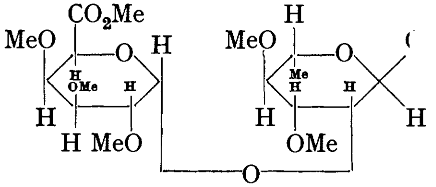
312. *Constitution of the Mucilage from the Bark of* Ulmus Fulva (*Slippery Elm Mucilage*). *Part I. The Aldobionic Acid obtained by Hydrolysis of the Mucilage.*

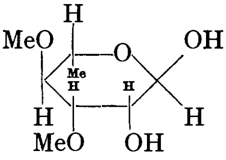
By R. E. Gill, E. L. Hirst, and J. K. N. Jones.

An aldobionic acid, 2-*d*-galacturonido-*l*-rhamnose, has been obtained by the partial hydrolysis of a mucilage which can be extracted from the inner bark of the tree *Ulmus fulva* (slippery elm bark). Methylation by the thallium process gave the fully methylated derivative, which on hydrolysis gave 2 : 3 : 4-trimethyl *d*-galacturonic acid and 3 : 4-dimethyl *l*-rhamnose in equimolecular proportion. It follows that the aldobionic acid is 2-*d*-galacturonido-*l*-rhamnose, and is therefore identical with an aldobionic acid isolated from flax seed mucilage by Tipson, Christman, and Levene (*J. Biol. Chem.*, 1939, **128**, 609).

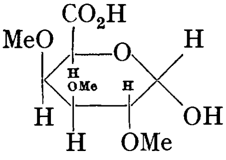
In connection with work on pectic materials we are investigating the structure of the well-known mucilage which can be extracted from the inner bark of the tree *Ulmus fulva*. Anderson (*J. Biol. Chem.*, 1933, **104**, 163) obtained from this substance an aldobionic acid which has the structure of a rhamnose galacturonide. We have now found that the aldobionic acid consists of a pyranose galacturonic acid residue united by its reducing group to the hydroxyl situated at C_2 of the rhamnopyranose portion. This conclusion follows from the identification of the hydrolysis products obtained from the fully methylated derivative (I) of the aldobionic acid. These were (*a*) 3 : 4-dimethyl rhamnose (II), recognised after oxidation by bromine water, which gave crystalline 3 : 4-dimethyl *l*-rhamnonolactone, the identity of which was confirmed by its conversion into 3 : 4-dimethyl *l*-rhamnonamide (Haworth, Hirst, and Miller, J., 1929, **246**; compare Tipson, Christman, and Levene, *J. Biol. Chem.*, 1939, **128**, 609); and (*b*) 2 : 3 : 4-trimethyl *d*-galacturonic acid (III), identified, after oxidation by bromine water and esterification of the product, as the crystalline methyl ester of 2 : 3 : 4-trimethyl mucic acid (Levene and Kreider, *J. Biol. Chem.*, 1937, **120**, 602).



(I.)



(II.)



(III.)

The aldobionic acid was therefore 2-*d*-galacturonido-*l*-rhamnose, in which both residues are present in the pyranose form. Whether the galacturonido link is α- or β- in type cannot at present be decided with certainty, but in view of the high positive rotation of the methylated aldobionic acid (which is a mixture of hexamethyl 2-*d*-galacturonido-α-methyl-*l*-rhamnoside and the corresponding β-form) it is probable that the galacturonic acid is bound to the rhamnose by an α-link. The same aldobionic acid has recently been found by Tipson, Christman, and Levene (*loc. cit.*) amongst the hydrolysis products of flax-seed mucilage. It is of interest to find in slippery elm mucilage yet another example of a naturally occurring polyuronide in which the uronic acid residue is united glycosidically to position 2 of a sugar residue. Other examples are damson gum (Hirst and Jones, J., 1938, 1178) and cherry gum (Jones, this vol., p. 558). In the two gums the corresponding aldobionic acid consists of *d*-glycuronic acid in its pyranose form, united through the glycosidic group to the hydroxyl at C_2 of a *d*-mannose residue.

Residues of 2-*d*-galacturonido-*l*-rhamnose are therefore a fundamental feature of the molecular structure of slippery elm mucilage. The uronic acid residue must be present in the mucilage in its pyranose form, but the evidence at present available does not permit of a decision regarding the ring structure of the rhamnose portion, which is capable of forming both furanoside and pyranoside derivatives, positions 4 and 5 being unsubstituted.

We hope to present shortly further evidence bearing on this point and on the nature of the galactose residue.

EXPERIMENTAL.

Preparation of Slippery Elm Mucilage.—The bark was extracted with boiling water for 24 hours and the liquid was then filtered through a large Buchner funnel (without filter-paper) and again through linen (to remove small quantities of bark); the clear solution was poured into 3 vols. of methylated spirits acidified with hydrochloric acid. The pure polysaccharide dissolved in water, forming very viscous acidic solutions. $[\alpha]_{\mathrm{D}}^{20^\circ}$ + 12° in water (*c*, 1·0). Equiv. wt. 460.

Isolation of an Aldobionic Acid (2-d-*Galacturonido*-l-*rhamnose*) *after Hydrolysis of the Mucilage.*—An aqueous solution of the polysaccharide when boiled underwent partial hydrolysis, with separation of about 10% of dark-coloured material which gave positive tests for C, H, N, and S, and became much less viscous. The filtrate was poured into alcohol, and the partially degraded polysaccharide collected, washed, and dried. $[\alpha]_{\mathrm{D}}^{20^\circ}$ + 68° in water (*c*, 1·0), equiv. wt. by titration 468, uronic anhydride (from carbon dioxide liberated by boiling 12% hydrochloric acid) 36·6% (corresponding to equiv. wt. 482), furfural and methylfurfural 18% (formed on boiling with 12% hydrochloric acid); methylfurfural approx. 12%.

Degraded mucilage (20 g.) was hydrolysed with N-sulphuric acid (200 c.c.) at 90° for 9 hours. The cooled solution was neutralised with barium carbonate, filtered, evaporated to 100 c.c., and poured into methyl alcohol. The precipitated barium salts were filtered off, washed with methyl alcohol, dried, dissolved in water, and purified by reprecipitation by ethyl alcohol. The precipitate (14 g.) was washed with alcohol and ether and dried (Found: Ba, 17·0. $C_{12}H_{19}O_{11}Ba_2$ requires Ba, 16·9%).

The *barium* salt (12 g.) was dissolved in water (50 c.c.), barium precipitated as sulphate by the gradual addition of N-sulphuric acid and removed on the centrifuge, and the solution (which still contained a little barium salt) evaporated to dryness at 40°/12 mm. after addition of N-thallous hydroxide (230 c.c.). The powdered solid was boiled with methyl iodide until it no longer gave an alkaline reaction, the excess of methyl iodide removed, and the solid extracted with alcohol. The extracts were concentrated to a syrup (8·0 g.), $n_{\mathrm{D}}^{19^\circ}$ 1·4745, which was methylated with Purdie's reagents. The product was isolated in the usual manner and some methylated monosaccharide was separated by distillation at 120°/0·01 mm. (bath temp.). The residue was methylated three times with Purdie's reagents, giving a product, $n_{\mathrm{D}}^{19^\circ}$ 1·4735, OMe 43·5%, a portion (1·2 g.) of which was distilled: fraction I (0·2 g.), b. p. 120—130°/0·01 mm. (bath temp.), $n_{\mathrm{D}}^{19^\circ}$ 1·4540 (mainly monosaccharides); fraction II, the methyl ester of 2-(2 : 3 : 4-trimethyl-*d*-galacturonido) 3 : 4-dimethyl methyl-*l*-rhamnopyranoside (0·85 g.), b. p. 170°/0·01 mm. (bath temp.), $n_{\mathrm{D}}^{19^\circ}$ 1·4722, $[\alpha]_{\mathrm{D}}$ + 76° (*c*, 3·2 in water) (Found: OMe, 46·2; equiv., 430. Calc. for $C_{19}H_{34}O_{11}$: OMe, 49·5%; equiv. 438).

The distillate (0·79 g.) was hydrolysed with 2N-hydrochloric acid (25 c.c.) at 95°. $[\alpha]_{\mathrm{D}}^{20^\circ}$ + 76° (initial value), falling to + 65° in 3 hours. The cooled solution was neutralised with silver carbonate and filtered, silver ions removed with hydrogen sulphide, the solution aerated to remove hydrogen sulphide, evaporated to a small volume, neutralised with barium carbonate, and filtered, and the filtrate evaporated to dryness, giving a product (A), which was exhaustively extracted with ether. Concentration of the extracts gave a syrup (0·20 g.), $n_{\mathrm{D}}^{19^\circ}$ 1·4730, $[\alpha]_{\mathrm{D}}^{19^\circ}$ + 20° (*c*, 2·0 in water) (Found: OMe, 31·2. Calc. for $C_8H_{16}O_5$: OMe, 32·2%).

The syrup (0·18 g.) was dissolved in water (10 c.c.) and oxidised with bromine (0·5 c.c.) until the solution was non-reducing (24 hrs.). Bromine was removed by aeration, and the solution worked up in the usual manner. The product (0·16 g.) crystallised almost completely and on recrystallisation from ether–light petroleum 3 : 4-dimethyl *l*-rhamnonolactone was obtained, m. p. 76—78°, $[\alpha]_{\mathrm{D}}^{20^\circ}$ − 150° (*c*, 3·0 in water) after 10 mins. and − 116° after 3 days (Found: OMe, 30·0; equiv., 194. Calc. for $C_8H_{14}O_5$: OMe, 32·6%; equiv. 190).

With liquid ammonia the lactone gave 3 : 4-dimethyl *l*-rhamnonamide, m. p. 153° (compare Haworth, Hirst, and Miller, *loc. cit.*), which with sodium hypochlorite yielded sodium *iso*cyanate (detected in the usual way as hydrazodicarbonamide, m. p. 258°). This positive Weerman reaction confirmed the presence of a free hydroxyl group at C_2 in the dimethyl rhamnose.

The residual barium salts from (A) were dissolved in water and oxidised with bromine water until non-reducing (2 days at 20°). The excess of bromine was removed by aeration, the solution neutralised with barium carbonate, filtered, and evaporated to dryness, and the residue boiled with 3% methyl-alcoholic hydrogen chloride (30 c.c.) for 12 hours. Hydrogen chloride was removed with silver carbonate, and the filtered solution evaporated to a solid, which was exhaustively extracted with ether. Concentration of the extracts gave a syrup (0·22 g.), which was distilled, b. p. 140°/0·01 mm. The distillate (0·2 g.) set solid and on recrystallisation from ether light–petroleum gave methyl 2 : 3 : 4-trimethyl mucate, m. p. and mixed m. p. with an authentic specimen 101° (Found : OMe, 58·0. Calc. for $C_{11}H_{20}O_8$: OMe, 55·4%).

The authors thank the Colston Research Society for the award of a Mardon Fellowship to one of them (R. E. G.).

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[*Received, July 27th,* 1939.]