Biological Evaluation of Crambe Seed Meals and Derived Products by Rat Feeding[1]

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ABSTRACT

Diverse hydrolytic reaction products have been identified that are derived from the major crambe seed thioglucoside, *epi*-progoitrin (*e*-PG), and the responsiveness of the reaction pathway to controllable environmental factors has been clarified. The principal product of thioglucoside breakdown by thioglucosidase(s) in crambe meal is either a mixture of three nitriles, the oxazolidinethione (*R*)-goitrin, or some combination of these substances. Such elucidation of products from thioglucosidase activity has made it possible to prepare for rat-feeding studies definitively characterized crambe meals, as well as compounds or fractions of known chemical composition derived from them.

Inclusion of 0.23% (*R*)-goitrin in rat diets decreased growth to 85% of that of a control group. A mild hyperplastic goiter and a mild degenerative nonspecific alteration of liver cells were the only microscopic changes detected. Similar growth restriction resulted when comparable levels of *e*-PG were fed, incorporated into the diet either as an isolate or in a crambe meal in which thioglucosidase enzyme(s) had been inactivated. In contrast, either autolyzed meal that contains the nitrile mixture, or the nitrile mixture itself after isolation from the meal by extraction, was more harmful. Both caused poorer growth or death, and resulted in bile-duct hyperplasia, fibrosis, and megalocytosis of hepatocytes and tubular epithelial cells in the kidney. Rats fed meal containing intact *e*-PG plus active thioglucosidase enzyme(s) had similar but milder lesions than did rats fed the nitrile mixture. Fibrosis was not observed; thyroid hyperplasia occurred.

Protein efficiency ratios (PER) were determined on defatted crambe meals that had been made nontoxic by aqueous acetone extraction without use of elevated temperatures. The PER's of 2.75 and 2.55 were higher than that of casein (2.50).

Hydrolysis of the major thioglucoside, *epi*-progoitrin I (*e*-PG), of crambe seed (1) in defatted meal by endogenous enzymes follows either pathway 1 (Fig. 1) to yield (*R*)-goitrin ((5*R*)-vinyloxazolidine-2-thione) II, pathway 2 to give (*S*)-1-cyano-2-hydroxy-3-butene III plus diastereomeric (2*S*)-1-cyano-2-hydroxy-3,4-epithiobutanes IV and V (2), or a combination of both pathways. During autolysis below 45°C. at pH 4.5 to 5.5 of crushed, recently harvested seed, the *e*-PG preferentially follows pathway 2 (3). Autolyzed meals that contain nitriles III, IV, and V are lethal to rats (4). Enantiomers of the products from crambe *e*-PG are formed from progoitrin in rapeseed (*Brassica napus*) (5). Defatted seed meals from this species of rape and from crambe are sources of animal feed that can be improved by removal or inactivation of these progoitrins or their hydrolysis products.

Rat-feeding experiments reported here show the relative toxicities of (a) crambe seed meals containing *e*-PG with and without the presence of active thioglucosidase enzymes, (b) meals containing nitriles or (*R*)-goitrin, and (c) isolated *e*-PG or products formed from *e*-PG. The livers, thyroids, and kidneys were weighed and examined grossly and histologically. Aqueous acetone extraction either before (6)

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MATERIALS AND METHODS

Assay of Meal Constituents

The amount of (*R*)-goitrin was estimated by partition into ethyl ether from hot-water extracts of the meals, and by measurement of its ultraviolet absorption. The *e*-PG content was determined similarly by measurement of the (*R*)-goitrin formed by myrosinase hydrolysis of *e*-PG in the hot-water extract at pH 7.0 (6). Total thioglucosides were estimated by titration of sulfate ion formed from their hydrolysis (6). The nitrile compounds were measured by their infrared absorption at 4.4 microns (7).

Thioglucosidase activity in meals was detected by measuring (*R*)-goitrin formation in a meal slurry after incubation at pH 7.0 at 45°C. for 3 hr. When no *e*-PG was present, 10 to 20 mg. of that substrate was added per g. of meal before a test for enzyme activity was run. As a control, a meal slurry with added mustard myrosinase was also run.

Preparation of Seed Meals

For meal 1 (Table I), dehulled crambe seed was passed through smooth iron rolls at 0.003-in. clearance to give flakes about 2 mm. in diameter and 0.2 mm. thick. The oil was removed by percolation of pentane-hexane at room temperature. The extracted meal was ground in a hammer mill. Such air-dried crambe meals contained less than 1% oil, 38 to 47% crude protein and 2.0 to 2.2% sulfur.

TABLE I. ANALYSIS OF TREATED CRAMBE MEALS[a]

Meal No. and Description	Total Thioglucosides[b] %	*epi*-Progoitrin[b] %	(*R*)-Goitrin %	Nitriles[c] %	Thioglucosidase Activity
1 Dehulled, defatted[d]	8.8 (8.2-9.4)	7.1 (6.8-7.6)	0.0	0.0	+
2 Heated meal 1[e]	8.7	7.1	0.0	0.0	—
3 Meal 1, autolyzed room temp.[e]	0.0	0.0	0.0	0.8	—
4 Meal 1, autolyzed room temp.	0.0	0.0	0.1	0.6	+
5 Meal 1, autolyzed 60°C., dil. soln.[e]	0.0	0.0	1.3	0.2	—
6 Meal 1, aq. acetone-extd.	0.0	0.0	0.0	0.0	+
7 Meal 1, autolyzed and aq. acetone-extd.	0.0	0.0	0.0	0.0	+
8 Heated meal 7	0.0	0.0	0.0	0.0	—
9 Meal 1, autolyzed and twice aq. acetone-extd.	0.0	0.0	0.0	0.0	+

[a]Percentage composition based on air-equilibrated meal.

[b]Calculated as *epi*-progoitrin potassium salt.

[c]Based on infrared analysis and calculated as 1-cyano-2-hydroxy-3-butene.

[d]Composition given is the mean value from three preparations; the range of values is given in parentheses.

[e]Meals treated with boiling water and dried by lyophilization; other preparations were air-dried.

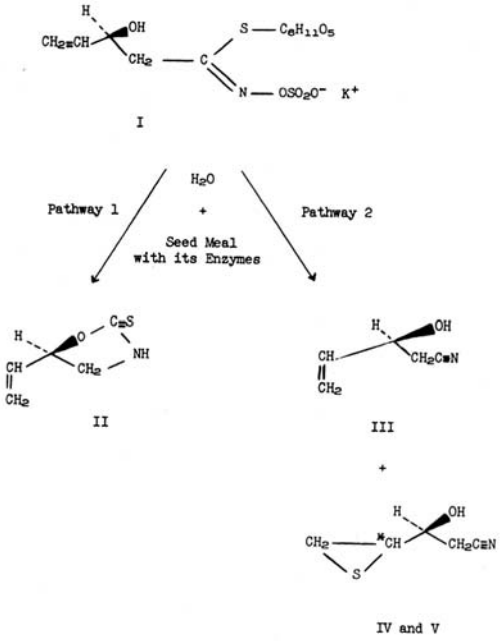

Fig. 1. Hydrolysis products from *epi*-progoitrin aglucon in crambe seed meal.

Meal 2 (Table I) was prepared by heating 30-g. amounts of meal 1 to above 80 ° C. in a 1-liter flask in a boiling-water bath, then adding 300 ml. of boiling water, and holding the meal slurry at 95°C. or above for 5 min. Water was removed by lyophilization.

Meal 3 was prepared by wetting 30 g. of meal 1 with 100 ml. of water in a 1-liter flask and allowing the wet paste to stand 30 min. Then 100 ml. of boiling water was added and the meal slurry was held at 95°C. or above for 5 min. The water was removed by lyophilization.

Meal 4 was prepared by wetting 500 g. of meal 1 with 1.6 liter of water. The wet paste was spread out in trays to 1/8-in. thickness and air-dried.

Meal 5 was prepared by slow addition with stirring of 60 g. of meal 1 at 60°C. to 600 ml. of water at 60° to 65°C. The meal slurry was stirred for 30 min. while being held at 60° to 65°C. The mixture was treated with boiling water and lyophilized as in the preparation of meals 2 and 3.

For meal 6, 500 g. of meal 1 was weighed into a 5-liter metal beaker with 3 liters of acetone (dry commercial grade). One liter of water was added dropwise to the stirred slurry. The slurry was filtered on a Buechner funnel through Whatman No. 54 filter paper. The meal was washed twice with 200 ml. of acetone per wash. This extraction was repeated four more times with 2 liters of acetone and 660 ml. of water each time. The average weight recovery from 12 preparations on air-dried, reground meal ranged from 70 to 73% of the starting meal. The combined air-dried preparation contained 46% crude protein and 1.4% sulfur. The acetone was removed from the extract under vacuum at less than 75°C. and the aqueous residue was used for the isolation of *e*-PG and (*R*)-goitrin.

Meal 7 was prepared as follows: meal 1 was autolyzed for 90 min. with 1 ml. of water per g. of meal; 8 ml. of acetone per g. of meal was added and the mixture was stirred for 30 min. The filtered meal was then extracted twice with 4 ml. acetone per g. of meal. For meal 8, meal 7 was spread to a depth of 2 cm. and steamed for 30 min. at 100°C. in a preheated autoclave and air-dried. Meal 9 was prepared like meal 7, except that it received a second aqueous acetone extraction with 4 ml. of 1 to 7 (v./v.) water-acetone per g. of meal. Crude protein contents of meals 7 through 9 were 49 to 50%; sulfur, 1.45%. Weight recoveries for the three meals were 86% of the starting meal. Meals 7, 8, and 9 were prepared for determination of PER's.

Preparation of Isolates

The isolation of (*R*)-goitrin is described in detail because of advantages in the present procedure over those previously reported for isolation of (*S*)-goitrin (8,9). After removal of acetone in the extract from preparation of meal 6, the aqueous residue containing the *e*-PG from 500 g. of meal was concentrated to 250 to 300 ml. and the precipitate removed by centrifugation. The aqueous solution was extracted three times with two volumes of peroxide-free ethyl ether per extraction. The aqueous phase was freed of dissolved ether on a rotary evaporator and adjusted to pH 7 to 7.5 with phosphate buffer and sodium hydroxide. The solution, diluted to 600 to 650 ml., was heated to 38° to 41°C. while being stirred with a magnetic stirrer. From 0.3 to 0.5 g. of mustard myrosinase (7) was added. Under these conditions about 10 hr. was required for complete hydrolysis of the *e*-PG to (*R*)-goitrin. Sodium hydroxide solution was added as required during the reaction to hold the pH between 6.5 and 7.5. After hydrolysis the solution was concentrated to 300 to 400 ml. at 45°C. and centrifuged. The (*R*)-goitrin was extracted from the centrifugate into ether by three extractions with 2 volumes of ether per extraction. The combined extracts were dried over anhydrous sodium sulfate. The filtered ether solution was concentrated to 15 to 20 ml.; from this the (*R*)-goitrin crystallized while it stood in the refrigerator. The crystalline material was washed with cold ether, freed of ether, dissolved in water, filtered, and recrystallized from water. Three preparations (6.7 to 7.0 g. each) for the rat-feeding studies provided sharp-melting goitrin which varied in melting point from 47°–48°C. to 50°–51°C. Reported melting points range from 47° to 49°C. (1). Elementary analyses: Found: C, 46.5 to 47.4; H, 5.7 to 5.9. Calculated for C_5H_7NOS: C, 46.5; H, 5.46. Molar absorptivities ranged from log ϵ = 4.25 to 4.47 at 244 millimicrons in ethanol, which were in agreement with literature values (4). Because the first ether extraction removes extraneous solubles before the hydrolysis, the (*R*)-goitrin crystallizes readily in good yield, an advantage not achieved in earlier procedures.

The *e*-PG was isolated essentially as reported earlier (1) but with the procedure scaled-up to handle larger batches. The acetone and some water from the aqueous acetone extract from 2 kg. of meal 1 were removed at less than 75°C. to give 1.3 to 1.8 liters of residue. The residue was centrifuged to remove solids. The crude *e*-PG was separated on a 4-kg. column of acidic alumina. Fractions containing the *e*-PG were detected and combined, and the *e*-PG was isolated. The noncrystalline, hygroscopic isolates assayed 85 to 90% *e*-PG potassium salt as shown by measurement of the (*R*)-goitrin formed by hydrolysis with mustard myrosinase at pH 6.5 to 7.

The mixture of nitriles was prepared from an ether extract of autolyzed meal 3 or 4 as described before (3). Samples used for rat feeding contained from 43 to 65%

nitrile calculated as 1-cyano-2-hydroxy-3-butene, 0.0% (*R*)-goitrin, and 16 to 18% sulfur.

The polypeptide crambin was isolated in a crystalline form as it was previously (10).

Feeding Experiments

Weanling rats were fed ad lib. an Addis diet (11) for experiments A and B, and Purina Rat Chow in experiment C (Table II). Organs from those animals fed 84 days or longer were weighed, and histological examinations were performed on tissues fixed and stained by standard procedures.

Organ and body weights of each group in which rats survived the experiment were tested statistically for significant differences from the controls by Fisher's "t" test. Average standard deviations for all groups in the experiment are also reported (Table II).

Under some conditions progoitrin is unstable to moisture (12). Each diet preparation containing *e*-PG (experiment A, Table II) was assayed for the amount of *e*-PG present immediately after mixing and just before its complete consumption by the animals. No loss of *e*-PG occurred during either mixing or storage. The control ration in experiment A, Table II, was tested for thioglucosidase activity. None was found.

The crystalline (*R*)-goitrin was dissolved in acetone, the solution mixed with the ration, and the acetone removed by air-drying. The nitrile fraction was kept below 0°C. before being mixed with the ration. After autolysis, meal 3 was lyophilized and stored at 0°C. before being mixed into the ration. Meal 4 was air-dried and stored at room temperature.

The PER's were determined for meals 7, 8, and 9 according to the procedure of Derse (13).

RESULTS

Biological Effects of Feeding Meals and Isolates: Experiment A

This feeding test was designed to compare the biological activities of *e*-PG and untreated meal fed at comparable levels of *e*-PG. When fed at the 0.5 and 1.5% levels, *e*-PG was approximately equal to that in meal 1 fed as 5 and 15% of the ration (Table II. Isolated *e*-PG inhibited growth, but to a much less extent than did meal 1. Meal 1 contained active thioglucosidase(s). The livers, kidneys, and thyroids of animals fed either the isolated *e*-PG or meal 1 were enlarged. Histological examination of the organs from the two groups showed mild hyperplasia of the thyroids. The liver and kidneys of one of the five rats fed 5% of meal 1 contained lesions identical with those described in experiment B where the effects of the nitrile fraction are assessed. Hydropic degeneration of the hepatic cells was observed in all rats fed 0.5% *e*-PG but not in those fed 1.5%. Hemoglobin and red and white blood cell counts of rats fed 1.5% *e*-PG were within the normal limits.

Rats fed the peptide crambin grew at normal rates, and no body tissue abnormalities were noted. Crambin was fed at a level approximating the amount the animals would receive when fed the defatted crambe meal at 30% of the ration.

Biological Effects of Feeding Meals and Isolates: Experiment B

This test was designed to compare the effects of feeding untreated meal, meal

TABLE II. WEIGHTS AND ORGAN WEIGHTS OF RATS FED CRAMBE MEALS OR DERIVED PRODUCTS

Test Substance[a]	Amt. in Ration %	Body Wt., Percent of Controls %	Organ Wt., per 100 g. Body Wt.		
			Liver g.	Kidney g.	Thyroid mg.
EXPERIMENT A[b] Control		100	3.2	0.61	8.5
Meal 1, dehulled defatted, 7.1% *e*-PG	5	82[c] **	4.5 **	0.79 **	16.8 **
Meal 1, dehulled defatted, 7.1% *e*-PG	15	c			
epi-Progoitrin	0.5	92	4.1**	0.64	11.2
epi-Progoitrin	1.5	71 **	4.1**	0.76**	13.9**
Crystalline polypeptide (crambin)	0.6	101	3.1	0.59	8.3
Std. dev.[d]		15.6	0.09	0.047	2.53
EXPERIMENT B[e] Control		100	2.7	0.65	4.4
Meal 1, dehulled defatted, 6.8% *e*-PG	5	76**	4.2**	0.68	7.5**
Meal 1, dehulled defatted, 6.8% *e*-PG	10	60**	5.1**	0.76	15.6**
Meal 4, autolyzed at r.t., air-dried	5	72**	4.3**	0.82*	7.7**
Meal 4, autolyzed at r.t., air-dried	10	61**	4.3**	0.78	7.7**
Nitrile mixture	0.1	17[f]**	5.6**	1.50**	6.1
Nitrile mixture	0.2	f			
Std. dev.[d]		11.2	1.02	0.108	1.96
EXPERIMENT C[g] Control		100	3.5	0.64	7.5
Meal 1, dehulled, defatted, 7.6% *e*-PG	10	41 [h]**	9.3**	1.54**	13.4**
Meal 2, meal 1 wet-heated	10	77**	4.5	0.86**	12.9**
Meal 3, autolyzed at r.t., lyophilized	10	h			
Meal 5, autolyzed at 60°C.	10	85**	3.7	0.68	20.8**
Meal 6, meal 1 aq.-acetone extd.	10	105	2.7	0.74	8.0
Meal 6, meal 1 aq.-acetone extd.	30	97	3.4	0.68	8.4
Meals 2, 3, and 5	3.3[i]	25**	5.9**	1.25**	7.4
epi-Progoitrin	0.85	85**	4.7*	0.81**	9.0
epi-Progoitrin	2.6	h			
(*R*)-Goitrin	0.23	85**	4.0*	0.62	14.7**
Std. dev.[d]		6.7	0.57	0.087	1.95

[a]Meals described in Table I.

[b]Five female rats per group on Addis ration. Average starting wt. was 50.4 g. Average wt. of controls was 194 g. at end (98 days) of experiment.

[c]Of the animals fed 5% meal, one died on the 20th day. Those fed 15% meal lost weight; two died within the first 19 days, at which time the experiment was discontinued.

[d]Average std. dev. for all groups in the experiment. The LSD between two means at 95% level is approx. 2.09 $\sqrt{2/5}$ (S.D.) or 4/3 S.D.

[e]Five male rats per group on Addis ration. Average starting wt. was 49 g. Average wt. of controls was 387 g. at end (106 days) of experiment.

[f]Two animals fed 0.1 nitrile fraction died within 84 days; the remaining three were then autopsied. All animals fed 0.2% nitrile fraction died within 14 days.

[g]Five female rats per group on Purina Chow. Average starting wt. was 58.0 g. Average wt. of controls was 267 g. at end (96 days) of experiment.

[h]Of those fed meal 1, four died within 35 days; all those fed meal 3 and 2.6% epi-progoitrin died within 21 and 56 days, respectively.

[i]Fed 3.3% of each meal; at 49 days two of the rats were sacrificed and remaining three fed 3.3% of meal 3 only, until end of experiment.

autolyzed to contain nitriles, and the isolated nitrile-containing fraction. Animals fed autolyzed meal (meal 4) at 5 and 10% levels grew at about the same rate as those fed like amounts of meal 1 (Table II). The nitrile mixture fed as 0.1% of the ration approximated the amount in a ration that contained 10% of autolyzed meal 3 or 4. The nitrile mixture fed at this level had greater biological activity than either meal 1 or 4 at the 10% level.

As in experiment A, animals fed meal 1 had enlarged livers, kidneys, and thyroids. These organs were also enlarged in the animals fed meal 4. The livers from rats fed meal 4 or the nitrile mixture were often dark and firm.

Microscopic examination of the thyroids from rats fed 10% of meal 1 showed hypertrophy and hyperplasia of the follicular epithelium with papillary formation and loss of colloid from the glands of all five animals. Liver and kidney lesions were similar to, but less severe than, lesions described below that were observed in animals fed the nitrile mixture. Those animals fed meal 4 showed mild thyroid hyperplasia in three of five animals fed 5% of the meal, but no thyroid hyperplasia in those animals fed 10%. Liver and kidney lesions were more severe in those fed 10% of meal 4 than in those fed 5%. The lesions were similar to those in animals fed the nitrile mixture.

Lesions of major significance were present in the liver and kidneys of the three surviving rats in the trial with the nitrile mixture. Similar lesions, although generally less severe, were observed in all animals fed meals containing the nitriles and meals containing both *e*-PG and active thioglucosidase enzymes. Some evidence of such lesions was also noted in animals fed *e*-PG, either isolated or in the meal without active thioglucosidase(s).

In the livers of the animals fed the nitrile mixture, the normal lobular architecture was disrupted, being replaced by various-sized groups and strands of hepatic cells which were separated by narrow, interlacing bands of moderately cellular, mature connective tissue. A few lymphocytic cells were scattered sparsely in some connective-tissue zones. Bile duct-ductule proliferation was very irregular and was usually found either near the capsule or adjacent to major vessels. There was no

evidence of tumor formation. One of each pair of liver sections from two rats contained zones of massive necrosis, owing to the compromising of vascular supply to these areas by cirrhosis.

In the kidneys of the rats fed the nitrile mixture, the epithelial cells of the convoluted tubules were swollen and contained fine brown granules of pigment in moderate quantities. A predominant feature of the renal lesion was the prominent enlargement of the nucleus of some tubular epithelial cells, together with less conspicuous cytoplasmic enlargement.

Red and white cell counts and hemoglobin contents of all the animals in experiment B were essentially normal.

Biological Effects of Feeding Meals and Isolates: Experiment C

The main purpose of this feeding trial was to compare performance of rats fed meal containing *e*-PG and active enzyme(s) (meal 1); meal containing *e*-PG and inactivated enzyme(s) (meal 2); nitriles (meal 3); (*R*)-goitrin (meal 5); and the meal with no *e*-PG or derived hydrolysis products (meal 6). The meals were fed as 10% of the ration. Isolated *e*-PG and (*R*)-goitrin were fed at comparable levels.

Growth and survival were poor on meal 1 in this experiment, in contrast to results with the meal 1 preparations fed in experiments A and B. This difference might have arisen, at least in part, from the higher *e*-PG content of the meal, which was 7.6% vs. 7.1 and 6.8% for meal 1 in experiments A and B. Death of all animals fed autolyzed meal 3 as 10% of the ration contrasts with the survival of rats fed autolyzed meal 4 as 10% of the ration in experiment B. This difference in response was caused, at least in part, by the higher nitrile content of meal 3 of 0.8% vs. 0.6% for meal 4. Also, freeze-drying and storage of meal 3 at 0°C. before incorporation into the ration may have prevented loss of toxic materials.

Those animals fed a mixture of meals 2, 3, and 5 as 3.3% of each in the ration did little more than maintain their starting weight. After 49 days on the experiment, two animals were sacrificed. The livers of these animals were dark in color and of firm consistency. The remaining three animals were fed 3.3% of meal 3 only, until the experiment was terminated. At autopsy these animals had enlarged livers and kidneys with lesions similar to those found in the animals fed the nitrile mixture. The thyroids were normal. The surviving rat on meal 1 had similar lesions in the enlarged liver and kidneys but also had lesions typical of mild hyperplastic goiter in the thyroid.

In contrast, rats fed 10% of meal 2 and those fed 0.85% *e*-PG, which was equivalent to the *e*-PG in meal 2 as 10% of the ration, grew to 77 and 85% of the weight of the controls, respectively. Mild bile duct proliferation in the liver and megalocytosis in the kidneys similar to the severe lesions in the rats receiving nitriles were noted in both groups. Although somewhat enlarged, the thyroids in the animals fed meal 2 were histologically normal. The thyroids of rats receiving 0.85% *e*-PG contained minimal lesions indicative of hyperplastic goiter.

Rats fed both 10% of meal 5 and 0.23% of (*R*)-goitrin weighed 85% of the weight of the controls at the end of the experiment. Lesions typical of mild hyperplastic goiter were observed in the thyroids of all animals in these two groups. A mild centrilobular hydropic-type degeneration observed in the livers of all animals in the two groups was also noted in those fed meal 6 as 10 and 30% of the ration. The abnormality may be an indication of a deleterious effect of the meal. However,

it was no more pronounced in animals fed meal 6 as 30% of the ration than when fed at the 10% level. The kidneys of the animals fed meal 2, meal 5, (*R*)-goitrin, and meal 6 appeared normal.

As in experiments A and B, hemoglobin content was within normal limits for all animals in experiment C.

Protein Efficiency Ratios

Since aqueous acetone extraction of crambe meals either before or after autolysis removes all major toxic substances (4,6), meals prepared in this manner can be evaluated for protein quality by feeding tests. Evaluation with rats as the test animal (Table III) shows that autolyzed aqueous acetone-extracted crambe meals contain as good-quality protein as does casein, or better. The corrected PER's of 2.55 and 2.75 for the unheated crambe meals compare favorably with the average corrected PER of 2.15 obtained by 11 collaborators on soy grits (13), which comprise a recognized good-quality oilseed protein. When heated under conditions required to inactivate trypsin inhibitor and possibly other deleterious substances in soybeans, aqueous acetone-extracted crambe meal gave a markedly lower PER (see meal 8, Table III).

DISCUSSION

Much of the prior literature on species of *Brassica*, which contain progoitrin, and on crambe, which contains *e*-PG, relates to the goitrogenic properties of seed meals from these plants because of the recognized (*R*)- and (*S*)-goitrins formed from the progoitrins (14). However, Tookey et al. (4) in 1965 showed that autolyzed crambe seed meals containing neither *e*-PG nor (*R*)-goitrin were lethal if the meals were not extracted to remove ethyl ether-soluble material, which we now know includes the nitriles III, IV, and V (2,7). Here we show that either crambe meals containing the nitriles or an isolated fraction containing them is more toxic than meals containing (*R*)-goitrin or isolated (*R*)-goitrin fed at equivalent levels.

TABLE III. PROTEIN EFFICIENCY RATIOS (PER) FOR EXTRACTED CRAMBE MEALS

Sample	Nitrogen, Air-Dry Basis %	Sample in Diet %	PER[a] Wt. Gain, g./Protein Eaten, g. Range	Average	Corrected[b]
Casein, control	13.60	11.8	2.52-3.52	3.18	2.50
Crambe meal 7	7.95	20.1	3.07-3.51	3.25	2.55
Crambe meal 8	7.84	20.4	1.67-2.11	1.92	1.51
Crambe meal 9	7.95	20.1	3.04-3.77	3.50	2.75

[a]Determined with 10 rats per group housed in individual wire-bottomed cages. All diets were 10% protein (NX 6.25).

[b]Corrected to an arbitrary value for casein of 2.50 (see ref. 13).

The more toxic meal or meal fraction causes major pathological lesions in the liver consisting of bile duct hyperplasia and in the kidney consisting of megalocytosis of the tubular epithelial cells. Lesions in the thyroid were minor or absent. The enantiomers of the nitriles from crambe are formed from progoitrin in rapeseed (*B. napus*) (5). There are reports in the literature of enlarged livers and kidneys ob-

served in rats and swine fed brassica seed meals (15,16). Thus it appears that major attention should be given to the toxicity caused by the alternate autolysis products formed instead of the goitrins from the progoitrins in these seed meals.

Attempts are under way to relate pathological manifestations of toxicity to specific compounds III, IV, and V and to isolate other toxic ethyl ether-soluble products of autolysis if present. The episulfides IV and V become insoluble (polymerize) easily, a property which complicates their isolation for rat-feeding experiments. Autolyzed meals that have been treated to partly polymerize IV and, V are less toxic (C. H. VanEtten and A. N. Booth, unpublished data). Meal 3, containing nitriles, that was dried and stored at low temperatures, was more toxic than meal 4 similarly prepared but dried and stored at room temperature. The toxicity of purified polymers of IV and V has not been demonstrated.

The essential amino acid content of crambe seed meal indicates high nutritional quality in comparison with many sources of seed protein (17), a result in agreement with the PER values obtained on the unheated aqueous acetone-extracted crambe meals reported here. Lowering PER by wet-heat-treatment indicates that minimum heating of the meal in processing is required to maintain nutritional quality of the protein.

Acknowledgments

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