72. *Constitution of the Mucilage from the Bark of* Ulmus Fulva (*Slippery Elm Mucilage*). *Part III. The Isolation of* 3-*Monomethyl* D-*Galactose from the Products of Hydrolysis.*

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The isolation and characterisation of 3-methyl D-galactose, a component of Slippery Elm mucilage, is described.

An investigation of the hydrolysis products of slippery elm mucilage by Anderson (*J. Biol. Chem.*, 1933, **104**, 163) revealed that D-galacturonic acid, D-galactose, L-rhamnose, and a partially methylated aldose were constituent monosaccharide units. Constitutional studies were

continued by Gill, Hirst, and Jones (Part I, *J.*, 1939, 1469) who, as the result of a graded hydrolysis of the mucilage, succeeded in isolating an aldobiuronic acid, identified as 2-D-galacturonosido-L-rhamnose. Conversion of the mucilage into the fully methylated derivative by the thallium procedure, followed by hydrolysis, gave a complex mixture of methylated monosaccharides containing only galactose, rhamnose, and galacturonic acid derivatives (Part II, *idem, ibid.*, 1946, 1025). The identity of the sugar containing the methoxy-groups was therefore unknown, and the present paper is concerned with the characterisation of this sugar.

The acidic polysaccharide obtained by repeated precipitation from acidified alcohol contained 3·3% of methoxyl groups. Acidic hydrolysis of this polysaccharide afforded a mixture of reducing sugars (*A*; see Experimental section), in addition to D-galacturonic acid and 2-D-galacturonosido-L-rhamnose. After separation from the acidic materials, the mixture of sugars was separated by paper partition chromatography, and from the rate of movement of the sugars and from the colours produced on reaction with a variety of specific spray reagents (Hough, Jones, and Wadman, *J.*, 1950, 1702), presumptive evidence was obtained for the presence of galactose, rhamnose, and 3-methyl galactose. This result was confirmed by the separation of the sugars on a column of cellulose, *n*-butanol–ethanol–water being used as the mobile phase (*idem, ibid.*, 1949, 2511), which led to the isolation of pure crystalline specimens of D-galactose, 3-methyl D-galactose, and L-rhamnose. Traces of at least three other sugars were detected, two of which appear to be glucose and fucose. 3-Methyl D-galactose isolated from slippery elm mucilage was identical in all respects with a synthetic specimen kindly supplied by Professor T. Reichstein. A preliminary account of these results has been given elsewhere (Hirst, Hough, and Jones, *Nature*, 1950, **165**, 34). 3-Methyl D-galactose has not been encountered hitherto in any natural product, and it is of interest to note the relation to the cardiac glycosides in which 3-methyl D-fucose (digitalose), 3-methyl 2-deoxy-D-allose (cymarose), and 3-methyl 2 : 6-dideoxy-D-allose (olleandreose) have been detected (cf. Elderfield, *Adv. Carbohydrate Chem.*, 1945, **1**, 147). It is noteworthy that the methylated sugars encountered so far in Nature are all of the 3-methyl type, thus raising an important phytochemical problem. In the case of the uronic acids, however, only one natural methyl derivative has so far been detected, namely, 4-methyl D-glucuronic acid in Mesquite gum (White, *J. Amer. Chem. Soc.*, 1948, **70**, 367).

The provisional formula for the general structure advanced by Gill, Hirst, and Jones (*loc. cit.*) for the carbohydrate portion of slippery elm mucilage still holds and it remains to determine which galactose residues are substituted on $C_{(3)}$ by methoxyl groups. A consideration of the analytical data for the purified mucilage, in conjunction with the results obtained for the hydrolysis products of the gum, indicates that the following monosaccharide residues are present, approximately in the proportions indicated: D-galactose (1 part), 3-methyl D-galactose (1 part), L-rhamnose (2 parts), and D-galacturonic acid (2 parts). A polysaccharide built up of these residues in the proportions indicated would contain 3·2% of methoxyl groups.

Experimental.

Hydrolysis of the Purified Polysaccharide.—The mucilage was extracted from slippery elm bark and purified by the method described in Part I (*loc. cit.*). The purified material had $[\alpha]_D^{20}$ +65° in water (*c*, 0·87) [Found: N, 0·5; sulphated ash, 1·9; OMe, 3·3%; equiv., 452 (by titration with alkali)]. The polysaccharide (5 g.) was dissolved in N-sulphuric acid (50 ml.) and heated in the boiling-water bath for 9 hours. The solution was then cooled, neutralised with a slurry of barium carbonate, and filtered, and the filtrate evaporated under reduced pressure. The residue was extracted with boiling methanol (4 × 150 ml.), and the extracts were combined and evaporated under reduced pressure to a thick syrup (*A*) (1·5 g.). The insoluble barium salts (*B*) were collected and dried *in vacuo* over phosphoric oxide at 60° (yield, 3·6 g.) (Found: Ba, 25·3; OMe, 2·2%).

Separation of the Mixture of Sugars. (A).—The syrup (*A*) (1·2 g.) was separated on a column of cellulose, *n*-butanol (95%)–ethanol (5%) nearly saturated with water being used as the mobile phase. After an examination of small portions of the eluate on the paper chromatogram, the eluate was divided in such a manner as to lead to the highest possible recovery of each component sugar. The solvent was removed by distillation under reduced pressure and the following fractions were obtained: Fraction I (0·17 g.) had R_G 0·30 and when crystallised gave L-rhamnose hydrate, m. p. 98—100°, $[\alpha]_D$ +9° (*c*, 1·2). Fraction II (0·005 g.) showed $[\alpha]_D$ +14° ± 10° (*c*, 0·2), and moved to the same position on the paper chromatogram as fucose; it also showed the same colour reactions when the paper chromatogram was sprayed with acidic solutions of diphenylamine, α-naphthylamine, or aniline and heated. This evidence is only indicative, however, and further evidence will be necessary for its conclusive identification. Fraction III (0·168 g.), R_G 0·17, crystallised from acetone–methanol, giving 3-methyl D-galactose, m. p. and mixed m. p. 140—141°, $[\alpha]_D^{18}$ +139° (10 minutes; *c*, 0·2 in water) ⟶ +86° (20 hours; constant value), $[\alpha]_D^{18}$ +62° (initial value; *c*, 0·2 in 4% methanolic hydrogen chloride) ⟶ −43° (16 hours; constant value) (Found: C, 43·3; H, 7·6; OMe, 15·2. Calc. for $C_7H_{14}O_6$: C, 43·3; H, 7·2; OMe,

16·0%); an *X*-ray powder photograph of the crystals was identical with that of a known specimen of 3-methyl D-galactose. Fraction IV (0·082 g.) showed $[\alpha]_D$ +70° (*c*, 1·6 in water) and consisted of a mixture of 3-methyl galactose (R_G 0·17) and an unidentified sugar (R_G 0·135). Fraction V (0·023 g.) showed $[\alpha]_D$ +33° (*c*, 0·9 in water). The rate of movement (R_G 0·135) on the paper chromatogram, and colour reactions with the specific spray reagents described above were not sufficient to characterise the sugar. Fraction VI (0·010 g.) showed $[\alpha]_D$ +33° (*c*, 0·4 in water), moved to the same position on the paper chromatogram as glucose (R_G 0·095), and gave an osazone (m. p. 204—205°) with a crystalline form typical of glucosazone. Fraction VII (0·07 g.) contained galactose (R_G 0·07) and traces of glucose (R_G 0·095). The syrup was dissolved in a little methanol and, on being kept, gave crystals of D-galactose {m. p. and mixed m. p. 166°; $[\alpha]_D$ +81·0° (equilibrium value; *c*, 0·5 in water); R_G 0·07}. Fraction VIII (0·368 g.) crystallised spontaneously to give D-galactose {m. p. and mixed m. p. 164—165°; $[\alpha]_D$ +81·8° (equilibrium value; *c*, 0·98 in water); R_G 0·07}.

Examination of the Barium Salts (B).—A portion was hydrolysed in 2N-sulphuric acid in a sealed tube immersed in a boiling-water bath. After neutralisation with barium carbonate, the solution was filtered, the filtrate evaporated, and the residue extracted with boiling methanol. The methanol extract was evaporated to a syrup which was examined on the paper chromatogram amd observed to contain mainly rhamnose (R_G 0·30), with only a small quantity of galactose (R_G 0·07) and 3-methyl galactose (R_G 0·17). The syrup crystallised overnight giving L-rhamnose hydrate {$[\alpha]_D$ +9° (*c*, 0·98 in water); m. p. 98—100°}. Another portion of the barium salts (*B*) was treated with Amberlite I.R. 100 and when free from barium the solution was evaporated to a syrup. The syrup was separated on the paper chromatogram, *n*-butanol (50 parts), acetic acid (25 parts), and water (25 parts) being used as the mobile phase, and developed with *p*-anisidine hydrochloride. Spots corresponding to rhamnose, 3-methyl galactose, galactose, galacturonic acid, and galacturonosidorhamnose were obtained.

Quantitative Examination of the Products of the Mucilage.—The dry, ash-free mucilage (269·7 mg.) was hydrolysed with 2N-sulphuric acid in a sealed tube immersed in a boiling-water bath for 12 hours. The tube was cooled and opened, and the contents added to ribose (68 mg.). The solution was neutralised with barium carbonate and filtered, and the filtrate evaporated to a syrup. The sugars were separated by paper chromatography and estimated by oxidation with periodate solution. The assumption was made that 3-methyl galactose is oxidised by this reagent in the same manner as 3-methyl glucose (Found: galactose, 0·544 mg.; 3-methyl galactose, 0·825 mg.; ribose, 0·625 mg.; rhamnose, 0·714 mg.). These figures correspond to the presence of 21·6% galactose (as $C_6H_{10}O_5$), 26·8% of 3-methyl galactose (as $C_7H_{12}O_5$), and 25·2% of rhamnose (as $C_6H_{10}O_4$) in the mucilage. Hydrolysis of the galacturonosidorhamnoside linkage is difficult and the rhamnose figure is low, probably because of incomplete hydrolysis (cf. Gill, Hirst, and Jones, *loc. cit.*).

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