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SYNTHESIS OF TWO FULLY METHYLATED PSEUDOALDOBIOURONIC ACIDS*

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ABSTRACT

Reaction of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**1**) with methyl (methyl 2-*O*-methyl-α-D-galactopyranosid)uronate (**2**) in the presence of silver carbonate yielded the crystalline pseudoaldobiouronic acid derivatives methyl [methyl 2-*O*-methyl-3-*O*-(**3**) and 4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-D-galactopyranosid]uronate (**4**). Deacetylation of **3** and **4** followed by Kuhn methylation afforded mainly crystalline, fully methylated products **5** and **9**. A by-product from **3** was methyl [methyl 4-deoxy-2-*O*-methyl-3-*O*-(2,3,4,6-tetra-*O*-methyl-β-D-glucopyranosyl)-β-L-*threo*-hex-4-enopyranosid]uronate (**6**), the structure of which was confirmed by mass spectrometry.

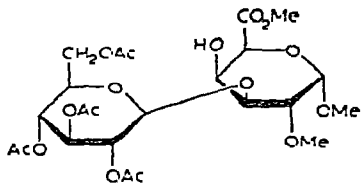
INTRODUCTION

The synthesis of uronic acid-containing oligosaccharides has been undertaken to obtain model compounds for studies of mass-spectrometric fragmentation and base-catalyzed degradation. In the preceding paper[1], we described the synthesis of methyl [methyl 3,4-di-*O*-methyl-2-*O*-(2,3,4,6-tetra-*O*-methyl-β-D-glucopyranosyl)-α-D-galactopyranosid]uronate, and we now report on the synthesis of fully methylated (1→3)- and (1→4)-linked pseudoaldobiouronic acids of the same type.

RESULTS AND DISCUSSION

Koenigs–Knorr condensation of tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**1**) with methyl (methyl 2-*O*-methyl-α-D-galactopyranosid)uronate[2] (**2**) gave two crystalline disaccharide derivatives in the ratio 3–4:1 (t.l.c.), the elemental analyses of which agreed with the structures **3** and **4**. Since HO-4 (axial) in **2** is less reactive[3–5] than HO-3 (equatorial), it was assumed that the (1→4)-linked product **4** was that formed in lower yield. The products **3** and **4** were isolated by column chromatography in yields of 50 and 14.6%, respectively, and their structures were established by mass spectrometry.

*Synthesis and Reactions of Uronic Acid Derivatives: Part XIII. For Part XII, see Ref. 1.



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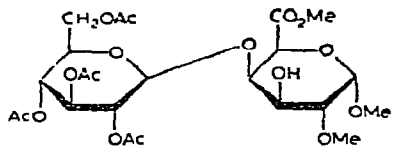

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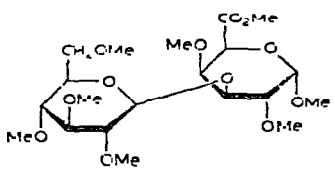
The molecular weight (566) for both substances was determined[6] from the baA_1, baE_1, and baA_2 ion peaks at *m/e* 535, 507, and 503, respectively. The structure of the neutral part of the molecule was confirmed[7] by the peaks of the aA series at *m/e* 331, 271, 229, 211, 187, 169, 127, and 99. The spectrum of **4** contained intense peaks of the bH_1 series at *m/e* 74 (*m/e* 75, after *O*-deuteration), which proved[8] the presence of a CH(OH).CH(OMe) group in the uronic acid residue*. In the spectrum of **3**, only a negligible peak at *m/e* 74 was present, but there was an intense peak at *m/e* 132 (no shift on attempted *O*-deuteration) characteristic[8] of hexuronic acid *O*-methyl derivatives having HO-4 unsubstituted.

Deacetylation of **3** and **4** with aqueous potassium hydroxide followed by chromatography gave the products as amorphous, hygroscopic materials which were methylated to give **5** and **9**. Purdie methylation was impractically slow[9] and the Hakomori procedure[10] was accompanied by side-reactions. Although the use of methyl iodide–silver oxide–*N*,*N*-dimethylformamide[11] caused some β-elimination to give olefinic products, it was the most satisfactory procedure. That the major products **5** and **9**, isolated crystalline in good yield by chromatography, were completely methylated followed[6,12] from the molecular weight of the substances calculated from



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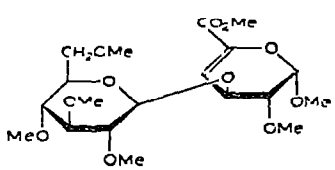

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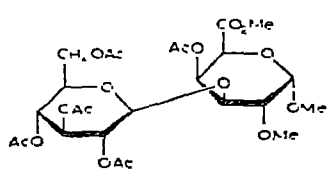

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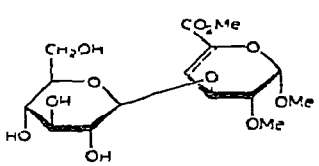

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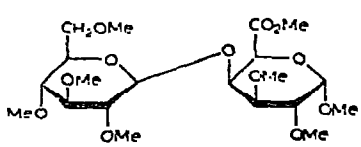

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*The correct elemental compositions of the p-ions at *m/e* 103 and 117, erroneously given in Ref. 8 as $C_5H_9O_3$ and $C_6H_{11}O_3$, are $C_4H_7O_3$ and $C_5H_9O_3$, respectively.

the *m/e* values of the A_1 ions found in their mass spectra (M = 219 + 233 + 16 = 468).

In agreement with the fact that acetal groups at position 4 in hexopyranuronates are good leaving-groups[1,2,13–15], β-elimination during methylation was more extensive (t.l.c.) for the (1→4)-linked than for the (1→3)-linked pseudoaldobiouronic acid derivative. Also, methylation of the latter compound was relatively less complete, presumably because of the low reactivity of HO-4 in the D-galacturonate moiety. The yields of **5** and **9** were similar. Small amounts of methyl 2,3,4,6-tetra-*O*-methyl-α- and β-D-glucopyranoside and methyl (methyl 4-deoxy-2,3-di-*O*-methyl-β-L-*threo*-hex-4-enopyranosid)uronate were also formed in the preparation of **9** and were identified by g.l.c.–m.s.

The olefin **6** was formed as a by-product of methylation of the (1→3)-linked pseudoaldobiouronic acid derivative. Its mass spectrum contained baA_1 (*m/e* 405), aA_1 (*m/e* 219), and bA_1 (*m/e* 201) ion peaks, which confirmed[6] the molecular weight of the substance (M = 405 + 31 = 436; M = 219 + 201 + 16 = 436). The main fragmentation pathway, characteristic[16] of methyl derivatives of 4-deoxy-β-L-*threo*-hex-4-enopyranosiduronic acid, was retro-Diels–Alder fragmentation of the olefinic ring, giving the ions bH_1 at *m/e* 88 and confirming the structure **6** except for the stereochemistry. Conclusive evidence for the structure **6** was provided by the formation of this compound by a β-elimination reaction of the acetate **7** followed by methylation of the deacetylated olefin **8**.

Reaction of **7** with methanolic sodium methoxide at room temperature gave a much lower yield of **8** than was expected by analogy with a number of similar reactions[4,17,18]. The main reaction was the deacetylation of AcO-4 of the uronic acid residue, and **8** was isolated in only 20% yield. A much higher yield of **8** from **7** was obtained when the reaction was carried out in 1,2-dimethoxyethane with sodium hydride or sodium methoxide generated *in situ*.

The possibility of depolymerization by β-elimination under the mild conditions of Kuhn methylation, as observed during preparation of **5** and **9**, should be taken into account in evaluating the results of methylation analysis of acidic polysaccharides and other uronic acid-containing substances.

EXPERIMENTAL

M.p.s. were determined on a Kofler hot-stage. Optical rotations were measured with a Perkin–Elmer automatic polarimeter Model 141. Mass spectra were obtained at 74 eV with a JMS-100-D spectrometer. The temperature at the site of evaporation was 130–140° and that in the ionizing chamber was 180°. For g.l.c.–m.s., spectra were obtained at 23 eV with the mass spectrometer connected to a JGC-20-K gas chromatograph [a stainless-steel column (200 × 0.3 cm) packed with 3% of OV-225 on Chromosorb W-AW-DMCS (100/200 mesh)]. Analyses were conducted isothermally at 160° and a helium inlet pressure of 98.1 kPa.

Deuterium exchange was effected by concentration (thrice) of solutions in methanol-*d*.

T.l.c. was performed on Silica gel G and column chromatography on dry-packed silica gel (Merck, 9385) with *A*, chloroform–acetone (8:1); *B*, carbon tetrachloride–acetone (2.5:1); *C*, chloroform–acetone (6:1); *D*, chloroform–methanol (4:1); *E*, benzene–acetone (5:1); *F*, chloroform–methanol (7:1); and *G*, benzene–acetone (6:1). Prior to packing, the silica gel was equilibrated with 40% (v/w) of the mobile phase[5]. Detection was effected by charring with sulfuric acid in ethanol, or spraying with 0.1% potassium permanganate in acetone. The latter reagent revealed olefinic substances immediately as yellow spots on a violet background.

Silver oxide and silver carbonate were freshly prepared[19,20] before use. *N,N*-Dimethylformamide was dried[21] and freshly distilled. Unless otherwise stated, the solutions were concentrated at 2 kPa (15 Torr) and 40°.

Methyl [methyl 2-O-methyl-3-O- (**3**) *and -4-O-(2,3,4,6-tetra-O-acetyl-β-*D*-gluco-pyranosyl)-α-*D*-galactopyranosid]uronate* (**4**). — A mixture of **2** (2 g, 8.46 mmol), silver carbonate (2.4 g), and Drierite (6 g) in dry benzene (30 ml) was stirred in the dark for 4 h at room temperature. After the addition of iodine (0.4 g) and **1** (3.5 g, 8.8 mmol), stirring was continued for 2 h at 40–45°, and then fresh portions of **1** (1.75 g, 4.4 mmol) and silver carbonate (1.2 g) were added. After a further 2 h, t.l.c. (solvent *A*) showed the absence of **1** (R_F 0.8) and the presence of **3** and **4** (R_F 0.3 and 0.15), together with unreacted **2** (R_F 0.1). A small amount of the product of hydrolysis (R_F 0.4) of **1** was also present. The reaction mixture was worked-up in the usual manner and the crude product was chromatographed on a column (95 × 4.5 cm) of silica gel. Solvent *B* removed the hydrolysis product of **1** and elution with solvent *C* then gave chromatographically homogeneous **3** (2.4 g, 50%) and **4** (0.7 g, 14.6%).

After three crystallisations from chloroform–ether (1:1), **3** had m.p. 82–85°, $[\alpha]_D^{22}$ +60° (*c* 1, chloroform). The melt solidified on standing and then had m.p. 118–120.5°, $[\alpha]_D^{22}$ +56° (*c* 1, chloroform). When a solution of this material was seeded with the higher-melting substance, the lower-melting modification was obtained. Both modifications gave identical mass spectra (Found: C, 48.58; H, 5.92. $C_{23}H_{34}O_{16}$ calc.: C, 48.76; H, 6.05%).

After two crystallisations from chloroform–isopropyl ether (1:1), **4** had m.p. 174–175°, $[\alpha]_D^{22}$ +35° (*c* 0.97, chloroform) (Found: C, 48.38; H, 6.06%).

*Methyl [methyl 2,4-di-*O*-methyl-3-*O*-(2,3,4,6-tetra-*O*-methyl-β-*D*-glucopyranosyl)-α-*D*-galactopyranosid]uronate* (**5**). — Water (12.5 ml) was added to a solution of **3** (2 g) in 1,2-dimethoxyethane (25 ml), the solution was cooled in ice, and M aqueous potassium hydroxide (6.3 ml) was added slowly with stirring. Another portion of M potassium hydroxide (25 ml) was added after 30 min, and the solution was kept at 55° for 90 min. After cooling in ice, the solution was deionized with Dowex-50W(H^+) resin, filtered, and concentrated with co-distillation with water to remove acetic acid, the residue was dissolved in methanol, and excess of diazomethane in ether was added. T.l.c. (solvent *D*) then revealed that, in addition to methyl (methyl 3-*O*-β-D-gluco-pyranosyl-2-*O*-methyl-α-D-galactopyranosid)uronate (R_F 0.25), small proportions of faster-moving, minor products (R_F 0.4 and 0.55) were also present. After purification on a column (32 × 3 cm) of silica gel with solvent *D*, the pure, deacetylated oligo-

saccharide (1.15 g, 2.9 mmol; 81.8%), obtained as an almost colourless, hygroscopic syrup, was dissolved in dry *N*,*N*-dimethylformamide (30 ml) and shaken in the dark with silver oxide (13.4 g; 57.8 mmol, 4 equiv./OH) and methyl iodide (3.6 ml; 57.8 mmol, 4 equiv./OH). Chloroform (15 ml) was added after 24 h, the mixture was filtered, the solids washed with chloroform, and the filtrate was concentrated (finally at 70° to remove *N*,*N*-dimethylformamide). T.l.c. examination (solvent *E*) of the residue showed the presence of **5** (R_F 0.3) and small amounts of products resulting from undermethylation (R_F ~0.1), together with the olefin **6** (R_F 0.45, detection with both reagents). Fractionation by column (32 × 3 cm) chromatography with solvent *E* gave pure **5** (0.93 g; 56.2% based on **3**, 68.9% based on the deacetylated oligosaccharide) and **6** (0.06 g, 4.75% based on the deacetylated oligosaccharide).

Compound **5**, when crystallized from ether–heptane (1:1, twice) at room temperature and then from isopropyl ether at 0°, had m.p. 85–86° (sintering at 82°), $[\alpha]_D^{22}$ +86° (*c* 1, chloroform) (Found: C, 51.38; H, 7.70. $C_{20}H_{36}O_{12}$ calc.: C, 51.27; H, 7.75%).

Olefin **6** had $[\alpha]_D^{22}$ +139° (*c* 1.03, chloroform) and was indistinguishable (t.l.c., m.s.) from the product described below.

Methyl [*methyl 2,3-di*-O-*methyl-4*-O-(*2,3,4,6-tetra*-O-*methyl*-β-D-*glucopyranosyl*)-α-D-*galactopyranosid*]*uronate* (**9**). — Deacetylation of **4** (0.56 g), as described for **3**, gave syrupy, hygroscopic methyl (methyl 4-*O*-β-D-glucopyranosyl-2-*O*-methyl-α-D-galactopyranosid)uronate (0.35 g, 89.3%), which was methylated under the conditions described in the preparation of **5**. The crude product contained **9** (R_F 0.25, solvent *E*), some undermethylated material, and substances having the same chromatographic mobility as methyl (methyl 4-deoxy-2,3-di-*O*-methyl-β-L-*threo*-hex-4-enopyranosid)-uronate (R_F 0.6) and methyl 2,3,4,6-tetra-*O*-methyl-αβ-D-glucopyranoside (R_F 0.55). Elution from a column (27 × 2.7 cm, solvent *E*) of silica gel gave first the unresolved mixture of the faster-moving components, which was examined by g.l.c.–m.s. The three components had retention times (relative to methyl 2,3,4,6-tetra-*O*-methyl-α-D-glucopyranoside) 0.65, 1.00, and 2.74. The mass spectra of the substances confirmed[16,22] the results of the t.l.c. analysis. Subsequently eluted was pure **9** (0.27 g, 58.3% based on **4**, or 65.8% based on the deacetylated oligosaccharide) having m.p. 87–88° (from isopropyl ether, twice), $[\alpha]_D^{22}$ +78.8° (*c* 1, chloroform) (Found: 51.0; H, 7.78%).

Methyl [*methyl 4*-O-*acetyl-2*-O-*methyl-3*-O-(*2,3,4,6-tetra*-O-*acetyl*-β-D-*glucopyranosyl*)-α-D-*galactopyranosid*]*uronate* (**7**). — Acetic anhydride (10 ml) was added to a solution of **3** (2 g) in dry pyridine (10 ml), and the reaction mixture was kept at 40° for 16 h. T.l.c. (solvent *E*) then showed that starting material (R_F 0.1) was still present. The crude product, isolated in the usual manner, was eluted from a column (35 × 3 cm) of silica gel to give **7** (2.1 g, 97.7%; R_F 0.25) as a white, amorphous solid, $[\alpha]_D^{22}$ +97° (*c* 1.06, chloroform) (Found: C, 49.65; H, 5.90. $C_{25}H_{36}O_{17}$ calc.: C, 49.34; H, 5.96%).

Methyl (*methyl 4-deoxy-3*-O-β-D-*glucopyranosyl-2*-O-*methyl*-β-L-*threo-hex-4-enopyranosid*)*uronate* (**8**). — (*a*) Compound **7** (0.2 g, 0.33 mmol) was added with

stirring to a mixture of dry methanol (6 ml), dry 2,2-dimethoxypropane (0.2 ml), and M methanolic sodium methoxide (0.4 ml, 0.4 mmol). The solution was kept at room temperature with the exclusion of atmospheric moisture and carbon dioxide for 15 min; t.l.c. then showed that no starting material (R_F 0.3, solvent *E*) was present. The solution was cooled in ice, deionized with Dowex-50W(H^+) resin, and filtered, and ethereal diazomethane was added to the filtrate. Concentration then gave a syrup containing (t.l.c., solvent *F*) the deacetylated starting material (R_F 0.1) and a small proportion of the olefin **8** (R_F 0.25, detection with both reagents). Fractionation on a column (20 × 2 cm) of silica gel gave **8** (25 mg, 20%) as a white, solid foam, $[\alpha]_D^{22}$ +135° (*c* 1.03, methanol) (Found: C, 47.57; H, 6.40. $C_{15}H_{24}O_{11}$ calc.: C, 47.36; H, 6.36%).

Subsequently eluted was methyl (methyl 3-*O*-β-D-glucopyranosyl-2-*O*-methyl-α-D-galactopyranosid)uronate (0.09 g, 69.2%).

(*b*) Compound **7** (0.4 g, 0.66 mmol) was added with stirring to dry 1,2-dimethoxyethane (15 ml) containing dry methanol (0.27 ml, 6.6 mmol) and sodium hydride (0.16 g, 6.6 mmol), and the mixture was stirred with the exclusion of atmospheric moisture and carbon dioxide for 30 min; t.l.c. then showed that no starting material was present. The reaction mixture was worked-up as described above, and the product contained (t.l.c.) olefin **8** and the deacetylated starting material in the ratio ~1:1, together with a small amount of faster-moving, olefinic substances (R_F 0.4 and 0.6) that were not further examined. Chromatography of the mixture on silica gel gave **8** (0.121 g, 48.4%) and methyl (methyl 3-*O*-β-D-glucopyranosyl-2-*O*-methyl-α-D-galactopyranosid)uronate (0.104 g, 40%).

(*c*) Compound **7** (0.25 g, 0.41 mmol) was added with stirring to a mixture of dry 1,2-dimethoxyethane (8 ml) and sodium hydride (0.05 g, 2 mmol). The mixture was stirred with the exclusion of atmospheric moisture and carbon dioxide at 20°; no reaction occurred (t.l.c.) during 2 h, and stirring was continued at 80° for 24 h, after which time no starting material was present. Work-up and chromatography of the product, as described in (*a*), gave **8** (0.141 g, 90.4%) identical in all respects with the product described in (*a*).

Methyl [*methyl 4-deoxy-2-*O*-methyl-3-*O*-(2,3,4,6-tetra-*O*-methyl-β-D-glucopyranosyl)-β-*L*-threo-hex-4-enopyranosid*]*uronate* (**6**). — Compound **8** (0.1 g) was methylated in *N*,*N*-dimethylformamide (3 ml) with methyl iodide (0.32 ml) and silver oxide (1.2 g), as described in the preparation of **5**. T.l.c. (solvent *G*) of the crude product showed that **6** (R_F 0.25) and some undermethylated material (R_F 0.15) were present. Chromatography on a column (18 × 2 cm) of silica gel with solvent *G* gave pure **6** (0.97 g, 84.4%), $[\alpha]_D^{22}$ +144° (*c* 1, chloroform) (Found: C, 52.14; H, 7.22. $C_{19}H_{32}O_{11}$ calc.: C, 52.28; H, 7.39%).

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