Carbohydrate Research, 56 (1977) 123–128

ISOLATION OF *Portulaca oleracea* (REGLA) MUCILAGE AND IDENTIFICATION OF ITS STRUCTURE

EL SAYED AMIN AND SANAA M. EL-DEEB

Department of Biochemistry, Alexandria University, Alexandria (Egypt)

(Received September 23rd, 1976; accepted for publication, December 3rd, 1976)

ABSTRACT

Portulaca oleracea leaves were found to contain 0.42% of a mucilage mixture. The mucilage was fractionated into an acidic and a neutral fraction. The acidic fraction consists of galacturonic acid residues joined by α-(1→4)-linkages; 60% of these residues are present as the calcium salt, and esterified galacturonic acid residues are absent. The neutral fraction is composed of 41% of arabinose and 43% of galactose residues, besides traces of rhamnose residues.

INTRODUCTION AND DISCUSSION

Portulaca oleracea, cultivated in Egypt, is an edible vegetable after being cooked. The aim of this work was to study the mucilage present; this was isolated by extraction of the leaves with 0.1M hydrochloric acid, and then fractionated with M calcium chloride, giving a white, amorphous substance designated mucilage A. To the clear filtrate was added acetone, to the ratio of 3:1 (v/v), giving a white, amorphous substance designated mucilage B.

Mucilage A was found to be insoluble in water; $[\alpha]_D^{23}$ +225°, η_{sp} 0.08 (*c* 0.05, 2M HCl); methoxyl, zero %; calcium, 22.4%. Acid hydrolysis followed by paper chromatography of the hydrolyzate indicated the presence of galacturonic acid, quantitative determination of which by the Lefèvre–Tollens method gave 55% of uronic acid. Complete methylation of mucilage A with dimethyl sulfate and 30% sodium hydroxide, followed by methyl iodide and silver oxide, gave a product having $[\alpha]_D^{23}$ +250° (*c* 0.1, chloroform); on hydrolysis, and neutralization with diazomethane, paper chromatography showed a main spot of methyl 2,3-di-*O*-methylgalacturonate and a faint spot of methyl 2,3,4-tri-*O*-methylgalacturonate, proving the presence of (1→4)-linkages. The highly positive rotation of the methylated product suggested α-linkages. Periodate oxidation showed that the mucilage molecule was composed of a chain of ~84 sugar residues; molecular weight, ~15,000.

Fraction B was a white, amorphous material, soluble in hot water, $[\alpha]_D^{23}$ +125°, η_{sp} 0.01 (*c* 0.01, M NaOH). Acid hydrolysis followed by paper chromatography indicated the presence of 41.1% of arabinose and 43.2% of galactose, besides traces of rhamnose, and no uronic acid. Complete methylation of the mucilage with di-

methyl sulfate and sodium hydroxide, followed by methyl iodide and silver oxide, gave a product having $[\alpha]_D^{23}$ +100°, η_{sp} 0.14 (*c* 0.1, chloroform). After hydrolysis, paper chromatography showed spots corresponding to 2,3,4,6-tetra-*O*-methylgalactose, 2,3,5-tri-*O*-methylarabinose (indicating terminal groups), and 2,3,6-tri-*O*-methylgalactose [indicating (1→4)-linkages], and two spots corresponding to 2,6-di-*O*-methylgalactose (indicating a branched structure) and 2,3-di-*O*-methylarabinose [indicating (1→5)-linkages]. The highly positive rotation of the methylated product suggested α-linkages. Periodate oxidation showed that the mucilage molecule was composed of a chain of ~14 sugar units; molecular weight, ~2,000.

EXPERIMENTAL

General. — Chromatography of sugar hydrolyzates and methylated sugars was conducted on Whatman No. 1 filter paper sheets by the descending method, using aniline hydrogen phthalate as the spray reagent. Evaporations were performed under diminished pressure at 50°. Specific rotations and viscosities were measured at 23°. Treatments with alkali were performed under nitrogen. Microanalyses were made by the Microanalytical Centre, Giza, Egypt.

Isolation and fraction of Portulaca oleracea *mucilage.* — The green leaves of *Portulaca oleracea* (5 kg), harvested in August, 1973 in Alexandria, were cut into thin slices, and minced[1] in 0.1M hydrochloric acid (10 liters) for 24 h. The mucilage, which was dispersed in the solution, was squeezed through muslin. The aqueous extract was centrifuged, decanted, and filtered under suction, giving a very clear, pale-yellow solution (10 liters) of pH 2.7. Two liters of M calcium chloride were added, and the solution was kept overnight, The white, amorphous precipitate obtained was centrifuged off, washed with water to remove the excess of calcium chloride, and then washed successively with ethanol, absolute ethanol, and ether, and dried (over anhydrous calcium chloride), giving a white, amorphous substance designated mucilage A; 19.2 g, 0.35% of the fresh leaves. Acetone[2] was added to the clear filtrate (obtained after centrifugation of mucilage A) in the ratio 3:1 (v/v), with stirring. The white, amorphous substance formed was centrifuged off, washed successively with ethanol, absolute ethanol, and ether, and dried (over anhydrous calcium chloride), giving mucilage B; 5.8 g, 0.12% of the fresh leaves.

Properties of mucilage A. — Mucilage A was a white, amorphous substance insoluble in organic solvents, cold or hot water, or alkali, but soluble in 2M hydrochloric acid. It had no action on Fehling or iodine solution. It had $[\alpha]_D^{23}$ +220° (*c* 0.05, 2M HCl) and η_{sp} 0.07 (*c* 0.04, 2M HCl). Found: moisture, 7.6; N (micro-Kjeldahl), zero; ash, 38.3%. The ash was examined by a spectrograph, which indicated calcium. Complexiметric determination of calcium in the ash, using standard EDTA, gave 37.2%. Flame-photometric determination of calcium in the ash indicated 37%.

Determination of uronic acid in mucilage A. — Mucilage A (50 mg) was heated with 2M hydrochloric acid (15 ml) and naphthoresorcinol (0.5 g) for 5 min in a

boiling-water bath; a blue-violet color was formed, proving the presence of uronic acid. Quantitative determination of uronic acid by the Lefèvre and Tollens[3] method gave 55.2% of uronic acid.

Test for methyl ester in mucilage A. — This test was conducted enzymically[4]. A portion (0.5 g) of mucilage A was suspended in distilled water (100 ml), and 2M sodium acetate (10 ml) and 2% sodium oxalate (10 ml) were added; then, the pH was adjusted to 7.5 with 0.5M sodium hydroxide from a buret. Tomato pectase extract (pre-adjusted to pH 7.5; 20 ml) was added. The contents were mixed by stirring, and kept for 2 h at room temperature. The pH was then 7.5, indicating the absence of esterified uronic groups.

Acid hydrolysis of mucilage A. — A portion (0.3 g) was heated[5,6] with 0.5M sulfuric acid (30 ml) in a sealed tube for 12 h at 100°. The mixture (in which insoluble material was present) was filtered, and the filtrate made neutral with barium carbonate (Methyl Orange end-point). The barium sulfate was filtered off, and the filtrate de-ionized with IR-120 (H^+) cation-exchange resin, and evaporated to dryness. The residue was extracted several times with boiling ethanol, and the extracts were combined, and evaporated to a yellow syrup (150 mg).

Chromatography and estimation of sugars in the hydrolyzate. — A portion of the acid hydrolyzate was examined by paper chromatography, using the upper layer of 4:1:5 1-butanol–acetic acid–water as the mobile phase and aniline hydrogen phthalate as the spray reagent. The chromatogram showed a main spot of galacturonic acid (R_F 0.14).

Preparation of galactaric acid from mucilage A. — Mucilage A (0.5 g) was dissolved in concentrated nitric acid (5 ml), water (5 ml) was added, and the solution was evaporated to dryness. Water (20 ml) was added, and the suspension was filtered. The white precipitate was purified by dissolving it in M sodium hydroxide (3 ml), and then reprecipitating it by acidification with M hydrochloric acid. The white crystals precipitated were washed with distilled water, and dried in a vacuum dessicator over anhydrous calcium chloride. The product was identified as galactaric acid, m.p. and mixed m.p. 214°.

Anal. Calc. for $C_6H_{10}O_8$: C, 34.3; H, 4.7. Found: C, 33.9; H, 4.8.

Acetylation[7] *of mucilage A.* — Powdered, dry mucilage A (0.5 g) was warmed with pyridine (20 ml) for 2 h at 70°, and the mixture was cooled, and kept overnight at room temperature. Acetic anhydride (10 ml) was then added dropwise during 30 min, and the mixture was kept for 3 days at 52°. The resulting, clear solution was diluted with acetic acid (20 ml), and poured into ethanol, with stirring. The precipitate was dried, giving 0.2 g of white, amorphous material, $[\alpha]_D^{23}$ +265° (*c* 0.01, chloroform); found, ash 0.0%.

Methylation of mucilage A. — Powdered, dry mucilage A (2 g) was suspended in water (20 ml) and allowed to swell for 2 h at 5°; then dimethyl sulfate[8] (15 ml) and 30% sodium hydroxide solution (30 ml) were added dropwise during 8 h, under an atmosphere of nitrogen. The base was then neutralized with 0.25M sulfuric acid, and the mixture was dialyzed against water for 24 h to remove inorganic salts.

Methylation was repeated eight times at room temperature in the presence of acetone (40 ml) to dissolve the methylated product and to allow further methylation. The solution was then acidified with 0.25M sulfuric acid, dialyzed against water, and extracted with chloroform. The extracts were combined, washed with distilled water, dried (anhydrous sodium sulfate), and evaporated to a brown syrup (yield, 0.8 g). The i.r. spectrum of the product had an OH band at 3500 cm^{-1}, so methylation was completed by treatment with methyl iodide and silver oxide (Purdie's reagents[9]), giving a brown substance (yield, 0.7 g); it had $[\alpha]_D^{23}$ +252°, η_{sp} 0.16 (*c* 0.1, chloroform), and the i.r. spectrum lacked an OH band.

Hydrolysis of methylated mucilage A. — The methylated product (0.5 g) was hydrolyzed with 0.5M hydrochloric acid (30 ml) for 12 h at 100°. The mixture was made neutral with cold, ethereal diazomethane[10], concentrated, and de-ionized by passing it through a mixture of Amberlite IR-120 (H^+) and IRA-400 (OH^-) ion-exchange resins. The effluent was evaporated to a syrup (320 mg).

Chromatography of the hydrolysis product of the methylated mucilage A. — Examination on a paper chromatogram obtained with the upper layer of 4:1:5 1-butanol–acetic acid–water as the mobile phase and aniline hydrogen phthalate as the spray reagent showed a main spot of methyl 2,3-di-*O*-methylgalacturonate (R_G 0.5) and a faint spot of methyl 2,3,4-tri-*O*-methylgalacturonate (R_G 0.6).

Periodate oxidation of mucilage A. — Eight portions (10 mg) of mucilage A were each suspended in water (10 ml), and treated with 5.6mM sodium metaperiodate[11] (5 ml) and acetate buffer (pH 5.2; 0.5 ml). The solutions were shaken at intervals; then 5% sodium hydrogencarbonate (2 ml) was added, followed by 20% potassium iodide solution (1 ml) and 6.9mM sodium arsenite (5 ml). The mixtures were kept for 15 min, and then the excess of arsenite was determined by titration against 7.1mM iodine solution. The results indicated that the maximum consumption of periodate was one molecular equivalent per sugar acid unit.

Test for formaldehyde during periodate oxidation of mucilage A. — Mucilage A (5 mg) was suspended in water (5 ml), acetate buffer of pH 5.2 (15 ml) was added, followed by 2 ml of 10% sodium metaperiodate solution, and the mixture was kept for 90 h. Dimedone solution (5%, in ethanol) was added, but no precipitate was formed.

Estimation of formic acid liberated by periodate oxidation of mucilage A. — Mucilage A (20 mg) was suspended in water (20 ml), and potassium chloride (0.5 g) and 0.37M sodium metaperiodate (2 ml) were added at room temperature. After 60 h, the excess of periodate was decomposed with ethylene glycol, and the formic acid liberated was titrated against 0.01M sodium hydroxide. The result indicated the formation of 6 moles of formic acid from one mole of mucilage A, and the presence of ~84 sugar acid units per end group.

Properties of mucilage B. — Mucilage B was a white, amorphous substance, soluble in boiling water to give a viscous solution. It dissolved readily in cold, dilute sodium hydroxide solution. It had $[\alpha]_D^{23}$ +125°, η_{sp} 0.07 (*c* 0.01, M sodium hydroxide); found: moisture, 7.8; ash, 6.2%. It had no action on Fehling or iodine solution.

After successive dialyses of mucilage B using different normalities of hydrochloric acid up to one normal, the ash was still 6.2%. This ash was examined by a spectrograph, which revealed the following elements: K, Na; Al, Co, Cu, Fe, Mg, Mn, Ni, Pb, Si, Sr, and Ti. Sodium and potassium were present in high concentrations, and the rest of the elements, in traces. Flame-photometric determination of sodium and potassium indicated 0.38 and 0.43%, respectively. MicroKjeldahl measurement gave N 0.25%, corresponding to 1.6% of protein. This protein was subjected to acid hydrolysis, followed by two-dimensional, paper chromatography of the hydrolyzate, giving the following amino acids: alanine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, serine, threonine, tyrosine, and valine.

Acid hydrolysis of mucilage B. — Mucilage B (0.5 g) was heated with 0.5M sulfuric acid (30 ml) in a sealed tube for 12 h at 100°. The mixture [in which was suspended a small amount (10 mg) of flocculent material] was filtered, and the filtrate was made neutral with barium carbonate. The barium sulfate was removed, the filtrate was evaporated to dryness, and the residue was extracted with boiling ethanol. The extracts were combined, de-ionized with a mixture of Amberlite IR-120 (H^+) and IRA-400 (OH^-) resins, and evaporated to a yellow syrup (280 mg).

Chromatography and estimation of sugars in mucilage B. — A portion of the acid hydrolyzate was examined by paper chromatography, using the upper layer of 5:1:4 1-butanol–ethanol–water as the mobile phase and aniline hydrogen phthalate as the spray reagent. It showed two main spots, corresponding to galactose (R_G 0.075) and arabinose (R_G 0.135), and a very faint spot corresponding to rhamnose (R_G 0.3).

Quantitative determination of the sugar components of mucilage B. — Mucilage B (0.42 g) was subjected to acid hydrolysis with 0.5M sulfuric acid. After neutralization, filtration of barium sulfate, and evaporation, the resulting syrup was extracted with ethanol, and the extract de-ionized, and evaporated to a pale-yellow syrup (C; 280 mg). A portion of syrup C (100 mg) was distributed on paper chromatograms, and, after development, the positions of arabinose and galactose were marked, and each band was eluted with distilled water. The two eluates were separately concentrated, and treated with 3,5-dinitrosalicylic acid[12]. The red color developed was measured colorimetrically, using calibration curves for standard D-galactose and L-arabinose, giving 43.2% of galactose and 41.1% of arabinose. Rhamnose was present in a very small proportion, which could not be determined quantitatively.

The yellow syrup C (150 mg) was distributed on paper chromatograms and developed. The bands of galactose and arabinose were extracted with distilled water, and each eluate was concentrated. They were respectively identified as: arabinosazone, m.p. and mixed m.p. 166°.

Anal. Calc. for $C_{17}H_{20}N_4O_3$: C, 62.1; H, 6.1; N, 17.0. Found: C, 62.3; H, 6.5; N, 17.2.

Galactosazone, m.p. and mixed m.p. 196°.

Anal. Calc. for $C_{18}H_{22}N_4O_4$: C, 60.3; H, 6.1; N, 15.6. Found: C, 60.7; H, 5.6; N, 15.9.

Acetylation of mucilage B. — Powdered, dry mucilage B (0.3 g) was warmed

with pyridine (14 ml) for 2 h at 70°, and the mixture was cooled and kept overnight at room temperature. Acetic anhydride (6 ml) was then added dropwise during 30 min, and the mixture was kept for 3 days at 52°. The resulting, clear solution was diluted with acetic acid (14 ml) and poured, with stirring, into ethanol. The white precipitate was dried; wt. 0.12 g. It had $[\alpha]_D^{23}$ +170° (*c* 0.01, chloroform). Found: ash, 0.0%.

Methylation of mucilage B. — Powdered mucilage B (1.5 g) was dissolved in water (30 ml). It was then methylated ten times[13] with dimethyl sulfate and sodium hydroxide solution under an atmosphere of nitrogen. The mixture was neutralized with 0.25M sulfuric acid, dialyzed to remove inorganic salts, and then extracted with chloroform. The extract was evaporated to a pale-yellow syrup (0.9 g), whose i.r. spectrum had an OH band at 3500 cm^{-1}. The partially methylated product (0.9 g) was further methylated with the Purdie reagents, giving a brown substance (0.8 g). It had $[\alpha]_D^{23}$ +100°, η_{sp} 0.14 (*c* 0.1, chloroform).

Hydrolysis of methylated mucilage B. — The methylated product (0.5 g) was heated for 12 h at 100° with 0.5M hydrochloric acid (30 ml). The mixture was made neutral with cold, ethereal diazomethane, concentrated, de-ionized by passing through a mixture of Amberlite IR-120 (H^+) and IRA-400 (OH^-) ion-exchange resins, and evaporated to a syrup (380 mg).

Chromatography of the hydrolysis product. — Examination on a paper chromatogram with the upper layer of 5:1:4 1-butanol–ethanol–water as the mobile phase and aniline hydrogen phthalate as the spray reagent showed the following spots: 2,6-di-*O*-methylgalactose (R_G 0.44), 2,3-di-*O*-methylarabinose (R_G 0.64), 2,3,6-tri-*O*-methylgalactose (R_G 0.7), 2,3,4,6-tetra-*O*-methylgalactose (R_G 0.88), and 2,3,5-tri-*O*-methylarabinose (R_G 0.95).

Periodate oxidation of mucilage B. — Periodate oxidation of mucilage B indicated the consumption of one mole of periodate per mole of mucilage B. The liberated formic acid was equivalent to 5 moles per mole of mucilage, indicating branches of ~14 sugar units.

REFERENCES

1 EL S. AMIN, *J. Chem. Soc.*, (1955) 2441–2445.
2 EL S. AMIN, *J. Chem. Soc.*, (1956) 828–832.
3 K. M. LEFÉVRE AND B. TOLLENS, *Ber.*, 40 (1907) 4518–4521.
4 C. H. HILLS, C. L. OGG, AND R. SPEISER, *Ind. Eng. Chem. Anal. Ed.*, 17 (1945) 507–512.
5 EL S. AMIN AND O. M. AWAD, *Carbohydr. Res.*, 7 (1968) 12–16.
6 EL S. AMIN AND M. EL SAYED, *Carbohydr. Res.*, 15 (1970) 159–161.
7 E. L. HIRST, J. K. N. JONES, AND A. J. ROUDIER, *J. Chem. Soc.*, (1948) 1779–1783.
8 S. LUCKETT AND F. SMITH, *J. Chem. Soc.*, (1940) 1106–1114.
9 T. PURDIE AND J. C. IRVINE, *J. Chem. Soc.*, 83 (1903) 1021–1026.
10 L. HOUGH, J. K. N. JONES, AND W. H. WADMAN, *J. Chem. Soc.*, (1952) 3393–3399.
11 J. G. HALSALL, E. L. HIRST, AND J. K. N. JONES, *J. Chem. Soc.*, (1948) 27–32.
12 K. H. MEYER, G. NEOLTING, AND P. BERNFELD, *Helv. Chim. Acta*, 31 (1948) 103–105.
13 C. E. BALLOU AND E. G. V. PERCIVAL, *J. Chem. Soc.*, (1952) 1054–1056.