281. *The Constitution of Pectic Acid. Part III. Hydrolysis of the Methyl Ester of Methylated Pectic Acid and the Isolation of the Methyl Ester of* 2 : 3-*Dimethyl* β-*Methylgalactopyruronoside.*

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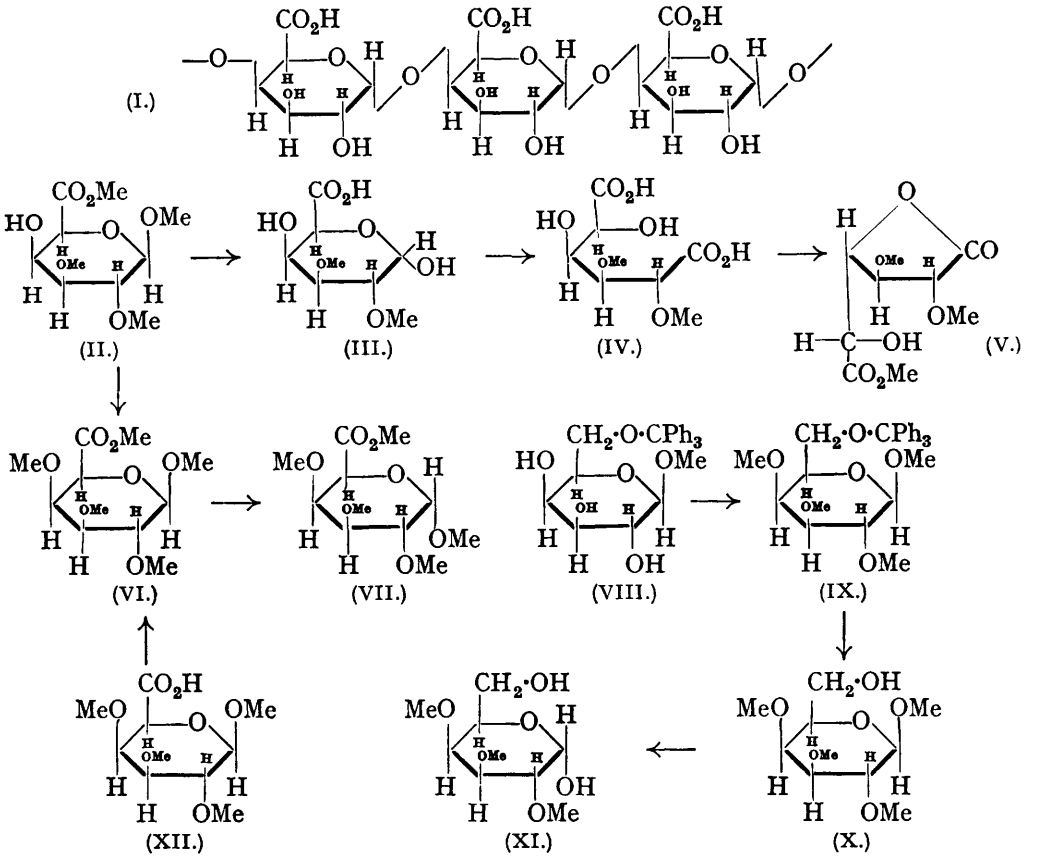
Hydrolysis of the methyl ester of methylated pectic acid by prolonged boiling with 2% methyl-alcoholic hydrogen chloride yields the crystalline *methyl* ester of 2 : 3-*dimethyl* β-*methylgalactopyruronoside* (II). The structure of the latter has been established by the fact that (*a*) on oxidation it gives 2 : 3-dimethyl mucic acid, identified as the crystalline γ-lactone methyl ester (V), and (*b*) on methylation with Purdie's reagents it affords the *methyl* ester of 2 : 3 : 4-*trimethyl* β-*methylgalactopyruronoside* (VI), the structure of which has been proved by synthesis.

In Part I (Luckett and Smith, this vol., p. 1106) attention was drawn to the stability towards hydrolysis of the methyl ester of methylated pectic acid. For complete hydrolysis it was found necessary to treat the methyl ester of methylated pectic acid with methyl-alcoholic hydrogen chloride in a sealed tube at 115°. This procedure gave the methyl ester of 2 : 3-dimethyl methylgalactofururonoside as the main product of the reaction (see also Beaven and Jones, *J. Soc. Chem. Ind.*, 1939, **58**, 363). It was also pointed out that the furanose structure of this 2 : 3-dimethyl methylgalacturonoside did not prove that pectic acid is composed of furanose residues of galacturonic acid. On the contrary the evidence available at that time suggested that the units of galacturonic acid present in pectic acid were of the pyranose type as shown in (I). The latter structure is

now supported by the observation that, when the methyl ester of methylated pectic acid is subjected to prolonged boiling with 2% methyl-alcoholic hydrogen chloride, partial hydrolysis takes place with the formation of the methyl ester of a dimethyl methylgalacturonoside (II) which has been shown to possess a pyranose structure.



The experimental evidence which led to the formulation (II) being assigned to this crystalline substance is as follows. The small negative rotation ($[\alpha]_D$ −11°) suggested that it was either a β-methylpyruronoside or an α-methylfururonoside, and its boiling point and refractive index lent support to the former possibility. When (II) was heated with dilute mineral acid, hydrolysis ensued and there was produced a dimethyl galacturonic acid (III) the rotation of which approximated to that of 2 : 3-dimethyl galacturonic acid previously prepared from the methyl ester of 2 : 3-dimethyl methylgalactofururonoside (Luckett and Smith, *loc. cit.*). Oxidation of (III) with nitric acid yielded a dimethyl mucic acid (IV), which was transformed by esterification and distillation into the crystalline lactone methyl ester (V), which proved to be identical with the γ-lactone methyl ester of 2 : 3-dimethyl mucic acid, the structure of which has been elucidated in Part I. It is clear, therefore, that the two ether methyl groups in (II) must occupy positions 2 and 3.

Methylation of (II) with Purdie's reagents gave a new crystalline trimethyl methylgalacturonoside (VI), the structure of which follows from the fact that, when (VI) was heated with methyl-alcoholic hydrogen chloride, it was converted into the crystalline methyl ester of 2 : 3 : 4-trimethyl α-methylgalactopyruronoside (VII) identical with an authentic specimen. This conversion was made possible as a result of the observation that the methyl ester of 2 : 3 : 4-trimethyl α-methylgalacturonoside is unaffected by boiling for several hours with 1% methyl-alcoholic hydrogen chloride. It follows that (VI) differs from the methyl ester of 2 : 3 : 4-trimethyl α-methylgalacturonoside only in the stereo-

chemistry of the hydrogen and methoxyl group at C_1. Consequently (VI) must be a β-glycoside and it is therefore designated the *methyl* ester of 2 : 3 : 4-*trimethyl* β-*methylgalactopyruronoside*. Since no change in ring structure could take place during the transformations (II) ⟶ (VI) ⟶ (VII), it is clear that (II), like (VII), must possess a pyranose structure and it must therefore be the *methyl* ester of 2 : 3-*dimethyl* β-*methylgalactopyruronoside*.

The pyranose structure of (II) and (VI) was confirmed as follows. When a solution of β-methylgalactopyranoside in pyridine was allowed to react with trityl chloride at room temperature, there was obtained 6-trityl β-methylgalactopyranoside (VIII). Methylation of the latter with methyl sulphate and sodium hydroxide solution gave 6-*trityl* 2 : 3 : 4-*trimethyl* β-*methylgalactopyranoside* (IX), from which the trityl group was removed by means of ethereal hydrogen chloride (Smith, J., 1939, 1724) to give crystalline 2 : 3 : 4-*trimethyl* β-*methylgalactopyranoside* (X). The structure of (X) follows from the fact that on methylation with Purdie's reagents it gave crystalline 2 : 3 : 4 : 6-tetramethyl β-methylgalactopyranoside and when subjected to hydrolysis with sulphuric acid (X) gave a trimethyl galactose (XI) which furnished a crystalline anilide identical with 2 : 3 : 4-trimethyl galactose anilide (McCreath and Smith, J., 1939, 387). Treatment of (X) with alkaline potassium permanganate according to the conditions employed in Part II (Luckett and Smith, this vol., p. 1114) oxidised the primary alcoholic group in position 6 to a carboxyl group and there resulted 2 : 3 : 4-trimethyl β-methylgalactopyruronoside (XII). Esterification of the latter with diazomethane yielded the corresponding crystalline methyl ester of 2 : 3 : 4-trimethyl β-methylgalactopyruronoside. This proved to be identical with that prepared from the 2 : 3-dimethyl derivative (II).

Furthermore, when 2 : 3 : 4-trimethyl galacturonic acid, prepared from the methyl ester of 2 : 3 : 4-trimethyl α-methylgalacturonoside by hydrolysis with dilute sulphuric acid, was methylated with methyl sulphate and sodium hydroxide solution, a preponderance of the β-glycoside was formed. After esterification of the 2 : 3 : 4-trimethyl methylgalacturonoside with diazomethane there was obtained a crystalline methyl ester which was identical with the methyl ester of the 2 : 3 : 4-trimethyl β-methylgalactopyruronoside derived from (II).

Experimental.

The Methyl Ester of Methylated Pectic Acid.—Pectic acid, prepared from citrus pectin (50 g.) by the method previously described (Ehrlich and Guttman, *Biochem. Z.*, 1933, **259**, 100; Luckett and Smith, this vol., p. 1106), was separated on the centrifuge and washed with water to remove the excess of hydrochloric acid and the soluble reducing substances. Whilst still containing water, the amorphous pectic acid was stirred vigorously with water (50 c.c.) and neutralised by the addition of a 15% solution of sodium hydroxide. The temperature was raised to 55° (bath temp.) and methyl sulphate (200 c.c.) and 30% sodium hydroxide solution were slowly added during 3 hours. Excess of sodium hydroxide must be avoided, otherwise the pectic acid is precipitated as a sodium salt. The methylation was completed by heating the mixture for 10 minutes at 70°. After cooling, the mixture was acidified with dilute sulphuric acid and subjected to dialysis at room temperature against a continuous stream of tap water to remove the excess of the sulphuric acid and most of the sodium sulphate. The dialysed solution was evaporated to a suitable volume (*ca.* 40 c.c.) under diminished pressure and the partially methylated pectic acid thus obtained was remethylated in the manner described above. After five such methylations the methylated pectic acid was isolated by dialysis and converted into the methyl ester of methylated pectic acid as described by Luckett and Smith (*loc. cit.*). Yield, 8 g. (Found : OMe, 44·8%).

Fractionation of the Methyl Ester of Methylated Pectic Acid.—To a solution of the crude material in acetone (20 c.c.), ether was slowly added with stirring to give a white flocculent amorphous precipitate (fraction I). To the liquid decanted from fraction I, more ether was added to give successively fractions II, III, and IV. Addition of more ether caused little or no precipitation, but on adding light petroleum there were obtained in turn fractions V, VI, and VII. Removal of the solvent from the mother-liquors gave a pale yellow residue (1·4 g.) which had OMe, 42·6%. Each of the fractions I to VII was dissolved in acetone, and the solution poured with stirring into light petroleum. In this way all the fractions were obtained as white amorphous powders which could be easily dried.

Fraction.	Weight, g.	$[\alpha]_D^{20°}$ in water.	% OMe.	Fraction.	Weight, g.	$[\alpha]_D^{20°}$ in water.	% OMe.
I	0·6	+209°	42·3	V	0·71	+209°	43·0
II	1·45	+212	43·2	VI	1·8	+208	44·0
III	0·8	+213	43·6	VII	0·72	+201	44·3
IV	0·55	+205	43·6				

The results of the osmotic pressure measurements of a chloroform solution of fraction V correspond to a molecular weight of approximately 1500 [Found for fraction VI: C, 49·2; H, 6·7. $(C_9H_{14}O_6)_n$ requires C, 49·5; H, 6·5%].

Hydrolysis of the Methyl Ester of Methylated Pectic Acid with 2% Methyl-alcoholic Hydrogen Chloride.—When a solution of the methyl ester of methylated pectic acid (fractions I, II, III, and IV; 3·4 g.) in 2% methyl-alcoholic hydrogen chloride (200 c.c.) was boiled, it showed: $[\alpha]_D$ +195° (initial value); + 187° (after 2 hours); + 181° (4 hours); + 176° (6 hours); + 172° (8 hours); + 168° (10 hours); + 165° (12 hours); + 159·5° (18 hours); + 158·5° (20 hours); +158° (22 hours); + 158° (28 hours). The solution was neutralised with silver carbonate, filtered, and evaporated under diminished pressure to give a pale yellow, glassy residue. Extraction of this with boiling ether gave a thick syrup ($n_D^{18°}$ 1·4720; OMe, 48·9%) and there remained a residue of incompletely hydrolysed material, $[\alpha]_D^{18°}$ + 177·5° in water (*c*, 0·7) (Found: OMe, 43·1%). Extraction of the thick syrup with ether at room temperature yielded a fairly viscous, pale yellow syrup (0·7 g.), $n_D^{18°}$ 1·4690 (Found: OMe, 49·8%). Distillation of this cold ether-soluble syrup gave: Fraction i (0·23 g.), b. p. (bath temp.) 100—120°/0·02 mm., $n_D^{20°}$ 1·4510, $[\alpha]_D^{18°}$ − 39·5° in methyl alcohol (*c*, 2·0) (Found: OMe, 54·4%); fraction ii (0·2 g.), b. p. (bath temp.) 140—160°/0·02 mm., $n_D^{18°}$ 1·4625. The second fraction crystallised spontaneously on keeping and after recrystallisation from ethyl alcohol–ether the *methyl* ester of 2 : 3-dimethyl β-methylgalactopyruronoside had m. p. 111°, $[\alpha]_D^{17°}$ − 11° in water (*c*, 1·1) (Found: C, 48·3; H, 7·1; OMe, 47·8; equiv. 256. $C_{10}H_{18}O_7$ requires C, 48·0; H, 7·2; OMe, 49·6%; equiv., 250).

When a solution of this methyl ester of 2 : 3-dimethyl β-methylgalacturonoside in 2% methyl-alcoholic hydrogen chloride was heated in a sealed tube for 16 hours on the boiling water-bath, the rotation changed from $[\alpha]_D$ − 12° to − 36°, probably owing to the transformation of the methylpyranoside into a methylfuranoside.

Conversion of the Methyl Ester of 2 : 3-Dimethyl β-Methylgalacturonoside into the Methyl Ester γ-Lactone of 2 : 3-Dimethyl Mucic Acid.—When a solution of the methyl ester of 2 : 3-dimethyl β-methylgalacturonoside (27 mg.) in 1% nitric acid was heated on the boiling water-bath, it showed $[\alpha]_D$ − 6° (initial value); − 2° (after 1 hour); + 9° (3½ hours); + 19° (5½ hours); + 25° (7½ hours); + 36° (10 hours); + 51° (15 hours); + 56° (19 hours); + 57° (29 hours); + 57° (32 hours). This solution was concentrated under reduced pressure almost to dryness and to the residual liquid a further amount (20 mg.) of the methyl ester of the 2 : 3-dimethyl β-methylgalacturonoside was added, followed by nitric acid (0·4 c.c., *d* 1·42). The solution was heated for ½ hour at 50° and for 1½ hours at 75°, diluted with water, and freed from nitric acid by distillation under diminished pressure, a process which was considerably facilitated by the simultaneous addition and distillation of small amounts (5 c.c.) of methyl alcohol. After removal of all traces of solvent the residue was dissolved in methyl alcohol (5 c.c.) and to the ice-cold solution of the 2 : 3-dimethyl mucic acid a slight excess of an ethereal solution of diazomethane was added. The excess of the diazomethane was immediately eliminated by concentration of the solution under reduced pressure. In this manner there was obtained a colourless syrup which crystallised almost completely when nucleated with the methyl ester γ-lactone of 2 : 3-dimethyl mucic acid. After recrystallisation from ethyl alcohol–ether the crystals had m. p. 92° alone or in admixture with an authentic specimen of the methyl ester γ-lactone of 2 : 3-dimethyl mucic acid previously prepared from the methyl ester of methylated pectic acid.

Methylation of the Methyl Ester of 2 : 3-Dimethyl β-Methylgalactopyruronoside with Purdie's Reagents.—One treatment of the methyl ester of 2 : 3-dimethyl β-methylgalacturonoside (20 mg.) with silver oxide and methyl iodide gave the corresponding 2 : 3 : 4-trimethyl derivative (18 mg.), which was isolated by means of acetone. After recrystallisation from ether–light petroleum the *methyl* ester of 2 : 3 : 4-trimethyl β-methylgalactopyruronoside had m. p. 102°, $[\alpha]_D^{18°}$ − 20° in methyl alcohol (*c*, 1·2). This crystalline compound was identical with specimens of the same substance prepared from the methyl ester of 2 : 3 : 4-trimethyl α-methylgalacturonoside and from 2 : 3 : 4-trimethyl β-methylgalactoside (see below) (Found: C, 50·2; H, 8·0; OMe, 58·0. $C_{11}H_{20}O_7$ requires C, 50·0; H, 7·65; OMe, 58·7%).

Transformation of the Methyl Ester of 2 : 3 : 4-Trimethyl β-Methylgalacturonoside into the Methyl Ester of 2 : 3 : 4-Trimethyl α-Methylgalacturonoside.—A solution of the methyl ester of 2 : 3 : 4-

trimethyl β-methylgalacturonoside (4·3 mg.), obtained from the previous experiment, in 2% methyl-alcoholic hydrogen chloride (0·4 c.c.) was heated in a sealed tube for 15 hours at 100°. The solution, which now showed $[\alpha]_D$ + 122°, was freed from solvent and hydrogen chloride by evaporation under reduced pressure over soda lime in a desiccator. The syrupy residue readily crystallised and after recrystallisation from ether–light petroleum there was obtained the methyl ester of 2 : 3 : 4-trimethyl α-methylgalactopyruronoside, m. p. alone and mixed with an authentic specimen 69°, $[\alpha]_D^{18°}$ + 172° in water (*c*, 1·2).

Preparation of the Methyl Ester of 2 : 3 : 4-*Trimethyl* α-*Methylgalacturonoside and its Conversion into the* β-*Methylgalacturonoside.*—Five treatments of the methyl ester of α-methylgalactopyruronoside (5 g.) with silver oxide and methyl iodide, the first of which required the addition of methyl alcohol to dissolve the material, gave the methyl ester of 2 : 3 : 4-trimethyl α-methylgalacturonoside, m. p. 72°, $[\alpha]_D^{18°}$ + 170° in water (*c*, 0·6) (after recrystallisation from ether–light petroleum) (Found : C, 50·2; H, 7·7; OMe, 59·1. Calc. for $C_{11}H_{20}O_7$: C, 50·0; H, 7·65; OMe, 58·7%).

Treatment of the methyl ester of 2 : 3 : 4-trimethyl α-methylgalacturonoside with methyl-alcoholic ammonia for 2 days at − 5° gave an amide, which crystallised on removal of the solvent. After recrystallisation from ethyl acetate–ether the amide of 2 : 3 : 4-trimethyl α-methylgalactopyruronoside had m. p. 154°, $[\alpha]_D^{21°}$ + 139° in water (*c*, 3·3) (Found : C, 48·2; H, 7·7; OMe, 49·1; N, 5·8. Calc. for $C_{10}H_{19}O_6N$: C, 48·2; H, 7·7; OMe, 49·8; N, 5·6%).

The crystalline methyl ester of 2 : 3 : 4-trimethyl α-methylgalacturonoside showed $[\alpha]_D^{18°}$ + 163° in 1% methyl-alcoholic hydrogen chloride. No change in rotation occurred when this solution was boiled for 3 hours and no change was observed after the hydrogen chloride content of the solution had been increased to 2% and the solution had been boiled for a further 5½ hours.

A solution of the methyl ester of 2 : 3 : 4-trimethyl α-methylgalacturonoside (1 g.) in N-sulphuric acid (50 c.c.) was heated for 4 days on the boiling water-bath. The solution was treated with N-sodium hydroxide (51 c.c.) and evaporated under diminished pressure almost to dryness. The residue containing the 2 : 3 : 4-trimethyl galacturonic acid was treated at room temperature with methyl sulphate (7 c.c.) and sodium hydroxide (21 c.c. of a 30% solution). The reagents were added with stirring during ¾ hour and after a further hour the solution no longer reduced Fehling's solution. The methylation was completed by heating the reaction mixture for ½ hour at 95° (bath temp.). After treatment of the pale yellow solution with a slight excess of dilute sulphuric acid it was evaporated under reduced pressure to remove dissolved carbon dioxide and then neutralised with N-sodium hydroxide. To the neutral solution, 0·1N-sulphuric acid (33 c.c.; calc., 37·8 c.c.) was added to liberate the 2 : 3 : 4-trimethyl β-methylgalacturonoside. The solution was evaporated to dryness under reduced pressure, and the methylated organic acid extracted from the residue by means of ether. Removal of the ether gave syrupy 2 : 3 : 4-trimethyl β-methylgalacturonoside, which was converted into the methyl ester by means of a slight excess of ethereal diazomethane. The syrup obtained on elimination of the excess of the solvent readily crystallised on nucleation and after recrystallisation from ether–light petroleum the methyl ester of 2 : 3 : 4-trimethyl β-methylgalactopyruronoside had m. p. 102°, $[\alpha]_D^{18°}$ − 21° in methyl alcohol, $[\alpha]_D^{18°}$ − 7° in water (*c*, 1·8) (Found : C, 50·3; H, 7·7; OMe, 58·4%).

Synthesis of the Methyl Ester of 2 : 3 : 4-*Trimethyl* β-*Methylgalactopyruronoside.*—A solution of β-methylgalactopyranoside (4·2 g.) in pyridine (45 c.c.) was treated with trityl chloride (5·7 g.). After keeping for 3 days at room temperature the reaction mixture was poured with stirring into water. The crystalline product was filtered off, washed with water, and dried in a vacuum over phosphoric oxide (yield, 9·2 g.). Repeated crystallisation from methyl alcohol gave the 6-trityl β-methylgalactoside in needles, m. p. 176° after softening at 83°, $[\alpha]_D^{16°}$ − 39° in chloroform (*c*, 1·9) (see Müller, *Ber.*, 1931, **64**, 1820).

The 6-trityl β-methylgalactoside, dissolved in acetone (35 c.c.), was treated with methyl sulphate (20 c.c.) and sodium hydroxide (60 c.c. of a 30% solution) at 45° (bath temp.) with stirring. The reagents were added simultaneously during 2 hours and acetone was added from time to time to replace that lost by evaporation in order to keep the trityl compound in solution. The stirring was continued until the acetone had been expelled and the partially methylated trityl compound, which had separated as a stiff syrup on the surface of the methylation mixture, was removed and remethylated as above. After five methylations in this manner the methylated 6-trityl β-methylgalactoside was isolated by extraction with chloroform. The chloroform solution was washed with water, dried over anhydrous calcium chloride, and evaporated to dryness to give a pale yellow, stiff syrup. Three treatments of this syrup with silver oxide and methyl iodide gave 6-*trityl* 2 : 3 : 4-*trimethyl* β-*methylgalactopyranoside* as a pale yellow glass

(7·6 g.) (after isolation with acetone), $[\alpha]_D^{17°}$ — 23° in chloroform (*c*, 2·2) (Found: OMe, 25·7. $C_{29}H_{34}O_6$ requires OMe, 25·9%).

When a solution of the 6-trityl 2 : 3 : 4-trimethyl β-methylgalactoside in ether (50 c.c.), cooled to 0°, was saturated with dry hydrogen chloride, the trityl group was readily eliminated. The ethereal solution was removed from the ice-bath, left at room temperature for ½ hour, and then exhaustively extracted with water. The combined aqueous extracts were neutralised with lead carbonate, filtered, and evaporated to dryness under reduced pressure. The product was purified by extraction with acetone and there was obtained a syrup (2·4 g.), which distilled giving: fraction i (chiefly 2 : 3 : 4 : 6-tetramethyl β-methylgalactoside) (0·8 g.), b. p. (bath temp.) 110—120°/0·04 mm., $n_D^{19°}$ 1·4520—1·4575; fraction ii (1·2 g.), b. p. (bath temp.) 120—130°/0·04 mm., $n_D^{19°}$ 1·4575—1·4640. The second fraction crystallised on keeping and after trituration with ether–light petroleum to remove adhering syrup, followed by recrystallisation from ether, the 2 : 3 : 4-*trimethyl* β-*methylgalactopyranoside* had m. p. 70—72°, $[\alpha]_D^{18°}$ + 11° in water (*c*, 1·0) (Found: C, 51·0; H, 8·6; OMe, 52·7. $C_{10}H_{20}O_6$ requires C, 50·7; H, 8·5; OMe, 52·6%).

One treatment of the 2 : 3 : 4-trimethyl β-methylgalactopyranoside (20 mg.) with Purdie's reagents gave 2 : 3 : 4 : 6-tetramethyl β-methylgalactopyranoside, m. p. and mixed m. p. 47° (after crystallisation from light petroleum).

When a solution of the 2 : 3 : 4-trimethyl β-methylgalactopyranoside (0·1 g.) in N-sulphuric acid (10 c.c.) was heated on the boiling water-bath for 8 hours, the rotation became constant ($[\alpha]_D^{17°}$ + 108°). After neutralisation of the sulphuric acid with barium carbonate and removal of the solvent under reduced pressure 2 : 3 : 4-trimethyl galactose was obtained as a syrup (0·095 g.). Treatment of this syrup with aniline (0·045 g.) in boiling ethyl alcohol for 2 hours gave 2 : 3 : 4-trimethyl galactose anilide, which crystallised on removal of the solvent, m. p. and mixed m. p. 166° (after recrystallisation from ethyl alcohol).

The crystalline 2 : 3 : 4-trimethyl β-methylgalactoside (0·17 g.) was dissolved in water (10 c.c.) and treated with a solution of potassium permanganate (0·26 g.) and potassium hydroxide (0·09 g.) in water (20 c.c.). After keeping for 2 days at room temperature, when the oxidation was complete, the solution was treated with a little charcoal, filtered, and neutralised with dilute sulphuric acid; a further addition of 0·1N-sulphuric acid (6·8 c.c.) was made in order to liberate the 2 : 3 : 4-trimethyl β-methylgalacturonoside. Evaporation of the solution to dryness under diminished pressure, followed by extraction of the residue with ether, yielded the organic acid as a colourless syrup, which was esterified by treatment with a slight excess of an ethereal solution of diazomethane. Removal of the solvent gave the methyl ester of 2 : 3 : 4-trimethyl β-methylgalactopyruronoside (0·15 g.), which distilled as a colourless liquid, b. p. (bath temp.) 130—135°/0·06 mm. The distillate crystallised on keeping and the crystals were separated on a tile. After recrystallisation from ether–light petroleum the methyl ester of 2 : 3 : 4-trimethyl β-methylgalactopyruronoside had m. p. 102°, $[\alpha]_D^{19°}$ — 21° in methyl alcohol (*c*, 1·3). This synthetic crystalline product was identical with the methyl ester of 2 : 3 : 4-trimethyl β-methylgalacturonoside prepared from the 2 : 3-dimethyl derivative.

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