47. *Synthesis of Uronic Acids. Part II.* 2 : 3 : 4-*Trimethyl Derivatives of Mannuronic, Glucuronic, and Galacturonic Acids.*

By F. Smith, M. Stacey, and P. I. Wilson.

The 2 : 3 : 4-trimethyl derivatives of mannuronic, glucuronic, and galacturonic acids have been synthesised by the oxidation of 2 : 3 : 4-trimethyl α-methylmannoside, 2 : 3 : 4-trimethyl β-methylglucoside, and 2 : 3 : 4-trimethyl α-methylgalactoside respectively with alkaline potassium permanganate.

The 2 : 3 : 4-trimethyl α-methylmannoside and the 2 : 3 : 4-trimethyl α-methylgalactoside were prepared according to the scheme : α-methylmannopyranoside (galactopyranoside) → 6-trityl α-methylmannopyranoside (galactopyranoside) → *6-trityl 2 : 3 : 4-trimethyl α-methylmannopyranoside* (galactopyranoside) → 2 : 3 : 4-trimethyl α-methylmannopyranoside (galactopyranoside). The 2 : 3 : 4-trimethyl β-methylglucopyranoside was obtained by the methylation of 6-trityl 1 : 2 : 3 : 4-tetra-acetyl glucose, followed by the elimination of the trityl group from the 6-trityl 2 : 3 : 4-trimethyl β-methylglucopyranoside.

Each of the 2 : 3 : 4-trimethyl hexuronic acids has been identified by conversion into crystalline derivatives.

Investigations into polysaccharides of microbiological, plant, and animal origin have revealed the presence in these substances of uronic acids. For example, glucuronic acid is a constituent of the important pneumococcus polysaccharides (Marrack, " Chemistry of Antigens and Antibodies," 1936); it occurs in cartilage (Levene,

" Hexosamines and Mucoproteins," 1925), in gum arabic (Butler and Cretcher, *J. Amer. Chem. Soc.*, 1929, **51**, 1519; Weinmann, *Ber.*, 1929, **62**, 1637; Challinor, Haworth, and Hirst, J., 1931, 258; Smith, J., 1939, 1724), in mesquite gum (Anderson and Otis, *J. Amer. Chem. Soc.*, 1930, **52**, 4461; Cunneen and Smith, unpublished work), in damson gum (Hirst and Jones, J., 1938, 1174) and in cherry gum (Jones, J., 1939, 558). Galacturonic acid has been found in pectic acid (Dore, *J. Amer. Chem. Soc.*, 1926, **48**, 232; Ehrlich and Schubert, *Ber.*, 1929, **62**, 1974; Beaven and Jones, *J. Soc. Chem. Ind.*, 1939, **58**, 363; Smith, *ibid.*, p. 363; Luckett and Smith, J., 1940, 1106), in gum tragacanth (Luckett and Smith, unpublished work) and it also occurs in mucilages (Gill, Hirst, and Jones, J., 1939, 1469; Christman, Levene, and Tipson, *J. Biol. Chem.*, 1939, **128**, 609). The third uronic acid, mannuronic acid, has been identified as the main constituent of alginic acid (mannucol) (Hirst *et al.*, J., 1939, 1880). Moreover, uronic acids appear to play a predominating rôle in the determination of the specificity of polysaccharides (Goebel, *Nature*, 1939, **143**, 77). It is therefore advantageous to have methods available whereby the identity of these uronic acids can be established. One method, employed in the determination of the structure of these complex polysaccharides containing uronic acid residues, involves methylation, followed by hydrolysis. Such a procedure may lead to the formation of uronic acids in which one or more of the hydroxyl groups are substituted by methoxyl groups. In order to facilitate the identification of the three uronic acids mentioned above, we have synthesised the 2 : 3 : 4-trimethyl derivatives of *d*-glucuronic, *d*-galacturonic, and *d*-mannuronic acid.

Hexuronic acids are derived from hexoses by the oxidation of the primary alcoholic group in position 6 to a carboxyl group. Direct oxidation is not as yet practicable (Jolles, *Biochem. Z.*, 1911, **34**, 242) and poor yields of uronic acids result from the oxidation of the glycosides (Bergmann and Wolff, *Ber.*, 1923, **56**, 1060). If, however, all the hydroxyl groups in a particular hexose, with the exception of that on C_6, are suitably protected, oxidation proceeds smoothly and the corresponding uronic acid is produced. Thus the oxidation of 1 : 2 : 3 : 4-diacetone galactose with potassium permanganate in alkaline solution affords 1 : 2 : 3 : 4-diacetone galacturonic acid (Ohle and Behrend, *Ber.*, 1925, **58**, 2585). The oxidation of 1 : 2 : 3 : 4-tetra-acetyl glucose with potassium permanganate in glacial acetic acid yields 1 : 2 : 3 : 4-tetra-acetyl glucuronic acid and this in turn can be transformed into glucuronic acid (Stacey, J., 1939, 1529). Similarly oxidation of 1 : 2-monoacetone 3 : 5-benzylidene glucose with alkaline potassium permanganate gives 1 : 2-monoacetone 3 : 5-benzylidene glucuronic acid (Zervas and Sessler, *Ber.*, 1933, **66**, 1326). In view of the fact that the protecting groups in these compounds can be readily eliminated, these methods lead to the synthesis of the free uronic acids. When methylated uronic acids are required it has been found convenient to commence the synthesis with a hexose in which the hydroxyl groups, with the exception of that at C_6, are protected by methyl residues (cf. Pryde and Williams, *Biochem. J.*, 1933, **27**, 1205; Reeves, *J. Amer. Chem. Soc.*, 1940, **62**, 1616; Jones, Peat, and Owen, J., 1941, 339). In this manner 2 : 3 : 5-trimethyl galacturonic acid was prepared from 2 : 3 : 5-trimethyl methyl-galactofuranoside (Luckett and Smith, J., 1940, 1114). We have now adopted this scheme for the synthesis of the 2 : 3 : 4-trimethyl derivatives of glucuronic, mannuronic, and galacturonic acids. Oxidation of 2 : 3 : 4-trimethyl α-methylmannoside and 2 : 3 : 4-trimethyl α-methylgalactoside has yielded 2 : 3 : 4-trimethyl α-methylmannuronoside and 2 : 3 : 4-trimethyl α-methylgalacturonoside respectively, and oxidation of 2 : 3 : 4-trimethyl β-methylglucoside has given rise to the corresponding 2 : 3 : 4-trimethyl β-methylglucuronoside.

The 2 : 3 : 4-trimethyl methylhexosides required for these syntheses were obtained as follows : Treatment of α-methylmannopyranoside with trityl chloride in anhydrous pyridine gave the 6-trityl α-methylmannopyranoside and this upon methylation with methyl sulphate and sodium hydroxide afforded 6-*trityl* 2 : 3 : 4-*trimethyl α-methylmannoside*. Removal of the trityl group was effected by the agency of ethereal hydrogen chloride (Smith, J., 1939, 1724), whereby there was produced the required 2 : 3 : 4-trimethyl α-methylmannopyranoside. Commencing with α-methylgalactopyranoside, the same procedure furnished 2 : 3 : 4-trimethyl α-methylgalactopyranoside. For the preparation of 2 : 3 : 4-trimethyl β-methylglucopyranoside, 6-trityl 1 : 2 : 3 : 4-tetra-acetyl glucopyranoside was subjected to direct methylation with methyl sulphate and sodium hydroxide. Detritylation of the resulting 6-trityl 2 : 3 : 4-trimethyl β-methylglucopyranoside with a solution of hydrobromic acid in glacial acetic acid (Helferich and Klein, *Annalen*, 1926, **450**, 219) gave the desired 2 : 3 : 4-trimethyl β-methylglucopyranoside.

When the 2 : 3 : 4-trimethyl α-methylmannopyranoside, the 2 : 3 : 4-trimethyl α-methylgalactopyranoside and the 2 : 3 : 4-trimethyl β-methylglucopyranoside were each treated with potassium permanganate in alkaline solution at room temperature, oxidation of the primary alcoholic group at C_6 took place and there resulted 2 : 3 : 4-trimethyl α-methylmannopyruronoside, 2 : 3 : 4-trimethyl α-methylgalactopyruronoside and 2 : 3 : 4-trimethyl β-methylglucopyruronoside respectively.

Hydrolysis of the 2 : 3 : 4-trimethyl α-methylmannuronoside with dilute hydrochloric acid yielded 2 : 3 : 4-trimethyl mannuronic acid, which upon oxidation with bromine gave 2 : 3 : 4-trimethyl mannosaccharic acid. Treatment of the latter with boiling methyl-alcoholic hydrogen chloride effected esterification and when this ester was allowed to react with methyl-alcoholic ammonia the characteristic crystalline diamide of 2 : 3 : 4-trimethyl mannosaccharic acid was produced (Haworth, Hirst, Isherwood, and Jones, J., 1939, 1878).

The 2 : 3 : 4-trimethyl derivative of glucuronic acid was identified as the crystalline 2 : 3 : 4-trimethyl β-methylglucopyruronoside (Haworth, Hirst, and Challinor, J., 1931, 258). The latter, when boiled with 3% methyl-alcoholic hydrogen chloride, was transformed into 2 : 3 : 4-trimethyl α-methylglucopyruronoside, which upon treatment with methyl-alcoholic ammonia yielded the characteristic amide of 2 : 3 : 4-trimethyl α-methylglucuronoside (Smith, *loc. cit.*).

The 2 : 3 : 4-trimethyl α-methylgalactopyruronoside was readily identified in the form of its crystalline methyl ester (Levene and Kreider, *J. Biol. Chem.*, 1937, **120**, 597; Luckett and Smith, J., 1940, 1506).

Experimental.

A. *Synthesis of* 2 : 3 : 4-*Trimethyl* α-*Methyl*-D-*mannuronoside.*

6-*Trityl* 2 : 3 : 4-*Triacetyl* α-*Methylmannoside.*—To a solution of α-methylmannopyranoside (50 g.) in dry pyridine (600 c.c.), trityl bromide (81 g.) was added and the mixture was shaken for 2 days at room temperature. Acetic anhydride (85 c.c.) was added, and the solution kept for a further 2 days at room temperature. The mixture was then poured with stirring into a large excess of cold water. The crisp yellow precipitate was collected, washed with water, and dried in the air. One crystallisation from boiling ethyl alcohol (1 l.) gave 6-trityl 2 : 3 : 4-triacetyl α-methylmannoside (94 g.). After recrystallisation from ethyl alcohol–light petroleum the compound showed m. p. 129°, $[\alpha]_D^{19°}$ + 35° in chloroform (*c*, 1·0). Treatment of this 6-trityl 2 : 3 : 4-triacetyl α-methylmannoside with hydrobromic acid in acetic acid according to the method of Helferich and Klein (*Annalen*, 1926, **450**, 219) gave 2 : 3 : 4-triacetyl α-methylmannoside, m. p. 97° (yield 80%).

6-*Trityl* 2 : 3 : 4-*Trimethyl* α-*Methylmannoside.*—6-Trityl α-methylmannoside (9·1 g.) was dissolved in acetone (60 c.c.) and methylated in the usual way with sodium hydroxide (310 c.c. of a 30% solution) and methyl sulphate (120 c.c.) at 35°. The reagents were added in tenths during 2 hours. While still being stirred, the reaction mixture was freed from acetone by heating on the water-bath. In this way the partially methylated product was obtained as a white granular mass. Two more methylations in the same way gave a white crystalline precipitate, which was collected and washed with hot water. After recrystallisation from methyl alcohol the 6-*trityl* 2 : 3 : 4-*trimethyl* α-*methylmannoside* had m. p. 149°, $[\alpha]_D^{20°}$ + 27° in chloroform (*c*, 1·05) (Found: C, 73·3; H, 7·1; OMe, 25·4. $C_{29}H_{34}O_6$ requires C, 72·9; H, 7·1; OMe, 25·9%).

2 : 3 : 4-*Trimethyl* α-*Methylmannoside.*—A solution of 6-trityl 2 : 3 : 4-trimethyl α-methylmannoside (7 g.) in ether (100 c.c.) was cooled in an ice-bath and saturated with dry hydrogen chloride. In order to remove some of the hydrogen chloride the ether was evaporated until trityl chloride began to crystallise and then the solution was extracted several times with water. The combined aqueous extracts were neutralised with lead carbonate, filtered, and evaporated under diminished pressure to dryness. Extraction of the residue with chloroform yielded 2 : 3 : 4-trimethyl α-methylmannoside as a colourless liquid (2·4 g.), b. p. (bath temp.) 130°/0·02 mm., $n_D^{24°}$ 1·4570, $[\alpha]_D^{17°}$ + 47° in water (*c*, 3·2) (Found: OMe, 50·0. Calc. for $C_{10}H_{20}O_6$: OMe, 52·5%) (cf. Haworth *et al.*, J., 1939, 1878).

2 : 3 : 4-*Trimethyl Mannose.*—A solution of 2 : 3 : 4-trimethyl α-methylmannoside (1 g.) in N-sulphuric acid (70 c.c.) was heated for 4 hours on the boiling water-bath. Hydrolysis had then been completed and the solution, which now showed $[\alpha]_D^{18°}$ + 10°, was neutralised with barium carbonate, filtered, and evaporated to dryness under reduced pressure. Extraction of the residue with acetone gave mainly syrupy 2 : 3 : 4-trimethyl mannose (0·88 g.), $n_D^{20°}$ 1·4780. Attempts to distil this substance in a high vacuum failed.

2 : 3 : 4-*Trimethyl* δ-*Mannonolactone.*—To a solution of 2 : 3 : 4-trimethyl mannose (0·75 g.) in water (10 c.c.), bromine (0·8 c.c.) was added. The solution was kept at room temperature for 2 days, oxidation then being complete. The solution was freed from excess of bromine by aeration, neutralised with silver oxide, filtered before and after treatment with hydrogen sulphide, and evaporated to dryness under reduced pressure. The colourless liquid (0·71 g.) thus produced, after being heated in a high vacuum at 120°, crystallised spontaneously (cf. Haworth *et al.*, *loc. cit.*). Crystallisation of the product from acetone–light petroleum gave 2 : 3 : 4-trimethyl δ-mannonolactone as a monohydrate, m. p. 74°, $[\alpha]_D^{20°}$ + 131° (initial value in water, *c*, 1·0), changing in 170 hours to + 80°, equilibrium value (Found: C, 45·6; H, 7·8; OMe, 38·2. Calc. for $C_9H_{16}O_6,H_2O$: C, 45·4; H, 7·6; OMe, 39·1%).

2 : 3 : 4-*Trimethylmannonamide.*—The liquid 2 : 3 : 4-trimethyl δ-mannonolactone (0·15 g.) was dried by heating for 1 hour in a vacuum at 100°, and then boiled for 6 hours with 2% methyl-alcoholic hydrogen chloride. The solution was neutralised with silver carbonate, filtered, and evaporated under diminished pressure. The syrup (0·11 g.) thus obtained was dissolved in methyl alcohol, and the solution, cooled to 0°, was saturated with dry ammonia. After the solution had been kept overnight at — 5°, the excess of the solvent was removed under reduced pressure in a desiccator. Recrystallisation of the solid residue from acetone gave the amide of 2 : 3 : 4-trimethyl mannonic acid in fine needles, m. p. 142°, $[\alpha]_D^{20°}$ + 5° in water (*c*, 0·9) (Found: C, 45·4; H, 8·1; OMe, 39·9; N, 5·9. Calc. for $C_9H_{19}O_6N$: C, 45·5; H, 8·0; OMe, 39·2; N, 5·9%).

2 : 3 : 4-*Trimethylmannosaccharodiamide.*—A solution of 2 : 3 : 4-trimethyl mannose (0·32 g.) in nitric acid (9 c.c., *d* 1·42) was heated for 4 hours at 50°. The nitric acid was removed by distilling water through the solution under diminished pressure and on complete removal of the solvent a syrup (0·33 g.) was obtained which reacted strongly acid to Congo-red paper. This product was esterified by boiling it for 8 hours with 2% methyl-alcoholic hydrogen chloride (50 c.c.). Neutralisation of the mineral acid with silver carbonate, followed by removal of the solvent, gave a syrupy product, which was distilled, giving: Fraction I (0·12 g.), b. p. (bath temp.) 135°/0·03 mm., $n_D^{20°}$ 1·4560 (Found: OMe, 54·2%). Fraction II (0·12 g.), b. p. (bath temp.) 160°/0·03 mm., $n_D^{20°}$ 1·4690 (Found: OMe, 56·0. Calc. for methyl 2 : 3 : 4-trimethyl mannosaccharate: OMe, 55·4%). Treatment of fraction II with methyl-alcoholic ammonia gave the diamide of 2 : 3 : 4-trimethyl mannosaccharic acid (0·1 g.), m. p. 228°, $[\alpha]_D^{20°}$ — 16° (*c*, 0·5 in water) (after recrystallisation from ethyl alcohol–ether) (Haworth *et al.*, *loc. cit.*, record m. p. 228°, $[\alpha]_D^{20°}$ — 17° in water, for this compound).

2 : 3 : 4-*Trimethyl* α-*Methylmannuronoside.*—2 : 3 : 4-Trimethyl α-methylmannoside (2·35 g.) was dissolved in water (100 c.c.) containing potassium hydroxide (1·18 g.). To this solution a solution of potassium permanganate (3·25 g.) in water (200 c.c.) was cautiously added. The volume was adjusted to 450 c.c., and the oxidation allowed to proceed at room temperature for 3 days. The mixture was then treated with charcoal, warmed to 50°, filtered, and neutralised with carbon dioxide. The filtrate was evaporated to dryness under diminished pressure. Extraction of the residue five times with boiling methyl alcohol gave a colourless glassy solid (3·05 g.) consisting mainly of the potassium salt of 2 : 3 : 4-trimethyl methylmannuronoside. This potassium salt was converted into the corresponding methyl ester by boiling it for 10 hours with 2% methyl-alcoholic hydrogen chloride (100 c.c.). The solution was filtered to remove potassium chloride, neutralised with silver carbonate, filtered again, and evaporated to dryness under diminished pressure. Extraction of the dry residue with boiling ether gave the methyl ester of 2 : 3 : 4-trimethyl methylmannuronoside (2 g.) as a colourless liquid, b. p. (bath temp.) 118°/0·02 mm., $n_D^{18°}$ 1·4515, $[\alpha]_D^{18°}$ + 45° in chloroform (*c*, 1·0) (Found: OMe, 57·1%) (cf. Haworth, Hirst, and Ault, J., 1935, 517).

2 : 3 : 4-*Trimethylmannuronic Acid.*—A solution of the methyl ester of 2 : 3 : 4-trimethyl methylmannuronoside (0·49 g.) in N-hydrochloric acid (10 c.c.) was heated on the boiling water-bath for 7 hours. The solution, which had $[\alpha]_D^{18°}$ + 50° initially, now showed $[\alpha]_D^{18°}$ + 32° (constant value). The solution was neutralised with silver carbonate, filtered before and after treatment with hydrogen sulphide, and evaporated to dryness under reduced pressure. The colourless viscid syrup (0·41 g.) thus produced was strongly reducing to Fehling's solution and reacted acid to Congo-red paper.

Oxidation of 2 : 3 : 4-*Trimethylmannuronic Acid by Bromine.*—To a solution of the syrupy 2 : 3 : 4-trimethylmannuronic acid (0·41 g.) in water (30 c.c.) bromine (0·7 c.c.) was added, and the mixture kept at room temperature until a test portion, when freed from bromine, no longer reduced Fehling's solution. The excess of bromine was then removed from the main bulk of the solution by aeration, and the solution neutralised with lead carbonate and filtered. Lead was removed as sulphide, and the filtrate freed from hydrogen sulphide by evaporation of some of the liquid under reduced pressure. The solution, which contained some hydrobromic acid, was neutralised with silver carbonate, filtered before and after treatment with hydrogen sulphide, and evaporated to dryness under reduced pressure. The syrup produced reacted acid to Congo-red paper, and was non-reducing to Fehling's solution (Found : equiv., 120. Calc. for 2 : 3 : 4-trimethyl mannosaccharic acid : equiv., 126). The acid was esterified by boiling with 2% methyl-alcoholic hydrogen chloride (50 c.c.) as described above. Distillation of the product gave : Fraction A (0·16 g.), b. p. (bath temp.) 135°/0·03 mm., $n_D^{20°}$ 1·4477. Fraction B (0·08 g.), b. p. (bath temp.) 150—160°/0·03 mm., $n_D^{20°}$ 1·4539. Treatment of fraction B with methyl-alcoholic ammonia in the manner previously described afforded the diamide of 2 : 3 : 4-trimethyl *d*-mannosaccharic acid, m. p. 228° (after recrystallisation from ethyl alcohol–light petroleum). This was identical with the diamide prepared directly from 2 : 3 : 4-trimethyl mannose.

B. *Synthesis of* 2 : 3 : 4-*Trimethyl β-Methyl-α-glucuronoside.*

6-*Trityl* 2 : 3 : 4-*Trimethyl β-Methylglucoside.*—6-Trityl 1 : 2 : 3 : 4-tetra-acetyl glucose (5·09 g.), prepared according to the directions given by Stacey (J., 1939, 1529), was dissolved in acetone and methylated with methyl sulphate and sodium hydroxide in the manner described previously for the methylation of 6-trityl 2 : 3 : 4-triacetyl α-methylmannoside. Five methylations gave 6-trityl 2 : 3 : 4-trimethyl β-methylglucoside as an amorphous, pale yellow powder. This was collected, washed with boiling water, and dried in a vacuum over phosphoric oxide. On removal of the trityl residue with a solution of hydrogen bromide in glacial acetic acid (Helferich and Klein, *loc. cit.*) there was obtained a syrup (1·9 g.). Purification by distillation gave a colourless liquid (1·65 g.), b. p. (bath temp.) 125—130°/0·04 mm., which underwent partial crystallisation when nucleated with 2 : 3 : 4-trimethyl β-methylglucoside. After draining on a porous tile, followed by recrystallisation from ether–light petroleum, the 2 : 3 : 4-trimethyl β-methylglucoside (0·82 g.) had m. p. and mixed m. p. 87°.

2 : 3 : 4-*Trimethyl β-Methylglucuronoside.*—To a solution of 2 : 3 : 4-trimethyl β-methylglucoside (1·0 g.) in water (50 c.c.) containing 0·1N-sodium hydroxide (10 c.c.) was added a solution of potassium permanganate (1 g.) in water (50 c.c.) during 7 hours at room temperature. After 30 hours the solution was treated with a little charcoal, filtered, neutralised with carbon dioxide, and evaporated to dryness under reduced pressure. A small amount of unchanged 2 : 3 : 4-trimethyl β-methylglucoside (*ca.* 50 mg.) was removed by extraction of the dry residue with chloroform. The residue was analysed for potassium and the exact amount of 0·1N-sulphuric acid required to convert the potassium into potassium sulphate was added. The solution was extracted several times with chloroform, and the combined extracts dried over anhydrous magnesium sulphate. Removal of the solvent gave crystalline 2 : 3 : 4-trimethyl β-methyl-glucuronoside (0·6 g.), m. p. and mixed m. p. 137° after recrystallisation from ether–light petroleum (Found : C, 47·8; H, 7·5; OMe, 48·8. Calc. for $C_{10}H_{18}O_7$: C, 47·8; H, 7·2; OMe, 49·4%). Esterification of this uronic acid by boiling with 2% methyl-alcoholic hydrogen chloride gave the methyl ester of 2 : 3 : 4-trimethyl methylglucuronoside as a liquid, from which the crystalline amide of 2 : 3 : 4-trimethyl α-methylglucuronoside was prepared by the action of methyl-alcoholic ammonia. This amide had m. p. and mixed m. p. 181° (after crystallisation from ethyl alcohol–ether).

C. *Synthesis of* 2 : 3 : 4-*Trimethyl α-Methyl-α-galacturonoside.*

To an ice-cold solution of potassium permanganate (3·0 g.) and potassium hydroxide (1·0 g.) in water (200 c.c.) there was slowly added during 1 hour a solution of 2 : 3 : 4-trimethyl α-methylgalactoside (2 g.) (prepared by the method of Smith, J., 1939, 1736) in water (30 c.c.). The ice-bath was removed, and the reaction mixture kept at room temperature for 3 days; all the permanganate had then been used up. The mixture was treated with a little charcoal, warmed to 50°, and filtered. The filtrate was neutralised with carbon dioxide, concentrated in a vacuum to half volume, and neutralised with dilute sulphuric acid, with methyl-orange as indicator. To this neutral solution N-sulphuric acid (8·0 c.c.) was added to liberate the organic acid and the solution was evaporated to dryness under diminished pressure. Extraction of the residue with chloroform, followed by removal of the solvent, gave an acidic syrup, which was esterified by boiling for 8 hours with 1% methyl-alcoholic hydrogen chloride (50 c.c.). Neutralisation of the mineral acid with silver carbonate, followed by removal of the excess of the solvent under diminished pressure, gave a colourless liquid which crystallised immediately upon nucleation with a specimen of the methyl ester of 2 : 3 : 4-trimethyl α-methylgalacturonoside prepared from galacturonic acid. The product was purified by distillation, b. p. 130°/0·03 mm. (yield, 1·5 g.). The distillate crystallised spontaneously and after recrystallisation from ether the methyl ester of 2 : 3 : 4-trimethyl α-methylgalacturonoside had m. p. and mixed m. p. 73°, $[\alpha]_D^{21°}$ + 169° in water (*c*, 0·65) (Found : C, 50·15; H, 7·7; OMe, 58·0. Calc. for $C_{11}H_{20}O_7$: C, 50·0; H, 7·65; OMe, 58·7%).

Treatment of the ester with methyl-alcoholic ammonia in the usual manner gave the amide of 2 : 3 : 4-trimethyl α-methylgalacturonoside, which was recrystallised from acetone–ether; m. p. and mixed m. p. with an authentic specimen 152°, $[\alpha]_D^{20°}$ + 120° (chloroform, *c*, 1·0) (cf. Levene and Kreider, *J. Biol. Chem.*, 1937, **121**, 158).

THE A.E. HILLS LABORATORIES,
THE UNIVERSITY, EDGBASTON, BIRMINGHAM.

[*Received, December 30th,* 1943.]