

legumes than on mature legumes or on grasses at any stage of growth. However, the highest percentage of pectic substances occurred at and after the bloom stage. The low pectic substances in bromegrass are in accord with its low bloat potential, but birdsfoot trefoil, which is not a bloat-producing forage, was found to be similar to alfalfa in its content of pectic substances.

The results of this investigation fail to show a direct relationship between level of pectic substances and occurrence of bloat in cattle. There is still the possibility, however, that these polysaccharides may be involved, in conjunction with other animal and microbial factors, in the etiology of bloat. Under certain conditions, pectins are capable of serving as foam-stabilizing agents and, in this way, may be related to bloat. Since the level of these substances does not seem

to be the important factor, perhaps the rate of their release during the early phases of rumen fermentation of bloat-producing legumes is of significance. Additional work is needed to ascertain the nature of the relationship, if any, between the pectic substances and bloat.

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NUTRIENTS IN SEEDS

Amino Acid Composition of Seeds from 200 Angiospermous Plant Species

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Amino acid compositions by ion exchange methods are reported on acid hydrolyzed seed meals from 134 plant species not previously analyzed. The data obtained, with those from 66 species reported earlier, are evaluated and interpreted together here. Lysine, methionine, arginine, proline, hydroxyproline, and glutamic acid showed the greatest variation. Less familiar amino acids and unidentified compounds were detected in many hydrolyzates. Percent of total nitrogen present as amino acids, ammonia, and unidentified nitrogen was estimated. The amino acid composition of seeds shows over-all similarities, but there are definite relationships between plant taxonomic classification and amount of each amino acid present. Detailed comparisons are given among legumes, crucifers, and composites. The results provide information on the distribution of nitrogenous constituents in a large variety of plant seeds.

DISCOVERY and development of sources of nutritionally high-quality vegetable protein are part of an extensive screening program by the Agricultural Research Service of the U. S. Department of Agriculture which is planned to find new crops that can profitably be grown by the farmer (33). A literature survey (28) on amino acid compositions of various plant seeds showed that relatively few have been analyzed for all the common amino acids and that the nutritionally essential amino acids have been determined on seeds from 89 species of 25 plant families. The availability of rapid and accurate ion exchange, chromatographic methods to determine all the amino acids in an acid hydrolyzate offers an efficient

means for such analyses. Because of the lack of published information concerning amino acid composition of plant seeds and because of the availability of suitable methods, such determinations were made a part of this screening program.

The amino acid content of seeds from previously selected species, including those of the Crucifer family, has been reported (18, 32). This paper provides information concerning the amino acid composition of seeds from 134 additional species, as well as an evaluation of data obtained from the entire 200. Amino acid compositions from 14 species of *Lesquerella* of the Crucifer family determined by the same methods (19) were not included.

Knowledge of amino acid composition makes possible an estimate of the nutritional quality of a seed protein by comparison with known amino acid requirements (9, 27, 22). Harper (13) indicates that nonessential amino acids may also be important in nutrition; hence their determination provides more information for gaining insight into the role they play in nutrition and in the nutritional evaluation of each seed meal.

In addition to chemical evaluation of seed from each species for nutritional quality, amino acid compositional data from seeds of a large number of different species permit study of possible fundamental relationships. From such data, the range limits of each amino acid,

interrelationships among the amino acids, and composition as related to taxonomic distribution among plant families may be examined. No extensive study of this kind has been made, presumably because of lack of reliable data (7). The accumulation of large amounts of analytical data in one laboratory under controlled conditions by the latest automatic ion exchange, chromatographic methods of analyses, as reported here, supplies the information for such a study.

The procedure also detected and determined the amount of several less familiar amino acids and detected unidentified compounds. Occurrence of these compounds is in many cases related to plant families or subfamilies. Several such compounds are of either nutritional or biological interest.

Loss of amino acids during acid hydrolysis may be a serious source of error (6, 10). The determination of all the amino acids and ammonia in an acid hydrolyzate offers a means for obtaining a nitrogen balance on the hydrolyzate. Such information, along with an estimation of the insoluble humin nitrogen formed during the acid hydrolysis, was obtained as a part of this investigation. Presentation and discussion of nitrogen distribution data are included because in the literature such information is limited and because it is valuable in estimating the accuracy of the determinations.

Materials and Methods

That part of the seed most easily prepared for processing was selected as the analytical sample. The sample was kernel alone, kernel and seed coat, or kernel plus seed coat and all or part of the pericarp. Almost all samples were from kernel and seed coat, with exceptions shown in the detailed amino acid composition table (Table IV).

All samples were ground, extracted with hexane, and acid-hydrolyzed with 6*N* hydrochloric acid for 24 hours as previously described (32). Nitrogen determinations were made on the seed meals, soluble hydrolyzates, and oven-dry insoluble humins by the Association of Official Agricultural Chemists' micro-Kjeldahl method (2). Amino acid compositions of the acid hydrolyzates previously reported (32) and of seed meals from nine species in this report were determined by the fraction-collector method of Moore, Spackman, and Stein (20). The amino acid compositions of the remaining 164 species, including those from the Cruciferae (18), were determined by the Spackman, Stein, and Moore automatic method (29) with a Model MS Beckman Spinco instrument.

Methionine sulfoxides when present were included as a part of the methionine.

Hydrolyzates analyzed within a week of preparation rarely contained significant amounts of the sulfoxides. The 150-cm. column was operated at 30° and 50° C. in order to separate hydroxyproline completely from aspartic acid. No corrections were made for likely small losses of serine and threonine that occurred during acid hydrolysis. Cystine values are not reported because of extensive destruction during acid hydrolysis (6, 10). In a few cases, trichloroacetic acid extracts were prepared by the method of Becker, Milner, and Nagel (3) in order to gain additional information concerning the nature of unusual amino acids present in the free form.

Measurement of tryptophan in natural products is difficult because of its destruction during alkaline, as well as acid, hydrolysis and because of the relatively small amount of this amino acid in most protein. Estimations of the tryptophan content of angiospermous seeds (6, 28) indicate that the amino acid is apt to be adequate in most seed meals in comparison with the nutritional requirement except in corn or seed from other species of Gramineae. For these reasons this amino acid was not estimated.

Amino acid compositions given in this report were determined on species from plant families as follows: Leguminosae, 35; Compositae, 13; Malvaceae, 7; Liliaceae, 5; Euphorbiaceae, 4; Boraginaceae, Chenopodiaceae, Ranunculaceae, Rosaceae, and Umbelliferae, 3 each; and the remaining 55 species from 46 different plant families. Ten species were Monocotyledoneae and the remainder, Dicotyledoneae.

Results and Discussion

Mean Compositions and Variation from the Mean. Crude protein and oil contents of the seeds are summarized in Table I. In most cases the seeds were selected on the basis of high crude protein and oil. For this reason the mean value given is higher than the protein and oil contents of seeds from randomly selected species.

Amino acid compositions of the 200 seed meals are summarized in Table II. Relative standard deviation offers a direct means of comparing the variation about the mean. Amounts of oil and crude protein both show greater variation among the seeds than do any of the amino acids, except hydroxyproline, despite the fact that almost all samples were selected because of their high oil and protein contents. The smaller variation in amino acid composition than in oil and in protein suggests that the amino acid composition of the protein in the seed is a more fundamental relationship common to all seeds.

Comparison of the relative standard deviation of the various amino acids

showed that lysine, methionine, arginine, glutamic acid, proline, and hydroxyproline had the greatest variation. Excluding hydroxyproline, the relative standard deviations of these amino acids ranged from 20.8 to 29.8. Hydroxyproline showed the greatest variation because it was found only in samples containing seed coat and pericarp (37). Relative standard deviation for the remaining amino acids ranged from 15.0 for histidine to 19.4 for glycine.

Of the amino acids showing the greatest variation, lysine and methionine are nutritionally essential and according to current knowledge (8) are the two most likely to be inadequate for nutrition in the protein of plant seeds. Because of the variability of these two amino acids, many plant seeds containing adequate amounts of them may be found. Also, selected combinations of seed protein from different plants, based on amino acid content, should give a protein mixture of high nutritional quality. Seed meals having high protein and high lysine contents, derived from species having favorable agronomic possibilities for the temperate zone, should be a good source of supplement protein for corn in the same manner that soybean meal is used.

McDermott and Pace (17) showed that lysine, arginine, glutamic acid, and proline had the greatest variation of any amino acid in wheat endosperm when they compared a low- and a high-protein-containing variety. The authors' data showed the greatest variation in the same amino acids of seeds from a wide variety of species. However, in contrast to these results, McDermott and Pace found no large variation in methionine content.

Analysis of cells from cultures for lysine, methionine, and tryptophan (27) of bacteria, yeasts, and molds from 492 different strains showed averages and variations from the averages of an order similar to that reported here from seeds of higher plants. Averages for the three classifications ranged from 6.5 to 7.5 grams per 16 grams nitrogen for lysine; 1.15 to 1.75 for methionine; and 0.32 to 0.80 for tryptophan. The relative standard deviation within each classification for each amino acid ranged from 15 to 37.

The averages and the extremes given in Table II indicate that the amino acid composition in all the seed meals analyzed tend to follow a general pattern. For example, isoleucine is nearly always present in a much smaller amount than leucine. The same is true for tyrosine with respect to phenylalanine and for aspartic with respect to glutamic acid. Hydroxyproline was the only amino acid not present in all samples.

Crude Protein and Amino Acid Content. Amino acid and ammonia

Table I. Protein and Oil Contents of Seed from 200 Angiospermous Species

Constituent, %	Mean ^{a, b}	Extremes, %	Relative	
			Std. Dev. ^a	Std. Dev. ^a
Protein (N × 6.25)	28.7	8.0-71.0	9.60	35.0
Oil	24.3	1.2-66.0	15.1	62.0

^a Statistical terminology recommended by *Analytical Chemistry* (7).

^b Calculated on the whole seed dry-basis.

Table III. Nitrogen Distribution Following Acid Hydrolysis

Distribution	Mean, %	Extremes, %	Std. Dev.	Relative Std. Dev.
Amino acids found in protein	74.8	52.0-92.0	6.93	9.26
Ammonia nitrogen	10.8	6.4-21.8	2.24	20.8
Insoluble nitrogen in acid hydrolyzate	3.42	0.1-19.6	2.65	77.5

nitrogen (Table III) were calculated from the ninhydrin color yield of the separate elution peaks of the amino acids found in protein and from the ammonia peak. The insoluble nitrogen formed during hydrolysis was estimated by micro-Kjeldahl analysis. On the average, 85.6% of the total nitrogen on which the crude protein content was based, was accounted for as amino acids found in protein and ammonia.

The insoluble nitrogen-containing material formed or not dissolved from the original sample during acid hydrolysis may or may not be derived from amino acids. Of the 200 seed meals, the percentage of insoluble nitrogen from 128 was less than the arithmetic mean of 3.42. The mean was strongly influenced by a few seed meals containing large amounts of insoluble nitrogen in their hydrolyzates. The insoluble nitrogen appeared to be associated with seed meals of low nitrogen content and with those containing large amounts of seed coat and pericarp. The seed coat and pericarp may contain other nitrogenous substances which form insoluble residues on acid hydrolysis.

The sum of the three means in Table III accounts for 89% of the nitrogen. Values for the remainder of the nitrogen were obtained by difference and are subject to accumulated analytical errors of the over-all procedure. Precision of the analytical method in our hands is about ±5% (18). Additional uncertainty is introduced by the acid hydrolysis. Despite the possible magnitude of these errors, the presence of large amounts of nitrogen in a seed meal may be detected which is not nitrogen of the common amino acids, ammonia, or insoluble nitrogen. For example, in a number of Leguminosae this nitrogen was mostly due to canavanine.

In other cases, where more than 15 or 20% of the nitrogen is unaccounted for as the amino acids commonly found in protein and ammonia, unidentified nitrogen-containing substances in the seed meal are likely present. The presence of such unknown sources of nitrogen lowers the amino acid composition of the seed when expressed in terms of grams per 16 grams of nitrogen or in percentage of crude protein. Many specific cases of this type can be found in Table IV.

Data of this type demonstrate the need for expressing dietary protein in terms of complete protein, as suggested by Howard, Bauer, and Block (14), rather than as crude protein. Analysis for cystine and tryptophan in seed meals that appear the most promising as a dietary source of protein will permit such an evaluation.

Those amino acids varying in amount by greater than one or two standard deviations from the mean given in Table II for 200 seed meals are marked in Table IV. This permits easy identification of a seed meal as either a rich or poor source of each amino acid. In evaluating each seed meal, one should consider the percentage of the total nitrogen present in the amino acids. On the basis of the analysis, many of the seed meals have less than 75% of their nitrogen in amino acids found in protein.

Of 17 seed meals more than one standard deviation below the mean in total amino acid nitrogen, 13 were also low in six or more of the amino acids reported in Table IV. Of these 17, the high canavanine content of six of those in the Leguminosae explains the large amount of nitrogen not found in the amino acids present in protein.

Of 20 seed meals more than one standard deviation above the mean in total

Table II. Summary of Amino Acid Compositions of Seed from 200 Angiospermous Species

Amino Acid	Grams per 16 Grams Nitrogen		Std. Dev.	Relative Std. Dev.
	Mean	Extremes		
Lysine	4.47	7.5-1.7	1.08	24.2
Methionine	1.47	3.2-0.5	0.436	29.8
Arginine	8.28	15.6-3.1	2.22	26.8
Glycine	4.66	7.7-2.6	0.905	19.4
Histidine	2.27	3.7-1.4	0.340	15.0
Isoleucine	3.56	5.8-1.9	0.545	15.3
Leucine	6.03	13.7-4.0	1.01	16.7
Phenylalanine	3.83	6.3-2.0	0.626	16.3
Tyrosine	2.85	4.8-1.9	0.480	16.8
Threonine	3.27	4.6-1.8	0.521	15.9
Valine	4.43	6.7-2.3	0.692	15.6
Alanine	3.90	8.8-1.5	0.729	18.7
Aspartic acid	8.42	14.5-5.4	1.48	17.6
Glutamic acid	16.42	33.1-8.6	3.41	20.8
Hydroxyproline ^a	1.10	5.7-0.0	0.99	90.0
Proline	4.21	11.3-2.2	1.16	27.5
Serine	4.14	6.7-2.7	0.682	16.5

^a Based on analysis of 105 seed meals.

amino acid nitrogen, six were also high in six or more of the amino acids of protein. Of these 20, 11 were high in arginine, the amino acid with the highest percentage of nitrogen.

Comparison of Amino Acid Composition of Mono- and Dicotyledoneae. Data obtained (Table V) indicate that monocots are a better source of methionine and dicots, a better source of lysine. Monocots appear high in alanine, dicots high in glycine although comparison is based on only 11 species from the monocots.

Comparison of Amino Acid Compositions Among Families. Those plant families from which the largest number of the 200 species were selected for examination were Cruciferae with 41, Leguminosae with 43, and Compositae with 16. Data from those species selected as most representative of each of these three families were tested for significant differences (Table VI). Only one species was selected from each genus. In selecting species from the Leguminosae, those seed meals containing large amounts of canavanine were not considered because of the high percentage of nitrogen in the seed that was from canavanine.

Significant differences at the 99% probability level were found among the three plant families for all the amino acids except isoleucine, leucine, and phenylalanine. Examples of differences shown by the evaluation are the high threonine and proline, and the low arginine and aspartic acid contents of seed from the Cruciferae when compared with those from either the Leguminosae or the Compositae. Leguminosae seeds when compared with those from Cruciferae and Compositae are low in methionine, glycine, and valine. Seeds from Compositae in

Table IV. Amino Acid Composition from Analysis of Seeds from 134 Plant Species^a

Seed Source	Protein, %	Oil, %	Grams Amino Acid per 16 Grams Nitrogen													% Nitrogen as amino acids				
			Lysine	Methionine	Arginine	Glycine	Histidine	Iso-leucine	Leucine	Phenylalanine	Tyrosine	Threonine	Valine	Alanine	Aspartic acid		Glutamic acid	Hydroxyproline	Proline	Serine
MONOCOTYLEDONS																				
GLUMIFLORAE																				
Gramineae																				
<i>Euchlaena mexicana</i> ^b	10	3.8	1.7	2.3	3.3	2.6	2.0	3.3	13.7	4.9	3.6	3.3	4.3	8.1	5.8	19.2	0.0	8.5	4.7	72
<i>Fingerhuthia sesteriaeformis</i>	33	4.0	1.8	2.0	3.1	2.6	1.7	4.0	8.9	4.8	3.1	3.4	5.4	5.0	5.4	33.1	0.0	5.3	3.3	70
Cyperaceae																				
<i>Carex crux-coropi</i> ^b	10	8.4	3.1	2.4	9.3	3.8	2.0	3.9	7.1	6.3	2.8	3.7	4.8	4.4	8.7	15.4	0.1	3.7	4.2	76
SPATHIFLORAE																				
Araceae																				
<i>Arisaema triphyllum</i>	15	2.8	4.5	1.6	9.9	5.4	2.3	3.1	5.8	4.4	2.5	3.0	4.8	5.5	6.4	16.0	0.1	3.5	4.0	77
LILIIFLORAE																				
Liliaceae																				
<i>Allium porrum</i>	28	14.0	4.5	3.2	10.8	4.0	2.0	2.8	4.8	3.6	2.6	3.1	5.4	3.8	6.4	20.6	0.8	3.3	4.1	80
<i>Cordylone australis</i>	20	48.0	3.7	2.3	12.1	4.4	2.4	3.8	6.1	4.2	3.2	3.0	4.9	3.7	8.4	19.5	0.3	4.1	4.6	84
<i>Dasylirion wheeleri</i>	50	22.0	3.6	1.6	11.5	3.8	1.7	3.0	4.6	3.1	2.6	2.9	3.8	3.7	10.7	15.0	0.1	3.0	3.8	75
<i>Hemerocallis fulva</i> ^c	39	27	3.3	1.2	7.2	3.3	1.6	3.7	5.1	3.6	2.0	2.4	4.6	3.5	10.1	13.9	0.0	2.8	3.7	67
<i>Yucca arizonica</i>	14	28	4.3	1.9	10.7	4.0	2.2	3.3	5.1	4.3	4.8	3.2	5.4	3.8	7.5	15.0	3.1	4.8	4.3	80
Iridaceae																				
<i>Iris germanica</i>	15	17	3.5	1.5	7.7	4.6	2.1	2.9	5.2	4.4	4.6	2.9	4.1	2.9	9.1	14.4	5.7	3.6	5.6	78
DICOTYLEDONS																				
URTICALLES																				
Moraceae																				
<i>Mactra pomifera</i> ^{c,d}	38	45	3.0	1.2	14.0	4.2	2.1	3.7	6.2	4.5	2.8	3.1	6.1	3.8	10.2	17.7	...	3.3	5.0	89
SANTALES																				
Santalaceae																				
<i>Gonolobus pallida</i> ^b	8	25	3.5	1.1	8.0	3.8	1.8	3.0	5.4	2.7	2.3	3.0	3.4	3.8	8.3	11.0	1.4	3.9	4.0	71
Oleaceae																				
<i>Ximelia americana</i>	20	62	5.2	1.2	10.0	3.7	2.6	3.6	7.9	4.0	2.7	3.9	5.0	4.2	8.9	14.4	2.2	4.6	3.8	81
POLYGONALES																				
Polygonaceae																				
<i>Polygonum esculentum</i> ^b	13	2.6	5.6	1.9	8.8	5.4	2.2	3.4	5.9	4.0	2.4	3.7	4.6	4.1	8.8	15.7	0.3	3.5	4.4	79
<i>Polygonum persyvanicum</i> ^b	10	4.4	4.6	1.8	6.8	4.1	2.2	3.6	6.0	3.7	2.3	3.3	4.6	4.0	7.3	12.4	0.1	3.4	4.0	71
CHENOPODIALES																				
Chenopodiaceae																				
<i>Chenopodium album</i> ^b	19	9.3	4.1	1.7	8.9	5.3	2.5	3.2	5.2	3.6	2.6	2.8	3.8	3.3	7.1	14.6	0.5	2.7	3.6	70
<i>Chenopodium quinoa</i> ^b	13	7.2	5.6	2.0	7.0	5.2	2.4	3.6	6.0	4.1	2.8	3.5	4.5	4.7	7.3	11.9	0.5	3.1	3.7	71
<i>Kochia scoparia</i>	28	16	5.5	1.7	7.1	5.1	2.4	3.5	5.9	3.8	3.6	3.3	4.6	3.9	8.0	12.3	0.0	3.4	4.0	70
Amaranthaceae																				
<i>Amaranthus retroflexus</i>	18	7.2	4.6	1.8	7.4	7.7	2.3	3.3	5.1	3.5	3.3	3.2	3.7	1.5	7.8	10.2	0.1	2.9	6.7	70
<i>Cedrela cristata</i>	18	13.4	4.5	1.9	10.8	6.5	2.6	3.6	5.7	3.8	3.8	3.4	4.4	4.0	8.2	16.5	0.5	2.2	3.8	80
Nyctaginaceae																				
<i>Mirabilis jalapa</i> ^b	17	3.9	4.8	1.4	6.4	5.9	2.7	3.6	5.7	4.2	3.0	3.5	4.6	3.5	8.8	14.7	0.7	3.9	4.0	74

Table IV. Amino Acid Composition from Analysis of Seeds from 134 Plant Species^a

Seed Source	Protein, %	Oil, %	Grams Amino Acid per 16 Grams Nitrogen													Nitrogen as amino acids %				
			Lysine	Methionine	Arginine	Glycine	Histidine	Iso-leucine	Leucine	Phenylalanine	Tyrosine	Threonine	Valine	Alanine	Aspartic acid		Glutamic acid	Hydroxyproline	Proline	Serine
<i>Crotalaria spectabilis</i> ^d	31	2.6	5.8	1.3	9.7	4.1	2.3	3.9	6.1	2.8	2.5	2.9	4.5	3.4	9.2	19.9	...	3.2	5.2	83
<i>Dalea oaxacana</i>	37	6.8	5.0	1.1	11.0	4.9	2.1	3.5	5.7	3.9	2.9	2.8	4.1	3.6	8.4	19.4	0.1	4.1	4.2	77
<i>Delonix regia</i> ^e	58	11.0	4.5	1.1	12.0	3.8	2.2	3.2	6.2	3.9	2.8	2.6	4.2	3.7	8.3	21.1	0.0	3.6	4.2	80
<i>Desmodium cinerascens</i>	38	12	6.2	1.2	6.7	3.2	2.4	3.4	6.2	3.2	2.5	3.1	3.7	3.4	9.7	13.0	0.2	3.9	3.9	71
<i>Genista monosperma</i> ^e	53	11	4.5	0.7	8.5	3.4	2.4	3.5	6.4	3.3	3.2	2.9	3.5	3.1	9.0	17.5	0.2	3.9	3.8	72
<i>Helysarum varium</i>	50	8.3	3.6	0.8	5.4	3.9	2.0	2.6	4.3	2.4	2.2	2.7	2.9	3.1	7.3	9.1	0.1	2.7	3.0	52
<i>Lathyrus ornatus</i>	33	1.4	5.6	0.7	6.7	3.5	2.3	3.2	5.5	3.4	2.5	3.0	3.4	3.1	8.4	14.8	0.2	3.4	3.6	68
<i>Lotus rigidus</i>	38	6.8	3.6	1.0	11.3	5.1	2.2	2.9	4.9	3.4	2.6	2.7	3.3	3.2	8.6	15.1	0.2	3.3	4.3	74
<i>Lotus scoparius</i>	35	8.4	3.9	1.0	9.1	5.2	2.2	3.0	5.1	3.4	2.8	2.8	3.3	3.2	8.3	13.9	0.1	3.5	4.2	71
<i>Lysiloma desmostachya</i>	41	9.1	4.4	0.6	3.8	2.6	...	2.4	4.9	2.5	2.5	2.1	2.7	2.6	6.2	8.6	0.2	3.3	3.0	...
<i>Medicago lupulina</i>	33	5.0	4.7	1.2	7.6	4.2	2.6	3.5	5.9	4.0	2.8	3.4	4.0	3.6	9.4	13.1	0.1	3.6	4.2	70
<i>Melilotus indica</i>	33	5.4	5.5	1.2	7.9	4.6	2.8	3.9	6.2	4.5	2.9	3.2	3.9	3.8	10.3	14.2	0.0	3.7	4.2	75
<i>Milletia ovalifolia</i>	27	37	5.1	0.8	4.2	4.0	1.6	2.8	6.2	4.0	2.7	3.1	3.9	3.1	8.7	11.4	0.0	3.8	4.0	60
<i>Onobrychis viciifolia</i>	41	7.6	5.6	1.3	10.4	4.1	3.7	3.2	5.6	3.0	2.5	3.0	3.7	3.7	9.0	15.6	0.4	3.7	4.1	79
<i>Onobrychis vulgaris</i>	41	6.8	5.5	1.3	9.9	4.0	3.5	3.1	5.4	2.9	2.4	3.1	3.6	3.2	9.4	15.7	0.1	3.8	4.1	77
<i>Oxytropis uralensis</i> ^d	27	4.3	4.6	1.0	9.3	4.2	2.2	3.7	6.2	3.6	3.0	2.8	4.4	4.0	9.5	15.6	...	3.4	4.3	76
<i>Robinia neomexicana</i>	41	13	3.6	0.7	8.0	3.6	2.0	2.4	4.8	2.8	2.3	2.4	3.2	2.8	6.5	12.4	0.1	3.0	3.5	60
<i>Sesbania macrocarpa</i>	38	6.4	4.9	0.9	8.2	4.3	2.6	3.2	5.7	3.7	2.7	2.9	3.5	3.2	7.9	14.3	0.1	3.8	4.0	69
<i>Tephrosia leucarpa</i>	44	11	5.0	0.9	7.2	3.2	2.2	3.0	6.2	3.7	2.6	2.6	3.3	3.0	8.9	13.2	0.1	3.8	3.8	70
<i>Vicia faba</i>	25	1.2	6.1	0.6	7.9	3.8	2.4	3.8	6.7	3.9	3.2	3.5	4.3	3.7	10.1	14.9	0.1	3.9	4.3	76
<i>Vigna sinensis</i>	26	1.4	6.4	1.2	7.3	4.1	3.2	4.0	7.2	6.0	3.4	3.6	4.7	4.4	10.6	16.9	0.5	3.5	4.5	80
GERANIALES																				
Zygophyllaceae																				
<i>Balanites aegyptiaca</i>	22	46	3.2	1.5	10.8	5.2	1.6	2.8	4.9	4.4	2.1	2.4	4.0	2.8	7.2	16.4	0.4	3.6	2.9	72
Simarubaceae																				
<i>Ailanthus altissima</i>	28	55	2.5	1.5	8.0	4.5	2.4	3.8	6.2	3.6	2.8	2.4	4.7	3.3	6.0	22.2	0.6	3.2	4.1	72
Euphorbiaceae																				
<i>Cnidioscolus angustidens</i>	42	44	3.3	1.5	7.6	3.8	1.9	3.2	6.0	3.9	2.5	3.4	6.4	4.1	9.5	14.9	0.1	4.3	4.1	75
<i>Euphorbia marginata</i> ^b	16	18	3.9	1.5	10.2	4.5	2.1	3.6	5.2	3.7	2.2	2.8	4.4	3.8	10.5	13.4	0.4	3.1	4.4	75
<i>Jatropha macrohiza</i>	36	53	3.2	1.6	12.4	4.1	2.4	4.7	6.9	4.3	2.8	3.6	4.6	4.5	9.3	14.9	0.4	4.6	4.5	84
<i>Ricinus communis</i> ^e	26	66	3.4	1.6	12.3	3.8	2.1	4.5	6.2	3.6	3.0	3.2	5.6	3.9	9.5	18.0	0.1	3.6	5.0	82
SAPINDALES																				
Buxaceae																				
<i>Buxus sempervirens</i>	21	42	3.7	1.2	8.7	3.8	1.6	3.5	6.2	3.7	2.8	3.1	4.3	3.9	8.8	14.8	0.6	3.9	3.7	72

Celastraceae	20	44.0	4.6	1.3	9.9	3.3	2.0	3.0	5.2	3.3	2.4	2.5	4.0	3.5	7.8	13.8	0.0	3.3	3.8	70
<i>Euonymus alatus</i>																				
Staphyleaceae	35	49	2.9	0.6	9.5	4.1	1.4	5.8	4.5	2.6	2.2	...	2.3	3.6	7.9	17.1	0.0	3.4	3.1	...
<i>Staphylea pinnata</i> ^c																				
Sapindaceae	31	35	5.7	1.4	9.6	4.3	1.7	3.1	5.3	4.1	2.6	2.7	4.3	3.9	6.5	17.2	0.0	4.3	3.6	75
<i>Sapindus mukorossi</i> ^c																				
Balsaminaceae	16	22	4.6	1.7	8.0	5.1	2.1	3.7	6.4	5.1	3.0	3.4	4.6	3.3	7.8	15.6	0.1	4.1	4.8	74
<i>Impatiens balsamina</i>																				
MALVACEAE																				
Malvaceae	26	13	3.9	1.2	6.9	4.4	2.4	2.3	4.1	3.2	2.2	2.3	3.3	2.9	6.7	11.9	0.1	2.6	3.1	60
<i>Althaea rosea</i> ^b																				
<i>Iibiscus cannabifolius</i>	28	18	4.7	1.4	13.3	4.4	1.9	2.9	6.1	4.0	2.6	3.1	4.0	4.0	9.0	16.9	0.0	3.5	4.3	83
<i>Hibiscus moscheutos</i>	22	13	4.1	1.4	8.7	5.0	2.2	3.0	5.4	4.1	2.5	3.1	4.1	4.2	9.0	15.4	0.1	3.6	4.2	73
<i>Hibiscus syriacus</i>	29	15	4.5	1.2	10.6	3.9	1.8	3.1	5.6	3.3	2.4	3.5	3.9	4.1	9.7	14.4	0.1	3.0	4.1	75
<i>Malope trifida</i> ^c	23	16	5.3	1.7	8.0	5.2	2.4	3.0	5.4	4.0	3.0	3.2	4.2	3.9	9.5	14.4	0.1	3.1	4.4	73
<i>Malva parviflora</i> ^b	21	13	5.1	1.6	7.2	5.0	2.9	3.0	5.2	3.6	2.7	3.3	3.9	3.8	9.8	13.0	0.7	3.3	4.6	71
<i>Urena lobata</i>	29	20	3.0	1.4	10.1	5.6	2.6	2.6	5.0	3.5	2.8	2.6	3.4	3.4	8.6	14.1	0.0	3.0	4.2	73
Bombacaceae	39	43	4.5	1.4	11.3	3.4	1.9	3.1	5.8	5.1	2.3	2.4	4.7	4.3	8.1	21.5	0.0	2.7	4.3	80
<i>Ceiba acuminata</i> ^c																				
Sterculiaceae	20	52	6.0	2.0	8.7	4.1	2.9	4.1	6.1	4.0	2.9	3.5	5.0	4.7	10.0	19.8	0.1	3.2	5.0	81
<i>Sterculia foetida</i> ^c																				
PARIETALES																				
Fouquieriaceae	43	31	4.1	1.5	9.2	3.5	2.2	3.3	5.9	3.3	2.4	2.9	4.0	3.8	7.5	18.6	1.0	3.6	3.9	73
<i>Fouquieria splendens</i>																				
Bixaceae	13	5	6.7	1.7	7.2	4.8	2.3	3.3	5.8	3.9	3.3	3.9	4.4	4.4	9.0	15.9	1.3	5.4	4.0	78
<i>Bixa orellana</i>																				
Dilleniaceae	16	22	2.9	2.7	10.3	4.7	2.0	4.2	6.8	3.5	2.2	3.3	5.2	4.6	9.1	19.2	0.0	3.9	3.8	77
<i>Actinidia arguta</i>																				
MYRTALES																				
Lythraceae	17	21	4.2	1.9	8.8	4.8	2.1	3.8	6.3	3.9	2.3	3.5	5.6	4.3	8.5	13.0	...	3.5	5.3	74
<i>Cyrtia ilicifolia</i> ^d																				
Onagraceae	29	36	2.8	2.8	12.1	6.1	2.4	3.3	7.0	4.8	2.7	2.7	5.2	4.6	9.5	18.5	...	3.0	5.1	86
<i>Clarkia elegans</i>																				
Umbelliferae	16	25	2.0	1.7	9.9	5.8	2.1	3.2	5.8	4.1	2.4	2.4	4.3	3.3	7.1	17.0	2.1	3.4	4.5	75
<i>Oenothera biennis</i>																				
UMBELLIFERAE																				
Araliaceae	17	46	4.6	2.1	7.6	4.9	2.7	4.4	7.3	4.2	3.3	3.6	5.1	4.4	9.6	20.6	0.8	5.4	5.3	83
<i>Aralia spinosa</i>																				
Umbelliferae	22	37	3.4	1.6	7.6	5.8	2.4	4.5	6.3	4.2	3.0	3.6	4.9	4.2	10.1	19.1	0.5	4.7	4.5	79
<i>Dipsacus caeruleus</i>																				
<i>Heracleum lanatum</i> ^b	21	18	4.5	1.3	5.0	3.8	1.5	3.5	5.4	3.6	2.4	3.5	4.8	4.4	11.3	11.2	0.8	3.7	3.6	63
<i>Pastinaca sativa</i> ^b	16	27	4.9	1.7	4.9	6.5	2.3	3.8	5.6	4.1	2.4	3.5	4.9	4.1	10.0	17.3	0.2	4.6	4.2	73
Ebenaceae	10	2.6	6.2	1.2	6.6	3.9	1.8	3.1	4.8	3.4	2.0	3.2	3.6	3.6	6.8	12.3	0.4	4.8	3.6	65
<i>Diospyros virginiana</i>																				
CONTORTIAE																				
Loganiaceae	19	28	3.6	2.3	10.1	4.9	2.3	4.2	6.2	4.6	3.2	3.7	4.6	4.2	8.0	17.2	0.1	3.9	4.5	80
<i>Buddleia davidii</i>																				

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Table IV. Amino Acid Composition from Analysis of Seeds from 134 Plant Species^a

Seed Source	Protein, %	Oil, %	Grams Amino Acid per 16 Grams Nitrogen													% Nitrogen as amino acids				
			Lysine	Methionine	Arginine	Glycine	Histidine	Isoleucine	Leucine	Phenylalanine	Tyrosine	Threonine	Valine	Alanine	Aspartic acid		Glutamic acid	Hydroxyproline	Proline	Serine
Asclepiadaceae	37	28	4.9	1.4	9.6	5.2	2.3	3.5	6.2	4.8	3.4	2.7	4.3	3.6	7.5	21.8	0.1	4.1	4.1	81
<i>Asclepias syriaca</i>																				
<i>Marsdenia edulis</i> ^{c,d}	27	47	3.3	1.3	9.8	5.7	2.8	4.4	7.7	5.4	3.3	3.1	5.1	4.4	7.3	25.4	...	3.4	5.5	88
POLEMONIALES																				
Convolvulaceae	23	11	4.6	1.4	6.1	4.2	2.2	3.2	6.0	4.0	2.8	3.5	4.3	4.1	8.1	14.6	1.2	3.2	4.2	68
<i>Ipomoea purpurea</i>																				
Hydrophyllaceae	18	10	4.9	1.6	7.9	6.1	2.2	4.2	5.8	3.9	3.5	3.8	4.6	4.5	9.3	15.3	0.0	3.6	4.8	78
<i>Phacelia tanacetifolia</i>																				
Borraginaceae	19	30	3.6	1.6	6.5	3.5	1.8	3.2	4.7	3.2	2.9	2.8	3.6	2.9	6.7	13.5	3.6	3.6	3.6	62
<i>Anchusa capensis</i> ^b																				
<i>Borago officinalis</i> ^b	21	38	4.2	1.7	8.2	4.6	2.0	3.9	5.5	3.6	3.5	3.3	4.3	3.5	8.2	14.6	2.8	4.0	4.2	74
<i>Myosotis alpestris</i> ^b	17	43	4.4	2.8	4.6	4.3	2.2	4.6	7.0	3.9	3.6	3.8	4.8	3.7	8.6	17.5	6.0	...
Solanaceae	16	18	4.8	1.5	7.2	4.4	2.0	3.4	5.7	3.7	2.5	3.7	4.4	3.9	14.5	15.9	2.1	4.8	4.1	76
<i>Capsicum frutescens</i>																				
<i>Solanum nigrum</i>	17	27	4.3	1.9	9.0	4.7	2.3	4.0	6.4	4.5	2.6	3.9	4.7	5.1	8.9	18.4	1.4	4.1	4.7	81
Scrophulariaceae	29	48	3.9	1.6	10.1	4.2	2.1	3.4	5.2	4.0	2.7	3.0	4.5	4.1	8.3	16.0	0.0	3.5	3.9	74
<i>Nemesia suttonii</i>																				
<i>Penstemon spectabilis</i>	11	30	4.0	1.5	6.5	5.0	2.0	4.0	6.1	3.8	3.2	3.7	4.6	4.5	7.8	12.3	2.0	4.2	4.7	71
Bignoniaceae	22	32	4.4	1.3	10.0	4.6	2.3	3.8	6.3	4.5	3.4	3.6	4.6	4.1	8.2	16.7	0.4	3.5	3.8	78
<i>Chilopsis linearis</i>																				
Martyniaceae	30	48	2.8	1.7	11.9	3.3	2.5	3.1	5.5	3.4	3.2	3.4	3.5	4.4	5.9	14.5	0.1	2.6	2.9	72
<i>Martynia parviflora</i> ^e																				
PLANTAGINALES																				
Plantaginaceae	16	5.9	4.3	1.9	7.3	7.0	2.3	4.1	6.3	3.9	2.8	3.8	4.7	5.4	8.1	23.8	0.6	3.8	4.2	77
<i>Plantago ovata</i>																				
RUBIALES																				
Rubiaceae	14	20	3.6	1.3	9.0	5.3	2.4	4.0	7.7	4.3	2.9	3.2	5.4	4.5	9.2	19.3	...	4.1	4.4	81
<i>Gardenia jasminoides</i>																				
CUCURBITALES																				
Cucurbitaceae	38	52	2.8	2.4	15.6	4.7	2.4	3.6	6.1	4.9	2.9	3.0	4.0	4.2	8.1	17.3	0.0	3.3	4.7	88
<i>Citrullus vulgaris</i> ^c																				
<i>Sicyos angulata</i> ^c	31	28	4.6	1.8	11.0	6.0	3.5	3.6	5.9	4.3	3.0	2.5	4.3	4.2	10.0	14.9	0.2	3.3	4.4	83
CAMPANULALES																				
Campanulaceae	22	47	3.5	2.2	11.7	4.8	2.3	4.3	6.6	4.5	3.3	3.5	5.1	4.2	10.5	20.8	0.5	4.3	4.4	87
<i>Lobelia erinus</i>																				
Compositae	43	39	3.3	1.2	6.6	3.6	1.7	3.6	5.8	3.4	2.3	2.7	4.1	3.3	7.2	17.3	0.2	3.9	3.4	64
<i>Actinomeris alternifolia</i>																				
<i>Ambrosia trifida</i> ^b	22	25	3.1	1.7	9.6	4.2	2.6	4.0	6.1	4.5	2.7	3.0	4.9	4.1	9.4	22.0	0.6	5.2	4.1	81
<i>Aster alpinus</i> ^b	19	23	3.5	1.6	7.2	5.0	2.2	3.8	5.7	4.1	2.1	3.2	4.4	3.8	8.2	21.3	0.7	4.0	4.1	73
<i>Cynara cardunculus</i> ^a	27	42	4.2	1.8	8.2	4.8	2.5	4.5	7.3	5.5	3.6	3.8	6.4	4.9	11.0	20.9	...	4.4	5.5	85
<i>Dimorpholheca sinuata</i>	42	34	3.8	1.4	...	5.4	2.0	3.9	5.8	3.3	2.8	3.3	4.5	3.7	9.3	16.7	0.1	4.2	3.7	...
<i>Gaillardia aristata</i> ^{c,d}	46	28	3.6	2.1	9.2	4.9	2.6	4.1	5.6	4.7	2.3	3.4	5.6	3.9	9.6	20.6	...	3.4	4.2	...

<i>Helianthus maximiliani</i> ^a	31	3.2	1.8	9.8	4.4	2.2	4.2	6.6	4.6	2.1	3.0	5.7	4.0	8.8	23.6	...	4.8	4.2	82
<i>Helichrysum bracteatum</i> ^b	22	3.1	1.8	6.7	5.7	1.5	3.2	5.3	3.3	2.2	3.4	4.0	4.3	8.1	16.9	0.7	3.4	4.8	60
<i>Kuhnia glutinosa</i> ^b	33	4.6	1.8	5.9	4.8	1.9	3.9	6.0	3.9	2.3	3.5	5.0	3.6	8.5	18.2	0.6	4.2	4.4	71
<i>Marchallia caespitosa</i> ^b	31	4.5	2.1	7.6	5.1	2.2	4.8	6.3	4.3	2.5	3.4	5.0	4.3	9.2	18.3	0.3	5.2	4.2	77
<i>Osteospermum ecklonis</i>	31	3.4	1.1	7.6	4.6	2.0	3.9	5.8	3.4	2.5	3.0	4.4	3.5	8.9	16.5	0.0	3.5	3.5	70
<i>Osteospermum spinosens</i>	40	3.3	1.5	8.0	4.1	2.1	3.7	5.2	3.6	2.3	2.8	4.1	3.3	8.3	17.0	0.0	3.3	3.6	73
<i>Rudbeckia bicolor</i> ^b	29	3.0	2.4	9.6	4.6	2.1	3.6	5.8	4.5	2.4	2.9	4.5	3.6	8.9	19.9	1.1	3.8	3.5	77

^a Those values underscored with one line are between one and two standard deviations above the mean for the 200 seed meals; those with two lines are more than two standard deviations above the mean. Those values underscored with one wavy line are between one and two standard deviations below the mean; those with two wavy lines are more than two standard deviations below the mean. Protein (N X 6.25) and oil are on the dry basis. ^b Sample consisted of seed and pericarp. All others consisted of seed except those noted as seed without seed coat. ^c Sample consisted of seed without seed coat. ^d Analyzed by the fraction collector method of Moore, Spachman, and Stein (20).

comparison with those from Leguminosae and Cruciferae are high in methionine and glutamic acid and low in lysine.

From the standpoint of nutrition, the percentage of methionine and lysine in a seed meal should be high. Cruciferae meals are more likely sources of both these amino acids than are meals from either Compositae or Leguminosae seeds. Seeds from Compositae are better sources of methionine and seed from Leguminosae of lysine. By selected combinations of seed meals from these two families, feeds should be found that are well balanced nutritionally with respect to amino acid content.

Based on data from a smaller number of species not statistically evaluated, seed from the Gramineae were high in methionine, leucine, phenylalanine, ala-

nine, and glutamic acid and low in lysine, arginine, glycine, and possibly aspartic acid when compared with the mean from the 200 species. Corn, wheat, rice, oats, and barley, all members of the Gramineae and major sources of food and feed, have an amino acid composition pattern (6) similar to Gramineae seeds given here.

Based on five species from the Liliaceae, relatively high methionine content may be associated with seed protein from this family. Seed meals from eight species of the Polygonaceae, Chenopodiaceae, and Amaranthaceae families, which are closely related to one another, have average or higher methionine and lysine contents. Previously reported values for seed from these families are also high for methionine and lysine (28). This information indicates these families contain a high-quality protein; however, the percentage crude protein in the seed is low. The low lysine and methionine contents of the seed meal from three species of the Rosaceae indicate this family to be a poor source of nutritionally high-quality protein.

Seeds from four species of the Ranunculaceae; from one of the Berberidaceae, a closely related family; and from *Argemone intermedia* of the Papaveraceae are high in tyrosine and low in phenylalanine in contrast to the higher phenylalanine found in seed from all other species analyzed. The low phenylalanine content of the seed from these six species suggests protein isolates from them may be lower in or devoid of phenylalanine. Such proteins might warrant investigation as a food source for treatment of phenylketonuria (23).

Of the Leguminosae, seed meals from nine species of seven genera contain

Table V. Significant Differences in Amino Acid Composition between Monocotyledoneae and Dicotyledoneae^a

Amino Acid	Mean, Grams per 16 Grams Nitrogen		Significant t Value
	Mono-cots	Dicots	
Lysine	3.35	4.51	3.90
Methionine	2.01	1.45	3.31
Glycine	3.80	4.71	4.14
Histidine	2.01	2.30	3.13
Leucine	7.06	5.94	3.86
Phenylalanine	4.42	3.78	2.43 ^b
Valine	4.86	4.38	2.40 ^b
Alanine	4.83	3.82	5.37

^a Calculations based on data from 11 monocotyledoneae and 189 dicotyledoneae. $T_{0.01} = 2.62$; $T_{0.05} = 1.98$.

^b Significantly different at the $T_{0.05}$ level only.

Table VI. Indicated Differences in Amino Acid Composition among Plant Families^a

Amino Acid	Mean, Grams per 16 Grams Nitrogen						Significant t Value		
	Compositae vs. Cruciferae			Compositae vs. Leguminosae			Compositae vs. Cruciferae	Compositae vs. Leguminosae	Cruciferae vs. Leguminosae
	Compositae	Cruciferae	Leguminosae	Cruciferae	Leguminosae	Leguminosae	Cruciferae	Leguminosae	Leguminosae
Lysine	3.5	5.0	5.2	4.71	7.32
Methionine	1.8	1.5	1.1	3.49	8.43	6.83
Arginine	8.5	7.1	9.1	3.03	...	4.66
Glycine	4.9	5.3	4.3	...	3.09	4.08
Histidine	2.2	2.4	2.5	2.12 ^b	2.78
Isoleucine	3.9	3.6	3.5	2.11 ^b	2.43 ^b
Leucine	6.0	6.0	6.2
Phenylalanine	4.2	3.7	3.7	2.74 ^b
Tyrosine	2.5	2.9	3.0	2.68 ^b	3.25
Threonine	3.2	3.8	3.1	4.42	...	6.09
Valine	5.0	4.7	4.0	...	5.10	7.42
Alanine	3.9	4.0	3.6	3.53
Aspartic acid	9.3	7.3	9.4	7.35	...	8.26
Glutamic acid	20.5	15.2	16.9	6.43	3.71	2.16 ^b
Proline	4.2	5.4	3.8	4.52	...	8.88
Serine	4.3	3.8	4.4	2.46 ^b	...	4.07

^a Calculations were based on data from seed meals of 12, 18, and 23 members of the Compositae, Cruciferae, and Leguminosae, respectively. Seed meals selected were considered more representative of protein amino acids from each family than were all the seed meals analyzed from each family. For Compositae vs. Cruciferae, $T_{0.01} = 2.76$, $T_{0.05} = 2.05$; Compositae vs. Leguminosae, $T_{0.01} = 2.74$, $T_{0.05} = 2.04$; Cruciferae vs. Leguminosae, $T_{0.01} = 2.70$, $T_{0.05} = 2.02$. ^b Significantly different at the $T_{0.05}$ level only.

Table VII. Canavanine Content of Seeds from Leguminosae

Genus and Species	Canavanine, Grams per 16 Grams Nitrogen
<i>Astragalus crassicaarpus</i> ^a	11.2
<i>A. mexicanus</i>	3.9
<i>Canavalia ensiformis</i>	11.8
<i>Colutea arborescens</i>	15.0
<i>Coursetia glandulosa</i>	14.6
<i>Desmodium cinerascens</i>	2.6
<i>Hedysarum varium</i> ^a	18.7
<i>Lotus rigidus</i> ^a	3.9
<i>L. scoparius</i> ^a	5.6
<i>Medicago lupulina</i>	3.6
<i>Oxytropis uralensis</i> ^a	4.2
<i>Robinia neomexicana</i> ^a	11.6
<i>Sesbania macrocarpa</i>	9.6

^a Not previously reported as containing canavanine.

large amounts (more than one standard deviation above the mean) of their nitrogen as the amino acids found in protein. This information shows the crude protein content for these nine species, determined by total nitrogen multiplied by a standard factor of 6.25, is a more accurate measure of the protein present than for most of the seed meals reported. Of the above Leguminosae, the two species from the *Cassia* genus contained relatively large amounts of lysine and methionine and were not low in any of the nutritionally essential amino acids. The remaining seven species—two from *Crotalaria* and one each from *Ceratonia*, *Cyamopsis*, *Lespedeza*, *Lupinus*, and *Trifolium* (32)—were low in methionine. The high crude protein, high lysine, and average methionine content of two species from the genus *Onobrychis* indicate seed meal from this genus to be a good source of protein. Seed meal from *Vigna sinensis* is also apparently of good protein quality.

Seed meal from *Staphylea pinnata* (bladder wort) from the Staphylaceae family had the most unusual amino acid composition of any. Isoleucine, 5.5 grams per 16 grams of nitrogen, was unusually high. Lysine, methionine, histidine, leucine, phenylalanine, tyrosine, valine, and serine were unusually low. Two large, unidentified, elution peaks showed a large amount of unidentified nitrogen. The seed appeared mature. The hydrolyzate contained only 0.5% of the total nitrogen in the insoluble form.

The hydrolyzate of those species from the Malvaceae contained large amounts of insoluble nitrogen. A mean of 6.0% of the nitrogen was insoluble with a standard deviation of 3.2. Apparently either substances were present in the seed which caused excessive destruction of the amino acids during acid hydrolysis or unknown material was present which formed insoluble nitrogen-containing products during the hydrolysis.

Table VIII. Major Elution Peaks from Unidentified and Less Familiar Amino Acids in Seed from Various Species

Seed Meal Source, Genus and Species	Elution Position ^a	Amount, ^b Grams per 16 Grams Nitrogen
<i>Acacia farnesiana</i>	$R_{meth.} = 0.98^c$	2.5
	$R_{meth.} = 0.97^d$	1.1
<i>A. willardiana</i>	$R_{asp.a.} = 0.95^e$	2.4
	$R_{ser.} = 1.06^{f/g}$	2.6
	$R_{gly.} = 0.91^h$	1.7
	$R_{meth.} = 0.98^c$	0.8
	$R_{meth.} = 0.97^d$	0.3
<i>Agrostemma githago</i>	$R_{asp.a.} = 0.74^f$	0.2
	$R_{meth.} = 0.98$	0.5
<i>Allium porrum</i>	$R_{meth.} = 0.99$	0.3
<i>Argemone intermedia</i>	$R_{ala.} = 1.06$	0.1
<i>Calliandra eriophylla</i>	$R_{ser.} = 1.05^{f/g}$	0.8
	$R_{meth.} = 0.96$	0.3
	$R_{meth.} = 0.98^c$	1.1
<i>Clarkia elegans virginiana</i>	$R_{glut.a.} = 1.03$	1.4
<i>Fouquieria splendens</i>	$R_{meth.} = 0.96$	0.5
<i>Hemerocallis fulva</i>	$R_{asp.a.} = 0.80$	9.8
<i>Ipomoeae purpurea</i>	$R_{hist.} = 0.92^f$	0.2
<i>Isomeris arborea</i>	$R_{lys.} = 0.80^f$	0.2
<i>Lathyrus ornatus</i>	$R_{meth.} = 0.99$	0.1
<i>Lysiloma desmostachya</i>	$R_{NH_3} = 1.16$	0.3
	$R_{ser.} = 1.04$	1.1
	$R_{gly.} = 0.90^h$	0.1
	$R_{meth.} = 0.99^c$	0.6
	$R_{meth.} = 0.98^d$	0.2
<i>Milletia ovalifolia</i>	$R_{lys.} = 0.79^f$	3.6
	$R_{asp.a.} = 0.76$	0.1
<i>Mirabilis jalapa</i>	$R_{asp.a.} = 0.77^f$	0.6
<i>Pastinaca sativa</i>	$R_{asp.a.} = 0.74^f$	0.5
<i>Plantago ovata</i>	$R_{ser.} = 1.06^{f/g}$	0.4
<i>Staphylea pinnata</i>	$R_{asp.a.} = 0.81$	10.0
	$R_{asp.a.} = 1.03$...
<i>Thalictrum revolutum</i>	$R_{isoleu.} = 0.99^f$	0.2
<i>Urena lobata</i>	$R_{NH_3} = 1.30$	0.2
<i>Vicia faba</i>	$R_{leu.} = 1.08$	0.1

^a $R_{amino\ acid}$ = ml. effluent to elution peak of unknown ml. per effluent to elution peak of known amino acid. ^b Calculated as leucine, if absorption was measured at 570 $m\mu$, and as proline, if absorption was measured at 440 $m\mu$. ^c Possible identity djenkolic acid. ^d Possible identity mesodjenkolic acid. ^e Possible identity willardiine. ^f Absorption measured at 440 $m\mu$ instead of 570 $m\mu$. ^g Possible identity 4-hydroxypipicolic acid. ^h Possible identity S-(β -carboxyisopropyl)L-cysteine.

Seed meals from *Sterculia foetida* and *Bixa orellana*, the only representatives analyzed of the Sterculaceae and Bixaceae, respectively, appeared to be of nutritionally high quality. The high methionine for the two members of the Cucurbitaceae are similar to previously reported values for seeds from this family (28, 32).

Canavanine in Seed. Canavanine has recently been reported in seeds from a number of Leguminosae (4, 5, 32) and has been isolated from several seed meals, as well as from species of the *Canavalia* genus from which it was first obtained (15). Seed meals from 13 of the 35 different species of Leguminosae in Table IV contained canavanine in amounts ranging from 2.6 to 14.6 grams per 16 grams of nitrogen (Table VII). These values were based on the elution peak which was identical with authentic canavanine ($R_{NH_3} = 1.15$) and were calculated from the color yield obtained with the pure compound.

In addition to the canavanine elution peak from the acid hydrolyzates of these seed meals, a minor elution peak occurred after serine ($R_{ser.} = 1.14$). This minor elution peak was also present on chromatography of authentic canavanine which had been acid-hydrolyzed in the same manner as the seed meals. For this reason, the unidentified compound was considered as a product of canavanine formed during acid hydrolysis. The elution position of the new product indicates it to be homoserine. Chromatograms from samples containing unusually large amounts of canavanine also contained a second, very minor, unidentified elution peak which immediately followed the peak tentatively identified as homoserine.

Trace amounts of canavanine were also indicated in seed meals from five additional Leguminosae and 25 of the remaining seed meals from families other than the Leguminosae. The small elution peak in the proper position for canavanine was observed because of the extreme sensitivity of the automatic ion exchange method of analysis. This information is given as evidence that canavanine may be present in seeds other than those from Leguminosae. Positive identification by chemical isolation would be difficult in view of the small amount indicated to be present.

Less Familiar Amino Acids from Seed of the Mimosoideae, a Subfamily of the Legumes. Seed from *Acacia farnesiana*, *A. willardiana*, and *Lysiloma desmostachya* contained a number of ninhydrin color-producing substances which, for the most part, eluted in the same position as six unfamiliar amino acids recently isolated (77, 72) from the nonprotein nitrogen of seeds from this subfamily. Chromatographic analysis of seed from the three species (Table VIII) gave a peak eluting before methionine and in the same position as djenkolic acid. Also preceding this peak was a shoulder which may be due to mesodjenkolic acid formed from the natural amino acid during acid hydrolysis. Unhydrolyzed nonprotein nitrogen extracts of the seed gave only one peak in this area, which was in the same position as djenkolic acid.

Authentic albizziin (α -amino- β -ureido-propionic acid) and 4-hydroxypipicolinic acid eluted in the same position as the peak following serine in *Acacia willardiana* and *Lysiloma desmostachya*. In these seed meals this peak could be a mixture of three or more compounds—namely, 4-hydroxypipicolinic acid showing maximum absorption at 440 $m\mu$, small amounts of acid-labile albizziin, and other unidentified, acid-stable amino acids.

Albizziin on acid hydrolysis yields α , β -diaminopropionic acid (12) which elutes with histidine on the short column. A paper chromatographic spot at the position of histidine and α , β -diaminopropionic acid from acid-hydrolyzed, nonprotein, nitrogen extract from *L. desmostachya* gave a negative test for histidine with diazotized sulfanilamide, which was evidence for the presence of the diamino compound. Ion-exchange chromatography of an unhydrolyzed, nonprotein, nitrogen extract of the seed showed the presence of albizziin based on elution position of the authentic compound ($R_{ser.}$ 1.05). Calculation from the color constant of the authentic compound showed 22% of the nitrogen in the seed was albizziin.

The unknown eluting before aspartic acid from *Acacia willardiana* was tentatively identified as willardiine [L -uracil β -(α -aminopropionic acid)-3]. With minor changes in eluting conditions, this unknown, as well as authentic willardiine, eluted with aspartic acid. Authentic S -(β -carboxyisopropyl)- L -cysteine eluted in the same position as the unknown eluting before glycine from *A. willardiana* and *Lysiloma desmostachya*. Authentic S -(β -carboxyethyl)- L -cysteine isolated from Mimosaceae (11) eluted with glutamic acid.

Acid hydrolyzates of seed meals which contain S -(β -carboxyethyl)- L -cysteine and albizziin, analyzed for amino acid composition by the method as described, will give high results for glutamic acid and histidine. As shown, the former compound elutes with glutamic acid; and α - β -diaminopropionic acid, the acid hydrolysis product of albizziin, elutes with histidine.

The unidentified peak from *L. desmostachya* that followed ammonia was not canavanine. No canavanine peak occurred when the material was analyzed with the 50-cm. column. This unidentified peak was not present when the unhydrolyzed, nonprotein, nitrogen fraction from the seed was analyzed. This omission indicated that the peak was due to a substance formed during acid hydrolysis. Analysis of the unhydrolyzed, nonprotein, nitrogen extract showed the presence of three unidentified acid-labile compounds which eluted before aspartic acid.

Unidentified Elution Peaks from Remaining Species. Seed from 20

different species gave unidentified elution peaks which contained 0.1 gram or more of amino acid per 16 grams of nitrogen when calculated as leucine or proline (Table VIII). Of these, the unidentified substance from *Calliandra eriophylla* and *Plantago ovata* ($R_{ser.}$ = 1.05–1.06, 440 $m\mu$ max.) elutes in the same position as authentic 4-hydroxypipicolinic acid. Since the ninhydrin color constant for this compound is small (0.35), large amounts of the amino acid are in the seed from these two species, if present at all. A number of unidentified peaks elute in the same position as djenkolic acid, but not all of them showed 440 $m\mu$ absorption typical of djenkolic acid.

Analysis of seed from *Hemerocallis fulva* and *Staphylea pinnata* showed a large elution peak before aspartic acid. A second unknown in *S. pinnata* eluted with threonine or as a shoulder before threonine. Seed from *Mirabilis jalapa* and *Pastinaca sativa* showed a 440 $m\mu$ peak before aspartic acid similar to that reported from soybean hull (25) and collagens of sponges (24).

In addition to the unidentified substances reported in Table VIII, a large number of minor unidentified elution peaks were observed, such as a small peak occurring in a large number of seed meals in the same position as that of the toxic compound γ , α -diamino butyric acid (26, 30). A small peak appeared after the buffer change but before methionine in analyses of seed from 45 species. This peak varied in elution position indicating it was due to more than one compound. Analyses of seed from 12 species contained a minor elution peak between isoleucine and leucine.

In all the chromatograms the elution peak of levulinic acid and associated compounds (34) has not been considered. The 150-cm. column was not run beyond phenylalanine in order to detect such compounds as β -alanine. The 15-cm. column was not continued beyond the elution of arginine.

This work shows the rather widespread occurrence of less familiar amino acids in plant seeds. Limited studies indicate these compounds to be part of the nonprotein nitrogen. Other unidentified amino acids may exist which either are acid-labile or elute under peaks of the known amino acids. Unusually high amino acid compositions reported may be in error due to the presence of other compounds that elute in the same position.

Total Ammonia in Relationship to Plant Families and to Glutamic and Aspartic Acids. The mean ammonia content of the acid hydrolyzates from the 41 Cruciferae (78) was 12.4% of the total nitrogen compared with 10.25% for the 159 seed meals from the remaining plant families. These mean values are significantly different at the 99%

probability level. The source of this ammonia nitrogen in the Cruciferae may be due in part to decomposition products formed on acid hydrolysis of sulfur-containing glucosides (16).

Simple regression analysis showed at the 99% level a positive correlation between glutamic acid and total ammonia of the acid hydrolyzate. In view of the common occurrence of the acidic amino acids in the amide form in plant protein, this relationship was not surprising. Regression analysis between aspartic acid and ammonia showed no positive correlation probably because of excessive amounts of glutamine.

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NATURALLY OCCURRING INSECTICIDES

Myristicin, an Insecticide and Synergist Occurring Naturally in the Edible Parts of Parsnips

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A chemical of insecticidal and strong synergistic nature was found in the edible parts of parsnips (*Pastinaca sativa* L.), which have been consumed for centuries by humans without causing any obvious harm. The insecticidal constituent, present at about 200 p.p.m., was isolated and identified as 5-allyl-1-methoxy-2,3-methylenedioxybenzene or myristicin. Its toxicity to various insects was established and compared with pyrethrum and aldrin. The knockdown effect, although definite, was not as great as that of pyrethrum. In tests with *Drosophila melanogaster* Meig., it acted as a repellent and also killed through fumigant action, characteristics not evident with pyrethrum. Comparison of the synergistic activity of myristicin and piperonyl butoxide with *Musca domestica* L., showed that piperonyl butoxide was better with the pyrethroids, and myristicin with the carbamates tested. With *Drosophila*, the synergistic activity of myristicin was superior with Sevin, similar with *p,p'*-DDT and allethrin, and inferior with pyrethrum as compared with piperonyl butoxide.

ONE of the most difficult aspects of the search for useful insect control chemicals is the attempt to evaluate the potential hazard of the compounds on prolonged ingestion by man. It is therefore intriguing to seek insecticidal components in the edible parts of plants long consumed by man and animals without causing any obvious harm. Such chemicals might be of further importance in naturally protecting the edible portions of certain crops from insect attack, thus reducing the necessity for applied methods of insect control.

In tests conducted at the University of Wisconsin, a chemical of insecticidal and also of strong synergistic properties was found in the edible parts of parsnips.

Experimental

Evidence of Insecticidal Activity. Parsnips (All American variety), *Pastinaca sativa* L. (*Umbelliferae*), were grown in a Carrington silt loam soil free from any insecticidal residues. After harvest, the edible part of the crops was washed with water and ground in a food grinder (Hobart, Model T-215 Food Cutter). Three grams of this material were placed

on wet filter paper within each of six small bioassay jars (5.0 cm. in diameter and 6.3 cm. deep). Fifty vinegar flies (*Drosophila melanogaster* Meig.) were then introduced into each of the six jars and also into six control jars containing only wet filter paper (6). After an exposure time of 24 hours, 40% of the flies exposed to the parsnip material were dead, while no control mortality occurred. Apparently, parsnips contained some insecticidal substance. The ground parsnip material was then extracted and purified by a method described for other plant materials (74). Aliquots of the extract were then pipetted into bioassay jars, the solvent was evaporated at the opening of a fume hood, and 50 vinegar flies were introduced into each of the jars. All of the flies were dead after having been exposed overnight to residues representing 2.6 grams of parsnip material. In another test, flies were exposed to the residue of an extract representing 4.4 grams of parsnip material. After an exposure period of 100 minutes, 90% of the flies were knocked down or dead. Since some flies stuck to the plant residue deposited on the glass bottom of the bioassay jar, a fine metal screen was placed in front of this

residue to prevent the insects from coming into direct contact with the insecticidal deposit. New flies were then introduced into the jars. After a 1-hour exposure time, 32% of the flies were motionless, and all had died after an additional 14-hour exposure period. No control mortality was observed. These preliminary experiments indicated that the substance derived from parsnips also killed by fumigant action.

Extraction and Purification. The following procedure was found to be the most efficient of several tried for extraction, purification, and isolation of the insecticidal principle.

After harvest, the edible parts of the parsnips were washed with water and then macerated in a food grinder. Four-hundred grams of the macerated crop material were then placed into a 2-quart, wide-mouthed Mason jar, and 900 ml. of redistilled acetone were added. After 1 hour of head-to-end tumbling, the supernatant liquid was decanted through filter paper, and the recovery volume was recorded. The acetone was then evaporated on a steam bath using a Vigreux reflux funnel. After the amount of the remaining water had been determined for correction of