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THE COMPOSITION OF GUM ARABIC[1,2]

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Although a vast amount of research has been done in the field of carbohydrate chemistry, comparatively little attention has been given to the group of substances which may be classified under the heading of acid polysaccharides. In this group we find such substances as pectins, plant gums, hemicelluloses, mucilages, soluble specific substances produced by bacteria and alginic acid from algae. Most of these substances appear to be formed by modification of cellulose under the influence of enzymes, few, if any, of them being produced directly by photo-synthesis. Acid polysaccharides apparently play a very important, though poorly understood, role in natural processes. Any information that may be obtained regarding the structure of these substances should be of value in the elucidation of their functions in the immunology of infectious diseases and the physiology of plants. They are also of interest in connection with the problems of ropy bread, beer and wine. Among the members of this class of substances which we are investigating in this Laboratory are the plant gums.

Under the name of gums, a variety of substances have been described which differ considerably from one another both chemically and physiologically. The plant gums, as is well known, are salts of very complex organic acids usually with calcium, magnesium and potassium. These complex acids are built up of hexose, pentose and methylpentose units in combination with the acidic part of the molecule. The facts that many of them liberate carbon dioxide on heating with 12% hydrochloric acid[3] and that they give the naphtho-resorcin test[4] indicate that they contain uronic acid units.[5] In fact Anderson and his co-workers have reported the presence of glucuronic acid in mesquite gum and galacturonic acid in cholla gum.[6]

But little work has been done on the acidic nucleus of gums since the early researches of O'Sullivan. This author hydrolyzed arabic acid—

[1] Presented before the Organic Chemistry Division of the American Chemical Society at the Swampscott Meeting, September, 1928.

[2] *Cf.* Cretcher and Butler, *Science*, **68**, 116 (1928), for a preliminary discussion on the nature of the acidic substance formed on the hydrolysis of this gum.

[3] (a) Nanji, Patton and Ling, *J. Soc. Chem. Ind.*, **44**, 253T (1925); (b) Anderson and Sands, THIS JOURNAL, **48**, 3172 (1926).

[4] Tollens, *Ber.*, **41**, 1788 (1908).

[5] Widsoe and Tollens, *ibid.*, **33**, 132 (1900); see also ref. 1.

[6] Abstract of papers presented before the Organic Chemistry Division of the American Chemical Society at the St. Louis Meeting, April, 1928.

from gum arabic—with dilute sulfuric acid and isolated a stable acid of lower molecular weight which he called λ-arabinosic acid.[7] He assigned to the substance the formula $C_{23}H_{38}O_{22}$. Similarly O'Sullivan prepared so-called C_{23} acids from gum tragacanth and from gedda gum.[8] Robinson, in 1906, also claimed to have isolated a C_{23} acid from the gum of *Cochlospermum Gossypium*.[9] Various other gums have been investigated, but as stated above with little, if any, emphasis placed on the acidic nucleus.

A botanically authentic sample of Gum Arabic Cordofan from *Acacia Senegal (L.) Willd.* was hydrolyzed with 2% sulfuric acid and the acidic reaction product isolated by methods which are set forth in the experimental part of this paper. Analysis of the dried, purified salt indicated an aldobionic acid of formula $C_{12}H_{20}O_{12}$. This acid was analytically identical with the λ-arabinosic acid of O'Sullivan,[10] who assigned to it the formula $C_{23}H_{38}O_{22}$ though his own analytical figures correspond as well to $C_{12}H_{20}O_{12}$. It gives a strong naphtho-resorcin test and reduces Fehling's solution. On boiling with 12% hydrochloric acid the correct amount of carbon dioxide is liberated, and the amount of iodine consumed in oxidation, as well as the percentage of calcium found, corresponds to the requirements of a compound of the formula given, containing one free aldehyde and one carboxyl group.

From a consideration of these facts the authors are led to wonder if the other C_{23} acids produced from gums and referred to in this paper are not in reality aldobionic acids. This point is being investigated.

The identity of the sugar constituent of this aldobionic acid was determined by hydrolysis with 5% sulfuric acid. This sugar had the correct rotation for *d*-galactose and formed mucic acid on oxidation. The acidic fraction was too small in amount to serve for purposes of identification due to the fact that, under the conditions of the hydrolysis, the uronic acid was largely destroyed. Simultaneous hydrolysis and oxidation of the aldobionic acid[11] was accomplished by boiling with hydrobromic acid in the presence of bromine. This led to the formation of saccharic acid, as shown by the isolation of acid potassium saccharate. The aldobionic acid is, therefore, galactoso-glucuronic. It is isomeric with the aldobionic acids isolated by Heidelberger and Goebel[11] from the polysaccharides produced by Pneumococcus Types II and III and from Friedlander Bacillus Types A, B and C.

In order to throw further light on the structure of this aldobionic acid, it was oxidized by the method of Goebel[12] to the dibasic acid, glucurono-

[7] O'Sullivan, *J. Chem. Soc.*, **45**, 41 (1884).

[8] O'Sullivan, *ibid.*, **59**, 1029 (1891); *ibid.*, **79**, 1164 (1901).

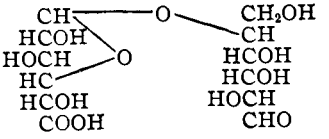
[9] Robinson, *ibid.*, **89**, 1496 (1906).

[10] Ref. 7, p. 45.

[11] Heidelberger and Goebel, *J. Biol. Chem.*, **74**, 613 (1927); *ibid.*, **74**, 619 (1927).

[12] Goebel, *ibid.*, **72**, 809 (1927).

galactonic. This substance was isolated and analyzed as the calcium salt. It gave a strong naphtho-resorcin test and on boiling with 12% hydrochloric acid liberated one molecular proportion of carbon dioxide. It did not reduce Fehling's solution. It is thus apparent that the uronic acid residue is intact in the oxidized acid and that the linkage between the two residues in the aldobionic acid is between the aldehyde of the glucuronic acid and a hydroxyl group of the galactose. Nothing, of course, is known as to which of the galactose hydroxyl groups enters into the glucosidic linking. The following formula is, therefore, purely hypothetical.



An interesting fact about this aldobionic acid is that, unless gum arabic is of bacterial origin, it is the first compound of this sort to have been found except in products of bacterial metabolism. It has been affirmed[13] and denied[14] that gums are of bacterial origin. Our results are interesting in this connection, though we do not offer them in evidence one way or the other.

Heidelberger, Avery and Goebel[15] have reported that occasional samples of gum arabic possess specific activity which they were able to enhance greatly by hydrolyzing until about 50% of the pentose contained in the gum was removed. The same authors have obtained an acid hydrolysis product of this gum which on analysis gave figures indicating that it was a somewhat impure aldobionic acid.[16] Since these investigators have shown that aldobionic acids are components of other specific carbohydrates,[11] the occurrence of this type of substance among the hydrolysis products of gum arabic does not seem surprising.

It has long been known that *d*-galactose and *l*-arabinose are formed on hydrolysis of this gum, or of arabic acid prepared from it.[17] Votoček and Vondraček[17c] have also reported the presence of *d*-glucose in the gum. Although the work done by us on the sugar fraction of the gum arabic hydrolysis product is not complete, the presence of *d*-galactose and *l*-arabinose has been confirmed, and the presence of the methylpentose,

[13] Smith, *Zentr. für Bakt.*, Abt. II, **10**, 61 (1903); *ibid.*, **11**, 698 (1904); *ibid.*, **15**, 380, 796 (1906); *J. Soc. Chem. Ind.*, **23**, 972 (1904); Prillieux and Delacroix, *Compt. rend.*, **118**, 1430 (1894); Aderhold, *Arbt. biol. Abt. Gesundheitsamt.*, **2**, 515 (1912).

[14] Rathay, *Zentr. für Bakt.*, Abt. II, **2**, 620 (1896); Sorauer, *Z. Pflanzenkrankh. Pflanzenschutz*, **25**, 71 (1915).

[15] Paper read before the Annual Meeting of the American Chemical Society, Philadelphia, September, 1926.

[16] Private communication.

[17] (a) Kiliani, *Ber.*, **13**, 2304 (1880); (b) *ibid.*, **15**, 34 (1882); (c) Votoček and Vondraček, *ibid.*, **37**, 3858 (1904).

rhamnose, has been established. We prepared arabic acid (the ash-free gum) according to the method of Neubauer,[18] and analyzed it for pentose and methylpentose by the furfural-phloroglucid method;[19] for galactose by oxidation and isolation of mucic acid:[20] for uronic acid carbon dioxide by the Lefevre method;[21] and for carbon and hydrogen. The results are shown in Table I. The figures correspond roughly to 1 molecule of aldobionic acid, 2 of galactose, 3 of arabinose and 1 of methylpentose.

TABLE I

RESULTS OF ANALYSES

Galactoso-glucuronic acid	28.3%
Hexose (as galactose)	29.5%
Pentose (as arabinose)	34.4%
Methylpentose (as rhamnose hydrate)	14.2%
Total	106.4%

Titration of the acid with sodium hydroxide solution gave an equivalent weight of 1030. Titration with alkali in hot and cold solution also showed that arabic acid exists to the extent of about 22% as lactone.

Experimental Part

Hydrolysis of Gum Arabic by Dilute Sulfuric Acid.—Five hundred grams of gum arabic, $[\alpha]_D^{25} = -34.05°$ (dry basis), containing 2.72% of ash and 9.89% of moisture was dissolved in one and one-half liters of water and to this solution was added a solution of 64 g. of sulfuric acid in a liter of water. The solution thus prepared contained arabic acid dissolved in 2% sulfuric acid. It was refluxed gently and the course of the hydrolysis was followed by polarimetric readings. When the observed rotation ($l = 1$) reached +9.4°, further heating caused but slight increase in rotation and the hydrolysis was considered to be complete. This required about twenty hours.

Isolation of Calcium Aldobionate.—An excess of calcium carbonate was then added, the mixture warmed for several hours on a water-bath and allowed to stand overnight. It was filtered and the precipitate washed with a little water. After two treatments with "Nuchar" a clear, pale yellow liquid was obtained. This was evaporated *in vacuo* to a sirup and a crude separation into acidic and non-acidic parts was made by addition of ethyl alcohol. Some colored impurities were separated by fractional precipitation from water with ethyl alcohol.[11]

Final purification was accomplished by several precipitations from a concentrated water solution by pouring with stirring into about 10 volumes of methyl alcohol. The yield of calcium salt was 44 g. An additional 10 g. of salt was obtained from the methyl alcoholic mother liquors (see page 1524) making the total yield 54 g.

This salt was quite soluble in water. A sample of 1.25 g. dissolved in water and made up to 25 cc. had an observed rotation of +0.18° in a 2-decimeter tube; $[\alpha]_D^{25} = +1.8°$. It reduced Fehling's solution and gave a strong naphtho-resorcin test.[22]

[18] Neubauer, *Ann.*, **102**, 105 (1857).

[19] Van der Haar, "Anleitung zum Nachweis, zur Trennung und Bestimmung der Monosaccharide und Aldehydesäuren," Gebrüder Borntraeger, Berlin, **1920**, p. 63.

[20] Ref. 19, p. 123.

[21] Ref. 19, p. 71.

[22] Ref. 2; ref. 19, p. 55.

Anal. Subs., 0.2437: CO_2, by Lefevre method, 0.0282. 0.2388 required 12.7 cc. of $N/10$ iodine for oxidation.[12,23] Subs., 0.1316: CO_2, 0.1830; H_2O, 0.0636. Subs., 0.9549: $CaSO_4$, 0.1872. Calcd. for $(C_{12}H_{19}O_{12})_2Ca$: CO_2, 11.7; $N/10$ iodine, 12.7 cc.; C, 38.33; H, 5.07; Ca, 5.33. Found: CO_2, 11.6; C, 37.91; H, 5.36; Ca, 5.76.

The alcoholic sugar solution resulting from the separation and purification of the calcium salt was saved for further investigation.

Free Aldobionic Acid.—Several attempts to prepare the free aldobionic acid were made. However, this acid has a strong tendency toward lactone formation. On evaporation of the solution obtained after removal of calcium from the salt by means of oxalic acid, a mixture of lactone and acid was obtained. This mixture—a white powder—was soluble in water and methyl alcohol.

On titrating 0.2000 g. with sodium hydroxide in the cold in the presence of phenolphthalein, 4.5 cc. of $0.1N$ sodium hydroxide was required. On warming the solution an additional 1.2 cc. of alkali was neutralized. These figures correspond to an equilibrium mixture of 79.5% of acid and 20.5% of lactone. The theoretical total titration for such a mixture is 5.6 cc. of $0.1N$ alkali.

Anal. Subs., 0.1411: CO_2, 0.2123; H_2O, 0.0730. Calcd. for 79.5% of $C_{12}H_{20}O_{12}$ and 20.5% of $C_{12}H_{18}O_{11}$: C, 40.95; H, 5.60. Found: C, 41.05; H, 5.78.

A 1.00-g. sample of calcium aldobionate dissolved in water, acidified with twice the calculated amount of hydrochloric acid and made up to 25 cc. had an initial observed rotation of $+0.19°$ in a 2-decimeter tube; $[\alpha]_D^{25} = +2.5°$ (calculated on the basis of free acid liberated). After seventeen hours the rotation was $[\alpha]_D^{25} = +1.71°$.

The Components of the Aldobionic Acid

The Identification of the Sugar.—The calcium was removed from about 1.0 g. of calcium aldobionate by the calculated amount of oxalic acid and the solution of free aldobionic acid was evaporated to dryness *in vacuo*. Fifty cubic centimeters of 1.0 *N* sulfuric acid was then added and the solution was refluxed gently for eighteen hours. The hydrolysis product was boiled with an excess of barium carbonate and after cooling was filtered. The filtrate was evaporated to dryness *in vacuo* and the residue extracted with alcohol, thus separating a small amount of alcohol-insoluble barium salt from the sugar formed on hydrolysis of the aldobionic acid. The alcoholic solution of the sugar was treated with a little Nuchar and evaporated to dryness. The observed rotation for $C = 0.6$ and $l = 2$ was $+0.94°$; $[\alpha]_D^{25} = +78.3°$; $[\alpha]_D$ (galactose) $= +80°$.

The sugar was submitted to oxidation with nitric acid. After cooling the reaction mixture and scratching the dish, fine crystals of mucic acid separated. The reaction mixture was allowed to stand overnight; the mucic acid was then filtered off, washed several times with saturated mucic acid solution and once with water and dried; m. p. 219–220° (corr.).

The Identification of the Acid Part of the Molecule.—The barium salt resulting from the hydrolysis above described was so crude and so small in amount that identification was impossible. A sample of aldobionic acid, prepared by decomposing 2.17 g. of the calcium salt with the calculated amount of oxalic acid, was, therefore, submitted to simultaneous hydrolysis and oxidation according to the method of Heidelberger and Goebel.[11] That mucic acid was not formed was shown by the fact that no insoluble dibasic acid separated out of the reaction mixture even after seeding with a few crystals of pure mucic acid. After neutralizing the concentrated solution with 50% potassium hydroxide solution, it was acidified with glacial acetic acid. After standing for several days in an ice box, 0.25 g. of crude acid potassium saccharate separated from solution. The material was recrystallized from 1.0 cc. of water, dried and analyzed.

[23] Willstätter and Schudel, *Ber.*, **51**, 780 (1918).

Anal. Subs., 0.1148: K_2SO_4, 0.0410. Calcd. for $COOH(CHOH)_4COOK$: K, 15.75. Found: K, 16.02.

The Stability of Galactoso-glucuronic Acid in 1 *N* Sulfuric Acid.—The very small amount of barium salt obtained by hydrolyzing galactoso-glucuronic acid with 1.0 *N* sulfuric acid indicated considerable decomposition of the aldobionic acid by the hot dilute sulfuric acid. An estimation of the extent to which this decomposition took place was obtained by weighing the carbon dioxide evolved during hydrolysis. A solution of 1.0 g. of calcium aldobionate in 50 cc. of 1.0 *N* sulfuric acid was refluxed gently for eighteen and one-half hours in the Lefevre carbon dioxide apparatus. The carbon dioxide evolved weighed 0.0520 g. Thus the aldobionate was decomposed to the extent of about 44%, since complete removal of carboxyl would yield 0.1173 g. of carbon dioxide.

Oxidation of the Aldobionic Acid to a Dibasic Acid.—A sample of galactoso-glucuronic acid was oxidized with barium hypo-iodite according to the method of Goebel.[12] Two grams of calcium aldobionate yielded about 1.5 g. of reprecipitated calcium glucurono-galactonate.

Anal. Subs., 0.5000: $CaSO_4$, 0.1681. Subs., 0.4000: CO_2, 0.0429 (Lefevre method). Calcd. for $C_{10}H_{18}O_9(COO)_2Ca$: Ca, 9.75; CO_2, 10.73. Found: Ca, 9.88; CO_2, 10.72.

Analysis of Arabic Acid.—This substance was prepared from gum arabic according to the method of Neubauer.[13] A sample of 0.9490 g. (dry basis) of acid containing 5.1% moisture made up to 25 cc. in water had an observed rotation of $-1.23°$ in a 1-decimeter tube; $[\alpha]_D^{25} = -32.36°$.

Anal. Subs. (dry), 0.1235: CO_2, 0.1978; H_2O, 0.0680. Found: C, 43.67; H, 6.15. Subs. (dry), 1.000: CO_2 (Lefevre method), 0.0353. Found: 3.53% of CO_2, equivalent to 14.1% of uronic acid anhydride. Subs. (containing 5.1% of moisture), 1.0537 (1.000 dry subs.) required 7.6 cc. of *N*/10 NaOH for neutralization at room temperature using phenolphthalein, and 9.7 cc. when titrated to a permanent end-point in hot solution. Equivalent weight based on hot titration, 1030. Subs., 0.2372: 0.0146 g. of alcohol-soluble phloroglucid and 0.0799 g. of alcohol-insoluble phloroglucid. From the latter figure was deducted 0.0112 g. to allow for the phloroglucid due to the uronic acid.[24] Found: methylpentose (as rhamnose hydrate), 14.2; pentose (as arabinose), 34.4. Subs., 0.9100, 0.9100: mucic acid, 0.2870, 0.2795.[25] Found: galactose, 42.9, 41.8, average, 42.4.

Preliminary Work on the Identification of the Sugars Formed on Hydrolysis of Gum Arabic

Galactose.—The presence of this sugar was shown both by analysis of arabic acid by the mucic acid method[24] and by isolation of the sugar. The methyl alcoholic mother liquors from the purification of the crude calcium aldobionate were concentrated to 100 cc. and poured into 350 cc. of methyl alcohol. An equal volume of ethyl alcohol was added and the mixture was allowed to stand to permit the precipitate to settle. An additional 10 g. of calcium salt was thus obtained.

The mother liquor from this precipitation was evaporated to about 75 cc.; it gave no further precipitate with methyl alcohol. The liquor was poured with stirring into 500 cc. of ethyl alcohol and a sirupy layer separated out. This became granular on grinding with absolute ethyl alcohol. The material, vacuum dried at 80°, was found to contain 1.07% of calcium, corresponding to 20.06% of calcium aldobionate. A

[24] Ref. 19, p. 75.

[25] Ref. 19, p. 123.

sample of 0.8762 g. made up to 25 cc. in water had an observed rotation of +4.33° in a 2-decimeter tube. If the small rotation of the aldobionate be neglected and allowance be made for 20% of (practically) inactive calcium salt, $c = 2.8$; $[\alpha]_D^{25} = +77.4°$; for galactose $[\alpha]_D = +80°$. On oxidation with nitric acid, mucic acid melting at 220° (corr.) was obtained. The substance was therefore impure galactose.

Arabinose and Rhamnose.—Practically pure arabinose of melting point 155–156° was isolated by the method of Anderson and Sands.[3] A sample of 1.00 g. made up to 25 cc. in water had an observed rotation of +8.16° in a 2-decimeter tube; $[\alpha]_D^{25} = +102°$.

The presence of both *l*-arabinose and rhamnose was demonstrated in the main bulk of mother liquor from the separation of crude calcium aldobionate after removal of two crops of crystals consisting of a mixture of pentose (probably arabinose) and hexose (probably galactose). A sample of the sirup was evaporated to dryness and treated with diphenylhydrazine according to Van der Haar.[26] A diphenylhydrazone separated, which after several recrystallizations melted at 210–202° (corr.), Van der Haar gives 204° as the melting point of pure arabinose diphenylhydrazone.[27]

A second sample of sirup was evaporated to dryness *in vacuo* and treated with *p*-bromophenylhydrazine, also according to Van der Haar.[28] A bright yellow *p*-bromophenylosazone was thus obtained which melted, after repeated washings with 90% ethyl alcohol and acetone, at 217–218°. The substance did not depress the melting point of a sample of pure rhamnose *p*-bromophenylosazone prepared from rhamnose which also melted at 217–218°. The observed rotation for $c = 2.47$ in a 20–80 pyridine–alcohol mixture and $l = 1$, was +1.0°; $[\alpha]_D^{25} = +40.5°$. The value found for rhamnose *p*-bromophenylosazone made from rhamnose was $[\alpha]_D^{25} = +0.95$ where $l = 1$ and $c = 2.34$; $[\alpha]_D^{25} = +40.6°$.

Anal. Subs., 0.2000: AgBr, 0.1510 by Stepanow's method. Calcd. for $C_{18}H_{20}O_3N_4Br_2$: Br, 31.96. Found: Br, 32.13.

Summary

1. Gum arabic and arabic acid prepared from this gum have been studied analytically and polarimetrically.

2. Rhamnose, *d*-galactose and *l*-arabinose have been identified in the sugar fraction of the hydrolysis product.

3. The acidic nucleus of the gum has been shown to be an aldobionic acid whose components are *d*-galactose and *d*-glucuronic acid.

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[26] Ref. 19, p. 249.

[27] Ref. 19, p. 178.

[28] Ref. 19, pp. 217, 262.