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Structure of aldobiouronic acid and glucuronic acid from *Agathis australis* degraded gum polysaccharide

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Abstract: *Agathis australis gum on acid hydrolysis with sulphuric acid yielded L-arabinose and D-galactose in 1:4 molar ratio with traces of L-fucose. The components of aldobiouronic acid and glucuronic acid were obtained by graded hydrolysis of degraded gum polysaccharide. The derivatives of aldobiouronic acid was obtained as methyl ester methyl glycoside.*

Key words: *Aldobiouronic acid, Glucuronic acid, Agathis australis gum polysaccharide*

Introduction

Agathis australis gum plant (Chadha, 1985) belongs to the family, Araucariaceae, is a large tree and is native to South Queensland and Kauri (Australia). In India, it occurs in F.R.I. Dehradun, Peechi and Devikulam in Kerala and locally known as Kauri. Gum a water soluble polysaccharide, was obtained with precipitation (ethanol) of filtrate which yielded sugars as D-galactose, L-arabinose and traces of L-fucose by paper chromatographic analysis, methylation studies for polysaccharide structure (Singh, 2004b), degraded polysaccharide structure (Singh, 2004a) and periodate oxidation studies (Singh, 2005). Present manuscript mainly deals with graded hydrolysis of degraded gum polysaccharide to obtain the aldobiouronic acid and to study the properties and structure of aldobiouronic acid and glucuronic acid.

Materials and Methods

Agathis australis gum was collected from the New Pratap Nursery and seeds stores, Pandit wari, Dehradun (Uttaranchal) in the form of clean nodules of yellowish orange colour which are practically free from dirt and bark. Aqueous solution of gum was precipitated with ethanol to form crude polysaccharide (Singh *et al*., 1999b). It was purified by precipitating with barium complex (Meier and Whistler, 1965), filtration and acidification of the aqueous solution of gum with alcohol (Fraction A). This product had sulphated ash, 0.26%, which was finally purified by cation exchange resin Duolite C-25 and anion exchange resin A-7 (Kanin, 1958). The structure of aldobiouronic acid and D-glucuronic acid was obtained by usual manner (Singh *et al*., 1999a) from the treatment of graded hydrolysates of degraded gum polysaccharide (Fraction B).

Results and Discussion

Aqueous solution of *Agathis australis* gum was acidic to undergo slow autohydrolysis (Baker and Hulton, 1920) which was heated on water bath. Hydrolysis was completed after 110 hr of heating as indicated by iodometric titration, which consumed 9.4 moles of iodine. The hydrolysed solution, after neutralization with barium carbonate was filtered and filtrate concentrated to syrup that was exhaustively extracted with methanol. The methanolic extract were concentrated (Fraction-B) and examined by paper chromatography (Partridge, 1946) on whatman no. 1 paper in upper layer of solvent mixture (v/v): (S_1) *n*-butanol, ethanol, water in 4:1:5 (Partridge and Westall, 1948) and using (R) *p*-anisidine phosphate (Mukherjee and Srivastava, 1952) as spray reagent and the spots corresponding to D-galactose, L-arabinose and traces of L-fucose were observed.

Autohydrolysis of *Agathis australis* gum mucilage: *Agathis australis* gum mucilage (40 g) was heated with water (800 ml) on water bath (110 hr) and course of hydrolysis was completed iodometrically by usual procedure. Autohydrolysate (2 ml) was taken out in a conical flask and iodine solution (0.1N, 20 ml) and sodium hydroxide solution (0.1N, 30 ml) were added. Content was acidified with sulphuric acid (1N) and excess iodine titrated against hypo solution (0.05N). Autohydrolysate was cooled and neutralized with barium hydroxide solution, filtered and filtrate evaporated to brown syrup. This was extracted exhaustively with methanol and the extract was concentrated to syrup (Fraction-A) which consisted of neutral sugars released on autohydrolysis. Solid amorphous residue (Fraction-B) consisting of barium salt of degraded gum was extracted with petroleum ether and the extract dried in vacuum (19 g).

Resolution of fraction (A) by column chromatography: Sugar fraction (A) were resolved into its components by column chromatography (Hough *et al*., 1950), using *n*-butanol half saturated with water as eluate (Chalson, *et al*., 1955). Glass column (14"x1") was washed with eluate and water till the washing becomes colourless. Sugar mixture was added slowly to the column and allowed to absorb the cellulose. Fractions were

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collected in several test tubes and results are given in Table 1. Each sugar fraction was examined by paper chromatography which showed the presence of D-galactose, L-arabinose and traces of L-fucose.

Graded hydrolysis of degraded gum (Fraction – B) to obtain aldobiouronic acid and uronic acid: Barium salt at degraded gum (Fraction-B, Ba 12.3%, 2.5 g) after autohydrolysis of gum was dissolved in sulphuric acid (0.1N, 500 ml) and resulting

Table - 1: Resolution of sugar fraction (A) by column chromatography

S.No.	Fr. No.	R_f value (S_1)	Wt. of sugars (gm)	Sugar present
1.	11-21	---	---	No sugar
2.	22-27	0.21	0.0080	L-fucose
3.	28-38	0.21 & 0.12	0.4698	L-fucose and L-arabinose
4.	39-62	0.12	0.9014	L-arabinose
5.	63-81	0.12 & 0.07	0.8426	L-arabinose and D-galactose
6.	82-105	0.07	1.4504	D-galactose

solution was heated on water-bath (35 hr). This solution was neutralized with barium carbonate, filtered and filtrate was evaporated to a syrup which was then extracted with boiling methanol. The methanolic extract after concentration to a syrup was examined by paper chromatography in solvent (S_1).

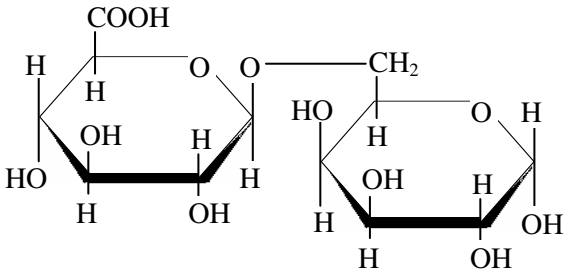

Fig. 1: Structure of aldobiouronic acid of *Agathis australis* gum polysaccharide

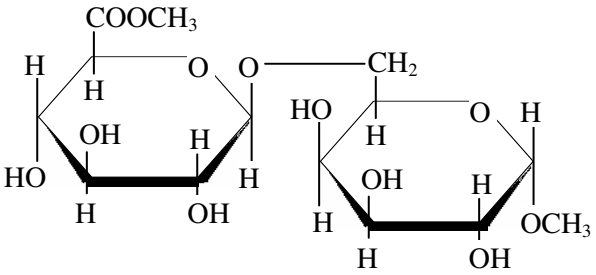

Fig. 2: Structure of methyl ester methyl glycoside derivative aldobiouronic acid of *Agathis australis* gum polysaccharide

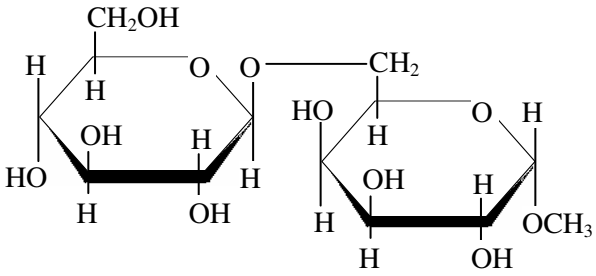

Fig. 3: Structure of neutral disaccharide of *Agathis australis* gum polysaccharide

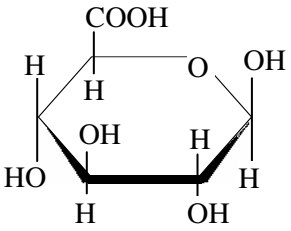

Fig. 4: Structure of D-glucuronic acid of *Agathis australis* gum polysaccharide

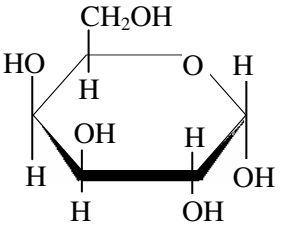

Fig. 5: Structure of D-galactose of *Agathis aurtralis* gum polysacchairde

Subsequently silver nitrate was used as spray reagent, a strong spot corresponding to D-galactose was observed. It was separated by column chromatography. The methanol insoluble fraction was the barium salt of an aldobiouronic acid $(C_{12}H_{19}O_{12})_2$, corresponding to 2% Ba. The barium salt was treated with sulphuric acid (1N) on water-bath (24 hr) at 100°C. After hydrolysis, the solution was allowed to cool and, then neutralized with barium carbonate and the hydrolysate was concentrated under reduced pressure and temperature (40-50°C), which showed strong spot of D-galactose on paper chromatogram. The methanol insoluble barium salt (Ba, 29%), barium hexuronate $(C_6H_9O_7)_2$. Ba (Ba, 26.5%) was treated with sulphuric acid (1N) and filtering out the precipitated barium sulphate. It did not give a brick red colour when treated with basic lead acetate, showing

that it was not glucuronic acid (Ehreich, 1932). Hexuronic acid was examined by paper chromatography on whatman no. 1 paper in solvent (S_1) and used (R) as spray reagent gave two pink spots corresponding to glucuronic acid (strong) and glucurone (faint) spot.

Complete acid hydrolysis: Purified gum (10 gm) was hydrolysed (Hamilton and Partlow, 1958) with sulphuric acid (1N, 200 ml) on water-bath (40 hr) at 100°C. After definite intervals of time, hydrolysate (2 ml) was taken and mixed with iodine solution (0.1N, 20 ml) in a conical flask. Sodium hydroxide (0.1N, 30 ml) was then added to it and after keeping for 20 minutes, acidified with H_2SO_4 (1N) and excess iodine titrated against hypo solution (0.05N) which showed the consumption of iodine (36%). Hydrolysate was neutralized with barium carbonate, filtered and filtrate concentrated to syrup. It was extracted with methanol and concentrated to syrup, which on paper chromatography in solvent (S_1) and used (R) as spray reagent on whatman no. 1 paper gave D-galactose, L-arabinose and L-fucose. These sugars were separated by column chromatography. Methanol insoluble barium salt of uronic acid was deionised to free from Ba by passing through a column of cation exchange resin duolite C-25. Uronic acid was further purified by absorbing it in a column of anion exchange resin Duolite A-101 (acetate form) and subsequently eluting the column with formic acid (0.05N). Eluate was concentrated to a syrup and identified as D-glucuronic acid by its migration rate on paper chromatogram.

Quantitative hydrolysis of gum: Molar ratio of manosaccharides present in *Agathis australis* gum (0.416 g) was estimated by carrying out quantitative hydrolysis (Hirst and Jones, 1949) with sulphuric acid (1.5N, 40 ml) in a sealed tube (40 hr). The liberated sugars were separated by paper chromatography on Whatman no.3 mm paper and paper strips corresponding to different sugars, were eluted with water (Dent, 1947). Sugars were then estimated by oxidising with sodium metaperiodate and titrating the liberated formic acid with sodium hydroxide (CO_2 free). Results indicated that the two major sugars L-arabinose and D-galactose were present in 1:4 proportion.

Reduction of aldobiouronic acid with lithium aluminium hydride and hydrolysis of disaccharide: Barium salt (1g) of aldobiouronic acid was refluxed with methanol hydrogen chloride (2%, 10 ml) for 8 hr. Reaction mixture was cooled and neutralized with silver carbonate (10% sodium carbonate to 10% silver nitrate), filtering the silver carbonate precipitate, washed with water and methanol afterwards it left for 4 hr. Precipitated silver chloride and unreacted silver carbonate were removed by filtration, washed with methanol and combined filtrate evaporated under reduced pressure to dryness to give methyl glycoside of methyl aldobiouronate.

Reduction of methyl glycosides and methyl ester with lithium aluminium hydride: A portion of resulting methyl ester methyl glycoside (500 mg) was dissolved in dry tetrahydrofuran (500 ml) and solution added drop wise (Adams and Bishop, 1956) over a period of 1hr to a stirred suspension of lithium aluminium hydride ($LiAlH_4$) in tetrahydrofuran (75 ml). Reaction mixture was heated (30min), cooled and excess lithium aluminium hydride was decomposed by ethyl acetate and water, filter and filtrate concentrated to a small volume. It was deionised with Duolite C-25 (H^+) and A.7 (OH) cation and anion exchange resin. Solution was obtained free form salt after anion exchange column and concentrated under reduced pressure (45-50°C) to give a disaccharide (0.3 g).

Structure of aldobiouronic acid and glucuronic acid: Degraded *Agathis australis* gum (500 mg) on hydrolysis with sulphuric acid (0.1N) furnished an aldobiouronic acid alongwith D-galactose. Aldobiouronic acid was purified and homogeneity of sample established by paper chromatography, when it showed a single spot (R_f 0.08). Pure aldobiouronic acid was found to carry one methoxyl group, when hydrolysed with H_2SO_4 (1N) to furnished D-galactose and D-glucuronic acid. This shows that the aldobiouronic acid is built up of D-galactose and D-glucuronic acid moieties. Observation is further confirmed by reduction of aldobiouronic acid with sodium borohydride to corresponding neutral disaccharide and its subsequent hydrolysis. Aldobiouronic acid (Fig. 1) was first converted it into methyl ester methyl glycosides (Fig. 2) by treatment with methanolic hydrogen chloride so as to protect the hemiacetal grouping of sodium borohydride. The reduction of $COOCH_3$ group of uronic acid in methyl ester methyl glycoside derivative of aldobiouronic acid (Fig. 2) was easily converted into CH_2OH. Thus giving rise to neutral disaccharide (Fig. 3) which was subsequently hydrolysed for identification of its component sugar. Since the glycosidic linkages in aldobiouronic acid shows the resistance of hydrolysis. Methyl glycoside of neutral disaccharide obtained was then hydrolysed into the corresponding monosaccharides. Paper chromatography of hydrolysate gave two spots corresponding to D-glucuronic acid (Fig. 4) and D-galactose (Fig. 5). Since the D-galactose was obtained by hydrolyzing the aldobiouronic acid itself, it follows that D-glucose was obtained as a reduction product of uronic acid component. Therefore, the uronic acid moiety of aldobiouronic acid (Fig. 1) was confirmed to be D-glucuronic acid (Fig. 4).

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