Comparative Studies of the Alkaloidal Composition of Two Mexican *Erythrina* Species and Nutritive Value of the Detoxified Seeds†

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The seeds of two species of *Erythrina* were studied in connection with their alkaloid content and nutritive value. The alkaloids present in the seeds were the main toxic components that impede the use of the seeds as food since the whole seeds killed the experimental animals. However, the detoxified seed flour showed a good-quality protein; although the process did not destroy the trypsin inhibitors present in the seed, it did not affect the growth of the weanling rats.

INTRODUCTION

Erythrina is a very distinct genus of the Fabaceae consisting of 108 species of wide distribution in the tropical regions of the world. As a result of the studies carried out by Krukoff and Barneby (1974), this genus of beautiful trees, shrubs, and a few herbs is probably better known in many respects than any other of comparable size and distribution. The current knowledge on these plants encompasses their morphology, distribution, chromosomes, palinology, and alkaloid and amino acid composition. Only in *Erythrina* can the alkaloids be considered typical; they have unusual structural features and exhibit a restricted distribution within the Fabaceae. Many species of *Erythrina* have been studied, and the alkaloidal data now available permit some grouping of species, but only a small fraction of them have been carefully examined (Hargreaves et al., 1974; Games et al., 1974).

In Mexico the genus is widely spread. There have been detected about 25 species distributed in all the country and in the Caribbean area (Neill, 1988). Most of them have alkaloids mainly in the seeds, but even in the flowers there are alkaloids, too (Aguilar et al., 1981). The flowers of *Erythrina americana* and *Erythrina mexicana* are edible in the southeast of Mexico, but they are boiled in water and then washed before the meal is prepared.

Although preliminary studies of the alkaloids present in the seeds of several *Erythrina* species, including *E. americana* and *E. breviflora*, have been done before (Folkers and Major, 1937; Abdullah et al., 1979), they have not been carefully examined; neither is there information about the nutritive value of the detoxified seed flour of both species. The objectives of this work were to compare the alkaloidal composition of the seeds of *E. americana* and *E. breviflora* and to determine the nutritive value of the detoxified seed flour.

MATERIALS AND METHODS

The seeds of *E. americana* were collected on the campus of the Universidad Nacional Autónoma de México (UNAM), in Mexico D.F., and those of *E. breviflora* were collected in Tuxpan, Michoacan, Mexico, and classified at the Biology Institute of the UNAM. The beans were dried in an oven at 50–55 °C and then milled in a Thomas Willey mill to pass a 2-mm sieve. The procedure to obtain the alkaloid fractions and the detoxified flour is shown in Figure 1.

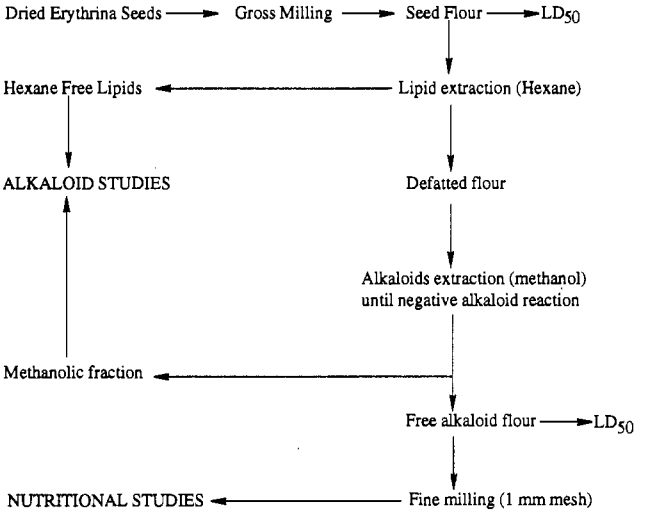

Figure 1. Procedure to obtain the alkaloid fractions and detoxification of the *Erythrina*.

Proximate analysis was done in the whole and detoxified flour according to the techniques described by the AOAC (1984).

Amino acid composition was determined after acid hydrolysis (Lucas and Sotelo, 1982) and alkaline hydrolysis for tryptophan determination (Lucas and Sotelo, 1980). A Technicon amino acid autoanalyzer Model 2P was used (Tarrytown, NY). The chemical score was also calculated (Pellet and Young, 1980).

Alkaloid Detection. This determination was done in the whole seeds, in the detoxified flour, and in the lipid fraction according to the technique described by Hulting and Torssell (1965).

Trypsin inhibitor determination was carried out according to the procedure of Kakade et al. (1974) using the synthetic substrate benzoyl-DL-arginine *p*-nitroanilide (BAPNA; Sigma, St. Louis, MO).

Hemagglutinin Determination. The technique described by Jaffé and Brucher (1972) was employed using hamster red blood cells.

Cyanogenic glucoside determination was done according to the technique described by Lucas and Sotelo (1984).

LD_{50} determination was done with intraperitoneal injection of saline extract of the whole or detoxified seeds, using 10 mice per dose (5 doses) (Litchfield and Wilcoxon, 1949).

Biological Protein Evaluation. The tests were performed in the detoxified flour (Pellet and Young, 1980) using weanling

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Table I. Composition of Experimental Diets[a]

ingredients	diet composition (g/100 g of total diet) control	*E. americana*	*E. breviflora*
casein (87% protein)[b]	11.2		
detoxified *E. americana* flour		23.5	
detoxified *E. breviflora* flour			25.9
sucrose	20.0	18.2	18.2
glucose[b]	20.0	18.2	18.2
dextrin[b]	20.0	18.2	18.2
lard	8.0	8.0	8.0
corn oil	7.0	6.0	6.0
mineral mix[c]	3.9	2.9	2.9
vitamin mix[d]	2.0	2.0	2.0
fiber cellulose type[b]	7.9	3.0	0.6

[a] N × 6.25 = 10.0 g of protein/100 g of total diet. Each diet was calculated to contain 422.4 kcal/100 g of diet. [b] Sigma, St. Louis, Mo. [c] Roger Harper mineral mix, ICN Pharmaceutical, Cleveland, OH. [d] ICN Pharmaceutical.

Wistar male rats 21–23 days old, weight average 36–60 g. Growth indices were determined: protein efficiency ratio (PER) and net protein ratio (NPR). Nitrogen balance indices were also determined: digestibility (D), net protein utilization (NPU), and biological value (BV) using metabolic cages for these determinations. The PER and NPR determinations lasted 21 and 10 days, respectively. For the NPU, BV, and D data, urine and feces were collected from the 12th to the 21st day (10 days). Both food and water were available *ad libitum*. Food consumption and the weight of each of the animals were recorded twice a week throughout the 21 days of the experiment. Isocaloric and isoproteic diets were prepared. Table I shows the composition of experimental diets.

Isolation and Analysis of Alkaloids. One kilogram of seeds was ground and placed in an adapted continuous extractor with hexane to remove the fat during 48 h. Thereafter, the defatted flour was placed in a 2-L Erlenmeyer flask and extracted with methanol, under magnetic stirring, and heated at 60–65 °C. The methanolic alkaloid fractions were removed every hour by adding fresh methanol until a negative alkaloid reaction was detected. The purpose of heating during the alkaloid extraction was to destroy the lectins and trypsin inhibitors. This process lasted 7 h.

Since the lipid fraction after the hexane was removed gave a positive alkaloid reaction in both *Erythrina* species, these alkaloids were removed also from the fat.

Isolation of the Alkaloid Fractions. The hexane free lipid fraction was washed with 2 N sulfuric acid, followed by neutralization of the aqueous acidic phase with sodium bicarbonate, and adjusted to pH 8 with solid sodium bicarbonate. Finally, a re-extraction into chloroform, gave the "free" alkaloid from the lipid hexane fraction. The methanol extracts from the flour extraction were evaporated under vacuum, combined, and taken up in 2 N sulfuric acid. The aqueous phase was saturated with sodium chloride and extracted with chloroform to remove traces of fat. Again the aqueous acidic phase was neutralized with sodium bicarbonate, adjusted to pH 8 with diluted sodium hydroxide solution, and extracted with chloroform to give the "free" alkaloid fraction. The remaining aqueous phase was reacidified to pH 2 with hydrochloric acid and heated on a steam bath for 8 h to hydrolyze the esterified alkaloids. The process of alkalinization, to pH 8, and extraction with chloroform was repeated to yield the "liberated" alkaloid fraction.

Gas Chromatography. In the preparation for GC analysis, the crude alkaloid mixture (1–2 mg) was derivatized as trimethylsilyl (TMSi) derivatives by treatment for 30 min with *N,O*-bis(trimethylsilyl)acetamide (25 μL) in acetonitrile (25 μL) using a Teflon-lined screw-cap vial to prevent evaporation. The mixture of alkaloids was then analyzed on a Pye Unicam S-204 gas chromatograph (Phillips, Cambridge, U.K.) equipped with a flame ionization detector and a 12 ft × 2 mm (i.d.) glass column packed with 3% OV-17 on gas Chrom Q 100–300 mesh (Pierce Chemical Co.). The injector and detector temperatures were maintained at 250 and 280 °C, respectively, while the oven temperature was programmed from 225 to 285 °C at a rate of 2 °C/min; the flow rate of the carrier gas (nitrogen) was maintained at 30 mL/min.

Table II. Proximate Composition (Grams per 100 g of Sample) of the Whole and Detoxified Seed Flour of the Two Mexican *Erythrina*

sample	moisture	protein	fat	ash	fiber	carbohydrate
Whole Seed Flour						
E. americana	5.8	27.2	17.7	3.6	15.3	30.4
E. breviflora	5.0	23.3	13.9	3.4	21.6	32.8
Detoxified Seed Flour						
E. americana	3.2	42.5	0.3	4.6	26.5	22.8
E. breviflora	4.7	38.5	0.1	4.2	31.4	21.1

Mass Spectrometry. Mass spectra were determined in a Varian CH-50 mass spectrometer (Palo Alto, CA) coupled with a GC via a two stage Watson–Biemann separator. The temperature of the ion source was 220 °C, and the accelerating and ionizing potentials were 3 kV and 70 eV, respectively. The spectra were recorded using a data system and, after subtraction of background peaks, were normalized and plotted on a fast printer.

Statistical Analysis. Data on the composition of the two *Erythrina* species are presented as mean values of three replicates. Biological studies were tested statistically by the one-way analysis of variance (ANOVA) and multiple range analysis (Duncan) procedure of the Statgraphic (v. 5)/PC 1991 software package. The LD_{50} was statistically analyzed according to the method of Litchfield and Wilcoxon (1949).

RESULTS AND DISCUSSION

The proximate composition in the two *Erythrina* species is shown in Table II. Both whole seeds showed high protein and fat content, while *E. americana* showed the highest concentration of both components. *E. breviflora* had higher fiber content. The protein and fiber contents were remarkably increased in the defatted and detoxified flour of both *E. americana* and *E. breviflora*.

The amino acid composition and chemical score of the two whole and detoxified flours of *Erythrina* are shown in Table III. As was expected, sulfur amino acids were limiting in both seeds, although in the detoxified *E. breviflora* seeds valine was the limiting amino acid followed very closely by sulfur amino acids.

The biological tests (Table IV) indicated that both detoxified flours had good protein quality, and the *E. americana* showed a PER value similar to that of the control diet. However, in the nitrogen balance indexes (BV, NPU) both seeds were similar to the casein diet, although the digestibility was lower in both legumes than in the control diet.

Previous to this paper information related to the nutritive value and protein quality of detoxified *Erythrina* flour was not found.

Table V shows the antinutritional and toxic factors present in the whole and detoxified flour as well as in the lipid fraction. It is important to mention that the trypsin inhibitor in the detoxified flour was almost the same as in the whole seeds, even during the alkaloid extraction when the samples were heated for 7 h at 60 °C. This means that the trypsin inhibitors were present in both *Erythrina* species but that they did not have adverse effect on the rats since the nutritional evaluation was not affected by them. Lectins were destroyed at that temperature, and the cyanogenic glycosides were not present in either of the seeds. The toxic effect of the *Erythrina* studied is due to the alkaloids present in the seeds since in a previous experiment (not published data) rats fed with cooked seeds were killed.

Alkaloid Structure. The distribution of various alkaloids characterized in the two species studied is shown

Table III. Amino Acid Composition (Grams of Amino Acid per 100 g of Protein)[a] and Chemical Score in the Whole and Detoxified Seed of *E. americana* and *E. breviflora*

	whole seed flour		detoxified seed flour		
amino acid (aa)	*E. americana*	*E. breviflora*	*E. americana*	*E. breviflora*	FAO/WHO pattern 1973
Met	2.3 ± 0.5	2.7 ± 0.2	2.6 ± 0.1	2.8 ± 0.2	3.5
Cys	1.1 ± 0.8	1.4 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	
Phe	3.3 ± 0.1	3.7 ± 0.3	4.4 ± 0.8	3.9 ± 0.2	6.0
Tyr	3.2 ± 0.5	3.5 ± 0.3	4.8 ± 0.4	4.0 ± 0.6	
Lys	10.2 ± 0.7	7.8 ± 1.0	8.3 ± 0.4	7.6 ± 0.8	5.5
Ile	3.7 ± 0.4	4.0 ± 0.8	4.9 ± 0.3	n4.2 ± 0.3	4.0
Leu	7.2 ± 0.9	7.4 ± 1.5	10.0 ± 0.9	7.5 ± 0.8	7.0
Val	3.8 ± 0.1	4.2 ± 0.1	5.0 ± 0.7	3.9 ± 0.9	5.0
Thr	3.3 ± 0.1	4.1 ± 0.7	4.8 ± 0.7	3.2 ± 0.4	4.0
Trp	0.9 ± 0.1	0.89 ± 0.1	1.07 ± 0.1	0.9 ± 0.1	1.0
His	2.9 ± 0.6	2.8 ± 0.2	2.7 ± 0.2	3.2 ± 0.4	
Asp	9.7 ± 1.4	12.16 ± 2.1	8.8 ± 0.8	4.9 ± 1.1	
Glu	15.4 ± 0.4	13.9 ± 1.4	15.8 ± 2.5	12.6 ± 2.3	
Ala	4.0 ± 0.4	4.2 ± 0.2	4.5 ± 0.1	3.7 ± 0.2	
Gly	4.0 ± 0.4	3.8 ± 0.8	4.4 ± 0.5	3.7 ± 0.2	
Pro	4.4 ± 0.8	4.3 ± 0.9	5.5 ± 0.5	4.1 ± 0.3	
Ser	5.9 ± 0.4	5.9 ● 0.1	5.4 ± 0.8	4.2 ± 0.2	
Arg	6.4 ± 0.5	4.5 ± 1.1	5.5 ± 0.6	5.1 ± 0.4	
total essential aa	37.9	38.3	46.0	38.0	
chemical score	66.6 ± 3.3	76.3 ± 4.6	75.1 ± 42	77.6 ± 10.4	
limiting aa	sulfur aa	sulfur aa	sulfur aa	valine	

[a] Values represent mean ± SD.

Table IV. Protein Quality of the Two Detoxified *Erythrina* Flours

	biological test[a]				
protein source	PER[b,c]	NPR[b]	BV[b]	NPU[b]	D[b]
casein	2.5 ± 0.2 A	4.2 ± 0.3 A	88.8 ± 4.4 A	94.4 ± 5.4 A	96 ± 1.8 A
E. americana	2.3 ± 0.2 AB	3.8 ± 0.7 A	93.9 ± 1.1 B	88.8 ± 5.5 A	86 ± 3.1 B
E. breviflora	2.1 ± 0.3 B	4.6 ± 0.4 A	91.6 ± 1.8 AB	86.3 ± 5.3 B	80 ± 4.5 C

[a] Average of six rats ± SD. Means in columns without common letters differ significantly ($P < 0.05$). [b] PER, protein efficiency ratio; NPR, net protein ratio; BV, biological value; NPU, net protein utilization; D, digestibility. [c] Adjusted indices to casein (2.50).

Table V. Antinutritional Factors and Toxic Content in the Whole Detoxified Flour and Lipid Fraction of the Two *Erythrina*

sample	trypsin inhibitors (TUI[a]/mg of dry sample)	lectins[b] hamster RBC	cyanogenic glucosides (mg of HCN/ 100 g of sample)	alkaloids	LD_{50}[a] (mg/kg)
E. americana					
whole flour	68.00 A	5	0	+	1375
detoxified flour	49.24 B	0	0	−	
lipid fraction				+	
E. breviflora					
whole flour	63.9 A	6	0	+	3900
detoxified flour	62.9 A	0	0	−	
lipid fraction				+	

[a] Trypsin units inhibited (Kakade et al., 1974). Means in column without common letters differ significantly ($P < 0.05$). [b] Titer: the highest dilution at which agglutination is found (Jaffe et al., 1972). [c] The test was done with saline extract intraperitoneally in mice.

Table VI. Occurrence of Alkaloids in the Two *Erythrina* Seeds Studied[a]

sample	1	2	3	6	9	12	13	U[b]
E. americana								
free (0.4%)[c]	0.28		3.0	0.81		12.4	83.4	
liberated (0.13)[c]	100							
E. breviflora								
free (0.54%)	43.8	30.3	5.5		1.0			18
liberated (0.23)								24.5

[a] Structures are shown in Figure 2. [b] Unknown. [c] The free alkaloids are those which occurred in the seeds as free bases, whereas those in the liberated fraction occurred as glycosides and were analyzed after acidic hydrolysis. The yield of free alkaloids obtained from the seeds (given in the first column) includes those in the hexane fraction. The relative proportions of the various alkaloids are calculated from the areas of the GC peaks.

in Table VI, and their structures are given in Figure 2. These results confirm the view that erysodine (1), erysopine (2), and erysovine (3) are the most widely distributed alkaloids. Erysodine and erysovine were found in both species and in *E. breviflora* were the most common alkaloids. *E. americana* contained both α-erythroidine and β-erythroidine (12 and 13) as major alkaloids; they were not present in the other species. In a recent paper (Aguilar et al., 1993), the presence of α-erythroidine but not of β-erythroidine in the seeds of *E. breviflora* was described, but this is uncommon because whenever the presence of α-erythroidine is detected, the presence of β-erythroidineis inferred since normally they are found together in the American *Erythrina* species that contain lactonic alkaloids.

We also included in the studies the analysis of the "hexane" fraction (lipids), since it has been described (Hargreaves et al., 1974) that the hexane fraction contains significant quantities of alkaloids. In the present study in this alkaloid fraction of *E. americana* the presence of the common alkaloids erysodine, erysovine, and the erythroidines was also detected, although in *E. breviflora* only trace amounts of erysodine were detected.

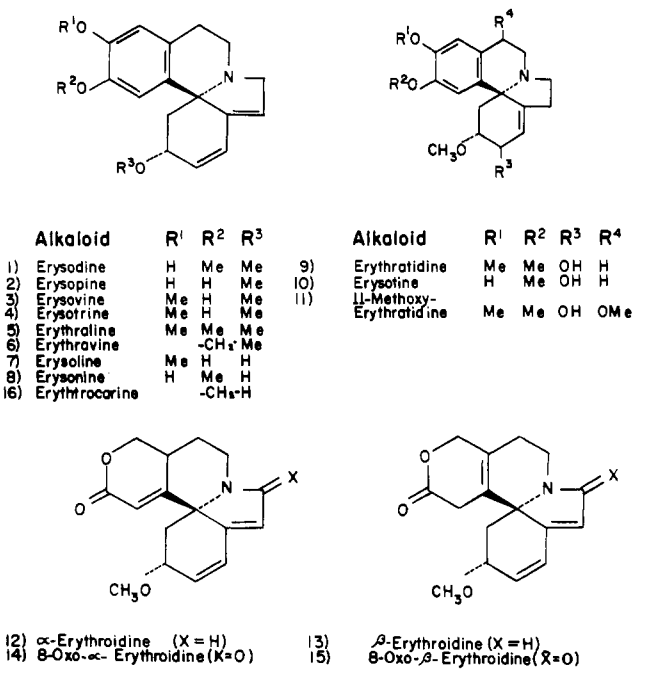

Figure 2. Structure of the (top) dienoid and alkenoid alkaloids and (bottom) lactonic alkaloids.

Conclusions. The distribution of seven dienoid and lactonic alkaloids in the two *Erythrina* species studied here is described. After heating, relatively stable (60 °C, 7 h) trypsin inhibitors were found in these seeds, but they did not affect the growth of weaning rats. Taking into consideration the high protein and fat concentration in these seeds, the adequate profile of amino acids, and the biological protein quality evaluation of the detoxified flour obtained, *Erythrina* is an interesting genus that requires future studies. A good process to eliminate the alkaloids is needed as is more knowledge about the biosynthesis of the alkaloids during the process of development of the pods and ripening of the seeds.

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