[CONTRIBUTION FROM THE WOOD CONVERSION LABORATORY OF THE UNIVERSITY OF IDAHO]

The Constitution of Mesquite Gum. II. Partial Hydrolysis of Mesquite Gum

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The preceding paper[1] showed that the polysaccharide known as Mesquite Gum consisted of *l*-arabinose, *d*-galactose and methoxy-*d*-glucuronic acid in the molar ratio of 4:2:1. One of the arabinose units was found to be terminal in character while the remainder were united at the first and second carbon atoms. All pentose units were present in the furanose form and therefore should be considerably more susceptible to acid hydrolysis than the other components of the gum. Previous studies by Anderson and co-workers[2] have shown this to be the case although quantitative data were not provided.

The present experiments were conducted in order to determine conditions for removal of the arabinose component of the gum with minimum concomitant hydrolysis of the more resistant portion. A number of preliminary trials served to establish a suitable reaction procedure. The crude gum was dissolved in water, filtered to remove extraneous material, acidified with sulfuric acid and heated on a water-bath. Samples of the hydrolyzing solution were removed at intervals and separated into fractions representing hydrolyzate and residual polysaccharide. Each fraction was analyzed for solids and for furfural, thus providing data for calculation of arabinose removal and concomitant hydrolysis of the other components of the gum. The results are given in Table I and are represented graphically in Fig. 1.

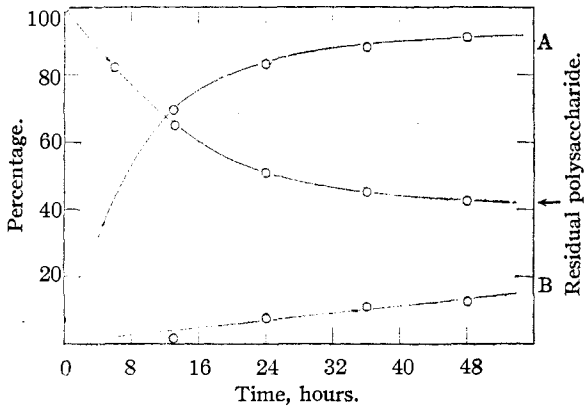

Fig. 1.—Hydrolysis of mesquite gum (acidity 0.15 *N* H_2SO_4; temp. 92°).

Hydrolysis of the arabinose component proceeds rapidly in 0.15 *N* acid (Curve A). The reaction is apparently first order. Simultaneously a slow hydrolysis of the more resistant portion of the molecule proceeds regularly (Curve B) and reaches 10% when 90% of the arabinose fraction has been removed. The residual polysaccharide remains of relatively high molecular weight and can be separated readily from the hydrolyzate by dialysis. It seems reasonable to conclude that the four arabinose units form a branch or tail attached to the remainder of the polysaccharide. This branch is of course terminated by a residue of arabofuranose and the individual units are joined through their first and second carbon atoms.[1]

As an extension of the study, the residual polysaccharide was examined in order to determine the position of linkage of the araban chain in the original gum. Thus, methylation of the hydrolysis resistant residue replacing available hydroxyl groups with methoxyl, including those newly formed by removal of the arabinose component, followed by methanolysis should give rise to trimethyl-methyl-galactoside. This fraction previously occurred as dimethyl-methyl-galactoside in the methanolysis products from the ether derivative of the original gum. Accordingly, the residual polysaccharide after thirty-six hours of hydrolysis was separated, treated with methyl sulfate and alkali and subjected to methanolysis. The resulting sirup, separated in the usual manner, furnished trimethyl-galactoside and this was analyzed for its isomeric components by a previously described method.[3] The relative proportion of these, namely, 63% of the 2 3,4-trimethyl derivative and 37% of the 2,4,6-isomer seemingly would not permit a clear decision as to the point of attachment of the araban chain. However, four arabinose residues must undergo hydrolysis on the average to furnish one new hydroxyl group in the more resistant portion of the repeating unit. The hydrolysis ratio of arabinose to residue determined by experiment as 9:1 therefore becomes 2.25:1 for araban to residue. The greater proportion of the 2,3,4-trimethyl isomer over the corresponding 2,4,6-derivative of the trimethyl-methyl-galactoside fraction thus definitely favors attachment of the araban chain at the three position of a galactose residue.

Experimental

Partial Hydrolysis of Mesquite Gum.—Two hundred grams of crude mesquite gum was dissolved in 1000 cc. of water and filtered to remove sand, seeds, bark, etc. The solution was then made 0.15 *N* with sulfuric acid and heated upon a water-bath at 92°. Samples of the hydrolyzing solution were removed at intervals, cooled, and a 10-cc. portion added slowly to rapidly stirred ethyl alcohol 0.2 *N* in sulfuric acid. The precipitate was settled in the centrifuge and the liquor decanted. The latter was titrated with barium hydroxide to the thymol blue endpoint, separated from barium sulfate and evaporated to 100 cc. volume. The precipitate was dissolved in water

(1) White, THIS JOURNAL, **68**, 272 (1946).

(2) Anderson and Otis, *ibid.*, **52**, 4461 (1930).

(3) White, *ibid.*, **64**, 2841 (1942).

and treated in a similar manner. Each solution was analyzed for solids and for furfural distilled by Tollens' method. The results are given in Table I together with the calculated percentage hydrolysis of the araban component and concomitant hydrolysis of the more resistant residue.

TABLE I

PARTIAL HYDROLYSIS OF MESQUITE GUM[a]

Acidity, 0.15 N H_2SO_4; temp., 92°

Time, hr.	Residue, g.	Residue, % Furfural	Hydrolyzate, g. Solids	Hydrolyzate, g. Arabinose	Percentage hydrolysis Arabinose	Percentage hydrolysis Residue
0	100	25.3				
6	82.0	23.2	21.2			
13	65.0	19.7	36.0	35.2	69.5	1.7
24	50.7	13.8	46.0	42.2	83.0	7.8
36	45.1	11.2	50.0	44.6	88.0	11.0
48	42.5	10.3	52.6	46.0	91.0	12.4

[a] Arabinose content 50.7%.

Separation of Residual Polysaccharide.—The above experiment showed that 90% of the araban component of mesquite gum could be removed by controlled hydrolysis with but a 10% change in the more resistant residue. Accordingly, in a separate experiment, a similarly prepared solution of the gum was hydrolyzed for thirty-six hours. The solution was then cooled, dialyzed against running water for two days to remove sulfuric acid, arabinose, etc., filtered with Super-Cel and evaporated to a sirup: yield 72 g.

Methylation of Residual Polysaccharide.—The residual polysaccharide sirup was treated in the usual manner with methyl sulfate and 30% hydroxide at 25°. The reagents were added simultaneously and dropwise with vigorous stirring over a period of three hours using 200 cc. of methyl sulfate and 400 cc. of alkali. After complete neutralization of the methyl sulfate, the reaction mixture was dialyzed to remove salt and excess alkali, filtered and distilled under reduced pressure to a sirup. After four similar treatments methylation was complete and the product was isolated as a friable resin by allowing the sirup to evaporate to dryness; yield 68 g. Found: OCH_3, 32.0.

This product proved to be a salt of the uronic acid component, soluble in water and alcohol but insoluble in other solvents. The free acid liberated therefrom could not be extracted readily and the salt was used directly in the following experiment.

Methanolysis of the Methylated Residual Polysaccharide and Separation of Trimethyl-methyl-galactoside.—Fifty grams of the methylated salt of the residual polysaccharide was dissolved in anhydrous methanol and methanol-hydrogen chloride added to furnish a 20% solution of the gum in 1 N acid. The precipitated inorganic salt was removed by centrifuging and the liquor, sealed in a glass tube, was heated at 100° for six hours in a rocking furnace. After cooling, the reaction was neutralized with silver carbonate, filtered and evaporated to a sirup. The uronic ester components were removed by saponification with barium hydroxide at 60° for two hours, followed by carbonation, filtration, evaporation to dryness and ether extraction of the glycosidic portion. The latter was distilled fractionally under high vacuum yielding mainly trimethyl-methyl-galactoside and dimethyl-methyl-galactoside together with a small quantity of trimethyl-methyl-arabinoside. The trimethyl-methyl-galactoside fraction was redistilled; yield, 6.8 g.

Anal. Calcd. for $C_{10}H_{20}O_6$: OCH_3, 52.5. Found: OCH_3, 52.4.

Analysis of Trimethyl-methyl-galactoside and Identification of the Components.—Five grams of trimethyl-methyl-galactoside from the above distillate was dissolved in 50 cc. of N sulfuric acid and heated on a boiling water-bath for nine hours. The hydrolysis products were separated in the usual manner and the free sugars, after ether extraction, were obtained as a sirup; yield 4.6 g.

Anal. Calcd. for $C_9H_{18}O_6$: OCH_3, 41.9. Found: OCH_3, 41.8.

Three grams of the free sugars was dissolved in 15 cc. of pyridine and treated with 4.0 g. of trityl chloride. After two days at room temperature a small quantity of water was added to dissolve pyridine hydrochloride and the reaction poured into rapidly stirred ice water. Following two days in the refrigerator and occasional stirring, the precipitate was filtered, washed, dissolved in acetone, dried and evaporated to a sirup; yield, 5.3 g. Found: OCH_3, 12.1. A sample, treated with aniline in the usual manner, furnished the anilide of 2,3,4-trimethyl-6-trityl galactose; m. p. 152°,[4] recrystallized from alcohol.

The filtrate from the tritylation reaction was neutralized with silver carbonate and filtered. Silver ion was removed as sulfide and the solution evaporated to dryness. The sirup was taken up in chloroform, filtered and excess solvent removed; yield, 0.9 g. Found: OCH_3, 41.9. A sample treated with aniline in the usual manner gave the anilide of 2,4,6-trimethyl-galactose; m. p. 178°,[5] recrystallized from the ether–ethanol.

The proportion of the two isomers in the original sirup was calculated on the basis of yield and methoxyl content, giving 63% of 2,3,4-trimethyl-methyl-galactoside and 37% of the 2,4,6-derivative.

Summary

1. The experimental conditions for preferential hydrolysis of the arabinose component of mesquite gum have been established.

2. Examination of the hydrolysis resistant residue by the methylation–methanolysis technique indicates that the araban fraction of the gum is attached to the remainder of the polysaccharide at the three position of a galactose anhydride unit.

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(4) White, *ibid.*, **64**, 1510 (1942).

(5) Percival and Somerville, *J. Chem. Soc.*, 1615 (1937).