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SCIENTIFIC PAPER

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KINETICS OF GLYCOALKALOID HYDROLYSIS AND SOLANIDINE EXTRACTION IN LIQUID–LIQUID SYSTEMS

The kinetics of glycoalkaloid hydrolysis and solanidine extraction in liquid–liquid systems were analyzed in this study. The extracts of glycoalkaloids obtained from dried and milled potato haulm, to which hydrochloric acid was added, is the first liquid phase, while chloroform, trichloroethylene or carbon tetrachloride is the second organic, liquid phase. The purpose of this paper was to combine the processes of glycoalkaloid hydrolysis to solanidine and solanidine extraction into one step, and to find the optimal liquid–liquid system for such a process.

Plants of the Solanum genus contain glycoalkaloids (GA) [1]. In the haulm of potato (*Solanum tuberosum* **L.**) glycoalkaloids are normally present in the range from 0.25–0.62% [2]. They are secondary plant metabolites [3], providing resistance to some fungi [4] and are also known as phytoalexins [5,6].

Two major glycoalkaloids, α–solanine and α–chaconine [7,8], which represent more than 95% of the total GA, are present in potato [9]. The 3β–O–glycosidic hydrolysis of glycoalkaloids by mineral acids is the cleavlage of the bond between the carbohydrate moiety and solanidine, yielding a carbohydrate moiety (solatriose from α–solanine and chacotriose from α–chaconine), as well as aglycon solanidine. Solanidine is an important precursor for hormone synthesis [2,10]. The chemical transformations of solanidine for obtaining 16–dehydropregnenolon acetate, the key intermediate in the industrial synthesis of progesterone and cortisone derivatives, are published in the literature [11]. According to some new information, glycoalkaloids and solanidine can be effectively used for removing precancerous actinic karatose and age spots [12].

Conventional acid hydrolysis, performed in one liquid phase, depends on the hydrolysis conditions (temperature, the concentration of mineral acid, type of used solvent, duration of hydrolysis etc). The β– and γ–forms are indicated as a product of the incomplete and mild acid hydrolysis of α–solanine and α–chaconine and, very often, under such conditions the aglycon solanidine was lost by converting it into solanthrene [13]. In our previous papers, the kinetics of hydrolysis of GA from potato haulm and sprouts in two–phase solid–liquid systems [14,15] and during fermentation of the plant material [16] were studied in order to define the optimal procedures of hydrolysis. Consequent solanidine extraction was required in both cases, making the procedures more complex and increasing the cost of industrial solanidine production.

The goal of the present study was to analyze the kinetics of glycoalkaloid hydrolysis and solanidine extraction in liquid–liquid systems and to combine in one step the processes of glycoalkaloid hydrolysis to solanidine and solanidine extraction and to determine an optimal liquid–liquid system for such an operation. Furthermore, the proposed procedure might be indicative that the effective protection of solanidine from a disintegration reaction is possible.

The model of glycoalkaloid hydrolysis and solanidine extraction in these systems is shown in Figure 1.

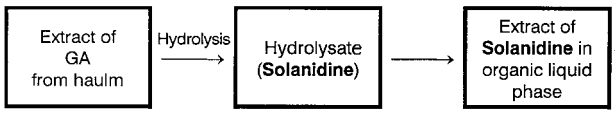

Figure 1. The mechanism of glycoalkaloid hydrolysis

The procedure is based on the basis of the difference in polarity between glycoalkaloids and their aglycones. The medium for hydrolysis contains aqueous or alhoholic acid solution and a non-polar organic solvent as two immiscible phases. The polar glycoalkaloid is hydrolysed in the acid phase and the non-polar aglycone formed is continuously withdrawn by the organic liquid phase. This procedure protects the aglycone from further disintegration. The generally accepted mechanism [17,18] of GA hydrolysis is the existence of an initial and fast pre–equilibrium condition which involves the protonation of the glycosidic oxygen. Such a condition is followed by slow cleavage of the glycosidic bond and the formation of a carbonium ion. The mechanism of glycoalkaloid hydrolysis to solanidine is given in Figure 2.

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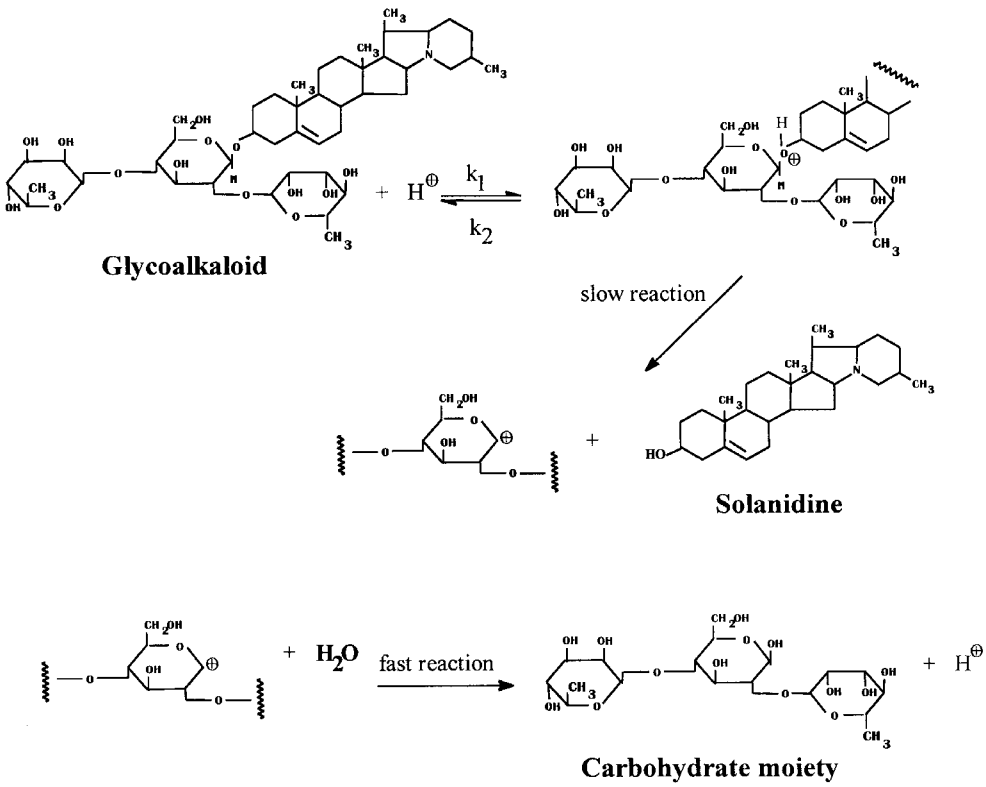

Figure 2. Model of glycoalkaloid hydrolysis and solanidine extraction in liquid–liquid systems

The carbonium ion is highly reactive and may further react with the solvent to form a free carbohydrate moiety and aglycon. The rate of hydrolysis is governed by the slow cleavage of the glycosidic bond.

EXPERIMENTAL SECTION

Plant material. The haulm of potato cv. Désirée was harvested in mid July 2001, dried at room temperature in trays for 21 days and milled to an average particle size of 0.14 mm. The moisture content was about 10 %.

Extraction of glycoalkaloids . The GA extract was obtained by extraction from potato haulm with: 2% w/v acetic acid; 10% w/v acetic acid; 50% vol. methanol or with 95% vol. ethanol, applying the ratio of the plant material and the corresponding solution 1:20, 1:20, 1:9 and 1:20 w/v, respectively. The obtained ethanolic extract was diluted by distilled water at a volume ratio of 1:1.

Glycoalkaloid hydrolysis and solanidine extraction in liquid–liquid systems. The hydrochloric acid was added to extracts of GA obtained by 2% w/v acetic acid, 10% w/v acetic acid, 50% vol. methanol to concentration of 5, 2, 10 and 10 % w/v, respectively, and the chloroform, trichloroethylene or carbontetrachloride were also added. The volume ratio of the liquid phases was 1:1. The flasks were placed in a water bath at boiling temperature. Aliquots of 1 cm^3 of the organic liquid phase were taken after 10, 15, 30, 45, 60, 90 and 120 minutes from each flask and the content of solanidine was determined by the using corresponding procedure [].

Glycoalkaloid hydrolysis and solanidine extraction in liquid–liquid systems with 96% ethanolic extract as the first liquid phase. The 96% vol. ethanolic extract of glycoalkaloids obtained by extaction from potato haulm was diluted with distilled water at a volume ratio of 1:1 and hydrochloric acid was added to the concentration of 10% w/v. The hydrolysis was carried out in a water bath at boiling temperature. Aliquots of 1 cm^3 of the hydrolysate were taken after 10, 15, 30, 45, 60, 90 and 120 minutes and the content of solanidine was determined as before by using the corresponding procedure [].

Content of solanidine. The organic liquid phase, the chloroform, trichloroethylene or carbon tetrachloride, was evaporated to dryness under vacuum. The dry residue was dissolved in 10 cm^3 of 2 % w/v acetic acid. The pH of the solutions was adjusted to 4.0 by adding a solution of sodium hydroxide (at first 50 and then 1% w/v). The solutions were transferred to a separating funnel for complex formation with methyl–orange, as described by Tuckalo i Tscarik [19]. The coloured complex was extracted by chloroform (5 times by 5 cm^3), dried with anhydrous sodium sulphate and its volume was adjusted to 25 cm^3. The absorbance of the extract was measured at 420 nm (UV–Vis Spectrophotometers, Lambda V Perkin Elmer). The content of solanidine was detemined by a standard calibration curve.

The content of total extractive matters. An aliquot of the organic liquid phase (1 cm^3) was placed on the disk plate of an electronic moisture analyzer (SCALTEC SMO 1, Scaltec Instruments GmbH, Germany). The content of total extractive matter at the end of the procedure was readout on the display.

TLC analysis. A 0.03 cm^3 aliquot of both liquid phases obtained after 10, 15, 30, 45, 60, 90 and 120 minutes, were applied to 20x20 cm plates covered with a 120 µm thick Silica gel G60 (Merck reagents) layer. The plates were developed to the height of 16 cm, with a lower layer of a mixture of methanol–chloroform–1% ammonium hydroxide (50:50:25 v/v). The spots were visualized when treated with 50% aqueous sulphuric acid and heated at 110°C for 30 minutes.

RESULTS AND DISCUSSION

The maximal degree of glycolakaloid hydrolysis (DH GA), the hydrolysis time, yield of solanidine, yield of total extractive matter and content of solanidine in the total extractive matter in different solid–liquid systems are given in Table 1. The DH GA was expressed as the ratio of the solanidine content in the organic liquid phase after a certain hydrolysis time to the maximal yield of solanidine which should be achieved from the used haulm.

The maximal yield of solanidine which should be achieved (0.24 g per 100 g dried haulm) was calculated according to the glycoalkaloid content in haulm (0.53 g per 100 g dried haulm) and to the ratio of α–solanine and α–chaconine in the total glycoalkaloids (1.27:1), considering that one mole of α–solanine or α–chaconine yields one mole of solanidine. The content of solanidine in the total extractive matter was calculated on the basis of the value of the solanidine yield (q_s) and on the value of the total extractive matter yield (q_{em}):

$$q_{sem} = k \cdot \frac{q_s}{q_{em}} \cdot 10^3$$

where k is a coefficient which depends on the used plant and solution ratio during the extraction of glycolakaloids from the haulm.

The best results of DH GA were achieved in liquid–liquid systems with chloroform, while the lowest DH GA was achieved by carbon tetrachloride as the second phase, independently of the used first liquid phase. In alcoholic extracts of GA as the first liquid phase, the results of DH GA were better than the results of DH GA in acetic acid extracts of GA as the first liquid phase.

The variations of DH GA in the ethanolic extract of GA from haulm by hydrochloric acid as the first liquid phase and chloroform, trichloroethylene and carbon tetrachloride as the second liquid phase during glycoalkaloid hydrolysis are shown in Figure 3. The highest DH GA (93.1%) was achieved in a system with chloroform as the second phase after 120 minutes of hydrolysis. As shown, the yield of solanidine depends on DH GA, so the highest yield of 0.20 g per 100 g of dried plant material was achieved under the same conditions as when the highest DH GA is achieved. The highest content of solanidine in the total extractive matter (53.00 mg per g of total extractive matter) was achieved in the same liquid–liquid system (Table 1).

Table 1. The results of the kinetic investigation of glycoalkaloid hydrolysis and solanidine extraction in liquid–liquid systems

First liquid phase	Second liquid phase		
	Chloroform	Trichloro-ethylene	Carbontetra-chloride
5% m/v HCl in 2% m/v acetic acid extract of GA	18.7[a] 120[b] 0.04[c] 0.26[d] 7.69[e]	7.3 30 0.02 0.33 3.03	4.2 60 0.01 0.06 8.33
5% m/v HCl in 10% m/v acetic acid extract of GA	25.9 120 0.05 0.28 11.07	23.1 120 0.05 0.38 6.71	14.4 30 0.03 0.06 26.66
10% m/v HCl in 50% vol. methanol extract of GA	78.0 60 0.17 0.43 44.7	72.3 90 0.16 0.58 30.65	45.6 120 0.09 0.13 86.3
10% m/v HCl in 50% vol. ethanol extract of GA	93.1 120 0.21 0.19 53.00	82,2 60 0.18 0.30 31.16	73,8 60 0.16 0.05 168.03

[a] the maximal degree of glycoalkaloid hydrolysis (DH GA) expressed as $q_S/q_{S\,max}\cdot 100$,

[b] time of hydrolysis (minutes) for obtaining the maximal DH GA,

[c] the yield of solanidine in the organic liquid phase (g/100 g of dried haulm),

[d] the yield of total extractive matter (g/g of organic liquid phase),

[e] the content of solanidine in the total extractive matter (mg/g of total extractive matter)

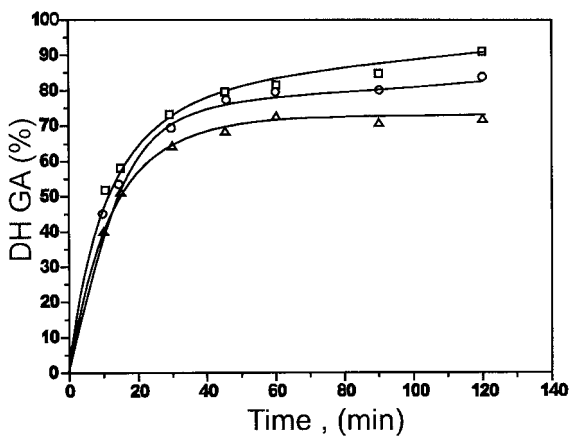

Figure 3. The variation of DH GA during glycoalkaloid hydrolysis in liquid–liquid systems (first liquid phase: 10 % w/v hydrochloric acid in 50 % vol. ethanol; second liquid phase: □ – chloroform; o – trichloroethylene; Δ – carbon tetrachloride; volume ratio of phases 1:1; temperature: app. 82°C)

The kinetic curves of glycoalkaloid hydrolysis in the investigated liquid–liquid systems do not show a maximum. This shape of the kinetic curves and TLC analysis in all the investigated cases signify that the solanidine is protected from further dehydration to solanthrene by the organic liquid phase. Such a conclusion was supported by TLC analysis. During conventional acid hydrolysis in liquid systems, (without the organic liquid phase) very often aglycon solanidine is lost by conversion into solanthrene [13]. Also, TLC analysis showed that under those conditions, the glycoalkaloids are directly hydrolized to solanidine without forming intermediate products such as the β_1–, β_2– and γ– forms of α–solanine and α–chaconine.

The equations for calculating the yield of solanidine in the organic liquid phase (q_S, expressed in g per 100 g dried haulm) and the equation for calculating the concentration of solanidine in the extract (C_s, expressed in mg per cm^3) as a function of hydrolysis time were solved by using Maple V Release Software:

$$q_S = 0.20\cdot(1-e^{-0.073t})$$

$$C_s = 9.72\cdot 10^{-2}(1-e^{-0.073t})$$

The variation of the rate of solanidine ($mol/dm^3\cdot s$) hydrolytic extraction in a liquid–liquid system containing 10% w/v hydrochloric acid in 50% vol ethanol as the first liquid phase and chloroform as the second liquid phase, as well as the rate of solanidine hydrolytic extraction in one liquid phase is given in Figure 4. The variation of the yield of solanidine in a two liqiud–liquid phase and in a one liquid system, on the right Y–axis, versus the hydrolysis time is also presented in Figure 4.

The highest rate of glycoalkaloid hydrolysis was achieved after 5 minutes of hydrolysis. The rate is 4.05×10^{-7} in a two liquid–liquid system and 6.21×10^{-7} ($mol/dm^3\cdot s$) in a one liquid system. By comparing the results, it way be concluded that only in the first 45 minutes of extraction these is a higher rate of solanidine extraction in the one liquid system compared to the situation when a two liquid–liquid system is applied. After 45 minutes the rates in the one and two liquid system become equal. The reason for such an effect is caused (two phase system) by the fact that the solanidine obtained by GA hydrolysis must be extracted by the organic liquid phase from the first liquid phase where solanidine is protected from the desintegration reaction. After 120 minutes, the complete obtained amount of solanidine (0.21 g/100 g dried haulm) is extracted by chloroform.

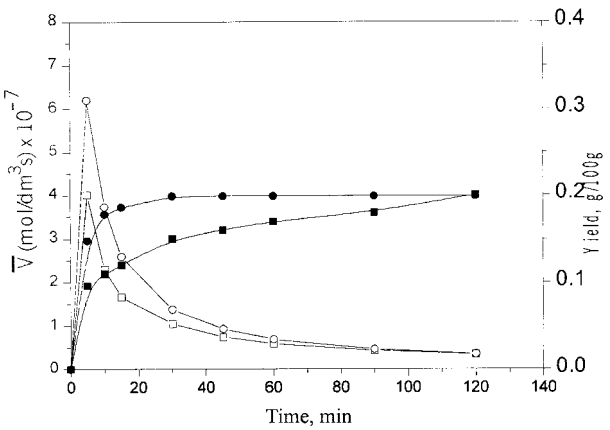

Figure 4. The rate of glycoalkaloid hydrolysis (left Y–axis): □–in a liquid–liquid system with 10 % w/v hydrochloric acid in a 50 % vol. ethanol extract of glycoalkaloids as the first and chloroform as the second liquid phase; o – in a liquid system with 10 % w/v hydrochloric acid in 50 % vol. ethanol extracts of glycoalkaloids. The yield of solanidine (right Y–axis): ■ – in the liquid–liquid sistem; ● – in the liquid system

CONCLUSION

The highest degree of glycoalkaloid hydrolysis (93.1%) was obtained in a two liquid–liquid system consisting of 10% w/v hydrochloric acid in a 50% vol. ethanol extract of glycoalkaloids and chloroform after 120 minutes of hydrolysis. The volume ratio of the liquid to liquid phase was 1:1 in that case. Also, the highest solanidine content in the total extractive matter (53.00 mg/g of total extractive matter) and the highest solanidine yield (0.21 g/100 g dried plant material) was achieved under that condition. The shape of the kinetic curves and TLC analysis in all the investigated cases indicated that the disintegration of solanidine to solanthrene was suppresed by the presence of the second organic (liquid) phase. The TLC analysis also showed that under those conditions glycoalkaloids are directly hydrolized to solanidine without forming the intermediate products of α–solanine and α–chaconine as in the case of glycoalkaloid hydrolysis performed in one liquid systems. The equation for calculating the yield of solanidine and the equation for calculating the concentration of solanidine in the extract in an optimal liquid–liquid system were determined.

NOTATION

DH GA – degree of glycoalkaloid hydrolysis, %
t – hydrolysis time, min
q_s – yield of solanidine, g/100 g of dried haulm
q_{smax} – maximal yield of solanidine, g/100 g of dried haulm
q_{em} – content of total extractive matter,
q_{Sem} – content of solanidine in the total extractive matter, mg/g
k – coefficient dependant on the plant material and solvent ratio during glycoalkaloid extraction
C_s – concentration of solanidine in the extract, mg/cm^3

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IZVOD

KINETIKA HIDROLIZE GLIKOALKALOIDA I EKSTRAKCIJA SOLANIDINA U TEČNO–TEČNO SISTEMU

(Naučni rad)

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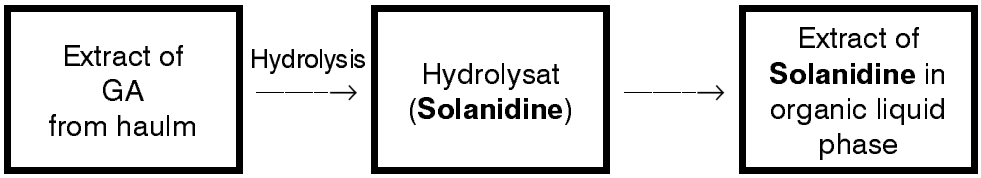
U radu je ispitana kinetika hidrolize glikoalkaloida i ekstrakcija solanidina u dvofaznim sistemima tečno–tečno. Ekstrakti glikoalkaloida iz nadzemnog dela krompira (*Solanum tuberosum* **L.**), dobijeni pod optimalnim uslovima ekstrakcije sa dodatkom hlorovodonične kiseline, čine jednu tečnu fazu, a organski rastvarač, hloroform, trihloretilen ili ugljentetrahlorid, drugu tečnu fazu. Cilj ispitivanja je bio da se proces hidrolize glikoalkaloida i proces ekstrakcije solanidina organskom fazom objedine u jedan proces i izabere optimalni tečno–tečni sistem. Najbolji stepen hidrolize glikoalkaloida od 93,1% ostvaren je u 50% vol. etanolnom ekstraktu glikoalkaloida sa hlorovodoničnom kiselinom u ekstraktu koncentracije 10% m/v kao jedne tečne faze i hloroforma kao druge tečne faze, posle 120 minuta hidrolize. Pri tome je ostvaren prinos solanidina u organskoj fazi od 0,21 g/100g suvog nadzemnog dela i sadržaj solanidina u ukupnim ekstraktivnim materijama od 53,00 mg/g. U uslovima hidrolize glikoalkaloida i ekstrakcije solanidina u tečno–tečnim sistemima za razliku od tečnih sistema, glikoalkaloidi se hidrolizuju do solanidina direktno, bez stvaranja intermedijernih formi, a dobijeni solanidin ne podleže transformaciji do solantrena.

Ključne reči: Krompir • Glikoalkaloidi • Hidroliza • Ekstrakcija • Solanidin •
Key words: Potato • Haulm • Glycoalkaloids • Hydrolysis • Solanidine • Extraction •


Extract of GA from haulm
Hydrolysis
Hydrolysat (**Solanidine**)
Extract of **Solanidine** in organic liquid phase

Hydrolysis