

# Modeling of Acid Hydrolysis of Solasodine Glycosides

## Part I: Formation of Solasodine by Glycoside Hydrolysis

Solasodine from *Solanum* plants is a potential raw material for steroid drug manufacture. Acid hydrolysis of the naturally occurring glycosides of solasodine is a major step in production of the aglycone. As part of a detailed study of the hydrolysis of solasodine glycosides, the hydrolysis procedure has been mathematically modelled. Two models are presented and have been used to obtain kinetic data for the reactions at a number of different reaction conditions. The subsequent conversion of solasodine to solasodine is considered in Part II of this paper; the combined reactions and guidelines for commercial hydrolysis are presented in Part III.

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### SCOPE

In the plants *Solanum aviculare* and *Solanum laciniatum*, solasodine is the only steroidal alkaloid present. This makes these two plants, both natives to Australia and New Zealand, attractive commercial sources of raw steroid. In these plants solasodine occurs as the triglycosides solasonine and solamargine, which must be hydrolyzed to release the solasodine. During hydrolysis, diglycosides and monoglycosides are formed as intermediates. The solasodine product may undergo further reaction to solasodine, an impurity which is difficult to remove.

No direct evaluation of reaction rates for hydrolysis of solasodine glycosides has been reported previously, and only a rough idea of the hydrolysis times necessary under various reaction conditions has been established. Many of the presented results and conclusions regarding the effects of changing the reaction

conditions are of doubtful validity.

To minimize the time for hydrolysis, while at the same time restricting the amount of solasodine formed to an acceptable limit, one must be able to predict accurately the end point of the hydrolysis. For this purpose it is necessary to know the rate constants for the various reactions involved. It is not sufficient to determine only an overall rate for the conversion of triglycoside to aglycone. The process will not be adequately described by an overall rate equation. In addition, the glycosidic mixture presented for hydrolysis may already be partially hydrolyzed by natural processes. If a kinetic model involving the conversions among the glycosides, the partial glycosides and the aglycone is developed and individual rate constants determined, the starting material can be analyzed and the hydrolysis time to reach a particular conversion accurately predicted.

### CONCLUSIONS AND SIGNIFICANCE

Because the hydrolysis of solasodine glycosides is carried out under strongly acidic conditions, reaction rates are not first order with respect to acid concentration, but can be described in terms of acidity functions. The consumption of acid which occurs in acidified aqueous alcohol solvents can be neglected in glycoside hydrolysis studies only if hydrochloric acid and isopropanol are used.

Hydrolysis of each of the two glycosides solasonine and solamargine proceeds independently and may be treated separately and analogously. Complete description of the individual reactions for either solasonine or solamargine hydrolysis in-

volves eight rate constants. Two simpler versions of this complete model have been proposed to facilitate experimental evaluation of the rate constants; in the "simplified model" only three different values are assigned to the eight rate constants, while four values are allowed in the "modified model." Procedures are presented for the use of experimental data to evaluate the kinetic parameters. Both models give a good description of the hydrolysis process. The modified model is more accurate but the simplified model is easier to use and is considered satisfactory for commercial use.

### INTRODUCTION

In recent years there has been a renewed interest in alternatives to diosgenin, which is extracted from the Central American plant

*Dioscorea mexicana*, as a raw material for manufacture of steroidal drugs. One of the alternative natural sources is solasodine from *Solanum* plants, particularly *S. aviculare* and *S. laciniatum*. The steroidal alkaloid solasodine is a nitrogen isomer of diosgenin and can be degraded in a similar way to pregnadienolone acetate, a starting point for production of corticosteroids, cardiovascular drugs, and therapeutic and contraceptive sex hormones.

Industrial-scale production of solasodine in Russia was reported by Chikov et al. (1976) and in New Zealand by Kloosterman (1979).

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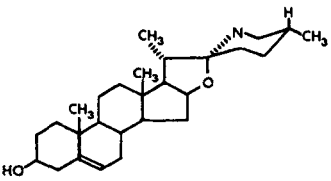
<u>AGLYCONES :</u>  <div style="text-align: center;">  </div>	<u>SOLASODINE</u>	<u>SOLASODIENE</u>
<u>NATURAL TRIGLYCOSIDES :</u>  <div style="display: flex; align-items: center;"> <div style="margin-right: 10px;"> <math>\alpha</math>-SOLASONINE  <math>\beta_1</math>-SOLASONINE  <math>\beta_2</math>-SOLASONINE  <math>\gamma</math>-SOLASONINE </div> <div> <math>\alpha</math>-SOLAMARGINE  <math>\beta_1</math>-SOLAMARGINE  <math>\beta_2</math>-SOLAMARGINE  <math>\gamma</math>-SOLAMARGINE </div> </div>	<div style="display: flex; align-items: center;"> <div style="margin-right: 10px;"> <math>\alpha</math>-SOLASONINE  <math>\beta_1</math>-SOLASONINE  <math>\beta_2</math>-SOLASONINE  <math>\gamma</math>-SOLASONINE </div> <div> <math>\alpha</math>-SOLAMARGINE  <math>\beta_1</math>-SOLAMARGINE  <math>\beta_2</math>-SOLAMARGINE  <math>\gamma</math>-SOLAMARGINE </div> </div>	<div style="display: flex; align-items: center;"> <div style="margin-right: 10px;"> <math>\alpha</math>-SOLASONINE  <math>\beta_1</math>-SOLASONINE  <math>\beta_2</math>-SOLASONINE  <math>\gamma</math>-SOLASONINE </div> <div> <math>\alpha</math>-SOLAMARGINE  <math>\beta_1</math>-SOLAMARGINE  <math>\beta_2</math>-SOLAMARGINE  <math>\gamma</math>-SOLAMARGINE </div> </div>
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<u>MONOGLYCOSIDES :</u>  $\alpha$ -SOLASONINE $\beta_1$ -SOLASONINE $\beta_2$ -SOLASONINE $\gamma$ -SOLASONINE	<u>MONOGLYCOSIDES :</u>  $\alpha$ -SOLAMARGINE $\beta_1$ -SOLAMARGINE $\beta_2$ -SOLAMARGINE $\gamma$ -SOLAMARGINE	<u>MONOGLYCOSIDES :</u>  $\alpha$ -SOLAMARGINE $\beta_1$ -SOLAMARGINE $\beta_2$ -SOLAMARGINE $\gamma$ -SOLAMARGINE

Figure 1. Structure of solasodine-related species.

Collins et al. (1976) considered the feasibility of steroid production from *Solanum* in Australia, but no industrial-scale work has been undertaken. Developmental work has been reported in many countries.

In *Solanum* plants, solasodine occurs as the triglycosides solasonine and solamargine. In industrial processes, after extraction, the cleavage of the sugars from these glycosides is a necessary step in the subsequent isolation of the aglycone solasodine. This hydrolysis step, achieved by batch processing with mineral acid in aqueous alcohol solution, constitutes a large proportion of the processing time in solasodine production. During acid hydrolysis, intermediate partial glycosides are formed: from solasonine,  $\beta_1$ - and  $\beta_2$ -solasonine (disaccharides) and  $\gamma$ -solasonine (monosaccharide). Similar nomenclature is used for the di- and mono-glycosides from solamargine. Under the hydrolysis conditions, solasodine is subject to slow reaction to solasodiene, which therefore constitutes an impurity in the final product. Figure 1 shows the structures of the compounds involved.

The effects of reaction conditions on hydrolysis rates and solasodine content have been studied by Labenskii and Koretskaya (1961), Labenskii and Kuzovkova (1965), Cionga et al. (1967, 1971), and Weston (1976). These reports contain several contradictory conclusions; Crabbe and Fryer (1978) and Crabbe (1980) have explained why this is so.

Following development of a method using high-pressure liquid chromatography for analysis of the various solasodine-related species (Crabbe and Fryer, 1979, 1980a), a detailed study of the hydrolysis of solasodine glycosides has been undertaken (Crabbe, 1980). An introduction to this work has been given by Crabbe and Fryer (1980b).

## MODELLING OF THE HYDROLYSIS REACTION

In acid hydrolysis of solasonine, the various glycosidic bonds have been shown to be broken in no particular order; i.e., the acid-induced cleavages occur randomly (Briggs et al., 1963). For sola-

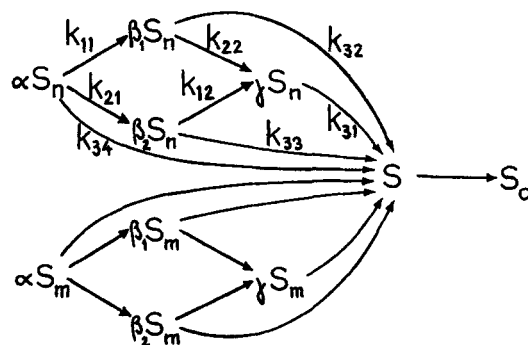


Figure 2. Overall reaction scheme for the conversion of solasodine glycosides to solasodine and solasodiene (complete model).

margine the existence of all products except the two different diglycosides has been reported after mild acid hydrolysis (Kuhn et al., 1955a). The existence of  $\beta_1$ - and  $\beta_2$ -forms for solamargine is supported however by the work of Kuhn et al. (1955b, 1955c) and of Schrieber (1968).

Thus, the hydrolysis of a mixture of  $\alpha$ -solasonine ( $\alpha S_n$ ) and  $\alpha$ -solamargine ( $\alpha S_m$ ) to give solasodine (S) may be represented schematically as in Figure 2. Figure 2 shows that the conversion of solasodine to solasodiene ( $S_d$ ) proceeds independently of the hydrolysis reactions and that the hydrolysis of solasonine and solamargine proceed independently of each other. In the following discussion only solasonine hydrolysis will be considered; equations for solamargine followed by direct analogy. The formation of solasodiene from solasodine is considered in Part II.

All the glycosidic bonds will be broken by a similar acid-catalyzed mechanism. Detailed analysis (Crabbe, 1980) of the generally accepted mechanism (BeMiller, 1967) provides the following rate equation for a glycoside substrate, A:

$$-\frac{d[A]}{dt} = k'h[A] \quad (1)$$

where  $h$  represents an acidity function, a measure of the ability of the solvent to protonate the glycosidic species. In weakly acidic solutions (e.g.,  $< 0.1$  M) this approximates the proton concentration  $[H^+]$ , but in stronger acid solutions  $h$  becomes much greater than  $[H^+]$ . This involvement of acidity functions in the rate equation has been discussed in much greater detail by Crabbe (1980). In the acid-catalyzed hydrolysis reaction there is no consumption of acid, so the group  $k'h$  may be replaced by  $k$ , a first-order rate constant, for any particular acid concentration.

### Complete Model

For solasonine hydrolysis it is probable that the bond cleavages corresponding to the eight pathways, Figure 2, proceed at different rates. This would lead to eight different first-order rate constants. For a closed system in which only these eight hydrolysis reactions are occurring, five independent rate equations based on the appearance and/or disappearance of each species may be written. These rate equations can be solved to give the concentration of each species as a function of time and initial concentration; if values of the eight rate constants were known, such equations could be used to predict the concentration-time profiles of each species in solution. However, it is not possible to use the solutions to the rate equations to evaluate the rate constants with experimental data obtained from the hydrolysis of  $\alpha$ -solasonine ( $\alpha S_n$ ) since there are eight unknown rate constants and only five independent equations.

The alternatives are to use multiple regression analysis to determine the undefined rate constants or to obtain further independent equations so that the system becomes solvable. This latter method would be preferred but is mathematically complex and requires extensive and difficult experimental support (Crabbe, 1980). It is preferable to simplify the kinetic model so that hydrolysis of  $\alpha$ -solasonine (or  $\alpha$ -solamargine) alone will permit determination of the rate constants. Two approaches have been considered, leading to a "modified model" and a "simplified model."

### SIMPLIFIED MODEL

#### Model Development

It may be noted from Figures 1 and 2 that the hydrolysis of solasonine involves cleavage of only three bonds, whereas the complete model assumes eight different rates for the process. Thus, reactions with rate constants  $k_{11}$  and  $k_{12}$  involve cleavage of the same bond (glucose-galactose), as do those with  $k_{21}$  and  $k_{22}$  (rhamnose-galactose), and those with  $k_{31}$ ,  $k_{32}$ ,  $k_{33}$  and  $k_{34}$  (galactose-solasodine). If it is assumed that the rate of the hydrolysis steps depends only on the bond being broken,  $k_{11} = k_{12} (=k_1)$ ,  $k_{21} = k_{22} (=k_2)$  and  $k_{31} = k_{32} = k_{33} = k_{34} (=k_3)$  leading to a greatly simplified model (Figure 3). This assumption means that the rate of cleavage of a particular bond depends only on the units (either a sugar molecule or the aglycone molecule) immediately adjacent to the bond. This assumption has been shown to be valid, for relatively simple glycosides, by Timell (1963), BeMiller and Mann, (1966), and Timell (1964).

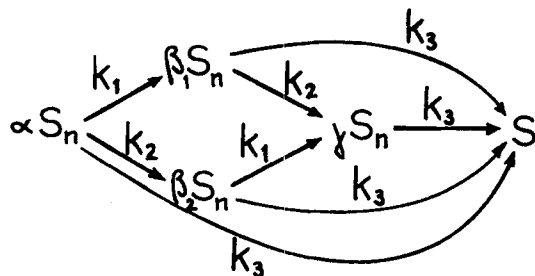


Figure 3. Simplified model reaction scheme (solasonine  $\rightarrow$  solasodine).

There is little evidence available to show whether these results can be extended to a more complex system such as solasonine, where three highly polar sugars are attached to the end of a large nonpolar aglycone. For such a system, steric, electronic and solvation effects could all be much more significant than in simple glycosides. The modified model developed in the next section makes more allowance for these effects.

### Model Equations

From Figure 3, for a closed system the rate equations are:

$$\frac{d[\alpha S_n]}{dt} = -(k_1 + k_2 + k_3)[\alpha S_n] \quad (2)$$

$$\frac{d[\beta_1 S_n]}{dt} = k_1[\alpha S_n] - (k_2 + k_3)[\beta_1 S_n] \quad (3)$$

$$\frac{d[\beta_2 S_n]}{dt} = k_2[\alpha S_n] - (k_1 + k_3)[\beta_2 S_n] \quad (4)$$

$$\frac{d[\gamma S_n]}{dt} = k_2[\beta_1 S_n] + k_1[\beta_2 S_n] - k_3[\gamma S_n] \quad (5)$$

$$\frac{d[S]}{dt} = k_3([\alpha S_n] + [\beta_1 S_n] + [\beta_2 S_n] + [\gamma S_n]) \quad (6)$$

where

$$\frac{d[\alpha S_n]}{dt} + \frac{d[\beta_1 S_n]}{dt} + \frac{d[\beta_2 S_n]}{dt} + \frac{d[\gamma S_n]}{dt} + \frac{d[S]}{dt} = 0 \quad (7)$$

For an initial concentration of  $\alpha$ -solasonine of  $[\alpha S_n]_i$  and zero initial concentration of all other components, combining the results for  $[\beta_1 S_n]$  and  $[\beta_2 S_n]$  the solutions are

$$[\alpha S_n] = [\alpha S_n]_i e^{-(k_1 + k_2 + k_3)t} \quad (8)$$

$$[\beta S_n] = [\alpha S_n]_i (e^{-k_1 t} + e^{-k_2 t} - 2e^{-(k_1 + k_2)t}) e^{-k_3 t} \quad (9)$$

$$[\gamma S_n] = [\alpha S_n]_i (1 + e^{-(k_1 + k_2)t} - e^{-k_1 t} - e^{-k_2 t}) e^{-k_3 t} \quad (10)$$

$$[S] = [\alpha S_n]_i (1 - e^{-k_3 t}) \quad (11)$$

For nonzero initial concentrations of the solasonine based species Eq. 11 becomes

$$[S] = ([\alpha S_n]_i + [\beta S_n]_i + [\gamma S_n]_i)(1 - e^{-k_3 t}) + [S]_i$$

It is because of the form of this equation that the simplified model would be so useful for commercial application. The formation of solasodine is described by the single rate constant  $k_3$  irrespective of the composition of the initial substrate. To measure the value of  $k_3$  it is necessary to follow the variation of  $([S] - [S]_i)/[G]_i$  with time where  $[G]_i = [\alpha S_n]_i + [\beta S_n]_i + [\gamma S_n]_i$ . This is analytically simple and may be achieved by chemical analysis, eliminating the need for high-pressure liquid chromatography.

#### Evaluation of Model Parameters

To determine the rate constants from experimental data the following procedure may be used. Equation 8 may be rewritten to give

$$\ln \frac{[\alpha S_n]}{[\alpha S_n]_i} = -(k_1 + k_2 + k_3)t \quad (12)$$

while Eq. 11 may be rewritten to give

$$\ln \left\{ 1 - \frac{[S]}{[\alpha S_n]_i} \right\} = -k_3 t \quad (13)$$

From Eqs. 9 and 8

$$\begin{aligned} \frac{[\beta S_n] + 2[\alpha S_n]}{[\alpha S_n]_i} &= e^{-(k_1 + k_2 + k_3)t} e^{k_2 t} + e^{-k_2 t} e^{-k_3 t} \\ &= e^{-(k_1 + k_2 + k_3)t} e^{k_1 t} + e^{-k_1 t} e^{-k_3 t} \end{aligned}$$

The last two forms of the equation are symmetric in  $k_1$  and  $k_2$ ; solution for either  $k_1$  or  $k_2$  will lead to the same result. If the term  $k_{1,2}$  is introduced to represent either  $k_1$  or  $k_2$  (but not both in the

same equation), incorporating Eqs. 8 and 11 the above equation becomes

$$[\alpha S_n](e^{k_{1,2}t})^2 - ([\beta S_n] + 2[\alpha S_n])e^{k_{1,2}t} + ([\alpha S_n]_i - [S]) = 0$$

This represents a quadratic equation in  $e^{k_{1,2}t}$  with solutions

$$e^{k_{1,2}t} = \frac{[\beta S_n] + 2[\alpha S_n] + ([\beta S_n]^2 - 4[\alpha S_n][\gamma S_n])^{1/2}}{2[\alpha S_n]} = \lambda_1 \quad (14)$$

and

$$e^{k_{1,2}t} = \frac{[\beta S_n] + 2[\alpha S_n] - ([\beta S_n]^2 - 4[\alpha S_n][\gamma S_n])^{1/2}}{2[\alpha S_n]} = \lambda_2 \quad (15)$$

since  $[\alpha S_n]_i = [\alpha S_n] + [\beta S_n] + [\gamma S_n] + [S]$  for all  $t$ . Thus

$$\ln \lambda_{1,2} = k_{1,2}t \quad (16)$$

There are two solutions to Eq. 16; one represents the value of  $k_1$ , the other the value of  $k_2$ . Because of the symmetry of the system definite assignment of values to  $k_1$  and  $k_2$  cannot be made. However, assignment of values to  $k_1$  and  $k_2$  will not affect the prediction of overall hydrolysis rates.

Equations 12, 13 and 16 are all of the general form

$$\ln \alpha = kt \quad (17)$$

where  $\alpha$  represents a function of the measured experimental concentrations, while  $k$  represents a linear function of the unknown rate constants. Values of  $k_1$ ,  $k_2$  and  $k_3$  may thus be obtained by linear regression of  $\ln \alpha$  vs.  $t$ .

## MODIFIED MODEL

### Model Development

If it is considered that substituents at a distance of more than *two* carbon atoms from the glycosidic bond have little effect on its rate of cleavage (a relaxation of the assumption leading to the simplified model), based on the structures in Figure 1,  $k_{31} = k_{33}$  and  $k_{32} = k_{34}$  in Figure 2. (For solamargine the situation is slightly different with the further equalities  $k_{11} = k_{22}$  and  $k_{21} = k_{22}$ .) This gives six unknown rate constants for solasonine and four for solamargine. If it is further assumed that for solasonine the presence of glucose does not effect the rate of cleavage of the rhamnose-galactose bond and the presence of rhamnose does not affect the rate of cleavage of the glucose-galactose bond,  $k_{11} = k_{12}$  and  $k_{21} = k_{22}$  and the number of rate constants reduces to four as is the case for solamargine. The validity of this assumption on steric grounds may be seen if a three-dimensional molecular model of solasonine is consulted. This shows that the galactose—solasodine bond may be blocked from reaction by the presence of the rhamnose unit, but that the other glycosidic bonds cannot be similarly blocked. This leads to a modified model based on four rate constants, Figure 4.

### Model Equations

The rate equations, again for a closed system are:

$$\frac{d[\alpha S_n]}{dt} = -(k_{11} + k_{21} + k_{32})[\alpha S_n] \quad (18)$$

$$\frac{d[\beta_1 S_n]}{dt} = k_{11}[\alpha S_n] - (k_{21} + k_{32})[\beta_1 S_n] \quad (19)$$

$$\frac{d[\beta_2 S_n]}{dt} = k_{21}[\alpha S_n] - (k_{11} + k_{31})[\beta_2 S_n] \quad (20)$$

$$\frac{d[\gamma S_n]}{dt} = k_{21}[\beta_1 S_n] + k_{11}[\beta_2 S_n] - k_{31}[\gamma S_n] \quad (21)$$

$$\frac{d[S]}{dt} = k_{31}([\beta_2 S_n] + [\gamma S_n]) + k_{32}([\beta_1 S_n] + [\alpha S_n]) \quad (22)$$

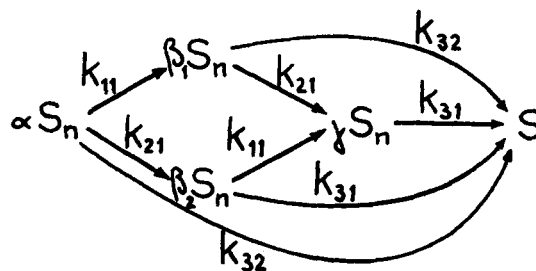


Figure 4. Modified model reaction scheme (solasonine  $\rightarrow$  solasodine).

Solution of these equations, again for an initial concentration of  $\alpha$ -solasonine of  $[\alpha S_n]_i$  and zero initial concentration of all other components, yields:

$$[\alpha S_n] = [\alpha S_n]_i e^{-Kt} \quad (23)$$

$$[\beta S_n] = [\alpha S_n]_i \{ (K' - 1)e^{-Kt} + e^{-(k_{21} + k_{32})t} - K'e^{-(k_{11} + k_{31})t} \} \quad (24)$$

$$[\gamma S_n] = [\alpha S_n]_i K' \{ e^{-(k_{11} + k_{31})t} + e^{-(k_{21} + k_{32})t} - e^{-k_{31}t} - e^{-Kt} \} \quad (25)$$

$$[S] = [\alpha S_n]_i \{ 1 - (1 + K')e^{-(k_{21} + k_{32})t} + K'e^{-k_{31}t} \} \quad (26)$$

where  $K = k_{11} + k_{21} + k_{32}$  and  $K' = k_{21}(k_{31} - k_{21} - k_{32})^{-1}$

### Evaluation of Model Parameters

The solutions above provide four independent equations with four unknown rate constants. However, rearrangement of the equations so that the rate constants may be evaluated from experimental data is now analytically difficult. To obtain the four rate constants from the hydrolysis of  $\alpha$ -solasonine only, the following procedure was adopted. Rewriting Eq. 23 gives

$$\ln \frac{[\alpha S_n]}{[\alpha S_n]_i} = -Kt \quad (27)$$

To obtain further estimates of rate constants, the rate of formation of  $S$  both initially and late in the reaction may be considered. Initially, before significant amounts of  $\beta_1 S_n$  and  $\beta_2 S_n$  are formed  $d[S]/dt = k_{32}[\alpha S_n]_i$ . Integration gives for  $[S]_i = 0$

$$[S] = k_{32}[\alpha S_n]_i t \quad (28)$$

Thus the initial slope of a plot of  $[S]/[\alpha S_n]_i$  vs.  $t$  will be  $k_{32}$ .

Once  $[\alpha S_n] \rightarrow 0$  and  $[\beta S_n] \rightarrow 0$ , from Eq. 22

$$\frac{d[S]}{dt} = k_{31}[\gamma S_n] = k_{31}([\alpha S_n]_i - [S])$$

giving

$$\ln \left\{ 1 - \frac{[S]}{[\alpha S_n]_i} \right\} = -k_{31}t + C \quad (29)$$

where  $C$  is a constant.

Equation 28 may also be written in this form:

$$\ln \left\{ 1 - \frac{[S]}{[\alpha S_n]_i} \right\} = \ln (1 - k_{32}t) \approx -k_{32}t \text{ as } k_{32}t \rightarrow 0 \quad (30)$$

Thus, according to the modified model, a plot of  $\ln \alpha$  vs.  $t$ , where  $\alpha = (1 - [S])/[\alpha S_n]_i$ , will have an initial slope  $-k_{32}$  and will become linear with slope  $-k_{31}$  once  $[\beta S_n] \rightarrow 0$ .

Using the above procedures  $K$ ,  $k_{31}$  and  $k_{32}$  may be estimated, but to establish values for  $k_{11}$  and  $k_{21}$  it is necessary to use a best fit technique. To do this, values for  $k_{11}$  and  $k_{21}$  were assumed, based on the calculated value of  $k_{11} + k_{21}$  ( $=K - k_{32}$ ). The sum of squares of the absolute deviations of the experimental relative concentrations from the predicted relative concentrations was then calculated and the values of  $k_{11}$  and  $k_{21}$  systematically varied until this sum of squares was minimized.

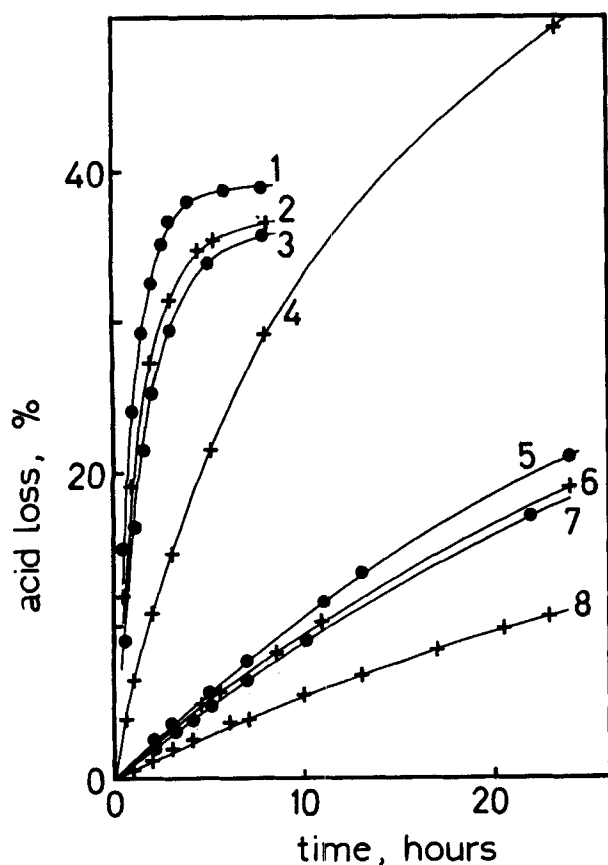


Figure 5. Acid loss occurring in solutions of HCl and  $\text{H}_2\text{SO}_4$  in various aqueous-alcohol mixtures. (All for 2N acid in 20 mol %  $\text{H}_2\text{O}$ /alcohol initially and reaction at  $70^\circ\text{C}$ ). 1: methanol/ $\text{H}_2\text{SO}_4$ ; 2: ethanol/ $\text{H}_2\text{SO}_4$ ; 3: n-propanol/ $\text{H}_2\text{SO}_4$ ; 4: methanol/HCl; 5: n-butanol/HCl; 6: ethanol/HCl; 7: n-propanol/HCl; 8: i-propanol/HCl.

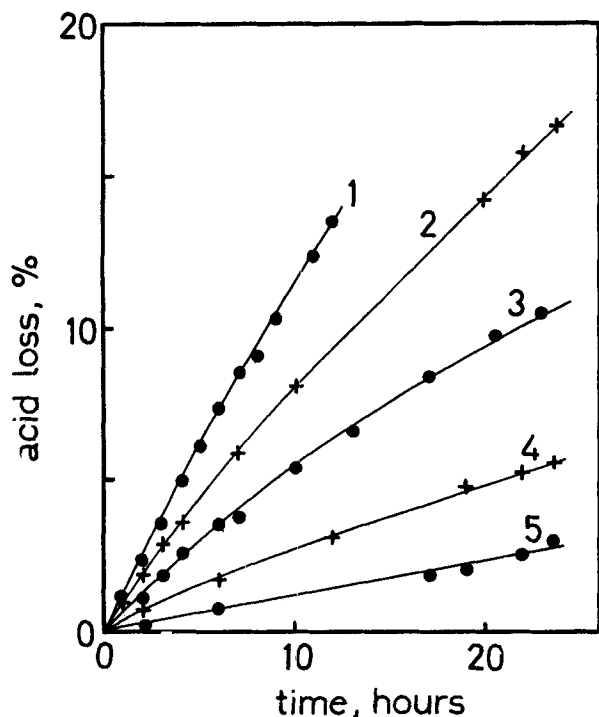


Figure 6. Effect of initial acid concentration on the acid loss in aqueous-isopropanol solvents (for HCl in 20 mol %  $\text{H}_2\text{O}$ /isopropanol and reaction at  $70^\circ\text{C}$ ). 1: 4.0 N; 2: 3.0 N; 3: 2.0 N; 4: 1.0 N; 5: 0.5 N.

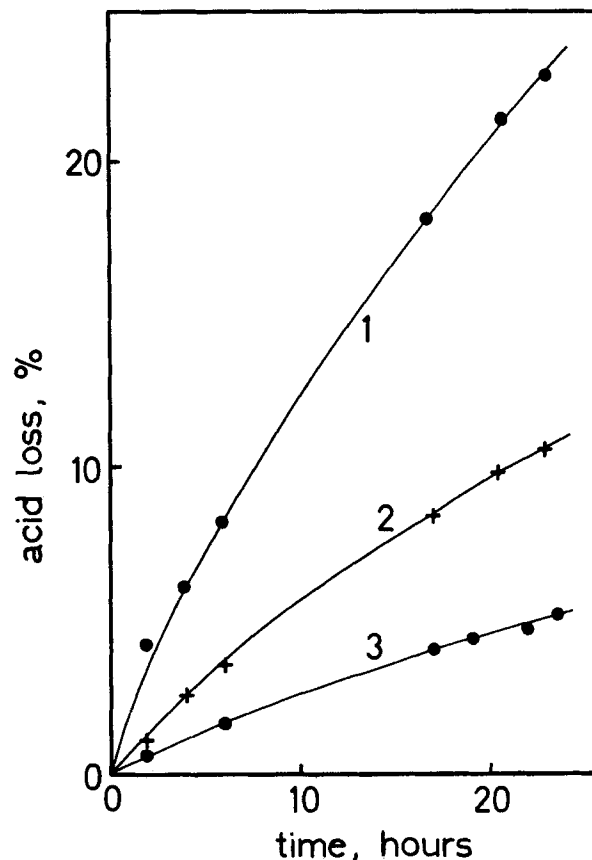


Figure 7. Effect of water content on the acid loss in aqueous-isopropanol solvents (for 2N HCl initially and reaction at  $70^\circ\text{C}$ ). 1: pure isopropanol; 2: 20 mole %  $\text{H}_2\text{O}$ /isopropanol; 3: 40 mole %  $\text{H}_2\text{O}$ /isopropanol.

#### ACID LOSS IN REACTION MEDIUM

Typically, acid hydrolysis of solasonine and solamargine has involved the reaction of a natural mixture of the glycosides with strong mineral acids in aqueous alcohol solvents. Alcohols commonly involved include methanol, ethanol or propanol and are added to the solvent medium to increase the solubility of the glycosides and of the intermediate and final products.

Whenever alcohols are used in an acidified medium, acid-induced substitution or elimination of the alcohol is possible (Roberts and Caserio, 1964). While this is a slow reaction, it soon became obvious in our studies that with certain combinations of alcohol and acid the acid loss was significant during the hydrolysis period. Such acid loss has not been previously reported. Loss of acid during the hydrolysis reaction is important. Commercially such loss of acid is uneconomical. If methanol or ethanol and HCl are involved, it may lead to dangerous pressure build-up. Experimentally, if kinetic rate data are to be accurately interpreted, this acid loss must be allowed in the determination of the rate constants. In particular, the first-order rate constants,  $k$  ( $=k'h$  from Eq. 1) will no longer be true constants. Figure 5 shows typical acid losses for various acid/alcohol combinations; HCl/isopropanol is seen to be the best. Figures 6 and 7 show the effect of acid strength and water content on the acid loss for the HCl/isopropanol system.

For the hydrolysis of solasodine glycosides using HCl in aqueous isopropanol, the acid loss over the period of the reaction is less than 1% and may be ignored for purposes of hydrolysis modelling. For the much slower solasodine to solasodine conversion, however, this is not true (Part II).

#### HYDROLYSIS EXPERIMENTS

A detailed account of the experimental techniques and procedures used has been reported by Crabbe (1980). The triglycosides solasonine and so-

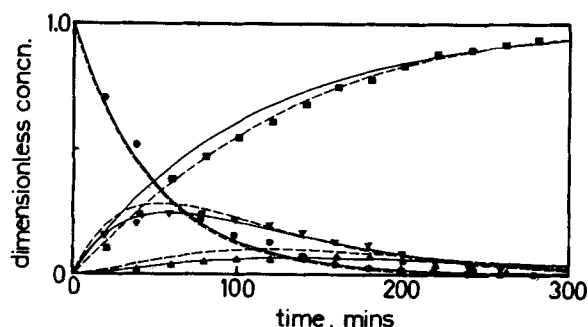


Figure 8. Concentration-time profiles for  $\alpha$ -solasone hydrolysis (reaction of  $2 \times 10^{-3}$  M solasone with 1 N HCl in 40 mole %  $\text{H}_2\text{O}$ /isopropanol at  $70^\circ\text{C}$ ).  $\bullet$   $[\alpha S_n]/[\alpha S_n]_i$ ;  $\nabla$   $[\alpha S_n]/[\alpha S_n]_i$ ;  $\blacktriangle$   $[\gamma S_n]/[\alpha S_n]_i$ ;  $\blacksquare$   $[S]/[\alpha S_n]_i$ ; solid lines: simplified model; broken lines: modified model.

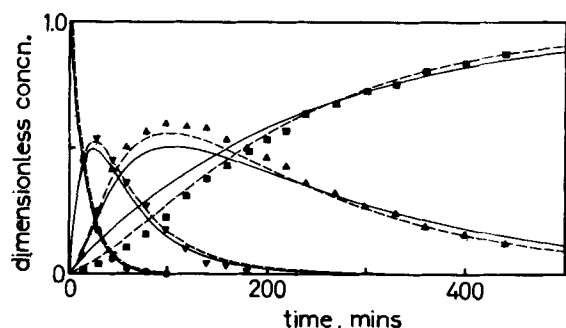


Figure 9. Concentration-time profiles for  $\alpha$ -solamargine hydrolysis (Reaction of  $2 \times 10^{-3}$  M solamargine with 1 N HCl in 40 mol %  $\text{H}_2\text{O}$ /isopropanol at  $70^\circ\text{C}$ ).  $\bullet$   $[\alpha S_m]/[\alpha S_m]_i$ ;  $\nabla$   $[\beta S_m]/[\alpha S_m]_i$ ;  $\blacktriangle$   $[\gamma S_m]/[\alpha S_m]_i$ ;  $\blacksquare$   $[S]/[\alpha S_m]_i$ ; solid lines: simplified model; broken lines: modified model.

lamargine were prepared from a natural mixture of the two using a modified version of the chromatographic procedure reported by Kuhn et al. (1955a). The acidified solvents were prepared by passing dry HCl gas into the required solvent mixture at low temperatures ( $-10^\circ\text{C}$ ). Determination of acid concentration was by titration against standard NaOH using phenolphthalein as the indicator. The experimental reactions were carried out in 7-mL glass vials capped with teflon-lined rubber septa. For the reaction the required amount of glycoside stock solution was measured into each vial and the solvent removed. The acidified solvent was added at  $0^\circ\text{C}$  just prior to the reaction and the reaction then carried out in a constant temperature water bath at 60 to  $75^\circ\text{C}$ . After the required period of time, the reaction mixture was cooled rapidly to  $0^\circ\text{C}$  and excess ammonia solution added. Analysis of the reaction products was made using high-pressure liquid chromatography (Crabbe and Fryer, 1979, 1980a).

## RESULTS AND MODEL PREDICTIONS

Hydrolysis experiments were 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. Figures 8 and 9 show typical results for the hydrolysis of pure solasone and pure solamargine respectively at one set of conditions, viz.,  $2 \times 10^{-3}$  M glycoside with 1 M HCl in 40 mol %  $\text{H}_2\text{O}$ /isopropanol at  $70^\circ\text{C}$ . In each case the experiment points and the predictions of the fitted models are shown. Both models provide good descriptions of the breakdown of the triglycoside, the appearance of the intermediates, and the production of solasodine. The values of  $[S]/[\alpha S_n]_i$  and  $[S]/[\alpha S_m]_i$  at the end of the reaction ( $[S]/[G]_i > 0.9$ ) are more accurately predicted by the modified model. Similar fit of the models was found under the other reaction conditions.

## EVALUATION OF RATE CONSTANTS

The rate constants used to compute the model predictions shown in Figures 8 and 9 were evaluated using Eqs. 12, 13, 16, 27 and

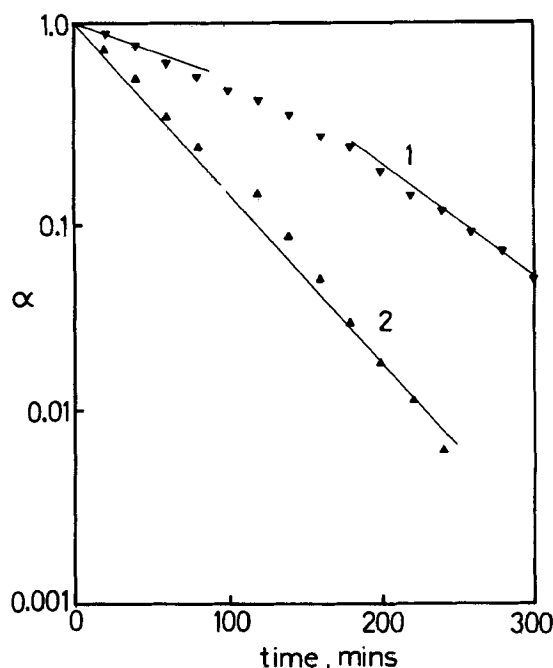


Figure 10. Evaluation of the rate constants used in Figure 8; use of Eqs. 12, 27 and 29/30. 1:  $\alpha \equiv 1 - [S]/[\alpha S_n]_i$ ; 2:  $\alpha \equiv [\alpha S_n]/[\alpha S_n]_i$ .

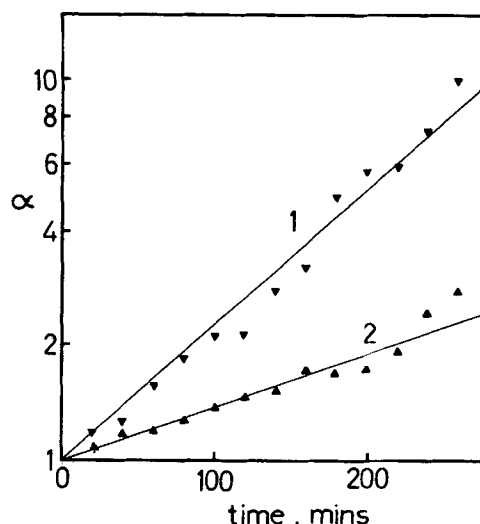


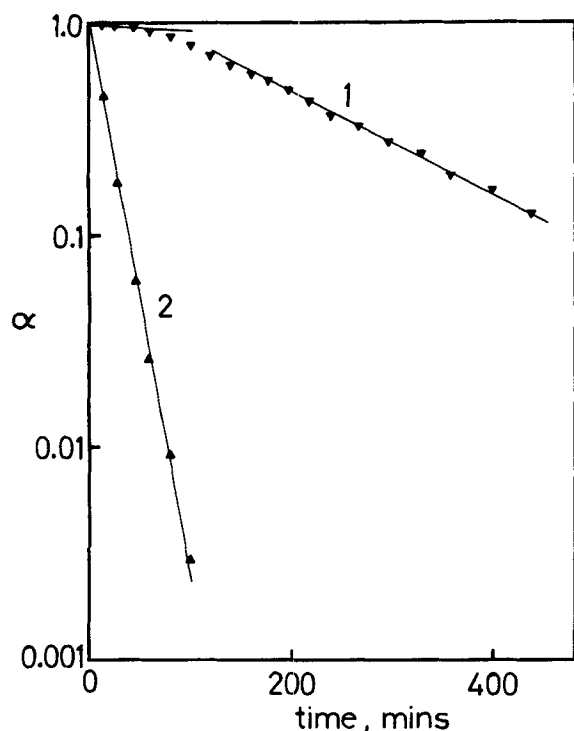
Figure 11. Evaluation of the rate constants used in Figure 8; use of Eq. 16. 1:  $\alpha \equiv \lambda_1$ ; 2:  $\alpha \equiv \lambda_2$ .

29/30. The relevant plots are shown in Figures 10 and 11 (for the solasone hydrolysis) and in Figures 12 and 13 (for solamargine). As expected, the plots are linear, except that of  $\ln \{1 - [S]/[G]_i\}$  vs.  $t$ . This plot behaves as predicted from the modified model, with an initial curved part and a final linear portion. This is better seen in the solamargine hydrolysis case (Figure 12) where both  $[\alpha S_m]$  and  $[\beta S_m]$  approach zero well within the period of the hydrolysis. Because the plot of  $\ln \{1 - [S]/[G]_i\}$  vs.  $t$  is nonlinear, Eq. 13 cannot be used to evaluate  $k_3$  in the simplified model. Instead  $k_1$  and  $k_2$  were determined by applying Eq. 16, then  $k_3$  was found from Eq. 12. In all the cases studied the shape of the  $\ln \{1 - [S]/[G]_i\}$  vs.  $t$  plot was such that  $k_{31} > k_{32}$ . This agrees with the assumptions leading to proposal of the modified model; i.e., the presence of rhamnose in  $\beta_1 S_n$ ,  $\beta_1 S_m$ ,  $\alpha S_n$  and  $\alpha S_m$  restricts attack on the galactose—solasodine bond.

In none of the experiments was there violation of the overall mass balance (Eq. 7) within the experimental error of the analysis ( $\pm 4\%$ ).

TABLE 1. GLYCOSIDE HYDROLYSIS: RATE CONSTANTS FOR SIMPLIFIED AND MODIFIED MODELS

Substrate	Reaction Conditions				Rate Constants <sup>1</sup>								
	[HCl] <sub>i</sub> (mol/L)	Solvent (mol % H <sub>2</sub> O/ isopropanol)	Temp. (°C)	[G] <sub>i</sub> (10 <sup>3</sup> mol/L)	Simplified Model				Modified Model				
					$k_1/k_T$	$k_2/k_T$	$k_3/k_T$	$k_T$ (min <sup>-1</sup> )	$k_{11}/k_T$	$k_{21}/k_T$	$k_{31}/k_T$	$k_{32}/k_T$	$k_T$ (min <sup>-1</sup> )
$\alpha S_m$	1.0	40	70	2	0.68	0.25	0.07	0.0608	0.63	0.27	0.084	0.016	0.0645
$\alpha S_n$	1.0	40	70	2	0.40	0.14	0.46	0.0205	0.21	0.21	0.39	0.19	0.0337
$\alpha S_n$	2.0	40	70	2	0.33	0.19	0.48	0.0894	0.11	0.30	0.36	0.23	0.139
$\alpha S_n$	0.5	40	70	2	0.42	0.18	0.40	0.00672	0.23	0.21	0.42	0.14	0.116
$\alpha S_n$	1.0	40	75	2	0.37	0.12	0.51	0.0392	0.20	0.23	0.41	0.16	0.0667
$\alpha S_n$	1.0	40	60	2	0.38	0.17	0.44	0.00437	0.12	0.30	0.38	0.19	0.00703
$\alpha S_n$	1.0	0	70	2	0.36	0.16	0.48	0.312	0.10	0.31	0.39	0.20	0.513
$\alpha S_n$	1.0	20	70	2	0.37	0.19	0.44	0.0557	0.11	0.39	0.35	0.15	0.0863
$\alpha S_n$	1.0	60	70	2	0.39	0.16	0.45	0.0102	0.09	0.35	0.40	0.16	0.017
$\alpha S_n$	1.0	40	70	1	0.39	0.15	0.46	0.0195	0.11	0.33	0.33	0.23	0.0293
$\alpha S_n$	1.0	40	70	5	0.39	0.14	0.47	0.0187	0.11	0.30	0.38	0.21	0.0307
AVERAGE <sup>2</sup>					0.38	0.16	0.46		0.14	0.29	0.38	0.19	
STD. DEV. <sup>2</sup>					0.02	0.02	0.03		0.05	0.06	0.03	0.03	

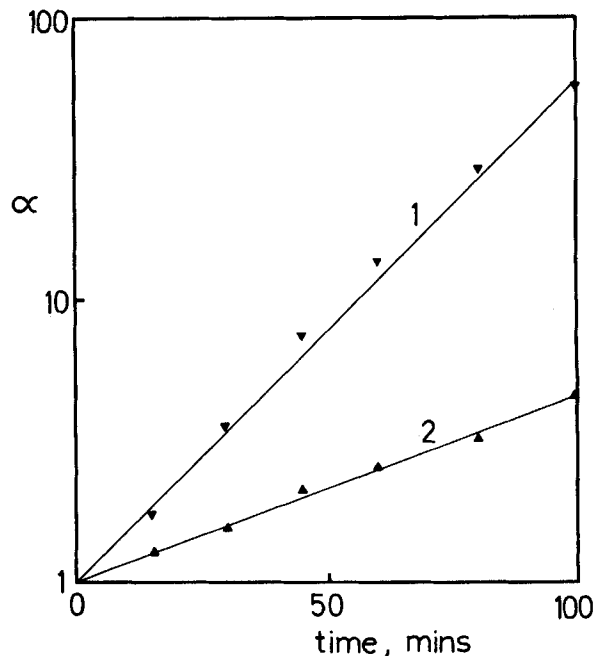
<sup>1</sup>  $k_T = k_1 + k_2 + k_3$  (simplified model) or  $k_T = k_{11} + k_{21} + k_{31} + k_{32}$  (modified model).<sup>2</sup> Based on  $\alpha S_n$  values only.Figure 12. Evaluation of the rate constants used in Figure 9; use of Eqs. 12, 27 and 29/30. 1:  $\alpha \equiv 1 - [S]/[\alpha S_m]_i$ ; 2:  $\alpha \equiv [\alpha S_m]/[\alpha S_m]_i$ .

The only exception was a small conversion of solasodine to solasodine as expected.

Rate constants for both models at the various reaction conditions, including those shown in Figures 8 and 9, are summarized in Table 1. The rate constants for both the simplified model and the modified model are included, written in terms of  $k_T$  ( $k_T = k_1 + k_2 + k_3$  or  $k_T = k_{11} + k_{21} + k_{31} + k_{32}$ ). The individual rate constants are seen to be approximately a constant proportion of  $k_T$  irrespective of the reaction conditions (mean and standard deviations given in Table 1) making  $k_T$  a suitable measure of the effect of the reaction conditions on the hydrolysis. The effects of the reaction conditions on the hydrolysis, as measured by the time for hydrolysis and the proportion of solasodine in the final product, are considered in Part III.

## CONCLUSION

Two models have been proposed to describe the sequence of reactions occurring during the hydrolysis of solasodine glycosides.

Figure 13. Evaluation of the rate constants used in Figure 9; use of Eq. 16. 1:  $\alpha \equiv \lambda_1$ ; 2:  $\alpha \equiv \lambda_2$ .

Procedures for evaluation of the rate constants have been applied to experimental data obtained by following the concentrations of the various solasodine-related species during batch hydrolysis. The models, fitted in this way, allow accurate prediction of hydrolysis rates and can be used to determine the time required for a specified conversion under known reaction conditions.

## NOTATION

[A]	= glycoside concentration
C	= integration constant, Eq. 29
[G]	= concentration of total glycoside
h	= acidity function
k'	= rate constant for glycoside hydrolysis
k	= $k'/h$ , first order rate constant at fixed acid concentration
$k_{11}, k_{21}, k_{12}, k_{22}, k_{31}, k_{32}, k_{33}, k_{34}$	= first-order rate constants in complete and modified models (Figures 2 and 4)
$k_1, k_2, k_3$	= first-order rate constants in simplified model (Figure 3)

$k_{1,2}$	= term used to represent either $k_1$ or $k_2$
$k_T$	= $k_1 + k_2 + k_3$ (in simplified model)
$k_T$	= $k_{11} + k_{21} + k_{31} + k_{32}$ (in modified model)
$K$	= $k_{11} + k_{21} + k_{32}$
$K'$	= $k_{21}(k_{31} - k_{21} - k_{32})^{-1}$
$[S]$	= concentration of solasodine
$[\alpha S_n]$	= concentration of $\alpha$ -solasonine
$[\beta_1 S_n]$	= concentration of $\beta_1$ -solasonine
$[\beta_2 S_n]$	= concentration of $\beta_2$ -solasonine
$[\beta S_n]$	= $[\beta_1 S_n] + [\beta_2 S_n]$
$[\gamma S_n]$	= concentration of $\gamma$ -solasonine
$t$	= reaction time

#### Subscript

$i$  = initial concentration

#### Greek Letter

$\lambda_1$  and  $\lambda_2$  = defined by Eqs. 14 and 15

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