Table IV. Comparison of Chemical Score Values of Essential Amino Acids of Okra Seeds with Those of Wheat and Soybean[a]

essential amino acids (EAA)	chemical score			
	soybean[b] (var. Clark)	wheat	okra seed variety Emerald	okra seed variety Ibtaira
leucine	98.99	77.50	75.06	79.10
isoleucine	85.00	74.20	54.31	57.41
cysteine and methionine	168.94	80.70	71.93	76.84
valine	65.81	74.30	54.05	67.03
tryptophan	74.00	60.00	64.00	56.67
phenylalanine	93.04	96.40	76.43	70.36
lysine	140.75	41.70	117.91	133.13
histidine	45.71	104.9	84.76	87.62
threonine	86.60	56.00	60.00	70.00
tyrosine				

[a] Data for EAA in the reference protein (whole egg) and EAA in wheat and the method used for calculation of the chemical score were reported by Osborne and Voogt (1978). [b] Al-Wandawi (1981).

Table V. Mineral Composition of Okra Seeds

element	mg/100 g of defatted seed flour for variety: Emerald	Ibtaira
calcium	$375.5^{y} \pm 10.04^{a,b}$	$268.8^{y} \pm 3.68^{a,b}$
copper	$<1^{y}$	$<1^{x}$
chromium	$<1^{y}$	$<1^{x}$
iron	$9.80^{y} \pm 0.96$	$7.30^{x} \pm 0.45$
magnesium	$643.8^{y} \pm 2.26$	$483.9^{y} \pm 3.68$
manganese	$4.85^{y} \pm 0.16$	$3.80^{y} \pm 0.20$
nickel	$1.70^{y} \pm 0.11$	$0.80^{y} \pm 0.21$
potassium	1309.00^{y} b 12.45	$1591.40^{x} \pm 8.20$
rubidium	$<1^{y}$	$<1^{x}$
strontium	$2.30^{y} \pm 0.28$	$1.90^{x} \pm 0.01$
sodium	$647.20^{y} \pm 4.81$	$475.60^{y} \pm 3.54$
zinc	$8.60^{y} \pm 0.07$	$7.00^{y} \pm 0.13$

[a] Mean ± SD. [b] Means followed by same letter on a horizontal line are significantly different according to an L.S.D. test ($P = 0.05$).

Gossypol and Cyclopropenoid Compounds. Gossypol, the phenolic compound found in cotton seed and known to cause undesirable physiological effects on nonruminants such as poultry and swine (Pons, 1977), was found in okra seed only as traces. Cyclopropenoid compounds, which are found in seed lipids of the order Malvales, were estimated in okra seed by using the Halpen test. These compounds were found in okra seed in an amount equal to half that present in cotton seed (determination of cyclopropenoid compounds in okra and cotton seed was carried out under identical conditions). It is worthwhile to mention that cotton seed oil contains concentrations of these cyclopropenoid compounds of 0.6–1.2% in crude oil and 0.1–0.5% in the processed oil (Mattson, 1973).

Registry No. Gossypol, 303-45-7; starch, 9005-25-8; Ca, 7440-70-2; Mg, 7439-95-4; K, 7440-09-7; Na, 7440-23-5; Cu, 7440-50-8; Cr, 7440-47-3; Fe, 7439-89-6; Mn, 7439-96-5; Ni, 7440-02-0; Rb, 7440-17-7; Sr, 7440-24-6; Zn, 7440-66-6.

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Received for review August 25, 1982. Revised manuscript received May 23, 1983. Accepted August 11, 1983.

COMMUNICATIONS

Ranunculin: A Toxic Constituent of the Poisonous Range Plant Bur Buttercup (*Ceratocephalus testiculatus*)

Toxic ranunculin has been isolated from bur buttercup (*Ceratocephalus testiculatus*), a range plant poisonous to grazing sheep. Analyses of various growth stages of the plant revealed that the "early flower" stage contained the highest concentration of ranunculin.

Bur buttercup (*Ceratocephalus testiculatus*) is a small gray-green woolly, early appearing (March–May) annual weed. Introduced into the western United States, the plant was first identified in Utah in 1932. It now grows in California, Colorado, Idaho, Nebraska, Nevada, Oregon, Utah, and Washington. Plants have been found on foot-

hills, sage slopes, old sheep bed grounds, and other waste places, and they seem to spread rapidly. Heavy stands have been reported on recently seeded crested wheatgrass ranges, and the weed is invading grain and alfalfa fields (Olsen et al., 1982).

Bur buttercup is not a true buttercup and has not been considered to be a poisonous plant. Its close relatives, the true buttercups (genus *Ranunculus*), however, have a different record. At least nine species of buttercups are poisonous, with some causing significant death losses in domestic animals (Watt and Brayer, 1932; Shearer, 1938; Ruijgrok, 1966).

The sudden death of about 150 ewes on grazing bur buttercup recently in Central Utah prompted Olsen et al. (1983) to investigate the toxicity of this plant to sheep. They found the weeds to be toxic to sheep with a minimum lethal dosage of 11 g (wet weight) of green plant material/kg. Experimental studies and field observations showed that clinical signs of bur buttercup poisoning are weakness, depression, diarrhea, labored breathing, anorexia, and occasional fever. Post-mortem findings included inflammation and edema of the rumen, hemorrhage in the left ventricle of the heart, congestion of the lungs, liver, and kidneys, and excessive fluid in the thoracic and abdominal cavities (Olsen et al., 1983). Even though it seems likely that animals on a normal grazing regimen or receiving supplemental feed would seldom be poisoned, livestock managers must be aware of the potential for significant livestock losses from bur buttercup.

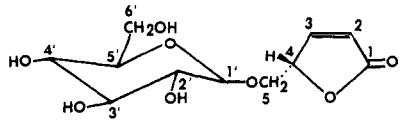
This paper reports the isolation of a toxic component of bur buttercup.

EXPERIMENTAL SECTION

The ^{13}C NMR, ^{1}H NMR, and IR spectra were obtained by using a JEOL JNM-PFT-100 (100 MHz), Varian EM-390 (90 MHz), and a Perkin-elmer 727-B spectrometer, respectively. Chemical shifts of the ^{13}C NMR and ^{1}H NMR spectra are reported relative to internal acetonitrile and sodium 3-(trimethylsilyl)propionate-*2,2,3,3-d*$_4$ (TSP), respectively. A multiplicity separation pulse sequence was employed when ^{13}C NMR spectra were obtained, which distinguishes quaternary and methylene from methyl and methine carbons (Le Cocq and Lallemand, 1981). The melting point is uncorrected.

Plant Collection. Plants were collected at Logan, UT, during April 1982. Plants werre selected for a uniform phenotype at each stage of growth. The plant parts were immediately put into plastic bags, weighed, and frozen within 2 h of collection. Two months later, aliquots of frozen plant were freeze-dried for subsequent analysis of ranunculin content of paired frozen and lyophilized samples. The water content of the frozen plant samples was determined by weighing before and after lyophilization. Early flower, full flower/early seed, and full seed samples contained 78%, 73%, and 75% water, respectively, consistent with values obtained with plant samples collected in 1980 and utilized in sheep toxicity tests.

Ranunculin (1). Ranunculin was first isolated in this



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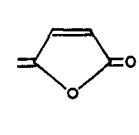

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study from a frozen sample of *C. testiculatus* collected at the full flower/early seed stage of growth by a slight modification of Hill and van Heyningen's procedure (1951). From 80 g of frozen plant was obtained 490 mg of crystalline ranunculin (1), which was recrystallized from methanol to afford colorless plates: mp 140–141 °C [lit. (Hill and van Heyningen, 1951) mp 141–142 °C]; ^{1}H NMR (Benn and Yelland, 1968; Boll, 1968) (D_2O) δ 7.78 (dd, J_{2-3} = 6.0 Hz, J_{2-4} = 1.5 Hz, 1 H, H-2), 6.29 (dd, J_{3-2} = 6.0 Hz, J_{3-4} = 2.0 Hz, 1 H, H-3), 5.47 (m, 1 H, H-4), 4.49 (d, $J_{1'-2'}$ = 8.0 Hz, 1 H, H-1'), 4.4–3.0 (m, 8 H, H-2'–H-6' and H-5); ^{13}C NMR (D_2O) δ 177 (C-1), 158.5 (C-2), 124.5 (C-3), 104.7 (C-4), 98.5 (C-1'), 78.5 (C-3' or C-5'), 78.2 (C-5' or C-3'), 75.6 (C-2'), 72.2 (C-4'), 66.0 (C-5), and 63.4 (C-6'); IR (KBr) ν_{max} 1750 cm^{-1} (C=O) [lit. (Bredenberg, 1961) ν_{max} 1750 cm^{-1}].

Table I. Ranunculin Concentration of *C. testiculatus*[a] at Various Growth Stages

growth stage	concentration, % wet weight	concentration, % dry weight
early flower	0.5	2.3
full flower/early seed	0.3	1.1
full seed	0.3	1.2

[a] Analyses were made on lyophilized samples stored at 0 °C. Values reported represent an average of the results of three analyses.

Extracts of the lyophilized and frozen samples at various growth stages were obtained from evaporation of the 50% aqueous ethanol wash of the activated charcoal used in the Hill and van Heyningen procedure and dried in vacuo. Measured amounts of the dried extracts were dissolved in 0.5 mL of 0.5% TSP in D_2O, and the integral of the doublet of ranunculin at δ 7.78 was compared with that of TSP. From these data the concentration of ranunculin for each growth stage could then be calculated. Three extracts were prepared from samples of each of the three growth stages, and the values reported in Table I represent the average of these. Table I reports only results obtained from lyophilized plant samples; the corresponding values for frozen samples are as follows: early flower, 0.4% (wet weight) and 1.9% (dry weight); full flower/early seed, 0.3% (wet weight) and 1.1% (dry weight); full seed, 0.3% (wet weight) and 1.2% (dry weight).

RESULTS AND DISCUSSION

We have isolated from *C. testiculatus* a significant amount of ranunculin (1) (Hill and van Heyningen, 1951), a toxic constituent of many species of true buttercup associated with livestock poisoning (Shearer, 1938; Ruijgrok, 1966). Ranunculin has not been previously isolated from bur buttercup. The acrid lactone protoanemonin (2) is enzymatically released from the precursor glycoside 1 once plant tissue is crushed and is believed to be the agent responsible for the observed toxicity of the true buttercups (Hellstrom, 1958; Hill and van Heyningen, 1951). The ^{1}H NMR spectrum and melting point of the isolate matched those reported (Benn and Yelland, 1968; Boll, 1968) in the literature for ranunculin, and the IR spectrum proved virtually identical with a published, complete spectrum of 1 (Bredenberg, 1961). In addition, the previously unreported ^{13}C NMR spectrum was consistent with the structure of ranunculin. The carbon signals of the sugar moiety were compatible with those of pure glucose, and the lactone moiety revealed a carbonyl carbon signal at δ 177.1, olefinic carbon signals at δ 158.5 (C-2) and δ 124.5 (C-3), and a methine carbon signal at δ 104.7 (C-4).

Samples of bur buttercup at various stages of growth were collected and analyzed for ranunculin to determine those with the greatest concentrations of the toxin. Those samples lyophilized after collection and stored in a freezer were generally found to retain ranunculin to a greater

degree than samples simply kept frozen. Therefore, the lyophilized samples were used for the comparison. Crude extracts of the various samples were dissolved in D_2O solutions containing a known concentration of TSP, and the integral of the isolated C-2 doublet signal (7.78 ppm) was compared to that of TSP in the ^1H NMR spectrum. This allowed for a determination of the total amount of ranunculin in a given crude extract, the results of which are reported in Table I. The growth stage with the highest concentration of ranunculin proved to be the "early flower" stage, accounting for 2.3% of the dry weight of the plant.

ACKNOWLEDGMENT

We acknowledge Dr. Russell J. Molyneux for helpful discussions, Calvin E. Jones for technical assistance, and Sue C. Witt for ^1H and ^{13}C NMR spectra.

Registry No. 1, 644-69-9.

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Ronald J. Nachman*[1]
John D. Olsen[2]

[1]Natural Products Chemistry Research
Western Regional Research Center
Agricultural Research Service
U.S. Department of Agriculture
Berkeley, California 94710
[2]Poisonous Plant Research Laboratory
Agricultural Research Service
U.S. Department of Agriculture
Logan, Utah 84322

Received for review April 21, 1983. Accepted June 9, 1983.

Amino Acid Composition of Developing Pigeon Pea (*Cajanus cajan*) Seeds

The amino acid composition of seed proteins and free amino acid pool of pigeon pea (*Cajanus cajan*) was investigated at different developmental stages after fertilization. The nitrogen content of pod covers increased up to 21 days followed by a gradual decline, while that of seeds increased sharply from 21 to 42 days. The free amino acid content of seeds was maximum between 21 and 28 days. The decrease in free amino acids after 28 days was accompanied by a rapid accumulation of protein up to 42 days in the developing seed. The amino acids cysteine, valine, isoleucine, and methionine were generally absent in the free pool after 28 days of seed development. The pigeon pea proteins were found to be very poor in methionine, tyrosine, and histidine.

Pigeon pea is an important source of dietary proteins in many developing countries, particularly in the semiarid regions. It is a well-recognized fact that to increase the productivity of legumes the foremost consideration is the stabilization of their yields by breeding techniques. However, to breed for their protein quality, it is necessary to understand the metabolic changes that occur in the seeds as they mature. Sulfur-containing amino acids and threonine are reported to be the most limiting amino acids in chickpea proteins (Kaul and Gassi, 1971). Since legumes are generally rich in the essential amino acid lysine, they play an important role in supplementation of cereal-rich diets (Chatterjee et al., 1977). In chickpea accumulation of protein fractions during development of cotyledons has been studied (Srivastava et al., 1981). However, there are no available data on the metabolic changes of developing pigeon pea seed. The present study was undertaken to ascertain the changes in the free amino acid pool, amino acid composition of seed proteins, protein content, and total nitrogen content of seeds and pod covers during their different stages of development.

MATERIALS AND METHODS

Plant Material. Pigeon pea (*Cajanus cajan*) cultivar "Sharda" was grown under field conditions on red soils at the ICRISAT farm at Patancheru. The flowers were tagged on the day of their opening. The pods for analysis were collected at 7, 14, 21, 28, 35, 42, and 49 days after the opening of flowers from different plants. The grains matured at 49 days after flowering.

Total Nitrogen. Seeds were separated from the pods by hand dissection, and the fresh weight was taken of both seeds and pod covers. Seeds and pod covers were oven-dried to constant weight. Dried seeds and pod covers were ground by a cyclone mill, and the total nitrogen was determined in duplicate by the micro-Kjeldahl method (AOAC, 1970).

Amino Acid Analysis and Protein Determination. Freshly weighed and counted seeds, collected at different maturity stages, were heated for 30 min at 80 °C in 5% trichloroacetic acid (TCA). The samples were homogenized (0.2 g/mL) in TCA in a Potter-Elvehjem homogenizer for 3 min and centrifuged at 2000*g* at room temperature for 15 min. The supernatant was saved and extracted with ether to remove TCA. Finally, the samples were evaporated to dryness under vacuum and dissolved in 0.2 M citrate buffer, pH 2.88, for quantitative determination of free amino acids. The pellet was washed twice by suspending in fresh 5% TCA and recentrifugation. After being washed, the TCA precipitate was dissolved in 1 N NaOH. Protein hydrolysis was performed as described earlier (Yadav et al., 1972). Amino acids in TCA super-

0021-8561/83/1431-1360$01.50/0