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Composition of Jimson Weed (*Datura stramonium*) Seeds

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Bulk commercial grain, such as soybeans and wheat, may be contaminated by nongrain impurities, including jimson weed seeds, that coexist with the crop to be harvested. The present study was undertaken to determine the content of the major alkaloids of jimson weed seeds, atropine and scopolamine, as well as protein, carbohydrate, fat, mineral, hemmagglutinin, and tannin. Combined GC–MS analysis of a jimson weed seed extract revealed the presence of atropine and scopolamine plus possibly three additional tropane-like alkaloids. An improved HPLC procedure showed that the alkaloid concentration in samples obtained from different parts of the United States varied by as much as 50%: 1.69–2.71 mg/g for atropine and 0.36–0.69 mg/g for scopolamine. The presence of a strongly fluorescent green compound of unknown structure is also described. Baking experiments with jimson weed seed fortified wheat flour showed that atropine and scopolamine largely survive bread-baking conditions. Jimson weed seeds do not contain protease or amylase inhibitors. These observations provide a rational basis for relating seed composition to biological effects in animals and for assessing the possible significance of low levels of the seeds in food-producing animals and in the human diet.

The plant *Datura stramonium* was grown in England in the 16th century from seeds that came from Constantinopole, Turkey (Claus, 1961). The English presumably imported the plant to the American colonies, as evidenced by the fact that when English soldiers, who were sent to quell Bacon's rebellion at Jamestown in Colonial Virginia, inadvertently ate the plant as part of a salad in 1676, some of them became ill and died. The name jimson weed or Jamestown weed derives from this episode of fatal poisoning (Claus, 1961; Duke, 1984 Feenghaty, 1982; O'Grady et al., 1983). This and related reports of poisonings by jimson weed seeds demonstrate that the plant exerts pharmacological and toxicological effects in animals and humans. In fact, Klein-Schwartz and Oderda (1984) suggest that jimson weed abuse is a potentially serious form of substance abuse in adolescents and young adults. The most common symptoms of jimson weed ingestion are altered perception of the environment, visual hallucinations, mydriasis (dilation of the eye pupils), and tachycardia (increase in heart rate) (O'Grady et al., 1983). High levels may cause depression of the central nervous system, with symptoms ranging from lethargy to coma (Klein-Schwartz and Oderda, 1984). Antidotes include the use of the anticholinesterase drug physostigmine, charcoal to slow down absorption, magnesium citrate to speed passage through the intestinal tract, and ipecac to induce vomiting (Orr, 1975; O'Grady et al., 1983).

The literature on jimson weed covers a variety of aspects including agronomic and botanical (Broekaert et al., 1988; Hagood et al., 1981; Kilpatrick et al., 1984; van De Velde et al., 1988; Weaver, 1986), chemical and pharmaceutical (Cordell, 1981; Duez et al., 1985; List and Spencer, 1976; List et al., 1979), and medical toxicological (Day and Dilworth, 1984; El Dirdiri, 1981; Fangauf and Vogt, 1961; Feenghaty, 1982; Flunker et al., 1987; Gururaja and Khare, 1987; Keeler, 1981; Levy, 1977; Mahler, 1975; Mikolich et al., 1975; Nelson et al., 1982; Shervette et al., 1979; Testa and Fontanelli, 1988; Urich et al., 1982; Weintraub, 1960; Williams and Scott, 1984; Worthington et al., 1981).

The objectives of this study were to (a) develop an improved HPLC procedure for the analysis of atropine and scopolamine in jimson weed seeds, (b) to demonstrate the presence of known and unknown alkaloids in the seeds by GC–MS analysis, (c) to measure the nutrient and antinutrient composition of the seeds, and (d) to assess the

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variation in the range of toxicant levels as affected by growing locale in order to estimate worst case concentrations.

MATERIALS AND METHODS

Atropine and scopolamine were obtained from Sigma Chemical Co. (St. Louis, MO). Apoatropine came from Adams Chemical Co. (Round Lake, IL). Seed samples were obtained from the Federal Grain Inspection Service (Kansas City, KS) and from Valley Seed Co. (Fresno, CA). Samples were first picked through to clean out any debris. They were then ground on a Wiley mill using a coarse screen (No. 10).

Proximate Composition. Analyses for nitrogen, moisture, fat, fiber, ash, carbohydrate, and mineral content were carried out by standard procedures (AOAC, 1975).

Amino Acid Composition. Three analyses with samples containing about 5 mg of protein (N × 6.25) were used to establish the amino acid composition of the jimson weed seed protein: (a) standard hydrolysis with 6 N HCl for 24 h in evacuated sealed tubes (Friedman et al., 1979); (b) hydrolysis with 6 N HCl after performic acid oxidation to measure half-cystine and methionine content as cysteic acid and methionine sulfone, respectively; (c) basic hydrolysis by barium hydroxide to measure tryptophan content (Friedman and Cuq, 1988).

Tannin Content. Tannin content was determined by the vanillin assay (Price et al., 1978) with 100 mg of jimson weed samples extracted with 5 mL of MeOH in capped vials with stirring for 20 min. The extracts were then centrifuged in a Beckman microfuge. The assay was carried out at 30 °C with reagents previously warmed to this temperature. The tannin content was calculated with the aid of a catechin standard curve.

Hemagglutination Assay. The assay for the presence of hemagglutinins (lectins) was carried out with 150-mg samples of jimson weed or soybean seed samples and human red blood cells, as described previously for lima bean and soybean flours (Wallace and Friedman, 1985; Friedman and Gumbmann, 1986).

Trypsin, Chymotrypsin, and α-Amylase Inhibition Assays. Titration by previously described techniques (Friedman and Gumbmann, 1986; Buoncore and Silano, 1986) demonstrated the absence of inhibitors of digestive enzymes in jimson weed seed extracts.

GC–MS Analysis of Jimson Weed Seed Extracts. The samples were defatted by extracting with hexane in a Soxhlet extractor for 18 h. The seed meal was air-dried and ground to a fine powder on a UDY cyclone mill. The defatted samples (100 mg) were extracted with methanol in a Soxhlet extractor for 18 h. The volume was reduced to a few milliliters, which was then filtered through a 0.45-μm membrane (Schleicher and Schuell). The extract was bought up to a volume of 10 mL with methanol and stored under refrigeration in an air-tight flask.

Preliminary studies showed that the same extracts of jimson weed seeds used for HPLC analysis described below were suitable for GC–MS analysis provided any HCl present was neutralized as follows: 0.5 mL of the methanol solution was dried under a stream of nitrogen. The residue was dissolved in 1.0 mL of 0.05 N NaOH. This solution was then mixed with 1 mL of chloroform, the two phases were vigorously stirred, and the chloroform phase containing the alkaloids was separated and used for analysis. The neutralization step was necessary because any HCl present was found to degrade the packing of the GC capillary column.

To minimize the appearance of large numbers of peaks associated with fatty acids and esters in the GC–MS chromatograms, samples were defatted twice with hexane before extraction with methanol.

HPLC Assay for Atropine and Scopolamine in Jimson Weed Seeds. An Alumina A Sep-Pak (Waters) was conditioned with 5 mL of chloroform. A 2-mL portion of the methanol extract followed by 5 mL of chloroform was then passed through the Sep-Pak. The eluants were combined and evacuated to dryness with an aspirator. The residue was then taken up in 1 mL of methanol containing 0.2 mg/mL cystamine as an internal standard. This solution was used for analysis by HPLC.

A Beckman Instruments (San Ramon, CA) 334 HPLC system with a 427 integrator and a 165 UV–vis variable-wavelength detector was used. The column was a C_8 Beckman Ultrasphere (4.6 × 250 mm) with a Beckman C_8 precolumn.

The mobile phase consisted of 64:36 water–methanol containing 0.02 M phosphate buffer (pH 3; 0.87 mL of 87% phosphoric acid plus 0.77 g of monosodium phosphate/L) and 0.01 M dibutylamine as a counterion. Solvent flow rate was 0.8 mL/min. Dibutylamine was chosen as a counterion because it has lower absorptivity at the wavelength used (200 nm) and produced a more stable base line than other amine modifiers, such as ethanolamine. The mixture of water and methanol proved to be stable on the column compared to other solvents such as acetonitrile. The low absorbtivity in the UV and the relatie low toxicity of this mobile phase were additional benefits.

The following compounds were evaluated as possible internal standards for the analysis of atropine and scopolamine by HPLC: benzylamine, cystamine, diaminopropionic acid, histamine, hydroxyethylamine, hydroxytryptamine, nicotinamide, nicotinic acid, nicotinic acid methyl ester, penicillamine, theobromine, theophylline, and tyramine. Cystamine was selected as the internal standard because its elution position, peak shape, and linear relationship of peak areas of concentration were superior to the other compounds tested.

Relative recovery of alkaloids was determined by spiking the ground jimson weed samples with atropine and scopolamine standards. The samples were then extracted and analyzed by HPLC.

Baking Experiments. Unbleached, unbrominated, malted, and enriched white wheat flour (Mellow Judith) was obtained from Con Agra Inc. (Oakland, CA). Fresh cake yeast (Fleischman's) was obtained at a local market.

The recipe for one loaf of bread consisted of 183.1 g of flour, 106.8 g of water, 3.5 g of salt, 6.1 g of yeast, and 25 g (12% of dry weight) of jimson weed seeds in the final mix. The jimson weed seeds were mixed whole with the flour, and the mixture was then ground in a Wiley mill. This overcame the difficulty of finely grinding the fatty seeds alone. The ingredients were combined, and the resulting dough was kneaded on a Hobart Model C100 kneader (Troy, OH) for a total of 8 min. The dough was allowed to rise twice in a fermentation chamber (National Co., Lincoln, NE) at 37 °C and 90% humidity for 45 min. The dough was placed in 14.6 × 7.6 × 7.4 cm nonstick-coated pans and baked 35 min at 215 °C. The crust was separated from the crumb with an electric knife. Both the crust and crumb were sliced, lyophilized, and ground in a Wiley mill with a 1-mm screen.

Green Fluorescent Compounds. In working with extracts of jimson weed seeds, it was noted that there was present a substance that under UV light (365 nm) exhibits an intense green fluorescence in neutral or acidic solutions and a bright yellow fluorescence in basic solution. The substance is present in such quantity that the interior portion of freshly ground seeds fluoresce without purification. The material is easily extracted with several portions of methanol.

The material was chromatographed on thin layer precoated silica gel plates (E. Merck). Twenty microliters of a methanol extract (1 g extracted with 10 mL of hot methanol) was spotted on plates 2 cm from bottom and allowed to run until the solvent front was 15 cm from the origin. In the solvent ethyl acetate–methanol–water (100:13.5:10), a large spot at R_f 0.05–0..10 was observed. In the solvent ethyl acetate–formic acid–glacial acetic acid–water (100:11:11:25), this green spot resolved itself into two components, a smaller spot at R_f 0.35 and a larger one at R_f 0.43.

RESULTS AND DISCUSSION

Seed Morphology and Contaminants. Seeds of the 25 species of *Datura* appear morphologically indistiguishable (J. Effengerger, Department of Food and Agriculture, State of California, Sacramento, private communication, May 16, 1985). *D. stramonium* (jimson weed) and *Datura ferox* (Chinese thornapple), for example, have seeds that are very similar in appearance. Generally, a genus name rather than that of a species should be used unless a grow-out test is carried out with the seed in question.

The nature of the contaminants found to be present in pure *Datura spp.* collected in the field are summarized in Table I. These need to be removed manually, as was done in our studies, before the seeds can be used for composi-

Table I. Contaminant Seeds in *Datura* Jimson Weed Seeds Collected in the Field[a]

	no. of seeds	%
Datura spp. seeds, pure	1303	78.78
contaminant		
broken seeds of *Glycine max* (soybean), caryopses of *Triticum* spp. (wheat), soil particles from microscopic to 7 mm long	243	14.69
immature shriveled seeds of *Fabaceae*, possibly *G. max.*	39	2.36
Abutilon spp. (Indian mallow)	35	2.12
Polygonum pennsylvanicum (Bigweed ladysthumb)	24	1.45
Triticum spp. (wheat)	5	0.30
Amaranthus spp. (pigweed)	2	0.12
Sida spinosa (prickly mallow)	1	0.06
Digitaria sanguinalis (crabgrass)	1	0.06
Atriplex spp. (saltbush)	1	0.06
	1654	100.00

[a] Test results by count. Some counts were made with an electronic seed counter and are approximate.

Table II. Comparison of Composition (%) of Jimson Weed and Some Commonly Contaminated Grains

material	unpurified jimson weed seed flour	defatted soy flour	full-fat soy flour	whole wheat pastry flour
nitrogen	3.1	8.0	5.4	2.2
H_2O	7.7	11.2	9.0	11.1
fat	18.1	0.9	20.7	0.6
fiber	17.8	1.5	4.5	1.7
ash	6.6	5.9	4.9	1.8
carbohydrate	31.9	37.8	31.5	81.6
starch	1.1	0.7	0.6	57.5
sugar	2.1	13.9	10.6	2.4
reducing sugar	0.3	0	0	0
glucose	0.16	0.13	0.12	0.06

[a] Detection limit 0.1%.

tional and toxicological studies.

Proximate Composition. Table II shows the nitrogen, moisture, fat, fiber, carbohydrate, and ash content of jimson weed seed flour, full-fat and defatted soy flour, and whole wheat flour for comparison. The value for nitrogen of 3.1%, corresponding to a protein content of about 20% (3.1 × 6.25), compares to 2.2% and 5.4% for wheat flour and soy flours, respectively. Thus, protein content of jimson weed is greater than that of wheat flour but much lower than of soy flour. The table also shows that the fat content of 18.1% in jimson weed seeds is similar to the 20.7% in soy flour. Wheat flour has a very low fat content. The crude fiber content of jimson weed seed flour was 17.8% compared to 4.5% for soy and 1.7% for wheat flour. The carbohydrate content of jimson weed seeds (31.9%) is identical with that of soy flour (31.5%) but less than half that of whole wheat flour (81.6%). A separate analysis for starch content revealed that both jimson weed and soy flours contain negligible amounts of this polysaccharide compared to a 57.5% content in whole wheat flour. These considerations suggest that, from a nutritional standpoint, jimson weed seeds are a good source of protein and an excellent source of fat and fiber.

Table III summarizes the content of minerals in jimson weed seeds, full-fat raw soy flour, defatted soy flour, and whole wheat flour. The data show that (a) the toxic trace elements cadmium, mercury, and selenium were not detected in any of the flours; (b) the iron content of jimson weed seed flour is about 40 times greater than in wheat flour and 14 and 17 times greater than in defatted and full-fat soy flour, respectively; (c) the chromium and manganese levels in jimson weed seeds are also greater than in the three grain flours; and (d) copper, zinc, magnesium, and calcium levels is the three flours do not differ significantly.

Table III. Mineral Content (ppm) of Jimson Weed Seeds and Other Grains

mineral	unpurified jimson weed seed flour	defatted soy flour	full-fat soy flour	whole wheat pastry flour
cadmium[a]	0	0	0	0
calium	2310	2680	2250	480
chromium	2.2	0.22	0.22	0.10
copper	16.4	16.0	14.5	4.4
iron	1305	76.9	91.2	33.9
magnesium	2890	2890	2500	1630
magnanese	134	36.4	28.0	40.7
mercury[b]	0	0	0	0
potassium	6540	2260	18700	4710
selenium[b]	0	0	0	0
sodium	175	221	271	54
zinc	59.5	63.1	60.6	38.8

[a] Detection limit 0.05 ppm. [b] Detection limit 0.5 ppm.

Table IV. Free Amino Acid Content of Jimson Weed Seeds

amino acid	content mg/100 g	content mg/16 g N	elution time, min
unknown[a]	51.0	230.0	40.2
γ-aminobutyric (GABA)	16.6	74.6	53.5
histidine	8.3	37.0	56.0
arginine	12.4	5.6	77.0

[a] Calculated as leucine equivalents.

Table V. Amino Acid Content (g/16 g of N) of Defatted Jimson Weed Seed Flour, Defatted Soy Flour, and Commercial Wheat Flour[a]

amino acid	jimson weed seed	soy flour	wheat flour	FAO[e]
Asp	7.74	11.74	4.11	
Thr	3.14	3.58	2.54	4.0
Ser	4.03	4.90	4.39	
Glu	13.05	18.59	33.1	
Pro	3.32	5.17	11.5	
Gly	3.87	4.04	3.48	
Ala	3.51	4.05	2.80	
Val	3.62	5.20	4.07	5.0
Cys[b]	2.00	1.14	2.20	
Met[c]	1.38	1.19	1.62	3.5[f]
Ile	3.22	4.66	3.53	4.0
Leu	5.31	7.74	6.73	7.0
Tyr	2.55	3.42	3.20	6.0[g]
Phe	3.47	5.05	4.78	
His	1.84	2.47	2.09	
Lys	3.19	5.82	1.85	5.5
Arg	6.54	7.27	3.65	
Try[d]	0.51	1.13	0.55	

[a] N content, %: defatted jimson weed seed flour, 3.56; defatted soy flour, 8.00; wheat flour, 2.18. [b] Determined as cysteic acid after performic acid oxidation. [c] Determined as methionine sulfone after performic acid oxidation. [d] Two separate determinations by ion-exchange chromatography after hydrolysis by barium hydroxide. [e] Provisional amino acid scoring pattern for an ideal protein (FAO, 1973). [f] Cys + Met. [g] Tyr + Phe.

Free and Protein Amino Acid Contents. Table IV lists the free amino acid content of jimson weed seeds. The seeds contained only histidine and arginine in the free form along with two unknown amino acids. One of these eluted in the same positions as γ-aminobutyric acid. The second elutes in the vicinity of cystine. It does not appear to be γ-glutamyl-L-aspartic acid that Ungerer et al. (1988) found in jimson weed seeds. This dipeptide elutes in the same position as methionine on our column. Elucidation of the

Table VI. Lectin Content of Jimson Weed Seeds Measured by Agglutination

jimson weed seeds[a]	activity[b] (n = 2)	activity 2 years later (n = 3)
1	0.63	0.63 ± 0
2	0.80	
3	2.50	
4	0.63	1.7 ± 0.3
5	1.25	
6	0.08	5.6 ± 1.0
7	0.10	
8	0.10	
9	0.04	

[a] See Table IX for origin of seeds. [b] Minimum amount of sample required to agglutinate human red blood cells (μg/50 μL). The lower the number, the greater the hemagglutinating activity.

structure of the second ninhydrin-positive compound awaits further studies.

Table V lists the amino acid composition of acid hydrolysates of defatted jimson weed seed flour, defatted soy flour, and commercial wheat flour. The values of the amino acid scoring pattern of the essential amino acids for an ideal protein, as defined by the Food and Agricultural Organization of the United Nations (FAO, 1973), are also shown for comparison. The data show that the amino acid pattern of jimson weed seeds, especially the essential amino acids, falls between those of a legume such as soy and a cereal such as wheat. The results show that jimson weed seeds meet the provisional requirements for the sulfur amino acids (Cys + Met) and for Tyr + Phe, but values for Thr, Val, Ile, and Lys are below those of the FAO pattern. In spite of these deficiencies, the amino acids of jimson weed could contribute significantly to protein nutrition, if it were possible to remove the toxic alkaloids either by chemical inactivation or through plant genetics. Thus, it may be possible in the future to develop jimson weed cultivars in which the genes controlling the biosynthesis of the alkaloids are suppressed or eliminated. The new varieties might then displace the ones currently growing in the field. A possible approach would be to use molecular biological techniques to insert DNA constructs capable of generating antisense RNA specific for genes involved in alkalid biosynthesis, resulting in blocked expression in the jimson weed plant (Walder, 1988). Instead of presenting a problem to the consumer, the new cultivars would contribute to the nutritional value of the grain. At this stage, more information is needed about specific enzymes involved in the biosynthesis of the alkaloids (Cordell, 1981) before it would be possible to control, via antisense RNA or otherwise, the genes coding for these compounds.

Hemagglutinin (Lectin) Content. Lectins, present in many plant seeds, are carbohydrate-binding proteins with differing sugar specificities. Normally, lectins are thermally unstable and are partly or fully denatured during cooking of foods. Lectins from different sources may, however, differ in heat stability. Inadequately cooked legumes containing lectins may cause gastrointestinal disturbances and adverse nutritional and toxic effects in humans (Reaidi et al., 1981; Liener, 1988).

D. stramonium seeds are reported to contain a lectin visualized by an immunocytochemical technique and quantified by agglutination assays (Broekaert et al., 1988). These authors also demonstrated a possible physiological function for the jimson weed seed lectin in the plant, involving mediation of cell–cell interactions.

Our agglutination assay of the jimson weed seed samples listed in Table VI revealed that the minimum amount of jimson weed seed flour required to cause agglutination of human red blood cells ranged from 0.04 to 2.5 μg/50 μL (the smaller the number, the more potent the hemagglutinating activity).

Table VII. Tannin Content of Jimson Weed Seeds

sample[a]	mg tannin/g seed[b]	sample[a]	mg tannin/g seed[b]
1	1.7 ± 0[c]	4	2.9 ± 0.05
2	1.4 ± 0	5	2.3 ± 0
3	1.9 ± 0	7	4.5 ± 0.02

[a] See Table IX for origin of seeds. [b] Expressed as catechin. [c] Average ± standard deviation from two separate determinations.

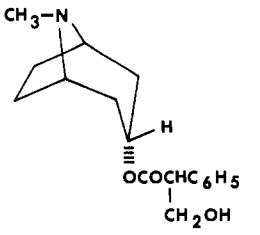
The reason for the wide variation in lectin content is unknown. An assay of the same three samples after a 2-year interval indicated an unchanged value for one and a lower content for two (Table V). The lectins of jimson weed seeds might be slowly inactivated during storage and exposure to heat and sunlight under field conditions, accounting for the variable results.

Tannin Content. Table VII lists the tannin contents of seven jimson weed samples obtained from different locations. The values ranged from 1.6 to 5.6 mg/g of seed. The reasons for the more than 3-fold variability in tannin content is not immediately apparent. Soil and climatic conditions may affect the biosynthesis of tannins (Deshpande et al., 1984).

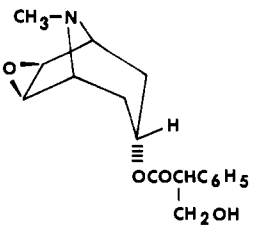
Tannins adversely affect the nutritional quality and safety of foods (Griffiths, 1986; Deshpande et al., 1984), presumably through chelation of essential trace elements such as iron and through inhibition of proteolytic digestive enzymes such as trypsin and chymotrypsin.

Atropine and Scopolamine Content. In their review of toxic weed seed contamination in soybeans during harvest, transport, and storage, List et al. (1979) state that jimson weed seeds are probably the more prevalent and most toxic compared to others such as castor (*Ricinus communis*), cocklebur (*Xanthium strumarium*), corn cockle (*Agrostemma githago*), cow cockle (*Saponaria vaccaria*), crotolaria (*Crotolaria spp.*), morning glory (*Ipomoea spp.*), nightshade (*Atropa belladonna*), and pokewood (*Phytolacca americana*). These authors also suggest that, aside from toxicity aspects, weed seed contamination may also adversely affect the appearance, organoleptic, functional, and nutritional properties of grain.

The major toxic principles present in jimson weed seeds are the alkaloids atropine and scoplamine. These solanaceous alkaloids are present in a number of other plants, including *Atropa belladonna* (nightshade) and *Hyoscyamus niger*. The alkaloids (see structures) have a bicyclic structure in which a five-membered pyrrolidine ring is fused to a six-membered pyridine ring. Atropine probably exists in nature as the optically active hyoscyamine isomer, which racemizes to the DL form (atropine) during isolation. The major difference between scopolamine, also known as hyoscine, and atropine is the prescence of an epoxide group in cis position to the N-bridge (Cordell, 1981):



ATROPINE (dl)
HYOSCYAMINE (l)



SCOPOLAMINE (dl)
HYOSCINE (l)

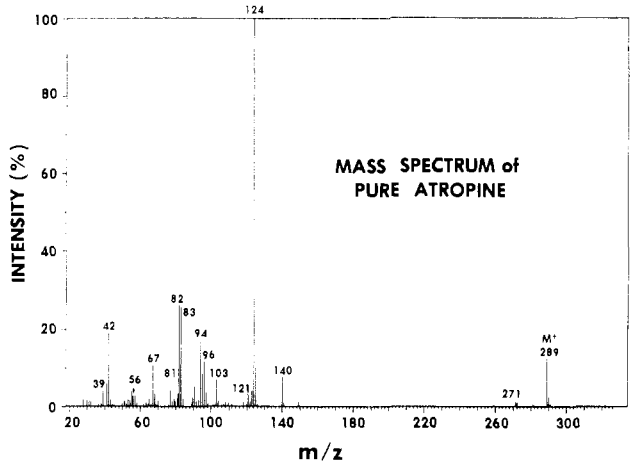


Figure 1. Mass spectrum of atropine.

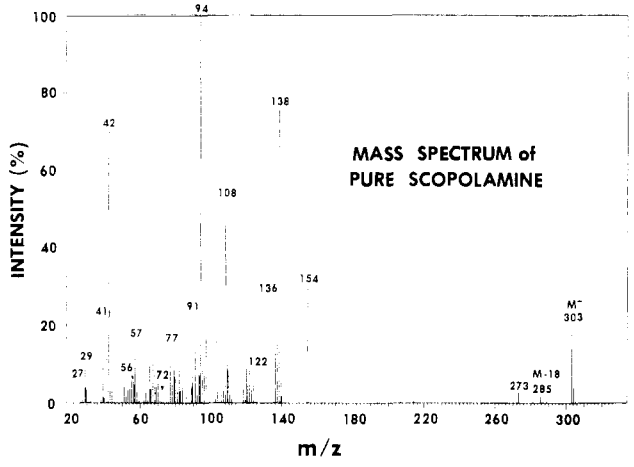


Figure 2. Mass spectrum of scopolamine.

Figures 1 and 2 show the mass spectral fragmentation patterns for authentic atropine and scopolamine obtained from commercial sources. The figures show the parent molecular ion peaks for both compounds: $M^+ = 289$ for atropine and $M^+ = 303$ for scopolamine. The presence of a parent peak in a mass spectrum generally defines the molecular weight of a compound (Friedman, 1977). The GC–MS chromatogram of a methanol extract of jimson weed seeds is shown in Figure 3. Alkaloids are present in the extract along with several other peaks associated with fatty acid esters. Note the clear separation of the atropine and scopolamine peaks at scans 374 and 422, respectively. These results indicate that GC–MS is useful for demonstrating the presence of atropine and scopolamine in plant extracts. However, this technique was found to be useful only for qualitative measurements. The method needs further study to assess its potential for a quantitative assessment of the alkaloid content.

In addition to atropine and scopolamine, Figure 3 suggests the presence of small amounts of three other akaloids designated as X, Y, and Z. X appears to be atropine-like in nature. Its molecular weight of 271 corresponds to the molecular weight of atropine minus 18 (H_2O), possible apotropine. The mass spectrum of authentic apoatropine was identical with that of X. Y, also atropine-like, has a molecular weight of 303, which corresponds to the molecular weight of atropine plus 14 (CH_3 group). Z may be scopolamine-like. Its molecular weight corresponds to that of scopolamine plus 14 (CH_3 group).

Extensive studies were carried out to develop a reliable HPLC procedure for measuring atropine and scopolamine in jimson weed seed extracts. Beneficial features of this

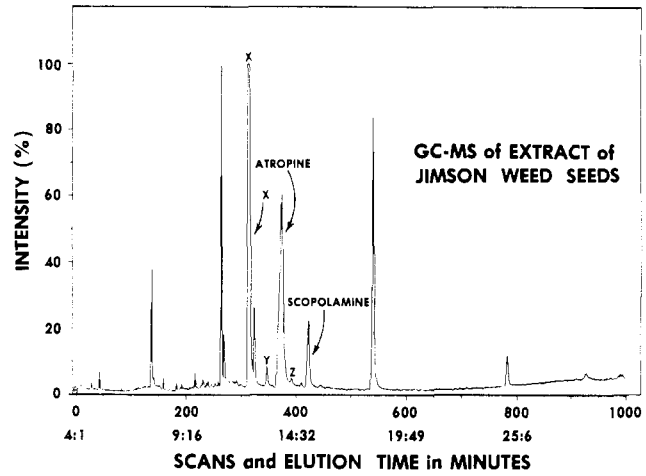


Figure 3. GC–MS analysis of a jimson weed seed extract showing linear plots of elution time on the gas chromatograph and scan numbers (scan rate 0.63 scans/s).

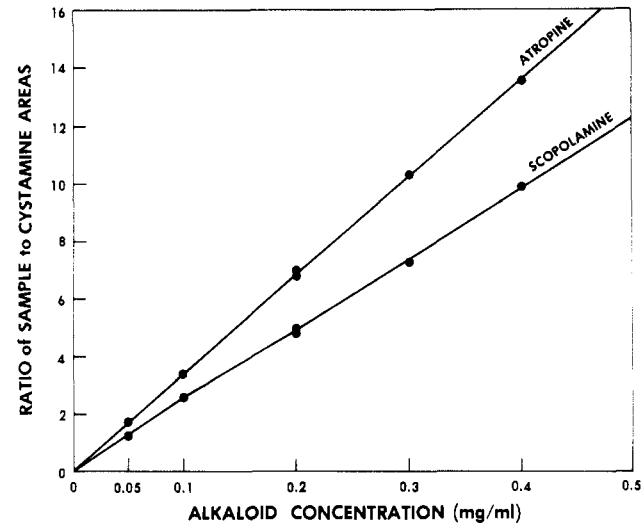


Figure 4. Plots of atropine and scopolamine concentrations against the ratio of areas of the HPLC peaks for the two alkaloids to the area for the internal standard cystamine.

method include (a) high sensitivity due to the use of a low detection wavelength (200 nm), (b) use of a solvent system with a stable base line even at this low wavelength, (c) use of a new internal standard that is well resolved from the complex matrix, and (d) rapid sample preparation using solid-phase extraction (Sep-Pak). Since tropane alkaloids are known to hydrolyze in aqueous solution, solid-phase extraction has an additional advantage over the traditional liquid–liquid extraction that utilizes acid–base solubility (List and Spencer, 1976).

Figure 4 shows that the HPLC column responds linearly to both atropine and scopolamine in the concentration range shown. As little as 20 ng of alkaloids was detected on the column. However, the response was not always linear with concentration below 200 ng of total alkaloids. Figure 5–7 demonstrate the clear separation of atropine and scopolamine from each other and from the internal standard cystamine. Spiking experiments, in which known amounts of a mixture of atropine and scopolamine were added to jimson weed flour and then reextracted, revealed that the recovery of atropine ranged from 92 to 97% of the amount added and of scopolamine from 87 to 92% (Table VIII).

Table IX shows that the atropine content of seven seeds obtained from different parts of the United States ranged from 1.69 to 2.71 mg/g seed, with an average ± SD = 2.27

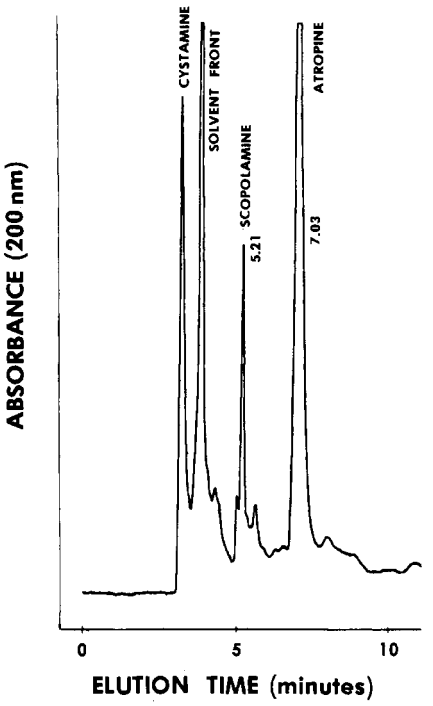


Figure 5. Typical HPLC chromatogram for atropine and scopolamine applied to the column as a mixture. Conditions: 0.05 mg of atropine + 0.05 mg of scopolamine + 0.2 mg of cystamine/mL. A 20-μL sample of this solution was injected onto the column. Flow rate: 0.8 mL/min. Solvent: 0.02 M phosphate buffer; 0.01 M dibutylamine; 36% methanol; pH 3.0.

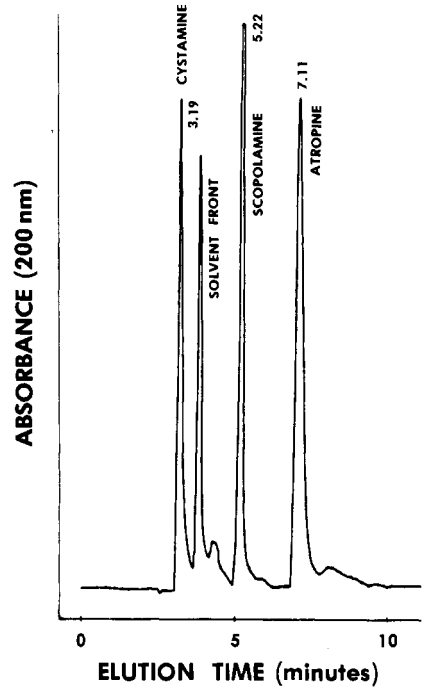


Figure 6. Typical HPLC chromatogram of a jimson weed seed extract. Conditions as in Figure 5.

± 0.36 mg/g. The corresponding range for scopolamine was from 0.36 to 0.69 mg/g, with an average ± SD = 0.53 ± 0.13 mg/g. The atropine and scopolamine values varied by as much as 50%, depending on origin. The cause of this variation is presently unknown but needs to be established. These results are similar to those observed by List and Spencer (1976), List et al. (1979), and Mirzamotov and Luftulin (1986) (who also report no significant differences in the biosynthesis rate of the two alkaloids in *D. stramonium* at different seasons of growth).

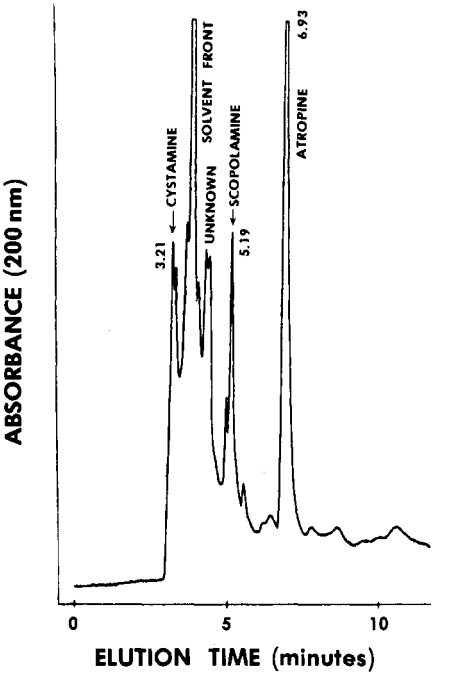


Figure 7. HPLC chromatogram of a methanol extract of a defatted diet containing 4% jimson weed seeds. Conditions as in Figure 4. This diet was used in animal feeding studies to assess the toxicity of jimson weed seeds.

Table VIII. Recovery of Atropine and Scopolamine Added to Jimson Weed Seed Flour[a]

material	atropine		scopolamine	
	mg/g	% rec	mg/g	% rec
jimson weed seed flour, control	2.69 ± 0.16		0.75 ± 0.04	
100 mg of jimson weed seed flour + 0.51 mg of atropine, 0.27 mg of scopolamine	3.09 ± 0.13	97	0.90 ± 0.04	88
100 mg of jimson weed seed flour + 1.01 mg of atropine, 0.54 mg of scopolamine	3.55 ± 0.21	96	1.19 ± 0.06	92
100 mg of jimson weed seed flour + 2.02 mg of atropine, 1.08 mg of scopolamine	4.31 ± 0.17	92	1.59 ± 0.05	87

[a] Average ± standard deviation from two separate determinations.

Table IX. Atropine and Scopolamine Content of Jimson Weed Seeds Obtained from Different Locations

sample no.	location	mg/g jimson weed seeds		ratio of atropine to scopolamine
		atropine	scopolamine	
1	Indianapolis, IN	1.69 ± 0.09	0.36 ± 0.03	4.7
2	Peoria, IL	2.07 ± 0	0.59 ± 0.02	3.5
3	Belle Chase, LA	2.09 ± 0.06	0.51 ± 0	4.1
4	Indianapolis, IN	2.26 ± 0.08	0.51 ± 0	4.4
5	Belle Chase, LA	2.41 ± 0.05	0.69 ± 0.02	3.5
6	Cedar Rapids, IA	2.68 ± 0.04	0.39 ± 0.05	6.9
7	Fresno, CA	2.71 ± 0.02	0.66 ± 0.05	4.1
	av ± SD	2.27 ± 0.36	0.53 ± 0.13	4.5 ± 1.16

[a] Average from two separate determinations ± standard deviation.

Since jimson weed seeds contaminate soybeans, corn, and wheat, it is of paramount interest to find out whether the tropane alkaloids survive the processing conditions to which these grains may be subjected before consumption. To obtain information on this question, we added jimson weed seed flour to wheat flour, which was then baked into bread. The bread was then separated into crust and crumb

Table X. Effect of Baking on Atropine and Scopolamine Content of Jimson Weed Seed Fortified Bread

material	atropine mg/g mixed flour	atropine % rec	scopolamine mg/g mixed flour	scopolamine % rec
wheat flour + 12% jimson weed seed flour, unbaked	0.408	100	0.115	100
bread crumb	0.304	75	0.100	87
bread crust	0.335	82	0.083	72

fractions. These were freeze-dried and milled into flours. Table X shows the following recovery of atropine and scopolamine from these flours compared to an unbaked control of wheat plus jimson weed flours: for atropine, bread crumb 75%, bread crust 82%; for scopolamine, bread crumb 87%, bread crust 72%. The findings show that both alkaloids in jimson weed seed flour largely survived the high temperature of bread-baking, in both the crumb and crust.

In a related study, List and Spencer (1976) describe the fate of the two alkaloids during processing of jimson weed contaminated soybeans. They report that extraction of a 50:50 mixture of soybeans and jimson weed seeds with petroleum ether produced a meal and crude oil fractions. Analyses of these fractions by a gas chromatographic procedure showed that virtually all of the atropine and scopolamine remained in the meal. They also found that alkali refining effectively removed atropine added to soybean oil.

Subchronic 90-day toxicity studies with laboratory rats were conducted to establish safe levels of jimson weed seeds (Dugan et al., 1989). Dose-related adverse effects were observed at 0.5% or more in the diet. An unanswered question is whether toxicological manifestations of jimson weed seeds can be predicted on the basis of their atropine and scopolamine contents or whether additional constituents, such as lectins, unknown alkaloids, and the described fluorescent compound(s), contribute to biological activity. If the former is true, then a rapid assay for tropane alkaloid content by the described HPLC method may be useful for predicting the safety of jimson weed seeds. Moreover, since dietary constituents and the process of digestion and metabolism can be expected to modify the adverse manifestations of atropine and scopolamine (Fuahiki et al., 1987), the described compositional information should contribute to a better understanding of toxicological manifestations of jimson weed seeds when fed as a part of a complete diet. These studies are expected to help provide a basis for establishing maximum tolerance levels in food and feed and in setting official grain standards.

ACKNOWLEDGMENT

We thank J. C. Halvorson and colleagues of the Federal Grain Inspection Service (FGIS) for collecting the seeds and for helpful advice, R. England and W. F. Haddon for the mass spectra, F. F. Bautistia and G. McDonald for excellent technical assistance, and M. R. Gumbmann for valuable contributions.

Registry No. GABA, 56-12-2; His, 71-00-1; Arg, 74-79-3; nitrogen, 7727-37-9; starch, 9005-25-8; glucose, 50-99-7; cadmium, 7440-43-9; calcium, 7440-70-2; chromium, 7440-47-3; copper, 7440-50-8; iron, 7439-89-6; magnesium, 7439-95-4; manganese, 7439-96-5; mercury, 7439-97-6; potassium, 7440-09-7; selenium, 7782-49-2; sodium, 7440-23-5; zinc, 7440-66-6; atropine, 51-55-8; scopolamine, 51-34-3.

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Red Squill Modified by *Lactobacillus acidophilus* for Rodenticide Use

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Red squill (*Urginia maritima*, Liliaceae) bulb and root preparations were treated with three strains of *Lactobacillus acidophilus*, fortifying the cultures with dry milk and oat, wheat, and rice flour. *Lactobacillus* growth with the production of a β-glucosidase converted bitter glucoside scilliroside to its tasteless aglycon scillirosidin. These products were blended into rat diets at 0.03% scillirosidin levels, and 95% of the female rats died. Although male rats usually ate more bait than the females, none ate enough for a lethal dose of scillirosidin. The rats learned to avoid the baits if they did not die after initial ingestion of these fast-acting rodenticides. Technical scillirosidin mixed into rat diets had a toxic effect on female rats similar to the *L. acidophilus* treated red squill products.

Red squill is being investigated as a new economic crop for the southwest United States where it grows well (Gentry et al., 1987). The bulb, roots, and other plant parts contain scilliroside [6β-(acetyloxy)-3β-(β-D-glucopyranosyloxy)-8,14-dihydroxybufa-4,20,22-trienolide], a highly toxic, emetic, and bitter bufadienolide glucoside (Verbiscar et al., 1986a, b). Because of high toxicity and the emetic safety factor, dried bulb powders have been used in rat baits for centuries. However, rats and mice, which are unable to vomit, may not eat a lethal amount of red squill baits when first exposed, resulting in formulation problems.

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Our initial attempts to improve acceptability involved conversion of scilliroside to its aglycon scillirosidin, which is tasteless and equally toxic. The aerobic fungus *Aspergillus niger* was used as a source of β-glucosidase to elicit this cleavage (Verbiscar et al., 1987). *A. niger* was grown in extracts of red squill, producing the enzyme necessary for the hydrolysis of scilliroside to scillirosidin. The resulting aglycon extracts were administered orally to Charles River rats. The scillirosidin aglycons were found to be more toxic to female rats than to males, which is also the case for scilliroside.

In addition to the *A. niger* study we tested 12 strains of *Lactobacillus bulgaricus* and *Lactobacillus acidophilus* on hand from a jojoba detoxification project. It seemed reasonable that because Lactobacilli cleave lactose, a galactosylglucose, the active enzyme could also cleave the glucose from scilliroside. The Lactobacilli are nontoxic and microaerobic, which facilitates processing. The Lactobacilli

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