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Analysis of tomato glycoalkaloids by liquid chromatography coupled with electrospray ionization tandem mass spectrometry

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Steroidal glycoalkaloids (SGAs) extracted from tomato leaves and berries (*Lycopersicon esculentum* Mill.) were separated and identified using optimized reversed-phase liquid chromatography with electrospray ionization (ESI) and ion trap mass spectrometry (ITMS). The ESI source polarity and chromatographic conditions were evaluated. The ESI spectra contain valuable information, which includes the mass of SGAs, the mass of the aglycones, and several characteristic fragment ions. Cleavage at the interglycosidic bonds proximal to the aglycones is the most prominent process in the ESI process. A protonated molecule, $[M+H]^+$, accompanied by a mixed adduct ion, $[M+H+Na]^{2+}$, was observed for α-tomatine (i.e., *m/z* 1034.7 and 528.9) and dehydrotomatine (i.e., *m/z* 1032.6 and 527.9) in positive ion mode spectra. The structures of these tomato glycoalkaloids were confirmed using tandem mass spectrometry. The identification of a new α-tomatine isomer glycoalkaloid, named filotomatine (MW 1033), which shares a common tetrasaccharide structure (i.e., lycotretraose) with α-tomatine and dehydrotomatine, and soladulcidine as an aglycone, is described for the first time. It occurs in significant amounts in the extracts of wild tomato foliage. Multistage mass spectrometry both of the protonated molecules and of the doubly charged ions was used for detailed structural elucidation of SGAs. Key fragmentations and regularities in fragmentation pathways are described and the fragmentation mechanisms involved are proposed.

Many *Solanaceae* plants, including potatoes, tomatoes, peppers and eggplants, produce toxic metabolites known as steroidal glycoalkaloids (SGAs), which are nitrogen-containing compounds bearing a sugar chain (three or four units) linked to a steroidal moiety (aglycone) by the 3-hydroxyl group.[1–3] The major components of a comprehensive family of SGAs are α-solanine and α-chaconine in potato plants (*Solanum tuberosum*), while α-tomatine and dehydrotomatine are spirosolane-type SGAs occurring in tomato plants (*L. esculentum*).[4–6] Toxic levels of these compounds in foliage and unripe fruit can prevent plant disease due to insects and other animals. It is very useful to obtain input on the various structural changes of SGAs as their content is regulated by several biotic and abiotic stimuli.[7] A complete survey of glycoalkaloids in the worldwide cultivated *L. esculentum* plants still, however, needs to be established. To discover the detailed functions of SGAs in berries, shoots and leaves of tomato plants, and to monitor their level during ripening of tomatoes, especially of novel breeds destined for human consumption, it is critical to develop a reliable, simple and sensitive method of SGA determination.

Several methods have been reported for the analysis of SGAs and their corresponding aglycones, including high-performance liquid chromatography (HPLC) coupled with ultraviolet (UV) detection[8–10] or pulsed amperometric detection (PAD),[11–13] gas chromatography (GC),[14,15] immunoassays,[16] matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry,[17] and capillary electrophoresis with UV detection.[18] Very recently, a novel analytical method has been reported which is based on the use of non-aqueous capillary electrophoresis coupled with electrospray ionization mass spectrometry (ESI-MS).[19,20] Although GC has previously been used as the main technique for the measurement of derivatized SGAs, we are developing a direct and sensitive method which should provide considerable advantages over current methodologies. Indeed, replacement of GC with LC allows the determination of thermally labile compounds without recurring to hydrolysis and derivatization steps, thus reducing the analysis time, eliminating predictable sources of error and hazardous/expensive chemicals.

Mass spectrometry is the most selective technique for the rapid qualitative determination of known compounds as well as the identification of unknown compounds from extracts of

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Compound		Molecular Formula	[M+H]$^+$	Stereo-chemical Confi-guration	Double Bonds	R	-X-	-Y-
1	α-Tomatine	$C_{50}H_{83}NO_{21}$	1034	22β*N*, 25S	-	-Lycotetraosyl	CH_2	NH
2	Tomatidine	$C_{27}H_{45}NO_2$	416	22β*N*, 25S	-	-OH	CH_2	NH
3	Dehydrotomatine	$C_{50}H_{81}NO_{21}$	1032	22β*N*, 25S	Δ^5	-Lycotetraosyl	CH_2	NH
4	Tomatidenol	$C_{27}H_{43}NO_2$	414	22β*N*, 25S	Δ^5	-OH	CH_2	NH
5	Soladulcidine	$C_{27}H_{45}NO_2$	416	22α*N*, 25R	-	-OH	NH	CH_2
6	U1, Filotomatine	$C_{50}H_{83}NO_{21}$	1034	22α*N*, 25R	-	-Lycotetraosyl	NH	CH_2
7	Solasodine	$C_{27}H_{43}NO_2$	414	22α*N*, 25R	Δ^5	-OH	NH	CH_2

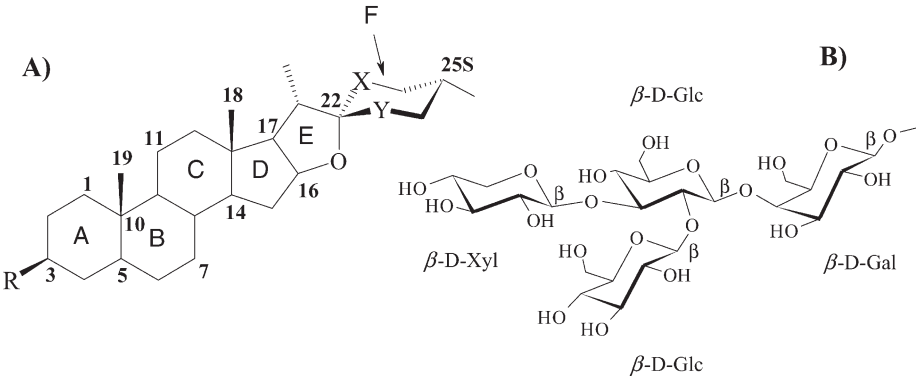


Figure 1. (A) Structures of steroidal spirosolane-type glycoalkaloids isolated from leaves and fruits of tomato, *Lycopersicon esculentum*, and (B) the lycotetraose sugar moiety. α-Tomatine: β-D-galactopyranoside (3β,5α,22β,25*S*)-spirosolan-3-yl-*O*-β-D-glucopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-*O*-β-D-glucopyranosyl-(1 → 4). Tomatidine: (3β,5*R*,22β,25*S*)-spirosolan-3-ol.

natural products. Generally, ESI-MS provides a mass spectrum with little or no fragmentation, and this technique is suitable for the characterization of not only a single compound, but also complex mixtures. In this study, an integrated approach consisting of LC/ESI-MS and tandem mass spectrometry (MS/MS) has been used for the identification of SGAs in leaves and berries extracts. The application of LC/MS to the determination of SGAs in *Solanaceae* plants has, till now, been very limited.[21–23] To the best of our knowledge, we report here for the first time a tomatine-like glycoalkaloid occurring in tomato leaves based on MS/MS analysis. Using ESI-MS, it has been possible to obtain structurally significant fragmentation ions of SGAs. The structures of spirosolane-type SGAs occurring in tomato plants are shown in Fig. 1, with the corresponding molecular formula and stereochemical configurations. The present approach is currently being applied to studies of the SGA content of crude extracts of tomato plants.

EXPERIMENTAL

Chemicals

Samples of authentic α-tomatine (99%) were supplied by Fluka Chemie (Buchs, Switzerland); solanidine (98%) and tomatidine hydrochloride were purchased from Sigma Chemical Co. (Steinheim, Germany); while dehydrotomatine and tomatidenol were present as impurities in α-tomatine and tomatidine standards, respectively.[24] Acetic acid, formic acid, acetonitrile, HPLC-grade methanol and water were obtained from Carlo Erba (Milan, Italy). All chemicals were used as received. Stock standard solutions of glycoalkaloids and aglycones were prepared from analytical reagent grade chemicals in methanol and were then stored at −20°C. Just before use, standard solutions were prepared from the stock solutions by dilution to the desired concentration with 0.1% formic acid in water and acetonitrile (65/35 v/v).

Instrumentation and separation conditions

LC/ESI-MS analysis was carried out using a LCQ Classic ion trap mass spectrometer (ThermoFinnigan, San Jose, CA, USA) equipped with a Spectra System P4000 binary pump and a solvent degasser. The column was a Supelcosil LC-ABZ, amide-C_{16} (5 μm, 250 × 4.6 mm) with a guard column of the same material (Supelco Inc., Bellefonte, PA, USA) and a mobile phase consisting of 0.1% formic acid in water (solvent A) and methanol (solvent B). The following gradient was applied: 30–43% B in 0–8 min; 43–60% B in 8–20 min and 60% B in 20–24 min. Prior to the next injection, the column was equilibrated for 6 min. The flow rate was 0.8 mL/min, which was split 3:1 after the analytical column to allow 200 μL/min to enter the ESI source.

Positive ion ESI-MS was chosen for the detection and quantification of SGAs. The instrument was tuned to facilitate the ionization process and achieve the highest sensitivity. The voltage on the ESI needle was set at 5 kV, producing a spray current of approximately 80 μA. The

capillary voltage was set at 14 V and the temperature of the heated capillary was 200°C. The sheath gas (N_2) flow rate used was 60 (arbitrary units) and the auxiliary gas was set to zero (arbitrary units). Total ion current (TIC) and selected ion monitoring (SIM) modes were used to record the abundances of the protonated molecules of SGAs and aglycones. In the full-scan mode, masses were scanned as centroid data from *m/z* 150–1200 at a rate of 2 scan/s. The instrumental control, data acquisition and data processing were performed using the Xcalibur software package (version 1.1, ThermoQuest).

Plant material and sample extraction

Freshly harvested green and red tomatoes (*Lycopersicon esculentum* Mill.) cherry cv. Camelia along with leaves of the same plant, grown on Barile Pozzolana substrate in a greenhouse of the 'Pantanello' Agricultural Experimental Station in Metaponto (Basilicata, Italy), were immediately stored at −20°C to arrest maturation. Then, 500 mg of each freeze-dried sample were placed in 10 mL of 1% acetic acid aqueous solution. To facilitate contact between plant tissue and extraction solvent, the suspension was stirred for about 2 h and then centrifuged at 4000 rpm for 20 min. The pellet obtained was suspended in 10 mL of 1% acetic acid, shaken, centrifuged and the two extracts were combined. To remove solid particles, this extract was filtered through a single-use 0.22 μm nylon filter (Whatman, Maidstone, UK) and 20 μL of sample were injected into the LC/MS system. As SGAs were present at relatively high levels in leaves and green tomatoes, an appropriate dilution with 0.1% formic acid in water and acetonitrile (65/35 v/v) was carried out before injection.

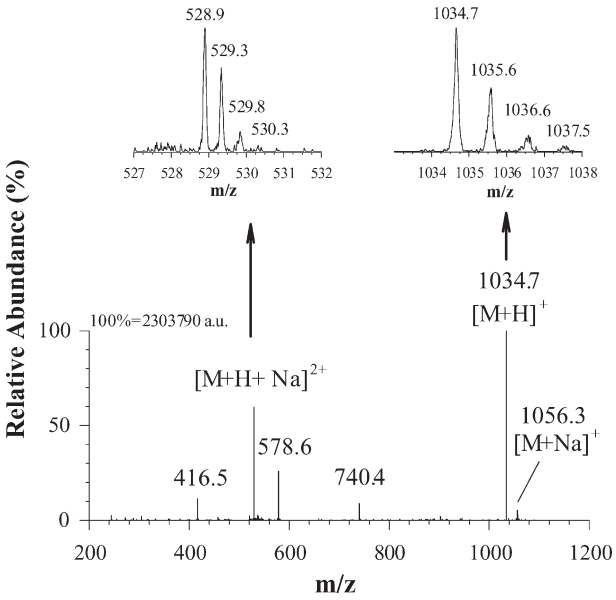


Figure 2. Full-scan positive ion ESI mass spectrum of a standard solution of α-tomatine. Solution infused, 1 mg/L α-tomatine in 0.1% formic acid aqueous solution and acetonitrile, 65/35 (v/v). Inset shows the mixed adduct ion, $[M+H+Na]^{2+}$, which exhibits a halving in spacing compared with the protonated molecule $[M+H]^+$ at *m/z* 1034.7. The charge status and *m/z* values of ions were checked in separate experiments using higher resolution scanning 'zoom scan'. Instrumental response was normalized to 100% but the absolute value in arbitrary units is provided in the heading of spectra.

RESULTS AND DISCUSSION

MS and MS/MS spectra of SGAs occurring in tomato plants

The positive ion ESI mass spectrum of an infused standard solution of α-tomatine (***1***, Fig. 1) is shown in Fig. 2, with the major six ions assigned. In addition to the $[M+H]^+$ ion (*m/z* 1034.7), relatively abundant fragment ions at *m/z* 578.6, 528.9 and 416.5 were also detected. It is suggested that the secondary amine of tomatidine is easily protonated during the ionization process. Ions at *m/z* 578.6 and 416.5 correspond to $[Tomatidine+Gal+H]^+$ and $[Tomatidine+H]^+$ which are mainly produced by eliminating the Xyl-Glc(-Glc)- moiety and the whole sugar chain (i.e., lycotetraose), respectively. The ion at *m/z* 528.9 (see inset of Fig. 2) is due to the formation of a doubly charged ion, $[M+H+Na]^{2+}$. Such an adduct is readily recognized because the ^{13}C isotope peak occurs about 0.5 Th higher than the corresponding ^{12}C peak.

In order to gain more structural information, MS/MS spectra of all the major ions occurring in Fig. 2 were recorded. Figure 3(A) shows the MS/MS spectrum of the protonated molecule, $[M+H]^+$; the most prominent product ion is *m/z* 1016.6 $[M+H-H_2O]^+$, formed by the loss of H_2O through a rearrangement process in the E ring, as suggested in Scheme 1.[25] The abundance of the *m/z* 1016.6 ion is much higher than that of the ion at *m/z* 416.5, which demonstrates that the $[M+H]^+$ ion preferentially expels a H_2O molecule. The same interpretation of loss of H_2O can be applied to the MS/MS spectrum of the ion at *m/z* 578.6 (see Fig. 3(B)), which yields an abundant product ion at *m/z* 560.5, i.e., $[Tomatidine+Gal+H-H_2O]^+$, and two other ions at *m/z* 273 and 255. MS/MS of the *m/z* 528.9 ion yields an abundant product ion at *m/z* 520.0, through loss of H_2O, $[M+H+Na-H_2O]^{2+}$ (Fig. 3(C)). In the same figure the reappearance can also be seen of the higher mass ions *m/z* 1034.5 and 1016.6, the protonated molecule and the ion formed after the loss of a molecule of H_2O, $[M+H-H_2O]^+$, respectively. Figures 3(A) and 3(C) display successive losses of xylose (132 Da) and a xylose-glucose unit (132 + 162 Da), yielding less abundant ions at *m/z* 902 and 740, respectively, which were of great importance for the determination of the sugar sequence of steroidal glycoalkaloids. A comprehensive rationalization of these processes is reported in Scheme 2. In addition to the product ion at *m/z* 398.6 corresponding to $[Tomatidine+H]^+-18$ (Fig. 3(D)), the ion at *m/z* 416.5, i.e., $[Tomatidine+H]^+$, yields two other main ions at *m/z* 273 and 255, involving cleavage of the E-ring. Such a fragmentation mechanism, which is similar to that of other steroidal saponins, is illustrated in Scheme 3.[26] It demonstrates that losses of H_2O are common to all ions containing the skeleton of spirosolane-type aglycones. Cleavage at the C–N bond of the spirosolane carbon is in agreement with the formation of the ions at *m/z* 273 and 255. These findings provide evidence that the dehydration occurs at a site other than the E-ring. Interestingly, all of the ions detected in this study could be structurally assigned.

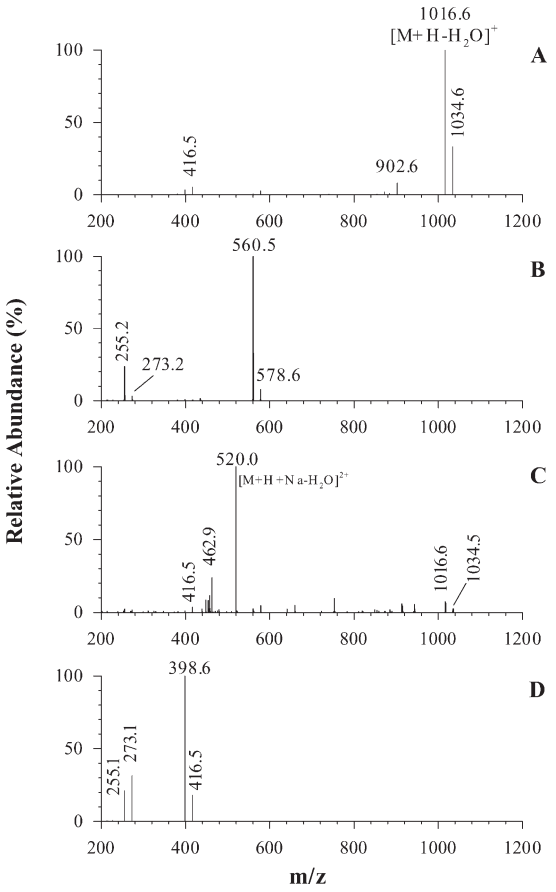
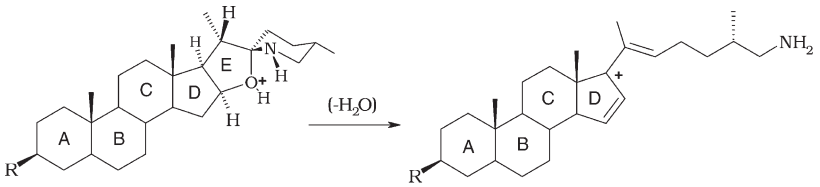


Figure 3. MS/MS spectra of α-tomatine ions at *m/z* 1034 (A), 578 (B), 529 (C) and 416 (D). Note the formation of the $[M+H-18]^+$ ion at *m/z* 1016.6 in spectrum (C). Relative collisional energy equal to 30%.

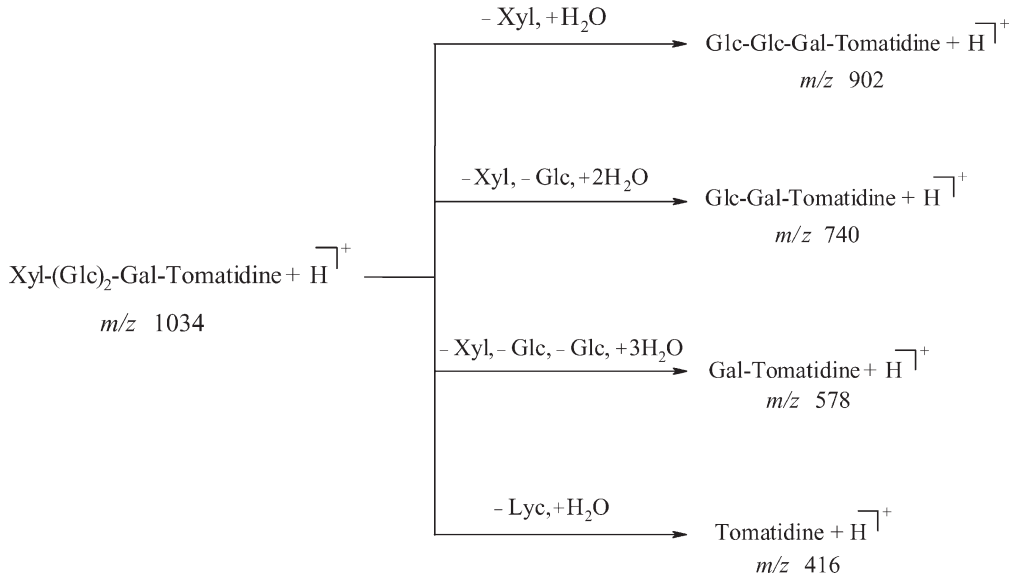
The second major glycoalkaloid found in tomato extracts is known as dehydrotomatine (**3**) with tomatidenol (**4**) as its aglycone (Fig. 1). Note that a standard compound of dehydrotomatine is not commercially available, but it is present as an impurity of α-tomatine. Thus the spectra illustrated here were acquired using the same standard solution prepared for α-tomatine. As can be seen from the spectrum in Fig. 4 the protonated molecule $[M+H]^+$ is at *m/z* 1032.5. Ions corresponding to [Tomatidenol+Gal+H]$^+$ at *m/z* 576.5 and $[M+H+Na]^{2+}$ at *m/z* 527.9 were also observed (see inset of Fig. 4). Unfortunately, the minor ion at lower mass values, i.e., [Tomatidenol+H]$^+$ at *m/z* 414.5, is not clearly distinguishable in the presence of a relatively large background noise. Here also, however, the MS/MS spectra were found to be very useful for structural elucidation. Figure 5(A) shows the MS/MS spectrum of the protonated molecule; the most prominent product ion is at *m/z* 1014.5, $[M+H-H_2O]^+$, followed by the [Aglycone+H]$^+$ ion at *m/z* 414.5 along with an ion at *m/z* 900.6. This latter loss of $1032-900=132$ Da corresponds to cleavage of a xylosil unit, as expected from the lycotetraose unit. The same issues discussed above for α-tomatine apply here for the ions at *m/z* 576.6 and 527.9 (see Figs. 5(B) and 5(C)). In addition to some product ions corresponding to fragments, MS/MS of *m/z* 527.9 yields the protonated molecule at *m/z* 1032.4.

Based on these findings, the mixed adduct ion of α-tomatine and dehydrotomatine, $[M+H+Na]^{2+}$, probably results from the sodiation of the tetrasaccharide moiety and the protonation of the nitrogen atom of the spirosolane ring, as suggested in Scheme 3. This may be considered as further confirmation that both α-tomatine and dehydrotomatine have the same carbohydrate side chain, i.e., the lycotretraose moiety.[27–30] The [Tomatidenol+H]$^+$ ion (*m/z* 414.5), produced by loss of the whole sugar chain from $[M+H]^+$, predominantly experiences cleavage of the E-ring, thus yielding two ions at *m/z* 271.2 and 253.2. These results show the difference of one unsaturation between tomatidenol and tomatidine, the former containing a double bond at position 5,6 in the steroidal skeleton (i.e., Δ^5). This set of observations clearly indicates that the structures of spirosolane-type SGA aglycones **2** and **4** are almost similar. The same ESI-MS features discussed above for a standard solution of α-tomatine and dehydrotomatine were also observed in the

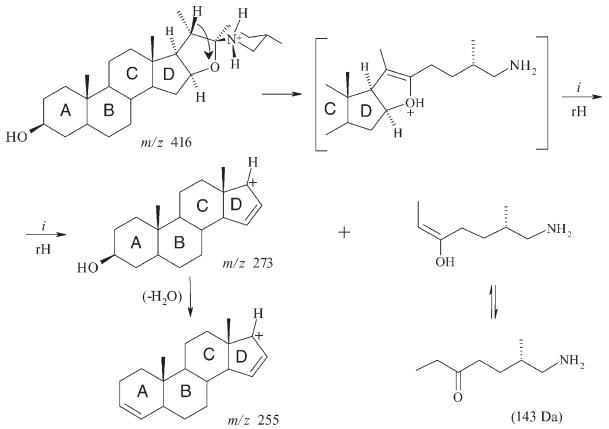


R	$[M+H]^+$ (*m/z*)	$[M+H-H_2O]^+$ (*m/z*)
Lycotreaose-	1034	1016
$(Glc)_2$-Gal-	902	886[a]
Glc-Gal-	740	722[a]
Gal-	578	560
HO-	416	398

Scheme 1. Rationalization of the common dehydration process of the α-tomatine fragments and tomatidine aglycone. Each of these ions shows a product ion mass spectra characterized by elimination of H_2O from the $[M+H]^+$.
[a]Low abundance ions.



Scheme 2. Main glycosidic cleavages of the lycotetraose moiety of α-tomatine after positive ion ESI-ITMS. Xyl: xylose; Glc: glucose; Gal: galactose.



Scheme 3. EF-ring cleavage of the steroidal spirosolane aglycone tomatidine (elimination reaction for loss of 143 Da). The neutral loss of H_2O is also illustrated (*m/z* 273 → 255).

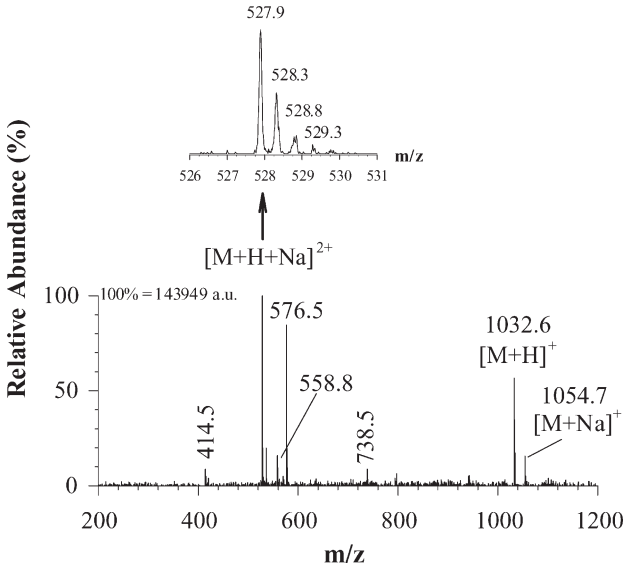


Figure 4. Full-scan positive ion ESI mass spectrum of dehydrotomatine. Inset shows the doubly charged ion $[M+H+Na]^{2+}$ at *m/z* 527.9. Experimental conditions as in Fig. 2.

mass spectra obtained from crude extracts of tomato leaves and berries (not shown).

In order to gain more structural information on the aglycone moieties of alkaloid steroids, accurate MS and MS/MS spectra were acquired of solanidine (397 Da) and tomatidine (415 Da) under the same experimental conditions as were used for the tomato glycoalkaloids, as illustrated in Figs. 6 and 7, respectively. Note that solanidine is the aglycone of α-solanine, which is typically found in potato extracts; it was chosen to show the very different fragmentation patterns in multistage MS experiments compared with those of tomatidine and tomatidenol. Whereas a definite product ion at *m/z* 383.5 was observed in the MS/MS spectrum of solanidine, probably derived from the protonated molecule at *m/z* 398.5 after a methyl radical loss (Fig. 6(B)), the MS/MS spectrum of tomatidine at *m/z* 416.5 exhibits a product ion at *m/z* 398.6, which is probably characteristic of E-ring scission with the positive charge localized on the D-ring (see Scheme 3). The same spectrum also revealed a specific loss of 143 Da yielding the product ions at *m/z* 273.1 and 255.1 from the precursor ions at *m/z* 416.5 and 398.6, respectively. In agreement with very recently data obtained in our laboratory, the fragmentation pattern of solanidine does not exhibit the loss of a H_2O molecule.[31,32] It should also be mentioned that the potato glycoalkaloids α-solanine and α-chaconine, under the present experimental conditions, exhibited very low abundances of mixed adduct ions (not shown). As a result, the occurrence of doubly charged $[M+Na+H]^{2+}$ ions appears to be very helpful for peak confirmation and the structural elucidation of tomato glycoalkaloids (*vide infra*).

LC/ESI-MS of tomato plant extracts

Friedman and co-workers have shown that the separation of tomato extracts can be achieved in reversed-phase liquid

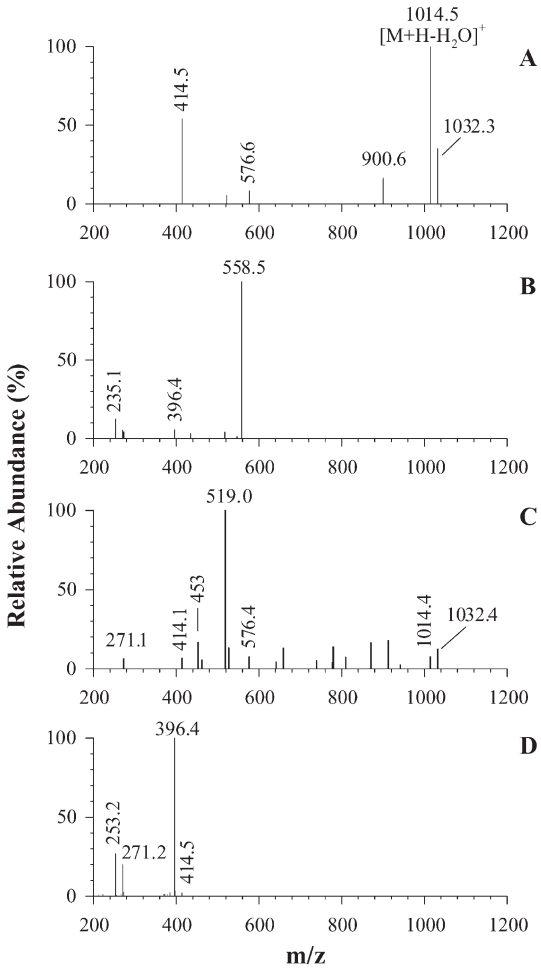


Figure 5. MS/MS spectra of dehydrotomatine ions at *m/z* 1032 (A), 576 (B), 528 (C) and 414 (D). Relative collisional energy equal to 30%.

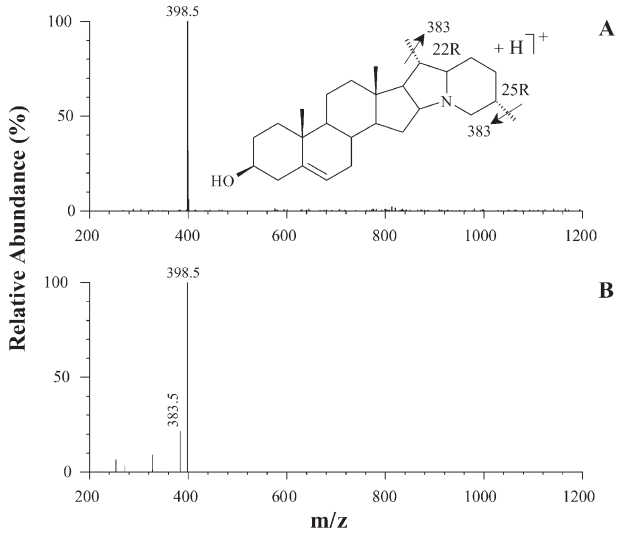


Figure 6. (A) Full-scan positive ion ESI mass spectrum of a standard solution of solanidine and (B) MS/MS spectrum of *m/z* 398, which exhibits a methyl radical loss (*m/z* 398.5 → 383.5). Note that solanidine is the aglycone of α-solanine. Relative collisional energy equal to 30%.

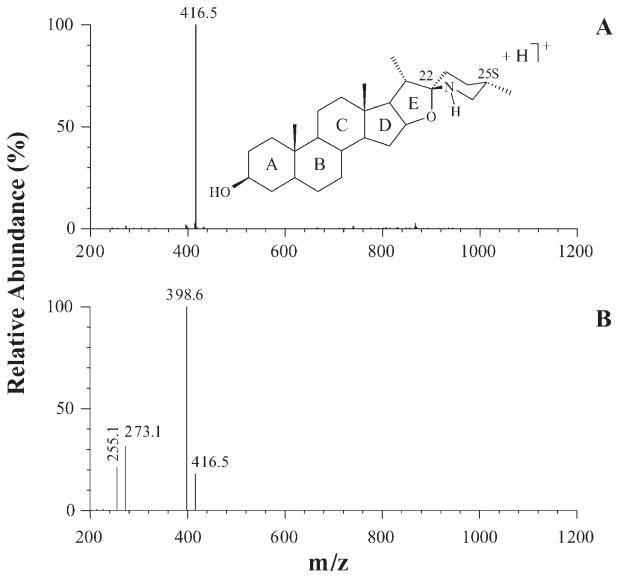


Figure 7. (A) Full-scan positive ion ESI mass spectrum of the aglycone tomatidine and (B) MS/MS spectrum of *m/z* 416. Experimental conditions as in Fig. 2.

chromatography (RP-LC) on amide-C_{16} columns.[11–13] A good separation of SGAs in a reasonable time was obtained using a Supelcosil LC-ABZ amide-C_{16} column, which was selected for subsequent experiments, using methanol and 0.1% formic acid in water as the mobile phase. A representative LC/MS total ion chromatogram (TIC) of the crude extract of tomato leaves is shown in Fig. 8. The $[M+H]^+$ ion of each component was obtained at the corresponding retention

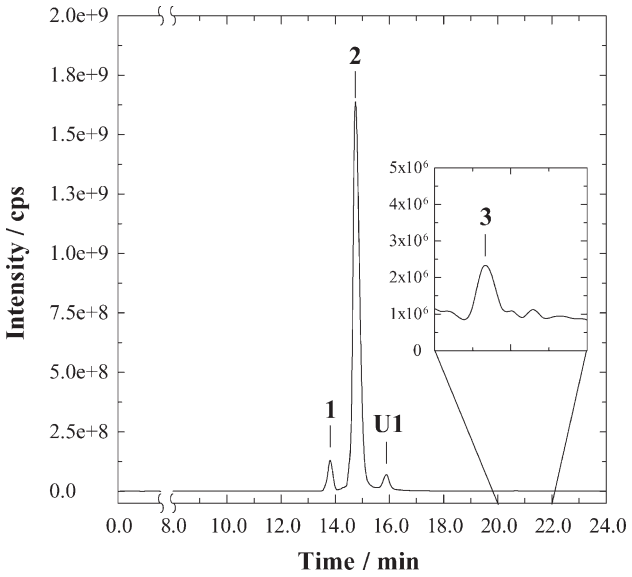


Figure 8. LC/ESI-MS chromatogram acquired in positive ion mode of an extract of tomato leaves. Peak numbers correspond to dehydrotomatine (1), α-tomatine (2), tomatidine (3) and unknown (U1). Chromatographic conditions: mobile phases, MeOH and 0.1% HCOOH in H_2O; column, Supelcosil LC-ABZ amide-C_{16} (250 × 4.6 mm, 5 μm); flow, 0.8 mL/min; loop, 20 μL.

time. Along with the prominent peak 2 of α-tomatine, the dehydrotomatine (peak 1), small amounts of tomatidine (peak 3) and a relatively intense peak at 16.0 min (U1) were observed. The relative intensities of peaks 1, 2 and U1 found in the foliage extracts were approximately 0.08, 1.00 and 0.07, respectively. A mass spectrum of the unknown compound U1 was acquired and its structure investigated. As can be seen from the spectrum in Fig. 9(A), the $[M+H]^+$ ion is at *m/z* 1034.5, which means that the nominal mass of the unknown U1 is 1033 Da. The minor ion at *m/z* 1056.4 is indicative of sodium adduction to give $[M+23]^+$. Moreover, the spectrum of compound U1 contains two fragment ions at *m/z* 528.9 and 578.8, which exhibit the same fragmentation behavior as α-tomatine, i.e., *m/z* 578.8 = $[Tomatidine+Gal+H]^+$, *m/z* 528.9 = $[M+H+Na]^{2+}$ and *m/z* 416.5 = $[Tomatidine+H]^+$, suggesting a tomatine-like structure. Figure 10 shows results from the MS/MS experiments on the ions at *m/z* 578.8, 528.9, and 416.5, which produced base peaks at *m/z* 560, 520, and 398, respectively, after the loss of a molecule of H_2O. The other product ions of *m/z* 528.9 at *m/z* 1016.6 and 740.4 are due to the neutral losses of 18 (H_2O) and 294. This last neutral loss is indicative of the presence in the molecule of a pentose-hexose unit (132 + 162). It has been reported that SGAs with branched sugar chains lose the terminal sugar moiety primarily.[31,32] Confirmation of the tetrasaccharide side chain incorporated within the parent glycoalkaloid U1 was obtained upon acid hydrolysis in 1 N HCl at 100°C for 2 h and subsequent separation by anion-exchange chromatography with pulsed amperometric detection.[33] The presence of galactose, glucose and xylose in the approximate molar ratios 1:2:1 was ascertained (not shown), which are the same ratios as those occurring in the oligosaccharide moiety of α-tomatine, within the experimental error of this analytical methodology.

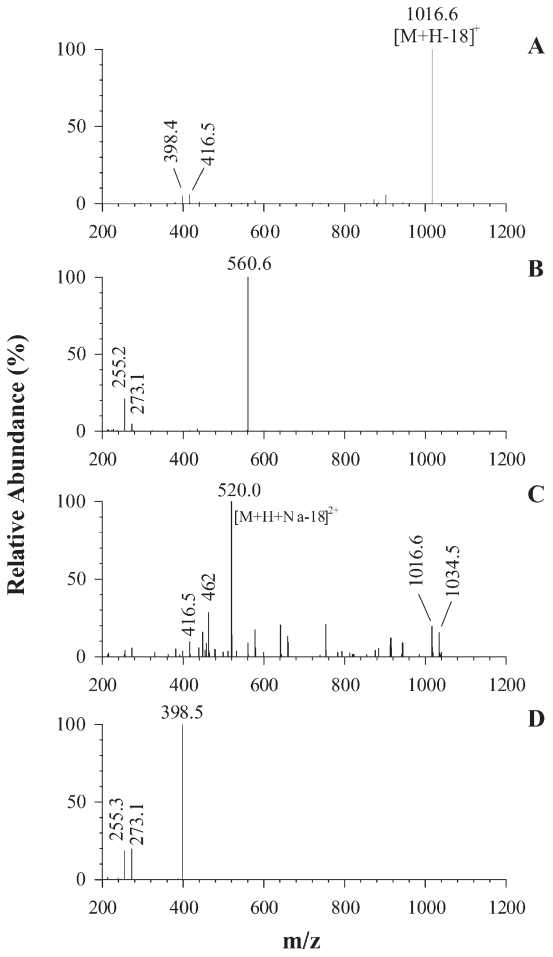


Figure 10. MS/MS spectra of filotomatine (U1) ions at *m/z* 1034 (A), 578 (B), 529 (C) and 416 (D). Note the formation of the $[M+H-18]^+$ ion at *m/z* 1016.6 in spectrum (C).

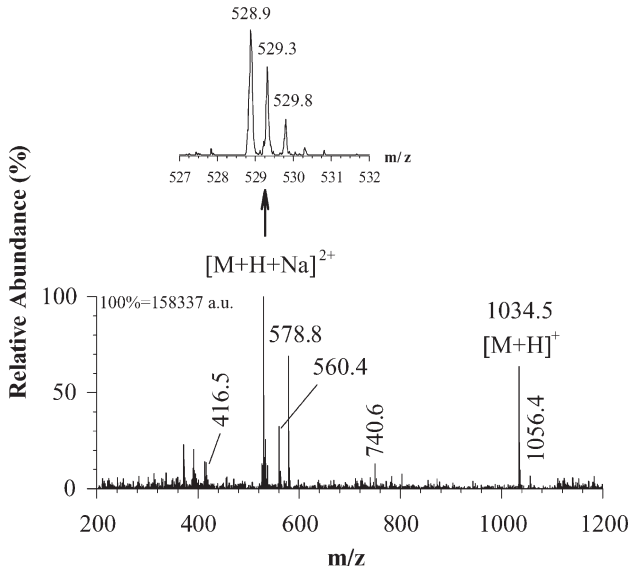


Figure 9. Positive ion ESI mass spectrum of the unknown peak U1, which is assigned to filotomatine. Inset shows the doubly charged ion $[M+H+Na]^{2+}$ which exhibits a halving in spacing compared with the protonated molecule $[M+H]^+$ at *m/z* 1034.7.

The $[Aglycone+H]^+$ ion (*m/z* 416.5), produced by loss of the whole sugar chain, undergoes cleavage of the E-ring to yield ions at *m/z* 271.1 and 253.3. It is probable that this aglycone is soladulcidine (see Fig. 1) which is closely related to tomatidine; they form a pair of isomers that have opposite configurations at C-22 and C-25 in the nitrogen-containing F-ring, which means that the C–N bond of the spirosolane carbon is in the α-configuration. The corresponding spirosolane aglycone containing a double bond at position 5,6 in the steroidal skeleton (i.e., Δ^5) is known as solasodine (**7**).[15,34,35] We could not elucidate the structure of the soladulcidine aglycone moiety based on MS/MS data. However, it is interesting to note that the *m/z* values of all the fragment ions of U1 and α-tomatine are identical, and slight differences with respect to relative product ion abundances in the MS/MS spectrum in the unknown U1 and in the reference MS/MS spectrum for α-tomatine can be explained as due to the relative quantitative level of these compounds. The similarities concerns also the double positively charged ion, $[M+H+Na]^{2+}$ at *m/z* 529.7. Work is underway to verify the formation of these mixed adduct ions within the family of SGAs in *Solanaceae* plants in terms of sugar units, classes of monosaccharides and alkali metal ions.

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From the analysis of the mass-measured ions discussed above, the following preliminary conclusion can be reached: the unknown U1 contains at least one N atom, its molecular weight is the same of that of tomatine with an equivalent fragmentation pattern and a strictly related tetrasaccharide moiety. By adding the condition of a different retention time in RP-LC, the only possibility is that this compound is an isomer of α-tomatine which has never previously been described. Probably the unknown U1 in the extracts of tomato leaves is composed of soladulcidine as an aglycone and lycotetreaose as the sugar linked to the hydroxyl group (see Fig. 1). This tomatine-like glycoalkaloid, which exhibits an inverse stereochemical configuration of the 22-carbon atom, is named filotomatine. Interestingly, the fragmentations of filotomatine can be rationalized based on the same mechanism illustrated in Schemes 1–3 for α-tomatine and its aglycone **2**. Unfortunately, these compounds did not give significant differences in product ion types, even in their sequential MS^3 and MS^4 spectra. Other spectroscopic techniques such as ^1H and ^{13}C NMR would be able to distinguish between the two SGAs. Considering, however, that α-tomatine and filotomatine are regioisomers, their chromatographic separation is satisfactory as their difference in retention time is about 1 min. We have systematically investigated the occurrence of this new glycoalkaloid in extracts of tomato leaves. Extracts of berries were also considered, but no peaks were observed at the retention time of U1, which is in agreement with previously reported data that the only major SGAs of tomatoes are α-tomatine and dehydrotomatine.[5] It would be interesting to know to what extent this glycoalkaloid is involved in host-plant resistance against phytopathogens.

CONCLUSIONS

Using LC/ESI-MS and MS/MS we have demonstrated the occurrence of a previously unidentified steroidal glycoalkaloid of tomato leaves which shares the same core aglycone structure as α-tomatine and dehydrotomatine, but where the differences from them appear to be confined to the F-ring of the steroidal moiety. For analysis of SGAs in crude extracts, we recommend initial application of positive ion ESI-MS to obtain the molecular mass information for the components via the $[M+H]^+$ ions, and to determine the sugar moiety at the C-3 position by acquiring MS/MS spectra. The observed clusters, which are mixed adduct ions of SGAs, $[M+H+Na]^{2+}$, were found very useful for peak confirmation. Although this method was developed specifically for tomato plants, it can be applied to evaluate the SGA composition of other vegetables, such as potatoes and sweet peppers. Construction of a library containing MS/MS spectra for known SGAs would greatly facilitate the identification of these compounds in real samples and would permit even more complete fingerprinting of SGAs arising from species differences between *Solanaceae* plant extracts.

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