Gums Separated from *Crotalaria intemerdia* and Other Leguminous Seeds by Dry Milling

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Galactomannan gums were separated by a dry-milling process from the leguminous seeds *Crotalaria intermedia, Cyamopsis tetragonoloba* (guar), and *Cassia marilandica*. The process included impact grinding, air-classification, and screening to separate coarse endosperm particles; then flaking and grinding of the endosperm to flour. The endosperm material from *Crotalaria intermedia* was heat-treated to stabilize the viscosity of aqueous dispersions. Yields of good quality gum from each of the three species amounted to over 20% of the original seed weight. Crotalaria gum may be dispersed in either cold or hot water to form viscous solutions that are stable from pH 5 to pH 9. The gum should be useful as a dispersing, stabilizing, or thickening agent. Laboratory experiments indicated its potential for use as a wet-end additive to increase strength properties of paper.

GALACTOMANNAN GUMS from leguminous seeds are useful for many industrial purposes such as sizing, thickening, and stabilizing agents. Gum from the locust bean (*Ceratonia siliqua*), one of the earliest materials used for these purposes, must be imported from the Mediterranean area. Guar gum (from *Cyamopsis tetragonoloba*) is largely imported from Asia. The recent introduction of guar into southwestern United States as a secondary crop provides a domestic source of galactomannan gum which currently supplies about 10% of the total U. S. consumption of guar gum.

During the past several years, USDA scientists have been investigating numerous uncultivated plant species as potential new crops. They have found that *Crotalaria intermedia* and *Cassia marilandica*, herbaceous plants of the legume family, yield seed that may be used as a raw material source for galactomannan gum (*11*). Several species of the genus *Crotalaria* (*C. sagittalis* and *C. spectabilis*) bear seeds that are highly poisonous because of their alkaloid content; however, the gum (endosperm) portion of *C. intermedia* seed is largely free of such toxic alkaloids. Industrial exploitation of *C. intermedia* as a source of polysaccharide gum will depend in part upon the acquisition of more complete toxicity data and an evaluation of the implications of this toxicity in agricultural practice, as well as the practicality either of restricting the gum to nonfood uses or demonstrating it to be acceptable in foods.

Industrial separation of guar gum may be accomplished by flaming the seeds to loosen the seedcoat, removing it by scouring or pearling, then milling and sifting to separate the gum from the germ (*4, 13*). The separated gum or endosperm particles are then tempered to 45% moisture, flaked on smooth rolls to disrupt cellular structure, and ground to flour in a hammer mill or similar grinder (*4*). Other methods suggested for recovering gum from leguminous seeds include: soaking in boiling dilute alkali, washing, and screening (*4*); soaking in boiling water, rubbing, and picking (*2*); soaking in water or borax solution, screening, drying, grinding, and screening (*8, 9*); soaking in aqueous solution of sulfuric acid containing glycerol, freezing, grinding, and screening (*5*); soaking in aqueous alcohol, boric acid, or alum, and separating the seedcoat (*6*); and extracting the whole seed with boiling water, filtering, and evaporating (*12*).

This paper describes the separation of galactomannan gums from *Crotalaria intermedia, Cyamopsis tetragonoloba* (guar), and *Cassia marilandica* by a dry-milling process that involves impact milling, air-classification, and screening. The dry-milling method affords a convenient means for preparing refined endosperm (gum) in sufficient quantities for testing in industrial applications. Properties of the crotalaria gum produced by heat treatment, flaking, and grinding of isolated endosperm are reported, together with certain properties of the cassia gum.

Experimental

Materials. *Crotalaria intermedia* and *Cassia marilandica* seeds were obtained through the cooperation of Quentin Jones, New Crops Research Branch, USDA. Guar seed was obtained through the courtesy of John Esser of General Mills, Inc. The seeds were cleaned and aspirated on a Federal dockage tester before processing.

Equipment. The whole seeds were ground in an Alpine 160Z Kolloplex pin mill operated at 18,000 r.p.m., with a feed rate of about 50 pounds per hour. The ground seeds were air-classified in a Pillsbury air-classifier, laboratory Model No. 1, operated at a feed rate of about 50 pounds per hour.

Screening was carried out in a standard Ro-Tap sifter. Endosperm fractions were aspirated in a Bates laboratory aspirator to remove small amounts of remaining seedcoat. Endosperm fragments were flaked in a set of 12-inch diameter × 6-inch length Wolf smooth rolls set at <0.003-inch clearance. Flaked endosperm was ground to flour finer than 100-mesh in a Raymond laboratory hammer mill equipped with 0.017-inch screens.

Procedures. Fifty-pound batches of whole seed were ground by one pass through the pin mill at 18,000 r.p.m. The ground seed was passed through the classifier at a cut point of about 35 microns. The residue was then passed again through the classifier at progressively higher cut points of about 45, 90, 100, and 110 microns to yield five fine fractions and a coarse fraction. The coarse fraction was screened on 40-, 50-, and 60-mesh screens to give a total of nine fractions of progressively increasing particle size. Fractions 8 and 9, respectively, from each seed were aspirated lightly in the Bates aspirator to remove dust and small amounts of seedcoat still present in these endosperm fractions. Thin layers (1/4 inch) of the coarse endosperm (Fraction 9) of *Crotalaria intermedia* were steamed 20 minutes at

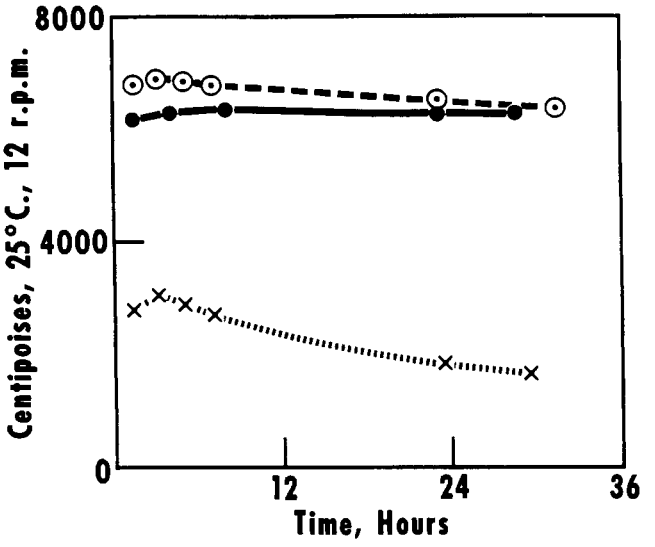

Figure 1. Viscosity of 1.0% solutions of *Crotalaria intermedia* endosperm flour after various treatments

Gum was suspended by 5 minutes of agitation at 80° C. and allowed to cool to room temperature
×- - ×, no treatment; ⊙——⊙, tempered and flaked before grinding; ●——●, steamed, tempered, and flaked before grinding

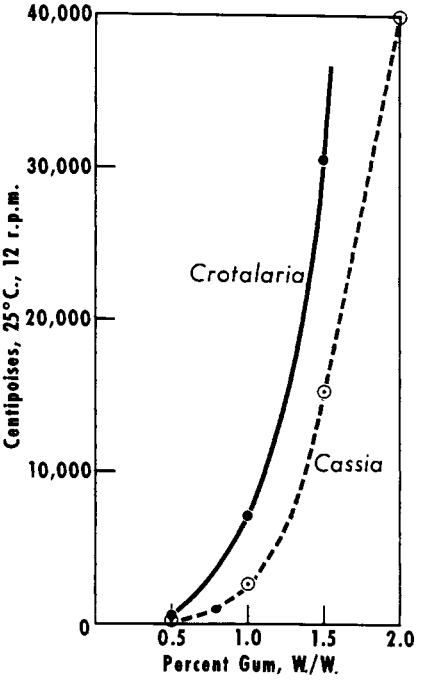

Figure 3. Viscosity *vs.* concentration curves for crotalaria and cassia gums

Gums were suspended at 80° C.; viscosity was measured after 16 to 20 hours

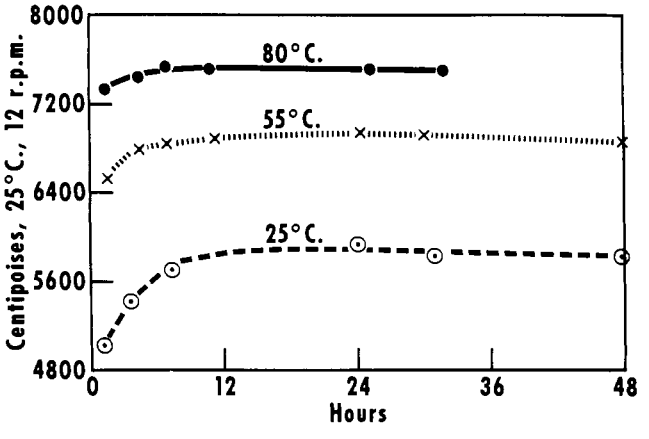

Figure 2. Viscosity stability of 1.0% crotalaria gum dispersions, prepared at different temperatures

Gum was suspended at indicated temperatures and cooled before measurement

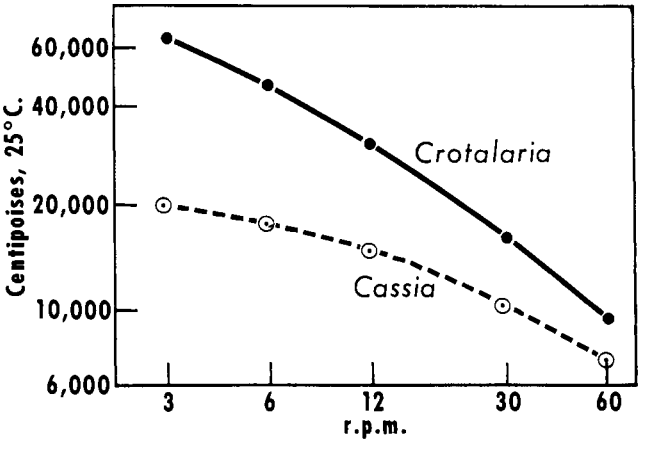

Figure 4. Viscosity changes of 1.5% crotalaria and cassia gum dispersions with changes of viscometer rotor speed

Gums were suspended at 80° C.; viscosity was measured after 16 to 20 hours

Table I. Fractionation of Ground Leguminous Seeds

Sample	*Crotalaria intermedia* Protein, %	*Crotalaria intermedia* Acid insolubles, %	*Crotalaria intermedia* Per cent of seed	*Guar* Protein, %	*Guar* Acid insolubles, %	*Guar* Per cent of seed	*Cassia marilandica* Protein, %	*Cassia marilandica* Acid insolubles, %	*Cassia marilandica* Per cent of seed
Ground seed	30.2	21.5	...	27.8	18.8	...	20.5	22.8	...
Fraction 1	58.1	18.8	22.7	57.4	16.1	26.0	46.8	20.3	22.0
2	58.8	17.6	7.3	53.0	16.7	10.2	37.9	21.0	8.4
3	45.9	27.6	6.4	36.5	20.9	7.0	20.6	26.1	6.3
4	39.6	27.0	3.2	30.3	25.1	3.5	19.2	31.0	4.1
5	36.8	30.3	1.9	28.2	26.9	2.5	16.2	34.2	3.2
6 (−60 mesh)	26.3	40.8	30.2	21.1	30.6	25.6	9.2	41.3	22.8
7 (+60 mesh)	14.9	35.1	2.8	10.9	13.0	1.7	6.8	32.2	2.3
8 (+50 mesh)	8.0	10.1	5.1	5.4	9.1	2.2	9.1	16.0	1.9
9 (+40 mesh)	4.5	3.2	17.7	3.6	3.0	18.0	8.4	6.5	27.5
Aspirated from Fraction 8	10.0	17.1	2.0	9.5	10.2	2.1	8.5	22.1	1.1
Aspirated from Fraction 9	6.5	6.2	0.7	7.0	5.0	1.2	7.9	19.0	0.4

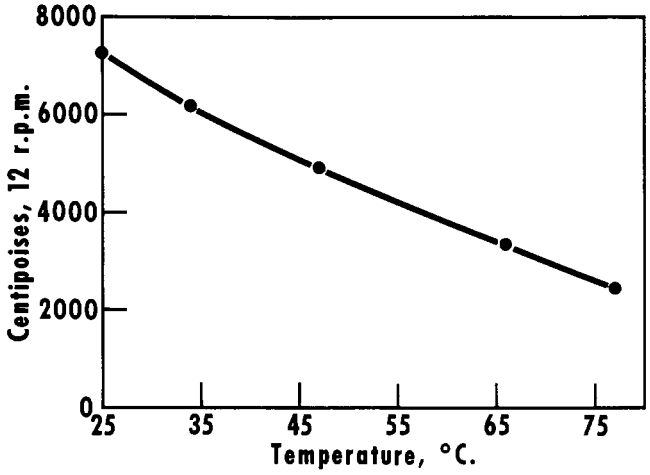

Figure 5. Viscosity vs. temperature curve for 1.0% crotalaria gum dispersion

Gum was suspended at 80° C.; viscosity measured after 16 to 20 hours

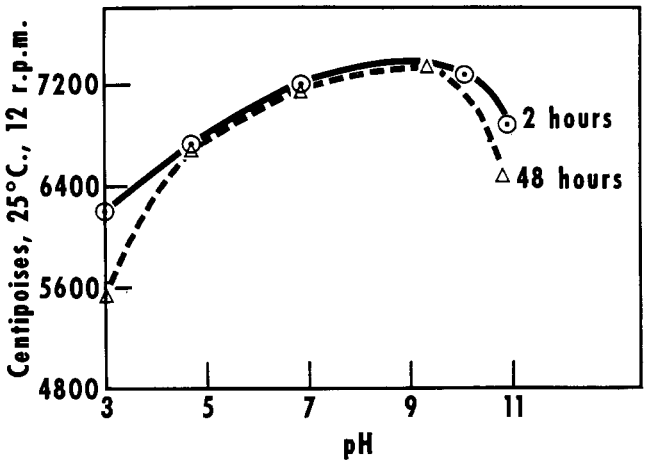

Figure 6. Viscosity stability of 1.0% crotalaria gum dispersions at different pH values

Gum was suspended at 80° C.

Table II. Handpicked Fractions of *Crotalaria intermedia*, Guar, and *Cassia marilandica* Seed

Seed	Seed Coat	Endosperm	Germ
Crotalaria			
Yield, %	22.2	37.2	40.6
Protein, %	6.8	4.5	71.8
Acid insolubles, %	63.2	3.2	18.8
Guar			
Yield, %	17.1	39.4	43.5
Protein, %	7.6	3.6	65.4
Acid insolubles, %	59.2	3.3	18.2
Cassia			
Yield, %	27.2	46.8	26.0
Protein, %	6.0	8.4	62.3
Acid insolubles, %	45.5	6.5	21.1

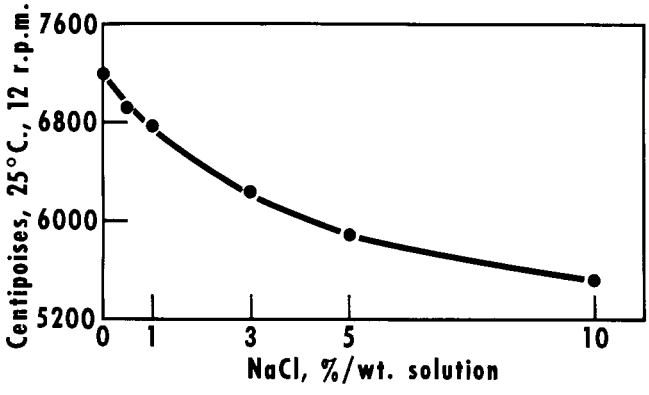

Figure 7. Viscosity changes of 1.0% crotalaria gum dispersion with added sodium chloride

Gum was suspended at 80° C.; viscosity measured after 16 to 20 hours

Table III. Seed Components in Fractions

	Crotalaria intermedia				*Guar*				*Cassia marilandica*			
	Per cent endosperm in fraction	Per Cent of Original Seed			Per cent endosperm in fraction	Per Cent of Original Seed			Per cent endosperm in fraction	Per Cent of Original Seed		
Fraction		Seed-coat	Endosperm	Germ		Seed-coat	Endosperm	Germ		Seed-coat	Endosperm	Germ
1	15.2	1.2	3.4	18.1	12.9	...	3.3	22.7	19.8	1.9	4.3	15.8
2	16.4	0.2	1.2	5.9	17.5	0.3	1.8	8.1	28.1	1.4	2.3	4.7
3	14.7	1.6	0.9	3.9	30.3	1.2	2.1	3.7	34.4	2.6	2.2	1.5
4	22.4	0.8	0.7	1.7	30.6	1.0	1.1	1.4	23.1	2.3	0.9	0.9
5	20.1	0.6	0.4	0.9	30.1	0.8	0.7	1.0	18.1	2.1	0.5	0.6
6	14.7	16.6	4.4	9.2	32.4	10.8	8.2	6.6	7.4	19.9	1.7	1.2
7	36.7	1.4	1.0	0.4	74.6	0.2	1.3	0.2	34.1	1.5	0.8	...
8	85.0	0.5	4.4	0.2	88.0	0.2	1.9	0.1	74.5	0.4	1.4	0.1
9	100.0	...	17.7	...	100.0	...	18.0	...	100.0	...	27.5	...
Aspirated from Fraction 8	73.0	0.4	1.5	0.1	80.7	0.3	1.7	0.1	58.0	0.5	0.6	...
Aspirated from Fraction 9	92.0	0.1	0.6	...	93.0	...	1.1	0.1	67.5	0.1	0.3	...
Total		23.4	36.2	40.4		14.8	41.2	44.0		32.7	42.5	24.8
Handpicked fractions		22.2	37.2	40.6		17.1	39.4	43.5		27.2	46.8	26.0

atmospheric pressure to stabilize the viscosity of aqueous solutions. The coarse endosperm fractions from all three species were tempered to 37.5% moisture (*4*), flaked by passage through smooth rolls, dried, and ground to a flour. The final products all had moisture contents of about 10%.

Small amounts of each whole seed were dissected and separated into germ, seedcoat, and endosperm fractions to determine approximate yields of these components and to furnish samples for chemical analyses needed in calculating component composition of each fraction.

Viscosity data for crotalaria and cassia gums were obtained with a Brookfield Model LVT viscometer.

Methods of Analysis. Moisture in the samples was determined by measuring the weight loss when 10-gram samples were heated at 130° C. for 30 minutes in a forced circulation oven. Protein (N × 6.25), ash, and fat were determined by Cereal Laboratory Methods 67.1, 9.1, and 31.5, respectively (*1*). Acid-insoluble material was determined by digesting 2 grams of sample on a steam bath for 6 hours in 150 ml. of a dilute sulfuric acid solution (25 ml. of concentrated sulfuric acid added to 2500 ml. of distilled water), maintaining volume during digestion with hot distilled water, filtering with the aid of 0.500 gram of dried Celite added to the solution, washing with hot distilled water, drying, and weighing. Results are reported on a dry-matter basis.

Results

Table I lists the analyses of the various seed fractions and the yield of each obtained from *Crotalaria intermedia*, guar, and *Cassia marilandica*. Table II lists the analyses and yields of the hand-picked fractions obtained from each seed. Analytical values from the hand-picked fractions were used to calculate (by means of simultaneous equations) the percentages of seedcoat, endosperm, and germ present in each fraction based on original seed. These figures are shown in Table III.

Effects of processing steps prior to grinding the endosperm (Fraction 9) of *Crotalaria intermedia* on the viscosity of gum solutions are shown in Figure 1. Viscosity curves obtained with processed crotalaria and cassia endosperm flours, similarly tempered and flaked, are shown in Figures 2 to 7. In Figure 2, viscosity of crotalaria gum dispersed at various temperatures is shown. Figure 3 shows the viscosity *vs.* concentration curves for both gums. Figure 4 illustrates viscosities of 1.5% gum dispersions at various speeds of the viscometer rotor. The variation of viscosity of crotalaria gum solutions with temperature appears in Figure 5; the stability of such solutions at various pH values in Figure 6, where the pH adjustment was made with either HCl or NaOH. Figure 7 shows how the viscosity of a 1.0% dispersion changes with added salt.

Table IV. Yields of Good Quality Gum Obtained by Dry-Milling Process

Gum	Combination of Fractions	Yield, % of Seed	Protein, %	Acid Insolubles, %	Fat, %	Ash, %
Crotalaria	8,9,9 Aspirate	23.5	5.3	4.8	0.4	1.7
Guar	8,9,8 and 9 Aspirates	23.5	4.5	4.3	0.2	0.7
Cassia	9	27.5	8.4	6.5	0.8	0.9

Discussion

As shown in Tables I through III. Fraction 9, the +40 mesh fraction, is relatively pure endosperm (gum), with little seedcoat or germ. Yields of good quality gum obtained by this dry-milling process are shown in Table IV.

Table III shows endosperm scattered throughout all fractions, with appreciable amounts in Fractions 1 and 6 from *Crotalaria intermedia* and guar, and in Fraction 1 from *Cassia marilandica*. Additional amounts of gum might be recovered from these fractions. Complete separation of seedcoat before impact milling by such means as soaking might permit recovery of more gum by requiring milder grinding and by permitting inclusion of more of the fractions in the gum portion. These refinements were not investigated since the purpose of this work was to indicate the general applicability of the processing procedure and to provide quantities of relatively pure gum from these seeds for use-testing.

Processing costs for producing stabilized and ground gum by the entire process described have been estimated at approximately $0.50 per hundred pounds of seed processed, in a plant handling 4000 pounds of seed per hour (20,000,000 pounds of seed per year). This cost includes all operating expenses and fixed charges, but does not include administrative and selling expenses. No credit is taken for any by-product hull or germ fractions.

A crotalaria gum solution prepared from endosperm (+40 mesh fraction), which had been ground without tempering or heat treatment, has a low initial viscosity which rapidly declines with time (Figure 1). Tempering and flaking of the endosperm yield a product with high viscosity. Steam treatment stabilizes this gain in viscosity (Figure 1). The loss in viscosity is thought to be caused by enzymatic degradation and may be partially prevented by lowering the pH below 4 or by treatment with citric acid at pH 5 (*7*), though ethylenediaminetetraacetic acid is not effective at this pH.

The steamed and tempered crotalaria gum may be dispersed in either cold or hot water to form stable, viscous solutions.

Table V. Rat Feeding Tests of *Crotalaria intermedia* Gum

Diet	Average Body Weight (Grams) Initial	After 35 days
Basal control[a]	39.2	171.8
+10% crotalaria gum	39.4	136.0
+10% guar gum	39.2	163.4
+10% alpha-cellulose	39.2	161.6

[a] 73% yellow corn meal, 10% crude casein, 10% linseed oil cake meal, 3% USP cod liver oil, 2% dehydrated alfalfa meal, 1.5% bone ash, and 0.5% NaCl.

As shown in Figure 2, suspension of the mucilage in hot water yields a higher viscosity at 25° C. than does suspension in cold water. The gum dispersions are stable from pH 5 to pH 9 (Figure 6)—outside this range the solutions lose viscosity on aging. The plot of viscosity *vs.* viscometer rotor speed (Figure 4) shows shear-thinning properties.

Crotalaria gum has a solution viscosity comparable to similarly prepared guar gum—approximately 7200 centipoises for crotalaria (Figure 3) and 6800 centipoises for guar in 1% solutions.

The gum product from *Cassia marilandica* seed forms stable solutions which are not as viscous as crotalaria gum (Figure 3), but are similar, and show shear-thinning properties as do other galactomannan gums (Figure 4).

Added salt moderately lowers the viscosity of the 1.0% crotalaria dispersion (Figure 7). A 1.0% dispersion is gelled by the addition of 100 mg. of borax per 100 ml. of solution. As little as 10 mg. of borax will form a soft gel (viscosity approximately 25,000 centipoises) if the pH is adjusted to be slightly alkaline.

Crotalaria gum may be useful where a dispersing or thickening agent is desired. Laboratory experiments indicate its potential as a superior material for use as a wet-end additive to increase strength properties of papers (*10*).

Several species of the *Crotalaria* genus (*C. sagittalis* and *C. spectabilis*) are highly poisonous because of alkaloid content. *C. intermedia* apparently has a lower degree of toxicity for rats than *C. spectabilis*. As little as 0.25% *C. spectabilis* seed in the diet causes 100% mortality in 2 weeks (*3*), whereas the authors have found that 100% mortality is not reached until 25 days on a diet containing 20.0% *C. intermedia* seed. *C.*

intermedia alkaloids were found localized in the germ or seedcoat rather than in the gum portion of the seed. Preliminary feeding tests of the gum indicate no short-term toxicity for rats other than retarded growth (Table V).

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PEANUT FLOUR CONSTITUENTS

Isolation and Identification of Pinitol from Peanut Flour

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Pinitol, a monomethyl ether of D-inositol has been isolated from peanut flour. Its identity was proved by elemental analysis, molecular weight determination, and comparison of the infrared spectra of the compound and of its derivative, diisopropylidene pinitol, with the infrared spectra of authentic samples of these substances.

A SYSTEMATIC INVESTIGATION of the minor constituents in peanuts is being made at this laboratory (*2*), the primary interest being in those that contribute to flavor and aroma. Many of the precursors to flavor factors are concentrated in the alcohol-soluble portion of defatted peanut flour; this extract represents about 3.5% of the blanched peanut. The present emphasis is on the isolation of constituents found in the water-soluble fraction of the alcohol extract, and this report deals with the isolation and identification of pinitol, a monomethyl ether of D-inositol, the structure of which has been established by Anderson (*1*).

Experimental

The fractionation procedure used in obtaining the alcohol-soluble fraction (D) in Figure 1 was the same as that used by Morris and Lee (*2*). This lyophilized material was extracted thoroughly with absolute ethyl alcohol; after removal of the solvent and lyophilization, a product (F) was obtained which represented 1.3% of the blanched peanut. Fifty-gram batches of the extract (F) were then refluxed with acetone on a steam bath. The acetone solution was decanted and filtered. More acetone was added and the procedure repeated until no residue remained on evaporation of the decanted solvent. After drying by lyophilization, this acetone-soluble portion (H) was shaken with chloroform and water. Three layers formed in the separatory funnel: a clear water-soluble layer, a clear chloroform-soluble layer, and an emulsion of the two at the interface. Heating on a steam bath followed by centrifuging broke this emulsion so that all of the material was water-soluble or chloroform-soluble. The water-soluble fraction (J) was washed with 1-butanol, and the resulting 1-butanol-insoluble fraction (J-w) represented 0.2% of the peanut. This fraction was the starting material for chromatographic separation.

Four grams of J-w (Figure 1) were placed on a column (9 cm. diam. × 40 cm.) of cellulose powder, and the column was developed with a mixture of 1-butanol–acetone–water (2:2:1). One hundred fractions, 70 ml. each, were collected, and J-w was resolved into four peaks by this procedure, as indicated by the curve obtained by plotting the weight of material eluted against the volume of eluant. These are Peaks I, II, III, and IV (Figure 1).

Crystals formed as the solvent was evaporated from fractions comprising Peak II. These fractions (Peak II) were combined and rechromatographed on the same column with the same developing solvent. Fractions in which crystals again formed represented 0.021% of the blanched peanut (II-B, Figure 1). The noncrystalline material in these fractions was dissolved in methanol and decanted. The crystals (Figure 2) remaining amounted to approximately 5.4% of II-B or 0.001% of the peanut.

The crystals (m.p. 187–188° C.) $[\alpha]\ D^{25} + 65.7°$ ($C=2$ in H_2O) had a sweet taste yet gave a negative Molisch test. Tests for acidic and ester groups were also negative. Qualitative elemental tests showed the absence of nitrogen, sulfur, phosphorus, magnesium, calcium, or halogen. Analysis: C, 43.36; H, 7.33; CH_3O, 16.15; mol. wt. 204 (differential vapor pressure osmometer method). Calculated for $C_7H_{14}O_6$: C, 43.29; H, 7.27; CH_3O, 15.98; mol. wt. 194.19.

With this information, tentative identification of the compound was established by reference to the Sadtler Index (*4*) which indicated only one compound with such a formula and melting point—i.e., pinitol, a monomethyl ether of