THE STRUCTURE OF ACACIA SUNDRA GUM

Part II. The Structure of the Degraded Gum

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It was shown in Part I\(^1\) that the Acacia sundra gum is composed of the following sugar residues, D-galactose, L-arabinose, L-rhamnose, D-glucuronic acid and a trace of another sugar probably xylose. Autohydrolysis of the gum removes L-rhamnose and most of L-arabinose residues with the formation of a degraded gum which is composed of D-galactose and D-glucuronic acid residues. Hydrolysis of the degraded gum with 0.1 N acid gave D-galactose, the aldobiouronic acid, 6-β-D-glucuronosyl-D-galactose and a small amount of L-arabinose which was probably present as an impurity.

This communication deals with the examination of the methylated sugars formed on hydrolysis of the methylated degraded gum. On the basis of the identification of these sugars and determination of their weights a tentative structure has been suggested for the degraded gum.

The degraded gum, prepared by autohydrolysis, was methylated first with dimethyl sulphate and then with silver oxide and methyl iodide. Methanalysis of the resulting methylated degraded gum yielded the methyl glycosides of methylated methyl glucuronate and galactose, which were separated as formerly described.\(^2\) The acid component, 2, 3, 4-tri-O-methyl-D-glucuronic acid (4 mol. props.) was identified as methyl 2, 3, 4-tri-O-methyl-D-glucarate-1, 5-lactone whereas the neutral components after hydrolysis were shown by paper chromatography to consist of 2, 3, 4-tri-(6 mol. props.), 2, 4, 6-tri-(1 mol. prop.) and 2, 4-di-O-methyl-D-galactose (3 mol. props.), all three of which were transformed into their characteristic aniline derivatives.

Since the equivalent weight of the degraded gum was 570 (calculated from the barium content of the barium salt of the degraded gum) it is suggested, in agreement with the above results, that the average repeating unit of the gum consists of about 10 units of D-galactose and 4 units of D-glucuronic acid.
The isolation\(^1\) of 6-\(\beta\)-D-glucuronosyl-D-galactose shows that the uronic acid units are joined to galactose by 1→6 bonds. Now if it be assumed that all of the aldobiouronic acid residues are linked in this manner and that they constitute side-chains, the formulation shown in I, which is analogous to that proposed\(^8\) for degraded arabic acid, may be put forward as one of a number of possible structures for the average repeating unit of degraded *sundra* gum acid.

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\begin{align*}
D-\text{Gal} & \xrightarrow{3} 6 \quad D-\text{Gal} & \xrightarrow{3} 6 \quad D-\text{Gal} & \xrightarrow{3} 6 \quad D-\text{Gal} & \xrightarrow{3} 6 \quad D-\text{Gal} \\
\uparrow & \quad \uparrow & \quad \uparrow & \quad \uparrow & \quad \uparrow \\
1 & \quad 1 & \quad 1 & \quad 1 & \quad 1 \\
D-\text{Gal} & \quad D-\text{Gal} & D-\text{Gal} & D-\text{Gal} & D-\text{Gal} \\
6 & \quad 6 & \quad 6 & \quad 6 & \quad 6 \\
\uparrow & \quad \uparrow & \quad \uparrow & \quad \uparrow & \quad \uparrow \\
1 & \quad 1 & \quad 1 & \quad 1 & \quad 1 \\
D-\text{GpA} & \quad D-\text{GpA} & D-\text{GpA} & D-\text{GpA} & D-\text{GpA}
\end{align*}
\]

*D-Gal* = D-Galactopyranose residue.


Neither the distribution of the aldobiouronic acid side-chains along the main galactose chain nor the position of their attachment to this chain is known, but it is apparent that the galactose units at which branching occurs correspond to those which give rise to 2, 4-di-O-methyl-D-galactose and consequently they are linked through C\(_1\), C\(_3\) and C\(_6\). The aldobiouronic acid side-chains may be attached either to C\(_3\) as shown in (I), or to C\(_6\), and it is also possible that both these structural features are present.

Of the remaining galactose units of the main chain it is evident that one is joined through C\(_3\) and the other two through C\(_6\); these postulations follow from the isolation of the 2, 4, 6-tri-O-methyl-D-galactose, and the remainder of the 2, 3, 4-tri-O-methyl-D-galactose not derived from the aldobiouronic acid side-chains. The presence of 1→3 linked D-galactose residues is supported by the previous observation\(^1\) that the gum contains galactose units which survive periodate oxidation.

**Experimental**

Unless otherwise stated, solutions were concentrated at 40–50\(^\circ\) under reduced pressure; paper chromatographic separations were carried out by descending method on Whatman No. 1 paper using 1-butanol: ethanol: water (5: 1: 4, by volume—upper layer) as the irrigating solvent and *p*-anisidine phosphate\(^4\) spray reagent was used to detect the sugars and uronic acids,
Isolation of barium salt of the degraded gum.—A solution of the purified gum (25 gm.) in water (500 ml.) was heated on a boiling water-bath for 80 hours. The hydrolysed solution was cooled and neutralised (barium hydroxide), filtered and evaporated to a thin syrup. It was then poured with stirring into methanol (500 ml.) and the precipitated material was washed with aqueous methanol and finally with methanol. It was dried in vacuum and the material so obtained was barium salt of the degraded gum (Ba 10·8%, yield 13 gm.). It reduced Fehling’s solution.

Methylation of the degraded gum.—To a solution of barium salt of the degraded gum (13 gm.) in water (50 ml.) dimethyl sulphate (75 ml.) was added followed by sodium hydroxide (225 ml., 30%), added dropwise during 8 hours with stirring. After stirring for another 12 hours, the solution, which was non-reducing to Fehling’s solution, was filtered and neutralised (sulphuric acid) when the partially methylated degraded gum precipitated out. It was again methylated by dissolving it in sodium hydroxide (200 ml., 30%) and adding dimethyl sulphate (105 ml.) as in the previous manner. The reaction mixture was acidified (sulphuric acid, acidic to congo red) when the methylated product precipitated out. It was dissolved in chloroform and filtered. The extract was methylated twice by the Purdie method to give a yellowish brown solid (8·9) (OCH₃ 42·4%). Its methoxyl content did not increase on further methylation.

Hydrolysis of the methylated degraded gum.—The methylated degraded gum (8·9 gm.) was dissolved in methanolic hydrogen chloride (180 ml.; 6·5%) and refluxed for 10 hours, cooled, neutralised (silver carbonate), filtered and the residue washed with methanol. The filtrate and washings were evaporated and the resulting syrup (9·2 gm.) was saponified with barium hydroxide (100 ml.; 0·3 N) at 55° for 2 hours. Excess of barium hydroxide in the solution was neutralised by passing carbon dioxide and the solution concentrated to a syrup. It was again saponified in the above manner, neutralised (carbon dioxide), filtered and the filtrate evaporated to dryness. Traces of moisture were removed by adding absolute alcohol repeatedly and distilling off the alcohol. The dried substance was extracted with ether. Fraction insoluble in ether was dissolved in water (150 ml.) and extracted with chloroform in a liquid-liquid extractor. Ether and chloroform extracts on evaporation gave a syrup (A) (5·8 gm.). The aqueous solution left after chloroform extraction was acidified (sulphuric acid, acidic to congo red) and again extracted with chloroform in a liquid-liquid extractor. The chloroform extract was evaporated to a syrup (B) (2·7 gm.).

Syrup (A) was hydrolysed (N hydrochloric acid, 100 ml.) by heating at 95–98° for 10 hours. The solution was neutralised (silver carbonate) and
filtered, and silver ions were removed from the filtrate by passage of hydrogen sulphide and filtration of precipitated silver sulphide. The filtrate was evaporated to a syrup (C) (5.7 gm.) and traces of moisture were removed from it by adding absolute alcohol repeatedly and distilling off the alcohol. Syrup (B) was hydrolysed (N hydrochloric acid, 75 ml.) in the same manner as described above and working up in the same way it yielded a syrup (D) (2.55 gm.).

Examination of syrup (C).—Syrup (C) which was a mixture of methylated sugars was examined on a paper chromatogram, when four spots were observed. The first one, very faint, red in colour and close to the origin, was due to a trace of uronic acid in the mixture. The second, third and fourth were yellow in colour and had R_G values 0.41, 0.62 and 0.68 respectively. These three fractions were separated on large sheets of filter-paper and the sugar-containing strips were eluted with water by Dent's method. The eluted solutions were concentrated separately to give the following fractions:

Fraction I.—(56.8 mgm.) _R_G_ 0.41; _OCH_3 29.1% calculated for di-O-methyl-D-galactose _OCH_3 29.8%. Its aniline derivative was prepared in the usual way and recrystallised from alcohol, m.p. 207° reported m.p. of 2, 4-di-O-methyl-N-phenyl-D-galactosylamine is 204–16°. A portion of the sugar was demethylated with hydroiodic acid (48% w/w) and worked up in the usual way. On paper chromatographic examination the demethylated product gave a spot corresponding to D-galactose together with other spots of higher _R_G_ values corresponding to methylated D-galactoses. The sugar was oxidised with periodic acid; the oxidate on treatment with dimeredone solution gave the crystalline dimeredone derivative of formaldehyde, m.p. and mixed m.p. 188°. It shows the presence of free hydroxyl group at C_6.

Fractions II.—(119.4 mgm.) _R_G_ 0.62; _OCH_3 40.9% calculated for tri-O-methyl-D-galactose, 41.9%. Its aniline derivative was prepared in the usual way and recrystallised from alcohol, m.p. and mixed m.p. with 2, 3, 4-tri-O-methyl-N-phenyl-D-galactosylamine 166°. Paper chromatographic examination of demethylated sugar gave spots of D-galactose and partially demethylated derivatives of 2, 3, 4-tri-O-methyl-D-galactose.

Fraction III.—(17.4 mgm.) _R_G_ 0.68. Its aniline derivative was prepared in the usual way and recrystallised from alcohol, m.p. and mixed m.p. with 2, 4, 6-tri-O-methyl-N-phenyl-D-galactosylamine 180°; _OCH_3 32.2%, calculated for tri-O-methyl-N-phenyl-D-galactosylamine 31.3%. The aniline derivative was demethylated and simultaneously hydrolysed with
hydroiodic acid (48% w/w) and worked up in the usual way. It gave spots of D-galactose and partially demethylated derivatives of 2, 4, 6-tri-O-methyl-D-galactose on a paper chromatogram.

Examination of syrup (D).—It was examined on paper chromatogram using 1-butanol : acetic acid : water (4 : 1 : 5, by volume—upper layer) as the irrigating solvent. It showed one main spot, \( R_G \) 0.82 (reported \( R_G \) 0.84 for 2, 3, 4-tri-O-methyl-D-glucuronic acid)\(^{13} \) and traces of two slower moving components \( R_G \) 0.73 and 0.61 respectively which gave red spots on paper chromatogram. The syrup (D) (OCH\(_3\) 38.4%; calculated for tri-O-methyl hexuronic acid OCH\(_3\) 39.5%) was oxidised with bromine and identified as methyl 2, 3, 4-tri-O-methyl-D-glucuronate-1, 5-lactone, (\( \alpha \)) \( D^{28} + 54^\circ \) (after 24 hours in methanol, C. 1%), m.p. 106\( ^\circ \) by the method described in the previous paper.\(^1 \)

**SUMMARY**

The degraded gum forms the basic nucleus of the whole gum; during autohydrolysis the labile sugar residues which are attached glycosidically to the main structure are removed and the degraded gum is obtained. The degraded gum is reducing in nature and consists of a repeating unit of ten D-galactose residues and four D-glucuronic acid residues. Methylation and subsequent hydrolysis of the resulting methyl ester of the methylated degraded polysaccharide furnished 2, 3, 4-tri-(6 mol. props.); 2, 4, 6-tri-(1 mol. prop.) and 2, 4-di-O-methyl-D-galactose (3 mol. props.) and 2, 3, 4-tri-O-methyl-D-glucuronic acid (4 mol. props.). On the basis of the identification of the methylated derivatives and their respective molecular proportions a tentative structure has been suggested for the degraded gum.

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**REFERENCES**

The Structure of Acacia sundra Gum—II

6. Dent, C. E. 

7. Hirst, E. L. and Jones, J. K. N.

   Ibid., 1953, 1631.

9. Smith, F.
   Ibid., 1939, 1737.

    Ibid., 1950, 1702.

11. Reeves, R. E.

12. Edington, R. A. and Percival, E.