Pfeiffer[22] has extended Werner's ideas. He argued that combination takes place probably in a similar fashion in all cases of molecular-compound formation. Phenols and amines with nitro compounds would therefore be represented as $R.NO_2$——C_6H_5OH, and $R.NO_2$——$C_6H_5NH_2$. The fact that deeper colors are obtained in the case of the phenol and amine compounds is used as evidence that the hydroxyl and amine groups are present in the free state. Our results in Tables I, II and III show this variation in color very strikingly.

Thus Kendall's ideas based on principal valence offer a satisfactory explanation for phenol and amine compounds but not for the hydrocarbon compounds. The Werner-Pfeiffer ideas based on subsidiary valence offer an explanation for all molecular combinations. Neither view, however, lends itself satisfactorily to the explanation of the structure of molecular compounds in other than equimolecular ratios.

Summary

New molecular organic compounds of *m*-dinitrobenzene, 2,4-dinitrotoluene and 2,4-dinitrophenol have been isolated.

A hydrate of a molecular organic compound has been isolated.

The structure of these compounds has been discussed.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF ARIZONA]

THE COMPOSITION OF MESQUITE GUM; THE ISOLATION OF *d*-GALACTOSE AND *l*-ARABINOSE

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In two previous publications[1] the authors have described the occurrence, physical properties and partial analysis of mesquite gum as well as the preparation of *l*-arabinose from it. The present contribution deals with further studies as a result of which it is now possible to calculate the approximate composition of the gum.

The Composition of Mesquite Gum.—A. W. van der Haar[2] has described the general procedure for determining the composition of plant gums and closely related substances. His monograph has been used freely in the present investigation.

[22] Pfeiffer, *Ann.*, **412**, 253 (1917); *Z. anorg. Chem.*, **112**, 81 (1920); *Z. angew. Chem.*, **34**, 350 (1921).

[1] Anderson and Sands, (a) Some Plant Gums of the Southwestern U. S., *Am. J. Pharm.*, **97**, 589 (1925); (b) The Preparation of *l*-Arabinose from Mesquite Gum, *Ind. Eng. Chem.*, **17**, 1257 (1925).

[2] van der Haar, "Anleitung zum Nachweis, zur Trennung und Bestimmung der Monosaccharide und Aldehydesäuren," Gebrüder Borntraeger, Berlin, **1920.**

The presence in mesquite gum of *l*-arabinose, *d*-galactose and an aldehyde acid belonging to the glucuronic acid group was established by isolating these substances from the products of its hydrolysis. The absence of glucose, mannose and levulose was shown by the fact that when the gum is hydrolyzed by acids, the resulting sirup is not readily fermented by pressed yeast. The absence of methylpentoses was established by the fact that when the furfural-phloroglucid precipitate is treated with ethanol according to the method of Tollens-Ellett-Haywood[3] it does not lose weight. The official methods were used in determining moisture, ash, nitrogen and pentosans.[4] Our results on the determination of galactose by the official method were unsatisfactory. Van der Haar[5] has described a modification of the official method for this determination. We obtained best results by a combination of the two methods, although our results are probably low. However, they agree approximately with the percentage of galactose which we have repeatedly isolated after hydrolysis of the gum. The percentage of the aldehyde acid mentioned above was determined very accurately by the method of K. U. Lefèvre.[6]

Our results indicate that *l*-arabinose is the only pentose present in mesquite gum. Since acids of the glucuronic acid group yield furfural during the pentosan determination, the amount of furfural-phloroglucid corresponding to this acid must be deducted from the total furfural-phloroglucid precipitate before calculating the percentage of arabinose. Lefèvre[7] has shown that three parts of glucuronic acid lactone correspond to one part of furfural-phloroglucid. In the results given below, this correction has been made. The composition of mesquite gum calculated from our analytical results is as follows: moisture, 11%; ash, 2.13%; nitrogen, 0.7%, corresponding to 4.37% of protein;[8] *d*-galactose, 18.7%; carbon dioxide by the Lefèvre method, 3.25%, corresponding to 13% of an aldehyde lactone of the glucuronic acid group; *l*-arabinose, 50.7%; total, 99.9%. This leaves between 5 and 10% unaccounted for, since the results of van der Haar[9] indicate that in general the percentages of the various hydrolytic products amount to between 105 and 110% of the weight of gum used.

[3] (a) Browne, "Handbook of Sugar Analysis," John Wiley and Sons, **1912,** p. 458. (b) Ref. 2, pp. 67–69. (c) Ellett and Tollens, *Z. deut. Zuckerind.*, **42,** 19 (1905). (d) Haywood, *Bull.*, **105,** p. 112 (1907).

[4] "Methods of Analysis, A. O. A. C."

[5] Ref. 2, pp. 123–130; *Biochem. Z.*, **81,** 263 (1917); *Chem. Weekblad,* **13,** 1204 (1916).

[6] Van der Haar, Ref. 2, pp. 71–76. Abderhalden, "Biochemisches Handlexikon," **2,** 518 (1911). Lefèvre and Tollens, *Ber.*, **40,** 4513 (1907). Tollens, *Z. physiol. Chem.*, **61,** 95 (1909).

[7] Lefèvre, "Untersuchungen über die Glucuronsäure," *Dissertation,* Göttingen, **1907.** See also Ref. 6.

[8] The nitrogen may be present in non-protein form. This point was not investigated.

[9] Ref. 2, pp. 319, 326, 336.

The General Procedure for Separating the Constituents of Mesquite Gum.[10]—In order to isolate the three main constituents of mesquite gum we subject it to a process of successive hydrolysis. The arabinose is first separated by hydrolysis with 4% sulfuric acid at 80°, the acids are neutralized by calcium carbonate and the arabinose is separated from the calcium salts. These salts are next hydrolyzed in the autoclave or boiling water-bath by 3% sulfuric acid, the acids neutralized by calcium carbonate and the galactose is separated from the calcium salt of the aldehyde acid. The details of the procedure are given below.

The Isolation of *l*-Arabinose.—While the method previously described[1b] by the authors for isolating arabinose gives good results, the following modifications of it increase very appreciably the yield and purity of the product.

Hydrolyze the gum at 80° for six hours.[11] Neutralize the acids by careful addition of 140 g. of calcium carbonate,[12] instead of barium hydroxide. Heat the solution with an excess of calcium carbonate in a boiling water-bath for an hour to complete the neutralization. Filter off the calcium sulfate and wash it with hot water. Concentrate the filtrate in an evaporating dish on the boiling water-bath to a volume of approximately 650 cc.[13] Transfer the solution to a 3-liter flask. The total volume should be approximately 700 cc. To this solution add, during vigorous shaking, twice its volume of 95% ethanol. Decant the solution from the gummy salts and extract the latter thrice under a reflux condenser, each time with 500 cc. of boiling ethanol. In order to remove all the arabinose from the salts dissolve them in 100 cc. of water, transfer the solution to an evaporating dish, add 400 cc. of 95% ethanol and triturate with a pestle. Decant the alcoholic extract and triturate the salts twice more, each time with 300 cc. of ethanol. Combine all the alcohol extracts and add 95% ethanol as long as any appreciable precipitate forms.[14] Allow the solution to stand for some hours until it is clear, decant the alcohol solution of arabinose and concentrate it in a vacuum on the boiling water-bath to a thin sirup.[15] Crystallization usually begins as soon as the sirup is cold, although

[10] Van der Haar, Ref. 2, pp. 300, 304 and 323, discusses the hydrolysis of gums and mentions that substances containing glucuronic acid are often difficult to hydrolyze. Morrow ["Extracts from Biochemical Research Methods," Pre-Print, C. A. Morrow, **1925,** Part II, p. 43] states that arabinose is easily hydrolyzed off from pectins while galactose is hydrolyzed off with difficulty.

[11] Most of the arabinose is hydrolyzed off during the first three hours' heating. The longer heating is necessary only in case the highest yield of arabinose is desired or the salts are to be used later for the preparation of galactose. Very little galactose is hydrolyzed off by heating to 80° for six hours. However, some of this sugar is hydrolyzed off at 100° and seriously interferes with the crystallization of the arabinose.

[12] The solution is apt to foam over during neutralization. This may be prevented by adding to the foaming solution from time to time small amounts of butyl alcohol to break up the bubbles. This method of neutralization was suggested by Dr. H. T. Clarke and is superior to that previously described by the authors.

[13] Foaming of the solution renders it difficult to distil in a vacuum at this point.

[14] Appreciable amounts of organic salts dissolve during the extraction of the sugar and interfere seriously with the crystallization of the latter. Most of the salts are precipitated by addition of 95% ethanol, leaving the sugar in solution.

[15] If all the water is removed at this stage by heating the gum in a vacuum for some time the sugar will not crystallize readily.

it is sometimes necessary to seed with arabinose. After crystallization has begun add a small volume of 95% ethanol, being careful not to precipitate an appreciable amount of gum. Set the solution in the refrigerator to crystallize. Filter off the crystalline arabinose and wash it with ethanol. To secure further crops of arabinose, distil the solvent in a vacuum on the boiling water-bath, dissolve the gum in 100 cc. to 200 cc. of hot methanol,[16] let the solution cool, seed with arabinose if necessary, set in the refrigerator for some hours and filter off the crystals of arabinose. After the first two crops of crystals have been secured the organic salts that originally dissolved with the arabinose begin to interfere with the crystallization. If one is to secure either a large yield or a pure product it is necessary to remove these salts. To accomplish this, distil the solvent in a vacuum on the boiling water-bath, dissolve the residue in approximately 200 cc. of boiling methanol and add ethanol slowly, during shaking, to precipitate the salts. Let the solution cool, decant the sugar solution from the gummy salts and distil the solvent from the sugar. Dissolve the sugar in a small volume of hot methanol and crystallize as directed above. By repeating this process a total yield of 45 to 50% of arabinose can be obtained. The first two crops of crystals are easily obtained and amount to approximately 33% of the gum used. The melting point of the crude sugar varies from 147° to 152°.

The Purification of *l*-Arabinose.—The crude powdered sugar can be purified[17] by heating it in the boiling water-bath with one and one-half times its weight of glacial acetic acid,[18] filtering off the crystals, washing them with 95% ethanol and recrystallizing them from a mixture of their weight of water and four times their weight of 95% ethanol.

TABLE I

THE HYDROLYSIS OF MESQUITE GUM AT 80° BY 4% SULFURIC ACID[19]

Mesquite gum used	Cryst. arabinose obtd.		Gum residue from arabinose[20]		Calcium salts	
G.	G.	%	G.	%	G.	%
500	220	44	20	4	250	50.0
500	232	46.4	20	4	254	50.8
50	25.1	50.2	3.7	7.4	25.7	51.4
500	212	42.4	44	8.8	270	54

The Calcium Salts Obtained by Hydrolysis of Mesquite Gum at 80°.—These salts are isolated as described above and amount to approximately one-half the weight of the gum used. They are a mixture of salts and contain various amounts of sugar. The main body of the salts which is first precipitated by alcohol, can be readily converted into a

[16] Methanol purified by distillation over quick lime is the most satisfactory solvent for use in crystallizing arabinose from gummy mixtures. Ethanol at this stage usually causes the precipitation of a gum.

[17] The crude sugar can also be purified by crystallization from water as previously described by the authors, Ref. 1 b.

[18] Hudson and Dale, THIS JOURNAL, **39**, 322 (1917).

[19] The data in Table I are representative of numerous experiments with the improved method of hydrolysis.

[20] Some gummy residue always remains after crystallizing the arabinose. Determinations of pentosans, mucic acid and carbon dioxide indicate that this consists chiefly of arabinose mixed with some of the more soluble salts. No methylpentose is present in it.

granular form but the small amount isolated during the crystallization of the arabinose does not readily become granular. The salts are easily soluble in water. The analytical results vary with the sample used. The specific rotation, $(\alpha)_D$, in 2% water solution varies from +10° to +20°. When the salt is ignited the oxide remaining varies from 6.25 to 8.1%. The carbon dioxide determined by the method of Lefèvre[6] varies from 5.5 to 6%, corresponding to between 22 and 24% of an aldehyde lactone of the glucuronic acid group. The *l*-arabinose present, calculated from the pentosan and carbon dioxide determinations,[7] varies from approximately 6 to 11%. The galactose, calculated from the mucic acid determination, varies from 38 to 42%. No methylpentose is present. The variation in the composition of the salt is undoubtedly due to incomplete hydrolysis of the gum and to incomplete removal of all the arabinose previously hydrolyzed off. The salt undoubtedly consists chiefly of the calcium salt of an aldehyde acid of the glucuronic acid group with which *d*-galactose is combined.

The Isolation of *d*-Galactose.—Dissolve the calcium salts obtained by the hydrolysis of mesquite gum at 80° in six times their weight of water.

Add enough sulfuric acid to precipitate all of the calcium and in addition leave a 3% solution of sulfuric acid. Heat the solution in an autoclave for eight hours at 1 atmosphere gage pressure or for 40 hours in the boiling water-bath.[21] Neutralize the

TABLE II

HYDROLYSIS OF THE CALCIUM SALTS IN THE AUTOCLAVE AT 1 ATM. GAGE PRESSURE

Calcium salts used[22] G.	H_2SO_4 concn., %	Galactose obtained[23] G.	Galactose obtained[23] %	Calcium salts obtained[24] G.	Calcium salts obtained[24] %	Non-crystalline gum from galactose[25] G.
50	2	16.2	16.2	13	13	14
50	2	17.2	17.2	11	11	14
50	3	18	18	8	8	14
50	4	17	17	8	8	14
23	2	8.2	18			
450	4	143	15.8			44

[21] The drastic treatment necessary to hydrolyze off the galactose decomposes a part of the aldehyde acid.

[22] Since 100 g. of mesquite gum yields approximately 50 g. of the salts, it is necessary only to double the amount of the salts used in order to obtain the corresponding weight of mesquite gum.

[23] The percentage of galactose and salts obtained is calculated on the weight of mesquite gum originally used.

[24] Decomposition of the aldehyde acid is least in 2% sulfuric acid and most in 4% sulfuric acid. Apparently, 3% sulfuric acid causes equally complete hydrolysis and less decomposition than 4% acid.

[25] Analysis of the non-crystalline gum indicates that it contains no methylpentose, approximately 10% of galactose and small amounts of arabinose and of the aldehyde acid.

acids with an excess of calcium carbonate as previously described, filter off the calcium sulfate and wash it with hot water. Clarify the solution with carbon and concentrate it in a vacuum on the boiling water-bath to a thin sirup. Add to this sirup approximately five times its volume of 95% ethanol, shake thoroughly, let stand until no longer turbid, and decant the sugar solution from the calcium salts. Dissolve the latter in a very small volume of hot water, transfer to a large evaporating dish, add 95% ethanol as long as a precipitate forms, triturate with a pestle and decant the clear sugar solution from the salts. Triturate the latter twice more with ethanol until they are granular. Filter off the salts and dry them on a porous plate. Combine all the alcohol solutions of the sugar, mix well, let stand until clear, decant the sugar solution from the gummy residue and distil the alcohol in a vacuum on the boiling water-bath. Dissolve the sugar in hot glacial acetic acid, let cool, seed with galactose if necessary and set in the refrigerator for 48 hours. Filter off the galactose, wash it with 95% ethanol and dry. Distil the solvent from the filtrate, in a vacuum on the boiling water-bath. Dissolve the residue in glacial acetic acid and secure a second crop of *d*-galactose. The yield of crystalline galactose is approximately 18% of the mesquite gum originally used. Its melting point is 154° to 156° and its specific rotation $(\alpha)_D$ in 2% water solution after treatment with a drop of ammonia is approximately +77°.

Purification of *d*-Galactose.[26]—Dissolve 100 g. of crude, crystalline *d*-galactose, m. p. 154° to 156°, in 100 cc. of hot water, add 100 cc. of 95% ethanol, clarify with charcoal, filter hot, add 300 cc. of 95% ethanol to the filtrate, seed with galactose and let stand in the refrigerator for some days. The sugar crystallizes slowly. The resulting crystals melt at 162° to 164°. In 2% water solution, after treatment with a drop of ammonia, they show $(\alpha)_D = +80°$, the known value for pure *d*-galactose.

The Calcium Salts Resulting from the Hydrolysis in the Autoclave at One Atmosphere Gage Pressure.—These salts are obtained as described above. They readily reduce Fehling's solution in the cold, and when heated with hydrochloric acid yield furfural and carbon dioxide. These facts indicate the presence of an aldehyde acid belonging to the glucuronic acid group. When the percentage of this acid is determined by the method of Lefèvre, and also from the amount of furfural-phloroglucid, the two results agree very closely. On oxidation of the salts by nitric acid, no mucic acid is produced. The substance is therefore not galacturonic acid. Work on the aldehyde acid is being continued and will be reported later.

Summary

Mesquite gum was hydrolyzed by sulfuric acid and the resulting products were found to be 50.7% of *l*-arabinose, 18.7% of *d*-galactose and 13% of an aldehyde acid belonging to the glucuronic acid group. These three products together with the moisture, ash and a small amount of nitrogenous material account for nearly all of the gum.

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[26] Tollens and Stone, *Ber.*, **21**, 1572 (1888). Ref. 3 a, p. 603. Abderhalden, "Handbuch d. bio. Arbeitsmethoden," **1922**, Abt. 1, Teil 5, p. 285.