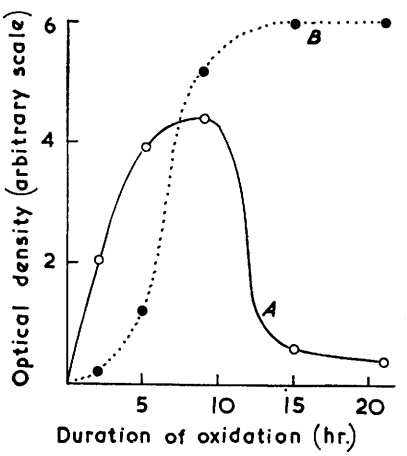
960. *Studies on Dextrans and Dextranases. Part V.*[1] *Synthesis of the Three Carboxylic Acids derived from Methyl β-Maltoside*

By D. Abbott and H. Weigel

The catalytic oxidation of methyl β-maltoside to give two monocarboxylic acids and one dicarboxylic acid is described. Evidence for their structures is presented.

In Part IV [1] we suggested that more than 18% of all branches of *Leuconostoc mesenteroides* (Birmingham) dextran consist of only one glucosyl unit. This view was based on the yields and structures of branched oligosaccharides produced when this α-1 ⟶ 3-branched dextran was treated with certain dextranases,[2] as well as on the mechanism of the action of the dextranases. A similar indication has now been obtained for the α-1 ⟶ 4-branched dextran of *L. mesenteroides* (NRRL B-1415).[3] It seemed to us that confirmatory evidence could be obtained by converting all primary hydroxyl groups of the dextrans into carboxyl groups and, after hydrolysis, isolation of the corresponding aldobiuronic acids. It thus became necessary to have available the uronic acids formed from disaccharides of D-glucose by oxidation of one or both primary hydroxyl groups. The recently reported synthesis of 4-*O*-(α-D-glucopyranosyluronic acid)-D-glucose [4] prompts us to describe our syntheses of all three uronic acids derived from maltose.[5]

The oxidation of methyl β-maltoside with oxygen in the presence of a platinum catalyst (Adams platinum dioxide reduced with hydrogen) yielded two acidic fractions (A and B, see Figure). Their mobilities during paper electrophoresis in phosphate solution



Relative concentrations of mono- (*A*) and dicarboxylic acids (*B*) produced from methyl β-maltoside

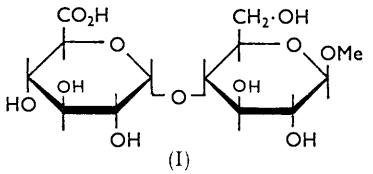
(pH 7·2) [A, $M_{GA}(P)$ 0·77; B, $M_{GA}(P)$ 1·33; for definition of all mobilities see Experimental section] and their rate of formation (and disappearance) on prolonged oxidation indicated that fraction A contained methyl 4-*O*-(α-D-glucopyranosyluronic acid)-β-D-glucopyranoside (I) and/or methyl 4-*O*-α-D-glucopyranosyl-β-D-glucopyranosiduronic acid (II) and that fraction B was methyl 4-*O*-(α-D-glucopyranosyluronic acid)-β-D-glucopyranosiduronic acid (III). For the preparation of the monocarboxylic acids (A) and the dicarboxylic acids (B) methyl β-maltoside was oxidised for 8·5 and 16 hr., respectively.

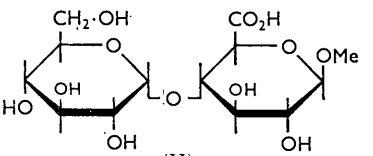
It was conceived that chromatographic resolution of the two monocarboxylic acids

[1] Part IV, D. H. Hutson and H. Weigel, *Biochem. J.*, 1963, **88**, 588.
[2] E. J. Bourne, D. H. Hutson, and H. Weigel, *Biochem. J.*, 1963, **86**, 555.
[3] D. Abbott and H. Weigel, unpublished results.
[4] G. G. S. Dutton and K. N. Slessor, *Canad. J. Chem.*, 1964, **42**, 1110.
[5] Presented at Internationales Symposium über die Chemie der Kohlenhydrate, Münster (Germany), July 1964.

(I and II) might be possible after treatment with benzaldehyde since only acid (II) could easily form a benzylidene derivative (involving the hydroxyl groups on C-4 and C-6 of the D-glucose portion). Indeed, this method gave the expected two components. Partial hydrolysis of the benzylidene derivative gave acid (II).

The following evidence, in addition to the above, shows that the component remaining unaffected by benzaldehyde was in fact acid (I) contaminated with small quantities of acid (II). Hydrolysis gave, as the main product, 4-*O*-(α-D-glucopyranosyluronic acid)-D-glucose (IV) and small quantities of materials which had R_G values identical with those of glucuronic acid, glucurone, glucose, and methyl β-D-glucopyranosiduronic acid (VII). Owing to the relative stabilities to acid hydrolysis of glycosides of D-glucuronic acid and D-glucose,[6] acid (IV) would indeed be expected as the main product from acid (I), whereas D-glucose and acid (VII) are expected from acid (II). Thus, the acid hydrolysis also degraded effectively the contamination (II), making the isolation of acid (IV) possible. Successive treatment of acid (IV) with (*a*) methanolic hydrogen chloride, (*b*) methyl iodide–silver oxide, and (*c*) lithium aluminium hydride gave a syrup which had OMe 47·5%, close to that expected for a methyl hexa-*O*-methylmaltoside (OMe, 49·3%). The hydrolysis products of this syrup were 2,3,6- and 2,3,4-tri-*O*-methyl-D-glucose. The former was


(I)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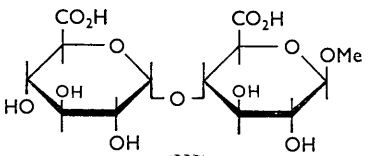
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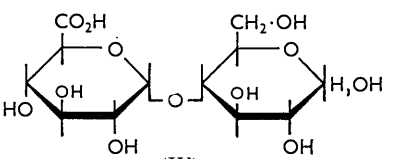
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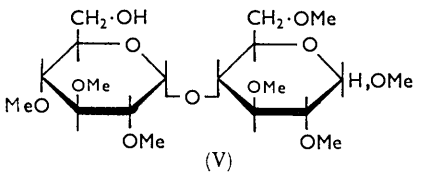
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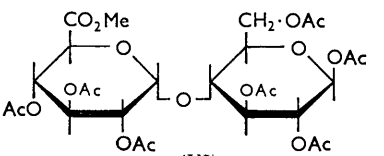
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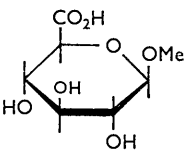
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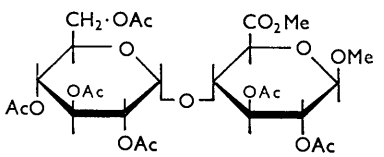
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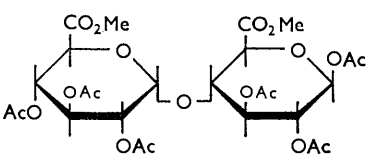
(IX)

obtained crystalline. The latter, obtained as a syrup, was converted into the crystalline 2,3,4-tri-*O*-methyl-*N*-*p*-nitrophenyl-D-glucosylamine. The tri-*O*-methyl-D-glucoses were further characterised by reduction, periodate oxidation, and subsequent demethylation to give products which were chromatographically and electrophoretically identical with threose (from 2,3,6-tri-*O*-methyl-D-glucose) and xylose (from 2,3,4-tri-*O*-methyl-D-glucose). This showed that the syrup was the methyl hexa-*O*-methylmaltoside (V). Acid (IV) also gave a methyl ester hepta-acetate which exhibited absorption at 835 and 885 cm.$^{-1}$,

[6] D. B. Easty, *J. Org. Chem.*, 1962, **27**, 2102.

indicative of α- and β-configurations at the anomeric carbon atoms [7] and expected for 1,2,3,6-tetra-*O*-acetyl-4-*O*-(methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosyluronate)-β-D-glucopyranose (VI).

Chromatography of the acid hydrolysate of acid (II) revealed the expected components. Acid (II) was esterified, methylated, and then reduced. The hydrolysate of the product contained two components which had migration rates (chromatography and electrophoresis) identical with those of 2,3,4,6-tetra- and 2,3-di-*O*-methyl-D-glucose. Acid (II) also gave a methyl ester hexa-acetate which exhibited absorption at 840 and 895 $cm.^{-1}$. These results show that acid (II) and its methyl ester hexa-acetate were in fact methyl 4-*O*-α-D-glucopyranosyl-β-D-glucopyranosiduronic acid (II) and methyl [4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)(methyl-2,3-di-*O*-acetyl-β-D-glucopyranosid)]uronate (VIII), respectively.

The equivalent weight (198) of the acid fraction B was close to that expected for a compound with structure (III) (192) and confirmed the conclusion drawn from electrophoresis. Hydrolysis with 90% formic acid produced a reasonable quantity of D-glucuronic acid (isolated as D-glucurone). Partial hydrolysis gave a dicarboxylic acid (electrophoretic evidence) which was converted into a diester (methyl) hexa-acetate. This exhibited absorption at 845 and 890 $cm.^{-1}$. Thus, acidic fraction B was methyl 4-*O*-(α-D-glucopyranosyluronic acid)-β-D-glucopyranosiduronic acid (III) and the di-ester hexa-acetate was methyl [1,2,3-tri-*O*-acetyl-4-*O*-(methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosyluronate)-β-D-glucopyran]uronate (IX).

Experimental

General.—(i) *Paper chromatography.* The solvents used were (*a*) ethyl acetate–acetic acid–formic acid–water (18 : 8 : 3 : 6); (*b*) butan-1-ol–ethanol–water (40 : 1 : 19); (*c*) butan-1-ol–ethanol–water–ammonia (40 : 10 : 49 : 1, organic phase); (*d*) ethyl acetate–acetic acid–water (9 : 2 : 2). Migration rates are expressed relative to the movements of D-glucose (R_G) and 2,3,4,6-tetra-*O*-methyl-D-glucose (R_{TMG}).

(ii) *Paper electrophoresis.* Migration rates are expressed relative to the movements of D-glucuronic acid [$M_{GA}(P)$], sorbitol [$M_S(Mo)$], D-ribose [$M_R(As)$], and D-glucose [$M_G(B)$]. The symbols *P*, *Mo*, *As*, and *B* refer to the electrolytes used: (*a*) 0·2M-sodium phosphate, adjusted to pH 7·2 (*P*); (*b*) 2% sodium molybdate dihydrate, adjusted to pH 5 (*Mo*); [8] (*c*) sodium arsenite (*As*), As_2O_3 (19·8 g.) dissolved in 0·13N-sodium hydroxide (1 l.); [9] 0·2M-sodium borate (*B*).[10]

(iii) *Spray reagents.* The spray reagents used for the detection of compounds were: (*a*) silver nitrate in acetone–ethanolic sodium hydroxide; [11] (*b*) *p*-anisidine–hydrochloric acid; [12] (*c*) potassium periodatocuprate–rosaniline; [13] (*d*) aniline hydrogen phthalate.[14]

Rates of Formation of Mono- and Di-carboxylic Acids from Methyl β-Maltoside.—Platinum catalyst [Adams platinum dioxide (0·5 g.) reduced with hydrogen [15]] was added to a solution containing methyl β-maltoside [16] (1·025 g.) and sodium hydrogen carbonate (0·1 g.) in water (25 ml.). The mixture was stirred at 60° and oxygen passed through. Additional quantities of sodium hydrogen carbonate were added to maintain pH 7·5—8·5. Aliquot portions were withdrawn at time intervals, treated with Amberlite resin IR-120(H^+), and examined by paper electrophoresis in electrolyte (*a*) using spray reagent (*a*). The stained electrophoretograms were rendered translucent by impregnation with liquid paraffin. The relative optical densities of the spots with $M_{GA}(P)$ 0·77 (fraction A) and 1·25 (fraction B) were then measured with an

[7] S. A. Barker, E. J. Bourne, M. Stacey, and D. H. Whiffen, *J.*, 1954, 171.
[8] E. J. Bourne, D. H. Hutson, and H. Weigel, *J.*, 1961, 35.
[9] J. L. Frahn and J. A. Mills, *Austral. J. Chem.*, 1959, **12**, 65.
[10] A. B. Foster, *J.*, 1953, 982.
[11] W. E. Trevelyan, D. P. Procter, and J. S. Harrison, *Nature*, 1950, **166**, 444.
[12] L. Hough, J. K. N. Jones, and W. H. Wadman, *J.*, 1950, 1702.
[13] T. G. Bonner, *Chem. and Ind.*, 1960, 345.
[14] S. M. Partridge, *Nature*, 1949, **164**, 443.
[15] K. Heyns and H. Paulsen, *Adv. Carbohydrate Chem.*, 1962, **17**, 169.
[16] T. J. Schoch, E. J. Wilson, and C. S. Hudson, *J. Amer. Chem. Soc.*, 1942, **64**, 2871.

EEL Scanner which incorporates a light source, a selenium cell, and a microammeter. The results are shown in the Figure.

Preparation of Monocarboxylic Acids from Methyl β-*Maltoside.*—(i) Methyl β-maltoside (4·1 g.) was oxidised in a manner similar to that described above except that the reaction was terminated after 8·5 hr. After cooling, the reaction mixture was filtered through " Celite." The solution was treated with Amberlite resin IR-120(H^+) followed by treatment with Duolite resin A4 (OH^-). From the latter acidic materials were eluted with N-sodium hydroxide. The eluate was freed from sodium ions by treatment with Amberlite resin IR-120(H^+) and then evaporated to a syrup. Preparative paper chromatography of the syrup using solvent (*a*) yielded two syrupy fractions: A (1·23 g.), with R_G 0·61 and $M_{GA}(P)$ 0·77; B (132 mg.), with R_G 0·73 and $M_{GA}(P)$ 1·25.

(ii) *Separation of monocarboxylic acids.* A mixture of the acidic fraction A (1·5 g.), benzaldehyde (4 g.), and freshly fused zinc chloride (1·5 g.) was shaken for 15 hr. Most of the excess of benzaldehyde was distilled off *in vacuo.* Ethanol (10 ml.) was added followed by light petroleum (b. p. 100—120°; 20 ml.). The lower layer of the two-phase mixture was concentrated *in vacuo.* Preparative paper chromatography using solvent (*b*) and spray reagent (*a*) yielded methyl 4-*O*-(α-D-glucopyranosyluronic acid)-β-D-glucopyranoside (I; 900 mg.; R_G 0·79), contaminated with small quantities of unchanged methyl 4-*O*-α-D-glucopyranosyl-β-D-glucopyranosiduronic acid (II) (see below), and the *O*-benzylidene derivative of acid (II) [365 mg. R_G 3·2 (streak)].

The *O*-benzylidene derivative of acid (II) (360 mg.) was heated in 0·1N-methanolic hydrogen chloride (10 ml.) at 60° for 30 min. After neutralisation with N-sodium methoxide, the solution was evaporated to dryness *in vacuo.* The residue was extracted with ethanol, the extract concentrated, and the syrupy residue heated *in vacuo* at 125° for 30 min. Fractionation by paper chromatography using solvent (*a*) gave acid (II) (150 mg.), with R_G 0·61 and $M_{GA}(P)$ 0·77, as the main component. In addition, small amounts of components with R_G values identical with those of glucuronic acid, glucose, and methyl β-D-glucopyranosiduronic acid were present in the hydrolysate.

Characterisation of Acid (I).—(i) Acid (I) could be detected on paper chromatograms and electrophoretograms with spray reagent (*a*) but not with (*b*).

(ii) *Partial hydrolysis.* Acid (I) (900 mg.) was partially hydrolysed with N-sulphuric acid (25 ml.) at 90° for 2 hr. After cooling, sulphuric acid was removed by extraction with a 5% solution of *NN*-di-n-octylmethylamine in chloroform (4 × 20 ml.). Preparative paper chromatography of the concentrated aqueous layer, using solvent (*d*) and spray (*a*), gave 4-*O*-(α-D-glucopyranosyluronic acid)-D-glucose (IV, 660 mg.) with R_G 0·29, $M_{GA}(P)$ 0·77, and $[\alpha]_D$ +113° (water). Small amounts of components with R_G values identical with those of glucuronic acid, glucurone, glucose [all revealed by spray (*a*)], and methyl β-D-glucopyranosiduronic acid [revealed by sprays (*b* and *c*)] were also present in the hydrolysate.

(iii) O-*Methyl-D-glucoses from acid* (IV). Acid (IV) (0·56 g.) was heated under reflux with 2% methanolic hydrogen chloride (150 ml.) for 6 hr. The solution was neutralised with silver carbonate, filtered, and concentrated *in vacuo.* The syrup was methylated according to the method of Kuhn, Baer, and Seeliger.[17] The methylated material was dissolved in a mixture of dioxan and ether (1 : 1 v/v; 80 ml.). A solution of lithium aluminium hydride (75 mg.) in the above solvent (80 ml.) was added dropwise with stirring. After *ca.* 18 hr. the excess of lithium aluminium hydride was destroyed by addition of ethyl acetate (1·5 ml.), followed by water (5 ml.). The reaction mixture was adjusted to pH 8 with N-sulphuric acid, filtered, and extracted with chloroform. Removal of the chloroform by distillation *in vacuo* left a clear syrup (0·49 g.) which had OMe, 47·5%.

The syrup (*ca.* 0·4 g.) was hydrolysed with 2N-hydrochloric acid (80 ml.) at 100° for 4 hr. The solution was neutralised with silver carbonate, filtered, and concentrated *in vacuo.* Paper chromatography of the residue using solvent (*c*) and spray (*b*) revealed the presence of only two, not well separated, components with R_{TMG} 0·90 and 0·88. The faster-moving component gave a yellow-brown stain, whereas the slower stained pink. The remainder of the residue was fractionated into two components on a charcoal–Celite column [18] (3·5 × 44 cm.) with aqueous ethyl methyl ketone as the eluant [linear gradient; 2·5% solution (1·5 l.), 5·5% solution (1·5 l.)].

[17] R. Kuhn, H. H. Baer, and A. Seeliger, *Annalen*, 1958, **611**, 236.
[18] B. Lindberg and B. Wickberg, *Acta Chem. Scand.*, 1954, **8**, 569.

The component which was eluted first stained pink with spray reagent (*d*) and was crystallised (90 mg.) from ether, m. p. 116°, $[\alpha]_D^{21}$ +70° (equilibrium, in water) (Found: C, 48·2; H, 8·0. Calc. for $C_9H_{18}O_6$: C, 48·6; H, 8·2%). Admixture with authentic 2,3,6-tri-*O*-methyl-D-glucose,[19] m. p. 115—116°, caused no depression in m. p.

The second component (145 mg., syrup) stained yellow-brown with spray reagent (*b*) and gave a crystalline *N*-*p*-nitrophenylglycosylamine, m. p. 220—222°. Admixture with authentic 2,3,4-tri-*O*-methyl-*N*-*p*-nitrophenyl-D-glucosylamine,[20] m. p. 219—222°, caused no depression in m. p.

(iv) *Further characterisation of the tri-*O*-methyl-D-glucoses.* The tri-*O*-methyl-D-glucoses (4—5 mg.) were separately reduced with sodium borohydride. Treatment of the deionised solutions with sodium metaperiodate in the dark at room temperature gave the following results. The reduced 2,3,6- and 2,3,4-tri-*O*-methyl-D-glucose respectively consumed 0·92 and 1·05 moles of periodate and gave 0 and 1·04 moles of formaldehyde per mole of tri-*O*-methylhexitol.

The excess of periodate in each solution was destroyed by addition of ethylene glycol. The deionised solutions were concentrated, and the residues dried *in vacuo* and demethylated with boron trichloride.[21] Paper chromatography and electrophoresis of the demethylated materials revealed in each case the presence of a single product. That from 2,3,6-tri-*O*-methyl-D-glucose had R_G 2·0 [solvent (*b*)] and $M_S(Mo)$ 0·56 identical with those of threose. The product from 2,3,4-tri-*O*-methyl-D-glucose had R_G 1·6 [solvent (c)] and $M_R(As)$ 0·18, identical with those of xylose.

(v) *Methyl ester hepta-acetate of acid* (IV). Acid (IV) (80 mg.) in methanol (7·5 ml.) was treated with a solution of diazomethane in ether [22] (*ca.* 30 ml.). After 30 min. the solution was evaporated to dryness. Acetic anhydride (15 ml.) and anhydrous sodium acetate (75 mg.) were added, the mixture was heated on the water-bath for 2 hr., and then poured into ice–water. The water was extracted with chloroform. The residue obtained after removal of chloroform was crystallised from methanol to give 1,2,3,6-tetra-*O*-acetyl-4-*O*-(methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosyluronate)-β-D-glucopyranose (VI, 60 mg.), m. p. 193—195°, $[\alpha]_D$ +79° (in chloroform) (Found: C, 48·8; H, 5·6; MeO, 4·9; Ac, 43·8. Calc. for $C_{27}H_{36}O_{19}$: C, 48·8; H, 5·5; MeO, 4·7; Ac, 45·3%). It exhibited absorption at 835 and 885 cm.$^{-1}$. Dutton and Slessor report m. p. 197—198°, $[\alpha]_D$ +77° (in chloroform).[4]

Characterisation of Acid (II).—(i) *Hydrolysis.* Acid (II) (5 mg.) was hydrolysed with N-sulphuric acid at 80° for 2 hr. Sulphuric acid was removed by extraction with 5% of *NN*-di-n-octylmethylamine in chloroform. Paper chromatography of the aqueous solution using solvent (*d*) revealed the presence of components with R_G values identical with those of acid (IV) (trace), glucuronic acid, glucose (large spot), methyl β-D-glucopyranosiduronic acid (large spot), and glucurone (trace).

(ii) O-*Methyl-D-glucoses from acid* (II). Acid (II) (150 mg.) was esterified by treatment with diazomethane, methylated, reduced, and hydrolysed under conditions similar to those described for acid (IV). Paper chromatography and electrophoresis of the hydrolysate revealed the presence of only two components which had R_{TMG} 1·0 and 0·68 [solvent (*c*)], M_G (*B*) 0 and 0·12, identical with those of 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3-di-*O*-methyl-D-glucose, respectively.

(iii) *Methyl ester hexa-acetate of acid* (II). Acid (II) (40 mg.) was esterified and acetylated as described for acid (IV) to give methyl [4-O-(2,3,4,6-*tetra*-O-*acetyl*-α-D-*glucopyranosyl*)(*methyl* 2,3-*di*-O-*acetyl*-β-D-*glucopyranosid*)]*uronate* (VIII; 22 mg.), m. p. 173—177°, $[\alpha]_D$ +80° (in chloroform) (Found: C, 49·2; H, 5·7; MeO, 9·6; Ac, 38·5. $C_{26}H_{36}O_{18}$ requires C, 49·1; H, 5·7; MeO, 9·8; Ac, 40·6%). It exhibited absorption at 840 and 895 cm.$^{-1}$.

Preparation of Acid (III).—Methyl β-maltoside (2·5 g.) was oxidised with oxygen in the presence of platinum catalyst, as described above except that the oxidation was continued for 18 hr. when acid (III) was the main acidic product (181 mg.). Its equivalent weight, determined by titration with alkali,[23] was 198.

Characterisation of Acid (III).—Acid (III) (181 mg.) was hydrolysed with N-sulphuric acid (90 ml.) at 100° for 15 hr. Sulphuric acid was extracted with 5% *NN*-di-n-octylmethylamine

[19] E. Schlüchterer and M. Stacey, *J.*, 1945, 776.
[20] J. W. Van Cleve, W. C. Schaefer, and C. E. Rist, *J. Amer. Chem. Soc.*, 1956, **78**, 4435.
[21] T. G. Bonner, E. J. Bourne, and S. McNally, *J.*, 1960, 2929.
[22] T. J. de Boer and H. J. Backer, *Rec. Trav. chim.*, 1954, **73**, 229.
[23] R. L. Whistler and M. S. Feater, "Methods in Carbohydrate Chemistry," ed. R. L. Whistler and M. L. Wolfrom, Academic Press Inc., New York, 1962, p. 467.

in chloroform. Preparative paper chromatography of the concentrated aqueous layer using solvent (*a*) and spray (*b*) gave a component (90 mg.) with R_G 0·31 and $M_{GA}(P)$ 1·3. (The hydrolysate contained also a small quantity of a component with chromatographic and electrophoretic properties identical with those of glucuronic acid.) This was esterified and acetylated as described for acid (IV) to give *methyl* [1,2,3-*tri*-O-*acetyl*-4-O-(*methyl* 2,3,4-*tri*-O-*acetyl*-α-D-*glucopyranosyluronate*)-β-D-*glucopyran*]*uronate* (IX, 42 mg.), m. p. 215—220° (from methanol), $[\alpha]_D$ +88° (in chloroform) (Found: C, 48·4; H, 5·4; MeO, 9·7; Ac, 38·4. $C_{26}H_{34}O_{19}$ requires C, 48·0; H, 5·3; MeO, 9·5; Ac, 39·7%).

Another sample of acid (III) (70 mg.) was hydrolysed with 90% formic acid (10 ml.) at 100° for *ca.* 15 hr. The solution was evaporated to dryness and the residue further hydrolysed with N-sulphuric acid (2 ml.) at 100° for 2 hr. Sulphuric acid was removed by extraction with 5% *NN*-di-n-octylmethylamine in chloroform. The aqueous solution was treated with Amberlite resin IR-4B(OH^-) and the resin eluted with 10% aqueous formic acid. Formic acid was extracted from the eluate with ether. After removal of water by distillation *in vacuo* the residue was crystallised from glacial acetic acid to give a product (15 mg.) with m. p. 174—175°, $[\alpha]_D^{18}$ +16° (in water). Admixture with authentic D-glucurone,[24] m. p. 174—175°, caused no depression in m. p.

We thank Professor E. J. Bourne for discussions.

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[24] J. K. N. Jones and M. B. Perry, *J. Amer. Chem. Soc.*, 1957, **79**, 2787.