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Studies on uronic acid materials and structure of *Acacia decurrens* gum polysaccharide

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Abstract

The polysaccharide exuded by *Acacia decurrens* trees contains residues of D-galactose , L-Arabinose , L-Fucose and D- glucuronic acid. An examination of the O- Methyl derivatives of the gum yields 2,3,4,6 tetra –O-methyl –D-galactose, 2,3 –di-O-Methyl –L-arabinose , 2,3,4-tri-O-methyl-D-galactose, 2,4-di-O-methyl-D-galactose together with 2,3,4 tri-O-methyl-D-glucuronic acid by acid hydrolysis. The degraded gum obtained after controlled acid hydrolysis is examined by linkage and methylation analysis. The O-methyl derivatives of the degraded gum yields; 2,3,4,tri-O-methyl-D-glucuronic acid, 2,4,6,tri-O-methyl-D-galactose, 2,3,4tri-O-methyl-D-galactose and 2,4 –di-O-methyl-D-galactose. These are examined by linkage and methylation analysis. The structural evidences suggests that *Acacia decurrens* gum molecules possess highly branched galactose framework to which D-glucuronic acid and residues of L-arabofuranose are attached , this conclusion is further supported by the methylation study of the Acacia gum.

Key Words: Uronic acid, Methylation, Gum, Structural elucidation & polysaccharide.

Introduction

Acacia decurrens belongs to the family leguminasae and spread through out the greater hilly parts of India. Plants gums have been known since very early times, due to their valuable characteristics they have been extensively utilized in both pharmacy and industry. The term plant gum is applied to exudations; composed of terpenoid resins, carbohydrate or a mixture of both types of materials, which are obtained from the fruits, trunks or branches of trees spontaneously or after mechanical injury of the plant by invasion of the bark or by removal of

the branch; or after invasion by species of bacteria or fungi. Commercial grade of gum exudates obtained from these species vary considerably in quality and quantity both. Different theory has been suggested for the exact physiological mechanism of gum formation by number of researchers. Only few species of gum and other polysaccharide have been chemically investigated so far for their chemical structure, structural features and uronic acid materials. Present study is an attempt to explore certain facts i.e. origin of gum, chemical structure, degradation of gum and methylation study of the gum.

Materials and Methods

Origin and purification of gum sample:-

Gum samples were collected by author during dry season Jan-April (1998-1999) from hilly parts of India, the identification of gum samples was confirmed by Forest Research Institute, Dehradun (UA). The gum polysaccharide was isolated from the collected gum by dissolving in water ,90%water in respect of ethanol and then precipitated with ethanol , resinous matter of the gum is removed by soxhet apparatus by extracting the gum with hot ethanol , powered resin free gum was dissolved in water , filtered to remove any insoluble impurities and the gum acid was recovered by acidifying its aqueous solution with HCl and precipitating with ethanol , this process is repeated 4 times with aqueous solution of the gum was de ionized by treatment with freshly regenerated cation exchange resin Duolit - 25 when a practically ashless sample was obtained in the form of white powder , purified gum did not reduce fehling solution , nitrogen, sulpher and halogens were found to be absent but pentosans 19.5%, pentoses 22.13% and furfural 11.4% were present in the gum sample (Table-1).

Table-1

Contents	Percentage
1. Pentoses	19.5 %
2. Pentoses	22.13 %
3. Furfural	11.4%
4. Equivalent weight (By direct titration)	1510

General methods

Specific rotations measured with a perkin elmer 343 polarimeter at 589 nm. Paper chromatography was carried out on whatman no.1 and 3 mm papers with the following solvents system v/v: (a) n-butanol-ethanol-water(4:1:5;upper layer): (b) butanol-acetic acid and water (4:1:5;upper layer) (c) butanol , ethanol,water (40:11:19) . Before solvent (c) was used the paper was pretreated with 0.3 M sodium di hydrogen phosphate solution and allowed to dry. Neutral sugars were measured by the phenol- sulphuric acid method (Dubois-Gilles Hamilton, Rebers & smith 1956) and uronic acids were determined by direct titration with NaOH solution on exhaustively electro dialyzed samples.

The neutral & acids components

Purified gum (1.2 gm) was hydrolysed with 1.5 N sulphuric acid (40 ml) for 30 hrs in a boiling water bath, after cooling and neutralization with barium carbonate and left over night then

filtered , filterate after concentration to syrups were exhaustively extracted with methanol, the eluted sugar solution mixed with sodium metaperiodate solution (0.1 ml) and liberated formic acid was treated with NaOH solution(0.1N) using methyl red indicator , yielded the ratio of neutral and acidic fraction respectively. After concentration to a syrup; neutral fraction was chromatographed in solvents (a) and (b) against authentic standards

Table-2

Sample	L – Arabinose	D- galactose
I	1	3.93
II	1	4.07
III	1	4.05

(in the ratio of 1:4)

The ratio was concentrated and repeated addition of water, followed by concentration to a syrup; paper chromatography was carried out in solvent (b). The aldobiouronic acids were fractioned on whatman 3 MM paper in solvent (b) and they were hydrolyzed with 0.1 N sulphuric acid on boiling water bath for 40 hrs. The hydrolysates were chromatographed in solvents (a), (b) and (c)

Partial acid hydrolysis

The gum 40 grm. was treated 1.5 N suphuric acid for 30 hrs. The hydrolysate was studies by paper chromatography p.c. in solvents (a) and (b). The isolate oligosaccharide were characterized by paper chromatography, specific rotation and sugar compositions.

Auto hydrolysis experiments:- A solution of purified gum sample (5%, P^H 5.5) was heated at 100^oC for 100 hrs, the polymer was isolated by vacuum drying. The results of auto hydrolysis are presented in table-3.

Preparation and studies of degraded gums A& B :

Experimental procedures used for the preparation and examination of degraded gum A & B were the same as those described previously , degraded gum (A) 2.35 gr. was obtained from purified gum 19 gr. by mild hydrolysis (1.5 N H_2SO_4 100^0 C for 30 hrs.). During the preparation of the polymer, dialysate was analysed by p.c. and polymer was isolated by freeze drying , preliminary small scale experiments showed that seven days were required for preparation of degraded gum (B) by periodate oxidation (0.1N) of degraded gum (A).

Preparation and studies of polysaccharide I – IV:

A series of three smith degradation was performed using the pure gum is the starting material 5 gm. afford polysaccharide-I (0.42 gm), this polymer (1.5 gm) yielded polysaccharide- II (0.51 gm) and from (0.62 gm) yielded polysaccharide-III (0.29 gm). The experimental conditions for the preparation and examination of these polymers were, in general as previously describe (leonde pinto etal 1994; matinez etal;1966).

Result and Discussion

The gum *Acacia decurrens* contains galactose , arabinose ,rhamnose , glucuronic acid and it's 4-O-methyl analogue. In order to establish the linkage of D-galactose and D-glucuronic acid residue in the degraded *Acacia decurrens* gum. It was subjected to methylation and periodate oxidation studies as presented in table -4. All the sugars were identified from the measurement of their specific rotation and melting points and their crystalline anilide derivatives.

Table -3

Time in hrs.	Volume in ml. of sodium thiosulphate solution (0.05N) required for excess of iodine (in mL.)
20	45.2
30	38.1
40	37.4
50	37.0
60	36.7
70	36.4
80	36.0
90	35.95
100	35.8
110	35.8

Table-4

Methylated degraded *Acacia decurrens* gum		**Methylated *Acacia decurrens* gum**
1.	2,4,6-tri-O-methyl-D-galactose	2,3,4,6 tetra –O-methyl-D-galactose(4moles)
2.	2,3,4-tri-O-methyl-D-galactose	2,3,4-tri-O-methyl-L-arabinose(4 moles)
3.	2,4di-O-methyl-D galactose (2moles)	2,3,4-tri-O-methyl-D-galactose(4 moles)
4.	2,3,4-tri-O-methyl-D-glucuronic acid (3moles)	2,4-di-O-methyl-D-galactose(8 moles)
		2,3,4tri-O-Methyl-D-glucuronic acid (4 moles)

(Hydrolysis products of methylated *Acacia decurrens* gum and degaraded *Acacia decurrens* gum)

The 2,3,4-tri-O-methyl-D-glucuronic acid , 2,4,6-tri-O-methyl-D-galactose, 2,3,4-tri-O-methyl-D-galctose and 2,4-di-O-methyl-D-galactose were found to be present in the ratio of 3:1:6:2. The

isolation of four cleavage fragments from methylated degraded *Acacia decurrens* gum indicated its branched chain character demonstrated that all D-galactose and D-gulcuronic acid units were of pyrano structure.

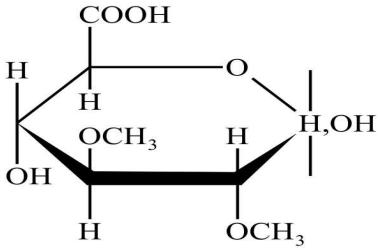

Fig-1

Isolation of two moles of 2,4 di-O-methyl- D- galactose as a hydrolysis product of the methylated degraded gum indicates that they may be regarded as the points of branching, and the two aldobiouronic acid units linked to C- 3 positions the absence of any tetra methyl sugar in the methanolysis product indicate that in the degraded *Acacia decurrens* gum branching probably starts from non reducing end of the main chain. Therefore, fig-2 can be proposed as a tentative structure of repeating unit of degraded *Acacia decurrens* gum.

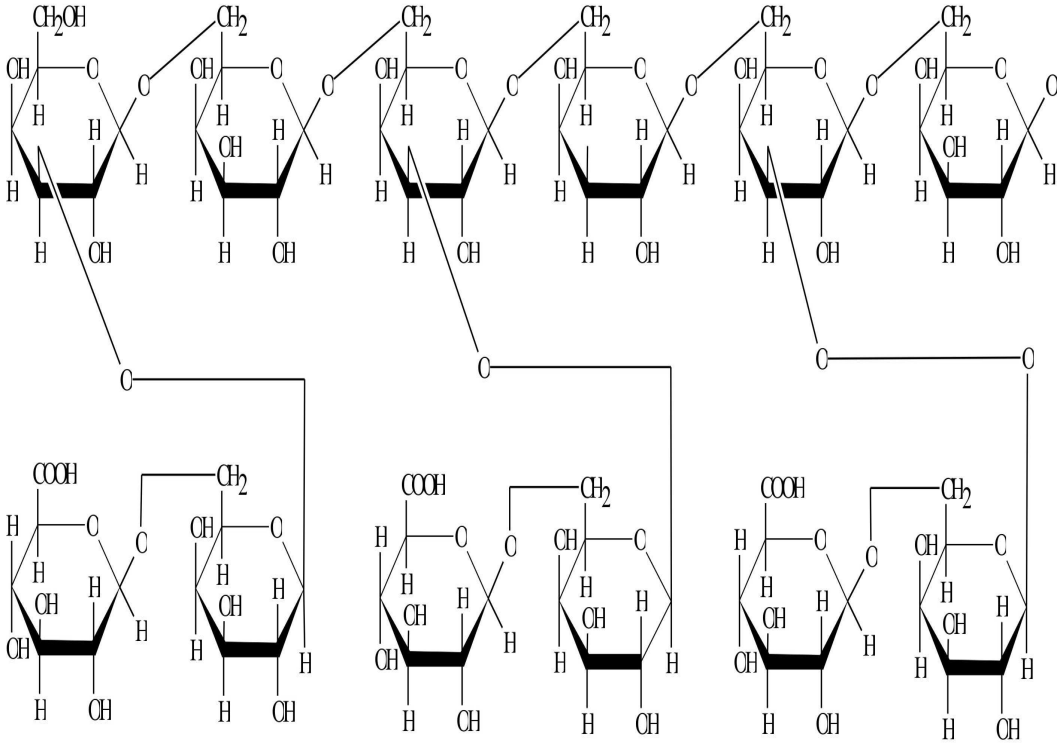

Fig-2

Further confirmation of the structure fig-3 degraded gum subjected to oxidation with sodium meta periodate, this resulted in the liberation of 2.43 moles of formic acid with concomitant consumption of 5.83 moles of periodate oxidized gum furnished on hydrolysis 0.89 mole of D-galactose per equivalent of degraded gum.

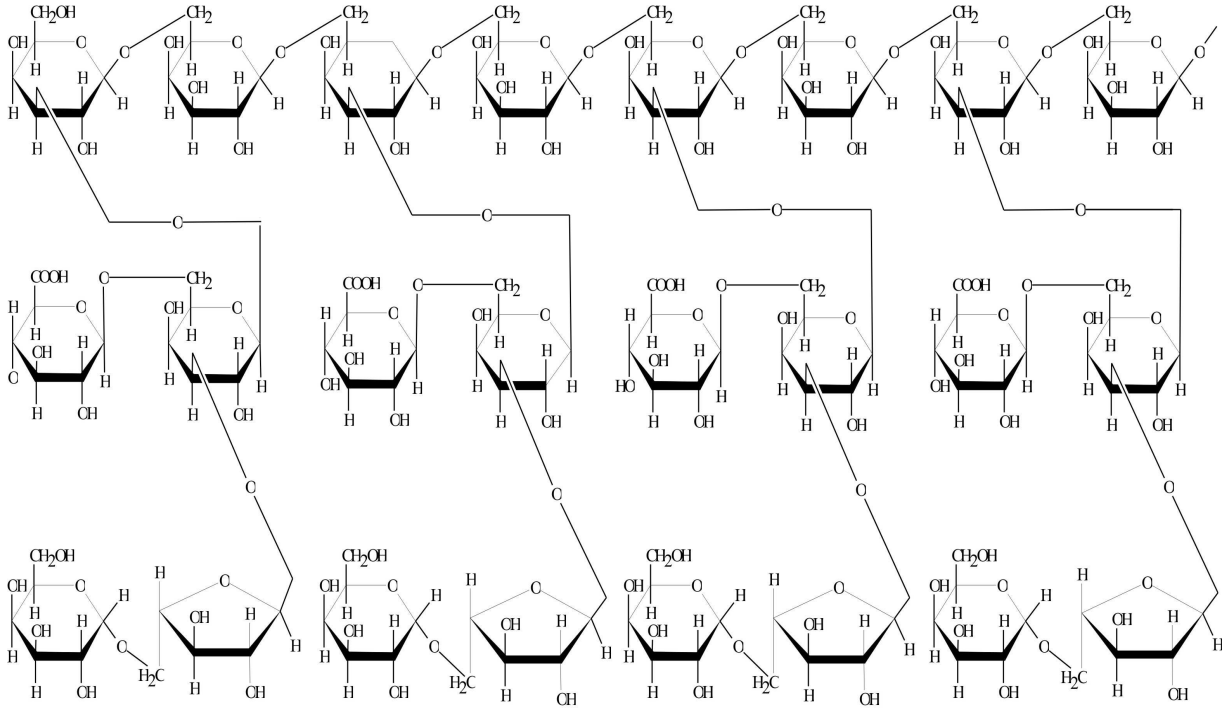

Fig-3

A careful examination of the table- 4 shows, that methylated degraded *Acacia decurrens* gum upon hydrolysis furnished 1 moles of 2,4,6-tri-O-methyl-D-galactose and 6 moles of 2,3,4-tri-O-methyl-D-galactose while from the cleavage products of fully methylated Acacia farnesiana gum only 4 moles of 2,3,4- tri-O-methyl-D-galactose could be isolated. This indicates that in the repeating unit of *Acacia decurrens* gum the lable sugar residue are attached in the form of side chain R as depicted in fig-3 in the structure to three of those galactose residues in degraded *Acacia decurrens* gum which gives rise to 2,3,4-tri-O-methyl-D-galactose after methylation and hydrolsis.

The nature of the side chain ‘R’ is also clear form the findings of the methylation experiments. The isolation of 2,3,4-tetra-O-methyl-D-galactose from the cleavage fragments of *Acacia decurrens* gum suggests that the side chain ’R’ is terminated by D-galactopyranose moiety. It may be inferred that in the parent gum the arabofuranose units are interposed between terminal galactose residues of the side chain R and nucleus of the degraded gum, therefore , side chain R in *Acacia decurrens* may be regarded as composed of L-arabofuranose unit joined to D-galactopyranose unit 5------1 linkage, according the R=L-Araf5-----1 D- Galp.

The presence of galactose as a hydrolysis product of the periodate oxidized Acacid decurrens gum may also be due to existence of 1-3 type of linkage in the molecule , a conclusion which has already been drawn form methylation results. Therefore fig -3 is an attempt to explain with reasonable accuracy the different type of linkage present in the gum.

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