Phytochemistry, Vol. 23, No. 2, pp. 337–338, 1984.
Printed in Great Britain.

0031–9422/84 $3.00 + 0.00

POLYSACCHARIDE COMPONENTS OF THE SEED-COAT MUCILAGE FROM *HYPTIS SUAVEOLENS*

D. CHANNE GOWDA

Department of Postgraduate Studies and Research in Chemistry, University of Mysore, Manasa Gangothri, Mysore 570006, India

(*Revised received* 4 *July* 1983)

Key Word Index—*Hyptis suaveolens*; Lamiaceae; seed coat; mucilaginous polysaccharide; fractionation; acidic and neutral polysaccharides; sugar composition.

Abstract—The mucilage isolated from the seed coat of *Hyptis suaveolens* contains L-fucose, D-xylose, D-mannose, D-galactose, D-glucose and 4-*O*-methyl-D-glucuronic acid in the mol ratios 1.0:2.5:1.5:7.0:12.5:1.1. Fractionation of the mucilage with Fehling's solution gave a neutral and an acidic polysaccharide. The neutral polysaccharide appears to be homogeneous and is composed of D-mannose, D-galactose and D-glucose in the mol ratios 1.0:4.5:7.5. The acidic polysaccharide is composed of L-fucose, D-xylose and 4-*O*-methyl-D-glucuronic acid in the mol ratios 1.0:2.5:1.1. It is homogeneous on gel filtration, DEAE-cellulose chromatography, sedimentation analysis and electrophoresis.

INTRODUCTION

Hyptis suaveolens Jacq. is a sweetly aromatic, medicinal plant [1]. The seeds of this plant have a transparent polysaccharide coat which readily swells in water to give a mucilage. This mucilage can be extracted quantitatively with water by mechanical stirring. No information is available so far on the nature and structure of this mucilage; this paper deals with its isolation, fractionation and preliminary characterization.

RESULTS AND DISCUSSION

The mucilage present in the seeds of *H. suaveolens* was extracted with water by mechanical stirring. The seeds were removed by squeezing the aqueous extract through a linen cloth. The mucilage was recovered in 20% yield by precipitation with ethanol. Hydrolysis with acid followed by PC indicated that the mucilage contained fucose, xylose, mannose, galactose, glucose and 4-*O*-methylglucuronic acid. Further, the neutral sugars were analysed as their alditol acetates by GLC [2]. The uronic acid content was estimated by the modified carbazole method [3]. These analyses suggested that fucose, xylose, mannose, galactose, glucose and 4-*O*-methylglucuronic acid were present in the mol ratios 1.0:2.5:1.5:7.0:12.5:1.1. Isolation of the individual sugars from the hydrolysate of the polysaccharide by preparative PC and determination of their specific rotations indicated that only fucose had the L-configuration while all the others had the D-configuration.

The polysaccharide was readily soluble in water to give a highly viscous solution. The intrinsic viscosity of the polysaccharide was 13 dl/g at 28°. While maximum viscosity was observed at pH 7–8, it decreased when the pH was either raised or lowered. A rapid decrease in the viscosity was also observed in the presence of added detergent cations or salts, and the major portion of the polysaccharide readily precipitated.

Fractionation [4] of the native polysaccharide with Fehling's solution gave two fractions, a neutral and an acidic polysaccharide, in 76 and 16% yield, respectively.

Acid hydrolysis of the neutral polysaccharide and analysis of the resulting sugars by PC, and their alditol acetates by GLC indicated that it was composed of D-mannose, D-galactose and D-glucose in the mol ratios 1.0:4.5:7.5. The neutral polysaccharide was readily and sharply precipitated from its aqueous solution by the addition of barium hydroxide, dilute hydrochloric acid, cetyltrimethylammonium bromide, aqueous sodium hydroxide, sodium chloride, etc. Its sugar composition remained unaltered even after several precipitations from the aqueous solution using the above reagents. Thus it appeared to be homogeneous.

Acid hydrolysis of the acidic polysaccharide and examination of the constituent sugars by PC indicated that it was composed of L-fucose, D-xylose and 4-*O*-methyl-D-glucuronic acid. The neutral sugars were analysed further as their alditol acetates by GLC. The uronic acid content was estimated by the modified carbazole method [3]. From these analyses it was inferred that the acidic polysaccharide was composed of L-fucose, D-xylose and 4-*O*-methyl-D-glucuronic acid in the mol ratios 1.0:2.5:1.1.

The homogeneity of the polysaccharide was shown by the following analysis: on ion-exchange chromatography with DEAE-cellulose employing an aqueous sodium chloride gradient the acidic polysaccharide was eluted as a single peak. On gel filtration using Sephadex G-200 it was eluted as a single peak. Sedimentation analysis of the 0.8% aqueous solution (pH 6.0) gave a single, symmetrical peak. On electrophoresis in 5% polyacrylamide gel using either 0.05 M Tris–glycine buffer (pH 8.9) or 0.1 M sodium tetraborate (pH 9.2) the acidic polysaccharide moved as a sharp, single band. All these analyses indicated that the acidic polysaccharide was homogeneous. Furthermore, the sugar composition of the acidic polysaccharide remained unaltered after gel filtration and DEAE-cellulose chromatography.

It is interesting to note the presence of L-fucose, which is rather a rare constituent of plant polysaccharides, in the

acidic polysaccharide from the seed-coat mucilage of *H. suaveolens*. 4-*O*-Methyl-D-glucurono-D-xylans [5–8] occur very widely in nature as components of plant hemicelluloses and often contain L-arabinose, but not L-fucose, as one of the constituent sugars.

EXPERIMENTAL

General methods. Unless stated otherwise, all hydrolyses were performed with 0.25 M H_2SO_4 at 100° for 6–8 hr. The hydrolysates were neutralized with $BaCO_3$, filtered and the clear filtrates deionized by passing through two successive columns containing Amberlite IR-120(H^+) and Amberlite IRA-400(CO_3^{2-}) resins. The neutral eluates and washings were concd under red. pres. below 45° and examined by PC. In addition, the neutral sugars were reduced with $NaBH_4$, acetylated using Ac_2O–C_5H_5N (1:1, v/v), and the resulting alditol acetates analysed by GLC [2]. The Amberlite IRA-400 column was eluted with 1 M HCOOH, evapd and the residue examined by PC for acidic sugars.

Descending PC was performed on Whatman No. 1 and 3 mm papers with the following solvent systems: (a) *n*-BuOH–C_6H_6–C_5H_5N–H_2O (5:1:3:3, upper layer); (b) *n*-BuOH–HOAc–H_2O (4:1:5, upper layer); and (c) *n*-BuOH–C_5H_5N–H_2O (6:4:3); sugars were detected with *p*-anisidine hydrochloride [9] and alkaline $AgNO_3$ [10].

GLC was carried out on a CIC (India) gas chromatograph fitted with a FID and a stainless-steel column (200 cm × 0.3 cm) containing 5% OV-225 on Gas Chrom Q (100–120 mesh). N_2 was used as the carrier gas.

Isolation of the polysaccharide. The seeds of *H. suaveolens* (5 g) were soaked in H_2O (400 ml) for 1 hr at room temp. and then stirred vigorously for 2 hr. The resulting viscous aq. extract was squeezed through a linen cloth. EtOH (6 vols.) was added. The polysaccharide was collected by centrifugation, washed with EtOH, Me_2CO and dried (P_2O_5); yield 1.0 g.

Sugar composition. The polysaccharide (30 mg) was hydrolysed first with 72% H_2SO_4 for 1 hr at room temp. and then after dilution to 0.5 M H_2SO_4 for 8 hr at 100°. The neutral sugars were analysed by PC, and their alditol acetates by GLC. The acidic sugars were examined by PC. For prep. PC, the polysaccharide (1.0 g) was hydrolysed as above. The neutral sugars were isolated using solvents (a) and (b) and the acidic sugar by solvent (b). The optical rotations of the purified sugars were determined.

Fractionation. The native polysaccharide (1.0 g) was dissolved in H_2O (500 ml) and Fehling's soln (20 ml) was added drop by drop with continuous stirring. The ppt. was removed by centrifugation, washed with H_2O containing a little Fehling's soln, acidified with cold dilute HCl and immediately precipitated with EtOH. The ppt. was washed with EtOH, Me_2CO and dried; yield 760 mg. This was designated the neutral polysaccharide. The combined aq. soln and the washings were concd (100 ml) under red. pres. below 40°, dialysed extensively and acidified with cold dilute HCl. EtOH (1 vol.) was added. The ppt. (~ 20 mg) formed was centrifuged off. Further addition of EtOH (4 vols.) precipitated the acidic polysaccharide. It was recovered by centrifugation, washed with EtOH, Me_2CO and dried; yield 160 mg. In subsequent lots, the above fractionation was performed directly on the aq. extracts of the seeds; the precipitation and dissolution of the native polysaccharide were avoided.

The neutral and the acidic polysaccharide fractions (20 mg each) were hydrolysed first with 72% H_2SO_4 at room temp. for 1 hr and then with 0.5 M H_2SO_4 for 8 hr at 100°. The hydrolysates were examined for neutral and acidic sugars by PC. Further, the neutral sugars were converted into their alditol acetates and analysed by GLC.

Estimation of the uronic aicd. The native and the acidic polysaccharide (10 and 2 mg, respectively) were separately hydrolysed as described above. After the usual work-up, the acidic sugars in the hydrolysates were recovered quantitatively by elution of the Amberlite IRA-400 columns with 1 M HCOOH. The eluates were evapd and the residues were diluted with H_2O (25 ml). The uronic acid content of these solns was determined by the modified carbazole method [3].

Gel filtration. The acidic polysaccharide (4 mg) was dissolved in 0.1 M NaCl (0.5 ml) and applied to a column (100 cm × 1.5 cm) containing Sephadex G-200 equilibrated with 0.1 M NaCl. The column was eluted with 0.1 M NaCl and elution was monitored using PhOH–H_2SO_4 reagent [11]. The eluate containing the polysaccharide was pooled, concd, dialysed and hydrolysed. The hydrolysate was examined by PC and by GLC.

DEAE-cellulose chromatography. The acidic polysaccharide (10 mg) dissolved in H_2O (0.5 ml) was applied to a column (15 cm × 2 cm) containing DEAE-cellulose (neutral form). The column was eluted with a linear gradient from 0.01 M to 1.2 M NaCl. The elution pattern was followed by PhOH–H_2SO_4 reagent [11]. The polysaccharide-containing fractions were pooled, concd, dialysed and hydrolysed. The resulting sugars were analysed by PC and GLC.

Sedimentation analysis. An 0.8% aq. soln (pH 6.0) of the acidic polysaccharide was analysed in a Beckman analytical ultracentrifuge model E at 25° and at a speed of 59780 rpm. The movement of the boundary was followed using the Schlieren-optic method.

Electrophoresis. Disc electrophoresis was carried out in 5% polyacrylamide gel using either 0.05 M Tris–glycine buffer (pH 8.9) or 0.1 M $Na_2B_2O_7$ (pH 9.2). The acidic polysaccharide (50–100 μg) was applied to each tube containing the polyacrylamide gel and electrophoresis was run at an applied current of 3 mA per tube for 3–4 hr. The gels were stained with H_5IO_6–Schiff's reagent [12].

Viscosity. The viscosity of the native polysaccharides in H_2O at several concns ranging from 0.1 to 0.01% was determined with H_2O as ref. at 28° using an Ubbelhode-type viscometer [13]. A plot of reduced viscosity vs the polysaccharide concn was made to determine its intrinsic viscosity.

Acknowledgements—I wish to thank Prof. T. R. Ramiah, Mr. K. S. Prakash and Miss M. Shylaja, Department of Biochemistry, University of Mysore, for electrophoresis and GLC analysis. This work was supported by financial assistance from the University of Mysore.

REFERENCES

1. (1959) *The Wealth of India—Raw Materials*, Vol. 5: H–K, p. 159. C.S.I.R., New Delhi.
2. Sloneker, J. H. (1972) *Methods Carbohydr. Chem.* **6**, 20.
3. Bitter, T. and Muir, H. M. (1962) *Analyt. Biochem.* **4**, 330.
4. Jones, J. K. N. and Stoodley, R. J. (1965) *Methods Carbohydr. Chem.* **5**, 36.
5. Aspinall, G. O. (1959) *Adv. Carbohydr. Chem.* **14**, 429.
6. Timell, T. E. (1964) *Adv. Carbohydr. Chem.* **19**, 247.
7. Timell, T. E. (1965) *Adv. Carbohydr. Chem.* **20**, 409.
8. Wilkie, K. C. B. (1979) *Adv. Carbohydr. Chem.* **36**, 215.
9. Hough, L., Jones, J. K. N. and Wadman, W. H. (1950) *J. Chem. Soc.* 1702.
10. Trevelyan, W. E., Procter, D. P. and Harrison, J. S. (1950) *Nature (London)* **166**, 444.
11. Dische, Z. (1947) *J. Biol. Chem.* **107**, 189.
12. Caldwell, R. C. and Pigman, W. (1965) *Arch. Biochem. Biophys.* **110**, 91.
13. Swenson, H. A. (1963) *Methods Carbohydr. Chem.* **6**, 20.