Short Communication

Solanidine isolation from Solanum tuberosum by centrifugal partition chromatography

The aim of this investigation was the preparative isolation of solanidine (aglycone of the two main potato glycoalkaloids: α-chaconine and α-solanine) from fresh Solanum tuberosum (cv. Pompadour) material by implementing a new preparation scheme using centrifugal partition chromatography (CPC). A setup for obtaining solanidine by hydrolysis of the glycoalkaloids found in the skin and sprouts of S. tuberosum was first developed. Then its isolation was carried out by the development of CPC conditions: the solvent system used for separation was ethyl acetate/butanol/water in the ratio 42.5:7.5:50 v/v/v, 0.6 g of crude extract were separated with a 8 mL/min flow rate of mobile phase while rotating at 2500 rpm. A run yielded 98 mg of solanidine (86.7 % recovery from the crude extract) in a one-step separation. The purity of the isolated solanidine was over 98%. Thus, CPC has proven to be the method of choice to get solanidine of very high purity from S. tuberosum biomass in large quantities.

Keywords: α-Chaconine / Glycoalkaloids / α-Solanine / Solanidine / Solanum tuberosum sprouts

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1 Introduction

The potato plant family (Solanaceae) is a very valuable crop providing high-quality nutrition [1]. In commercial potatoes (Solanum tuberosum), two major steroidal glycoalkaloids (GAs) are found: α-chaconine and α-solanine, both trisaccha-rides of the common aglycon solanidine. GAs are usually distributed in all plant organs with a main localization in sprouts, flowers, and skin [2] and are involved in plant resistance to pests and predators [3]. These secondary metabolites have been shown to be toxic to a wide range of organisms from fungi to humans [4, 5]. GAs present major toxic properties that are due to (i) the ability of GAs to bind with membrane 3β-hydroxy sterols and to disrupt membrane function and (ii) the ability to inhibit acetylcholinesterase [5–7].

GAs and aglycons may also have beneficial effects, antibi-otic activities against pathogenic bacteria, viruses, and fungi have been described [5] as well as their ability to inhibit the growth of human colon (HT29) and liver (HepG2) cancer cells [8]. These studies demonstrated that biological activity was influenced by the structure of the aglycon and by the nature and the number of carbohydrate groups making up the side chain attached to the aglycon. In this context, the solani-dine skeleton has been regarded as a basis for the design of new potential anticancer agents.

Alongside these numerous biological assessments, many analytical methods of GAs and their aglycons were developed and published. They generally include three stages: extraction, purification, and analysis. The main methods used to detect, quantify and isolate GAs are chromatographic tech-niques such as GC, HPLC, or LC–ESI-MS [9–12]. However, because of their complex chemical structures (hydrophobic 27-carbon skeleton of steroidal alkamine attached to a hydrophilic trisaccharide) serious technical difficulties are associated to GAs quantitative analysis and isolation. Problems can also arise because of their weak absorption and low sensi-tivity in the short UV wavelengths (200–215 nm) due to their lack of strong chromophores. In addition, HPLC analysis of GAs requires buffers to set a suitable pH to observe efficient detection and separation. This strong constraint limits the use of HPLC for the development of easy preparative procedures since a final extraction step is necessary to remove salts and to recover isolated products. Therefore, only few setups for GAs preparative isolation are described in the literature [13, 14].

Centrifugal partition chromatography (CPC) has many advantages over HPLC techniques including (i) no nonspe-cific adsorption to a solid support, (ii) higher selectivity, (iii) higher sample loading capacity, (iv) less solvent used, and (v) shorter separation time. In addition, with this technique a scaling can easily be considered, which is a key point to be able to isolate large quantities of natural products. However, as a counterpoint to all these advantages, this technique requires a preliminary and sometimes tedious determination of solvent...
mixture that will serve as stationary and mobile phases. This determination of a suitable two-phase system is monitored by a parameter called “distribution constant” \( K_D \) calculated according to the ratio: concentration of a compound in its single definite form in the stationary phase/concentration of the compound in the same form in the mobile phase [15, 16]. To ensure a proper separation, the compound of interest should be almost equally distributed between the two phases \( 0.5 < K_D < 2 \) [17].

In the present paper, we describe an efficient method for the isolation and purification of solanidine, the common aglycon of \( \alpha \)-chaconine and \( \alpha \)-solanine, from \textit{S. tuberosum} (cv. Pompadour). Our work forms part of a very broad purpose to recover byproducts from the potato processing industry. A focal point of this large project is to modify the saccharide part of GAs to induce modulation of their stability and biological activity on different target organisms since both the nature and order of attachment of the carbohydrate residues appear to influence biological activity. To achieve this objective, large amounts of solanidine of high purity are needed. Moreover the setup developed to isolate and produce solanidine should be as simple as possible in order to consider an easy scaleup.

Starting from some of the literature results, our aim was to develop a setup that would reduce the overall number of steps from raw plant material up to the obtention of pure solanidine. This objective has been applied to skin and sprouts of \textit{S. tuberosum} cv. Pompadour to determine the best biomass for the recovery of the largest quantities of solanidine. Finally a setup of isolation and purification of solanidine by CPC was developed which is to our knowledge the first described in the literature.

2 Materials and methods

2.1 Apparatus

The CPC instrument used in this study is a SPOT CPC 100 Light (Armen Instrument) fitted with a rotor of ten circular partition disks (1000 partition cells: 0.1 mL per cell; total column capacity of 100 mL). Rotation speed can be chosen from 0 to 4000 rpm. The effluent was monitored by a Lash 06 DAD detector (ECOM, Prague) equipped with a preparative flow cell operating at 202 nm and 210 nm and collected by a LS 5600 (Armen) fraction collector.

The HPLC used was a Shimadzu HPLC System including a LC-20AT pump and a SPD-M20A diode-array detector. LC–MS spectra were performed on a Waters 2695 Alliance coupled with a quadrupole mass spectrometer ZQ (Waters-Micromass, Manchester, UK) equipped with an electrospray ion source (ESI-MS). The capillary voltage was \( \pm 3.5 \) kV and a cone voltage range from \( \pm 20 \) to \( \pm 60 \) V was used. Data acquisition and processing were performed with MassLynx V4.0 software.

NMR spectra were recorded at 300 K on a Bruker (Wissembourg, France) Avance 300 spectrometer, operating at 300.13 MHz for \(^1\)H spectra and 75.47 MHz for \(^13\)C spectra. Pyridine was used as the solvent, and tetramethylsilane was used as the internal standard.

2.2 Materials and reagents

\( \alpha \)-Chaconine and \( \alpha \)-solanine used as reference standards were purchased from Extrasynthese (France). Solanidine was prepared from pure \( \alpha \)-chaconine and identified by comparison of reported NMR data [18]. All organic solvents were analytical grade and purchased from Prolabo (France).

2.3 Preparation of crude extract

Unless specified in the text below, the same setup was applied to skin and sprouts from \textit{S. tuberosum} cv. Pompadour. A total of 50 g of fresh potato material was ground and treated with 500 mL of \( \text{CH}_3\text{OH} / \text{H}_2\text{O} (50:50 \text{v/v}) \). Hydrochloric acid was added to a 3% w/v concentration. The mixture was heated under stirring for 2 h at 80°C and after cooling centrifuged at 5000 rpm for 30 min. The liquid phase was then extracted with \( \text{CHCl}_3 \). The organic phases were concentrated and the residue was dissolved in \( \text{CH}_3\text{OH} \).

For skin samples, the precipitate formed was filtered. Then the same volume of \( \text{NH}_4\text{OH} \) (commercial solution at 30%) was added under cooling and this solution was left overnight at \(-10^\circ\text{C}\). The precipitate (hereafter “crude extract”) was separated by centrifugation at 5000 rpm for 30 min. The crude extract was redissolved in \( \text{CH}_3\text{OH} \) and stored at \( 4^\circ\text{C}\) for subsequent analysis and separation. The operation was repeated three times for either fresh skin and sprouts.

2.4 HPLC and LC-MS analyses

HPLC analyses of the crude extract and of the CPC peak fractions were conducted at 202 nm on a 250 × 4.6 mm, 5 \( \mu \)m, Prevail reverse-phase C18 column (Grace) using a linear binary gradient of \( \text{H}_2\text{O} / \text{H}_3\text{KPO}_4 \) 0.1 M (solvent A) and \( \text{CH}_3\text{CN} \) (solvent B) with a flow rate of 1 mL/min as follows: 20–40% B (0–15 min), 40–80% B (15–30 min), 80% B (30–35 min), 80–20% B (35–40 min). A total of 20 \( \mu \)L were used for injection that was repeated three times. LC-MS analyses of the samples were conducted at 202 nm on a 250 × 4.6 mm, 5 \( \mu \)m, Prevail reverse-phase C18 column (Grace) using a linear binary gradient of \( \text{H}_2\text{O} \) (solvent A) and \( \text{CH}_3\text{CN} \) (solvent B) both containing 0.1% v/v formic acid, with a flow rate of 1 mL/min as follows: 20–40% B (0–15 min), 40–80% B (15–30 min), 80% B (30–45 min), 80–20% B (35–40 min). The calibration curve was prepared using six different concentrations of solanidine in \( \text{CH}_3\text{OH} \). 20 \( \mu \)L of solution ranging from 1 to 0.03125 mg/mL (twofold serial dilutions) were injected in triplicate in the column. Calibration graph was plotted based on linear regression analysis of the peak area versus concentration, the curve showed good linearity \( (r^2 = 0.989) \).
2.5 Selection of the two-phase solvent system

The solvent system was selected according to the distribution constant $K_D$ of solanidine. The $K_D$ value was determined by HPLC analysis. Solanidine was dissolved in the tested solvent system and vortexed for 30 s. After separation and evaporation under reduced pressure, the residue of each layer was dissolved in 500 μL of methanol for HPLC analysis. The $K_D$ values were calculated according to the ratio: concentration of solanidine in the stationary phase/concentration of solanidine in the mobile phase [16].

2.6 CPC separation

The solvent system used for separation was ethyl acetate/butanol/water in the ratio 42.5:7.5:50 v/v/v. Biphasic system was prepared just before use by thoroughly mixing volumes of solvent in the above ratio. After the equilibration was established, the stationary phase (lower phase in the ascending mode) was pumped into the column at a flow rate of 30 mL/min while the apparatus was run at 500 rpm according to the equilibration mode of the apparatus. After injection of the sample (0.6 g of crude extract in 10 mL of the stationary phase), the mobile phase was perfused at 2500 rpm at a flow rate of 8 mL/min under 26–28 b during the runs. The eluent was monitored at 202 nm and fractions of 20 mL were collected and analyzed by HPLC. Fractions collected before the fraction number 4 contained no compound of interest.

3 Results and discussion

In literature, the few procedures reported for the obtention of solanidine include a preliminary extraction of GAs from plant material and are of two main types: those which consist in sequential steps of hydrolysis of GAs followed by solanidine extraction by organic liquid phase and those that consist in a “one-pot” process where concomitant hydrolysis and extraction of solanidine occur in a two-phase system [19–23]. Concerning plant material itself, high GA levels are found in potato tuber sprouts and skin (2–3% of tuber) [2]. To determine the most convenient source of solanidine, as a valuable starting material for chemical synthesis of modified GAs, these two potato plant parts from S. tuberosum cv. Pompadour were extracted on the basis of the conditions described by Nikolic et al. [23]. However, to anticipate a future implementation at an industrial scale, we modified the single step extraction process that involves the use of an organic solvent heated to reflux.

Moreover, since it avoids the time and energy-consuming drying steps, fresh plant material was used as the solid phase and was subjected to acid hydrolysis. Some samples were dried only to evaluate the yield and to express the result in “g/dry weight (DW)”. Acid hydrolysis usually requires to set up some hydrolysis conditions such as temperature, acid concentration and time. With our plant material, the challenge was to establish industrially transposable operating conditions that could result in total GAs hydrolysis while preventing the dehydration side reaction of solanidine to solanthrene, which are conflicting objectives from a chemical point of view. In literature, the few studies concerning the isolation of GA aglycones showed that the development of optimal conditions, taking into account these two constraints, is very difficult and require many experiences [20–22].

Therefore, in order to quickly implement an effective procedure, the conditions of Nikolic et al. [23] i.e. 2% HCl, 50% MeOH and 2 h of reaction were chosen in a first experiment. Under these conditions, the analysis of the reaction mixture by HPLC showed the presence of four main peaks with retention times ($R_1$) values of 10.0, 16.8, 24.1, and 26.7 min (Fig. 1A). These peaks were assigned on the basis of their MS spectra (Fig. 1B) and with the knowledge of the different steps of the chemical process of hydrolysis (Fig. 1C). First, as expected in these HPLC conditions, the more polar products, i.e. the diglycosylated, $\beta_1/\beta_2$-chacine and $\beta_1/\beta_2$-solanine are less retained on the column and exhibit the smallest retention times. Then, the retention times increase with the reduction of the polarity of the products and the more apolar compound, which is also an undesired secondary compound, i.e. solanthrene, shows the highest $R_1$. These semi-empirical results were confirmed according to the MS spectra of all the compounds formed during the hydrolysis reaction (Fig. 1B). On the basis of the values of the MS [M + H]$^+$ molecular ion peaks, we definitively assigned the structures of the main hydrolysis products (Fig. 1A).

To complete the hydrolysis reaction, both the amount of acid and reaction time while remaining within a range of values described by Nikolic et al. [23] were increased. The results are shown on Fig. 2: the peak corresponding to the diglycosylated product has disappeared, there is still the monoglycoside and the relative quantity of solanidine has increased. The amount of solanidine obtained in these conditions was then quantified by analytical HPLC and was 29.04 mg/50 g fresh weight (FW) for skin and 41.13 mg/50 g FW for sprouts (Table 1). In a previous study [24], we showed that the average content of GAs present in sprouts from S. tuberosum cv Pompadour was about 108 mg/50 g FW which corresponds to 45 mg/50 g FW of solanidine considering the molecular weight difference. Based on these data showing that these conditions allow the hydrolysis of all the GAs, we decided to work with sprouts and to use the hydrolysis conditions mentioned above, i.e. 50% methanol, 3% HCl and 3 h of reaction.

A first assay to isolate solanidine by preparative HPLC was performed but the results were not satisfactory: a very low amount of solanidine was obtained after two sequential steps of HPLC separation and subsequent extraction with chloroform to remove salts that could interfere with the further chemical transformations planned to obtain new derivatives of solanidine. Therefore, we decided to implement a separation by CPC that allows separating natural products according to their partition behaviors between immiscible solvents used as a mobile and stationary phases. The suitability of a given solvent system is empirical and generally estimated using its
Figure 1. (A) Compounds formed during the acid hydrolysis of potato GAs from Solanum tuberosum sprouts (cv. Pompadour). (B) Chromatogram of the crude extract of S. tuberosum sprouts by analytical HPLC in the preliminary hydrolysis conditions: 2% HCl, 50% MeOH and 2 h of reaction. (C) Major fragment ions (m/z) of the compounds formed during acid hydrolysis observed in MS spectra.

key parameter—the distribution constant (K_D)—of the target compound(s) between the two phases. There are a variety of appropriate solvent systems available; a mixture of hexane, ethyl acetate, methanol, and water in different ratios is often used as a starting point for the separation of natural products [17]. Variations of this solvent system include the replacement of methanol with butanol. In the present study, two solvent systems were selected. The K_D values from this solvent system are listed in Table 2. The mixture of ethyl acetate/butanol/water (42.5:7.5:50 v/v/v) was chosen as solvent system as it led to a K_D of 0.7, which is a value in the range of 0.5–2 generally accepted as necessary to ensure a successful separation by CPC [17].

Under these conditions of CPC purification, we never observed usual chromatographic peaks but chromatograms similar to those obtained during the implementation of pH-zone-refining CPC [25]. This might be explained by the column loading, which is an important factor. In our separation conditions, we introduced 600 mg of sample on a column of 100 mL, i.e. a loading value of 6 mg/mL, which is high compared to the average loading of 2.2 mg/mL observed in the literature and could contribute to produce peak broadening and overlapping [26]. However, the unusual shape of the chromatogram is not linked to the quality of the separation since this rather unusual form of peaks together with a successful separation of GAs was already observed [24]. Each fraction collected during the separation process was analyzed by HPLC [Fig. 3 (F5) and (F7)]. It is noteworthy that the elution order is reversed between the two techniques, HPLC and CPC. The less polar compound, i.e. solantherene, is initially eluted and the increasing polarity causes a higher affinity to the stationary phase for solanidine leading to a longer retention time. For each fraction, analysis of HPLC calibration curves allowed to determine the amount of solanidine as well as its purity. After evaporation, the amount of isolated solanidine from the purest fractions, F6 and F7, was 98 mg. The solanidine obtained as a yellowish solid was of high purity (> 98%) as determined by HPLC analysis using the calibration graph. F5, F8, and F9 fractions were collected and stored for further purification. The efficiency of the procedure can be calculated from the analytical quantification of the solanidine given in Table 1: for 600 mg of crude extract 113 mg of solanidine
Figure 2. HPLC chromatogram of the residue obtained from *Solanum tuberosum* (cv. Pompadour) sprouts after 3 h of reaction with 3% HCl, 50% MeOH and called “crude extract.”

Table 1. Results obtained by extraction of 50 g (FW) of skin and sprouts from *Solanum tuberosum* (cv. Pompadour) (*n* = 3)

<table>
<thead>
<tr>
<th></th>
<th>Skin</th>
<th>Sprouts</th>
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<tr>
<td>Dry weight (DW) (g)</td>
<td>8.725 ± 0.175</td>
<td>8.94 ± 0.184</td>
</tr>
<tr>
<td>Solid residuea) (mg)</td>
<td>52.16</td>
<td>—</td>
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<tr>
<td>Crude extractb) (mg)</td>
<td>44.01 ± 5.07</td>
<td>218.10 ± 26.22</td>
</tr>
<tr>
<td>Solanidine contentc) (mg)</td>
<td>29.04 ± 0.33</td>
<td>41.13 ± 0.82</td>
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a) Obtained after CH$_3$OH addition.
b) Obtained after NH$_4$OH addition.
c) Determined from the crude extract by analytical HPLC (3 injections).

Studies to isolate GAs or GAs aglycones from potato plant material are often unique studies and it is therefore difficult to have points of comparison, however our results are quite satisfactory within the context of isolation of natural products from plant biomass.

4 Conclusion

In the present study, CPC was successfully implemented for the isolation of solanidine whereas its isolation was almost unfeasible by semipreparative HPLC. Despite a somewhat atypical chromatogram, we obtained the alkaloid of interest from *S. tuberosum* (cv. Pompadour) with a very good yield and high purity (higher than 98%). These results demonstrate the high effectiveness of this methodology for providing significant amounts of this bioactive steroidal alkaloid for further chemical modulations and biological studies. The conditions developed in this study allow the isolation of large amounts of complex compounds within a few hours, without any preliminary cleanup or concentration steps and avoids the poor UV detection of alkaloids. Moreover, a large-scale CPC production of solanidine, which is a major bottleneck to
achieve our research objectives can be developed from our findings.

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5 References

