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Note

Synthesis of an aldotriouronic acid derivative related to (4-*O*-methylglucurono)xylans*

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Acetylation of methyl 4-*O*-(2-*O*-benzyl-β-D-xylopyranosyl)-β-D-xylopyranoside (**2**) followed by catalytic debenzylation of the product **3**, gave methyl 2,3-di-*O*-acetyl-4-*O*-(3,4-di-*O*-acetyl-β-D-xylopyranosyl)-β-D-xylopyranoside (**4**). Reaction of the nucleophile **4** with methyl 2,3-di-*O*-benzyl-1-chloro-1-deoxy-4-*O*-methyl-α,β-D-glucopyranuronate (**1**) in the presence of silver perchlorate and *sym*-collidine afforded the 4-*O*-methyl-α-D-glucuronic acid-containing trisaccharide derivative **5a** as the major product. The target methyl ester methyl glycoside **7a** was obtained from **5a** by successive catalytic deacetylation and debenzylation. The structures of **7a** and its β anomer **7b** were confirmed by interpreting their ^{13}C-n.m.r. spectra and by analysis of mass-spectral fragmentation patterns of the corresponding permethyl ethers **8a** and **8b**. Criteria for distinguish between xylan-type aldotriouronic acids bearing the uronic acid at the reducing and nonreducing end of the molecule have been established by comparing mass spectra of **8a** and its positional isomer **19**. Treatment of **1** with its hydrolysis product gave the trehalose type, 4-*O*-methyl-D-glucuronic acid-containing, disaccharide derivatives **9**–**11**, which were also isolated from products of the reaction of **1** with **4**. Structures **9**–**11** were determined by further chemical conversions, and by ^{13}C-n.m.r. and mass spectrometry.

In addition to D-xylose, xylo-oligosaccharides and a small amount of 4-*O*-methyl-D-glucuronic acid, graded acid hydrolysis of hardwood (4-*O*-methylglucurono)xylans yields a homologous series of linear aldouronic acids having 4-*O*-methyl-α-D-glucuronic acid linked to O-2 of a nonreducing D-xylose end-group. With the aim of studying various properties of oligosaccharides that reflect closely the structure of natural polysaccharides, we have previously prepared, by controlled syntheses, a number of methyl β-glycosides of oligosaccharides related to xylans. We now report a synthesis of the methyl ester methyl β-glycoside **7a** related to (4-*O*-methylglucurono)-

*Synthesis and Reactions of Uronic Acid Derivatives, Part XX. For Part XIX see ref. 1. For a preliminary communication on a portion of this work see ref. 2.

xylans. Synthesis of the positionally isomeric, branched derivative **18** has been described elsewhere in this Series[3].

RESULTS AND DISCUSSION

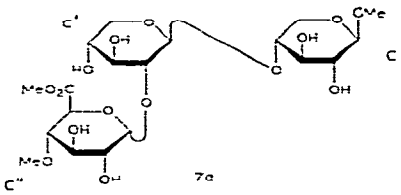
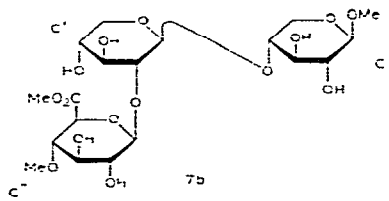
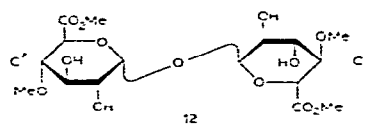
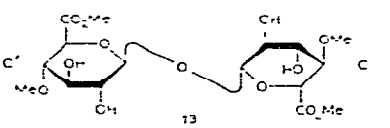
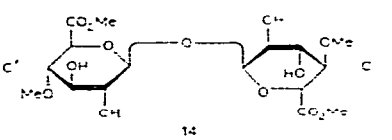
The point of departure in the present synthesis was methyl 4-*O*-(2-*O*-benzyl-β-D-xylopyranosyl)-β-D-xylopyranoside[4] (**2**), which was conventionally converted (by acetylation and debenzylation) into crystalline methyl tetra-*O*-acetyl-β-xylobioside (**4**), which has only HO-2′ unsubstituted.

Treatment of **4** with an excess of the glycosyl halide **1** was conducted under conditions[1,5] known to yield mainly 1,2-*cis* glycosides. The nucleophile **4** reacted almost completely (t.l.c.), and chromatography of the crude product gave three, non-reducing disaccharides (**9–11**), and an unresolved mixture of trisaccharides (**5a** and **5b**). To identify the non-reducing disaccharides, compounds **9–11** were converted into the hydroxyl derivatives **12–14**, and these were trideuteriomethylated to afford compounds **15–17**, which produced qualitatively identical mass spectra. The spectra confirmed that **15–17** were isomeric methyl [(methyl 2,3-di-*O*-trideuteriomethyl-4-*O*-methylhexopyranosyluronate)-2,3-di-*O*-trideuteriomethyl-4-*O*-methylhexopyranosid]uronates. The (1→1)-linkage was indicated by peaks of intense F_1 ions at *m/z* 104 and weak peaks of ions of the H_1 and J_1 series (*m/z* 94, 78, and 81). Further ions diagnostic of the structures were reflected by peaks at *m/z* 459 and 424 ($[M - CD_3OH]^+$ and $[M - 2CD_3OH]^+$, respectively) and, by analogy with ions at *m/z* 263, 231, 219, and 187, present[6] in the spectrum of 2,3,4-tri-*O*-methyl-α-D-xylopyranosyl 2,3,4-tri-*O*-methyl-β-D-xylopyranoside, at *m/z* 333, 298, 286, and 251. Configurations of the glycosidic linkage in **9–11** and products of their further conversions were tentatively assigned on the basis of specific optical rotations observed for **12–14**, and the structures were finally confirmed by analyzing their ^{13}C-n.m.r. spectra (Table I). By analogy with ^{13}C-n.m.r. spectra[7] of α,α- and β,β-trehalose, ^{13}C-n.m.r. chemical shifts observed for both pyranoid rings in the α,α- and β,β-linked non-reducing disaccharides **12** and **13** were identical and diagnostic of magnetic equivalence of the respective carbon atoms in both saccharide rings. The differences in ^{13}C-chemical shifts found in the spectrum of **14**, as compared with those in the spectra of **12** and **13**, is indicative of a different angle between the planes of the two pyranoid rings, resulting in different shielding effects to which carbon atoms in this α,β-linked dimer are exposed.

The trisaccharide derivatives **5a** and **5b** were obtained in combined yield of 95.7%. They appeared as one spot several t.l.c. systems and were only partially resolved by column chromatography. The last fractions of the mixture of **5a** and **5b** eluted from the silica-gel column were enriched in **5b**, some of which could be crystallized. The unresolved mixture of **5a** and **5b** was deacetylated, yielding a product whose t.l.c. showed two poorly-separated spots, of which the faster-moving one was indistinguishable from **6b** obtained by deacetylation of crystalline **5b** (that is, compared with the acetates **5a** and **5b**, the glycosides **6a** and **6b** showed reversed chro-

TABLE I

^{13}C-N.M.R. SPECTRAL DATA FOR **7a**, **7b** AND **12–14**

Compound	*Ring*	*Chemical shifts*								
		C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*MeO-1*	*MeO-4*	*MeO-6*
 **7a**	C	105.21	74.18	75.60	77.55	64.18	—	58.59	—	—
	C′	103.01	78.46	75.22	70.80	66.38	—	—	—	—
	C″	99.37	72.23	73.53	82.62	70 80	173.39	—	61.14	54.57
 **7b**	C	105.34	74.18	75.73	77.94	64.31	—	58.59	—	—
	C′	101.71	83.27	76.25	71.08	66.12	—	—	—	—
	C″	104.95	74.57	75.73	82.49	71.08	172.22	—	61.45	54.70
 **12**	C	95.87	71.72	73.15	82.63	71.20	172.88	—	61.46	54.71
	C′	95.87	71.72	73.15	82.63	71.20	172.88	—	61.46	54.71
 **13**	C	100.30	74.46	76.02	82.38	73.55	172.11	—	61.47	54.72
	C′	100.30	74.46	76.02	82.38	73.55	172.11	—	61.47	54.72
 **14**	C	101.85	73.40	74.50	82 20	71.20	172.70	—	61.36	56 60
	C′	104.33	73.80	75.60	82.20	71.20	172.10	—	61.36	56.60

matographic mobilities). Chromatographically purified **6a** and **6b** were debenzylated to afford the final compounds **7a** and **7b**, which were converted into the fully methylated products **8a** and **8b** that afforded qualitatively identical mass spectra. Molecular weights of the compounds could be calculated from adA_1 and bA_1 ion-peaks according to the equation: $M = adA_1 + bA_1 + 16 = 393 + 175 + 16 = 584$.

Characteristic of xylan-type aldouronic acids is a 4-*O*-methyl-α-D-glucuronic[8] or α-D-glucuronic[9–12] acid residue linked to O-2 of D-xylose residues. To find criteria for distinguishing this type of aldotriouronic acid bearing the uronic acid units at the reducing and non-reducing end, compound **18** (ref. 3) was methylated and the mass spectrum of **19** thus obtained was compared with that of **8a**. For convenience

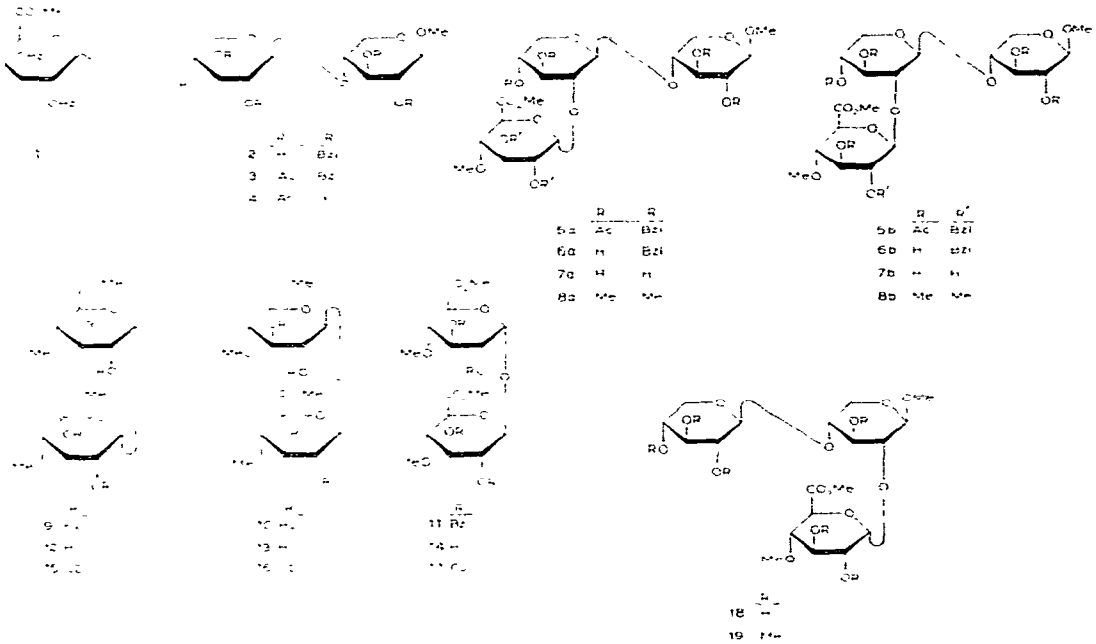
TABLE II

MASS SPECTRA (12 eV) OF **8a** AND **19**

m/z	d→2a→4b (8a)		a→4b2←d (19)		m/z	d→2a→4b (8a)		a→4d2←d (19)	
	%Σ45 × 100	Symbol	%Σ45 × 100	Symbol		%Σ45 × 100	Symbol	%Σ45 × 100	Symbol
521			3	$badA_2$	173	45	dC_2	36	dC_2
479	5	$badF_1$	52	$badF_1$	169	123	dA_3	73	dA_3
453			191	$abdJ_1$	159	27		27	
447			30	$badF_2$	157	33		33	
395	75	$dabJ_1$	62	$dabJ_1$	145	75		398	
393	27	adA_1	94	bdA_1	143	249	bA_2	1044	aA_2
375			43		142	81			
361	856	adA_2	151	bdA_2	141	49	dC_3		
329	17	adA_3	11	bdA_3	131	31		23	
319	73	adF_1			129	19		33	
305			54		115	115	bC_2	102	aC_2
303	49		19		114	55		159	
287	61	adF_2			111	71	bA_3	159	aA_3
263			27		101	1554	F_1	1929	F_1
249			22		99	33		87	
245	13		19		88	577	H_1	613	H_1
235	239	abJ_1			85	229	dK_2	23	dK_2
233	577	dA_1	621	dA_1	83	328	bC_3	14	aC_3
219	37				75	518	bJ_1	414	bJ_1
215			17		71	103		44	
201	2570	dA_2	2391	dA_2	59	11		14	
189	23		54		58	17	aK_1	19	aK_1
183			73		57			22	
175	956	bA_1	717	aA_1	45	15		19	
174			47						

of interpretation, the component sugars are designated *d*→2*a*→4*b* (**8a**) and *a*→4*b*2←*d* (**19**), where *a* and *b* = per-*O*-methyl-β-D-xylopyranose, and *d* = methyl per-*O*-methyl-α-D-glucopyranuronate. Aided by the known fragmentation of fully methylated aldobiouronic acids[13] and isomeric xylo-oligosaccharides[6], the origin of all structurally significant ion-species formed in the fragmentation of **8a** and **19** could be identified. From data in Table II it follows that the presence of a hexopyranosyluronic acid at C-2′ (ring *a*) in fully methylated xylan-type aldotriouronic acids is characterized by the formation of adF_1, adF_2, and abJ_1 ions (*m/z* 319, 287, and 235, respectively), whereas ions $abdJ_1$ (*m/z* 453) are formed from compounds bearing the uronic acid residue at O-2 (ring *b*).

Structures **7a** and **7b** fully confirmed data obtained from their ^{13}C-n.m.r. spectra (Table I), the interpretation of which was based on analyzed ^{13}C-n.m.r. spectra of a series of isomeric xylo-oligosaccharides[14], taking into account ^{13}C-n.m.r. chemical shifts found in the spectra of **12–14**.



EXPERIMENTAL

General methods. — Melting points were determined on a Kofler hot-stage. Optical rotations (*c* 1, 22°, chloroform, unless stated otherwise) were measured with a Perkin–Elmer Model 141 automatic polarimeter. Noise-decoupled, ^{13}C-n.m.r. spectra for solutions in D_2O (25°, internal standard 1,4-dioxane) were recorded with a Jeol FX-60 FT-NMR spectrometer. The ^{13}C chemical-shift of 1,4-dioxane *vs.* Me_4Si (67.96 p.p.m.) was determined separately. The spectra were measured by using a repetition time of 5.0 sec, a pulse-width of 4 μsec (4° flip angle), a sweep width of 4000 Hz, and 8 K real data points. The average number of accumulations was 5000. Chemical shifts (Table I) are given relative to Me_4Si. Mass spectra (70 and 12 eV) were recorded at an emission of 300 μA by using a JMS 100 D instrument. The temperature in the site of evaporation was, according to the volatility of the compounds, 180–220°, and that in the ionizing chamber was 200°.

T.l.c. was performed on Silica gel G and column chromatography on dry-packed silica gel (Merck, 9385) with A, 4:1 benzene–acetone; B, 4:1 benzene–ethyl acetate; C, 12:1 chloroform–methanol; and D, 4:1 chloroform–methanol. Detection was effected by charring with 5% sulfuric acid in ethanol.

Microanalyses were performed with a Perkin–Elmer Model 240 automatic analyzer. Solutions were dried with anhydrous sodium sulfate and concentrated at 40°/2kPa.

Chromatographically pure **8a**, **8b**, **15–17**, and **19** were obtained by methylation of **7a**, **7b**, **12–14**, and **18** with methyl iodide or trideuteriomethyl iodide and silver oxide in *N*,*N*-dimethylformamide.

Methyl 2,3-di-O-acetyl-4-O-(3,4-di-O-acetyl-β-D-xylopyranosyl)-β-D-xylopyranoside (**4**). — Conventional acetylation of **2** (7 g) with 1:2 pyridine–acetic anhydride (66 mL) gave crystalline methyl tetra-*O*-acetyl-2′-*O*-benzyl-β-xylobioside (**3**, 9.2 g, 91.5%), m.p. 145–147° (from ethanol), $[\alpha]_D^{22}$ −50° (Found: C, 56.27; H, 6.26. $C_{26}H_{34}O_{13}$ calc.: C, 56.30; H, 6.18).

The foregoing compound **3** (8.9 g) in 1:1 ethanol–acetone (250 mL) was hydrogenated at room temperature over 5% palladium-on-charcoal (1 g) until the starting material disappeared (~6 h), as showed by t.l.c. (solvent *A*). The product (R_F 0.4, compare 0.6 for the starting material) was isolated conventionally and crystallized from ethanol–isopropyl ether, m.p. 84–93°, $[\alpha]_D$ −77°. When dried at 30°/133 Pa, crystalline **4** (6.95 g, 93.3%) gave analytical data consistent with the substance's being a hemihydrate (Found: C, 48.23; H, 6.08. $C_{19}H_{28}O_{13} \cdot 0.5\ H_2O$ calc.: C, 48.20; H, 6.17). Drying for 2 h at 110°/2kPa gave an amorphous solid, $[\alpha]_D$ −79°, which gave analytical data consistent with the compounds being a methyl tetra-*O*-acetylpentobioside. (Found: C, 49.18; H, 5.96. $C_{19}H_{28}O_{13}$ calc.: C, 49.13; H, 6.08). To avoid possible migration of acetyl groups during dehydration at elevated temperature, the hemihydrate was used subsequently.

Methyl 4-O-[2-O-(methyl 4-O-methyl-α- (**7a**) *and β-D-glucopyranosyluronate)-β-D-xylopyranosyl]-β-D-xylopyranoside* (**7b**). — A solution of chloride **1** (75 mL of 0.2M stock solution[1], 15 mmol) was added at −10° to a stirred mixture of **4** (2.4 g, 5 mmol), *sym*-collidine (2.5 mL, 18.7 mmol) and silver perchlorate (3.12 g, 15 mmol) in dichloromethane (25 mL). Cooling was discontinued and, after 30 min, t.l.c. (solvent *B*) showed that no chloride (R_F 0.9) and only traces of **4** (R_F 0.1) were present, and that products having R_F 0.35, 0.6, 0.7 and 0.8 had been formed. A small amount of hydrolysis product of **1** (R_F 0.4) was also present. The mixture was processed as described[1], and the crude product was eluted from a column of silica gel to afford, in order, **9**, **10**, **11**, and an unresolved mixture of **5a** and **5b** (4.2 g, 95.7).

Compound **9**, which was not obtained chromatographically pure, was debenzylated and, after purification by chromatography, the resultant, amorphous **12** showed $[\alpha]_D$ +143° (water) (Found: C, 45.18; H, 6.14. $C_{16}H_{26}O_{13}$ calc.: C, 45.06; H, 6.15).

Compound **10** had m.p. 115–116° (from ethanol), $[\alpha]_D$ +13° (Found: C, 67.32; H, 6.46. $C_{44}H_{50}O_{13}$ calc.: C, 67.16; H, 6.40).

Compound **11** had m.p. 119–120° (from methanol), $[\alpha]_D$ +59.2° (Found: C, 67.35; H, 6.53. $C_{44}H_{50}O_{13}$ calc.: C, 67.16; H, 6.40).

Hydrogenolysis of **10** and **11** gave compound **13**: m.p. 212–213° (from acetone), $[\alpha]_D$ −75.5° (water) (Found: C, 44.94; H, 6.30. $C_{16}H_{26}O_{13}$ calc.: C, 45.06; H, 6.15), and compound **14**: m.p. 190–191° (from methanol–acetone), $[\alpha]_D$ +59° (water) (Found: C, 45.01; H, 6.18. $C_{16}H_{26}O_{13}$ calc.: C, 45.06; H, 6.15.

From the late fractions of the eluted mixture of **5a** and **5b**, a portion of **5b** (0.9 g) could be crystallized from methanol, m.p. 163–164°, $[\alpha]_D$ −50° (Found: C, 58.53; H, 6.31. $C_{41}H_{52}O_{19}$ calc.: C, 58.01; H, 6.18).

The mother liquor remaining after crystallization of **5b** was combined with the

unresolved mixture of **5a** and **5b**, and the material (3.3 g) was deacetylated (Zemplén). T.l.c. (solvent *C*, double development) showed presence of two, poorly separated spots (**6a** and **6b**) in ~3:1 ratio (R_F ~0.25 and 0.3) of which the faster-moving was indistinguishable from **6b** obtained by deacetylation of crystalline **5b**. Repeated chromatography yielded chromatographically pure, syrupy **6a** (1.8 g, yield of the α-linked trisaccharide, 53.4%, based on the amount of isolated **5a** + **5b**) plus **6b** (0.65 g, total yield of the β-linked trisaccharide, 40%). A portion of **6a**, when acetylated with acetic anhydride–pyridine, gave **5a** as a colorless foam, $[\alpha]_D$ +5.2° (Found: C, 57.91; H, 6.35. $C_{41}H_{52}O_{19}$ calc.: C, 58.01; H, 6.18).

Catalytic hydrogenolysis of **6a** and **6b** afforded: compound **7a**; colorless foam, R_F 0.4 (solvent *D*), $[\alpha]_D$ +19.5° (water) (Found: C, 45.70; H, 6.53. $C_{19}H_{32}O_{15}$ calc.: C, 45.60; H, 4.45); and compound **7b**: m.p. 241–242° (from methanol), R_F 0.4 (solvent *D*); $[\alpha]_D$ −66° (water) (Found: C, 45.44; H, 6.50. $C_{19}H_{32}O_{15}$ calc.: C, 45.60; H, 6.45).

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