

353. *The Constitution of Arabic Acid. Part II. Degraded Arabic Acid.*

By F. SMITH.

Degraded arabic acid obtained from arabic acid by autohydrolysis or by hydrolysis with 0.01N-sulphuric acid is regarded as the basic nucleus of arabic acid to which the labile sugar residues are attached by glycosidic links. It possesses a free reducing group and the repeating unit consists of 9 residues of galactose and 3 residues of glucuronic acid. Hydrolysis of the methyl ester of methylated degraded arabic acid furnishes 2 : 3 : 4 : 6-tetramethyl galactose (1 molecular proportion), 2 : 3 : 4-trimethyl galactose (5 molecular proportions), 2 : 4-dimethyl galactose (3 molecular proportions), and 2 : 3 : 4-trimethyl glucuronic acid (3 molecular proportions) the structures of which are proved. The identification of these methylated derivatives shows that 1 : 6- and 1 : 3-glycosidic links are present in degraded arabic acid and that the sugar units, all of which have pyranose rings, are joined in a branched chain type of structure probably having four terminal or "end" residues.

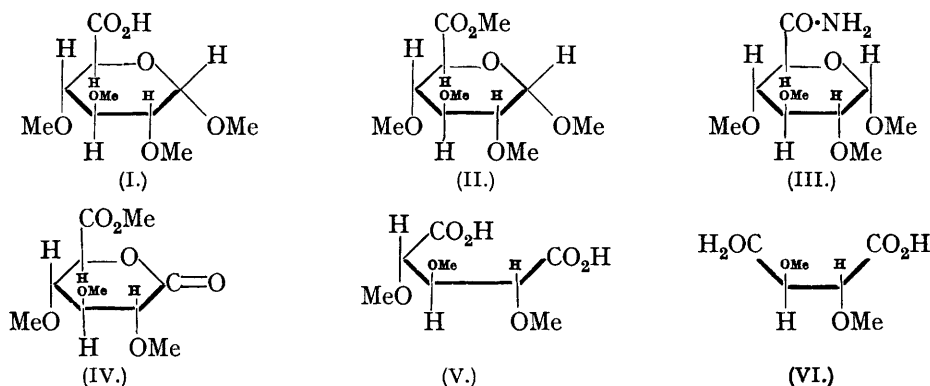
AUTOHYDROLYSIS of arabic acid or hydrolysis with 0.01N-sulphuric acid leads to the formation of a mixture of sugars (*l*-arabinose, *l*-rhamnose, 3-*d*-galactosido-*l*-arabinose) and degraded arabic acid in about equal proportions (Part I, this vol., p. 744). Degraded arabic acid is relatively stable to further hydrolytic cleavage, and it seems that it represents the basic nucleus of arabic acid, to which are attached side chains which may consist of one or more labile sugar residues. An essential preliminary step towards the elucidation of the structure of arabic acid thus lies in the determination of the constitution of the basal unit, degraded arabic acid.

The barium salt of degraded arabic acid, obtained by the method outlined in the previous paper, has a barium content which corresponds to an equivalent weight of 670, and the free acid prepared from the barium salt by means of dilute sulphuric acid has an equivalent of 660 (determined by titration). Since degraded arabic acid contains only galactose and glucuronic acid residues (see below), it will be seen that the above figures indicate the presence of a repeating unit composed of one glucuronic acid and three galactose units. Both the barium salt and the free degraded arabic acid reduce Fehling's solution and it would seem that this portion of the arabic acid molecule is of finite size and relatively small in comparison with the parent polysaccharide acid, arabic acid, and with highly complex polysaccharide molecules such as starch or cellulose. The comparatively small molecular size of the degraded arabic acid is borne out by the osmotic pressure results, which give a molecular weight of approximately 4800 for methylated degraded arabic acid. It is, however, worthy of note that the iodine number of degraded arabic acid corresponds to a

molecular weight of approximately 2000 and this figure is in agreement with the size of the repeating unit as determined chemically by the method outlined below.

In order to ascertain the nature and mode of linking of the residues obtaining in degraded arabic acid, the latter was subjected to methylation with methyl sulphate and sodium hydroxide solution, and a well-defined methylated derivative (equiv. 830) which still contained free carboxyl groups was obtained. Esterification and completion of the methylation with Purdie's reagents gave a fully methylated degraded arabic acid which appeared to be essentially homogeneous, since fractional precipitation failed to disclose the presence of degraded material.

The methyl ester of methylated degraded arabic acid undergoes hydrolysis with boiling methyl-alcoholic hydrogen chloride, a procedure which also transforms the methylated galactoses and the methylated glucuronic ester into the corresponding glycosides. This mixture is then treated with barium hydroxide in order to convert the methyl ester of methylated glucuronic acid into a barium salt, and from the dry mixture of the barium salt and the methylated galactosides the latter are extracted with ether, the barium salt of a trimethyl methylglucuronoside remaining insoluble. The mixture of the tetramethyl methylgalactoside, trimethyl methylgalactoside, and dimethyl methylgalactoside so obtained is resolved into its constituents by fractional distillation. This separation is simplified by the fact that the dimethyl methylgalactoside crystallises in both α - and β -modifications. The trimethyl methylglucuronoside was identified as 2 : 3 : 4-trimethyl methylglucuronoside, the tetramethyl methylgalactoside as 2 : 3 : 4 : 6-tetramethyl methylgalactoside, the trimethyl methylgalactoside as 2 : 3 : 4-trimethyl methylgalactoside, and the dimethyl methylgalactosides as 2 : 4-dimethyl methylgalactoside, by the series of investigations described below.



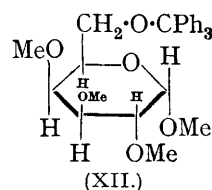
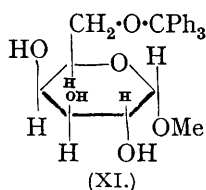
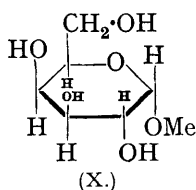
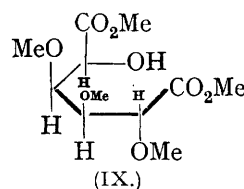
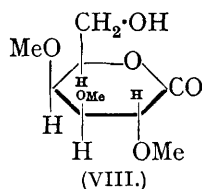
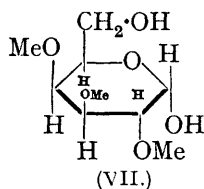
The structure of the trimethyl methylglucuronoside (I) is deduced from the following considerations. The methyl ester of trimethyl methylglucuronoside (II), which can be obtained from both the acid (I) and the barium salt of (I) by boiling with methyl-alcoholic hydrogen chloride, readily forms a crystalline *amide* (III) the rotation of which (+ 138°) indicates that it is derived from the α -glucuronoside. This view is supported by the fact that the methyl ester of 2 : 3 : 4-trimethyl β -methylglucuronoside yields an *amide* with a negative rotation (− 47°). When 2 : 3 : 4-trimethyl β -methylglucuronoside is esterified by boiling with methyl-alcoholic hydrogen chloride, the resulting methyl ester (a mixture of the α - and the β -form of 2 : 3 : 4-trimethyl methylglucuronoside) furnishes the amide of the α -form identical with (III) (compare Hirst and Jones, J., 1938, 1174). The glycosidic methoxyl group of (I) or of its methyl ester (II) is eliminated, although with some difficulty, by the agency of dilute mineral acid, and the corresponding 2 : 3 : 4-trimethyl glucuronic acid is produced; the stability of the uronide methyl residue suggests a pyranoside structure for (I). When the trimethyl glucuronic acid is oxidised with bromine, 2 : 3 : 4-trimethyl saccharolactone is produced and this on esterification and subsequent distillation gives the characteristic crystalline 2 : 3 : 4-trimethyl saccharolactone methyl ester (IV). The latter proved to be identical in every respect with a specimen prepared by the nitric acid oxidation

1726 *Smith: The Constitution of Arabic Acid. Part II.*

of 2 : 3 : 4-trimethyl β -1 : 6-anhydroglucose, followed by esterification and subsequent distillation. The same compound has also been obtained by Charlton, Haworth, and Herbert (J., 1931, 2855), Robertson and Waters (*ibid.*, p. 1709), and by Hirst and Jones (*loc. cit.*). Oxidation of the methyl ester of 2 : 3 : 4-trimethyl methylglucuronoside (II) with nitric acid furnishes additional evidence of its structure, since *i*-xylotrimethoxyglutaric acid (V) and *l* (+)-threodimethoxysuccinic acid (*d*-dimethoxysuccinic acid) (VI) are obtained in addition to 2 : 3 : 4-trimethyl saccharolactone methyl ester (IV). The compounds (V) and (VI) are identified by the isolation of their characteristic crystalline amides.

The tetramethyl methylgalactoside is identified as 2 : 3 : 4 : 6-tetramethyl methylgalactoside by converting it into the corresponding reducing sugar, which furnishes the highly characteristic 2 : 3 : 4 : 6-tetramethyl galactose anilide on treatment with ethylalcoholic aniline. This was identified by its melting point and comparison with an authentic specimen (Haworth and Leitch, J., 1918, **113**, 197).

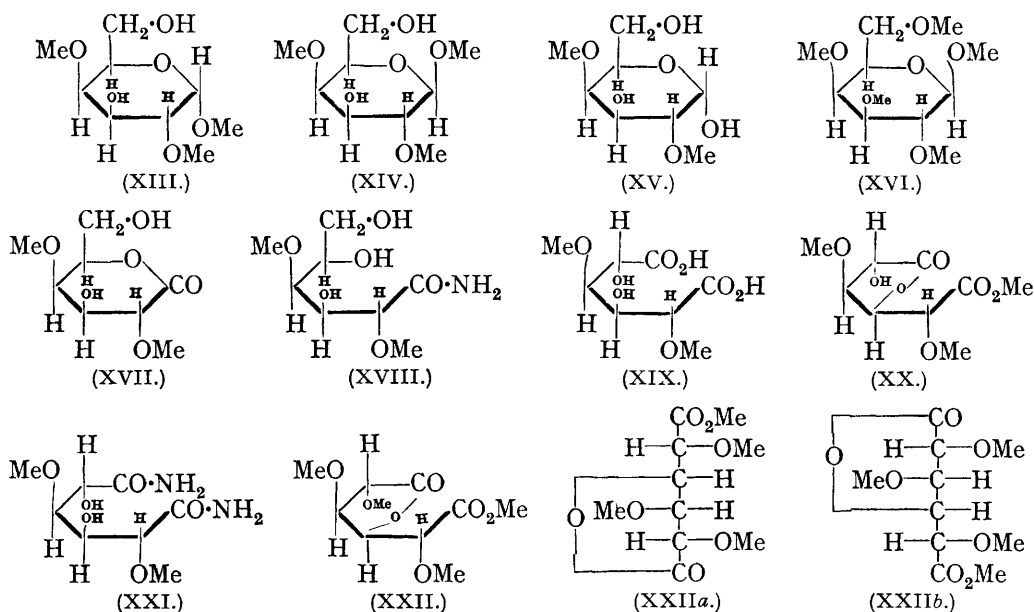
Hydrolysis of the trimethyl methylgalactoside yields a trimethyl galactose (VII), which can be readily isolated in the form of a crystalline monohydrate; it is easier, however, to identify this trimethyl galactose in the form of its crystalline anilide (McCreath and Smith, this vol., p. 387). Oxidation (VII) with bromine gives a trimethyl galactonolactone (VIII), an aqueous solution of which exhibits relatively rapid mutarotation, thus indicating that it belongs to the δ -series of lactones. A free hydroxyl group would therefore appear to be located in position 5 and since the conditions in the lactone preparation are favourable for the formation of γ -lactones it seems reasonable to conclude that a methoxyl residue is substituted for the hydroxyl in position 4. The lactone forms a crystalline *amide*, and also a crystalline phenylhydrazide which gives no depression of the melting point when in admixture with authentic 2 : 3 : 4-trimethyl galactonic acid phenylhydrazide (McCreath and Smith, *loc. cit.*). Further oxidation of (VIII) introduces a carboxyl group in position 6



without loss of a methoxyl group with the formation of 2 : 3 : 4-trimethyl mucic acid, which was identified as the crystalline dimethyl ester (IX) (Haworth, Hirst, and Challinor, J., 1931, 258). This 2 : 3 : 4-trimethyl mucic acid can also be further characterised by forming its crystalline *diamide* and *bismethylamide*. It is clear, therefore, that the methyl groups in (VII) can only be in the positions 2, 3, and 4. This conclusion was further verified in the following way. When α -methylgalactopyranoside (X) in pyridine solution is allowed to react with trityl chloride, 6-trityl α -methylgalactopyranoside (XI) is produced and this on methylation with methyl sulphate and sodium hydroxide yields 6-trityl 2 : 3 : 4-trimethyl α -methylgalactopyranoside (XII). Treatment of the latter with an ethereal solution of hydrogen chloride gives 2 : 3 : 4-trimethyl α -methylgalactopyranoside (cf. Haworth, Hirst, Smith, and Wilson, J., 1937, 829), and when this is hydrolysed by heating with dilute sulphuric acid, 2 : 3 : 4-trimethyl galactose is obtained. This synthetic 2 : 3 : 4-trimethyl galactose yields a crystalline monohydrate and a crystalline anilide identical with those produced from (VII).

The position of the methyl residues in the two crystalline forms of the dimethyl methylgalactoside is ascertained in the following way. Both the α -form (XIII) and the β -form

(XIV) yield on hydrolysis with dilute sulphuric acid the same dimethyl galactose (XV), and hence (XIII) and (XIV) differ only in the stereochemical arrangement of the groups at C₁. The dimethyl galactose crystallises as a *monohydrate* and the anhydrous dimethyl galactose readily yields a crystalline *anilide*. Complete methylation of the *dimethyl β-methylgalactoside* (XIV) with Purdie's reagents furnishes 2 : 3 : 4 : 6-tetramethyl β-methylgalactoside (XVI), showing that (XIII) and (XIV) are derivatives of galactose and that substitution of methoxyl at C₅ in (XIII) and (XIV) is excluded. The two methyl residues may therefore be attached to any two of the four positions C₂, C₃, C₄, and C₆. Now when the dimethyl galactose (XV) is treated with phenylhydrazine and acetic acid, an *osazone* is produced with the loss of one methoxyl group. This methoxyl group, thus eliminated, with the formation of 4-methyl galactose phenylosazone, must be in position 2. The formation of an osazone in an analogous manner with the elimination of a methoxyl group on C₂ has been observed several times in the galactose series (see Robertson and Lamb, J., 1934, 1321; Percival and Ritchie, J., 1936, 1765; Percival and Somerville, J., 1937, 1615; Oldham and Bell, J. Amer. Chem. Soc., 1938, 60, 323; McCreath and Smith, *loc. cit.*). When the anhydrous dimethyl galactose (XV) is treated with cold 1% methyl-alcoholic hydrogen chloride, the rotation slowly increases to a maximum and simultaneously the *dimethyl α-methylgalactoside*



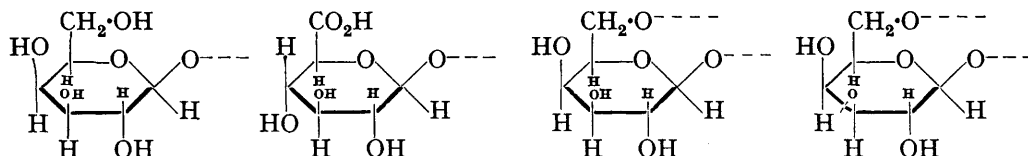
(XIII) is produced. Since the conditions employed are favourable for furanose formation, which usually takes place at a rapid rate and, in the case of galactose, would probably be accompanied by a fall and not a rise in rotation (see Haworth, Ruell, and Westgarth, J., 1924, 125, 2468), it would appear that the galactoside produced possesses a pyranose ring and hence a methyl residue is probably substituted in position 4. From these results it was deduced that the two methyl residues occupy positions 2 and 4. Confirmation of the structure of the sugar as being 2 : 4-dimethyl galactose is forthcoming from the following experiments. Oxidation of the dimethyl galactose (XV) with bromine gives the corresponding crystalline *lactone* (XVII), which is characterised by the formation of a crystalline *phenylhydrazide* and a crystalline *amide* (XVIII). The lactone (XVII) mutarotates relatively quickly in aqueous solution, showing that it is of the δ-type and consequently suggests the presence of a free hydroxyl group in position 5, and furthermore the absence of γ-lactone ring formation also supports the view that a methoxyl residue is substituted for the hydroxyl group on C₄. The 2 : 4-dimethyl galactonamide (XVIII) prepared from the lactone by means of alcoholic ammonia gives a negative Weerman test for α-hydroxy-amides (*Rec. Trav. chim.*, 1917, 36, 16), showing that a methyl residue is also substituted for the hydroxyl

group on C_2 . Now when the 2 : 4-dimethyl galactose (XV), either of the two galactosides (XIII) and (XIV), or the lactone (XVII) is treated with nitric acid, oxidation takes place without loss of any methoxyl groups and 2 : 4-dimethyl mucic acid (XIX) is formed. Esterification and subsequent distillation of the last furnishes the crystalline *methyl ester lactone* of 2 : 4-dimethyl mucic acid, the slow mutarotation of which in aqueous solution suggests that it contains a 3 : 6- or γ -lactone ring as shown in (XX); the latter formulation is proved by the fact that methylation of this methyl ester lactone of 2 : 4-dimethyl mucic acid with Purdie's reagents gives a *methyl ester lactone* of the trimethyl mucic acid (XXII or XXIIa) which proved to be the enantiomorph of the methyl ester lactone of 2 : 3 : 5-trimethyl mucic acid (XXIIb) (Smith, *Chem. and Ind.*, 1939, 58, 363). The ether methyl groups in (XXII) must therefore occupy positions 2, 4, and 5, hence the lactone ring in (XX) engages C_6 and C_3 . Further evidence of a free hydroxyl group on C_5 in the dimethyl galactose is forthcoming from the fact that the 2 : 4-dimethyl mucic acid diamide (XXI) obtained from (XX) by means of methyl-alcoholic ammonia shows a positive Weerman test for α -hydroxy-amides. The foregoing facts prove that the formula (XV) assigned to the 2 : 4-dimethyl galactose isolated is correct, and it is of interest that this is identical with the dimethyl galactose obtained by Baldwin and Bell from a methylated galactosan (J., 1938, 1465) (see also Schlubach and Loop, *Annalen*, 1937, 532, 228).

The identification of the four hydrolytic products derived from methylated degraded arabic acid, namely, 2 : 3 : 4 : 6-tetramethyl galactose, 2 : 3 : 4-trimethyl galactose, 2 : 4-dimethyl galactose, and 2 : 3 : 4-trimethyl glucuronic acid, clearly shows that the degraded polysaccharide acid is composed of only galactose and glucuronic acid residues all of which must have the pyranose structure. Since the whole of the glucuronic acid and a portion of the galactose were isolated in the form of completely methylated derivatives, these must exist in the complex as terminating or "end" residues. Therefore the molecule of degraded arabic acid must be of the branched chain type (Smith, *Chem. and Ind.*, 1939, 58, 203).

The relative proportions of the hydrolytic products from two experiments are approximately 1 of 2 : 3 : 4 : 6-tetramethyl galactose, 3 of 2 : 4-dimethyl galactose, 3 of 2 : 3 : 4-trimethyl glucuronic acid, and 5 of 2 : 3 : 4-trimethyl galactose. A structure composed of these 12 units joined by glycosidic links and having its single free reducing group protected with a methyl residue would have a molecular weight of 2494 and an equivalent of 831 (Found : 830 by titration) and the methyl ester would have a methoxyl content of 46.4 (Found : 45.2%). The unmethylated degraded arabic acid calculated on this basis would have a molecular weight of 2003 (Found : 2000 by iodine number) and an equivalent weight of 663 (Found : 660 by direct titration and 670 by estimation of the barium content of its barium salt).

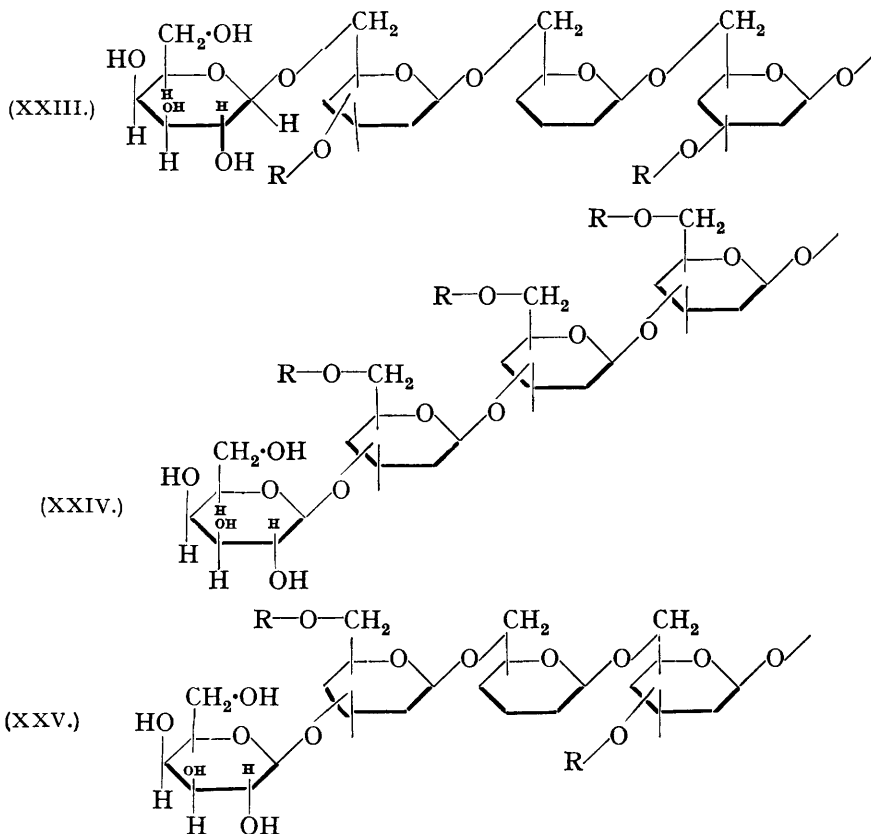
The degraded arabic acid reduces Fehling's solution and on the above data the minimum molecular size would consist of 12 units, 9 being *d*-galactose and 3 being *d*-glucuronic acid. The mode of attachment of these units is indicated by the isolation of their various methylated derivatives. Thus in the methylated degraded arabic acid the 2 : 3 : 4 : 6-tetramethyl galactose and the 2 : 3 : 4-trimethyl glucuronic acid can respectively be linked only through these reducing groups C_1 , the 2 : 3 : 4-trimethyl galactose through positions C_6 and C_1 , and the 2 : 4-dimethyl galactose may be joined to certain parts of the chain through positions C_1 , C_3 , and C_6 (see below).



The isolation of 1 molecular proportion of 2 : 3 : 4 : 6-tetramethyl galactose and 3 of 2 : 3 : 4-trimethyl glucuronic acid shows clearly that there must be four "ends" in the structure of the repeating unit of 12 sugar residues. The constitution can therefore be represented by a terminated chain of galactose residues to which are attached three side

chains each terminated by a glucuronic acid unit; the side chains may be attached to any but the terminal galactose unit which furnishes the 2 : 3 : 4 : 6-tetramethyl galactose, the point of linking being indicated by the isolation of 2 : 4-dimethyl galactose (see formulæ below). This view is supported by the isolation of equal amounts of 2 : 3 : 4-trimethyl glucuronic acid and 2 : 4-dimethyl galactose.

The evidence suggests that the galactose residues in the main chain are mutually joined by 1 : 6-glycosidic links as in (XXIII) or by 1 : 3-links as in (XXIV) or even by 1 : 6- and 1 : 3-links, one example of which is shown in (XXV). In the same way it follows that the side chains R each containing a terminal glucuronic acid residue may be attached by 1 : 3-links as in (XXIII) or by 1 : 6-links as in (XXIV) and at this stage the possibility that they are joined by 1 : 3- and 1 : 6-links as in (XXV) cannot be excluded.



Examination of the above formulæ (XXIII, XXIV, and XXV) shows that the 2 : 4-dimethyl galactose will result from those galactose units in the main chain to which the side chains R are attached. The side chains R may be constituted by single units of glucuronic acid or there may be one or more units of galactose interposed between the terminal glucuronic acid residue and the main galactose chain. From the fact that the trimethyl galactose, obtained from methylated degraded arabic acid, was identified as the 2 : 3 : 4-trimethyl derivative, it follows that any galactose residues in the side chains R must be joined by 1 : 6-links. In the case of formula (XXIV) the 2 : 3 : 4-trimethyl galactose can only arise from galactose units in the side chains R which are joined by 1 : 6-links; it cannot arise from galactose units in the main chain because they are all joined by 1 : 3-links. In formulæ (XXIV) and (XXV), however, the 2 : 3 : 4-trimethyl galactose may be produced from galactose units in the side chains R or from those in the main galactose chain which are joined by 1 : 6-links.

The three formulæ (XXIII, XXIV, and XXV) give expression to the experimental

1730 *Smith : The Constitution of Arabic Acid. Part II.*

results obtained in this work and they represent in a general way the type of structure which may be present in degraded arabic acid.

EXPERIMENTAL.

Degraded Arabic Acid.—The barium salt of degraded arabic acid prepared from arabic acid by hydrolysis with 0.01N-sulphuric acid was identical with that obtained by autohydrolysis (see Smith, this vol., p. 744). It was a white powder and had $[\alpha]_D^{20} = 9^\circ$ in water (*c*, 1.0); equiv., 670 (determined from the barium content). The free degraded arabic acid, liberated from the barium salt in aqueous solution by a slight excess of dilute sulphuric acid, was after filtration obtained as a colourless precipitate by pouring the filtrate into vigorously stirred alcohol. Purification by reprecipitation from an aqueous solution with alcohol gave a powder which had $[\alpha]_D^{20} = 9^\circ$ in water (*c*, 1.0); equiv., 660 (determined by titration with 0.1N-sodium hydroxide); iodine number, *ca.* 10.0. Both degraded arabic acid and its barium salt reduced Fehling's solution on boiling. Fractional precipitation of the free acid and the barium salt from an aqueous solution by means of alcohol showed that both were essentially homogeneous.

Methylation of Degraded Arabic Acid.—The barium salt of degraded arabic acid (15 g.) in water (50 c.c.) was methylated with methyl sulphate (200 c.c.) and sodium hydroxide (450 c.c. of a 30% aqueous solution) in the presence of a little acetone. The reagents were added in tenths during 3 hours at 20° . After 12 hours the methylation was completed by heating for 15 minutes at 80° . No insoluble methylated material separated and since trial experiments had shown that only a small proportion could be extracted with chloroform from an acidified aqueous solution, the pale yellow solution containing the sodium salt of partly methylated degraded arabic acid was cooled to 0° and almost neutralised with 5N-sulphuric acid. The sodium sulphate was filtered off, and the slightly alkaline aqueous solution concentrated under diminished pressure almost to dryness. The product contained in this residue was subjected to further methylation under the same conditions as above. After three methylations the solution containing sodium sulphate and the partly methylated degraded arabic acid was acidified with 5N-sulphuric acid and then extracted several times with chloroform. The chloroform extracts were combined, washed twice with an aqueous solution of sodium sulphate to remove mineral acid, dried over anhydrous magnesium sulphate, filtered, and evaporated to dryness, giving a pale yellow glassy solid. In this manner 90 g. of the barium salt of degraded arabic acid yielded 82 g. of the partly methylated compound. Its aqueous solution was acid to litmus and it did not reduce Fehling's solution. It was soluble in alcohol and acetone but insoluble in ether and light petroleum (Found : OMe, 35.0%).

The partly methylated material was divided into four portions, and each portion was methylated in the presence of acetone, the same quantities of reagents as employed above being used, at 30 – 35° for 8 hours, acetone being added from time to time to replace that lost by evaporation in order to keep the methyl derivative in solution. After each methylation the solution was cooled and acidified with sulphuric acid, and the product extracted with chloroform. Five methylations under these conditions gave a product (58 g.) which had $[\alpha]_D^{20} = 54.4^\circ$ in chloroform (*c*, 1.1); equiv., 817 (determined by titration of an aqueous alcoholic solution of the substance with sodium hydroxide) (Found : OMe, 39.0%). After nine methylations the material (47 g.) appeared to be completely methylated. The methylated degraded arabic acid, a pale yellow solid, had $[\alpha]_D^{20} = 54.0^\circ$ in chloroform (*c*, 1.4); equiv., 825 (Found : OMe, 39.5%).

Fractionation of the Methylated Degraded Arabic Acid.—The glassy solid was dissolved in chloroform, and fractional precipitation effected by gradual addition of light petroleum with stirring. Three fractions were obtained having almost identical properties, $[\alpha]_D^{20} = 55^\circ$ in chloroform (*c*, 1.0); equiv., 830 (Found : OMe, 39.5%).

Methylation with Silver Oxide and Methyl Iodide.—When a solution of methylated degraded arabic acid (27 g.) in methyl iodide (40 c.c.) was treated with silver oxide, esterification proceeded smoothly without the application of external heat. The methylation was completed by boiling under reflux for 6 hours. The solution was filtered, and the residue washed well with hot acetone. The filtrate and washings were combined and evaporated to dryness. After three such treatments the product (26 g.) had $[\alpha]_D^{20} = 54^\circ$ in chloroform (*c*, 1.2) (Found : OMe, 44.6%).

Fractionation of the Methyl Ester of Methylated Degraded Arabic Acid.—The methyl ester of methylated degraded arabic acid (26 g.) was dissolved in chloroform (80 c.c.) and precipitated in three fractions by the addition of light petroleum slowly with stirring : Fraction (I) (16.2 g.), $[\alpha]_D^{20} = 58.8^\circ$ in chloroform (*c*, 1.0), η_{sp}^{20} 0.117 in *m*-cresol (*c*, 1.0) (Found : OMe, 44.8%); fraction (II) (7 g.), $[\alpha]_D^{20} = 58.2^\circ$ in chloroform (*c*, 1.0), η_{sp}^{20} 0.118 in *m*-cresol (*c*, 1.0) (Found : C, 51.8; H, 7.4; OMe, 45.0%); fraction (III) (2 g.), $[\alpha]_D^{20} = 46.4^\circ$ in chloroform (*c*, 1.0) (Found :

OMe, 43.0%). The last fraction, being incompletely methylated, was rejected. Since the "shape-factor" will obviously play an important part in the viscosity of solutions of this branched chain molecule, it is evident that the above figures for the specific viscosity cannot be used to calculate the molecular weight of the methyl ester of methylated degraded arabic acid.

Hydrolysis of the Methyl Ester of Methylated Degraded Arabic Acid.—Fractions I and II were combined and the material (22 g.) was boiled for 10 hours with 6.5% methyl-alcoholic hydrogen chloride (600 c.c.); the specific rotation had then become constant ($[\alpha]_D^{20}$ ca. +103°). The solution was neutralised with silver carbonate, filtered, and evaporated at 40° under diminished pressure to give a non-reducing syrup (25 g. approx.). This syrup was dissolved in water (200 c.c.) and heated with 0.3N-barium hydroxide (200 c.c.) for 2 hours at 55°. The solution was neutralised with carbon dioxide and filtered, and the residue washed several times with hot water. The filtrate and washings were combined and concentrated to a volume of 200 c.c. under diminished pressure at 40°. The treatment with 0.3N-barium hydroxide (200 c.c.) was repeated to ensure complete saponification of the ester. The solution was again neutralised with carbon dioxide, filtered, and evaporated to dryness under reduced pressure and the syrupy product, from which all traces of solvent were removed by heating in a vacuum at 60° for 2 hours, was exhaustively extracted with alcohol-free dry ether. The barium salt (B) was filtered off, washed with ether, and dried (yield, 7.5 g.). The ethereal extracts and washings were combined and freed from solvent, giving a syrup (A).

Examination of the syrupy mixture of galactosides (A). This syrup (A) slowly deposited crystals, and these, after keeping for several days, were triturated with ether to remove adhering syrup, filtered off, and washed with ether (yield, 0.8 g.). Crystallisation from acetone-ether gave 2 : 4-dimethyl β -methylgalactopyranoside (XIV), m. p. 165–166°. The ethereal solution and washings were combined and evaporated under diminished pressure; the syrup thus produced was subjected to fractional distillation, giving :

Fraction.	Weight (g.).	B. p. (bath temp.).	n_D^{19} .	$[\alpha]_D$ in water.	OMe, %.
I	3.16	120°/0.07 mm.	1.4540	+112.9°	56.2
II	4.64	120—130/0.04 mm.	1.4650	135.6	51.0
III	1.7	130—135/0.04 mm.	1.4670	139.5	49.6
IV	2.44	135—140/0.04 mm.	1.4730	140.5	44.3
V	1.42	140—160/0.04 mm.	1.4740	139.0	42.9

The undistilled residue (ca. 2 g.) was rehydrolysed by boiling with 5% methyl-alcoholic hydrogen chloride (150 c.c.) for 8 hours. The syrupy product isolated as above gave on distillation :

Fraction.	Weight (g.).	B. p. (bath temp.).	n_D^{19} .	$[\alpha]_D$ in water.	OMe, %.
VI	1.02	120°/0.04 mm.	1.4500	+90.8°	55.6
VII	0.98	140—170/0.04 mm.	1.4730	112.5	42.2

The undistillable dark residue (0.2 g.) was rejected.

The fractions IV, V, and VII, which crystallised spontaneously, were dissolved in a small quantity of hot acetone, and light petroleum added until a slight turbidity appeared. On keeping, 2 : 4-dimethyl α -methylgalactoside (XIII) separated in needles, m. p. 102° after crystallisation from the same mixture of solvents (yield, 3.09 g.). Removal of solvent from the mother-liquors gave a syrup (S), which was included in the following distillation.

Redistillation of fractions I, II, III, VI, and the syrup (S). Fractions I and VI were combined and distilled until the refractive index of the distillate reached that of the first drop which was collected in Fraction II; the latter was then added and distillation continued. Similarly, when the refractive index of the distillate rose to that of the first drop collected in Fraction III, this was also added and finally the syrup (S) was also subjected to redistillation. In this way the following fractions were obtained :

Fraction.	Weight (g.).	B. p. (bath temp.).	n_D^{20} .	OMe, %.
C	1.92	115—120°/0.02 mm.	1.4505	—
D	0.20	120—125°/0.02 mm.	1.4540	—
E	7.76	140—160°/0.02 mm.	1.4650	51.8
F	0.22	160—165°/0.02 mm.	1.4685	49.5
G	1.39	165—170°/0.02 mm.	1.4735	—

Fraction G yielded 2 : 4-dimethyl α -methylgalactoside (0.65 g.), m. p. 102°, and a syrup, 2 : 3 : 4-trimethyl methylgalactoside (0.74 g.), b. p. (bath temp.) 160°/0.02 mm., n_D^{20} 1.4675 (Found : OMe, 49.9%).

1732 *Smith: The Constitution of Arabic Acid. Part II.*

Fractions C and D were combined and redistilled, giving: fraction (i) (1.84 g.), 2:3:4:6-tetramethyl methylgalactoside, b. p. (bath temp.) $90^{\circ}/0.05$ mm., n_D^{20} 1.4500 (Found: OMe, 60.0%), and fraction (ii) (0.17 g.), 2:3:4-trimethyl methylgalactoside, b. p. (bath temp.) $140^{\circ}/0.05$ mm., n_D^{20} 1.4650 (Found: OMe, 51.7%).

Assuming that the refractive indices of mixtures of α - and β -forms of tetra-, tri-, and dimethyl methylgalactosides are n_D^{20} 1.4500, 1.4650, and 1.4750 respectively, it can be calculated that the approximate amounts of these present in the fractions after the final distillation are as follows: 2:3:4:6-tetramethyl methylgalactoside, 1.84 g.; 2:3:4-trimethyl methylgalactoside, 8.63 g.; 2:4-dimethyl methylgalactoside, 4.8 g. (including the crystalline α - and β -forms of 2:4-dimethyl methylgalactoside which had been separated). The acid portion of the molecule, consisting of the barium salt of 2:3:4-trimethyl methylglucuronoside, amounted to 7.5 g. In molecular proportions these yields correspond approximately to 2:3:4:6-tetramethyl methylgalactoside (1), 2:3:4-trimethyl methylgalactoside (5), 2:4-dimethyl methylgalactoside (3), and 2:3:4-trimethyl methylglucuronoside (3). The amount of 2:3:4:6-tetramethyl methylgalactoside obtained was 8.4% of the methyl ester of the methylated degraded arabic acid hydrolysed.

In another series of experiments (with J. Jackson), degraded arabic acid, prepared solely by autohydrolysis of arabic acid in aqueous solution, was methylated as described above. The methylated degraded arabic acid had $[\alpha]_D^{18} - 55^{\circ}$ in chloroform (*c.* 1.0), n_D^{20} 0.031 in *m*-cresol (*c.* 0.4) (Found: OMe, 45.5%). The molecular weight determined by osmotic pressure measurements (carried out by Dr. S. R. Carter and Dr. W. T. Chambers) was approximately 4800. The product (15.38 g.) gave on hydrolysis with 6% methyl-alcoholic hydrogen chloride (300 c.c.), 2:3:4-trimethyl methylglucuronoside, 3.95 g. (*ca.* 3 mol. prop.); 2:3:4:6-tetramethyl methylgalactoside, 1.28 g. (*ca.* 1 mol. prop.); 2:3:4-trimethyl methylgalactoside, 5.93 g. (*ca.* 5 mol. prop.); 2:4-dimethyl methylgalactoside, 3.84 g. (*ca.* 3 mol. prop.). The amounts of these substances were calculated from the refractive index of the fractions after three distillations. The tetramethyl methylgalactoside amounts to 8.3% of the methylated derivative hydrolysed.

Structure of the 2:3:4-Trimethyl Methylglucuronoside (I).—The ether-insoluble barium salt (B) was easily soluble in water and in methyl alcohol. It was neutral and non-reducing [Found: OMe, 37.1. ($C_{10}H_{17}O_7$)₂Ba requires OMe, 39.05%]. The free acid (I) was prepared from the barium salt (B) (1 g.) by dissolving it in water (20 c.c.) and adding a slight deficiency of 0.1N-sulphuric acid. The barium sulphate was filtered off, and the filtrate evaporated to dryness under diminished pressure. The organic acid was extracted with chloroform and after drying over anhydrous magnesium sulphate the chloroform solution gave on evaporation a syrup (0.75 g.), which was freed from solvent by heating for 2 hours at 70° under reduced pressure. The syrup dissolved in water, giving an acid solution which did not reduce Fehling's solution. It had $[\alpha]_D^{17} + 80^{\circ}$ in water (*c.* 0.9). When the acid was boiled with dilute hydrochloric acid for a few minutes, hydrolysis took place and the solution, which now contained 2:3:4-trimethyl glucuronic acid, readily reduced Fehling's solution (Found: OMe, 48.2; equiv., 270. Calc. for $C_{10}H_{16}O_7$: OMe, 49.6%; equiv., 250).

Methylation of the barium salt. There was no increase in methoxyl content when the barium salt (B) (1 g.) was methylated with sodium hydroxide (30 c.c. of a 30% solution) and methyl sulphate (10 c.c.) in the presence of acetone (12 c.c.) at 35° . The 2:3:4-trimethyl methylglucuronoside isolated from the methylation mixture had $[\alpha]_D^{20} + 79^{\circ}$ in water (*c.* 0.94; equiv., 275 (Found: OMe, 49.0%).

Methyl Ester of 2:3:4-Trimethyl Methylglucuronoside (II).—The free acid (0.7 g.), prepared from the barium salt by the action of sulphuric acid as described above, was boiled for 8 hours with 3% methyl-alcoholic hydrogen chloride (100 c.c.). The solution was neutralised with silver carbonate, filtered, and concentrated to a thin syrup, which was distilled, giving a colourless liquid (0.6 g.), b. p. (bath temp.) $120^{\circ}/0.02$ mm., n_D^{19} 1.4470, $[\alpha]_D^{18} + 87^{\circ}$ in water (*c.* 1.0). It was neutral and did not reduce Fehling's solution. This methyl ester was also prepared directly from the barium salt by boiling the latter with methyl-alcoholic hydrogen chloride and was isolated in the same way (Found: C, 50.0; H, 7.7; OMe, 58.0; CO_2Me , 22.0. Calc. for $C_{11}H_{20}O_7$: C, 50.0; H, 7.65; OMe, 58.7; CO_2Me , 22.35%). When treated with methyl-alcoholic ammonia, the methyl ester (285 mg.) gave the amide of 2:3:4-trimethyl α -methylglucuronoside (250 mg.) (III), which separated well from ethyl alcohol-ether; m. p. 183° , $[\alpha]_D^{20} + 137.5^{\circ}$ in water (*c.* 0.7) (Found: C, 48.4; H, 7.8; OMe, 50.2; N, 5.7. $C_{10}H_{16}O_6N$ requires C, 48.2; H, 7.7; OMe, 49.8; N, 5.6%).

Methylation of Glucuronic Acid.—Glucuronolactone (1.0 g.) was methylated according to the

directions given by Challinor, Haworth, and Hirst (*loc. cit.*) and the 2 : 3 : 4-trimethyl methylglucuronoside (0.7 g.) was esterified by boiling for 6 hours with 3% methyl-alcoholic hydrogen chloride (100 c.c.). The methyl ester distilled as a colourless liquid (0.6 g.), b. p. (bath temp.) $125^{\circ}/0.2$ mm., n_D^{20} 1.4467, $[\alpha]_D^{20} + 81^{\circ}$ in water (c , 0.8). It was neutral to litmus and did not reduce Fehling's solution (Found : OMe, 58.2; CO₂Me, 21.8. Calc. for C₁₁H₂₀O₇ : OMe, 58.7; CO₂Me, 22.35%). On treatment with methyl-alcoholic ammonia at 0° for 24 hours the ester yielded the amide of 2 : 3 : 4-trimethyl α -methylglucuronoside (III), m. p. 183° (after recrystallisation from ethyl alcohol–light petroleum), $[\alpha]_D^{20} + 138^{\circ}$ in water (c , 0.6). It gave no depression of the m. p. when mixed with the amide of 2 : 3 : 4-trimethyl methylglucuronoside obtained by hydrolysis of the methyl ester of methylated degraded arabic acid (Found : C, 48.4; H, 8.0; OMe, 50.0; N, 5.8%).

When 2 : 3 : 4-trimethyl β -methylglucuronoside (m. p. 134°) was esterified by titration with ethereal diazomethane, there was produced, on removal of the excess of the solvent, a syrupy methyl ester which failed to crystallise. This methyl ester was then treated with methyl-alcoholic ammonia in the normal way and after 2 days the excess of solvent was removed, leaving a crystalline residue, which was recrystallised from acetone–ether. The amide of 2 : 3 : 4-trimethyl β -methylglucuronoside had m. p. 193° , $[\alpha]_D^{20} - 47^{\circ}$ in water (c , 1.2) (Found : N, 5.7. C₁₀H₁₈O₆N requires N, 5.6%).

2 : 3 : 4-Trimethyl Saccharolactone Methyl Ester (IV).—A solution of the methyl ester of 2 : 3 : 4-trimethyl methylglucuronoside (0.5 g.) in *N*-sulphuric acid (30 c.c.) was heated on the boiling water-bath for 20 hours. The solution, which then contained 2 : 3 : 4-trimethyl glucuronic acid, showed $[\alpha]_D^{18} + 45^{\circ}$ and reduced Fehling's solution. (The ester methoxyl as well as the glycosidic methoxyl is probably removed in this hydrolysis.) The solution was neutralised with barium carbonate, filtered, and evaporated to dryness under reduced pressure. Inorganic impurities were separated by extracting the barium salt of the methylated uronic acid with methyl alcohol. Removal of the solvent gave a pale yellow, glassy solid which reduced Fehling's solution and gave a positive test for barium. The barium salt was then dissolved in water (5 c.c.), and bromine (1 c.c.) added. The bromine oxidation was allowed to proceed at room temperature until a portion of the solution, when freed from bromine, no longer reduced Fehling's solution. Excess of the bromine was removed by aeration and the solution was neutralised with silver oxide. The silver bromide was filtered off, and the filtrate treated with hydrogen sulphide and again filtered to remove silver sulphide. The solution was then evaporated to dryness under diminished pressure, and the syrupy residue esterified by boiling for 8 hours with 1% methyl-alcoholic hydrogen chloride (100 c.c.). The solution was neutralised with silver carbonate, filtered, and evaporated to dryness, giving a syrup; this was distilled in a high vacuum, giving a colourless distillate, which crystallised immediately. After crystallisation from ethyl alcohol–ether–light petroleum the 2 : 3 : 4-trimethyl saccharolactone methyl ester (IV) had m. p. 107° alone or in admixture with authentic 2 : 3 : 4-trimethyl saccharolactone methyl ester prepared from 2 : 3 : 4-trimethyl β -1 : 6-anhydroglucose (see below). The 2 : 3 : 4-trimethyl saccharolactone methyl ester showed $[\alpha]_D^{24} + 32^{\circ}$ (equilibrium value in water); $[\alpha]_D^{21} + 102^{\circ}$ (initial value in methyl alcohol, c , 1.0), changing to $+ 52^{\circ}$ (equilibrium value) (Found : C, 48.5; H, 6.5; OMe, 49.9. Calc. for C₁₀H₁₆O₇ : C, 48.4; H, 6.5; OMe, 50.0%).

Oxidation of 2 : 3 : 4-Trimethyl Methylglucuronoside with Nitric Acid.—A solution of the methyl ester of 2 : 3 : 4-trimethyl methylglucuronoside (II) (2.5 g.) in nitric acid (35 c.c., d 1.42) was heated for $1\frac{1}{2}$ hours at 50° and for 3 hours at 95° . The solution was then diluted with water and freed from nitric acid by distillation under diminished pressure at 45° , water being added from time to time to facilitate this process. The final traces of nitric acid were removed by addition of methyl alcohol and evaporation to dryness. The non-reducing acid syrup was esterified by boiling for 6 hours with 1% methyl-alcoholic hydrogen chloride (250 c.c.). The solution was cooled, neutralised with silver carbonate, filtered, and evaporated to a thin syrup, which was distilled, giving : Fraction (i) [a mixture of methyl *l*(+)-threedimethoxysuccinate and methyl *i*-xylotrimethoxyglutarate] (1.5 g.), b. p. (bath temp.) $120^{\circ}/0.02$ mm., n_D^{18} 1.4392—1.4414, $[\alpha]_D^{20} + 32.4^{\circ}$ (in methyl alcohol, c , 1.5) (Found : OMe, 60.0. Calc. for C₈H₁₄O₆ : OMe, 60.2%. Calc. for C₁₀H₁₈O₇ : OMe, 62.0%). Fraction (ii) (0.5 g.), b. p. (bath temp.) $140^{\circ}/0.02$ mm., n_D^{20} 1.4457. Fraction (I) was treated with methyl-alcoholic ammonia and after 2 days at -5° the separation of crystalline material appeared to be complete. The supernatant liquor (M) was decanted and the crystals were washed with methyl alcohol, ether, and then dried. They were insoluble in ether and light petroleum, sparingly soluble in methyl and ethyl alcohol and slightly soluble in cold water, m. p. 295° (decomp.) (after recrystallisation from hot water) alone or in admixture with authentic *l*(+)-threedimethoxysuccinamide (*d*-dimethoxysuccin-

1734 *Smith : The Constitution of Arabic Acid. Part II.*

amide), and showed $[\alpha]_D^{18} + 95.2^\circ$ in water (*c*, 1.1) (Found : C, 41.0; H, 6.7; OMe, 35.6; N, 15.7. Calc. for $C_6H_{12}O_4N_2$: C, 40.9; H, 6.9; OMe, 35.2; N, 15.9%). Removal of the excess of the solvent from the mother-liquor (M) gave a crystalline mass, which was triturated with ether-ethyl alcohol to remove adhering syrup; after recrystallisation from alcohol-ether, the m. p. was 190° (decomp.) alone or in admixture with authentic *i*-xylotrimethoxyglutaramide. This amide was optically inactive (Found : C, 43.5; H, 7.3; OMe, 43.0; N, 12.6. Calc. for $C_8H_{16}O_5N_2$: C, 43.6; H, 7.35; OMe, 42.3; N, 12.7%).

Fraction (ii) deposited large crystals on keeping; these, after being washed with ether and light petroleum, dried, and twice crystallised from ether-light petroleum, had m. p. 107° alone or in admixture with authentic 2 : 3 : 4-trimethyl saccharolactone methyl ester (IV).

Oxidation of 2 : 3 : 4-Trimethyl β -1 : 6-Anhydroglucose with Nitric Acid.— β -1 : 6-Anhydroglucopyranose, prepared by the destructive distillation of starch (Pictet and Sarascin, *Helv. Chim. Acta*, 1918, 1, 87), was converted into the corresponding 2 : 3 : 4-trimethyl β -1 : 6-anhydroglucopyranose by the action of Purdie's reagents (Irvine and Oldham, J., 1921, 119, 1744). A solution of 2 : 3 : 4-trimethyl β -1 : 6-anhydroglucopyranose (3 g.) in nitric acid (20 c.c., *d* 1.42) was heated on the water-bath for $\frac{1}{2}$ hour at 60° and for 3 hours at 90° . The solution was then diluted with water and freed from nitric acid by distillation under diminished pressure; the removal of the nitric acid was facilitated by simultaneous addition and distillation of water, and when only a small amount of nitric acid remained, methyl alcohol was added and the solution was evaporated to dryness. Esterification of the dry syrupy acid product was effected by boiling it (for 8 hours) with 2% methyl-alcoholic hydrogen chloride (150 c.c.); the solution was cooled, neutralised with silver carbonate, filtered, and evaporated to dryness, giving a thin syrup, which was distilled : Fraction (I) [mainly methyl *l*(+)-threodimethoxysuccinate (methyl *d*-dimethoxysuccinate) and some *i*-xylotrimethoxyglutaric acid]; fraction (II), b. p. (bath temp.) $150^\circ/0.05$ mm., n_D^{20} 1.4520; this fraction crystallised spontaneously. The crystals of 2 : 3 : 4-trimethyl saccharolactone methyl ester were freed from adhering syrup by trituration with ether-light petroleum and then crystallised from ethyl alcohol-ether-light petroleum; m. p. 107° , $[\alpha]_D^{18} + 103^\circ$ (initial value in water; *c*, 1.0) (Found : OMe, 49.8. Calc. for $C_{10}H_{16}O_7$: OMe, 50.0%).

The Structure of the 2 : 3 : 4 : 6-Tetramethyl Methylgalactoside.—A solution of the colourless mobile syrup in *N*-sulphuric acid was heated on the boiling water-bath until the rotation became constant ($[\alpha]_D^{20}$ ca. $+100^\circ$). The solution was neutralised with barium carbonate, filtered, and evaporated under diminished pressure to a syrup, which was freed from a little inorganic impurity by extraction with alcohol. The syrup obtained on removal of the solvent distilled as an oil, b. p. (bath temp.) $150^\circ/0.5$ mm., which crystallised on keeping (yield, almost quantitative). After recrystallisation from ether-light petroleum the material had m. p. 75° alone or in admixture with authentic 2 : 3 : 4 : 6-tetramethyl α -galactopyranose (Found : C, 51.0; H, 8.2; OMe, 52.3. Calc. for $C_{10}H_{20}O_6$: C, 50.85; H, 8.6; OMe, 52.5%). On boiling in alcoholic solution with aniline (1 mol.) the characteristic 2 : 3 : 4 : 6-tetramethyl galactopyranose anilide was obtained, m. p. 192° alone or in admixture with an authentic specimen (Found : C, 61.5; H, 8.3; OMe, 40.5; N, 4.7. Calc. for $C_{16}H_{26}O_5N$: C, 61.7; H, 8.1; OMe, 39.9; N, 4.5%).

The Structure of 2 : 3 : 4-Trimethyl Methylgalactoside. 2 : 3 : 4-Trimethyl Galactose (VII).—A solution of trimethyl methylgalactoside (2.4 g.) in *N*-sulphuric acid (100 c.c.) was heated on the boiling water-bath until the rotation became constant. The solution was then neutralised with barium carbonate, filtered, and evaporated to dryness under reduced pressure at 40° . A small amount of barium carbonate was removed by extracting the 2 : 3 : 4-trimethyl galactose with ethyl alcohol; removal of the solvent gave a colourless reducing syrup which failed to crystallise. On exposure to the air, however, or on the addition of a little water, crystalline 2 : 3 : 4-trimethyl galactose monohydrate was readily obtained and could be crystallised from acetone-ether-light petroleum, $[\alpha]_D^{18} + 152^\circ$ (initial value in water, *c*, 1.0) changing in $2\frac{1}{2}$ hours to $+114^\circ$ (equilibrium value); m. p. 80° after sintering at 74° , not depressed by a synthetic specimen prepared either from β -1 : 6-anhydrogalactose (McCreath and Smith, *loc. cit.*) or from 6-trityl α -methylgalactoside by the method described below (Found : OMe, 38.4. Calc. for $C_9H_{16}O_6 \cdot H_2O$: OMe, 38.8%).

When the anhydrous 2 : 3 : 4-trimethyl galactose was treated with aniline (1 mol.) in boiling ethyl alcohol, the corresponding anilide was produced, m. p. 167° after crystallisation from ethyl alcohol (Found : C, 60.8; H, 7.7; OMe, 31.7; N, 4.8. Calc. for $C_{15}H_{23}O_5N$: C, 60.6; H, 7.75; OMe, 31.35; N, 4.7%).

2 : 3 : 4-Trimethyl 8-Galactonolactone (VIII).—The trimethyl galactose (1 g.) in water (20 c.c.) was treated with bromine (1 c.c.) at 30° until the reducing power of the solution had disappeared.

Excess of the bromine was removed by aeration, and the solution was neutralised with silver oxide, filtered before and after treatment with hydrogen sulphide, and evaporated to dryness under reduced pressure. The syrupy product was extracted with ether and distilled, giving 0.6 g., b. p. (bath temp.) 140—150°/0.09 mm., n_D^{17} 1.4757; $[\alpha]_D^{18} + 134^\circ$ (after 10 mins.), changing to $+ 24^\circ$ (after 9 hours; equilibrium value in water, c , 1.0). The lactone was acid to Congo-red, reacted readily with 0.1N-sodium hydroxide, and did not reduce Fehling's solution.

When the lactone (130 mg.) was heated with phenylhydrazine (75 mg.) first in boiling ethereal solution for 15 minutes and finally for 2 hours at 85—90° in the absence of solvent, a crystalline phenylhydrazide was obtained. The crystals were washed by decantation with ether and recrystallised from alcohol-ether (yield, 110 mg.), m. p. 175—176° alone or in admixture with a specimen of the phenylhydrazide of 2 : 3 : 4-trimethyl galactonic acid. The latter was also prepared from a pure specimen of the methyl ester of heptamethyl 6- β -D-glucuronosido-D-galactose, m. p. 94°, $[\alpha]_D^{25} - 21^\circ$ in water (c , 1.6) (cf. Challinor, Haworth, and Hirst, *loc. cit.*) (Found: C, 54.9; H, 7.3; OMe, 28.4; N, 8.8. Calc. for $C_{15}H_{24}O_8N_2$: C, 54.9; H, 7.4; OMe, 28.4; N, 8.5%).

The amide of 2 : 3 : 4-trimethyl galactonic acid was prepared by treating the lactone (40 mg.) with methyl-alcoholic ammonia at $- 5^\circ$ for 24 hours. After recrystallisation from absolute alcohol it had m. p. 165°, $[\alpha]_D^{18} + 32^\circ$ in water (c , 2.1) (Found: C, 45.7; H, 7.8; OMe, 39.0; N, 5.8. $C_9H_{19}O_6N$ requires C, 45.6; H, 8.1; OMe, 39.5; N, 5.9%).

Methyl 2 : 3 : 4-Trimethyl Mucate (IX).—A solution of trimethyl methylgalactoside (1.3 g.) in nitric acid (10 c.c., d 1.42) was heated for 1 hour at 50° and for 2 hours at 80°. The solution was diluted with water and freed from nitric acid and water as previously described. The acid thus obtained was esterified by boiling for 6 hours with 1% methyl-alcoholic hydrogen chloride (100 c.c.). The solution was neutralised with silver carbonate, filtered and evaporated to dryness under reduced pressure. The ester thus produced crystallised on keeping and after recrystallisation from acetone-ether-light petroleum had m. p. 102—103° alone or in admixture with authentic methyl 2 : 3 : 4-trimethyl mucate; $[\alpha]_D^{25} + 36^\circ$ in water (c , 1.7). Its aqueous solution was neutral to litmus and it did not reduce Fehling's solution (Found: C, 47.2; H, 7.2; OMe, 54.8. Calc. for $C_{11}H_{20}O_8$: C, 47.1; H, 7.2; OMe, 55.3%).

When the crystalline dimethyl ester of 2 : 3 : 4-trimethyl mucic acid was treated with methyl-alcoholic ammonia, two amides were obtained. The crystals which separated from the methyl-alcoholic ammonia were washed with alcohol-ether and recrystallised from methyl alcohol, giving the *diamide* of 2 : 3 : 4-trimethyl mucic acid, m. p. 273° (decomp.) (Found: C, 43.4; H, 6.9; OMe, 36.7; N, 11.5. $C_9H_{18}O_6N_2$ requires C, 43.2; H, 7.3; OMe, 37.2; N, 11.2%). Removal of the excess of solvent from the mother-liquors gave a crystalline mass, which was recrystallised from alcohol-ether-light petroleum, giving a *monoamide* of methyl 2 : 3 : 4-trimethyl mucic acid, m. p. 156°, $[\alpha]_D^{18} + 34^\circ$ in water (c , 3.0). This monoamide gave a negative Weerman test for α -hydroxy-amides and the amide group is therefore considered to be in position 1 (Found: C, 45.0; H, 7.0; OMe, 45.8; N, 5.3. $C_{10}H_{19}O_7N$ requires C, 45.3; H, 7.2; OMe, 46.8; N, 5.3%).

The *bismethylamide* of 2 : 3 : 4-trimethyl mucic acid was obtained by treating the dimethyl ester of 2 : 3 : 4-trimethyl mucic acid with methyl-alcoholic methylamine for 3 days at 15°. Removal of the solvent gave a crystalline product, which separated well from ethyl alcohol-ether, apparently as a monohydrate, m. p. 205°, $[\alpha]_D^{18} + 7.5^\circ$ in water (c , 1.3) (Found: C, 44.4; H, 8.25; OMe, 31.0; N, 9.5. $C_{11}H_{22}O_6N_2 \cdot H_2O$ requires C, 44.6; H, 8.2; OMe, 31.4; N, 9.5%).

Synthesis of 2 : 3 : 4-Trimethyl Galactose (VIII).— α -Methylgalactopyranoside, which probably contained some β -methylgalactopyranoside, was dehydrated by heating at 110° in a vacuum for several hours. The clear colourless syrup was dissolved in dry pyridine, trityl chloride (1 mol.) added, and after 5 days the syrupy crystalline mass was warmed and then poured into water. The pyridine was removed, most of it by repeated washing of the stiff syrup with water, and the last traces by washing a chloroform solution of the crude 6-trityl α -methylgalactoside several times with N-sulphuric acid. The chloroform solution was then washed with sodium bicarbonate solution, followed by water, dried over anhydrous magnesium sulphate, and filtered. On removal of the solvent under reduced pressure there was obtained a pale yellow, glassy residue of 6-trityl α -methylgalactopyranoside (XI), $[\alpha]_D^{18} + 30^\circ$ in acetone (c , 2.0) (yield, almost quantitative) (Found: OMe, 6.1. $C_{26}H_{28}O_6$ requires OMe, 7.1%).

The 6-trityl α -methylgalactopyranoside (65 g.) was dissolved in acetone and subjected to methylation with methyl sulphate (150 c.c.) and sodium hydroxide (450 c.c. of a 30% solution) in the usual way at 35—40°, acetone being added from time to time to replace that lost by evapor-

1736 *Smith: The Constitution of Arabic Acid. Part II.*

ation in order to keep the trityl compound in solution. After $1\frac{1}{2}$ hours the methylation was completed by heating the mixture for 20 minutes at 70° to expel the acetone, and the partially methylated 6-trityl α -methylgalactoside separated as a syrup which on cooling became sufficiently stiff to allow of its easy transference to another flask for a further methylation by the same method. After six methylations in this way, the crude material was dissolved in chloroform and the solution was washed several times with water, dried over anhydrous magnesium sulphate, and evaporated to dryness under reduced pressure, giving a pale yellow, glassy product, which was subjected to two treatments with Purdie's reagents. The 6-trityl 2 : 3 : 4-trimethyl α -methylgalactoside (XII) isolated by means of acetone had $[\alpha]_D^{18} + 44^\circ$ in chloroform (*c*, 2.0) (Found : OMe, 20.2. $C_{29}H_{34}O_6$ requires OMe, 25.9%). The low methoxyl value is probably due to the presence of triphenylmethylcarbinol, which at this stage would be in the form of its methyl ether.

A solution of 6-trityl 2 : 3 : 4-trimethyl α -methylgalactopyranoside (68 g.) in ether (400 c.c.) was cooled to 10° and saturated with dry hydrogen chloride. After keeping for 2 hours at 10 – 15° , as much hydrogen chloride as possible was removed by distillation under reduced pressure at room temperature. The ethereal solution was then exhaustively extracted with water and the combined aqueous extracts were neutralised with lead carbonate, filtered, and evaporated to dryness under reduced pressure. The 2 : 3 : 4-trimethyl α -methylgalactoside extracted from the residue with acetone was distilled, giving 25 g., b. p. (bath temp.) $160^\circ/0.05$ mm., $n_D^{20} 1.4640$, $[\alpha]_D^{18} + 160^\circ$ in water (*c*, 1.3) (Found : OMe, 51.0. Calc. for $C_{10}H_{20}O_6$: OMe, 52.5%).

A solution of 2 : 3 : 4-trimethyl methylgalactoside (10 g.) in *N*-sulphuric acid (100 c.c.) was heated on the boiling water-bath until the rotation became constant ($[\alpha]_D^{18} + 115^\circ$). It was then neutralised with barium carbonate, filtered, and evaporated to dryness under reduced pressure, giving a syrup, which was freed from a little inorganic matter by extraction with acetone. Distillation of the acetone gave 2 : 3 : 4-trimethyl galactose as a syrup, which was crystallised as its monohydrate, m. p. 79° after sintering at 73° ; $[\alpha]_D^{18} + 154^\circ$ (initial value in water, *c* 1.2), changing in 2 hours to $+114^\circ$ (equilibrium value) (Found : OMe, 39.0. Calc. for $C_9H_{18}O_6 \cdot H_2O$: OMe, 38.8%).

On treating the anhydrous 2 : 3 : 4-trimethyl galactose with aniline (1 mol.) in boiling ethyl alcohol for 4 hours, the corresponding anilide was obtained, m. p. 167° after crystallisation from ethyl alcohol; $[\alpha]_D^{18} - 65^\circ$ (initial value in methyl alcohol, *c* 1.1), changing to $+43^\circ$ (equilibrium value) (Found : N, 4.9. Calc. for $C_{15}H_{23}O_5N$: N, 4.7%).

Structure of the 2 : 4-Dimethyl Methylgalactosides.—(a) 2 : 4-Dimethyl β -methylgalactopyranoside (XIV). The crystalline material (0.8 g.) which separated from the syrup (A) was recrystallised from acetone–ether, and 2 : 4-dimethyl β -methylgalactoside, m. p. 165 – 166° , obtained. The crystalline material was non-reducing, but after boiling with dilute hydrochloric acid (5*N*) for several minutes the solution readily reduced Fehling's solution. It showed no appreciable rotation in water (*c*, 1.8) (Found : C, 48.9; H, 8.5; OMe, 41.0. $C_9H_{18}O_6$ requires C, 48.6; H, 8.2; OMe, 41.9%).

The dimethyl methylgalactoside (0.360 g.) was completely methylated by one treatment with methyl iodide and silver oxide and the syrup isolated by means of acetone was distilled, giving a colourless liquid (0.3 g.), b. p. (bath temp.) $110^\circ/0.15$ mm. The distillate crystallised spontaneously and after recrystallisation from light petroleum the 2 : 3 : 4 : 6-tetramethyl β -methylgalactoside (XVI) had m. p. 48° alone or in admixture with authentic 2 : 3 : 4 : 6-tetramethyl β -methylgalactopyranoside; $[\alpha]_D^{18} + 19.1^\circ$ in water (*c*, 3.5) (Found : OMe, 61.9. Calc. for $C_{11}H_{22}O_6$: OMe, 62.0%).

A solution of 2 : 4-dimethyl β -methylgalactoside (177 mg.) in *N*-sulphuric acid (10 c.c.) was heated for 6 hours on the boiling water-bath until the rotation became constant ($[\alpha]_D^{20} + 84^\circ$). The solution was neutralised with barium carbonate, filtered, and evaporated to dryness under diminished pressure at 50° , giving 2 : 4-dimethyl galactose (XV), which crystallised as the monohydrate on exposure to the air or on the addition of a little water. It separated well from ethyl alcohol–acetone–ether, m. p. 103° , $[\alpha]_D^{18} + 122^\circ$ (after 10 minutes), changing to $+85.6^\circ$ (equilibrium value in water, *c* 1.7) (Found : C, 42.5; H, 8.2; OMe, 27.9. $C_8H_{16}O_6 \cdot H_2O$ requires C, 42.5; H, 8.0; OMe, 27.4%).

(b) 2 : 4-Dimethyl α -methylgalactopyranoside (XIII). The second crystalline dimethyl methylgalactoside which was separated during the fractional distillation of the mixture of methylated galactosides was recrystallised from acetone–light petroleum. The product, 2 : 4-dimethyl α -methylgalactoside, had m. p. 105° , $[\alpha]_D^{18} + 142^\circ$ in water (*c*, 1.1). It was much more soluble in alcohol, acetone, and ether than the corresponding 2 : 4-dimethyl β -methylgalactoside but, like the latter, it was insoluble in light petroleum. It did not reduce Fehling's solution until

subjected to hydrolysis by boiling with dilute hydrochloric acid (Found : C, 48.7; H, 7.8; OMe, 41.2. $C_9H_{18}O_6$ requires C, 48.6; H, 8.2; OMe, 41.9%).

A solution of the 2 : 4-dimethyl α -methylgalactoside (0.69 g.) in *n*-sulphuric acid (20 c.c.) was heated on the boiling water-bath for $3\frac{1}{2}$ hours, the specific rotation (initial value $+120^\circ$) then becoming constant ($+79^\circ$). The solution was neutralised with barium carbonate, and the 2 : 4-dimethyl galactose isolated as in the previous case (0.7 g.). It readily gave the monohydrate, m. p. 103° , $[\alpha]_D^{25} + 122.7^\circ$ (after 5 minutes), changing to $+87.5^\circ$ (equilibrium value in water, *c* 1.8), and it was identical with the 2 : 4-dimethyl galactose monohydrate prepared from 2 : 4-dimethyl β -methylgalactoside (Found : C, 42.6; H, 7.8; OMe, 27.4%).

2 : 4-Dimethyl Galactose Anilide.—When anhydrous 2 : 4-dimethyl galactose was allowed to react for 3 hours with aniline (1 mol.) in boiling ethyl alcohol, the corresponding *anilide* was readily obtained, m. p. 216° (after crystallisation from methyl alcohol). It was sparingly soluble in ethyl alcohol and insoluble in acetone and ether (Found : C, 59.45; H, 7.8; OMe, 22.2; N, 4.8. $C_{14}H_{21}O_5N$ requires C, 59.4; H, 7.5; OMe, 21.9; N, 4.9%).

4-Methyl Galactose Phenyllosazone.—Crystalline dimethyl galactose (0.3 g.), dissolved in water (10 c.c.) containing glacial acetic acid (2 c.c.), was heated with phenylhydrazine (0.5 g.) for 3 hours at 80° . The crystalline osazone was filtered off, washed with dilute acetic acid, water, benzene, and ether, and recrystallised from aqueous alcohol (60%); it then had m. p. 150° . It was insoluble in benzene, ether, and light petroleum but readily soluble in acetone and alcohol (Found : C, 61.0; H, 6.9; OMe, 7.7; N, 15.0. $C_{19}H_{24}O_4N_4$ requires C, 61.3; H, 6.5; OMe, 8.3; N, 15.1%). When this 4-methyl galactose phenyllosazone was kept in the solid state, the m. p. gradually fell until after about 3 years it was approx. 130° . When it was crystallised from aqueous methyl alcohol, a product, m. p. 158° (decomp.), was obtained, the analysis of which indicates that it is a 4-methyl anhydrogalactose phenyllosazone (Found : C, 64.6; H, 6.5; OMe, 8.8; N, 15.9. $C_{19}H_{22}O_3N_4$ requires C, 64.4; H, 6.3; OMe, 8.8; N, 15.8%).

2 : 4-Dimethyl Galactonolactone (XVII).—Dimethyl galactose (1 g.) in water (15 c.c.) was allowed to react with bromine (1 c.c.) for 2 days at 20° until the solution became non-reducing. The solution was freed from excess of the bromine, neutralised with silver oxide, filtered before and after treatment with hydrogen sulphide, and evaporated to dryness under reduced pressure, giving a syrupy 2 : 4-dimethyl galactonolactone which was acid to litmus and did not reduce Fehling's solution. It showed $[\alpha]_D^{18} + 100^\circ$ (initial value in water, *c* 1.2), changing in 18 hours to $+62^\circ$ (equilibrium value) (Found : OMe, 30.1%). On treatment with phenylhydrazine (1 mol.) by the method previously used, the phenylhydrazide of 2 : 4-dimethyl galactonic acid was obtained in good yield, m. p. 183° after crystallisation from alcohol-ether (Found : C, 53.3; H, 7.2; OMe, 19.7; N, 8.7. $C_{14}H_{22}O_6N_2$ requires C, 53.5; H, 7.1; OMe, 19.75; N, 8.9%). The lactone was regenerated from the phenylhydrazide by heating the latter at 80° for 10 hours with excess of 0.1*N*-sulphuric acid. The solution was neutralised with barium carbonate, filtered, and freed from phenylhydrazine by extraction with ether. The barium was removed by means of sulphuric acid and after filtration the solution was evaporated under diminished pressure, giving a syrup, which was distilled in a high vacuum. The distillate crystallised spontaneously and after recrystallisation from ether-acetone the 2 : 4-dimethyl δ -galactonolactone had m. p. 113° , $[\alpha]_D^{15} + 162.2^\circ$ in water (*c*, 0.54) (initial value), changing in 20 hours to $+52.6^\circ$ (equilibrium value) (Found : OMe, 30.0. $C_8H_{14}O_6$ requires OMe, 30.1%). **2 : 4-Dimethyl galactonamide (XVIII).** prepared from the lactone by the action of methyl-alcoholic ammonia, had m. p. 167° after crystallisation from ethyl alcohol; $[\alpha]_D^{18} + 59^\circ$ in water (*c*, 0.8). This amide gave a negative Weerman reaction (Found : C, 43.2; H, 7.5; OMe, 27.6; N, 6.2. $C_8H_{17}O_6N$ requires C, 43.1; H, 7.7; OMe, 27.8; N, 6.3%).

The Methyl Ester γ -Lactone of 2 : 4-Dimethyl Mucic Acid (XX).—A solution of 2 : 4-dimethyl galactose (0.91 g.) in nitric acid (30 c.c., *d* 1.2) was heated for 24 hours at 50 – 55° ; it was then diluted with water and freed from nitric acid by distillation under diminished pressure, water and finally methyl alcohol being added to facilitate this process. The dry syrupy acid thus obtained was boiled for 6 hours with 1% methyl-alcoholic hydrogen chloride (50 c.c.). The solution was cooled, neutralised with silver carbonate, filtered, and evaporated to a syrup, which was distilled, giving a colourless liquid (0.4 g.), b. p. (bath temp.) $190^\circ/0.12$ mm. The distillate of the methyl ester of 2 : 4-dimethyl mucic acid 3 : 6-lactone crystallised spontaneously. It separated well from acetone-ether-light petroleum in needles, m. p. 111° ; $[\alpha]_D^{14} + 120^\circ$ (initial value in water, *c* 1.0), $+112^\circ$ (after 1 day), $+104^\circ$ (3 days), $+93^\circ$ (8 days), $+89^\circ$ (10 days), $+86^\circ$ (12 days), $+83.5^\circ$ (14 days) (mutarotation still incomplete) (Found : C, 46.1; H, 6.0; OMe, 39.3. $C_9H_{14}O_7$ requires C, 46.15; H, 6.0; OMe, 39.75%). A freshly prepared solution in water was neutral to litmus but developed acidity after several days. The sub-

1738 *Smith: The Constitution of Arabic Acid. Part II.*

stance behaved as a γ -lactone, reacting slowly with sodium hydroxide (1 equiv.) in the cold but when warmed at 50° for 30 minutes it reacted with two equivs. [19.69 mg. required 16.8 c.c. of 0.01N-sodium hydroxide, giving an equiv. wt. 117 (approx.) or M , 234. $C_9H_{14}O_7$ requires M , 234].

The presence of one ester methoxyl group was shown in the following way. The compound (ca. 30 mg.) was heated with 0.15N-barium hydroxide (5 equivs.) at 50° for 1 hour. The solution was neutralised with carbon dioxide and evaporated to dryness under diminished pressure. All these operations were effected in a Zeisel apparatus and a methoxyl estimation was carried out on the dry residue consisting of barium carbonate and the barium salt of the organic acid [Found: OMe, 26.2. Calc.: OMe (for loss of 1 methoxyl group), 26.3%]. The methyl ester γ -lactone of 2:4-dimethyl mucic acid, m. p. 111° , $[\alpha]_D^{15} + 119.0^\circ$ (initial value in water, c 1.3), was also obtained (a) when 2:4-dimethyl δ -galactonolactone was similarly oxidised with nitric acid (d 1.2), and (b) by the nitric acid oxidation of both the α - and the β -form of 2:4-dimethyl methylgalactoside.

On treatment with methyl-alcoholic ammonia for 24 hours at 0° the ester lactone readily gave the *diamide* (XXI) of 2:4-dimethyl mucic acid, which separated well from hot aqueous alcohol; m. p. 229° (decomp.), $[\alpha]_D + 30^\circ$ in water (c 1.0). It showed a positive Weerman reaction (Found: OMe, 26.3; N, 11.8. $C_8H_{14}O_6N_2$ requires OMe, 26.3; N, 11.9%).

Similarly, when the 2:4-dimethyl mucic lactone methyl ester was allowed to react with methyl-alcoholic methylamine for 3 days at room temperature, the *bismethylamide* was produced. The crystalline product obtained on removing the excess of the solvent was recrystallised from methyl alcohol-ether and had m. p. 214° , $[\alpha]_D^{15} + 27^\circ$ in water (c 1.5) (Found: OMe 22.4; N, 10.7. $C_{10}H_{20}O_6N_2$ requires OMe, 23.5; N, 10.6%).

The Methyl Ester Lactone of 2:4:5-Trimethyl Mucic Acid (XXII).—The methyl ester of the γ -lactone of 2:4-dimethyl mucic acid (0.5 g.) was methylated twice with Purdie's reagents in the usual way. The product, isolated by means of acetone, was dissolved in a small volume of ether; on keeping at room temperature, rectangular plates of methyl 2:3:4:5-tetramethyl mucate separated, m. p. 109° alone or in admixture with an authentic specimen (Karrer and Peyer, *Helv. Chim. Acta*, 1922, 5, 577, record m. p. 103°). This dimethyl ester showed no rotation in aqueous solution (Found: C, 49.1; H, 7.7; OMe, 63.2. Calc. for $C_{12}H_{22}O_8$: C, 49.0; H, 7.6; OMe, 63.3%). Light petroleum was added to the ethereal mother-liquor until a faint turbidity was produced. On cooling the solution to -5° , long needles separated; these on repeated fractional crystallisation gave the pure *methyl ester lactone of 2:4:5-trimethyl mucic acid*, m. p. $63-64^\circ$, $[\alpha]_D^{15} + 85^\circ$ in water (c 1.2) (Found: C, 48.7; H, 6.4; OMe, 49.9. $C_{10}H_{20}O_7$ requires C, 48.4; H, 6.5; OMe, 50.0%). When mixed with its enantiomorph, the methyl ester lactone of 2:3:5-trimethyl mucic acid [m. p. 63° , $[\alpha]_D^{15} - 86^\circ$ in water (c 1.3) (Smith, *Chem. and Ind.*, 1939, 58, 363)], the m. p. rises to ca. 75° . This elevation in m. p. agrees with the observation that the racemic mixture of the methyl ester γ -lactones of 2:3:5- and 2:4:5-trimethyl mucic acid prepared by the action of Purdie's reagents on the monolactone of mucic acid (Fischer, *Ber.*, 1891, 24, 2136) has m. p. 81° (Haworth and Smith, unpublished results).

When the methyl ester γ -lactone of 2:4:5-trimethyl mucic acid was treated with methyl-alcoholic ammonia, the corresponding *diamide* of 2:4:5-trimethyl mucic acid was produced, m. p. 225° (decomp.) after crystallisation from water. This amide was sparingly soluble in cold water and insoluble in most organic solvents (Found: C, 43.35; H, 7.4; OMe, 36.4; N, 11.0. $C_9H_{18}O_6N_2$ requires C, 43.2; H, 7.3; OMe, 37.2; N, 11.2%).

Similarly, the action of methyl-alcoholic methylamine on the methyl ester γ -lactone of 2:4:5-trimethyl mucic acid furnished the *bismethylamide*, m. p. 232° (decomp.) after crystallisation from ethyl alcohol, $[\alpha]_D^{15} + 23^\circ$ (in water, c 1.3) (Found: C, 47.6; H, 8.2; OMe, 32.2; N, 10.1. $C_{11}H_{22}O_6N_2$ requires C, 47.5; H, 8.0; OMe, 33.4; N, 10.1%).

The author thanks Professor W. N. Haworth, F.R.S., for his interest in this work.

THE A. E. HILLS LABORATORIES,
THE UNIVERSITY, EDGBASTON, BIRMINGHAM, 15.

[Received, September 8th, 1939.]