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SCIENTIFIC PAPER

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SOLID-LIQUID SYSTEMS FOR THE HYDROLYSIS OF GLYCOALKALOIDS FROM POTATO (*Solanum tuberosum* L.) TUBER SPROUTS AND SOLANIDINE ISOLATION

Solanidine ($C_{17}H_{43}ON$) is a steroidal aglycone of glycoalkaloids and an important precursor for the synthesis of hormones and some pharmacologically active compounds. Glycoalkaloids are hydrolysed by mineral acid yielding solanidine and a carbohydrate moiety. In this paper the kinetics of hydrolysis of glycoalkaloids from potato (*Solanum tuberosum* L.) tuber sprouts by using solid-liquid systems were studied as well as solanidine isolation from the liquid phase of the system. The dried and milled tuber sprouts of potato were used as the solid phase and solutions of hydrochloric acid of different concentration in 96 % vol. ethanol, mixed with chloroform in a volume ratio of 2:3, 1:1, 3:2 and 4:1, were used as the liquid phase. The aim of the paper was to choose the optimal concentration of hydrochloric acid in ethanol, the volume ratio of hydrochloric acid in ethanol to chloroform in the liquid phase and the time for solanidine hydrolytic extraction, as well as to isolate solanidine from the liquid phase.

Potato sprouts are a conventional starting material for the production of steroid hormones [1,2]. They contain steroidal glycoalkaloids (GA) possessing solanidine as the aglycone part and a carbohydrate moiety [3]. The two major GA of potato sprouts, which represent more than 95% of the total GA, are α -solanine and α -chaconine [4,5]. Upon the hydrolysis of GA by the action of mineral acid, a carbohydrate moiety and the aglycon solanidine are recovered as the result of cleaving the β -O-glycosidic bond [3]. There is data in the literature that there is a chemical transformation of solanidine to 16-dehidropregnenolon acetate [6], a key intermediate for the industrial synthesis of progesterone and cortisone derivatives.

The procedure for obtaining solanidine from plant material is complicated and requires glycoalkaloid extraction from the plant material, hydrolysis to solanidine and the extraction of solanidine by an organic liquid phase. By using a solid-liquid phase, the process of glycoalkaloid extraction from plant material and hydrolysis are united in a single step. The obtained solanidine remains in the same liquid phase from where it can be isolated.

In our previous paper the kinetics of GA hydrolysis and solanidine extraction from potato haulm by using solid-liquid systems [7] as well as liquid-liquid systems [8] and solid-liquid-liquid systems [9], have already been considered.

In this paper potato tuber sprouts are used as the plant material instead haulm. Glycoalkaloids are normally present in dried haulm in the range 0.25–0.62%, while in dried potato sprouts in the range 0.56–5.03 % [10]. A higher content of glycoalkaloids in tuber sprouts than in haulm is important for the more economic, simpler and faster obtaining of solanidine.

The model of glycoalkaloid hydrolysis in a solid-liquid system is shown in Figure 1. The aim of the paper was to study the kinetics of glycoalkaloid extraction and hydrolysis by using solid-liquid systems in order to determine the optimal conditions for obtaining solanidine from tuber sprouts and to isolate solanidine from the liquid phase of the system.

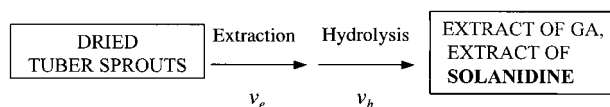


Figure 1. The model of glycoalkaloid hydrolysis in a solid-liquid system

MATERIALS AND METHODS

Plant material. The tuber sprouts of potato cv. Désirée were grown at 20–22°C for 60 days in the dark and at a relative humidity of 60% [10]. They were dried at 40°C for 4 h and milled to an average particle size of 0.21 mm.

Glycoalkaloid hydrolysis in solid-liquid systems. Dried and milled tuber sprouts (40 g) were treated by 800 mL of a mixture consisting of 2, 5 or 10 % w/v hydrochloric acid of 96% vol. ethanol and chloroform in separate flasks. The volume ratio of hydrochloric acid in 96% vol ethanol and chloroform in the mixture was 2:1, 1:1, 3:2 and 4:1. The flasks were placed in a bath with boiling water and connected to a reflux condenser. Aliquots of 1 mL of the filtered liquid phase were taken at 10, 15, 30, 45, 60, 90 and 120 minute intervals, from each flask and the content of solanidine was determined.

Content of solanidine. The liquid phase was evaporated to dryness under vacuum. The dry residue was dissolved in 10 mL of 2% w/v aqueous acetic acid. The pH of the solutions was adjusted to 4.0 by adding aqueous sodium hydroxide (at first by 50 and then by 1% w/v). The solutions were transferred to a separatory

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Paper received: May 30, 2003
Paper accepted: June 21, 2003

funnel for complex formation with methyl-orange, as described by Tukalo and Tsarik [11]. The coloured complex was extracted by chloroform (5 times by 5 mL), dried with anhydrous sodium sulphate and the volume was adjusted to 25 mL. The absorbance of the extract was measured at 420 nm (UV-Vis Spectrophotometers, Lambda V Perkin Elmer). The content of solanidine was determined using a standard curve.

The content of total extractive matter. An aliquot of the liquid phase (1 cm³) 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 read out on the display.

TLC analysis. A 0.03 mL aliquot of the liquid phase obtained after 10, 15, 30, 45, 60, 90 and 120 minutes, was applied to 20x20 cm plates of 120 µm thick Silica gel G 60 (Merck reagents). 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 by spraying the chromatogram when treated with a 50% v/v aqueous solution of sulphuric acid and heated at 110°C for 30 minutes [12].

Isolation of solanidine. The dried tuber sprouts (100 g) were treated by 2000 mL of a mixture of 10% m/v hydrochloric acid in 96% vol. and chloroform in a volume ratio of 1:1. The flask was placed in a bath with boiling water, for 120 minutes. The liquid phase was then separated from the system by filtration. Activated carbon was added and the extract filtered hot through a 2 cm bed of Celite and filterpaper circles, type 391 (Filtrak, Germany). The filtrate was evaporated to dryness under vacuum. The dry residue was dissolved in a minimum amount of hot 96 % ethanol and solanidine was precipitated by adding concentrated aqueous ammonia. The precipitate was separated by centrifuging (3500 min⁻¹) and washed several times by distilled water.

IR spectrophotometry. The IR spectra were recorded at room temperature on a Bomem MB-100 (Hartmann & Brunn) Michelson FTIR spectrometer using the KBr technique.

MS spectrometry. A Varian 3700 gas chromatograph-mass spectrometer (MAT 311 A) was used to obtain the electron impact mass spectrum. A 30 m x 0.30 µm column was packed with OV-101. The column temperature was programmed from 150-290°C at a rate of 2°C/min. Helium was used as the carrier gas at a linear velocity of 30 m/s. The mass spectrometer ionization was set at 70 eV and the source temperature was 180°C.

RESULTS AND DISCUSSION

The maximal achieved degree of glycoalkaloid hydrolysis (DH GA), the hydrolytic extraction time (t), yield of solanidine (q_s), yield of the total extractive matter

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

Concentration of HCl in 96% vol. ethanol (w/v)	Ratio of HCl etanolic solution and chloroform in the liquid phase (v/v)			
	2:	3	1:	3:
2	31.5 ^(a)	37.5	40.0	36.5
	120 ^(b)	35	120	120
	0.49 ^(c)	0.59	0.63	0.57
	3.02 ^(d)	3.87	3.92	4.00
	8.18 ^(e)	7.59	8.04	7.16
5	55.0	58.0	60.5	62.5
	120	120	120	120
	0.86	0.91	0.95	0.98
	3.62	3.83	4.03	4.12
	11.92	11.87	11.77	11.91
10	75.5	82.7	58.3	37.2
	120	120	90	30
	1.19	1.29	0.91	0.58
	3.85	4.00	4.22	4.30
	15.39	16.18	10.84	6.79

^(a)the maximal degree of glycoalkaloid hydrolysis (DH GA) (%),

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

^(c)the yield of solanidine in the liquid phase (g per 100 g of dried potato sprouts),

^(d)the yield of total extractive matter (g/g of liquid phase)

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

(q_m) and the content of solanidine in total extractive matters (q_{sem}) in different solid-liquid systems is given in Table 1. DH GA was expressed as the ratio of the solanidine content in the liquid phase after certain hydrolytic time to the maximal yield of solanidine which may be achieved from the used tuber sprouts (the content of glycoalkaloids was 3.38 g per 100 g dried sprouts and the maximal yield of solanidine was calculated to be 1.56 g per 100 g).

The yield of solanidine was calculated according to DH GA and the maximal possible yield of solanidine from the used plant material while the content of solanidine in the total extractive matter was calculated based on the yield of solanidine and the yield of total extractive matter.

The variation of DH GA during the time of hydrolysis by 10% w/v HCl in 96% vol. ethanol mixed with chloroform in different volume ratios, is shown in Figure 2.

The best DH GA of about 83.0% was achieved by 10% w/v hydrochloric acid in 96% vol. ethanol mixed with chloroform in a volume ratio of 1:1, after 120 minutes of hydrolysis. The yield of solanidine depends on the DH GA and the best yield of 1.29 g per 100 g dried and milled potato sprouts was achieved by using the same system when the best DH GA was achieved. The content of solanidine in the total extractive matter,

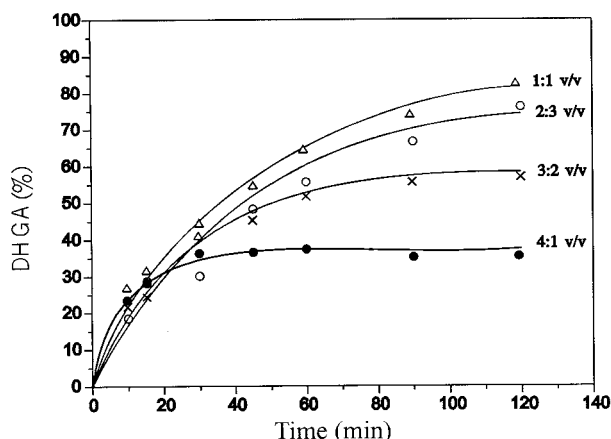


Figure 2. The variation of degree of GA hydrolysis (DH GA, %) in solid-liquid systems with 10% w/v HCl in 96% vol. ethanol mixed with chloroform in different volume ratio, versus the hydrolytic extraction time

was also the best using the same system and its value was 16.18 mg per g of total extractive matter (Table 1).

The equation for calculating the DH GA depending on the hydrolytic extraction time, t (min), according to the kinetic curve for the optimal system, was obtained by using the Maple V Release program:

$$DH = 0.46 + 3.02 \times 10^{-3} t$$

According to the previous equation, the equation for calculating the yield of solanidine, q_s (g solanidine per 100 g dried and milled potato sprouts) as a function of the hydrolytic extraction time is:

$$q_s = 0.72 + 4.75 \times 10^{-3} t$$

The rate of GA hydrolysis calculated as the moles of solanidine per dm^3 per second, in the optimal solid-liquid system (left Y-axis) and the rate of GA

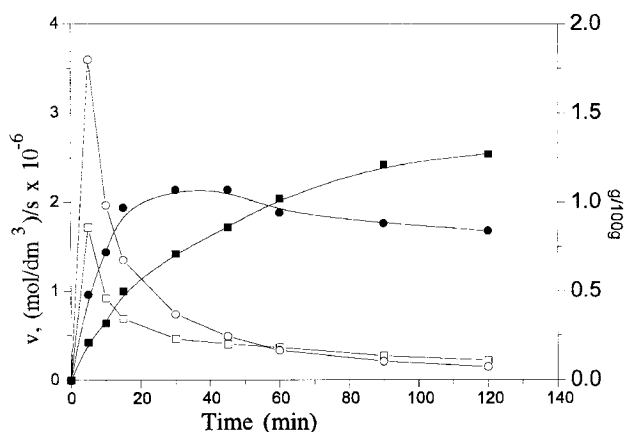


Figure 3. The rate of glycoalkaloid hydrolysis (left Y-axis): – in a solid-liquid system without chloroform in the liquid phase; O – in a solid-liquid system with chloroform in liquid phase. The yield of solanidine (right Y-axis): – in a solid-liquid system without chloroform; ● – in a solid-liquid system with chloroform in the liquid phase.

hydrolysis in the corresponding solid-liquid systems, but without mixing the ethanolic solution of HCl with chloroform for the liquid phase, versus the hydrolytic extraction time, are presented in Figure 3. The corresponding yield of solanidine (right Y-axis) versus the hydrolytic extraction time, are presented in same figure. The highest rate of GA hydrolysis is achieved after 5 minutes of hydrolysis and its value is 3.6×10^{-6} in the system without chloroform in the liquid phase, while the value of the rate of GA hydrolysis in the optimal solid-liquid system with chlorophorm in liquid phase is 1.7×10^{-6} mol solanidine per dm^3 per second.

The rate of glycoalkaloid hydrolysis in solid-liquid systems after 5 minutes of hydrolytic extraction is nearly two times lower than the rate of GA hydrolysis in the system without chloroform in the liquid phase. The presence of chloroform in the liquid phase makes the rate of glycoalkaloid hydrolysis lower, but protects the solanidine from further dehydration to solanthrene [13]. This was confirmed by TLC analysis: solanthrene was not detected in the liquid phase of the optimal solid-liquid system.

In the corresponding solid-liquid system without chloroform in the liquid phase, the aglycone solanidine may be lost by converting into the dehydration product, solanthrene. That is the reason why the yield of solanidine in this system decreases after 60 minutes of hydrolysis. The chloroform in the liquid phase of the optimal solid-liquid system, probably protects the solanidine from the dehydration reaction and the yield of solanidine constantly increases during hydrolysis. This conclusion is in agreement with literature data [13]. About 74% of the solanidine was isolated from the liquid phase. A solution of solanidine in 2% w/v aqueous acetic acid reacted positively with Dragendorff's and Mayer's reagent.

GC/MS analysis largely agrees with the literature data (Figure 4) [14]. There is a parent peak at 397 supporting the solanidine molecular formula and a molecular weight of 397.3 (Figure 4). Fragments at m/z 204 and m/z 150 are also diagnostic for fragment

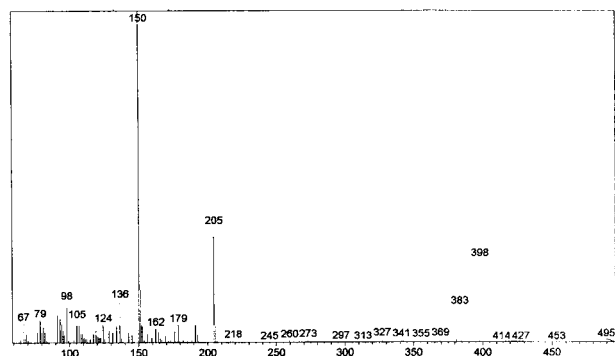


Figure 4. MS spectrum of the isolated solanidine

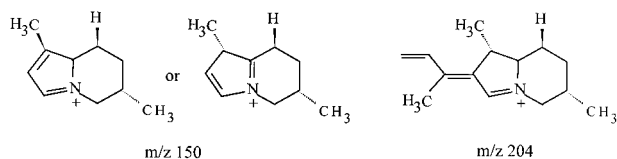


Figure 5. Fragments of solanidine

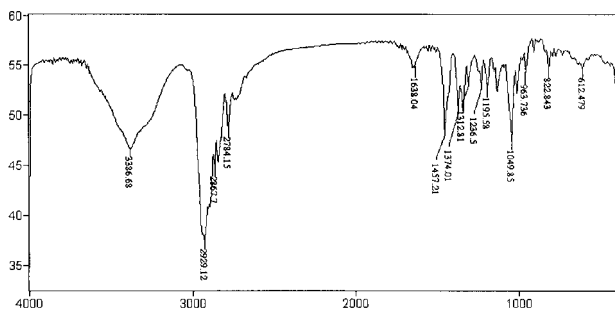


Figure 6. IR spectrum of the isolated solanidine

formulas of $C_{14}H_{22}N$ and $C_{10}H_{16}N$ respectively (Figure 5) [15]. The other fragments are at m/z : 67, 79, 98, 105, 124, 136, 162, 179, 383, 495.

The IR spectrum given, in Figure 6 shows the major functional groups at 3386 cm^{-1} of ν (OH), 2929 and 2867 cm^{-1} of ν (CH), 1638 cm^{-1} of ν (C=C), 1457 cm^{-1} of δ (CH), 1374 cm^{-1} of ν (CN) vibrations, bands at 1236 , 1164 and 1049 cm^{-1} of γ (C–O) and γ (C–C).

CONCLUSION

The best degree of glycoalkaloid hydrolysis (DH GA) of 83.0% from tuber sprouts in solid–liquid systems was achieved by 10% w/v hydrochloric acid in 96% vol. ethanol mixed with chloroform in the volume ratio of 1:1, after 120 minutes of hydrolysis. Chloroform in the liquid phase of the optimal solid–liquid system protects solanidine from dehydrating to solanthrene and the yield of solanidine constantly increase during hydrolysis. The yield of solanidine depends on the DH GA and the best yield of 1.29 g per 100 g dried and milled potato sprouts was achieved by using the same system when the best DH GA was also achieved. Then the content of solanidine in the total extractive matter was 16.18 mg per g of total extractive matter, which was also the best value. Solanidine was isolated from liquid phase of the optimal solid–liquid system in the yield of 71% compared to the calculated yield. GC/MS and IR analysis of the isolated solanidine largely agree with the literature data.

NOTATION

DH GA – degree of glycoalkaloid hydrolysis, %
 t – time of hydrolysis, min

Q_s – yield of solanidine, g/100 g of dried potato sprouts
 Q_S – yield of solanidine, g per 100 g of dried potato sprouts,
 Q_{em} – yield of total extractive matter, g/g of liquid phase
 Q_{Sem} – content of solanidine in the total extractive matter, mg/g of total extractive matter

ACKNOWLEDGMENT

This work was supported under the project 0029 by the Ministry of Science and Technology of the Republic of Serbia, Serbia and Montenegro.

We thank dr Goran Nikolić for his assistance with the IR spectra.

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IZVOD

ČVRSTO-TEČNI SISTEMI ZA HIDROLIZU GLIKOALKALOIDA IZ KLICA KROMPIRA (*Solanum tuberosum* L.) I IZOLOVANJE SOLANIDINA

(Naučni rad)

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Biljke familije *Solanaceae* među kojima je i krompir (*Solanum tuberosum* L.) sadrže glikoalkaloide koji predstavljaju sekundarne metabolite biljaka. U klicama krompira njihov sadržaj je znatno veći nego u ostalim delovima biljke (0,56–5,03%). U ovom radu ispitana je kinetika hidrolize glikoalkaloida klica krompira (*Solanum tuberosum* L.) u čvrsto-tečnim sistemima. Za ispitivanja su korišćene suve samlevene klice krompira kao čvrsta faza i smeša rastvora hlorovodonične kiseline u 96% vol. etanolu i hloroforma u zapreminskom odnosu 2:3, 1:1, 3:2 i 4:1, kao tečna faza sistema. Hidrolizom glikoalkaloida dobija se solanidin, steroidni aglikon glikoalkaloida. Solanidin je važan prekursor u sintezi hormona i drugih farmakološki aktivnih jedinjenja. Cilj istraživanja bio je da se izabere optimalni čvrsto-tečni sistem za hidrolizu glikoalkaloida i da se iz tečne faze optimalnog sistema izoluje solanidin. Najbolji stepen hidrolize glikoalkaloida (DH GA) od 83%, ostvaren je sa smešom rastvora 10% m/v hlorovodonične kiseline u 96% vol. etanolu i hloroforma u zapreminskom odnosu 1:1, za 120 minuta hidrolize. Teorijski prinos solanidina pod ovim uslovima je 1.29 g/100 g suvih samlevenih klica, a sadržaj solanidina u ukupnim ekstraktivnim materijama je 16.18 mg/100g suvih klica. TLC analiza je pokazala da u tečnoj fazi sistema nema solantrena kao dehidratacionog produkta solanidina, što ukazuje na zaključak da prisustvo hloroforma u tečnoj fazi sistema onemogućava ovu reakciju. Iz tečne faze sistema izdvojen je solanidin u prinosu od 71% u odnosu na sračunati prinos. GC/MS i IR analiza izolovanog solanidina pokazuje dobro slaganje sa literaturnim podacima.

Ključne reči: Krompir • Klice • Glikoalkaloide • Hidroliza • Solanidin • Izolovanje •
Key words: Potato sprouts • Glycoalkaloids • Hydrolysis • Solanidine • Isolation •