203. *The Constitution of Pectic Acid. Part I. Methylation of Pectic Acid and the Isolation of the Methyl Ester of 2 : 3-Dimethyl Methyl-galacturonoside.*

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A pectic acid or polygalacturonic acid, prepared from citrus pectin by the action of dilute hydrochloride acid, has been converted into the *methyl* ester of *methylated pectic acid*. Hydrolysis of the latter with methyl-alcoholic hydrogen chloride yielded

the *methyl* ester of 2 : 3-*dimethyl methylgalactofururonoside* (II) as the main product. The structure of (II) has been proved by its oxidation to 2 : 3-dimethyl mucic acid, which formed a crystalline *γ-lactone methyl* ester (VIII) and by the fact that on methylation (II) gave the *methyl* ester of 2 : 3 : 5-*trimethyl* β-*methylgalactofururonoside* (IV), which after oxidation furnished the *γ-lactone methyl* ester of 2 : 3 : 5-*trimethyl mucic acid* (IX), obtainable also from (VIII) by methylation.

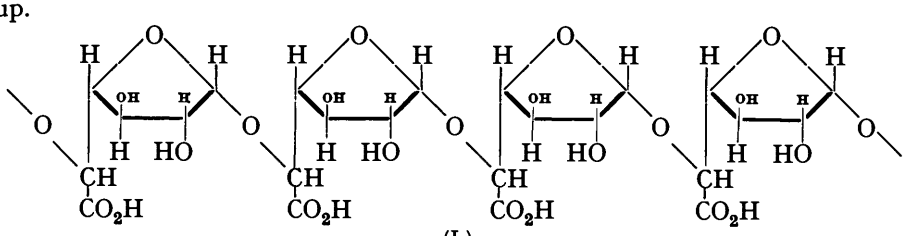
It is suggested that citrus pectic acid is composed of pyranose residues of galacturonic acid joined by 1 : 4-α-glycosidic links as in (I*a*). The molecule of the methyl ester of methylated pectic acid appears to be relatively small, the size as determined by osmotic pressure measurements being about 13 units.

A PECTIC acid corresponding to Ehrlich's " tetragalacturonic acid " has been prepared from citrus pectin (Ehrlich and Guttman, *Biochem. Z.*, 1933, **259**, 100) and subjected to repeated methylation. Frequent treatment of this pectic acid with methyl sulphate and sodium hydroxide solution furnished a partially methylated product, which was insoluble in organic solvents but readily soluble in water; consequently the isolation of the material could not be effected by the usual methods of solvent extraction, but it was found possible to remove inorganic impurities by dialysis in acid solution. Methylation of the partially methylated pectic acid, so obtained, by the thallium method (Fear and Menzies, J., 1926, 929; Purves and Hudson, *J. Amer. Chem. Soc.*, 1937, **59**, 49, 1170; Hirst and Jones, J., 1938, 496), followed by treatment with silver oxide and methyl iodide, gave the *methyl* ester of *methylated pectic acid*. The latter was also obtained from the methylated pectic acid by treatment of its silver salt first with methyl iodide and then, when esterification was complete, with Purdie's reagents.

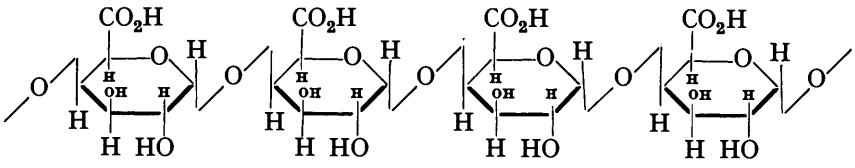
The methyl ester of methylated pectic acid was relatively stable inasmuch as prolonged boiling of it with 1% methyl-alcoholic hydrogen chloride caused only slight hydrolysis. When it was treated, however, with 1% methyl-alcoholic hydrogen chloride in a sealed tube at 120°, simultaneous hydrolysis and glycoside formation took place with the formation of the *methyl* ester of the *dimethyl methylgalacturonoside* (II) as the main product of the reaction (Smith, *Chem. and Ind.*, 1939, **58**, 363).

The structure of this methyl ester of dimethyl methylgalacturonoside (II) is based upon the following experimental facts. Treatment of (II) with methyl-alcoholic ammonia afforded a crystalline *amide* (III), the rotation ($[\alpha]_D$ — 150°) and analysis of which suggested that it was derived from the β-form of a dimethyl methylgalacturonoside containing a furanoside ring. This view was supported by the fact that on methylation with Purdie's reagents, (II) gave the crystalline *methyl* ester (IV) of trimethyl methylgalacturonoside, which in turn yielded the corresponding crystalline *amide* (V). The methyl groups in this methyl ester (IV) and in the amide (V) were present in positions 2, 3, and 5, inasmuch as (IV) and (V) were found to be identical with synthetic specimens of the methyl ester of 2 : 3 : 5-trimethyl β-methylgalactofururonoside and its amide respectively (see succeeding paper). It is clear, therefore, that the two ether methyl groups in (II) must occupy positions 2 : 3, 2 : 5, or 3 : 5. Saponification of the methyl ester (II) with an aqueous solution of barium hydroxide yielded the barium salt, from which the dimethyl methylgalacturonoside (VI) was obtained by means of sulphuric acid. When (VI) was heated with dilute sulphuric acid, the glycosidic methyl group was eliminated and there was produced a dimethyl galacturonic acid (VII). The rate of hydrolysis of (VI) is slow when compared, for example, with that of methylarabofuranoside, but it is relatively rapid when compared with the rate of hydrolysis of 2 : 3 : 4-trimethyl methylgalactopyruronoside. This fact and the observation that during the conversion of (VI) into (VII) the rotation changes from a negative value to a relatively high positive one, suggest that a furanoside ring is present in (VI) and therefore in (II). Oxidation of the dimethyl galacturonic acid (VII) with bromine gave a dimethyl mucic acid, from which the crystalline *lactone methyl* ester (VIII) was prepared by esterification and subsequent distillation. This ester-lactone (VIII) was shown to contain a 1 : 4-γ-lactone ring because on methylation with Purdie's reagents it gave the crystalline 1 : 4-*γ-lactone methyl* ester (IX) of 2 : 3 : 5-*trimethyl mucic acid* and this yielded the corresponding characteristic *diamide* of 2 : 3 : 5-trimethyl mucic acid. The ester-lactone (IX) and its diamide were identical with synthetic

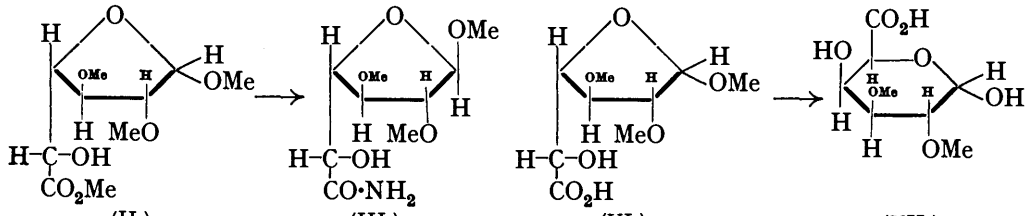
specimens of the 1 : 4-γ-lactone methyl ester of 2 : 3 : 5-trimethyl mucic acid and its diamide respectively, prepared from 2 : 3 : 5-trimethyl methylgalactofuranoside (succeeding paper). Furthermore it was found that (IX) and its diamide were the enantiomorphs of the 3 : 6-γ-lactone methyl ester of 2 : 4 : 5-trimethyl mucic acid and its diamide respectively (Smith, J., 1939, 1724). It is clear, therefore, that position 4 in (II) is not occupied by a methoxyl group.



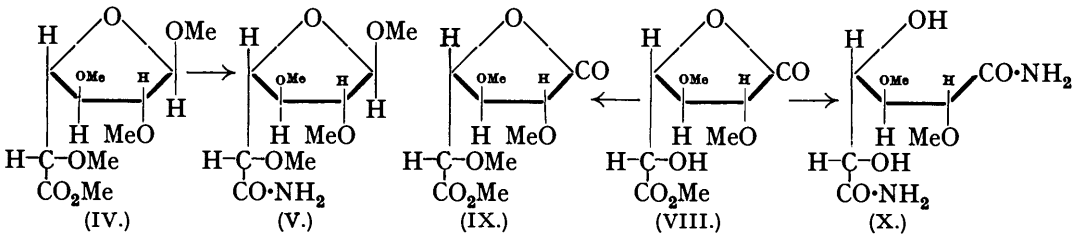
(I.)



(Ia.)



(II.) (III.) (VI.) (VII.)



(IV.) (V.) (IX.) (VIII.) (X.)

The possibility of the two methyl groups being in the positions 2 and 5 was eliminated for the reason that the diamide (X) of the dimethyl mucic acid, obtained from the ester-lactone (VIII) by the agency of methyl-alcoholic ammonia, showed a positive Weerman test for α-hydroxy-amides (*Rec. Trav. chim.*, 1917, **36**, 16); this result demonstrated that there must be a free hydroxyl group in position 2 or 5 and hence it follows that (X) and therefore (II) must be either 2 : 3- or 3 : 5-dimethyl derivatives. Conclusive proof that the two methyl groups occupy the positions 2 and 3 was forthcoming from the observation that 2 : 3-dimethyl galactose, prepared by the method of Robertson and Lamb (J., 1934, 1321), gave on oxidation with nitric acid, followed by esterification and subsequent distillation, a lactone methyl ester of 2 : 3-dimethyl mucic acid which was identical with the lactone methyl ester (VIII) of the dimethyl mucic acid obtained from (II). The position of the two methyl groups in the 2 : 3-dimethyl mucic acid has also been ascertained by Beaven and Jones (*J. Soc. Chem. Ind.*, 1939, **58**, 363), who showed that on treatment of the dimethyl mucic acid with periodic acid, followed by bromine, there was obtained *l*(+)-threo-dimethoxysuccinic acid (*d*-dimethoxysuccinic acid).

The identification of the methyl ester of the dimethyl methylgalacturonoside as the 2 : 3-dimethyl derivative demonstrates that pectic acid is composed of galacturonic acid residues, joined by links which do not involve positions 2 and 3. This condition can be satisfied in two ways; the pectic acid may be composed either of furanose units of galacturonic acid joined by 1 : 5-linkages as in (I) or of pyranose residues of galacturonic acid joined by 1 : 4-glycosidic links as shown in (I*a*).

Although the methyl ester of the 2 : 3-dimethyl methylgalacturonoside obtained above from the methyl ester of the methylated pectic acid was shown to have a furanose structure, it does not follow that furanose galacturonic acid residues pre-exist in pectic acid; in fact it now appears that 2 : 3-dimethyl galacturonic acid, like galactose (Cunningham, J., 1918, **113**, 596), shows a tendency preferentially to form a methylfuranoside when treated with acid methyl alcohol under the conditions employed in this work.

The high positive rotations of the pectic acid ($[\alpha]_D$ + 260° in dilute sodium hydroxide solution) and of the methylated derivative ($[\alpha]_D$ + 225°), together with the stability of the latter to boiling methyl-alcoholic hydrogen chloride, are best explained on the view that the pectic acid consists of pyranose units of galacturonic acid joined by 1 : 4-α-glycosidic links as shown in (I*a*); thus it appears that, as far as the glycosidic links are concerned, pectic acid is related to starch and not to cellulose (cf. Schreider and Bock, *Ber.*, 1937, **70**, 1617). This fundamental feature of the structure of citrus pectic acid has also been shown to apply to a degraded strawberry pectic acid by Jones and Beaven (*loc. cit.*). This view has also been put forward by Levene and Kneider (*J. Biol. Chem.*, 1937, **120**, 591) on the basis of the formation of *d*(−)-threo-dihydroxysuccinic acid (*l*-tartaric acid) by the action of periodic acid, followed by bromine, upon the methyl ester of a degraded pectic acid. More recently, however, Levene, Meyer, and Kuna (*Science*, 1939, **39**, 370) have examined the rate of hydrolysis of the methyl ester of a methylated pectic acid and they suggest that pectic acid is composed of furanose units of galacturonic acid joined by 1 : 5-glycosidic linkages.

Measurements of the osmotic pressure of a solution of the methyl ester of methylated pectic acid in chloroform (carried out in these laboratories by Dr. S. R. Carter and Dr. W. T. Chambers) indicate that it has a molecular size of about thirteen units (cf. Link, *J. Biol. Chem.*, 1934, **105**, 4). The relatively small molecular size of the methylated pectic acid is supported by viscosity measurements in *m*-cresol and also by the results of molecular weight determinations by the depression of the melting point of camphor.

It is of interest to note that a terminal or " end " group (the methyl ester of a trimethyl methylgalacturonoside) was not detected among the cleavage fragments and, although this result may be explained by the fact that pectic acid consists of a long chain of galacturonic acid units as in cellulose, it is not unlikely that the methylated pectic acid is composed of galacturonic acid residues arranged in the form of a loop (see Levene, Meyer, and Kuna, *loc. cit.*).

Experimental.

Pectic Acid.—Citrus pectin (100 g.) was converted into pectic acid by treatment with 5% hydrochloric acid at 75—80° for 1¾ hours (Ehrlich and Guttman, *loc. cit.*). The flocculent white precipitate was separated on the centrifuge and washed with water, aqueous ethyl alcohol (70%), absolute alcohol, and finally with ether. After drying in a vacuum the material was obtained as a white powder which appeared to be insoluble in dilute acids and only slightly soluble in cold water; it was insoluble in organic solvents. The pectic acid dissolved in boiling water and was not precipitated on cooling; this solution reacted strongly acid to Congo-paper and showed $[\alpha]_D^{19°}$ + 250° (*c*, 1·0). A suspension of the polygalacturonic acid in water dissolved when 1 equiv. of sodium hydroxide was added (Found: equiv., 203) and the neutral solution showed $[\alpha]_D^{19°}$ + 262° (*c*, 0·4). On the addition of excess of 5N-sodium hydroxide to the neutral solution of the pectic acid, a yellow sodium salt was precipitated and when heated both the precipitate and the solution became coloured orange. The pectic acid reduced Fehling's solution on prolonged boiling.

When a solution of the pectic acid (2 g.) in water (200 c.c.) was boiled, autohydrolysis took place with the ultimate formation of galacturonic acid. The solution had an iodine number of 5·5, $[\alpha]_D$ + 265° (after 1 hour); iodine number 9 (after 3 hours); 12, + 262° (4 hours); 14·5 (5 hours); 18 (6 hours); 21, + 246° (7 hours); 31 (9 hours); 34·5 (11 hours); 44·5 (14½

hours); 68, + 196° (17½ hours); 80, + 170° (22 hours); iodine number 105 (27 hours); 140 (35 hours); 165 (42 hours); 175 (46 hours); 188 (52 hours); 198 (56 hours); 202 (60 hours); 223 (72 hours); 230 (81 hours); 225 (91 hours).

The solution was treated with charcoal, filtered, and evaporated to dryness under reduced pressure. To a solution of the residue in methyl alcohol, acetone and ether were added with stirring to give a small acidic precipitate of degraded pectic acid, which was centrifuged off, washed with ethyl alcohol–ether and dried (Found : equiv., 206). Removal of the solvent from the mother-liquors yielded a glassy solid, which showed $[\alpha]_D^{18°}$ + 24° in water (*c*, 1·0) (Found : equiv. 192). The nature of the products produced during this autohydrolysis is now being investigated.

In another experiment pectic acid (5 g.) was added to boiling water (500 c.c.). The solution showed $[\alpha]_D$ + 235° (after 5 minutes); + 35° (70 hours). After being shaken with a little charcoal, the solution was filtered and evaporated under diminished pressure to a glassy solid, which was extracted with ethyl alcohol. Removal of the solvent yielded a pale yellow, glassy solid. When a solution of a portion of this product (1·9 g.) in 2·8% methyl-alcoholic hydrogen chloride (100 c.c.) was kept at room temperature, it showed $[\alpha]_D$ + 34° (initial value); + 5·5° (6 hours); + 9·5° (16 hours); + 12° (22 hours); + 25° (40 hours); + 29° (52 hours); + 34° (63 hours); + 43° (87½ hours); + 51° (136 hours). After being kept for a further 72 hours, the solution, now non-reducing to Fehling's solution, was neutralised with silver carbonate, filtered, and freed from solvent. The product was dissolved in the minimum quantity of methyl alcohol and treated with Purdie's reagents. After three more Purdie methylations using silver oxide and methyl iodide alone, the product was isolated by means of acetone and distilled, giving a colourless liquid (1·1 g.), b. p. (bath temp.) 130—140°/0·04 mm., $n_D^{21°}$ 1·4650, $[\alpha]_D^{21°}$ + 35° in water (*c*, 0·8). On keeping, crystals of the methyl ester of 2 : 3 : 4-trimethyl α-methylgalacturonoside separated. The crystals were freed from adhering syrup by tiling and then recrystallised from ether–light petroleum; m. p. and mixed m. p. 70—71°.

Methylation of Pectic Acid.—The flocculent pectic acid prepared from pectin (50 g.) was washed on the centrifuge to remove impurities and while still containing water it was treated at room temperature for 3 hours with methyl sulphate (250 c.c.) and 30% sodium hydroxide solution as required, with vigorous stirring. (A large excess of sodium hydroxide should be avoided, otherwise the sodium salt is precipitated and methylation is inhibited.) The vigorous stirring was continued for a further 9 hours and then the mixture was acidified with sulphuric acid and freed from sodium sulphate by dialysis against a continuous stream of tap water. The solution was made slightly alkaline by the addition of sodium hydroxide and evaporated almost to dryness under diminished pressure. The methylation was then repeated. After three methylations in this manner the product (Found : OMe, 11·7%) was given two further methylations at 35—40°, the material being isolated as before by dialysis in acid solution.

A solution of the crude pale yellow solid sodium salt in water (120 c.c.) was dialysed for 4 days, fresh additions of sulphuric acid being made during the first 2 days to ensure that all the sodium salt had been converted into the free methylated acid. When the solution no longer gave a positive sulphate test it was evaporated under diminished pressure to give a pale yellow, glassy solid (9·1 g.), which had $[\alpha]_D^{16°}$ + 252° in water (*c*, 1·0) [Found : OMe, 22·5%; equiv., 208. The dimethyl derivative of a polygalacturonic acid $(C_8H_{12}O_6)_n$ requires OMe, 27·4%; equiv. 204].

A solution of the methylated pectic acid (4 g.) in water (10 c.c.) was treated with a moderate excess of 0·3N-thallium hydroxide. No precipitate was produced, but on the addition of excess of ethyl alcohol the heavy thallium salt was precipitated as a cream-coloured powder. This salt was centrifuged off, washed with ethyl alcohol, and dried in a vacuum over phosphoric oxide (yield, 9 g.). By the same procedure the rest of the methylated pectic acid was transformed into 14 g. of thallium salt.

The thallium salt (23 g.) was then boiled with methyl iodide (60 c.c.) in the presence of dry methyl alcohol (20 c.c.) until esterification was complete, as indicated by the complete disappearance of the turbidity in the solution (12 hours). The solution was treated with a little charcoal, filtered, and evaporated under diminished pressure to a pale yellow, crisp solid (8·6 g.). Three treatments of this neutral product with Purdie's reagents yielded the crude methyl ester of methylated pectic acid, $[\alpha]_D^{18°}$ + 202° in methyl alcohol (*c*, 0·4) (isolated by means of acetone) (Found : OMe, 39·6%). After a further treatment with silver oxide and methyl iodide the material (8·4 g.) had $[\alpha]_D^{21°}$ + 213° in water (*c*, 0·4) [Found : OMe, 39·5. The methyl ester of a methylated polygalacturonic acid $(C_9H_{14}O_6)_n$ requires OMe, 42·6%].

The methylated pectic acid (OMe, 22·5%) was also converted into the methyl ester as follows. Neutralisation of an aqueous solution of the methylated polygalacturonic acid with silver oxide,

followed by filtration and evaporation, gave the silver salt, which was boiled with methyl iodide containing dry methyl alcohol. When esterification was complete, fresh additions of silver oxide and methyl iodide were made and the experiment was continued as for an ordinary Purdie methylation. The material was isolated by means of methyl alcohol and remethylated with Purdie's reagents. When a solution of the almost colourless glassy solid in acetone was poured into light petroleum, the *methyl* ester of *methylated pectic acid* was obtained as a white powder, which had $[\alpha]_D^{18°}$ + 201° in water (*c*, 0·3); $[\alpha]_D^{18°}$ + 202° in methyl alcohol (*c*, 0·3) (Found : OMe, 39·0%).

Fractionation of the Methyl Ester of Methylated Pectic Acid.—To a solution of the methylated polygalacturonic acid (8·4 g.) in acetone (50 c.c.), ether was added with shaking to give fraction I (0·83 g.) as a greyish-white amorphous powder. This fraction contained the inorganic impurities (colloidal silver iodide and charcoal) introduced during the working up of the methylation product (Found : OMe, 38·7%). Further addition of ether to the liquid decanted from fraction I gave successively fraction II (1·27 g.; OMe, 41·1%); fraction III (1·41 g.; OMe, 41·2%); fraction IV (0·95 g.; OMe, 41·0%). After the separation of fraction IV the acetone–ether mother-liquors were poured with stirring into excess of light petroleum to give fraction V (1·81 g.; OMe, 43·3%).

Fraction IV had $[\alpha]_D^{20°}$ + 223·5° in water (*c*, 0·4); $[\alpha]_D^{20°}$ + 224° in chloroform (*c*, 0·6); equiv., 252 (by heating with N/50-sodium hydroxide); *M*, *ca.* 800 (by the depression of the m. p. of camphor); $\eta_{sp.}^{20°}$ 0·096 in *m*-cresol (*c*, 0·836) (using $K = 10^{-3}$, this corresponds to *M*, 2500). Measurement of the osmotic pressure of a solution of the methyl ester of methylated pectic acid (fraction IV) gave a value corresponding to *M*, *ca.* 2900.

Hydrolysis of the Methyl Ester of Methylated Pectic Acid.—When a solution of the methyl ester of methylated pectic acid (3·18 g.) (fractions III and V) in 5% methyl-alcoholic hydrogen chloride (150 c.c.) was heated on the boiling water-bath, it showed $[\alpha]_D$ + 225° (initial value); + 150° (7 hours); + 130° (18 hours). The solution was cooled, neutralised with silver oxide, filtered, and evaporated under diminished pressure to give a glassy product. This product was only partly soluble in ether and since this indicated incomplete hydrolysis the material was treated with 1% methyl-alcoholic hydrogen chloride (50 c.c.) for 18 hours in a sealed tube at 115°. After neutralisation with silver carbonate, the solution was evaporated under reduced pressure to give a fairly mobile liquid (2·9 g.).

Fractions II and IV of the methyl ester of methylated pectic acid (2·4 g.) were also hydrolysed by means of 1% methyl-alcoholic hydrogen chloride in a sealed tube; this product was combined with the syrup obtained from fractions III and V, making a total of 4·7 g., and distilled, giving : Fraction I (the *methyl* ester of 2 : 3-*dimethyl methylgalacturonoside*) (3·01 g.), b. p. (bath temp.) 125—130°/0·01 mm., $n_D^{18°}$ 1·4540 (Found : OMe, 49·2%; equiv., 250, by heating with N/50-sodium hydroxide). Fraction II (a mixture of the methyl esters of 2 : 3-dimethyl methylgalacturonoside and of a monomethyl methylgalacturonoside) (1·2 g.), b. p. (bath temp.) 160—170°/0·02 mm., $n_D^{19°}$ 1·4695, $[\alpha]_D^{15°}$ + 34·5° in water (*c*, 0·4) (Found : OMe, 43·9%; equiv., 244). There remained an undistillable residue of incompletely hydrolysed material (0·43 g.). Retreatment of this with methyl-alcoholic hydrogen chloride (30 c.c. of 2%) for 18 hours at 115° in a sealed tube gave a liquid, isolated by the method previously employed, b. p. (bath temp.) 160—170°/0·02 mm., $n_D^{18°}$ 1·4680 (Found : OMe, 40·8%).

Fraction I was then slowly redistilled through a fractionating column, giving : Fraction IA (0·29 g.), b. p. (bath temp.) 120—125°/0·04 mm., $n_D^{17°}$ 1·4540, $[\alpha]_D^{15°}$ − 64° in water (*c*, 0·7) (Found : OMe, 49·1. The methyl ester of a dimethyl methylgalacturonoside, $C_{16}H_{18}O_7$, requires OMe, 49·6%. $C_{11}H_{20}O_7$ requires OMe, 58·7%). There was no variation of the refractive index during the distillation of this fraction. Fraction IB (0·71 g.), b. p. (bath temp.) 125°/0·04 mm., $n_D^{17°}$ 1·4532, $[\alpha]_D^{15°}$ − 65·5° in water (*c*, 0·6) (Found : OMe, 49·9%). Fraction IC (1·91 g., the residual liquid in the flask), $n_D^{17°}$ 1·4532, $[\alpha]_D^{15°}$ − 46° in water (*c*, 1·0) (Found : OMe, 49·3%). There does not appear to be any methyl ester of a trimethyl methylgalacturonoside present in fraction IA, IB, or IC. The difference between the rotations of fractions IC and IA and IB is probably due to a variation in the amount of α- and β-forms of the methyl ester of 2 : 3-dimethyl methylgalacturonoside.

An examination of the monomethyl methylgalacturonoside present in fraction II above will form the subject of a later communication.

Determination of the Structure of the 2 : 3-*Dimethyl Methylgalactofururonoside.*

The Amide of 2 : 3-*Dimethyl* β-*Methylgalactofururonoside* (III).—When specimens of each of the fractions IA, IB, and IC were separately treated with methyl-alcoholic ammonia for

3 days at − 5°, an amide was produced which crystallised after removal of the solvent at room temperature in an evacuated desiccator. The *amide* of 2 : 3-*dimethyl* β-*methylgalactofururonoside* had m. p. 124°, $[\alpha]_D^{17°}$ − 151° in water (*c*, 1·5) (after recrystallisation from ethyl acetate) (Found : C, 46·2; H, 7·4; N, 6·2; OMe, 39·3. $C_9H_{17}O_6N$ requires C, 46·0; H, 7·3; N, 6·0; OMe, 39·6%).

The Methyl Ester of 2 : 3 : 5-*Trimethyl* β-*Methylgalactofururonoside* (IV).—Two treatments of fraction IB (0·5 g.) with Purdie's reagents gave a liquid (0·5 g.), b. p. (bath temp.) 130°/0·03 mm., $n_D^{17°}$ 1·4445, $[\alpha]_D^{17°}$ − 85° in water (*c*, 0·8) (Found : OMe, 58·6%; equiv., 275). The distillate crystallised on keeping and after removal of adhering syrup from the crystals by trituration with ice-cold light petroleum the *methyl* ester of 2 : 3 : 5-*trimethyl* β-*methylgalactofururonoside* had $[\alpha]_D^{12°}$ − 123° in methyl alcohol (*c*, 1·0), m. p. 42° alone or in admixture with a synthetic specimen prepared by the oxidation of 2 : 3 : 5-trimethyl methylgalactofuranoside (see succeeding paper) (Found : C, 49·9; H, 7·0; OMe, 58·1. $C_{11}H_{20}O_7$ requires C, 50·0; H, 6·8; OMe, 58·7%).

Treatment of the crystalline methyl ester of 2 : 3 : 5-trimethyl β-methylgalactofururonoside with methyl-alcoholic ammonia for 2 days at − 5° gave an amide which crystallised on removal of the excess of the solvent. After recrystallisation from ether the *amide* of 2 : 3 : 5-trimethyl β-methylgalactofururonoside (V) had m. p. 106° alone or in admixture with a synthetic specimen; $[\alpha]_D^{14°}$ − 151·5° in water (*c*, 1·0) (Found : C, 48·1; H, 7·4; N, 5·5; OMe, 49·0. $C_{10}H_{19}O_6N$ requires C, 48·2; H, 7·6; N, 5·6; OMe, 49·8%).

Oxidation of the Methyl Ester of 2 : 3 : 5-*Trimethyl Methylgalactofururonoside with Nitric Acid and the Isolation of the* γ-*Lactone Methyl Ester of* 2 : 3 : 5-*Trimethyl Mucic Acid* (IX).—A solution of the crystalline methyl ester of 2 : 3 : 5-trimethyl β-methylgalacturonoside (140 mg.) in nitric acid (1·5 c.c., *d* 1·42) was heated for ½ hour at 50° and for 2 hours at 80°. The solution was then diluted with water and freed from nitric acid by distillation under diminished pressure, the process being facilitated initially by the simultaneous addition and distillation of water; the last traces of nitric acid and of solvent were eliminated by the addition and distillation of methyl alcohol. The dry acidic product was then esterified by boiling for 8 hours with 1% methyl-alcoholic hydrogen chloride (20 c.c.); the solution was cooled, neutralised with silver carbonate, filtered, and evaporated under reduced pressure to a syrup, which crystallised on standing. After recrystallisation from ether the γ-lactone methyl ester of 2 : 3 : 5-trimethyl mucic acid had m. p. 62° alone or in admixture with a synthetic specimen; $[\alpha]_D^{20°}$ − 83° in water (*c*, 1·0).

When this γ-lactone methyl ester of 2 : 3 : 5-trimethyl mucic acid was allowed to react with methyl-alcoholic ammonia at room temperature for 2 days, it readily gave the *diamide* of 2 : 3 : 5-trimethyl mucic acid, m. p. and mixed m. p. 255° (decomp.). This diamide showed a negative Weerman reaction (Found : C, 43·2; H, 7·1; N, 11·1; OMe, 37·3. $C_9H_{18}O_6N_2$ requires C, 43·2; H, 7·3; N, 11·2; OMe, 37·2%).

The γ-*Lactone Methyl Ester of* 2 : 3-*Dimethyl Mucic Acid* (VIII).—*Method* (*a*). A solution of the methyl ester of 2 : 3-dimethyl methylgalacturonoside (0·95 g. of fraction IC) in water (10 c.c.) was warmed with 0·244N-barium hydroxide (20 c.c.) for 1 hour at 60°. The free 2 : 3-dimethyl methylgalacturonoside was liberated from the barium salt by the addition of 0·1N-sulphuric acid (50 c.c.; calc., 48·8 c.c.). After the solution had been warmed with a little charcoal, the barium sulphate was filtered off and washed with water; the filtrate was concentrated under diminished pressure to a suitable volume (28 c.c.), and N-sulphuric acid (7 c.c.) added. When the solution, which contained the 2 : 3-dimethyl methylgalacturonoside, was heated on the boiling water-bath, it showed $[\alpha]_D$ − 41° (initial value); − 22° (after 1 hour); − 7·5° (2 hours); + 13·5° (3 hours); + 23·5° (4 hours); + 32·5° (5 hours); + 38° (6 hours); + 48·6° (8 hours); + 59° (10 hours); + 66° (12 hours); + 71° (14 hours); + 73·5° (16 hours); + 77·5° (18 hours); + 79·5° (20 hours); + 80° (22 hours); + 80·3° (24 hours); + 79·5° (27 hours). The solution, which now reduced Fehling's solution actively, was neutralised while still hot with barium carbonate, treated with a little charcoal, filtered, and evaporated to dryness under diminished pressure. The residue was dissolved in water (15 c.c.), filtered to remove a little barium carbonate, and treated with bromine (0·7 c.c.) at room temperature for 2 days. After removal of the excess of the bromine by aeration, the solution was neutralised with silver oxide, filtered before and after treatment with hydrogen sulphide, and evaporated under diminished pressure to give the acid barium salt of 2 : 3-dimethyl mucic acid. Esterification of the latter was brought about by boiling it for 8 hours with 1·5% methyl-alcoholic hydrogen chloride (40 c.c.). The solution was cooled, neutralised with silver carbonate, filtered, and evaporated to a syrup, which was purified by extraction with ether. The product distilled

as a colourless liquid (0·7 g.), b. p. (bath temp.) 160—165°/0·02 mm., $n_D^{21°}$ 1·4600; $[\alpha]_D^{18°}$ — 43° (initial value in water, *c*, 0·7); — 39° (after 15½ hours) — 9° (18 days); — 8° (22 days); — 7° (33 days). The distillate crystallised on keeping and after recrystallisation from ethyl alcohol–ether–light petroleum the *γ-lactone methyl* ester of 2 : 3-*dimethyl mucic acid* had m. p. 92° alone or in admixture with a specimen prepared from 2 : 3-dimethyl galactose (see below); $[\alpha]_D^{17°}$ — 55·8° (initial value in water, *c*, 0·6); — 54° (after 1 day); — 12·5° (14 days); — 10·5° (18 days); — 4° (29 days, constant value). The rotation solution, originally neutral, was now strongly acid to Congo-paper (Found: C, 46·1; H, 5·8; OMe, 39·3. $C_9H_{14}O_7$ requires C, 46·15; H, 6·0; OMe, 39·8%).

Method (*b*). A solution of the methyl ester of 2 : 3-dimethyl methylgalacturonoside (1 g.) in nitric acid (12 c.c., *d* 1·42) was heated for ½ hour at 50° and for 3½ hours on the boiling water-bath. The dimethyl mucic acid thus produced was freed from nitric acid as above and esterified by boiling it for 8 hours with 1% methyl-alcoholic hydrogen chloride (100 c.c.). After neutralisation of the solution, followed by removal of the solvent, the product distilled, giving the γ-lactone methyl ester of 2 : 3-dimethyl mucic acid, b. p. (bath temp.) 130—140°/0·03 mm., $n_D^{15°}$ 1·4600. The distillate crystallised and showed m. p. 92°, $[\alpha]_D^{15°}$ — 56°, initial value in water (*c*, 1·0) (after recrystallisation from ethyl alcohol–ether).

Treatment of the crystalline γ-lactone methyl ester of 2 : 3-dimethyl mucic acid with methyl-alcoholic ammonia for 2 days at room temperature gave the *diamide* (X) of 2 : 3-dimethyl mucic acid, m. p. 228° (decomp.) (after recrystallisation from water). When this diamide (20 mg.) was treated with a standard solution of sodium hypochlorite according to the directions previously used (Smith, J., 1939, 753), a Weerman degradation occurred with the formation of sodium *iso*cyanate, the presence of which was proved by the formation of hydrazodicarbonamide, m. p. 256°, when the solution was treated with sodium acetate and semicarbazide hydrochloride (Found: C, 41·0; H, 7·0; N, 11·7; OMe, 26·1. $C_8H_{16}O_6N_2$ requires C, 40·7; H, 6·8; N, 11·9; OMe, 26·3%).

Similarly when the lactone methyl ester of 2 : 3-dimethyl mucic acid was allowed to react with methylamine in methyl alcohol for 2 days at room temperature, the corresponding methyl-amide was produced, which crystallised on removal of the excess of the solvent. After recrystallisation from ethyl alcohol the *bismethylamide* of 2 : 3-dimethyl mucic acid had m. p. 184° alone or in admixture with a synthetic specimen; $[\alpha]_D^{17°}$ — 7·5° in water (*c*, 2·5) (Found: C, 45·2; H, 7·8; N, 10·4; OMe, 23·5. $C_{10}H_{20}O_6N_2$ requires C, 45·4; H, 7·65; N, 10·6; OMe, 23·5%).

Methylation of the Lactone Methyl Ester of 2 : 3-*Dimethyl Mucic Acid.*—The crystalline lactone methyl ester of 2 : 3-dimethyl mucic acid (75 mg.) was given one treatment with silver oxide and methyl iodide. The product was isolated by means of acetone and crystallised from ethyl alcohol–ether–light petroleum to give methyl 2 : 3 : 4 : 5-tetramethyl mucate, m. p. 109° (optically inactive) (Karrer and Peyer, *Helv. Chim. Acta*, 1922, **5**, 577; Smith, J., 1939, 1724) (Found: C, 49·1; H, 7·7; OMe, 62·4. Calc. for $C_{12}H_{22}O_8$: C, 49·0; H, 7·6; OMe, 63·3%). Purification of the crystalline material obtained from the mother-liquors by recrystallisation from ether gave the *γ-lactone methyl* ester of 2 : 3 : 5-*trimethyl mucic acid* (IX), m. p. 62°, $[\alpha]_D^{17°}$ — 83° in water (*c*, 1·3) (Found: OMe, 50·1. $C_{10}H_{16}O_7$ requires OMe, 50·0%).

Synthesis of the γ-Lactone Methyl Ester of 2 : 3-*Dimethyl Mucic Acid* (VIII).—4 : 6-Benzylidene α-methylgalactopyranoside (7 g.), prepared by the method of Robertson and Lamb (*loc. cit.*), was given three methylations with Purdie's reagents, the first one being carried out in the presence of acetone to dissolve the benzylidene α-methylgalactoside. After isolation by means of acetone, followed by recrystallisation from ether, the 4 : 6-benzylidene 2 : 3-dimethyl α-methylgalactoside had m. p. 125° (yield, 4·5 g.).

4 : 6-Benzylidene 2 : 3-dimethyl α-methylgalactoside (4·1 g.) was hydrolysed by heating with N-sulphuric acid (50 c.c.) for 8 hours on the boiling water-bath. The solution was concentrated to half its volume under diminished pressure to remove benzaldehyde and then freed from benzoic acid by filtration and extraction of the filtrate with ether. After neutralisation of the filtrate with barium carbonate, followed by removal of the solvent under reduced pressure, the 2 : 3-dimethyl galactose was obtained as a syrup, $[\alpha]_D^{17°}$ *ca.* — 2° in water (*c*, 2·0) (Found: OMe, 30·4. Calc. for $C_8H_{16}O_6$: OMe, 29·8%).

A solution of 2 : 3-dimethyl galactose (1·5 g.) in nitric acid (10 c.c., *d* 1·42) was heated for ½ hour at 55—60° and for 2 hours at 75°. The solution was diluted with water and freed from nitric acid as in previous instances. The dry acidic residue was boiled for 10 hours with 1% methyl-alcoholic hydrogen chloride (100 c.c.). The solution was cooled, neutralised with silver carbonate, filtered, and evaporated to a syrup, which distilled, giving a colourless liquid (1·2 g.),

b. p. (bath temp.) 150°/0·03 mm., $n_D^{20°}$ 1·4610. This distillate crystallised spontaneously and after recrystallisation from ethyl acetate–ether–light petroleum the *lactone methyl* ester of 2 : 3-*dimethyl mucic acid* had m. p. 91°; $[\alpha]_D^{18°}$ — 56° (initial value in water, *c*, 0·8); — 44·5° (after 2 days); — 37° (4 days); — 15·5° (13 days); — 6° (36 days, const. value) (Found : C, 46·4; H, 6·0; OMe, 39·2. $C_9H_{14}O_7$ requires C, 46·15; H, 6·0; OMe, 39·8%).

When this synthetic γ-lactone methyl ester of 2 : 3-dimethyl mucic acid was treated with methyl-alcoholic ammonia, it gave the corresponding diamide (X) of 2 : 3-dimethyl mucic acid, m. p. 226° (decomp.) (after recrystallisation from water) (Found : C, 40·4; H, 7·1; N, 11·8; OMe, 27·0. Calc. for $C_8H_{16}O_6N_2$: C, 40·7; H, 6·8; N, 11·9; OMe, 26·3%).

Treatment of this synthetic lactone methyl ester with methyl-alcoholic methylamine for 2 days at room temperature gave the crystalline bismethylamide of 2 : 3-dimethyl mucic acid in good yield, m. p. 184°, $[\alpha]_D^{18°}$ — 7·5° in water (*c*, 2·1) (after recrystallisation from ethyl alcohol) (Found : C, 45·45; H, 7·8; N, 10·5; OMe, 22·9. Calc. for $C_{10}H_{20}O_6N_2$: C, 45·4; H, 7·65; N, 10·6; OMe, 23·5%).

Methyl 2 : 3 : 4 : 5-*Tetramethyl Mucate*.—A solution of the *dl*-γ-lactone methyl ester of trimethyl mucic acid (prepared by the methylation of the monolactone of mucic acid with Purdie's reagents) (1 g.) in water (5 c.c.) was treated with methyl sulphate (10 c.c.) and sodium hydroxide (30 c.c. of a 30% solution) at 40°. The reagents were added simultaneously during 2 hours. The mixture was then heated at 80° for ½ hour, cooled, and neutralised by the addition of dilute sulphuric acid. After removal of the sodium sulphate by filtration, the solution was treated with N-sulphuric acid (8·0 c.c.) in order to liberate the tetramethyl mucic acid, and evaporated to dryness under diminished pressure. Extraction of the residue with ethyl alcohol gave syrupy 2 : 3 : 4 : 5-tetramethyl mucic acid, which was esterified by boiling for 8 hours with 1% methyl-alcoholic hydrogen chloride (50 c.c.). After neutralisation with silver carbonate, the solution was filtered and freed from solvent, giving methyl 2 : 3 : 4 : 5-tetramethyl mucate, m. p. 109° (after recrystallisation from ether). The crystals were optically inactive (Found : C, 49·2; H, 7·6; OMe, 62·7. Calc. for $C_{12}H_{22}O_8$: C, 49·0; H, 7·5; OMe, 63·3%).

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