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Photo-induced synthesis of tomatidenol-based glycoalkaloids in *Solanum phureja* tubers

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Abstract

The effect of light exposure on the steroidal glycoalkaloid content of *Solanum phureja* tubers has been investigated and compared with that in domesticated potato (*Solanum tuberosum*) tubers. The results indicated that the increase in the concentration of solanidine-based glycoalkaloids, α-solanine and α-chaconine was broadly similar in both species. However, in the *S. phureja* tubers, light exposure also induced the synthesis of tomatidenol-based glycoalkaloids. These have been identified as α- and β-solamarine. These glycoalkaloids were not detected in tubers continually stored in darkness.

Keywords: Glycoalkaloids; α-Solanine; α-Chaconine; α-Solamarine; Light exposure; *S. phureja*; *S. tuberosum*

1. Introduction

Steroidal glycoalkaloids are commonly found in the foliage and tubers of species from within the Solanaceae (Van Gelder, Vinke & Scheffer, 1988; Petersen, Molgaard, Nyman & Olsen, 1993). In the foliage their presence has been linked to pest resistance, whilst the accumulation of these compounds in the tubers may represent a potential toxic hazard to both animals and man (Friedman & McDonald, 1999). In the domesticated potato (*Solanum tuberosum*), the predominant glycoalkaloids in the tubers are α-solanine and α-chaconine, both of which are differently glycosylated forms of the aglycone, solanidine (Friedman & McDonald, 1997). In wild *Solanum* species, often used as the parental material to introduce new attributes to commercial potato breeding programmes, solanidine-based glycoalkloids would appear to predominate. However, a number of glycoalkaloids containing other aglycones including solasodine, tomatidenol, demissidine and tomatidine have also been identified as both the major and minor glycoalkaloid constituents in tubers of certain accessions from some wild species (Van Gelder, Vinke & Scheffer, 1988; Petersen et al., 1993).

S. phureja is a close relative of *S. stenotomum*, which is regarded as an ancestor of the domesticated potato (*S. tuberosum*) (Hawkes, 1990). In addition to being grown as a cultivated species in South America, it has been widely used in genetic research and in breeding programmes where it has been utilised as a bridging species to transfer desirable characteristics into *S. tuberosum* from other incompatible wild species; e.g. late-blight resistance from *S. bulbocastanum* (Hermsen, 1984). Little information appears available regarding the glycoalkaloid content of *S. phureja* tubers although in two independent studies (Van Gelder & Jonker, 1986; Petersen et al., 1993), only solanidine-based glycoalkaloids were detected.

Post-harvest treatments can significantly alter tuber glycoalkaloid concentrations in *S. tuberosum*. Exposure to direct sunlight (Percival, Dixon & Sword, 1994) or

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to artificial lighting (Dale, Griffiths, Bain & Todd, 1993) significantly increased tuber glycoalkaloid concentration with the magnitude of the observed increase being cultivar dependent (Griffiths, Dale & Bain, 1994). This can result in the production of tubers with glycoalkaloid contents well above 20 mg 100 g^{-1} fresh weight, the maximum concentration recommended for human consumption (Sinden & Webb, 1972).

In view of the current interest in developing *S. phureja* as a crop adapted for European conditions (De Maine, Carroll & Torrance, 1993), it would appear timely to investigate not only the levels of glycoalkaloids in non-stressed tubers but also to compare the effects of light exposure on glycoalkaloid synthesis in *S. phureja* and *S. tuberosum* tubers.

2. Results and discussion

2.1. Effect of light on solanidine-based glycoalkaloids

Tubers from *S. tuberosum* cultivars Eden and Brodick have previously been identified respectively as slow and rapid accumulators of steroidal glycoalkaloids in response to light exposure (Dale et al., 1993). Consequently, in order to compare the relative rates of glycoalkaloid accumulation in *S. phureja* accessions with that of commercially available *S. tuberosum* cultivars these two cultivars were selected as controls. Analysis of tubers, which had been stored continually in a light-free environment revealed (Table 1, dark) that in both species only two glycoalkaloids, ᾱ-solanine and α-chaconine were detected in quantifiable amounts. In both species the two solanidine-based glycoalkaloids were present at comparable levels and in all cases the total glycoalkaloid concentration was well below the maximum concentration (20 mg 100 g^{-1} fresh weight) recommended for human consumption. It was, however of interest to note that in both *S. phureja* accessions the concentration of α-solanine was greater than that of α-chaconine whilst as previously reported for a wide range of *S. tuberosum* cultivars (Dale et al., 1993; Percival et al., 1994), the converse was found in the tubers of Brodick and Eden.

Analysis of the tuber samples exposed to light for a period of 120 h revealed (Table 1) that, as expected, the total concentration of the solanidine-based glycoalkaloids increased significantly in both species. In the *S. tuberosum* cultivars, the greatest total increase (31.5 mg 100 g^{-1} fresh weight) was seen in Brodick tubers, whilst light exposure increased the total glycoalkaloid content of Eden tubers by only 7.3 mg 100 g^{-1} FW. In both cultivars the greatest increase was in the concentration of α-solanine, suggesting that enzyme systems involved in the biosynthesis of the added solatriose side chain are more sensitive to the consequences of light exposure than those involved in the addition of chacotriose required for the synthesis of α-chaconine.

In the *S. phureja* tubers, light exposure increased the concentration of solanidine-based glycoalkaloids in accessions DB299/14 and 85/T8 exMS86(13) by 8.3 and 14.4 mg 100 g^{-1} FW respectively. As with the two *S. tuberosum* cultivars the greatest increase was seen in ᾱ-solanine concentration and indeed in accession DB299/14 the small increase observed in the ᾱ-chaconine level was not statistically significant. The total increases fell within the range found for the *S. tuberosum* cultivars, suggesting that any potential hazards arising from the consumption of solanidine-based glycoalkaloids in light-exposed *S. phureja* tubers should be no greater than that from similarly treated *S. tuberosum*. However, as with new *S. tuberosum* cultivars it would appear prudent with regard to public safety to check both the normal levels and the rate of accumu-

Table 1
The effect of light exposure on the concentration (mg 100 g^{-1} FW) of individual and total glycoalkaloids in S. tuberosum and S. phureja tubers

	α-Solanine		α-Chaconine		α-Solamarine	Total	
	Dark	Light	Dark	Light	Light[a]	Dark	Light
S. tuberosum							
Brodick	2.8	20.3	3.1	17.1	nd	5.9	37.4
Eden	2.1	6.6	2.1	4.9	nd	4.2	11.5
S. phureja							
DB299/14	4.0	11.5	1.9	2.6	12.1	5.9	26.3
85/T8exMS86(13)	6.2	18.2	2.3	4.7	11.2	8.5	34.1
LSD ($P > 0.05$)[b]	1.66		1.26		NS	3.33	
Mean	3.8	14.1	2.4	7.4		6.2	27.3
LSD ($P > 0.05$)[c]	0.83		0.63			1.65	

[a] α-Solamarine only detected in light-exposed tubers.
[b] Least significant difference (Treatment × Cultivar).
[c] Least significant difference (Treatment Means).

lation of glycoalkaloids in any new cultivars destined for human consumption.

2.2. Identification of light-induced glycoalkaloids

Comparison of the chromatograms produced by high performance liquid chromatography from extracts prepared from light-exposed *S. phureja* tubers with those from tubers continually stored in the dark, revealed that the former, in addition to peaks corresponding to α-solanine (R_t = 11.8 min) and α-chaconine (R_t = 12.5 min), contained two additional peaks (R_t = 8.1 min and R_t = 8.6 min). The area of the earlier eluting peak was of a similar magnitude as that of α-solanine, whilst the later eluting peak was detected in only trace amounts and was not quantifiable. Analysis by liquid chromatography–atmospheric pressure chemical ionisation–mass spectrometry (LC–APCI–MS) revealed that as expected the two later eluting peaks produced spectra identical to those of commercially available samples of α-solanine and α-chaconine (Sigma, Poole, UK). In both spectra a small but detectable peak corresponding to the protonated molecular $[M+H]^+$ ion was detected (Table 2) as were diagnostic fragment ions, whose empirical formulae masses were consistent with the formation of positive charged ions resulting from the sequential loss of the appropriate carbohydrate molecule from α-solanine and α-chaconine. In the spectra of both compounds the base peak (m/z = 398) theoretically corresponded with a positively charged ion with a formula weight identical to that of the common aglycone, solanidine. It should, however, be stressed that the identification of the fragmentary ions in these spectra is at best tentative.

The LC–MS spectrum of the earliest eluting peak (R_t = 8.1) was consistent with that of a spirosolene-based glycoalkaloid with a solatriose side chain (Table 2, unknown 1). In addition to the $[M+H]^+$ ion (m/z = 885), fragments corresponding with the sequential loss of the individual carbohydrate molecules in a manner analogous to that found in the spectra of α-solanine and α-chaconine were also detected. Similarly as in the spectra of the solanidine-based glycoalkaloids, the base peak (m/z = 414) appeared to be a positively charged ion with an empirical weight equal to that of a spirosolene aglycone. The low concentration of the other unidentified peak (R_t = 8.6 min) did not facilitate its identification from a full scan spectrum. However, by using single ion monitoring all the predicted diagnostic ions of a chacotriose-spirosolene glycoalkaloid were detected (Table 2, unknown 2).

Previously reported studies (Shih & Kuć, 1974) demonstrated the presence of both α- and β-solamarine, the respective solatriose and chacotriose containing glycosides of the spirosolene aglycone, tomatidenol (Fig. 1), in the leaves of the domesticated potato (*S. tuberosum*) cultivar, Kennebec. Analysis by HPLC and by LC–APCI–MS of extracts prepared from freeze-dried leaves of Kennebec produced chromatograms with four peaks with retention times and mass spectra identical to those of extracts from the light-exposed *S. phureja* tubers.

These results strongly suggest that the two originally unidentified peaks in the chromatogram of extracts prepared from light exposed *S. phureja* were α- and β-solamarine. However, similarly glycosylated glycoalkaloids based on the spirosolene aglycone, solasodine have also been identified in over a 100 *Solanum* species (Friedman & McDonald, 1999). Solasodine can be considered as an epimer of tomatidenol (Fig. 1), differing only in the spatial orientation of the nitrogen atom. Both aglycones have identical formula weights and it is unlikely that similarly glycosylated glycoalkaloids containing tomatidenol would be distinguishable on the basis of their mass spectra produced by LC-APCI-MS from those containing solasidine.

Table 2
The tentative identification of the ions detected in the liquid chromatograpic–atmospheric pressure chemical ionisation-mass spectra (LC–APCI–MS) of α-solanine, α-chaconine and two unidentified peaks observed in extracts prepared from light-exposed *S. phureja* tubers

Solanidine-based glycoalkaloids

Carbohydrate side chain	MW	$[M+H]^+$	Observed ions	
			α-Solanine	α-Chaconine
Solatriose	868.0	869.0	869	
Chacotriose	852.0	853.0		853
Galactose–rhamnose	705.9		706	
Glucose-rhamnose	705.9			706
Galactose–glucose	721.9		722	
Galactose	559.8		560	
Glucose	559.8			560
None	397.6		398	398

Spirosolene[a]-based glycoalkaloids

Carbohydrate side chain	MW	$[M+H]^+$	Observed ions	
			Unknown 1	Unknown 2
Solatriose	884.0	885.0	885	
Chacotriose	868.0	869.0		869
Galactose–rhamnose	721.9		722	
Glucose-rhamnose	721.9			722
Galactose–glucose	737.9		738	
Galactose	575.8		576	
Glucose	575.8			576
None	413.6		414	414

[a] Either solasidine or tomatidenol.

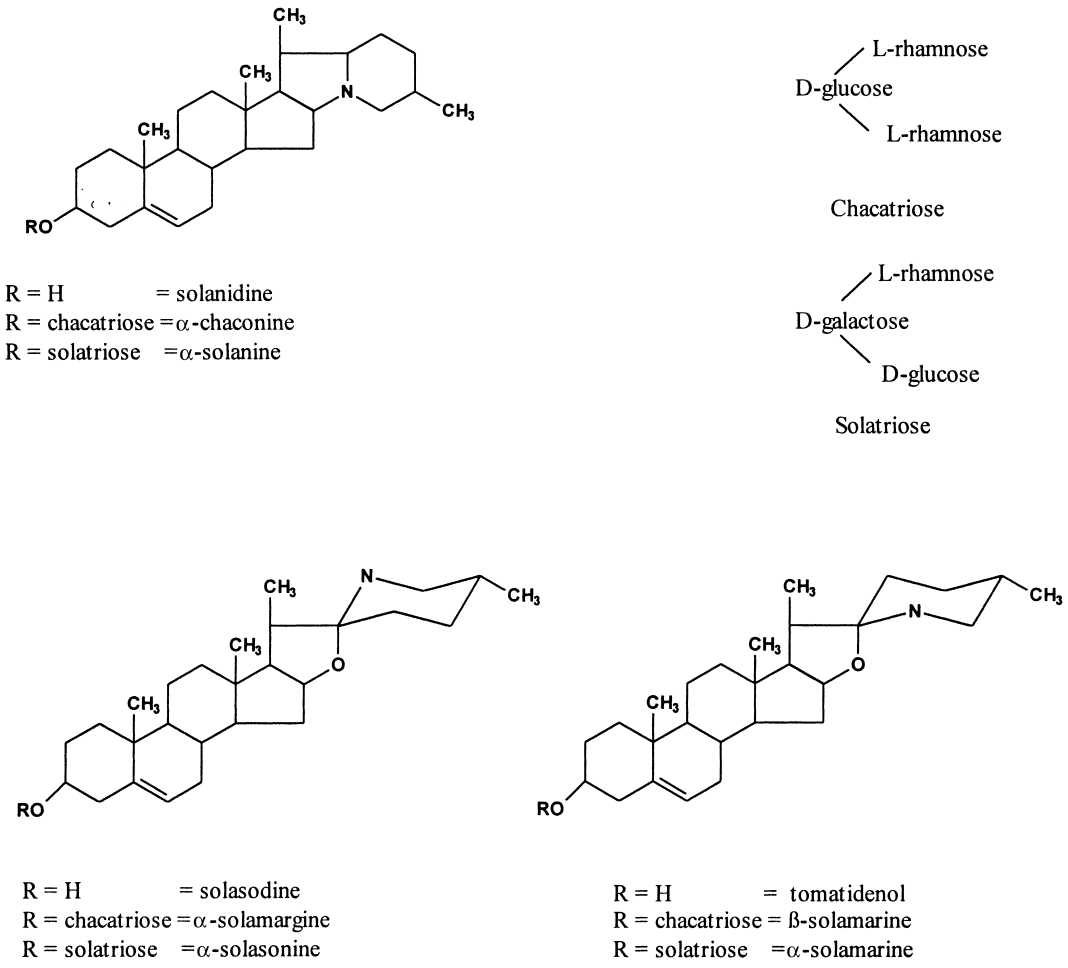

Fig. 1. The structures of solanidine-, tomatidenol- and solasodine-based glycoalkaloids.

Consequently, to eliminate the possibility that the *S. phureja* tubers synthesised solasodine rather than tomatidenol-based glycoalkaloids, extracts from the light-exposed tubers and from Kennebec leaves were hydrolysed. Analysis of the aglycones produced from the Kennebec leaf extracts by thin layer chromatography (TLC) revealed two major components, one of which had identical R_f values to that of commercially available solanidine, whilst the other aglycone previously identified as tomatidenol (Shih & Kuć, 1974) was distinguishable from commercially available solasodine in both solvent systems used (Table 3). Similarly, trimethylsilyl (TMSi) derivatives prepared from the hydrolysed Kennebec leaf extracts produced two major peaks when analysed by gas chromatography–mass spectrometry (GC–MS). The earlier eluting peak had identical retention time and mass spectrum to that of a similarly prepared TMSi derivative of commercially available solanidine. The later eluting peak, although having a mass spectrum almost identical to that of the TMSi derivative of commercially available solasodine, did not have the same retention time (Table 3). Thus, this later eluting peak was identified as the TMSi derivative of tomatidenol.

TLC analysis of the hydrolysed extracts prepared from the light exposed *S. phureja* tubers also revealed the presence of two major components, which on the basis of their R_f values in both solvent systems were identified as solanidine and tomatidenol. The results of GC–MS studies of the TMSi-derivatised hydrolysed

Table 3
The R_f values of glycoalkaloid aglycones and the retention times and mass spectral data for their trimethylsilyl derivatives

Aglycone	TLC		GC-MS	
	R_f[a]	R_f[b]	R_t[c]	Major ions
Solanidine	0.39	0.37	19.9	469[M+], 454, 204, 150
Solasidine	0.54	0.12	24.7	485[M+], 470, 138, 114
Tomatidenol	0.56	0.32	25.4	485[M+], 470, 138, 114

[a] R_f in ethylacetate:acetic acid:water (11:2:2).
[b] R_f in ethyl acetate:cyclohexane (3:1).
[c] R_t in retention time (min).

extracts confirmed these findings with the two major peaks having identical mass spectra and retention times to those found in similarly derivatised Kennebec leaf extracts.

The study of the hydrolysed extracts confirmed that the additional glycoalkaloids induced by light exposure in the tubers of *S. phureja* were the tomatidenol based glycoalkaloids, ᾱ- and β-solamarine.

2.3. Effect of light on tomatidenol-based glycoalkaloids

The exposure of *S. phureja* tubers to light resulted in the synthesis of significant quantities of α-solamarine. The amount produced was of the same order of magnitude as the increase observed in α-solanine (Table 1). However, light exposure induced the synthesis of only small, non-quantifiable amounts of the chacotriose derivative of tomatidenol, β-solamarine. It is of interest to note that light exposure also resulted in only minor increases in the levels of ᾱ-chaconine, the chacotriose derivative of solanidine. This would again suggest that the enzymatic systems involved in either the biosynthesis of chacotriose-based glycoalkaloids was significantly less sensitive to light than the those involved in the synthesis of their solatriose equivalents.

The effect of light exposure on the total glycoalkaloid content of tubers from both *S. phureja* accessions (Table 1) was sufficient to produce levels significantly higher than the recommended maximum concentration for human consumption (20 mg 100 g^{-1} fresh weight). As there appears to be little if any toxicological data available for the tomatidenol-based glycoalkaloids the potential hazards to consumers is currently impossible to determine. However, it would seem advisable to limit their introduction into commercial cultivars until such studies have been undertaken. Similarly, prior to the introduction of desirable breeding characteristics, such as disease resistance from wild *Solanum* species into the domesticated potato, it would appear prudent to examine not only the levels and types of glycoalkaloids present at harvest in the tubers of the wild species but also to determine whether, as has been found in the two *S. phureja* accessions studied here, previously undetected glycoalkaloids may be induced in response to light exposure or indeed by other forms of stress.

3. Experimental

3.1. Plant material

Tubers from the two *S. phureja* accessions and the two domesticated potato cultivars, Eden and Brodick, were harvested from replicated field trials grown at Mylnefield, Dundee in 1997. Post-harvest, the tubers were stored for two weeks at ambient temperature and then transferred for a further eight weeks to a temperature-controlled store set at 10°C. At the end of this period, 20 average-sized tubers were selected from each cultivar and divided into four replicates, each consisting of five tubers.

The methodology used to evaluate the effect of light on tuber glycoalkaloid content was similar to that previously reported (Dale et al., 1993; Griffiths, Dale & Bain, 1994; Griffiths, Bain & Dale, 1998). The tubers were longitudinally cut in half, and one half placed cut surface down, on a tray lined with moist paper toweling. The trays were then transferred to an environmental chamber (20°C; ambient humidity) illuminated with high-pressure sodium lights (predominant wavelengths 550–660 nm) adjusted to produce a photon flux of 140 μmol $m^{-2}s^{-1}$ at tray level. The remaining half tubers were also placed on lined trays, transferred into light-proof bags and then stored in the same environmental chamber. After 120 h the tubers were removed from the environmental chamber, a 2 mm slice discarded from the cut surface of each of the tubers, which were then quartered and opposite quarters bulked by replicate and treatment. The samples were manually diced, frozen in liquid nitrogen, freeze-dried and after milling stored at −20°C prior to analysis.

3.2. Extraction of glycoalkaloids

Glycoalkaloids were extracted from freeze-dried samples using 2% aqueous acetic acid containing 0.5 g sodium bisulphite per 100 ml. After centrifugation, the supernatant was loaded onto pre-equilibrated 100 mg C18 reverse-phase column, washed with 15:85 v/v acetonitrile/0.05 M phosphate buffer and the glycoalkaloids eluted with 30:70 v/v acetonitrile/0.05 M phosphate buffer (Hellenäs, 1986). The glycoalkaloid contents of the resulting semi-purified extracts were then determined by HPLC.

The extracts used for LC–APCI–MS analysis and for subsequent hydrolysis studies were similarly prepared and differed only in the fact that the during the C18 purification stage, the 0.05 M phosphate used in the washing and elution buffers was replaced by water.

3.3. Hydrolysis and derivatisation

The extracted glycoalkaloids were hydrolysed to their constituent aglycones by the method of Lawson, Erb & Miller, 1992. The trimethyl silyl derivatives of the aglycones were prepared by evaporating an aliquot (500 μl) of the hydrolysis products to dryness, 200 μl of *N*, *O*-bis (trimethylsilyl)-trifluoroacetamide was then added and the sealed vial heated at 140°C for 45 min. The resulting solution was blown to dryness using a stream of gaseous nitrogen and the residue re-sus-

pended in dichloromethane. The resulting solution was analysed by GC–MS after 72 h storage at 4°C.

3.4. Quantitative analysis of glycoalkaloids

The concentrations of the individual glycoalkaloids in the semi-purified extracts were determined by a isocratic high performance liquid chromatographic method (Dale et al., 1993) based on that of Hellenäs, 1986.

3.5. Liquid chromatography–atmospheric pressure chemical ionisation–mass spectrometry

All liquid chromatography–mass spectrometry (LC–MS) was performed on a Finnigan MAT SSQ 710C single quadrupole instrument with an atmospheric pressure-chemical ionisation (APCI) interface (Thermo Quest, Hemel Hempstead, UK). The instrument was configured for positive ion chemical ionisation with a coronal discharge of ~5 μA. The samples were loaded via a 20 μl loop onto a reversed phase Hypersil C18 HPLC column (250 × 4.5 mm i.d., Jones Chromatography, Hengoed, Wales, UK). The glycoalkaloids were isocratically eluted at 1.2 ml min^{-1} with 38% aqueous-acetonitrile containing 0.05% ethanolamine (pH adjusted to 4.6 using trifluoroacetic acid). Flow to the APCI interface was reduced to 25% using a 3 : 1 flow splitter post-column. Full scan spectra were acquired over the range 250–1000 m/z with a scan time of 1.5 s.

3.6. Gas chromatography–mass spectrometry

The trimethyl silyl derivatives of the aglycones were characterised using a Hewlett Packard 5989B quadrupole mass spectrometer. The samples (1 μl) were directly introduced by cold on-column injection, onto a DB5-ms capillary column (30 m × 0.25 mm i.d., 0.25 μm film; J&W Scientific, Fulsom, California, USA). Oven temperature was held for 3 min at 55°C, then increased at 20°C min^{-1} to 280°C; after being held at this temperature for 20 min, the oven temperature was finally increased at 5°C min^{-1} to 325°C. The mass spectrometer scanned the mass range 35–700 a.m.u. at 1 scan s^{-1} and was used in electron impact mode (ionisation energy, 70 eV.; trap current, 300 μA; source temperature, 250°C). The data were processed using the Hewlett Packard G1034C MS Chemstation software package.

3.7. Thin-layer chromatography

Samples from the hydrolysed glycoalkaloid extracts were spotted onto aluminium-backed silica plates (Silica gel 60, Merck Ltd, Poole, UK). The solvent systems employed were as described by Shih & Kuć, 1974. The separated components were visualised using molybdophosphoric acid followed by heating at 120°C in a temperature-controlled oven.

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