METHYL ETHERS OF URONIC ACIDS

by

R. A. Edington

A thesis for the degree of Doctor of Philosophy

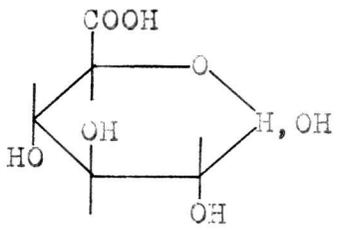
Edinburgh

October, 1954

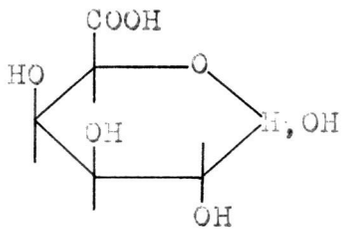
C O N T E N T S

INTRODUCTION

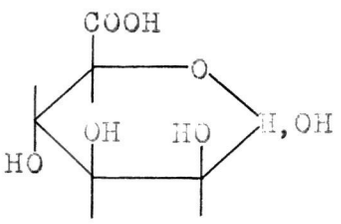
The uronic acids are hexose derivatives which occur widely in nature and which differ from the simple sugars in having a carboxylic group in the terminal position. Three unsubstituted uronic acids, D-glucuronic, D-galacturonic, and D-mannuronic acids, have been identified in natural products. D-Glucuronic



D-Glucuronic Acid



D-Galacturonic Acid



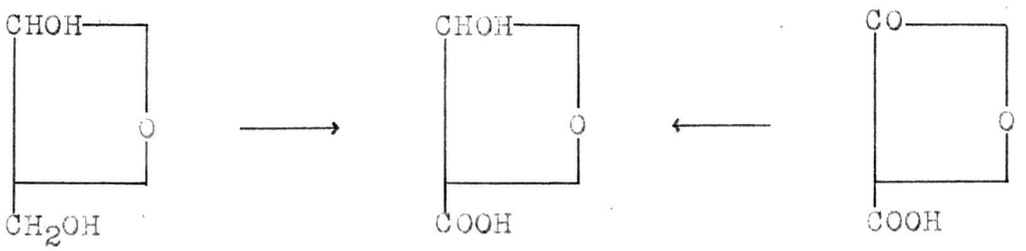
D-Mannuronic Acid

acid occurs in certain bacterial polysaccharides[1], in the gums and hemicelluloses of higher plants[2,3,4], in the mucopolysaccharides of animals[5], and in the products of detoxification of phenols and sterols by mammals[6]. D-Galacturonic acid is found in the gums[7], seed mucilages[3], and pectic materials[7] of many higher plants and in a number of bacterial polysaccharides[1]. Alginic acid[8,9], a mucilaginous polysaccharide confined to the Algae, is the only known natural source of D-mannuronic acid.

Two partly methylated uronic acids have also been found in natural products. A monomethyl glucuronic acid which has been isolated from several sources has been characterised as 4-methyl-D-glucuronic acid[10]. In addition, an unidentified monomethyl ether of galacturonic acid has been found by Adams and Castagne[11] in the hydrolysate of an unmethylated polysaccharide from the hemicelluloses of wheat. Hydrolysates of methylated polysaccharides, however, have

been the source of the majority of the known methylated uronic acids. 2:3-Dimethyl derivatives of D-glucuronic[12], D-galacturonic[13], and D-mannuronic[8] acids have all been isolated from the hydrolysates of methylated polysaccharides and have generally been characterised, after bromine oxidation, as the crystalline diamides of the corresponding glycaric acids. The 2:3:4-trimethyl ethers of all three uronic acids[8,14,15] and 2:3:5-trimethyl-D-galacturonic acid[13] have been obtained and characterised in a similar manner. Finally, the only other methylated uronic acid of known constitution to be derived from natural sources is 3:4-dimethyl-D-glucuronic acid, which has recently been obtained from two sources, methylated glycyrrhinic acid[16], a disaccharide composed of two glucuronic acid residues, and methylated sapote gum[17]. The crystalline acid obtained from the latter source is the first partly methylated uronic acid to be obtained in crystalline condition.

The three unsubstituted uronic acids may be prepared by hydrolysis of natural products[18] or by synthetic means. Synthetically the two alternative methods are the reduction of the monolactone of a glycaric acid[19] or the oxidation of the primary alcoholic group of an aldohexose:



Aldohexose — Uronic Acid — Glycarolactone

In practice, the latter procedure has been the more useful. In its original form the method involved the oxidation, usually with alkaline

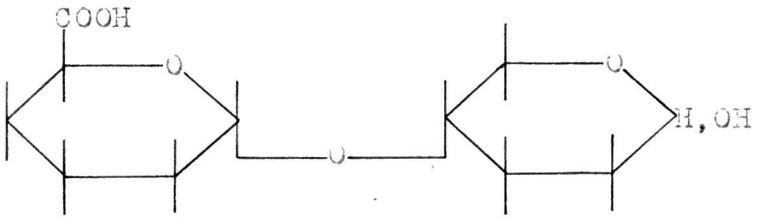
permanganate, of an aldohexose derivative having the terminal hydroxyl free and all the others suitably protected[20,21,22]. More recently, however, methods have been devised for the specific oxidation of the primary alcoholic group of glycosides and non-reducing acetals, in which the secondary hydroxyl groups may be unprotected.[23]

There are two principal methods for the synthesis of a methyl ether of a uronic acid; either a suitable uronic acid derivative may be methylated, or else a methylated hexose may be oxidised. A third method, the reduction of a methylated glycarolactone, has been employed in one instance[24], but it is of very limited application. Of the six possible fully methylated uronic acids, five have been synthesised. 2:3:4-Trimethyl-D-glucuronic acid[25], 2:3:4-trimethyl-D-mannuronic acid[25], and 2:3:4- and 2:3:5-trimethyl-D-galacturonic acids[25,26] have been prepared by oxidation of the corresponding methyl trimethyl-hexosides. In addition, 2:3:4-trimethyl-D-mannuronic acid[27], 2:3:4-trimethyl-D-galacturonic acid[15,28], and 2:3:4-trimethyl-D-glucuronic acids[29] have been synthesised by methylation of the appropriate glycosiduronic acids. In most instances these trimethyl uronic acids were isolated as the crystalline trimethyl glycosiduronamides. In the case of 2:3:5-trimethyl-D-glucuronic acid and 2:3:4-trimethyl-D-mannuronic acid, however, the only crystalline derivatives known at the commencement of this work were the trimethyl glycaramides formed by bromine oxidation of the uronic acid followed by esterification and ammonolysis.

The position of the partly methylated uronic acids, however, is much less satisfactory, Of the twenty seven mono- and di-methyl uronic acids theoretically possible, only three have been synthesised. 3-Methyl-D-glucuronic acid has been prepared in two ways. In 1924,

Levene and Meyer[24] reduced 3-methyl-D-glucarolactone, presumably to 3-methyl-D-glucuronic acid, but they were unable to characterise their product satisfactorily. More recently, Marsh[30] subjected 3-methyl-1:2-isopropylidene-D-glucofuranose to catalytic dehydrogenation with platinum black in the presence of oxygen. Again the product was a syrup which was difficult to characterise. 2:5-Dimethyl-D-glucuronic acid has been prepared on several occasions[31,32] by methylation of D-glucurone, the stable lactone of D-glucuronic acid, or of the derived methyl furanoside. Lastly, in 1947, Jones and Stacey[33] synthesised 2-methyl-D-galacturonic acid by a method which consisted essentially of methylation of methyl 3:4-isopropylidene-α-D-galactosiduronic methyl ester, followed by hydrolysis.

The properties of a uronic acid combine those of an aldohexose with those of a polyhydroxycarboxylic acid. The reducing group reacts with alcohols in the usual manner, and examples of furanosides and pyranosides[13], and of both anomeric forms[34], are known. The pyranosides are remarkably stable to acid hydrolysis, and this has made work on the polyuronides exceptionally difficult, since the conditions which are necessary for complete hydrolysis generally cause extensive decomposition. On the other hand, the stability of the glycosiduronic linkage has facilitated work on certain polysaccharides containing uronic acids since, by controlled hydrolysis, it has often been possible to isolate an aldobiuronic acid, a disaccharide containing one uronic acid and one pentose or hexose unit. In every aldobiuronic acid which has been studied the linkage has involved the glycosidic centre of the uronic acid and it is almost certainly the inherent stability of the glycosiduronic linkage which has ensured the survival of this particular fragment of the polysaccharide molecule.



Aldobiuronic Acid

Several aldobiuronic acids have been isolated from natural sources and two have been synthesised[35,36]. In 1952, for instance, Bishop[36] synthesised an aldobiuronic acid containing D-glucuronic acid linked to the 3 position of D-xylose by condensing the acetylglycosyl bromide of D-glucuronic acid with an appropriately substituted derivative of D-xylose. He was unable, however, to characterise his product by the isolation of crystalline derivatives.

The methyl furanosides of the uronic acids appear to be similar in properties to the normal aldofuranosides, but no reliable evidence has been advanced for the existence of furanosiduronic acid groups in natural products.

Certain reactions typical of aldoses are less easily applicable to uronic acids because of the modifying effect of the carboxylic group. The important group of nitrogeneous derivatives, including osazones, hydrazones, and arylglycosylamines, is less satisfactory for the characterisation of uronic acids since the product is apparently a mixture of the desired product with the corresponding amine salt or amide. When the reducing group of a uronic acid is protected by glycoside formation the product behaves as a simple hydroxy-carboxylic acid. If it is sterically possible a lactone[37] is formed, and the metallic salts, alkyl esters, amides, and substituted amides, are

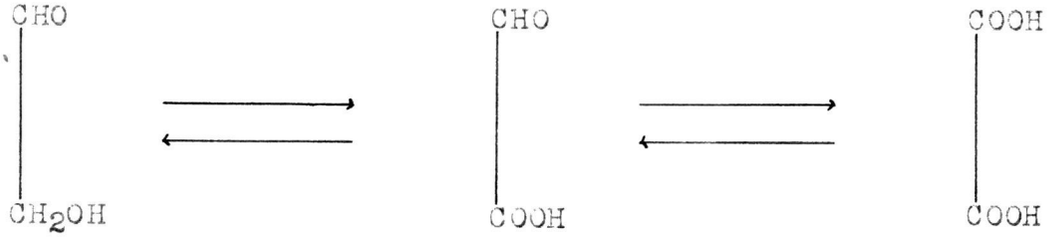
obtainable by classical methods. The methyl glycosiduronamides have, in general, proved to be the most easily crystallisable derivatives of the uronic acids, and are quite suitable for characterisation; they are stable, readily recrystallised, have sharp melting points, and are easily converted back into the glycosiduronic acid. The comparative stability of the glycosidic group of the glycosiduronic acids has been very useful, for it is generally possible to perform a variety of chemical operations upon the rest of the molecule without altering the configuration of the glycosidic centre.

The secondary alcoholic groups of a uronic acid react to form esters, ethers, and acetals in the usual manner. Acetylglycosyl halides have been prepared[35,36] and the halogen atom appears to have approximately the same reactivity as in the corresponding derivatives of aldohexoses.

The reducing group of a uronic acid may be oxidised by several reagents, the product being the corresponding glycaric acid. Oxidation of the free uronic acid with aqueous bromine[34], or of the glycosiduronic acid or ester with nitric acid[12], was, until the introduction of lithium aluminium hydride reduction, the most widely used method for the characterisation of uronic acids. The resulting glycaric acids generally give easily crystallisable diamides, and they may conveniently be synthesised for comparison by nitric acid oxidation of the corresponding aldoses. The glycaramides, however, are not perfect derivatives for characterisation, since they are sometimes not easily recrystallised and since the melting points of different isomers are generally not greatly different from one another. In the galactaric acid series, for instance, the three known dimethyl-

galactaramides[13,34,38] have melting points between 228° and 230°. Moreover, the "melting points" are in fact decomposition temperatures and are therefore not entirely suitable for mixed melting point observations.

A more recent method for the characterisation of uronic acids and their methyl ethers is the reduction of the carboxylic group to primary alcohol. This is in effect a reversal of the method of synthesis from the corresponding aldohexose and it is therefore possible to carry out all four reactions correlating the aldohexoses, uronic acids, and glycaric acids:



Aldohexose Uronic Acid Glycaric Acid

The reagents which have been used for the reduction of uronic acids are lithium aluminium hydride[16] and sodium borohydride[39]. In both cases it is essential that the reducing group be protected by glycoside formation. The immediate product is, therefore, the aldoside, from which the corresponding aldohexose may be obtained by acid hydrolysis. This method is particularly convenient since the methyl ethers of the common aldohexoses are relatively well-known.

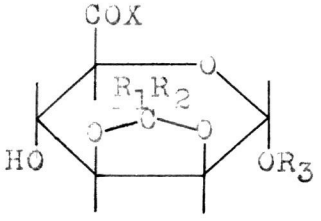
The synthesis of crystalline derivatives of the various methylated uronic acids and the establishment of physical constants is, however, very desirable since in many investigations of natural products it would obviate the carrying out of a reduction and subsequent investigation of the hexose derivative produced. Furthermore, even when reduction is contemplated, it is obviously desirable that the uronic

acid should be isolated as a crystalline, and consequently single, substance before reduction[10]. Unfortunately, however, the number of crystalline derivatives of methylated uronic acids which were known at the commencement of this work was small, and since moreover, the periodate oxidation of uronic acids had been reported to give anomalous results,[40] it was decided to attempt the preparation of a number of new methylated derivatives of the three common uronic acids and to study their general properties, including periodate oxidation.

DERIVATIVES OF D-MANNURONIC ACID

DISCUSSION

4-Methyl-D-mannuronic acid may in theory be prepared by methylation of a 2:3-acetal of D-mannuronic acid:



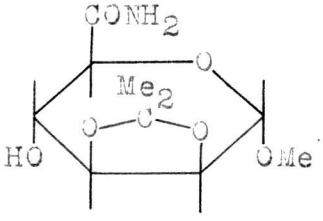
The only derivative of this type known at the commencement of this work was an amorphous potassium salt prepared by Ault, Haworth, and Hirst[27] by oxidation of methyl 2:3-isopropylidene-α-D-mannopyranoside. Methyl α-D-mannopyranoside, prepared conveniently from carob gum, was condensed with acetone under the conditions employed by Ault, Haworth, and Hirst, but only the 2:3-4:6-di-acetal was isolated. In further preliminary experiments, attempts were made to remove any mono-isopropylidene derivative from the vicinity of the catalyst (copper sulphate or cation exchange resin in a Soxhlet thimble) as soon as it was formed and before further condensation could occur, but again no mono-isopropylidene derivative was obtained. In order to prevent formation of the 2:3-4:6-di-acetal the 6-toluene-p-sulphonyl ester of methyl α-D-mannopyranoside was prepared and condensed with acetone in the usual manner. Neither this isopropylidene derivative nor the corresponding 2:3-benzylidene acetal was crystalline, however, and

in view of the fact that the methyl 6-toluene-p-sulphonyl-α-D-mannopyranoside was also a syrup this scheme was abandoned.

Attention was next directed to the preparation of a derivative of mannuronic acid which might be condensed with acetone. In 1944, Stacey and Wilson[37] oxidised methyl 2:3:4-triacetyl-α-D-mannoside to the corresponding uronic acid. Their product was a syrup from which a crystalline lactone was obtained after deacetylation. In the present work a number of preliminary experiments were carried out in order to investigate the possibility of preparing and oxidising the corresponding 2:3:4-tribenzoate, which might reasonably be expected to give more easily crystallisable derivatives. Methyl 2:3:4-tribenzoyl-6-toluene-p-sulphonyl-α-D-mannoside, a substance first obtained by Haskins, Hann, and Hudson[57], was prepared in improved yield, but attempts to remove the toluene-p-sulphonyl group by reduction, as described for certain carbohydrate sulphonates by Kenner and Murray[58], were unsuccessful except when conditions were drastic enough to cause more extensive changes. The toluene-p-sulphonyl group was easily replaced by iodine[59] but the latter could not be replaced directly by hydroxyl. Eventually, methyl 2:3:4-tribenzoyl-α-D-mannoside was prepared by replacement of the iodo group by nitrate[59], followed by reductive de-nitration[60], but in the meantime the same substance had been synthesised more conveniently through the crystalline 6-triphenylmethyl ether.

In the scheme finally employed, methyl α-D-mannopyranoside was treated successively with triphenylchloromethane and benzoyl chloride in pyridine. The product, methyl 2:3:4-tribenzoyl-6-triphenylmethyl-α-D-mannoside, crystallised out in good yield from the reaction mixture. As originally obtained or after recrystallisation from aqueous pyridine

the product contained one molecule of firmly held pyridine of crystallisation and analogous compounds containing chloroform or acetone of crystallisation were easily obtained by recrystallisation from the corresponding solvent. The pyridine of crystallisation did not interfere with the removal of the trityl residue with hydrogen bromide in glacial acetic acid[66] and the crystalline tribenzoate was obtained in high yield. Oxidation with potassium permanganate in acetic acid-acetone-water[21] gave crystalline methyl 2:3:4-tribenzoyl-α-D-mannosiduronic acid, from which the crystalline methyl ester was easily obtained. In preliminary experiments debenzoylation by the Zemplén procedure[61], using a catalytic amount of sodium methoxide, followed by esterification, gave syrupy methyl α-D-mannopyranosiduronic methyl ester, ammonolysis of which gave a crystalline amide. In a later experiment debenzoylation and amide formation were carried out simultaneously with methanolic ammonia. The amide, on condensation with acetone, gave crystalline methyl 2:3-isopropylidene-α-D-mannopyranosiduronamide, which was also prepared



from the syrupy methyl ester by acetone condensation followed by ammonolysis. Methylation with silver oxide and methyl iodide gave a crystalline product which analysed as methyl 4-methyl-2:3-isopropylidene-α-D-mannosiduronic methylamide. The presence of the methylamide grouping was confirmed by conversion to the methyl ester

from which the original crystalline substance was regenerated by treatment with methylamine. Acid hydrolysis, followed by bromine oxidation, gave 4-methyl-D-mannaric acid (which by reason of the symmetry of the mannaric acid molecule is identical with 3-methyl-D-mannaric acid), isolated as the crystalline diamide.

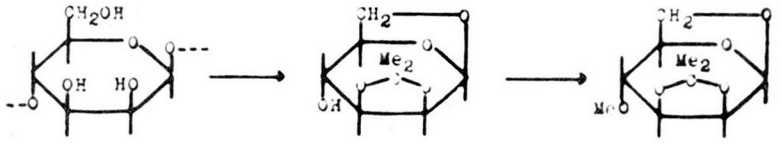
By methylation with methyl iodide and silver oxide of methyl α-D-mannopyranosiduronamide, methyl 2:3:4-trimethyl-α-D-mannosiduronic methylamide was prepared as a crystalline solid. 2:3:4-Trimethyl-D-mannuronic acid has been synthesised on two previous occasions[25,27], but no crystalline derivatives except the trimethyl glycaramide were isolated. In the present instance the methylamide was further characterised, after hydrolysis and oxidation, as crystalline 2:3:4-trimethyl-D-mannaramide, m.p. 211° (decomp.), undepressed on admixture with an authentic specimen.

As the first stage in the preparation of 2:3-dimethyl-D-mannuronic acid, the preparation of the toluene-p-sulphonyl ester of methyl 2:3-isopropylidene-α-D-mannopyranosiduronamide seemed to be the obvious route to follow. The conditions which were employed successfully in the preparation of methyl 3:4-isopropylidene-2-toluene-p-sulphonyl-α-D-galactosiduronic methyl ester were, however, quite unsuccessful in the present instance. From numerous experiments under widely varying conditions only a minute quantity of solid was isolated on two occasions and this synthesis was abandoned in the meantime.

DERIVATIVES OF D-MANNURONIC ACID

PRELIMINARY EXPERIMENTS

(a)

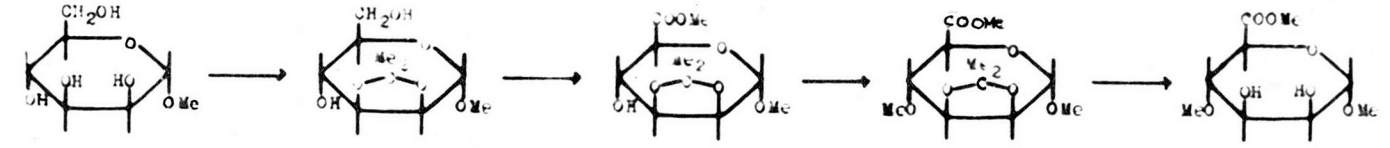


β-D-Mannosan

2:3-Isopropylidene-D-mannosan<1:6>β<1:5>

4-Methyl-2:3-isopropylidene-D-mannosan <1:6>β<1:5>

(b)



Methyl α-D-Mannoside

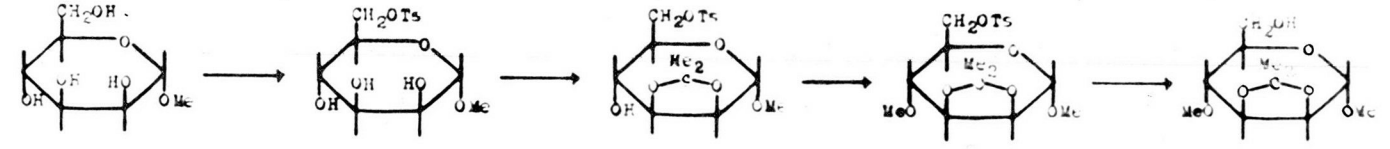
Methyl 2:3-Isopropylidene-α-D-mannoside

Methyl 2:3-isopropylidene-α-D-mannosiduronic Methyl Ester

Methyl 4-Methyl-2:3-isopropylidene-α-D-mannosiduronic Methyl Ester

Methyl 4-Methyl-α-D-mannosiduronic Methyl Ester

(c)



Methyl α-D-Mannoside

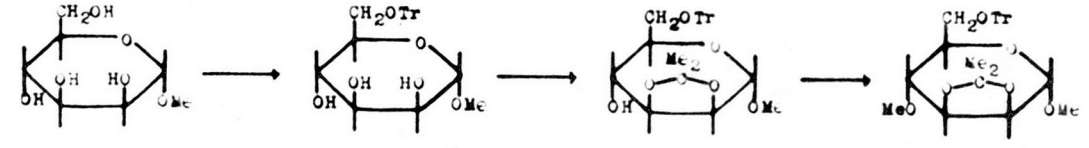
Methyl 6-Tosyl-α-D-mannoside

Methyl 2:3-Isopropylidene-6-tosyl-α-D-mannoside

Methyl 4-Methyl-2:3-isopropylidene-6-tosyl-α-D-mannoside

Methyl 4-Methyl-2,3-isopropylidene-α-D-mannoside

(d)



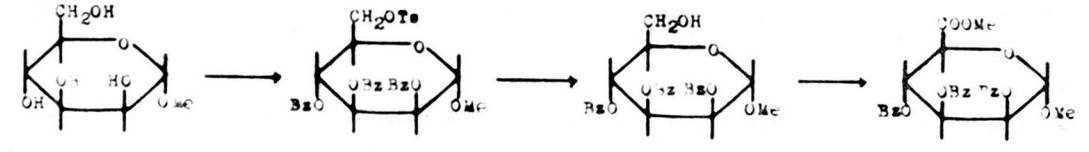
Methyl α-D-Mannoside

Methyl 6-Trityl-α-D-mannoside

Methyl 2:3-Isopropylidene-6-trityl-α-D-mannoside

Methyl 4-Methyl-2:3-isopropylidene-6-trityl-α-D-mannoside

METHYL 2:3:4-TRIBENZOYL-α-D-MANNOSIDURONIC METHYL ESTER

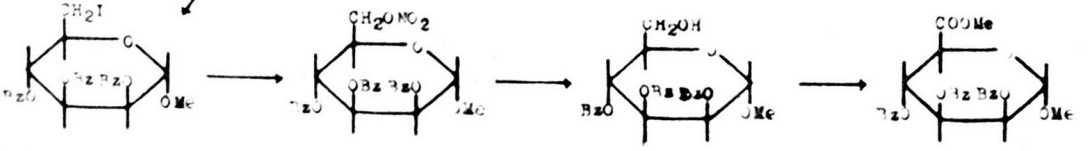


Methyl α-D-Mannoside

Methyl 2:3:4-Tribenzoyl-6-tosyl-α-D-mannoside

Methyl 2:3:4-Tribenzoyl-α-D-mannoside

Methyl 2:3:4-Tribenzoyl-α-D-mannosiduronic Methyl Ester



Methyl 2:3:4-Tribenzoyl-6-deoxy-6-iodo-α-D-mannoside

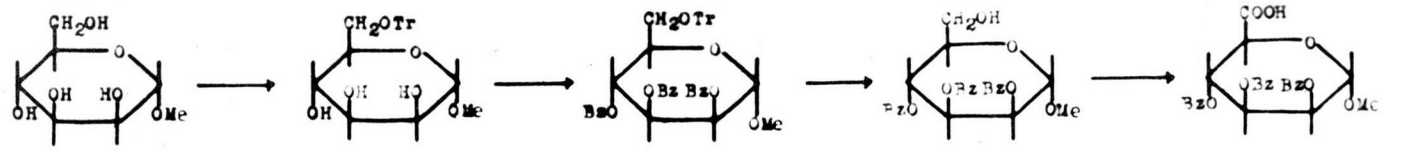
Methyl 2:3:4-Tribenzoyl-α-D-mannoside-6-nitrate

Methyl 2:3:4-Tribenzoyl-α-D-mannoside

Methyl 2:3:4-Tribenzoyl-α-D-mannosiduronic Methyl Ester

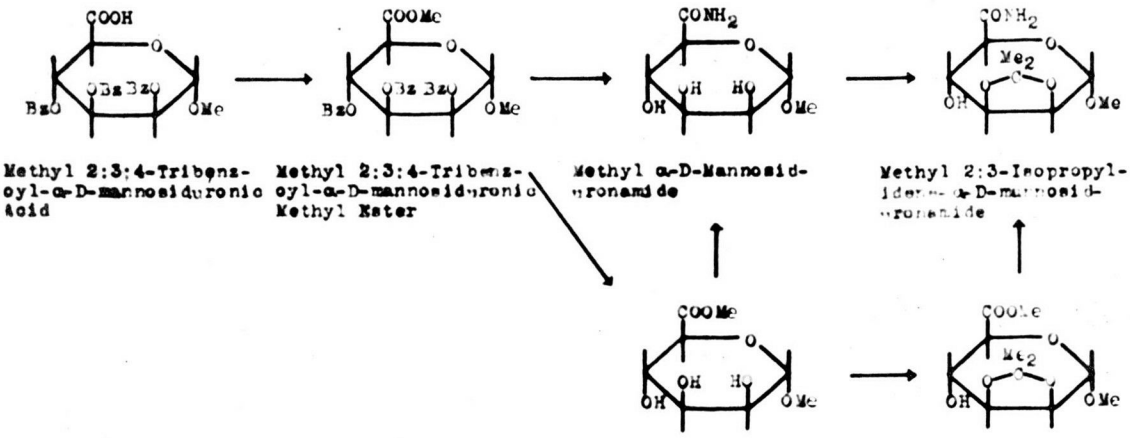
DERIVATIVES OF D-MANNURONIC ACID (CONT.)

(b)



Methyl α-D-Mannoside | Methyl 6-Trityl-α-D-mannoside | Methyl 2:3:4-Tribenzoyl-6-trityl-α-D-mannoside | Methyl 2:3:4-Tribenzoyl-α-D-mannoside | Methyl 2:3:4-Tribenzoyl-α-D-mannosiduronic Acid

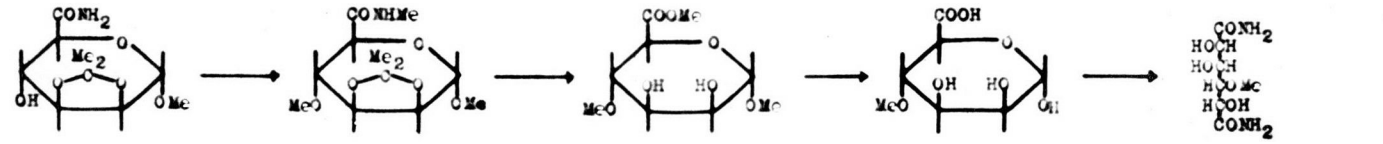
METHYL 2:3-ISOPROPYLIDENE-α-D-MANNOSIDURONAMIDE



Methyl 2:3:4-Tribenzoyl-α-D-mannosiduronic Acid | Methyl 2:3:4-Tribenzoyl-α-D-mannosiduronic Methyl Ester | Methyl α-D-Mannosiduronamide | Methyl 2:3-Isopropylidene-α-D-mannosiduronamide

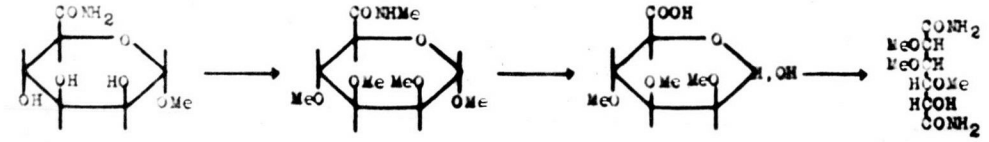
Methyl α-D-Mannosiduronic Methyl Ester | Methyl 2:3-Isopropylidene-α-D-mannosiduronic Methyl Ester

METHYL 4-METHYL-α-D-MANNOSIDURONIC METHYL ESTER



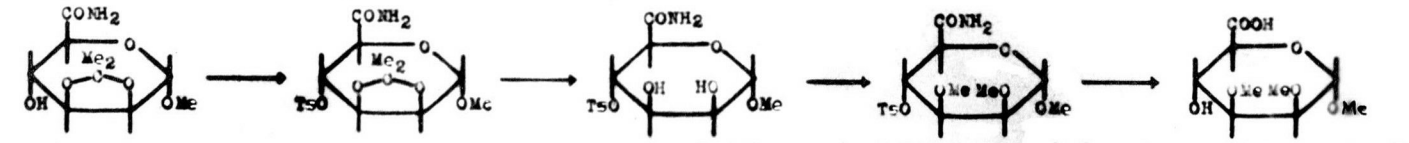
Methyl 2:3-Isopropylidene-α-D-mannosiduronamide | Methyl 4-Methyl-2:3-isopropylidene-α-D-mannosiduronic methylamide | Methyl 4-Methyl-α-D-mannosiduronic Methyl Ester | 4-Methyl-D-mannuronic Acid | 4-Methyl-D-mannaramide (3-Methyl-D-mannaramide)

METHYL 2:3:4-TRIMETHYL-α-D-MANNOSIDURONIC METHYLAMIDE



Methyl α-D-Mannosiduronamide | Methyl 2:3:4-Trimethyl-α-D-mannosiduronic thylamide | Methyl 2:3:4-Trimethyl-D-mannuronic Acid | 2:3:4-Trimethyl-D-mannaramide

ATTEMPTED SYNTHESIS OF 2:3-DIMETHYL-D-MANNURONIC ACID



Methyl 2:3-Isopropylidene-α-D-mannosiduronamide | Methyl 2:3-Isopropylidene 4-tosyl-α-D-mannosiduronamide | Methyl 4-Tosyl-α-D-mannosiduronamide | Methyl 2:3-Dimethyl-4-tosyl-α-D-mannosiduronamide | Methyl 2:3-Dimethyl-α-D-mannosiduronic Acid

EXPERIMENTAL.

Pyrolysis of Ivory Nut Mannan.

Ivory nuts, in the form of fine turnings, were heated with a full Meker flame under 15 mm. pressure, using the apparatus of Knauf, Hann, and Hudson[62]. Two batches of 100 g. turnings were treated in this way, two hours being required for complete pyrolysis in each case. The combined pyrolysates were shaken with charcoal, filtered, and evaporated to a dark syrup which was shaken for 120 hours with acetone and anhydrous copper sulphate. Filtration of the copper salts and removal of the acetone gave a dark syrup which failed to crystallise. In order to reduce the time required for complete pyrolysis, the ivory nut shavings were hammer-milled, giving a fine powder which on pyrolysis reacted completely within 45 minutes. Again, however, the final product was a dark syrup which did not crystallise. In a further experiment the pyrolysate was neutralised with barium carbonate before evaporation and condensation with acetone. The product was a brown syrup which partly crystallised: the crystals could not be separated successfully from the tarry syrup, and in view of the low yield (5 g. from 100 g. ivory nut) the product was not further examined.

Preparation of Methyl α-D-Mannopyranoside from Carob Gum.

0·5N Sulphuric acid (500 ml.) was added rapidly to a paste of carob gum (100 g.) in industrial methylated spirit (100-200 ml.) and the mixture heated on the steam-bath for 12 hours[63]. After filtration, the residue was washed with hot water and the combined filtrates neutralised with calcium carbonate. The calcium salts were removed and washed and the neutral filtrate and washings evaporated to a glass

which was heated under reflux for 18 hours with 2% methanolic hydrogen chloride (400 ml.). The solution was concentrated (150-200 ml.), allowed to stand overnight at 0°, and the resulting crystals filtered off and washed with cold methanol (50 ml.) and acetone (250 ml.) and dried in vacuo over potassium hydroxide. After recrystallisation from ethanol or aqueous acetone, methyl α-D-mannopyranoside had m.p. 194-195°.

Found: OMe, 15·8. Calc. for $C_7H_{14}O_6$: OMe, 16·0%.

The carob gum was supplied by Messrs. Ellis Jones, Stockport, and appeared to be of variable quality. From three different batches of gum the yields of pure methyl α-D-mannopyranoside from 100 g. of the undried gum were: 30-40 g. (from gum of Cyprian origin); 10-15 g. (from gum of unspecified origin); and 20-25 g. (from gum of unspecified origin). Smith[63] obtained 44-50 g./100 g. dry gum. The water content of the gum (determined by drying at 110° to constant weight) was about 15% and the residue obtained after hydrolysis accounted for about 20-30% of the undried gum.

Preparation of Methyl α-D-Mannopyranoside from D-Mannose

D-Mannose (98 g.) was heated under reflux for 18 hours with 0·5% methanolic hydrogen chloride (1500 ml.). The solution was cooled and the resulting crystals (78·5 g.) filtered off, washed with methanol (100 ml.) and dried in vacuo over potassium hydroxide. The filtrate and washings were neutralised with silver carbonate and the neutral filtrate and washings concentrated (100 ml.). After standing overnight the crystalline solid (22 g. Total 100·5 g., 95% of theoretical) was filtered off, washed with methanol (50 ml.) and dried.

Condensation of Methyl α-D-Mannopyranoside with Acetone

(a) Methyl α-D-mannopyranoside was shaken with acetone, anhydrous copper sulphate and acetaldehyde for 120 hours.[41] The product was a syrup from which methyl 2:3-4:6-di-O-isopropylidene-α-D-mannoside was obtained as a crystalline solid, m.p. 73-76° (yield 80% and 85% of theoretical in different experiments). No other product was obtained.

(b) Finely powdered methyl α-D-mannopyranoside (1·0 g.), mixed intimately with anhydrous copper sulphate (10-15 g.), was placed in a Soxhlet thimble and extracted for three hours with dry acetone, the boiling flask containing a few grams of barium carbonate. Filtration, followed by evaporation of the acetone, gave a syrup which was extracted with light petroleum under reflux. The extract was discarded and the residual syrup extracted with cold acetone containing 5% light petroleum. A crystalline residue of methyl α-D-mannopyranoside, m.p. 194°, remained. The acetone-light petroleum extract was evaporated to a colourless syrup (ca. 0·5 g.) which, on paper chromatography using butanol-ethanol-water (4:1:5) as eluant and aniline oxalate containing 3% phosphoric acid as spray, showed a single spot of the same speed as authentic methyl 2:3-4:6-di-O-isopropylidene-α-D-mannoside.

(c) Finely powdered methyl α-D-mannopyranoside (10 g.) was mixed intimately with cation exchange resin Dowex 50 (17 g., 200-400 mesh; washed with acetone and dried in vacuo). The mixture was placed in a Soxhlet thimble and extracted for 24 hours with dry acetone from a flask containing barium carbonate (ca. 5 g.). Solids were removed by filtration and washed and the combined filtrates evaporated to small volume:

crystalline methyl α-D-mannopyranoside (1·35 g., m.p. 191-194°) was filtered off and the filtrate evaporated to a syrup which was exhaustively extracted with light petroleum under reflux: the extracts on cooling gave a strongly reducing crystalline solid (1·35 g., m.p. 121-123°) identified as 2:3-5:6-di-O-isopropylidene-D-mannofuranose. Concentration of the mother liquor yielded crystalline methyl 2:3-4:6-di-O-isopropylidene-α-D-mannoside (2·6 g.). The syrup (3·1 g.) remaining after extraction with light petroleum was examined by paper chromatography as in (b). Four components were identified, by comparison with controls, as mannose (trace; R_G 0·12); methyl α-mannopyranoside (R_G 0·30); 2:3-5:6-di-O-isopropylidene-mannofuranose (R_G 1·00); and methyl 2:3-4:6-di-O-isopropylidene-α-mannopyranoside (R_G 1·04). A fifth component, R_G 0·60 was not identified An attempt to separate the component of R_G 0·60 by fractional distillation in high vacuum was unsuccessful.

Preparation of Methyl 6-O-Toluene-p-sulphonyl-α-D-Mannopyranoside.

Methyl α-D-mannopyranoside (21 g.) was stirred with dry pyridine (200 ml.) for 30 minutes at room temperature, cooled to 0° and a solution of toluene-p-sulphonyl chloride (22 g., 1·05 equiv.) in pyridine (50 ml.) added with constant stirring and cooling during 9 hours. After stirring had continued for a further one hour at room temperature the mixture was cooled to 0° and water (200 ml.) added with vigorous stirring during 20 minutes. More water (300 ml.) was added and the solution extracted with chloroform (4 x 125 ml.). The chloroform extracts were washed twice with water, dried, and evaporated to a pale yellow syrup (28·7 g., 76% of theoretical).

Found: S, 9·2; $NaOSO_2C_7H_8$, 54·3.[57]

$C_{14}H_{21}O_8S$ requires S, 9·2; $NaOSO_2C_7H_8$, 55·8%.

Attempted Preparation of a Crystalline Acetal of Methyl 6-O-toluene-p-Sulphonyl-α-D-Mannopyranoside.

(a) Methyl 2:3-O-isopropylidene-6-O-toluene-p-sulphonyl-α-D-mannopyranoside.- Methyl 6-O-toluene-p-sulphonyl-α-D-mannopyranoside was condensed with acetone in the presence of anhydrous copper sulphate and acetaldehyde. The syrupy product gave a positive iodoform test for the isopropylidene group. This isopropylidene compound was acetylated, using acetic anhydride in pyridine, and benzoylated, using benzoyl chloride in pyridine, but in both cases the product failed to crystallise.

(b) Methyl 2:3-O-benzylidene-6-O-toluene-p-sulphonyl-α-D-mannopyranoside.- Methyl 6-O-toluene-p-sulphonyl-α-D-mannopyranoside was condensed with benzaldehyde in the presence of anhydrous zinc chloride as catalyst[64] and gave a product which did not crystallise.

Preparation of Methyl 2:3:4-Tri-O-benzoyl-6-O-Toluene-p-Sulphonyl-α-D-Mannoside.

Methyl α-D-mannopyranoside (107 g.) was converted into the 6-toluene-p-sulphonyl ester as described above and the latter dissolved in pyridine (400 ml.) and treated with benzoyl chloride (258 ml., 4 equiv.). The mixture was kept at room temperature for 24 hours, water (20 ml.) added, and the solution poured into saturated aqueous sodium bicarbonate (2 l.). The deep red gum thus obtained was washed with water (2 x 2 l.) by decantation, drained, and heated on the steam-bath with ethanol (300 ml.) until only a white solid remained. The solid was filtered off and after recrystallisation from chloroform-

ethanol gave colourless plates (I) (157 g., 50% of theoretical), m.p. 198°, $[\alpha]_D^{16}$ -104° (c, 1·2 in $CHCl_3$). (cf. Haskins, Hann, and Hudson[57], yield 37%, m.p. 197-199°, $[\alpha]_D^{20}$ -102·4° in $CHCl_3$)

Found: C, 63·5; H, 4·9; S, 5·1.

Calc. for $C_{35}H_{32}O_{11}S$: C, 63·6; H, 4·9; S, 4·9%.

Preparation of Methyl 2:3:4-Tri-O-benzoyl-6-Deoxy-6-Iodo-α-D-Mannoside

The above crystalline compound (I) (6·0 g.) and sodium iodide (6·0 g.) in dry acetone (100 ml.) were heated in a sealed tube at 100° for 2 hours[57]. After cooling, sodium toluene-p-sulphonate was filtered off, the filtrate evaporated, and the crystalline residue extracted with water. The dry residue (5·57 g.) was recrystallised from acetone as large prisms (II) (5.05 g., 90% of theoretical), m.p. 199-201°, $[\alpha]_D^{17}$ -106° (c, 1·0 in $CHCl_3$)

Found: C, 55·1; H, 4·1; I, 20·6.

Calc. for $C_{28}H_{25}O_8I$: C, 54·5; H, 4·1; I, 20·6%.

Attempted Replacement of the 6-Iodo Group by Hydroxyl

(a) Methyl 2:3:4-tri-O-benzoyl-6-deoxy-6-iodo-α-D-mannoside (II) was shaken for 7 days with dry silver oxide in dry benzene. On filtration and evaporation the starting material was recovered in good yield.

(b) The experiment was repeated using moist silver oxide and again unchanged material was recovered quantitatively.

(c) The iodo compound (II) and moist silver oxide in benzene were heated in a sealed tube at 100° for 2 hours. After filtration and evaporation of the solvent a dark syrup which failed to crystallise

and was strongly reducing to Fehling's solution was obtained.

Methyl 2:3:4-Tri-O-benzoyl-α-D-Mannoside 6-Nitrate

Methyl 2:3:4-tri-O-benzoyl-6-deoxy-6-iodo-α-D-mannoside (II) (13 g.) was dissolved in acetonitrile (200 ml.) and heated under reflux for 4 hours with powdered silver nitrate (4 g.). The cooled solution was treated with a solution of sodium iodide (1·0 g.) in acetone, the mixture filtered, the filtrate diluted with chloroform (300 ml.), and extracted with water (3 x 300 ml.). Evaporation of the dried chloroform layer gave a crystalline solid. By fractional crystallisation from ethanol unchanged starting material (6·2 g., m.p. 193°) was separated from the more soluble nitrate, which was obtained from 90% ethanol as large prisms (III) (5·5 g., 91% of theoretical), m.p. 103-104°, $[\alpha]_D^{16}$ -116° (c, 1·5 in $CHCl_3$).

Found: C, 61·1; H, 4·5; N, 2·5.

$C_{28}H_{25}O_{11}N$ requires C, 61·0; H, 4·6; N, 2·5%.

Methyl 2:3:4-Tri-O-benzoyl-α-D-Mannoside

The above nitrate (III) (2·0 g.), dissolved in glacial acetic acid (10 ml.) and benzene (20 ml.), was treated at room temperature with a mixture of equal quantities of zinc and iron powders until a portion of the solution no longer gave a pink coloration on treatment with diphenylbenzidine in concentrated sulphuric acid. The mixture was filtered, the residue washed with benzene, the benzene solution washed with water, dried, and evaporated to a crystalline solid (IV) (1·3 g., 71% of theoretical) which had, after recrystallisation from ethanol, m.p. 143°, undepressed on admixture with a specimen prepared by detritylation of the 6-trityl ether.

Attempted Removal of the Toluene-p-Sulphonyl Group by Hydrogenation

Unchanged material was recovered in good yield after attempted hydrogenation of (I) with Raney nickel in ethyl acetate under the following conditions:

(a) 30 p.s.i. H_2, 20°, 6 hours with shaking

(b) 30 p.s.i. H_2, 20°, 72 " " "

(c) 90 p.s.i. H_2, 20°, 72 " " "

(d) 1500 p.s.i. H_2, 20°, 12 " " stirring

(e) Mozingo conditions, 77°, 6 hours[58].

(f) " " 77°, 6 hours with stream of H_2.

When hydrogenation was carried out at 1500 p.s.i. pressure and 100° for 12 hours the product was a syrup, $[\alpha]_D^{18} \pm 0°$ (c, 1·0 in $CHCl_3$), which was non-reducing to Fehling's solution and gave a negative qualitative test for primary toluene-p-sulphonyl ester [the substance (10 mg.) and sodium iodide (10 mg.) in dry acetone (0·2 ml.) were heated in a sealed tube at 110° for 1 hour. No crystalline precipitate of sodium toluene-p-sulphonate was observed on cooling the tube].

Found: C, 63·0; H, 7·4; S, 2·4; OMe, 6·7%; equiv., 184.

$C_{28}H_{26}O_9$ requires C, 66·4; H, 5·2; OMe, 6·1%; equiv., 169

$C_{28}H_{44}O_9$ requires C, 64·1; H, 8·1; OMe, 5·8%; equiv., 175.

($C_{28}H_{26}O_9$ = Methyl 2:3:4-tri-O-benzoyl-α-D-mannoside.

$C_{28}H_{44}O_9$ = Methyl 2:3:4-tri-O-hexahydrobenzoyl-α-D-mannoside).

Preparation of Methyl 6-O-Triphenylmethyl-α-D-Mannopyranoside

Methyl α-D-mannopyranoside (4·0 g.) and triphenylchloromethane (8·0 g.) in pyridine (60 ml.) were heated on a boiling water-bath with occasional shaking for 3 hours[65]. The solution was allowed to cool,

water added to turbidity, and the mixture left overnight. Crystalline triphenylcarbinol was filtered off and the filtrate poured with stirring into ice-water. The powdery solid (V) which was obtained was stirred and washed by decantation with water until it was granular enough to be filtered off. Yield 9·5 g., 106% of theoretical. m.p. 84-124°.

A calcium chloride addition compound was prepared by adding a solution of the trityl ether in ethanol to a solution of anhydrous calcium chloride in ethanol. The crystalline solid obtained gave positive tests for calcium and chloride ions and for the trityl group and had m.p. 143-147°.

Attempted Condensation of Methyl 6-O-Trityl-α-D-Mannopyranoside with Acetone.

(a) Condensation of (V) with acetone in the presence of anhydrous copper sulphate and acetaldehyde gave a syrupy product which gave a negative iodoform test for the isopropylidene group.

(b) Methyl 6-O-trityl-α-D-mannopyranoside (V) was shaken with acetone, anhydrous copper sulphate, and acetaldehyde for 4 days. Concentrated sulphuric acid (0·05%, v/v) was added and shaking continued for 12 hours. After filtration, neutralisation with barium carbonate, re-filtration and evaporation, a syrupy product was obtained. By trituration with ethanol-light petroleum, crystals of triphenylcarbinol (m.p. 158°) were obtained. Evaporation of the mother liquor gave a syrupy product which gave a positive iodoform test for the isopropylidene group.

(c) Methyl 6-O-trityl-α-D-mannopyranoside (V) was treated as in (b)

except that the concentration of acid during the last 12 hours was 0·01% (v/v). Again, triphenylcarbinol (m.p. 152-154°) was obtained. Later crops of crystals (m.p. 138-142°; 78-81°; 78-80°) contained the trityl but not the isopropylidene group. The mother liquor was evaporated to a syrup which was extracted with water. Evaporation of the aqueous extracts gave a syrup from which methyl 2:3-4:6-di-O-isopropylidene-α-D-mannoside (m.p. 74°) was obtained.

Methyl 2:3:4-Tri-O-benzoyl-6-O-Triphenylmethyl-α-D-Mannoside

(a) Methyl 6-O-trityl-α-D-mannopyranoside (V) (50 mg.), dissolved in pyridine (0·7 ml.), was treated with benzoyl chloride (0·15 ml.) at room temperature. The crystalline solid which separated immediately was filtered off and washed with pyridine and then with ethanol. After recrystallisation from acetone the large prisms (60 mg., 70% of theoretical), m.p. 100-110°, gave positive qualitative tests for the trityl and benzoyl groups and were reducing to Fehling's solution after saponification and acid hydrolysis.

Found: C, 74·5; H, 5·9.

$C_{47}H_{40}O_9 \cdot C_3H_6O$ requires C, 74·3; H, 5·7%.

(b) Methyl α-D-mannopyranoside (105·5 g.), triphenylchloromethane (175 g.), and pyridine (1050 ml.) were heated at 50° with occasional shaking, until all solid had dissolved (6 hours). The solution was left at room temperature for 18 hours, after which benzoyl chloride (317 ml.) was added rapidly and without cooling. The mixture was set aside at room temperature for 24 hours and the crystalline solid filtered off and washed with pyridine (100 ml.), with ethanol (2 x 100 ml.), with water (3 l.), and again with ethanol (2 x 100 ml.). After

drying, the product was a colourless, crystalline solid (368 g., 82% of theoretical).

(i) With Pyridine of Crystallisation.- The product obtained above was found to contain pyridine of crystallisation, which was retained on recrystallisation from aqueous or ethanolic pyridine but lost on recrystallisation from acetone or from ethanol-chloroform. The substance (VI) showed a double melting point, 100-120°, recrystallising 120-125°, final m.p. 188-188·5°. $[\alpha]_D^{18}$ -110° (c, 1·3 in $CHCl_3$).

Found: C, 75.1; H, 5·3; N, 1·9.

$C_{47}H_{40}O_9 \cdot C_5H_5N$ requires C, 75.4; H, 5.5; N, 1·7%.

(ii) With Acetone of Crystallisation.- Recrystallisation of the above substance (VI) from acetone gave stout prisms, containing acetone of crystallisation, m.p. 100-115°, recrystallising 115-120°, final m.p. 187-188°, $[\alpha]_D^{20}$ -114° (c, 1·2 in $CHCl_3$).

Found: C, 74·2; H, 6·1.

$C_{47}H_{40}O_9 \cdot C_3H_6O$ requires C, 74·3; H, 5·7%.

(iii) With Chloroform of Crystallisation.- Recrystallisation of either of the above substances from ethanol-chloroform gave colourless plates, containing chloroform of crystallisation, m.p. 100-115°, recrystallising 115-120°, final m.p. 187-188°, $[\alpha]_D^{21}$ -107° (c, 1·1 in $CHCl_3$).

Found: C, 67·9; H, 5·0; Cl, 9·3.

$4C_{47}H_{40}O_9 \cdot 3CHCl_3$ requires C, 68·4; H, 4·9; Cl, 9·5%.

(iv) Without Solvent of Crystallisation.- When the substance

containing chloroform of crystallisation was dried in vacuo, the following losses of weight were observed:-

20°/15 mm./72 hours	: loss of weight,	0·08%
50°/15 mm./ 3 hours	: " " "	0.03%
100°/15 mm./ 2 hours	: " " "	1.10%
100°/15 mm./15 hours	: " " "	10.4%
($4C_{47}H_{40}O_9.3CHCl_3$ requires	" " "	10.7%).

The product was a fine white powder, m.p. 189-191°, $[\alpha]_D^{20}$ -121° (c, 1·1 in $CHCl_3$).

Found : C, 75·4; H, 5·3.

$C_{47}H_{40}O_9$ requires C, 75·4; H, 5·4%.

(c) Methyl α-D-mannopyranoside (4·35 g.), triphenylchloromethane (8·7 g.), and pyridine (43 ml.) were shaken at room temperature until solution was complete (11 days). Benzoyl chloride (13 ml.) was added and the mixture left at room temperature for 24 hours. The product (15·1 g., 81% of theoretical), isolated as in (b), had m.p. 100-115°, recrystallising 120°, final m.p. 187-188°.

(d) Methyl α-D-mannopyranoside (10·0 g.), triphenylchloromethane (20·0 g.), and pyridine (100 ml.) were heated on the boiling water-bath until all solid had dissolved (3¼ hours). The solution was cooled to room temperature, benzoyl chloride (30 ml.) added, and the mixture left at room temperature for 24 hours. The product (32 g., 75% of theoretical) had m.p. 105-115°, recrystallising 115°, final m.p. 187-188°.

Methyl 2:3:4-Tri-O-benzoyl-α-D-Mannoside

(a) Methyl 2:3:4-tri-O-benzoyl-6-O-trityl-α-D-mannoside (VI) (pyridine solvate) (20 g.) was shaken vigorously for 45 seconds with 10% (w/v) hydrogen bromide in glacial acetic acid (33 ml.) and filtered immediately into water (500 ml.)[66]. The aqueous mixture was extracted with chloroform (3 x 100 ml.) and the chloroform extracts washed with water, dried, and evaporated to a syrup. Trituration with ethanol gave a solid (2 g.) identified by m.p. (100-110°, 187-188°) and mixed m.p. as starting material. Evaporation of the mother liquor gave a crystalline solid (8·6 g., 79% of theoretical), m.p. 142-145°.

(b) When the above procedure was modified by shaking vigorously for 4 minutes the product was a syrup which was entirely soluble in ethanol. Evaporation of the ethanolic solution gave a crystalline solid (8·0 g. from 20·0 g., 65% of theoretical) m.p. 142-145°.

(c) Methyl 2:3:4-tri-O-benzoyl-6-O-trityl-α-D-mannoside (VI) (pyridine solvate) (20 g.) was shaken vigorously for 90 seconds with 10% (w/v) hydrogen bromide in glacial acetic acid (33 ml.) and filtered immediately through a sintered glass filter (porosity no. 1) into a mixture of water (1500 ml.) and chloroform (1000 ml.). Fourteen further portions (each 20 g.) were treated in the same way and all were filtered into the same chloroform-water. Finally the chloroform layer was separated and the aqueous layer extracted with chloroform (2 x 400 ml.). The combined chloroform extracts were washed with water until the wash liquid was neutral, dried, and evaporated to a crystalline solid which was dissolved in ethanol. After standing at

room temperature overnight, crystalline starting material (5 g., m.p. 95-120°, 187-189°) was filtered off. Evaporation of the mother liquor gave a crystalline solid (VII) (172 g., 95% of theoretical), which, after recrystallisation from aqueous ethanol, had m.p. 143-145°, $[\alpha]_D^{15}$ -160° (c, 1·0 in $CHCl_3$).

Found: C, 66·6; H, 5·3.

$C_{28}H_{26}O_9$ requires C, 66·4; H, 5·2%.

Methyl 2:3:4-Tri-O-benzoyl-α-D-Mannosiduronic Acid

A solution of methyl 2:3:4-tri-O-benzoyl-α-D-mannoside (VII) (150 g.) in acetone (1500 ml.), acetic acid (1500 ml.), and water (150 ml.) was stirred at room temperature, and potassium permanganate (160 g.) added in small portions over 30 hours[21]. Stirring was continued for a further 18 hours and the solution was then decolourised by the slow addition, with stirring, of aqueous potassium sulphite solution. 4N Sulphuric acid (150 ml.) was added, the solution extracted with chloroform (3 x 1000 ml.) and the chloroform extracts washed with water (6 x 5 l.), dried, and evaporated to a syrup (131 g., 85% of theoretical) which crystallised on storage. After recrystallisation from chloroform-light petroleum the product (VIII) had m.p. 180-181·5°, $[\alpha]_D^{15}$ -140° (c, 1·9 in $CHCl_3$).

Found: C, 64·5; H, 4·7.

$C_{28}H_{24}O_{10}$ requires C, 64·6; H, 4·6%.

Methyl 2:3:4-Tri-O-benzoyl-α-D-Mannosiduronic Methyl Ester

(a) A solution of methyl 2:3:4-tri-O-benzoyl-α-D-mannosiduronic

acid (VIII) (6·65 g.) in 2% methanolic hydrogen chloride (60 ml.) was kept at 20° for 24 hours. The crystalline solid (3·70 g.) which separated was filtered off, washed with methanol, and dried over potassium hydroxide. The filtrate and washings were neutralised with silver carbonate, filtered, and the neutral filtrate and washings evaporated to a crystalline solid (IX), which was washed with methanol (total yield, 5·75 g., 84% of theoretical). After recrystallisation from methanol the product had m.p. 143-144°.

(b) A solution of methyl 2:3:4-tri-O-benzoyl-α-D-mannosiduronic acid (VIII) (130 g.) in 0·5% methanolic hydrogen chloride (1500 ml.) was maintained at 20° for 72 hours. The resulting crystalline solid (80 g.) was filtered off, washed with methanol (100 ml.), and dried. The filtrate and washings were evaporated (15-20°/15 mm.) to a crystalline solid which was washed with methanol (total yield 123 g., 92% of theoretical). After recrystallisation from methanol as large prisms the product (IX) had m.p. 143-144°, $[\alpha]_D^{19}$ -127° (c, 0·6 in $CHCl_3$).

Found: C, 65·8; H, 5·2.

$C_{29}H_{26}O_{10}$ requires C, 65·1; H, 4·9%.

Methyl α-D-Mannopyranosiduronamide.

(a) Methyl 2:3:4-tri-O-benzoyl-α-D-mannosiduronic methyl ester (IX) (3·8 g.) was dissolved by vigorous shaking in a 0·2% solution of sodium in methanol (33 ml.)[61]. The solution was kept at room temperature until the rotation was constant ($[\alpha]_D^{18}$ +17°, 18 hours) and then passed slowly through a column (10 x 350 mm.) of cation

exchange resin (Amberlite IR-120-H). The eluate was evaporated to a syrup which was dissolved in 2% methanolic hydrogen chloride (100 ml.) and kept at room temperature for 24 hours. Neutralisation with silver carbonate, followed by evaporation of the neutral filtrate and washings, gave a syrup which was repeatedly extracted with dry ether. Methyl α-D-mannopyranosiduronic methyl ester remained as a colourless syrup (X) (1·2 g.), n_D^{19} 1·4842, $[\alpha]_D^{22}$ +80° (c, 0·5 in H_2O). Treatment of this methyl ester (0·20 g.) with methanolic ammonia at room temperature for 24 hours, followed by evaporation, gave a colourless syrup which crystallised on storage. After recrystallisation from ethanol-acetone, methyl α-D-mannopyranosiduronamide (XI) (0·11 g., 45% of theoretical yield from IX) had m.p. 182-183°, $[\alpha]_D^{15}$ +63° (c, 0·9 in H_2O).

(b) Methyl 2:3:4-tri-O-benzoyl-α-D-mannosiduronic methyl ester (IX) (100 g.) was dissolved in saturated methanolic ammonia (1100 ml.) and the solution allowed to stand at room temperature for 24 hours. Evaporation gave a syrup, from which benzamide was removed by repeated extraction with ether (250 ml. portions). The crystalline residue was washed once with acetone (100 ml.) and recrystallised from aqueous acetone as colourless prisms (XI) (20·1 g., 52% of theoretical), m.p. 182-183°, $[\alpha]_D^{18}$ +66° (c, 1·1 in H_2O).

Found: C, 41·0; H, 6·3; N, 6·6.

$C_7H_{13}O_6N$ requires C, 40·6; H, 6·3; N, 6·7%.

Methyl 2:3-O-Isopropylidene -α-D-Mannopyranosiduronamide.

(a) Methyl α-D-mannopyranosiduronic methyl ester (X) (0.20 g.)

was shaken with acetone (10 ml.), anhydrous copper sulphate (3 g.) and a small drop of acetaldehyde for four days. Sulphuric acid (0·03%) was added and shaking continued for a further 24 hours. The product, isolated in the usual manner, was a syrup (0.05 g.), n_D^{18} 1·4763, $[\alpha]_D^{18}$ +65° (c, 0·5 in H_2O). Treatment with methanolic ammonia at 0° for 48 hours gave a syrup which crystallised on storage. Methyl 2:3-O-isopropylidene-α-D-mannopyranosiduronamide (XII) (0·02 g., 9% of theoretical) had m.p. 177°, $[\alpha]_D^{15}$ +24° (c, 1·0 in H_2O).

(b) Methyl α-D-mannopyranosiduronamide (XI) (20 g.), anhydrous copper sulphate (100 g.), and acetone (750 ml.) were shaken for 2 days, concentrated sulphuric acid (0·22 ml.) added, and shaking continued for a further 2 days. The supernatant liquid was decanted off and neutralised with barium carbonate and the residual solids shaken for a further 2 days with acetone (500 ml.) containing 0·03% (v/v) sulphuric acid. When the volume of the combined neutral extracts was 4 l. the barium salts were filtered off, washed, and the filtrate and washings evaporated to a crystalline solid which was recrystallised from acetone-light petroleum as large, colourless prisms (XII) (15·0 g., 63% of theoretical), m.p. 177·5-179°, $[\alpha]_D^{15}$ +14° (c, 1·0 in H_2O).

Found: C, 49·0; H, 6·8; N, 5·1.

$C_{10}H_{17}O_6N$ requires C, 48·5; H, 6·9; N, 5·7%.

Methyl 4-O-Methyl-2:3-O-Isopropylidene-α-D-Mannosiduronic Methylamide

Methyl 2:3-O-isopropylidene-α-D-mannopyranosiduronamide (XII)

(1·00 g.) was methylated three times with methyl iodide (23 ml.) and silver oxide (12 g.), the first methylation being conducted in the presence of dry acetone (23 ml.). The final product, a colourless syrup (1·09 g., 93% of theoretical), n_D^{15} 1·4650, crystallised on storage and after recrystallisation from light petroleum, (XIII) had m.p. 151-153°, $[\alpha]_D^{18}$ +23° (c, 0·6 in H_2O).

Found: C, 52·4; H, 7·7; N, 5·3.

$C_{12}H_{21}O_6N$ requires C, 52·4; H, 7·6; N, 5·1%.

A sample of the above substance (XIII) was treated with 2N sodium hydroxide at 100° for 3 hours, the solution cooled to -5° and acidified by careful addition of 4N sulphuric acid, and the acid solution extracted with ice-cold chloroform. The chloroform extracts were washed with water and evaporated to a syrup which was esterified with ethereal diazomethane. Treatment of this methyl ester with ethanolic methylamine gave a crystalline product identical with the starting material.

Methyl 4-O-Methyl-α-D-mannosiduronic Methyl Ester.

Methyl 4-O-methyl-2:3-O-isopropylidene-α-D-mannosiduronic methylamide (XIII) (2·0 g.) was heated at 100° for 30 minutes with N sodium hydroxide (20 ml.). The solution was cooleed to -5°, acidified with 4N sulphuric acid (7 ml.), and the solution extracted with chloroform (4 x 50 ml.). The chloroform extracts were washed with water (3 x 50 ml.) and evaporated to a syrup which was dissolved in 1% methanolic hydrogen chloride and left at room temperature for 48 hours. Neutralisation with silver carbonate, followed by filtration and evaporation of the filtrate and washings gave a syrup (XIV)

(1·5 g.), n_D^{17} 1·4727, $[\alpha]_D^{19}$ +84° (c, 1·0 in H_2O).

Found: C, 45·3; H, 7·3.

$C_9H_{16}O_7$ requires C, 45·8; H, 6·8%.

Methylation of a small quantity of the above product with methyl iodide and silver oxide in two operations, followed by treatment with ethanolic methylamine, gave crystalline methyl 2:3:4-tri-O-methyl-α-D-mannosiduronic methylamide (XV), m.p. 103-105°, undepressed on admixture with a specimen prepared by methylation of methyl α-D-mannopyranosiduronamide.

4-O-Methyl (or 3-O-Methyl) -D-Mannaramide.

Methyl 4-O-methyl-α-D-mannosiduronic methyl ester (XIV) (0·097 g.) was heated in a sealed tube at 100° for 24 hours with N hydrochloric acid (5 ml.). Bromine (0·3 ml.) was added to the cooled solution and the mixture left at 40° for 7 days. Bromine was removed by aeration, and water and hydrohalic acids by repeated distillation with ethanol under reduced pressure. The residue was treated with 1% methanolic hydrogen chloride at 30° for 48 hours and the solution neutralised, filtered, and evaporated to a syrup which was distilled (b.p. 160-170°/0·1 mm.). Treatment of the distillate with methanolic ammonia gave a crystalline diamide (0·030 g.), m.p. 192-193°, $[\alpha]_D^{15}$ -16° (c, 0·6 in H_2O).

The compound is being analysed.

Found : C, 38.0; H, 6.2; N, 12.5.

$C_7H_{14}O_6N_2$ requires C, 37.8; H, 6.4; N, 12.6%

Methyl 2:3:4-Tri-O-methyl-α-D-Mannosiduronic Methylamide.

Methyl α-D-mannopyranosiduronamide (XI) (1·99 g.) was methylated

three times with methyl iodide (23 ml.) and silver oxide (12 g.), dioxan being added as a solvent in the first operation. The product was a colourless syrup (2·618 g., 103% of theoretical), n_D^{15} 1·4661, which crystallised on trituration with light petroleum. After recrystallisation from light petroleum the methylamide (XV) had m.p. 103-105°, $[\alpha]_D^{19}$ +42° (c, 1·4 in H_2O).

Found: C, 50·4; H, 8·0; N, 4·8.

$C_{11}H_{21}O_6N$ requires C, 50·2; H, 8·1; N, 5·3%.

A sample of the above substance was treated with 2N sodium hydroxide at 100° for 3 hours, the solution acidified with sulphuric acid, exhaustively extracted with chloroform and the chloroform extracts washed with water. Evaporation of the chloroform extracts gave an acid syrup which was esterified by means of 1% methanolic hydrogen chloride at room temperature for 48 hours. Treatment of the resulting syrup with ethanolic methylamine gave in good yield a methylamide, m.p. 103-105°, undepressed on admixture with a specimen of the original substance (XV).

2:3:4-Tri-O-methyl-D-Mannaramide

Methyl 2:3:4-tri-O-methyl-α-D-mannosiduronic methylamide (XV) (0·10 g.) was saponified with 2N sodium hydroxide at 100° for 1 hour, the solution acidified, exhaustively extracted with chloroform, and the chloroform extracts evaporated. The residue was heated with N hydrochloric acid (5 ml.) at 100° for 24 hours, and bromine, water, and volatile acids removed in the manner already described. The residue was esterified with 1% methanolic hydrogen chloride (5 ml.)

at 30° for 24 hours, and the product, isolated in the usual manner, distilled in high vacuum (b.p. 130-140°/0.1 mm.). The distillate, on treatment with methanolic ammonia, gave a crystalline diamide (0·030 g.), m.p. 211° (decomp.), unchanged by repeated recrystallisation from methanol and undepressed on admixture with a specimen of the synthetic product of Haworth, Hirst, Isherwood, and Jones[73], which itself was found to have a m.p. 211°, on the Kofler apparatus or in a capillary tube.

Attempted Preparation of Methyl 2:3-O-Isopropylidene-4-O-Toluene-p-Sulphonyl-α-D-Mannosiduronamide.

(a) Methyl 2:3-O-isopropylidene-α-D-mannopyranosiduronamide (XII) (0·99 g.) in pyridine (20 ml.) containing "Drierite" (13 g.) was treated with toluene-p-sulphonyl chloride (1·7 g.) under exactly the conditions used for the preparation of methyl 3:4-O-isopropylidene-2-O-toluene-p-sulphonyl-α-D-galactosiduronic methyl ester. No precipitate was obtained on addition of water, and chloroform extraction gave only a small amount (15-20 mg.) of a syrup.

The preparation was repeated twice, with the same result.

(b) A solution of XII (0.95 g.) in pyridine (20 ml.) containing "Drierite" (12 g.) was treated with toluene-p-sulphonyl chloride (1·6 g.) in pyridine (10 ml.) at room temperature for 48 hours. The mixture was filtered, the filtrate treated with water (0·10 ml.) and poured slowly, with stirring, into ice-water. A small quantity (20 mg.) of a powdery solid was obtained, which after recrystallisation from ethanol, had m.p. 118-120°, $[\alpha]_D^{15}$ ±0° (c, 2.0 in $CHCl_3$). A carbazole test for uronic acid was positive.(90)

Found: C, 52·9; H, 5·8; N, 4·3; S, 9·3.

$C_{17}H_{23}O_8NS$ requires C, 50·9; H, 5·8; N, 3·5; S, 8·0%.

(c) A solution of XII (0.45 g.) in acetone (3 ml.) containing toluene-p-sulphonyl chloride (0·36 g.) and pyridine (0·15 ml.) was kept at room temperature for 14 days. Samples were removed daily, poured into water, and the aqueous solution extracted with chloroform. No chloroform-soluble product was obtained.

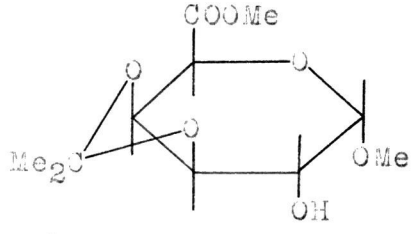
(d) A similar experiment conducted at 30° for 10 days also gave negative results.

(e) XII (1·110 g.) and toluene-p-sulphonyl chloride (1·115 g.) were dissolved in pyridine (35 ml.) containing "Drierite" (12 g.). Samples (3 ml.) were withdrawn after 1 and 2 days at 0°, after an additional 1, 2, 4, and 8 days at room temperature, and after a further 1, 5, and 10 days at 30°. Each sample was poured with stirring into ice-water: from the third sample 4-5 mg. of a powdery solid was obtained. All other samples gave negligible quantities of solid product on pouring into ice-water.

DERIVATIVES OF D-GALACTURONIC ACID.

DISCUSSION

In principle, 2-methyl-D-galacturonic acid may be prepared by methylation of any derivative of D-galacturonic acid with a free hydroxyl at C2, while for the preparation of the 3:4-dimethyl ether a derivative having free hydroxyl groups at positions 3 and 4 is required. Methyl 3:4-isopropylidene-α-D-galactosiduronic methyl ester, which was prepared by Jones and Stacey in 1947,[33] appeared



to be a suitable intermediate, since methylation followed by hydrolysis of the isopropylidene group would give the 2-methyl ether, while the 3:4-dimethyl ether could be prepared by suitable masking of the hydroxyl at C2, followed by hydrolytic removal of the isopropylidene residue, methylation, and removal of the blocking group.

For their synthesis of methyl 3:4-isopropylidene-α-D-galactosiduronic methyl ester, Jones and Stacey[33] prepared D-galacturonic acid both by enzymic hydrolysis of pectin and by synthesis from D-galactose.[21] Since the yields obtained by Stacey's synthetic method were low, and in the present work the enzymic method was not convenient, other synthetic routes were investigated. Ault, Haworth, and Hirst in 1935[41] described the preparation of methyl 3:4-

isopropylidene-α-D-galactoside and its oxidation with alkaline permanganate to the potassium salt of methyl 3:4-isopropylidene-α-D-galactosiduronic acid, which was isolated as an amorphous powder. In the present work, as a preliminary experiment, potassium methyl 3:4-isopropylidene-α-D-galactosiduronate, prepared according to the method of Ault, Haworth, and Hirst, was acidified and immediately esterified with diazomethane. From the resulting mixture, crystalline methyl 3:4-isopropylidene-α-D-galactosiduronic methyl ester was isolated in an overall yield from D-galactose of 8% of theoretical. In another preliminary experiment methyl α-D-galactopyranoside[42] was subjected to catalytic dehydrogenation by means of platinum black in the presence of oxygen.[43,44] The reaction did not appear to be complete even after 24 hours, however, and after acidification and esterification the yield of methyl α-D-galactopyranosiduronic methyl ester was only 11% of theoretical.

The scheme which finally proved to be most satisfactory was a modification of that used by Link and his co-workers,[45] following earlier work by Ohle and collaborators[46]. D-Galactose was condensed with acetone to give the 1:2-3:4-diisopropylidene derivative which was oxidised in dilute aqueous solution with alkaline potassium permanganate to potassium 1:2-3:4-diisopropylidene-D-galacturonate. Link had previously prepared methyl α-D-galactopyranosiduronic methyl ester by treatment of this potassium salt with boiling methanolic hydrogen chloride which effected simultaneous removal of the potassium ion, hydrolysis of the isopropylidene groups, esterification, and glycoside formation. In the presnt work, however, certain modifications were introduced. 1:2-3:4-Diisopropylidene-D-galacturonic acid

was prepared in good yield by treatment of the potassium salt with cation exchange resins. Thereafter, removal of isopropylidene residues, esterification, and glycoside formation were effected with either hydrogen chloride or cation exchange resins as catalysts. In both cases, the product of first treatment was a mixture of isomeric glycosides from which about 33% of the crystalline α-pyranoside was isolated. Several re-treatments of the mother liquor were therefore necessary to obtain a satisfactory yield (66% of theoretical). Condensation with acetone in the presence of anhydrous copper sulphate[41] gave methyl 3:4-isopropylidene-α-D-galactosiduronic methyl ester in an overall yield from D-galactose of 29% of theoretical. Jones and Stacey[33] prepared this substance in a yield of 7,8% (calculated from the yield quoted by Stacey[21] for his synthetic D-galacturonic acid).

For the methylation of the free hydroxyl group at C2, the Purdie technique, using silver oxide and methyl iodide, was preferred to the Haworth method, since the alkali used in the latter would cause saponification of the ester grouping. The product, methyl 2-methyl-3:4-isopropylidene-α-D-galactosiduronic methyl ester, was a syrup which was easily distilled in high vacuum and gave a crystalline amide. Jones and Stacey[33] removed the isopropylidene residue from their methylated product by means of hot acetic acid. Their product, however, had a low methoxyl content, presumably due to partial hydrolysis of the ester grouping, and even after re-esterification the methoxyl content was still low. A more satisfactory procedure was therefore sought, and the use of cation exchange resins in moist methanol at comparatively low temperatures (15°, 40°) was found to be entirely

satisfactory. The products were neutral, non-reducing to Fehling's solution, contained no isopropylidene residue, and had a high positive rotation ($[\alpha]_D$ +113°). The use of boiling methanolic hydrogen chloride gave a product which differed in having a much lower rotation ($[\alpha]_D$ +4°), presumably owing to the presence of a considerable proportion of the furanosides, as was observed by Luckett and Smith[13] in the case of 2:3-dimethyl-D-galacturonic acid. Methyl 2-methyl-α-D-galactopyranosiduronic methyl ester was a distillable syrup which gave a crystalline amide. Hydrolysis with dilute mineral acid gave 2-methyl-D-galacturonic acid, which was oxidised with bromine water to the corresponding glycaric acid, isolated as the crystalline diamide. Direct nitric acid oxidation of methyl 2-methyl-3:4-isopropylidene-α-D-galactosiduronic methyl ester, followed by esterification and ammonolysis gave 2-methyl-D-galactaramide, identical with the product obtained through bromine oxidation. The melting point of this diamide was 205° (decomp.), whereas Jones and Stacey[33] reported a melting point of 200° (decomp.) for their synthetic product. Brown, Hirst, and Jones[47] obtained a diamide, m.p. 195° by oxidation of 2-methyl-D-galacturonic acid isolated from methylated cholla gum, while Hough and Jones[48] isolated a monomethylgalactaramide of m.p. 207° from the methylated gum of Sterculia Setigera.

Experiments on the stability of methyl 2-methyl-α-D-galactosiduronic methyl ester in aqueous solution to cation exchange resins indicated that the pyranosiduronic linkage is at least eighty times as stable at room temperature as the glycosidic linkage in a typical methyl hexopyranoside.

In preliminary work on the synthesis of 3:4-dimethyl-D-galacturonic acid, several attempts were made to prepare 1:2-isopropylidene-D-galacturonic acid by partial hydrolysis of the 1:2-3:4-diisopropylidene acid, by analogy with the preparation of 1:2-isopropylidene-D-galactose from 1:2-3:4-diisopropylidene-D-galactose.[49] No mono-isopropylidene derivative could be isolated, however, and there was no evidence that one acetal group was more rapidly hydrolysed than the other.

For the preparation of the 3:4-dimethyl ether, it was, therefore, necessary to substitute position 2 of methyl 3:4-isopropylidene-α-D-galactosiduronic methyl ester prior to hydrolysis of the isopropylidene group and subsequent methylation. The toluene-p-sulphonyl group appeared to be suitable,[50] since it is relatively stable to acid hydrolysis, is non-migratory, and may be removed without difficulty. Treatment of methyl 3:4-isopropylidene-α-D-galactosiduronic methyl ester with toluene-p-sulphonyl chloride in pyridine under the usual conditions,[51] however, led to a very low yield (6-7%) of crystalline product. After a number of experiments, a satisfactory yield (85%) was obtained by careful purification of the reagents and by allowing the reaction to proceed for a prolonged period at a relatively high temperature in the presence of an internal desiccant. In addition, the crystalline product was isolated by careful addition of water to the pyridine solution instead of pouring the latter into water. The apparently low reactivity of this substituted uronic acid is difficult to explain, since, in a glycoside, position 2 is normally the most reactive of the secondary groups.[52] Hydrolytic removal of the isopropylidene group was satisfactorily accomplished with either

hydrogen chloride or cation exchange resins in moist methanol, and two interesting points emerge from a comparison of this reaction with the removal of the isopropylidene groups from methyl 2-methyl-3:4-isopropylidene-α-D-galactosiduronic methyl ester. Firstly, hydrolysis with resins is much slower in the case of the 2-toluene-p-sulphonyl derivative, probably owing to the bulkiness of the arylsulphonyl group. Secondly, whereas the use of boiling methanolic hydrogen chloride on the 2-methyl derivative gave a product of comparatively low specific rotation, in the case of the 2-toluene-p-sulphonyl derivative the product was a crystalline substance having a high positive rotation. This may be a further example of the stabilisation of glycosides by a toluene-p-sulphonyl group at C2 recorded by Percival and Zobrist.(53) By methylation with methyl iodide and silver oxide, crystalline methyl 3:4-dimethyl-2-toluene-p-sulphonyl-α-D-galactosiduronic methyl ester was obtained in good yield. Toluene-p-sulphonyl groups are generally removed by reductive fission with sodium amalgam,(50) after which the methylated sugar may generally be extracted with chloroform. In the present instance, however, the expected products of the treatment with sodium amalgam were the sodium salts of methyl 3:4-dimethyl-α-D-galactosiduronic acid and toluene-p-sulphinic acid. Since the solubilities of these sodium salts or of the free acids appeared to be similar, separation was accomplished by absorbing both acids on an anion exchange resin and subsequently eluting the weaker uronic acid with a suitable acid eluant. A dilute methanolic solution of formic acid was effective and had the advantage of being easily removed from the eluant by evaporation. After reductive fission

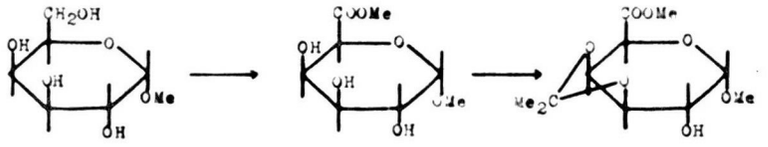
with sodium amalgam and separation of the acids in this manner, methyl 3:4-dimethyl-α-D-galactosiduronic acid was obtained in 87% yield as a crystalline solid. Esterification with methanolic hydrogen chloride at room temperature or with diazomethane gave a crystalline methyl ester, in an overall yield from D-galactose of 19% of theoretical. Treatment of the methyl ester with methanolic ammonia gave the crystalline amide in rather poor yield: in contrast, with methanolic methylamine, the crystalline methylamide was obtained in quantitative yield.

Methyl 3:4-dimethyl-α-D-galactosiduronic methyl ester was hydrolysed by means of aqueous acid to 3:4-dimethyl-D-galacturonic acid which was oxidised with bromine water. The oxidation, followed polarimetrically, was complete after 11 days at 30°. Removal of water and hydrogen bromide by repeated distillation with ethanol gave the crystalline diethyl ester of 3:4-dimethylgalactaric acid. The dimethyl ester and diamide were also obtained as crystalline solids. All three galactaric acid derivatives were optically inactive, as would be expected in symmetrically substituted derivatives of galactaric acid. The original dimethyl galacturonic acid, therefore could have been the 2:5 or the 3:4-dimethyl ether. The 2:5 possibility was eliminated by the observation that the dimethyl galactaramide gave a positive Weermann test,[54] and further evidence for the 3:4-substitution was furnished by the isolation of methyl 2:3:4-trimethyl-α-D-galactosiduronic methyl ester after methylation with the Purdie reagents.

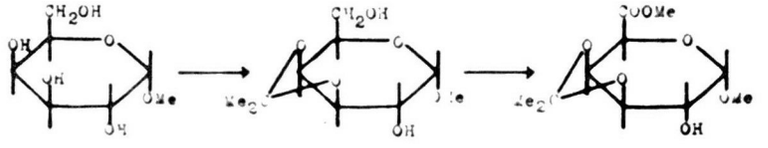
DERIVATIVES OF D-GALACTURONIC ACID

METHYL 3:4-ISOPROPYLIDENE-α-D-GALACTOSIDURONIC METHYL ESTER

(a)

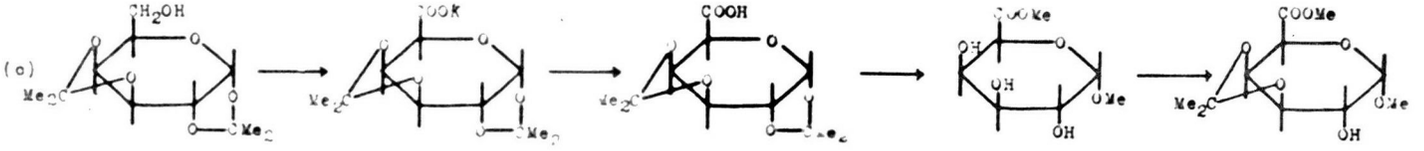


Methyl α-D-Galactoside — Methyl α-D-Galactosiduronic Methyl Ester — Methyl 3:4-Isopropylidene-α-D-galactosiduronic Methyl Ester



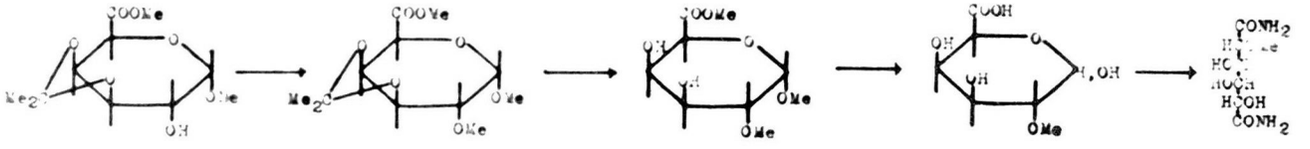
Methyl α-D-Galactoside — Methyl 3:4-Isopropylidene-α-D-galactoside — Methyl 3:4-Isopropylidene-α-D-galactosiduronic Methyl Ester

(c)



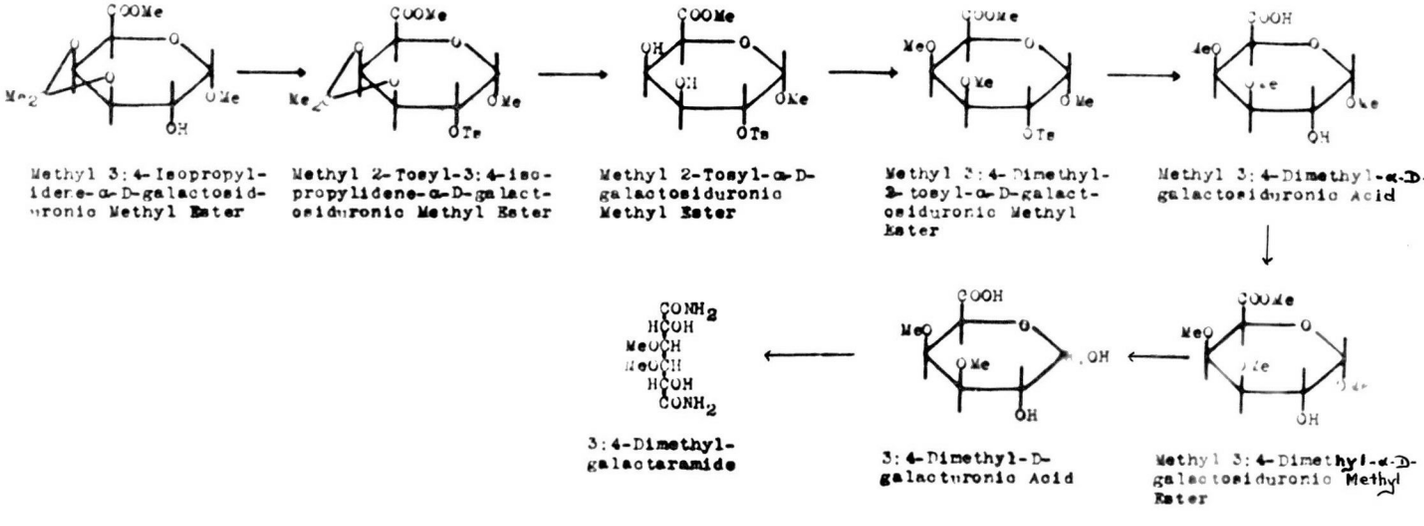
1:2-3:4-Diisopropylidene-D-galactose — Potassium 1:2-3:4-Diisopropylidene-D-galacturonate — 1:2-3:4-Diisopropylidene-D-galacturonic Acid — Methyl α-D-Galactosiduronic Methyl Ester — Methyl 3:4-Isopropylidene-α-D-galactosiduronic Methyl Ester

METHYL 2-METHYL-α-D-GALACTOPYRANOSIDURONIC METHYL ESTER



Methyl 3:4-Isopropylidene-α-D-galactosiduronic Methyl Ester — Methyl 2-Methyl-3:4-isopropylidene-α-D-galactosiduronic Methyl Ester — Methyl 2-Methyl-α-D-galactosiduronic Methyl Ester — 2-Methyl-D-galacturonic Acid — 2-Methyl-D-galactaramide

METHYL 3:4-DIMETHYL-α-D-GALACTOSIDURONIC METHYL ESTER



Methyl 3:4-Isopropylidene-α-D-galactosiduronic Methyl Ester — Methyl 2-Tosyl-3:4-isopropylidene-α-D-galactosiduronic Methyl Ester — Methyl 2-Tosyl-α-D-galactosiduronic Methyl Ester — Methyl 3:4-Dimethyl-2-tosyl-α-D-galactosiduronic Methyl Ester — Methyl 3:4-Dimethyl-α-D-galactosiduronic Acid

3:4-Dimethyl-galactaramide — 3:4-Dimethyl-D-galacturonic Acid — Methyl 3:4-Dimethyl-α-D-galactosiduronic Methyl Ester

EXPERIMENTAL

Methyl α-D-Galactopyranosiduronic Methyl Ester by Catalytic Dehydrogenation of Methyl α-D-Galactopyranoside.

Methyl α-D-galactopyranoside was prepared according to the method of Robertson and Lamb[42] by refluxing D-galactose (25 g.) with 1% methanolic hydrogen chloride (200 ml.) for 10 hours. After neutralisation, filtration and evaporation the product (14 g., 50% of theoretical) had m.p. 109-110°.

To a solution of methyl α-D-galactopyranoside (3·5 g.) in water (100 ml.) was added sodium bicarbonate (0·40 g.) and the catalyst, platinum black on activated charcoal (Darco G60) (0·5 g.), prepared in the manner described by Mehltretter.[43] A stream of oxygen purified by passage through sulphuric acid and water was bubbled into the vigorously stirred mixture at 50°. When the pH had fallen from 9·0 (initially) to 7·0 (2 hours), further sodium bicarbonate (0·40 g.) was added. After 10 hours, when the pH was 7·0-7·5, a further portion (0·40 g.) of bicarbonate was added. After 24 hours (pH 7·0-7·5) the catalyst was filtered off and washed briefly with distilled water (10 ml.). The filtrate and washings were passed slowly through a column of cation exchange resin (Amberlite IR-100-H), evaporated to 20 ml., and neutralised with ethereal diazomethane. Evaporation gave a yellow syrup from which by trituration with ethanol a crystalline solid (0·50 g., 11% of theoretical) was obtained. This was identified by m.p. (147°) and mixed m.p. as methyl α-D-galactopyranosiduronic methyl ester monohydrate.

Methyl 3:4-O-Isopropylidene-α-D-Galactosiduronic Methyl Ester from Methyl α-D-Galactopyranoside.

Methyl α-D-galactopyranoside[42] (5·0 g.) was condensed with acetone by the method of Percival and Percival.[51] The product, a viscous syrup (5 g.), was dissolved in water (250 ml.) and treated with potassium hydroxide (3·6 g.) and potassium permanganate (9·7 g.) for 24 hours at room temperature. The mixture was filtered, decolourised with potassium metabisulphite solution, acidified with hydrochloric acid (2N; 33 ml.) and neutralised with ethereal diazomethane. Evaporation of the dried ethereal layer gave a syrup which partly crystallised. After separation and recrystallisation from acetone-light petroleum the crystalline product (0·52 g., 8% of theoretical) was identified by m.p. (107-108°) and mixed m.p. as methyl 3:4-O-isopropylidene-α-D-galactosiduronic methyl ester.

1:2-3:4-Di-O-isopropylidene-D-Galactose.

D-Galactose (80·0 g.) was converted into 1:2-3:4-di-O-isopropylidene-D-galactose by shaking at room temperature for 30 hours with acetone containing 3% (v/v) of concentrated sulphuric acid, as described by Ohle and Berend.[46] The mixture was filtered (weight of recovered galactose, 30·0 g.), the filtrate was neutralised with sodium carbonate, filtered again, and the combined neutral filtrate and washings were evaporated to a syrup which was distilled. The colourless product (55·3 g., 76% of theoretical) had b.p. 130-170°/ 0·03 mm., n_D^{25} 1·4657, $[\alpha]_D^{19}$ -51° (c, 1·2 in H_2O).

Found: Me_2CO, 44·9. Calc.for $C_{12}H_{20}O_6$: Me_2CO, 44·6%.

Potassium 1:2-3:4-Di-O-isopropylidene-D-Galacturonate.

A solution of 1:2-3:4-di-O-isopropylidene-D-galactose (55·0 g.) in water (5 l.) containing potassium hydroxide (26 g.) was treated with potassium permanganate (74 g.), according to the conditions of Ohle and Berend.[46] After removal of manganese dioxide the pink filtrate was decolourised by careful addition of potassium metabisulphite solution and finally neutralised with carbon dioxide. The white solid obtained by evaporating the solution was extracted with ethanol (3 x 500 ml.) under reflux. On concentrating the extracts to about 150 ml., potassium 1:2-3:4-di-O-isopropylidene-D-galacturonate monohydrate (I) crystallised as colourless needles (48·0 g., 70% of theoretical), m.p. 200° (decomp.), $[\alpha]_D^{21}$ -70° (c, 2·0 in H_2O).

Found: C, 43·3; H, 5·5; Me_2CO, 36·0.

Calc. for $C_{12}H_{17}O_7K.H_2O$: C, 43·6; H, 5·8; Me_2CO, 35·2%.

1:2-3:4-Di-O-isopropylidene-D-Galacturonic Acid.

(a) Preliminary Experiment.- An aqueous solution of potassium 1:2-3:4-di-O-isopropylidene-D-galacturonate monohydrate (4·0 g.) was shaken vigorously with cation exchange resin (Amberlite IR-100-H) (6 g.) at room temperature for three hours. The solution was filtered and the filtrate treated as before with a fresh portion of resin (6 g.). The product (3·0 g., 86% of theoretical) obtained on evaporation of the filtrate had m.p. 157° and contained no potassium ion.

(b) Large Scale Preparation.- Potassium 1:2-3:4-di-O-isoproplydene-D-galacturonate monohydrate (16·0 g.) in water (200 ml.) was shaken for

six hours at room temperature with cation exchange resin (Amberlite IR-100-H) (24 g.). The mixture was filtered and the filtrate passed slowly through a column (250 x 18 mm.) of the same resin. Concentration of the eluate gave 1:2-3:4-di-O-isopropylidene-D-galacturonic acid monohydrate as large colourless crystals (12·7 g., 91% of theoretical), m.p. 158-159°, $[\alpha]_D^{17}$ -79° (c, 0·9 in $CHCl_3$). (Niemann and Link[45] record m.p. 158°, $[\alpha]_D$ -84° for 1:2-3:4-di-O-isopropylidene-D-galacturonic acid).

Found: C, 50·1; H, 6·9; Me_2CO, 40·0; CO_2, 16·1.
Calc. for $C_{12}H_{18}O_7.H_2O$: C, 49·3; H, 6·9; Me_2CO, 39·8; CO_2, 15·1%.

Attempted Preferential Hydrolysis of the 3:4-O-Isopropylidene Residue from 1:2-3:4-Di-O-isopropylidene-D-Galacturonic Acid.

The course of hydrolysis of 1:2-3:4-di-O-isopropylidene-D-galacturonic acid in 2% aqueous solution containing 0·05% and 0·2% sulphuric acid at room temperature was followed polarimetrically. In both cases the graph of rotation against time showed no break in the curve and no evidence that one group was hydrolysed more easily than the other. 1:2-3:4-Di-O-isopropylidene-D-galacturonic acid was shaken in moist acetone with cation exchange resin (Amberlite IR-100-H) and portions of the solution removed at intervals of one day and evaporated to dryness. From the first six samples only starting material (m.p. 157°) was isolated; from the next eight the latter and D-galacturonic acid (m.p. 108°) were both obtained; after 14 days only D-galacturonic acid was present. In another experiment 1:2-3:4-di-O-isopropylidene-D-galacturonic acid monohydrate was shaken in dry chloroform with dry cation exchange resin (Amberlite IR-100-H) for 28 days. After

filtration and evaporation of the filtrate, crystalline D-galacturonic acid was obtained: no other product could be detected.

Methyl α-D-Galactopyranosiduronic Methyl Ester.

(a) Potassium 1:2-3:4-di-O-isopropylidene-D-galacturonate monohydrate (I) (4·8 g.) was refluxed with 2% (w/v) methanolic hydrogen chloride (100 ml.) for 10 hours.[45] After cooling, potassium chloride, which had appeared on the addition of hydrogen chloride solution, was removed by filtration, the filtrate concentrated, and further potassium chloride removed. The filtrate was neutralised with silver carbonate and evaporated to a dark-coloured syrup which partly crystallised on trituration with ethanol. The crystals (0·40 g., 12% of theoretical), m.p. 136-137°, were contaminated with potassium and silver chlorides. The mother liquors were dark-coloured and on standing for several weeks failed to deposit crystals.

(b) The potassium chloride produced by the addition of 2% methanolic hydrogen chloride (100 ml.) to potassium 1:2-3:4-di-O-isopropylidene-D-galacturonate monohydrate (I) (3·8 g.) was removed immediately by filtration and the filtrate refluxed for 10 hours. The solution was neutralised with silver carbonate, filtered, and the filtrate and washings evaporated to a partly crystalline syrup. The crystals (0·7 g., 26% of theoretical), m.p. 136-137°, were again contaminated with inorganic material. The residual syrup was brown in colour and had $[\alpha]_D^{20}$ -13° (c, 0·8 in H_2O).

(c) 1:2-3:4-Di-O-isopropylidene-D-galacturonic acid monohydrate (I) (1·8 g.) was refluxed for 9 hours with 1% methanolic hydrogen chloride (60 ml.). Ethereal diazomethane was added to the cooled solution until a faint yellow colour persisted. After standing for 45 minutes, the solution was evaporated, giving a yellow syrup which crystallised on trituration with ethanol. The crystals (0·5 g., 34% of theoretical), m.p. 134-136° were pale yellow in colour. On evaporation of the mother liquor a yellow, viscous, syrup (0·9 g., 61% of theoretical) was obtained.

(d) 1:2-3:4-Di-O-isopropylidene-D-galacturonic acid monohydrate (I) (15·3 g.) was refluxed for 10 hours with 1% methanolic hydrogen chloride (250 ml.) and the solution neutralised with silver carbonate. Filtration, followed by evaporation of the filtrate, gave a pale yellow syrup which crystallised on trituration with ethanol. The crystals (4·2 g.) were filtered off and the filtrate evaporated to a syrup which was treated again with 1% methanolic hydrogen chloride (200 ml.) for 10 hours. After four such treatments the total yield of crystalline material, m.p. 145°, was 8·3 g. (66% of theoretical).

(e) 1:2-3:4-Di-O-isopropylidene-D-galacturonic acid monohydrate (I) (13·9 g.) was boiled for 24 hours with dry methanol (200 ml.) containing dry washed cation exchange resin (Amberlite IR-100-H) (14 g.). The resin was removed by filtration and washed with methanol and the filtrate and washings evaporated to a colourless syrup from which a crystalline solid (3·6 g.) was obtained by trituration with ethanol. The mother liquor was evaporated to a nearly colourless syrup which was dissolved in methanol (150 ml.) and re-

treated with the same batch of resin. After five treatments with the same sample of resin the total yield of crystalline products was 7·48 g. (66% of theoretical). After recrystallisation from ethanol, methyl α-D-galactopyranosiduronic methyl ester monohydrate (II) had m.p. 145°, $[\alpha]_D^{16}$ +121° (c, 1·0 in H_2O). (Jones and Stacey[33] record m.p. 147°, and Niemann and Link[45] $[\alpha]_D$ +121° for the anhydrous substance.

Found: C, 39·9; H, 6·6; OMe, 26·5.

Calc. for $C_8H_{14}O_7.H_2O$: C, 40·0; H, 6·7; OMe, 25·8%.

Methyl 3:4-O-Isopropylidene-α-D-Galactosiduronic Methyl Ester.

(a) Methyl α-D-galactopyranosiduronic methyl ester monohydrate (II) (1·20 g.) was shaken for 72 hours with dry acetone (50 ml.), anhydrous copper sulphate (12 g.), and acetaldehyde (1 drop), as described for methyl α-D-rhamnoside by Percival and Percival.[51] Sulphuric acid (0·05 ml.) was added and shaking continued for a further 24 hours. The mixture was filtered, the copper salts extracted with dry acetone (4 x 30 ml.), the filtrate and washings neutralised with barium carbonate, and the neutral filtrate and washings evaporated to a yellow solid which was recrystallised from acetone-light petroleum as colourless needles, m.p. 109-110° (1·15 g., 81% of theoretical).

(b) Methyl α-D-galactopyranosiduronic methyl ester monohydrate (II) (3·60 g.) was shaken for 120 hours with dry acetone (250 ml.), anhydrous copper sulphate (30 g.), and acetaldehyde (2 drops).[41] Evaporation of the filtered solution and washings gave a white solid which was recrystallised from light petroleum (1100 ml.) as colourless

needles (III) (3·62 g., 94% of theoretical), m.p. 113-114°, $[\alpha]_D^{18}$ +117° (c, 1·2 in H_2O). (Jones and Stacey[33] record m.p. 107°, $[\alpha]_D^{20}$ +118° for this substance).

Found: C, 50·8; H, 6·7; OMe, 23·2.

Calc. for $C_{11}H_{18}O_7$: C, 50·4; H, 6·9; OMe, 23·7%.

Methyl 2-O-Methyl-3:4-O-Isopropylidene-α-D-Galactosiduronic Methyl Ester.

(a) Methyl 3:4-O-isopropylidene-α-D-galactosiduronic methyl ester (III) (0·63 g.) was dissolved in the minimum quantity of acetone, methyl iodide (23 ml.) added, and the solution refluxed gently on a water bath. Dry silver oxide (12 g.) was added in small (1 g.) portions every 30 minutes, with frequent shaking. The mixture was filtered, the silver residues extracted with acetone, the filtrate and washings evaporated, and the resulting syrup (n_D^{16} 1·4622) kept in vacuo overnight. After one further treatment, using methyl iodide (23 ml.) alone as solvent, the product was a pale yellow viscous syrup (0·55 g., 83% of theoretical), n_D^{17} 1·4630.

Found: OMe, 33·3. Calc. for $C_{12}H_{20}O_7$: OMe, 33·7%.

(b) Methyl 3:4-O-isopropylidene-α-D-galactosiduronic methyl ester (III) (3·80 g.) was methylated three times by the procedure described above, using 33 ml. methyl iodide and 20 g. of silver oxide for each operation. The product (3·24 g., 87% of theoretical) was decolourised with charcoal-Filtercel and distilled in presence of a trace of barium carbonate. Methyl 2-O-methyl-3:4-O-isopropylidene-α-D-galactosiduronic methyl ester (IV) was obtained as a colourless

syrup (2·70 g., 72% of theoretical), b.p. 120-130°/0·1 mm., n_D^{17} 1·4622, $[\alpha]_D^{16}$ +103° (c, 0·7 in H_2O), +114° (c, 0·8 in MeOH). (Jones and Stacey[33] record n_D^{26} 1·4210, $[\alpha]_D$ +116° (c, 1·1 in H_2O) for this substance). n_D^{26} 1·4210 is certainly an error.

Found: C, 52·8; H, 7·4; OMe, 33·3.

Calc. for $C_{12}H_{20}O_7$: C, 52·2; H, 7·3; OMe, 33·7%.

Methyl 2-O-Methyl-3:4-O-Isopropylidene-α-D-Galactosiduronamide.

The foregoing ester (IV) (0·20 g.) was treated with saturated methanolic ammonia (5 ml.) at 0° for 48 hours. On evaporation, a white crystalline solid (0·20 g.) was obtained. After recrystallisation from ethanol-light petroleum, the product, methyl 2-O-methyl-3:4-O-isopropylidene-α-D-galactosiduronamide, had m.p. 123-124°, $[\alpha]_D^{19}$ +70° (c, 1·1 in H_2O).

Found: C, 49·9; H, 7·1; N, 5·0; OMe, 23·7.

$C_{11}H_{19}O_6N$ requires C, 50·6; H, 7·3; N, 5·4; OMe, 23·8%.

Methyl 2-O-Methyl-α-D-Galactopyranosiduronic Methyl Ester.

(a) A solution of methyl 2-O-methyl-3:4-O-isopropylidene-α-D-galactosiduronic methyl ester (IV) (2·48 g.) in dry methanol (50 ml.) containing water (0·20 ml.) was shaken with dry cation exchange resin (Amberlite IR-100-H) (1·5 g.) at room temperature for 6 days. The resin was removed by filtration, washed with methanol, and the filtrate and washings evaporated to a colourless syrup (V) (2·18 g., 98% of theoretical), n_D^{17} 1·4680, $[\alpha]_D^{18}$ +105° (c, 0·7 in MeOH), $[\alpha]_D^{16}$ +113° (c, 0·8 in H_2O). (Jones and Stacey[33] record n_D^{21} 1·4732

EX BIBL. UNIV. GRONINGEN

and $[\alpha]_D^{20}$ +80° (c, 1·1 in H_2O) for their synthetic specimen, and Brown, Hirst, and Jones[47] record n_D^{20} 1·4695, $[\alpha]_D$ +21° (in H_2O) for this substance isolated from methylated Cholla gum).

Found: C, 45·3; H, 6·9; OMe, 39·7.

Calc. for $C_9H_{16}O_7$: C, 45·8; H, 6·8; OMe, 39·4%.

(b) Methyl 2-O-methyl-3:4-O-isopropylidene-α-D-galactosiduronic methyl ester (IV) (0·30 g.) was dissolved in dry methanol (10 ml.) containing water (0·03 ml.) and heated with cation exchange resin (Amberlite IR-100-H) (0·3 g.) at 50° for 48 hours. The product was isolated as above as a colourless syrup (V) (0·271 g., 98% of theoretical), n_D^{17} 1·4679, $[\alpha]_D^{20}$ +97° (c, 0·7 in MeOH). It was neutral, non-reducing to Fehling's solution, and contained no isopropylidene group.

(c) A solution of methyl 2-O-methyl-3:4-O-isopropylidene-α-D-galactosiduronic methyl ester (IV) (0·25 g.) in 0·5% methanolic hydrogen chloride (10 ml.) was heated at 70° for 70 minutes. After neutralisation with silver carbonate, filtration, extraction of the silver residues with dry methanol, and evaporation of the filtrate and washings, the product was a colourless syrup (V) (0·22 g., 98% of theoretical), n_D^{17} 1·4679, $[\alpha]_D^{17}$ +1·2° (c, 1·6 in MeOH), +4° (c, 1·7 in H_2O), which was neutral and non-reducing and gave a negative test for the isopropylidene group.

Found: OMe, 39·4. Calc. for $C_9H_{16}O_7$: OMe, 39·4%.

Stability of the Glycosidic Group to Hydrolysis.

A solution of methyl 2-O-methyl-α-D-galactopyranosiduronic methyl

ester (V) (80 mg.) in water (10 ml.) was shaken at room temperature with cation exchange resin (Amberlite IR-100-H) (0·2 g.). Initially, the solution had $[\alpha]_D^{16}$ +113°, pH 7, and was non-reducing. After 14 days, the solution had $[\alpha]_D^{16}$ +110° and pH 2 and was non-reducing. After standing for fourteen months with occasional shaking the solution was still non-reducing. Under similar conditions, methyl D-xylofuranoside became strongly reducing within 24 hours and methyl α-D-mannopyranoside within 120 hours.

2-O-Methyl-D-Galactaramide.

(a) Methyl 2-O-methyl-3:4-O-isopropylidene-α-D-galactosiduronic methyl ester (IV) (120 mg.) was dissolved in nitric acid (d, 1·3; 5 ml.). The temperature of the solution was raised from 40° to 80° during 15 minutes and maintained at 80° for 10 minutes. The solution was diluted with water and nitric acid removed by distillation under reduced pressure with frequent additions of water. The resulting acid syrup was refluxed with 4% methanolic hydrogen chloride (10 ml.) for 6 hours, neutralised with silver carbonate, filtered, the silver residues extracted, and the filtrate and washings evaporated. Distillation gave a colourless syrup (70 mg.), b.p. 160-180°/0·1 mm. On treatment of this syrup with saturated methanolic ammonia at 0° for 48 hours, crystalline 2-O-methyl-D-galactaramide (40 mg.), m.p. 205° (decomp.), was obtained. (Jones and Stacey[33] record m.p. 200° for their synthetic product, Brown, Hirst, and Jones[47] m.p. 195° for the same product from methylated Cholla gum, and Hough and Jones[48] m.p. 207° for the product from the methylated gum of Sterculia setigera).

(b) A solution of methyl 2-O-methyl-3:4-O-isopropylidene-α-D-galactosiduronic methyl ester (IV) (307 mg.) in 0·2N sulphuric acid (10 ml.) was heated at 100° for 48 hours ($[\alpha]_D$ +36°, constant), neutralised with barium carbonate, filtered, the residue extracted with water, and the filtrate passed through a column (160 x 12 mm.) of cation exchange resin (Amberlite IR-120-H). Evaporation of the eluate gave 2-O-methyl-D-galacturonic acid as a colourless syrup (250 mg.), $[\alpha]_D^{18}$ +42° (c, 1·0 in H_2O). An aqueous solution of the latter was oxidised with bromine at 30° until the solution was non-reducing (120 hours). Excess bromine was removed by aeration, and water and hydrogen bromide by repeated distillation with ethanol. The resulting syrup was refluxed with 2% methanolic hydrogen chloride for 8 hours and the product isolated in the usual way as a syrup (120 mg.), n_D^{15} 1·4640, $[\alpha]_D^{18}$ +34° (c, 3·0 in H_2O). The diamide, prepared as described under (a), had m.p. 205° (decomp.).

Found: C, 37·8; H, 6·2; N, 11·6; OMe, 14·9.

Calc. for $C_7H_{14}O_6N_2$: C, 37·8; H, 6·4; N, 12·6; OMe, 14·0%.

Methyl 3:4-O-Isopropylidene-2-O-Toluene-p-Sulphonyl-α-D-Galactosiduronic Methyl Ester.

The starting material for preparations (a), (b), (c), and (d) was recrystallised from acetone-light petroleum and had m.p. 108-109°, whilst that for preparation (e) was recrystallised from light petroleum and had m.p. 113-114°.

(a) A solution of methyl 3:4-O-isopropylidene-α-D-galactosiduronic methyl ester (III) (1·30 g.) in pyridine (5 ml.) was cooled to 0° and powdered toluene-p-sulphonyl chloride (1·8 g.) added in small portions over several hours. After standing for 48 hours at room temperature the solution was poured with vigorous stirring into ice-water. The resulting flocculent precipitate was washed several times with water by decantation and filtered, giving an amorphous solid (130-150 mg., 6-7% of theoretical) which became sticky on exposure to the air. No further material was obtained by chloroform extraction of the aqueous washings.

(b) In another experiment, carried out under similar conditions, a granular solid (40 mg. from 201 mg., 13% of theoretical) was obtained. After recrystallisation from ethanol the product had m.p. 157-158°, $[\alpha]_D^{18}$ +85° (c, 1·3 in $CHCl_3$).

Found: C, 48·0; H, 5·3; S, 8·1.

$C_{18}H_{24}O_9S$ requires C, 51·9; H, 5·8; S, 7·8%.

(c) A solution of methyl 3:4-O-isopropylidene-α-D-galactosiduronic methyl ester (III) (1·085 g.) in pyridine (15 ml.) was cooled to 0° and allowed to stand in contact with "Drierite" (1·5 g.) for

30 minutes. A solution of toluene-p-sulphonyl chloride (1·50 g.) in pyridine (10 ml.) was added in small amounts over several hours and the mixture kept at room temperature for 96 hours. After filtration of the "Drierite," water (0·5 ml.) was added with vigorous stirring, the mixture allowed to stand for 30 minutes and then diluted with chloroform (100 ml.). The chloroform solution was extracted twice with water, dried, and evaporated to a syrup which partly crystallised. The crystals (0·284 g., 17% of theoretical) were separated by means of a little methanol and had m.p. 158-159°, $[\alpha]_D^{18}$ +122° (c, 1·3 in MeOH).

Found: C, 52·3; H, 6·0; S, 8·1.

$C_{18}H_{24}O_9S$ requires C, 51·9; H, 5·8; S, 7·8%.

The mother liquor failed to crystallise further on storage or after a second treatment with toluene-p-sulphonyl chloride in pyridine.

(d) Methyl 3:4-O-isopropylidene-α-D-galactosiduronic methyl ester (1·30 g.), treated exactly as in (c) above, gave a syrup which did not crystallise even on prolonged storage. Re-treatment of this syrup with toluene-p-sulphonyl chloride failed to give a crystalline product, although the syrup isolated gave positive tests for sulphur and for the isopropylidene group.

(e) Methyl 3:4-O-isopropylidene-α-D-galactosiduronic methyl ester (0·931 g., m.p. 113-114°), dissolved in dry pyridine (20 ml.), was allowed to stand in contact with "Drierite" (12 g.) at 0° for 20 hours. A solution of recrystallised toluene-p-sulphonyl chloride (1·6 g.) in dry pyridine (10 ml.) was added in small amounts during several hours, the mixture being maintained at 0°. The solution was kept at 0° for

24 hours, at 15° for 24 hours, and at 30° for 72 hours. After cooling to 0°, the "Drierite" was filtered off and water (100 ml.) was added cautiously and with constant cooling. After standing at 0° for one hour, the crystalline solid (1·028 g.) was separated and washed with water. Methyl 3:4-O-isopropylidene-2-O-toluene-p-sulphonyl-α-D-galactosiduronic methyl ester (VI) had m.p. 157-158°, unchanged on recrystallisation from methanol, $[\alpha]_D^{20}$ +122° (c, 1·1 in MeOH), $[\alpha]_D^{19}$ +117° (c, 2·2 in $CHCl_3$).

Found: C, 52·0; H, 5·5; S, 8·1; OMe, 14·6.

$C_{18}H_{24}O_9S$ requires C, 51·9; H, 5·8; S, 7·8; OMe, 14·9%.

The aqueous mother liquor was extracted twice with chloroform (200 ml.), the chloroform extracts washed with water (3 x 400 ml.), dried, and evaporated to a syrup which crystallised spontaneously. After recrystallisation from methanol, crystals (0·23 g.), identical with the main product, were obtained. Total yield 1·255 g., 85% of theoretical.

Methyl 2-O-Toluene-p-Sulphonyl-α-D-Galactopyranosiduronic Methyl Ester.

(a) Methyl 3:4-O-isopropylidene-2-O-toluene-p-sulphonyl-α-D-galactosiduronic methyl ester (VI) (1·345 g.) was dissolved in 1% methanolic hydrogen chloride (130 ml.) and kept at 30° for 30 hours, $[\alpha]_D^{15}$ +122° (initially); +91° (7 hours); +79·5° (26 hours); +79° (28 hours); +79° (30 hours). Neutralisation with silver carbonate and evaporation of the filtrate and washings gave a colourless glass (1·180 g., 97% of theoretical) which crystallised completely from aqueous methanol. After recrystallisation from aqueous methanol, methyl 2-O-toluene-p-sulphonyl-α-D-galactopyranosiduronic methyl ester

monohydrate (VII) had m.p. 71°, $[\alpha]_D^{15}$ +61° (c, 1·1 in $CHCl_3$).

Found: C, 45·8; H, 5·7; S, 8·4; OMe, 15·2.

$C_{15}H_{20}O_9S.H_2O$ requires C, 45·7; H, 5·6; S, 8·1; OMe, 15·7%.

An identical product was obtained:

(b) in 96% yield by treatment of the isopropylidene derivative with 1% methanolic hydrogen chloride at 70° for 60 minutes,

(c) in approximately 90-95% yield by shaking in methanolic solution with the cation exchange resin (Amberlite IR-120-H) for 21 days.

Methyl 2-O-Toluene-p-Sulphonyl-α-D-Galactopyranosiduronamide.

Treatment of the above methyl ester (VII) (210 mg.) with methanolic ammonia (10 ml.) at 0° for 48 hours, followed by evaporation of the solvent, gave a crystalline amide (195 mg.), which after recrystallisation from ethanol had m.p. 94-95°, $[\alpha]_D^{18}$ +67° (c, 0·5 in $CHCl_3$).

Found: C, 45·1; H, 5·6; N, 3·5; S, 8·1; OMe, 8·2.

$C_{14}H_{19}O_8NS.H_2O$ requires C, 44·3; H, 5·6; N, 3·7; S, 8·4; OMe, 8·2%.

Methyl 3:4-Di-O-methyl-2-O-Toluene-p-Sulphonyl-α-D-Galactosiduronic Methyl Ester.

Methyl 2-O-toluene-p-sulphonyl-α-D-galactopyranosiduronic methyl ester monohydrate (VII) (1·180 g.) was methylated four times with methyl iodide (23 ml.) and silver oxide (15 g.). The product (VIII) (1·218 g., 96% of theoretical) had, after recrystallisation from aqueous methanol m.p. 82·5-83°, n_D^{20} 1·4900, $[\alpha]_D^{17}$ +82° (c, 1·1 in $CHCl_3$),

+88° (c, 2·4 in EtOH).

Found: C, 50·2; H, 5·8; S, 8·1; OMe, 29·5.

$C_{17}H_{24}O_9S$ requires C, 50·5; H, 6·0; S, 7·9; OMe, 30·7%.

Methyl 3:4-Di-O-methyl-α-D-Galactosiduronic Acid.

In order to test the separation of uronic acid from aryl sulphonyl acid by preferential elution from anion exchange resin, the following experiment was carried out. A solution of potassium 1:2-3:4-di-O-isopropylidene-D-galacturonate (I) (100 mg.) and sodium toluene-p-sulphonate (100 mg.) in methanol (30 ml.) was passed slowly through a column (300 x 12 mm.) of anion exchange resin (Amberlite IRA-400-OH). The eluate was alkaline and optically inactive. The column was washed with methanol and eluted with 1% formic acid in methanol (200 ml.). Evaporation of the eluate gave a colourless syrup (ca. 50 mg.), which gave a negative test for sulphur and a strongly positive naphtharesorcinol test for uronic acid,[55] [the substance (2 mg.) and naphtharesorcinol (5-10 mg.) were boiled for a few minutes with concentrated hydrochloric acid (10 ml.), cooled, and extracted with benzene: a blue-purple colour in the benzene layer indicated the presence of uronic acid]. Further elution of the resin with 2N aqueous hydrochloric acid gave an optically inactive eluate.

(a) Preliminary Experiment.- Methyl 3:4-di-O-methyl-2-O-toluene-p-sulphonyl-α-D-galactosiduronic methyl ester (VIII) (125 mg.) was dissolved in methanol (10 ml.), water (5 ml.) added, and the solution stirred at room temperature and sodium amalgam (4%, 3 g.) added in small portions over 6 hours.[56] After stirring for a further 18 hours at

room temperature the mixture was filtered, neutralised with carbon dioxide, and evaporated to dryness. The residual white solid was extracted with methanol (4 x 50 ml.) under reflux and the methanolic extracts evaporated to a white solid (323 mg.) which was dissolved in methanol (30 ml.) and passed through a column (300 x 12 mm.) of cation exchange resin (Amberlite IR-120-H), previously washed with methanol. The acid eluate was then passed slowly through a similar column of anion exchange resin (Amberlite IRA-400-OH). The alkaline eluate was passed twice more through the two columns: the final eluate was neutral and gave no appreciable residue on evaporation. The column of anion exchange resin was washed with methanol (100 ml.) and then slowly eluted (6 hours) with 2% formic acid in methanol (200 ml.). Evaporation of the eluate gave a syrup (30 mg.) which crystallised on storage (m.p. 151-153°). The product gave a negative test for sulphur and a positive naphtharesorcinol test for uronic acid.

(b) Large Scale Preparation.- Anion exchange resin (Amberlite IRA-400-OH), previously regenerated by passage of N sodium hydroxide for 1 hour and thorough washing with distilled water, was packed into short glass tubes (60 x 13 mm. and with a constriction at one end). Four of these tubes, containing ca. 20 c.c. of resin in all, were placed alternately with three similar tubes containing cation exchange resin (Amberlite IR-120-H), and held in position by means of short rubber sleeves. The composite column was washed with distilled water (300 ml.), with ethanol (200 ml.), and with methanol (200 ml.).

A solution of methyl 3:4-di-O-methyl-2-O-toluene-p-sulphonyl-α-D-galactosiduronic methyl ester (VIII) (1·200 g.) in methanol (20 ml.) was stirred at room temperature with 2N aqueous sodium hydroxide (1·5

ml.). After 30 minutes, water (10 ml.) was added, followed by sodium amalgam (4%; 15 g.) in small portions over 6 hours. After stirring had continued for a further 18 hours the solids and mercury were filtered off and washed with methanol. The combined filtrates were neutralised with carbon dioxide, evaporated to dryness, and the resulting white solid exhaustively extracted with hot, dry methanol (4 x 50 ml.). The cooled methanolic extracts were passed through the composite column described above at the rate of 3-4 ml. per minute and the eluate recycled through the column, which was finally washed with methanol (100 ml.). The combined eluate and washings were evaporated to a syrup (0·03 g.) which was discarded. The composite column was dismantled and the portions containing anion exchange resin re-assembled and eluted by passage during 60 hours of 2% formic acid in methanol (500 ml.). Evaporation of the eluate gave a colourless syrup (0·613 g., 87% of theoretical) which crystallised spontaneously. After recrystallisation from ethanol-light petroleum, methyl 3:4-di-O-methyl-α-D-galactosiduronic acid (IX) had m.p. 154-155°, $[\alpha]_D^{15}$ +158° (c, 1·3 in $CHCl_3$), +156° (c, 1·3 in MeOH), +163° (c, 1·3 in H_2O).

Found: C, 46·4; H, 7·0; OMe, 38·4.

$C_9H_{16}O_7$ requires C, 45·8; H, 6·8; OMe, 39·4%.

Methyl 3:4-Di-O-methyl-α-D-Galactosiduronic Methyl Ester.

(a) A solution of methyl 3:4-di-O-methyl-α-D-galactosiduronic acid (IX) (0·375 g.) in 1% methanolic hydrogen chloride (32 ml.) was kept at 30° for 48 hours and then neutralised with silver carbonate. After filtration and extraction of the silver salts, evaporation of the filtrate and washings gave a crystalline solid (X) which was

recrystallised from light petroleum as long silky needles (0·378 g., 95% of theoretical), m.p. 113-114°, $[\alpha]_D^{16}$ +165° (c, 0·4 in $CHCl_3$).

Found: C, 48·0; H, 7·1; OMe, 49·8.

$C_{10}H_{18}O_7$ requires C, 48·0; H, 7·3; OMe, 49·6%.

(b) An identical product was obtained in 94% yield by treatment of a solution of methyl 3:4-di-O-methyl-α-D-galactosiduronic acid (IX) in ethanol with ethereal diazomethane. Evaporation of the solution and recrystallisation from light petroleum gave needles, m.p. 113-114°.

Methyl 3:4-Di-O-methyl-α-D-Galactosiduron-amide and -methylamide.

Treatment of the above methyl ester (X) with methanolic ammonia at 0° for 48 hours, followed by evaporation, gave a syrup, $[\alpha]_D^{17}$ +108° (c, 1·1 in EtOH), from which by trituration with ethanol a crystalline solid, m.p. 130-131°, was obtained in poor yield. Similar treatment of the ester (X) (110 mg.) with methanolic methylamine gave the crystalline methylamide (109 mg.) which was recrystallised from acetone in the form of large prisms, m.p. 205°, $[\alpha]_D^{15}$ +116° (c, 0·6 in H_2O).

Found: C, 48·5; H, 7·5; N, 5·8; OMe, 38·8.

$C_{10}H_{19}O_6N$ requires C, 48·2; H, 7·7; N, 5·6; OMe, 37·4%.

Methyl 2:3:4-Tri-O-methyl-α-D-Galactosiduronic Methyl Ester.

Methyl 3:4-di-O-methyl-α-D-galactosiduronic methyl ester (X) (18 mg.) was methylated twice with methyl iodide and silver oxide. The product (19 mg.) had m.p. 71-72° (after sublimation in vacuo) alone and

admixed with a specimen prepared from methyl α-D-galactopyranosiduronic methyl ester as described below, $[\alpha]_D^{16}$ +172° (c, 1·0 in H_2O).

For comparison, methyl α-D-galactopyranosiduronic methyl ester was methylated with silver oxide and methyl iodide in two operations.[15,28] The product crystallised and after purification by sublimation in vacuo had m.p. 71-72°.

Diethyl 3:4-Di-O-methylgalactarate.

A solution of methyl 3:4-di-O-methyl-α-D-galactosiduronic methyl ester (X) (294 mg.) in 0·2N sulphuric acid (20 ml.) was heated on the boiling water bath for 51 hours: $[\alpha]_D$ +160° (initially); +118° (3·5 hours); +112° (6·5 hours); +100° (24 hours); +90° (31 hours); +84° (48 hours); +84° (51 hours, constant). The solution was neutralised with barium carbonate, filtered through charcoal-Filtercel, the barium salts washed and the filtrate and washings passed through a column (300 x 10 mm.) of cation exchange resin (Amberlite IR-120-H). Evaporation of the eluate gave a colourless syrup (242 mg.), n_D^{11} 1·4615, $[\alpha]_D^{17}$ +87° (c, 1·2 in EtOH), $[\alpha]_D^{15}$ +93° (c, 1·4 in H_2O). A solution of this syrup (220 mg.) in water (15 ml.) was oxidised at 40° with bromine (2 ml.). The course of the reaction was followed polarimetrically, excess bromine being removed by aeration before each observation and added again afterwards: $[\alpha]_D$ +93° (initially); +80° (1 day); +60° (3 days); +23° (5 days); +15° (8 days); +15° (11 days). After 11 days, water and hydrogen bromide were removed by repeated distillation with ethanol under reduced pressure, the residue dissolved in 0·2N hydrochloric acid (10 ml.) and the solution heated at 100° for 50 hours ($[\alpha]_D$ +8°). Bromine (2 ml.) was added and after 3 days at 40°

the rotation was zero. Water and hydrogen bromide were removed as before, and a crystalline solid (205 mg.) which was insoluble in water, was obtained. After recrystallisation from aqueous acetone, diethyl 3:4-di-O-methylgalactarate (XI) was obtained as flat plates, m.p. 148-149°, $[\alpha]_D^{18}$ ±0°.

Found: C, 49·4; H, 7·4; OR (as OMe), 42·2.

$C_{12}H_{22}O_8$ requires C, 49·0; H, 7·5; OR (as OMe), 42·2%.

Dimethyl 3:4-Di-O-methylgalactarate.

Treatment of the above diethyl ester (XI) with 1·5% methanolic hydrogen chloride at 60° for 16 hours, followed by neutralisation with silver carbonate, and evaporation of the filtrate and washings gave the crystalline dimethyl ester (XII), which was recrystallised from acetone-light petroleum as needles, m.p. 172-173°, $[\alpha]_D^{18}$ ±0°.

Found: C, 44·8; H, 6·7.

$C_{10}H_{18}O_8$ requires C, 45·1; H, 6·8%.

3:4-Di-O-methylgalactaramide.

Treatment of the dimethyl ester (XII) in the usual way with methanolic ammonia gave the crystalline diamide, which, after recrystallisation from methanol, had m.p. 230° (decomp.), $[\alpha]_D^{18}$ ±0°. A Weermann test[54] was positive.

Found: N, 11·9. $C_8H_{16}O_6N_2$ requires N, 11·9%.

DERIVATIVES OF D-GLUCURONIC ACID

DISCUSSION

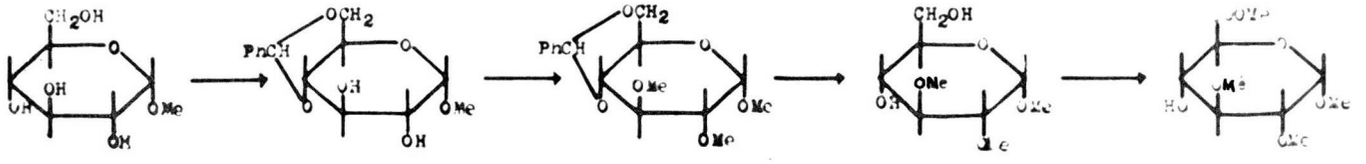
Methyl 2:3-dimethyl-α-D-glucopyranoside has been prepared[64,67] by methylation of several different acetals of methyl α-D-glucopyranoside and it was considered suitable for oxidation to the glycoside of 2:3-dimethyl-D-glucuronic acid by means of alkaline permanganate. Methyl 4:6-benzylidene-α-D-glucoside[64] was chosen as starting material, and methylation, followed by removal of the benzylidene residue with dilute acid at room temperature gave crystalline methyl 2:3-dimethyl-α-D-glucopyranoside in good yield. Oxidation with dilute aqueous alkaline permanganate, followed by esterification, gave methyl 2:3-dimethyl-α-D-glucopyranosiduronic methyl ester as a syrup. Treatment with phenylhydrazine gave the crystalline phenylhydrazide previously prepared by Smith[12] from the hydrolysate of methylated arabic acid, and esterification with p-nitrobenzoyl chloride in pyridine gave the beautifully crystalline p-nitrobenzoate, shown by mixed melting point determinations to be identical with a specimen prepared by Smith[12] from methylated arabic acid. Hydrolysis and bromine oxidation led to the production of 2:3-dimethyl-D-glucaric acid, characterised as the crystalline 1→4-lactone-6-methyl ester and as the crystalline diamide.

An attempt was made to carry out an unambiguous synthesis of 4-methyl-D-glucuronic acid by a modification of the method used for the synthesis of 4:6-dimethyl-D-glucose.[68,69] Crystalline methyl 2:3-dibenzyl-α-D-glucopyranoside was prepared by benzylation of methyl 4:6-benzylidene-α-D-glucoside, followed by mild acid hydrolysis

to remove the benzylidene group. Oxidation at room temperature with potassium permanganate in neutral acetone solution resulted in a mixture which was diluted with water, acidified, and extracted with chloroform. Evaporation of the chloroform and removal of a considerable quantity of benzoic acid by extraction with light petroleum left a residue which gave positive tests for uronic acid and was therefore methylated with dimethyl sulphate and sodium hydroxide. The product of methylation, however, was a very mobile liquid which contained no uronic acid. The aqueous solution remaining after extraction with chloroform gave a strongly positive test for uronic acid, probably indicating the presence of a quantity of uronic acid which had lost its benzyl substituents and was therefore useless in the present synthesis. It seems probable that the dibenzyl methyl glycoside was extensively degraded by the action of neutral permanganate and that very little, if any of the desired product, methyl 2:3-dibenzyl-α-D-glucosiduronic acid, was obtained. Since the corresponding dimethyl ether was successfully oxidised to the uronic acid by means of the more vigorous alkaline permanganate it is unlikely that degradation commenced at the unprotected hydroxyl on C4. It may therefore be inferred that the benzyl ether grouping, which is known to be relatively stable to acid and alkali and to be readily removable by mild reduction, is unstable under comparatively mild oxidising conditions.

DERIVATIVES OF D-GLUCURONIC ACID

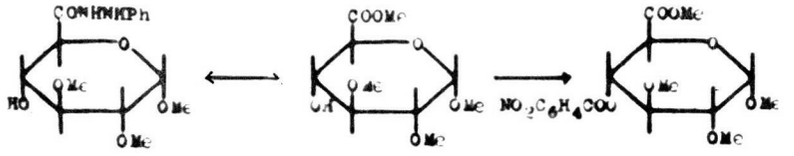
METHYL 2:3-DIMETHYL-α-D-GLUCOPYRANOSIDURONIC METHYL ESTER



Methyl α-D-Glucoside — Methyl 4:6-Benzylidene-α-D-glucoside — Methyl 4:6-Benzylidene-2:3-dimethyl-α-D-glucoside — Methyl 2:3-Dimethyl-α-D-glucoside — Methyl 2:3-Dimethyl-α-D-glucosiduronic methyl Ester

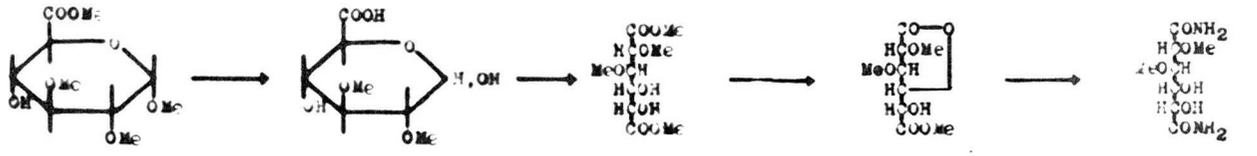
CHARACTERISATION OF METHYL 2:3-DIMETHYL-α-D-GLUCOSIDURONIC METHYL ESTER

(a)



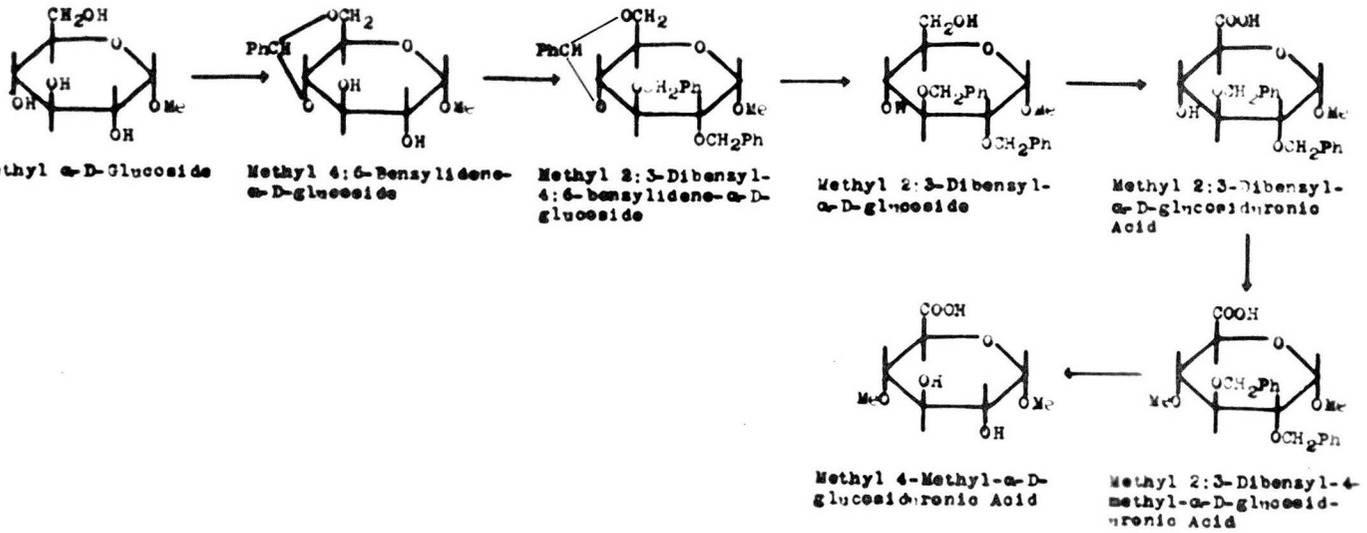
Methyl 2:3-Dimethyl-α-D-glucosiduronic Phenylhydrazide — Methyl 2:3-Dimethyl-α-D-glucosiduronic Methyl Ester — Methyl 2:3-Dimethyl-4-p-nitrobenzoyl-α-D-glucosiduronic Methyl Ester

(b)



Methyl 2:3-Dimethyl-α-D-glucosiduronic Methyl Ester — 2:3-Dimethyl-D-glucuronic Acid — Dimethyl 2:3-Dimethyl-D-glucarate — 2:3-Dimethyl-D-glucaro-1:4-lactone-6-methyl Ester — 2:3-Dimethyl-D-glucaramide

ATTEMPTED SYNTHESIS OF 4-METHYL-D-GLUCURONIC ACID



Methyl α-D-Glucoside — Methyl 4:6-Benzylidene-α-D-glucoside — Methyl 2:3-Dibenzyl-4:6-benzylidene-α-D-glucoside — Methyl 2:3-Dibenzyl-α-D-glucoside — Methyl 2:3-Dibenzyl-α-D-glucosiduronic Acid — Methyl 2:3-Dibenzyl-4-methyl-α-D-glucosiduronic Acid — Methyl 4-Methyl-α-D-glucosiduronic Acid

EXPERIMENTAL

Methyl α-D-Glucopyranoside was prepared from D-glucose by the action of 5% methanolic hydrogen chloride.[70] M.p. 163-165°, $[\alpha]_D^{17}$ +158° (c, 1·8 in H_2O).

Methyl 4:6-O-Benzylidene-α-D-Glucoside, was prepared by a modification of the method of Freudenberg.[64] Methyl α-D-glucopyranoside (100 g.), powdered anhydrous zinc chloride (75 g.), and benzaldehyde (500 ml.) were shaken for 4 hours. The solution was then poured with stirring into a mixture of saturated aqueous sodium bicarbonate (1200 ml.) and light petroleum (1200 ml.). The white solid which was deposited was filtered, washed with water (1000 ml.), with light petroleum (1000 ml.), and drained overnight. The resulting moist solid was extracted with boiling water (1500 ml.) and the aqueous extract filtered hot. On cooling the product was obtained as long needles (102 g., 70% of theoretical), m.p. 164-165°, $[\alpha]_D^{15}$ +108° (c, 1·1 in $CHCl_3$).

Found: C, 59·0; H, 6·6.

Calc. for $C_{14}H_{18}O_6$: C, 59·6; H, 6·4%.

Methyl 4:6-O-Benzylidene-2:3-Di-O-methyl-α-D-Glucoside.

A solution of methyl 4:6-O-benzylidene-α-D-glucoside (50 g.) in acetone (300 ml.) was stirred at 55-60° and 30% aqueous sodium hydroxide (320 ml.) and dimethyl sulphate (130 ml.) added in 1/10 portions at 10 minute intervals.[71,72] The solution was stirred for a further 20 minutes, the acetone removed under reduced pressure, and

the residue chilled. The resulting crystalline solid was filtered off, dissolved in acetone (300 ml.) and methylated as before. The product (51·5 g., 94% of theoretical) had, after recrystallisation from ethanol, m.p. 121-122°, $[\alpha]_D^{18}$ +95° (c, 3·0 in $CHCl_3$).

Found: C, 61·8; H, 7·0.

Calc. for $C_{16}H_{22}O_6$: C, 61·9; H, 7·2%.

Methyl 2:3-Di-O-methyl-α-D-Glucopyranoside.

A solution of methyl 4:6-O-benzylidene-2:3-di-O-methyl-α-D-glucoside (38·4 g.) in a mixture of acetone (800 ml.) and 4N sulphuric acid (200 ml.) was kept at room temperature for 72 hours: $[\alpha]_D$ +95° (initially); +109° (53 hours); +110° (72 hours, constant). The solution was neutralised with barium carbonate, filtered, the barium salts washed with water, and the neutral filtrate and washings evaporated to a syrup from which benzaldehyde was removed by repeated distillation with water under reduced pressure. The crystalline residue (23·5 g., 98% of theoretical) had, after recrystallisation from carbon tetrachloride, m.p. 81-82°, $[\alpha]_D^{18}$ +153° (c, 1·0 in H_2O).

Found: C, 48·9; H, 8·0.

Calc. for $C_9H_{18}O_6$: C, 48·6; H, 8·1%.

Methyl 2:3-Di-O-methyl-α-D-Glucopyranosiduronic Methyl Ester.

A solution of methyl 2:3-di-O-methyl-α-D-glucopyranoside (27·3 g.) in water (2 l.), containing potassium hydroxide (14 g.), was stirred at room temperature, and potassium permanganate (37 g.) added in small portions over 8 hours. Stirring was continued for a further

16 hours, excess permanganate decomposed by careful addition of aqueous potassium metabisulphite, the mixture filtered, and the residue washed with hot water (4 x 150 ml.). The filtrate was neutralised with solid carbon dioxide and evaporated to dryness. The resulting white solid was extracted, first with ether (2 x 300 ml.) and then with ethanol (3 x 300 ml.) under reflux and the cooled ethanolic extracts passed slowly through a column (17 x 700 mm.) of cation exchange resin (Amberlite IR-120-H). The column was washed with water (200 ml.) and the eluate and washings evaporated to a syrup (20·5 g.) which was dissolved in 1% methanolic hydrogen chloride (500 ml.) and left at room temperature for 48 hours. The solution was neutralised with silver carbonate, filtered, and the filtrate and washings evaporated to a colourless syrup (20·9 g., 68% of theoretical), b.p. 130-140°/0·5 mm., n_D^{18} 1·4441, $[\alpha]_D^{18}$ +111° (c, 1·1 in H_2O).

Methyl 2:3-Di-O-methyl-4-O-p-Nitrobenzoyl-α-D-Glucosiduronic Methyl Ester.

To a solution of methyl 2:3-di-O-methyl-α-D-glucopyranosiduronic methyl ester (2·2 g.) in pyridine (7 ml.), p-nitrobenzoyl chloride (3·0 g.) was added in small portions over 2 hours.[12] The mixture was left at room temperature for 48 hours and water (0·20 ml.) added cautiously, with shaking and cooling. The resulting crystalline solid (4·8 g.) was filtered off and washed with aqueous sodium bicarbonate (200 ml.) and water (300 ml.). After recrystallisation from ethanol, the p-nitrobenzoate had m.p. 156-158°, $[\alpha]_D^{20}$ +69° (c, 1·8

in $CHCl_3$). Smith[12] records m.p. 157°.

Found: C, 51·4; H, 5·4; N, 4·1.

$C_{17}H_{21}O_{10}N$ requires C, 51·1; H, 5·3; N, 3·5%.

A specimen (kindly supplied by Professor F. Smith) of the p-nitrobenzoate prepared from 2:3-di-O-methyl-D-glucuronic acid isolated from the hydrolysate of methylated arabic acid had m.p. 154°, and a mixture of the two specimens had m.p. 154-157°.

Methyl 2:3-Di-O-methyl-α-D-Glucopyranosiduronic Phenylhydrazide.

Methyl 2:3-di-O-methyl-α-D-glucopyranosiduronic methyl ester (2·0 g.) and freshly distilled phenylhydrazine (0·78 ml.) were heated together in an atmosphere of carbon dioxide in a sealed tube at 110° for 18 hours. The resulting mixture was extracted with ether (3 x 20 ml.) under reflux and the crystalline residue (1·85 g.) recrystallised from benzene as prisms, m.p. 195-197°, $[\alpha]_D^{18}$ +85° (c, 1·1 in $CHCl_3$). Smith[12] reports m.p. 225-227° for the phenylhydrazide obtained from the dimethyl uronic acid isolated from the hydrolysate of methylated arabic acid.

Found: C, 55·2; H, 6·8; N, 9·0.

$C_{15}H_{22}O_6N_2$ requires C, 55·2; H, 6·8; N, 8·6%.

2:3-Di-O-methyl-D-Glucaramide.

Methyl 2:3-di-O-methyl-α-D-glucopyranosiduronic methyl ester (2·0 g.) was heated in a sealed tube at 100° for 24 hours with N hydrochloric acid (40 ml.). Bromine (5 ml.) was added to the cooled solution and the mixture kept at 40° for 6 days. Bromine was removed

by aeration and hydrohalic acids by repeated distillation with ethanol under reduced pressure. The syrupy residue was esterified with 1% methanolic hydrogen chloride at 30° for 3 days, and the product, isolated in the usual manner, distilled in vacuo (b.p. 120-130°/0·1 mm.). The distillate crystallised spontaneously and after recrystallisation from benzene 2:3-di-O-methyl-D-glucaro-1 → 4-lactone-6-methyl ester had m.p. 99-100°, $[\alpha]_D^{18}$ +17° (c, 1·2 in H_2O). Smith[12] records m.p. 101°, $[\alpha]_D^{18}$ +12° in H_2O for this compound.

Treatment of the above lactone ester with methanolic ammonia, followed by evaporation, gave crystalline 2:3-di-O-methyl-D-glucaramide, which after recrystallisation from methanol had m.p. 154-155°, undepressed on admixture with an authentic specimen.

Methyl 2:3:4-Tri-O-methyl-α-D-Glucosiduronamide.

Methyl 2:3-di-O-methyl-α-D-glucopyranosiduronic methyl ester (70 mg.) was methylated with methyl iodide and silver oxide in two operations. The product (70 mg.) was distilled (b.p. 130-140°/0·1 mm.) and the distillate treated with methanolic ammonia. The resulting amide, after recrystallisation from benzene, had m.p. 180°, undepressed on admixture with an authentic specimen, $[\alpha]_D^{15}$ +147° (c, 0·7 in H_2O).

Methyl 2:3-Di-O-benzyl-4:6-O-benzylidene-α-D-Glucoside.

Methyl 4:6-O-benzylidene-α-D-glucoside (50 g.) was dissolved in a mixture of benzyl chloride (134 ml.) and toluene (1500 ml.).[69] Powdered sodium hydroxide (350 g.) was added and the mixture heated on the boiling water-bath, with vigorous stirring, for 5 hours.

Water (1200 ml.) was added to the cooled mixture and the toluene layer separated and washed with water. The toluene solution was evaporated and benzyl chloride removed from the residue by repeated distillation with water under reduced pressure. Finally, the solid residue was washed with water and recrystallised from aqueous ethanol or from light petroleum as fine needles (53 g., 65% of theoretical) m.p. 94-95°, $[\alpha]_D^{18}$ -30° (c, 2·3 in $CHCl_3$).

Found: C, 72·3; H, 6·8.

Calc. for $C_{28}H_{30}O_6$: C, 72·7; H, 6·5%.

Methyl 2:3-Di-O-benzyl-α-D-Glucopyranoside.

A solution of methyl 2:3-di-O-benzyl-4:6-O-benzylidene-α-D-glucoside (48 g.) in acetone (800 ml.) and 4N sulphuric acid (200 ml.) was kept at room temperature for 72 hours: $[\alpha]_D$ +20° (initially); +45° (7 hours); +67° (23 hours); +72° (53 hours, constant). The solution was neutralised with barium carbonate, filtered, the barium salts washed with acetone, and the neutral filtrate evaporated to a syrup from which benzaldehyde was removed by repeated distillation with water under reduced pressure. The crystalline residue (35·5 g., 92% of theoretical) had, after recrystallisation from aqueous ethanol, m.p. 77-78°, $[\alpha]_D^{19}$ +26° (c, 4·3 in $CHCl_3$).

Found: C, 67·1; H, 6·8.

Calc. for $C_{21}H_{26}O_6$: C, 67·4; H, 7·0%.

Oxidation of Methyl 2:3-Di-O-benzyl-α-D-Glucopyranoside.

To a solution of methyl 2:3-di-O-benzyl-α-D-glucopyranoside (50 g.) in acetone (1500 ml.) containing water (100 ml.), potassium permanganate (100 g.) was added in small portions with constant stirring during 48 hours. The mixture was acidified (170 ml. of 4N sulphuric acid) with stirring and cooling, filtered, and the residue washed with acetone (2 x 200 ml.). The filtrate was diluted with water (3 l.), extracted with chloroform (4 x 500 ml.) and the chloroform extracts washed with water (3 x 400 ml.). Evaporation of the chloroform solution gave a syrup which was extracted with light petroleum (4 x 200 ml.) under reflux. From the light petroleum extracts benzoic acid (15 g.) was obtained on cooling. The residual syrup (8 g.) had $[\alpha]_D$ +103° (c, 1·8 in $CHCl_3$) and gave a positive naphtharesorcinol test for uronic acid. Two methylations with dimethyl sulphate and sodium hydroxide gave a very mobile liquid, isolated by acidification and chloroform extraction, which gave negative naphtharesorcinol and carbazole tests for uronic acid. After debenzylation with sodium methoxide as described by Bell and Lorber[69] naphtharesorcinol and carbazole tests were still negative. The aqueous solution remaining after extraction of the oxidation mixture with chloroform gave a positive test for uronic acid.

NOTES

Melting points were determined on the Kofler hot-stage microscope. All boiling points recorded are bath temperatures. Rotations were measured in 1 dm. polarimeter tubes. Elementary analyses were performed by Drs. Weiler and Strauss, Oxford. Methoxyl[78] and acetone[79] determinations were carried out by the author, using volumetric methods. For analysis and the determination of physical constants, crystalline substances were dried in vacuo (0·1 mm.) over phosphorus pentoxide. All evaporations were carried out under reduced pressure at a temperature not higher than 50°. Salts of silver and barium were removed by filtration through layers of charcoal and Filtercel on a sintered glass filter and the residue on the filter washed at least thrice with a suitable warm solvent. In the case of the silver salts from Purdie methylations, boiling acetone was most effective. All light petroleum used was the fraction of b.p. 60-80°.

The conventions of the Journal of the Chemical Society, with regard to new compounds,[80] are observed throughout the experimental sections. The analyses of new compounds are therefore reported in the form:

Found: C, -.-; H, -.-.

$C_{12}H_{16}O_6$ requires C, -.-; H, -.-%.

whereas for compounds already adequately described in the literature the form is:

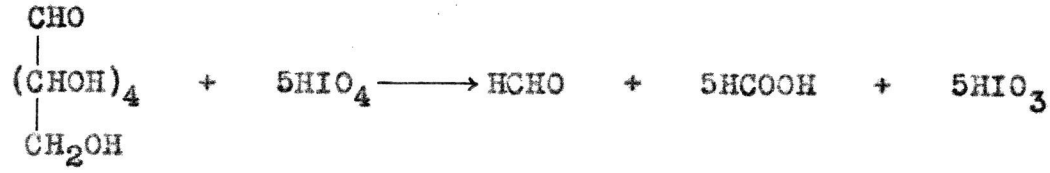
Found: C, -.-; H, -.-.

Calc. for $C_{12}H_{16}O_6$: C, -.-; H, -.-%.

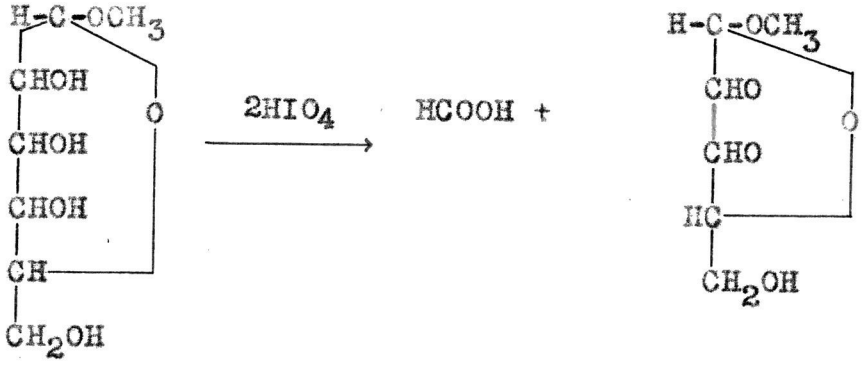
Through the experimental sections the nomenclature is in accordance with the recommendations of the Joint Committee on Nomenclature.[81] In the Introduction and Discussion, however, a simplified nomenclature is used for the sake of clarity.

PERIODATE OXIDATION OF URONIC ACIDS

In 1928 Malaprade[84] showed that α-glycols were smoothly oxidised by periodic acid with the production of formic acid from a secondary alcohol group and formaldehyde from a primary alcohol residue. When this reaction was applied to hexoses it was found that 5 molecules of periodic acid are consumed and that the hexoses break down with the liberation of 5 molecules of formic acid and one of formaldehyde.

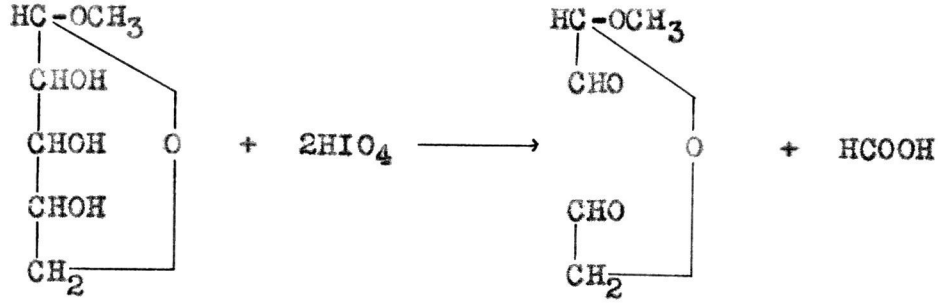


Although this represents the hexoses in the aldehydic form periodic acid was also found to react with the α-glycol groups in the cyclic glycosides[85] in a similar way; the chain of carbon atoms is broken whilst the oxide ring of the sugar is left intact. Thus with a methyl α-D-hexoside a molecule of formic acid is released and a derivative of diglycollic aldehyde produced. Aslightly different result is obtained with the β-hexosides; the oxidation product has



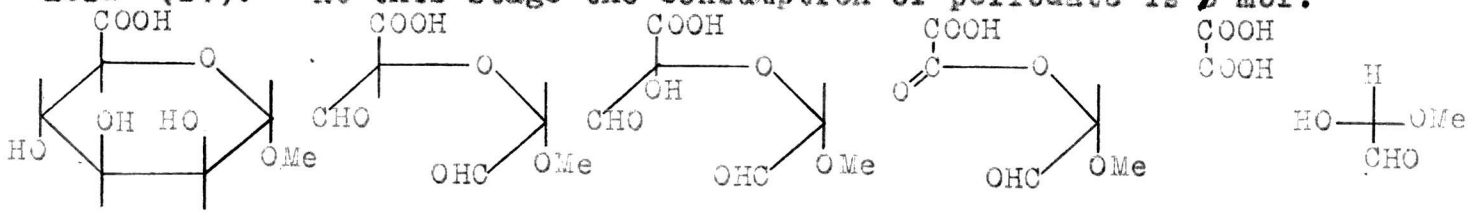
the configuration of C1 reversed. When a pure methyl α- or β-D-pentofuranoside is oxidised the same products are obtained, with the

difference that only one molecule of periodic acid is consumed and no formic acid is produced. On the other hand the methyl pentopyranosides under the same treatment give monosubstituted diglycollaldehyde derivatives. In this way periodic acid oxidation

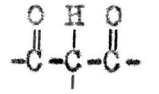


has proved a valuable method for the determination of ring structures and for the correlation of the configuration of the glycosidic centre of glycosides, and has provided many valuable results in spite of the fact that the original simple concept of specificity for the α-diol structure has been modified considerably. Link and co-workers[86] recorded that zinc bornyl glucosiduronate and methyl α-D-galactosiduronic methyl ester gave rise to more than the theoretical amount of formic acid. Halsall, Hirst and Jones[40] confirmed these results. These latter workers found that if they used potassium metaperiodate at pH 4 and kept the concentration of formic acid low the methyl glycosides of hexoses, pentoses and the reducing disaccharides gave the normal quantity of formic acid, while the methylglycosides of uronic acids and of hexofuranosides and the reducing sugars all underwent further oxidation. Link and his colleagues had previously explained the over-oxidation of the uronosides on the assumption that after the formation of the dialdehyde II the hydrogen situated on carbon 5 is activated by the adjacent carboxyl and aldehydic groups

and is oxidised to a hydroxyl group (III). "This would result in the formation of a substance which in its hydrated form contains hydroxyl groups on adjacent carbon atoms and would undergo further oxidation with periodate with the formation of an ester of oxalic acid" (IV). At this stage the consumption of periodate is 4 mol.



I II III IV V VI

for each mol. of uronic acid. If the conditions are such that the ester (IV) is hydrolysed, ~~both products, oxalic acid (V) and~~ the hemi-acetal of glyoxal (VI) would be oxidised further and the total consumption of periodate would be 5 mol. Sprinson and Chargaff[87] investigated the conversion of the aldehyde (II) into the hydroxyl aldehyde (III) and proved that substances such as malonic acid which contain a hydrogen atom combined to a carbon atom situated between 2 carbonyl groups  can be oxidised to the corresponding hydroxy compound which then undergoes further oxidation. Hirst and his co-workers found that this explanation could be applied to the various compounds they investigated where over-oxidation occurred. They also observed that where this type of oxidation occurred free iodine was invariably released and ascribed this to the reaction between sodium iodide and sodium iodate in acid solution, the sodium iodide being formed by reduction of sodium iodate during oxidation on the activated carbon atom.

In the present work methyl α-D-galactopyranosiduronic methyl ester was oxidised under different conditions of pH and temperature.

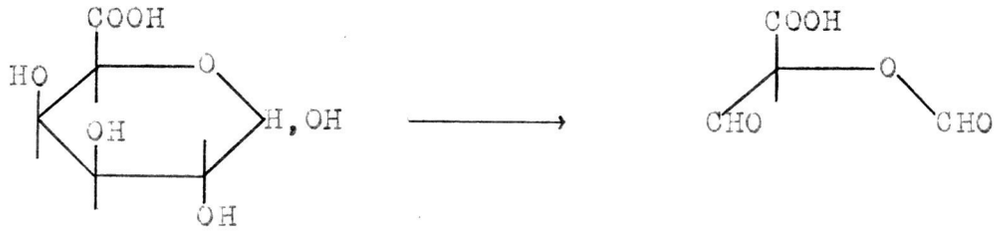
Within 20 hours under all the conditions employed more than the 2 mol. required by the simple oxidation had been consumed. In 66 hours the consumption in all cases was 3 mol. or more and the reaction generally appeared to become slower after this point. In 300 hours reaction appeared to be complete at a consumption of 5·0-5·1 mol. except in four cases, notably at pH 4·5 at 0° and 18°, in which the consumption was still only 4·0-4·1 mol. From the results it appears that the over-oxidation postulated by the previous workers occurs under all the conditions studied and that, apart from the figure of 5 mol. representing complete over-oxidation, the only significant arrest in the progress of oxidation occurs at 3·0 mol., most evident at 0° with no buffer or with a buffer of pH 4·5. It would appear, therefore, if the previous explanation of over-oxidation is correct, that hydrolysis of the oxalyl ester (IV) is relatively slow. No liberation of iodine occurred under any of the conditions employed nor with any of the other derivatives investigated.

In order to investigate the behaviour of methylated glycosiduronic acids, each was oxidised with periodate at 0° in a buffer of pH 4·5 and at 15° with a buffer of pH 7·0. Of the four glycosiduronic acid derivatives studied in which over-oxidation was expected it did indeed occur, but the numerical results are difficult to reconcile with theory, At 0° and a pH of 4·5 the over-oxidation was small even after 300 hours, methyl α-D-galactosiduronamide and mannosiduronamide consumed respectively 2·5 and 2·7 mol. periodate for each sugar molecule, although the postulated over-oxidation would require 5 mol. Methyl 2-methyl-α-D-galactosiduronic methyl ester consumed 2 mol. mid-way between normal oxidation and complete over-oxidation. No over-oxidation occurred

with methyl 4-methyl-α-D-mannosiduronic methyl ester which consumed a single mol. of periodate for each mol. of sugar; on the current theories this is to be expected as the methyl group on C4 would prevent oxidation between C3 and C4 and consequently the formation of an active hydrogen atom. This is also in agreement with the results of Smith[88] who found that methyl 4-methyl-α-D-glucosiduronic methyl ester consumed only one mol. of periodate. With two dimetnyl and three trimethyl uronosidic methyl esters where no oxidation of any kind was expected the actual results fitted expectations.

A number of free uronic acids were oxidised at 0° and pH 4.5. The periodate consumption after 300 hours was in every case roughly 1 mol. less than would be expected if the uronic acid reacted in the straight-chain (aldehydo) form. In every experiment the glycosiduronic ester was hydrolysed with sulphuric acid at 100° for 24 hours and no attempt was made to isolate the product before oxidation with periodate and it might be argued that low consumption of periodate was due to loss of sugar during hydrolysis. However, hydrolysis of methyl 3:4-dimethyl-α-D-galactosiduronic methyl ester under the same conditions gave a 97% yield of the free acid. White[17] found that 3:4-dimethyl-D-glucuronic acid consumed only one mol. of periodate which is in agreement with the present results for 3:4-dimethyl-D-galacturonic acid. Greville and Northcote[89] found that 3:4-dimethyl-D-glucose consumed only 1 instead of 2 mol. of periodate which could be explained by assuming that the sugar reacts in the pyranose configuration, although these authors consider that the immunity of C5 and C6 to oxidation may only partly be due to the impossibility of

the sugar assuming the furanose configuration. Application of this concept to the uronic acids oxidised in the present work gives figures which fit experimental results in the case of 4-methyl-D-mannuronic acid, 3:4-dimethyl-D-galacturonic acid, and 2:3:4-trimethyl-D-glucuronic acid. In considering the results of the oxidation of the remaining uronic acids it must be borne in mind that if an uronic acid having hydroxyls at C3 and C4 reacts in the pyranose form one of the intermediates is a substance which contains activated hydrogen and over-oxidation is therefore to be expected.



This over-oxidation would be expected with D-galacturonic acid, D-mannuronic acid, and 2-methyl-D-galacturonic acid, in all of which the periodate consumption actually exceeds the figure predicted on the basis of pyranose configuration without over-oxidation. 2:3-Dimethyl-D-glucuronic acid also consumes more periodate than would be expected from the pyranose form although in this case over-oxidation due to activated hydrogen is hardly to be expected.

EXPERIMENTAL

Buffer solutions were prepared according to Vogel,[82] except that sodium salts were used in all cases. The following buffer solutions were used: (total molarity ~ 0·1 M)

Buffer A (pH 2·0) Toluene-p-sulphonic acid-sodium toluene-p-sulphonate.

Buffer B (pH 4·5) Acetic acid-sodium acetate.

Buffer C (pH 5·3) Acetic acid-sodium acetate.

Buffer D (pH 5·3) Disodium hydrogen phosphate-sodium dihydrogen phosphate.

Buffer E (pH 7·0) Sodium dihydrogen phosphate-sodium hydroxide.

A typical oxidation is described in detail below.

The substance (10-50 mg.; sufficient to give a back titration difference of 10-20 ml.) was weighed accurately and dissolved in about 40 ml. of the buffer solution at the appropriate temperature. Sodium metaperiodate solution (0·097M; 5 ml.) was added and the volume made up to 50 ml. with buffer solution. At suitable intervals, portions of 10 ml. were withdrawn, saturated with sodium bicarbonate, 10 ml. of 0·1N sodium arsenite and 1 g. of potassium iodide added. The mixture was allowed to stand for 15 minutes after the addition of the potassium iodide and then titrated quickly with 0·1N iodine solution, using starch indicator, until addition of 1 drop of iodine gave a blue colour which persisted for 5 seconds with shaking. A blank experiment was treated similarly.

RESULTS

Uptake of Periodate (mol.) by Methyl α-D-Galactopyranosiduronic Methyl Ester.

Temperature	18°						0°		
Buffer	None	A	B	C	D	E	None	B	E
pH	-	2.0	4.5	5.3	5.3	7.0	-	4.5	7.0
Time (hrs.)									
20	3.5	2.9	2.9	3.0	3.1	4.2	2.5	2.4	3.1
66	4.0	3.2	3.2	3.8	4.7	4.9	3.0	3.0	4.7
140	4.7	3.5	3.3	4.9	5.0	5.0	3.1	3.0	4.9
300	5.1	3.8	4.1	5.1	5.1	5.1	4.0	4.0	5.0

Uptake of Periodate (Mol.) by Uronic Acid Derivatives.

Uronic Acid	Mol. Expected		Buffer B pH 4·5 0°		Buffer E pH 7·0, 18°	
	A	B	90 hrs	300 hrs	90 hrs	300 hrs
Me-α-D-galactopyranosiduronic Me ester	2	5	3·0	4·0	5·0	5·1
Me-α-D-galactopyranosiduronamide	2	5	2·3	2·5	2·8	4·7
Me-α-D-mannopyranosiduronamide	2	5	2·3	2·7	2·8	4·7
Me 2-Me-α-D-galactosiduronic Me ester	1	3	1·4	2·0	2·0	3·0
Me 4-Me-α-D-mannosiduronic Me ester	1	1	0·9	1·0	1·0	1·1
Me 3:4-Me_2-α-D-galactosiduronic Me ester	0	0	0·0	0·0	0·0	0·0
Me 2:3-Me_2-α-D-glucosiduronic Me ester	0	0	0·0	0·0	0·0	0·0
Me 2:3:4-Me_3-α-D-glucosiduronic Me ester	0	0	0·0	0·0	0·0	0·0
Me 2:3:4-Me_3-α-D-mannosiduronic methyl-amide	0	0	0·0	0·0	0·0	0·0
	C	D E				
D-Galacturonic acid	5	3 5	3·3	3·6		
D-Mannuronic acid	5	3 5	3·9	4·2		
4-Me-D-mannuronic acid	3	2 2	2·0	2·1		
2-Me-D-galacturonic acid	3	1 3	1·5	1·9		
3:4-Me_2-D-galacturonic acid	2	1 1	1·1	1·2		
2:3-Me_2-D-glucuronic acid	2	0 0	0·9	1·2		
2:3:4-Me_3-D-glucuronic acid	1	0 0	0·0	0·0		

A Without over-oxidation

B With over-oxidation

C Open-chain configuration

D Pyranose, without over-oxidation

E Pyranose, with over-oxidation.

PAPER CHROMATOGRAPHY OF URONIC ACIDS

Although the paper chromatography of uronic acids has been mentioned in the literature on a number of occasions,[74] there are remarkably few reliable records of the R_G values of the uronic acids and their methyl ethers. There are probably two principal reasons for this, firstly the scarcity of authentic specimens of the uronic acids, and, secondly, the practical difficulties of "trailing" and poorly defined spots. The various uronic acids prepared in the course of this work were, therefore, examined on the paper chromatogram using various solvent systems and spray reagents. From preliminary experiments it appeared that all the systems containing acetic acid behaved rather similarly and that for most purposes the two types of solvent system available were, on the one hand, those containing acetic acid, typified by butanol-acetic acid-water, and, on the other hand, the butanol-formic acid-water system proposed by Akher, Smith, and Spriestersbach.[75] The properties of both of these solvent mixtures change on standing owing to esterification of the acid by the butanol. Smith and Spriestersbach[83] used paper impregnated with alginic acid in order to overcome this difficulty but in the present work consistent results were obtained by using an aged or otherwise equilibrated mixture. Although rhamnose has been proposed[76] as a control substance for the paper chromatography of uronic acids, it was found in the present work to be hopelessly erratic, its R_G value sometimes varying by over 50% under apparently identical conditions. Tetramethyl-D-glucose was found to give reasonably consistent results, the derived R_G values being generally within 3% of one

another on different papers and at different times, using butanol-aqueous formic acid. In the case of butanol-acetic acid-water, however, results were less consistent the maximum variation in the R_G value being about 6%. The formic acid system has certain other advantages over the systems containing acetic acid: it is faster, gives better separations, and is much less liable to cause badly shaped spots or trails through incomplete de-ionisation. It is noteworthy that the "heart-shaped spot" which is occasionally mentioned in the literature[77] as being characteristic of uronic acids has, in the present work been observed only with acetic acid-containing solvents in the presence of inorganic cations. As a rule, the spots are discrete and spherical or elliptical in outline. Aqueous aniline oxalate and butanolic p-anisidine hydrochloride were found to be the most generally useful spray reagents. With uronic acids having a free hydroxyl at C2 these spray reagents give a brown or reddish-brown colour, whereas with acids substituted at C2 a brilliant red or purple colour is produced.

The following table summarises the results obtained. Methyl glycosiduronic acids were generally hydrolysed, prior to paper chromatography, with N sulphuric acid at 100° for 24 hours in a sealed tube, followed by neutralisation with barium carbonate, filtration, and de-ionisation with cation exchange resin (Amberlite IR-100-H or IR-120-H). Butanol-acetic acid-water (B) (40:10:50) and butanol-formic acid-water (A) (500:115:385) were either kept at room temperature for 14 days or boiled under reflux for 1 hour before use.

Uronic Acid	Colour with Aniline Oxalate	Colour with p-Anisidine HCl	R_G in A	R_G in B
D-Galacturonic Acid	Brown	Brown	0·03	0·15
D-Mannuronic Acid	Brown	Brown	0·05	0·16
D-Mannuronolactone	Brown	Brown	0·13	0·29
2-Me-D-Galacturonic Acid	Orange-Red	Red-Purple	0·20	0·22
D-Glucuronolactone	Brown	Brown	0·21	0·37
4-Me-D-Mannuronic Acid	Red-Brown	Red-Brown	0·25	0·29
3:4-Me_2-D-Galacturonic Acid	Red-Brown	Red-Brown	0·43	0·46
2:3-Me_2-D-Glucuronic Acid	Orange-Red	Red-Purple	0·47	0·56
2:3:4-Me_3-D-Galacturonic Acid	Orange-Red	Red-Purple	0·63	0·61
2:3:4-Me_3-D-Mannuronic Acid	Orange-Red	Red-Purple	0·79	0·80
2:3:4-Me_3-D-Glucuronic Acid	Orange-Red	Red-Purple	0·84	0·84

SUMMARY

(1) Crystalline derivatives of 4-methyl-D-mannuronic acid have been prepared by methylation of methyl 2:3-isopropylidene-α-D-mannosiduronamide.

(2) Crystalline derivatives of 2:3:4-trimethyl-D-mannuronic acid have been prepared by methylation of methyl α-D-mannosiduronamide.

(3) Attempts to prepare the 4-toluene-p-sulphonate of methyl 2:3-isopropylidene-α-D-mannosiduronamide as an intermediate in the synthesis of 2:3-dimethyl-D-mannuronic acid have been unsuccessful.

(4) Crystalline derivatives of 2-methyl-D-galacturonic acid have been prepared by methylation of methyl 3:4-isopropylidene-α-D-galactosiduronic methyl ester.

(5) Crystalline derivatives of 3:4-dimethyl-D-galacturonic acid have been prepared by methylation of methyl 2-toluene-p-sulphonyl-α-D-galactosiduronic methyl ester, followed by removal of the blocking group.

(6) Crystalline derivatives of 2:3-dimethyl-D-glucuronic acid have been prepared by oxidation of methyl 2:3-dimethyl-α-D-glucopyranoside.

(7) An attempt to prepare methyl 2:3-dibenzyl-α-D-glucosiduronic acid by permanganate oxidation of methyl 2:3-dibenzyl-α-D-glucoside was unsuccessful, the benzyl group being apparently unstable under these conditions.

(8) The uronic acids and certain of their derivatives prepared in the course of this work have been oxidised with periodate under various conditions.

(9) The paper chromatography of the uronic acids and their methyl ethers has been studied.

REFERENCES

(1) Evans and Hibbert, Adv. Carbohydrate Chem., 1946, 2, 203.
Norman, "Biochemistry of Cellulose etc.", Oxford, 1937, 197.

(2) Anderson and Sands, Adv. Carbohydrate Chem., 1945, 1, 329.

(3) Jones and Smith, Adv. Carbohydrate Chem., 1949, 4, 243.

(4) Whistler, Adv. Carbohydrate Chem., 1950, 5, 269.

(5) Stacey, Adv. Carbohydrate Chem., 1946, 2, 161.

(6) Bray, Adv. Carbohydrate Chem., 1953, 8, 251.

(7) Hirst, J., 1942, 70.

(8) Hirst, Jones, and Jones, J., 1939, 1880.

(9) Chanda, Hirst, Percival, and Ross, J., 1952, 1833.

(10) Smith, J., 1951, 2646.

(11) Adams and Castagne, Canad. J. Chem., 1951, 29, 109; 1952, 30, 515.

(12) Smith, J., 1940, 1035.

(13) Luckett and Smith, J., 1940, 1106.

(14) Smith, J., 1939, 1724.

(15) Luckett and Smith, J., 1940, 1506.

(16) Lythgoe and Trippett, J., 1950, 1983.

(17) White, J. Amer. Chem. Soc., 1953, 75, 4692.

(18) Weinmann, Ber., 1929, 62B, 1637.
Morell, Baur, and Link, J. Biol. Chem., 1934, 105, 15.
Frush and Isbell, J. Res. Nat. Bur. Stand., 1946, 37, 321.

(19) Niemann and Link, J. Biol. Chem., 1933, 100, 407; 1932, 95, 203.
Kiliani, Ber., 1925, 58, 2344.
Mehltretter, Adv. Carbohydrate Chem., 1953, 8, 233.

(20) Ohle and Berend, Ber., 1925, 58, 2585.

(21) Stacey, J., 1939, 1529.

(22) Zervas and Sessler, Ber., 1933, 66, 1326.

(23) Mehltretter, Adv. Carbohydrate Chem., 1953, 8, 231.

(24) Levene and Meyer, J. Biol. Chem., 1924, 60, 173.

(25) Smith, Stacey, and Wilson, J., 1944, 131.

(26) Luckett and Smith, J., 1940, 1114.

(27) Ault, Haworth, and Hirst, J., 1935, 517.

(28) Levene and Kreider, J. Biol. Chem., 1937, 120, 597.

(29) Challinor, Haworth, and Hirst, J., 1931, 258.

(30) Marsh, J., 1952, 1578.

(31) Pryde and Williams, Biochem. J., 1933, 27, 1205.

Reeves, J. Amer. Chem. Soc., 1940, 62, 1616.

Smith, J., 1944, 584.

(32) Owen, Peat, and Jones, J., 1941, 339.

(33) Jones and Stacey, J., 1947, 1340.

(34) Smith, J., 1939, 1724.

(35) Hotchkiss and Goebel, J. Biol. Chem., 1936, 115, 285.

(36) Bishop, Canad. J. Chem., 1953, 31, 134.

(37) Stacey and Wilson, J., 1944, 587.

(38) Present work, J., 1953, 2473.

(39) Wolfrom and Kimiko Anno, J. Amer. Chem. Soc., 1952, 74, 5583.

(40) Halsall, Hirst, and Jones, J., 1947, 1427.

(41) Ault, Haworth, and Hirst, J., 1935, 1012.

(42) Robertson and Lamb, J., 1934, 1321.

(43) Mehltretter, Alexander, Mellies, and Rist, J. Amer. Chem. Soc., 1951, 73, 2424.

(44) Marsh, J., 1952, 1578.

(45) Niemann and Link, J. Biol. Chem., 1934, 104, 197.

(46) Ohle and Berend, Ber., 1925, 58, 2585.

(47) Brown, Hirst, and Jones, J., 1949, 1761.

(48) Hough and Jones, J., 1950, 1199.

(49) Levene and Meyer, J. Biol. Chem., 1931, 29, 257.

(50) Tipson, Adv. Carbohydrate Chem., 1953, 8, 108.

(51) Percival and Percival, J., 1950, 690.

(52) Sugihara, Adv. Carbohydrate Chem., 1953, 8, 1.

(53) Percival and Zobrist, J., 1952, 4306.

(54) Weermann, Rec. Trav. chim., 1917, 36, 16.

(55) Tollens, Ber., 1908, 41, 1788.

(56) Tipson, Adv. Carbohydrate Chem., 1953, 8, 161.

(57) Haskins, Hann, and Hudson, J. Amer. Chem. Soc., 1946, 68, 628.

(58) Kenner and Murray, J., 1949, S178.

(59) Oldham and Rutherford, J. Amer. Chem. Soc., 1932, 56, 366.

(60) Dewar and Fort, J., 1944, 492.

(61) Zemplen and Pacsu, Ber., 1929, 62, 1613.

(62) Knauf, Hann, and Hudson, J. Amer. Chem. Soc., 1941, 63, 1447.

(63) Smith, J., 1948, 1989.

(64) Freudenberg, Toepffer, and Anderson, Ber., 1928, 61, 1750.

(65) Watters, Hockett, and Hudson, J. Amer. Chem. Soc., 1939, 61, 1528.

(66) Helferich and Klein, Ann., 1926, 450, 219.

(67) Brederick, Ber., 1935, 68, 777.

(68) Freudenberg and Plankenhorn, Ber., 1940, 73, 621.

(69) Bell and Lorber, J., 1940, 453.

(70) Org. Synth., Coll. Vol.I, John Wiley and Sons, New York, 2nd Ed., 1941, p.364.

(71) Percival and Somerville, J., 1937, 1615.

(72) Bell, J., 1936, 859.

(73) Haworth, Hirst, Isherwood, and Jones, J., 1939, 1878.

(74) Lederer and Lederer, "Chromatography," Elsevier, 1953, pp.158-171.

(75) Akher, Smith, and Spriestersbach, J., 1952, 3637.

(76) Prof. J. K. N. Jones, private communication to Prof. E. L.Hirst,F.R.S.

(77) Partridge, Biochem. J., 1948, 42, 238.

Reid, J. Sci. Food Agric., 1950, 1, 234.

Chanda, Hirst, and Percival, J., 1951, 1240.

(78) Viebock and Brecker, Ber., 1930, 63, 3207.

Elek, Anal., 1939, 11, 174.

(79) Cole, "Practical Physiological Chemistry," Heffer, Cambridge, 9th Ed., 1933, p.335.

(80) "The Presentation of Papers," The Chemical Society, London, 1952.

(81) Editorial Report on Nomenclature, J., 1952, 5108.

(82) Vogel, "Quantitative Inorganic Analysis," Longmans, Green, & Co., London, 1939, p.808.

(83) Smith and Spriestersbach, Nature, 1954, 174, 466.

(84) Malaprade, Bull. Soc. Chim., 1928, 43, 683; 1934, (5), 1, 833.

(85) Jackson and Hudson, J. Amer. Chem. Soc., 1937, 54, 994.

(86) Huebnar, Lohmar, Dimler, Moore, and Link, J. Biol. Chem., 1945, 159, 502.

(87) Sprinson and Chargaff, J. Biol. Chem., 1946, 164, 411.

(88) Smith, J., 1951, 2646.

(89) Greville and Northcote, J., 1952, 1945.

(90) Gurin and Hood, J. Biol. Chem., 1941, 139, 775.

INDEX

The author is very grateful to Professor E. L. Hirst and Dr. E. E. Percival for their guidance, to the Department of Scientific and Industrial Research for a Maintenance Grant, and to the University of Edinburgh for the award of a Vans Dunlop Scholarship. He also wishes to record his gratitude to his wife for her constant support.

Preprinted from the Journal of the Chemical Society, August 1953, (501), pages 2473—2477.

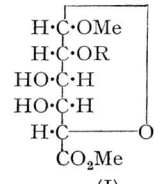
501. *Synthesis of* 2-O-*Methyl- and* 3 : 4-*Di*-O-*methyl*-D-*galacturonic Acid.**

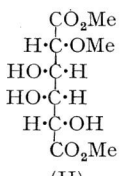
By R. A. Edington and Elizabeth E. Percival.

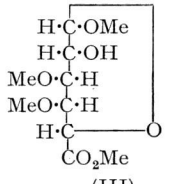
Crystalline methyl (methyl 3 : 4-*O*-*iso*propylidene-α-D-galactosid)uronate (B) has been synthesised from D-galactose. Methylation of (B) gave methyl (methyl 2-*O*-methyl-3 : 4-*O*-*iso*propylidene-α-D-galactosid)uronate (C) which was characterised by isolation of a crystalline amide, by oxidation to dimethyl 2-*O*-methyl-D-galactarate and by formation of the known crystalline diamide thereof. Hydrolysis of (C) gave 2-*O*-methylgalacturonic acid which on appropriate treatment gave the same diamide.

The crystalline 2-toluene-*p*-sulphonyl derivative of (B) was prepared, and removal of the *iso*propylidene residue followed by methylation and reductive fission of the toluene-*p*-sulphonyl group gave methyl 3 : 4-di-*O*-methyl-D-galactosiduronic acid. The methyl ester and its corresponding amide and methylamide were also obtained.

Apart from their intrinsic interest the methyl ethers of galacturonic acid are of great importance in the structural studies of the polysaccharides occurring in plant gums and pectins and in certain bacterial polysaccharides. Methylation followed by hydrolysis of these polysaccharides has led to a number of partially methylated galacturonic acid derivatives (Luckett and Smith, *J.*, 1940, 1106, 1506; Hirst, Hough, and Jones, *J.*, 1949, 3145; Brown, Hirst, and Jones, *J.*, 1949, 1761; Hough and Jones, *J.*, 1950, 1199). In addition G. A. Adams *et al.* (*Canad. J. Res.*, 1950, **28**, *B*, 753; *Canad. J. Chem.*, 1951, **29**, 109) isolated a monomethyl galacturonic acid from the hydrolysis products of the unmethylated polysaccharide from the hemicelluloses of wheat. The present work is concerned with the synthesis of 2-*O*-methyl- and 3 : 4-di-*O*-methyl-galacturonic acid. Methyl (methyl 2-*O*-methyl-α-D-galactopyranosid)uronate (I; R = Me) has been synthesised


(I)


(II)


(III)

previously by Jones and Stacey (*J.*, 1947, 1340) from 1 : 2 : 3 : 4-tetra-*O*-acetyl-6-*O*-tritylgalactose, which on removal of the trityl residue and oxidation gave 1 : 2 : 3 : 4-tetra-*O*-acetylgalacturonic acid. Hydrolysis of the acetyl groups and treatment with 1% methanolic hydrogen chloride then gave methyl (methyl α-D-galactosid)uronate (I; R = H). The hydroxyl groups at $C_{(3)}$ and $C_{(4)}$ were blocked by an *iso*propylidene residue and methylation followed by hydrolysis gave methyl (methyl 2-*O*-methyl-D-galactosid)-uronate in approximately 7·5% overall yield. The present synthesis differs in the earlier stages and gives a 20% overall yield. 1 : 2-3 : 4-Di-*O*-*iso*propylidene-D-galactose was oxidised with permanganate, giving crystalline potassium 1 : 2-3 : 4-di-*O*-*iso*propylidene-D-galacturonate monohydrate. This was converted into the crystalline free acid monohydrate which when boiled in dry methanol with cation-exchange resins furnished crystalline methyl (methyl α-D-galactosid)uronate. Although different conditions for the sub-

* For nomenclature, see *J.*, 1952, 5108, especially rules 9, 20, 26, 27, and 28.

stitution and for the removal of the *iso*propylidene residue have been used, the subsequent stages were the same as those employed by Jones and Stacey (*loc. cit.*). In the present experiments the syrupy methyl (methyl 2-*O*-methyl-3 : 4-*O*-*iso*propylidene-α-D-galactopyranosid)uronate was characterised by the isolation of a crystalline amide and by oxidation to dimethyl 2-*O*-methylgalactarate (II). This, on appropriate treatment, gave the known crystalline diamide. Hydrolysis of the *iso*propylidene and glycosidic and ester methoxyl groups from methyl (methyl 2-*O*-methyl-3 : 4-*O*-*iso*propylidene-α-D-galactopyranosid)uronate and oxidation of the resulting 2-*O*-methyl-D-galacturonic acid with bromine water gave the same dimethyl ester which after treatment with methanolic ammonia furnished the above-mentioned crystalline diamide.

Crystalline methyl (methyl 3 : 4-di-*O*-methyl-α-D-galactopyranosid)uronate (III) has been synthesised by blocking position 2 in crystalline methyl (methyl 3 : 4-*O*-*iso*propylidenegalactosid)uronate with a toluene-*p*-sulphonyl group. Hydrolysis of the *iso*propylidene residue followed by methylation gave crystalline methyl (methyl 3 : 4-di-*O*-methyl-2-*O*-toluene-*p*-sulphonylgalactosid)uronate. Reductive fission of the toluene-*p*-sulphonyl group with sodium amalgam and separation from the toluenesulphinic acid and metallic ions by adsorption on ion-exchange resins followed by preferential elution led to the isolation of crystalline methyl 3 : 4-di-*O*-methylgalactosiduronic acid. Treatment with methanolic hydrogen chloride furnished the methyl ester (III) as long needles, and appropriate treatment gave a crystalline amide and methylamide. It was converted into crystalline methyl (methyl-2 : 3 : 4-tri-*O*-methyl-α-D-galactopyranosid)uronate. Hydrolysis of (III) followed by oxidation gave crystalline diethyl 3 : 4-di-*O*-methylgalactarate. The corresponding crystalline dimethyl ester and diamide were isolated.

Experimental

Methyl (Methyl α-D-Galactopyranosid)uronate.—D-Galactose (80·0 g.) was converted into 1 : 2-3 : 4-di-*O*-*iso*propylidene-D-galactose by the method described by Ohle and Berend (*Ber.*, 1925, **58**, 2585). The syrupy product, when distilled in a high vacuum, had b. p. 130—170°/0·03 mm., n_D^{25} 1·4657 (yield, 55·3 g., 76%), $[\alpha]_D^{19}$ −51° (*c*, 1·2 in H_2O) [Found : $COMe_2$, 44·9. Calc. for $C_{12}H_{20}O_6$: $COMe_2$, 44·6%).

Oxidation of this syrup (55·0 g.) by potassium permanganate as described by Ohle and Berend (*loc. cit.*) gave potassium 1 : 2-3 : 4-di-*O*-*iso*propylidene-D-galacturonate monohydrate (48 g., 70%), m. p. 200° (decomp.), $[\alpha]_D^{21}$ −70° (*c*, 2·0 in H_2O) (Found : C, 43·3; H, 5·5; $COMe_2$, 36·0. Calc. for $C_{12}H_{17}O_7K,H_2O$: C, 43·6; H, 5·8; $COMe_2$, 35·2%). 1 : 2-3 : 4-Di-*O*-*iso*propylidene-D-galacturonic acid was obtained by treatment of this (16 g.) with cation-exchange resins (Amberlite I.R. 100-H) (24 g.) in distilled water (200 c.c.) for 6 hr. The resins were removed by filtration and the filtrate was passed through a column (250 × 18 mm.) of the same resin. Removal of part of the water gave the acid as colourless crystals (12·7 g., 91%), m. p. 158°, $[\alpha]_D^{17}$ −79° (*c*, 0·9 in $CHCl_3$) (Niemann and Link, *J. Biol. Chem.*, 1934, **104**, 197, record m. p. 157°, $[\alpha]_D$ −84°) (Found : C, 50·1; H, 6·9; $COMe_2$, 40·0; CO_2, 16·1. Calc. for $C_{12}H_{18}O_7,H_2O$: C, 49·3; H, 6·9; $COMe_2$, 39·8; CO_2, 15·1%).

1 : 2-3 : 4-Di-*O*-*iso*propylidene-D-galacturonic acid monohydrate (13·9 g.) was boiled for 24 hr. with dry methanol (200 c.c.) containing cation-exchange resins (Amberlite I.R. 100-H). Removal of the resins and evaporation gave a colourless syrup which on trituration with ethanol partly crystallised (3·6 g., 32%). Repeated treatment of the mother-liquor with methanol and resins brought the total yield of crystalline methyl (methyl α-D-galactosid)uronate to 7·48 g. (66%), m. p. 145°, $[\alpha]_D^{16}$ +121° (*c*, 1·0 in H_2O) (Jones and Stacey, *loc. cit.*, record m. p. 147°, and Niemann and Link, *loc. cit.*, $[\alpha]_D$ +121°) (Found : C, 39·9; H, 6·6; OMe, 26·5. Calc. for $C_8H_{14}O_7,H_2O$: C, 40·0; H, 6·7; OMe, 25·8%).

Methyl (Methyl 3 : 4-O-isoPropylidene-α-D-galactosid)uronate.—Methyl (methyl α-D-galactosid)uronate monohydrate (3·6 g.) was shaken for 120 hr. with dry acetone (250 c.c.) containing acetaldehyde (2 drops) and anhydrous copper sulphate (30 g.). A white solid was obtained which on recrystallisation from light petroleum (b. p. 60—80°) gave colourless needles (3·62 g., 94%), m. p. 113—114°, $[\alpha]_D$ +117° (*c*, 1·2 in H_2O) (Jones and Stacey, *loc. cit.*, record m. p. 107°, $[\alpha]_D^{20}$ +118°) (Found : C, 50·8; H, 6·7; OMe, 23·2. Calc. for $C_{11}H_{18}O_7$: C, 50·4; H, 6·9; OMe, 23·7%).

Methyl (Methyl 2-O-Methyl-α-D-galactopyranosid)uronate.—The foregoing *iso*propylidene compound (3·8 g.) was methylated thrice with methyl iodide and silver oxide, and the product

distilled (b. p. 120—130°/0·1 mm.) as a colourless syrup (*A*) (2·70 g., 72%), n_D^{17} 1·4622, $[\alpha]_D^{16}$ +103° (*c*, 0·7 in H_2O), +114° (*c*, 0·8 in MeOH) (Found: C, 52·8; H, 7·4; OMe, 33·3. Calc. for $C_{12}H_{20}O_7$: C, 52·2; H, 7·3; OMe, 33·7%). This was characterised by conversion into the crystalline *amide* by treatment with methanolic ammonia. After recrystallisation from ethanol–light petroleum (b. p. 60—80°) the crystals had m. p. 123—124°, $[\alpha]_D^{19}$ +70° (*c*, 1·1 in H_2O) (Found: C, 49·9; H, 7·1; N, 5·0; OMe, 23·7. $C_{11}H_{19}O_6N$ requires C, 50·6; H, 7·3; N, 5·4; OMe, 23·8%).

Mild hydrolysis of the *iso*propylidene residue was carried out in three ways: (1) A solution of the syrup (*A*) (2·48 g.) in methanol (50 c.c.) containing water (0·20 c.c.) was shaken with dry cation-exchange resins (Amberlite I.R. 100-H) (1·5 g.) for 6 days. Filtration and removal of the solvent gave methyl (methyl 2-*O*-methyl-α-D-galactopyranosid)uronate as a colourless syrup (2·18 g., 98%), n_D^{17} 1·4680, $[\alpha]_D$ +105° (*c*, 0·7 in MeOH), +113° (*c*, 0·8 in H_2O) (Jones and Stacey, *loc. cit.*, record n_D 1·4732, $[\alpha]_D^{20}$ +80°) (Found: C, 45·3; H, 6·9; OMe, 39·7. Calc. for $C_9H_{16}O_7$: C, 45·8; H, 6·8; OMe, 39·4%). (2) Similar treatment of (*A*) for 48 hr. at 50° gave a syrup (98%), $[\alpha]_D$ +97° (*c*, 0·7 in MeOH). (3) Treatment of (*A*) with 0·5% methanolic hydrogen chloride at 70° for 70 min. gave a syrup (98%), n_D^{17} 1·4679, $[\alpha]_D^{17}$ +4·0° (*c*, 1·7 in H_2O) (Brown, Hirst, and Jones, *J.*, 1949, 1761, record $[\alpha]_D$ +21° in H_2O). The derived amide, after recrystallisation from ethanol–ether, had m. p. 174—175°, $[\alpha]_D^{15}$ +60° (*c*, 1·3 in H_2O) (Jones and Stacey, *loc. cit.*, record m. p. 174°, $[\alpha]_D^{18}$ +55° in EtOH) (Found: C, 43·8; H, 6·6; N, 6·8; OMe, 28·4. Calc. for $C_8H_{15}O_6N$: C, 43·4; H, 6·8; N, 6·3; OMe, 28·1%).

Characterisation of Methyl (*Methyl* 2-O-*Methyl*-3: 4-O-iso*propylidene- and* 2-O-*Methyl*-α-D-*galactosid*)*uronate*.—A portion of the syrup (*A*) (0·12 g.) was oxidised with nitric acid (*d* 1·3) by raising the temperature from 40° to 80° during 15 min., and then kept at 80° for 10 min. After removal of the nitric acid by distillation under diminished pressure, with frequent additions of water, the product was esterified by boiling it for 6 hr. with methanolic hydrogen chloride (4%). The resulting ester, dimethyl 2-*O*-methyl-D-galactarate, gave on distillation a syrup (0·07 g.), b. p. 160—180°/0·1 mm., from which a crystalline diamide, m. p. 205° (decomp.), was obtained on treatment with methanolic ammonia for 48 hr. at 0° (Jones and Stacey, *loc. cit.*, record m. p. 200°; Brown, Hirst, and Jones, *loc. cit.*, give m. p. 195° for this product from methylated *Cholla* gum; Hough and Jones, *loc. cit.*, give m. p. 207° for the same derivative from methylated gum from *Sterculia setigera*).

The syrup (*A*) (0·3 g.) was hydrolysed with 0·2N-sulphuric acid at 100° until the rotation was constant ($[\alpha]_D$ +36°; 48 hr.). Neutralisation was effected with barium carbonate and 2-*O*-methyl-D-galacturonic acid was obtained as a colourless syrup ($[\alpha]_D^{18}$ +42°), after elution through a column (160 × 12 mm.) of cation-exchange resin (Amberlite I.R. 120-H). Oxidation with bromine, followed by ester formation, furnished dimethyl 2-*O*-methyl-D-galactarate which had n_D^{15} 1·4640, $[\alpha]_D^{18}$ +34° (*c*, 3·0 in H_2O). The diamide prepared as above had m. p. 205° (decomp.) (Found: C, 37·8; H, 6·2; N, 11·6; OMe, 14·9. Calc. for $C_7H_{14}O_6N_2$: C, 37·8; H, 6·4; N, 12·6; OMe, 14·0%).

Methyl (*Methyl* 3: 4-O-iso*Propylidene*-2-O-*toluene*-p-*sulphonyl*-α-D-*galactosid*)*uronate*.—The above-mentioned *iso*propylidene derivative, m. p. 113—114° (0·931 g.), was dissolved in dry pyridine (20 c.c.) and kept with "Drierite" (12 g.) at 0° for 20 hr. Toluene-*p*-sulphonyl chloride (1·6 g.) in dry pyridine (10 c.c.) was added in small portions during several hours, the mixture being kept at 0°. The solution was then set aside for 24 hr. at 0°, for 24 hr. at 15°, and for 72 hr. at 30° (unless these conditions are observed the yield is much diminished). The mixture was cooled to 0°, the "Drierite" removed by filtration, and water (100 c.c.) cautiously added with constant cooling. *Methyl* (*methyl* 3: 4-O-iso*propylidene*-2-O-*toluene*-p-*sulphonyl*-α-D-*galactosid*)*uronate* (*B*) was deposited as colourless needles (1·02 g.), m. p. 157—158°, $[\alpha]_D^{20}$ +122° (*c*, 1·1 in MeOH), +117° (*c*, 2·2 in $CHCl_3$) (Found: C, 52·0; H, 5·5; S, 8·1; OMe, 14·6. $C_{18}H_{24}O_9S$ requires C, 51·9; H, 5·8; S, 7·8; OMe, 14·9%). After removal of the crystals, extraction of the aqueous filtrate with chloroform and removal of the solvent gave a further yield (0·23 g.) of crystals (total yield, 1·255 g., 85%).

Methyl (*Methyl* 3: 4-*Di*-O-*methyl*-2-O-*toluene*-p-*sulphonyl*-α-D-*galactosid*)*uronate*.—The crystals (*B*) (1·345 g.), dissolved in 1% methanolic hydrogen chloride (130 c.c.), were kept at 30° for 30 hr. After neutralisation with silver carbonate *methyl* (*methyl* 2-O-*toluene*-p-*sulphonyl*-α-D-*galactosid*)*uronate* (*C*) (1·18 g., 97%) was obtained. After recrystallisation from aqueous methanol it had m. p. 71°, $[\alpha]_D^{18}$ +61° (*c*, 1·1 in $CHCl_3$) (Found: C, 45·8; H, 5·7; S, 8·4; OMe, 15·2. $C_{15}H_{20}O_9S,H_2O$ requires C, 45·7; H, 5·6; S, 8·1; OMe, 15·7%). Methanolic anhydrous ammonia quantitatively converted the ester into the *amide* which crystallised on removal of the solvent. The amide after recrystallisation from methanol had m. p. 94—95°, $[\alpha]_D^{18}$ +67°

(*c*, 0·5 in $CHCl_3$) (Found: C, 45·1; H, 5·6; N, 3·5; S, 8·1; OMe, 8·2. $C_{14}H_{19}O_8NS,H_2O$ requires C, 44·3; H, 5·6; N, 3·7; S, 8·4; OMe, 8·2%).

The product (*C*) (1·18 g.) was methylated four times with methyl iodide and silver oxide. The resultant *dimethyl ether* (1·21 g., 96%) was dissolved in warm ethanol and crystallisation induced by the addition of water. After recrystallisation from aqueous methanol it had m. p. 83°, n_D^{20} 1·4900, $[\alpha]_D^{17}$ +82° (*c*, 1·1 in $CHCl_3$), +88° (*c*, 2·4 in EtOH) (Found: C, 50·2; H, 5·8; OMe, 29·5; S, 8·1. $C_{17}H_{24}O_9S$ requires C, 50·5; H, 6·0; OMe, 30·7; S, 7·9%).

Methyl 3 : 4-*Di*-O-*methyl*-α-D-*galactosiduronic Acid*.—Anion-exchange resin (Amberlite I.R.A. 400-OH) was packed into four short glass tubes (60 × 10 mm.) which were arranged alternately with three similar tubes containing cation-exchange resin (Amberlite I.R. 120-H) to form a column, and the whole washed with distilled water (300 c.c.), with ethanol (200 c.c.), and with methanol (200 c.c.).

Methyl (methyl 3 : 4-di-*O*-methyl-2-*O*-toluene-*p*-sulphonyl-α-D-galactosid)uronate (1·2 g.), dissolved in methanol (20 c.c.), was stirred at room temperature with 0·25N-aqueous sodium hydroxide (12 c.c.) during 30 min.; thereafter sodium amalgam (4%; 15 g.) was added during 6 hr. with continuous stirring. The mixture was stirred for a further 18 hr. and then the solids were removed by filtration and washed with methanol. After treatment with solid carbon dioxide the combined filtrates were evaporated to dryness and a white solid was obtained which was repeatedly extracted with dry methanol under reflux. The cooled methanolic extracts (200 c.c.) were passed through a column, prepared as described above, 3—4 c.c. of eluate being collected during a minute, and, after complete elution, the eluate was recycled through the column which was finally washed with methanol (100 c.c.). Removal of the solvent from the combined eluate and washings gave a syrup (0·03 g.) which was discarded. The column was dismantled and the portions containing the anion-exchange resin reassembled and eluted by slow passage (60 hr.) of 2% formic acid in methanol (500 c.c.). Removal of solvent from the eluate, under reduced pressure, gave *methyl* 3 : 4-*di*-O-*methyl*-α-D-*galactosiduronic acid* as a colourless syrup (0·613 g., 87%) which crystallised spontaneously. Recrystallised from ethanol–light petroleum (b. p. 60—80°) it had m. p. 154—155°, $[\alpha]_D^{15}$ +158° (*c*, 1·3 in $CHCl_3$), +156° (*c*, 1·3 in MeOH), +163° (*c*, 1·3 in H_2O) (Found: C, 46·4; H, 7·0; OMe, 38·4. $C_9H_{16}O_7$ requires C, 45·8; H, 6·8; OMe, 39·4%).

Methyl (*Methyl* 3 : 4-*Di*-O-*methyl*-α-D-*galactosid*)*uronate*.—Methyl 3 : 4-di-*O*-methyl-α-D-galactosiduronic acid (0·375 g.), dissolved in methanolic hydrogen chloride (1%; 32 c.c.), was kept at 30° for 48 hr. The solution was neutralised with silver carbonate, and the filtrate after evaporation to dryness at 40°/15 mm. furnished a crystalline ester. Recrystallisation from light petroleum (b. p. 60—80°) gave needles of *methyl* (*methyl* 3 : 4-*di*-O-*methyl*-α-D-*galactosid*)-*uronate* (0·378 g., 95%), m. p. 113—114°, $[\alpha]_D^{16}$ +165° (*c*, 0·4 in $CHCl_3$) (Found: C, 48·0; H, 7·1; OMe, 49·8. $C_{10}H_{18}O_7$ requires C, 48·0; H, 7·25; OMe, 49·6%). The crystalline amide and *methylamide* were prepared by treating the ester, in the usual manner, with methanolic ammonia, and with methanolic methylamine respectively. The amide, after trituration with ethanol, had m. p. 130—131°, $[\alpha]_D^{17}$ +108° (*c*, 1·1 in EtOH). The methylamide, obtained as prisms on recrystallisation from acetone, had m. p. 205°, $[\alpha]_D^{15}$ +116° (*c*, 0·6 in H_2O) (Found: C, 48·5; H, 7·5; N, 5·8; OMe, 38·8. $C_{10}H_{19}O_6N$ requires C, 48·2; H, 7·7; N, 5·6; OMe, 37·4%).

The ester (0·018 g.) was methylated twice with methyl iodide and silver oxide, and crystalline methyl (methyl 2 : 3 : 4-tri-*O*-methyl-α-D-galactopyranosid)uronate (0·019 g.) was obtained. It had m. p. 71—72° (after sublimation *in vacuo*) alone and admixed with an authentic specimen.

Diethyl 3 : 4-*Di*-O-*methylgalactarate*.—The foregoing dimethyl ether ester (0·294 g.) was hydrolysed at 100° with sulphuric acid (20 c.c., 0·2N), the rotations observed being $[\alpha]_D$ +160° (0 hr.), +118° (3·5 hr.), +112° (6·5 hr.), +100° (24 hr.), +90° (31 hr.), +84° (48 hr., const.). The solution was neutralised with barium carbonate and filtered through a well-washed bed of charcoal–"Filter Cel," and barium ions were removed by passage of the filtrate through a column (300 × 10 mm.) of Amberlite (I.R. 120-H) ion-exchange resin. The clear eluate was concentrated to a colourless syrup, n_D^{11} 1·4615, $[\alpha]_D^{16}$ +37° (*c*, 1·2 in EtOH), +93° (*c*, 1·3 in H_2O). The syrup (0·22 g.) in water (15 c.c.) was oxidised with bromine (2 c.c.) at 40°, the rotations observed being $[\alpha]_D$ +93° (0 hr.), 80° (1 day), 60° (3 days), 23° (5 days), 15° (8 days, constant). After removal of the water and hydrobromic acid under diminished pressure with frequent addition of ethanol the syrupy residue was heated in hydrochloric acid (10 c.c.; 0·2N) at 100° for 50 hr. by which time the rotation had fallen to +8°. Further treatment with bromine (2 c.c.) for 3 days at 40° gave a solution with $[\alpha]_D$ ±0°. Removal of the water and hydrobromic acid as above gave crystalline *diethyl* 3 : 4-*di*-O-*methylgalactarate*. Recrystallisation from

aqueous acetone gave flat plates, m. p. 148—149°, $[\alpha]_D$ ±0° [Found: C, 49·4; H, 7·4; OR (as OMe), 42·2. $C_{12}H_{22}O_8$ requires C, 49·0; H, 7·5; OR (as OMe), 42·2%]. Treatment with methanolic hydrogen chloride (1·5%) at 60° for 16 hr. gave a crystalline product. Recrystallisation from acetone–light petroleum (b. p. 60—80°) furnished *dimethyl* 3 : 4-*di*-O-*methylgalactarate* as needles, m. p. 172—173°, $[\alpha]_D$ ±0° (Found: 44·8; H, 6·7. $C_{10}H_{18}O_8$ requires C, 45·1; H, 6·8%). The derived *diamide*, after recrystallisation from methanol, had m. p. 230° (decomp.), $[\alpha]_D$ ±0° (Found: N, 11·9. $C_8H_{16}O_6N_2$ requires N, 11·9%).

The authors are very grateful to Professor E. L. Hirst, F.R.S., for his interest and advice, and thank the University of Edinburgh for the award of a Vans Dunlop Scholarship, the Department of Scientific and Industrial Research for the award of a maintenance grant to one of them (R. A. E.), and the Distillers Company Limited for a grant.

UNIVERSITY OF EDINBURGH. [*Received, May 4th,* 1953.]

PRINTED IN GREAT BRITAIN BY RICHARD CLAY AND COMPANY, LTD.,
BUNGAY, SUFFOLK.