square of the concentration except near the melting point or when M_w is small indicates that, with the latter exceptions, the ratio of useless to total bonds is independent of concentration. Either $G \propto (n_t - n_a)$, as would be expected for rubberlike elasticity, and $n_t \propto c^2$, following a binary association; or $G \propto (n_t - n_a)^2$, which might correspond to a stiff strutted structure, and $n_t \propto c$, a peculiar type of association recently postulated by Doty for polyvinyl chloride solutions.[20] At present it is not possible to distinguish between these two alternatives.

Decrease in rigidity with increasing temperature must be due primarily to decrease in n_t. At the same time, the change is probably enhanced, at least near the melting point, by an increase in n_a, since the contributions to n_a from loose ends remain constant and those in the sol fraction should increase.

The decrease in rigidity with decreasing average molecular weight is clearly not due solely to the increase in loose ends, since in this case the total number of bonds should be constant and either G or $\sqrt{G}$ should be a linear function[19] of $1/M_n$. The total number of bonds evidently varies with average molecular weight, and with molecular weight distribution. Further work will be needed to explain the form of the empirical relationship given in equation (1).

(20) P. Doty, H. Wagner and S. Singer, *J. Phys. Colloid Chem.*, **51**, 32 (1947).

Summary

1. The rigidity of a gelatin gel at a given temperature reaches a constant value more rapidly if the temperature is approached from below than from above.

2. For a sample of slight degradation (M_n = 45,000), the rigidity was closely proportional to the square of the concentration up to 60 g./l. At higher concentrations, it increased somewhat less rapidly; for a sample of higher degradation, somewhat more rapidly, than with the square of the concentration.

3. For all samples and at all concentrations, the rigidity decreased gradually with increasing temperature from 0° to the melting point.

4. The rigidity decreased markedly with increasing degradation, or decreasing average molecular weight.

5. An empirical equation for the dependence of rigidity on temperature and weight average molecular weight is given.

BOSTON, MASS. RECEIVED SEPTEMBER 30, 1947

[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL CHEMISTRY, PURDUE UNIVERSITY AGRICULTURAL EXPERIMENT STATION]

Chemical Composition and Properties of Guar Polysaccharides[1,2]

BY EILEEN HEYNE AND ROY L. WHISTLER

Endosperm of the guar seed consists principally of a galactomannan polysaccharide. It is thus analogous to the endosperm of locust beans which are widely used in commerce, and to galactomannans from other sources. Guar, a drouth-resistant legume of the genus *Cyamopsis*, is native to India where it is used for food and feed. The endosperm can be employed industrially in many ways, such as a size for paper and textiles, a dispersing agent, and a thickener. These uses have resulted in the recent growing of the guar plant in commercial quantities in the United States. As yet, however, little information is available with regard to the fundamental composition and structure of the endosperm polysaccharides. This report covers a preliminary investigation of the general composition and properties of the guar endosperm and particularly of the water soluble component which constitutes the major portion of the endosperm.

Experimental

Material.—Guar flour, produced by grinding the endosperm of the decorticated guar seed, was obtained through the kindness of General Mills, Inc. The grayish-white flour contained 0.60% nitrogen, 0.06% phosphorus, 1.06% ash and 1.5% ethanol solubles from twenty-four hours of Soxhlet extraction.

Analytical Methods.—Galactose anhydride content was determined by the following procedure: One gram of polysaccharide material ground to pass a 60-mesh sieve was dissolved in 150 ml. of 5% nitric acid by heating the mixture to 100°. After hydrolysis for three and one-half hours at this temperature, the solution was concentrated on a steam-bath to a volume of 25 ml. and 5.6 ml. of concentrated nitric acid was added to make a solution concentration of 25%. The solution was then oxidized according to the methods proposed by Tollens,[3] Van der Haar,[4] and Wise and Peterson.[5] The weight of the solution was reduced to 20 g. by heating on a steam-bath. Because of the high galactose content of the preparations, it was not found necessary to add the 500 mg. of pure mucic acid recommended by earlier works. The crystallization of mucic acid was allowed to proceed for forty-eight hours at a temperature of 0° ± 0.1° obtained with a large constant temperature bath. The solubility of mucic acid at 0° is 0.0175 g. per 100 ml. The amount of galactose anhydride equivalent to mucic acid was found by multiplying the weight of mucic acid obtained in this procedure by the factor 1.33. This factor was computed from data obtained on the analysis of a mixture of galactose and mannose combined in such proportions as to give the same yield of mucic acid as the guar samples.

(1) Journal Paper No. 319 of the Purdue University Agricultural Experiment Station.

(2) Paper presented before the Division of Sugar Chemistry and Technology at the 111th meeting of the American Chemical Society, Atlantic City, 1947.

(3) B. Tollens, *Ann.*, **227**, 223 (1885); **232**, 187 (1886).

(4) A. W. Van der Haar, *Biochem. Z.*, **81**, 263 (1917).

(5) L. E. Wise and F. C. Peterson, *Ind. Eng. Chem.*, **22**, 362 (1930).

Mannose anhydride content was determined through isolation of mannose phenylhydrazone by Nowotnowna's[6] modification of Schorger's[7] and Tollens'[8] procedure.

Both mannose and galactose are of the D-series as evidenced by the optical rotations of their derivatives. Mannose phenylhydrazone from guar showed the same optical mutarotation as the derivative of D-mannose[9] changing from $[\alpha]^{25}D$ +21 (1 hour) → −3 (33 hours) → +35 (216 hours) (*c*, 1 in pyridine). Melting point and mixed melting point with D-mannose phenylhydrazone were 182.5°. The benzimidazole of galactose prepared from guar hydrolyzate after D-mannose had been removed as the phenylhydrazone agreed with the known rotation of D-galactose benzimidazole[10] $[\alpha]^{25}D$ +43.3 (*c*, 0.5 in 5% citric acid).

Pentosans were determined by isolation of the furfural-phloroglucinol complex.[11]

Viscosities were determined at approximately 0.2% concentrations in 1 *N* sodium hydroxide solution at 25°. Solution of the sample was obtained by shaking it overnight in an atmosphere of nitrogen. Viscosity measurements were made twenty hours after the addition of alkali.

Water Fractionation.—An 0.8% aqueous suspension of guar flour was prepared by sprinkling 4-g. portions of the flour into 500-ml. portions of distilled water stirred in a Waring Blendor and by repeating this procedure until 8 liters were obtained. The viscous dispersion was autoclaved at 15 pounds pressure for three hours (*p*H 5.8) and immediately centrifuged in a supercentrifuge (40,000 r. p. m.) to remove the insoluble component. Longer periods of autoclaving did not appreciably change the ratio of soluble to insoluble fractions. The insoluble component was gradually added to ethanol stirred in a Waring Blendor, filtered, and stirred three further times with fresh portions of ethanol in the Blendor. After the final washing and filtration, the brown flocculent precipitate was dried over calcium chloride in a vacuum desiccator. The residue represented approximately 7.8% of guar flour.

The soluble component was recovered by adding an equal volume of ethanol with rapid stirring to the centrifugate; preferably the ethanol was added to small portions of solution stirred in a Waring Blendor. Precipitated material was filtered and washed four successive times with fresh portions of ethanol in the Blendor. The white fibrous precipitate was dried as described above and represented approximately 86.5% of the guar flour. A small amount of carbohydrate was not recovered by this method of treatment. The isolated material contained 0.15% nitrogen, 0.3% or less pentosan, 35.6% D-galactose anhydride and 63.1% D-mannose anhydride. The absence of glucose was indicated by the fact that after nitric acid oxidation of the hydrolyzate and separation of mucic acid, it was not possible to crystallize potassium acid saccharate from the mother liquor. Neither was it possible to isolate glucose as the benzimidazole from the sugar hydrolyzate. Intrinsic viscosity in 1 *N* sodium hydroxide was 5.57; $[\alpha]^{25}D$ +54.5 (*c*, 1 in 1 *N* sodium hydroxide). No uronic acid was detected by the quantitative method of Whistler, Martin and Harris.[12]

Ethanol Fractionation.—Subfractionation of the water soluble component was accomplished by gradual addition of ethanol to the aqueous centrifugate recovered from the separation of insoluble component. Ethanol was added drop by drop from a separatory funnel to 11 l. of the strongly stirred centrifugate. At the first appearance of precipitate the addition of ethanol was stopped and the mixture was supercentrifuged. The solution was allowed to stand for twenty-four hours to allow any additional precipitate to form. If a precipitate formed, the solution was allowed to stand an additional twenty-four hours. If no precipitate occurred, the solution was stirred and further addition of ethanol was made. In this manner a number of fractions were obtained as shown in Fig. 1.

The first precipitate occurred at an alcohol concentration of 20% by volume and the last at 40%. When the alcohol concentration reached 31%, an additional increase of 2% concentration brought about a slow but continuous precipitation which continued for three days. This precipitate which accumulated from 31 to 33% alcohol concentration represented 58% of the total soluble component. Approximately 93% of the soluble component was precipitated by ethanol. Precipitated fractions were washed through four fresh portions of ethanol and dried as described above.

After separation of the above individual fractions, the solution was concentrated under reduced pressure to 1.5 l. and 0.4 l. of ethanol was added. This procedure precipitated fraction number 15. The centrifugate from the precipitation was concentrated under reduced pressure to 280 ml. and 3 l. of ethanol was added to produce fraction number 16. On concentration of the final centrifugate to dryness on the steam bath, fraction 17 was obtained.

Preparation of Guaran.—Material of uniform chemical composition was separated from the water soluble component by discarding the first 10% of material which precipitated up to an ethanol concentration of 25% and then collecting all material which precipitated up to an ethanol concentration of 40% by volume. While only the first 3–5% of ethanol precipitated material appeared to be different from the central fraction, the first 10% which precipitated was discarded simply as a precaution against possible contamination of the central fraction. This material separating between ethanol concentrations of 25–40% and called guaran contained 34.5% D-galactose anhydride, 63.4% D-mannose anhydride and 0.1% nitrogen. The rotation was $[\alpha]^{25}D$ +53 (*c*, 1 in 1 *N* sodium hydroxide). Guaran is not oxidized by Fehling solution. Addition of small amounts of Fehling solution to aqueous solutions of guaran causes the precipitation of a polysaccharide–copper complex.

Acid Hydrolysis of Guaran.—A 1% water solution of guaran was prepared by dispersing 5.50 g. of guaran of known moisture content in 500 ml. of distilled water and by heating this dispersion in an oil-bath at 100° until solution was accomplished. Twenty-five per cent. by weight sulfuric acid was added to make a 1% acid solution. Hydrolysis was carried out at 100°. Rotation measurements were made on 15-ml. aliquots which were removed from the reaction flask and cooled to 25°. The specific rotation changed from an initial value of about +59 to a final value of +37.

Enzymatic Hydrolysis of Guaran.—A 1% water solution prepared as above was treated with a commercial diastase in the proportion of 0.1 g. of diastase for each 100 ml. of solution. Viscosity measurements taken in an Ostwald-Cannon-Fenske tube indicated a decrease to about one-third the initial value in a period of eighteen hours.

Esterification of Guaran.—Freshly precipitated guaran was freed of ethanol by stirring with glacial acetic acid for fifteen minutes and by filtering off the excess reagents. To the pretreated guaran was added 33 parts pyridine and 33 parts acetic anhydride. The mixture was heated in an oil-bath with stirring for four to five hours at 105°. The acetate was then precipitated by pouring the solution into excess ethanol stirred in a Waring Blendor. The precipitate was filtered and washed in the Blendor four successive times with fresh portions of ethanol. The final product was air dried. It was a white, very fibrous material closely resembling amylose triacetate in appearance.

(6) A. Nowotnowna, *Biochem. J.*, **30**, 2177 (1936).

(7) A. W. Schorger, *Ind. Eng. Chem.*, **9**, 748 (1917).

(8) B. Tollens, *Ber.*, **23**, 2990 (1890).

(9) C. L. Butler and L. H. Cretcher, THIS JOURNAL, **53**, 4358 (1931).

(10) S. Moore and K. P. Link, *J. Biol. Chem.*, **133**, 300 (1940).

(11) N. C. Pervier and R. A. Gortner, *Ind. Eng. Chem.*, **15**, 1167 (1923); B. Tollens and E. Krober, *J. Landw.*, **48**, 355 (1900); **49**, 7 (1901); see also Browne and Zerban, "Sugar Analysis," 3rd ed., p. 904.

(12) R. L. Whistler, A. R. Martin and M. Harris, *Bur. Standards J. Research*, **24**, 13 (1940).

Acetyl content, 44.8%; theory, 44.78%; $[\alpha]^{25}D$ 34° (*c*, 1 in chloroform), m. p. 224–226°.

The acetate could be cast into a film by standard methods.[13] The films were lustrous, clear, strong, and flexible. When placed in water at 95–100°, films containing 20% dibutyl phthalate plasticizer could be elongated 550%. The stretched films were birefringent in polarized light but showed no detectable crystallinity on examination with X-rays. When broken under stress, the elongated films developed many longitudinal cracks.

A 2% solution of guaran triacetate dissolved in chloroform was fractionally precipitated by dropwise addition of ethanol with rapid stirring. Seven fractions were obtained each of which possessed a constant rotation of $[\alpha]^{25}D$ 34° (*c*, 1 in chloroform).

Results and Discussion

Guar flour consists principally of carbohydrate material. There is present only 1.5% fatty material or other substances extractable by ethanol. The low nitrogen value indicates the presence of but 3.5–4.0% protein. Only very small amounts of phosphorus-containing compounds are present. From this principally carbohydrate material it is, therefore, not surprising that a water soluble polysaccharide can be easily separated in 86–7% yield. The polysaccharide contains 35.6% D-galactose anhydride and 63.1% D-mannose anhydride. No ketoses[14,15] or uronic acids have been detected in the polysaccharide material. The absence of uronic acids differentiates the polysaccharide from the great majority of plant gums and mucilages.

Information with regard to the homogeneity of the soluble component is obtained by ethanol fractionation of its aqueous solution and comparison of the various fractions. The per cent. of the material received in each fraction is shown in Fig. 1, and the amount precipitated at different concentrations of ethanol is shown in Fig. 2. Comparison of the various fractions as to mannose anhydride content, specific optical rotation, and intrinsic viscosity are shown in Figs. 3, 4 and 5, respectively. These graphs indicate that the first 2.5% of the soluble component to be precipitated is of slightly different composition and lower viscosity than the main portion of the soluble component whose fractions are similar. The lower mannose anhydride content (40.2%) of the first material precipitated may relate it to the water insoluble component which has a mannose anhydride content of 39.1%. It is possible that some of the insoluble component is solubilized by the autoclave treatment. The graphs also indicate that there is present in the soluble component about 3% of very low molecular weight material which judged by its low mannose anhydride content and negative rotation is quite different from the main polysaccharide fractions.

Collectively, the graphs suggest that the water soluble component of guar endosperm consists principally (90–95%) of a polysaccharide which precipitates in a narrow range of ethanol concentration. Further evidence for the homogeneity of the polysaccharide material is the observation that when its acetate in chloroform solution is fractionated into seven parts by gradual ethanol addition, all fractions are found to possess identical optical rotations. Since the polysaccharide contains 34.5% D-galactose anhydride and 63.4% D-mannose anhydride, it may properly be termed a galactomannan. For convenience in designation, this particular polysaccharide fraction in this and future papers is given the name "guaran."

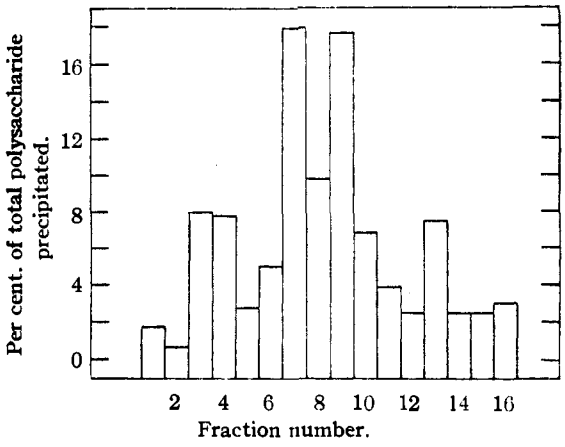

Fig. 1.—Per cent. of guar in each fraction.

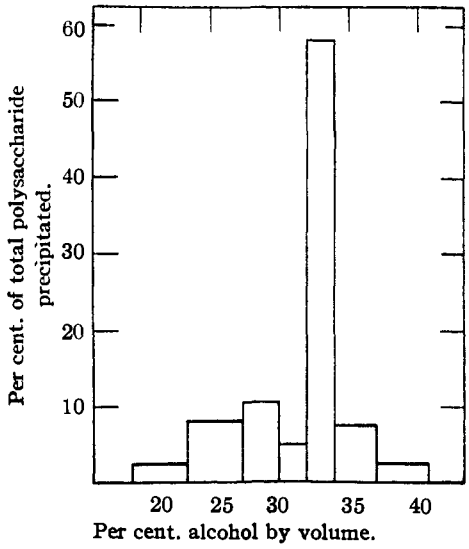

Fig. 2.—Per cent. polysaccharide precipitated at various alcohol concentrations.

On heating guaran in acid solution it undergoes hydrolysis and the specific optical rotation changes from a value of about +59 to +37. This change from a positive to a less positive value is indicative of the predominance of α-D-glycosidic links and is in agreement with the view of Lew and Gortner[16] who suggest the presence of alpha linkages in the galactomannan of carob bean endosperm. A further indication of the predominance of alpha link-

(13) R. L. Whistler and G. E. Hilbert, *Ind. Eng. Chem.*, **36**, 796 (1944).

(14) B. Th. Seliwanoff, *Ann. Chim. Applicata*, **21**, 535 (1931).

(15) R. Ofner, *Chem. Zig.*, **53**, 682 (1929).

(16) B. W. Lew and R. A. Gortner, *Arch. Biochem.*, **1**, 325 (1943).

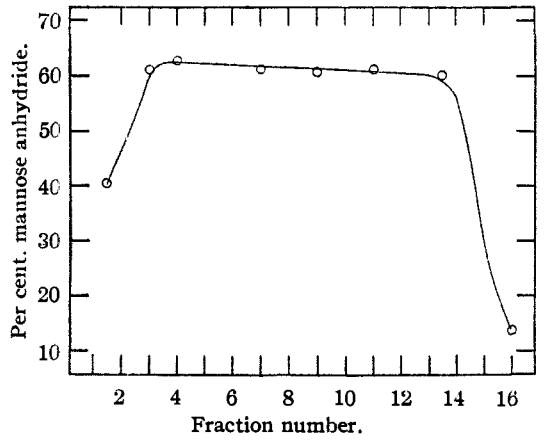

Fig. 3.—Per cent. mannose anhydride in guar fractions.

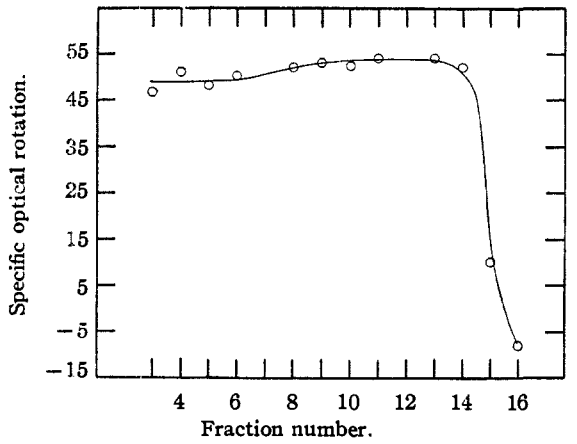

Fig. 4.—Specific optical rotation of guar fractions.

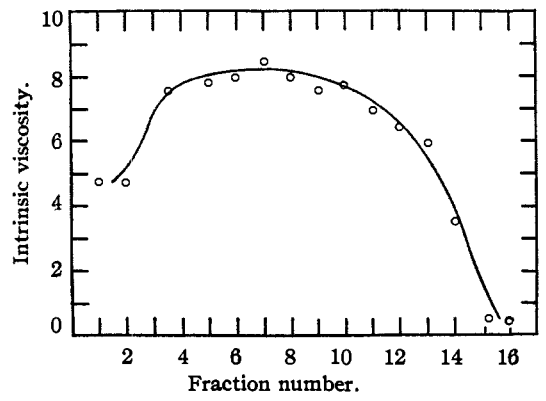

Fig. 5.—Intrinsic viscosity of guar fractions.

ages in guaran is the fact that its solutions are hydrolyzed by diastase, the viscosity decreasing to a value about one-third or less of the original.

Films produced by casting guaran acetates are clear, lustrous, strong, and pliable. Films plasticized with 20% dibutyl phthalate may be easily stretched in water at 100° to elongations of about 550%. During the stretching the film properties change from isotropic to anisotropic. The elongated films when broken under stress tend to shatter in lines parallel to the direction of elongation. Furthermore, a pronounced birefringence is developed during the process of elongation. These occurrences are all indications of the presence of anisodimensional and perhaps linear molecules which are orientated when the film is subjected to plastic flow. However, while the presence of linear molecules is indicated, X-ray analysis of the elongated films failed to give evidence of crystallinity. Films of uniform linear molecules would be expected to produce a fiber pattern or evidence some degree of crystallinity. Failure to obtain this effect may be accounted for by assuming that the guaran chains consist of D-galactose and D-mannose units arranged in random order or that the principal chain may possess branches of very short length. Therefore, although the chains become ordered when the films undergo plastic flow, an orderly three dimensional arrangement of sugar units is not brought about.

Acknowledgment.—The authors wish to express their appreciation to Dr. H. J. Yearian for the X-ray work and to Mrs. Ann Kimmell and Mrs. Helen Gleason for their assistance in part of the analytical determinations.

Summary

Guar flour can be readily separated into a water soluble (86–87%) and a water insoluble component. The water soluble component contains only small amounts of nitrogen and phosphorus impurities.

By gradual addition of ethanol to an aqueous solution of the water soluble component, it is separated into 17 fractions. Analyses of the fractions indicate that the third to the twelfth fractions are identical as to composition and represent a galactomannan polysaccharide which contains 34.5% D-galactose anhydride and 63.4% D-mannose anhydride. This polysaccharide is given the name "guaran."

Presence of α-D-glycosidic linkages in guaran is indicated by its rapid acid hydrolysis with accompanying decrease in specific rotation and by its hydrolysis under the action of diastase.

Guaran may be esterified easily to the triacetate which may be cast into a strong, flexible film. On stretching to 550% the film becomes strongly birefringent but yields only an amorphous X-ray pattern. It is assumed that the guaran molecules are linear or highly anisodimensional but have either a random distribution of D-galactose and D-mannose units in the molecular chains or that the chains have very short branches.

LAFAYETTE, INDIANA RECEIVED NOVEMBER 5, 1947