3. Albright, J. D., Mitscher, L. A. et Goldmann, L. (1963) *J. Org. Chem.* **28**, 38.
4. Mamatas-Kalamaras, S., Sevenet, T., Thal, C. et Potier, P. (1975) *Phytochemistry* **14**, 1849.
5. Le Hir, A., Janot, M.-M. et Goutarel, R. (1953) *Bull. Soc. Chim. Fr.* 1027.
6. Poisson, J., Neuss, N., Goutarel, R. et Janot, M.-M. (1958) *Bull. Soc. Chim. Fr.* 1195.
7. Douzoua, L., Mansour, M., Debray, M., Le Men-Olivier, L. et Le Men, J. (1974) *Phytochemistry* **13**, 1994.
8. Gabetta, B. et Mustich, G. (1975) *Spectral Data of Indole Alkaloids*. Inverni della Beffa, Milan.
9. Wenkert, E. et Roychaudhuri, D. K. (1958) *J. Am. Chem. Soc.* **80**, 1613.
10. Bartlett, L., Dastoor, N. J., Hrbek J., Jr., Klyne, W., Schmid, H. et Snatzke, G. (1971) *Helv. chim. Acta* **54**, 1238.

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SUBCELLULAR LOCALIZATION OF STEROIDAL GLYCOALKALOIDS IN VEGETATIVE ORGANS OF *LYCOPERSICON ESCULENTUM* AND *SOLANUM TUBEROSUM*

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Key Word Index—*Lycopersicon esculentum*; *Solanum tuberosum*; Solanaceae; tomato; potato; subcellular localization; steroidal glycoalkaloids; α-tomatine, α-solanine, α-chaconine.

Abstract—Cell fractions from the major vegetative organs of tomato and potato plants were obtained by homogenization and differential centrifugation. In both species, steroidal glycoalkaloids were found to accumulate mainly in the soluble fraction, with smaller amounts in the microsomal fraction. Alkaloids appeared sporadically in the mitochondrial fractions but were only detected in lower fractions as an artefact.

INTRODUCTION

In a previous communication [1] it was shown that in unripe tomato pericarp α-tomatine is most abundant in the soluble phase of the cells, with only small amounts being associated with certain particulate fractions. However, since developing fruits show progressive degradation of tomatine [2] while developing vegetative organs synthesize and accumulate the alkaloid [2,3], localization of tomatine within vegetative cells cannot reasonably be deduced by extrapolating from studies conducted with reproductive cells. This communication provides information on the distribution of α-tomatine in vegetative cells of tomato and also on glycoalkaloid (α-solanine and α-chaconine) distribution in vegetative cells of potato. The data presented here complement and extend those reported elsewhere [4].

RESULTS AND DISCUSSION

Although glycoalkaloids varied in amount in different organs, their subcellular distribution was essentially the same in all tested organs of both species with the highest concentration (per unit of protein) in the 105000*g* supernatant and (with the exception of potato root) smaller amounts in the 105000 g pellet (Table 1). These data therefore suggest that, in vegetative cells of potato and tomato, as in tomato fruit cells, glycoalkaloids are located principally in the soluble phase, a conclusion which tends to be supported by the close correspondence found between expressed sap alkaloid levels and concentration in whole organs [4]. Although not studied here, it seems probable that the alkaloids of reproductive cells of potato show a similar distribution at the subcellular level to that in vegetative cells. The presence of glycoalkaloids (although in much smaller quantities) in, and, in some cases, their apparent absence from, the microsomal and mitochondrial fractions may reflect a variety of causes and conditions e.g. sites of synthesis, contamination, limits of detection, technical problems, etc., as has already been discussed [4].

No glycoalkaloids were detected in the 2500*g* or 500*g* pellets from potato cells or from tomato root cells, but in each of the first two tomato leaf fractionations, significant amounts of tomatine were observed in both these fractions. In view of the actual and relative amounts of alkaloid in these two fractions, the large amounts present in the leaf as a whole, and the fact that tomatine is not

Table 1. Distribution of glycoalkaloids in cell fractions from tomato and potato

		Tissue residue	500 *g* pellet	2500 *g* pellet	16000 *g* pellet	105000 *g* pellet	Supernatant
Tomato	Tomatine (mg)	10.6	N.D. (16.5†)	N.D. (3.6†)	Trace‡	1.5	137.6§
Leaf	Protein (g)	5.66	0.33	0.19	0.16	0.15	1.31
(50 g)*	Tomatine concn (mg/g protein)	1.9	—	—	Trace	9.9	105.0
Tomato	Tomatine (mg)	6.3	N.D.	N.D.	N.D.	Trace	2.9
Root	Protein (g)	3.82	0.11	0.12	0.11	0.11	1.20
(51 g)*	Tomatine concn (mg/g protein)	1.7	—	—	—	Trace	2.4
Potato	Alkaloid (mg)‖	10.3	N.D.	N.D.	0.7	0.9	19.3
Leaf	Protein (g)	10.57	0.12	0.16	0.35	0.22	1.42
(60 g)*	Alkaloid concn (mg/g protein)	1.0	—	—	2.0	4.1	13.6
Potato	Alkaloid (mg)‖	18.3	N.D.	N.D.	N.D.	N.D.	1.3
Root	Protein (g)	9.98	0.14	0.02	0.14	0.18	0.55
(83 g)*	Alkaloid concn (mg/g protein)	1.8	—	—	—	—	2.4
Potato	Alkaloid (mg)‖	0.9	N.D.	N.D.	0.05	0.09	4.7
Tuber	Protein (g)	9.75	0.11	0.11	0.14	0.10	1.60
(202 g)*	Alkaloid concn (mg/g protein)	0.1	—	—	0.4	0.9	2.9

N.D. Not detectable by assay or TLC; * Fresh weight of tissue extracted; † Alkaloid deposited in the fraction but not a genuine component of it; ‡ Below limits of assay method but detectable by TLC; § Includes values in parentheses for 500 *g* and 2500 *g* pellets ‖Calculated as α-solanine.

particularly soluble above pH 6, it was suspected that the alkaloid might be coming out of solution and being deposited in these pellets. When the fractionation was repeated using a similar buffer but of pH 5.3, tomatine could not be detected in either of the first two pellets, although it was still present in approximately the same amounts as previously in the 105000 *g* and 16000 *g* pellets and in larger amounts in the supernatant. It was therefore concluded that tomatine is not a major component of the cell material present in the 500 *g* and 2500 *g* fractions and that the relatively large quantities of alkaloid observed in these fractions had originated in the soluble fraction. However, the pH 5.3 buffer appeared to cause a certain amount of disintegration in some of the organelles and so, in order to obtain meaningful data, the fractionation was conducted at pH 7.4 and the amounts of alkaloid found in the first two fractions (Table 1, data in parentheses) were added to the supernatant value.

The findings of this work do not resolve, but rather confirm, the previously-discussed [1] disparity concerning the intracellular distribution of steroidal glycoalkaloids (mainly soluble) [1] and steroidal saponins (mainly particulate [5]. Of significance, however, may be the recent finding by Nickell and Staba [6] that in cells of *Digitalis lanata* and *D. purpurea* the cardiac (steroid) glycoside digoxin accumulates mainly, but not exclusively, in the soluble phase.

EXPERIMENTAL

Methods were as previously described [1] but with the following modifications

Plant material. Tomato (*Lycopersicon esculentum* cv. Best of All) and potato (*Solanum tuberosum* cv. Majestic) plants were pot-grown in glasshouses and were 2–3 months old at the time of harvest. Leaf lamina tissue was weighed immediately on excision whereas roots and tubers were first washed to remove adhering particles then surface-dried and weighed. Tubers were not peeled before homogenization.

Fractionation of tissues. Tissues were homogenized in 0.1 M Pi buffer in the ratio of 2 ml per g fr. wt. (except that no vols < 100 ml were used). Following homogenization in a blender, root tissues were further broken using a mortar and pestle and sand. The tissue remaining after filtering through muslin was washed twice with the same vol. of buffer. None of the fractions was subjected to sonication.

Extraction of glycoalkaloids. Following ammonification to pH 10, potato extracts were heated at 80° for 30 min after which they were cooled as previously. After centrifuging ammonified extracts, all pellets were dried, extracted with MeOH and the combined MeOH extracts evaporated to dryness. Extracts were then made to vol., in 96% EtOH in the case of tomato, and in MeOH in the case of potato.

Assay of potato alkaloids. Total glycoalkaloids of potato tissues were estimated as α-solanine by the colorimetric method of Shih *et al.* [7]. This involved treating with acidified EtOH and 1% HCHO, then measuring absorbance at 562 nm. Blanks were treated with H_2O instead of HCHO. The amount of alkaloid was calculated using a standard curve prepared with authentic α-solanine.

TLC. In all extracts, presence or absence of glycoalkaloids was confirmed by TLC. Solvent systems used were *iso*-PrOH–HCOOH–H_2O (73:3:24); *n*-BuOH–HOAc–H_2O (4:1:5) upper phase and 10:3:1); 95% EtOH. Developed plates were sprayed with modified Dragendorff's reagent [8].

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REFERENCES

1. Roddick, J. G. (1976) *Phytochemistry* **15**, 475.
2. Sander, H. (1956) *Planta* **47**, 374.
3. Roddick, J. G. (1974) *Phytochemistry* **13**, 1459.
4. Roddick, J. G. in *Symposium on the Biology and Taxonomy of the Solanaceae* (Hawkes, J. G. and Lester, R. N., eds.). Academic Press, London (in press).

5. Akahori, A., Yasuda, F., Kagana, K., Ando, M. and Togami, M. (1970) *Phytochemistry* **9**, 1921.
6. Nickell, S. and Staba, E. J. in *Plant Tissue Culture and its Biotechnological Application* (Barz, W., Reinhard, E. and Zenk, M. H., eds.). Springer-Verlag, Berlin (in press).
7. Shih, M., Kuć, J. and Williams, E. B. (1973) *Phytopathology* **63**, 821.
8. Cromwell, B. T. (1955) in *Modern Methods of Plant Analysis* (Paech, K. and Tracey, M. V., eds.) Vol. IV, p. 367. Springer-Verlag, Berlin.