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SHORT COMMUNICATION

EXAMINATION OF THE GUM FROM *LANNEA COROMANDELICA*

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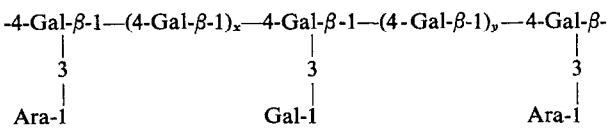
Abstract—The gum isolated from the tree *Lannea coromandelica* has been found to be a neutral polysaccharide composed of D-galactose and L-arabinose in the ratio 4 1. Hydrolysis of the methylated polysaccharide gave 2,3,4,6-tetra-*O*-methyl-D-galactose (1 mole); 2,3,6-tri-*O*-methyl-D-galactose (4 moles); 2,6-di-*O*-methyl-D-galactose (3 moles); and 2,3,5-tri-*O*-methyl-L-arabinose (2 moles). Acetyl and methoxyl groups were found to be absent.

THE CHEMICAL composition of the flowers and stem-bark of *Lannea coromandelica* (Hindi-Jhingan; Anacardiaceae) has been studied earlier.[1] The gum of *L. grandis* (Hindi-Shemat or Modal), a closely related species has been examined in detail.[2]

The gum from *L. coromandelica*, was obtained as an amorphous white powder by pouring its thick mucilagenous solution into ethanol. The ash (1·8%) was found to contain iron, calcium and phosphate. The purified polysaccharide, $(\alpha)_D^{30}$ +45° (water) consisted of galactose and arabinose in the molecular proportion 4:1. Graded hydrolysis with 0·1 N sulphuric acid and the chromatographic examination at various intervals indicated that arabinose was the first sugar released; galactose was released in detectable amounts only after 60 min. This suggests that the polysaccharide consists of a main chain of galactose and a branch chain containing predominantly arabinose in the acid-labile furanose form.

Hydrolysis of methylated polysaccharide yielded 2,3,4,6-tetra-*O*-methyl-D-galactose, 2,3,6-tri-*O*-methyl-D-galactose, 2,6-di-*O*-methyl-D-galactose and 2,3,5-tri-*O*-methyl-L-arabinose in the molar ratio 1:4:3:2. The methylated products were separated on paper chromatograms and identified by the preparation of crystalline derivatives.

From periodate oxidation, 1 mole of formic acid was liberated and 8·2 moles of periodate was consumed per repeating unit of the polysaccharide which is in close agreement to that expected from the methylation studies. The structure of the polysaccharide can thus be represented as (I)



$x+y=4$

(I)

(Gal, D-galactopyranose; Ara, L-arabofuranose)

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All specific rotations are equilibrium values and all m.p. are uncorrected. Chromatographic separations were carried out at room temperature by descending method using the non-aqueous phase of any one of the following combinations of solvents: Butanol:acetic acid:water (4:1:5)—Solvent "A": Butanol:ethanol: ammonia:water (40:10:1:49)—Solvent "B".

The spray reagent used was aniline hydrogen phthalate in *n*-butanol.

Isolation of Polysaccharide from the Crude Gum

The thick mucilagenous solution of the gum nodules (20 g) dissolved in water (500 ml) was poured into ethanol (2 l.) with continuous stirring. A white product settled on keeping and was filtered and dried when amorphous white powder (18·2 g) was obtained, $(\alpha)_D^{30}+45°$ (water).

Hydrolysis of the polysaccharide. The polysaccharide (50 mg) was hydrolysed at 100° with 25 ml of 1·5 N H_2SO_4 for 24 hr. After neutralization ($BaCO_3$) the derived syrup, on paper chromatographic analysis, revealed spots with mobilities of galactose and arabinose (Solvent "A"). The presence of the above sugars were further confirmed by preparing their crystalline osazones after separating them on Whatman No. 1 paper. The molecular proportion of the sugars, determined by periodate oxidation,[3] was 4·00 (galactose) to 1·00 (arabinose).

Homogeneity of the polysaccharide. 2 g of the polysaccharide dissolved in 50 ml of water was poured into 100 ml of ethanol with continuous stirring The precipitate was filtered (Sample "A") and the filtrate diluted with 1 l. of ethanol to precipitate the rest of the polysaccharide (Sample "B"). Both samples were hydrolysed separately and the quantitative estimation of sugars revealed that they were essentially similar.

Acetylation and deacetylation finally established the polysaccharide to be homogeneous

Graded hydrolysis. 50 mg of the polysaccharide in 20 ml of 0·1 N H_2SO_4 was hydrolysed at 100° and the hydrolysate examined chromatographically after 15, 30, 60 and 100 min. Galactose could be detected only after 60 min.

Periodate Oxidation

Liberation of formic acid.[4] To the polysaccharide (201·7 mg) in 10 ml water was added 2·8 g KCl and 0·25 M $NaIO_4$ (10 ml) and the solution made up to 100 ml. The oxidation was conducted in the dark at room temperature, 5-ml samples were drawn at various intervals (up to 72 hr) and were titrated by 0·1 N NaOH after reduction of the periodate by ethylene glycol. The amount of formic acid liberated (48 hr) corresponds to one terminal hexose unit per repeating unit of the polysaccharide.

Uptake of periodate.[5] To the polysaccharide 114·6 mg in 10 ml of water was added 10 ml of 0·25 M $NaIO_4$ and the volume was made up to 100 ml with water. 5 ml aliquots were titrated against 0·0502 N $Na_2S_2O_3$ at various intervals. 1 20 ml of $Na_2S_2O_3$ was consumed after 60 hr, which corresponds to 8·2 mole of periodate per repeating unit of the polysaccharide. After 100 hr the polysaccharide was hydrolysed and examined for the presence of unoxidized galactose and arabinose. Both were found to be absent.

Methylation of the polysaccharide. The polysaccharide (3 g) was methylated by the method followed by Andrews *et al.*,[6] when pale-yellow crispy solid (1 g) $(\alpha)_D^{30}+32°$ ($CHCl_3$) OMe, 42 per cent was obtained.

Hydrolysis of the methylated polysaccharide. The methylated polysaccharide (800 mg) was hydrolysed with methanolic HCl and then with N HCl (after evaporating methanol) for 24 hr and the methylated sugars were separated on Whatman No. 3 paper sheets using Solvent "B".

Fraction I— R_G 0 49, 2,6-di-*O*-methyl galactose identified as anilide, m.p. 119–120, lit.,[7] 121–122°.
Fraction II— R_G 0·70, 2,3,6-tri-*O*-methyl galactose, identified as lactone, m.p. 98–99°, lit.[8] m.p. 99–100°.
Fraction III—R_G 0·89, 2,3,4,6-tetra-*O*-methyl galactose, identified as anilide, m.p. 185–187°, lit.[9] m p. 187–188°.
Fraction IV—R_G 0 95, 2,3,5-tri-*O*-methyl arabinose, identified as 2,3,5-tri-*O*-methyl arabonamide, m.p. 135–136°, lit.[10] m.p. 138°.

Quantitative Estimation of Methylated Sugars

36·2 mg of the methylated polysaccharide was hydrolysed in 2 per cent methanolic HCl (15 ml) and 13 5 mg of glucose was dissolved in it, finally the mixture was completely hydrolysed in 10 ml of N HCl. The methylated

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sugars were separated by Solvent "B". The sugars were estimated by alkaline hypoiodite,[11] 0·1 N iodine (2 ml) being used in each case, and the reaction mixture kept for 20 hr. After acidification the excess of iodine was titrated with 0·01 N $Na_2S_2O_3$. The recovery of methylated sugars was calculated on the basis of complete recovery of glucose.

Found (results expressed as ml of 0·01 N $Na_2S_2O_3$ consumed). Dimethyl galactose 1·43; tri-methyl galactose 1·90; tetramethyl galactose 0·42; tri-methyl arabinose 1·05; glucose 2. These figures correspond to an average dimethyl galactose:trimethyl galactose:tetramethyl galactose:trimethyl arabinose—Ratio 3:4:1:2.

[11] E. L. HIRST and J. K. N. JONES, *J. Chem. Soc.* 928 (1949).