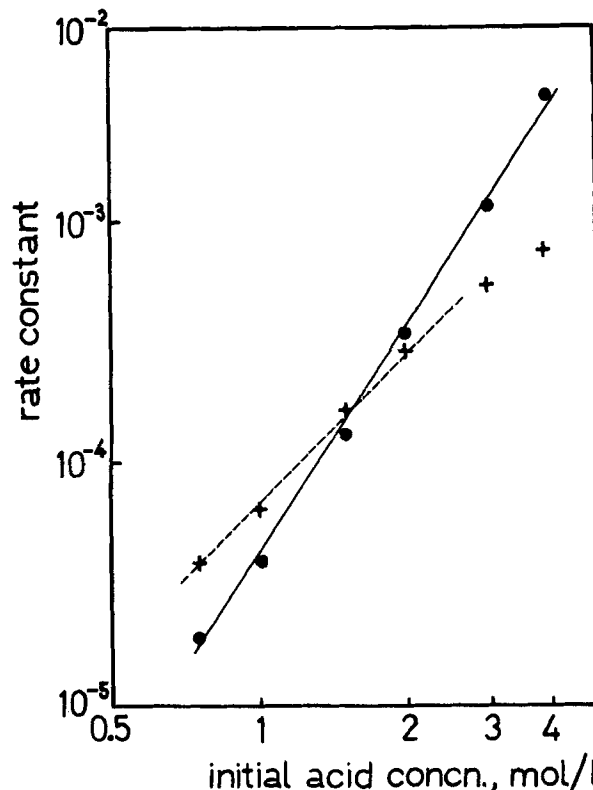
TABLE I. RATE CONSTANTS AND ASSOCIATED REGRESSION ERRORS FOR SOLASODINE CONVERSION AND ACID LOSS

| Reaction Conditions | | | | Rate Constants and Associated Errors | |
|---|---|---|---|---|---|
| $[HCl]_i$ (mol/L) | Solvent (mol % $H_2O$/isopropanol) | Temperature (°C) | $[S]_i$ ($10^3$ mol/L) | Solasodine Loss $10^5 \, k_s$ (min$^{-1}$) | Acid Loss $10^5 \, k_a$ (min$^{-1}$) |
| 0.75 | 20 | 70 | 1.0 | 1.88 (± 2.8%) | 3.95 (± 0.5%) |
| 1.0 | 20 | 70 | 1.0 | 3.92 (± 1.8%) | 6.50 (± 0.7%) |
| 1.5 | 20 | 70 | 1.0 | 13.1 (± 1.9%) | 16.5 (± 1.3%) |
| 2.0 | 20 | 70 | 1.0 | 34.6 (± 1.9%) | 29.0 (± 1.0%) |
| 3.0 | 20 | 70 | 1.0 | 116 (± 2.1%) | 54.8 (± 0.5%) |
| 4.0 | 20 | 70 | 1.0 | 329 (± 1.2%) | 74.6 (± 1.7%) |
| 1.0 | 20 | 75 | 1.0 | 7.46 (± 1.0%) | 14.9 (± 1.1%) |
| 1.0 | 20 | 65 | 1.0 | 1.81 (± 0.5%) | 3.27 (± 1.6%) |
| 1.0 | 0 | 70 | 1.0 | 11.4 (± 1.1%) | 41.0 (± 1.0%) |
| 1.0 | 40 | 70 | 1.0 | 2.56 (± 0.6%) | 3.14 (± 2.0%) |
| 1.0 | 60 | 70 | 1.0 | 3.00 (± 1.6%) | 1.59 (± 0.8%) |
| 1.0 | 20 | 70 | 0.5 | 3.89 (± 1.6%) | 3.95 (± 0.5%) |
| 1.0 | 20 | 70 | 2.0 | 3.84 (± 1.6%) | 3.95 (± 0.5%) |

using the rate constant values discussed below. The fit of the model was similar under the other reaction conditions and in all cases the predicted $[S]/[S]_i$ and $h/h_i$ curves closely follow the experimental data.

Table 1 tabulates the calculated rate constants and associated regression errors. Values of $k_s$ and $k_a$ for reactions with different initial acid concentrations (other reaction conditions the same) were calculated by the iterative procedure outlined above. The iterative procedure converged within two iterations using Eq. 6 (a total of three evaluations of $k_s$). When the values of $k_s$ and $k_a$ so obtained in 20 mol % $H_2O$/isopropanol at 70°C are plotted against $[HCl]_i$ on log-log scale, straight lines are obtained (Figure 4). The equations of these lines are

$$k_s = 4.1 \times 10^{-5} [HCl]_i^3 \quad \text{for} \quad 0.75 < [HCl]_i \leqslant 3.0N \quad (10)$$

and

$$k_a = 7.0 \times 10^{-5} [HCl]_i^2 \quad \text{for} \quad 0.75N < [HCl]_i \leqslant 3.0N \quad (11)$$

The error in the use of Eq. 10 to predict values of $k_s$ range from $-8.0\%$ to $+5.3\%$ and the errors in the use of Eq. 11 to predict $k_a$ vary from $-4.2\%$ to $+15.0\%$.

Combining Eqs. 10 and 11 with Eq. 6 gives a single equation for the prediction of solasodine loss by conversion to solasodiene in 20 mol % $H_2O$/isopropanol at 70°C:

$$\frac{[S]}{[S]_i} = \exp\{-0.59[HCl]_i(1 - \exp(-7.0 \times 10^{-5}[HCl]_i^2 t))\}$$

$$\text{for } 0.75N < [HCl]_i \leqslant 3.0N \quad (12)$$

The advantage of Eq. 12 is that it does not require plots of $k_s$ or $k_a$ against $[HCl]_i$ for the evaluation of $[S]/[S]_i$. The $[S]/[S]_i$ line predicted from Eq. 12 is included on Figure 3.

## CONCLUSION

The conversion of solasodine to solasodiene is described by a simple rate equation using an acidity function as a measure of the effect of acid strength. With an expression for acid loss incorporated into the rate equation, the model provides an adequate description of solasodiene formation. Experiments at different initial acid concentrations provide sufficient data for the rate constants to be evaluated; the results can then be combined with the model equations in a single expression for solasodine conversion to solasodiene at specified reaction conditions.

## NOTATION

$c, n, p$ =- constants
$h$ = acidity function
$h_{d_i}$ = Hammett acidity function (Hammett, 1970)
$h_R$ = modified Deno's acidity function (Hammett, 1970)
$k_a'$ = rate constant for acid loss reaction
$k_s'$ = rate constant for solasodiene formation
$k_s$ = $k_s' h_i$
$k_a$ = $n k_a' [HA]_i^{p-1}$
$[S]$ = concentration of solasodine
$[S_d]$ = concentration of solasodiene
$t$ = reaction time

## Subscript

$i$ = initial concentration or acidity function

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