177. *The Mucilages of* Hibiscus esculentus (*Okra or Bamia fellahi*) *and* Corchorus olitorius (*Mulukhia*).

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The purification and monosaccharide degradation products of these two mucilages are reported.

Okra (Family Malvaceæ, Order Malvales) is widely cultivated and the pods are used as common food in Egypt. In water the minced pods yield a mucilage indistinguishable in appearance from solutions of other plant gums and mucilages. Partial hydrolysis of the mucilage gave three galactobioses, one of which has been proved to be 4-*O*-D-galactopyranosyl-D-galactose.[1] The following oligosaccharides were found[2] as hydrolytic fragments, suggesting that the linkages involved are present in the polysaccharide: 2-*O*-(D-galactopyranosyluronic acid)-L-rhamnose, galactosyl → (galactosyluronic acid) → rhamnose, and (galactosyluronic acid) → rhamnosyl → galactose.

In our work, the mucilage was extracted with water at room temperature and purified by reprecipitation from alcohol, formation of a water-insoluble copper complex, and dialysis. Attempts to separate the pentoses and uronic acid by fractional precipitation were unsuccessful. The substance had a low positive optical rotation and a slight reducing action on Fehling's solution. Hydrolysis of the pure mucilage by mineral acid and paper

[1] Whistler and Conrad, *J. Amer. Chem. Soc.*, 1954, **76**, 1673.
[2] *Idem, ibid.*, p. 3544.

chromatography gave galactose (80%), rhamnose (10%), and a little arabinose (3%) and galacturonic acid (6%). The mucilage was then acetylated, deacetylated, and methylated with methyl sulphate and sodium hydroxide followed by methyl iodide and silver oxide. Fractionation and hydrolysis of the main fraction yielded 2 : 3 : 4 : 6-tetra- (12%), 2 : 3 : 6-tri- (60%), and 2 : 6-di-*O*-methylgalactose (6%), 3 : 4-di-*O*-methylrhamnose (8%), and (?)-*O*-methyl-L-rhamnose but no arabinose derivative. This suggests that the main linkage among the hexose units is 1 : 4, and that rhamnose is linked through $C_{(1)}$ and $C_{(2)}$ and is in the pyranose form. The dimethylgalactose may result from incomplete methylation or from demethylation of the methylated sugar during methanolysis.

Corchorus (Family Tiliaceæ, Order Malvales) is also widely cultivated and the leaves are a popular food in Egypt. When the leaves are minced in water, a mucilage is dispersed, the viscosity of which is greatly diminished by boiling. The mucilage was prepared by extraction with water at room temperature, and purified in the same way as the okra mucilage. It gave a white amorphous substance difficultly soluble in water, with a low specific rotation and slight reducing action on Fehling's reagent. Pentoses could not be separated by fractional precipitation. Hydrolysis of the mucilage by mineral acid and paper chromatography gave galactose (60%), rhamnose (20%), arabinose (12%), xylose (6%), and uronic acid (1·2%). Methylation as above and fractionation gave a main fraction with a low specific rotation. Hydrolysis yielded 2 : 3 : 4 : 6-tetra- (3·5%), 2 : 3 : 6-tri- (58%), and 2 : 6-di-*O*-methylgalactose (8%), 3 : 4-di-*O*-methylrhamnose (14%), and (?)-*O*-methylrhamnose (10%). Xylose and arabinose derivatives were absent. The above derivatives prove that the hexose units are mainly linked through $C_{(1)}$ and $C_{(4)}$, and rhamnose through $C_{(1)}$ and $C_{(2)}$, being present in the pyranose form.

Experimental

Chromatography was carried out on Whatman No. 1 filter-paper sheets by the descending method [3] at room temperature, with butan-1-ol–ethanol–water (5 : 1 : 4 v/v). Sugars and methyl-sugars were detected with *p*-anisidine hydrochloride as spray reagent. Evaporations were done under reduced pressure at 50° unless otherwise stated. Specific rotations and viscosities were measured at 20°. Treatments with alkali were under nitrogen. The results were calculated on an ash-free basis.

Preparation of Okra Mucilage.—Okra pods (5 kg.), harvested in August, 1953, at Alexandria, Egypt, were cut into thin slices and minced in 0·1N-hydrochloric acid (9 l.) for 24 hr. The mucilage which was dispersed in the aqueous solution was squeezed through a sieve (mesh 2 mm.). Re-extraction was carried out and the residue discarded. The aqueous extract was shaken with charcoal for 4 hr. at room temperature, then twice centrifuged (3500, then 24,000 r.p.m.). The clear liquor was poured into 96% ethyl alcohol (5 parts) with stirring, giving a white fibrous material. To reduce the ash content (2%) the product was redispersed in water, 5% hydrochloric acid was added to a final normality of 1, and the mucilage dialysed for 24 hr. (yield 210 g., 2%). Further purification was effected by dissolution in N-sodium hydroxide, acidification with acetic acid, centrifugation, and precipitation with alcohol (yield 200 g., 4%). The yield of the mucilage was not quantitative as much of it remained in the unruptured cells. The product was dissolved in ammonia (*ca.* 2N), and a neutral solution was treated with 25% aqueous copper chloride. The resultant insoluble copper complex was removed on the centrifuge and washed with water and alcohol, followed by ethanolic hydrogen chloride (5%) until the washings were copper-free, and finally with ethanol until chloride-free. The product was dried to constant weight (160 g.) in a vacuum at 50°. The mother-liquor from the copper complex was concentrated to 200 c.c. and treated with alcohol (5 parts). The precipitate was treated as above, giving an amorphous white polysaccharide (4·5 g.).

Properties of Okra Mucilage.—It was a white amorphous substance insoluble in organic solvents, and dissolved with difficulty in water, but easily in alkali, giving a viscous solution. It had little reducing action on Fehling's reagent, and was unaffected by saliva during 10 min. (Found : sulphated ash, 0·1; N, 0·08%); it had $[\alpha]_D$ +26° (*c* 0·8 in N-NaOH), $\eta_{sp.}$ 0·22 (*c* 0·1 in N-NaOH), and gave 6% of uronic acid by Lefèvre and Tollens's method.[4] According to a report from Prof. Dr. M. S. Ridi, Kasr El Aini, Faculty of Medicine, Cairo, the composition of Okra pods was : water 85·7, carbohydrate 6·3, protein 3·0, ash 1·3, fat 0·2%, Cross cal./100 g. 40·0.

[3] Partridge, *Biochem. J.*, 1948, **42**, 238.
[4] Lefèvre and Tollens, *Ber.*, 1907, **40**, 4513.

Acid Hydrolysis of Okra Mucilage.—A portion (420 mg.) was heated with N-sulphuric acid (30 c.c.) at 100° in a sealed tube for 12 hr. The mixture in which was suspended a small amount of flocculent material (10 mg.) was filtered and the filtrate neutralised with 0·01N-barium hydroxide, barium sulphate removed, and the solution evaporated to dryness. The residue was extracted with boiling methanol until the extracts were non-reducing, leaving a residue of barium salts (*A*) (30 mg.). The methanol extracts were de-ionised with a mixture of Amberlite resins IR-120 and IRA-400 and concentrated to a syrup (*B*) (280 mg.). Examination of a portion on the paper chromatogram gave spots corresponding to galactose (R_G 0·07), rhamnose (R_G 0·30), and arabinose (R_G 0·12). Quantitative determination of the sugars [5] indicated galactose 80, arabinose 3, and rhamnose 10%.

The syrup *B* (120 mg.) was separated on the paper chromatogram, and appropriate fractions were extracted with hot methanol. The first fraction had $[\alpha]_D$ +78° (*c* 0·9 in N-H_2SO_4) and gave D-galactose methylphenylhydrazone [6] (60 mg.), m. p. and mixed m. p. 185° (decomp.), and mucic acid,[7] m. p. and mixed m. p. 215°. The second fraction, $[\alpha]_D$ +9° (equil.; *c* 1·0), was treated with alcoholic benzoylhydrazine,[6] giving crystalline L-rhamnose benzoylhydrazone (8 mg.), m. p. and mixed m. p. 183° (decomp.). The third fraction, $[\alpha]_D$ +104° (equil.; *c* 0·3 in H_2O), gave L-arabinose α-benzoylphenylhydrazone [6] (3 mg.), m. p. and mixed m. p. 170°.

Examination of the barium salt (A). A portion (30 mg.) was treated with dilute hydrochloric acid, then chloride ion was removed by silver carbonate. After filtration, hydrogen sulphide was passed into the filtrate, the insoluble silver sulphide removed, and the filtrate concentrated to a syrup (4 mg.). This was examined on the paper chromatogram alongside galacturonic acid. There was one spot corresponding to galacturonic acid. The syrup gave a brick-red precipitate when warmed with basic lead acetate, indicating the presence of galacturonic acid, a test not given by glucuronic acid or mannuronic acid.[8]

Acetylation of Okra Mucilage.—The dry powdered polysaccharide (5 g.) was warmed with pyridine (100 c.c.) for 2 hr. at 70°, then cooled and kept overnight at room temperature. Acetic anhydride (40 c.c.) was added dropwise during ½ hr. The mixture was then incubated at 52° for 3 days. The clear solution was diluted with acetic acid (100 c.c.) and poured in stirred ethyl alcohol (2 l.). The white amorphous material was centrifuged, washed with alcohol and ether, and dried (yield, 4 g.). Reacetylation was carried out in the same way, giving a product (3·2 g.) having $[\alpha]_D$ +40° (*c* 0·8 in $CHCl_3$).

Methylation of Okra Mucilage.—(*a*) A portion (6·5 g.) was suspended in water (40 c.c.) and allowed to swell for 2 hr. The air was displaced by nitrogen, and the flask was cooled to 5°. Sodium hydroxide solution (30%; 80 c.c.) was added with vigorous stirring. After 2 hr., dimethyl sulphate (40 c.c.) was added during 8 hr. Stirring was continued overnight, and the reaction was completed at 50° for an hour. The mixture was cooled and treated with 30% sodium hydroxide solution (100 c.c.), followed by methyl sulphate (50 c.c.) as before. It was then neutralised with 0·5N-sulphuric acid and dialysed for 24 hr. The methylation was repeated six times in presence of acetone (80 c.c. each time) at room temperature. The final product was extracted with chloroform, and the chloroform extract twice methylated as above, then twice treated with methyl iodide (80 c.c.) and silver oxide (40 g.) for 12 hr., giving a product (4·2 g.), $[\alpha]_D$ +52° (*c* 0·8 in $CHCl_3$) (Found: OMe, 37·5%).

(*b*) A portion (3 g.) of the acetylated mucilage was dispersed in acetone (150 c.c.) and exhaustively methylated as above {yield 2·4 g.; $[\alpha]_D$ +60° (*c* 1 in $CHCl_3$) (Found: OMe, 39%)}.

The methylated product was fractionated by successive extractions under reflux with mixtures (50 c.c.) of light petroleum (b. p. 60—80°) and chloroform in the following proportions: 19 : 1; 10 : 1; 17 : 3; 4 : 1. The product dissolved almost completely in the last mixture, and the fraction obtained was unchanged in methoxyl content.

Hydrolysis of Methylated Okra Mucilage.—A portion (0·6 g.) was heated for 12 hr. at 100° with 3% methanolic hydrogen chloride, then neutralised with cold ethereal diazomethane, concentrated at room temperature, and refluxed with 4% hydrochloric acid for 7 hr. The solution was neutralised with silver carbonate, de-ionised with a mixture of Amberlite resins IR-120 and IRA-400, and evaporated to a syrup (540 mg.). Examination on the paper chromatogram indicated the presence of 2 : 3 : 4 : 6-tetra-*O*-methylgalactose, 3 : 4-di-*O*-methylrhamnose (R_G 0·86), 2 : 3 : 6-tri- (R_G 0·71) and 2 : 6-di-*O*-methylgalactose (R_G 0·41), and (?)-*O*-methylrhamnose (R_G 0·57). Quantitative determination by the alkaline hypoiodite

[5] Flood, Hirst, and Jones, *J.*, 1948, 1679; Nicolet and Shinn, *J. Amer. Chem. Soc.*, 1941, **63**, 1456.
[6] Hirst, Jones, and Woods, *J.*, 1947, 1048.
[7] Heyne and Whistler, *J. Amer. Chem. Soc.*, 1948, **70**, 2249.
[8] Stacey, *J.*, 1939, 1529.

procedure [9] gave 2 : 3 : 4 : 6-tetra-*O*-methylgalactose and 3 : 4-di-*O*-methylrhamnose (20%), 2 : 3 : 6-tri- (60%) and 2 : 6-di-*O*-methylgalactose (6%), and (?)-*O*-methylrhamnose (9%).

Another portion (200 mg.) of the hydrolysate was separated on the paper chromatogram, and fractions were extracted with methanol. The first fraction (Found : OMe, 44·3. Calc. for $C_{10}H_{20}O_6$: OMe, 52·5; for $C_8H_{16}O_5$: OMe, 32·3%) appeared to contain 2 : 3 : 4 : 6-tetra-*O*-methylgalactose (12%) and 3 : 4-di-*O*-methylrhamnose (8%). That fraction was heated with methanolic 2% hydrogen chloride under reflux for 7 hr.; fractional distillation in a vacuum then gave: fraction (*a*), b. p. 120—125°/0·001 mm., hydrolysed by N-sulphuric acid for 6 hr. to a syrup, which gave 2 : 3 : 4 : 6-tetra-*O*-methyl-*N*-phenyl-D-galactosylamine (6 mg.), m. p. and mixed m. p. 194°; and fraction (*b*), b. p. 130—140°/0·001 mm., hydrolysed as above to a syrup, which was oxidised with bromine water to 3 : 4-di-*O*-methyl-L-rhamnolactone (3 mg.), m. p. and mixed m. p. 78°, $[\alpha]_D$ −114° (equil.; *c* 0·2 in H_2O). The second fraction had $[\alpha]_D$ +87° (*c*, 0·9) (Found : OMe, 38·5. Calc. for $C_9H_{18}O_6$: OMe, 41·8%) and was oxidised with bromine [10] to 2 : 3 : 6-tri-*O*-methyl-D-galactonolactone (from ether–light petroleum) (6 mg.), m. p. and mixed m. p. 99°, $[\alpha]_D$ −40·5° (*c* 0·8 in H_2O).

Corchorus *Mucilage*.—The leaves (5 kg.) were freshly harvested in August, 1953, at Alexandria, Egypt. They were cut and minced in 0·1N-hydrochloric acid (9 l.) for 24 hr. The mucilage was then isolated as for Okra mucilage (yield 100 g., 2%). The yield of polysaccharide from the soluble copper complex was 0·5 g. The yield of mucilage was not quantitative as some still remained in the unruptured cells.

Properties of Corchorus *Mucilage*.—These were similar to those of Okra mucilage (Found : sulphated ash, 0·1; N, 0·03%); the material had $[\alpha]_D$ +39° (*c* 0·6 in N-NaOH), $\eta_{sp.}$ 0·1 (*c* 0·1 in N-NaOH), and gave 3% of uronic acid.

Hydrolysis of Corchorus *Mucilage*.—A portion (500 mg.) was heated with N-sulphuric acid (35 c.c.) at 100° for 24 hr. in a sealed tube. Flocculent material (8 mg.) was filtered off and the filtrate neutralised with barium carbonate. It was de-ionised as above and concentrated to a syrup (320 mg.), which on the paper chromatogram gave spots corresponding to galactose (R_G 0·07), rhamnose (R_G 0·30), arabinose (R_G 0·12), and xylose (R_G 0·15). Quantitative determination [5] of the sugars indicated galactose (60%), rhamnose (20%), arabinose (12%), and xylose (6%).

A portion (150 mg.) of the hydrolysate was separated on the paper chromatogram and the fractions were extracted with hot methanol. The first fraction had $[\alpha]_D$ +78° (*c*, 0·9 in N-H_2SO_4) and gave D-galactose methylphenylhydrazone (40 mg.), m. p. and mixed m. p. 185°, and mucic acid, m. p. and mixed m. p. 215°. The second fraction had $[\alpha]_D$ +9·4° (equil; *c* 0·3) and gave L-rhamnose benzoylhydrazone (15 mg.), m. p. and mixed m. p. 183° (decomp.). The third fraction had $[\alpha]_D$ +106° (equil.; *c* 0·2 in H_2O) and gave L-arabinose α-benzoylphenylhydrazone (5 mg.), m. p. and mixed m. p. 170°. The fourth fraction gave di-*O*-benzylidene-D-xylose dimethyl acetal [11] (3 mg.), m. p. and mixed m. p. 211°.

Acetylation of Corchorus *Mucilage*.—A portion (5·5 g.) was twice acetylated in the same way as Okra mucilage (yield 3 g.). It had $[\alpha]_D$ +46° (*c* 0·9 in $CHCl_3$).

Methylation of Corchorus *Mucilage*.—(*a*) A portion (7 g.) was exhaustively methylated with methyl sulphate and sodium hydroxide, then esterified by two treatments with methyl iodide and silver oxide (yield 4·3 g.). The product had $[\alpha]_D$ +58° (*c* 0·6 in $CHCl_3$) (Found : OMe, 36%). The methylated product was fractionated by successive extractions with light petroleum–chloroform as before. The main fraction was soluble in the 4 : 1 mixture and had the same methoxyl content as the other fractions and $[\alpha]_D$ +51° (*c* 0·7 in $CHCl_3$).

Hydrolysis of Methylated Corchorus *Mucilage*.—A portion (560 mg.) was heated for 12 hr. at 100° with methanolic hydrogen chloride, then neutralised with cold ethereal diazomethane, concentrated, and refluxed with 4% hydrochloric acid for 7 hr. The solution was neutralised with silver carbonate, de-ionised as usual, and evaporated to a syrup (yield 490 mg.). Examination on the paper chromatogram indicated presence of 2 : 3 : 4 : 6-tetra-*O*-methylgalactose, 3 : 4-di-*O*-methylrhamnose (R_G 0·86), 2 : 3 : 6-tri- (R_G 0·71) and 2 : 6-di-*O*-methylgalactose (R_G 0·41), and (?)-*O*-methylrhamnose (R_G 0·57). Quantitative determination [9] gave 2 : 3 : 4 : 6-tetra-*O*-methylgalactose and 3 : 4-di-*O*-methylrhamnose (17·5%), 2 : 3 : 6-tri- (58%) and 2 : 4-di-*O*-methylgalactose (6%), and (?)-*O*-methylrhamnose (10%). Another portion of the hydrolysate (180 mg.) was chromatographed, the methylated sugars were located, and sections of the different fractions extracted with hot methanol. The first fraction (Found : OMe, 36·3.

[9] Hirst, Hough, and Jones, *J.*, 1949, 928.
[10] Hough and Jones, *J.*, 1950, 1199.
[11] Breddy and Jones, *J.*, 1945, 738.

Calc. for $C_{10}H_{20}O_6$: OMe, 52·5; for $C_8H_{16}O_5$: OMe, 32·3%) contained 2 : 3 : 4 : 6-tetra-*O*-methyl-galactose (3·5%) and 3 : 4-di-*O*-methylrhamnose (14%). This fraction was heated with methanolic 2% hydrogen chloride under reflux for 7 hr. The methylated sugars were fractionally distilled in a vacuum, giving fractions: (*a*) b. p. 120—125°/0·001 mm., hydrolysed by N-sulphuric acid (6 hr.) to a syrup (Found: OMe, 49·2%), and yielding 2 : 3 : 4 : 6-tetra-*O*-methyl-*N*-phenyl-D-galactosylamine (4 mg.), m. p. and mixed m. p. 194°; and (*b*) b. p. 130—140°/0·001 mm., hydrolysed as above to a syrup, which was oxidised with bromine to 3 : 4-di-*O*-methyl-L-rhamnolactone, m. p. and mixed m. p. 78° $[\alpha]_D$ −116° (equil; *c* 0·3 in H_2O).

The second fraction had $[\alpha]_D$ +87° (*c* 0·9) (Found: OMe, 39·2. Calc. for $C_9H_{18}O_6$: OMe, 41·8%). It was oxidised with bromine[10] to 2 : 3 : 6-tri-*O*-methyl-D-galactonolactone (10 mg.), m. p. and mixed m. p. 99°, $[\alpha]_D$ −40° (*c* 1 in H_2O).

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