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High-Performance Liquid Chromatographic Separation of Eastern Black Nightshade (*Solanum ptycanthum*) Glycoalkaloids

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The glycoalkaloids present in Eastern black nightshade were isolated, purified, and identified. An analytical system using high-performance liquid chromatography has been developed for their quantitation. The total glycoalkaloid content of nightshade berries was 12-14 mg/g and the alkaloids found were β-solamargine (2.4 mg/g), α-solamargine (3.3 mg/g), α-solasonine (4.9 mg/g), α-chaconine (0.5 mg/g), and α-solanine (1.0 mg/g).

A recent review (Rogers and Ogg, 1981) on the biology of weeds in the *Solanum nigrum* complex in North America describes four common species of nightshades. The four species are discussed in greater detail in another publication (Ogg et al., 1981). The nightshades have been considered minor weeds in most areas, but in recent years they have become pests in various crops in many parts of the world. The most recent crop to be infested has been the soybean. Since nightshade is a broad-leafed plant, its control by herbicides in soybeans is difficult (Jennings and Fawcett, 1977). Judicious use of herbicides and crop rotation is very important for nightshade control. Nightshade species are members of the Solanaceae family, which contains glycoalkaloids that can cause poisoning and even death when ingested in sufficient quantities.

In 1981, infestation of soybeans occurred in many of the growing areas of the United States. For 3 months in the fall of 1981, the Federal Grain Inspection Service of the U.S. Department of Agriculture conducted a survey on the incidence of eastern black nightshade berries (*Solanum ptycanthum* Dun) in soybeans (Hoy, 1982). On the basis of the results of the survey, the percentage of infested samples is small and the nightshade problem appears to be localized. In Mobile, AL, 0.3% of the soybean samples contained nightshade berries, whereas inspectors at Cedar Rapids, IA, and Fort Dodge, IA, found 0.6 and 1.9% infested samples, respectively.

We have identified the alkaloids present in eastern black nightshade, and this communication describes their isolation and purification. A high-performance liquid chromatographic (HPLC) procedure for quantitation was developed using methanol for the extraction and nicotine as an internal standard.

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MATERIALS AND METHODS

Materials. α-Solanine, α-chaconine, and nicotine standards were purchased from Sigma Co., St. Louis, MO. Solasonine and solamargine standards were generously supplied by Dr. Stanley Osman, Eastern Regional Research Center, U.S. Department of Agriculture, Philadelphia, PA. Dried nightshade berries separated from contaminated soybeans were supplied by Dr. Manjit Misra, Department of Plant Pathology, Iowa State University, Ames, IA. Plants grown from seeds separated from these berries were identified as Eastern black nightshade (*S. ptycanthum* Dun) by Dr. A. G. Ogg, Jr., Irrigated Agriculture Research and Extension Center, Prosser, WA, and Dr. E. E. Shilling, Jr., University of Tennessee, Knoxville, TN.

Dried nightshade berries were ground for 1-3 min in a Varco (Bellville, NJ) electric grinder, Model 228100. The full-fat flour from this grinder contained particles, 90% of which passed a 40-mesh screen. The full-fat powder was extracted for 6 h with *n*-hexane in a Soxhlet extractor to give a defatted material. After extraction, the hexane was stripped from the oil, and the oil was then extracted with methanol. No glycoalkaloids were detected in the methanol-soluble fraction, indicating a defatting process in which nearly all of the glycoalkaloids were left in the defatted residue.

HPLC Analysis. A Waters Associates (Milford, MA) HPLC system comprised of a M-45 solvent delivery system and a Model 450 variable-wavelength detector was used. All columns were protected with guard columns of Porasil μBondapak. The solvent flow rate was usually 2 mL/min and the absorption was measured at 200 nm. Solvents were spectral grade, and distilled water was deionized before use. All solvent ratios are on a volume basis.

HPLC conditions were according to Morris and Lee (1981), with some minor modifications. A 1-g defatted ground sample was extracted with 20 mL of methanol, containing approximately 0.20 mg of nicotine/mL as an internal standard, by refluxing on a steam bath for 2 h.

All extracts were filtered through 0.45-μm Millipore filters before injection on the HPLC. The sample was chromatographed on a radially packed silicic acid column, 8-mm i.d., by using acetonitrile–water (77.5:22.5) with 0.01% ethanolamine, titrated to pH 4.0 with phosphoric acid. For our use, C_8, C_{18}, and NH_2 columns with CH_3CN–H_2O, tetrahydrofuran (THF)–H_2O–CH_3CN, and $(CH_3)_2CHOH$–CH_3OH solvents were all unsatisfactory.

Total Glycoalkaloid Content by Titration. Total glycoalkaloid content of our defatted nightshade was also determined by the titration method of Fitzpatrick and Osman (1974) and Fitzpatrick et al. (1978). A sample (20 g) was extracted with 100 mL of CH_3OH–$CHCl_3$ (2:1) in a Waring Blendor for 5 min and filtered through No. 1 Whatman, and then the extract was mixed with 100 mL of 0.8% Na_2SO_4. The mixture was shaken in a separatory funnel and allowed to settle overnight. The upper (CH_3OH) layer was separated, brought to 250 mL with CH_3OH, and taken to dryness in the presence of Antifoam A to yield a gummy residue, which was dissolved in 15 mL of 2 N H_2SO_4. The solution was then heated for 2 h on a steam bath and made basic with 10 mL of 4 N NaOH. The aglycons were extracted with three 15-mL portions of benzene. The benzene was then stripped off, and the residue was taken up in 5 mL of CH_3OH. Samples were titrated with a solution of 0.067% bromphenol blue and 10% phenol in absolute methanol, against a blank of methanol. The total glycoalkaloids were calculated by using a standard curve prepared with known concentrations of α-solanine and α-chaconine in methanol.

Preparation and Purification of α-Solamargine, β-Solamargine, and α-Solasonine. The major glycoalkaloids in a methanol extract of nightshade were found to be α-solamargine, β-solamargine, and α-solasonine, so in order to isolate these compounds we tried a general flocculation procedure for glycoalkaloids following the procedure of Bushway et al. (1980), who originally used the method to isolate chaconine and solanine from potato tubers. Bushway's procedure with some modifications was used on both full-fat and defatted ground nightshade berries. Defatted nightshade (20–50 g) was mixed (1:10 w/v) with CH_3OH–$CHCl_3$ (2:1) and blended in a Waring Blendor for 5 min. The solution was vacuum filtered through Whatman No. 1, and the residue was reextracted with fresh solvent (1:5). Filtrates were combined, evaporated to 10–25 mL, and acidified with 0.2 N HCl. The mixture was sonicated for 5 min and centrifuged at 10000 rpm for 15 min. The supernatant was decanted and NH_4OH was added to make the solution alkaline, pH 10.4 or above. The solution was heated at 70 °C for 30 min and then refrigerated overnight to further enhance flocculation. The cold mixture was centrifuged, and the resulting pellet (~0.01 g/g of nightshade) was dissolved in tetrahydrofuran–water–acetone (5:3:2).

For further purification, the flocculated sample was streaked on preparative TLC plates (silica gel 60 F 254, 2 mm) and developed in Boll's (1962) solvent ($CHCl_3$–CH_3OH–1% aqueous NH_4OH, 2:2:1) by using two ascensions with the same solvent for optimum separation. Bands were visualized under long-range (~360 nm) fluorescent light by ANS (8-anilinonaphthalene-1-sulfonate) spray (Gitler, 1972). Fluorescing bands were scraped off and eluted with CH_3OH–$CHCl_3$ (1:1). Other solvent systems, such as $CHCl_3$–CH_3OH–H_2O (65:75:4), and sprays (anisaldehyde, $SbCl_3$) were examined but were less successful.

Identification of Glycoalkaloids. The three purified components were compared to standards by TLC, GC, and HPLC. TLC conditions were the same as for purification, except that samples were chromatographed on regular silica plates (0.25 mm) with only one ascension. GC of the separated monosaccharides of each component was done according to the method described earlier of Eldridge et al. (1979).

The three components isolated by TLC and the purchased samples were also hydrolyzed (1 N ethanolic HCl, steam bath for 45 min, 90 °C) and extracted with benzene, and their aglycons were identified by TLC and HPLC. The structures of the five glycoalkaloids found in Eastern black nightshade are shown in Figure 1.

Quantitation of Glycoalkaloids. Quantitation of the five glycoalkaloids was achieved by comparing the peak area of the glycoalkaloid with that of a known amount of internal standard. Response factors relative to nicotine

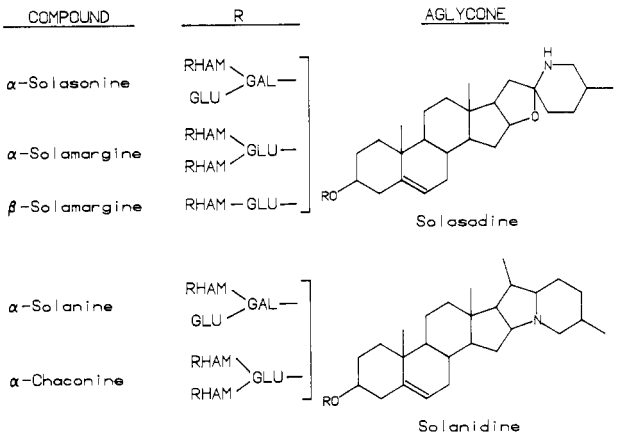

Figure 1. Structures of five glycoalkaloids found in Eastern black nightshade.

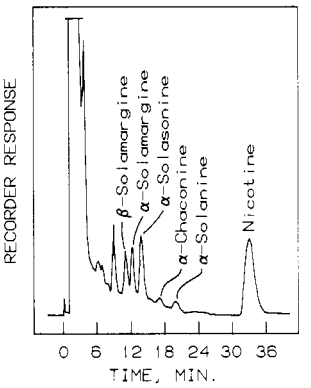

Figure 2. HPLC elution diagram of CH_3OH extracts of *S. ptycanthum* with added nicotine.

Table 1. Extraction of Glycoalkaloids from Defatted Nightshade (*S. ptycanthum*) with Varying Solvent Systems and Extraction Times[a]

solvent	total glycoalkaloid content, mg/g, for hours refluxed			
	1	2	3	4
100% CH_3OH	13.9	14.0	12.0	13.2[b]
50% CH_3OH	6.5	6.8	7.7	8.8
100% CH_3CN	0.0	0.0	0.0	0.0
50% CH_3CN	9.3	9.7	13.1	8.6
100% THF	1.9	2.7	2.6	3.2
50% THF	8.2	9.0	9.0	NA[c]
THF-H_2O-$(CH_3)_2CO$ (5:3:2)	13.2	12.6	NA	NA

[a] Solvent:berry ratio for extraction = 20:1 (v/w) in all cases. [b] Total glycoalkaloid content by titration yielded 13.1 mg/g. [c] Not analyzed.

Table II. Glycoalkaloids (mg) Recovered from a Methanolic Extract of Defatted Nightshade

compound	mg/g of nightshade extract	mg added	theoretical	found	% recovery
β-solamargine	2.4	5.8	8.2	8.2	100.0
α-solamargine	3.3	7.7	11.0	11.1	100.9
α-solasonine	4.9	9.5	14.4	14.4	100.0
chaconine	0.5	10.0	10.5	10.6	101.0
solanine	1.0	10.0	11.0	11.5	104.6
	12.1[a]				

[a] Standard deviation: 2.08.

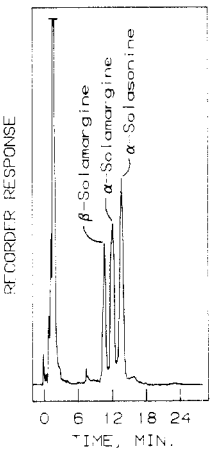

Figure 3. HPLC elution pattern of glycoalkaloids flocculated from *S. ptycanthum*.

were calculated as 4.74 for solanine, 3.97 for chaconine, and 5.28 for both solasonine and solamargine. Concentrations of the glycoalkaloids were then determined by computer integration of peak areas to their respective response factors by the use of an in-house computer system described by Butterfield et al. (1978).

RESULTS AND DISCUSSION

Figure 2 shows an HPLC elution diagram of a methanol extract of Eastern black nightshade containing added nicotine. Five of the peaks were tentatively identified on the basis of retention times as β-solamargine, α-solamargine, α-solasonine, α-chaconine, and α-solanine.

The three major peaks were identified conclusively by separating the glycoalkaloids by flocculation and then isolating and characterizing the individual compounds. Figure 3 is the HPLC elution diagram of glycoalkaloids flocculated from *S. ptycanthum* by Bushway's procedure (Bushway et al., 1980). The peaks were isolated by preparative TLC and preparative HPLC. The isolated peaks were identified by comparing the R_f's and cochromatography on TLC, by cochromatography on HPLC with known compounds, and by sugar analysis.

TLC of the three major components purified by Boll's solvent system gave R_f values of 0.54, 0.37, and 0.24 for β-solamargine, α-solamargine, and α-solasonine, respectively. R_f values for standards of the three components were 0.55, 0.39, and 0.24, respectively.

HPLC elution profiles show the three components with relative retention times (RRT) (relative to nicotine) of 0.338, 0.381, and 0.419 for β-solamargine, α-solamargine, and α-solasonine, respectively. α-Solanine and α-chaconine gave RRT's of 0.483 and 0.537, respectively.

Hydrolysis of the isolated components and sugar analysis by GC provided a qualitative method to examine the sugars attached to each individual component. The first eluting peak contained rhamnose and glucose in a 1:1 ratio, the second eluting peak contained rhamnose and glucose in a 2:1 ratio, and the third eluting peak contained rhamnose, glucose, and galactose in a 1:1:1 ratio.

Extraction of glycoalkaloids from defatted Eastern nightshade berries with various solvents was investigated. Table I gives the results obtained. Boiling methyl alcohol or a mixture of tetrahydrofuran–water–acetone (5:3:2) gave the largest amount of glycoalkaloids. Values of 12.0–14.0 mg of glycoalkaloids/g of nightshade berry were found. We preferred to use the methyl alcohol for convenience and to avoid the possibilty of peroxides when THF is used. Also included in Table I are data obtained when defatted nightshade berries were analyzed by our HPLC and the TGA procedure. Defatted nightshade contains 13–14 mg/g total glycoalkaloid as determined by both HPLC and TGA.

Table II presents data on the amount of individual glycoalkaloids found in defatted berries. Also included in the table is the recovery of added glycoalkaloids. The data suggest that the quantitation of the glycoalkaloids is good because excellent recoveries of the added glycoalkaloids were obtained.

This analytical procedure now is being applied to the quantitative determination of these biologically active compounds in blends of soybeans and nightshade, and the results will be reported elsewhere.

Registry No. β-Solamargine, 32449-98-2; α-solamargine, 20311-51-7; α-solasonine, 19121-58-5; α-chaconine, 20562-03-2; α-solanine, 20562-02-1.

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