[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF ARIZONA]

THE COMPOSITION OF CHOLLA GUM. I. THE ISOLATION OF *l*-ARABINOSE, *d*-GALACTOSE AND *l*-RHAMNOSE

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Until recently not much work has been done upon plant gums since the work of O'Sullivan[1] and Tollens.[2] It is only lately that they have come into a position of importance more in keeping with their significance. Butler and Cretcher[3] have classed them as acid polysaccharides and, as the name suggests, they present a phase of carbohydrate chemistry quite distinct from that connected with starch and cellulose.

These substances occur in nature as the salts of a complex acid, the molecule of which seems to be built about a nucleus of uronic acids. The monosaccharides, arabinose, galactose and frequently rhamnose, are attached in such a manner that it is possible to hydrolyze off one kind of monosaccharide more or less completely under suitable conditions without detaching another kind. For example, arabinose may be removed almost quantitatively from cholla gum while a part of the galactose and virtually all of the rhamnose remain in combination with the acid nucleus. The galactose, in turn, can be hydrolyzed off while the rhamnose remains attached to the uronic acid group. In like manner mesquite gum[4] was found to release its arabinose while the galactose-uronic acid complex remained intact.

Whether these various monosaccharides are attached to the acid nucleus in long chains arranged in the order in which they are removed or whether the relative ease of hydrolysis depends on the nature of the linking which holds them to the nucleus, we cannot say as yet. Since the original cholla gum does not reduce Fehling's solution, all of the aldehyde groups must be involved in the linkings that hold the molecule together. All hydrolytic products reduce Fehling's solution but no product reduces it vigorously in the cold until after it is hydrolyzed in the autoclave, a procedure which removes the most difficultly hydrolyzable sugar, rhamnose. Evidently the aldehyde group of the uronic acid in the cholla gum is joined to the rhamnose by a glucosidic linking. The other linkings remain a subject of conjecture.

The occurrence and general properties have been described by Anderson and Sands.[5] The present communication describes work preliminary

[1] O'Sullivan, *J. Chem. Soc.*, **45**, 41 (1884); *ibid.*, **59**, 1029 (1891); *ibid.*, **79**, 1164 (1901).

[2] (a) Flint and Tollens, *Ber.*, **25**, 2917 (1892); (b) Widtsoe and Tollens, "Dissertation," Göttingen, **1899**; *Ber.*, **33**, 132 (1900); (c) Tollens, *ibid.*, **41**, 1788 (1908).

[3] Butler and Cretcher, THIS JOURNAL, **51**, 1519 (1929).

[4] Anderson and Sands, *ibid.*, **48**, 3172 (1926).

[5] Anderson and Sands, *Am. J. Pharm.*, **97**, 589 (1925).

to the determination of the structure of the molecule and includes the isolation of *l*-arabinose, *d*-galactose and *l*-rhamnose and the identification of *d*-galacturonic acid as the uronic acid present.

Cholla gum as it comes from the plant is not a single compound. When it was ground to pass a forty-mesh sieve, ethanol dissolved 1% of its weight. Although the gum has a light yellow color and a faint, not unpleasant odor, this alcoholic extract upon evaporation of the solvent, was dark brownish-red and highly odoriferous. A part of this extract was ether-soluble and a part could be saponified with alkali, showing that this fraction contained the lipins and the fat-soluble substances and was not carbohydrate. The remainder of the gum is carbohydrate in composition but can be separated into two fractions. After extraction with 140 times its weight of water during a period of forty-eight hours,[6] about one sixth of the total volume contained the greatly swelled, water-insoluble portion amounting to 80% of the gum, while the remainder was a slimy, opalescent solution containing the rest of the gum. This procedure gave an imperfect separation, it is true, but analysis of the two fractions showed them to contain the same components though in somewhat different amounts. The water-soluble fraction was nearly twice as high in uronic acid as the water-insoluble fraction, while the latter fraction contained practically all of the non-hydrolyzable, cellulose-like fraction of the gum.

Analysis of the gum on a water-free basis gave the following results: ash, 8.4%; uronic acid,[7] 11.5%; arabinose,[8] 53.2%; rhamnose,[9] 5.5%; galactose,[10] 8.4%.

[6] The gum was thoroughly mixed with 80 times its weight of water and allowed to stand for twenty-four hours. Three-fourths of the volume was siphoned off and replaced with water. After another twenty-four hours the supernatant liquid was again siphoned off and the very mobile suspension of water-insoluble material was concentrated by centrifuging.

[7] The uronic acid was determined by the method of Lefèvre: (a) Van der Haar, "Anleitung zum Nachweis, zur Trennung and Bestimmung der Monosaccharide und Aldehydesäuren," Gebrüder Borntraeger, Berlin, **1920**, p. 71–76; (b) Lefèvre and Tollens, *Ber.*, **40**, 4513 (1907); (c) Tollens, *Z. physiol. Chem.*, **61**, 95 (1909); (d) Lefèvre, "Untersuchungen über die Glucuronsäure," "Dissertation," Göttingen, **1907.**

[8] Pentoses were determined according to "Methods of Analysis," A. O. A. C., **1925**, and calculated as arabinose. Since furfural is liberated from the uronic acid as well as from the arabinose during a pentosan determination, the arabinose in the substance under consideration had to be calculated by correcting the total amount of furfural phloroglucide for that which was derived from the acid. To our knowledge, the relationship between galacturonic acid and its furfural-phloroglucide production has not been determined, so for this purpose the factor given by Lefèvre, expressing the relationship between glucuronic acid and its phloroglucide production, was used [Van der Haar, Ref. 7a, p. 71]. The accuracy of such a method is questionable. Any error from this source together with that resulting from the inaccuracy of the pentosan determination itself when applied to difficultly hydrolyzable substances must be kept in mind in consideration of data determined by this method.

General Procedure for Separating the Constituents of Cholla Gum.—In order to separate the constituents, the cholla gum was subjected to a series of hydrolyses. After the liberation of arabinose by hydrolysis at 80°, the partially degraded gum acid was turned into its calcium salt (calcium salt "A") and separated from the sugar. Calcium salt "A" was further hydrolyzed at 100° and the calcium salt of the still simpler acid (calcium salt "B") was separated from the galactose. Calcium salt "B" was next hydrolyzed in the autoclave and the calcium salt of the resulting acid (salt "C") was separated from the rhamnose. Details of the procedure follow.

Initial Hydrolysis of the Gum.—The gum was ground to pass a 40-mesh sieve and extracted with ethanol to remove the lipoid substances. One kilo was mixed with ten liters of 2% sulfuric acid, placed in three-liter flasks and heated for thirty hours in water-baths kept at 80°.[11] After hydrolysis there remained a residue of a cellulose-like substance amounting to 10% of the weight of the gum, which resisted further decomposition even in the autoclave at 120°. It was filtered off and discarded.

The solution was neutralized with calcium carbonate, decolorized with norit and the resulting almost colorless solution was concentrated in the presence of excess calcium carbonate in large evaporating dishes over the free flame.[12] At low volume it was filtered and concentrated to 45% solids. The sirup was poured into 3 to 4 times its volume of 95% ethanol, slowly and with vigorous stirring. The light yellow, curdy precipitate was filtered off and washed with hot 95% alcohol. To ensure the complete removal of any sirup that was occluded, the precipitate was dissolved in water and after diluting to 26% solids[13] it was poured into 3 to 4 times its volume of ethanol; it came down again as a flaky precipitate. This was washed with hot alcohol and then ether. It gave 286 g. of powdery light yellow calcium salt "A."

Isolation of *l*-Arabinose.—Since any sugar which hydrolyzed off the gum remained

[9] Methyl pentoses were determined by the method of Ellett and Tollens and of Haywood and calculated as rhamnose: (a) Ellett and Tollens, *Z. deut. Zuckerind.*, **42**, 19 (1905); (b) Haywood, Bulletin 105, U. S. Bureau of Chemistry, **1907**, p. 112.

[10] Galactose was determined according to van der Haar, Ref. 7a, pp. 123–130. The total amount of mucic acid resulting from a determination represented that produced from the galacturonic acid present as well as that from the galactose. We have found no statement of the exact relationship between galacturonic acid and its mucic acid production, but Ehrlich obtained from tetra-galacturonic acid a yield of 70% or more of pure mucic acid [*Chem.-Ztg.*, **41**, 198 (1917)]. Since galactose gives about 75% of its weight of mucic acid, van der Haar determined galacturonic acid by multiplying the weight of mucic acid by the same factor that was used to determine galactose (Ref 7a, p. 336). In this work the total weight of mucic acid was converted to galactose by use of the table compiled by van der Haar, and this value minus the weight of galacturonic acid as determined by the method of Lefèvre was taken as the weight of galactose in the original sample.

[11] The acid concentration and time of hydrolysis here and in later instances were chosen as optimum after hydrolyzing samples of the gum and the various salts under a variety of conditions.

[12] Evaporation at reduced pressure was not feasible because of the tendency of the solution to foam.

[13] The optimum concentration of the salt solutions for precipitation with alcohol was, in all cases, the most concentrated solution that could be added to the alcohol and give the desired finely divided precipitate in place of droplets of sirup which would coalesce into a gummy mass and thus carry down sugar. The volume of alcohol used was sufficient to prevent coalescence of the flaky precipitate as the watery sirup was added.

dissolved in the alcohol, all filtrates and washings were combined, allowed to stand overnight to permit any calcium salt remaining in colloidal suspension to settle and then concentrated to 84–86% of solids. The sirup was thinned with 200 cc. of methanol,[14] cooled and seeded with arabinose. Crystallization began within an hour, but the sirup was allowed to stand in the refrigerator for a week with occasional stirring in order that as much of the sugar as possible might separate. Enough 95% ethanol was added to make the thick mass into a thin paste and the crystals were filtered off with suction. The sugar was washed by triturating it with a mixture of one part of glacial acetic acid and three parts of 95% ethanol and later with ethanol alone. This gave 250 g. of light-colored, fairly pure product.

When the mother liquor from the arabinose was evaporated approximately to dryness *in vacuo*, there remained 328 g. of a vitreous, dark red, transparent sirup. Analysis showed the presence of 5.3% of uronic acid, 7.7% of galactose, 3.7% of rhamnose and 4.7% of ash. These substances doubtless were present as a calcium salt of a complex acid resembling that in salt "A," which owed its presence to its appreciable solubility in alcohol. In addition the sirup contained arabinose to the extent of 34.4% or 113 g. However, the second crop of arabinose never amounted to more than a few grams. The procedure followed in the case of mesquite gum, namely, the purification of the sirup by the precipitation of a second crop of salts in order to permit the further crystallization of the sugar, failed in this case. Bertrand's method failed to give cadmium xylonate, and the sirup was not fermented by yeast, showing the absence of both xylose and glucose. Why the mixture resisted separation has not been determined.

Identification of *l*-Arabinose.—When the sugar referred to above was purified by the method of Anderson and Sands[4] it melted at 155–157° and in 4% solution gave a specific rotation of $[\alpha]_D$ +101.9°. The hydrazone, after recrystallization from 95% alcohol, melted at 150–151° with decomposition.[15] A portion of the sugar was oxidized with bromine to arabonic acid. The phenylhydrazide of this acid was a creamy white substance melting at 212–213° and having a specific rotation of $[\alpha]$ +15.2° in 1% solution. Fischer[16] gave 215° as the melting point and Hudson[17] gave the rotation of the hydrazide of *d*-arabonic acid as $[\alpha]$ −16.09°. These facts, together with the fact that seeding with arabinose caused crystallization of the sirup, identified the sugar as arabinose.

Nature of Calcium Salt "A."—Analysis of the salt gave the following results: ash, 9.4%; uronic acid, 33.9%; arabinose, 5.5%; galactose, 5.1%; rhamnose, 27.9%.

The success with which the arabinose was removed by hydrolysis at 80° was evidenced by the reduction of the arabinose content from 53.2% in the original gum to 5.5% in salt "A." A rather large portion of the galactose was lost, however. The 5.1% of salt "A" corresponds to 14.5 g. of galactose, while the mother liquor left after the removal of the arabinose contained 25.3 g. of galactose. The high rhamnose content of salt, together with the small amount of rhamnose found in the mother sirup mentioned above (3.7%), indicated the success with which this sugar was left undisturbed. Evidently this salt was the calcium salt of fragments of the original gum molecules derived by the loss of the arabinose, a part of the galactose and possibly a little of the rhamnose.

Hydrolysis of Calcium Salt "A."—The 280 g. of calcium salt "A" was dissolved in

[14] Methanol was more suitable for diluting this sirup than ethanol since the calcium salt which remained dissolved in the sugar fraction was more soluble in the methanol and consequently showed less tendency to precipitate as a gummy mass.

[15] Van der Haar, Ref. 7a, p. 145.

[16] E. Fischer, "Untersuchungen über Kohlenhydrate und Fermente," Vol. I, p. 329.

[17] Hudson, THIS JOURNAL, 39, 462 (1917).

ten times its weight of 1% sulfuric acid plus enough sulfuric acid to precipitate the calcium and heated in the boiling water-bath for six hours. The solution was then neutralized with calcium carbonate, decolorized and evaporated in the presence of excess calcium carbonate. At low volume it was filtered, the concentration adjusted to 55% solids, and when cold the sirup was precipitated with ethanol as in the case of salt "A." The light yellow granular precipitate (calcium salt "B") amounted to 200 g.

Isolation and Identification of *d*-Galactose.—The alcoholic filtrates and washings obtained in the isolation of salt "B" were concentrated as much as possible *in vacuo* and then mixed with an equal volume of glacial acetic acid and seeded with galactose. Crystallization followed within a few minutes. After standing for a few days in the refrigerator, the galactose was filtered off and washed with a mixture of one part of glacial acetic acid and three parts of 95% alcohol. The yield of crude sugar was 18 g.[18] After purification[4] the product melted at 163–165°, gave a specific rotation of $[\alpha]_D$ 79.7° in 4% solution and produced approximately the quantitative yield of mucic acid when oxidized with nitric acid. These data identified the sugar as *d*-galactose.

Nature of Calcium Salt "B."—Analysis of this salt was as follows: ash, 9.5%; uronic acid, 40.8%; galactose, 0%; arabinose, 2.7%; rhamnose, 28.5%.

The hydrolysis at 100° appeared successful in the complete removal of galactose and, in view of the inaccuracy involved in the arabinose determination, the small percentage of that sugar found in salt "B" might as well be interpreted to indicate its absence as not. The fact that further hydrolysis of the salt in the autoclave left its pentose content unchanged substantiated the assumption that arabinose was completely removed from the gum by this second hydrolysis. The high rhamnose content of this salt, together with the fact that only 4.3% of rhamnose was found in the mother liquor from the galactose, showed that rhamnose was not conspicuously disturbed by hydrolysis at 100°. Calcium salt "B" differed from salt "A" mainly in that galactose and the last remnant of arabinose had been removed from the molecule.

Hydrolysis of Calcium Salt "B."—One hundred ninety-five grams of salt "B," dissolved in ten times its weight of 2% sulfuric acid with a sufficient excess to precipitate the calcium, was hydrolyzed in the autoclave at one atmosphere gage pressure or 120° for eight hours. The solution was neutralized, decolorized, concentrated to 50% solids and the salts were precipitated with alcohol exactly as in the two preceding cases. Eighty-six grams of light brown calcium salt "C" resulted.

Isolation and Identification of *l*-Rhamnose Hydrate.—Again the alcoholic filtrates and washings were evaporated *in vacuo* to a very thick sirup and mixed with an equal volume of glacial acetic acid. Without seeding the sirup showed no evidence of crys-

[18] The fact that the yield of galactose was larger than the total galactose content of salt "A" as shown by analysis is not surprising in view of the lack of reliability of the method used when applied to galactans in the presence of foreign material. Schorger found that one sample of galactan from the larch gave values of galactan content varying from 44.56 to 93.77%. Dore found that varying the sample of pure galactose from 0.2 to 2 g. caused the yield of mucic acid to range from 54 to 90%. Lippman has shown that foreign organic substances may prevent entirely the separation of mucic acid. In the work on mesquite gum in this Laboratory it was difficult to get the analytical results to show a total galactose content which equaled the galactose actually isolated from the gum. The application of the method to duplicate samples of the hydrolytic products of cholla gum gave results that checked within 2–3% and led to conclusions that seemed reasonable in the light of other experimental results. It was with these facts in mind that the results of these determinations were used in this article: (a) Schorger, *J. Ind. Eng. Chem.*, **8**, 498 (1916); (b) Dore, *ibid.*, **7**, 721 (1915); (c) Lippman, "Chemie der Zuckerarten," 3d ed., Vol. I, p. 274.

tallization at the end of three weeks, but upon seeding with rhamnose, crystals formed rapidly with the liberation of heat. After several days the sugar was filtered off and washed with the acetic acid–alcohol mixture. The yield was 27 g. The sugar was purified by dissolving it in water, decolorizing, concentrating to 86% solids and adding half its volume of glacial acetic acid. The sugar obtained melted at 92–94° and formed a phenylosazone which melted at 178–180°. In 4% solution the specific rotation was $[\alpha]_D$ −5.1° after five minutes and $[\alpha]_D$ 0° after ten minutes, while the final constant value was $[\alpha]_D$ +7.8°. The sugar was thus identified as *l*-rhamnose hydrate.

Nature of Calcium Salt "C."—Analysis of the salt gave: ash, 17.0%; uronic acid, 36.1%; galactose, 0%; arabinose, 2.9%; rhamnose, 10.5%.

The rhamnose was incompletely hydrolyzed off but was reduced from 28.5% in salt "B" to 10.5% in salt "C." Therefore salt "C" approached very closely in composition the calcium salt of the nucleus of the gum. Work on the constitution of this salt as well as the salts "A" and "B" is in progress in this Laboratory. The destruction of the uronic acid during the vigorous hydrolysis required to remove the rhamnose was shown by its reduction from 81.6 g. in salt "B" to 31.1 g. in this final salt.

Identification of *d*-Galacturonic Acid.—The calcium salt "C" reduced Fehling's solution almost immediately in the cold and gave the naphthoresorcinol test, properties peculiar to the uronic acids. Oxidation with nitric acid produced mucic acid in amounts corresponding to the galacturonic acid as determined by the method of Lefèvre. When this salt was treated with bromine water in the cold, a white precipitate of mucic acid formed which was soluble in sodium hydroxide, reprecipitated upon acidification with hydrochloric acid and melted at 215°. When oxidized with bromine water at 100°, the salt yielded 17.7% of mucic acid, an amount corresponding in magnitude to 20.7% obtained by oxidation with nitric acid. Galactose will not give appreciable quantities of mucic acid when oxidized with bromine, whereas Ehrlich[19] found tetra-galacturonic acid to yield 60–70% of its weight of mucic acid. These facts proved that the uronic acid in cholla gum was *d*-galacturonic acid. The absence of glucuronic acid was established by failure to obtain potassium acid saccharate from the oxidation products of calcium salt "C."

Summary

1. Cholla gum, by a method of successive hydrolyses, was changed into salts of three distinct acids, each differing from its precursor in the loss first of arabinose, then galactose and finally rhamnose.

2. *l*-Arabinose, *d*-galactose and *l*-rhamnose hydrate were isolated and identified.

3. *d*-Galacturonic acid was definitely proved to be the uronic acid in cholla gum.

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[19] Ehrlich, *Chem.-Ztg.*, **41**, 198 (1917).