almost identical with that of the pure substance (*A*, Figure 2) to the relatively featureless spectrum *E* at 1.9×10^5 rads. Some of the molecular species represented by the latter spectrum are evidently changed considerably from the original naphthoquinone structure. It is possible that the changes taking place in menadione, irradiated anaerobically, do not interfere with its reaction with 2,4-dinitrophenylhydrazine as drastically as they apparently reduce its biological potency (*10*).

The curved retention slope, Figure 1, III, given by menadione irradiated under oxygen, might imply that in this system irradiation products are more successful than menadione in competing for free radicals. An alternative explanation is that these products are being reconverted, either to menadione, or to naphtho- or benzoquinones with spectra closely resembling that of the vitamin. More critical chemical methods and/or bioassay would be needed to distinguish between these effects.

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PLANT PROTEIN CONSTITUENTS

Hydroxyproline Content of Seed Meals and Distribution of the Amino Acid in Kernel, Seed Coat, and Pericarp

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Hydroxyproline was found in 63 of 99 samples of solvent-extracted, acid-hydrolyzed seed meals obtained from a wide variety of plants in amounts ranging from 0.1 to 5.7 grams per 16 grams of nitrogen. Eleven seed meals derived from the kernel alone contained no hydroxyproline. Eight samples of only seed coat or pericarp from seven plant families had hydroxyproline contents ranging from 3.1 to 10.0 grams per 16 grams of nitrogen. L-Hydroxyproline was isolated from the seed coat of *Iris germanica* and its identity established by classical methods. Limited solubility studies indicate that the compound is part of the protein in seed coat and pericarp.

THE AMINO ACID content of numerous seed meals is being determined as one phase of a screening program (*17*) to find new crops that could be profitably grown by the farmer. In making such determinations it was observed that most seed meals contained hydroxyproline. This amino acid was frequently present in amounts greater than 1 gram per 16 grams of nitrogen.

Little information was found in the literature concerning hydroxyproline in plants (*7, 9*). Piez, Irreverre, and Wolff (*8*) reported small amounts in the pericarp of dates. The authors reported that soybean seed coat contains 7.6 grams of this amino acid per 16 grams of nitrogen (*10*). Kleinshmidt reported its presence in the poppy plant (*4*).

Hydroxyproline is formed in situ from proline after proline has become part of the peptide chains in collagen formation (*13*). Steward and co-workers (*9, 14, 15*) in a series of papers show that in plant tissue cultures hydroxyproline is formed similarly from proline. Their work indicates it to be part of a stable protein not subject to "carbon turnover." More recently, Dougall and Shimbayashi (*3*) and Lamport and Northcote (*5*) have reported the compound in plant tissue cultures and have used its measurement as a means of following metabolic processes. No reference has been found in the literature to isolation and characterization of the compound from plant sources. Radikrishnan and Giri (*11*) isolated *allo*-hydroxyproline from *Santalum album* in which it was present uncombined. The paucity of information on the presence of the amino acid in proteins from naturally growing plants and its apparently unusual method of formation from proline prompted this further investigation of hydroxyproline in plant materials.

Experimental

Materials. Amino acid analyses were made on solvent-extracted meals derived from seeds, including in most cases seed coat or seed coat and pericarp because these parts could not be easily separated. When it became apparent that hydroxyproline was associated with seed coat and pericarp, selected separate preparations of these tissues were also analyzed. All preparations were ground, extracted with petroleum ether in order to remove oil, and acid-hydrolyzed before analysis as previously described (*16*).

Methods of Analysis. The amount of each amino acid present in the hydrolyzate was determined by the ion exchange, chromatographic automatic analysis method of Spackman, Stein, and Moore (*12*), using the Beckman Spinco, Model No. MS instrument. By operating the long column at 30° C. during the first part of the run, well

Table I. Hydroxyproline Content as Related to Structural Components Present in Meals of 99 Species

Plant Part Analyzed	Number of Species Having Indicated Hydroxyproline Content: 0 or trace[a]	0.1 to 1.0[a]	>1.0[a]
Kernel[b]	11	0	0
Kernel and seed coat	21	32	19
Kernel, seed coat, and pericarp	4	7	5

[a] Grams/16 grams of nitrogen.
[b] Kernel used throughout to mean a seed with its seed coat removed.

Table II. Seed Meals Containing Over 2.0 Grams of Hydroxyproline per 16 Grams of Nitrogen

Sample	Plant Family	Hydroxyproline[a]
Iris germanica[b]	Iridaceae	5.7
Nandina domestica[b]	Berberidaceae	4.2
Scabiosa atropurpurea[b]	Dipsacaceae	4.1
Anchusa capensis[c]	Boraginaceae	3.6
Lesquerella lasiocarpa[b]	Cruciferae	3.5
Yucca arizonica[b]	Liliaceae	3.1
Borago officinalis[c]	Boraginaceae	2.8
Isatis tinctoria[c]	Cruciferae	2.4
Lesquerella lindheimeri[b]	Cruciferae	2.4
Lunaria annua[b]	Cruciferae	2.3
Ximenia americana[b]	Olacaceae	2.2
Capsicum frutescens[b]	Solanaceae	2.1
Oenothera biennis[b]	Onagraceae	2.0

[a] Grams/16 grams of nitrogen.
[b] Seed meal consisted of kernel and seed coat.
[c] Seed meal consisted of kernel, seed coat, and pericarp.

Table III. Hydroxyproline Content of Soluble Acid Hydrolyzates from Pericarp or Seed Coat of Selected Plants

Sample	Plant Family	Nitrogen in Sample, %	Hydroxyproline[a]
Iris germanica[b]	Iridaceae	2.1	10.0[c]
Citrullus vulgaris[b]	Cucurbitaceae	0.2	6.9
Sapindus mukorossi[d]	Sapindaceae	0.3	6.3
Sapindus mukorossi[b]	Sapindaceae	1.5	5.1
Acacia farnesiana[b]	Leguminosae	1.0	4.1
Ceiba acuminata[b]	Bombacaceae	1.0	3.9
Calycanthus floridus[d]	Calycanthaceae	0.3	3.1
Crambe abyssinica[d]	Cruciferae	1.6	3.1

[a] Grams/16 grams nitrogen.
[b] Seed coat.
[c] Obtained by isolation.
[d] Pericarp.

separated symmetrical elution peaks in the same elution position as authentic L-hydroxyproline were obtained. Absorption maximum was at 440 mμ. The color yield constant was 2.25 by the calculation described by Spackman, Stein, and Moore (*12*). In many cases, the hydrolyzates were qualitatively examined by paper strip chromatography. Water–1-butanol–acetic acid (4:4:1) was used as the developing solvent. Proline was detected with isatin, and hydroxyproline with *p*-dimethylaminobenzaldehyde in hydrochloric acid (*2*). Trichloroacetic acid extracts of the meal were made according to the method of Becker, Milner, and Nagel (*1*). Extractions were carried out with 0.8*N* trichloroacetic acid using three successive 40-ml. volumes per gram of material.

Characterization of L-Hydroxyproline. Positive identification of the compound was accomplished by isolation from acid hydrolyzate of seed coat from *Iris germanica* using a procedure similar to that described by Levine (*6*). Alpha-amino acids were destroyed by heating with nitrous acid. After acid hydrolysis of the nitrosoamines of L-proline and L-hydroxyproline, these two imino acids were separated on a Dowex 50 column with dilute hydrochloric acid as elutriant. L-Hydroxyproline was converted from its hydrochloride to the free base form by adsorption on a Dowex 50 column in the acid form and by elution with dilute ammonia. It was crystallized from water by adding alcohol. From 10 grams of seed coat containing 2.0% of nitrogen 120 mg. of material was crystallized. The isolated part gave an x-ray pattern identical with that of an authentic sample of L-hydroxyproline crystallized in the same way. Its optical rotation was $[\alpha]^{26°}_{D}$ −75.6 (water, c 1). Literature value for L-hydroxyproline: $[\alpha]^{25.5°}_{D}$ −75.2 (*6*). Elementary analysis found: C, 45.7%; H, 6.96%; N, 10.5%. Theoretical: C, 45.7%; H, 6.87%; N, 10.68%.

Trichloroacetic Acid Extractions. Trichloroacetic acid extracts from the meal of *Eruca sativa*, seed coat of *Iris germanica*, and pericarp of *Crambe abyssinica* gave negative tests for hydroxyproline. Acid hydrolyzates of the residues gave strong positive tests for the compound. These experiments showed that hydroxyproline was not in readily extractable form. The nature of the protein or proteinlike structure of which it is part requires further extensive study.

Results

Analysis of Seed Meals. Table I is a compilation of single analyses from 99 different species, distributed among 50 plant families. From one to 23 species per family were analyzed. As seen in Table I, 11 seed meals consisted of kernel, 72 of kernel and seed coat, and 16 of kernel, seed coat, and pericarp. Hydroxyproline, when present, appears to be associated with those materials containing seed coat or pericarp. However, not all meals containing seed coat or seed coat and pericarp contained hydroxyproline. Except for traces, which could easily be due to slight contamination from seed coat or pericarp because of difficulty in their separation from the kernel, no hydroxyproline was found in meals derived from kernels alone. These were: *Acacia farnesiana*, *Ailanthus altissima*, *Ceiba acuminata*, *Ceratonia siliqua*, *Citrullus vulgaris*, *Hemerocallis sp.*, *Iris germanica*, *Lunaria annua*, *Martynia parviflora*, *Sapindus mukorossi*, and *Sterculia foetida*. Seed meals that contained the largest amounts of hydroxyproline are shown in Table II. These 13 species are distributed among nine diverse plant families.

The largest number sampled for a single family was 23 species of the Cruciferae. Their hydroxyproline content ranged from 0.3 to 3.5 grams per 16 grams of nitrogen. Seed meals consisting of kernel and seed coat from different cultivated varieties of two species of *Brassica* were analyzed. Three varieties each from *B. campestris* and *B. napus* ranged from 0.8 to 1.3 and 0.6 to 1.5 grams of hydroxyproline per 16 grams of nitrogen, respectively.

Analysis of Pericarp and Seed Coat. Because analysis of the meals showed that hydroxyproline was more likely to be present in the seed coat or pericarp, 12 samples of pericarp, eight of seed coat, and eight probably containing both seed coat and pericarp, from species other than those included in Table I, were qualitatively examined for hydroxyproline by paper strip chromatography. Pericarp obtained from No. 2 commercial yellow corn was among the samples containing hydroxyproline. All except three species gave positive tests. Samples of bran from Conley hard red spring wheat and of pericarp from *Kochia scoparia* (sample also contained calyx) and from *Ulmus americana* were negative.

Eight samples of pericarp or seed coat were analyzed quantitatively for their hydroxyproline content by ion exchange chromatography (Table III).

The first four species, which contained the largest amounts of hydroxyproline, were among the ones found to contain no hydroxyproline in meals derived from

kernel alone. Percentages reported on seed coat or pericarp are subject to error because of the large loss of nitrogen (16 to 40%) in the insoluble humin formed during acid hydrolysis of this material.

Discussion

The authors' data show the widespread occurrence of hydroxyproline in seed meals containing seed coat or seed coat and pericarp. Its presence in a number of separated seed coat and pericarp preparations, and its isolation from seed coat of *Iris germanica*, from which it could not be extracted with trichloroacetic acid, support the conclusion that hydroxyproline is a part of normal plant protein. Its presence in plants is not restricted to formation under plant tissue culture conditions.

Since hydroxyproline is found in collagens (structural proteins) of animals, its presence in the seed coat and pericarp of plants suggests that these parts of plants contain structural protein. The protective nature of seed coverings may be partly due to presence of protein of the structural type.

Available evidence (*9*, *13*) indicates that hydroxyproline formation in protein synthesis is different from that of most, if not all, remaining amino acids. It is formed from proline after proline becomes part of the proteinlike material. For this reason, information presented here might aid in such areas of plant physiological research as the study of processes associated with seed coat and surrounding tissues in both seed formation and germination, and in the isolation of their constituent proteins.

Presence of the compound in the large numbers and varieties of seed meals examined indicates that hydroxyproline is common in food and feed derived from plant sources. Practical use of these results might be made in testing meals and similar products for contamination by "hull material" that contains hydroxyproline.

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THIAMINE IN SOYBEAN MEAL

The Alleged "Thiamine-Destroying Factor" in Soybeans

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For 15 years, the literature has contained an unrefuted postulation that soybeans contain a thiamine-destroying factor. This postulation is based on loss of thiamine added to aqueous slurries of soybean meal, and an analogous observation with oriental millet meal which caused thiamine deficiency symptoms on bioassay. Data presented here show that the reported loss of thiamine is based on an unreliable thiochrome assay procedure, and that the analogy with millet meal is untenable. Thiamine in soybean meal exists as 40% free and 60% bound, presumably as cocarboxylase. Enzymes in unheated meal readily convert cocarboxylase to thiamine. Thiamine stability at neutral pH is decreased by addition of phenolic compounds, and increased by absence of air or addition of (ethylenedinitrilo)tetraacetic acid.

Many investigations of thiamine destruction in biological materials have been prompted either by poor recovery of thiamine added to assay samples or through evidence of thiamine deficiency symptoms when these materials are used in a diet. Thiaminase activity of bracken fern (*Pteris aquilina*) has been associated with the poisoning of cattle (*15*), horses (*18*), and rats (*5*) because steaming the fern destroyed activity and abolished toxicity for all species (*21*).

In previous studies (*11*) involving the measurement of thiamine in trichloroethylene-extracted soybean meals, we found no apparent discrepancy in thiamine content of different meals as determined by chemical analysis using the accepted thiochrome method described by Johnson (*10*). Known amounts of thiamine added to all assay samples were quantitatively recovered, and results were confirmed by microbiological assay. These findings led us to question the report by Bhagvat and Devi (*3*) that soybeans contain a factor that destroys thiamine.

Bhagvat and Devi used a thiochrome method in which thiamine was extracted