

GLYCOALKALOIDS IN TISSUE CULTURE OF *SOLANUM* SPECIES.
DEHYDROCOMMERSIONINE FROM CULTURED ROOTS OF *SOLANUM*
CHACOENSE

R.M. ZACHARIUS and S.F. OSMAN

Eastern Regional Research Center*, Philadelphia, Pa. 19118 (U.S.A.)

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SUMMARY

Dehydrocommersonine, a newly described glycoalkaloid, was isolated from root tissue initiated from a callus culture of *Solanum chacoense*. Tuber tissue of this plant introduction line did not contain commersonine or its dehydro compound. Callus of *S. chacoense* and three cultivars of *Solanum tuberosum* that failed to initiate roots were devoid of glycoalkaloids. Callus of *S. tuberosum* varieties Merrimack and Wauseon which had developed some roots were found to contain solanine and chaconine.

INTRODUCTION

Wild species of *Solanum* have been useful in developing new cultivars of *Solanum tuberosum* for the purpose of incorporating pest resistant characteristics which may be attributed to steroid glycoalkaloids [1]. During a study of the glycoalkaloid composition of tuber-bearing wild *Solanum* species, Osman et al. [2] recently reported that selected plant introduction lines of *Solanum chacoense* and *Solanum commersonii* contain two major glycoalkaloids, demissine and a newly described compound, commersonine.

Glycoalkaloid production in cultured tissues of species of *Solanum* has not received much attention. Although plant cell and tissue cultures, in general, have not been noted for readily producing secondary metabolites as the native tissue of origin, cultured callus tissue from tubers provides a potential means of studying the biosynthesis and accumulation of potato glycoalkaloids with

*Agricultural Research Service, U.S. Department of Agriculture.

Abbreviations: GLC, gas-liquid chromatography; PI, plant introduction, TLC, thin-layer chromatography.

manipulative variables which tubers do not afford. In this study glycoalkaloids were not found in the callus of *S. chacoense* or of any of the cultivars of *S. tuberosum* which had not shown macroscopic evidence of root initiation. On the other hand, roots initiated from callus of a selected plant introduction (PI) line of *S. chacoense* produced a new glycoalkaloid, dehydrocommersonine. Callus from *S. tuberosum* which had initiated roots produced the major glycoalkaloids of the explant tuber tissue.

MATERIALS AND METHODS

Tissue culture of S. chacoense and S. tuberosum

Callus was initiated from explants of tubers of *S. chacoense* PI 320281. Tubers were washed in 1% sodium hypochlorite solution for 15 min and borings of tissue were removed under sterile conditions with a No. 2 cork borer. Slices were cut with a scalpel; those thin enough to be translucent (0.2–0.4 mm) were selected for culture. The explants were cultured on an 0.8% agar medium (RM-1964) of Linsmaier and Skoog [3] modified by the replacement of indoleacetic acid with 1.0 mg/l α -naphthalene acetic acid and the inclusion of 0.2 mg/l kinetin. The medium was adjusted with 1 N NaOH so that a pH of 5.7 resulted after 15 min autoclaving. The planted explants were grown in Petri dishes at 28°C in an unlighted incubator. After 4 weeks the callus tissues were transferred to a fresh medium and incubated under the same conditions. After 10 weeks some of the enlarged calli initiated roots, which continued to proliferate until a large mass of fine white roots covered most of the container. Some of the calli that failed to initiate roots were exposed to continuous light from Gro-Lux* fluorescent lamps for a period of up to 6 weeks with another medium transfer. Those exposed to fluorescent light became partially green but did not initiate roots.

Calli of *S. tuberosum* var. Kennebec, var. Wauseon and var. Merrimack were initiated and grown as above. Roots did not develop from the callus tissues of the Kennebec variety. Some were exposed to the above light conditions. One-half of the Merrimack and Wauseon calli developed some root tissue.

Glycoalkaloid isolation

The tissue culture roots of *S. chacoense* PI 320281 (~750 mg) were macerated and stirred in 100 ml of a CHCl_3 – CH_3OH solution (1 : 1) for 3 h at room temperature. The solution was filtered and 100 ml of 0.8% Na_2SO_4 solution was added to the filtrate. The aqueous CH_3OH layer was separated from the CHCl_3 layer and concentrated in vacuo. The residue was dissolved in 3 ml 0.5 M H_2SO_4 and adjusted to pH 10 with concentrated NH_4OH . The alkaline solution was heated for 30 min at 70°C and chilled overnight; the resulting precipitate was centrifuged, removed and dried. The dry weight of the precipitate was approx. 50 mg.

*Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

TABLE I

GLC/MS IDENTIFICATION OF AGLYCONE^a

Compound	GLC t_R	t_R^b (TLC)	[M+]	Major ions ^c		
Unknown				150	379	204
Peak 1	12.0	0.96	379	150	204	397
Peak 2	13.8	0.64	397	150	379	204
Solanthrene	12.1	0.96	379	150	204	397
Solanidine	13.8	0.64	397	150	204	397

^a GLC/MS were obtained on a LKB-9000. GLC conditions: 3% OV-17 coated on Gas-Chrom Q (100–120 mesh) packed in 1/8" × 10' glass column; oven temperature programmed from 200 to 280°C @ 6°C min; He flow 30 ml/min.

^b TLC conditions: 250 μm silica gel G (Analtech); developing solvent: cyclohexane–ethylacetate (1 : 1, V/V).

^c Three most intense ions in spectrum in order.

TABLE II

IDENTIFICATION OF GLYCOSIDIC LINKAGES

GLC t_R^a		MS fragments	Identification
Ref	Obs		
1.0	1.0	117,161,205	2,3,4,6-tetramethyl-1,5-diacetyl glucose
2.42	2.43	117,233	2,3,6-trimethyl-1,4,5-triacetyl galactose
4.02	4.03	161,261	4,6-dimethyl-1,2,3,5-tetracetyl glucose

^a GLC conditions: column 1/8" × 6'; 3% ECNSS-M on Gas-Chrom Q, 100–120 mesh; column temp., 180°C; He flow rate, 40 ml/min. Relative retention time to 2,3,4,6-tetramethyl-1,5-diacetyl glucose.

Thin-layer chromatographic (TLC) analysis on silica gel G plates employing CHCl₃–CH₃OH (1 : 1) saturated with 1% NH₄OH yielded a single spot (R_F , 0.25) with I₂ vapor; and gas–liquid chromatographic (GLC) analysis [4] also yielded a single peak.

All other callus tissues were extracted for glycoalkaloids and subjected to TLC and GLC as above.

Characterization of glycoalkaloid

A portion of the glycoalkaloid isolated from the tissue culture roots was hydrolyzed in 1 N H₂SO₄ for 2 h at 100°C. The hydrolysate was neutralized with concentrated NH₄OH and extracted with benzene. The benzene extract was concentrated and analyzed by GLC/MS. The two main components (representing ~96% of total) were solanidine and solanthrene (Table I). The

latter is a product of the dehydration of solanidine glycoalkaloids under the above hydrolysis conditions [5]. Demissidine was not detected by GLC. A second portion of the glycoalkaloid was hydrolyzed under the same conditions and neutralized with BaCO₃. The BaSO₄ was removed by centrifugation and the supernatant concentrated to dryness. The sugars in the residue were converted to the aldonitrile acetate derivatives by the method of Varma et al. [6] and analyzed by GLC. Glucose and galactose in a 3 : 1 ratio were the only sugars present. The sugar sequence was determined by the permethylation technique and GLC [7] (Table II).

RESULTS AND DISCUSSION

The glycoalkaloid isolated from the tissue culture of roots of *S. chacoense* differs from commersonine only in the unsaturated C₅-C₆ bond of the aglycone (i.e. solanidine) (Fig. 1). We have given it the name dehydrocommersonine. From approx. 750 mg of cultured roots, approx. 50 mg of dehydrocommersonine were isolated. Moreover, no other glycoalkaloids could be detected. An explanation of this unusually high yield of glycoalkaloid in cultured roots is presently being investigated.

An unidentified glycoalkaloid (courtesy of S. Sinden, ARS, Beltsville, Md., U.S.A.) isolated from leaves, stems and tubers of *Solanum spegazzinii* was found to be identical with dehydrocommersonine from root callus culture by the above criteria. Neither commersonine nor the dehydro compound have been found in the native tuber tissue of *S. chacoense* PI 320281 which contained solanine and chaconine (S.L. Sinden, unpublished). Callus tissue and callus with root initials from explants of this same PI line did not demonstrate the presence of any other glycoalkaloids whether grown in light or dark.

Roots that were allowed to develop on stored tubers of *S. chacoense* (PI line unknown) were found to contain solanine and chaconine with smaller amounts of commersonine and/or dehydrocommersonine and demissine, although the tuber yielded only solanine and chaconine. This evidence suggests that the roots of *S. chacoense* are capable of synthesizing glycoalkaloids not found in the tuber. Shih and Kuć [8] have described the solamarines in the leaves of the Kennebec potato which were not found in the unstressed tuber. Moreover, because of the nature and sequence of the sugars in the trisaccharide glycosides, solanine and chaconine, their derivation from the root tetrasaccharide glycoside, dehydrocommersonine by simple enzymatic hydrolysis is not possible.

We have as yet to demonstrate the ability of the roots or tubers to enzymatically interconvert dehydrocommersonine and commersonine.

As in *S. chacoense* callus, no glycoalkaloids could be detected in any of the *S. tuberosum* calli (devoid of roots) either grown entirely in the dark or later transferred to the light where greening occurred. However, the Merrimack and Wauseon calli that developed roots were found to contain measurable levels of solanine and chaconine, both of which are normally present in the tubers.

Roddick and Butcher [9] found evidence that the steroidal glycoalkaloid

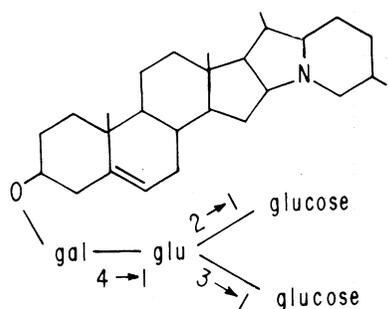


Fig. 1. Dehydrocommersonine.

tomatine can occur in tomato callus as well as roots initiated from callus although the latter had a higher tomatine content. Further, 3-year-old hypocotyl callus containing detectable levels of tomatine had never produced organized structures. The appearance of tomatine in callus grown on two types of media but not the third was offered as evidence that certain components of the media are capable of affecting accumulation of the alkaloid. Raj Bhandary et al. [10] observed that the principal alkaloids of *Atropa belladonna* are produced only in association with organized root structures (as in the whole plant), but considered the possibility that a given chemical environment may be capable of activating alkaloid biosynthesis without the prior development of organized structures. Nevertheless, since cell differentiation and early structure organization is not always readily discernible, this distinction may be an ambiguous one.

REFERENCES

- 1 K. Schreiber, in R.H.F. Manske (Ed.), *The Alkaloids*, Vol. 10, Academic Press, New York, 1968, p. 1.
- 2 S.F. Osman, S.F. Herb, T.J. Fitzpatrick and S.L. Sinden, *Phytochemistry*, 15 (1976) 1065.
- 3 E.M. Linsmaier and F. Skoog, *Physiol. Plant.*, 18 (1965) 100.
- 4 S.F. Herb, T.J. Fitzpatrick and S.F. Osman, *J. Agric. Food Chem.*, 23 (1975) 520.
- 5 R. Kuhn and I. Low, *Chem. Ber.*, 95 (1962) 1748.
- 6 R. Varma, R.S. Varma and A.H. Wardi, *J. Chromatogr.*, 77 (1973) 222.
- 7 H. Bjorndal, C.G. Hellerqvist, B. Lundberg and S. Svenson, *Angew. Chem. Int. Ed. Engl.*, 9 (1970) 610.
- 8 M. Shih and J. Kuć, *Phytochemistry*, 13 (1974) 997.
- 9 J.G. Roddick and D.N. Butcher, *Phytochemistry*, 11 (1972) 2019.
- 10 S.B. Raj Bhandary, H.A. Collin, E. Thomas and H.E. Street, *Ann. Bot. (London)*, 33 (1969) 647.