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Solanidine Hydrolytic Extraction and Separation from the Potato (*Solanum tuberosum* L.) Vines by Using Solid−Liquid−Liquid Systems

NADA C. NIKOLIC* AND MIHAJLO Z. STANKOVIC

Faculty of Technology, University of Nis, 16000 Leskovac, Bulevar oslobodjenja 124, Yugoslavia

Solanidine is a steroidal aglycon of potato (*Solanum tuberosum* L.) glycoalkaloids and a very important precursor for the synthesis of hormones and some pharmacologically active compounds. Glycoalkaloids are hydrolyzed by mineral acid, yielding solanidine. This paper deals with the kinetics of solanidine hydrolytic extraction in different solid−liquid−liquid systems. The dried and milled potato (*S. tuberosum* L.) vines were used as a source of glycoalkaloids and as the solid phase. The solutions of hydrochloric acid in 2 and 10% (w/v) aqueous acetic acid, in 50% (volume) aqueous methanol, and in 50% (volume) aqueous ethanol were first liquid phase, and the medium for glycoalkaloid extraction from potato vines and their hydrolysis to solanidine. The chloroform, trichloroethylene, or carbon tetrachloride were the second, organic, liquid phase and the medium for solanidine extraction. This procedure combines three different processes: extraction of glycoalkaloids from potato vines, their hydrolysis to solanidine, and the extraction of solanidine, in a single step. The term hydrolytic extraction of solanidine was used for these processes. The purpose of the paper was to choose an optimal solid−liquid−liquid system for solanidine extraction and to define the procedure for its isolation from the organic liquid phase. The best degree of solanidine hydrolytic extraction (DHE) of more than 98% was achieved when 10% (w/v) hydrochloric acid in 50% (volume) methanol were the first liquid phase and chloroform was the second liquid phase, after 90 min. The yield of solanidine (q_S) under these conditions is calculated to be 0.24 g/100 g of potato vines. Approximately 78% of the maximal possible yield of solanidine was isolated from chlorofom liquid phase. The IR and MS spectra of isolated solanidine were recorded.

KEYWORDS: Potato vines; glycoalkaloids; solanidine; solathrene; extraction; hydrolysis; isolation

INTRODUCTION

In the potato (*Solanum tuberosum* L.) vines, glycoalkaloids are present as secondary plant metabolites (*1*). They synthesize under the conditions of bud activity and stress, such as exposure to light and mechanical injury (*2*, *3*). Glycoalkaloids are normally present in dried potato vines in the range of 0.25−0.62% and in dried potato sprouts in the range of 0.56−5.03% (*4*). There are two major glycoalkaloids, α-solanine and α-chaconine, which represent more than 95% of the total glycoalkaloids (*5*, *6*). They provide the plant resistance to some fungi (*7*) and are also known as phytoalexins (*8*).

Glycoalkaloids are considered to be among the most toxic components in the human diet (*9*). They interfere with anesthesia by inhibiting two important enzymes: butyrylcholinesterase and acetylcholinesterase (*10*). Because of their human toxicity, the glycoalkaloid content is limited to 20 mg/100 g of fresh potato tuber (*11*), and glycoalkaloid content analysis is necessary. Since glycoalkaloids are toxic, and their aglycon solanidine is less toxic, the carbohydrate part appears to be paramount in influencing physiological and toxicological activities (*12*).

By hydrolysis of glycoalkaloids by mineral acid, the 3β-*O*-glycosidic bond between carbohydrate moiety and solanidine is cleft, yielding a carbohydrate moiety and solatriose from α-solanine and chacotriose from α-chaconine, as well as aglycon solanidine (*13*). According to BeMiller (*14*) and Capon (*15*), the acceptable mechanism of acid-catalyzed hydrolysis is the reaction in which fast protonation of the 3β-*O*-glycosidic oxygen atom yields carbonium ion. Carbonium ion is very reactive and yields the carbohydrate moiety and solanidine (**Figure 1**).

The conventional acid hydrolysis depends on the hydrolysis conditions such as temperature, the mineral acid concentration, the solvent, the hydrolytic time, etc. During the mild acid hydrolysis, the β- and γ-forms are denoted as products of α-solanine and α-chaconine incomplete hydrolysis. Very often, aglycon solanidine was lost when it was converted into solanthrene (*16*) (**Figure 2**). Solanidine is an important precursor for hormone synthesis. Chemical transformations of solanidine for obtaining 16-dehydropregnenolon acetate, the key intermediate in industrial synthesis of progesterone and cortisone derivates, are presented in the literature (*17*).

A starting point for this research was the hydrolysis technique using two phase systems for estimating the total amount of

* To whom correspondence should be addressed. Phone: +381-16-247-203. Fax: +381-16-242-859. E-mail: nikolic_n2002@yahoo.com.

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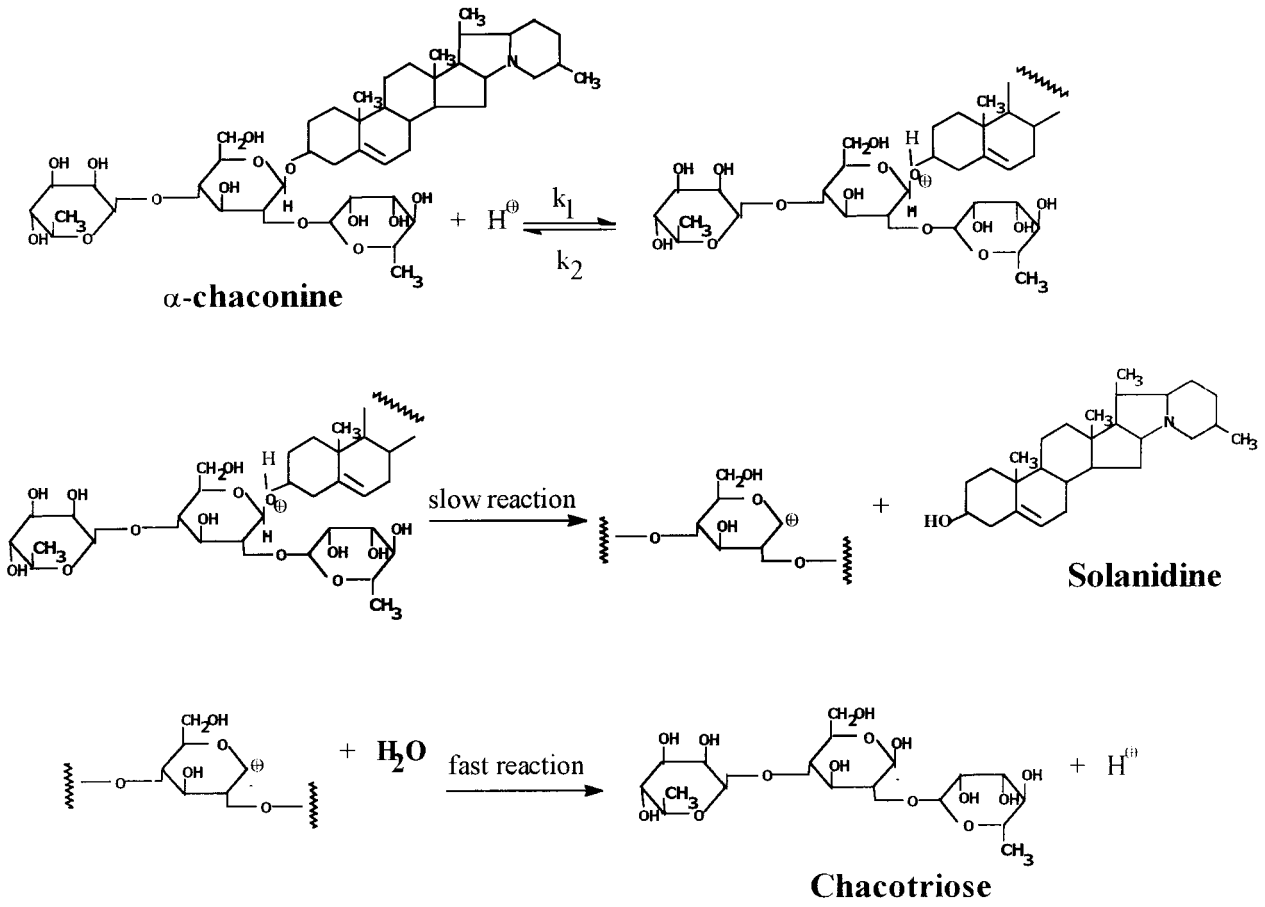

Figure 1. Solanidine obtained by mineral acid hydrolysis of α-chaconine.

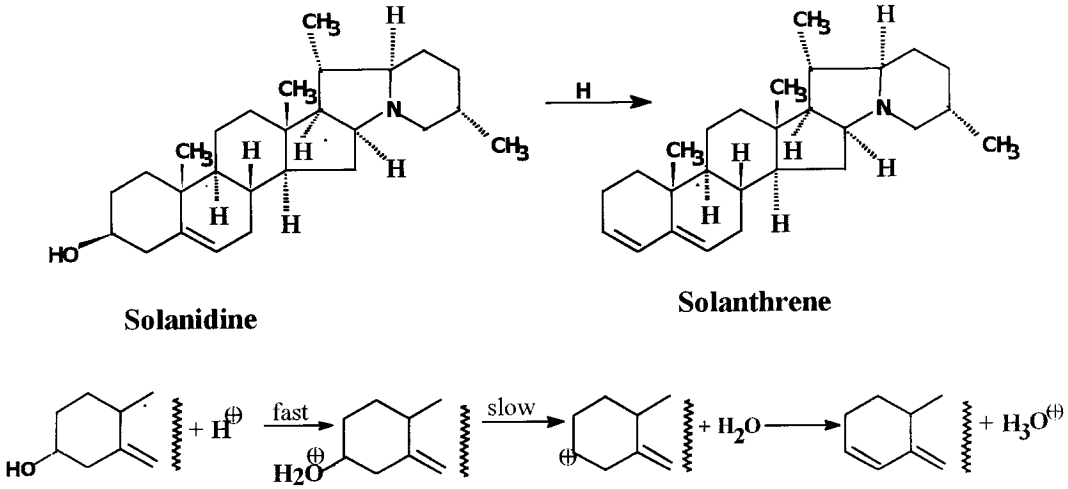

Figure 2. Reaction of solanidine dehydratation to solanthrene.

glycoalkaloids in *Solanum* spp. (*18*). The procedure exploits the difference in polarity between glycoalkaloids and their aglycones. The hydrolysis medium consists of aqueous acid and a nonpolar organic solvent, resulting in two immiscible phases. The polar glycoalkaloid is hydrolyzed in the acid phase, and the nonpolar aglycone that is formed is continuously withdrawn from the acid by the organic liquid phase in which it is protected from further disintegration. In the literature, the procedures for solanidine isolation from potato vines and sprouts, mainly by acid hydrolysis of GA with mineral acid solutions, for analytical purposes, are known (*11*, *19*, *20*). There are fewer data for solanidine isolation from fermented plant material after hydrolysis of GA by specific enzymes from plant material (*21*). All of these procedures needed several phases: extraction of GA from plant material, separation of extracts, GA hydrolysis in extracts, solanidine reextraction by the organic liquid phase, and then isolation. In the case of acid hydrolysis, the process of GA acid hydrolysis can be carried out during GA extraction from the plant material (process of hydrolytic extraction). In the case of enzymic hydrolysis, the solanidine has to be extracted from fermented plant material, reextracted with an organic solution, and then isolated.

The research in this paper concerns the kinetics of solanidine extraction from potato vines in different solid−liquid−liquid systems, with an aim of choosing an optimal system for the extraction and isolation of solanidine. In these systems, three

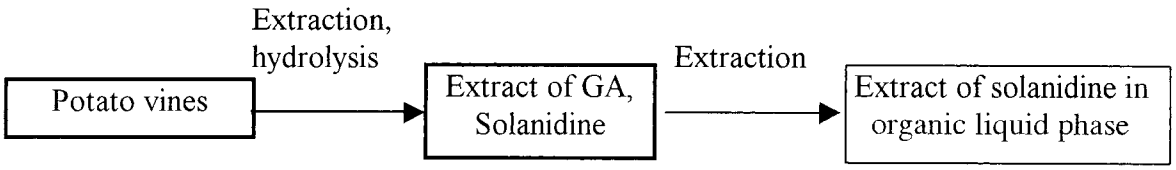

Figure 3. Model of solanidine hydrolytic extraction from the potato vines in solid–liquid–liquid systems.

different processes, extraction of glycoalkaloids from potato vines, their hydrolysis to solanidine, and extraction of solanidine, were combined into a single step. This can make the procedure for solanidine isolation simpler, faster, and more economic. The dried and milled potato vines were used as the solid phase; the solutions of hydrochloric acid in aqueous acetic acid and in an aqueous alcoholic solution were used as the first liquid phase, and chloroform, trichloroethylene, or carbon tetrachloride was used as the second, organic liquid phase. The model of these processes is presented in **Figure 3**. The term hydrolytic extraction of solanidine was used for these processes.

MATERIALS AND METHODS

Plant Material. Potatoes (*S. tuberosum* L. cv. Désirée) were grown during the year 2000 in a 1 ha plot on the Vlasina lake. The plot consisted of 150 rows, each of 300 plants. The soil analysis showed the presence of the following constituents: 1.98% humus, 0.10% nitrogen, 11.5% P_2O_5, and 8.0% K_2O (pH 6.70). Mineral fertilizer (NPK 8:16:24, 325 kg/ha) was applied in mid-June 2000. The plot was not irrigated. The potato vines (40 t/ha) were harvested in mid-July, dried at room temperature in trays for 21 days, and milled to an average particle size of 0.14 mm. The moisture content was ~10%; the content of glycoalkaloids was 0.53 g/100 g, and the ratio of α-solanine to α-chaconine was 1.27:1.

Kinetics of Solanidine Hydrolytic Extraction. Dried and milled potato vines (40 g) were treated with 5 and 10% (w/v) hydrochloric acid in 2% (w/v) aqueous acetic acid in a ratio of potato vines to solution of 1:20 (w/v), with 2% (w/v) hydrochloric acid in 10% (w/v) aqueous acetic acid in a ratio of 1:20 (w/v), with 10% (w/v) hydrocloric acid in 50% (volume) aqueous methanol in a ratio of 1:9 (w/v), and with 10% (w/v) hydrocloric acid in 50% (volume) aqueous ethanol in a ratio of 1:20 (w/v). Then the organic phase (chloroform, trichloroethylene, or carbon tetrachloride) in a volume ratio to the acid phase of 1:1 was added in three different flasks for each solution. The flasks were placed in the bath with boiling water and connected with a reflux condenser. The temperature of the lower layer (chloroform, trichloroethylene, and carbon tetrachloride) was approximately 65, 90, and 80 °C, respectively, and the temperature of the upper layer was approximately 70, 95, and 85 °C, respectively. Aliquots of 1 mL of organic liquid phase were taken at 10, 15, 30, 45, 60, 90, and 120 min intervals, from each flask, to determine the content of solanidine.

Content of Solanidine. The organic phase was evaporated until dry under vacuum. The dry residue was dissolved in 10 mL of 2% (w/v) aqueous acetic acid by using the water bath at boiling temperature and a reflux condenser. The pH of the cooled solutions was adjusted to 4.0 by adding aqueous sodium hydroxide [at first by 50 and then 1% (w/v)]. The solutions were transferred to a separatory funnel, and 2 mL of 0.05% (w/v) aqueous methyl orange for complex formation was added (*19*, *20*). The solanidine formed the yellow colored complex with the methyl orange which is soluble in chloroform. The complex was extracted with chloroform (five times by 5 mL). The chloroform layer was dried with anhydrous sodium sulfate and placed in a volumetric flask (25 mL). The flask was filled to the mark with chloroform, and the absorbance of the extract was read at 420 nm (UV−vis spectrophotometer, Lambda V, Perkin-Elmer). The content of solanidine was detemined on the standard curve.

Isolation of Solanidine. The dried and milled potato vines (200 g) were treated with 1800 mL of 10% (w/v) hydrochloric acid in 50% (w/v) methanol and with 1800 mL of chloroform, in the bath with boiling water by using the reflux condenser, over the course of 90 min. Then the system was allowed to cool, and the chloroform layer was separated from the systems, using the separatory funnel. The activated carbon was added (1 g) to the chloroform's extract and heated on a water bath at boiling temperature with a reflux condenser. Then the extract was hot filtered through a 2 cm bed of Celite and filter paper circles sorte 391 (Filtrak, Niedersclag 1, Brenstein, Germany), and the filtrate was evaporated until dry under vacuum. The dry residue was dissolved in a minimum amount of 96% ethanol by heat on a water bath at boiling temeperaure and by using the reflux condenser. The solanidine was precipitated by adding concentrated aqueous ammonia to a pH of 10. The precipitate was separated by centrifugation for 15 min at 1700*g*. The precipitate was washed twice with 30 mL of distilled water and centrifuged again.

TLC Analysis. A 0.03 mL aliquot of 10% hydrochloric acid in 50% (volume) methanol extract and corresponding chloroform phase, obtained after 10, 15, 30, 45, 60, 90, and 120 min, were applied to the 20 cm × 20 cm plates, 120 μm thick Silica gel G 60 (Merck reagents). The plates were developed to the height of 16 cm, with the lower layer of the mixture being methanol, chloroform, and 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 sulfuric acid and heated at 110 °C for 30 min (*22*).

IR Spectrophotometry. The IR spectra of isolated solanidine were recorded at room temperature on a Bomem MB-100 (Hartmann & Brunn) Michelson FTIR spectrometer, by using the KBr technique.

MS Spectrophotometry. A Varian 3700 gas chromatograph−mass spectrometer (MAT 311 A) was used to obtain the electron impact mass spectrum. A 30 m × 0.30 mm capillary column was packed with OV-101. The column temperature was programmed from 150 to 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

Kinetics of Solanidine Hydrolytic Extraction. The variations of the degree of solanidine hydrolytic extraction (DHE) during the hydrolytic extraction time by 10% (w/v) hydrochloric acid in 50% (volume) methanol as the first liquid phase and chloroform, trichloroethylene, or carbon tetrachloride as the other liquid phase are shown in **Figure 4**. DHE was expressed as the ratio of solanidine content in the organic liquid phase after a certain amount of hydrolytic extraction time to the maximal yield of solanidine which should be achieved from the used plant material. The maximal yield of solanidine which should be achieved from potato vines was calculated according to the glycoalkaloid content and the ratio of α-solanine and α-chaconine in potato vines, considering that 1 mol of α-solanine or α-chaconine yields 1 mol of solanidine.

The maximal DHE, the hydrolytic extraction time, and the yield of solanidine (q_S) achieved with different systems are given in **Table 1**. The maximal DHE of 98.6% was realized with 10% (w/v) hydrochloric acid in 50% (volume) methanol as the first liquid phase and chloroform as the other liquid phase after 90 min. The lowest value of DHE was achieved with carbon tetrachloride as the organic phase, independent of the first liquid phase.

The results of Wim van Gelder (*18*) in a two-phase system with chloroform instead of carbon tetrachloride showed that solanidine quantitatively remained in the protective chloroform phase, resulting in a much less extensive dehydratation reaction

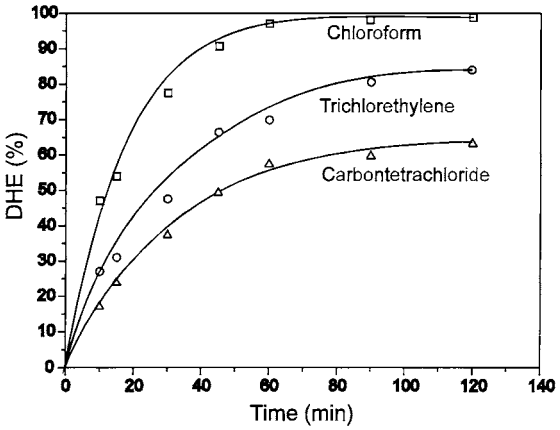

Figure 4. Variation of the degree of solanidine hydrolytic extraction (DHE) from the potato vines during solanidine hydrolytic extraction at water bath boilling temperature with 10% (w/v) hydrochloric acid in 50% (volume) methanol as the first and chloroform, trichloroethylene, or carbon tetrachloride as the other liquid phase, in a 1:1 volume ratio.

Table 1. Maximal Achieved Degree of Solanidine Hydrolytic Extraction from Potato Vines[a]

	second liquid phase		
first liquid phase	chloroform	trichloro-ethylene	carbon tetrachloride
5% (w/v) HCl in 2% (w/v) acetic acid	58.5 120 0.14	32.2 120 0.08	30.0 120 0.07
10% (w/v) HCl in 2% (w/v) acetic acid	42.5 120 0.10	28.5 120 0.07	18.2 120 0.04
2% (w/v) HCl in 10% (w/v) acetic acid	73.5 120 0.18	33.5 90 0.08	24.5 90 0.06
10% (w/v) HCl in 50% (volume) methanol	98.6 90 0.24	83.8 120 0.20	63.5 120 0.15
10% (w/v) HCl in 96% (volume) ethanol	69.5 120 0.17	40.0 60 0.10	36.3 60 0.09

[a] The first row of values for each condition contains DHE values in percent, the second row for each condition solanidine hydrolytic extraction times in minutes, and the third row for each condition the calculated yields of solanidine in grams per 100 g of dried potato vines. The values were determined by using different solid–liquid–liquid systems.

to solanthrene. In our system, if the reaction of solanidine dehydratation to solanthrene occurs, the content of solanthrene is technologically negligible.

The yield of solanidine depends on DHE, and the best yield is achieved by using the same solid−liquid−liquid systems when the best DHE is achieved. The yield of solanidine was calculated according to DHE and the maximal posible yield of solanidine which can be obtained from the used potato vines. The equations for calculating DHE expressed in percent and q_S expressed in grams of solanidine per 100 g of dried and milled potato vines, by using 10% (w/v) hydrochloric acid in 50% (volume) methnol and chloroform as liquid phases, depending on the hydrolytic extraction time in the Maple V Release program, were obtained:

$$\mathrm{DHE} = 98(1 - \mathrm{e}^{-0.065t})$$

$$q_s = 0.24(1 - \mathrm{e}^{-0.065t})$$

where t is the hydrolytic extraction time of solanidine expressed in minutes.

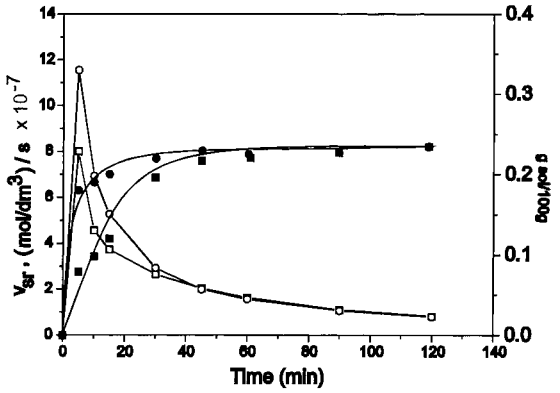

Figure 5. Rate of solanidine hydrolytic extraction (left *Y*-axis) from the potato vines in a solid–liquid–liquid system by 10% (w/v) hydrochloric acid in 50% (volume) methanol as the first and chloroform as the other liquid phase (□) and in a solid–liquid system with 10% (w/v) hydrochloric acid in 50% (volume) methanol (○), vs solanidine extraction time. The yield of solanidine (right *Y*-axis) in the solid–liquid–liquid system (■) and in the solid–liquid system (●), vs solanidine extraction time.

The process of GA extraction from potato vines, its hydrolysis to solanidine, and the extraction of solanidine in the chlorophorm phase are sequenced one after another. According to Ponomarjev (*23*), there are two periods of hydrolytic extraction: a fast period and a slow period. In the fast period of extraction, over the course of 55 min, ∼97% of GA from potato vines is extracted, hydrolyzed to solanidine, and extracted in the chlorophorm phase, and in the slow period of extraction, only ∼1.6% is processed.

In **Figure 5**, the variation of the rate of solanidine hydrolytic extraction calculated as the moles of solanidine per square decimole per second, in solid−liquid−liquid systems, and the rate of solanidine hydrolytic extraction in solid−liquid systems, on the left *Y*-axis, versus hydrolytic extraction time are presented. For the solid−liquid system, the potato vines were used as the solid and 10% hydrochloric acid solutions in 50% (volume) aqueous methanol as the liquid phase. The variation of the yield of solanidine with the corresponding three- or two-phase system on the right *Y*-axis, versus hydrolytic extraction time, is also presented in **Figure 5**. The maximal rate of solanidine hydrolytic extraction is achieved after hydrolytic extraction for 5 min. Its value is 8.02×10^{-7} mol of solanidine $\mathrm{dm}^{-3}\ \mathrm{s}^{-1}$ in three phase system and 11.54×10^{-7} mol of solanidine $\mathrm{dm}^{-3}\ \mathrm{s}^{-1}$ in the two-phase system. By comparing the results, we can conclude that the maximal rate of solanidine hydrolytic extraction in solid−liquid systems (3.52×10^{-7}) is greater than the maximal rate of solanidine hydrolytic extraction in solid−liquid−liquid systems. It is because the rate in the solid−liquid system involved only reactions of GA extraction and their hydrolysis to solanidine, while the rate in the solid−liquid−liquid system beside these two reactions also involved the reaction of solanidine extraction by the chloroform phase. During the hydrolytic extraction period from 5 to 55 min, the diference in the values of the rate of solanidine hydrolytic extraction decreased. The duration of the period of hydrolytic extraction time where the value of the rate of solanidine hydrolytic extraction in the solid−liquid system is larger than the rate in the solid−liquid−liquid system is concurred by a fast period of extraction. After this period, the period of slow extraction comes and molecular diffusion controls the process of hydrolytic extraction (*23*). After hydrolytic extraction for 55 min, the rates of solanidine hydrolytic extraction in these systems become equal. During this period, the whole amount of solanidine obtained by extraction of glycoalkaloids from

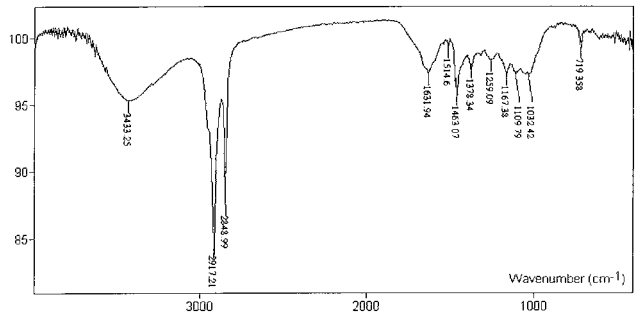

Figure 6. IR spectrum of isolated solanidine.

potato vines and by their hydrolysis is extracted by chloroform in practically the same time.

Also, the calculated yield of solanidine is greater in the solid−liquid system during the first 55 min of hydrolytic extraction. It means that solanidine is obtained in the acid phase, but it is not extracted by chloroform. After hydrolytic extraction for 55 min, the yields of solanidine in the solid−liquid system and in the solid−liquid−liquid system became equal. The maximal value of solanidine yield is 0.21 g of solanidine/100 g of dried and milled potato vines.

Isolation of Solanidine. Solanidine ($S_{27}H_{43}ON$) was also known as solanidine t and solatubine. Solanidine t had been used to differentiate the solanidine of *S. tuberosum* L. from aglycone of the alkaloid of *Solanum sodemeum* L., which is now known as solasodine. Solanidine has one basic tertiary nitrogen and one secondary hydroxyl as well as a double bond. It is a white crystalline powder or looks like long needles. Its dissociation constant pK_B is 5.38; $[\alpha]^{20}_D = -28.5$ (*c* 0.5 in chloroform), and it melts at 219 °C. The solanidine $R_f = 0.97$ in the chloroform/methanol/1% (w/w) ammonia mixture, lower layer, after staying overnight, as the mobile phase (*22*).

Approximately 78% of the possible solanidine yield was isolated from the chloroform phase. The solution of solanidine in chloroform had an R_f of 0.98 in the mobile phase as before and reacted positively with Dragendorff's and Mayer's reagent. The melting point was determined on Tile's apparatus and was found to be 220.5 °C.

GC−MS analysis largely agrees with the literature data (*24*). There is a parent peak at *m*/*z* 397 supporting the solanidine molecular formulas and molecular mass of 397.3345. Fragments at *m*/*z* 204 and 150 are also diagnostic of fragment formulas $C_{14}H_{22}N$ and $C_{10}H_{16}N$, respectively. The other fragments are as follows: *m*/*z* (relative abundance) 45 (80%), 55 (65%), 67 (42%), 79 (43%), 98 (40%), 110 (28%), 136 (15%), 178 (44%), 195 (37%) 217 (3%), 259 (8%), 272 (7%), 326 (8%), 341 (19%), 354 (17%), 368 (17%), and 382 (69%).

IR spectrum given in **Figure 6** shows the major functional groups: IR (ν) 3435 (OH), 2917 and 2849 (CH), 1632 (C═C), 1463 (δ, CH), and 1378 cm^{-1} (CN) vibrations, bands at 1261, 1164, and 1032 cm^{-1} of γ (C−O) and γ (C−C), and a band at 717 cm^{-1} (probably a CH bending vibration).

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