[CONTRIBUTION FROM THE WILLIAM G. KERCKHOFF LABORATORIES OF THE BIOLOGICAL SCIENCES, CALIFORNIA INSTITUTE OF TECHNOLOGY]

A Mucilage from Aloe Vera[1]

BY ELIZABETH ROBOZ[2] AND A. J. HAAGEN-SMIT

The approximately 190 species of the genus *Aloe* are chiefly natives of Africa, a few species being found on other continents. While some species are widely cultivated for use as ornamentals, several are grown for their drug content. Large plantations of *Aloe vera* in Hawaii and elsewhere furnish much of the commercial aloes used as a laxative. The split leaves are used in native pharmacology as wound and burn dressings. A limited commercial use of the leaves for X-ray burns exists in this country. The material known to the pharmacist as aloe consists of the dried latex of the aloe leaf.

In contrast to the extensive research on the latex only qualitative tests have been carried out on the other portions of the plant.[3] In the present work approximate analyses for gross constituents were made on the plant after removal of the latex. The mucilaginous layer was removed mechanically from the leaves and purified by precipitation from water–ethanol. The material so obtained had a high ash content (12.9%) which was essentially removed by dialysis. The mucilage so isolated was a white amorphous product that exhibited an elementary analysis corresponding essentially to a hexosan. Hydrolysis yielded mainly glucose and mannose in about equal amount. A small content (2.37%) of uronic acid was determined analytically. This amount would not essentially change the analytical data for a hexosan. Mannose was determined by the phenylhydrazone method and glucose as the phenylosazone. Ketoses were absent.

Experimental

Preparation of Plant Material.—The plants selected for investigation were of the species *Aloe vera* from Hawaii. The average weight of each plant was about 6 kg. Before extraction of the mucilage was made, the latex was drained off by cutting the leaves near the basal end (bleeding). The aloe leaves were cut in half, parallel to the blade of the leaf and the mucilaginous layer was removed by scraping. In a plant of 6.6 kg., the weight of mucilage parenchyma was 1.30 kg. (20%). In a single average leaf of 400 g., the yield of parenchyma was 186 g. (45%). The dry substance of the parenchyma varied, according to the shipments, between 1.98 and 2.50%. The mucilage content of the parenchyma was 0.20%, that of the assimilatory tissue 1.73%, while the stem did not contain any. The results of the analytical data are recorded in Table I.

Extraction and Purification.—The finely ground mucilaginous material prepared as described above was extracted at room temperature with an equal weight of 95% ethanol, followed by five extractions with 50% ethanol. If this extract was exposed to air, pink oxidation products were formed which were difficult to remove in the subsequent steps of purification. Therefore, contact with air was avoided. The filtration of the viscous extract was carried out rapidly by using a large covered Buchner funnel without filter paper.

TABLE I

ANALYSIS OF HAWAIIAN ALOE VERA

Constituents	% (Dry basis) Leaf parenchyma	Assimilatory tissue	Stem
Protein	2.87	2.62	2.32
Fat	4.76	2.49	1.93
Fiber	5.09	12.20	16.30
Sugars	25.50	9.02	23.30
Ash	8.63	6.94	8.55
Mucilage	30.00	16.90	0
Crude aloin, oil and resin	22.30		

From the first viscous extract, the mucilage was precipitated by the addition of four volumes of 95% ethanol. The other more diluted extracts were concentrated in a nitrogen atmosphere at 50° before precipitation. The crude mucilage was repeatedly dissolved in hot water and reprecipitated with ethanol. After every reprecipitation the mixture was kept at 0°. When the precipitate settled as a fibrous mass, it was centrifuged, dehydrated with absolute ethanol, dried under reduced pressure and finally extracted with ether. The ethanolic filtrate, which was discarded, contained the aloin that had not been completely removed from the plant by bleeding. Since the analysis of the crude mucilage showed a high ash content of 12.90% and a nitrogen content of 1.40%, it was further purified by dialysis. The mucilage was dissolved in 0.01 *N* hydrochloric acid and dialyzed for forty-eight hours against distilled water. After precipitation with four parts of 95% ethanol, the mucilage was filtered, dried, redissolved and reprecipitated with an equal volume of 95% ethanol. This operation was repeated twice. Then the precipitate was washed with cold distilled water to remove the last traces of hydrochloric acid, washed with absolute ethanol and ether and dried under reduced pressure at 80°.

Properties of the Mucilage.—The purified aloe mucilage was a white amorphous powder. It dissolved slowly in hot water to form a highly viscous solution. The mucilage was soluble in water and was insoluble in the common organic solvents. It did not have a sharp melting point but decomposed at 271–276°; $[\alpha]^{20}D$ 0° (*c* 1, water).

Anal.[4] Calcd. for $(C_6H_{10}O_5)_n$: C, 44.40; H, 6.10. Found: C, 44.54; H, 6.17; ash, 0.11.

Hydrolysis and Identification of the Monosaccharide Constituents.—The mucilage was hydrolyzed with 4% sulfuric acid by heating and stirring on the boiling water-bath for seven hours. During this time the opaque solutions became clear, indicating a complete hydrolysis. The acid solution was neutralized with barium carbonate and the following qualitative tests were performed on the filtrate: pentose (Bial orcinol[5] and phloroglucinol[5] tests); ketose (Seliwanoff[6] test); galactose (mucic acid[5] formation

(1) This investigation was supported by a grant from Alexander Baldwin, Ltd., Hawaii.

(2) Present address: Stanford Research Institute, Stanford University, Calif.

(3) F. Prollius, *Arch. Pharm.*, **22**, 553 (1884).

(4) The authors wish to express their thanks to Dr. G. Oppenheimer for carrying out the microanalyses.

(5) C. A. Morrow and W. M. Sandstrom, "Biochemical Laboratory Methods," 2nd ed., John Wiley & Sons, Inc., 1935, pp. 152, 154, 164.

(6) T. Seliwanoff, *Ber.*, **20**, 181 (1887).

on nitric acid addition); rhamnose (Rosenthaler test[7]). All of these tests were negative.

The phenylosazone test was carried out with the hydrolyzed mucilage at 0° using freshly purified phenylhydrazine and glacial acetic acid. Pure D-mannose and D-glucose were used as a control. The hydrolyzed mucilage and D-mannose yielded a creamy white, crystalline precipitate. After standing for one hour in the cold the mannose phenylhydrazone was removed by filtration. The crystals, when examined under the microscope were homogeneous and had the same crystal form as stated by Hassid[8] for mannose phenylhydrazone. The phenylhydrazone of the mucilage hydrolyzate was recrystallized twice from 60% ethanol; m. p. 186–188°, unchanged on admixture with an authentic specimen of D-mannose phenylhydrazone.

After filtration of the mannose phenylhydrazone from the hydrolyzate, the solution was heated on the water-bath for thirty minutes and cooled to room temperature. At this point yellow colored crystals separated. These crystals had the same shape as a simultaneously prepared D-glucose phenylosazone; they were recrystallized twice from 60% ethanol; m. p. 205–206°, unchanged on admixture with an authentic specimen of D-glucose phenylosazone.

(7) R. Rosenthaler, *Z. anal. Chem.*, **48**, 165 (1909).

(8) W. Z. Hassid and R. M. McCready, *Ind. Eng. Chem., Anal. Ed.*, **14**, 683 (1942).

Quantitative Assay of Constituents.—Quantitative assays showed that essentially all of the sugars present in the mucilage hydrolyzate were aldoses with no fructose or other ketoses being present since the total reducing sugar as determined by the Hassid ferrocyanide method[9] was 87.80% and aldose sugars as determined by the Willstätter–Schudel[10] hypoiodite method was 89.0%. Uronic acid as determined by the method of Lefèvre and Tollens[11] was 2.37%.

Mannose was determined quantitatively by weighing the mannose phenylhydrazone (using freshly distilled phenylhydrazine and glacial acetic acid); 46.90% was found.

Summary

1. A mucin has been isolated from the medicinal plant *Aloe vera*. The substance has been shown to consist essentially of about equal parts of glucose and mannose together with a small amount of uronic acid.

(9) W. Z. Hassid, *ibid.*, **8**, 138 (1936); **9**, 228 (1937).

(10) R. Willstätter and G. Schudel, *Ber.*, **51**, 780 (1918); G. W. Pucher and M. W. Finch, *J. Biol. Chem.*, **76**, 331 (1928).

(11) K. V. Lefèvre and B. Tollens, *Ber.*, **40**, 4513 (1907).

GENEVA, N. Y. RECEIVED MAY 1, 1948

[CONTRIBUTION FROM THE DIVISION OF CHEMICAL ENGINEERING, UNIVERSITY OF MINNESOTA, AND THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF BIRMINGHAM, ENGLAND]

The Constitution of Carob Gum

BY F. SMITH

Carob gum is the galactomannan polysaccharide obtainable from the carob bean (*Ceratonia Siliqua L.*) by extraction of the seeds with water or aqueous alkaline solutions. It appears to be known also as Swine's bread, gum Hevo, gum Gatto, Jandagum, Lakoe gum, Lupogum, Luposol, Rubigum, Tragon and Tragasol. The bean is grown in the Mediterranean region and used as a food called St. John's bread. In Europe and the United States the gum from the seeds is used in the sizing of textiles, tanning of leather, in the paper industry and as a mucilage in pharmaceutical products. The nature of the polysaccharide carob gum forms the subject of this investigation and is one of a series directed to the study of plant gums and related substances.

The gum is shown herein to be composed of D-galactose (20%) and D-mannose (80%), the presence of which have already been established by Effront, Van Ekenstein, Bourquelot and Herissey and by Iglesias.[1,2,3,4] The polysaccharide resembles the plant mucilages in the manner in which it forms gels.[5] Like other mannan polysaccharides, for example yeast mannan,[6] carob gum forms a copper hydroxide complex when treated with Fehling solution; it appears also to be sensitive to borates.[5,7]

Compared with ivory nut mannan,[8] carob gum was found to be relatively easy to hydrolyze and from the cleavage products α-methyl-D-mannoside was isolated in good yield. For this reason it has been recommended as an excellent source of D-mannose.[9] The presence of D-mannose in the hydrolysis product of carob gum was also established by the production in good yield of the phenylhydrazone and the anilide of mannose; D-galactose can be identified as a constituent of the gum by nitric acid oxidation of either the acid hydrolysis products of the gum or of the gum itself.

Graded hydrolysis of the polysaccharide gum with 0.2 *N* sulfuric acid afforded D-galactose and a mixture of oligosaccharides which appeared to be of varying molecular size. There was no arrest point in the hydrolysis of the carob gum as is the case with plant gums such as gum arabic,[10] damson gum,[11] cherry gum[12] and mesquite gum,[13] and there appears to be no evidence to support the view that the rest of the molecular complex re-

(1) J. Effront, *Compt. rend.*, **125**, 38, 309 (1897).

(2) A. Van Ekenstein, *ibid.*, **125**, 719 (1897).

(3) E. Bourquelot and H. Herissey, *ibid.*, **129**, 228, 391 (1899).

(4) G. Iglesias, *Anales soc. españ. fis. quím.*, **33**, 114 (1935).

(5) R. Hart, *Ind. Eng. Chem., Anal. Ed.*, **2**, 329 (1930).

(6) W. N. Haworth, E. L. Hirst and F. A. Isherwood, *J. Chem. Soc.*, 784 (1937).

(7) A. L. Williams, *Analyst*, **53**, 411 (1928).

(8) C. S. Hudson, "Organic Syntheses," Coll. Vol. I, 1946, p. 371.

(9) F. Smith, *J. Chem. Soc.*, in press.

(10) F. Smith, *ibid.*, 744 (1939).

(11) E. L. Hirst and J. K. N. Jones, *ibid.*, 1174 (1938).

(12) J. K. N. Jones, *ibid.*, 558 (1939).

(13) E. Anderson and Louise Otis, THIS JOURNAL, **52**, 4461 (1930).