The Synthesis of Methyl Ethers of Mannuronic and Glucuronic Acid, and their Reaction with Periodate.

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4-*O*-Methyl-D-mannuronic and 2 : 3-di-*O*-methyl-D-glucuronic acid have been synthesised for the first time and characterised by crystalline derivatives. 2 : 3 : 4-Tri-*O*-methyl-D-mannuronic acid has also been characterised as the crystalline methyl 2 : 3 : 4-tri-*O*-methyl-α-D-mannosiduronomethylamide. The action of periodate under varying conditions on these substances and on the two methyl ethers of D-galacturonic acid (Edington and Percival, *J.*, 1953, 2473) has been studied.

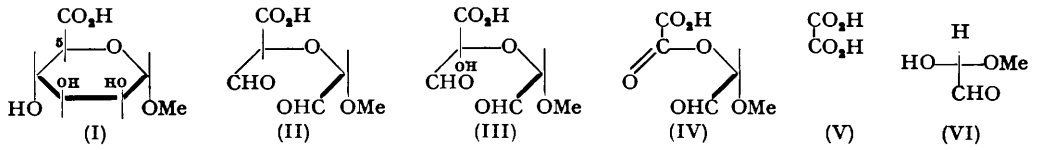
In continuation of our work on the synthesis of methyl ethers of D-galacturonic acid (Edington and Percival, *J.*, 1953, 2473) we have synthesised the 4-methyl ether of D-mannuronic acid and the 2 : 3-dimethyl ether of D-glucuronic acid. The first crystalline derivative of 2 : 3 : 4-tri-*O*-methyl-D-mannuronic acid has also been obtained. As the preparation of pure D-mannuronic acid derivatives from alginic acid proved exceedingly difficult it was considered preferable in the present work to prepare authentic crystalline derivatives of methyl α-D-mannoside and oxidise these to the uronic acid. Attempts to prepare crystalline methyl 2 : 3-*O*-*iso*propylidene-α-D-mannopyranoside (Ault, Haworth, and Hirst, *J.*, 1935, 517) and methyl 2 : 3-*O*-*iso*propylidene- and 2 : 3-*O*-benzylidene-6-*O*-toluene-*p*-sulphonyl-α-D-mannopyranoside were all unsuccessful. Accordingly crystalline methyl 2 : 3 : 4-*O*-tribenzoyl-6-*O*-toluene-*p*-sulphonyl-α-D-mannopyranoside (Haskins, Hann, and Hudson, *J. Amer. Chem. Soc.*, 1946, **68**, 628) was utilised, and although the removal of the toluene-*p*-sulphonyl group by reduction (Kenner and Murray, *J.*, 1949, S178) could not be achieved this group was easily replaced by iodine; the latter was resistant to direct substitution by the hydroxyl group, but was easily replaced by nitrate. Reductive denitration then gave crystalline methyl 2 : 3 : 4-tri-*O*-benzoyl-α-D-mannoside. Parallel experiments on the successive treatment with triphenylmethyl chloride and benzoyl chloride of methyl α-D-mannopyranoside in pyridine gave methyl 2 : 3 : 4-tri-*O*-benzoyl-6-*O*-triphenylmethyl-α-D-mannoside and this with hydrogen bromide in glacial acetic acid gave the same crystalline tribenzoate in high yield. As originally obtained or after recrystallisation from aqueous pyridine, methyl 2 : 3 : 4-tri-*O*-benzoyl-6-*O*-triphenylmethyl-α-D-mannoside contained one molecule of firmly held pyridine of crystallisation and analogous compounds containing chloroform or acetone of crystallisation were obtained by recrystallisation from the corresponding solvent. The pyridine of crystallisation did not interfere with the removal of the trityl residue. Dr. C. A. Beevers kindly undertook an examination of these crystals with the polarising microscope and by *X*-ray analysis. Oxidation of the tribenzoate with potassium permanganate gave crystalline methyl 2 : 3 : 4-tri-*O*-benzoyl-α-D-mannosiduronic acid, which gave a crystalline methyl ester. Removal of the benzoyl residues by two different methods led to crystalline methyl α-D-mannopyranosiduronamide. Condensation of this amide with acetone gave crystalline methyl 2 : 3-*O*-*iso*propylidene-α-D-mannopyranosiduronamide and methylation with Purdie's reagents gave a crystalline product which was analysed as methyl 4-*O*-methyl-2 : 3-*O*-*iso*propylidene-α-D-mannosiduronomethylamide. The fact that the amide group had also been methylated was confirmed by conversion into the methyl ester and regeneration of the original substance by treatment with methylamine. This was further characterised by acid hydrolysis, followed by bromine oxidation and the isolation, as its crystalline diamide, of 4-*O*-methyl-D-mannaric acid (equivalent, by reason of the symmetry, to 3-*O*-methyl-D-mannaric acid).

Although 2 : 3 : 4-tri-*O*-methyl-D-mannuronic acid has been synthesised previously (Smith, Stacey, and Wilson, *J.*, 1944, 131; Ault, Haworth, and Hirst, *loc. cit.*) no crystalline derivative except the trimethylmannaramide was isolated. In the present work methylation with Purdie's reagents of methyl α-D-mannopyranosiduronamide gave crystalline

methyl 2 : 3 : 4-tri-*O*-methyl-α-D-mannosiduronomethylamide. Again proof that the amide group had been methylated was obtained by hydrolysis and regeneration by treatment with methylamine. The regenerated material was further characterised, after hydrolysis and oxidation, as crystalline 2 : 3 : 4-tri-*O*-methyl-D-mannardiamide.

Although 2 : 3-di-*O*-methyl-D-glucuronic acid was isolated by Smith (*J.*, 1940, 1035) from methylated arabic acid no authentic synthesis of it has been reported. Methylation of methyl 4 : 6-*O*-benzylidene-α-D-glucoside (Freudenberg, Toepffer, and Andersen, *Ber.*, 1928, **61**, 1750) gave the crystalline 4 : 6-*O*-benzylidene-2 : 3-di-*O*-methyl derivative from which the benzylidene residue was removed by dilute acid at room temperature. Oxidation of the crystalline product with dilute aqueous alkaline permanganate, followed by esterification, led to syrupy methyl (methyl 2 : 3-di-*O*-methyl-α-D-glucopyranosid)uronate, which gave a crystalline *p*-nitrobenzoate identical (mixed m. p.) with a specimen prepared by Smith (*loc. cit.*) from the hydrolysate of methylated arabic acid. The crystalline phenylhydrazide of the 2 : 3-dimethyl ether was also prepared and had m. p. 195—197°. Smith (*loc. cit.*) records m. p. 225—227° for this derivative and we are grateful to him for a re-determination : his material had changed on storage to m. p. 207° (205° after resolidifying) and gave a mixed m. p. with our material of 196—200° (197° after resolidifying). The existence of two crystalline forms is not new in carbohydrate chemistry (cf. 2 : 3-di-*O*-methyl-*N*-phenyl-D-xylosamine, m. p. 144° and 126°; Smith *et al.*, *J. Amer. Chem. Soc.*, 1952, **74**, 1341). The dimethyl ether was further characterised by conversion into the known methyl 2 : 3 : 4-tri-*O*-methyl-α-D-glucosiduronamide and 2 : 3-di-*O*-methyl-D-glucaramide.

In spite of the fact that the original concept of specificity for the α-diol structure has been modified considerably, periodate remains a valuable tool. The presence of uronic acid residues in many polysaccharides makes it desirable, therefore, that precise information should be available concerning the reaction of these substances and their derivatives with periodate. Link and his co-workers (*J. Biol. Chem.*, 1945, **159**, 502) recorded that zinc bornyl glucosiduronate and methyl (methyl α-D-galactosid)uronate gave more than the theoretical amount of formic acid under the action of periodate. Halsall, Hirst, and Jones (*J.*, 1947, 1427) confirmed this and found that if they used potassium metaperiodate at pH 4 and kept the concentration of formic acid low the uronic acids underwent further oxidation. Link and his colleagues had explained the over-oxidation of the uronosides on the assumption that after the formation of the dialdehyde (II) the hydrogen situated on the original $C_{(5)}$ is activated by the adjacent carboxyl and aldehydic groups and is oxidised to a hydroxyl group (as in 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. per mol. of uronic



acid. If the conditions are such that the ester (IV) is hydrolysed, the hemiacetal (VI) of glyoxal would be oxidised further and the total consumption of periodate would be 5 mol. Sprinson and Chargaff (*J. Biol. Chem.*, 1946, **164**, 435) investigated the conversion of the aldehyde (II) into the hydroxy-aldehyde (III) and proved that substances such as malonic acid which contain a hydrogen atom on a carbon atom situated between two carbonyl groups can be oxidised to the corresponding hydroxy-compound which then undergoes further oxidation.

In the present work methyl (methyl α-D-galactopyranosid)uronate was oxidised under different conditions of pH and temperature (see Table 1). Within 20 hours under all the conditions employed more than the 2 mol. of periodate required by the simple oxidation had been consumed. In 66 hours the consumption in all cases was 3 mol. or more and the

TABLE 1. *Uptake of periodate (mol.) by methyl* α-D-*galactopyranosiduronic methyl ester.*

Temp. :	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 (hr.)									
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

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. Meyer and Rathgeb (*Helv. Chim. Acta*, 1949, **32**, 1102) state that, if the oxidation is carried out at 0° and the pH kept between 5·7 and 4·2, then the hydrolysis of formyl esters is negligible. It appears probable therefore that the oxidations carried out at pH 4·5 are arrested at the ester (IV). From the results it appears that some overoxidation occurs under all the conditions studied and that, apart from the figure of 5 mol. representing complete overoxidation, the only significant arrest in the progress of oxidation occurs at the α-hydroxy-aldehyde (III) after the consumptiom of 3·0 mol. of oxidant. According to Halsall, Hirst, and Jones (*loc. cit.*) this is to be expected as the rate of oxidation of the α-hydroxy-aldehyde is comparatively slow.

Of the four glycosiduronic acid derivatives studied (see Table 2) in which overoxidation

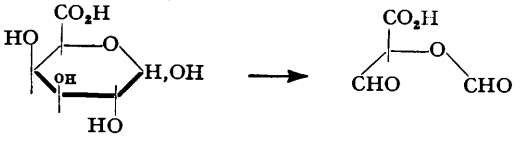
TABLE 2. *Uptake of periodate (mol.) by uronic acid derivatives.*

	Mol. expected			Buffer B pH 4·5, 0°		Buffer E pH 7·0, 18°	
Substance	(*a*)	(*b*)		90 hr.	300 hr.	90 hr.	300 hr.
Me (Me α-D-galactopyranosid)uronate	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 (Me 2-*O*-methyl-α-D-galactosid)uronate	1	3		1·4	2·0	2·0	3·0
Me (Me 4-*O*-methyl-α-D-mannosid)uronate	1	1		0·9	1·0	1·0	1·1
Me (Me 3 : 4-di-*O*-methyl-α-D-galactosid)uronate	0	0		0·0	0·0	0·0	0·0
Me (Me 2 : 3-di-*O*-methyl-α-D-glucosid)uronate	0	0		0·0	0·0	0·0	0·0
Me (Me 2 : 3 : 4-tri-*O*-methyl-α-D-glucosid)uronate	0	0		0·0	0·0	0·0	0·0
Me 2 : 3 : 6-tri-*O*-methyl-α-D-mannosiduronomethylamide	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-*O*-Methyl-D-mannuronic acid	3	2	2	2·0	2·1	—	—
2-*O*-Methyl-D-galacturonic acid	3	1	3	1·5	1·9	—	—
3 : 4-Di-*O*-methyl-D-galacturonic acid	2	1	1	1·1	1·2	—	—
2 : 3-Di-*O*-methyl-D-glucuronic acid	2	0	0	0·9	1·2	—	—
2 : 3 : 4-Tri-*O*-methyl-D-glucuronic acid	1	0	0	0·0	0·0	—	—

(*a*) Without overoxidation. (*b*) With overoxidation. (*c*) Open-chain configuration. (*d*) Pyranose, without overoxidation. (*e*) Pyranose, with overoxidation.

was expected it did occur, but the numerical results are difficult to reconcile with theory. At 0° and pH 4·5 the overoxidation was small even after 300 hours, methyl α-D-galactosiduronamide and mannosiduronamide consuming respectively 2·5 and 2·7 mol. of periodate instead of the postulated 5 mol. Methyl (methyl 2-*O*-methyl-α-D-galactosid)uronate consumed 2 mol., mid-way between normal oxidation and complete overoxidation. No overoxidation occurred with methyl (methyl 4-*O*-methyl-α-D-mannosid)uronate; on the current theories this is to be expected as the 4-methyl group would prevent oxidation between positions 3 and 4 and consequently the formation of an active hydrogen atom. This is also in agreement with the results of Smith (*J.*, 1951, 2646) who found that methyl (methyl 4-*O*-methyl-α-D-glucosid)uronate consumed only 1 mol. of periodate. With two dimethyl- and two trimethyl-uronosidic methyl esters where no oxidation of any kind was expected the results fitted expectations.

A number of free uronic acids were oxidised at 0° and pH 4·5 (see Table 2); under these conditions, if a formic ester is produced, it is unlikely to be hydrolysed (Meyer and Rathgeb, *loc. cit.*). The periodate consumption after 300 hours was in every case about 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 N-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 (methyl 3 : 4-di-*O*-methyl-α-D-galactosid)-uronic under the same conditions gave a 97% yield of the free acid. White (*J. Amer. Chem. Soc.*, 1953, **75**, 4692) found that 3 : 4-di-*O*-methyl-D-glucuronic acid consumed only 1 mol. of periodate which is in agreement with the present results for 3 : 4-di-*O*-methyl-D-galacturonic acid. Greville and Northcote (*J.*, 1952, 1945) found that 3 : 4-di-*O*-methyl-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 $C_{(5)}$ and $C_{(6)}$ to oxidation may only partly be due to the impossibility of the sugar's 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-*O*-methyl-D-mannuronic acid, 3 : 4-di-*O*-methyl-D-galacturonic acid, and 2 : 3 : 4-tri-*O*-methyl-D-glucuronic acid. For the remaining uronic acids it must be borne in mind that if a uronic acid having 3- and 4-hydroxyl groups reacts in the pyranose form one of the intermediates contains activated hydrogen and overoxidation is therefore to be expected.



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

Experimental

All solvents were removed under reduced pressure and below 50°. M. p.s were determined on the Kofler hot-stage microscope. Silver and barium salts were removed by filtration through layers of charcoal and Celite and the residue on the filter washed at least thrice with a suitable warm solvent. Optical rotations were determined at 18° ± 2° in $CHCl_3$ unless otherwise stated. Light petroleum was the fraction of b. p. 60—80°.

Methyl 2 : 3 : 4-*Tri*-O-*benzoyl*-6-O-*triphenylmethyl*-α-D-*mannoside*.—Methyl α-D-mannopyranoside, m. p. 194—195° (105·5 g.), isolated from carob gum (supplied by Messrs. Ellis Jones, Stockport) (Smith, *J.*, 1948, 1989) was heated with triphenylmethyl chloride (175 g.) and dry pyridine (1050 ml.) at 50° with occasional shaking, until all the solid had dissolved (6 hr.), then kept at room temperature for 18 hr., after which benzoyl chloride (230 ml.) was added rapidly without cooling. The mixture was set aside at room temperature for 24 hr. and the crystalline solid filtered off, and washed with pyridine (100 ml.), with ethanol (2 × 100 ml.), with water (3 l.), and again with ethanol (2 × 100 ml.). After drying, the *product* was a colourless crystalline solid (368 g., 82%), m. p. 100—120°, $[\alpha]_D$ −110° (Found : C, 75·1; H, 5·3; N, 1·9. $C_{47}H_{40}O_9,C_5H_5N$ requires C, 75·4; H, 5·5; N, 1·7%). Recrystallisation from aqueous or ethanolic pyridine gave an unchanged product. Recrystallisation from acetone gave stout prisms, m. p. 100—115°, $[\alpha]_D$ −114° (Found : C, 74·2; H, 6·1. $C_{47}H_{40}O_9,C_3H_6O$ requires C, 74·3; H, 5·7%). Recrystallisation from ethanol–chloroform gave plates, m. p. 100—115°, $[\alpha]_D$ −107° (Found : C, 67·9; H, 5·0; Cl, 9·3; loss of wt. at 100°/15 mm. in 15 hr., 10·4. $4C_{47}H_{40}O_9,3CHCl_3$ requires C, 68·4; H, 4·9; Cl, 9·5; $CHCl_3$, 10·7%). The crystals from each of these solvents, after melting, recrystallised between 115—125° and had a final m. p. 188—189°. After removal of the solvent *in vacuo* the *product* was a fine white powder, m. p. 189—191°, $[\alpha]_D$ −121° (Found : C, 75·4; H, 5·3. $C_{47}H_{40}O_9$ requires C, 75·4; H, 5·4%).

Examination with the polarising microscope [Dr. Beevers]. Crystals from $CHCl_3$: flat

prisms showing an extinction at 45° to their length. Crystals from acetone: prisms, some with parallel and some with a 45° extinction. Crystals from aqueous pyridine: small prisms, a woolly mass, 22° extinction. Crystals from $CHCl_3$–EtOH: opaque mass, no single crystals visible. Conclusion: These crystals are of low symmetry, and perhaps show more than one crystal form.

X-*Ray Examination* [Dr. BEEVERS]. Oscillation and Weissenberg photographs (zero and first layer-line) were taken of two crystals from chloroform. Both specimens showed a similar phenomenon, a doubling of the spots, more pronounced in some directions than in others, indicating a twinning of two slightly different lattices very similar to one another. A detailed analysis of the two lattices has not been made, although it is thought that both are either monoclinic or triclinic. If the doubled spots are treated as one, an "average" lattice can be deduced, and has the following properties: monoclinic, $a = 9{\cdot}07$, $b = 24{\cdot}3$, $c = 10{\cdot}4$ Å, $\beta = 105°\ 27'$. This cell is body-centred (though of course it can be transformed into an A or a C face-centred lattice). The volume of the cell is 2209 Å^3, and this value gives 2·05 of (molecule + $\frac{1}{4}CHCl_3$) per cell. Since this refers to an "average" cell it is quite possible that there are 2 molecules of chloroform in one kind of cell and 1 in the other, although it might also be that there are 1·5 molecules in both kinds of cell. The two cells appear to be equally numerous, *i.e.*, the average intensity of the two sets of spots is about the same.

It is hoped that an opportunity may arise for the lengthy detailed study of these twins, especially as a large single crystal of the product from acetone is available.

Methyl 2 : 3 : 4-*Tri*-O-*benzoyl*-6-O-*toluene*-p-*sulphonyl*-α-D-*mannoside*.—Methyl α-D-mannoside (107 g.) was stirred with dry pyridine (1000 ml.) for 30 min. at room temperature, then cooled to 0°, and a solution of toluene-*p*-sulphonyl chloride (112 g., 1·05 equiv.) in pyridine (250 ml.) was added with stirring and cooling to 0° during 9 hr. After a further hour's stirring at room temperature the mixture was cooled to 0° and water (1000 ml.) added with vigorous stirring during 20 min. More water (1500 ml.) was added and the solution extracted with chloroform (4 × 600 ml.). The extracts were washed with water and dried. Removal of the solvent gave a yellow syrup (146 g., 76%) (Found: S, 9·2; $C_7H_7O_3SNa$, 54·3. $C_{14}H_{21}O_8S$ requires S, 9·2; $C_7H_7O_3SNa$, 55·8%). The syrup (146 g.) in pyridine (400 ml.) was treated with benzoyl chloride (258 ml., 4 equiv.) and set aside at room temperature for 24 hr. After treatment with water (20 ml.) the mixture was poured into saturated aqueous sodium hydrogen carbonate, a red gum separating. Repeated extraction under reflux with ethanol left a white residue of tribenzoate which recrystallised from chloroform–ethanol as colourless plates (157 g., 50% from methyl α-D-mannoside), m. p. 198°, $[\alpha]_D$ −104° (Haskins, Hann, and Hudson, *loc. cit.*, record 38% yield and m. p. 197—199°, $[\alpha]_D$ −102°) (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%).

Methyl 2 : 3 : 4-*Tri*-O-*benzoyl*-α-D-*mannoside*.—(*a*) Methyl 2 : 3 : 4-tri-*O*-benzoyl-6-*O*-triphenylmethyl-α-D-mannoside (20 g.) was shaken vigorously for 90 sec. with hydrogen bromide (10%; w/v) in glacial acetic acid (33 ml.) and filtered immediately 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 mixture. The aqueous layer was extracted with chloroform (2 × 400 ml.) and the combined chloroform extracts, after being washed and dried, gave a crystalline solid on evaporation. When an ethanol solution of this solid was kept overnight at room temperature crystalline starting material (5 g.; m. p. 95—120°, 187—189°) was deposited. Evaporation of the mother-liquors gave a crystalline *tribenzoylmannoside* (172 g., 95%), which after recrystallisation from aqueous alcohol had m. p. 143—145°, $[\alpha]_D$ −160° (Found: C, 66·6; H, 5·3. $C_{28}H_{26}O_9$ requires C, 66·4; H, 5·2%).

(*b*) The foregoing toluene-*p*-sulphonate was recovered unchanged and in good yield after hydrogenation with Raney nickel in ethyl acetate at 1 and 3 atm. during 72 hr. with shaking at 20°, at 100 atm. during 12 hr. with stirring, and under the conditions recorded by Mozingo *et al.* (*J. Amer. Chem. Soc.*, 1945, **67**, 2092) for 6 hr. at 77° with or without a stream of hydrogen. In a final experiment at 100°/100 atm. for 12 hr. a non-reducing syrup, $[\alpha]_D$ ±0°, was isolated (Found: C, 63·0; H, 7·4; S, 2·4; OMe, 6·7%; equiv., 184). It gave a negative test for primary toluene-*p*-sulphonyl ester (Tipson, Clapp, and Cretcher, *J. Org. Chem.*, 1947, **12**, 133).

(*c*) The toluene-*p*-sulphonate (6 g.) was converted into the 6-deoxy-6-iodo-derivative according to Oldham and Rutherford's method (*J. Amer. Chem. Soc.*, 1932, **54**, 366). Recrystallisation from acetone gave large prisms of the 6-iodide (5·05 g., 90%), m. p. 199—201°, $[\alpha]_D$ −106° (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%). Treatment of this in dry benzene with dry or with moist silver oxide with vigorous shaking for 7 days, followed by filtration and evaporation, gave the starting material in quantitative yield. Heating

with moist silver oxide in benzene in a sealed tube at 100° for 2 hr. gave, after filtration and evaporation, a dark, reducing syrup.

The iodide (13 g.), in acetonitrile (200 ml.), was heated under reflux for 4 hr. with powdered silver nitrate (4 g.). Excess of silver nitrate was removed by treating the cooled solution with sodium iodide (1·0 g.) in acetone. After removal of the precipitated silver iodide the solution was diluted with chloroform (300 ml.) and extracted with water (3 × 300 ml.). Evaporation of the dried chloroform layer gave crystals from which unchanged starting material (6·2 g.; m. p. 193°) was removed by fractional crystallisation from ethanol. The more soluble *nitrate* was obtained from 90% ethanol as large prisms (5·5 g., 91%), m. p. and mixed m. p. with a specimen provided by Dr. G. O. Aspinall (prepared by direct nitration of methyl 2 : 3 : 4-tri-*O*-benzoyl-α-D-mannoside, unpublished work) 103—104°, $[\alpha]_D$ −116° (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%). A solution of this nitrate (2·0 g.) in glacial acetic acid (10 ml.) and benzene (20 ml.) was treated at room temperature with equal quantities of zinc and iron powders until the solution no longer gave a pink colour with diphenylbenzidine in concentrated sulphuric acid. Filtration and extraction with benzene, followed by aqueous washing of the benzene extracts and evaporation, gave crystals (1·3 g., 71%), m. p. 143° alone or mixed with the previous end-product (after recrystallisation from ethanol).

Methyl α-D-Mannopyranosiduronamide.—A solution of methyl 2 : 3 : 4-tri-*O*-benzoyl-α-D-mannoside (150 g.) in acetone (1500 ml.), glacial acetic acid (1500 ml.), and water (150 ml.) was stirred at room temperature, and potassium permanganate (160 g.; "AnalaR") was added in small portions during 30 hr. (Stacey, *J.*, 1939, 1529). After a further 18 hr. the solution was decolorised by aqueous potassium metabisulphite. 4N-Sulphuric acid (150 ml.) was added, and chloroform extraction (3 × 1000 ml.) followed by evaporation gave *methyl 2 : 3 : 4-tri-O-benzoyl-α-D-mannosiduronic acid* (131 g., 85%) which crystallised on storage and after recrystallisation from chloroform–light petroleum had m. p. 180—181·5°, $[\alpha]_D$ −140° (Found: C, 64·5; H, 4·7. $C_{28}H_{24}O_{10}$ requires C, 64·6; H, 4·6%). Treatment with methanolic hydrogen chloride (0·5%) at 20° for 72 hr. led to crystalline methyl (methyl 2 : 3 : 4-tri-*O*-benzoyl-α-D-mannosid)-uronate (123 g., 92%) which after recrystallisation from methanol had m. p. 143—144°, $[\alpha]_D$ −127° (Found: C, 65·2; H, 4·9. $C_{29}H_{26}O_{10}$ requires C, 65·1; H, 4·9%). This ester (100 g.) was dissolved in saturated methanolic ammonia (1100 ml.) and set aside at room temperature for 24 hr. Evaporation gave a syrup from which benzamide was removed by repeated extraction with ether (250-ml. portions). The crystalline residue of *methyl α-D-mannopyranosiduronamide* was washed once with acetone and recrystallised from aqueous acetone as colourless prisms (20·1 g., 52%), 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%). Removal of the benzoyl groups from the previous tribenzoate with sodium methoxide (Zemplén and Pacsu, *Ber.*, 1929, **62**, 1613), followed by esterification with methanolic hydrogen chloride (2%), gave syrupy methyl (methyl α-D-mannopyranosid)uronate, n_D^{19} 1·4842, $[\alpha]_D^{22}$ +80° (*c*, 0·5 in H_2O); the derived amide (45% yield from the tribenzoate ester) had m. p. and mixed m. p. 182—183°, $[\alpha]_D^{15}$ +63° (*c*, 0·9 in H_2O).

Methyl (Methyl 4-O-Methyl-α-D-mannosid)uronate.—The foregoing amide (20 g.) was condensed with acetone (750 ml.) (Percival and Percival, *J.*, 1950, 690). After 4 days the supernatant liquid was decanted off and neutralised with barium carbonate, and the residual solids were shaken for a further 2 days with acetone (500 ml.) containing sulphuric acid (0·03% v/v). Condensation of the residual solids with acetone was repeated a further 6 times after which the barium salts were removed from the combined neutral extracts. Evaporation of the acetone gave crystalline *methyl 2 : 3-O-isopropylidene-α-D-mannosiduronamide*, which recrystallised from acetone–light petroleum as large colourless prisms (15·0 g., 63%), m. p. 177·5—179°, $[\alpha]_D$ +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%). Threefold methylation with methyl iodide and silver oxide gave *methyl 4-O-methyl-2 : 3-O-isopropylidene-α-D-mannosiduronomethylamide* (1·09 g. from 1 g.; 93%) which crystallised and after recrystallisation from light petroleum 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%). Hydrolysis with 2N-sodium hydroxide at 100° for 3 hr., followed by esterification of the resulting syrup with ethereal diazomethane and then treatment with ethanolic methylamine, regenerated the crystalline methylamide. This methylamide (2·0 g.) was heated at 100° for 30 min. with N-sodium hydroxide (20 ml.), then cooled to −5° and acidified with 4N-sulphuric acid (7 ml.); the solution was extracted with chloroform (4 × 50 ml.); after being washed with water the chloroform extracts were evaporated to a syrup which was dissolved in 1% methanolic hydrogen chloride and kept at room temperature for 48 hr. Appropriate treatment gave *methyl (methyl*

4-O-*methyl*-α-D-*mannosid*)*uronate* as a syrup (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 twice with methyl iodide and silver oxide, followed by treatment with ethanolic methylamine, gave methyl 2 : 3 : 4-tri-*O*-methyl-α-D-mannosiduronomethylamide, m. p. 103—105° alone or mixed with a specimen prepared by direct methylation of methyl α-D-mannopyranosiduronamide. After hydrolysis of the preceding ester (0·097 g.) with N-hydrochloric acid at 100° for 24 hr. in a sealed tube, oxidation with bromine water (7 days at 40°), esterification, distillation (b. p. 160—170°/0·1 mm.) and treatment with methanolic ammonia gave 2 : 3 : 4-*tri*-O-*methyl*-D-*mannardiamide* (0·030 g.) m. p. 192—193°, $[\alpha]_D^{15}$ −16° (*c*, 0·6 in H_2O) (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-*mannosiduronomethylamide*.—Methyl α-D-mannopyranosiduronamide (1·99 g.) was methylated thrice with methyl iodide (23 ml.) and silver oxide (12 g.), dioxan being added as a solvent in the first operation. A colourless syrup (2·618 g., 103%), n_D^{15} 1·4661, which crystallised on trituration with light petroleum was obtained. After recrystallisation from light petroleum the *methylamide* 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%). Hydrolysis with sodium hydroxide, esterification, and treatment with ethanolic methylamine regenerated it in good yield. Treatment of the methylamide (0·10 g.) with 2N-sodium hydroxide, then with N-hydrochloric acid, oxidation with bromine water, esterification, distillation of the derived ester (b. p. 130—140°/0·1 mm.), and treatment with methanolic ammonia gave the trimethylmannardiamide (0·030 g.), m. p. 211° (decomp.) unchanged by repeated recrystallisation from methanol or on admixture with a synthetic specimen prepared by Haworth, Hirst, Isherwood, and Jones (*J.*, 1939, 1878) which was now found to have m. p. 211° on the Kofler apparatus and in a capillary tube.

Methyl 2 : 3-*Di*-O-*methyl*-α-D-*glucopyranoside*.—Methyl α-D-glucopyranoside, m. p. 163—165° $[\alpha]_D^{17}$ +158° (*c*, 1·8 in H_2O) (100 g.), was converted into the 4 : 6-*O*-benzylidene derivative according to the method of Freudenberg *et al.* (*loc. cit.*). The crude product was extracted with boiling water (1500 ml.), and the aqueous extract filtered whilst hot. On cooling, the product was obtained as long needles (102 g., 70%), m. p. 164—165°, $[\alpha]_D$ +108° (Found: C, 59·0; H, 6·6. Calc. for $C_{14}H_{18}O_6$: C, 59·6; H, 6·4%). A solution of it (50 g.) in acetone (300 ml.) was methylated twice with aqueous 30% sodium hydroxide (320 ml.) and methyl sulphate (130 ml.) (Bell, *J.*, 1936, 859). Methyl 4 : 6-*O*-benzylidene-2 : 3-di-*O*-methyl-α-D-glucoside (51·5 g., 94%) had, after recrystallisation from ethanol, m. p. 121—122°, $[\alpha]_D$ +95° (Found: C, 61·8; H, 7·0. Calc. for $C_{16}H_{22}O_6$: C, 61·9; H, 7·2%). A solution of this (38·4 g.) in acetone (800 ml.) and 4N-sulphuric acid (200 ml.) was kept at room temperature until the rotation of the solution became constant, $[\alpha]_D$ +110° (72 hr.). After neutralisation (barium carbonate) the combined filtrate and washings were concentrated to a syrup from which benzaldehyde was removed by repeated distillation with water. The residue of methyl 2 : 3-di-*O*-methyl-α-D-glucopyranoside (23·5 g., 86%), recrystallised from carbon tetrachloride, had m. p. 81—82°, $[\alpha]_D^{18}$ +153° (*c*, 1·0 in H_2O) (Found: 48·9; H, 8·0. Calc. for $C_9H_{18}O_6$: C, 48·6; H, 8·1%).

Methyl (*Methyl* 2 : 3-*Di*-O-*methyl*-α-D-*glucopyranosid*)*uronate*.—The last mentioned glucoside (27·3 g.) dissolved in aqueous potassium hydroxide (14 g. in 2 l.) was stirred at room temperature and potassium permanganate (37 g.) added in small portions during 8 hr.; stirring was continued for a further 16 hr. Excess of permanganate was decomposed by aqueous potassium metabisulphite, the mixture filtered, and the residue washed with hot water (4 × 150 ml.). The combined filtrate and washings were neutralised with solid carbon dioxide and evaporated to dryness. The resultant white solid was extracted with ether (2 × 300 ml.) and then with boiling ethanol (3 × 300 ml.), and the ethanolic extracts were passed through a column (17 × 700 mm.) of Amberlite resin IR-120. The column was washed with water (200 ml.) and evaporation of the combined eluate and washings gave a syrup (20·5 g.) which was converted into the ester by treatment with 1% methanolic hydrogen chloride (500 ml.) at room temperature for 48 hr. Distillation of the product gave methyl (methyl 2 : 3-di-*O*-methyl-α-D-glucopyranosid)uronate as a syrup (20·9 g., 68%), b. p. 130—140°/0·5 mm., n_D^{18} 1·4441, $[\alpha]_D^{18}$ +111° (*c*, 1·1 in H_2O). The derived 4-*O*-*p*-nitrobenzoyl derivative (Smith, *J.*, 1940, 1035) (3·5 g. from 2·2 g.) had m. p. 156—158° alone or mixed m. p. with a specimen kindly supplied by Professor F. Smith, $[\alpha]_D$ +69° (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%). The derived uronophenylhydrazide, isolated as a crystalline residue (1·85 g.) after the ester (2·0 g.) had been heated with freshly distilled phenylhydrazine (0·78 ml.) in carbon dioxide in a sealed tube at 110° for 18 hr. and extracted with ether (3 × 20 ml.) under reflux, had m. p. 195—197° (from benzene), $[\alpha]_D$ +85°. Smith (*loc. cit.*) records m. p. 225—227°. The

mixed m. p., kindly determined by Professor F. Smith with his specimen, was 196—200°, 197° (after resolidification) (Found: C, 55·2; H, 6·8; N, 9·0. Calc. for $C_{15}H_{22}O_6N_2$: C, 55·2; H, 6·8; N, 8·6%). Hydrolysis of the methyl uronate with N-hydrochloric acid at 100° for 24 hr., followed by oxidation with bromine water, esterification, and distillation, gave syrupy 2 : 3-di-*O*-methyl-D-glucaro-l→4-lactone 6-methyl ester, b. p. 120—130°/0·1 mm., which crystallised after distillation and on recrystallisation from benzene had m. p. 99—100°, $[\alpha]_D^{18}$ +17° (*c*, 1·2 in H_2O). The derived amide had m. p. and mixed m. p. 154—155°. Two Purdie methylations of the methyl uronate (70 mg.), distillation, amide formation, and recrystallisation from benzene, gave 2 : 3 : 4-tri-*O*-methyl-α-D-glucosiduronamide, m. p. and mixed m. p. 180°, $[\alpha]_D^{15}$ +147° (*c*, 0·7 in H_2O).

Oxidation by Periodate.—Buffer solutions were prepared according to Vogel ("Quantitative Inorganic Analysis," Longmans Green and Co., London, 1939, p. 808), except that sodium salts were used in all experiments. The following buffer solutions were used (total molarity ~ 0·1M): A (pH 2·0), toluene-*p*-sulphonic acid–sodium toluene-*p*-sulphonate. B (pH 4·5), acetic acid–sodium acetate. C (pH 5·3), acetic acid–sodium acetate. D (pH 5·3), disodium hydrogen phosphate–sodium dihydrogen phosphate. E (pH 7·0), sodium dihydrogen phosphate–sodium hydroxide.

A typical oxidation is described in detail. The weighed substance (10—50 mg.; sufficient to give a back-titration difference of 10—20 ml.) was dissolved in about 40 ml. of the buffer solution at the correct temperature. 0·097M-Sodium metaperiodate (5 ml.) was added, the volume made up to 50 ml. with buffer solution, and the whole set aside in the dark at the appropriate temperature. At suitable intervals, portions of 10 ml. were saturated with sodium hydrogen carbonate, and 10 ml. of 0·1N-sodium arsenite and 1 g. of potassium iodide were added. This mixture was then set aside for 15 min. and finally titrated quickly with 0·1N-iodine (starch) until addition of 1 drop of iodine gave a blue colour which persisted for 5 sec. with shaking. A control experiment was conducted similarly. The detailed results for methyl α-D-galactopyranosiduronic methyl ester are given in Table 1 and the summarised results for all the derivatives oxidised are given in Table 2.

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