223. *Constitution of the Mucilage from the Bark of* Ulmus Fulva (*Slippery Elm Mucilage*). *Part II. The Sugars formed in the Hydrolysis of the Methylated Mucilage.*

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

The protein-free mucilage has been methylated with thallium hydroxide and methyl iodide. The methyl derivative on hydrolysis gave a mixture of sugars among which 2 : 3 : 4 : 6-tetramethyl, 2 : 4 : 6-trimethyl, and 2 : 3 : 6-trimethyl *d*-galactose, 3 : 4-dimethyl and 4-methyl *l*-rhamnose, and 2 : 3 : 4-trimethyl and 2 : 3-dimethyl *d*-galacturonic acid have been identified. A quantitative examination of these sugars has been made and a provisional structural formula for the carbohydrate portion of the mucilage is suggested.

" Slippery elm bark is obtained from *Ulmus Fulva* a tree native to North America. The inner bark, which forms the commercial drug, is marketed in the form of broad flat strips. In addition to the usual anatomical elements (sieve tubes, parenchyma, bast fibres, and rays), slippery elm bark contains special mucilage-containing sacs, considerably larger than the surrounding cells, visible in cross sections under the microscope as rounded cavities scattered irregularly through the tissues " (Solereder's " Systematic Anatomy of the Dicotyledons," English Translation, p. 768). On stirring the bark with boiling water, the mucilage, together with resins, oils, and calcium oxalate, was extracted as a viscous brown solution with a characteristic odour. Addition of alcohol to the extract precipitated the crude polysaccharide as a brown fibrous material which contained calcium oxalate and other impurities. It was purified by dissolving in cold dilute hydrochloric acid and pouring into alcohol; the ash-free, acidic mucilage was thus obtained as a white fibrous precipitate, containing carbon, hydrogen, nitrogen, and sulphur. It dissolved in water giving a colourless viscous solution which did not reduce Fehling's solution.

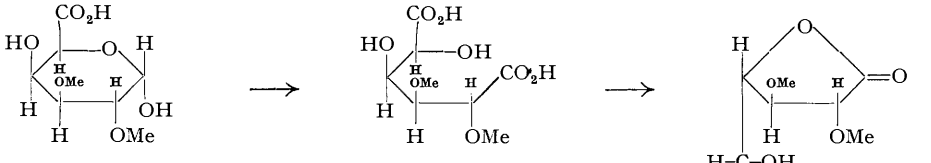
On boiling an aqueous solution of the ash-free polysaccharide it underwent autohydrolysis with considerable reduction in viscosity and precipitation of a brown protein-like solid which contained sulphur and nitrogen. After filtration the solution was poured into alcohol; the sulphur- and nitrogen-free polysaccharide was thus precipitated as a white powder, freely soluble in water and slighly reducing Fehling's solution. This degraded polysaccharide underwent further hydrolysis with N-acid with the formation of reducing sugars (*d*-galactose, *l*-rhamnose, *d*-galacturonic acid) and an aldobionic acid.

In Part I of this series (Gill, Hirst, and Jones, *J.*, 1939, 1469) the isolation of this aldobionic acid from slippery elm mucilage was described. It was shown that complete methylation followed by hydrolysis gave 2 : 3 : 4-trimethyl *d*-galacturonic acid and 3 : 4-dimethyl *l*-rhamnose, and it followed, therefore, that the aldobionic acid present in the mucilage was 2-*d*-galacturonosido-*l*-rhamnose, identical with the aldobionic acid present in flax seed mucilage (Tipson, Christman, and Levene, *J. Biol. Chem.*, 1939, **128**, 609).

It was not found possible to prepare a satisfactory acetyl derivative, and methylation of the mucilage with sodium hydroxide and methyl sulphate resulted in the removal of the protein-like portion of the mucilage

with formation of a partially methylated polysaccharide closely resembling that formed from the methylation of autohydrolysed mucilage. Attempts were made, therefore, to methylate the autohydrolysed polysaccharide. Direct methylation with sodium hydroxide and methyl sulphate was unsatisfactory, as was an attempt to methylate the nitrate ester of the mucilage with the same reagents. Methylation was achieved, however, by the use of thallium hydroxide, thallium ethoxide, and methyl iodide (Menzies, *J.*, **1926**, 937; Hirst and Jones, *J.*, **1938**, 496). The fully methylated material was isolated finally as a homogeneous, crisp, pale cream powder, soluble in benzene, alcohol, acetone, chloroform, and cold water.

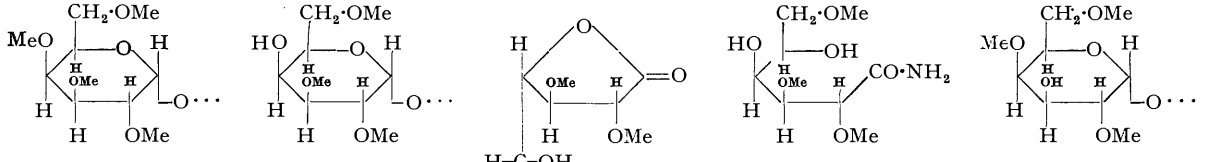
The isolation, estimation, and identification of the sugars present in the methylated polysaccharide was not a simple matter. Complete hydrolysis was difficult and was accompanied by much decomposition, and isolation of pure fractions of methylated sugars was complicated by the formation of constant-boiling mixtures. The free sugars and their glycosides did not crystallise well and it was necessary, therefore, to convert the sugars into well-characterised, crystalline derivatives such as anilides, lactones, and amides. Since, of necessity, the yields of these derivatives were not quantitative, estimates only of the relative amounts of sugars present could be made. Where possible these estimated amounts were checked by polarimetric observations. The calculated amounts of the various sugars identified indicated that they were present in the approximate proportions indicated below and from these figures one of many possible formulæ which will fit the known facts has been suggested (Hirst, *J.*, **1942**, 78). (*a*) 2 : 3-Dimethyl *d*-galacturonic acid (4 parts), (*b*) 2 : 3 : 4 : 6-tetramethyl *d*-galactose (2 parts), (*c*) 2 : 3 : 6-trimethyl *d*-galactose (1 part), (*d*) 2 : 4 : 6-trimethyl *d*-galactose (1 part), (*e*) 3 : 4-dimethyl *l*-rhamnose (2 parts), (*f*) 4-methyl *l*-rhamnose (2 parts), and (*g*) 2 : 3 : 4-trimethyl *d*-galacturonic acid (trace). The constitution assigned to the above sugars rests upon the following facts: (*a*) 2 : 3-Dimethyl *d*-galacturonic acid (I) was oxidised to 2 : 3-dimethyl mucic acid (II), identified as its crystalline ester lactone (III) (Beavan and Jones, *Chem. and Ind.*, **1939**, **58**, 363; Smith, *ibid.*, p. 363; Luckett and Smith, *J.*, **1940**, 1107).



(I.) (II.) (III.)

(*b*) 2 : 3 : 4 : 6-Tetramethyl *d*-galactose (IV) was identified as its well-characterised crystalline anilide.

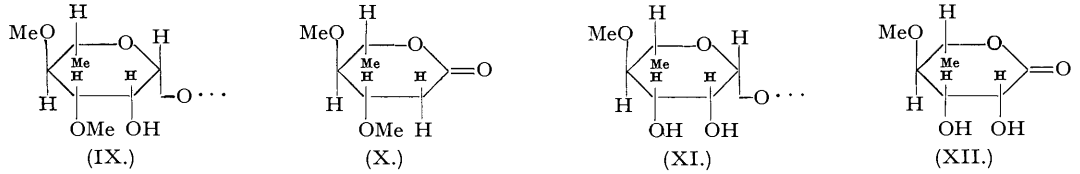
(*c*) 2 : 3 : 6-Trimethyl *d*-galactose (V) was recognised by its ability to form a furanose derivative with downward change of rotation with cold methyl alcoholic hydrogen chloride. After oxidation with bromine water it yielded crystalline 2 : 3 : 6-trimethyl γ-*d*-galactonolactone (VI) which with liquid ammonia gave the corresponding amide (VII) (Haworth, Hirst, and Stacey, *J.*, **1932**, 2481).



(IV.) (V.) (VI.) (VII.) (VIII.)

(*d*) 2 : 4 : 6-Trimethyl *d*-galactose (VIII) was identified as its well-characterised crystalline anilide.

(*e*) 3 : 4-Dimethyl *l*-rhamnose (IX) was identified by its conversion into 3 : 4-dimethyl *l*-rhamnonolactone (X) (Haworth, Hirst, and Miller, *J.*, **1929**, 2469).

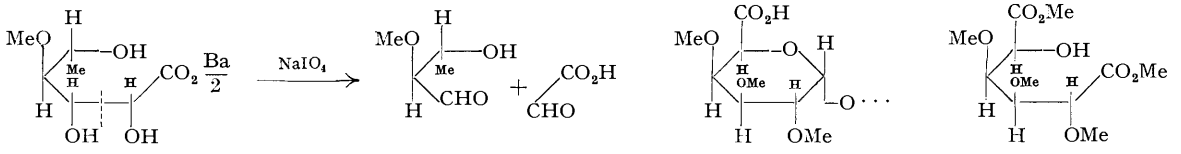


(IX.) (X.) (XI.) (XII.)

(*f*) The monomethyl *l*-rhamnose (XI) was oxidised by bromine water to a crystalline lactone (XII) which from its rotation and rate of mutarotation appeared to be a δ-lactone. The barium salt (XIIa) of the acid was oxidised by sodium periodate with the formation of glyoxylic acid (XIIb). These two observations indicate that there must be a methoxyl group on C_4. Further, the amide obtained by the action of methyl alcoholic ammonia on the lactone (XII) gave a positive Weerman test proving the presence of a hydroxyl group on C_2. Since acetaldehyde was not produced on oxidation of the barium salt (XIIa) with sodium periodate there cannot be a methoxyl group on C_3. It follows that the sugar is 4-methyl *l*-rhamnose (XI)

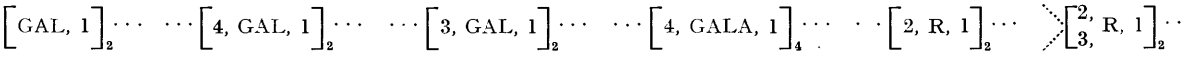
which was first reported by Levene and Compton (*J. Biol. Chem.*, 1936, **114**, 16). The crystalline lactone obtained by oxidation of (XI) was therefore 4-methyl *l*-rhamnonolactone (XII).

(*g*) 2 : 3 : 4-Trimethyl *d*-galacturonic acid (XIII) was identified after oxidation and esterification as the dimethyl ester of 2 : 3 : 4-trimethyl mucic acid (XIV).



(XIIa.) (XIIb.) (XIII.) (XIV.)

From the above evidence it is apparent that the repeating unit of slippery elm mucilage must be built up of the following residues linked together in the positions indicated, the precise order of arrangement being as yet undefined :



(IV.) (V.) (VIII.) (I.) (IX.) (XI.)

GAL = *d*-galactose residue. R = *l*-rhamnose residue. GALA = *d*-galacturonic acid residue.

It is of interest to note that in slippery elm mucilage the 1 : 3-linkage occurs along with 1 : 4- and 1 : 2-linkages, and that galacturonic acid and not glucuronic acid is responsible for the acidity of the molecule. In this respect the polysaccharide appears to be a link between the plant gums and the pectin materials. The polysaccharide is similar to other mucilages in that it appears to have properties closely resembling those of the mucoproteins and bacterial polysaccharides (Stacey, *Chem. and Ind.*, 1943, **62**, 110).

Experimental.

Methylation.—The autohydrolysed polysaccharide (nitrogen and sulphur free) (Gill, Hirst, and Jones, *loc. cit.*) had $[\alpha]_D^{20°}$ + 68° in water (*c*, 1·0), equiv. wt. by titration 486, uronic anhydride 36·6%, furfural and methylfurfural 18%, methylfurfural approximately 12%. Preliminary attempts to methylate the autohydrolysed product by means of 30% sodium hydroxide and methyl sulphate were unsuccessful. With fuming nitric acid the mucilage gave an acetone-soluble nitro-derivative. Attempts to methylate this by means of 30% sodium hydroxide and methyl sulphate were also unsuccessful and the following method of methylation was therefore adopted.

The polysaccharide (40 g.) was dissolved in water (200 c.c.) and a hot solution of 4·2N-thallous hydroxide (330 c.c.) was added. The precipitated thallium complex was filtered off, washed with methyl alcohol, and dried in a vacuum at 40° for 18 hours. The solid (252 g.) was powdered (120 mesh) and boiled under reflux with methyl iodide for 15 hours. Excess of methyl iodide was distilled off and 1·4N-thallous hydroxide (300 c.c.) was added to the solid residue and the whole evaporated to dryness at 50°/12 mm. The residue was powdered (120 mesh) and boiled with methyl iodide for 20 hours. At the end of this time the insoluble material reacted neutral to litmus. Excess of methyl iodide was distilled off, and to the residue dissolved in alcohol N-thallium ethoxide in benzene (700 c.c.) was added. The solvents were removed under reduced pressure and the powdered product was boiled with methyl iodide for 15 hours. After distillation of excess of methyl iodide the solid (*X*) was exhaustively extracted with acetone and with chloroform. Evaporation of the solvent gave a partially methylated mucilage (37·4 g.) (Found : OMe, 37·5%). The solid residue (*X*, above) was extracted with water and the extracts concentrated and added to the partially methylated material (37·4 g.) obtained above. The combined products were evaporated to dryness with a benzene solution of N-thallium ethoxide (280 c.c.) and the residue boiled with methyl iodide for 15 hours. The methylated polysaccharide (37·5 g.) was isolated in the usual manner (Found : OMe, 40; ash, 0·01%).

Fractionation.—The methylated polysaccharide (37·4 g.) was dissolved in chloroform (80 c.c.) and fractionally precipitated by gradual addition of light petroleum (b. p. 40—60°) giving *Fraction I* (2·7 g.) : $[\alpha]_D^{20°}$ + 52° in water (*c*, 1·47); OMe, 39·6%. *Fraction II* (9·3 g.) : $[\alpha]_D^{20°}$ + 52° in water (*c*, 1·59); equiv. wt. (by titration with 0·01N-sodium hydroxide), 644; uronic anhydride (from carbon dioxide liberated on boiling with 12% hydrochloric acid), 27·3; OMe, 38·0%. *Fraction III* (17·9 g.) : $[\alpha]_D^{20°}$ + 55° in water (*c*, 1·64); equiv. wt., 595; uronic anhydride, 29·6; OMe, 40·7%. *Fraction IV* (6·2 g.) : $[\alpha]_D^{20°}$ + 55° in water (*c*, 1·5); equiv. wt., 587; uronic anhydride, 30·0; OMe, 39·5%. A completely methylated polysaccharide containing equimolecular amounts of *l*-rhamnose, *d*-galactose, and *d*-galacturonic acid would have equiv. wt., 596; uronic anhydride, 29·5; OMe, 41·6%. From the constants cited for the above four fractions, it was concluded that the methylated material was essentially homogeneous and they were therefore combined.

Hydrolysis of the Methylated Polysaccharide.—Complete hydrolysis of the methylated material was very difficult and after a number of trials the method given below was found to be the most satisfactory. The polysaccharide (32·3 g.) was boiled with 3% methyl alcoholic hydrogen chloride (100 c.c.) for 8 hours. Owing to darkening of the solution, it was not possible to follow the course of the hydrolysis with the polarimeter. The cooled solution was neutralised with silver carbonate, filtered, and evaporated at 40°/12 mm. to a syrup (32·6 g.). Extraction of this with light petroleum gave a soluble syrup (*A*, 25·05 g.) [$n_D^{16°}$ 1·4600; $[\alpha]_D^{20°}$ + 38° in water (*c*, 1·2); equiv. wt., 549; OMe, 48·9%] and an insoluble residue (8·06) which was further hydrolysed by boiling with 3% methyl alcoholic hydrogen chloride. The solution was neutralised with silver carbonate and worked up in the usual manner and the resulting syrup (8·01 g.) extracted with ether giving a red viscous syrup (*B*, 7·25 g.) {$[\alpha]_D^{20°}$ + 39° in methyl alcohol (*c*, 1·5); equiv. wt., 375; OMe, 36·9%} and a solid residue (0·77 g.) which was not further examined.

The syrup (*A*, 25·0 g.) was dissolved in a slight excess of 0·4N-barium hydroxide (150 c.c.) and the solution heated at 100° for 5 hours. The hot solution was neutralised with carbon dioxide, filtered, and the filtrate exhaustively extracted with ether; concentration of the extracts gave a yellow mobile syrup (1·67 g.). The aqueous solution was then evaporated at 40°/12 mm. to a small volume (30 c.c.) and exhaustively extracted with chloroform. The chloroform extracts were combined with the ether extracts (1·67 g.) and the solvent evaporated under reduced pressure, leaving an orange syrup (*C*, 12·54 g., $n_D^{17°}$ 1·4580). Concentration of the aqueous solution gave the barium salts (14·1 g.) as a light brown powder. The barium salts (*D*) were exhaustively extracted with ether to remove the last traces of sugar (*E*, 0·74 g.; $n_D^{13°}$ 1·4718)

(obtained on evaporation of the solvent), and then dried in a vacuum for 8 hours (12·47 g.) (Found : Ba, 21·0; OMe, 31·0. Calc. for the barium salt of 2 : 3-dimethyl methyl-*d*-galacturonoside : Ba, 22·6; OMe, 30·6%).

The syrup (*B*, 7·2 g.) was heated with 0·3N-barium hydroxide (60 c.c.) for 5 hours, and the hot solution neutralised with carbon dioxide and the material separated into sugars (F_1, 1·20 g., $n_D^{15°}$ 1·4630) and barium salts (G_1, 7·08 g.) (Ba, 18·0; OMe, 25%) (for details of separation, see above). It was apparent from these analytical figures that some sugar was attached to the uronic acid in the barium salt (G_1) and this was therefore boiled with 4% methyl alcoholic hydrogen chloride (150 c.c.) for 24 hours. The cooled solution was then neutralised with silver carbonate, filtered, and evaporated to a solid which was exhaustively extracted with acetone. Concentration of the extracts gave a syrup (6·0 g.), which was heated with 0·3N-barium hydroxide (100 c.c.) at 100° for 5 hours. The solution, worked up as described above, gave an ether-soluble syrup (F_2, 1·20 g.) and ether-insoluble barium salts (G_2, 5·0 g.).

Identification of the Barium Salts.—A portion (12·4 g.) of the barium salts (*D*) from fraction *A* was boiled with 4% methyl alcoholic hydrogen chloride (200 c.c.) for 5 hours. The cooled solution was neutralised with silver carbonate, filtered, and the filtrate evaporated at 40°/20 mm. to a syrup which was exhaustively extracted with acetone. The extracts gave on concentration a syrup (9·96 g.), n_D^{19} 1·4627 (Found : equiv. wt., 280. Calc. for the dimethyl ester of 2 : 3-dimethyl methyl-*d*-galacturoside : equiv. wt., 250). The syrup was fractionally distilled in a vacuum giving *Fraction* 1 : a mixture of the methyl esters of 2 : 3 : 4-trimethyl methyl-*d*-galacturonoside and 2 : 3-dimethyl methyl-*d*-galacturonoside (0·39 g.); b. p. 156° (bath temp./0·07 mm.); $n_D^{18°}$ 1·4490; $[\alpha]_D^{20°}$ − 13° in water (Found : equiv. wt., 254; OMe, 52·0. Calc. for $C_{11}H_{20}O_7$: equiv. wt., 264; OMe, 58·7%). *Fraction* 2 (3·00 g.) : methyl ester of 2 : 3-dimethyl methyl-*d*-galacturonoside; b. p. 158° (bath temp.)/0·05 mm.; $n_D^{18°}$ 1·4532; $[\alpha]_D^{20°}$ − 15° (in water) (equiv. wt., 266; OMe, 52·2%). *Fraction* 3 (2·38 g.) : methyl ester of 2 : 3-dimethyl methyl-*d*-galacturonoside; b. p. 165° (bath temp.)/0·05 mm.; $n_D^{18°}$ 1·4553; $[\alpha]_D^{20°}$ − 18° (in water) (equiv. wt., 260; OMe, 52·0%). *Fraction* 4 (0·54 g.) : b. p. 170° (bath temp.)/0·005 mm.; $n_D^{18°}$ 1·4572; $[\alpha]_D^{20°}$ ±0° (in water) (equiv. wt., 255; OMe, 50·0%). *Fraction* 5 (1·10 g.) : b. p. 190° (bath temp.)/0·03 mm.; $n_D^{18°}$ 1·4701; $[\alpha]_D^{20°}$ + 52° (in water) (equiv. wt., 262; OMe, 50·0%). *Fraction* 6 (1·07 g.) : b. p. 230° (bath temp.)/0·04 mm.; $n_D^{18°}$ 1·4761; $[\alpha]_D^{20°}$ + 50° (in water) (equiv. wt., 330; OMe, 47·8%). *Fraction* 7 : still residue, 1·07 g.

The constants for fraction 6 indicated that it was mainly disaccharide in nature while those for fractions 1, 2, 3, 4, and 5 were very near to those required for the methyl ester of 2 : 3-dimethyl methylgalacturonoside (Calc. : OMe, 49·6%). The methoxyl values of the first five fractions suggest, however, that they are contaminated with small amounts of the methyl ester of 2 : 3 : 4-trimethyl methylgalacturonoside (Calc. : OMe, 58·7%).

The fractions were hydrolysed individually with N-hydrochloric acid. Fraction 1 changed from $[\alpha]_D^{20°}$ − 13° to + 54° in 2½ hours. Fractions 2, 3, 4, 5, and 6 each became constant at $[\alpha]_D^{20°}$ + 67° after 3 hours.

The solutions obtained from the hydrolysis of the first five fractions were combined, neutralised with silver carbonate, filtered before and after the passage of hydrogen sulphide, and evaporated to a syrup. Fraction 6 was neutralised with barium carbonate, filtered, and evaporated to dryness. The solid residue was exhaustively extracted with ether, some syrupy sugar (0·1 g.) being thereby removed. The extracted barium salt was dissolved in water, barium removed as sulphate by the addition of the calculated quantity of N-sulphuric acid, and the filtered solution added to the syrup obtained from fractions 1, 2, 3, 4, and 5 and the whole evaporated to a syrup (5·65 g.), $[\alpha]_D^{14°}$ + 62° in water (*c*, 1·07) (Found : equiv. wt., 224; OMe, 29. Calc. for 2 : 3-dimethyl *d*-galacturonic acid : equiv. wt., 222; OMe, 27·9%).

The syrup (5·59 g.) was dissolved in water (20 c.c.) and oxidised with bromine (5 c.c.) for 10 hours. Excess of bromine was removed by aeration; the solution was then neutralised with silver carbonate and filtered before and after the passage of hydrogen sulphide. The solvent was then removed at 40°/20 mm. and the residual syrup boiled with 2% methyl alcoholic hydrogen chloride (150 c.c.) for 6 hours. The cooled solution was neutralised with silver carbonate and worked up in the usual manner to give a syrup (4·7 g.), which was distilled in a vacuum giving : *Fraction* 8 (0·37 g.) : mainly the dimethyl ester of 2 : 3 : 4-trimethyl *d*-mucic acid; b. p. 140° (bath temp.)/0·001 mm.; $n_D^{19°}$ 1·4545 (Found : OMe, 50%). This fraction crystallised on standing and gave the dimethyl ester of 2 : 3 : 4-trimethyl *d*-mucic acid (0·1 g.), m. p. and mixed m. p. 101° after recrystallisation from ether. *Fraction* 9 (4·1 g.) : γ-lactone methyl ester of 2 : 3-dimethyl *d*-mucic acid, b. p. 150° (bath. temp.)/0·001 mm.; $n_D^{19°}$ 1·4640 (Found : OMe, 39·1. Calc. for $C_9H_{14}O_7$: OMe, 39·8%). This fraction crystallised on nucleation with an authentic specimen, giving the γ-lactone methyl ester of 2 : 3-dimethyl mucic acid, m. p. and mixed m. p. 92° after recrystallisation from ether; $[\alpha]_D^{20°}$ − 39°, falling to − 34° (76 hours); − 27° (100 hours); − 22° (124 hours); − 13° (11 days); − 4° (17 days).

From the above methoxyl values, it may be calculated that the acids in fraction (*D*) consisted approximately of 2 : 3 : 4-trimethyl *d*-galacturonic acid (10%) and 2 : 3-dimethyl *d*-galacturonic acid (90%).

The barium salts (G_2) were combined with fraction 7 (1·07 g.) and the whole refluxed with 4% methyl alcoholic hydrogen chloride (150 c.c.) for six hours. The solution was neutralised with silver carbonate and worked up in the manner described above and the syrup (6·1 g.) distilled in a vacuum giving : *Fraction* 10 (2·20 g.) : the methyl ester of 2 : 3-dimethyl *d*-galacturonoside, b. p. 135° (bath temp.)/0·001 mm.; $n_D^{19°}$ 1·4579; $[\alpha]_D^{20°}$ + 7° in water (*c*, 1·15) (equiv. wt., 257; OMe, 49·0%). *Fraction* 11 (1·10 g.) : b. p. 165° (bath temp.)/0·001 mm.; $n_D^{19°}$ 1·4652; $[\alpha]_D^{20°}$ − 2° in water (*c*, 1·12) (equiv. wt., 261; OMe, 43·6%). The still residue (2·6 g.) appeared to consist mainly of unhydrolysed polysaccharide.

These two fractions were combined and hydrolysed with N-hydrochloric acid (50 c.c.) for 5 hours; $[\alpha]_D^{20°}$ rose from + 2° to + 65° (constant value). The free acids were isolated, oxidised with bromine water, esterified with methyl alcoholic hydrogen chloride (see above for details), and the resulting esters (3·0 g.) distilled in a vacuum, giving the γ-lactone methyl ester of 2 : 3-dimethyl *d*-mucic acid (2·0 g.), b. p. 160° (bath temp.)/0·001 mm.; $n_D^{21°}$ 1·4545 (superfused liquid); OMe, 37·8%; m. p. and mixed m. p. 92° with an authentic specimen, after recrystallisation from ether. The dimethyl ester of 2 : 3 : 4-trimethyl *d*-mucic acid appeared to be absent from this fraction.

It would appear from these experimental results that the acidic fractions obtained after hydrolysis of the methylated polysaccharide consisted mainly of 2 : 3-dimethyl *d*-galacturonic acid along with some 7% of 2 : 3 : 4-trimethyl *d*-galacturonic acid which may have been present as an end-group in the original methylated polysaccharide.

Identification of the Sugars.—The methyl glycosides (*C*, 12·47 g.), $n_D^{17°}$ 1·4580, were fractionally distilled in a vacuum giving *Fraction* 12 (9·27 g.) : tetramethyl and trimethyl methyl-*d*-galactosides, b. p. 120—130° (bath temp.)/0·001 mm.; $n_D^{18°}$ 1·4530; $[\alpha]_D^{20°}$ + 80° in water; OMe, 56·8%. *Fraction* 13 (2·68 g.) : trimethyl methyl-*d*-galactoside, dimethyl-*l*-rhamnoside, and monomethyl methyl-*l*-rhamnoside, b. p. 130—150° (bath temp.)/0·001 mm.; $n_D^{18°}$ 1·4620; $[\alpha]_D^{20°}$ + 29° in water; OMe, 44·3%. Still residue, 0·40 g.

Fraction 12 was dissolved in N-hydrochloric acid (100 c.c.) and heated at 90—95° for 3 hours. $[\alpha]_D^{19°}$ rose from + 80° to + 110° (20 mins.) and then fell to + 90° (constant value). The solutions were neutralised with silver carbonate, filtered before and after the passage of hydrogen sulphide, and evaporated at 40°/12 mm. to a syrup (*H*) (8·2 g.), $n_D^{18°}$ 1·4700; $[\alpha]_D^{19°}$ + 44° rising to + 66° in 1% methyl alcoholic hydrogen chloride (after 26 hours); OMe, 46·0%.

The syrup (*H*) (0·96 g.) on heating with aniline (0·41 g.) in alcohol (30 c.c.) for 2 hours gave on cooling a mixture of anilides. By fractional crystallisation from alcohol 2 : 3 : 4 : 6-tetramethyl *d*-galactose anilide (0·65 g.) was isolated, m. p. and mixed m. p. 194° (Found : OMe, 40·6. Calc. for $C_{16}H_{25}O_5N$: OMe, 39·9%), and 2 : 4 : 6-trimethyl *d*-galactose

anilide (0·10 g.), m. p. and mixed m. p. 179° (Found : OMe, 29·2. Calc. for $C_{15}H_{23}O_5N$: OMe, 31·3%). (Proof of identity was confirmed by an *X*-ray analysis of the crystals for which we are indebted to Dr. T. Malkin.)

Another sample of the syrup (*H*) (1·5 g.) was dissolved in water (10 c.c.) and oxidised with bromine (2 c.c.) at 50° for 6 hours. The mixed galactonic acids were isolated as a syrup (1·45 g.) in the usual manner and distilled in a vacuum giving 2 : 3 : 4 : 6-tetramethyl, 2 : 4 : 6-trimethyl, and 2 : 3 : 6-trimethyl *d*-galactonolactones as a syrup (1·0 g.), b. p. 125—150° (bath temp.)/0·001 mm.; $[\alpha]_D^{20°}$ + 85° in water (*c*, 1·0, initial value); + 6° (24 hours, constant value) (Equiv. wt., 225; OMe, 48·0%). The syrup crystallised on standing and was drained on a porous tile giving 2 : 3 : 6-trimethyl *d*-galactonolactone (0·40 g.), m. p. 98° (after sublimation in a vacuum), not depressed on admixture with an authentic specimen; $[\alpha]_D^{20°}$ — 40° (initial value, *c*, 2·1 in water); — 35° (30 hours); — 30° (3 weeks) (Found : OMe, 43·0. Calc. for $C_9H_{16}O_6$: OMe, 42·3%).

2 : 3 : 4-Trimethyl *d*-galactose was absent from the mixture since the syrup (*H*) (1·54 g.) after conversion into the mixed glycosides with methyl alcoholic hydrogen chloride did not give a positive reaction for the presence of a $-CH_2 \cdot OH$ group on heating its *p*-toluenesulphonyl derivative with sodium iodide in acetone (Oldham and Rutherford, *J. Amer. Chem. Soc.*, 1932, **54**, 366).

These results, taken in conjunction with refractive indices, methoxyl values, and rotations of the mixed sugars, mixed glycosides, and mixed lactones, indicate the presence of some 50% of 2 : 3 : 4 : 6-tetramethyl *d*-galactose in fraction 12, together with some 50% of trimethyl *d*-galactose of which approximately half is 2 : 3 : 6-trimethyl *d*-galactose and the remainder 2 : 4 : 6-trimethyl *d*-galactose.

Fraction 13 was hydrolysed with N-hydrochloric acid and the sugars (2·1 g.) were isolated in the usual manner; $[\alpha]_D^{19°}$ + 31° in water (*c*, 1·0); OMe, 33·5%. This syrup gave no crystalline anilide on heating with an alcoholic solution of aniline, showing the absence of any large percentage of 2 : 4 : 6-trimethyl, 2 : 3 : 4-trimethyl, or 2 : 3 : 4 : 6-tetramethyl *d*-galactose. The syrup (1·7 g.) was therefore oxidised with bromine water and the resulting acids, isolated in the usual manner, were lactonised by heating at 100°/5 mm. for 3 hours; the lactones then crystallised. The crystals (1·2 g.) which were mainly, if not entirely, a mixture of 3 : 4-dimethyl *l*-rhamnonolactone and 2 : 3 : 6-trimethyl *d*-galactonolactone, had m. p. 70—90°; $[\alpha]_D^{20°}$ — 57° in water (*c*, 3·1, initial value) falling to — 27° (8 days). By solution of the mixed lactones in liquid ammonia, a mixture of the corresponding crystalline amides was obtained. This was separated into its components by fractional crystallisation from methyl alcohol–acetone–ether giving 3 : 4-dimethyl *l*-rhamnonamide, m. p. and mixed m. p. 153°, and 2 : 3 : 6-trimethyl *d*-galactonamide, m. p. and mixed m. p. 135° (Haworth, Raistrick, and Stacey, *Biochem. J.*, 1937, **31**, 644), respectively. With sodium hypochlorite the amides gave cyanic acid, proving the presence of an amide with a free α-hydroxyl group (Weerman, *Rec. Trav. chim.*, 1917, **37**, 16).

The mixed lactones (0·30 g.) on oxidation with periodic acid (a reagent diagnostic for αβ-glycols) gave no formaldehyde or acetaldehyde (dimedon), thereby proving the absence of 2 : 3-dimethyl *l*-rhamnonolactone and 2 : 3 : 4-trimethyl *d*-galactonolactone.

The mixture of lactones was separated into its components by a tedious process of repeated crystallisation and fractional sublimation giving small quantities of 3 : 4-dimethyl *l*-rhamnonolactone, m. p. 74°, mixed m. p. with an authentic specimen, 76°, $[\alpha]_D^{20°}$ — 158° (initial value in water), and 2 : 3 : 6-trimethyl *d*-galactonolactone, m. p. and mixed m. p. 98°, $[\alpha]_D^{20°}$ — 40° in water.

From an examination of the methoxyl values and refractive indices it may be calculated that fraction 13 contains some 75% of 3 : 4-dimethyl *l*-rhamnose and 25% of 2 : 3 : 6-trimethyl *d*-galactose

Fractions *E* (0·74 g.), F_1 (1·20 g.), F_2 (1·20 g.), the syrup from fraction 6 (0·10 g.), and the residue (0·40 g.) from the distillation of fraction *C* were combined, boiled under reflux with 2% methyl alcoholic hydrogen chloride, neutralised with silver carbonate, and the product (3·40 g.), isolated in the usual manner, was distilled in a vacuum giving 3·0 g., b. p. 140—150° (bath temp.)/0·001 mm.; $n_D^{18°}$ 1·4670; $[\alpha]_D^{20°}$ — 27° (*c*, 1·0 in water) [Found : OMe, 33·0. Calc. for $C_8H_{16}O_5$ (methyl methylrhamnoside) : OMe, 32·3%].

The distillate was dissolved in N-hydrochloric acid (50 c.c.) and heated at 90—95° for 4 hours. $[\alpha]_D^{20°}$ rose from — 27° (initial value) to + 28° (constant value). The cooled solution was neutralised with silver carbonate, filtered before and after the passage of hydrogen sulphide, and concentrated to a syrup (2·80 g.), $[\alpha]_D^{20°}$ + 22° in water (*c*, 1·10), which consisted mainly of 4-methyl rhamnose (Found : OMe, 20·3. Calc. for $C_7H_{14}O_5$: OMe, 17·4%).

This material (2·80 g.) was oxidised with bromine (2 c.c.) in water (10 c.c.) for 8 hours at 20°. Excess of bromine was removed by aeration. The product, isolated in the usual manner, crystallised on standing. Recrystallisation from acetone–light petroleum gave the *4-methyl δ-l-rhamnonolactone* in the form of long needles, m. p. 82°; $[\alpha]_D^{20°}$ — 141° in water (*c*, 1·06) falling to — 115° (14 hours, constant value) (Found : C, 47·2; H, 7·1; OMe, 17·2; equiv. wt., 182. $C_7H_{12}O_5$ requires C, 47·7; H, 7·0; OMe, 17·7%; equiv. wt., 176). On reaction with methyl alcoholic ammonia the lactone gave 4-methyl *l*-rhamnonamide as a syrup which gave a positive Weerman raction.

Oxidation of the Barium Salt of 4-Methyl l-Rhamnonic Acid with Sodium Periodate.—The crystalline lactone (13·4 mg.) was titrated with 0·01N-barium hydroxide (7·5 c.c.) and sodium periodate (0·236M, 1 c.c. excess) added. Carbon dioxide was bubbled through the solution and then through 2 : 4-dinitrophenylhydrazine solution. No acetaldehyde derivative formed during two hours. Rhamnose hydrate (19·6 mg.) gave, under the same conditions, a copious precipitate of acetaldehyde 2 : 4-dinitrophenylhydrazone, m. p. 161°. The solution of the salt of 4-methyl *l*-rhamnonic acid, after oxidation, was freed from periodate and iodate by the addition of barium chloride, filtered, and the filtrate tested for the presence of glyoxylic acid with naphtharesorcinol (Fearon test); a strong positive reaction was obtained.

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

THE UNIVERSITY, BRISTOL.
THE UNIVERSITY, MANCHESTER.

[*Received, March 7th,* 1946.]