Methods for Determining Thioglucosides in *Crambe abyssinica*[1]

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Abstract

Four methods are described for the determination of thioglucosides in *Crambe abyssinica*: sulfate ion, sulfur balance, silver complexing, and hot-water extraction. The analytical results of all four methods agree closely as to the thioglucoside content in dehulled, defatted meal, from 11–12%. Any one of these methods should be useful in studying new plant species because approximate thioglucoside content is obtained even though the specific thioglucosides and isothiocyanates involved may not be known.

Introduction

CRAMBE ABYSSINICA is under study by botanists, agronomists, and chemists in the Department of Agriculture as a potential new industrial crop for the United States. Its high erucic-acid content, agronomic characteristics, and apparent good protein quality have already brought crambe to the fore as another domestic crop that might provide a profitable market without competing with those long established. But like other members of the Cruciferae family, crambe contains thioglucosides that are associated in some plants with unpalatability and goitrogenicity. Consequently, thioglucoside content must be accurately determined.

When ground crambe meal is moistened, thioglucosides are readily hydrolyzed by enzymes occurring naturally in it. The following thioglucoside hydrolysis reaction is typical:

$$\text{Thioglucoside} + \text{Water} \xrightarrow{\text{Enzyme}} \text{Aglycone} + \text{Glucose} + KHSO_4$$

Of the hydrolysis products, the aglycone is the compound generally responsible for the undesirable properties that thioglucosides contribute to feed meals. For example, in oriental mustard the aglycone is allyl isothiocyanate, which renders meals unpalatable unless removed somehow (4). In rapeseed, the aglycone is *l*-5-vinyl-2-thiooxazolidone, demonstrated to be goitrogenic to certain animals (1). Determination of thioglucosides by analysis for the aglycones released by enzyme hydrolysis is complicated because the aglycones differ in chemical and physical properties and because their identities are often unknown.

At least in crambe, thioglucoside determination by reducing sugar analysis of hydrolyzed meals is impractical. Not only are combined free sugars and glucose present but other reducing sugars are formed by the enzyme hydrolysis of the compounds in the seed meal. Because inorganic sulfate accompanies all known thioglucoside hydrolysis reactions regardless of the chemical nature of the aglycone, it is a better standard to use. Three other analytical methods were selected—sulfur balance, silver complexing, and hot-water extraction—based upon physical or chemical properties of the thioglucosides. All four procedures determine thioglucosides as a group, so that specific knowledge as to the identity of each thioglucoside is not required. Any one of the analyses is equally useful.

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Experimental

Materials and Methods

Seed lots of crambe were obtained from 1961 and 1962 crops grown in Montana, Nebraska, Texas and Wyoming.

Meal Preparation

The crambe seed was cracked in a single pass through corrugated rolls at 0.035-in. clearance and aspirated to remove hulls. Flakes produced by passing the dehulled grits through smooth rolls were hexane-extracted, air-dried, and ground to pass a 40-mesh screen. The various analyses reported are corrected for moisture content and residual fat.

Sulfate Ion Determination

Ten grams of crambe defatted air-dried meal were added to 250 ml of distilled water. The homogenous mixture was hydrolyzed at 54C for 1 hr and then boiled 2 additional hr while keeping the volume constant by addition of water. After filtering the solution, the cake was slurried three times with 50 ml of hot water, and this wash water was then added to the initial filtrate and the total volume was made up to 600 ml. The barium sulfate was precipitated hot with excess 5% barium chloride solution, digested on a steam bath a few hours or overnight, and removed on ashless filter paper. The weight of the precipitate was obtained after ashing.

$$\frac{(\text{m wt thioglucoside})\ (\text{Wt of } BaSO_4)}{(\text{M wt } BaSO_4)\ (\text{Sample wt})} \times 100 = \%\ \text{Thioglucoside}$$

The free sulfate content of the meal before thioglucoside hydrolysis was estimated by adding the sample to boiling water for 5 min to deactivate the natural enzymes, cooling rapidly, and performing the remaining steps before ashing at room temperature. In these steps heating was minimized to avoid chemical hydrolysis of the thioglucosides, particularly in the presence of barium chloride. Since the amount of sulfate found by this method was small (0.029%) and of questionable origin, no correction was made for it in the sulfate ion determination.

Sulfur Balance

Total sulfur in the seed was determined by the method of Shaw (6), and amino acid sulfur, from the cystine and methionine present by the method of Spackman et al. (7). Thioglucoside content was obtained from the following expression:

$$\frac{(\text{Total sulfur-amino acid sulfur})\ (\text{M wt thioglucoside})}{2(\text{A wt sulfur})\ (\text{Sample wt})} \times 100 = \%\ \text{Thioglucoside}$$

Silver Complexing

This method is based upon the reaction of silver with thioglucosides according to the Schmid and Karrer (5) reaction. Ten grams of meal were enzyme deactivated by dropping into 250 ml of boiling water for 5 min. The enzyme deactivation step should not continue longer than 5 min to avoid chemical breakdown of the thioglucosides. After enzyme deactivation the solution was filtered on a Buchner funnel. The cake was slurried three times in 50 ml of hot water and filtered each time in the same manner. The combined washes and initial filtrate were then made up to 500 ml. From this a 25-ml aliquot was added to a 100-ml flask containing 10 ml of 0.1N $AgNO_3$ solu-

TABLE I
Thioglucoside Content as Percent of Defatted Dry Meal

Crambe meal sample	Methods[a]			
	Sulfate ion	Sulfur balance	Silver complex	Water extraction
1	10.9	11.1	11.3	11.1
2	10.5	11.5	10.3	11.1
3	10.8	11.4	11.1	11.5
4	12.2	12.1	11.5	12.1
Mean	11.1	11.5	11.1	11.5

[a] Standard deviation = 0.318.

tion and 25 ml of 95% ethanol. The solution was refluxed 45 min on a water bath equipped with a 2-ft long air condenser, it was cooled, made up to 100 ml with distilled water, and centrifuged. The unreacted silver was then determined volumetrically by the Volhard method. By this method a 25-ml aliquot of the centrifugate was added to a 125-ml flask containing 2 ml of 6N nitric acid and 6 ml of 8% ferric ammonium sulfate solution, and the homogenous mixture was titrated to a pale salmon color with a solution of 0.01M potassium thiocyanate. A blank was run with each determination. The following equation yields the percentage of thioglucoside:

$$\frac{(\text{Blank-titration})\ (4)\ (0.01)\ (\text{M wt thioglucoside})\ (\text{Total volume})}{(1000)\ (2)\ (\text{Sample wt})\ (25)} \times 100 = \%\ \text{Thioglucoside}$$

Water Extraction

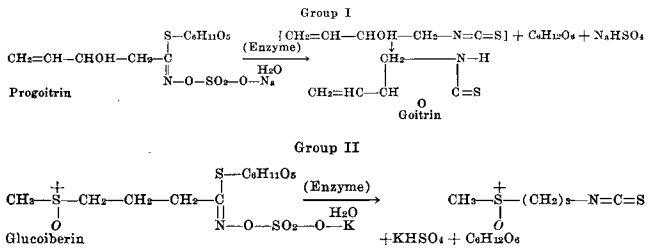
Fifty grams of air-dried defatted flakes were added to 250 ml of boiling water and boiled for 5 min to deactivate the natural enzymes. Then the temperature was reduced to 80C and held for 20 min. After the hot solution was filtered on a Buchner funnel, the residue meal was extracted four additional times, with filtering after each extraction, and dried 1.5 hr in a forced draft oven at 80C. One gram of the meal was analyzed for sulfur by the oxidation method previously mentioned. From the weight of the meal before and after extraction and the total sulfur values, the grams of sulfur extracted by hot water can be calculated and substituted into the following expression.

$$\frac{(\text{Grams of sulfur extracted})\ (\text{M wt thioglucoside})}{2\ (\text{A wt sulfur})\ (\text{Sample wt})} \times 100 = \%\ \text{Thioglucoside}$$

Thiooxazolidone content of crambe hydrolyzates was determined by a modification of the Wetter method for simultaneous determination of volatile isothiocyanates and thiooxazolidone. By this modification a disodium phosphate-citric acid buffer system at pH 5.9 replaced the citric acid-sodium hydroxide pH 4.0 buffer system used by Wetter.

Results and Discussion

Results obtained by applying the four methods to crambe are shown in Table I. Mean values for all methods are in the range of 11–12% thioglucoside for the four seed lots analyzed. Standard deviation of the sulfate ion method, which was 0.310, agrees well with the 0.318 standard deviation in all four methods; statistical evaluation of these data shows no significant difference in precision among methods. Variation of thioglucoside content between seed lots is significant at the 1% level. The results are expressed on a weight-percent basis assuming a thioglucoside molecular weight of 411, which is the molecular weight of (R)-2-hydroxy-3-butenyl glucosinolate recently identified by other workers (3) at this laboratory as the major thioglucoside in crambe. We have found that this thioglucoside constitutes at least 75% of the total and that an additional 5% can be attributed to precursors of volatile isothiocyanates having molecular weights in the same range. The major thioglucoside was calculated from the quantity of thiooxazolidone released by the natural enzyme at pH 5.9 by a modified Wetter procedure. The quantity of thiooxazolidone obtained at pH 5.9 is more than 2.5 times the amount obtained with the standard Wetter procedure at pH 4.0. The increase apparently results from minimizing side reactions that can occur during conversion and cyclization. If other changes were made in the analytical procedure even more of the major thioglucoside in crambe may be demonstrated. Application of the sulfur balance and water-extraction results requires an assumption concerning the moles of sulfur in the aglycone of the thioglucoside. Two groups of thioglucosides were identified on the basis of their sulfur content: those containing 1 mole of sulfur and those containing 2 moles of sulfate *released* in conversion (2). Examples are:



The predominate thioglucoside in crambe was determined by a sulfur balance of the equation for group I. The data derived from sulfate ion, amino acid, and total sulfur analyses show that the thioglucosides of crambe are like group I containing 1 mole of aglycone sulfur per mole of sulfate. This structure is consistent with the recent identification by Daxenbichler et al. (3).

The four methods should be useful in determining the thioglucoside content of other Cruciferae as well. Certain sources of error unique to each method should be considered in such application. For example, coprecipitation of carbonate or protein matter in the sulfate ion method could give high thioglucoside results. Vegetable proteins are coagulable by heating; consequently if intensive heating occurs during the initial extraction period, the filtrate should be relatively free of protein. In analyzing new oilseeds by the sulfur balance method, the presence of sulfur other than amino acids or thioglucosides could give high results. Application of this method also requires a knowledge of the number of moles of sulfur per mole of thioglucoside. This number can readily be determined from a sulfur balance as illustrated earlier with crambe (Table II). The sulfur balance method requires an amino acid analysis. In many cases the general amino acid content of the seed under study is known, or a single sample can be run, and the same amino acid content assumed for all other samples of the same seed species. Such an assumption was made for our work on crambe. Amino acid analyses were obtained on only one seed lot, and the same content of sulfur contained in amino acids per gram of nitrogen was assumed for all other samples. Where this

TABLE II
Crambe Meal Sulfur Balance

Method	Sulfur content g/100 g moisture and fat-free basis
Sulfate sulfur[a]	0.95
Isothiocyanate sulfur	0.95
Amino acid sulfur	0.54
Total sulfur by summation	2.44
Total sulfur by analysis	2.44

[a] Based on an average of five analyses of a single seed lot.

assumption is made, only nitrogen values need be obtained for most samples.

Silver complexing should be applicable to new oilseeds although recognition of the end point requires some practice to achieve the desired accuracy. This analytical procedure could become further complicated if color bodies unique to a certain oilseed are present. In addition, the stability of thioglucosides in boiling water is not known and may differ according to type. A short initial boiling period is necessary in the thioglucoside isolation step of this method to inactivate enzymes, but boiling should not continue beyond 5 min to avoid a breakdown of thioglucosides.

The water-extraction method is also subject to error if thioglucosides are broken down during extraction. In its application to oilseeds other than crambe, the presence of water-soluble sulfur compounds other than thioglucosides is a source of error. Such compounds include water-soluble peptides or proteins containing significant quantities of sulfur amino acids. As with the sulfate ion method, the solution should be thoroughly boiled to insolubilize the protein.

With these precautions in mind, the methods outlined should be useful in determining the thioglucoside content of members of the Cruciferae. The sulfate ion and silver complex methods are particularly well suited to screening programs where a large number and variety of thioglucosides may be encountered.

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Oil and Protein Content, and Oil Composition of the Seeds of Some Plants of the Canadian Prairies

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Abstract

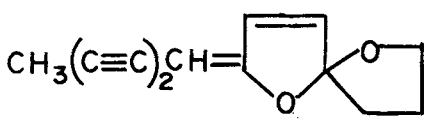
The oil and protein content are reported for the seeds of 19 plant species selected for their possible crop potential for the Canadian prairie region. Data on seed oil composition are reported for the 12 species which contained greater than 15% seed oil.

Introduction

THE SEEDS OF A NUMBER of plant species, mostly uncultivated plants of the Canadian prairie region, were investigated for oil and protein content. The composition of the oil was also determined for species with seeds containing more than 15% oil. Only species which were believed to have relatively high yields of seed, which held their seed well and which appeared reasonably easy to harvest, were examined. The combination of high yields of seeds, high content of useful oils in the seeds and recognizable hardiness in the plants are attributes which should enhance the potential for field crop consideration and possible development. Familiarity with the composition of the oils and with the oil content of wild plant seeds might lead also to by-product utilization of such seeds separated from more orthodox seed production during harvesting and cleaning operations.

Previous investigations, notably those of Earle and his associates (1) (2), have included data on many plant species of the Canadian prairie region. The present investigation has sought to supplement the literature with respect to species not previously studied, as well as to contribute information where data on particular species appeared incomplete.

Of the 19 plant species investigated, 12 were found to contain sufficient seed oil to warrant examination of its composition. As judged by the spectroscopic and GLC retention data, the major fatty acids of most of these oils were the usual C_{16}, C_{18} and C_{20} fatty acids, and only small amounts of epoxy, hydroxy or conjugated dienoid fatty acids were detected. The seed oil of *Hackelia americanum* (Boraginaceae family) contained substantial amounts of the $\Delta^{6,9,12}C_{18}$ trienoic and the $\Delta^{6,9,12,15}C_{18}$ tetraenoic fatty acids. These have already been reported by Craig and Bhatty (4) and by Kleiman et al. (5) to be present in the seed oil of many species of the Boraginaceae family. Both the seed and the other above ground parts of *Artemisia biennis* were found to contain significant amounts of the heterocyclic polyyne I, tentatively identified on the basis of spectroscopic evidence. This particular polyyne has already been isolated from two *Matricaria* species by Bohlmann and his associates (6).



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Materials and Methods

Seeds of *Artemisia biennis, Hackelia americanum, Atriplex hortensis, Axyris amaranthoides* and *Rumex fennicus* were obtained from plants collected near Saskatoon during the fall and winter of 1963 and the fall of 1964. Seeds of *Gypsophila paniculata, Saponaria vaccaria, Chenopodium rubrum, Chenopodium hybridum* var. *gigantospermum, Galeopsis tetrahit, Moldavica parviflora* (*Dracocephalum parviflorum*), and *Lappula echinata* were supplied from the weed seed collection, Plant Ecology Department, University of Saskatchewan. The seeds of all the other species investigated were supplied by commercial seed houses. In the case of *Coreopsis tinctoria,* commercial seed