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DEGRADED POLYSACCHARIDE STRUCTURE OF *Agathis australis* GUM POLYSACCHARIDE BY METHYLATION TECHNIQUE

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ABSTRACT

Agathis australis degraded polysaccharide was methylated by Brown *et al.,* and Purdie;s method which yielded degraded methyl sugars as: 2,4,6-tri-0-methyl-D-galactose, 2,3,4-tri-0-methyl-D-galactose, 2,4-di-0-methyl-D-galactose and 2,3,4-tri-0-methyl-D-glucuronic acid in the molar ratio of 1:6:2:3.

Keywords: Degraded methyl sugars, *Agathis australis,* gum polysaccharide.

INTRODUCTION

Plant of *Agathis australis*[1] belongs to the family Araucariaceae and is native to South Queensland and Australia (Kauri). Plant is planted in Forest Research Institute, Dehradun (Uttaranchal) and other parts of Southern India. It is called as Kauri, the gums of plant contain a water soluble polysaccharide as L-arabinose and D-galactose in the molar ratio of 1:4 and traces of L-fucose. The present investigation mainly deals with the methylation of degraded gum polysachharide along with a degraded gum polysaccharide structure of *A. australis*[1] gum.

EXPERIMENTAL

The degraded methyl sugars of *A. australis* gum polysaccharide were separated and identified by the descending of paper chromatography[2] on Whatman No. 1 and 3 MM filter paper sheet. The following solvent mixture (v/v) were used for the detection of degraded methyl sugars as: (A) n-butanol, ethanol water (4:1:5, upper phase)[3] and (B) n-butanol, acetic acid, water (4:1:5, upper phase)[3]. The (R) p-anisidine phosphate[4] was used a spray reagent for the appearance of the degraded methyl sugars.

Isolation of the barium salt of degraded gum:

Degraded *A. australis* gum (40 gm) was prepared with distilled water (800 ml) for 100 hrs. The autohydrolysate[5] was cooled, neutralized with barium carbonate slurry when the barium salt of degraded gum was obtained as an amorphous powder. This was freed from the adhering sugar impurities by boiling four times with fresh methanol (100 ml, each time). The sample was then dried and analysed, yielded (19 gm), Ba 12.3%, equivalent weight from barium percentage 656.

Methylation of degraded gum:

The degraded gum (free from L-arbinose) was methylated extensively with dimethyl sulphate and sodium hydroxide[6]. The entire experiment was carried out in an atmosphere of nitrogen. To a solution of barium salts of the degraded gum (10 ml) in water (50 ml), dimethyl sulphate (120 ml) was slowly added. A solution of the sodium hydroxide (30%, 350 ml) was added in such a manner that the whole addition took place in 6.5 hrs. After stirring for another 12 hrs, the reaction product was neutralized with dil. H_2SO_4 in cold. The precipitated sodium sulphate was filtered off and extracted with methanol. The filtrate and methanolic extract was combined and concentrated. This was again methylated by dissolving in sodium hydroxide (30%, 350 ml) and sodium sulphate (120 ml). The reaction product was extracted with chloroform in a liquid-liquid extractor at pH 8.0 to remove the neutral methylated sugars. The aqueous solution was then acidified (pH 3-4) and extract exhaustively with chloroform and it on concentration left a residue (9.4 gm). The residue was dissolved in methanol (25 ml) and mixed with iodine (50 ml). The mixture was refluxed gently and freshly prepared silver oxide (22.5 gm) was added to it in small portion during 7 hrs. The methylated product was filtered

and the residue extracted with hot methanol. The combined filtrate was concentrated and again methylated three times with Purdie's reagent[7], did not increase it methoxyl content yielded 7.85 gm, -OCH^3, 40.8%.

Hydrolysis of fully methylated degraded gum:

The fully methylated degraded gum (5 gm) was dissolved in methanolic hydrogen chloride (6.5%, 120 ml) and refluxed for 10 hrs. The reaction mixture was cooled, neutralized with freshly prepared silver carbonate (Ag_2O_3), filtered and evaporated to syrup. The resulting syrup was saponified with barium hydroxide (0.3 N, 75 ml) for 2 hrs at 55°C. The excess barium hydroxide ($Ba(OH)_2$) was removed by passing CO_2. The precipitated barium carbonate was removed by filtration and washed with water. The combined filtrate was concentrated to a syrup which was again saponified with barium hydroxide and extracted with liquid-liquid extractor. The chloroform extract consisting of methyl glycosides of neutral methylated sugars was evaporated to a syrup (Fraction A yielded 2.49 gm). The aqueous solution left after chloroform extraction was acidifed with dil. H_2SO_4 (acidic to congo red) in cold and again extracted with chloroform. The chloroform extract was evaporated to a syrup to obtain methylated uronic acid moiety (Fraction - B, 0.85 gm).

Examination of neutral methylated sugars (Fraction - A):

The mixture of methyl glycosides of neutral methylated sugar (Fraction - A) was hydrolyzed with hydrochloric acid (1M, 50 ml) for 10 hrs on a boiling water bath. The hydrolysate was neutralized with silver carbonate, filtered and silver ions removed from the filtrate by passing hydrogen sulphide and concentrated to a syrup (yield, 2.4 gm). It was resolved into three fractions by paper partition chromatography on Whatman No. 3 MM filter paper sheet using solvent mixture (A) and paper strips corresponding to individual methyl sugars were eluted with water by Dent's method[8]. The elutaed methyl sugars were concentrated separately to furnished three fractions were identified as follows:

Fraction-I: 2,4-di-0-methyl-D-galactose:

The syrup (185 gm) on paper chromatographic examination gave a single spot (Rf 0.40) in solvent (A). It had $[\alpha]_D^{29}$ + 83°C (H_2O), Found: -OCH_3, 29.85%, calculated for $C_8H_{16}O_6$ required, -OCH_3, 29.8%. Sugar (35 mg) was refluxed for 2 hrs with absolute alcohol and freshly distilled aniline (15 mg). After the refluxing was over and alcoholic solution ws concentrated when crystals of aniline derivative were separated out. This upon recrystallization from ethanol furnished crystals of 2,4,di-0-methyl-N-phenyl-D-glactopyranosyl-amine, had m.p. 206-207°C, Lit. m.p. 20°C[9].

A portion of the methylated sugars (15 mg) was oxidized[10] with sodium metaperiodate (0.3 M, 1 ml), water (100 ml) and sodium bicarbonate (1N, 1 ml) and allowed to stand for 25 hrs. Hydrochloric acid (1N, 1.5 ml) and sodium arsenite (1.2 N, 1 ml) were then added to the reaction mixture, when the yellow colour of the solution had completely disappeared, sodium acetate (1M, 1 ml) and 2 ml dimedon reagent (5,5-dimethyldihydroresorcinol 80 ml per mole of 90% alcohol) was added. The resulting crystalline dimedon derivative of formaldehyde filtered out and dried, had m.p. 188°C. This demonstrates that the hydroxyl group at C_6 of the sugar is unmethylated.

Fraction-II: 2,3,4,tri-0-methyl-D-galactose:

Syrup (540 mg) gave a single spot of D-galactose (R_f 0.62) in solvent (A) when examined on a paper chromatogram. It had $[\alpha]_D^{31}$ + 140°C $\rightarrow$ + 115°C (H_2O), Found: -OCH_3, 39.05%, calculated for $C_9H_{18}O_6$, requires, -OCH_3, 41.9%. Derivative was prepared by usual manner as 2,3,4-tri-0-methyl-N-phenyl-D-galactosylamine m.p. 163°C[11].

Fraction-III: 2,4,6-tri-0-methyl-D-galactose:

Syrup (0.091 gm) gave a single spot parallel to D-galactose (R_f 0.60) in solvent (A) on paper chromatogram. It had $[\alpha]_D^{31}$ + 116°C $\rightarrow$ + 88°C (H_2O), Found: -OCH_3, 40.25%, calculated for $C_9H_{18}O_6$, requires, -OCH_3, 41.9%. Derivative was prepared as 2,4,6-tri-0-methyl-N-phenyl-D-galactosylamine m.p. 178°C[12].

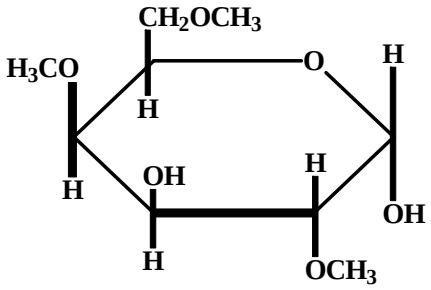
Acidic methylated sugar (Fraction-B):

Fraction -IV: 2,3,4-tri-0-methyl-D-glucuronic acid:

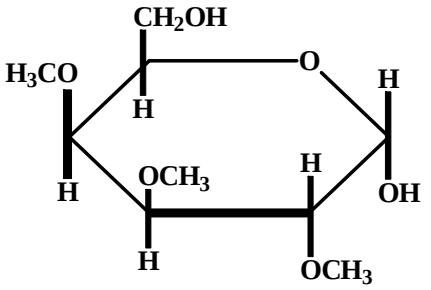
Methylated sugar fraction (B) was hydrolyzed with hydrochloric acid (1N, 20 ml) for 18 hrs. on water bath. Hydrolysate was cooled, neutralized with silver carbonate and worked up in usual manner to yield methylated uronic acid. It gave single spot (R_f 0.82) in solvent (B). Found: -OCH_3, 38.9%, calculated for C9H18O8, requires -OCH3, 39.4%. Its amide derivative was prepared, on recrystallization with ethanol and pet. ether mixture gave crystals of methyl, 2,3,4-tri-0-methyl-D-glucopyranoside uronamide, m.p. 182-183°C, Lit. m.p. 183°C[11].

RESULTS AND DISCUSSION

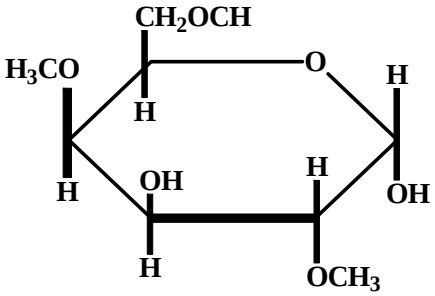
Agathis australis degraded polysaccharide (eq. wt. 656) was methylated by Brown and Purdie's method. Methyl sugar mixture was resolved by partition paper chromatography on Whatman No. 3 MM filter paper. The individual methyl sugars components (Figure 1) were characterized as 2,4,6-tri-0-methyl-D-galactose; 2,3,4-tri-0-methyl-D-galactose, 2,4-di-0-methyl-D-galactose and 2,3,4-tri-0-methyl-D-glucuronic acid were present in the molar ratio of 1:6:2:3. The isolation of four cleavage fragments from the methylated degraded gum indicated its branched chain character and also demonstrated that all D-galactose and D-glucuronic acid units were of pyranose structure.



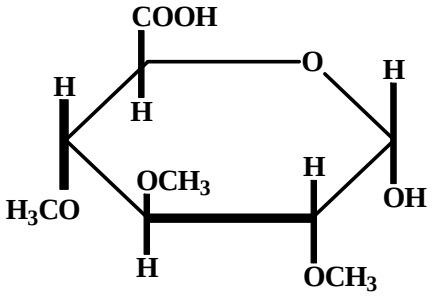
(I) 2,4,6-tri-0-methyl-D-galactose



(II) 2,3,4-tri-0-methyl-D-galactose



(III) 2,4-di-0-methyl-D-galactose



(IV) 2,3,4-tri-0-methyl-D-glucuronic acid

Figure - I : Structure of methylated degraded *A. australis* gum polysaccharide

On the basis of above results, an average repeating unit composed of nine-D-galactose residues and three D-glucuronic acid residues can be built up for degraded gum. Isolation of 3 moles of 2,3,4-tri-0-methyl-D-glucuronic acid suggested that it formed a part of the aldobiouronic acid units and the constitution of which has already been settled (b-D-glucopyranosyl-uronic acid-D-galactose). In the repeating units of the degraded gum, the three aldobiouronic acid occurred as side chain and were linked the main chain of D-galactose through C1 of their D-galactose moieties, which gave rise to 6 moles of 2,3,4-tri-0-methyl-D-galactose in the main chain after methylation and hydrolysis. The isolation of 2 moles of 2,4-di-0-methyl-D-galactose as a hydrolysis product of the methylated degraded gum indicates that they may be regarded as the points of branching point and 2 aldobiouronic acid are linked to C2 position. The absence of any tetra methyl sugars in the methanolysis product indicates that the degraded *Agathis australis* gum, the branching probably started from the non-reducing end of the main chain. Considering all the above aspects (Figure - 2) can be proposed as a tentative structure for the repeating unit of the degraded *A. australis* gum polysachharide which accomodates all the known type of linkages.

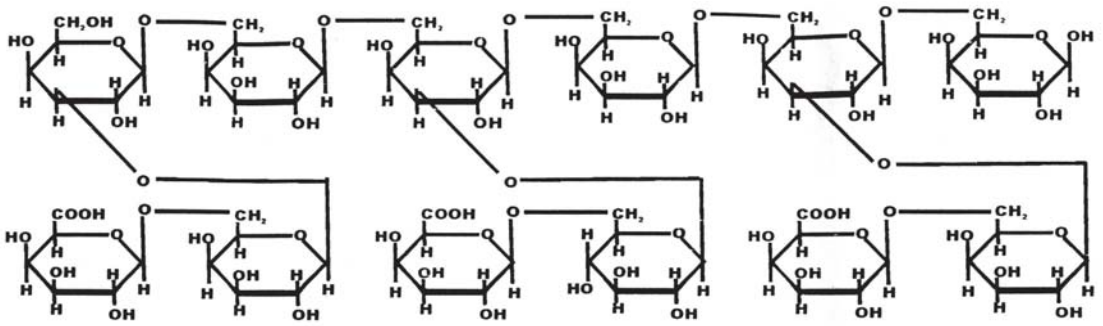


Figure - 2 : Structure of degraded *A. australis* gum polysaccharide

REFERENCES

1. Chadha, Y.R., *The Wealth of India, Raw Materials,* CSIR Pub. New Delhi, IA 102 (1985)
2. Partridge, S.M., *Nature* (London), **158,** 270 (1946)
3. Partridge, S.M. and Westall, R.G., *Brochem. J.,* **42,** 238 (1948)
4. Mukherjee, S. and Srivastava, H.C., *Nature* (London), **169,** 330 (1952)
5. Baker, J.L., and Hulton, H.F.E., *Biochem. J.,* **14,** 754 (1920)
6. Brown, F., Hirst, E. L. and Jones, J.K.N., *J. Chem. Soc.,* 1761 (1949)
7. Purdie, T. and Irvine, J.C., *J. Chem. Soc.,* **83,** 1021 (1903)
8. Dent, C.E., *Biochem. J.,* **41,** 240 (1947)
9. Hirst, E.L. and Jones, J.K.N., *J. Chem. Soc.,* 1969 (1949)
10. Reeves, R.E., *J. Amer. Chem. Soc.,* **63,** 1476 (1941)
11. Bose, S. and Gupta, K.C., *Indian J. Chem.,* **2,** 156 (1964)
12. Bose, S. and Dutta, A.S., *J. Indian Chem. Soc.,* **40,** 557 (1963)