Composition of Sicklepod (*Cassia obtusifolia*) Toxic Weed Seeds

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Bulk commercial grain, such as soybeans and wheat, may be contaminated with nongrain impurities, including sicklepod seeds (*Cassia obtusifolia*), that coexist with the crop to be harvested. The present study was undertaken to determine the content of the major anthraquinone constituents of sicklepod seeds, as well as carbohydrate, fat, mineral, and amino acid (protein) content. Thin-layer chromatography, ultraviolet spectroscopy, and combined gas chromatography–mass spectrometry revealed the presence of known and unknown anthraquinone derivatives. Known compounds include chrysophanic acid, physcion, obtusofolin, emodin, questin, obtusin, chrysoobtusin, aurantioobtusin, and others. Rapid thin-layer chromatographic procedures were developed to screen sicklepod seeds for anthraquinone derivatives. These procedures also revealed the presence of β-sitosterol and of flavonoids, UV-quenching compounds, and fluorescent blue compounds of unknown structure. The described methodology should facilitate screening large numbers of samples both in the laboratory and under practical field conditions. The data provide a rational basis for relating seed composition to reported muscle-damaging and other toxicological effects of sicklepod, for assessing the significance of low levels of the seeds in diets of food-producing animals and humans, and for setting grain standards.

INTRODUCTION

Soybeans and other field crops are often contaminated with possibly toxic weed seeds such as sicklepod (*Cassia obtusifolia*) (Banks, 1986) and jimson weed seeds (*Datura stramonium*) (Dugan et al., 1989). This has become especially important of late as many major markets for soybeans will not accept them if there is a possibility of contamination. One way to eliminate toxic seeds is to mechanically remove all foreign seeds. This is time-consuming and not always cost effective. Another way is to develop strains of the major contaminant plants that do not contain toxic materials and yet will thrive and eliminate the poisonous varieties. To pursue this second alternative as well as to provide compositional data for risk assessment, it is necessary to identify such toxic compounds in these seeds and to develop a quick screening method.

The extract of dried Cascara bark (*Rhamnus purshiana*) has been used as a purgative, but so have dried leaflets of *Cassia senna* L. and seed preparations of sicklepod (*C. obtusifolia*) and *Cassia tora* in Japan and China. Parenthetically, there seems to be some confusion in the literature differentiating *C. tora* and *C. obtusifolia*. Poethke et al. (1968) concluded that a Vietnamese *C. tora* was identical with *C. obtusifolia*, and other investigations have shown the two to be remarkably similar, if not interchangeable. Koshioka et al. (1978) and Upadhyaya and Singh (1986), however, found several differences in chemical makeup. Upadhyaya and Singh suggest *C. tora* may be derived from *C. obtusifolia*. Koshioka et al. also point out that regional differences cause a wide variance within a species in amounts of anthraquinones, which cause the purgative effects of *Cassia* species.

C. obtusifolia is a leading cause of livestock poisoning in the southern United States (McCormack and Neisler, 1980). Patel et al. (1965) reported an LD-50 of 10 g/kg in mice for *Cassia fistula*. The anthraquinones in these plants are partly free but mostly glycosides (with the aglycon occurring usually as a reduced form, e.g., anthrones, dianthrones, or oxanthrone). The cathartic effect decreases in the order anthrone glycoside, free anthrone, and the aglycon of anthraquinones (Fairbairn, 1970). Higher molecular weights or greater degrees of glycosylation are related to increases in activity. The aglycon of anthraquinones actually does not survive metabolism in the gut. The dianthrones and glycosides are more protected and survive intact to reach the large intestine. Here they are hydrolyzed to the aglycon after oxidation to the anthraquinone form. Studies indicate that anthraquinone aglycons such as emodin are mutagenic as well (Brown, 1980; Masude and Ueno, 1984; Friedman and Henika, 1991).

Aglycons that have been identified and characterized in *C. obtusifolia* are chrysophanic acid, physcion, obtusifolin, obtusin, chrysoobtusin, aurantioobtusin, and emodin (Takido, 1958, 1960). Many other investigators have found numerous other anthraquinone-like compounds, including toralactone (Takahashi, 1973), torachrysone (Shibata et al., 1969), and others (Wong et al., 1989a).

Chromatographic methods that have been used successfully to identify and quantitate anthraquinones in *Cassia* extracts include column chromatography on Sephadex LH-20 (Koshioka et al., 1978), capillary GC–MS (Van Eijk and Roeijmans, 1984), fused silica capillary GC (Lewis and Shibamoto, 1985), and HPLC (Oshima et al., 1986; Van Den Berg and Labadie, 1985). These approaches are relatively time-consuming; the GC methods involved derivatizing, so they were not appropriate for screening a large number of samples quickly. Thin-layer chromatography (TLC) has been used for separating anthraquinones (Poethke et al., 1968; Rai and Shok, 1981) and could be readily adapted for running many samples in a day. Furthermore, TLC can be somewhat more flexible than GC or HPLC.

This study addresses the task of providing compositional data on the seeds of sicklepod by adapting TLC methodologies and other suitable techniques. The findings complement related compositional studies of jimson and morning glory toxic weed seeds (Friedman and Levin, 1989; Friedman and Dao, 1990; Friedman et al., 1989).

MATERIALS AND METHODS

Chrysophanic acid, emodin, rhein, and frangulin standards were obtained from Sigma Chemical Co. (St. Louis, MO). Physcion came from Atomergic Chemetals Corp. (Farmingdale,

Table I. Proximate Composition of Different Lots of Sicklepod Seeds Found as Contaminants among Commercial Grain Seeds

material	sicklepod seed (FSY-1013), %	sicklepod seed (FSY-1134), %
nitrogen	3.11	2.22
H_2O	9.72	9.68
fat	5.29	6.30
ash	4.20	4.81
carbohydrate	66.5	68.9
starch	0.2	0.2
sugar	8.1	8.3
reducing sugar	0.1	0.1

Table II. Mineral Content of Different Lots of Sicklepod Seeds Found as Contaminants among Commercial Grain Seeds[a]

mineral	sicklepod seed (FSY-1013), ppm	sicklepod seed (FSY-1134), ppm
cadmium	0	0
calcium	4100	6590
copper	31.5	9.5
iron	29.6	36.8
magnesium	3220	2820
manganese	14.3	10.9
mercury	0	0
potassium	9120	9200
selenium	0	0
sodium	930	770
zinc	75.5	10.9

[a] Each value is an average of three separate determinations.

NY). Seed samples were obtained from Azlin Seed Service (Leland, MS) and Valley Seed Service (Fresno, CA). Seeds were mixed with dry ice chips to keep fat from separating and ground to 20 mesh in a Wiley mill.

Proximate Composition. Analyses for nitrogen, moisture, fat, ash, carbohydrate, and mineral content were carried out by standard procedures (AOAC, 1980). The historical reproducibility of these analyses is estimated to be ±3% or better (Tables I and II).

Extraction of Seeds. The seeds were defatted by extracting with hexane in a Soxhlet extractor for 16 h. The seed meal was air-dried and ground to 60 mesh in a Wiley mill.

Since many of the compounds in *Cassia* species are known to be glycosides, a comparison of a neutral extraction to an acid-hydrolyzed extraction containing the aglycons is important.

(*a*) *Neutral Extraction.* Defatted samples (10 g) were mixed with 100 mL of 80% methanol and heated with stirring on a hot plate. The solution was filtered through a medium-coarseness fritted-glass funnel, and the residue was exhaustively re-extracted similarly nine more times. The filtrates were combined and evaporated to dryness. Nearly all of the compounds were extracted after about three times. However, it was necessary to carry out 8–10 extractions to isolate the sparingly soluble di- and triglycosides.

(*b*) *Acid Hydrolysis.* Defatted samples (10 g) were refluxed for 4 h with 100 mL of HCl in methanol. After filtration, the residue was re-extracted as above two times with 1 N HCl in methanol and three times with 80% methanol. The combined filtrate was neutralized with 6 N NaOH and evaporated to dryness.

Preparation of Extracts for TLC. Dried extracts were dissolved in water (25 mL) and partitioned with ethyl acetate, giving two fractions. The ethyl acetate fraction contains most of the compounds, especially the anthraquinone aglycons. This ethyl acetate fraction was successively partitioned with 5% $NaHCO_3$, 5% Na_2CO_3, and 5% NaOH. Resulting basic solutions were neutralized and repartitioned with ethyl acetate or benzene, giving five fractions. Benzene can substitute for ethyl acetate with similar results. However, benzene requires special handling because of possible health hazards.

Thin-Layer Chromatographic (TLC) Methods. The following solvent mixtures were used: Solvent A (benzene–methanol, 4:1) is described by Rai and Shok (1981); however, this paper lists a 1:4 ratio. Our studies suggest that the ratio should be reversed. Solvent B (ethyl acetate–methanol–water, 100:13.5:10) is described by Wagner et al. (1984). Solvent C (ethyl acetate–acetone–methanol–water, 5:5:1:1) is a new combination developed for this study. Solvent D (benzene–ethyl acetate, 4:1) is a modification of the 3:1 mixture recommended by Poethke et al. (1968). Solvent E (acetone–methanol–water, 10:1:1) was developed for this study. Solvent F (petroleum ether–ethyl acetate, 4:1) is a modification of the 9:1 mixture recommended by Danilovic and Naumovic-Stevanov (1965).

All plates were precoated with silica gel 60 from E. Merck. Qualitative and quantitative work was on 0.25-mm thickness, preparative plates were 0.50- or 2.0-mm thickness. Plates were spotted 2.5 cm from the bottom and allowed to develop until the solvent front was 15 cm from the origin. Plates were developed in TLC tanks lined with filter paper and saturated for at least 1 h. For preparative work the spots were scraped off the plate and eluted with either hot benzene–methanol (1:1), hot methanol, or hot 80% ethanol, depending on solubility. With benzene it is important to take safety precautions to avoid exposure to liquid or vapor.

Spray reagents used for visualizing spots were as follows: (a) 5% methanolic KOH (anthraquinones); (b) 1.0 mL of anisaldehyde, 10 mL of glacial acetic acid, 5 mL of H_2SO_4 and 85 mL of methanol with the plates then heated at 110 °C for 10 min (sugars, essential oils); (c) 1% methanolic diphenylboric acid 2-aminoethyl ester followed by 5% ethanolic polyethylene glycol 4000 (PEG) with the plates viewed under UV light at 365 nm (flavonoids, coumarins, carboxylic acids); (d) Dragendorf reagent—1.7 g of bismuth subnitrate and 20 g of tartaric acid dissolved in 80 mL of water that had been mixed with 16.0 g of KI previously dissolved in 40 mL of water to give stock solution. For spraying 1 mL of stock solution was mixed with 10 mL of water and 2 g of tartaric acid (alkaloids).

Mass Spectral Analysis. Samples were isolated and purified by TLC as described above. The mass spectra of the sample solutions were determined by direct probe using a VG Micromass 7070 F mass spectrometer.

Ion-Exchange Chromatography of Carbohydrates. Carbohydrates were analyzed on a Dionex ion chromatograph with a Carbopac PA 1 column and a pulsed amperometric detector. The eluant for the first 4 min was 0.01 M NaOH followed by water with a postcolumn addition of 0.3 M NaOH.

Fatty Acid Composition. Fatty acids were methylated by using boron trifluoride–methanol and then analyzed by GLC analysis using a Varian 5040A gas chromatograph modified for capillary injection under the following conditions: fused silica capillary column from J&W Scientific Inc. (Rancho Cordova, CA); liquid phase, DB-Wax, 0.25-μm thickness, 30 m × 0.245 mm; initial temperature 150 °C; final temperature 220 °C; program 2 °C/min for 20 min, total time 55 min; injector temperature 220 °C; FID 300 °C; helium carrier gas flow 30 cm/s; split ratio 25:1. The reproducibility of these analyses is estimated to be ±10% (Crawford, 1989).

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 *Cassia* seed protein: (a) standard hydrolysis with 6 N HCl for 22 h in evacuated sealed tubes (Friedman and Levin, 1989); (b) hydrolysis with 6 N HCl after performic acid oxidation to measure half-cystine and methionine content as cysteic acid and methionine sulfone, respectively (Friedman et al., 1979); (c) basic hydrolysis to measure tryptophan content (Hugli and Moore, 1972). The reproducibility of these analyses is estimated to be about ±3% (Friedman et al., 1979).

RESULTS AND DISCUSSION

Proximate Composition. Table I shows the nitrogen, moisture, fat, ash, and carbohydrate content of two different lots of sicklepod seed flours. The only notable difference in the sicklepod samples is the nitrogen content, which corresponds to a value of 13.9% protein (N × 6.25) for FSY-1013 compared to 19.4% protein for FSY-1134. This difference is probably due to different growing conditions. FSY-1013 was a contaminant of soybean crops,

Table III. Thin-Layer Chromatography of Extracts of Composite Lots of Sicklepod Seeds

		appearance			
	spot	visible	treatment	UV_{365nm}	identification (tentative)
Ma[a]	A1	yellow	red-violet	yellow	chrysophanic acid
Ma	A2	yellow	red-violet	yellow	physcion
	B	yellow	orange	orange	chrysophanic acid 8-Me ether
Ma	C1	yellow	orange-brown	orange	obtusifolin
Ma	C2	yellow	orange-brown	quenching	
Ma	C4	yellow	red	quenching	
Ma	D	yellow-orange	red-brown	yellow	emodin
Ma	E1	yellow	orange	red-orange	questin
Ma	E2	yellow	yellow-brown	quenching	
	F	red-brown	brown		
	G			green	
Ma	H1	yellow	orange	orange	(MW = 284+) unknown anthraquinone
Ma	H2	yellow	orange-brown	quenching	
	J			blue	
Ma	K	yellow-brown	red	orange-brown	(MW 344 + 358 + 330) anthraquinone mixture (see text)
	P1	yellow	orange-red	orange	anthraquinone di- or triglycoside or dimer
	Q1	yellow	orange-red	orange	anthraquinone di- or triglycoside or dimer
	Q2	yellow	orange-red	orange	anthraquinone di- or triglycoside or dimer
	R1			blue	hydroxycoumarin
	R2			blue-green	hydroxycoumarin + unknown
	R3			blue	hydroxycoumarin
	S			blue	hydroxycoumarin
	U1	yellow	orange-red	orange	anthraquinone triglycoside
	U2	yellow	orange-red	orange	anthraquinone triglycoside
	V			blue	hydroxycoumarin
	W1	yellow	orange-red	orange	anthraquinone triglycoside
	W2	yellow	orange-red	orange	anthraquinone triglycoside
	W3	yellow	orange-red	orange	anthraquinone triglycoside
	W4	yellow	orange-red	orange	anthraquinone triglycoside
chrysophanic acid		yellow	red-violet	orange	
physcion		yellow	red-violet	orange	
emodin		yellow-orange	orange-brown	orange	
rhein		yellow	red-brown	orange	
frangulin		light yellow	yellow-orange	yellow-orange	

[a] Ma, major component.

whereas FSY-1134 was pure-grown. Whereas the fat content of sicklepod is moderate for seeds, the carbohydrate value appears to be fairly high. After a complete hydrolysis of sicklepod seed (cold 72% H_2SO_4 for 16 h), free sugars were run by ion-exchange chromatography. Found were arabinose (4%), galactose (6%), glucose (33%), xylose (3%), and mannose (54%). The high mannose value is in line with the work of Varshney et al. (1973), who found a complex polysaccharide of galactose, glucose, mannose, and xylose in the molar ratio 2:2:7:1. Free sugars were mostly sucrose with small amounts of raffinose and traces of stachyose.

Mineral Content. Table II shows the mineral content of the two samples of sicklepod seed. Sicklepod seed is higher in calcium and sodium compared to normal levels of most seeds.

TLC. Sicklepod seed extract proved to be a complex mixture of compounds. Over 50 separate spots were isolated by TLC using various combinations of solvents. Table III lists 11 major components (estimated to be present in amounts from 0.1 to 2%) and 18 minor components (estimated to be present in amounts from 0.01 to 0.1%) of the seed extracts. The table also shows the appearance of each spot in visible light before and after spraying with 5% methanolic KOH and in UV light at 365 nm. A tentative identification is assigned to these spots. In addition, over 20 other spots were present in trace amounts, either in too small a quantity or too unstable to obtain meaningful data on this scale. Table IV lists the R_f values of these spots in six different solvent systems. Solvents A, B, and F were particularly effective in separating the anthraquinone aglycons. Solvent C was useful for the anthraquinone diglycosides or bianthraquinones. Solvent E was the only solvent listed that would move the triglycosides with distinct separation. Other systems of acetone–water such as 85:15, 87:13, or 95:5 or of acetone–methanol–water such as 9:1:1 could be used for separating some spots but were usually specific for one compound only. Table V lists the mass spectral and UV spectroscopic data obtained from the isolated and purified spots. The compounds in this table are grouped into distinct classes distinguished by appearance of the spots. These classes are discussed in the following sections.

Anthraquinones. Sicklepod seeds, like those of other *Cassia* species, contain a relatively large amount of anthraquinones. Anthraquinones and related compounds are easily identifiable by TLC as yellow spots in normal light and yellow-orange or orange spots in UV light. After spraying with 5% methanolic KOH, they are red-violet in normal light and orange or orange-red in UV light. The free anthraquinones (Table V) easily identified in the extract are chrysophanic acid (spot A1), physcion (A2), chrysophanic acid 8-methyl ester (B), obtusifolin (C1), emodin (D), and questin (E1). An unknown anthraquinone of molecular weight 284 (H1) and a mixture of the higher weight obtusin, chrysoobtusin, and auriantoobtusin (K) were also found. Rhein, reported to be in some *Cassia* species, was not present. Table VI shows the total of the aglycons in the seed as determined by TLC. These represent about 0.1–0.2% of weight in the fresh seed and seem to increase somewhat over several months, especially if the seeds are stored at ambient or elevated temperatures. According to Fairbairn (1959) storage reduces the potency of the drug preparations used as purgatives. *Cassia* purgatives are allowed to age for at least a year to reduce cramping (Singleton and Kratzer, 1969).

The major portion of anthraquinones in sicklepod seed exists, however, as the di- or triglycosides. Table VI also

Table IV. R_f Values of TLC Spots of Sicklepod Weed Seed Extracts in Six Solvent Systems[a]

TLC spot	R_f A	B	C	D	E	F
A1	0.74	0.78		0.65		0.55
A2	0.73	0.74		0.65		0.43
B	0.70	0.70		0.55		0.22
C1	0.65	0.74		0.53		0.30
C2	0.65	0.73		0.47		
C4	0.65	0.72		0.38		
D	0.60	0.74		0.45		0.14
E1	0.56	0.70		0.32		0.04
E2	0.55	0.72		0.27		
F	0.40	0.25–0.60				
G	0.33	0.37	0.61			
H1	0.36	0.33	0.57			
H2	0.31	0.28	0.54			
J	0.23	0.27	0.51			
K	0.22	0.29	0.55			
P	0.10	0.08	0.37		0.56	
Q1	0.03	0.02	0.22		0.34	
Q2	0.03	0.02	0.20		0.34	
R1	0.08	0.08	0.35		0.57	
R2	0.08	0.07	0.34		0.56	
R3	0.04	0.05	0.32		0.53	
S	0.02	0.01	0.15		0.51	
U1		0.14		0.09		
U2		0.13		0.08		
V		0.10		0.07		
W1		0.12		0.08		
W2		0.11		0.07		
W3		0.10		0.07		
W4		0.09		0.06		
chrysophanic acid	0.75	0.79		0.66		0.57
physcion	0.74	0.78		0.65		0.44
emodin	0.61	0.74		0.46		0.15
rhein	0.10	0.26	0.48			

[a] Solvents: A, benzene–methanol, B, ethyl acetate–methanol–water, C, ethyl acetate–acetone–methanol–water, D, benzene–ethyl acetate, E, acetone–methanol–water, F, petroleum ether–ethyl acetate, 4:1; 100:13.5:10; 5:5:1:1; 4:1; 10:1:1; 4:1.

shows the amount of anthraquinones after hydrolysis. Total anthraquinones make up about 1–2% by weight of the seed. All the aglycon compounds increase, especially chrysophanic acid, which accounts for almost half the anthraquinone present. Unhydrolyzed seed extract yielded a number of glycosides or bianthraquinones that upon hydrolysis yield aglycons—P, Q1, Q2, U1, U2, W1, W2, W3, and W4. These were not identifiable as single compounds but seemed to be mixtures. They could not be further resolved by simple methods, but some conclusions could be drawn. No monoglycosides of the frangulin type were detected. All were either di- or triglycosides and upon hydrolysis yielded only glucose. There also seemed to be small amounts of bianthraquinones that upon hydrolysis gave chrysophanic acid or emodin or both, but not glucose (Kitanaka and Takido, 1983; Dutta et al., 1964).

The UV data in Table V were inconclusive as to hydrolysis products. However, all compounds exhibited a shift either from 406–412 to 425–432 nm or from 405 to 411 nm.

UV-Quenching Compounds. Spots C2, C4, E2, and H2 (Table V) were all yellow spots that quenched fluorescence in UV light at 365 nm. After spraying with 5% KOH, they appeared orange-brown or red-brown. The spots seemed to have a molecular weight of 330, 344, or 358. Each compound showed two large UV peaks, one at around 220 nm and a larger one at around 284 nm. These compounds are unaffected by hydrolysis.

Fluorescent Blue Compounds. Spots J, R1, R3, S, and V (Table V) were all colorless under normal light and fluoresced blue-violet under 365-nm UV light. The compounds were relatively stable when refrigerated. After hydrolysis, however, the compounds fluoresced light blue and were unstable, losing all fluorescence after 24 h even when refrigerated. All these compounds had essentially only one UV peak, at 264 nm. Mass spectral data were inconclusive, and no molecular ion was established. These spots and several similar trace spots were either hydroxycoumarins or flavonoids.

(*a*) *Hydroxycoumarins.* Extracts were screened for hydroxycoumarins by TLC. Plates were developed in toluene–ether (90:10) saturated with 10% acetic acid. Standards were aesculetin, scopoletin, and umbelliferone. Spots were visible under UV light (365 nm) without treatment. Results were essentially negative, although one small spot, probably caffeic acid, was observed.

(*b*) *Flavonoids.* Plates were developed in ethyl acetate–formic acid–acetic acid–water (100:11:11:27). Standards were rutin, chlorogenic acid, and caffeic acid as phenolcarboxylic acids are also detected by this approach. Spots were visualized by spraying with 1% diphenylboric acid β-ethylamino ester followed by 5% PEG. This produced five relatively intense blue-white spots at R_f 0.05, 0.15, 0.25, 0.70, and 0.95. The spot at the solvent front was probably caffeic acid. The other spots are unidentified but are not flavones, flavonols, or flavonones, which typically give yellow, orange, or yellow-green spots by this treatment. Thus, kaempferol, which has been reported in several *Cassia* species (Mahesh, 1984), could not be confirmed in sicklepod seed. These compounds were also not the usual plant acids (chlorogenic, isochlorogenic, and neochlorogenic acids), although traces of these compounds were also detected.

Miscellaneous Compounds. Three other compounds are found in the seed extracts in relatively large amounts. β-Sitosterol was isolated by TLC or by precipitation with digitonin in amounts of 0.1–0.2%. The R_f values of β-sitosterol in solvents A, B, and D are very similar to those of spots C1, C2, and C4 and sometimes interfered with detection (see Table IV).

The other two compounds were unstable, especially when dried. The first had an R_f of about 0.40 in solvent A and streaked badly in other solvents. It was a yellow-brown spot that appeared purple when made basic. Attempts to collect and purify this compound resulted in complete loss. The second compound streaked somewhat in all solvents. It was normally colorless, but was a brillant red under UV light. In basic solution, it appeared green and did not fluoresce. In neutral solution it fluoresced red, but decomposed to a gray powder after several hours at room temperature. In acid solution, it decomposed rapidly. This may be a chlorophyll-related compound.

Sicklepod seed extract was also screened for essential oils, saponins, and alkaloids by TLC. Separate plates were run in ethyl–methanol–water (100:13.5:10) and toluene–ethyl acetate (93:7) and sprayed with anisaldehyde. Results were negative for essential oils and saponins. A plate was run in toluene–ethyl acetate–diethylamine (70:20:10) with standards of atropine and scopolomine and sprayed with Dragendorf reagent followed by 5% sodium nitrate. Results were again negative. No alkaloids were present.

Fatty Acid Content. The results of fatty acid distribution analysis of sicklepod are shown in Table VII. Distribution analyses of other seeds are also shown for comparison. The fatty acid profiles are reasonably similar in all of the seeds including the edible seed soybean, with linoleic acid the most abundant fatty acid in all seeds (43–67%). Soybean and morning glory also contain moderate levels of an ω − 3 fatty acid (linolenic acid).

Table V. Mass Spectra and Ultraviolet Absorption Data of Sicklepod Components Isolated from TLC Plates

TLC spot	MW from mass spectra[a]	UV spectra max,[b] nm	UV spectra max after hydrolysis,[c] nm
		Anthraquinones	
A1	254	225, 255, 277, 287, 429	
A2	284	222.5, 251.5, 264.5, 281, 432.5	
B	268	221.5, 255.5, 414.5	
C1	284	225.5, 275.5, 400	
D	270	221.5, 253, 265.5, 287, 436.5	
E1	284	223.5, 248, 284, 427	
H1	284	220.5, 263.5, 403	
K	330, 344, 358	223.5, 277, 431	
		Anthraquinone Glycosides and Bianthraquinones	
P		218.5, 258.5, 409.5	224, 256.5, 286, 430
Q1	(254, 255, 354)	222, 259, 410	224.5, 257.5, 277, 427
Q2	(254, 284)	222, 259, 411	224.5, 260, 277, 423.5
U1	(354, 419)	222, 259, 405	224, 258, 429.5
U2	(398)	222, 260, 404.5	224, 259.5, 276.5, 411.5
W1	(326)	220.5, 258.5, 410	222.5, 257.5, 428.5
W2	(254)	222, 259, 407.5	223.5, 258.5, 410
W3	(394)	222, 259, 404.5	224.5, 258.5, 276.5, 42
W4		222, 260.5, 275.5, 404.5	224, 259.5, 276.5, 411.5
		UV Quenching Spots	
C2	344	223, 284, 315.5, 385	
C4	358	218, 283.5, 307, 358	
E2	330	218, 286, 313.5, 389	
H2		224.5, 282.5, 311, 394	
		Blue Spots on 365 nm	
R1		262.5, 278	
R3		262, 359	
S		261.5, 354	
V		258.5, 410	

[a] Single numbers are identifiable as molecular ions. Two or more numbers signify impure compounds. Numbers in parentheses are significant peaks but are not molecular ions. [b] Dissolved is absolute methanol. [c] 1 N HCl at 100 °C for 10 min, evaporate, and dissolve in absolute methanol.

Table VI. Amounts of Anthraquinone Aglycons in Sicklepod Estimated by TLC

spot	compd	FSY-1134, mg/g unhydrolyzed	FSY-1134, mg/g hydrolyzed
A1	chrysophanic acid	0.2	6.0
A2	physcion	0.04	1.0
B	chrysophanic acid 8-Me ester	0.02	0.4
C1	obtusifolin	0.3	1.0
D	emodin	0.1	1.8
E1	questin	0.2	1.5
H1	anthraquinone (MW 284)[a]	0.3	1.0
K	mixture of obtusin, chrysoobtusin, auriantoobtusin, etc.	0.1	1.0
	total	1.26	12.7

[a] Unknown structure.

Table VII. Fatty Acid Distribution (g/100 g) in Weed and Soybean Seeds

	sicklepod	morning glory	jimson weed	soybean
C16:0	16.9	21.9	13.1	11.2
C18:0	6.7	7.5	2.6	4.5
C18:1ω9	26.2	18.6	26.5	22.3
C18:1ω7	1.5	0.8	1.0	1.4
C18:2ω6	43.7	42.7	56.9	53.6
C18:3ω3	1.8	3.8		7.0
C20:0	1.6	1.4		
C20:1	0.3	0.7		
C22:0	0.9			
% fat	5.3	11.2	18.0	20.0

Amino Acid Content. Table VIII shows the amino acid composition of hydrolysates of sicklepod 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 sicklepod, especially for essential amino acids, exceeds the provisional requirements for (a) aromatic amino acids (phenylalanine, tyrosine, tryptophan), (b) basic amino acids (lysine), (c) neutral amino acids (isoleucine, leucine, valine), and (d) sulfur amino acids (half-cystine and methionine). If it were possible to remove the toxic principles of sicklepod weed seeds by food processing or by controlling the genes in the plant that biosynthesize these compounds, then sicklepod seeds could serve as a nutritional source of a good-quality protein.

Table VIII. Amino Acid Content of Sicklepod (*C. obtusifolia*) Seeds

amino acid	g/100 g of flour	g/16 g of N[d]	FAO,[e] g/16 g of N
Ala	0.65	4.8	
Arg	1.02	7.6	
Asp	1.50	11.1	
Cys[a]	0.244	1.8	3.5[f]
Glu	2.13	15.8	
Gly	0.689	5.1	
His	0.359	2.7	
Ile	0.596	4.4	4.0
Leu	1.09	8.1	7.0
Lys	0.95	7.0	5.5
Met[b]	0.227	1.7	3.5[f]
Phe	0.653	4.8	6.0[g]
Pro	0.536	4.0	
Ser	0.759	5.6	
Thre	0.594	4.4	4.0
Try[c]	0.218	1.6	
Tyr	0.442	3.3	6.0[g]
Val	0.742	5.5	5.0
total	13.4	99.3	

[a] Half-cystine determined as cysteic acid after performic acid oxidation. [b] Determined as methionine sulfone after performic acid oxidation. [c] Determined after 48-h alkaline hydrolysis at 135 °C (Hugli and Moore, 1972). [d] N content, 2.16%. [e] Provisional amino acid scoring pattern for an ideal protein (FAO, 1973; Owens and Pettigrew, 1989). [f] Cys + Met. [g] Tyr + Phe.

SIGNIFICANCE FOR FOOD SAFETY

Sicklepod seeds, which have been recommended as a poultry feed (Katoch and Bhomik, 1983; Talpada et al., 1980), contain a large number of known and unknown anthraquinone and other pharmacologically and toxicologically active compounds. Although such compounds are still being used medically as purgatives, the reported adverse effects of sicklepod seeds on muscular and other functions in rabbits, rodents, calves, and cattle (Chan et al., 1976; Crawford and Friedman, 1990; Fairbarn and Moss, 1970; Henson et al., 1965; Henson and Dollahite, 1966; Hebert et al., 1983; Ishii et al., 1988; Koo et al., 1976; Lewis and Shibamoto, 1989; McCormack and Neisler, 1980; Mercer et al., 1967; Nicholson et al., 1986; O'Hara et al., 1969; Pal and Pal, 1978; Patel et al., 1965; Putnam et al., 1988; Tarparia and Talmale, 1978; Wong et al., 1989b) suggest that these seeds should be largely removed from food grain such as soybeans and wheat before consumption. The rapid TLC screening procedures described in this study should make it possible to measure the anthraquinone and thus sicklepod content of large numbers of samples of contaminated grain both in the laboratory and inexpensively under practical field and grain elevator conditions. This in turn should help set safety standards to minimize the consumption of possibly unsafe sicklepod-contaminated grain.

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