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STUDIES OF A CARBOHYDRATE-CONTAINING POLYMER FROM *CORDIA MYXA*

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A carbohydrate-containing polymer has been isolated from the mucilage of *Cordia myxa* and purified by gel filtration and ion exchange chromatography. The polymer has been shown to contain xylose, glucose, galactose, mannose and N-acetylglucosamine in addition to small amounts of amino acids. Hemagglutination inhibition studies and compositional analysis of this material suggest that the polymer is of the glycoprotein type.

INTRODUCTION

Cordia myxa (Vern. -Lasuri, Eng. - Sebestan plum), belongs to the plant family Boraginaceae. It is distributed throughout Pakistan and India. The fruits of the plant find use in the local materia medica as a remedy against coughs, chest infections and irritation of the urinary tract [1].

The chemical nature of the mucilage has been recently elucidated in two independent studies employing paper chromatographic techniques. Whereas Kassem *et al.* [2] have shown that the mucilage is composed of glucose, fructose and galacturonic acid, Ifzal and Qureshi [3] have reported the mucilage to consist of glucose, galacturonic acid, arabinose and xylose.

The present communication describes the isolation, purification and some structural features of the carbohydrate-containing ploymer obtained from *Cordia myxa* and presents results indicating a significantly different composition of the mucilage.

EXPERIMENTAL

The ripe *Cordia myxa* fruits were collected in the month of July. The fruits were macerated in water and the aqueous solution of the pulp was filtered through muslin cloth. The filtrate was acidified with HCl (0.5%) and the polysaccharide was precipitated by adding 95% ethanol. The precipitate was filtered through a sintered glass funnel, washed with ethanol and dried under low pressure at 40°. The dried powder was used for subsequent investigations.

The powder (0.5 g) was dispersed in 10 *mM* NaOH (100 ml) and the mixture was stirred for 16 hr at 4° under nitrogen. The insoluble material was removed by centrifugation (10,000 rev/min) and the solution was dialyzed against three exchanges of distilled water at 4°. The non-diffusible material was freeze-dried to give a residue (100 mg).

The residue (70 mg) in 5 *mM* tris-HCl (pH 7.3; 3 ml) was applied to a column (2 x 40 cm) of Bio-gel P-100. The carbohydrate-containing fractions were combined and freeze dried. A portion of the residue (40 mg) in 50 *mM* sodium phosphate (pH 7.0, 2 ml) was applied to a column (2.2 x 50 cm) of DEAE-cellulose. The column was eluted with a gradient of 50 *mM*-1*M* sodium phosphate (pH 7.0; 250 ml) followed by 0.05-1*M* LiCl. The carbohydrate-containing fractions were combined and freeze-dried to give the polymer (25 mg). The eluates from both the columns were examined for carbohydrates by the phenol–sulphuric acid procedure [4] and the presence of amino acids was detected by absorption at 280 nm. The sugar residues were identified after methanolysis as trimethylsilyl derivatives according to the procedure of Reinhold [5].

Canavalia ensiformis hemagglutinin [6], *Ricinus communis* hemagglutinin [7] and *Triticum vulgaris* hemagglutinin [8] were purified by affinity chromatography. *Glycine max* hemagglutinin was used as a crude extract. The titration and inhibition assays were performed with human erythrocytes using the method of Matsumoto and Osawa [9]. The cells used for inhibition assays on concanavalin and glycine max hemagglutinin were trypsin treated [9].

RESULTS AND DISCUSSION

The material examined in these studies was dark brown

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Table 1. Composition of the extract obtained.

Sugar	Retention time*		Molar ratio†
D - Xylose	0.66	0.71	1
D - Glucose	0.91	0.93	6
D - Galactose	0.85	0.87	22
D - Mannose	0.79	0.82	2
2 - Acetamido-2-deoxy-D-glucose	1.15	1.18	2
Unidentified	0.60	0.61	
Unidentified	1.00	1.05	+++++
Unidentified	1.09	1.10	++

*Retention time relative to inositol

†Molar ratio relative to D – xylose

and mainly contained carbohydrates (Table 1). Column chromatography on Bio-gel P-100 showed the presence of a single macromolecular component containing carbohydrate and a small amount of protein. Ion exchange chromatography on DEAE cellulose also showed the presence of a homogenous material consisting mainly of sugar residues, though the presence of amino acids was also indicated. The proportion of sugars to amino acids in the macromolecule was approximately 9:1 and the two components together accounted for only 35% of the total weight. It is possible that the dark brown substance, present in the extract and eluted with the carbohydrate-containing material from the Bio-gel P-100 and DEAE cellulose columns, contributed to the remainder of the weight. The nature of this residue will be reported subsequently.

Gas liquid chromatography of the sugar residues showed the presence of a pentose, a mixture of hexoses, a 2-acetamido-2-deoxy-hexose and unidentified sugars (R_{ins} 0.60, 0.61, 1.00 1.05, 1.09 and 1.10 Table 1).

The inhibition studies on this polymer indicated weak inhibitory activity against *Glycine max* hemagglutinin, concanavalin A, *Triticum vulgaris* hemagglutinin and *Solanum tuberosum* hemagglutinin, but no activity was observed in the inhibition of *Ricinus communis* hemagglutinin. The known specificities of the above hemagglutinins [10] suggest that the polymer contains an α-D-glucopyranosyl and/or α-D-mannopyranosyl residue, a 2-acetamido-2-deoxy-β-D-glucopyranosyl residue and a non-reducing terminal 2-acetamido-2-deoxy-β-D-galactopyranosly residue, but it does not have a β-D-galactopyranosyl residue as a terminal sugar.

These results are consistent with the compositional data (Table 1) except in that no detectable amount of 2-acetamide-2-deoxy-D-galactose was identified by GLC. Inhibitory hemagglutination activity with concanavalin A is in agreement with the fair proportion of glucose and mannose present. The inhibition specificities based upon mono- and disaccharides do not always correspond to the polymeric structure [11]. The results, however, suggest certain structural similarities. Insignificant activity against *Ricinus communis* hemagglutinin despite the large proportion of galactose in the polymer suggest two possible structural features of this sugar residue.

It is either located in a terminal position in an inactive α-configuration or is attached in an inactive sequence. Both the Cal→GlcNAc and Cal→Glc linked 1,4 would be expected to be active. The composition of this polymer is unusual and is unlike those of the known polysaccharides of plant origin, it is possible that this may constitute a plant glycoprotein.

REFERENCES

1. W. Dymock, *Pharmacographia indica,* Education Society Press, Bombay, reprinted by the Journal of the Institute of Health and Tibbi (Medical) Research, Karachi.
2. A. Kassem Aly, S.K. Wahba, A.R. EL Gendy and M.M. Badaway, J. Drug Res.,(U.A.R.),**2**, 109 (1969).
3. Syed M. Ifzal and Anis Qureshi, Pakistan J. Sci. Ind. Res., **19** , 64 (1976).
4. M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebber and F. Smith, Anal. Chem., **28**, 350 (1956).
5. V.N. Reinhold, Methods in Enzymol.,**25**, 244 (1972).
6. B.B.L. Agrawal and I.J. Goldstein, Biochim. et. Biophys. Acta, **147**, 262 (1967).
7. M. Tomita, T. Kurokawa, K. Onosaki, N. Ichiki, T. Osawa and T. Ukita, Experiential,**28**, 84 (1972).
8. V.T. Marchesi, Methods in Enzymol., **28** 254 (1972).
9. I. Matsumoto and T. Osawa, Biochemistry, **13**, 582 (1974).
10. G.L. Nicolson, Intern. Rev. Cytol., **39**, 89 (1974).
11. T. Osawa, T. Terao, T.Kawaguchi, M. Fukuda, S.Toyoshima and T. Irimura, *Actes du Coloque International n^{O} 221 Sur La Methodologie de la Structure et du Metabolisme des Glyconjugues* (1974), 765-776, Editions du Centre National de La Recherche Scientifique, Paris.