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The Structure of *Salmalia Malabarica* Gum*. Part III. A Tentative Structure from Methylation Studies

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Methylated *Salmalia malabarica* gum on hydrolysis has been found to yield 2, 3, 4, 6-tetra-, 2, 6-di-, and 2,4-di-*O*-methyl-D-galactose and 2,3,5-tri- and 2,5-di-*O*-methyl-L-arabinose. A probable structure for the gum has been suggested in the light of these and previous results.

Previous investigations[1] of *Salmalia malabarica* gum have shown that the gum is composed of D-galactose, L-arabinose, and D-galacturonic acid residues together with traces of rhamnose. Graded hydrolysis of the gum furnishes an aldobiouronic acid and a degraded gum, the structure of which have already been reported[1]. In the present communication, methylated sugars, obtained from hydrolysis of the methylated *Salmalia malabarica* gum, have been examined and their significance on the structure of the gum has been discussed. The structure of aldobiouronic acid, obtained earlier[1] from the gum, has been further confirmed by reducing its fully methylated derivative.

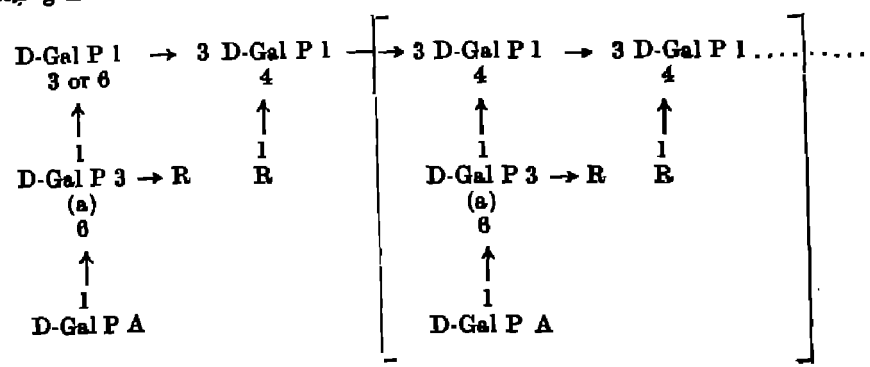
Reduction of the fully methylated aldobiouronic acid with sodium borohydride furnished a disaccharide which on mild acid hydrolysis yielded 2,3,4-tri-*O*-methyl-D-galactose. The sugar was identified by preparing its crystalline anilide, m.p. 164°. Isolation of a single methylated galactose residue, namely, 2,3,4-tri-*O*-methyl-D-galactose, confirms that the uronic acid moiety of the aldobiouronic acid is D-galacturonic acid and is linked through C_6 of another D-galactose residue.

Purified *Salmalia malabarica* gum was converted to its fully methylated derivative. The methylated polysaccharide was hydrolysed and the hydrolysate after treatment with barium hydroxide was separated into ether-soluble fraction (A) and ether-insoluble residue (B). The neutral methylated sugars present in (A) were fractionated chromatographically on Whatman No. 3 MM filter paper sheets to reveal presence of six components of which the following five were identified: 2,3,4,6-tetra-*O*-methyl-D-galactose, 2,6-di-and 2,4-di-*O*-methyl-D-galactose, 2,3,5-tri-*O*-methyl-L-arabinose, and 2,5-di-*O*-methyl-L-arabinose. The remaining sugar residue, which was obtained in a very small amount, appeared to be a mono-*O*-methyl-D-galactose from its rate of migration, but it could not be identified definitely. The extremely poor yield of mono-*O*-methyl-D-galactose excludes its possibility as a major structural unit of the gum. Fraction (B) consisting of barium salt of methylated uronic acid was made barium free and characterised as 2,3,4-tri-*O*-methyl-D-galacturonic acid from its rate of movement on the paper chromatogram and by preparing the corresponding crystalline α-methylpyranoside amide, m.p. 152°.

*N.S.I. communication No. 16 on the structure of plant polysaccharides.

1. Bose and Dutta, this *Journal*, 1963, **40**, 257, 557.

A quantitative estimate of the ratio of the methylated sugar moieties present in the methylated gum was not attempted as the hydrolysis of the methylated gum was accompanied by considerable decomposition and the yields of pure sugars isolated were rather low as a result of number of steps involved in their separation. The existence of all the methylated sugar moieties can, however, be accommodated by advancing a provisional partial structure (I) for the *Salmalia malabarica* gum after taking into consideration the structure of the degraded *Salmalia malabarica* gum[1] which consists of a framework of D-galactopyranose residues with main chain linked 1 → 3 and with side chain of the aldobiouronic acid attached by 1 → 4 linkage. The absence of tri-*O*-methyl-D-galactose residues in the cleavage products of methylated *Salmalia malabarica* gum shows that the labile sugar residues are attached in the form of side chains R, as shown in structure (I), to those galactose residues in degraded *Salmalia malabarica* gum which give rise to 2,3,4-tri-*O*-methyl-D-galactose and 2,4,6-tri-*O*-methyl-D-galactose on hydrolysis of the methylated degraded gum.



(I)

R = L-Araf 1 ...., D-Gal P 1, —→ 3L-Araf 1 ....
L-Araf 1 —→ 3L-Araf 1 ....
GalP = Galactopyranose.
Araf = Arabofuranose.
GalPA = Galacturonic acid.

The residues 'R' are obviously composed of those sugars which are eliminated from the stable nucleus of the degraded *Salmalia malabarica* gum during mild acid hydrolysis. The isolation of 2,3,5-tri-*O*-methyl-L-arabinose in a greater yield than 2,3,4,6-tetra-*O*-methyl-D-galactose indicates that most of the side chains R are terminated by L-arabofuranose units, whereas a few of them are terminated by D-galactopyranose units. This conclusion is supported by the fact that prolonged autohydrolysis of the gum liberates both L-arabinose and D-galactose, contrary to L-arabinose alone, as reported previously[1]. The occurrence of 2,3,4-tri-*O*-methyl-D-galacturonic acid in the cleavage product of the methylated gum indicates that it is present as the terminal residue in the gum and no other substituent is attached to it. A portion of arabinose units in the methylated *Salmalia malabarica* gum appears as 2,5-di-*O*-methyl-L-arabinose in the products of hydrolysis. This shows that some of the arabinose residues are interposed between the terminal groups of side chains R and the nucleus of the degraded gum. The point of attachment of these 'R' residues with the main-chain galactose residues is not yet known, but it may be assumed

that these are linked to the main chain in the same manner as the aldobiouronic acid residues, because this is generally the case with majority of gums. The possibility of R residues being linked through position C_3 of the main-chain galactose residue cannot, however, be eliminated altogether. As far as the attachment of R groups to the galactose moiety of the aldobiouronic acid side chains designated by (a) are concerned, the only possibility is that it may be attached through position C_3 of the galactose residues (a), thus giving rise to 2,4-di-*O*-methyl-D-galactose after methylation and hydrolysis. In structure (I), units of rhamnose are not included, although the sugar was obtained in trace amounts because hydrolysis of the methylated gum furnished no derivative of rhamnose. One striking feature of *Salmalia malabarica* gum and degraded *Salmalia malabarica* gum is that in both the cases, the aldobiouronic acid branching starts from the non-reducing end of the main galactan chain. Such a kind of attachment of aldobiouronic acid side chains has also been observed in the case of mesquite[2] and *Acacia Sundra*[3] gums. Like *Salmalia malabarica* gum, these gums also do not furnish any tetramethylgalactose derivative after methylation and hydrolysis.

The tentative structure (I) is obviously an outline of the type of possible structure for *Salmalia malabarica* gum and has been put forward only to explain the varied types of linkages present in the gum. The speculation as to the detailed molecular structure of *Salmalia malabarica* gum can only be more definitely decided by further quantitative evaluation of the methylation and periodic acid oxidation results.

EXPERIMENTAL

Paper chromatographic analysis was carried out by the descending method on Whatman No. 1 and Whatman No. 3 MM filter paper sheets using non-aqueous phase of any one of the following solvent systems: (a) *n*-butanol: ethanol: water (5:1:4), and (b) *n*-butanol: acetic acid: water (4:1:5); *p*-anisidine phosphate was used as a spray reagent. The specific rotations reported are equilibrium values and the melting points are uncorrected. Unless otherwise stated, all evaporations were carried out at 40-50° under reduced pressure and the methylations were carried out in an atmosphere of nitrogen. Demethylation of the methylated sugars was carried out by the procedure of Hough *et al.*[4] Aniline derivatives of methylated sugars were prepared by refluxing the sugar in ethanolic aniline for 2 hr.

Reducation of the Fully Methylated Aldobiouronic Acid with Sodium Borohydride.— The aldobiouronic acid, obtained by the graded hydrolysis of the *Salmalia malabarica* gum, was converted to its fully methylated derivative by the same procedure as described previously[1]. The fully methylated aldobiouronic acid (0.5 g.) was dissolved in water (40 ml) and the solution was added slowly to a solution of sodium borohydride (1 g.) in water (40 ml) with continuous stirring. Excess of sodium borohydride was destroyed after 4 hr. by adding dilute acetic acid. The solution was then deionised by passing through the columns of freshly regenerated cation-exchange, Duolite C-25, and anion-exchange, Duolite

2. Jones and Smith, "Advances in Carbohydrate Chemistry", Vol. IV, Academic Press Inc., New York, 1949, p. 253.
3. Mukherjee and Shrivastava, *Proc. Ind. Acad. Sci.*, 1959, **50A**, 374.
4. *J. Chem. Soc.*, 1950, 1705.

A-7, resins. The eluate from the columns was concentrated and was hydrolysed with 4% methanolic HCl (30 ml) for 20 hr. on a boiling water bath. After removal of the methanol, hydrolysis was continued by heating at 100° with *N*-HCl (30 ml) for 20 hr. The hydrolysate, after neutralisation with silver carbonate, was freed of silver ions by passing H_2S. The excess H_2S was removed by aeration and the solution was concentrated to a syrup (0.32 g.). Paper chromatographic examination of the syrup (solvent a) showed a single spot (R_f 0.64) corresponding to 2,3,4-tri-*O*-methyl-D-galactose. (Found: OMe, 41.6. Calc. for tri-*O*-methyl-D-galactose, $C_9H_{18}O_6$:OMe, 41.9%). The sugar was finally identified as 2,3,4-tri-*O*-methyl-D-galactose by conversion to 2,3,4-tri-*O*-methyl-*N*-phenyl-D-galactosylamine, m.p. 164°; m.p. of the compound remained undepressed when mixed with a sample obtained earlier[1].

Methylation of the Gum.—Purified *Salmalia malabarica* gum (20 g.) was methylated four times with dimethyl sulphate and 30%NaOH solution and the reaction product after being worked up in the manner, described earlier[1], afforded a partly methylated gum (8.2 g., OMe, 36.5%). This was further methylated three times with Purdie's reagent to provide a fully methylated product. Yield 7.22g., OMe 40.85%, $[\alpha]_D^{26}$ —26.2° (*c*, 0.84 in water). Treatment of this product with Purdie's reagent once more did not increase its methoxyl content.

Hydrolysis of the Methylated Gum.—The fully methylated gum (7 g.) after being refluxed with 5% methanolic HCl (180 ml) for 30 hr. was evaporated to dryness. The syrup thus obtained was heated with aqueous *N*-HCl (200 ml) on a boiling water bath for 40 hr. The hydrolysate was neutralised with silver carbonate, filtered, and the silver ions were removed from the filtrate by passing H_2S. It was again filtered and excess H_2S removed by aeration. It was then neutralised with barium carbonate, filtered, and evaporated to dryness. The residue (6.1 g.) was thoroughly extracted with dry ether to provide an ether-soluble fraction (A) (yield 3.93 g.) and an ether-insoluble fraction (B) (yield 1.72 g.).

Ether-soluble Fraction (A)

A part of fraction (A) consisting of neutral methylated sugars was resolved into six components by paper partition chromatography on Whatman No. 3 MM filter paper sheets, using solvent (a). Strips corresponding to individual sugars were eluted with water by Dent's method[5]. The eluted components were concentrated separately to provide the following six fractions;

Fraction (I).—The sugar was obtained in a very low yield and furnished galactose on demethylation. It appeared to be monomethylgalactose from its paper chromatographic examination (R_f 0.20). The sugar could not be identified further due to paucity of material.

Fraction (II).—The syrup (0.160 g.) on paper chromatographic examination (solvent a) exhibited a single spot (R_f 0.40). It had $[\alpha]_D^{21}$ +84.9° (*c*, 1.59 in water) and afforded galactose on demethylation. (Found; OMe, 29.58. Calc. for di-*O*-methyl-D-galactose, $C_8H_{16}O_6$: OMe, 29.8%). The sugar was identified as 2,4-di-*O*-methyl-D-galactose by conversion to 2,4-di-*O*-methyl-*N*-phenyl-*D*-galactosylamine, m.p. 210° (*lit.*[6] m.p. 209-10°).

5. *Biochem. J.*, 1947, **41**, 248.

Fraction (III).—The paper chromatographic examination of the syrup (0.285 g.) having $[\alpha]_D^{31}$ +86° (*c*, 1.2 in water) showed the presence of a major component (R_f 0.44) together with traces of another sugar (R_f 0.40) which may be due to 2,4-di-*O*-methyl-D-galactose. Demethylation of the sugar afforded galactose only. (Found: OMe, 29.7. Calc. for di-*O*-methyl-D-galactose, $C_8H_{16}O_6$: OMe, 29.8%). The sugar was identified as 2, 6-di-*O*-methyl-D-galactose by conversion to 2,6-di-*O*-methyl-*N*-phenyl-D-galactosylamine; m.p. and mixed m.p. with a sample obtained earlier[1], 121°.

Fraction (IV).—The sugar syrup (0.310 g.) having $[\alpha]_D^{31}$ +56° (*c*, 1 in water) showed the presence of a major component (R_f 0.81) and a minor component (R_f 0.86) when examined paper chromatographically (solvent a). The minor component was present in traces and may be due to 2,3,4,6-tetra-*O*-methyl-D-galactose. Demethylation of the syrup yielded arabinose together with a very small amount of galactose. (Found: OMe, 34.88. Calc. for di-*O*-methyl-L-arabinose, $C_7H_{14}O_5$: OMe, 34.80%). The main component was identified as 2,5-di-*O*-methyl-L-arabinose by preparing its amide derivative. The 2, 5-di-*O*-methyl-L-arabonamide derivative was prepared by oxidising the syrup (0.2 g.) with bromine (0.4 ml) in water (1 ml) for 2 days at the room temperature. Excess of bromine was removed and the reaction product was repeatedly extracted with chloroform to obtain the lactone in the form of a syrup. It was then treated with ethanol, saturated with ammonia at 0°. This was then kept in a refrigerator for 40 hr. On removing excess of ethanol, crystals of 2,5-di-*O*-methyl-L-arabonamide, m.p. 126°, were obtained (lit.[7] m.p. 125-26°).

Fraction (V).—Paper chromatography of the syrup (0.080 g.) having $[\alpha]_D^{18}$ +112° (*c*, 0.64 in water) showed the presence of a main component having R_f 0.86 and a trace of another sugar (R_f 0.92) which may be due to 2,3,5-tri-*O*-methyl-L-arabinose. Demethylation yielded galactose together with traces of arabinose. (Found: OMe, 52.12. Calc. for tetra-*O*-methyl-D-galactose, $C_{10}H_{20}O_6$: OMe, 52.50%). The main component was identified as 2,3,4,6-tetra-*O*-methyl-D-galactose by conversion to 2,3,4,6-tetra-*O*-methyl-*N*-phenyl-D-galactosylamine, m.p. 190° (lit.[8] m.p. 192°).

Fraction (VI).—The sugar syrup (0.180 g.) having $[\alpha]_D^{18}$ −35.5° was found to consist of a main component (R_f 0.92) together with traces of another component (R_f 0.86) which may be due to 2,3,4,6-tetra-*O*-methyl-D-galactose. (Found: OMe, 48.89. Calc. for 2,3,5-tri-*O*-methyl-L-arabinose, $C_8H_{16}O_5$: OMe, 48.8%). Demethylation of the sugar syrup provided arabinose together with traces of galactose. The identity of the main component was confirmed by conversion to 2,3,5-tri-*O*-methyl-L-arabonamide, m.p. 135° (lit.[9] m.p. 138°).

Ether-insoluble Fraction (*B*)

Paper chromatographic analysis of fraction (B) using solvent (b) revealed the presence of 2, 3, 4-tri-*O*-methyl-D-galacturonic acid (R_f 0.64) together with small amounts of unidentified methylated sugars. Resolution of the mixture was then carried out by parti-

6. Aspinall and Hirst, *J. Chem. Soc.*, 1953, 1631.
7. Kreider and Levene, *J. Biol. Chem.*, 1937, 121, 155.
8. Smith, *J. Chem. Soc.*, 1940, 1035.
9. Jones, *Ibid.*, 1947, 1055.

tion chromatography, using solvent (b) on Whatman No. 3 MM filter paper sheets, and the paper strips containing the methylated uronic acid only were cut and eluted with water to obtain pure uronic acid. (Found: OMe, 38.95. Calc. for tri *O*-methyl-D-galacturonic acid, $C_9H_{16}O_7$: OMe, 39.40%). The uronic acid was identified by preparing the crystalline methyl-2,3,4-tri-*O*-methyl-D-galactopyranosiduronamide; m.p. and mixed m.p. with a sample obtained earlier[1], 152°.

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