ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. 55, nr 4, 653-657
1986

Steroidal alkaloids of *Solanum lycopersicoides*

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(Received: December 27, 1985. Accepted: January 31, 1986)

Abstract

Tomatine was identified as the sole glycoalkaloid of *Solanum lycopersicoides* leaves. Its quantity in field-grown plants was about 3.2–3.5% of leaf dry matter. This finding gives more evidence of the strong affinity of *S. lycopersicoides* to the genus *Lycopersicon*. Furthermore, it suggests that this species can be a useful germplasm in tomato breeding programs.

Key words: *Solanum lycopersicoides*, *Solanaceae*, *steroid alkaloids*, *tomatine*, *tomato*

INTRODUCTION

Solanum lycopersicoides Dun. is a perennial shrub that grows in southern Peru and is known under the local name "tomatillo". It belongs to the genus *Solanum*, series *Juglandifolia*. It possesses a strong morphological affinity to *Lycopersicon* species (Rick 1979) and crossing with the tomato has been successful (Rick 1951). *S. lycopersicoides* is of interest to tomato breeders because of its disease resistance (Phills et al. 1977a) and cold tolerance (Robinson and Phills 1977, Phills et al. 1977b). However, it is commonly known that some nightshades contain toxic concentrations of glycoalkaloids (Zintak 1977). These glycoalkaloids, if transferred to tomato, may be of help in insect and disease resistance, but also may prove to be a health hazard (Rick 1951, Roddick 1974).

Much concern has already been directed to the wild tuberforming *Solanum* species that are used in potato breeding (Osman et al. 1978), but virtually nothing is known about glycoalkaloid occurrence in *Solanum*

lycopersicoides. In the preliminary brief note (Oleszek et al. 1980), a much higher foliar tomatine concentration in *S. lycopersicoides* than in commercial tomato cultivars was reported. This paper provides data to support this finding.

MATERIAL AND METHODS

EXTRACTION AND ISOLATION OF ALKALOIDS

Leaves of *S. lycopersicoides* were collected from field-grown plants, freeze-dried and finely powdered. A 300 g sample of this material was extracted three times by maceration with 2 dm^3 of ethanol containing 5% (v/v) acetic acid. The extract was condensed under reduced pressure, and steroidal alkaloids were precipitated with NH_4OH at pH 10. The precipitate was separated from the solution by centrifugation for 10 min at 10000 × g, dried and defatted with methylene chloride and then extracted with ethanol in a Soxhlet apparatus. The volume of the ethanol extract was reduced and alkaloids precipitated with excess ammonified water and centrifuged. This yielded 4 g of slightly greenish, crude alkaloids (CA). A preliminary test of their composition was made by TLC.

ACID HYDROLYSIS

One g of CA was dissolved in 60 cm^3 of ethanol, and 40 cm^3 of 2.5 N HCl was added. This was heated in a steam bath for 3 h and then the alcohol was removed in vacuo at 40° C. A greenish crystalline compound precipitated. It was filtered and washed with a small volume of ethanol and ether. A small portion (5 cm^3) of filtrate was evaporated a few times until the HCl was completely removed, then dissolved in 1 cm^3 of 10% isopropanol and chromatographed for sugars. The washed precipitate was redissolved in methanol and ether was added. This furnished 260 mg of a crystalline compound with mp. 252–255° C, MS m/e (rel int.) 415 (11), 400 (3), 387 (18), 372 (1), 358 (2), 316 (2), 287 (1), 273 (2), 180 (2), 152 (5), 138 (69), 125 (10), 114 (100), 36 (11) which was identified as tomatine hydrochloride.

This compound was dissolved in 75% ethanol and the solution was ammonified with ammonium hydroxide. This yielded 170 mg of crystalline tomatidine mp. 205–206° C, MS m/e (rel int.) 415 (19), 400 (5), 387 (13), 372 (1), 358 (2), 316 (3), 287 (3), 273 (10), 255 (2), 180 (3), 161 (13), 152 (10), 138 (87), 125 (20), 114 (100), 93 (24), 79 (25).

THIN LAYER CHROMATOGRAPHY

The CA were chromatographed together with commercial tomatine (Sigma) on silica gel (DC-Fertigeplatten Kiselgel 60 Merck, 0.25 mm) in 4 solvent systems: isopropanol-formic acid-water (IFW 37: 3: 24), chloroform-methanol (96: 4), n-butanol-acetic acid-water (BAW, 4: 1: 5), ethanol-ethyl acetate-diethylamine (15: 5: 1). Tomatidine was chromatographed with an original sample (Sigma) in 2 solvents: ethyl acetate-n-heptane-diethylamine (7: 3: 1) and n-hexane-ethyl acetate (1: 1). Tomatine and tomatidine were visualized by spraying the plates with 50% (v/v) H_2SO_4 and heating at 100° C. TLC of sugars was performed on cellulose (DC-Fertigeplatten Cellulose, Merck) and developed with benzene-n-butanol-pyridine-water (1: 5: : 3: 3 upper layer). This was visualized by spraying with an ammonified silver nitrate solution.

QUANTIFICATION OF TOMATINE

The foliar tomatine content was evaluated with gravimetric and colorimetric methods. For this purpose 50 g of the youngest fully expanded leaves were homogenized three times in a Waring blender with 100 cm^3 of 3% acetic acid followed by centrifugation at 10000 × g. The combined supernatants were ammonified with ammonium hydroxide to give a pH above 10, heated to 70° C for 5 min and stored overnight. It was then filtered on Whatman 2 paper and dried at 60° C. The dry sample was extracted in a Soxhlet with 95% methanol for 1 h and, after removing the solvent, the dry residue was dissolved in 20 cm^3 of 96% ethanol. 20 mm^3 of this solution was added to 2 cm^3 of 2% chloramine-T in concentrated sulfuric acid and tomatine was quantified colorimetrically at 474 nm using a standard curve (unpublished data). To the remainder of the ethanol solution, 2 cm^3 of 4% cholesterol in 95% ethanol were added and the tomatine-cholesterol complex was allowed to precipitate overnight at room temperature. The precipitates were filtered on preweighed filter paper, washed with small portions of ethanol, dried at 60° C and weighed. Tomatine was estimated from the cholesterol-tomatine ratio.

RESULTS AND DISCUSSION

The thin layer chromatography of crude alkaloids (CA) revealed the appearance of a single spot in all of the systems used. The R_f values of this spot were in all cases the same as for commercial tomatine.

The chemical and spectral analysis of hydrolysis products gave further proof of this finding. Acid hydrolysis yielded a crystalline compound with a melting point value of 205–206° C and glucose, galactose and xylose as sugar chain components. These data and mass spectrograms of the aglycone moiety are in good agreement with the results obtained for tomatine and its hydrolysis products by Sato et al. (1952) and Barber et al. (1968) and reviewed by Roddick (1974). Thus it was proven that tomatine is the only foliar glycoalakloid of *S. lycopersicoides* leaves.

It was previously reported (Roddick 1974, Schreiber 1968) that tomatine is restricted in its taxonomic distribution to the family *Solanaceae* and that in the genus *Solanum* it is most often accompanied by other glycoalkaloids, whereas in the genus *Lycopersicon* it is usually the only steroidal alkaloid present. Thus, the occurrence of tomatine as the sole alkaloid in *Solanum lycopersicoides* leaves provides further proof of the great affinity between this species and the genus *Lycopersicon*.

The foliar tomatine content of *S. lycopersicoides* was surprisingly high. With the gravimetric method, based on the ability of tomatine to complex with cholesterol in vitro (Roddick 1979), the leaf tomatine content was found to be 3.5% of dry matter. With the colorimetric method a result of 3.2% was obtained. These values are much higher than the leaf tomatine content of commercially available tomato cultivars (Roddick 1974). They are only comparable to the glycoalkaloid content of some wild *Solanum* species being used as raw material for synthesis of steroidal hormones (Bradley et al. 1978).

This high foliar tomatine content may make *S. lycopersicoides* useful germplasm for breeding tomatos with an increased tomatine level and thereby resistant to different parasites. At the same time, it is unlikely that such new cultivars would present a hazard to human health; no glycoalkaloid more toxic than tomatine would be introduced, and the increased foliar concentration of tomatine would not necessarily be accompanied by an increase of fruit alkaloid content. It has been shown that tomatine is not transported from the vegetative organs to fruits, but is accumulated in fruits entirely due to synthesis (Elthayeb and Roddick 1985). Even if the green fruit tomatine content of new cultivars is higher, the enzyme system of tomato fruits is able to reduce the tomatine content to zero during fruit ripening (Roddick 1974). Therefore, *S. lycopersicoides* glycoalkaloids do not appear to present a health hazard if introduced into tomato breeding stocks.

REFERENCES

Barber M., Green B. N., Wolstenholme W. A., 1968. Fragmentation maps and their use in structural analysis. Adv. Mass Spectrom. 4: 89–98.

Bradley V., Collins D. J., Crabbe P. G., Eastwood F. W., Irvine M. C., Swan J. M., Symon D. E., 1978. A survey of Australian *Solanum* plants for potentially usefull sources of solasodine. Aust. J. Bot. 26: 723–754.

Eltayed E. A., Roddick J. G., 1985. Biosynthesis and degradation of α-tomatine in developing tomato fruits. Phytochemistry 24: 253–257.

Oleszek W., Shannon S., Robinson R. W., 1980. Glycoalkaloid composition of *Solanum* species of interest for tomato breeding. Tomato Gen. Coop. Report 30: 28.

Osman S. F., Herb S. F., Fitzpatrick T. J., Schmiediche P., 1978. Glycoalkaloid composition of wild and cultivated tuber-bearing *Solanum* species of potential value in potato breeding programs. J. Agric. Food Chem. 26: 1246–1248.

Phills B. R., Provvidenti R., Robinson R. W., 1977a. Reaction of *Solanum lycopersicoides* to viral diseases of the tomato. Tomato Gen. Coop. Report 27: 18.

Phills B. P., Robinson R. W., Short J. W., 1977b. Resistance to early blight and other fungal diseases in *Solanum lycopersicoides*. Tomato Gen. Coop. Report 27: 18–19.

Rick C. M., 1951. Hybrids between *Lycopersicon esculentum* Mill. and *Solanum lycopersicoides* Dun. Proc. Nat. Acad. Sci. USA 37: 741–744.

Rick C. M., 1979. Biosystematic studies in *Lycopersicon* and closely related species of *Solanum*. Proc. Int. Symp. Biol. Chem. Solanaceae, Academic Press N. Y. pp. 667–677.

Robinson R. W., Phills B. R., 1977. *Solanum lycopersicoides*: a source of cold tolerance for the tomato. Tomato Gen. Coop. Raport 27: 25.

Roddick J. G., 1974. The steroidal glycoalkaloid α-tomatine. Phytochemistry 13: 9–25.

Roddick J. G., 1979. Complex formation between Solanaceous steroidal glycoalkaloids and free sterols in vitro. Phytochemistry 18: 1467–1470.

Sato Y., Katz A., Mosetting E., 1952. Degradation of tomatidine. J. Amer. Chem. Soc. 74: 538–539.

Schreiber K., 1968. Steroid alkaloids: The *Solanum* group. In: The alkaloids. Manske (ed.). Academic Press, New York.

Zintak A., 1977. Steroids and capsaicinoids of Solanaceous food plants. In: Nightshades and health. Childers N. F. and Russo G. M. (eds.). Somerset Press Inc. Somerville N. J. pp. 41–91.

Alkaloidy sterydowe Solanum lycopersicoides

Streszczenie

Wykazano, że tomatyna jest jedynym glikoalkaloidem występującym w liściach *Solanum lycopersicoides*. Jej zawartość w roślinach rosnących w warunkach polowych wynosiła 3.2–3.5% suchej masy. Wyniki te są dodatkowym dowodem świadczącym o bliskim pokrewieństwie *S. lycopersicoides* do rodzaju *Lycopersicon*. Ponadto sugerują one, że gatunek ten może być dobrym i bezpiecznym źródłem cech odpornościowych w hodowli pomidora.